

Wastewater Lab

Week 1

Course #2212



September 12-16, 2016

Fleming Training Center



Wastewater Treatment Lab - Week 1

Course #2212

Instructor: Barbara Loudermilk

Week 1

September 12 - 16, 2016



Monday, September 12:

8:30 Introductory Crossword

Lab Basics:

Lab Rules

Safety (GHS-SDS)

Lab Scavenger Hunt

Lab Equipment

Sampling

Tuesday, September 13:

8:30 QA/QC Program and Method Update Rule

Approved Methods of Analyses

40CFR136 excerpts, table IA, IB, II, 136.7

NPDES Permit excerpts

Common Deficiencies in the WWTP Laboratory

Dissolved Oxygen Analysis

Biochemical Oxygen Demand (BOD)

Prep Filters for TSS

Wednesday, September 14:

8:30 pH Analysis and Calibration

Whole Effluent Toxicity Testing

Solids Analysis: Total Suspended Solids; Settleable Solids (Imhoff)

Thursday, September 15:

8:30 Read Results of TSS

Alkalinity

Chemical Oxygen Demand (COD)

Solutions Chemistry

Friday, September 16:

8:30 Exam and Course Evaluation

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2022 Blanton Dr.
Murfreesboro, TN 37129

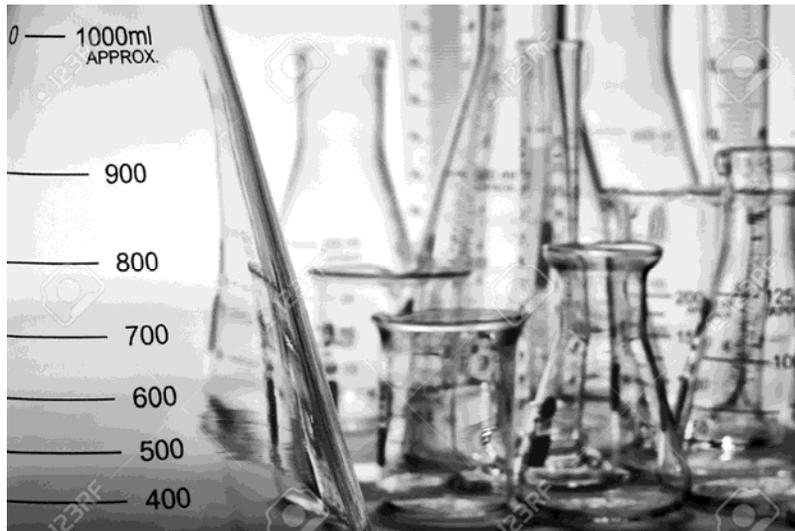
Phone: 615-686-9755
Fax: 615-898-8064
E-mail: Barbara.Loudermilk@tn.gov

Wastewater Lab Course - Week 1

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Section 1 Lab Basics



Lab Policies

1. No horse play.
2. No shorts or open-toed shoes.
3. No smoking, eating, dipping or drinking in the lab.
4. Put broken glass in broken glass container, NOT IN THE TRASH.
5. Do no pipet by mouth.
6. Each day after class:
 - ◆ All used glassware will be washed in hot soapy water, rinsed in tap water, then distilled water.
 - ◆ All counter top will be wiped clean with disinfectant.
 - ◆ Balance room must be clean.
7. Used pipets are placed in containers containing detergent immediately after use, tip up.
8. Acid spills must be cleaned up immediately.
9. Pipet bulbs must be cleaned immediately after overpipeting.
10. Wear safety glasses when performing any experiment.
11. Wear aprons in the lab at all times.
12. Wear gloves when performing any experiment or washing glassware.
13. Wash your hands before leaving the laboratory.
14. Know where the eye wash stations are located and how to use them.
15. Know where the emergency shower is and how to use it.
16. Know where each fire extinguisher is located and how to use them.
17. Read carefully the Material Safety Data Sheets for all chemicals used in the laboratory.

OSHA's Change Over to the Globally Harmonized System (GHS) of Classification Labeling of Chemicals and SDS

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Compliance Dates

Effective Dates

The table below summarizes the phase-in dates required under the revised Hazard Communication Standard (HCS):

Effective Completion Date	Requirement(s)	Who
December 1, 2013	Train employees on the new label elements and safety data sheet (SDS) format.	Employers
June 1, 2015*	Compliance with all modified provisions of this final rule, except: The Distributor shall not ship containers labeled by the chemical manufacturer or importer unless it is a GHS label	Chemical manufacturers, importers, distributors and employers
December 1, 2015		
June 1, 2016	Update alternative workplace labeling and hazard communication program as necessary, and provide additional employee training for newly identified physical or health hazards.	Employers
Transition Period to the effective completion dates noted above	May comply with either 29 CFR 1910.1200 (the final standard), or the current standard, or both	Chemical manufacturers, importers, distributors, and employers

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Training

- Why?
 - Some suppliers/distributors are already using the new Safety Data Sheet format and labels
 - To ensure all employees are able to interpret the new labels and Safety Data Sheets
 - Unique to each facility and is provided by the **employer**
- Who?
 - Essentially any employee *potentially exposed* to chemicals as part of their routine job. That means everyone.
 - e.g. an employee occasionally picking up a bottle of Windex to wipe down a door would not need training; however, an employee who uses Windex regularly would

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WHAT IS A HAZARDOUS CHEMICAL UNDER GHS?

Hazard Classification

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Hazardous Chemical

- A chemical is defined as hazardous when it is classified as one of the following:
 - Health hazard
 - Physical hazard
 - Simple asphyxiant
 - Combustible dust
 - Pyrophoric gas
 - Hazard not otherwise classified

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Health Hazard Classification

- A chemical is classified as a health hazard if it poses one of the following effects:
 - Acute oral toxicity (any route)
 - Skin corrosion or irritation
 - Serious eye damage or eye irritation
 - Respiratory or skin sensitization
 - Germ cell mutagenicity
 - Carcinogenicity
 - Reproductive toxicity
 - Specific target organ toxicity
 - Aspiration hazard

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Physical Hazard Classification

- A chemical that poses one of the following hazardous effects:
 - Explosive
 - Flammable
 - Oxidizer
 - Self-reactive
 - Pyrophoric
 - Self-heating
 - Organic peroxide
 - Corrosive to metal
 - Gas under pressure
 - In contact with water emits flammable gas

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Simple Asphyxiant Classification

- A chemical is classified as such if it displaces oxygen in the ambient atmosphere and can cause oxygen deprivation leading to unconsciousness and death
- For example:
 - Nitrogen
 - Carbon dioxide
 - Hydrogen
 - Methane

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Combustible Dust

- NFPA 654 (2006) and NEP Definitions
 - **Combustible Dust** A combustible particulate solid that presents a fire or deflagration hazard when suspended in air or some other oxidizing medium over a range of concentrations, regardless of particle size or shape
 - **Combustible Particulate Solid** Any combustible solid material, composed of distinct particles or pieces, regardless of size, shape or chemical composition
- NFPA 69 (2002), and 499 (2004) Definitions
 - **Combustible Dust.** Any finely divided solid material 420 microns* or less in diameter (i.e., material passing through a U.S. No 40 Standard Sieve) that presents a fire or explosion hazard when dispersed

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Combustible Dusts

Agricultural Products Egg white Milk, powdered Milk, nonfat, dry Soy flour Starch, corn Starch, rice Starch, wheat Sugar Sugar, milk Sugar, beet Tapioca Whey Wood flour	Cottonseed Garlic powder Gluten Grass dust Green coffee Hops (malted) Lemon peel dust Lemon pulp Linseed Locust bean gum Malt Oat flour Oat grain dust Olive pellets Onion powder Parsley (dehydrated) Peach Peanut meal and skins Peat Potato Potato flour Potato starch Raw yucca seed dust Rice dust Rice flour Rice starch Rye flour Semolina	Soybean dust Spice dust Spice powder Sugar (100) Sunflower Sunflower seed dust Tea Tobacco blend Tomato Walnut dust Wheat flour Wheat grain dust Wheat starch Xanthan gum	Chemical Dusts Adipic acid Anthraquinone Ascorbic acid Calcium acetate Calcium stearate Carboxy-methylcellulose Dextrin Lactose Least stearate Methyl-cellulose Paraformaldehyde Sodium ascorbate Sodium stearate Sulfur	Epoxy resin Melamine resin Melamine, molded (phenol-cellulose) Melamine, molded (wood flour and mineral filled phenol-formaldehyde) (poly) Methyl acrylate (poly) Methyl acrylate, emulsion polymer Phenolic resin (poly) Propylene Terpenes-phenol resin Urea-formaldehyde/cellulose, molded (poly) Vinyl acetate/ethylene copolymer (poly) Vinyl alcohol (poly) Vinyl butyral (poly) Vinyl chloride/ethylene/vinyl acetylene suspension copolymer
Agricultural Dusts Alfalfa Apple Beet root Carrageen Carrot Cocoa bean dust Cocoa powder Coconut shell dust Coffee dust Corn meal Cornstarch Cotton	Carbonaceous Dusts Charcoal, activated Charcoal, wood Coal, bituminous Coke, petroleum Lampblack Lignite Peat, 22%+H ₂ O Soot, pine Cellulose Cellulose pulp Cork Corn	Metal Dusts Aluminum Bronze Iron carbonyl Magnesium Zinc	Plastic Dusts (poly) Acrylamide (poly) Acrylonitrile (poly) Ethylene (low-pressure process)	

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Pyrophoric Gas Classification

- A chemical in a gaseous state that will ignite spontaneously in air at a temperature of 130°F
- For example:
 - Arsine
 - Silane
 - Metal carbonyls (dicobalt octacarbonyl, nickel carbonyl)
 - Diborane

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Hazard Not Otherwise Classified Classification

- A chemical is classified as such when there is an adverse physical or health effect identified through evaluation of scientific evidence that does not meet the specified criteria for the physical and health hazard classes
- Not required on the label, but should be on the MSDS
- Does not apply to adverse physical and health hazards under a GHS category that was not adopted by OSHA, such as acute toxicity Category 5

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Signal Word

- Used to indicate the relative level of severity of hazard and alert the reader to a potential hazard
- One, but not both, of the following
 - Danger**—more severe hazard
 - Warning**—less severe hazard



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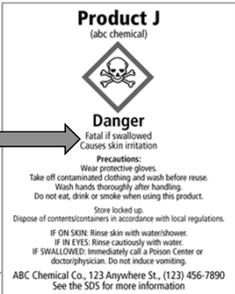
Hazard Statement

- Assigned to a hazard class and hazard category and describes the nature of the hazard
- Examples
 - Fatal if swallowed
 - May cause damage to *kidneys* through prolonged or repeated exposure
 - May cause or intensify fire
 - Extremely flammable liquid or vapor
 - Heating may cause an explosion

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Hazard Statement



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Precautionary Statements

- A phrase that describes recommended measures that should be taken to minimize or prevent adverse effects resulting from exposure or improper storage or handling
- Prevention
- Response
- Storage
- Disposal
- They can be combined or consolidated to save space on the label

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Precautionary Statement



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Pictograms

- Nine are designated by GHS
- Eight are adopted by OSHA
- No duplicates or blank diamonds allowed on the label
- Correct name for the diamond is “squares-on-point”



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Pictogram

Red frame

Black hazard symbol

White background

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Health Hazard

- Carcinogen
- Mutagenicity
- Reproductive Toxicity
- Respiratory Sensitizer
- Target Organ Toxicity
- Aspiration Toxicity

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Skull and Crossbones

- Acute Toxicity

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Flame

- Flammables
- Pyrophorics
- Self-Heating
- Emits Flammable Gas
- Self Reactives
- Organic Peroxides

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Flame Over Circle

- Oxidizers

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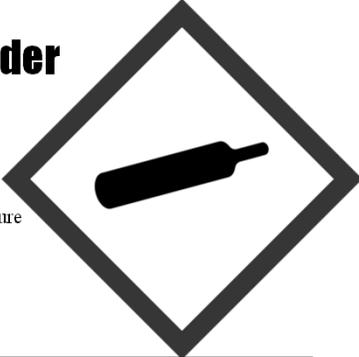
Corrosion

- Skin Corrosion/Burns
- Eye Damage
- Corrosive to Metals

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Gas Cylinder

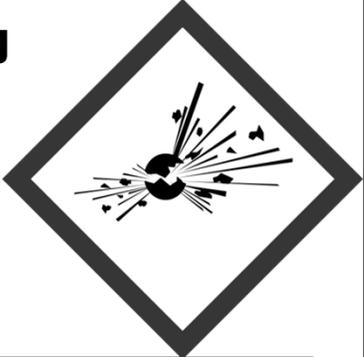


- Gases Under Pressure

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Exploding Bomb



- Explosives
- Self-Reactives
- Organic Peroxides

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Exclamation Mark



- Irritant (skin and eye)
- Skin Sensitizer
- Acute Toxicity-low
- Narcotic Effects
- Respiratory Tract Irritant
- Hazardous to Ozone Layer (-non-mandatory)
- (Low degree health hazard)

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Environmental (non-mandatory)

OSHA Does Not Enforce This One



- Aquatic Toxicity

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HCS Pictogram & Hazards

<ul style="list-style-type: none"> • Carcinogen • Mutagenicity • Reproductive Toxicity • Respiratory Sensitizer • Target Organ Toxicity • Aspiration Toxicity 	<p>Flame</p> <ul style="list-style-type: none"> • Flammables • Self-Heating • Oxidizing • Organic Peroxides 	<p>Exclamation Mark</p> <ul style="list-style-type: none"> • Irritant (skin and eye) • Skin Sensitizer • Acute Toxicity • Narcotic Effects • Respiratory Tract Irritant • Hazardous to Ozone Layer (-non-mandatory) • (Low degree health hazard)
<p>Under Pressure</p>	<p>Corrosive to Metals</p>	<p>Self-Reactives</p> <ul style="list-style-type: none"> • Organic Peroxides
<p>Flame Over Circle</p>	<p>Environment (non-mandatory)</p>	<p>Skull and Crossbones</p>

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Pictogram

HCS Pictograms and Hazards

Health Hazard	Flame	Exclamation Mark
<ul style="list-style-type: none"> • Carcinogen • Mutagenicity • Reproductive Toxicity • Respiratory Sensitizer • Target Organ Toxicity • Aspiration Toxicity 	<ul style="list-style-type: none"> • Flammables • Pyrophorics • Self-Heating • Emits Flammable Gas • Self-Reactives • Organic Peroxides 	<ul style="list-style-type: none"> • Irritant (skin and eye) • Skin Sensitizer • Acute Toxicity • Narcotic Effects • Respiratory Tract Irritant • Hazardous to Ozone Layer (-non-mandatory) • Low degree health hazard
<p>Gas Cylinder</p> <ul style="list-style-type: none"> • Gases Under Pressure 	<p>Corrosion</p> <ul style="list-style-type: none"> • Skin Corrosion/Burns • Eye Damage • Corrosive to Metals 	<p>Exploding Bomb</p> <ul style="list-style-type: none"> • Explosives • Self-Reactives • Organic Peroxides
<p>Flame Over Circle</p> <ul style="list-style-type: none"> • Oxidizers 	<p>Environment (non-mandatory)</p> <ul style="list-style-type: none"> • Aquatic Toxicity • OSHA Does Not Enforce This One 	<p>Skull and Crossbones</p> <ul style="list-style-type: none"> • Acute Toxicity (fatal or toxic)

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Workplace Labels (Transfer containers)

- The employer shall ensure that each container is labeled with either
 - Product identifier
 - Signal word
 - Hazard statement(s)
 - Pictogram
- Or**
- Product identifier and
- Adequate information about the hazards
- Employers must comply by June 1, 2016

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Transfer Container Labeling Exemption Continues

- Portable containers**
 - Identity and hazard information (or product identifier, signal word, hazard statement, signal word, pictogram) must be transferred unless the portable container is:
 - Under the control at all times of the employee making the transfer from the labeled container and
 - Contents used up in one shift

Employers must comply by June 1, 2016

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Alternative Labeling



- Permitted when employer's overall program proven effective
- Must ensure employees fully aware of hazards/use and understanding of labeling system
- Employer bears burden of establishing that employee awareness equals or exceeds conventional labeling system

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Workplace Labeling

- Can HMIS or NFPA system be used?
- While, the hazard category does not appear on the label, consider

GHS		HMIS/NFPA	
Category	Hazard	Category	Hazard
1	highest	1	slight
2	high	2	moderate
3	medium	3	serious
4	low	4	severe

→

NFPA categories were intended for emergency response, not workplace hazards; only considers acute effects, does not consider chronic effects

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Labeling Effective Dates

- Chemical manufacturers, importers, and employers
 - Will not ship containers without GHS labeling/SDS by June 1, 2015
- Employers
 - By June 1, 2016
 - Update alternative workplace labeling and hazard communication program as necessary, and provide additional employee training for newly identified physical or health hazards.

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SAFETY DATA SHEETS (SDS)

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Safety Data Sheet Info

- In English
- New 16-section format
- Compliance date for chemical manufactures, imports and distributors —June 1, 2015
- **Example pH 7 below**

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Safety Data Sheet Sections

- Section 1 Identification
 - Section 2 Hazard(s) identification
 - Section 3 Composition/information on ingredients
 - Section 4 First-aid measures
 - Section 5 Fire-fighting measures
 - Section 6 Accidental release measures
 - Section 7 Handling and storage
 - Section 8 Exposure controls/personal protection
 - Section 9 Physical and chemical properties
 - Section 10 Stability and reactivity
 - Section 11 Toxicological information
 - Section 12 Ecological information
 - Section 13 Disposal considerations
 - Section 14 Transport information
 - Section 15 Regulatory information
 - Section 16 Other information, including date of preparation or last revision
- Information in these sections will not be enforced by OSHA

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Section 1

Section 1 – Chemical Product and Company Identification

Catalog Numbers: 40475
Product Identity: Buffer Soln. pH 7.00

Manufacturer's Name: AquaPhoenix Scientific, Inc., 9 Barnhart Dr., Hanover, PA 17331
Emergency Contact Number (24hr): InfoTrac (800) 535-5053

Identification Of The Substrate Or Mixture And Of The Supplier

- GHS product identifier
- Other means of identification
- Recommended use of the chemical and restrictions on use
- Supplier's details
 - Name, address, phone #
- Emergency phone number

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Section 2

Section 2 – Composition, Information on Ingredients

Sodium Phosphate, Dibasic, CAS# 7558-79-4, <3% w/v, ACGIH TLV: NA, OSHA PEL: NA
Potassium Phosphate, Monobasic, CAS# 7778-77-0, <2% w/v, ACGIH TLV: NA, OSHA PEL: NA
Water, purified, CAS# 7732-18-5, >95% w/v, ACGIH TLV: NA, OSHA PEL: NA

Hazards Identification

- GHS classification of the substance/mixture and any national or regional information
- GHS Label elements, including precautionary statements
 - Hazard symbols may be provided as a graphical reproduction of the symbols in black and white or the name of the symbol,
 - e.g. flame, skull and crossbones
- Other hazards which do not result in classification or are not covered by the GHS

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Section 3

Section 3 – Hazard Identification

Emergency Overview: Non-flammable, non-corrosive, non-toxic. Does not present significant health hazards. Wash areas of contact with water.

Target Organs: Eyes, skin.

Potential Health Effects

Eyes: May cause slight irritation.

Skin: May cause slight irritation.

Ingestion: Large doses may cause upset stomach

Inhalation: Not likely to be a hazard

Chronic Effect / Carcinogenicity: None (IARC, NTP, OSHA)

Composition/Information Ingredients

- Substance
 - Chemical identity
 - Common name, synonyms, etc.
 - CAS number, EC number, etc.
 - Impurities and stabilizing additives which are themselves classified and which contribute to the classification of the substance
- Mixture
 - The chemical identity and concentration or concentration ranges of all ingredients which are hazardous within the meaning of the GHS and are present above their cutoff levels

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Section 4

Section 4 – First Aid

Eyes: Immediately flush eyes with water for at least 15 minutes. Immediately get medical assistance.

Skin: Flush with water for 15 minutes. Get medical assistance if irritation develops.

Ingestion: Dilute with water or milk. Get medical assistance.

Inhalation: Remove to fresh air. Give artificial respiration if necessary. If breathing is difficult, give oxygen.

First Aid Measures

- Description of necessary measures, subdivided according to the different routes of exposure
 - i.e. inhalation, skin and eye contact, and ingestion
- Most important symptoms/effects, acute and delayed
- Indication of immediate medical attention and special treatment needed, if necessary

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Section 5

Section 5 – Fire Fighting Measures

Flash Point: NA
 Extinguishing Media: Use means suitable to extinguishing surrounding fire.
 Fire & Explosion Hazards: Not considered to be a fire or explosion hazard.
 Fire Fighting Instructions / Equipment: Use normal procedures. Poisonous gases may be produced in fire. Use protective clothing. Use NIOSH-approved breathing equipment.
 NFPA Rating: (estimated) Health: 1; Flammable: 0; Reactivity: 0

Firefighting Measures

- Suitable (and unsuitable) extinguishing media
- Specific hazards arising from the chemical
 - e.g. nature of any hazardous combustion products
- Special protective equipment and precautions for firefighters

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Section 6

Section 6 – Accidental Release Measures

Absorb with suitable material. Always obey local regulations.

Accidental Release Measures

- Personal precautions, protective equipment and emergency procedures
- Environmental precautions
- Methods and materials for containment and cleaning up

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Section 7

Section 7 – Handling and Storage

Handling: Wash hands after handling. Avoid contact with skin and eyes.
 Storage: Protect from freezing and physical damage.

Handling and Storage

- Precautions for safe handling
- Conditions for safe storage, including any incompatibilities

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Section 8

Section 8 – Exposure Controls, Personal Protection

Engineering Controls: Normal ventilation is adequate.

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Respiratory Controls: Normal ventilation is adequate.

Skin Protection: Chemical resistant gloves.

Eye Protection: Safety Glasses or goggles.

Exposure Controls/Personal Protection

- Control parameters
 - e.g. occupational exposure limit values or biological limit values
- Appropriate engineering controls
- Individual protection measures, such as PPE

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Section 9

Section 9 – Physical and Chemical Properties

Appearance: Clear, yellow liquid
 pH: 5.8-8
 Boiling Point: Approx 100C
 Melting Point: Approx 0 C

Odor: Odorless
 Solubility in Water: Infinite
 Specific Gravity: Approx 1
 Vapor Pressure: NA

Physical and Chemical Properties

- Appearance
- Odor
- Odor threshold
- pH
- Melting point/freezing point
- Initial boiling point and boiling range
- Flash point
- Flammability
- Upper/lower flammability or explosive limits
- Vapor pressure
- Vapor density
- Relative density
- Solubility(ies)
- Partition coefficient
- Autoignition temperature
- Decomposition temperature

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Section 10

Section 10 – Stability and Reactivity

Chemical Stability: Stable under normal conditions of use and storage.
 Incompatibility: None Identified.
 Hazardous Decomposition Products: Oxides of Phosphorus
 Hazardous Polymerization: Does not occur

Stability and Reactivity

- Chemical stability
- Possibility of hazardous reactions
- Conditions to avoid
 - e.g. static discharge, shock or vibration
- Incompatible materials
- Hazardous decomposition products

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Section 11

Section 11 – Toxicological Information

LD50 orl-rat: 17 g/kg (Sodium Phosphate, Dibasic)
LC50 inhalation-rat: >4640 mg/kg (Potassium Phosphate, Monobasic)

Toxicological Information

- Concise but complete comprehensible description of the various toxicological (health) effects and the available data used to identify those effects
- Includes:
 - Information on the like routes of exposure
 - Symptoms related to the physical, chemical and toxicological characteristics
 - Delayed and immediate effects and also chronic effects from short and long term exposures
 - Numerical measures of toxicity

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Information in this section will not be enforced by OSHA

Section 12

Section 12 – Ecological Information

Ecotoxicity: NA

Ecological Information

- Eco-toxicity
- Persistence and degradability
- Bio-accumulative potential
- Mobility in soil
- Other adverse effects

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Section 13

Section 13 – Disposal Considerations

Dilute with water.

All chemical waster generators must determine whether a discarded chemical is classified as hazardous waste.

Comply with all local, state, and federal regulations.

Disposal Considerations

- Description of waste residues and information on their safe handling and methods of disposal
 - Including the disposal of any contaminated packaging

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Section 14

Section 14 – Transport Information

DOT - Not Regulated

Transport Information

- UN number
- UN proper shipping name
- Transport hazard class(es)
- Packing group, if applicable
- Marine pollutant (yes/no)
- Special precautions which a user needs to be aware of or needs to comply with in connection with transport or conveyance either within or outside their premises

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Section 15

Regulatory Information

- Safety, health and environmental regulations specific for the product in question

Section 15 – Regulatory Information (not meant to be all inclusive)

OSHA Status: These chemicals are not considered hazardous by OSHA.
TSCA: The components of this solution are listed on the TSCA Inventory
SARA Title III Section 313: Not Applicable
RCRA Status: NA
CERCLA Reportable Quantity: Sodium Phosphate, Dibasic – 5,000 lbs.
WHMIS: NA

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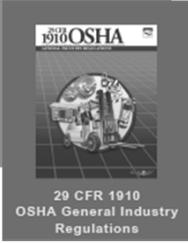
Section 16

Other Information Including Information on Preparation and Revision of the SDS

Section 16 – Additional Information

Issue Date: 12/28/06
Revision Date: 6/5/08, 11/19/09

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EFFECTS ON OTHER STANDARDS

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Flammable Liquids

GHS FL Category	Flashpoint Deg F	Boiling Point Deg F	Old OSHA Class	Flashpoint Deg F	Boiling Point Deg F
1	<73.4	≤95	IA	<73	<100
2	<73.4	<95	IB	<73	≥100
3	≥73.4 and ≤140		IC II	≥73 and <100 ≥100 and <140	
4	>140 and ≤199.4		IIIA	≥140 and <200	
None			IIIB	>200	62

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WHAT-TO-DO BOOKLET
A Template for Compliance With
(29 CFR 1910.1200 and 29 CFR 1926.59)
Hazard Communication Standard
And
(800-1-1-09)
The Tennessee Hazardous Chemical Right-To-Know Law



GHS **GHS**

Revised November, 2012 63

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Resources



Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

- CDC works 24/7 to protect America from health, safety and security threats, both foreign and in the U.S. Whether diseases start at home or abroad, are chronic or acute, curable or preventable, human error or deliberate attack, CDC fights disease and supports communities and citizens to do the same. www.cdc.gov

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Resources

- OSHA (www.osha.gov)
 - "No one should have to sacrifice their life for their livelihood, because a nation built on the dignity of work must provide safe working conditions for its people."
-Secretary of Labor Thomas E. Perez
 - Under federal law, you are entitled to a safe workplace. Your employer must provide a workplace free of known health and safety hazards. If you have concerns, you have the right to speak up about them **without fear of retaliation**. You also have the right to:

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Resources

- OSHA (www.osha.gov)
 - Be trained in a language you understand
 - Work on machines that are safe
 - Be provided required safety gear, such as gloves or a harness and lifeline for falls
 - Be protected from toxic chemicals
 - Request an OSHA inspection, and speak to the inspector
 - Report an injury or illness, and get copies of your medical records
 - See copies of the workplace injury and illness log
 - Review records of work-related injuries and illnesses
 - Get copies of test results done to find hazards in the workplace

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Resources

• TOSHA

- Tennessee OSHA improves occupational safety and health through enforcement of the general industry, construction and agricultural occupational safety and health standards in workplaces. - See more at:

<https://www.tn.gov/workforce/section/tosha>

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Resources

• TOSHA

- TOSHA's mission is to assure the safety and health of Tennessee's working men and women
- by promulgating and enforcing standards and regulations; providing training, outreach, and education;
- establishing cooperative programs; and encouraging continual improvement in workplace safety and health
- as well as the development of comprehensive safety and health management systems. Effective and
- efficient use of resources requires careful, flexible planning. In this way, the overall goal of hazard
- abatement and employee protection is best served.

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TN Department of Environment and Conservation

Resources

- OSHA www.osha.gov

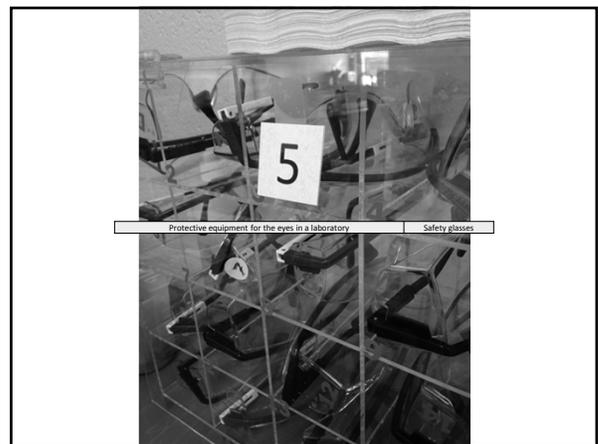
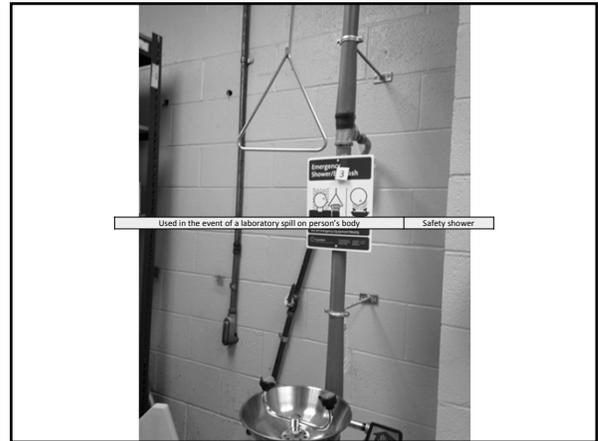
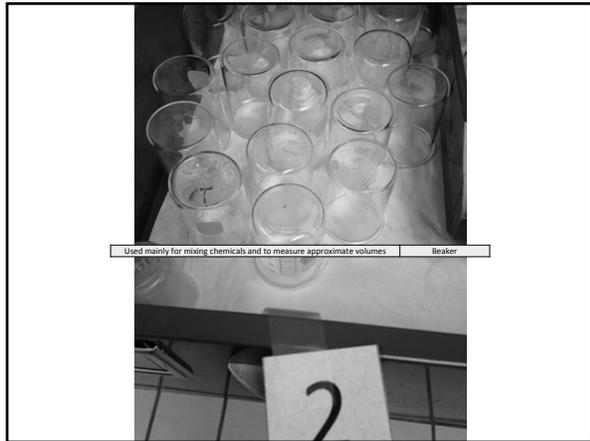
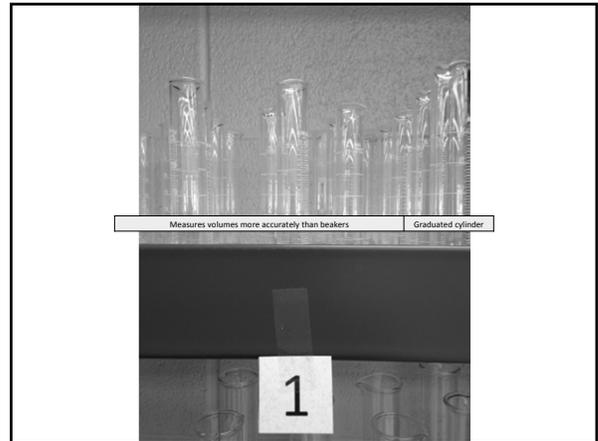
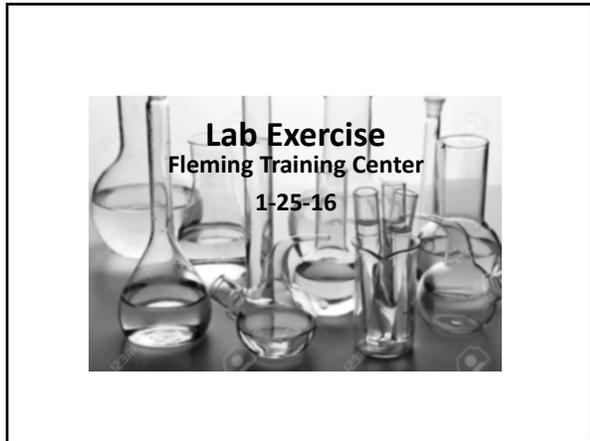
- CDC www.cdc.gov

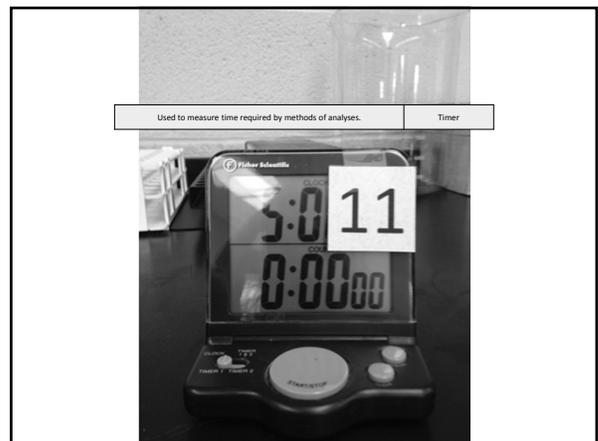
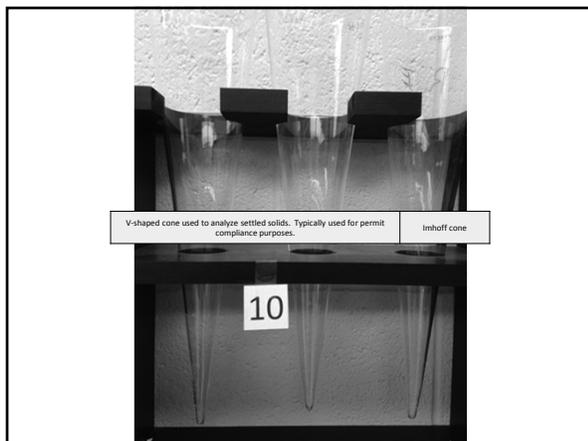
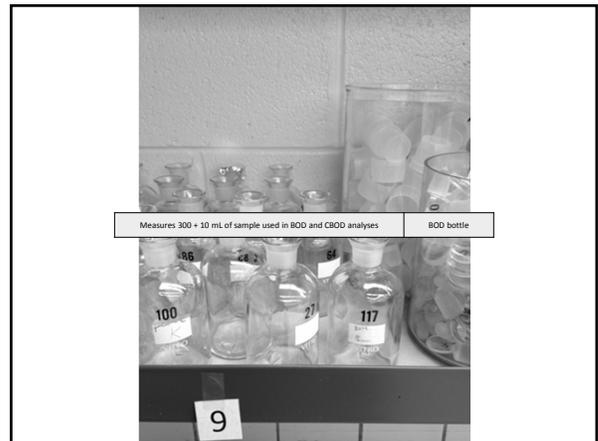
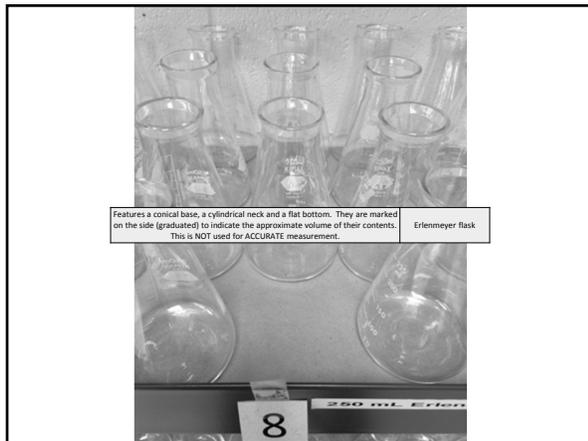
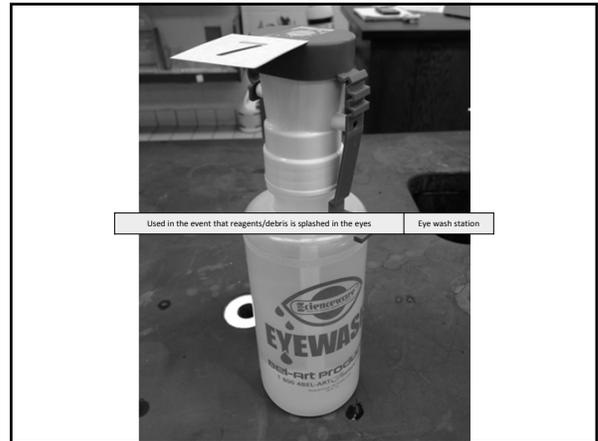
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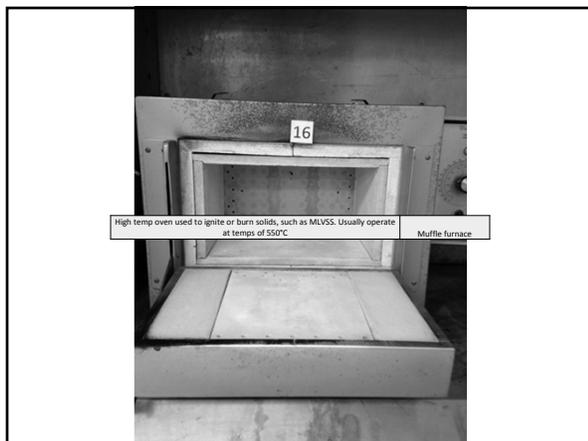
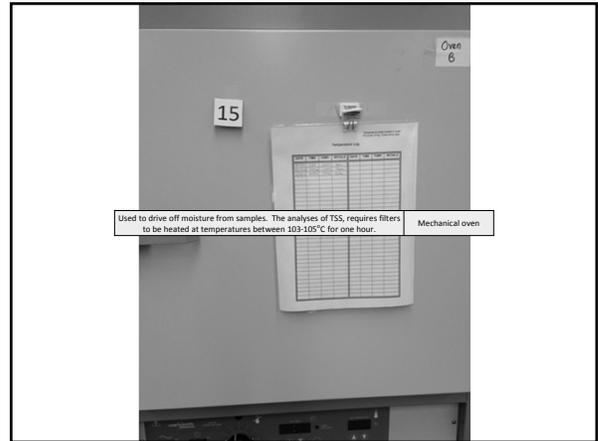
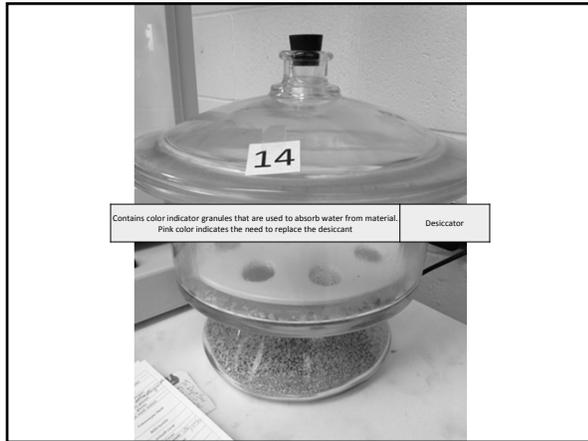
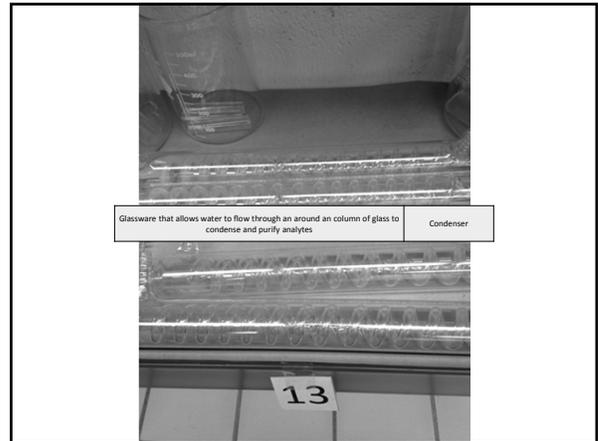
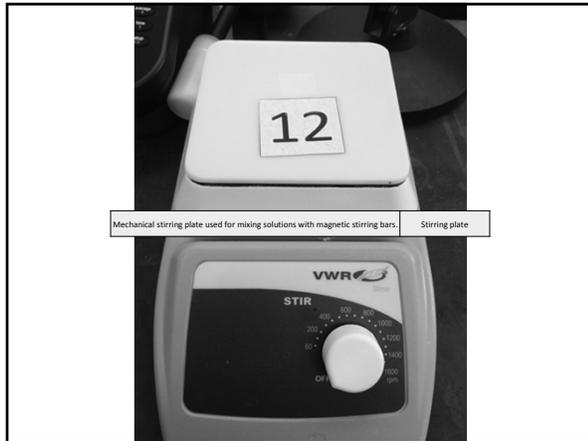
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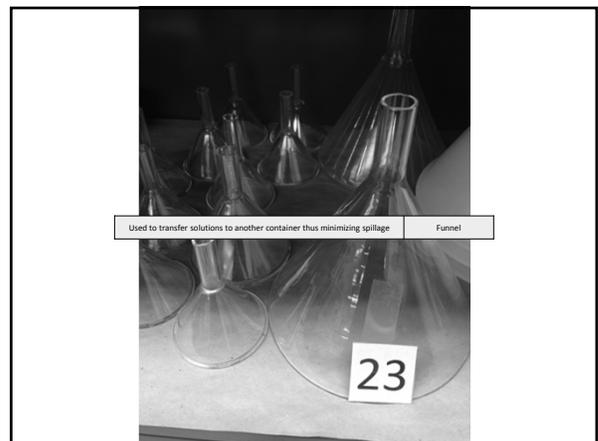
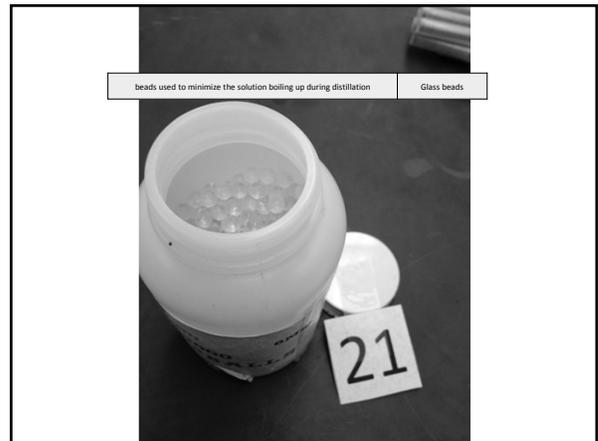
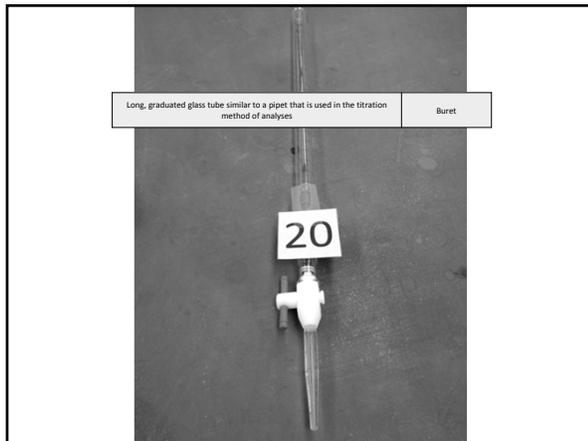
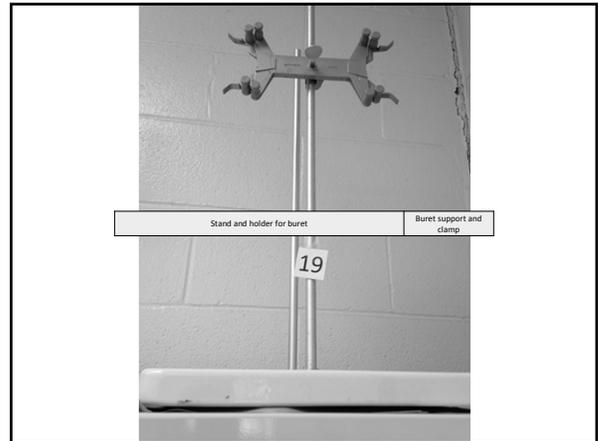
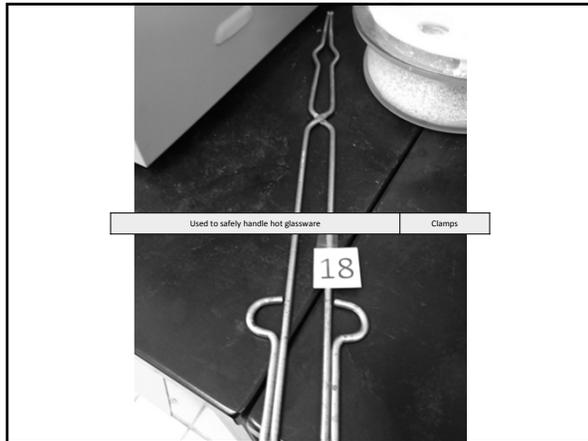
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- Jackson Office 731-423-5641
- Nashville Office 615-741-2793
1-800-249-8510
- Knoxville Office 865-594-6180
- Kingsport Office 423-224-2042
- Chattanooga 423-634-6424

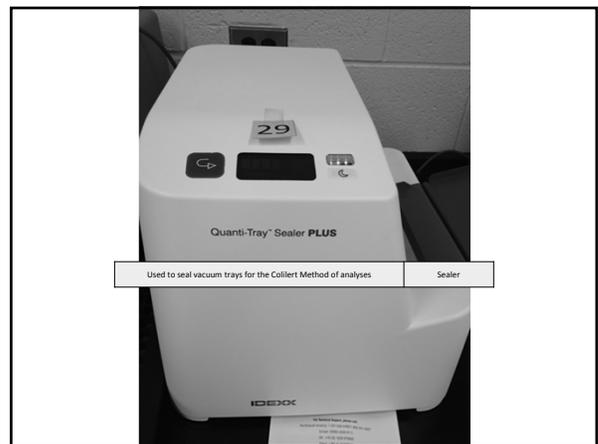
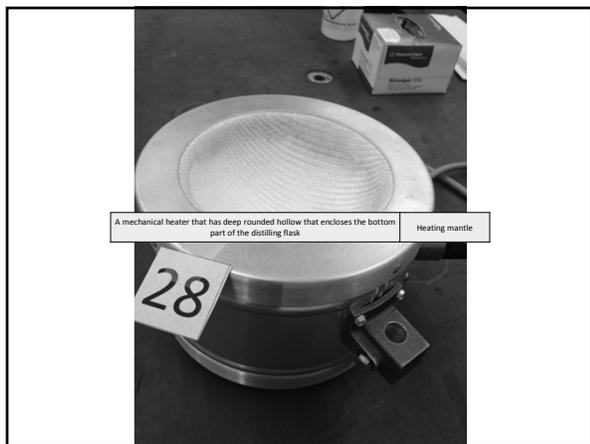
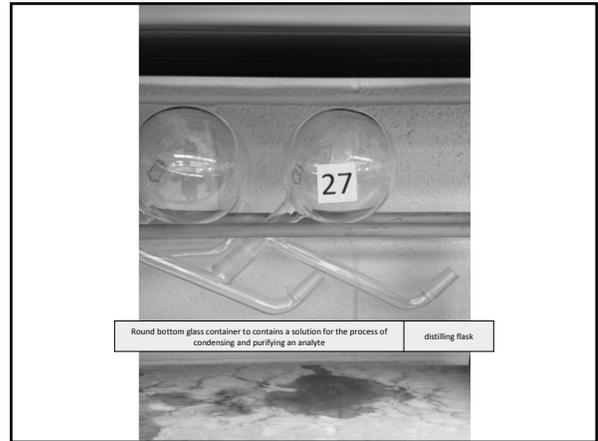
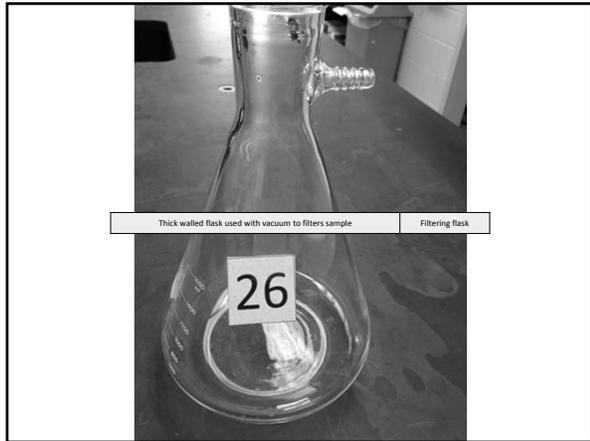
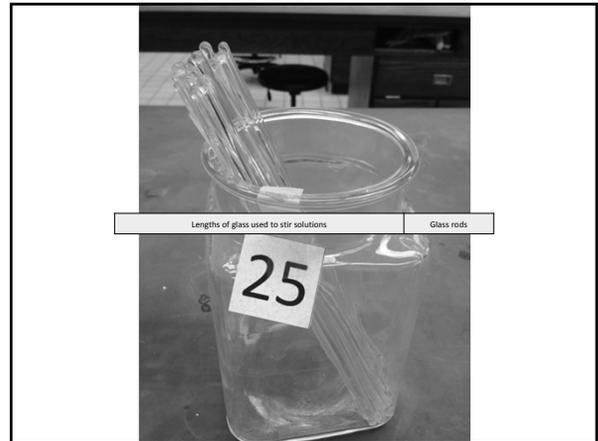
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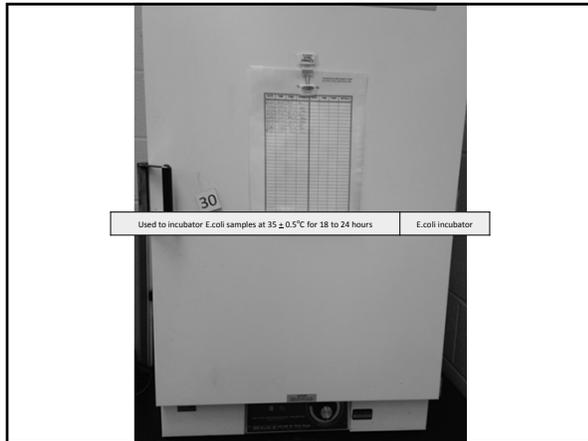




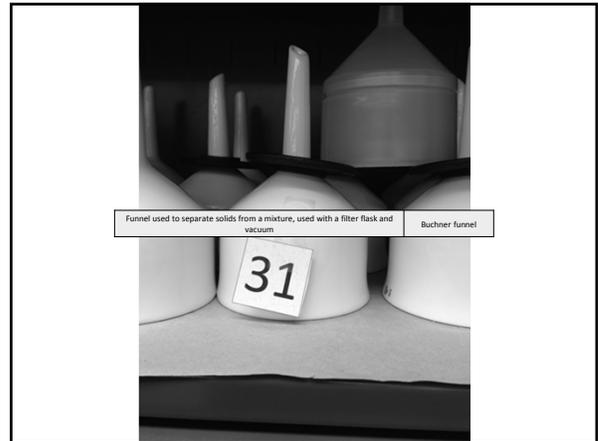




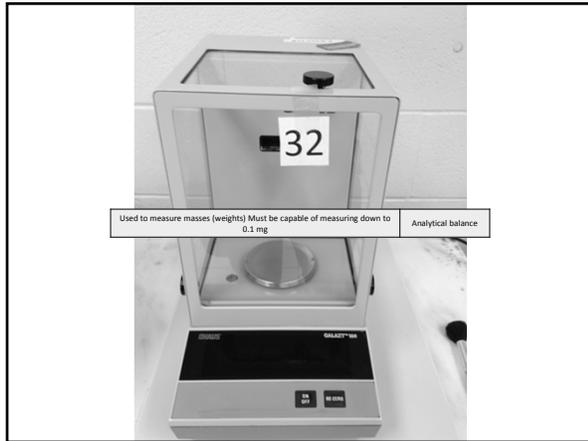




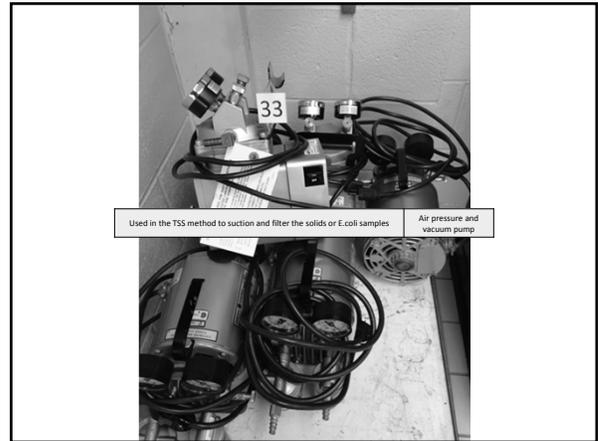
Used to incubator E.coli samples at $35 \pm 0.5^\circ\text{C}$ for 18 to 24 hours E.coli incubator



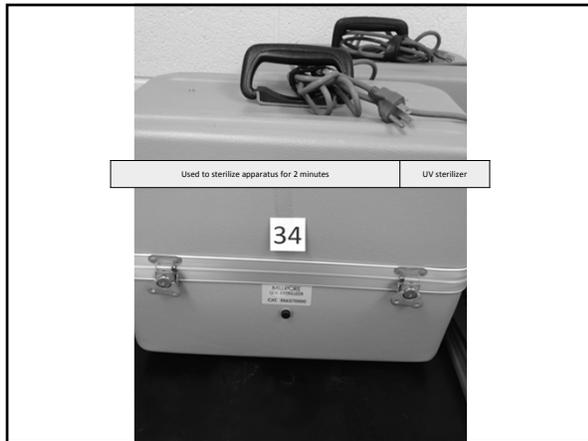
Funnel used to separate solids from a mixture, used with a filter flask and vacuum Buchner funnel



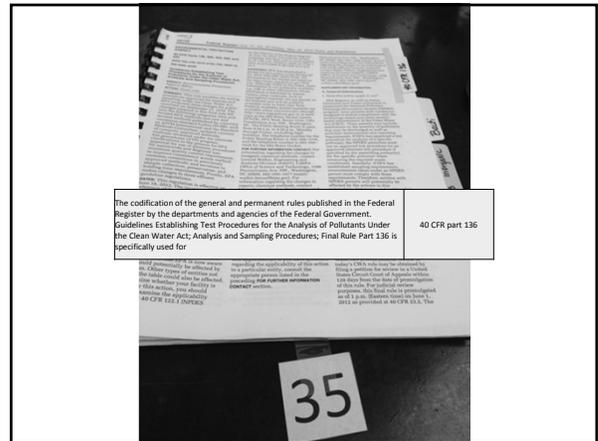
Used to measure masses (weights) Must be capable of measuring down to 0.1 mg Analytical balance



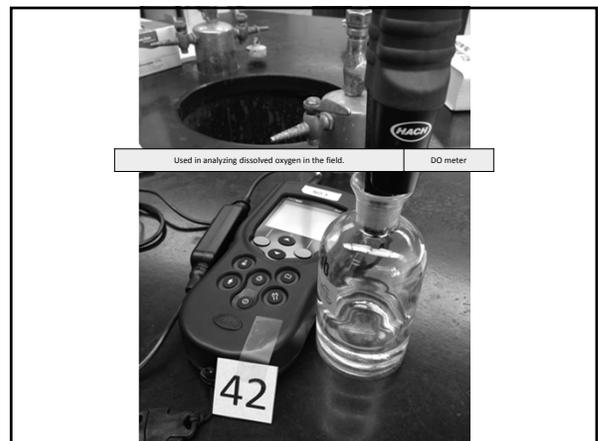
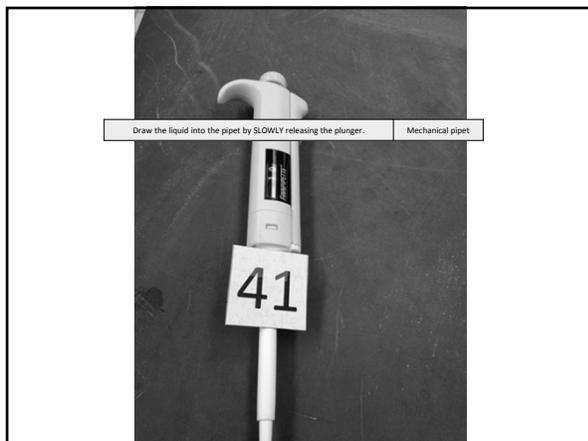
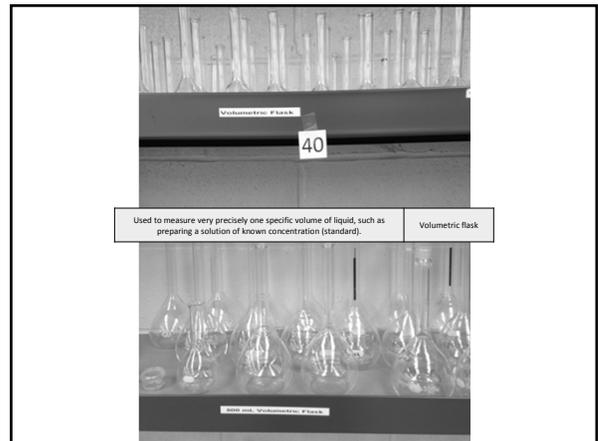
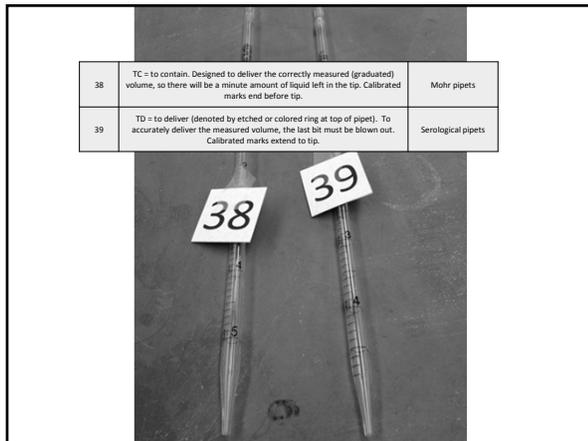
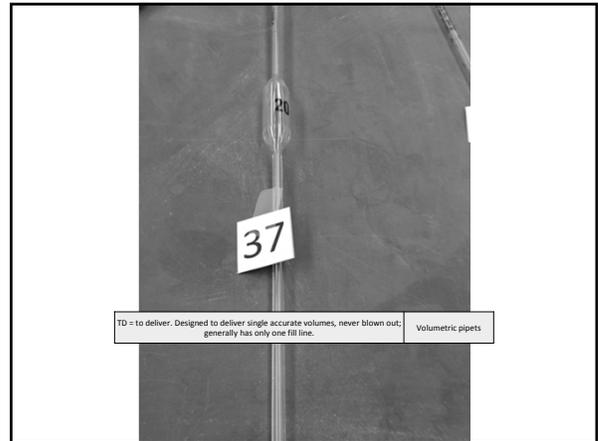
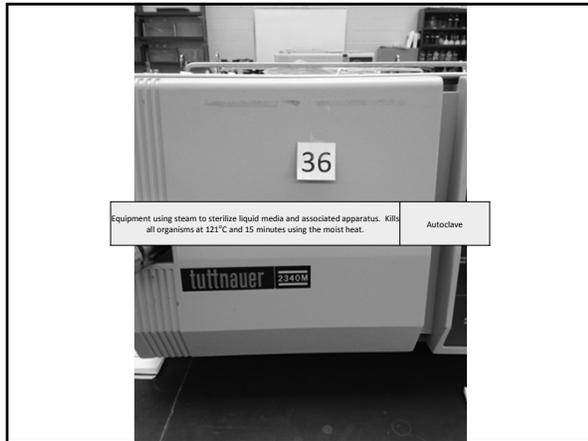
Used in the TSS method to suction and filter the solids or E.coli samples Air pressure and vacuum pump

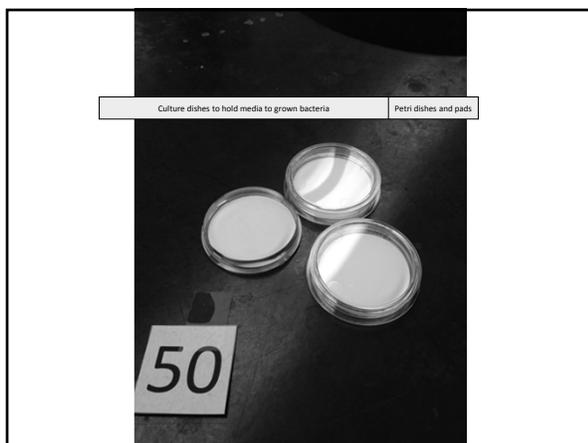
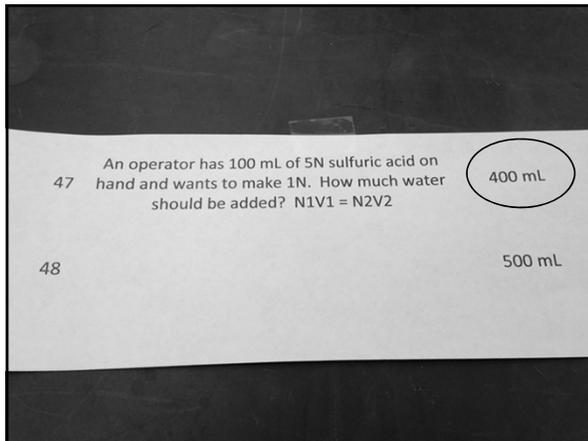
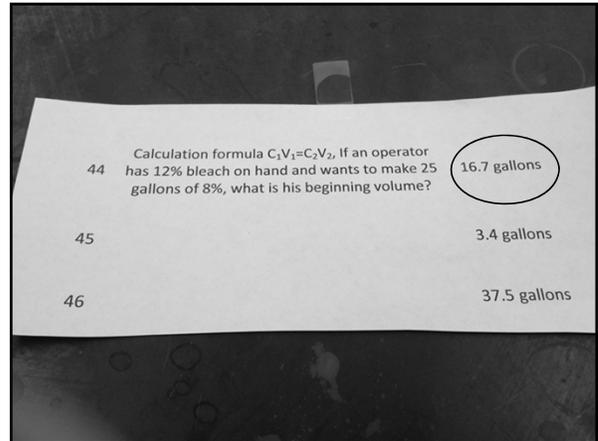
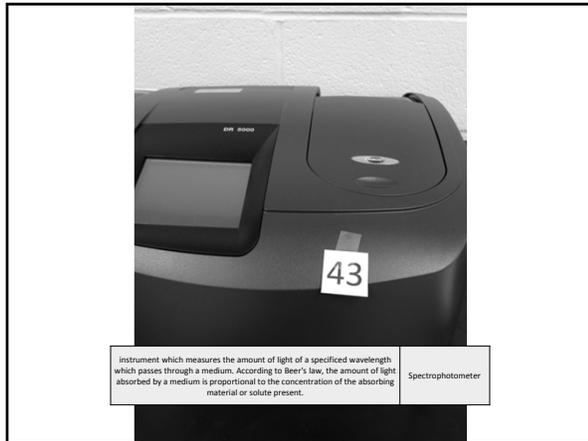


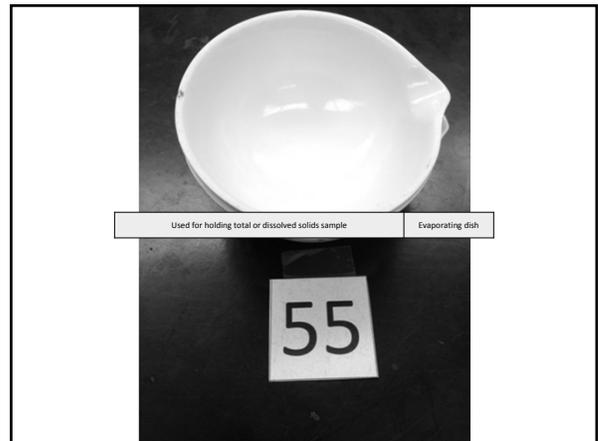
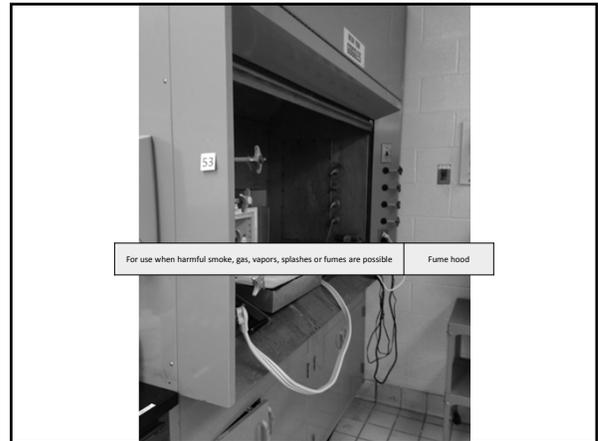
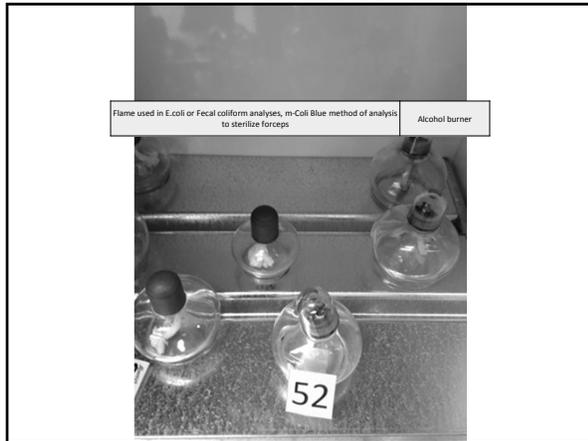
Used to sterilize apparatus for 2 minutes UV sterilizer

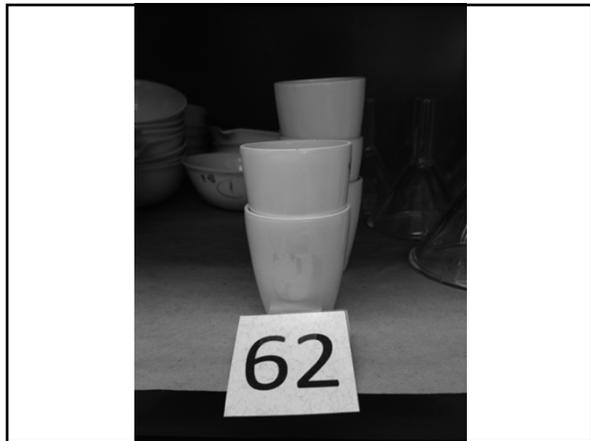
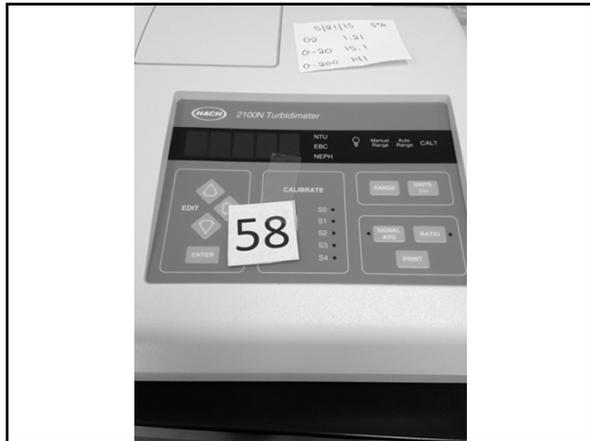


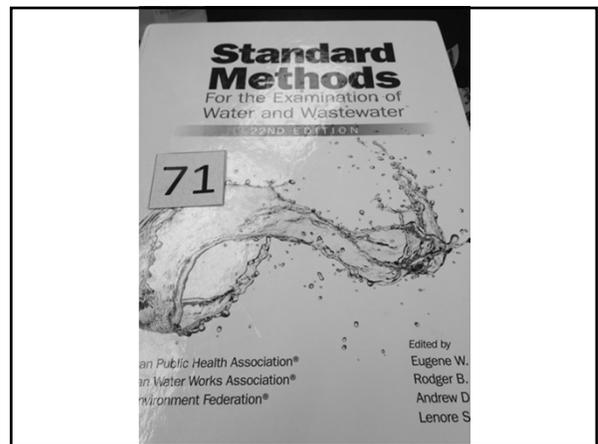
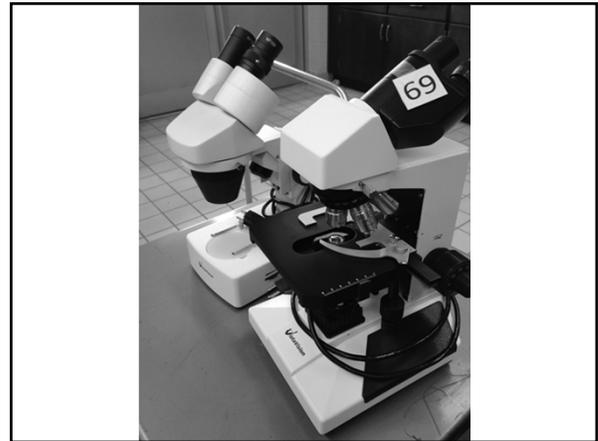
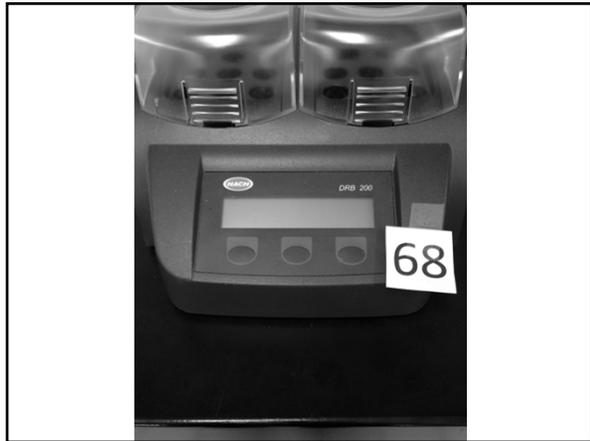
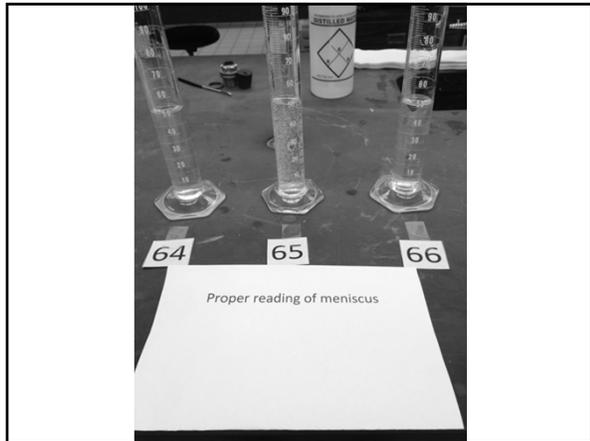
The codification of the general and permanent rules published in the Federal Register by the departments and agencies of the Federal Government. Guidelines. Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Analysis and Sampling Procedures; Final Rule Part 136 is specifically used for 40 CFR part 136

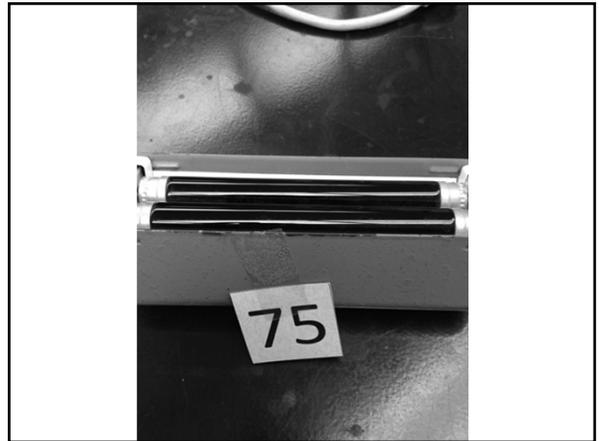














Laboratory Equipment

Identification, Handling, Cleaning
and Safety



TDEC - Fleming Training Center 1

Objectives

- Identify equipment commonly used in water treatment and wastewater laboratory
- Discuss accuracy and use of glassware
- Discuss how to maintain analytical equipment

TDEC - Fleming Training Center 2

Beakers

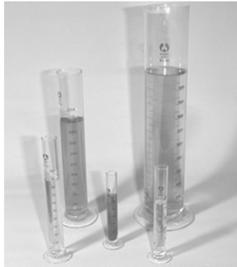
- Used for:
 - Mixing
 - Measuring approximate volumes
 - ~10% accuracy



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Graduated Cylinders

- Accurate to ~1%
- Measures liquid volumes more accurately than beakers, but still not the most accurate
- Measure quicker



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Volumetric Flasks

- Most accurate way to measure volume
- Disadvantage:
 - Only can measure one volume
 - Not used for storing or heating solutions



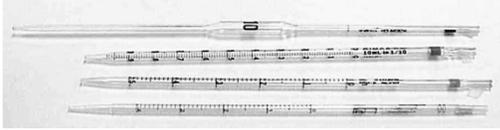
TDEC - Fleming Training Center 5

What Are Pipets?

- Pipets are glass or plastic tubes, usually open at both ends, which are used to transfer specific amounts of liquid from one container to another.
- They are usually used for volumes between 1 and 100 milliliters.

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Types of Pipets



- Volumetric
- Measuring
 - Mohr
 - Serological

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Volumetric Pipets

- Used to deliver a single specific volume of liquid, usually between 1 and 100 ml.
- Shaped like rolling pins with a large belly, one blunt end, the neck, and one tapering end, the tip.



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Volumetric Pipets

- Used for accurate measurements, since it is designed to deliver only one volume and is calibrated at that volume.
- Should be used when accuracy and reproducibility are crucial, because these can achieve accuracy to four significant figures.

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9

Specifications on a Volumetric Pipet

- When emptying a volumetric pipet, the liquid is allowed to drain out
 - It is NOT forced out.
- After it is emptied, the small amount of liquid which remains in the tip should not be blown out.
- Volumetric pipets are NOT blow-out pipets

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Measuring Pipets

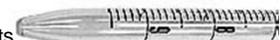
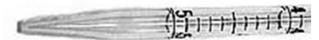
- They are straight glass or plastic tubes with one tapering end.
- Calibrated into small divisions so that various amounts of liquid can be measured with the same pipet.
- Usually used to measure any amount between 0.1ml and 25.0ml.
- They are not as accurate due to the fact that any imperfection in their internal diameter will have a greater effect on the volume delivered.

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Mohr and Serological Pipets

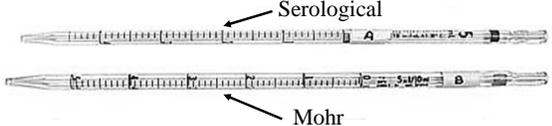
- Measuring pipets are divided into:
 - Mohr Pipets
 - Graduations on these always end before the tip
 - Serological Pipets
 - Graduation marks continue to the tip



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12

Examine pipets A and B.
Which is the serological and which is the Mohr?



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Specifications on a Measuring Pipet



- Maximum volume of liquid that can be transferred
- Size of the divisions on the pipet
- Temperature at which calibrations were made
- If the pipet is a "to deliver" (TD) or "to contain" (TC) pipet.

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5 in 1/10 ml TD 20°C



- Specifications on a pipet as shown above indicate that the pipet is calibrated in 1/10ml divisions and will deliver up to 5.0 ml within published tolerance levels at 20°C.

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1 ml in 1/100 TD 20°C



- These specifications indicate that the pipet is calibrated in 1/100 ml divisions and it will deliver up to 1.00 ml within published tolerance levels at 20°C.

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Handling and Disposing of Pipets

- Chipped and cracked pipets should be replaced as they are unsafe and may affect the accuracy of measurements.
- NEVER mouth pipet.
- Hold the pipet by the upper third of the tube and keep the tip from touching anything.



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Handling and Disposing of Pipets

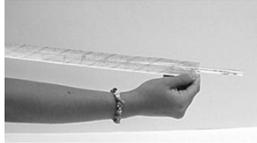
- Dispose dirty pipets by placing in soapy water solution in a tray or pipet washer.
- Place disposable pipets in a cardboard holder.
- Do not leave pipets on counters or sinks.




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Handling Sterile Pipets

- When using sterile pipets, be sure to use proper sanitary techniques.
- If you have a sterile package of disposable pipets, tear only a small corner of the package open and push one pipet out of this opening, then immediately close the package to prevent contamination.



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Handling Sterile Pipets

- If you are using sterile pipets in a pipet canister, place the canister on its side, slide off the cover, pull out one pipet and replace the cover immediately.



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20

Transferring a Precise Volume of Liquid

- A pipet bulb is used to draw liquid up into the pipet.
- There are many types of pipet bulbs.



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Transferring a Precise Volume of Liquid

- You should observe the meniscus at eye-level
- Touch the tip of the pipet to the inside of the container when the meniscus is at the desired level



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Transferring a Precise Volume of Liquid

- Squeeze bulb and touch it to the mouth of the pipet.
- Place other end of the pipet in liquid to be transferred and slowly release pressure on bulb.
- Draw liquid up past desired level, quickly replacing bulb with index finger.

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Transferring a Precise Volume of Liquid

- Let liquid drain until bottom of meniscus lines up with desired level on pipet.
- Touch tip of pipet to inside of beaker to remove any adhering drops.
- Transfer liquid to second beaker and touch tip to inside of beaker and let liquid drain out of pipet.

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Other Pipet Bulbs

- Other pipet bulbs that are often used include the Vadosa pipet filler, seen on the left, and the pipet Pumper, on the right.



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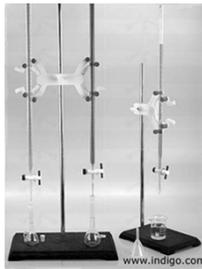
Other Pipet Types

- Transfer of uncalibrated volumes up to 2.5 ml can be accomplished using glass "transfer" or "Pasteur" pipets shown below. These may be sterilized before use.
- Roughly calibrated volumes of 1 and 2 ml can be transferred with the one piece plastic transfer pipets which may be purchased as sterile or non-sterile units.



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Burettes and Titrations



- Burettes
 - Used for titrations
 - Treat like a Mohr pipet, do not let liquid completely drain out
 - Also, make sure to remove air bubble in tip before titrating

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Flasks

- Distilling Flask
- Florence (Flat Bottom) Flask



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Flasks

- Erlenmeyer Flasks
- Filter Flask

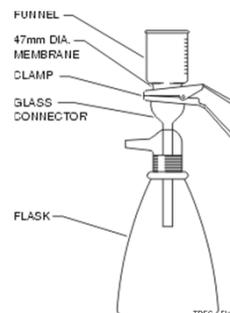


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www.indigo.com
Filter Flask & Funnels

29

Filter Apparatus



- Vacuum Pump



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Bottles

- Dilution Bottle
- Sample Bottles



TDEC - Fleming Training Center 31

Bottles

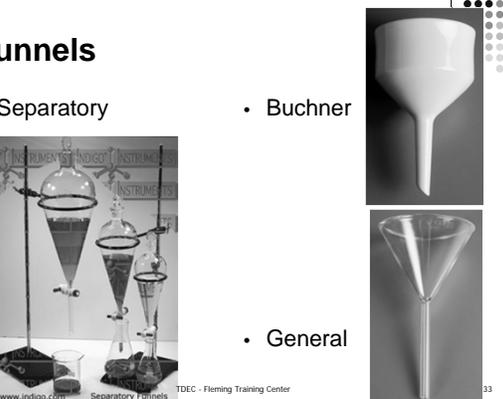
- Reagent Bottle
- Weighing Bottles



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Funnels

- Separatory
- Buchner
- General



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- Petri Dish
 - Culturing container
- Desiccators
 - Dust and moisture free



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- Evaporating Dish
- Crucible



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Centrifuge

- Used to separate materials of different density



TDEC - Fleming Training Center 36

Autoclave

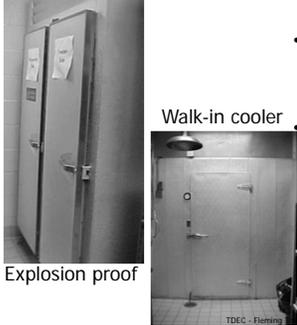
- Pressure cooker used to sterilize glassware, bottles, membrane filter equip, culture media and contaminated material to be discarded.
- Standard temperature is set at 121°C and 15 PSI



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Refrigerators

- Sample storage should maintain between 1-5 °C
- Never store samples and chemicals together



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Incubators

- Artificially heated container used for growing bacteria cultures
- Dry-Heat types hold temperatures to $\pm 0.5^\circ\text{C}$
- For E. coli and Total coliform = $35 \pm 0.5^\circ\text{C}$
- Water Bath for fecals = $44.5 \pm 0.2^\circ\text{C}$



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Incubators

- For BOD incubation at $20 \pm 1^\circ\text{C}$
- Do not store chemical solutions and samples in same refrigerator



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UV Sterilizer

- Use in Bac'T Lab to sterilize test equipment



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Drying Oven

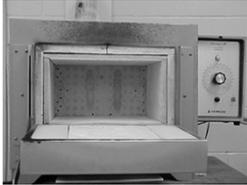
Used more often in wastewater labs

For solids testing set oven at 103-105°C



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Muffle Furnace



- High temp oven used to ignite or burn solids.
- Usually operate at temps of 550°C.
- More often used in Wastewater lab work.

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Fume Hood



- Can prevent serious accidents
- Use whenever heat is used in a test procedure
- Fumes vented out of lab
- Use when harmful smoke, gas, vapors, splashes or fumes are possible

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Water Still



- Produces distilled water for lab tests and rinsing washed glassware
- Removes dissolved minerals, organic and inorganic nonvolatile compounds
- Does not sterilize

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Heating and Stirring Samples




- Combo Heat/Stir Plate
 - Can be used to stir or heat and stir samples
 - Safer than heating with an open flame
- Gas Burner
 - Uses natural gas

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Balances

- Top Loading
 - Weighs to the nearest 0.01 g
- Analytical
 - Precise to 0.0001 g




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pH Meter



- Use buffer solutions to calibrate
- Store electrodes properly
- Calibrate daily
- Maintain records on daily calibrations

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Spectrophotometer

- HACH DR 4000
 - Factory pre-set programs for lab chemical analysis
- Very versatile



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Colorimeters

- Determine the concentration of many chemicals
- Most commonly used is chlorine type colorimeter
- Portable and battery powered



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Amperometric Titrator

- Chlorine analysis
- Accurate and unaffected by sample color or turbidity
- Takes greater skill to use than DPD method with colorimetric devices



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Turbidimeter

- Desk top and continuous on-line monitoring
- Position away from direct sunlight and have extra light bulb on hand
- Ensure sample bottles maintained; no scratches; acid clean if necessary



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Chemical Storage

- Do not store volatile chemicals together
- Have separate storage cabinets for acids and bases/caustics



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Flammable Cabinet

- Flammable chemicals should be kept in a flammable cabinet.



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Safety Equipment



PPE (Personal Protective Equipment):

- Goggles
- Gloves
- Aprons
- Wear safety clothing.

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Eye Wash and Shower



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Cleaning Glassware

- Just because it looks clean does not mean residues are not left behind
- Results need to be accurate to use data for process control and/or reporting to the State
- Detergents, such as Alconox, may be sufficient
 - Should be phosphate-free

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- Residues of minerals and other substances can build up on glassware, causing erroneous test results

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Steps for Washing

- Clean glassware using laboratory detergent (phosphate-free)
- Rinse with tap water
- Rinse at least three times with distilled water
- Let air dry

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Steps for Acid-Washing

- Clean glassware using laboratory detergent (phosphate-free)
- Rinse with tap water
- Rinse with 1:1 hydrochloric acid or nitric acid
 - 1:1 means equal parts distilled water and acid
- Rinse well with distilled water
- Let air dry

Note: always use gloves and goggles when handling acids

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Chemical Safety - Storage



Label and close the chemical containers properly

A rusty surface indicates containers are not closed properly

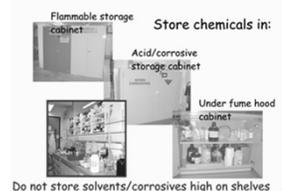
- Current chemical inventory available
- No expired chemicals.
 - Disposed of out-dated chemicals
- Chemical containers properly labeled, in good condition and closed properly
- Only compatible chemicals are stored together
 - Everything not stored in alphabetical order

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Chemical Safety - Storage

- Secondary containment for stored chemicals as necessary.
 - Polyethylene trays for separate storage of acids and bases
- Chemicals stored at safe levels, in cabinets or on stable shelving (but not on high levels)



Do not store solvents/corrosives high on shelves

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Chemical Safety - Flammables



Store flammable chemicals in flammable storage cabinet or explosion-proof/flammable storage refrigerator

- Stored in flammable cabinet and/or explosion-proof or flammable storage refrigerator
- Stored away from sources of heat and ignition
- Not stored along path of egress or in aisle space

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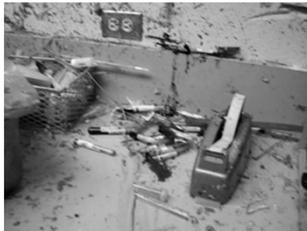
Autoclave Safety

- To prevent bottles from shattering during pressurization, the caps of containers with liquids must be loosened before loading
- Before removing autoclaved items, wait 5 minutes for loads containing only dry glassware, and 10 minutes for autoclaved liquid loads
- Remember: these are not used to steam your lunch!!

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Autoclave Safety



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Lab Hygiene

- Food and drink are not to be stored or prepared in laboratories or chemical storerooms
- Use appropriate personal protective equipment and wash your hands regularly when working with chemical reagents, especially before meals or snacks.
- Smoking in laboratories is prohibited.

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Lab Hygiene

- Loose sleeves are a hazard and should not be worn in the lab.
- If you have long hair, ensure that it is properly tied back.
- Wearing of contact lenses in the lab is strongly discouraged.
 - If it is unavoidable, advise your supervisor and co-workers so that this information is known in the event of a chemical splash in the eyes.

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Personal Practices

- No inappropriate clothing and shoes (shorts, sandals, slippers, etc.)
- Gloves removed before handling telephone, door handle or leaving laboratory
- No pipeting by mouth



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Personal Practices

- Lab coats and safety glasses/goggles worn by all where necessary
- Proper gloves are used as needed
- Other personal protective equipment used properly as needed



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Safety Glasses

- Unbreakable lenses of plastic or tempered glass
- For light-to-moderate work
- Can be prescription glasses
- Do not interfere with contact lenses



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Goggles

- Work with significant risk of splash of chemicals or projectiles
- Can be worn over prescription glasses



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Face Shield



- Work with significant risk of splash on face or possible explosion
- Face shield protects face adequately but not eyes
 - Should be worn with safety glasses and/or goggles to protect eyes

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Pregnancy

- Women who are pregnant should discuss their work assignments with their supervisors to seek alternate work assignments if the potential for exposure to teratogens exist
 - Teratogens are reproductive toxins that may cause damage to the fetus
 - THM Plus method by Hach for determining Trihalomethanes deals with chloroform, a teratogen

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Fume Hood



- All work generating toxic/hazardous vapor, fume, or aerosol performed in hood
- Front sash at appropriate level when hood is in use/not in use
- Storage within the hood minimized and containers kept sealed

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Fume Hood

- Verify that the fume cupboard is working properly.
- Locate work at least 6 inches inside the hood.
- Do not block the face of the hood, e.g. with shielding or large equipment.
- Do not block the space between tapered metal front lip and the work surface.
- Do not block rear exhaust slot. Place bulky items to rear and sides.
- Secure papers and other light weight materials to prevent their entrainment in the exhaust.

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Lab Work Area

- The work bench is to be kept clean at all times, and free from chemicals and apparatus which are not required.
- Before starting an experiment, make sure you are familiar with all the procedures and the potential hazards of the starting materials and products.
 - Determine the appropriate safeguards and remedies.
 - Know the procedures for emergency shut off as well as the person and phone numbers to contact in case of emergency.
 - If anything unexpected occurs during your experiment, or if you are in any doubt, consult your supervisor immediately.

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Handling Glassware

- Examine all glassware before use.
 - Discard any broken glass apparatus in the appropriate sharps container.
- Never store damaged glassware in cupboards.
 - Damaged glassware should either be sent for repair properly or disposed in a separate labeled container for sharps disposal.
- Use gloves when sweeping up broken glass, do not use bare hands.
 - Pick up fine glass particles with wet paper toweling.

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Handling Glassware



- Cut ends of glass rods and tubing should always be fire-polished before use.
- Use a cloth for protection when inserting glass tubing, rods or thermometers into bungs or tubing
 - Use a lubricant or water where necessary.

Chemical Spills



- Small spills (generally less than 100 mL) can usually be cleaned up safely by the employees involved.
- The hazardous properties of the material must be considered when deciding whether it is a “small” spill or not, and therefore whether unassisted clean-up should be attempted.
- Employees must be trained in advance to handle cleanup of even small spills



SAMPLING

Why Sample?

- Meet compliance requirements
- Process control
- Ensure public safety/protect the environment

Considerations

- The way the sample is collected
- The sample volume required
- The way the sample is stored
- The selection of sample points
- The sampling frequency

Grab Samples

- Single volume of water
- Representative of water quality at exact time and place of sampling
- Grab samples are used to test for unstable parameters that could change if the sample were allowed to stand for any length of time
 - DO
 - pH
 - Chlorine residual
 - Temperature
 - E. coli and/or fecal coliform

Composite Sample

- Collected at regular intervals
- In proportion to existing flow
- Combined to form sample representative of entire flow for period

Composite Sample

- Refrigerated; thoroughly mixed
- Measure flow and sample volume
- Examples:
 - BOD
 - Total N
 - Settleable solids

Sampling Guidelines

- Representative
- Proper container
- Do not contaminate the lid
- Preservative/ dechlorinating agent

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Sampling Guidelines

- Hold by base
- Turn into current
- Avoid air bubbles
- Label containers with sampler name; date and time; method; test to run; preservatives.

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Sample Volume

- Depends on test procedure
- Headspace for mixing
- Preservative
- QA/QC comparisons

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Sampling Point Selection

- Flow well mixed
- Exclude large particles (>1/4 inch)
- Exclude floating matter
- Readily accessible & in safe area

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Sampling Devices



- **Automatic:**
 - Timesavers
 - Clean intake line regularly to prevent growth of bacteria

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Sampling Devices



- **Manual:**
 - Dippers
 - Weighted bottle sampler
 - Whirl-pak® bags
 - Jugs

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Sources of Error

- Improper sampling
- Poor or improper sample preservation
- Lack of sufficient mixing during compositing and testing

Preservation Techniques

- Refrigeration at 6°C
- pH<2:
 - Using HCl
 - Using H₂SO₄
 - Using HNO₃
- pH>9 using NaOH
- pH>12 using NaOH

Section 2
QA/QC & Method Update Rule

QA/QC

2014 Updates for 40CFR136

Method Update Rule
February 5, 2014
Fleming Training Center

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NPDES Permit

- * Section 1.2.3 Test Procedures
 - * b. Unless otherwise noted in the permit, all pollutant parameters shall be determined according to methods prescribed in Title 40, Code of Federal Regulations, Part 136, as amended, promulgated pursuant to Section 304 (h) of the Act.
- * Section 2.1.4 Proper O&M
 - * a. ...proper O&M also includes ... appropriate quality assurance procedures.

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You Have Heard it All Before

- * More Rules
- * More Testing
- * More Paperwork
- * More Cost



- * But everything we do is regulated.

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2012 Update of 136

- * Standard Methods approved by date not Edition
- * Section 136.7 Quality Assurance and Quality Control.



Federal Register May 18, 2012

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Routinely run parameter	New approval year for parameter method in Std Methods	Method #	Associated methods	Found in 20 th Ed. Std Methods
Oxygen	2001	4500-O	B,C,D, E, F	No
Phenols	2005	5530-phenols	B, D	No
Oil & Grease	2001	5520-Oil and Grease	B, F	No
Total Phosphorus	1999	4500-P	E,F, G, H	No
Nitrate	2000	4500-NO ₃	D	No
TSS	1997	2540-TSS	B,C,D,E,F	Yes
Ammonia as N	1997	4500-NH ₃	B,C,D,E,F,G,H	Yes
Cyanide	1999	4500-CN	G	No
Hydronium ion, pH	2000	4500-H	B	No
CBOD, BOD	2001	5210	B	No
Total residual Chlorine	2000	4500-Cl	D,E,B,C,F,G	No

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40 CFR 136 03-12-2007

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES

Parameter	Methodology ¹	Reference method number or page ²				ASTM
		EPA ³ 816	Standard methods (18th, 19th)	Standard methods (20th)	Standard methods online	
1. Acidity, as CaCO ₃ , mg/L	Electrometric endpoint or phenolphthalein endpoint		210 B(4)	210 B(4)	210 B(4)-97	D107-02, 02
2. Alkalinity, as CaCO ₃ , mg/L	Electrometric or colorimetric titration automatic		200 B	200 B	200 B-97	D107-02, 02
3. Aluminum—Total, mg/L	Digestion ⁴ followed by AA direct aspiration ⁵ AA furnace	310.3 (Rev. 12/4)	3113 B		3113 B-99	
	STGAA ⁶	200 B, Rev. 2.2 (1994)			3113 B-99	
	ICP-AES ⁷	200.7, Rev. 4.4 (1994)	3120 B	3120 B	3120 B-99	
4. Ammonia (as N), mg/L	ICP-AES ⁷	200.8, Rev. 5.4 (1994)				D673-03
	Direct Current Plasma (DCP) ⁸		5000-AI D	5000-AI B	5000-AI B-01	D4190-04, 99
	Colorimetric (Electrode system F)		4500-NH ₃ B	4500-NH ₃ D	4500-NH ₃ B-97	
	Manu. distillation (at pH 9.5) ⁹ followed by Nesslerization	300.1, Rev. 2.0 (1992)	4500-NH ₃ C (18th and 19th)	4500-NH ₃ C	4500-NH ₃ C-97	D1426-98, 03 (A)
Titration			4500-NH ₃ D (19th) and 4500-NH ₃ E (18th)	4500-NH ₃ D or E	4500-NH ₃ D or E-97	D1426-98, 03 (B)
	Electrode		4500-NH ₃ G (19th) and 4500-NH ₃ F or G (18th)	4500-NH ₃ G	4500-NH ₃ G-97	
Automated phenals, or		300.1 ¹⁰ , Rev. 2.0 (1992)	4500-NH ₃ G (19th) and 4500-NH ₃ H (18th)	4500-NH ₃ G	4500-NH ₃ G-97	
Automated electrode						D673-03
Ion Chromatography						D673-03

1. Full- or partial-standards method
2. EPA 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000

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12 Quality Control Elements

1. DOC – demonstration of capability
 2. MDL – method detection level
 3. LRB/MB – method blank
 4. LFB – laboratory fortified blank (standard)
 5. LFM/LFMD – laboratory fortified matrix/duplicate (spike)
 6. Internal standards, surrogate standards or tracer – only applies to organic analysis and radiochemistry
 7. Calibration- initial and continuing
 8. Control charts or other trend analysis
 9. Corrective action – root cause analysis
 10. QC acceptance criteria
 11. Definition of a batch (preparation and analytical)
 12. Minimum frequency for conducting all QC elements
 13. Unwritten 13th Step – SOP – Standard Operating Procedures need to be written and followed for all lab sampling and analyses
- Not all of these items apply to all tests, there are many exceptions!



Can you defend what you do?

- * How do you interpret your Permit language or the Rule?
- * Can you defend that interpretation, will a judge or jury support you?
- * What do Regulators say and what is written?
 - * Is it clear?
 - * Don't be afraid to ask Why?
 - * Don't be afraid to ask for directives in writing.



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What You Are Already Doing



- * Most Labs are doing lots of QA/QC stuff
- * Write down what you do....SOP
- * Summarize QC Data
 - * Table Form
 - * Average, Max, Min.
 - * Control Charts

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Demonstration of Capability

- * DOC
- * Standard Methods 1020.B.1
 - * As a minimum, include a reagent blank and at least 4 LFBs at a concentration between 10 times the MDL and the midpoint of a calibration curve.
- * Standard Methods 2020B.1.a, 4020B.1.a. & 5020.B.1.a
 - * Run a laboratory-fortified blank (LFB) at least four times and compare to the limits listed in the method
 - * LFB initial recovery limits = $\text{Mean} \pm (5.84 \times \text{Standard Deviation})$

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Demonstration of Capability

- * What tests does this apply to?
 - * Ammonia, BOD/CBOD, Chlorine, pH, DO, Total Phosphorus, TSS
- * Analyst needs to make up this standard, cannot be bought premade
- * Example: for ammonia, the analyst needs to make up 1.0 mg/L, not purchase pre-made 1.0 mg/L
 - * Analyst can make 1 L of 1.0 mg/L by diluting down from 100 mg/L or 1000 mg/L and then pour up 4-100 mL aliquots

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Demonstration of Capability

- * How often?
 - * Once for each analyst.
 - * Recommended yearly for backup analyst who does not perform tests frequently
 - * EPA highly recommends running every 2-3 years for every analyst
 - * Each analyst should have a file kept on their training within and for the lab.
 - * Something to keep along with these records is a signed form (documentation) that analyst has read and understands all appropriate SOPs and Methods.

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Demonstration of Capability

- * **2014 Update**
- * **DMRQA's were removed as acceptable DOC**
- * **Analyst have had a year, there should be at least 4 standards that have been analyzed and within limits to demonstrate capability.**

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Method Detection Level

- * MDL
- * Standard Methods 1020.B.4
 - * As a starting point for selecting the concentration to use when determining the MDL, us an estimate of five times the estimated true detection level
 - * Ideally, prepare and analyze at least seven portions of this solution over a 3-day period to ensure the MDL determination is more representative of routine measurements as performed in the laboratory

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Method Detection Level

- * Standard Methods 1020.B.4 - continued
- * Recommended that the replicate measurements should be in the range of one to five times the estimated MDL, and recoveries of the known addition should be between 50-150% , with RSD (relative standard deviation) values $\leq 20\%$
- * Standard Methods 4020.B.1.b
 - * Ideally use pooled data from several analysts rather than data from one analyst

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Method Detection Level

- * What tests does this apply to?
 - * Ammonia, Chlorine, Total Phosphorus
- * How often?
 - * Annually

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What the heck IS an MDL study?

- * It is a calculation that statistically gives the lowest concentration that a lab/facility can "see", that is detect an analyte
- * Not practical for many analyses
- * It is a bit tricky the first time, but KEEP RECORDS so next year it will be a breeze.
- * Fresh samples prepared daily are preferred and it is recommended that samples are run over 3 days to give a more accurate account of how samples are run.

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How MDL Studies are Performed

- * Make seven very low level blank spikes (can be lower than the lowest point on your curve)
- * Analyze all seven over several days and calculate the standard deviation
- * Multiply the standard deviation by the "student t" for 7 values (3.14)
- * You cannot "cherry pick" your results, they must be 7 samples in a row

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MDL Calculations

- * The result is the MDL (method detection level)
- * The MDL must be greater than 1/10 the concentration of each spike
 - * Example: if the spike was 3, the MDL cannot be lower than 0.3 (3 divided by 10)
- * Keep up with the best spike value used for your MDL study so you don't have to go through several attempts each year
- * **2014 Update – this is your reporting limit**

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Good MDL Values for Cl₂

Date	Analyst	Number	True Value	Value Read	% Recovery (50-150%)
1/28/2013	SEP	1	0.05	0.09	180.00
1/28/2013	SEP	2	0.05	0.07	140.00
1/28/2013	SEP	3	0.05	0.07	140.00
1/30/2013	SEP	4	0.05	0.08	160.00
1/30/2013	SEP	5	0.05	0.08	160.00
2/1/2013	SEP	6	0.05	0.07	140.00
2/1/2013	SEP	7	0.05	0.08	160.00
Standard Deviation				0.007559289	
Relative Standard Deviation (RSD)				9.7990789	(Needs to be ≤ 20%)
MDL				0.0237362	

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MDL is NOT good because it is < 1/10th of the spike of 1.00. 1/10th of the spike is 0.10 mg/L and the calculated MDL is less than this and therefore unacceptable.

NOT Good MDL Values for Cl₂

Date	Analyst	Number	True Value	Value Read	% Recovery (50-150%)
1/28/2013	SEP	1	1.00	1.00	100.00
1/28/2013	SEP	2	1.00	0.99	99.00
1/28/2013	SEP	3	1.00	1.00	100.00
1/30/2013	SEP	4	1.00	1.00	100.00
1/30/2013	SEP	5	1.00	0.98	98.00
2/1/2013	SEP	6	1.00	1.01	101.00
2/1/2013	SEP	7	1.00	1.00	100.00
Standard Deviation				0.009511897	
Relative Standard Deviation (RSD)				0.9539152	(Needs to be ≤ 20%)
MDL				0.0298674	

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Laboratory Reagent Blank

- * LRB
- * Also known as Method Blank
- * Standard Methods 1020.B.5
 - * A reagent blank (method blank) consists of reagent water and all reagents that normally are in contact with a sample during the entire analytical procedure (distillation, incubation, etc.)
- * What tests does this apply to?
 - * Ammonia, BOD/CBOD, Chlorine, Total Phosphorus, TSS
- * How often?
 - * Depends on method QA/QC

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Laboratory Fortified Blank

- * LFB
- * Standard Methods 1020.B.6
 - * A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added
 - * Sample batch = 5% basis (see batch size for more information)
 - * Use an added concentration of at least 10 times the MDL, or less than or equal to the midpoint of the calibration curve

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Laboratory Fortified Blank

- * Standard Methods 2020.B.2.e – TSS
 - * Using stock solutions, prepare fortified concentrations so they are within the calibration curve
- * Standard Methods 4020.B.2.e – Ammonia, BOD/CBOD, Chlorine, Phosphorus
 - * Calculate percent recovery, plot control charts and determine control limits
 - * **More control chart info later**
- * What tests does this apply to?
 - * Ammonia, BOD/CBOD, Chlorine, Total Phosphorus, TSS

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Laboratory Fortified Blank

- * How often?
- * For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria:
 - * Influent and Effluent are 2 different samples
 - * If a permit stated that 3 analyses per week, we would allow for a LFB to be analyzed at least once per month.
 - * Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - * If a permit stated 5 analyses per week, we would allow twice a month.
 - * Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

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Laboratory Fortified Matrix and Duplicate

- * LFM/LFMD
- * Also known as a spike and spike dup
- * Standard Methods 1020.B.7
 - * A laboratory matrix (LFM) is an additional portion of a sample to which a known amount of the analyte of interest is added before sample preparation
 - * The LFM is used to evaluate analyte recovery in a sample
 - * Sample batch = 5% basis
 - * Add a concentration less than or equal to the midpoint of the calibration curve
 - * Preferably the same concentration as the LFB (laboratory fortified blank)

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Laboratory Fortified Matrix and Duplicate

- * Standard Methods 4020.B.2.g
 - * When appropriate for the analyte, include at least one LFM/LFMD ... with each batch of 20 samples
 - * Add a known concentration of analyte (ideally from a second source) to a randomly selected routine sample. Calculate percent recovery and relative percent difference, plot control charts and determine control limits for spikes at different concentrations
- * What tests does this apply to?
 - * Ammonia and Total Phosphorus

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Laboratory Fortified Matrix and Duplicate

- * How often?
- * For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria:
 - * Influent and Effluent are 2 different samples
 - * If a permit stated that 3 analyses per week, we would allow for a spike to be analyzed at least once per month.
 - * Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - * If a permit stated 5 analyses per week, we would allow twice a month.
 - * Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

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Laboratory Fortified Matrix and Duplicate

- * Also called a Matrix Spike/Matrix Spike Duplicate (MS/MSD)
 - * Calculate RPD between Spike and Spike Dup
- * Shows if there are interferences in the effluent matrix
- * **2014 Update – Spike volume should be less than 1% of the volume.**
 - * Example: spike with 1 mL of 1000 mg/L into 100 mL sample will equal a 10 mg/L increase in ammonia concentration.

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Duplicate

- * Dup
- * Not a part of the 12 Steps of QA, an addition from the State of TN
- * Standard Methods 1020.B.8
 - * As a minimum, include one duplicate sample with each sample set or on a 5% basis
- * Standard Methods 1020.B.12
 - * Calculate the RPD (relative percent difference)
 - * Equal to or less than 20% RPD

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Duplicate – TSS & Sett. Solids

- * Standard Methods 2020.B.2.f
 - * Include at least one duplicate for each matrix type daily or with each batch of 20 or fewer samples
- * Standard Methods 2540.A.2
 - * To aid in quality assurance, analyze samples in duplicate.
 - * Dry samples to constant weight if possible.
 - * This entails multiple drying-cooling-weighing cycles for each determination... more info later in presentation.
- * Standard Methods 2540.D.3.c
 - * Analyze at least 10% of all samples in duplicate

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Duplicate – Cl₂, pH and DO

- * Standard Methods 4020.B.2.f
 - * Randomly select routine samples to be analyzed twice
 - * Process duplicate sample independently through the entire sample preparation and analysis
 - * Include at least one duplicate for each matrix type daily or with each batch of 20

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Duplicate – BOD/CBOD

- * Standard Methods 5020.B.2.f
 - * Randomly select routine samples to be analyzed twice
 - * Process duplicate sample independently through the entire sample preparation and analysis
 - * Include at least one duplicate for each matrix type daily or with each batch of 20

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Duplicate

- * What tests does this apply to?
 - * BOD/CBOD, Chlorine, pH, DO, TSS and Settleable Solids
- * How often?
- * For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria: (10% would be once every 10 samples for TSS)
 - * If a permit stated that 3 analyses per week, we would allow for a dup to be analyzed at least once per month.
 - * Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - * If a permit stated 5 analyses per week, we would allow twice a month.
 - * Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

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Initial Calibration Verification & Continuing Calibration Verification

- * ICV
 - * Standard Methods 1020.B.11.b
 - * Perform initial calibration using at least three concentrations of standards for linear curves
 - * Calibrate meter (DO, pH or ISE) or verify balance, thermometer and colorimeter/spectrophotometer

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Initial Calibration Verification & Continuing Calibration Verification

- * CCV
 - * Standard Methods 1020.B.11.c
 - * Analysts periodically use a calibration standard to confirm that the instrument performance has not changed significantly since initial calibration.
 - * Verify calibration by analyzing one standard at a concentration near or at the mid-point of the calibration range.
 - * Verify the calibration (especially if preset by manufacturer) at beginning of day, after every 10 readings and at the end of the batch
 - * Daily (day of)

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ICV - TSS



- * Standard Methods 2020.B.2.a
- * Check instrument balances daily
- * Standard Methods 9020.B.4.b
- * Service balances annually or more often as conditions change or problems occur
 - * Check balances routinely, preferably daily before use, with at least two working weights that bracket the normal usage range (e.g. ANSI/ASTM Class 1 or NIST Class S accompanied by appropriate certificate) for accuracy, precision and linearity.
 - * Record results along with date and technicians initials
 - * Recertify reference weights as specified in the certificate of calibration or at least every 5 years.

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ICV – Temperature

- * Standard Methods 9020.B.4.a
 - * Annually, or preferably, semiannually check accuracy of all working temperature sensing devices, such as liquid-in-glass thermometers, thermocouples and temperature-recording instruments at the use temperature against a certified National Institute of Standards and Technology (NIST) thermometer or one traceable to NIST and conforming to NIST specifications.
 - * Record calibration results, along with the date and the technician's signature, in a quality control logbook.
 - * Mark the necessary calibration correction factor on each temperature measuring device so that only calibrated-corrected temperature values are recorded.
 - * Verify accuracy of the reference certified thermometer as specified on the certificate of calibration or at least every 5 years.

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ICV –Ammonia, BOD/CBOD, Chlorine, pH, DO, Phosphorus

- * Standard Methods 4020.B.2.a
- * Calibrate initially with at least one blank and three calibration standards
- * The appropriate linear correlation coefficient for standard concentration-to-instrument response should be greater than or equal to 0.995.
- * The back-calculated and true concentrations should agree within $\pm 10\%$.

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CCV –Ammonia, BOD/CBOD, Chlorine, pH, DO, Phosphorus

- * Standard Methods 4020.B.2.b
 - * Verify calibration by periodically analyzing a calibration standard and calibration blank during a run – typically after each batch of 10 samples and at the end of the run.
 - * For the calibration verification to be valid, check standards must be within 10% of its true value

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ICV –Probes for Ammonia

- * Standard Methods 4500-NH₃ D.4.a.
 - * Prepare a series of standard solutions covering the concentrations of 1000, 100, 10, 1 and 0.1 mg NH₃-N/L
- * Standard Methods 4500-NH₃ D.4.b.
 - * Calibrate from lowest to highest concentration.
 - * Wait until the reading has stabilized (at least 2-3 min) before recording millivolts for standards and samples containing ≤ 1 mg NH₃-N/L.
- * Standard Methods 4500-NH₃ D.4.c.
 - * If the electrode is functioning properly a tenfold change of NH₃-N concentration produces a potential change of about 59 mV.

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ICV/CCV –Chlorine

- * Prepare a set of Chlorine Standards or Potassium Permanganate (KMnO₄) standards in accordance with "Guidance for Secondary Standards use in Calibration" monthly.
- * Initial – Chlorine Standards or Potassium Permanganate (KMnO₄) standards monthly.
- * Continuing – Chlorine Standards, Potassium Permanganate (KMnO₄) standards or gel standards daily (day of).

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ICV/CCV –Chlorine

- * Secondary standards (gel standards) are specifically designed to verify the instrument’s calibration and to check the instrument’s performance.
- * They are not intended to be used to create calibration curves or to calibrate the instrument.
- * Because the DPD reagent cannot be mixed with the gel standards, the quality and the reaction time of the reagent cannot be assessed.
- * For these reasons gel standards cannot take the place of primary standards.

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ICV/CCV – Phosphorus

- * ICV/CCV – does not go through digestion
- * LFM and LFB – does go through digestion

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Accuracy Control Charts

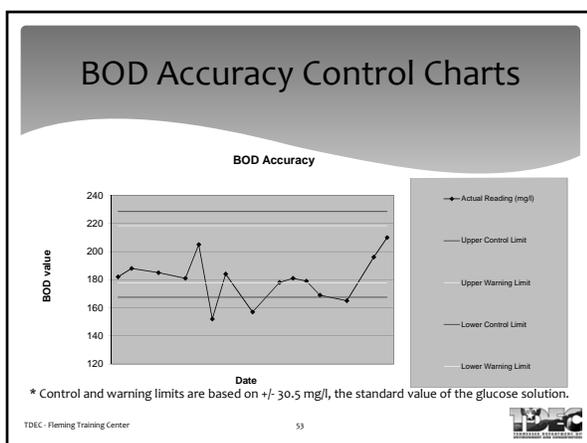
- * Standard Methods 1020 B.13.a
- * The accuracy chart for QC samples (e.g., reagent blanks, LFBs, calibration check standards and LFMs) is constructed from the average and standard deviation of a specified number of measurements of the analyte of interest.
- * The accuracy chart includes upper and lower warning levels (WL) and upper and lower control levels (CL).
- * Common practice is to use $\pm 2s$ and $\pm 3s$ limits for the WL and CL, respectively, where s represents standard deviation.

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BOD Accuracy Control Charts

Date	Reading (mg/l)	Standard Value	Difference	*Upper Limit	*Lower Limit	*2/3 Upper	*2/3 Lower
8/22	182	198	-16	228.5	167.5	218.33	177.67
8/23	188	198	-10	228.5	167.5	218.33	177.67
8/25	185	198	-13	228.5	167.5	218.33	177.67
8/27	181	198	-17	228.5	167.5	218.33	177.67
8/28	205	198	7	228.5	167.5	218.33	177.67
8/29	152	198	-46	228.5	167.5	218.33	177.67
8/30	184	198	-14	228.5	167.5	218.33	177.67
9/1	157	198	-41	228.5	167.5	218.33	177.67
9/3	178	198	-20	228.5	167.5	218.33	177.67
9/4	181	198	-17	228.5	167.5	218.33	177.67
9/5	179	198	-19	228.5	167.5	218.33	177.67
9/6	169	198	-29	228.5	167.5	218.33	177.67
9/8	165	198	-33	228.5	167.5	218.33	177.67
9/10	196	198	-2	228.5	167.5	218.33	177.67
9/11	210	198	12	228.5	167.5	218.33	177.67

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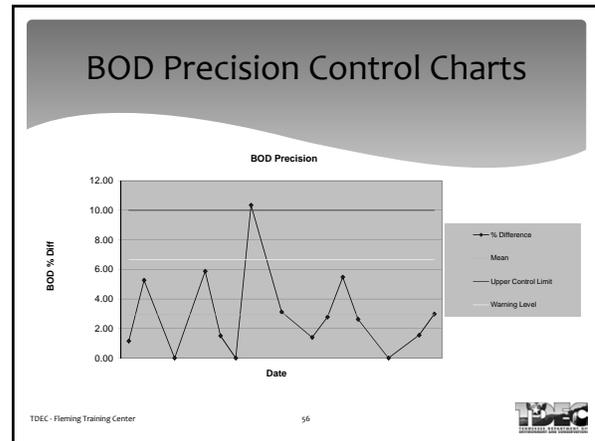
Precision Control Charts

- * Standard Methods 1020 B.13.b
- * The precision chart also is constructed on the average and standard deviation of a specified number of measurements (e.g., %RSD [relative standard deviation] or RPD) for a replicate or duplicate analyses of the analyte of interest.

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BOD Precision Control Charts

Date	#1 (mg/l)	#2 (mg/l)	Avg.	Diff. (Range)	% Diff.	2 X SD	3 X SD	Mean	Max Variat.	2/3 Max Var.	
8/22	87	86	86.5	1.0	1.16	5.63	8.45	2.94	10.00	6.67	
8/23	78	74	76.0	4.0	5.26	5.63	8.45	2.94	10.00	6.67	
8/25	62	62	62.0	0.0	0.00	5.63	8.45	2.94	10.00	6.67	
8/27	70	66	68.0	4.0	5.88	5.63	8.45	2.94	10.00	6.67	
8/28	67	66	66.5	1.0	1.50	5.63	8.45	2.94	10.00	6.67	
8/29	76	76	76.0	0.0	0.00	5.63	8.45	2.94	10.00	6.67	
8/30	61	55	58.0	6.0	10.34	5.63	8.45	2.94	10.00	6.67	
9/1	65	63	64.0	2.0	3.13	5.63	8.45	2.94	10.00	6.67	
9/3	72	71	71.5	1.0	1.40	5.63	8.45	2.94	10.00	6.67	
9/4	73	71	72.0	2.0	2.78	5.63	8.45	2.94	10.00	6.67	
9/5	75	71	73.0	4.0	5.48	5.63	8.45	2.94	10.00	6.67	
9/6	77	75	76.0	2.0	2.63	5.63	8.45	2.94	10.00	6.67	
9/8	83	83	83.0	0.0	0.00	5.63	8.45	2.94	10.00	6.67	
9/10	65	64	64.5	1.0	1.55	5.63	8.45	2.94	10.00	6.67	
9/11	66	68	67.0	2.0	2.99	5.63	8.45	2.94	10.00	6.67	
TDEC - Fleming Training Center					2.94	mean					
					2.82	St.Dev.					



Control Charts

- * **2014 Update** - Create and maintain control charts if you have 20-30 data points within 90 days.
- * If you do not meet the above criteria, follow QC Acceptance Criteria below.
 - * Blanks < MDL
 - * LFB ± 15%
 - * ICV/CCV ± 10%
 - * LFM/LFMD ± 20%
 - * RPD < 20%
 - * Reporting limit = MDL

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Corrective Action

- * Standard Methods 1020 B.15
- * QC data that are outside the acceptance limits or exhibit a trend are evidence of unacceptable error in the analytical process.
- * Take corrective action promptly to determine and eliminate the source of error.
- * Do not report data until the cause of the problem is identified and either corrected or qualified (see Table 1020:11)

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Corrective Action

- * The corrective action plan needs to be in your SOP for each method on what to do if your QC tests fail or are out of range
- * If you have a "boo boo", write down how you fixed it
- * Any issues should be recorded and a sentence on how it can be prevented, if possible, in the future
- * Common problems and their corrections should be covered in your Standard Operating Procedures (SOP)
 - * If you see things frequently, you can give them qualifiers that are noted in your SOP;
 - * R = rain event
 - * D = bad dilution, etc.

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QC Acceptance

- * Have in SOP for each method the acceptance ranges for standards, duplicates, spikes, etc. and make sure they match the method requirements.
- * If not mentioned in method, these are the accepted criteria for QC:
 - * Blank < reporting limit
 - * LFB ± 15%
 - * MS/MSD ± 20%
 - * ICV/CCV ± 10%
 - * RPD < 20%
 - * Reporting limit = MDL

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Batch Size

- * Each "Batch" could be daily (day of), every 10 samples or every 20 samples.
- * Check method
- * Influent and Effluent are 2 different samples
- * If you sample only once a month, need to run QC each time.
 - * Once per month is minimum requirement

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Batch Size

- * For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria:
 - * If a permit stated that 3 analyses per week, how many samples would that be a week?
 - * TSS and BOD would be 6, Cl₂ would be 3
 - * If a permit stated 5 analyses per week, how many samples would that be a week?
 - * TSS and BOD would be 10, Cl₂ would be 5

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QC Frequency

- * Usually lumped in with the definition of a "batch" and should be in the SOP of some kind

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Standard Operating Procedure

- * Here's that "13th Step", your SOP
- * All procedures must be documented in some type of SOP
- * It can be very simple but must provide the information necessary for someone who is not familiar with the test to perform it
 - * Step by step instructions on how and where to collect the samples, how to run the test and how to report values.
- * It must include the QC Acceptance Criteria, the definition of a "Batch" and the minimum frequency of QC checks

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Initial Demonstration of Capability (DOC)

- 2020 B.1 - each analyst must run a known standard concentration at least four times and compare limits listed in the method (under Precision). Table 2020:II lists duplicates and MB for QC only.
- Recommend running replicates and compare results and calculate the standard deviation to compare with that reported in 2540 D.5.
- **Real people language - each operator running this test needs to analyze 4 samples of a TSS Standard**
 - **Keep a folder for each analyst, keep a copy here**
 - **Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.**
 - **Recommend backup analyst do this once a year.**

Method Detection Limit (MDL)

- NONE

Initial Calibration Verification (ICV)

- 2020 B.2.a.– check instrument balance daily as stated below.
- 9020.B.4.b. Service balances annually or more often as conditions change or problems occur...

Check balance routinely, preferably daily before use, with at least two working weights that bracket the normal usage range. (e.g., ANSI/ASTM Class 1 or NIST Class S accompanied by appropriate certificate) for accuracy, precision, and linearity. Record results along with date and technician's initials.

Recertify reference weights as specified in the certificate of calibration or at least every 5 years.

- 2540 B.2. analytical balance, with a sensitivity of 0.1 mg
- **Real people language – check balance daily (day of) with at least 2 working weights that bracket the normal usage range and record results on bench sheet or separate log book.**

Method Blank

- 2020 B.2.d.– include at least 1 method blank (MB) daily or with each batch of 20 or fewer samples, whichever is more frequent.
- **Real people language – on a 5% basis (see batch size for more information) filter 100 mL of distilled water.**
 - **Should be less than 2.5 mg/L.**

Laboratory Fortified Blank (LFB)

- 1020 B.6.– A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added.
 - Sample batch = 5% basis

12 Steps of Lab Quality Assurance

Parameter	Method	DOC	MDL	Method Blank	LFB	LFM / LFMD	Dup	ICAL / CCV	Control Charts	Corrective Action	QC Acceptance	Batch Size	*QC Frequency
Ammonia	SM4500-NH3 D - 1997	X	X	X	X	X		X, Calibrate meter daily	X	X	X	20	Depends on Permit
BOD ₅ / CBOD ₅	SM5210 B - 2001	X		X	X		X	X, Calibrate meter daily	X	X	X	20	Depends on Permit
Chlorine, TR	SM4500-Cl G - 2000	X	X	X	X		X	X, verify meter daily w Secondary Gel Standards	X	X	X	20	Depends on Permit
pH	SM4500-H+ B - 2000	X					X	X, Calibrate meter daily		X	X	20	Depends on Permit
Oxygen, dissolved	SM4500-O G - 2001	X					X	X, Calibrate meter daily & verify with air-saturated water		X	X	20	Depends on Permit
	Hach Method 10360 Luminescence Oct. 2011	X					X	X, Calibrate meter daily & verify with air-saturated water		X	X	20	Depends on Permit
Phosphorus, total	SM4500-P B and E - 1999	X	X	X	X	X		X, verify meter	X	X	X	20	Depends on Permit
TSS	SM2540 D - 1997	X		X	X		X	X, verify scale daily		X	X	20	Depends on Permit
Sett. Solids	SM2540 F - 1997						X			X		20	Depends on Permit
Temperature	SM2550 B - 2000							X, verify against NIST thermometer		X			Annually

DOC – Demonstration of Capability

- Each analyst should have a file kept from where they have calibrated and analyzed 4 standards to demonstrate they can accurately run this test
- Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods
- Recommend backup analyst do this once a year

MDL – Method Detection Limit

- Annually run at least 7 samples at low levels

Method Blank – aka Laboratory Reagent Blank (LRB)

- Analyze distilled/deionized water as a sample

LFB – Laboratory Fortified Blank

- Analyze a known standard

LFM/LFMD – Laboratory Fortified Matrix/Laboratory Fortified Matrix Duplicate

- Analyze a sample with a known amount of standard added (spike)

Dup – Duplicate

- Analyze the same sample twice

ICAL/CCV – Initial Calibration/Continuing Calibration Verification

- Calibrate meter (DO, pH, ISE) or verify balance, thermometer and colorimeter/spectrophotometer
- Verify the calibration (especially if preset by manufacturer) at beginning of day and/or after every 10 readings, whichever comes first.

Control Charts

- Create and maintain control charts if you have 20-30 data points within 90 days.
- If you do not meet the above criteria, follow QC Acceptance Criteria below.

Corrective Action

- Have corrective action plan in SOP for each method on what to do if QC tests fail or are out of range.
- For example, if standards fail, re-calibrate and run test again.

QC Acceptance

- Have in SOP for each method the acceptance ranges for standards, duplicates, spikes, etc. and make sure they match the method requirements.

Batch Size

- Each batch could be daily, every 10 samples or every 20 samples. Check method.

*QC Frequency (depends on permit) – at least once a month

- For samples that need to be analyzed on a 5% basis or once for every 20 samples, follow these criteria:
 - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
 - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, we would allow twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
 - **Please note that influent and effluent samples count as two separate samples. For example, if you are supposed to run 3 BODs a week, that should be counted as running 6 samples for that week.**
- For samples that need to be analyzed on a 10% basis or once for 10 samples, follow these criteria:
 - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least twice per month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, we would allow a duplicate to be analyzed once per week.
 - Pick a date and be consistent, every Monday or Wednesday. Mark your calendar!!
 - **Please note that influent and effluent samples count as two separate samples. For example, if you are supposed to run 5 TSSs a week, that should be counted as running 10 samples for that week and you should run your duplicates once a week.**

Standard Operating Procedure

- Here's that "13th Step", your SOP
- All procedures must be documented in some type of SOP
- It can be very simple but must provide the information necessary for someone who is not familiar with the test to perform it
 - Step by step instructions on how and where to collect the samples, how to run the test and how to report the values.
- It must include the QC Acceptance Criteria, the definition of a "Batch" and the minimum frequency of QC checks

Section 2020 B.2.e. – Using stock solutions, prepare fortified concentrations so they are within the calibration curve.

- **Real people language – analyze TSS Standard sample that can be prepared from recipe below or bought premade.**
 - **Run on a 5% basis (see batch size for more information).**

TSS Standard Samples

To prepare TSS check samples from dry reference material:

1. Dry the reference material* in the desiccator
2. On an analytical balance, weigh 0.1000 gram of the dry powder, put it in a 1000 mL volumetric flask, bring it to the mark with distilled or deionized water and shake well until well suspended.
3. Measure 100 mL and process as usual for environmental samples.
4. A difference of 10 mg should be obtained.
5. Calculation:

$$\frac{(A - B) (1000)}{\text{Vol. used}} = \frac{(10 \text{ mg}) (1000)}{100 \text{ mL}} = 100 \text{ mg/L}$$

*Example of material available from Fisher

- Celite 545 Filtler Aid (Powder), Fisher Chemical, 500 gram bottle – Cat#C212-500

Procedure to Omit Re-drying/Re-cooling/Re-weighing Cycle

How to acquire acceptable results for the total suspended solids comparability data:

- The maximum holding time for a total suspended solids sample prior to analysis is 7 days if stored at temperatures of 6 °C and below (not 0 °C). (40CFR part 136, Table II)
- EPA recommends that 4-7 different samples, in duplicate, be collected and analyzed for this procedure in order to prove that the step for “reheating, recooling, and reweighing” is unnecessary. “Different” could mean samples collected 4-7 consecutive days or 4-7 samples run in one day. These 4-7 samples are dried **overnight** at 103-105°C.
- The next morning, the filters are removed from the oven, allowed to cool in the desiccator and weighed.
- The samples are then returned to the drying oven for one hour, re-cooled and reweighed.
- The resulting data should be examined to determine if the difference between the overnight values and the redried values are less than 4% or 0.5 mg, whichever is less. If so, the redrying step may be omitted for a normal set of samples.
- This procedure excludes atypical samples. (i.e. high fat, oil and grease samples).
- The operator may choose not to perform this study and continue to follow the procedure for redrying/recooling/reweighing cycle as stated the method (SM 2540 D.3.c.).

The study should be re-evaluated at least once per year or whenever a change in sample characteristics occurs and kept on file at the treatment plant.

Duplicate

- 1020 B.8. states as a minimum to include one duplicate sample with each sample set or on a 5% basis whichever is more frequent.
- 2020 B.2.f. states to include at least one duplicate for each matrix type daily or with each batch of 20 or fewer samples.
- 2540 A.2. “To aid in quality assurance, analyze samples in duplicate. Dry samples to constant weight if possible. This entails multiple drying-cooling-weighing cycles for each determination.”
- 2540 D.3.c. Analyze at least 10% of all samples in duplicate.
- **Real people language – analyze 2 samples for TSS.**

Total Suspended Solids

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S. Pratt, January 2014



- **For example, filter 100 mL of effluent through filter pad A then filter another 100 mL of effluent through filter pad B. Dry, cool and weigh. Figure RPD for both samples.**
 - **Target value should be close to the first value and have a small RPD (less than 15%)**
 - **Analyze a duplicate at a 10% rate (see batch size for more information).**
- **For reporting purposes, average sample and duplicate.**

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD)

- NONE

Control Charts

- NONE

Corrective Action - 1020 B.5., B.8., & B.15.

QC Acceptance Criteria

- Blanks < 2.5 mg/L
- LFB \pm 15%
- RPD \pm 15%

Batch Size

- Influent and Effluent are 2 different samples
- For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria:
 - If a permit stated that 3 analyses per week, that would be 6 samples per week, we would allow for a blank and LFB to be analyzed at least twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, that would be 10 samples per week, we would allow once a week.
 - Pick a date and be consistent, every Monday. Mark your calendar!!
- For samples that need to be analyzed on a 10% basis or once for every 10 samples follow these criteria:
 - If a permit stated that 3 analyses per week, that would be 6 samples per week, we would allow for a duplicate to be analyzed at least twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, that would be 10 samples per week, we would allow once a week.
 - Pick a date and be consistent, every Monday. Mark your calendar!!

Total Suspended Solids

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Calculations

- % Recovery for LFB
 - = $\frac{\text{LFB concentration}}{\text{Expected concentration}} \times 100\%$
- RPD – relative percent differences for duplicates and LFM/LFMD
 - = $\frac{\text{Difference between sample and duplicate}}{\text{Average of the sample and duplicate}} \times 100\%$

Initial Demonstration of Capability (DOC)

- Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.

Method Detection Limit (MDL)

- NONE

Initial Calibration Verification (ICV)

- NONE

Method Blank

- NONE

Laboratory Fortified Blank (LFB)

- NONE

Duplicate –

- 2540 A.2. *“To aid in quality assurance, analyze samples in duplicate.*
- **Real people language – analyze 2 samples for Sett. Solids.**
 - **For example, pour up 1000 mL of effluent into Imhoff then pour up another 1000 mL of effluent in another Imhoff. Wait 45 min, stir, wait 15 min, read. Figure RPD for both samples.**
 - **Target value should be close to the first value and have a small RPD (less than 20%)**
 - **Run on a 5% basis (see batch size for more information).**
 - **For reporting purposes, average sample and duplicate.**

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD)

- NONE

Control Charts

- NONE

Corrective Action - 1020 B.5., B.8., & B.15.

QC Acceptance Criteria

- RPD < 20%
- Reporting Limit = lowest graduation mark on Imhoff cone

Batch Size

- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:

- Section 2
- If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
 - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, we would allow twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

Calculations

- RPD – relative percent differences for duplicates
 - = $\frac{\text{Difference between sample and duplicate}}{\text{Average of the sample and duplicate}} \times 100\%$

Initial Demonstration of Capability (DOC)

- Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.

Method Detection Limit (MDL)

- NONE

Initial Calibration Verification (ICV)

- 2020 B.2.a.– Verify calibration **annually**
- 9020.B.4.a. – Annually or, preferably, semiannually check accuracy of all working temperature-sensing devices, such as liquid-in-glass thermometers, thermocouples, and temperature-recording instruments at the use temperature against a certified National Institute of Standards and Technology (NIST) thermometer or one traceable to NIST and conforming to NIST specifications.
 - Record calibration results, along with the date and the technician’s signature, in a quality control logbook.
 - Mark the necessary calibration correction factor on each temperature measuring device so that only calibrated-corrected temperature values are recorded.
 - Verify accuracy of the reference certified thermometer as specified on the certificate of calibration or at least every 5 years.
- **Real people language – have thermometers verified annually by a NIST thermometer.**

Method Blank

- NONE

Laboratory Fortified Blank (LFB)

- NONE

Duplicate

- NONE

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD)

- NONE

Control Charts

- NONE

Corrective Action - 1020 B.5., B.8., & B.15.

Batch Size

- NONE

Minimum Detectable Concentration – 4500-Cl G.1.c. – approximately 10 µg/L (0.010 mg/L)

Initial Demonstration of Capability (DOC)

- 4020 B.1.a. - each analyst must run a known standard concentration at least four times and compare limits listed in the method.
- **Real people language – each operator running this test needs to analyze 4 samples of a chlorine or potassium permanganate (KMnO₄) standard at a concentration of 0.5 mg/L**
 - **Keep a folder for each analyst, keep a copy here**
 - **Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.**
 - **Recommend backup analyst do this once a year.**
 - **Only good for that type of instrument you are using at that plant. If you have a backup instrument made by a different manufacturer or you work at another plant with a different make/model, you would need another DOC.**
 - **DOCs demonstrate you are proficient with that one instrument.**

Method Detection Limit (MDL)

- 1020 B. 4 – As a starting point for selecting the concentration to use when determining the MDL, use an estimate of five times the estimated true detection level (5 x 0.010 mg/L = 0.050 mg/L).
 - Ideally, prepare and analyze at least seven (7) portions of this solution over a 3-day period to ensure that the MDL determination is more representative of routine measurements as performed in the laboratory.
 - The replicate measurements should be in the range of one to five times the estimated MDL, and recoveries of the known addition should be between 50 and 150%, with %RSD (relative standard deviation) values ≤ 20%.
- 4020 B.1.b. – Verify MDL at least **annually**.
 - Ideally use pooled data from several analysts rather than data from one analyst.
- **Real people language – have several operators, who run this test, analyze chlorine or Potassium Permanganate (KMnO₄) standards at a concentration of 0.05 mg/L over several days with a total of at least 7 samples**
 - **Joe analyzes 3 samples on Monday**
 - **Bob analyzes 3 samples on Tuesday**
 - **Mary analyzes 3 samples on Wednesday**
- **Run this once a year**

Initial Calibration Verification (ICV)

- 1020 B.11.b. – Perform initial calibration using at least three concentrations of standards for linear curves.
- 4020.B.2.a. – Calibrate initially with at least one blank and three calibration standards.
 - The appropriate linear correlation coefficient for standard concentration-to-instrument response should be greater than or equal to 0.995.
 - The back-calculated and true concentrations should agree within ± 10%.

- **Real people language – prepare a set of chlorine or potassium permanganate (KMnO_4) standards in accordance with [Guidance for Secondary Standards Use in Calibration 12-19-2013](#) monthly.**

Method Blank

- 1020 B.5.– A reagent blank (method blank) consists of reagent water and all reagents that normally are in contact with a sample during the entire analytical procedure.
- 4020 B.2.d. – Include at least one method blank *daily* or with each batch of 20 or fewer samples, whichever is more frequent.
 - If any method blanks measurements are at or above the reporting level, take immediate corrective action.
- **Real people language – analyze distilled water as a sample by adding a DPD powder pillow and waiting the 3-6 minutes before reading**
 - **Target value is less than reporting limit**
 - **Reporting limit will be equal to your Method Detection Limit (MDL)**
 - **Run on a 5% basis (see batch size for more information).**

Laboratory Fortified Blank (LFB)

- 1020 B.6.– A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added.
 - Sample batch = 5% basis = 1 every 20 samples
 - Use an added concentration of at least 10 times the MDL, less than or equal to the midpoint of the calibration curve.
- 4020 B.2.e. – Calculate percent recovery, plot control charts and determine control limits
- **Real people language – analyze chlorine or potassium permanganate standard at a concentration of 0.5 mg/L**
 - **Run on a 5% basis (see batch size for more information).**

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD)

- NONE

Duplicate

- 1020 B.12.f. – Calculate RPD (relative percent difference)
- 4020 B.2.f. – Randomly select routine samples to be analyzed twice.
 - Process duplicate sample independently through the entire sample preparation and analysis.
 - Include at least one duplicate for each matrix type daily or with each batch of 20 or fewer samples.
- **Real people language – on a 5% basis (see batch size for more information) analyze 2 samples for chlorine, after reading one, pour out sample and start with a fresh sample**
 - **For reporting purposes, average sample and duplicate.**
 - **Target value should be close to the first value and have a small RPD (less than 20%)**

Continuing Calibration Verification (CCV)

- 1020 B.11.c. – Analysts periodically use a calibration standard to confirm that the instrument performance has not changed significantly since initial calibration.
 - Verify calibration by analyzing one standard at a concentration near or at the mid-point of the calibration range.
- 4020.B.2.b. – Verify calibration by periodically analyzing a calibration standard and calibration blank during a run – typically after each batch of 10 samples and at the end of the run.
 - For the calibration verification to be valid, check standards must not exceed 10% of its true value
- **Real people language**
 - **Read Secondary Standards in accordance with [Guidance for Secondary Standards Use in Calibration 12-19-2013](#) daily (day of).**
 - **OR run a chlorine or potassium permanganate standard daily.**

Control Charts – 1020 B.13.

- **Real people language**
 - **Create and maintain control charts if you have 20-30 data points within 90 days.**
 - **If you do not meet the above criteria, follow QC Acceptance Criteria below.**

Corrective Action - 1020 B.5., B.8., & B.15.**QC Acceptance Criteria**

- Blanks < Method Detection Limit (MDL)
- LFB \pm 15%
- ICV/CCV \pm 10%
- RPD < 20%
- Reporting Limit = MDL

Batch Size

- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:
 - If a permit stated 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
 - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, we would allow twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
 - If sampling only once a month, need to run QC once a month (when samples are analyzed).

Calculations

- % Recovery for LFB
 - = $\frac{\text{LFB concentration}}{\text{Expected concentration}} \times 100\%$

Total Residual Chlorine

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- RPD – relative percent differences for duplicates
 - = $\frac{\text{Difference between sample and duplicate}}{\text{Average of the sample and duplicate}} \times 100\%$

The Use of Secondary Standards for Spectrophotometer/Colorimeter Calibration

Secondary standards (gel standards) are specifically designed to verify the instrument's calibration and to check the instrument's performance. They are not intended to be used to create calibration curves or to calibrate the instrument. Because the DPD reagent cannot be mixed with the gel standards, the quality and the reaction time of the reagent cannot be assessed. For these reasons gel standards cannot take the place of primary standards.

The analyst is responsible for the following:

- Preparing the calibration curve for each instrument ***once per month*** at a minimum with chlorine standards or potassium permanganate (see instructions below for KMnO_4), before the use of new DPD reagents, or the use of new gel standards
- Recording reagent lot #'s for reagents and standards
- Recording calibration concentrations
- Verifying the calibration curve using a minimum of one blank and two gel standards that bracket the expected sample concentration
- Recording all verification data

POTASSIUM PERMANGANATE (KMnO_4) STOCK STANDARD SOLUTION

0.891 grams of reagent grade KMnO_4 in 1000 mL vol. flask made to mark with deionized water. Deionized water must never be stored in plastic containers or exposed to airborne contamination. Store the stock solution in an amber bottle in a cool area. The typical shelf life of the stock solution is six (6) months. If solids appear in the solution, **do not use**.

*****Avoid leaving the cap off for extended periods of time and avoid contamination.*****

INTERMEDIATE (WORKING) STANDARD SOLUTION (10 mg/L)

10 mL of *STOCK* made in 1000 mL vol. flask made to mark with deionized water. The flask should be labeled with the name, KMnO_4 , date of preparation, and initials of who made it.

This information should also be entered into a logbook.

****The intermediate stock solution should be stable for approximately 5 days if kept cool and away from light.****

Care should be taken that the pipette and glassware are clean and thoroughly rinsed with deionized water to avoid contamination. Store only in a glass container (preferably amber glass) never in plastic containers. The working solution should be remade if solids appear in the bottom of the container.

CALIBRATION STANDARD SOLUTIONS

If using KMnO_4 , four to five calibration standard solutions should be made according to the table below with the addition of DPD to create a calibration curve ***once per month*** at a minimum. The correlation coefficient of the curve should correlate to 0.995 or better. This curve is then used to check instrument calibration. Gel standards are run against the curve and must agree to within $\pm 10\%$.

****The working solution should be stable for approximately 2 hours and will fade rapidly (within 15 minutes) if chlorine demand-free water is not used.****

A target value (e.g. permit value for a facility) should be known, and three gel standards, 0.00 mg/L (blank) and two other standards (a low and a high standard) that bracket the target value should be chosen. Gel standards are run against the curve and must agree to within $\pm 10\%$.

mL Working Standard Diluted w/Deionized water	Chlorine Equivalent mg/L
20 mL (vol. Pipette) to 100 mL (vol. flask)	2.0 mg/L
10 mL (vol. Pipette) to 100 mL (vol. flask)	1.0 mg/L
5 mL (vol. Pipette) to 100 mL (vol. flask)	0.5 mg/L
1 mL (vol. Pipette) to 100 mL (vol. flask)	0.1 mg/L
1 mL (vol. Pipette) to 200 mL (vol. flask)	0.05 mg/L
1 mL (vol. Pipette) to 500 mL (vol. flask)	0.02 mg/L
100 mL of deionized water	0.00 mg/L

Initial Demonstration of Capability (DOC)

- 4020 B.1.a. - each analyst must run a known standard concentration at least four times and compare limits listed in the method.
- **Real people language – each operator running this test needs to calibrate instrument and analyze 4 buffers at a pH of 7**
 - **Keep a folder for each analyst, keep a copy here**
 - **Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.**
 - **Recommend backup analyst do this once a year.**
 - **Only good for that type of instrument you are using at that plant. If you have a backup instrument made by a different manufacturer or you work at another plant with a different make/model, you would need another DOC.**
 - **DOCs demonstrate you are proficient with that one instrument.**

Method Detection Limit (MDL)

- NONE

Initial Calibration Verification (ICV)

- 1020 B.11.b. – Perform initial calibration
- **Real people language – calibrate daily (day of) with fresh buffers by following manufacturer’s instructions.**
- **Analyze 7 buffer solution as a sample after calibration and before samples to verify initial calibration (ICV), should be within ± 0.2 s.u.**

Method Blank

- NONE

Laboratory Fortified Blank (LFB)

- NONE

Duplicate

- 1020 B.12.f. – Calculate RPD (relative percent difference)
- 4020 B.2.f. – Randomly select routine samples to be analyzed twice.
 - Process duplicate sample independently through the entire sample preparation and analysis.
 - Include at least one duplicate for each matrix type daily or with each batch of 20 or fewer samples.
- **Real people language – on a 5% basis (see batch size for more information) analyze 2 samples for pH, after reading one, pour out sample and start with a fresh sample**
 - **Example, grab sample in bucket and dip pH probe in twice to get a duplicate reading**
 - **Target value should be close to the first value (within ± 0.2 s.u.)**

- **For reporting purposes, all duplicates should be reported according to your permit limits. If your permit sets a minimum or maximum limit such as pH, then the minimum or maximum value should be reported even if falls outside your permit limit.**

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD)

- NONE

Continuing Calibration Verification (CCV)

- 1020 B.11.c. – Analysts periodically use a calibration standard to confirm that the instrument performance has not changed significantly since initial calibration.
 - Verify calibration by analyzing one standard at a concentration near or at the mid-point of the calibration range.
- 4020.B.2.b. – Verify calibration by periodically analyzing a calibration standard during a run – typically after each batch of 10 samples and at the end of the run.
- **Real people language – read 7 Buffer after analyzing samples daily (day of and within \pm 0.2 pH units).**

Control Charts

- NONE

Corrective Action - 1020 B.5., B.8., & B.15.

QC Acceptance Criteria

- ICV/CCV within \pm 0.2 s.u.
- Duplicates within \pm 0.2 s.u.

Batch Size

- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:
 - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
 - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, we would allow twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

Calculations

- NONE

40 CFR 136 Table 1B says the approved methodology is manual distillation⁶ or gas diffusion (pH>11) followed by any of the following: Nesslerization, titration, electrode, manual phenate or automated phenate. Footnote 6 states: “Manual distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary: however, manual distillation will be required to resolve any controversies. **In general, the analytical method should be consulted regarding the need for distillation.** If the method is not clear, the laboratory may compare a minimum of 9 different sample matrices to evaluate the need for distillation. For each matrix, a matrix spike and matrix spike duplicate are analyzed both with and without the distillation step. (A total of 36 samples, assuming 9 matrices). If results are comparable, the laboratory may dispense with the distillation step for future analysis. Comparable is defined as < 20% RPD for all tested matrices). Alternatively the two populations of spike recovery percentages may be compared using a recognized statistical test.”

Standard Methods

- 4500-NH₃ A.1 – In general, direct manual determination of low concentrations of ammonia is confined to drinking waters, clean surface or groundwater and good-quality nitrified wastewater effluent.
- 4500-NH₃ D.1.b. – Sample distillation is unnecessary.

Tennessee recommends that one sample is run yearly to compare the distilled and undistilled results and that the results are within 20% of each other.

- Note – if distilled sample and undistilled sample are below detection limit, you cannot calculate the percent difference.

Initial Demonstration of Capability (DOC)

- 1020 B. 1 – As a minimum, include a reagent blank and at least 4 LFBs at a concentration between 10 times the MDL and the midpoint of a calibration curve.
- 4020 B.1.a. - each analyst must run a known standard concentration at least four times and compare limits listed in the method.
- **Real people language – each operator running this test needs to calibrate the probe and analyze 4 samples of an NH₃ Standard at a concentration around 1.0 mg/L**
 - **Keep a folder for each analyst, keep a copy here**
 - **Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.**
 - **Recommend backup analyst do this once a year.**

Method Detection Limit (MDL)

- 1020 B. 4 – As a starting point for selecting the concentration to use when determining the MDL, use an estimate of five times the estimated true detection level (5 x 0.03 mg/L = 0.15 mg/L).
 - Ideally, prepare and analyze at least seven (7) portions of this solution over a 3-day period to ensure that the MDL determination is more representative of routine measurements as performed in the laboratory.
 - The replicate measurements should be in the range of one to five times the estimated MDL, and recoveries of the known addition should be between 50 and 150%, with %RSD (relative standard deviation) values ≤ 20%.

- Ideally use pooled data from several analysts rather than data from one analyst.
- **Real people language – have several operators, who run this test, analyze an NH₃ Standard at a concentration of 0.15 mg/L over several days with a total of at least 7 samples**
 - **Joe analyzes 3 samples on Monday**
 - **Bob analyzes 3 samples on Tuesday**
 - **Mary analyzes 3 samples on Wednesday**
- **Run this once a year**

Initial Calibration Verification (ICV)

- 1020 B.11.b. – Perform initial calibration using at least three concentrations of standards for linear curves.
- 4020 B.2.a. – Calibrate initially with at least one blank and three calibration standards.
 - The appropriate linear correlation coefficient for standard concentration-to-instrument response should be greater than or equal to 0.995.
 - The back-calculated and true concentrations should agree within $\pm 10\%$.
- 4500-NH₃ D.4.a. – Prepare a series of standard solutions covering the concentrations of 1000, 100, 10, 1 and 0.1 mg NH₃-N/L
- 4500-NH₃ D.4.b. – calibrate from lowest to highest concentration. Wait until the reading has stabilized (at least 2-3 min) before recording millivolts for standards and samples containing ≤ 1 mg NH₃-N/L.
- 4500-NH₃ D.4.c. – If the electrode is functioning properly, a tenfold change of NH₃-N concentration produces a potential change of about 59 mV.
- **Real people language – calibrate according to manufacturer's instructions with at least 3 standards that will bracket your sample range daily (day of).**
- **Analyze a 10 mg/L standard as a sample after calibration and before samples to verify initial calibration (ICV)**

Method Blank – goes through distillation if you distill

- 1020 B.5.– A reagent blank (method blank) consists of reagent water and all reagents that normally are in contact with a sample during the entire analytical procedure.
- 4020 B.2.d. – Include at least one method blank daily or with each **batch of 20** or fewer samples, whichever is more frequent.
 - If any method blank measurements are at or above the reporting level, take immediate corrective action.
- **Real people language – analyze distilled water as a sample by going through distillation (if you still distill samples) and using ISA (ion strength adjuster).**
 - **Target value is less than reporting limit**
 - **Reporting limit will be equal to your Method Detection Limit (MDL)**
 - **Run on a 5% basis (see batch size for more information).**

Laboratory Fortified Blank (LFB) – goes through distillation if you distill

- 1020 B.6.– A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added.

- Sample batch = 5% basis = 1 every 20 samples
 - Use an added concentration of at least 10 times the MDL, less than or equal to the midpoint of the calibration curve.
- 4020 B.2.e. – Calculate percent recovery, plot control charts and determine control limits (see Control Charts below)
- **Real people language – analyze an NH₃ standard at a concentration of 5.0 mg/L**
 - **Run on a 5% basis (see batch size for more information).**

Duplicate

- NONE

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD) – goes through distillation if you distill

- 1020 B.7.– A laboratory fortified matrix (LFM) is an additional portion of a sample to which a known amount of the analyte of interest is added before sample preparation
 - The LFM is used to evaluate analyte recovery in a sample
 - Sample batch = 5% basis
 - Add a concentration that is at least 10 times the MRL (minimum reporting level), less than or equal to the midpoint of the calibration curve.
 - Preferably use the same concentration as the LFB
- 4020 B.2.g. – When appropriate for the analyte, include at least one LFM/LFMD daily or with each batch of 20 or fewer samples
 - Add a known concentration of analyte to a randomly selected routine sample
 - Calculate percent recovery and relative percent difference, plot control charts and determine control limits for spikes at different concentrations (see Control Charts below)
- **Real people language – add a known amount of ammonia to a sample and expect that amount to be added to your sample concentration and repeat process for LFMD.**
 - **Run on a 5% basis (see batch size for more information).**
 - **Calculate RPD between spiked sample and spiked duplicate, target value should be close to the first value and have a small RPD (less than 20%).**
 - **Spike volume should be less than 1% of the volume.**
 - **Example: spike with 1 mL of 1000 mg/L into 100 mL sample will equal a 10 mg/L increase in ammonia concentration.**

Continuing Calibration Verification (CCV)

- 1020 B.11.c. – Analysts periodically use a calibration standard to confirm that the instrument performance has not changed significantly since initial calibration.
 - Verify calibration by analyzing one standard at a concentration near or at the mid-point of the calibration range.
- 4020.B.2.b. – Verify calibration by periodically analyzing a calibration standard and calibration blank during a run – typically after each batch of 10 samples and at the end of the run.
 - For the calibration verification to be valid, check standards must be within 10% of its true value
- **Real people language – analyze 10 mg/L at the end of all samples daily (day of).**

Ammonia

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- **Real people language**
 - **Create and maintain control charts if you have 20-30 data points within 90 days.**
 - **If you do not meet the above criteria, follow QC Acceptance Criteria below.**

Corrective Action - 1020 B.5., B.8., & B.15.

QC Acceptance Criteria

- Blanks < MDL
- LFB ± 15%
- ICV/CCV ± 10%
- LFM/LFMD ± 20%
- RPD < 20%
- Reporting limit = MDL

Batch Size

- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:
 - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
 - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, we would allow twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
 - If sampling only once a month, need to run QC once a month.

Calculations

- % Recovery for LFB
 - = $\frac{\text{LFB Result}}{\text{Expected Concentration}} \times 100\%$
- RPD – relative percent differences for duplicates and LFM/LFMD
 - = $\frac{\text{Difference between sample and duplicate}}{\text{Average of the sample and duplicate}} \times 100\%$
- % Recovery for LFM – when using less than or equal to 1% spike volume compared to sample volume
 - = $\frac{\text{LFM Result} - \text{Sample Result}}{\text{Actual Concentration of spike}} \times 100\%$

Initial Demonstration of Capability (DOC)

- Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
- Calibrate daily according to manufacturer's instructions
- Hach Method 10360 9.2.1 – Prepare and measure four samples of air-saturated water according to section 7.2.
 - 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
 - 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately $20 \pm 3^{\circ}\text{C}$.
 - 7.2.3 – With a steady gentle stream of filtered air ($\approx 10\text{-}40$ mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.
 - 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature ($20 \pm 3^{\circ}\text{C}$) for 30 minutes and note the barometric pressure [uncorrected] of the laboratory during preparation. The barometric pressure reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.
 - 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
 - 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman...
- **Real people language – each analyst should calibrate the probe, in accordance with manufacturer's instruction, prepare dilution water that is air-saturated and analyze four bottles and compare to the theoretical dissolved oxygen concentration (± 0.2 mg/L).**
 - **Theoretical dissolved oxygen can be found at USGS's website at <http://water.usgs.gov/software/DOTABLES/> or by using a DO Saturation Table.**

Method Detection Limit (MDL)

- None

Initial Calibration Verification (ICV)

- 7.1.1 – Add approximately 1 inch (2.54 cm) of reagent water to a clean BOD bottle and stopper).
- 7.1.2 – Shake vigorously for ~ 10 seconds.
- 7.1.3 – Allow for the BOD bottle and its contents to equilibrate to room temperature. Room temperature should be approximately $20 \pm 3^{\circ}\text{C}$.
- 7.1.4 – The stopper may now be removed from the BOD bottle and the LBOD probe inserted for calibration purposes.
- **Real people language – calibrate daily (day of) by following manufacturer's instructions.**

Method Blank

- NONE

Laboratory Fortified Blank (LFB)

- NONE

Duplicate

- **Real people language – on a 5% basis (see batch size for more information) analyze 2 samples for DO.**
 - **First sample is result, second sample is duplicate**
 - **Target value is to be close to the first value and have a small difference (≤ 0.2 mg/L)**
- **For reporting purposes, all duplicates should be reported according to your permit limits. If your permit sets a minimum, then the minimum value should be reported even if falls outside your permit limit.**

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD)

- NONE

Continuing Calibration Verification (CCV)

- 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
- 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately $20 \pm 3^{\circ}\text{C}$.
- 7.2.3 – With a steady gentle stream of filtered air ($\approx 10\text{-}40$ mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.
- 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature ($20 \pm 3^{\circ}\text{C}$) for 30 minutes and note the barometric pressure of the laboratory during preparation. The barometric pressure reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.
- 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
- 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman...
- 9.3.1 – Upon air calibration, prepare a calibration verification standard with each analytical batch of 20 samples or less in an 8-hour period.
- 9.4.3 – Initially and at the end of each analytical batch of samples, analyze a dilution water sample that is air-saturated
- **Real people language – prepare dilution water that is air-saturated and analyze bottles and compare to the theoretical dissolved oxygen concentration (± 0.2 mg/L) after daily samples or with each batch of 20 samples or less in an 8-hour period.**
 - **Theoretical dissolved oxygen can be found at USGS's website at <http://water.usgs.gov/software/DOTABLES/> or by using a DO Saturation Table.**

Control Charts

- NONE

Dissolved Oxygen – Luminescence Measurement Hach Method

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Batch Size

- 9.3.1 – ... with each analytical batch of 20 samples or less in an 8 hour period.
- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:
 - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
 - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, we would allow twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

Initial Demonstration of Capability (DOC)

- Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
- Calibrate daily according to manufacturer's instructions
- Follow Hach Method 10360 9.2.1 – Prepare and measure four samples of air-saturated water according to section 7.2.
 - 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
 - 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately $20 \pm 3^{\circ}\text{C}$.
 - 7.2.3 – With a steady gentle stream of filtered air ($\approx 10\text{-}40$ mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.
 - 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature ($20 \pm 3^{\circ}\text{C}$) for 30 minutes and note the barometric pressure [uncorrected] of the laboratory during preparation. The barometric pressure reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.
 - 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
 - 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman...
- **Real people language – each analyst should calibrate the probe, in accordance with manufacturer's instruction, prepare dilution water that is air-saturated and analyze four bottles and compare to the theoretical dissolved oxygen concentration (± 0.2 mg/L).**
 - **Theoretical dissolved oxygen can be found at USGS's website at <http://water.usgs.gov/software/DOTABLES/> or by using a DO Saturation Table.**

Method Detection Limit (MDL)

- None

Initial Calibration Verification (ICV)

- 1020 B.11.b. – Perform initial calibration
- **Real people language – calibrate daily (day of) by following manufacturer's instructions.**

Method Blank

- NONE

Laboratory Fortified Blank (LFB)

- NONE

Duplicate

- 1020 B.12.f. – Calculate RPD (relative percent difference)
- 4020 B.2.f. – Randomly select routine samples to be analyzed twice.

Dissolved Oxygen – Membrane Electrode Method

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S. Pratt, January 2014



- TDEC - Fleming Training Center
- Section 2
- Process duplicate sample independently through the entire sample preparation and analysis.
 - Include at least one duplicate for each matrix type daily or with each batch of 20 or fewer samples.
 - **Real people language – on a 5% basis (see batch size for more information) analyze 2 samples for DO.**
 - **First sample is result, second sample is duplicate**
 - **Target value is to be close to the first value and have a small difference (≤ 0.2 mg/L)**
 - **For reporting purposes, all duplicates should be reported according to your permit limits. If your permit sets a minimum, then the minimum value should be reported even if falls outside your permit limit.**

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD)

- NONE

Continuing Calibration Verification (CCV)

- Follow Hach Method 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
- 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately $20 \pm 3^{\circ}\text{C}$.
- 7.2.3 – With a steady gentle stream of filtered air ($\approx 10\text{-}40$ mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.
- 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature ($20 \pm 3^{\circ}\text{C}$) for 30 minutes and note the barometric pressure of the laboratory during preparation. The barometric temperature reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.
- 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
- 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman...
- 9.3.1 – Upon air calibration, prepare a calibration verification standard with each analytical batch of 20 samples or less in an 8 hour period.
- 9.4.3 – Initially and at the end of each analytical batch of samples, analyze a dilution water sample that is air-saturated
- **Real people language – prepare dilution water that is air-saturated and analyze bottles and compare to the theoretical dissolved oxygen concentration (± 0.2 mg/L) after daily samples or with each batch of 20 samples or less in an 8-hour period.**
 - **Theoretical dissolved oxygen can be found at USGS’s website at <http://water.usgs.gov/software/DOTABLES/> or by using a DO Saturation Table.**

Control Charts

- NONE

Corrective Action - 1020 B.5., B.8., & B.15.

Batch Size

- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:
 - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
 - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, we would allow twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

Minimum Detectable Concentration – 4500-P E.1.c. – approximately 10 µg/L (0.010 mg/L)

Initial Demonstration of Capability (DOC)

- 1020 B. 1 – As a minimum, include a reagent blank and at least 4 LFBs at a concentration between 10 times the MDL and the midpoint of a calibration curve.
- 4020 B.1.a. - each analyst must run a known standard concentration at least four times and compare limits listed in the method.
- **Real people language – each operator running this test needs to analyze 4 samples of Phosphorus standard at a concentration of about 0.5 mg/L**
 - **Keep a folder for each analyst, keep a copy here**
 - **Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.**
 - **Recommend backup analyst do this once a year.**

Method Detection Limit (MDL)

- 1020 B. 4 – As a starting point for selecting the concentration to use when determining the MDL, use an estimate of five times the estimated true detection level ($5 \times 0.010 \text{ mg/L} = 0.050 \text{ mg/L}$).
 - Ideally, prepare and analyze at least seven (7) portions of this solution over a 3-day period to ensure that the MDL determination is more representative of routine measurements as performed in the laboratory.
 - The replicate measurements should be in the range of one to five times the estimated MDL, and recoveries of the known addition should be between 50 and 150%, with %RSD (relative standard deviation) values $\leq 20\%$.
- 4020 B.1.b. – Verify MDL at least **annually**.
 - Ideally use pooled data from several analysts rather than data from one analyst.
- **Real people language – have several operators, who run this test, analyze a Phosphorus standard at a concentration of 0.05 mg/L over several days with a total of at least 7 samples**
 - **Joe analyzes 3 samples on Monday**
 - **Bob analyzes 3 samples on Tuesday**
 - **Mary analyzes 3 samples on Wednesday**
- **Run this once a year**

Initial Calibration Verification (ICV) – does not go through digestion

- 1020 B.11.b. – Perform initial calibration using at least three concentrations of standards for linear curves.
- 4020.B.2.a. – Calibrate initially with at least one blank and three calibration standards.
 - The appropriate linear correlation coefficient for standard concentration-to-instrument response should be greater than or equal to 0.995.
 - The back-calculated and true concentrations should agree within $\pm 10\%$.

- **Real people language – prepare a set of Phosphorus standards (4-5 standards) to verify the factory pre-set calibration curve **monthly** or more frequently if reagent lot # changes.**

Method Blank – goes through digestion

- 1020 B.5.– A reagent blank (method blank) consists of reagent water and all reagents that normally are in contact with a sample during the entire analytical procedure.
- 4020 B.2.d. – Include at least one method blank daily or with each batch of 20 or fewer samples, whichever is more frequent.
 - If any method blank measurements are at or above the reporting level, take immediate corrective action.
- **Real people language – analyze distilled water as a sample by going through all digestion and reagent addition before reading.**
 - **Target value is less than reporting limit.**
 - **Reporting limit will be equal to your Method Detection Limit (MDL)**
 - **Run on a 5% basis (see batch size for more information).**

Laboratory Fortified Blank (LFB) – goes through digestion

- 1020 B.6.– A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added.
 - Sample batch = 5% basis = 1 every 20 samples
 - Use an added concentration of at least 10 times the MDL, less than or equal to the midpoint of the calibration curve.
- 4020 B.2.e. – Calculate percent recovery, plot control charts and determine control limits (see Control Charts below)
- **Real people language – analyze Phosphorus standard at a concentration of 0.5 mg/L**
 - **Run on a 5% basis (see batch size for more information).**

Duplicate –

- NONE

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD) – goes through digestion

- 1020 B.7.– A laboratory fortified matrix (LFM) is an additional portion of a sample to which a known amount of the analyte of interest is added before sample preparation
 - The LFM is used to evaluate analyte recover in a sample
 - Sample batch = 5% basis = 1 every 20 samples
 - Add a concentration that is at least 10 times the MRL (minimum reporting level), less than or equal to the midpoint of the calibration curve.
 - Preferably use the same concentration as the LFB
- 4020 B.2.g. – When appropriate for the analyte, include at least one LFM/LFMD daily or with each batch of 20 or fewer samples
 - Add a known concentration of analyte to a randomly selected routine sample
 - Calculate percent recovery and relative percent difference, plot control charts and determine control limits for spikes at different concentrations (see Control Charts below)

- **Real people language – add a known amount of phosphorus to a sample and expect that amount to increase your sample concentration**

- Run on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent), see batch size for more information
- Calculate RPD between spiked sample and spiked duplicate, target value should be close to the first value and have a small RPD (less than 20%).
- Spike volume should be less than 1% of the volume.
 - Example: spike with 0.1 mL of 100 mg/L into 10 mL sample will equal a 1 mg/L increase in phosphorus concentration.

Continuing Calibration Verification (CCV) – does not go through digestion

- 1020 B.11.c. – Analysts periodically use a calibration standard to confirm that the instrument performance has not changed significantly since initial calibration.
 - Verify calibration by analyzing one standard at a concentration near or at the mid-point of the calibration range.
- 4020.B.2.b. – Verify calibration by periodically analyzing a calibration standard and calibration blank during a run – typically after each batch of 10 samples and at the end of the run.
 - For the calibration verification to be valid, check standards must not exceed 10% of its true value
- **Real people language – analyze mid-range Phosphorus standard daily (day of).**

Control Charts – 1020 B.13.

- **Real people language**
 - Create and maintain control charts if you have 20-30 data points within 90 days.
 - If you do not meet the above criteria, follow QC Acceptance Criteria below.

Corrective Action - 1020 B.5., B.8., & B.15.

QC Acceptance Criteria

- Blanks < MDL
- LFB \pm 15%
- ICV/CCV \pm 10%
- LFM/LFMD \pm 20%
- RPD < 20%
- Reporting Limit = MDL

Batch Size –

- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:
 - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
 - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, we would suggest twice a month.

- Pick a date and be consistent, the 1st and 15th of every month on the 1st and 3rd Thursday of every month. Mark your calendar!!

Calculations –

- % Recovery for LFB
 - = $\frac{\text{LFB concentration}}{\text{Expected concentration}} \times 100\%$
- RPD – relative percent differences for duplicates and LFM/LFMD
 - = $\frac{\text{Difference between sample and duplicate}}{\text{Average of the sample and duplicate}} \times 100\%$
- % Recovery for LFM – when using less than or equal to 1% spike volume compared to sample volume
 - = $\frac{\text{LFM concentration} - \text{Sample concentration}}{\text{Concentration of spike}} \times 100\%$

Initial Demonstration of Capability (DOC)

- 1020 B. 1 – As a minimum, include a reagent blank and at least 4 LFBs
- 4020 B.1.a. – Before analysts run any samples, verify their capability with the method. Run a laboratory fortified blank at least four times and compare to the limits listed in the method.
 - If no limit is specified, use the following procedure to establish limits.
 - Calculate the standard deviation of the four samples. Then calculate the LFB's recovery limits (see formula at end)
- **Real people language – each operator running this test needs to analyze 4 samples of GGA at a concentration of 198 ±30.5 mg/L**
 - **Keep a folder for each analyst, keep a copy here**
 - **Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.**
 - **Recommend backup analyst do this once a year.**

Method Detection Limit (MDL)

- NONE

Initial Calibration Verification (ICV)

- 1020 B.11.b. – Perform initial calibration
- Hach's Method: 7.1.1 – Add approximately 1 inch (2.54 cm) of reagent water to a clean BOD bottle and stopper).
- 7.1.2 – Shake vigorously for ~ 10 seconds.
- 7.1.3 – Allow for the BOD bottle and its contents to equilibrate to room temperature. Room temperature should be approximately 20 ± 3°C.
- 7.1.4 – The stopper may now be removed from the BOD bottle and the LBOD probe inserted for calibration purposes.
- **Real people language – calibrate DO probe daily (day of) by following manufacturer's instructions.**

Method Blank

- 1020 B.5.– A reagent blank (method blank) consists of reagent water and all reagents that normally are in contact with a sample during the entire analytical procedure.
- 5020 B.2.d. – Include at least one method blank daily or with each batch of 20 or fewer samples, whichever is more frequent.
- 5210 B.6.c. – With each batch of samples, incubate one or more bottles of dilution water that contains nutrient, mineral and buffer solutions but no seed or nitrification inhibitor.
 - The DO uptake in 5 days must not be more than 0.20 mg/L and preferably not more than 0.10 mg/L, before making seed corrections.
- **Real people language – analyze dilution water blanks daily (day of), preferably one at beginning and one at end**
 - **Target value is less than 0.20 mg/L (preferably less than 0.10 mg/L)**

Laboratory Fortified Blank (LFB)

- 1020 B.6.– A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added.
 - Sample batch = 5% basis
- 5020 B.2.e. – Calculate percent recovery, plot control charts and determine control limits (see Control Charts below)
 - When appropriate, include at least one LFB daily or per each batch of 20 or fewer samples.
- **Real people language – analyze GGA sample at a concentration of 198 ±30.5 mg/L**
 - **Run on a 5% basis (see batch size for more information).**
 - **If permit requires cBOD, add nitrification inhibitor (NI) to one GGA bottle once/quarter (or more often if the Lot # of NI changes), which should be equal to 164 ±30.7 mg/L**

Duplicate

- 1020 B.12.f. – Calculate RPD (relative percent difference)
- 5020 B.2.f. – Randomly select routine samples to be analyzed twice.
 - Process duplicate sample independently through the entire sample preparation and analysis.
 - Include at least one duplicate for each matrix type daily or with each batch of 20 or fewer samples.
- **Real people language – analyze 2 samples for BOD or cBOD, run an extra sample dilution bottle**
 - **Example: if you run 100mL, 200mL and 300mL for your effluent, run a second 300mL sample. You will have 4 bottles total for your effluent dilutions.**
 - **Target value should be close to the first value (same dilution) and have a small RPD (less than 20%)**
- **For reporting purposes, average results that meet method criteria.**

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD)

- NONE

Continuing Calibration Verification (CCV)

- Hach Method 10360 7.2 and 9.4 – Calibration Verification for membranes and LDO probes
 - 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
 - 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately 20 ± 3°C.
 - 7.2.3 – With a steady gentle stream of filtered air (≈ 10-40 mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.
 - 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature (20 ± 3°C) for 30 minutes and note the barometric pressure of the laboratory during preparation. The barometric temperature reading is used in the calculation and

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determination of the theoretical DO concentration for the preparation of air-saturated water.

- 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
- 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman...
- 9.4.3 – Initially and at the end of each analytical batch of samples, analyze a dilution water sample that is air-saturated
- **Real people language – prepare dilution water that is air-saturated and analyze bottles and compare to the theoretical dissolved oxygen concentration (± 0.2 mg/L).**
 - **Theoretical dissolved oxygen can be found at USGS's website at <http://water.usgs.gov/software/DOTABLES/> or by using a DO Saturation Table.**

Control Charts – 1020 B.13.

- **Real people language**
 - **Create and maintain control charts if you have 20-30 data points within 90 days.**
 - **If you do not meet the above criteria, follow QC Acceptance Criteria below.**

Corrective Action - 1020 B.5., B.8., & B.15.

- 5210 B.7.b. – Identify results in the test reports when any of the following quality control parameters is not met:
 - Dilution water exceeds 0.20 mg/L (5210B.6c)
 - Glucose-glutamic acid check falls outside of acceptable limits (5210B.6b)
 - Test replicates show more than 30% difference between high and low values
 - Seed control samples do not meet the above criteria in all dilutions (5210B.6d) or
 - Minimum DO is less than 1.0 mg/L (5210B.7a3)

QC Acceptance Criteria

- Blanks < 0.20 mg/L
- GGA = 198 ± 30.5 mg/L (if running cBOD, add NI to one bottle once/quarter or more often if NI Lot# changes, and it should = 164 ± 30.7 mg/L)
- RPD < 20%
- Minimum of three dilutions for each sample, at least one sample must have valid data with at least 2.0 mg/L depletion and a residual of 1.0 mg/L

Batch Size

- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:
- Influent and Effluent are 2 different samples
 - If a permit stated that 3 analyses per week, that would be 6 samples per week, we would allow for a duplicate to be analyzed at least twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, that would be 10 samples per week, we would allow once a week.
 - Pick a date and be consistent, every Monday. Mark your calendar!!

Calculations

- % Recovery for LFB
 - = $\frac{\text{LFB concentration}}{\text{Expected concentration}} \times 100\%$
- RPD – relative percent differences for duplicates
 - = $\frac{\text{Difference between sample and duplicate}}{\text{Average of the sample and duplicate}} \times 100\%$
- Unseeded - BOD₅, mg/L = $\frac{D_1 - D_2}{P}$
- Seeded - BOD₅, mg/L = $\frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$
 - Where:
 - D₁ = Initial Dissolved Oxygen Concentration in Sample, mg/L
 - D₂ = Final Dissolved Oxygen Concentration in Sample, mg/L
 - B₁ = Initial Dissolved Oxygen Concentration in Seed Control, mg/L
 - B₂ = Final Dissolved Oxygen Concentration in Seed Control, mg/L
 - P = Sample Concentration, % (expressed as a decimal)
 - f = $\frac{\text{Seed in Sample, \%}}{\text{Seed in Seed Control, \%}}$

phosphorus, as long as complete oxidation can be demonstrated.

(xxii) Use of an axially viewed torch with Method 200.7.

■ 7. Add new § 136.7 to read as follows:

§ 136.7 Quality assurance and quality control.

The permittee/laboratory shall use suitable QA/QC procedures when conducting compliance analyses with any Part 136 chemical method or an alternative method specified by the permitting authority. These QA/QC procedures are generally included in the analytical method or may be part of the methods compendium for approved Part 136 methods from a consensus organization. For example, Standard Methods contains QA/QC procedures in the Part 1000 section of the Standard Methods Compendium. The permittee/laboratory shall follow these QA/QC procedures, as described in the method or methods compendium. If the method lacks QA/QC procedures, the permittee/laboratory has the following options to comply with the QA/QC requirements:

(a) Refer to and follow the QA/QC published in the “equivalent” EPA method for that parameter that has such QA/QC procedures;

(b) Refer to the appropriate QA/QC section(s) of an approved Part 136 method from a consensus organization compendium;

(c)(1) Incorporate the following twelve quality control elements, where applicable, into the laboratory’s documented standard operating procedure (SOP) for performing compliance analyses when using an approved Part 136 method when the method lacks such QA/QC procedures. One or more of the twelve QC elements may not apply to a given method and may be omitted if a written rationale is provided indicating why the element(s) is/are inappropriate for a specific method.

(i) Demonstration of Capability (DOC);

(ii) Method Detection Limit (MDL);

(iii) Laboratory reagent blank (LRB), also referred to as method blank (MB);

(iv) Laboratory fortified blank (LFB), also referred to as a spiked blank, or laboratory control sample (LCS);

(v) Matrix spike (MS) and matrix spike duplicate (MSD), or laboratory fortified matrix (LFM) and LFM duplicate, may be used for suspected matrix interference problems to assess precision;

(vi) Internal standards (for GC/MS analyses), surrogate standards (for organic analysis) or tracers (for radiochemistry);

(vii) Calibration (initial and continuing), also referred to as initial

calibration verification (ICV) and continuing calibration verification (CCV);

(viii) Control charts (or other trend analyses of quality control results);

(ix) Corrective action (root cause analysis);

(x) QC acceptance criteria;

(xi) Definitions of preparation and analytical batches that may drive QC frequencies; and

(xii) Minimum frequency for conducting all QC elements.

(2) These twelve quality control elements must be clearly documented in the written standard operating procedure for each analytical method not containing QA/QC procedures, where applicable.

■ 8. Revise Appendix C to Part 136 to read as follows.

APPENDIX C TO PART 136— DETERMINATION OF METALS AND TRACE ELEMENTS IN WATER AND WASTES BY INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROMETRY METHOD 200.7

1.0 Scope and Application

1.1 Inductively coupled plasma-atomic emission spectrometry (ICP-AES) is used to determine metals and some nonmetals in solution. This method is a consolidation of existing methods for water, wastewater, and solid wastes.¹⁻⁴ (For analysis of petroleum products see References 5 and 6, Section 16.0). This method is applicable to the following analytes:

Analyte	Chemical abstract services registry number (CASRN)
Aluminum (Al)	7429-90-5
Antimony (Sb)	7440-36-0
Arsenic (As)	7440-38-2
Barium (Ba)	7440-39-3
Beryllium (Be)	7440-41-7
Boron (B)	7440-42-8
Cadmium (Cd)	7440-43-9
Calcium (Ca)	7440-70-2
Cerium ^a (Cr)	7440-45-1
Chromium (Cr)	7440-47-3
Cobalt (Co)	7440-48-4
Copper (Cu)	7440-50-8
Iron (Fe)	7439-89-6
Lead (Pb)	7439-92-1
Lithium (Li)	7439-93-2
Magnesium (Mg)	7439-95-4
Manganese (Mn)	7439-96-5
Mercury (Hg)	7439-97-6
Molybdenum (Mo)	7439-98-7
Nickel (Ni)	7440-02-0
Phosphorus (P)	7723-14-0
Potassium (K)	7440-09-7
Selenium (Se)	7782-49-2
Silica ^b (SiO ₂)	7631-86-9
Silver (Ag)	7440-22-4
Sodium (Na)	7440-23-5
Strontium (Sr)	7440-24-6
Thallium (Tl)	7440-28-0
Tin (Sn)	7440-31-5
Titanium (Ti)	7440-32-6

Analyte	Chemical abstract services registry number (CASRN)
Vanadium (V)	7440-62-2
Zinc (Zn)	7440-66-6

^aCerium has been included as method analyte for correction of potential interelement spectral interference.

^bThis method is *not* suitable for the determination of silica in solids.

1.2 For reference where this method is approved for use in compliance monitoring programs [e.g., Clean Water Act (NPDES) or Safe Drinking Water Act (SDWA)] consult both the appropriate sections of the Code of Federal Regulation (40 CFR Part 136 Table 1B for NPDES, and Part 141 § 141.23 for drinking water), and the latest **Federal Register** announcements.

1.3 ICP-AES can be used to determine dissolved analytes in aqueous samples after suitable filtration and acid preservation. To reduce potential interferences, dissolved solids should be <0.2% (w/v) (Section 4.2).

1.4 With the exception of silver, where this method is approved for the determination of certain metal and metalloids contaminants in drinking water, samples may be analyzed directly by pneumatic nebulization without acid digestion if the sample has been properly preserved with acid and has turbidity of <1 NTU at the time of analysis. This total recoverable determination procedure is referred to as “direct analysis”. However, in the determination of some primary drinking water metal contaminants, preconcentration of the sample may be required prior to analysis in order to meet drinking water acceptance performance criteria (Sections 11.2.2 through 11.2.7).

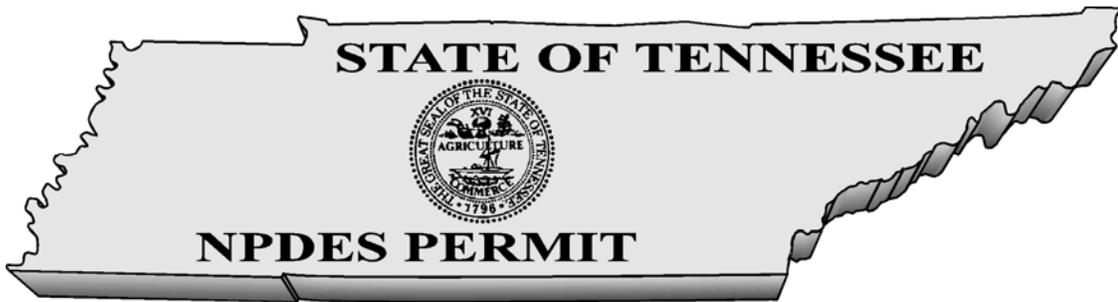
1.5 For the determination of total recoverable analytes in aqueous and solid samples a digestion/extraction is required prior to analysis when the elements are not in solution (e.g., soils, sludges, sediments and aqueous samples that may contain particulate and suspended solids). Aqueous samples containing suspended or particulate material 1% (w/v) should be extracted as a solid type sample.

1.6 When determining boron and silica in aqueous samples, only plastic, PTFE or quartz labware should be used from time of sample collection to completion of analysis. For accurate determination of boron in solid samples only quartz or PTFE beakers should be used during acid extraction with immediate transfer of an extract aliquot to a plastic centrifuge tube following dilution of the extract to volume. When possible, borosilicate glass should be avoided to prevent contamination of these analytes.

1.7 Silver is only slightly soluble in the presence of chloride unless there is a sufficient chloride concentration to form the soluble chloride complex. Therefore, low recoveries of silver may occur in samples, fortified sample matrices and even fortified blanks if determined as a dissolved analyte or by “direct analysis” where the sample has not been processed using the total recoverable mixed acid digestion. For this reason it is recommended that samples be digested prior to the determination of silver.

Section 3
Approved Methods and NPDES Permit

40 CFR 136





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Part II

Environmental Protection Agency

40 CFR Parts 136, 260, et al.

Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Analysis and Sampling Procedures; Final Rule

TABLE IA—LIST OF APPROVED BIOLOGICAL METHODS FOR WASTEWATER AND SEWAGE SLUDGE

Parameter and units	Method ¹	EPA	Standard methods	AOAC, ASTM, USGS	Other
Bacteria:					
1. Coliform (fecal), number per 100 mL or number per gram dry weight.	Most Probable Number (MPN), 5 tube, 3 dilution, or	p. 132 ³ 1680 ^{11,15} . 1681 ^{11,20} .	9221 C E–2006.		
	Membrane filter (MF) ² , single step	p. 124 ³	9222 D–1997	B–0050–85 ⁴ .	
2. Coliform (fecal) in presence of chlorine, number per 100 mL.	MPN, 5 tube, 3 dilution, or	p. 132 ³	9221 C E–2006.		
	MF ² , single step ⁵	p. 124 ³	9222 D–1997.		
3. Coliform (total), number per 100 mL.	MPN, 5 tube, 3 dilution, or.	p. 114 ³	9221 B–2006.		
	MF ² , single step or two step.	p. 108 ³	9222 B–1997	B–0025–85 ⁴	
4. Coliform (total), in presence of chlorine, number per 100 mL.	MPN, 5 tube, 3 dilution, or	p. 114 ³	9221 B–2006		
5. <i>E. coli</i> , number per 100 mL ²¹ .	MF ² with enrichment ⁵ MPN ^{6,8,16} multiple tube, or.	p. 111 ³	9222 (B + B.5c) – 1997 9221B.1–2006/9221F–2006 ^{12,14} .		
	multiple tube/multiple well, or	9223 B–2004 ¹³	991.15 ¹⁰	Colilert ^{®13,18} Colilert-18 ^{®13,17,18} mColiBlue-24 ^{®19}
	MF ^{2,6,7,8} single step ...	1603 ²²	
6. Fecal streptococci, number per 100 mL.	MPN, 5 tube 3 dilution, or	p. 139 ³	9230 B–2007.		
	MF ² , or	p. 136 ³	9230 C–2007	B–0055–85 ⁴	
	Plate count	p. 143 ³	D6503–99 ⁹	Enterolert ^{®13,24}
7. Enterococci, number per 100 mL ²² .	MPN ^{6,8} , multiple tube/multiple well, or		
	MF ^{2,6,7,8} single step or	1600 ²⁵	9230 C–2007		
	Plate count	p. 143 ³		
8. <i>Salmonella</i> , number per gram dry weight ¹¹ .	MPN multiple tube	1682 ²³		
Aquatic Toxicity:					
9. Toxicity, acute, fresh water organisms, LC ₅₀ , percent effluent.	<i>Ceriodaphnia dubia</i> acute.	2002.0. ²⁶			
	<i>Daphnia pulex</i> and <i>Daphnia magna</i> acute.	2021.0. ²⁶			
	Fathead Minnow, <i>Pimephales promelas</i> , and Bannerfin shiner, <i>Cyprinella leedsi</i> , acute.	2000.0. ²⁶			
	Rainbow Trout, <i>Oncorhynchus mykiss</i> , and brook trout, <i>Salvelinus fontinalis</i> , acute.	2019.0. ²⁶			
10. Toxicity, acute, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, LC ₅₀ , percent effluent.	Mysid, <i>Mysidopsis bahia</i> , acute.	2007.0. ²⁶			

TABLE IA—LIST OF APPROVED BIOLOGICAL METHODS FOR WASTEWATER AND SEWAGE SLUDGE—Continued

Parameter and units	Method ¹	EPA	Standard methods	AOAC, ASTM, USGS	Other
11. Toxicity, chronic, fresh water organisms, NOEC or IC ₂₅ , percent effluent.	Sheepshead Minnow, <i>Cyprinodon variegatus</i> , acute.	2004.0 ²⁶			
	Silverside, <i>Menidia beryllina</i> , <i>Menidia menidia</i> , and <i>Menidia peninsulae</i> , acute.	2006.0 ²⁶			
	Fathead minnow, <i>Pimephales promelas</i> , larval survival and growth.	1000.0 ²⁷			
	Fathead minnow, <i>Pimephales promelas</i> , embryolarval survival and teratogenicity.	1001.0 ²⁷			
	Daphnia, <i>Ceriodaphnia dubia</i> , survival and reproduction.	1002.0 ²⁷			
12. Toxicity, chronic, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, NOEC or IC ₂₅ , percent effluent.	Green alga, <i>Selenastrum capricornutum</i> , growth.	1003.0 ²⁷			
	Sheepshead minnow, <i>Cyprinodon variegatus</i> , larval survival and growth.	1004.0 ²⁸			
	Sheepshead minnow, <i>Cyprinodon variegatus</i> , embryolarval survival and teratogenicity.	1005.0 ²⁸			
	Inland silverside, <i>Menidia beryllina</i> , larval survival and growth.	1006.0 ²⁸			
	Mysid, <i>Mysidopsis bahia</i> , survival, growth, and fecundity.	1007.0 ²⁸			
	Sea urchin, <i>Arbacia punctulata</i> , fertilization.	1008.0 ²⁸			

Table IA notes:

¹ The method must be specified when results are reported.

² A 0.45- μ m membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth.

³ Microbiological Methods for Monitoring the Environment, Water, and Wastes, EPA/600/8-78/017. 1978. US EPA.

⁴ U.S. Geological Survey Techniques of Water-Resource Investigations, Book 5, Laboratory Analysis, Chapter A4, Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples. 1989. USGS.

⁵ Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies.

⁶ Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample.

⁷ When the MF method has been used previously to test waters with high turbidity, large numbers of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results.

⁸ To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.

⁹ Annual Book of ASTM Standards—Water and Environmental Technology, Section 11.02. 2000, 1999, 1996. ASTM International.

¹⁰ Official Methods of Analysis of AOAC International. 16th Edition, 4th Revision, 1998. AOAC International.

¹¹ Recommended for enumeration of target organism in sewage sludge.

¹² The multiple-tube fermentation test is used in 9221B.1-2006. Lactose broth may be used in lieu of lauryl tryptose broth (LTB), if at least 25 parallel tests are conducted between this broth and LTB using the water samples normally tested, and this comparison demonstrates that the false-positive rate and false-negative rate for total coliform using lactose broth is less than 10 percent. No requirement exists to run the completed phase on 10 percent of all total coliform-positive tubes on a seasonal basis.

¹³ These tests are collectively known as defined enzyme substrate tests, where, for example, a substrate is used to detect the enzyme β-glucuronidase produced by *E. coli*.
¹⁴ After prior enrichment in a presumptive medium for total coliform using 9221B.1–2006, all presumptive tubes or bottles showing any amount of gas, growth or acidity within 48 h ± 3 h of incubation shall be submitted to 9221F–2006. Commercially available EC–MUG media or EC media supplemented in the laboratory with 50 µg/mL of MUG may be used.
¹⁵ Method 1680: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation Using Lauryl-Tryptose Broth (LTB) and EC Medium, EPA–821–R–10–003. April 2010. U.S. EPA.
¹⁶ Samples shall be enumerated by the multiple-tube or multiple-well procedure. Using multiple-tube procedures, employ an appropriate tube and dilution configuration of the sample as needed and report the Most Probable Number (MPN). Samples tested with Colilert® may be enumerated with the multiple-well procedures, Quanti-Tray®, Quanti-Tray®/2000, and the MPN calculated from the table provided by the manufacturer.
¹⁷ Colilert-18® is an optimized formulation of the Colilert® for the determination of total coliforms and *E. coli* that provides results within 18 h of incubation at 35 °C rather than the 24 h required for the Colilert® test and is recommended for marine water samples.
¹⁸ Descriptions of the Colilert®, Colilert-18®, Quanti-Tray®, and Quanti-Tray®/2000 may be obtained from IDEXX Laboratories, Inc.
¹⁹ A description of the mColiBlue24® test, is available from Hach Company.
²⁰ Method 1681: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using A–1 Medium, EPA–821–R–06–013. July 2006. U.S. EPA.
²¹ Recommended for enumeration of target organism in wastewater effluent.
²² Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (modified mTEC), EPA–821–R–09–007. December 2009. U.S. EPA.
²³ Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium, EPA–821–R–06–014. July 2006. U.S. EPA.
²⁴ A description of the Enterolert® test may be obtained from IDEXX Laboratories Inc.
²⁵ Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI), EPA–821–R–09–016. December 2009. U.S. EPA.
²⁶ Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA–821–R–02–012. Fifth Edition, October 2002. U.S. EPA.
²⁷ Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA–821–R–02–013. Fourth Edition, October 2002. U.S. EPA.
²⁸ Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. EPA–821–R–02–014. Third Edition, October 2002. U.S. EPA.

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES

Parameter	Methodology ⁵⁸	EPA ⁵²	Standard methods	ASTM	USGS/AOAC/Other
1. Acidity, as CaCO ₃ , mg/L.	Electrometric endpoint or phenolphthalein endpoint.	2310 B–1997	D1067–06	I–1020–85. ²
2. Alkalinity, as CaCO ₃ , mg/L.	Electrometric or Colorimetric titration to pH 4.5, Manual.	2320 B–1997	D1067–06	973.43 ³ , I–1030–85. ²
3. Aluminum—Total, ⁴ mg/L.	Automatic	310.2 (Rev. 1974) ¹	I–2030–85. ²
	Digestion, ⁴ followed by any of the following: AA direct aspiration ³⁶	3111 D–1999 or 3111 E–1999. 3113 B–2004.	I–3051–85. ²
	AA furnace
	STGFAA	200.9, Rev. 2.2 (1994).
	ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B–1999	D1976–07	I–4471–97. ⁵⁰
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B–2009	D5673–05	993.14, ³ I–4471–97. ⁵⁰
4. Ammonia (as N), mg/L.	Direct Current Plasma (DCP) ³⁶	D4190–08	See footnote. ³⁴
	Colorimetric (Eriochrome cyanine R).	3500–Al B–2001.
	Manual distillation ⁶ or gas diffusion (pH > 11), followed by any of the following:	350.1, Rev. 2.0 (1993).	4500–NH ₃ B–1997	973.49 ³ .
	Nesslerization	D1426–08 (A)	973.49 ³ , I–3520–85. ²
	Titration	4500–NH ₃ C–1997.
	Electrode	4500–NH ₃ D–1997 or E–1997.	D1426–08 (B).
	Manual phenate, salicylate, or other substituted phenols in Berthelot reaction based methods.	4500–NH ₃ F–1997	See footnote. ⁶⁰
	Automated phenate, salicylate, or other substituted phenols in Berthelot reaction based methods.	350.1 ³⁰ , Rev. 2.0 (1993).	4500–NH ₃ G–1997 4500–NH ₃ H–1997.	I–4523–85. ²

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology ⁵⁸	EPA ⁵²	Standard methods	ASTM	USGS/AOAC/Other
5. Antimony—Total, ⁴ mg/L.	Automated electrode	Ion Chromatography	D6919–09	See footnote. ⁷
	Digestion, ⁴ followed by any of the following:				
	AA direct aspiration ³⁶	3111 B–1999.		
	AA furnace	3113 B–2004.		
	STGFAA	200.9, Rev. 2.2 (1994).			
6. Arsenic—Total, ⁴ mg/L.	ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B–1999	D1976–07	I–4471–97. ⁵⁰
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B–2009	D5673–05	993.14, ³ I–4471–97. ⁵⁰
	Digestion, ⁴ followed by any of the following:	206.5 (Issued 1978) ¹ .			
	AA gaseous hydride	3114 B–2009 or	D2972–08 (B)	I–3062–85. ²
	AA furnace	3114 C–2009	D2972–08 (C)	I–4063–98. ⁴⁹
7. Barium—Total, ⁴ mg/L.	STGFAA	200.9, Rev. 2.2 (1994).	3113 B–2004		
	ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B–1999	D1976–07.	
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B–2009	D5673–05	993.14, ³ I–4020–05. ⁷⁰
	Colorimetric (SDDC)	3500–As B–1997	D2972–08 (A)	I–3060–85. ²
	Digestion ⁴ , followed by any of the following:				
8. Beryllium—Total, ⁴ mg/L.	AA direct aspiration ³⁶	3111 D–1999		I–3084–85. ²
	AA furnace	3113 B–2004	D4382–02(07).	
	ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B–1999		I–4471–97. ⁵⁰
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B–2009	D5673–05	993.14, ³ I–4471–97. ⁵⁰
	DCP ³⁶	See footnote. ³⁴
9. Biochemical oxygen demand (BOD ₅), mg/L.	Digestion, ⁴ followed by any of the following:				
	AA direct aspiration	3111 D–1999 or	D3645–08 (A)	I–3095–85. ²
	AA furnace	3111 E–1999		
	STGFAA	200.9, Rev. 2.2 (1994).	3113 B–2004	D3645–08 (B).	
	ICP/AES	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B–1999	D1976–07	I–4471–97. ⁵⁰
10. Boron—Total, ³⁷ mg/L.	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B–2009	D5673–05	993.14, ³ I–4471–97. ⁵⁰
	DCP	D4190–08	See footnote. ³⁴
	Colorimetric (aluminon).	See footnote ⁶¹ .		
	Dissolved Oxygen Depletion.	5210 B–2001		973.44 ³ , p. 17. ⁹ , I–1578–78, ⁸ See footnote. ^{10,63}
	Colorimetric (curcumin)	4500–B B –2000		I–3112–85. ²
11. Bromide, mg/L	ICP/AES	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B–1999	D1976–07	I–4471–97. ⁵⁰
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B–2009	D5673–05	993.14, ³ I–4471–97. ⁵⁰
	DCP	D4190–08	See footnote. ³⁴
	Electrode	D1246–05	I–1125–85. ²
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1–1, Rev 1.0 (1997).	4110 B–2000, C–2000, D–2000.	D4327–03	993.30. ³
12. Cadmium—Total, ⁴ mg/L.	CIE/UV	4140 B–1997	D6508–00(05)	D6508, Rev. 2. ⁵⁴
	Digestion, ⁴ followed by any of the following:				

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology ⁵⁸	EPA ⁵²	Standard methods	ASTM	USGS/AOAC/Other	
13. Calcium—Total, ⁴ mg/L.	AA direct aspiration ³⁶	3111 B–1999 or 3111 C–1999	D3557–02(07) (A or B).	974.27, ³ p. 37, ⁹ I–3135–85 ² or I–3136–85 ²	
	AA furnace	3113 B–2004	D3557–02(07) (D) ...	I–4138–89. ⁵¹	
	STGFAA	200.9, Rev. 2.2 (1994).	
	ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B–1999	D1976–07	I–1472–85 ² or I–4471–97. ⁵⁰	
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B–2009	D5673–05	993.14, ³ I–4471–97. ⁵⁰	
	DCP ³⁶	D4190–08	See footnote. ³⁴	
	Voltametry ¹¹	D3557–02(07) (C).	
	Colorimetric (Dithionite).	3500–Cd–D–1990.	
	Digestion, ⁴ followed by any of the following:	
	AA direct aspiration	3111 B–1999	D511–08(B)	I–3152–85. ²	
14. Carbonaceous biochemical oxygen demand (CBOD ₅), mg/L ¹² .	ICP/AES	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B–1999	I–4471–97. ⁵⁰	
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B–2009	D5673–05	993.14. ³	
	DCP	See footnote. ³⁴	
	Titrimetric (EDTA)	3500–Ca B–1997 ...	D511–08 (A).	
	Ion Chromatography	D6919–09.	
	Dissolved Oxygen Depletion with nitrification inhibitor.	5210 B–2001	See footnote. ^{35,63}	
	15. Chemical oxygen demand (COD), mg/L.	Titrimetric	410.3 (Rev. 1978) ¹ ..	5220 B–1997 or C–1997	D1252–06 (A)	973.46, ³ p. 17, ⁹ I–3560–85. ²
		Spectrophotometric, manual or automatic.	410.4, Rev. 2.0 (1993).	5220 D–1997	D1252–06 (B)	See footnotes. ^{13,14} I–3561–85. ²
	16. Chloride, mg/L	Titrimetric: (silver nitrate)	4500–Cl [–] B–1997 ...	D512–04 (B)	I–1183–85. ²
		(Mercuric nitrate)	4500–Cl [–] C–1997 ...	D512–04 (A)	973.51, ³ I–1184–85. ²
Colorimetric: manual	I–1187–85. ²	
Automated (Ferricyanide)	4500–Cl [–] E–1997	I–2187–85. ²	
Potentiometric Titration	4500–Cl [–] D–1997.	
Ion Selective Electrode	D512–04 (C).	
Ion Chromatography		300.0, Rev 2.1 (1993) and 300.1–1, Rev 1.0 (1997).	4110 B–2000 or 4110 C–2000	D4327–03	993.30 ³ , I–2057–90. ⁵¹	
17. Chlorine—Total residual, mg/L.		CIE/UV	4140 B–1997	D6508–00(05)	D6508, Rev. 2. ⁵⁴
		Amperometric direct	4500–Cl D–2000	D1253–08.
		Amperometric direct (low level).	4500–Cl E–2000.
	Iodometric direct	4500–Cl B–2000.	
	Back titration ether endpoint ¹⁵	4500–Cl C–2000.	
	DPD–FAS	4500–Cl F–2000.	
	Spectrophotometric, DPD	4500–Cl G–2000.	
	Electrode	See footnote. ¹⁶	
	17A. Chlorine—Free Available, mg/L.	Amperometric direct	4500–Cl D–2000	D1253–08.
		Amperometric direct (low level).	4500–Cl E–2000.
DPD–FAS	4500–Cl F–2000.	
Spectrophotometric, DPD	4500–Cl G–2000.	
18. Chromium VI dissolved, mg/L.	0.45-micron Filtration followed by any of the following:	
	AA chelation–extraction.	3111 C–1999	I–1232–85. ²	
	Ion Chromatography	218.6, Rev. 3.3 (1994).	3500–Cr C–2009	D5257–03	993.23.	
19. Chromium—Total, ⁴ mg/L.	Colorimetric (Diphenyl–carbazide).	3500–Cr B–2009	D1687–02(07) (A) ...	I–1230–85. ²	
	Digestion, ⁴ followed by any of the following:	

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology ⁵⁸	EPA ⁵²	Standard methods	ASTM	USGS/AOAC/Other	
20. Cobalt—Total, ⁴ mg/L.	AA direct aspiration ³⁶	3111 B—1999	D1687—02(07) (B)	974.27, ³ I—3236—85. ²	
	AA chelation—extraction.	3111 C—1999.			
	AA furnace	3113 B—2004	D1687—02(07) (C)	I—3233—93. ⁴⁶	
	STGFAA	200.9, Rev. 2.2 (1994).				
	ICP/AES ³⁶	200.5, Rev 4.2 (2003), ⁶⁸ 200.7, Rev. 4.4 (1994).	3120 B—1999	D1976—07	I—4471—97. ⁵⁰	
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B—2009	D5673—05	993.14, ³ I—4020—05. ⁷⁰	
	DCP ³⁶	D4190—08	See footnote. ³⁴	
	Colorimetric (Diphenyl-carbazide). Digestion, ⁴ followed by any of the following:	3500—Cr B—2009.		
	AA direct aspiration	3111 B—1999 or 3111 C—1999.	D3558—08 (A or B) ..	p. 37, ⁹ I—3239—85. ²	
	AA furnace	3113 B—2004	D3558—08 (C)	I—4243—89. ⁵¹	
STGFAA	200.9, Rev. 2.2 (1994).					
ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B—1999	D1976—07	I—4471—97. ⁵⁰		
ICP/MS	200.8, Rev. 5.4 (1994).	3125 B—2009	D5673—05	993.14, ³ I—4020—05. ⁷⁰		
DCP	D4190—08	See footnote. ³⁴		
21. Color, platinum cobalt units or dominant wavelength, hue, luminance purity.	Colorimetric (ADMI)	See footnote. ¹⁸	
	(Platinum cobalt) Spectrophotometric. Digestion, ⁴ followed by any of the following:	2120 B—2001	I—1250—85. ²	
22. Copper—Total, ⁴ mg/L.	AA direct aspiration ³⁶	3111 B—1999 or 3111 C—1999	D1688—07 (A or B) ..	974.27, ³ p. 37, ⁹ I—3270—85. ² or I—3271—85. ²	
	AA furnace	3113 B—2004	D1688—07 (C)	I—4274—89. ⁵¹	
	STGFAA	200.9, Rev. 2.2 (1994).				
	ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B—1999	D1976—07	I—4471—97. ⁵⁰	
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B—2009	D5673—05	993.14, ³ I—4020—05. ⁷⁰	
	DCP ³⁶	D4190—08	See footnote. ³⁴	
23. Cyanide—Total, mg/L.	Colorimetric (Neocuproine). (Bathocuproine)	3500—Cu B—1999.	See footnote. ¹⁹	
	Automated UV digestion/distillation and Colorimetry. Segmented Flow Injection, In-Line Ultraviolet Digestion, followed by gas diffusion amperometry.	Kelada—01. ⁵⁵	
	Manual distillation with MgCl ₂ , followed by any of the following:	D7511—09.		
	Flow Injection, gas diffusion amperometry.		
	Titrimetric	4500—CN ⁻ B—1999 or C—1999.	D2036—09(A), D7284—08.	10—204—00—1—X. ⁵⁶	
	Spectrophotometric, manual.	D2036—09(A) D7284—08.		
	Spectrophotometric, manual.	4500—CN ⁻ D—1999	D2036—09(A)	p. 22, ⁹	
	Spectrophotometric, manual.	4500—CN ⁻ E—1999	D2036—09(A)	I—3300—85. ²	
	Semi-Automated ²⁰ ..	335.4, Rev. 1.0 (1993) ⁵⁷	10—204—00—1—X, ⁵⁶ I—4302—85. ²	
	Ion Chromatography	D2036—09(A).		

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology ⁵⁸	EPA ⁵²	Standard methods	ASTM	USGS/AOAC/Other
24. Cyanide—Available, mg/L.	Ion Selective Electrode.	4500-CN ⁻ F-1999	D2036-09(A).	
	Cyanide Amenable to Chlorination (CATC); Manual distillation with MgCl ₂ , followed by Titrimetric or Spectrophotometric.	4500-CN ⁻ G-1999	D2036-09(B).	
		Flow injection and ligand exchange, followed by gas diffusion amperometry ⁵⁹	D6888-09
24.A Cyanide-Free, mg/L.	Automated Distillation and Colorimetry (no UV digestion).	Kelada-01. ⁵⁵
	Flow Injection, followed by gas diffusion amperometry.	D7237-10	OIA-1677-09. ⁴⁴
25. Fluoride—Total, mg/L.	Manual micro-diffusion and colorimetry.	D4282-02.	
	Manual distillation, ⁶ followed by any of the following:	4500-F ⁻ B-1997.		
	Electrode, manual	4500-F ⁻ C-1997 ...	D1179-04 (B).	
	Electrode, automated.	I-4327-85. ²
	Colorimetric, (SPADNS).	4500-F ⁻ D-1997 ...	D1179-04 (A).	
26. Gold—Total, ⁴ mg/L.	Automated complexone.	4500-F ⁻ E-1997.		
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1-1, Rev 1.0 (1997).	4110 B-2000 or C-2000.	D4327-03	993.30. ³
	CIE/UV	4140 B-1997	D6508-00(05)	D6508, Rev. 2. ⁵⁴
	Digestion, ⁴ followed by any of the following:
27. Hardness—Total, as CaCO ₃ , mg/L.	AA direct aspiration	231.2 (Issued 1978) ¹	3111 B-1999.		
	AA furnace	200.8, Rev. 5.4 (1994).	3113 B-2004.	D5673-05	993.14. ³
	ICP/MS	3125 B-2009	See footnote. ³⁴
	DCP
	Automated colorimetric ...	130.1 (Issued 1971) ¹
28. Hydrogen ion (pH), pH units.	Titrimetric (EDTA)	2340 C-1997	D1126-02(07)	973.52B, ³ I-1338-85. ²
	Ca plus Mg as their carbonates, by inductively coupled plasma or AA direct aspiration. (See Parameters 13 and 33)..	2340 B-1997.		
	Electrometric measurement.	4500-H ⁺ B-2000	D1293-99 (A or B) ..	973.41, ³ I-1586-85. ²
29. Iridium—Total, ⁴ mg/L.	Automated electrode	150.2 (Dec. 1982) ¹	See footnote, ²¹ I-2587-85. ²
	Digestion, ⁴ followed by any of the following:
30. Iron—Total, ⁴ mg/L	AA direct aspiration	3111 B-1999.		
	AA furnace	235.2 (Issued 1978) ¹
	ICP/MS	3125 B-2009.		
	Digestion, ⁴ followed by any of the following:
	AA direct aspiration ³⁶	3111 B-1999 or	D1068-05 (A or B) ..	974.27, ³ I-3381-85. ²
	AA furnace	3111 C-1999
29. Iridium—Total, ⁴ mg/L.	STGFAA	200.9, Rev. 2.2 (1994).	3113 B-2004	D1068-05 (C).	
	ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B-1999	D1976-07	I-4471-97. ⁵⁰
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B-2009	D5673-05	993.14. ³

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology ⁵⁸	EPA ⁵²	Standard methods	ASTM	USGS/AOAC/Other	
31. Kjeldahl Nitrogen ⁵ —Total, (as N), mg/L.	DCP ³⁶	D4190-08	See footnote. ³⁴	
	Colorimetric (Phenanthroline).	3500-Fe-1997	D1068-05 (D)	See footnote. ²²	
	Manual digestion ²⁰ and distillation or gas diffusion, followed by any of the following:	4500-N _{org} B-1997 or C-1997 and 4500-NH ₃ B-1997.	D3590-02(06) (A)	I-4515-91. ⁴⁵	
	Titration	4500-NH ₃ C-1997	973.48. ³	
	Nesslerization	D1426-08 (A).	
	Electrode	4500-NH ₃ D-1997 or E-1997.	D1426-08 (B).	
	Semi-automated phenate.	350.1 Rev 2.0 1993	4500-NH ₃ G-1997.	
	Manual phenate, salicylate, or other substituted phenols in Berthelot reaction based methods.	4500-NH ₃ H-1997. 4500-NH ₃ F-1997	See footnote. ⁶⁰	
	Automated Methods for TKN that do not require manual distillation					
	32. Lead—Total, ⁴ mg/L.	Automated phenate, salicylate, or other substituted phenols in Berthelot reaction based methods colorimetric (auto digestion and distillation).	351.1 (Rev. 1978) ¹	I-4551-78. ⁸
Semi-automated block digester colorimetric (distillation not required).		351.2, Rev. 2.0 (1993).	4500-N _{org} D-1997 ...	D3590-02(06) (B)	I-4515-91. ⁴⁵	
Block digester, followed by Auto distillation and Titration.		See footnote. ³⁹	
Block digester, followed by Auto distillation and Nesslerization.		See footnote. ⁴⁰	
Block Digester, followed by Flow injection gas diffusion (distillation not required).		See footnote. ⁴¹	
Digestion, ⁴ followed by any of the following:		
AA direct aspiration ³⁶	3111 B-1999 or 3111 C-1999.	D3559-08 (A or B) ..	974.27, ³ I-3399-85. ²	
AA furnace	3113 B-2004	D3559-08 (D)	I-4403-89. ⁵¹	
STGFAA		200.9, Rev. 2.2 (1994).	
ICP/AES ³⁶		200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B-1999	D1976-07	I-4471-97. ⁵⁰	
33. Magnesium—Total, ⁴ mg/L.	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B-2009	D5673-05	993.14, ³ I-4471-97. ⁵⁰	
	DCP ³⁶	D4190-08	See footnote. ³⁴	
	Voltametry ¹¹	D3559-08 (C).	
	Colorimetric (Dithionite).	3500-Pb B-1997.	
	Digestion, ⁴ followed by any of the following:	
	AA direct aspiration	3111 B-1999	D511-08 (B)	974.27, ³ I-3447-85. ²	
	ICP/AES	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B-1999	D1976-07	I-4471-97. ⁵⁰	
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B-2009	D5673-05	993.14. ³	
	DCP	See footnote. ³⁴	
	34. Manganese—Total, ⁴ mg/L.	Gravimetric.
Ion Chromatography		D6919-09.	

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology ⁵⁸	EPA ⁵²	Standard methods	ASTM	USGS/AOAC/Other	
35. Mercury—Total, ⁴ mg/L.	AA direct aspiration ³⁶	3111 B—1999	D858—07 (A or B)	974.27, ³ I—3454—85. ²	
	AA furnace	3113 B—2004	D858—07 (C).		
	STGFAA	200.9, Rev. 2.2 (1994).				
	ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B—1999	D1976—07	I—4471—97. ⁵⁰	
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B—2009	D5673—05	993.14, ³ I—4471—97. ⁵⁰	
	DCP ³⁶	D4190—08	See footnote. ³⁴	
	Colorimetric (Persulfate) (Periodate)	3500—Mn B—1999	920.203. ³	
	Cold vapor, Manual	245.1, Rev. 3.0 (1994).	3112 B—2009	D3223—02(07)	See footnote. ²³ 977.22, ³ I—3462—85. ²	
	Cold vapor, Automated ..	245.2 (Issued 1974) ¹		
	Cold vapor atomic fluorescence spectrometry (CVAFS).	245.7 Rev. 2.0 (2005) ¹⁷	I—4464—01. ⁷¹	
36. Molybdenum—Total, ⁴ mg/L.	Purge and Trap CVAFS Digestion, ⁴ followed by any of the following:	1631E ⁴³ .				
	AA direct aspiration	3111 D—1999	I—3490—85. ²	
	AA furnace	3113 B—2004	I—3492—96. ⁴⁷	
	ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B—1999	D1976—07	I—4471—97. ⁵⁰	
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B—2009	D5673—05	993.14, ³ I—4471—97. ⁵⁰	
37. Nickel—Total, ⁴ mg/L.	DCP	See footnote. ³⁴	
	Digestion ⁴ followed by any of the following:				
	AA direct aspiration ³⁶	3111 B—1999 or	D1886—08 (A or B) ..	I—3499—85. ²	
	AA furnace	3111 C—1999		
	STGFAA	200.9, Rev. 2.2 (1994).	3113 B—2004	D1886—08 (C)	I—4503—89. ⁵¹	
	ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B—1999	D1976—07	I—4471—97. ⁵⁰	
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B—2009	D5673—05	993.14, ³ I—4020—05. ⁷⁰	
	DCP ³⁶	D4190—08	See footnote. ³⁴	
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1—1, Rev 1.0 (1997).	4110 B—2000 or C—2000.	D4327—03	993.30. ³	
	38. Nitrate (as N), mg/L.	CIE/UV	4140 B—1997	D6508—00(05)	D6508, Rev. 2. ⁵⁴
Ion Selective Electrode.		4500—NO ₃ ⁻ D—2000.		
Colorimetric (Brucine sulfate).		352.1 (Issued 1971) ¹	973.50, ³ 419D ^{1,7} , p. 28. ⁹	
Nitrate-nitrite N minus Nitrite N (See parameters 39 and 40).		See footnote. ⁶²	
39. Nitrate-nitrite (as N), mg/L.		Cadmium reduction, Manual.	4500—NO ₃ ⁻ E—2000	D3867—04 (B).	
		Cadmium reduction, Automated.	353.2, Rev. 2.0 (1993).	4500—NO ₃ ⁻ F—2000	D3867—04 (A)	I—2545—90. ⁵¹
		Automated hydrazine.	4500—NO ₃ ⁻ H—2000.	
		Reduction/Colorimetric.	See footnote. ⁶²
		Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1—1, Rev 1.0 (1997).	4110 B—2000 or C—2000.	D4327—03	993.30. ³
40. Nitrite (as N), mg/L		CIE/UV	4140 B—1997	D6508—00(05)	D6508, Rev. 2. ⁵⁴
	Spectrophotometric: Manual.	4500—NO ₂ ⁻ B—2000	See footnote. ²⁵	
	Automated (Diazotization).	I—4540—85 ² , See footnote. ⁶²	

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology ⁵⁸	EPA ⁵²	Standard methods	ASTM	USGS/AOAC/Other
41. Oil and grease— Total recoverable, mg/L.	Automated (*bypass cadmium reduction).	353.2, Rev. 2.0 (1993).	4500-NO ₃ ⁻ F-2000	D3867-04 (A)	I-4545-85. ²
	Manual (*bypass cadmium reduction).	4500-NO ₃ ⁻ E-2000	D3867-04 (B).	
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1- 1, Rev 1.0 (1997).	4110 B-2000 or C- 2000.	D4327-03	993.30. ³
	CIE/UV	4140 B-1997	D6508-00(05)	D6508, Rev. 2. ⁵⁴
42. Organic carbon— Total (TOC), mg/L.	Hexane extractable ma- terial (HEM): n- Hexane extraction and gravimetry.	1664 Rev. A; 1664 Rev. B ⁴² .	5520 B-2001 ³⁸ .		
	Silica gel treated HEM (SGT-HEM): Silica gel treat- ment and gravim- etry.	1664 Rev. A; 1664 Rev. B ⁴² .	5520 B-2001 ³⁸ and 5520 F-2001 ³⁸ .		
43. Organic nitrogen (as N), mg/L.	Combustion	5310 B-2000	D7573-09	973.47 ³ , p. 14. ²⁴
	Heated persulfate or UV persulfate oxida- tion.	5310 C 2000	D4839-03	973.47 ³ , p. 14. ²⁴
44. Ortho-phosphate (as P), mg/L.	Total Kjeldahl N (Param- eter 31) minus ammo- nia N (Parameter 4).	Ascorbic acid meth- od:			
45. Osmium—Total ⁴ , mg/L.	Automated	365.1, Rev. 2.0 (1993).	4500-P F-1999 or G-1999.	973.56 ³ , I-4601-85. ²
	Manual single rea- gent.	4500-P E-1999	D515-88(A)	973.55. ³
	Manual two reagent Ion Chromatography	365.3 (Issued 1978) ¹ . 300.0, Rev 2.1 (1993) and 300.1- 1, Rev 1.0 (1997).	4110 B-2000 or C- 2000.	D4327-03	993.30. ³
	CIE/UV	4140 B-1997	D6508-00(05)	D6508, Rev. 2. ⁵⁴
46. Oxygen, dissolved, mg/L.	Digestion ⁴ , followed by any of the following: AA direct aspiration, AA furnace	252.2 (Issued 1978) ¹ .	3111 D-1999.		
	Winkler (Azide modifica- tion).	4500-O B-2001, C- 2001, D-2001, E- 2001, F-2001.	D888-09 (A)	973.45B ³ , I-1575- 78. ⁸
47. Palladium—Total, ⁴ mg/L.	Electrode	4500-O G-2001	D888-09 (B)	I-1576-78. ⁸
	Luminescence Based Sensor.	D888-09 (C)	See footnote ⁶³ See footnote. ⁶⁴
	Digestion ⁴ , followed by any of the following: AA direct aspiration AA furnace	253.2 ¹ (Issued 1978).	3111 B-1999.		
48. Phenols, mg/L	ICP/MS	3125 B-2009.		See footnote. ³⁴
	DCP		
	Manual distillation ²⁶ , fol- lowed by any of the following: Colorimetric (4AAP) manual.	420.1 ¹ (Rev. 1978) ...	5530 B-2005	D1783-01.	
49. Phosphorus (ele- mental), mg/L.	Automated colori- metric (4AAP).	420.1 ¹ (Rev. 1978) ...	5530 D-2005 ²⁷	D1783-01 (A or B).	
	Gas-liquid chroma- tography.	See footnote. ²⁸
50. Phosphorus— Total, mg/L.	Digestion ²⁰ , followed by any of the following: Manual	4500-P B(5)-1999	973.55. ³
	Automated ascorbic acid reduction.	365.3 ¹ (Issued 1978) 365.1 Rev. 2.0 (1993).	4500-P E-1999	D515-88 (A).	
	ICP/AES ^{4, 36}	200.7, Rev. 4.4 (1994).	4500-P F-1999, G- 1999, H-1999.	973.56 ³ , I-4600-85. ²
	3120 B-1999	I-4471-97. ⁵⁰

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology ⁵⁸	EPA ⁵²	Standard methods	ASTM	USGS/AOAC/Other
51. Platinum—Total, ⁴ mg/L.	Semi-automated block digester (TKP digestion).	365.4 ¹ (Issued 1974)	D515-88 (B)	I-4610-91. ⁴⁸
	Digestion ⁴ followed by any of the following: AA direct aspiration	3111 B-1999.
	AA furnace	255.2 (Issued 1978) ¹ .	3125 B-2009.
52. Potassium—Total, ⁴ mg/L.	Digestion ⁴ , followed by any of the following: AA direct aspiration	3111 B-1999	973.53 ³ , I-3630-85. ²
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B-1999.
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B-2009	D5673-05	993.14. ³
53. Residue—Total, mg/L.	Flame photometric	3500-K B-1997.
	Electrode	3500-K C-1997.
54. Residue—filterable, mg/L.	Ion Chromatography	D6919-09.
	Gravimetric, 103-105°	2540 B-1997	I-3750-85. ²
55. Residue—non-filterable (TSS), mg/L.	Gravimetric, 180°	2540 C-1997	D5907-03	I-1750-85. ²
	Gravimetric, 103-105° post washing of residue.	2540 D-1997	D5907-03	I-3765-85. ²
56. Residue—settleable, mg/L.	Volumetric, (Imhoff cone), or gravimetric.	2540 F-1997.
57. Residue—Volatile, mg/L.	Gravimetric, 550°	160.4 (Issued 1971) ¹	2540-E-1997	I-3753-85. ²
58. Rhodium—Total, ⁴ mg/L.	Digestion ⁴ followed by any of the following: AA direct aspiration,	3111 B-1999.
	or. AA furnace	265.2 (Issued 1978) ¹ .	3125 B-2009.
	ICP/MS
59. Ruthenium—Total, ⁴ mg/L.	Digestion ⁴ followed by any of the following: AA direct aspiration,	3111 B-1999.
	or. AA furnace	267.2 ¹ .	3125 B-2009.
	ICP/MS
60. Selenium—Total, ⁴ mg/L.	Digestion ⁴ , followed by any of the following: AA furnace	3113 B-2004	D3859-08 (B)	I-4668-98. ⁴⁹
	STGFAA	200.9, Rev. 2.2 (1994).
	ICP/AES ³⁶	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B-1999	D1976-07.
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B-2009	D5673-05	993.14 ³ , I-4020-05. ⁷⁰
61. Silica—Dissolved, ³⁷ mg/L.	AA gaseous hydride	3114 B-2009, or 3111 C-2009.	D3859-08 (A)	I-3667-85. ²
	0.45-micron filtration followed by any of the following: Colorimetric, Manual	4500-SiO ₂ C-1997 ..	D859-05	I-1700-85. ²
	Automated (Molybdosilicate).	4500-SiO ₂ E-1997 or F-1997.	I-2700-85. ²
	ICP/AES	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B-1999	I-4471-97. ⁵⁰
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B-2009	D5673-05	993.14. ³
62. Silver—Total, ^{4, 31} mg/L.	Digestion ^{4, 29} , followed by any of the following: AA direct aspiration	3111 B-1999 or 3111 C-1999	974.27 ³ , p. 37 ⁹ , I-3720-85. ²
	AA furnace	3113 B-2004	I-4724-89. ⁵¹
	STGFAA	200.9, Rev. 2.2 (1994).

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology ⁵⁸	EPA ⁵²	Standard methods	ASTM	USGS/AOAC/Other
63. Sodium—Total, ⁴ mg/L.	ICP/AES	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B—1999	D1976—07	I—4471—97. ⁵⁰
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B—2009	D5673—05	993.14 ³ , I—4471—97. ⁵⁰
	DCP	See footnote. ³⁴
	Digestion ⁴ , followed by any of the following: AA direct aspiration	3111 B—1999	973.54 ³ , I—3735—85. ²
	ICP/AES	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B—1999	I—4471—97. ⁵⁰
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B—2009	D5673—05	993.14. ³
64. Specific conductance, micromhos/cm at 25°C.	DCP	See footnote. ³⁴
	Flame photometric	3500—Na B—1997.
65. Sulfate (as SO ₄), mg/L.	Ion Chromatography	D6919—09.
	Wheatstone bridge	120.11(Rev. 1982) ...	2510 B—1997	D1125—95(99) (A)	973.40 ³ , I—2781—85. ²
66. Sulfide (as S), mg/L.	Automated colorimetric ...	375.2, Rev. 2.0 (1993).	4500—SO ₄ ²⁻ F—1997 or G—1997.
	Gravimetric	4500—SO ₄ ²⁻ C—1997 or D—1997.	925.54. ³
	Turbidimetric	4500—SO ₄ ²⁻ E—1997.	D516—07.
	Ion Chromatography ..	300.0, Rev 2.1 (1993) and 300.1—1, Rev 1.0 (1997).	4110 B—2000 or C—2000.	D4327—03	993.30 ³ , I—4020—05. ⁷⁰
67. Sulfite (as SO ₃), mg/L.	CIE/UV	4140 B—1997	D6508—00(05)	D6508, Rev. 2. ⁵⁴
	Sample Pretreatment	4500—S ²⁻ B, C—2000.
	Titrimetric (iodine)	4500—S ²⁻ F—2000	I—3840—85. ²
	Colorimetric (methylene blue).	4500—S ²⁻ D—2000.
68. Surfactants, mg/L	Ion Selective Electrode.	4500—S ²⁻ G—2000 ...	D4658—08.
	Titrimetric (iodine-iodate)	4500—SO ₃ ²⁻ B—2000.
69. Temperature, °C ..	Colorimetric (methylene blue).	5540 C—2000	D2330—02.
70. Thallium—Total, ⁴ mg/L.	Thermometric	2550 B—2000	See footnote. ³²
	Digestion ⁴ , followed by any of the following: AA direct aspiration	3111 B—1999.
71. Tin—Total, ⁴ mg/L ..	AA furnace	279.21(Issued 1978)	3113 B—2004.
	STGFAA	200.9, Rev. 2.2 (1994).
	ICP/AES	200.7, Rev. 4.4 (1994); 200.5 Rev. 4.2 (2003) ⁶⁸ .	3120 B—1999	D1976—07.
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B—2009	D5673—05	993.14 ³ , I—4471—97. ⁵⁰
	Digestion ⁴ , followed by any of the following: AA direct aspiration	3111 B—1999	I—3850—78. ⁸
	AA furnace	3113 B—2004.
72. Titanium—Total, ⁴ mg/L.	STGFAA	200.9, Rev. 2.2 (1994).
	ICP/AES	200.5, Rev 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B—2009	D5673—05	993.14. ³
	Digestion ⁴ followed by any of the following: AA direct aspiration	3111 D—1999.
AA furnace	283.21(Issued 1978).	
ICP/AES	200.7, Rev. 4.4 (1994).	
ICP/MS	200.8, Rev. 5.4 (1994).	3125 B—2009	D5673—05	993.14. ³	

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology ⁵⁸	EPA ⁵²	Standard methods	ASTM	USGS/AOAC/Other
73. Turbidity, NTU ⁵³ ...	DCP	See footnote. ³⁴
	Nephelometric	180.1, Rev. 2.0 (1993).	2130 B-2001	D1889-00	I-3860-85. ² See footnote. ⁶⁵ See footnote. ⁶⁶ See footnote. ⁶⁷
74. Vanadium—Total, ⁴ mg/L.	Digestion ⁴ , followed by any of the following:				
	AA direct aspiration	3111 D-1999.		
	AA furnace	3113 B-2004	D3373-03(07).	
	ICP/AES	200.5, Rev. 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B-1999	D1976-07	I-4471-97. ⁵⁰
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B-2009	D5673-05	993.14 ³ , I-4020-05. ⁷⁰
	DCP	D4190-08	See footnote. ³⁴
75. Zinc—Total ⁴ , mg/L	Digestion ⁴ , followed by any of the following:				
	AA direct aspiration ³⁶	3111 B-1999 or 3111 C-1999.	D1691-02(07) (A or B).	974.27 ³ , p. 37 ⁹ , I-3900-85. ²
	AA furnace	289.2 ¹ (Issued 1978).
	ICP/AES ³⁶	200.5, Rev. 4.2 (2003) ⁶⁸ ; 200.7, Rev. 4.4 (1994).	3120 B-1999	D1976-07	I-4471-97. ⁵⁰
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B-2009	D5673-05	993.14 ³ , I-4020-05. ⁷⁰
	DCP ³⁶	D4190-08	See footnote. ³⁴
76. Acid Mine Drainage.	Colorimetric (Zincon)	3500 Zn B-1997	See footnote. ³³
	1627 ⁶⁹

Table IB Notes:

¹ Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020. Revised March 1983 and 1979, where applicable. U.S. EPA.

² Methods for Analysis of Inorganic Substances in Water and Fluvial Sediments, Techniques of Water-Resource Investigations of the U.S. Geological Survey, Book 5, Chapter A1., unless otherwise stated. 1989. USGS.

³ Official Methods of Analysis of the Association of Official Analytical Chemists, Methods Manual, Sixteenth Edition, 4th Revision, 1998. AOAC International.

⁴ For the determination of total metals (which are equivalent to total recoverable metals) the sample is not filtered before processing. A digestion procedure is required to solubilize analytes in suspended material and to break down organic-metal complexes (to convert the analyte to a detectable form for colorimetric analysis). For non-platform graphite furnace atomic absorption determinations a digestion using nitric acid (as specified in Section 4.1.3 of Methods for the Chemical Analysis of Water and Wastes) is required prior to analysis. The procedure used should subject the sample to gentle, acid refluxing and at no time should the sample be taken to dryness. For direct aspiration flame atomic absorption determinations (FLAA) a combination acid (nitric and hydrochloric acids) digestion is preferred prior to analysis. The approved total recoverable digestion is described as Method 200.2 in Supplement 1 of "Methods for the Determination of Metals in Environmental Samples" EPA/600R-94/111, May, 1994, and is reproduced in EPA Methods 200.7, 200.8, and 200.9 from the same Supplement. However, when using the gaseous hydride technique or for the determination of certain elements such as antimony, arsenic, selenium, silver, and tin by non-EPA graphite furnace atomic absorption methods, mercury by cold vapor atomic absorption, the noble metals and titanium by FLAA, a specific or modified sample digestion procedure may be required and in all cases the referenced method write-up should be consulted for specific instruction and/or cautions. For analyses using inductively coupled plasma-atomic emission spectrometry (ICP-AES), the direct current plasma (DCP) technique or the EPA spectrochemical techniques (platform furnace AA, ICP-AES, and ICP-MS) use EPA Method 200.2 or an approved alternate procedure (e.g., CEM microwave digestion, which may be used with certain analytes as indicated in Table IB); the total recoverable digestion procedures in EPA Methods 200.7, 200.8, and 200.9 may be used for those respective methods. Regardless of the digestion procedure, the results of the analysis after digestion procedure are reported as "total" metals.

⁵ Copper sulfate or other catalysts that have been found suitable may be used in place of mercuric sulfate.

⁶ Manual distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary; however, manual distillation will be required to resolve any controversies. In general, the analytical method should be consulted regarding the need for distillation. If the method is not clear, the laboratory may compare a minimum of 9 different sample matrices to evaluate the need for distillation. For each matrix, a matrix spike and matrix spike duplicate are analyzed both with and without the distillation step. (A total of 36 samples, assuming 9 matrices). If results are comparable, the laboratory may dispense with the distillation step for future analysis. Comparable is defined as < 20% RPD for all tested matrices). Alternatively the two populations of spike recovery percentages may be compared using a recognized statistical test.

⁷ Industrial Method Number 379-75 WE Ammonia, Automated Electrode Method, Technicon Auto Analyzer II. February 19, 1976. Bran & Luebbe Analyzing Technologies Inc.

⁸ The approved method is that cited in Methods for Determination of Inorganic Substances in Water and Fluvial Sediments, Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 5, Chapter A1. 1979. USGS.

⁹ American National Standard on Photographic Processing Effluents. April 2, 1975. American National Standards Institute.

¹⁰ In-Situ Method 1003-8-2009, Biochemical Oxygen Demand (BOD) Measurement by Optical Probe. 2009. In-Situ Incorporated.

¹¹ The use of normal and differential pulse voltage ramps to increase sensitivity and resolution is acceptable.

¹² Carbonaceous biochemical oxygen demand (CBOD₅) must not be confused with the traditional BOD₅ test method which measures "total BOD." The addition of the nitrification inhibitor is not a procedural option, but must be included to report the CBOD₅ parameter. A discharger whose permit requires reporting the traditional BOD₅ may not use a nitrification inhibitor in the procedure for reporting the results. Only when a discharger's permit specifically states CBOD₅ is required can the permittee report data using a nitrification inhibitor.

¹³ OIC Chemical Oxygen Demand Method. 1978. Oceanography International Corporation.

¹⁴ Method 8000, Chemical Oxygen Demand, Hach Handbook of Water Analysis, 1979. Hach Company.

¹⁵ The back titration method will be used to resolve controversy.

¹⁶ Orion Research Instruction Manual, Residual Chlorine Electrode Model 97-70. 1977. Orion Research Incorporated. The calibration graph for the Orion residual chlorine method must be derived using a reagent blank and three standard solutions, containing 0.2, 1.0, and 5.0 mL 0.00281 N potassium iodate/100 mL solution, respectively.

¹⁷ Method 245.7, Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, EPA-821-R-05-001. Revision 2.0, February 2005. US EPA.

¹⁸ National Council of the Paper Industry for Air and Stream Improvement (NCASI) Technical Bulletin 253, December 1971.

¹⁹ Method 8506, Biocinchoninate Method for Copper, Hach Handbook of Water Analysis. 1979. Hach Company.

²⁰ When using a method with block digestion, this treatment is not required.

²¹ Industrial Method Number 378-75WA, Hydrogen ion (pH) Automated Electrode Method, Bran & Luebbe (Technicon) Autoanalyzer II. October 1976. Bran & Luebbe Analyzing Technologies.

²² Method 8008, 1,10-Phenanthroline Method using FerroVer Iron Reagent for Water. 1980. Hach Company.

²³ Method 8034, Periodate Oxidation Method for Manganese, Hach Handbook of Wastewater Analysis. 1979. Hach Company.

²⁴ Methods for Analysis of Organic Substances in Water and Fluvial Sediments, Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 5, Chapter A3, (1972 Revised 1987) p. 14. 1987. USGS.

²⁵ Method 8507, Nitrogen, Nitrite-Low Range, Diazotization Method for Water and Wastewater. 1979. Hach Company.

²⁶ Just prior to distillation, adjust the sulfuric-acid-preserved sample to pH 4 with 1 + 9 NaOH.

²⁷ The colorimetric reaction must be conducted at a pH of 10.0 ± 0.2 .

²⁸ Addison, R.F., and R.G. Ackman. 1970. Direct Determination of Elemental Phosphorus by Gas-Liquid Chromatography, *Journal of Chromatography*, 47(3):421-426.

²⁹ Approved methods for the analysis of silver in industrial wastewaters at concentrations of 1 mg/L and above are inadequate where silver exists as an inorganic halide. Silver halides such as the bromide and chloride are relatively insoluble in reagents such as nitric acid but are readily soluble in an aqueous buffer of sodium thiosulfate and sodium hydroxide to pH of 12. Therefore, for levels of silver above 1 mg/L, 20 mL of sample should be diluted to 100 mL by adding 40 mL each of 2 M Na₂S₂O₃ and NaOH. Standards should be prepared in the same manner. For levels of silver below 1 mg/L the approved method is satisfactory.

³⁰ The use of EDTA decreases method sensitivity. Analysts may omit EDTA or replace with another suitable complexing reagent provided that all method specified quality control acceptance criteria are met.

³¹ For samples known or suspected to contain high levels of silver (e.g., in excess of 4 mg/L), cyanogen iodide should be used to keep the silver in solution for analysis. Prepare a cyanogen iodide solution by adding 4.0 mL of concentrated NH₄OH, 6.5 g of KCN, and 5.0 mL of a 1.0 N solution of I₂ to 50 mL of reagent water in a volumetric flask and dilute to 100.0 mL. After digestion of the sample, adjust the pH of the digestate to >7 to prevent the formation of HCN under acidic conditions. Add 1 mL of the cyanogen iodide solution to the sample digestate and adjust the volume to 100 mL with reagent water (NOT acid). If cyanogen iodide is added to sample digestates, then silver standards must be prepared that contain cyanogen iodide as well. Prepare working standards by diluting a small volume of a silver stock solution with water and adjusting the pH ≤ 7 with NH₄OH. Add 1 mL of the cyanogen iodide solution and let stand 1 hour. Transfer to a 100-mL volumetric flask and dilute to volume with water.

³² "Water Temperature-Influential Factors, Field Measurement and Data Presentation," Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 1, Chapter D1. 1975. USGS.

³³ Method 8009, Zincon Method for Zinc, Hach Handbook of Water Analysis, 1979. Hach Company.

³⁴ Method AES0029, Direct Current Plasma (DCP) Optical Emission Spectrometric Method for Trace Elemental Analysis of Water and Wastes. 1986-Revised 1991. Thermo Jarrell Ash Corporation.

³⁵ In-Situ Method 1004-8-2009, Carbonaceous Biochemical Oxygen Demand (CBOD) Measurement by Optical Probe. 2009. In-Situ Incorporated.

³⁶ Microwave-assisted digestion may be employed for this metal, when analyzed by this methodology. Closed Vessel Microwave Digestion of Wastewater Samples for Determination of Metals. April 16, 1992. CEM Corporation

³⁷ When determining boron and silica, only plastic, PTFE, or quartz laboratory ware may be used from start until completion of analysis.

³⁸ Only use n-hexane (n-Hexane—85% minimum purity, 99.0% min. saturated C6 isomers, residue less than 1 mg/L) extraction solvent when determining Oil and Grease parameters—Hexane Extractable Material (HEM), or Silica Gel Treated HEM (analogous to EPA Methods 1664 Rev. A and 1664 Rev. B). Use of other extraction solvents is prohibited.

³⁹ Method PAI-DK01, Nitrogen, Total Kjeldahl, Block Digestion, Steam Distillation, Titrimetric Detection. Revised December 22, 1994. OI Analytical.

⁴⁰ Method PAI-DK02, Nitrogen, Total Kjeldahl, Block Digestion, Steam Distillation, Colorimetric Detection. Revised December 22, 1994. OI Analytical.

⁴¹ Method PAI-DK03, Nitrogen, Total Kjeldahl, Block Digestion, Automated FIA Gas Diffusion. Revised December 22, 1994. OI Analytical.

⁴² Method 1664 Rev. B is the revised version of EPA Method 1664 Rev. A. U.S. EPA. February 1999, Revision A. Method 1664, n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry. EPA-821-R-98-002. U.S. EPA. February 2010, Revision B. Method 1664, n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry. EPA-821-R-10-001.

⁴³ Method 1631, Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, EPA-821-R-02-019. Revision E. August 2002, U.S. EPA. The application of clean techniques described in EPA's Method 1669: *Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels*, EPA-821-R-96-011, are recommended to preclude contamination at low-level, trace metal determinations.

⁴⁴ Method OIA-1677-09, Available Cyanide by Ligand Exchange and Flow Injection Analysis (FIA). 2010. OI Analytical.

⁴⁵ Open File Report 00-170, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Ammonium Plus Organic Nitrogen by a Kjeldahl Digestion Method and an Automated Photometric Finish that Includes Digest Cleanup by Gas Diffusion. 2000. USGS.

⁴⁶ Open File Report 93-449, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Chromium in Water by Graphite Furnace Atomic Absorption Spectrophotometry. 1993. USGS.

⁴⁷ Open File Report 97-198, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Molybdenum by Graphite Furnace Atomic Absorption Spectrophotometry. 1997. USGS.

⁴⁸ Open File Report 92-146, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Total Phosphorus by Kjeldahl Digestion Method and an Automated Colorimetric Finish That Includes Dialysis. 1992. USGS.

⁴⁹ Open File Report 98-639, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Arsenic and Selenium in Water and Sediment by Graphite Furnace-Atomic Absorption Spectrometry. 1999. USGS.

⁵⁰ Open File Report 98-165, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Elements in Whole-water Digests Using Inductively Coupled Plasma-Optical Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry. 1998. USGS.

⁵¹ Open File Report 93-125, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Inorganic and Organic Constituents in Water and Fluvial Sediments. 1993. USGS.

⁵² Unless otherwise indicated, all EPA methods, excluding EPA Method 300.1-1, are published in U.S. EPA. May 1994. Methods for the Determination of Metals in Environmental Samples, Supplement I, EPA/600/R-94/111; or U.S. EPA. August 1993. Methods for the Determination of Inorganic Substances in Environmental Samples, EPA/600/R-93/100. EPA Method 300.1 is US EPA. Revision 1.0, 1997, including errata cover sheet April 27, 1999. Determination of Inorganic Ions in Drinking Water by Ion Chromatography.

⁵³ Styrene divinyl benzene beads (e.g., AMCO-AEPA-1 or equivalent) and stabilized formazin (e.g., Hach StabCal™ or equivalent) are acceptable substitutes for formazin.

⁵⁴ Method D6508, Test Method for Determination of Dissolved Inorganic Anions in Aqueous Matrices Using Capillary Ion Electrophoresis and Chromate Electrolyte. December 2000. Waters Corp.

⁵⁵ Kelada-01, Kelada Automated Test Methods for Total Cyanide, Acid Dissociable Cyanide, and Thiocyanate, EPA 821-B-01-009, Revision 1.2, August 2001. US EPA. Note: A 450-W UV lamp may be used in this method instead of the 550-W lamp specified if it provides performance within the quality control (QC) acceptance criteria of the method in a given instrument. Similarly, modified flow cell configurations and flow conditions may be used in the method, provided that the QC acceptance criteria are met.

⁵⁶ QuikChem Method 10-204-00-1-X, Digestion and Distillation of Total Cyanide in Drinking and Wastewaters using MICRO DIST and Determination of Cyanide by Flow Injection Analysis. Revision 2.2, March 2005. Lachat Instruments.

⁵⁷ When using sulfide removal test procedures described in EPA Method 335.4-1, reconstitute particulate that is filtered with the sample prior to distillation.

⁵⁸ Unless otherwise stated, if the language of this table specifies a sample digestion and/or distillation "followed by" analysis with a method, approved digestion and/or distillation are required prior to analysis.

⁵⁹ Samples analyzed for available cyanide using OI Analytical method OIA-1677-09 or ASTM method D6888-09 that contain particulate matter may be filtered only after the ligand exchange reagents have been added to the samples, because the ligand exchange process converts complexes containing available cyanide to free cyanide, which is not removed by filtration. Analysts are further cautioned to limit the time between the addition of the ligand exchange reagents and sample filtration to no more than 30 minutes to preclude settling of materials in samples.

⁶⁰ Analysts should be aware that pH optima and chromophore absorption maxima might differ when phenol is replaced by a substituted phenol as the color reagent in Berthelot Reaction ("phenol-hypochlorite reaction") colorimetric ammonium determination methods. For example when phenol is used as the color reagent, pH optimum and wavelength of maximum absorbance are about 11.5 and 635 nm, respectively—see, Patton, C.J. and S.R. Crouch. March 1977. Anal. Chem. 49:464-469. These reaction parameters increase to pH > 12.6 and 665 nm when salicylate is used as the color reagent—see, Krom, M.D. April 1980. The Analyst 105:305-316.

⁶¹ If atomic absorption or ICP instrumentation is not available, the aluminum colorimetric method detailed in the 19th Edition of *Standard Methods* may be used. This method has poorer precision and bias than the methods of choice.

⁶² Easy (1-Reagent) Nitrate Method, Revision November 12, 2011. Craig Chinchilla.

⁶³ Hach Method 10360, Luminescence Measurement of Dissolved Oxygen in Water and Wastewater and for Use in the Determination of BOD₅ and cBOD₅, Revision 1.2, October 2011. Hach Company. This method may be used to measure dissolved oxygen when performing the methods approved in Table IB for measurement of biochemical oxygen demand (BOD) and carbonaceous biochemical oxygen demand (CBOD).

⁶⁴ In-Situ Method 1002-8-2009, Dissolved Oxygen (DO) Measurement by Optical Probe. 2009. In-Situ Incorporated.

⁶⁵ Mitchell Method M5331, Determination of Turbidity by Nephelometry. Revision 1.0, July 31, 2008. Leck Mitchell.

⁶⁶ Mitchell Method M5271, Determination of Turbidity by Nephelometry. Revision 1.0, July 31, 2008. Leck Mitchell.

⁶⁷ Orion Method AQ4500, Determination of Turbidity by Nephelometry. Revision 5, March 12, 2009. Thermo Scientific.

⁶⁸ EPA Method 200.5, Determination of Trace Elements in Drinking Water by Axially Viewed Inductively Coupled Plasma-Atomic Emission Spectrometry, EPA/600/R-06/115. Revision 4.2, October 2003. US EPA.

⁶⁹ Method 1627, Kinetic Test Method for the Prediction of Mine Drainage Quality, EPA-821-R-09-002. December 2011. US EPA.

⁷⁰ Techniques and Methods Book 5-B1, Determination of Elements in Natural-Water, Biota, Sediment and Soil Samples Using Collision/Reaction Cell Inductively Coupled Plasma-Mass Spectrometry, Chapter 1, Section B, Methods of the National Water Quality Laboratory, Book 5, Laboratory Analysis, 2006. USGS.

⁷¹ Water-Resources Investigations Report 01-4132, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Organic Plus Inorganic Mercury in Filtered and Unfiltered Natural Water With Cold Vapor-Atomic Fluorescence Spectrometry, 2001. USGS.

TABLE IC—LIST OF APPROVED TEST PROCEDURES FOR NON-PESTICIDE ORGANIC COMPOUNDS

Parameter ¹	Method	EPA ^{2,7}	Standard methods	ASTM	Other
1. Acenaphthene	GC	610.			
	GC/MS	625, 1625B	6410 B-2000		See footnote ⁹ , p. 27.
2. Acenaphthylene	HPLC	610	6440 B-2000	D4657-92 (98)	
	GC	610.			
3. Acrolein	GC/MS	625, 1625B	6410 B-2000		See footnote ⁹ , p. 27.
	HPLC	610	6440 B-2000	D4657-92 (98).	
4. Acrylonitrile	GC	603.			
	GC/MS	624 ⁴ , 1624B.			
5. Anthracene	GC	603.			
	GC/MS	624 ⁴ , 1624B.			
6. Benzene	GC	610.			
	GC/MS	625, 1625B	6410 B-2000		See footnote ⁹ , p. 27.
7. Benzidine	HPLC	610	6440B-2000	D4657-92 (98).	
	GC	602	6200 C-1997.		
8. Benzo(a)anthracene	GC/MS	624, 1624B	6200 B-1997.		
	Spectro-photometric.				See footnote ³ , p.1.
9. Benzo(a)pyrene	GC/MS	625 ⁵ , 1625B	6410 B-2000.		
	HPLC	605.			
10. Benzo(b)fluoranthene	GC	610.			
	GC/MS	625, 1625B	6410 B-2000		See footnote ⁹ , p. 27.
11. Benzo(g,h,i)perylene	HPLC	610	6440 B-2000	D4657-92 (98).	
	GC	610.			

provided to assure such changes in sample preservation, containers or holding times do not adversely affect the integrity of the sample. The Regional ATP Coordinator or permitting authority will review the application and then notify the applicant and the appropriate

State agency of approval or rejection of the use of the alternate test procedure. A decision to approve or deny any request on deviations from the prescribed Table II requirements will be made within 90 days of receipt of the application by the Regional

Administrator. An analyst may not modify any sample preservation and/or holding time requirements of an approved method unless the requirements of this section are met.

TABLE II—REQUIRED CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES

Parameter number/name	Container ¹	Preservation ^{2,3}	Maximum holding time ⁴
Table IA—Bacterial Tests:			
1–5. Coliform, total, fecal, and <i>E. coli</i>	PA, G	Cool, <10 °C, 0.0008% Na ₂ S ₂ O ₃ ⁵ .	8 hours. ^{22,23}
6. Fecal streptococci	PA, G	Cool, <10 °C, 0.0008% Na ₂ S ₂ O ₃ ⁵ .	8 hours. ²²
7. Enterococci	PA, G	Cool, <10 °C, 0.0008% Na ₂ S ₂ O ₃ ⁵ .	8 hours. ²²
8. <i>Salmonella</i>	PA, G	Cool, <10 °C, 0.0008% Na ₂ S ₂ O ₃ ⁵ .	8 hours. ²²
Table IA—Aquatic Toxicity Tests:			
9–12. Toxicity, acute and chronic	P, FP, G	Cool, ≤6 °C ¹⁶	36 hours.
Table IB—Inorganic Tests:			
1. Acidity	P, FP, G	Cool, ≤6 °C ¹⁸	14 days.
2. Alkalinity	P, FP, G	Cool, ≤6 °C ¹⁸	14 days.
4. Ammonia	P, FP, G	Cool, ≤6 °C ¹⁸ , H ₂ SO ₄ to pH <2.	28 days.
9. Biochemical oxygen demand	P, FP, G	Cool, ≤6 °C ¹⁸	48 hours.
10. Boron	P, FP, or Quartz	HNO ₃ to pH <2	6 months.
11. Bromide	P, FP, G	None required	28 days.
14. Biochemical oxygen demand, carbonaceous	P, FP, G	Cool, ≤6 °C ¹⁸	48 hours.
15. Chemical oxygen demand	P, FP, G	Cool, ≤6 °C ¹⁸ , H ₂ SO ₄ to pH <2.	28 days.
16. Chloride	P, FP, G	None required	28 days.
17. Chlorine, total residual	P, G	None required	Analyze within 15 minutes.
21. Color	P, FP, G	Cool, ≤6 °C ¹⁸	48 hours.
23–24. Cyanide, total or available (or CATC) and free.	P, FP, G	Cool, ≤6 °C ¹⁸ , NaOH to pH >10 ^{5,6} , reducing agent if oxidizer present.	14 days.
25. Fluoride	P	None required	28 days.
27. Hardness	P, FP, G	HNO ₃ or H ₂ SO ₄ to pH <2 ..	6 months.
28. Hydrogen ion (pH)	P, FP, G	None required	Analyze within 15 minutes.
31, 43. Kjeldahl and organic N	P, FP, G	Cool, ≤6 °C ¹⁸ , H ₂ SO ₄ to pH <2.	28 days.
Table IB—Metals:⁷			
18. Chromium VI	P, FP, G	Cool, ≤6 °C ¹⁸ , pH = 9.3–9.7 ²⁰ .	28 days.
35. Mercury (CVAA)	P, FP, G	HNO ₃ to pH <2	28 days.
35. Mercury (CVAFS)	FP, G; and FP-lined cap ¹⁷	5 mL/L 12N HCl or 5 mL/L BrCl ¹⁷ .	90 days. ¹⁷
3, 5–8, 12, 13, 19, 20, 22, 26, 29, 30, 32–34, 36, 37, 45, 47, 51, 52, 58–60, 62, 63, 70–72, 74, 75. Metals, except boron, chromium VI, and mercury.	P, FP, G	HNO ₃ to pH <2, or at least 24 hours prior to analysis ¹⁹ .	6 months.
38. Nitrate	P, FP, G	Cool, ≤6 °C ¹⁸	48 hours.
39. Nitrate-nitrite	P, FP, G	Cool, ≤6 °C ¹⁸ , H ₂ SO ₄ to pH <2.	28 days.
40. Nitrite	P, FP, G	Cool, ≤6 °C ¹⁸	48 hours.
41. Oil and grease	G	Cool to ≤6 °C ¹⁸ , HCl or H ₂ SO ₄ to pH <2.	28 days.
42. Organic Carbon	P, FP, G	Cool to ≤6 °C ¹⁸ , HCl, H ₂ SO ₄ , or H ₃ PO ₄ to pH <2.	28 days.
44. Orthophosphate	P, FP, G	Cool, to ≤6 °C ^{18,24}	Filter within 15 minutes; Analyze within 48 hours.
46. Oxygen, Dissolved Probe	G, Bottle and top	None required	Analyze within 15 minutes.
47. Winkler	G, Bottle and top	Fix on site and store in dark.	8 hours.
48. Phenols	G	Cool, ≤6 °C ¹⁸ , H ₂ SO ₄ to pH <2.	28 days.
49. Phosphorous (elemental)	G	Cool, ≤6 °C ¹⁸	48 hours.
50. Phosphorous, total	P, FP, G	Cool, ≤6 °C ¹⁸ , H ₂ SO ₄ to pH <2.	28 days.
53. Residue, total	P, FP, G	Cool, ≤6 °C ¹⁸	7 days.
54. Residue, Filterable	P, FP, G	Cool, ≤6 °C ¹⁸	7 days.

TABLE II—REQUIRED CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES—Continued

Parameter number/name	Container ¹	Preservation ^{2,3}	Maximum holding time ⁴
55. Residue, Nonfilterable (TSS)	P, FP, G	Cool, ≤6 °C ¹⁸	7 days.
56. Residue, Settleable	P, FP, G	Cool, ≤6 °C ¹⁸	48 hours.
57. Residue, Volatile	P, FP, G	Cool, ≤6 °C ¹⁸	7 days.
61. Silica	P or Quartz	Cool, ≤6 °C ¹⁸	28 days.
64. Specific conductance	P, FP, G	Cool, ≤6 °C ¹⁸	28 days.
65. Sulfate	P, FP, G	Cool, ≤6 °C ¹⁸	28 days.
66. Sulfide	P, FP, G	Cool, ≤6 °C ¹⁸ , add zinc acetate plus sodium hydroxide to pH >9.	7 days.
67. Sulfite	P, FP, G	None required	Analyze within 15 minutes.
68. Surfactants	P, FP, G	Cool, ≤6 °C ¹⁸	48 hours.
69. Temperature	P, FP, G	None required	Analyze.
73. Turbidity	P, FP, G	Cool, ≤6 °C ¹⁸	48 hours.
Table IC—Organic Tests: ⁸	.	.	.
13, 18–20, 22, 24–28, 34–37, 39–43, 45–47, 56, 76, 104, 105, 108–111, 113. Purgeable Halocarbons.	G, FP-lined septum	Cool, ≤6 °C ¹⁸ , 0.008% Na ₂ S ₂ O ₃ ⁵ .	14 days.
6, 57, 106. Purgeable aromatic hydrocarbons	G, FP-lined septum	Cool, ≤6 °C ¹⁸ , 0.008% Na ₂ S ₂ O ₃ ⁵ , HCl to pH 2 ⁹ .	14 days. ⁹
3, 4. Acrolein and acrylonitrile	G, FP-lined septum	Cool, ≤6 °C ¹⁸ , 0.008% Na ₂ S ₂ O ₃ , pH to 4–5 ¹⁰ .	14 days. ¹⁰
23, 30, 44, 49, 53, 77, 80, 81, 98, 100, 112. Phenols ¹¹ .	G, FP-lined cap	Cool, ≤6 °C ¹⁸ , 0.008% Na ₂ S ₂ O ₃ .	7 days until extraction, 40 days after extraction.
7, 38. Benzidines ^{11,12}	G, FP-lined cap	Cool, ≤6 °C ¹⁸ , 0.008% Na ₂ S ₂ O ₃ ⁵ .	7 days until extraction. ¹³
14, 17, 48, 50–52. Phthalate esters ¹¹	G, FP-lined cap	Cool, ≤6 °C ¹⁸	7 days until extraction, 40 days after extraction.
82–84. Nitrosamines ^{11,14}	G, FP-lined cap	Cool, ≤6 °C ¹⁸ , store in dark, 0.008% Na ₂ S ₂ O ₃ ⁵ .	7 days until extraction, 40 days after extraction.
88–94. PCBs ¹¹	G, FP-lined cap	Cool, ≤6 °C ¹⁸	1 year until extraction, 1 year after extraction.
54, 55, 75, 79. Nitroaromatics and isophorone ¹¹	G, FP-lined cap	Cool, ≤6 °C ¹⁸ , store in dark, 0.008% Na ₂ S ₂ O ₃ ⁵ .	7 days until extraction, 40 days after extraction.
1, 2, 5, 8–12, 32, 33, 58, 59, 74, 78, 99, 101. Polynuclear aromatic hydrocarbons ¹¹ .	G, FP-lined cap	Cool, ≤6 °C ¹⁸ , store in dark, 0.008% Na ₂ S ₂ O ₃ ⁵ .	7 days until extraction, 40 days after extraction.
15, 16, 21, 31, 87. Haloethers ¹¹	G, FP-lined cap	Cool, ≤6 °C ¹⁸ , 0.008% Na ₂ S ₂ O ₃ ⁵ .	7 days until extraction, 40 days after extraction.
29, 35–37, 63–65, 107. Chlorinated hydrocarbons ¹¹ .	G, FP-lined cap	Cool, ≤6 °C ¹⁸	7 days until extraction, 40 days after extraction.
60–62, 66–72, 85, 86, 95–97, 102, 103. CDDs/CDFs ¹¹
Aqueous Samples: Field and Lab Preservation	G	Cool, ≤6 °C ¹⁸ , 0.008% Na ₂ S ₂ O ₃ ⁵ , pH <9.	1 year.
Solids and Mixed-Phase Samples: Field Preservation.	G	Cool, ≤6 °C ¹⁸	7 days.
Tissue Samples: Field Preservation	G	Cool, ≤6 °C ¹⁸	24 hours.
Solids, Mixed-Phase, and Tissue Samples: Lab Preservation.	G	Freeze, ≤ –10 °C	1 year.
114–118. Alkylated phenols	G	Cool, <6 °C, H ₂ SO ₄ to pH <2.	28 days until extraction, 40 days after extraction.
119. Adsorbable Organic Halides (AOX)	G	Cool, <6 °C, 0.008% Na ₂ S ₂ O ₃ HNO ₃ to pH <2.	Hold at least 3 days, but not more than 6 months.
120. Chlorinated Phenolics	Cool, <6 °C, 0.008% Na ₂ S ₂ O ₃ H ₂ SO ₄ to pH <2.	30 days until acetylation, 30 days after acetylation.
Table ID—Pesticides Tests:	.	.	.
1–70. Pesticides ¹¹	G, FP-lined cap	Cool, ≤6 °C ¹⁸ , pH 5–9– ¹⁵ ..	7 days until extraction, 40 days after extraction.
Table IE—Radiological Tests:	.	.	.
1–5. Alpha, beta, and radium	P, FP, G	HNO ₃ to pH <2	6 months.
Table IH—Bacterial Tests:	.	.	.
1. <i>E. coli</i>	PA, G	Cool, <10 °C, 0.0008% Na ₂ S ₂ O ₃ ⁵ .	8 hours. ²²
2. Enterococci	PA, G	Cool, <10 °C, 0.0008% Na ₂ S ₂ O ₃ ⁵ .	8 hours. ²²
Table IH—Protozoan Tests:	.	.	.
8. <i>Cryptosporidium</i>	LDPE; field filtration	1–10 °C	96 hours. ²¹
9. <i>Giardia</i>	LDPE; field filtration	1–10 °C	96 hours. ²¹

¹ “P” is for polyethylene; “FP” is fluoropolymer (polytetrafluoroethylene (PTFE); Teflon®), or other fluoropolymer, unless stated otherwise in this Table II; “G” is glass; “PA” is any plastic that is made of a sterilizable material (polypropylene or other autoclavable plastic); “LDPE” is low density polyethylene.

²Except where noted in this Table II and the method for the parameter, preserve each grab sample within 15 minutes of collection. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sample; see 40 CFR 122.21(g)(7)(i) or 40 CFR Part 403, Appendix E), refrigerate the sample at ≤ 6 °C during collection unless specified otherwise in this Table II or in the method(s). For a composite sample to be split into separate aliquots for preservation and/or analysis, maintain the sample at ≤ 6 °C, unless specified otherwise in this Table II or in the method(s), until collection, splitting, and preservation is completed. Add the preservative to the sample container prior to sample collection when the preservative will not compromise the integrity of a grab sample, a composite sample, or aliquot split from a composite sample within 15 minutes of collection. If a composite measurement is required but a composite sample would compromise sample integrity, individual grab samples must be collected at prescribed time intervals (e.g., 4 samples over the course of a day, at 6-hour intervals). Grab samples must be analyzed separately and the concentrations averaged. Alternatively, grab samples may be collected in the field and composited in the laboratory if the compositing procedure produces results equivalent to results produced by arithmetic averaging of results of analysis of individual grab samples. For examples of laboratory compositing procedures, see EPA Method 1664 Rev. A (oil and grease) and the procedures at 40 CFR 141.34(f)(14)(iv) and (v) (volatile organics).

³When any sample is to be shipped by common carrier or sent via the U.S. Postal Service, it must comply with the Department of Transportation Hazardous Materials Regulations (49 CFR part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirement of Table II, the Office of Hazardous Materials, Materials Transportation Bureau, Department of Transportation has determined that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater); Nitric acid (HNO₃) in water solutions at concentrations of 0.15% by weight or less (pH about 1.62 or greater); Sulfuric acid (H₂SO₄) in water solutions at concentrations of 0.35% by weight or less (pH about 1.15 or greater); and Sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

⁴Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before the start of analysis and still be considered valid. Samples may be held for longer periods only if the permittee or monitoring laboratory has data on file to show that, for the specific types of samples under study, the analytes are stable for the longer time, and has received a variance from the Regional Administrator under Sec. 136.3(e). For a grab sample, the holding time begins at the time of collection. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR part 403, Appendix E), the holding time begins at the time of the end of collection of the composite sample. For a set of grab samples composited in the field or laboratory, the holding time begins at the time of collection of the last grab sample in the set. Some samples may not be stable for the maximum time period given in the table. A permittee or monitoring laboratory is obligated to hold the sample for a shorter time if it knows that a shorter time is necessary to maintain sample stability. See 136.3(e) for details. The date and time of collection of an individual grab sample is the date and time at which the sample is collected. For a set of grab samples to be composited, and that are all collected on the same calendar date, the date of collection is the date on which the samples are collected. For a set of grab samples to be composited, and that are collected across two calendar dates, the date of collection is the dates of the two days; e.g., November 14–15. For a composite sample collected automatically on a given date, the date of collection is the date on which the sample is collected. For a composite sample collected automatically, and that is collected across two calendar dates, the date of collection is the dates of the two days; e.g., November 14–15. For static-renewal toxicity tests, each grab or composite sample may also be used to prepare test solutions for renewal at 24 h, 48 h, and/or 72 h after first use, if stored at 0–6 °C, with minimum head space.

⁵ASTM D7365–09a specifies treatment options for samples containing oxidants (e.g., chlorine). Also, Section 9060A of Standard Methods for the Examination of Water and Wastewater (20th and 21st editions) addresses dechlorination procedures.

⁶Sampling, preservation and mitigating interferences in water samples for analysis of cyanide are described in ASTM D7365–09a. There may be interferences that are not mitigated by the analytical test methods or D7365–09a. Any technique for removal or suppression of interference may be employed, provided the laboratory demonstrates that it more accurately measures cyanide through quality control measures described in the analytical test method. Any removal or suppression technique not described in D7365–09a or the analytical test method must be documented along with supporting data.

⁷For dissolved metals, filter grab samples within 15 minutes of collection and before adding preservatives. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR Part 403, Appendix E), filter the sample within 15 minutes after completion of collection and before adding preservatives. If it is known or suspected that dissolved sample integrity will be compromised during collection of a composite sample collected automatically over time (e.g., by interchange of a metal between dissolved and suspended forms), collect and filter grab samples to be composited (footnote 2) in place of a composite sample collected automatically.

⁸Guidance applies to samples to be analyzed by GC, LC, or GC/MS for specific compounds.

⁹If the sample is not adjusted to pH 2, then the sample must be analyzed within seven days of sampling.

¹⁰The pH adjustment is not required if acrolein will not be measured. Samples for acrolein receiving no pH adjustment must be analyzed within 3 days of sampling.

¹¹When the extractable analytes of concern fall within a single chemical category, the specified preservative and maximum holding times should be observed for optimum safeguard of sample integrity (i.e., use all necessary preservatives and hold for the shortest time listed). When the analytes of concern fall within two or more chemical categories, the sample may be preserved by cooling to ≤ 6 °C, reducing residual chlorine with 0.008% sodium thiosulfate, storing in the dark, and adjusting the pH to 6–9; samples preserved in this manner may be held for seven days before extraction and for forty days after extraction. Exceptions to this optional preservation and holding time procedure are noted in footnote 5 (regarding the requirement for thiosulfate reduction), and footnotes 12, 13 (regarding the analysis of benzidine).

¹²If 1,2-diphenylhydrazine is likely to be present, adjust the pH of the sample to 4.0 ± 0.2 to prevent rearrangement to benzidine.

¹³Extracts may be stored up to 30 days at < 0 °C.

¹⁴For the analysis of diphenylnitrosamine, add 0.008% Na₂S₂O₃ and adjust pH to 7–10 with NaOH within 24 hours of sampling.

¹⁵The pH adjustment may be performed upon receipt at the laboratory and may be omitted if the samples are extracted within 72 hours of collection. For the analysis of aldrin, add 0.008% Na₂S₂O₃.

¹⁶Place sufficient ice with the samples in the shipping container to ensure that ice is still present when the samples arrive at the laboratory. However, even if ice is present when the samples arrive, immediately measure the temperature of the samples and confirm that the preservation temperature maximum has not been exceeded. In the isolated cases where it can be documented that this holding temperature cannot be met, the permittee can be given the option of on-site testing or can request a variance. The request for a variance should include supportive data which show that the toxicity of the effluent samples is not reduced because of the increased holding temperature. Aqueous samples must not be frozen. Hand-delivered samples used on the day of collection do not need to be cooled to 0 to 6 °C prior to test initiation.

¹⁷Samples collected for the determination of trace level mercury (< 100 ng/L) using EPA Method 1631 must be collected in tightly-capped fluoropolymer or glass bottles and preserved with BrCl or HCl solution within 48 hours of sample collection. The time to preservation may be extended to 28 days if a sample is oxidized in the sample bottle. A sample collected for dissolved trace level mercury should be filtered in the laboratory within 24 hours of the time of collection. However, if circumstances preclude overnight shipment, the sample should be filtered in a designated clean area in the field in accordance with procedures given in Method 1669. If sample integrity will not be maintained by shipment to and filtration in the laboratory, the sample must be filtered in a designated clean area in the field within the time period necessary to maintain sample integrity. A sample that has been collected for determination of total or dissolved trace level mercury must be analyzed within 90 days of sample collection.

¹⁸Aqueous samples must be preserved at ≤ 6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. Also, for purposes of NPDES monitoring, the specification of " ≤ 6 °C" is used in place of the " 4 °C" and " < 4 °C" sample temperature requirements listed in some methods. It is not necessary to measure the sample temperature to three significant figures (1/100th of 1 degree); rather, three significant figures are specified so that rounding down to 6 °C may not be used to meet the ≤ 6 °C requirement. The preservation temperature does not apply to samples that are analyzed immediately (less than 15 minutes).

¹⁹An aqueous sample may be collected and shipped without acid preservation. However, acid must be added at least 24 hours before analysis to dissolve any metals that adsorb to the container walls. If the sample must be analyzed within 24 hours of collection, add the acid immediately (see footnote 2). Soil and sediment samples do not need to be preserved with acid. The allowances in this footnote supersede the preservation and holding time requirements in the approved metals methods.

²⁰To achieve the 28-day holding time, use the ammonium sulfate buffer solution specified in EPA Method 218.6. The allowance in this footnote supersedes preservation and holding time requirements in the approved hexavalent chromium methods, unless this supersession would compromise the measurement, in which case requirements in the method must be followed.

²¹Holding time is calculated from time of sample collection to elution for samples shipped to the laboratory in bulk and calculated from the time of sample filtration to elution for samples filtered in the field.

²²Sample analysis should begin as soon as possible after receipt; sample incubation must be started no later than 8 hours from time of collection.

²³For fecal coliform samples for sewage sludge (biosolids) only, the holding time is extended to 24 hours for the following sample types using either EPA Method 1680 (LTB-EC) or 1681 (A-1): Class A composted, Class B aerobically digested, and Class B anaerobically digested.

²⁴The immediate filtration requirement in orthophosphate measurement is to assess the dissolved or bio-available form of orthophosphorus (*i.e.*, that which passes through a 0.45-micron filter), hence the requirement to filter the sample immediately upon collection (*i.e.*, within 15 minutes of collection).

■ 4. Section 136.4 is revised to read as follows:

§ 136.4 Application for and approval of alternate test procedures for nationwide use.

(a) A written application for review of an alternate test procedure (alternate method) for nationwide use may be made by letter via email or by hard copy in triplicate to the National Alternate Test Procedure (ATP) Program Coordinator (National Coordinator), Office of Science and Technology (4303T), Office of Water, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave. NW., Washington, DC 20460. Any application for an alternate test procedure (ATP) under this paragraph (a) shall:

(1) Provide the name and address of the responsible person or firm making the application.

(2) Identify the pollutant(s) or parameter(s) for which nationwide approval of an alternate test procedure is being requested.

(3) Provide a detailed description of the proposed alternate test procedure, together with references to published or other studies confirming the general applicability of the alternate test procedure for the analysis of the pollutant(s) or parameter(s) in wastewater discharges from representative and specified industrial or other categories.

(4) Provide comparability data for the performance of the proposed alternative test procedure compared to the performance of the reference method.

(b) The National Coordinator may request additional information and analyses from the applicant in order to determine whether the alternate test procedure satisfies the applicable requirements of this Part.

(c) *Approval for nationwide use.* (1) After a review of the application and any additional analyses requested from the applicant, the National Coordinator will notify the applicant, in writing, of acceptance or rejection of the alternate test procedure for nationwide use in

CWA programs. If the application is not approved, the National Coordinator will specify what additional information might lead to a reconsideration of the application, and notify the Regional Alternate Test Procedure Coordinators of such rejection. Based on the National Coordinator's rejection of a proposed alternate test procedure and an assessment of any approvals for limited uses for the unapproved method, the Regional ATP Coordinator or permitting authority may decide to withdraw approval of the method for limited use in the Region.

(2) Where the National Coordinator approved an applicant's request for nationwide use of an alternate test procedure, the National Coordinator will notify the applicant that the National Coordinator will recommend rulemaking to approve the alternate test procedure. The National Coordinator will notify the Regional ATP Coordinator or permitting authorities that they may consider approval of this alternate test procedure for limited use in their Regions based on the information and data provided in the applicant's application. The Regional ATP Coordinator or permitting authority will grant approval on a case-by-case basis prior to use of the alternate test procedure for compliance analyses until the alternate test procedure is approved by publication in a final rule in the **Federal Register**.

(3) EPA will propose to amend 40 CFR Part 136 to include the alternate test procedure in § 136.3. EPA shall make available for review all the factual bases for its proposal, including any performance data submitted by the applicant and any available EPA analysis of those data.

(4) Following public comment, EPA shall publish in the **Federal Register** a final decision on whether to amend 40 CFR Part 136 to include the alternate test procedure as an approved analytical method.

(5) Whenever the National Coordinator has approved an applicant's request for nationwide use of an

alternate test procedure, any person may request an approval of the method for limited use under § 136.5 from the EPA Region.

■ 5. Section 136.5 is revised to read as follows:

§ 136.5 Approval of alternate test procedures for limited use.

(a) Any person may request the Regional Alternate Test Procedure (ATP) Coordinator or permitting authority to approve the use of an alternate test procedure in the Region.

(b) When the request for the use of an alternate test procedure concerns use in a State with an NPDES permit program approved pursuant to section 402 of the Act, the requestor shall first submit an application for limited use to the Director of the State agency having responsibility for issuance of NPDES permits within such State (*i.e.*, permitting authority). The Director will forward the application to the Regional ATP Coordinator or permitting authority with a recommendation for or against approval.

(c) Any application for approval of an alternate test procedure for limited use may be made by letter, email or by hard copy. The application shall include the following:

(1) Provide the name and address of the applicant and the applicable ID number of the existing or pending permit and issuing agency for which use of the alternate test procedure is requested, and the discharge serial number.

(2) Identify the pollutant or parameter for which approval of an alternate test procedure is being requested.

(3) Provide justification for using testing procedures other than those specified in Tables IA through IH of § 136.3, or in the NPDES permit.

(4) Provide a detailed description of the proposed alternate test procedure, together with references to published studies of the applicability of the alternate test procedure to the effluents in question.

phosphorus, as long as complete oxidation can be demonstrated.

(xxii) Use of an axially viewed torch with Method 200.7.

■ 7. Add new § 136.7 to read as follows:

§ 136.7 Quality assurance and quality control.

The permittee/laboratory shall use suitable QA/QC procedures when conducting compliance analyses with any Part 136 chemical method or an alternative method specified by the permitting authority. These QA/QC procedures are generally included in the analytical method or may be part of the methods compendium for approved Part 136 methods from a consensus organization. For example, Standard Methods contains QA/QC procedures in the Part 1000 section of the Standard Methods Compendium. The permittee/laboratory shall follow these QA/QC procedures, as described in the method or methods compendium. If the method lacks QA/QC procedures, the permittee/laboratory has the following options to comply with the QA/QC requirements:

(a) Refer to and follow the QA/QC published in the “equivalent” EPA method for that parameter that has such QA/QC procedures;

(b) Refer to the appropriate QA/QC section(s) of an approved Part 136 method from a consensus organization compendium;

(c)(1) Incorporate the following twelve quality control elements, where applicable, into the laboratory’s documented standard operating procedure (SOP) for performing compliance analyses when using an approved Part 136 method when the method lacks such QA/QC procedures. One or more of the twelve QC elements may not apply to a given method and may be omitted if a written rationale is provided indicating why the element(s) is/are inappropriate for a specific method.

(i) Demonstration of Capability (DOC);

(ii) Method Detection Limit (MDL);

(iii) Laboratory reagent blank (LRB), also referred to as method blank (MB);

(iv) Laboratory fortified blank (LFB), also referred to as a spiked blank, or laboratory control sample (LCS);

(v) Matrix spike (MS) and matrix spike duplicate (MSD), or laboratory fortified matrix (LFM) and LFM duplicate, may be used for suspected matrix interference problems to assess precision;

(vi) Internal standards (for GC/MS analyses), surrogate standards (for organic analysis) or tracers (for radiochemistry);

(vii) Calibration (initial and continuing), also referred to as initial

calibration verification (ICV) and continuing calibration verification (CCV);

(viii) Control charts (or other trend analyses of quality control results);

(ix) Corrective action (root cause analysis);

(x) QC acceptance criteria;

(xi) Definitions of preparation and analytical batches that may drive QC frequencies; and

(xii) Minimum frequency for conducting all QC elements.

(2) These twelve quality control elements must be clearly documented in the written standard operating procedure for each analytical method not containing QA/QC procedures, where applicable.

■ 8. Revise Appendix C to Part 136 to read as follows.

**APPENDIX C TO PART 136—
DETERMINATION OF METALS AND
TRACE ELEMENTS IN WATER AND
WASTES BY INDUCTIVELY COUPLED
PLASMA-ATOMIC EMISSION
SPECTROMETRY METHOD 200.7**

1.0 Scope and Application

1.1 Inductively coupled plasma-atomic emission spectrometry (ICP-AES) is used to determine metals and some nonmetals in solution. This method is a consolidation of existing methods for water, wastewater, and solid wastes.¹⁻⁴ (For analysis of petroleum products see References 5 and 6, Section 16.0). This method is applicable to the following analytes:

Analyte	Chemical abstract services registry number (CASRN)
Aluminum (Al)	7429-90-5
Antimony (Sb)	7440-36-0
Arsenic (As)	7440-38-2
Barium (Ba)	7440-39-3
Beryllium (Be)	7440-41-7
Boron (B)	7440-42-8
Cadmium (Cd)	7440-43-9
Calcium (Ca)	7440-70-2
Cerium ^a (Cr)	7440-45-1
Chromium (Cr)	7440-47-3
Cobalt (Co)	7440-48-4
Copper (Cu)	7440-50-8
Iron (Fe)	7439-89-6
Lead (Pb)	7439-92-1
Lithium (Li)	7439-93-2
Magnesium (Mg)	7439-95-4
Manganese (Mn)	7439-96-5
Mercury (Hg)	7439-97-6
Molybdenum (Mo)	7439-98-7
Nickel (Ni)	7440-02-0
Phosphorus (P)	7723-14-0
Potassium (K)	7440-09-7
Selenium (Se)	7782-49-2
Silica ^b (SiO ₂)	7631-86-9
Silver (Ag)	7440-22-4
Sodium (Na)	7440-23-5
Strontium (Sr)	7440-24-6
Thallium (Tl)	7440-28-0
Tin (Sn)	7440-31-5
Titanium (Ti)	7440-32-6

Analyte	Chemical abstract services registry number (CASRN)
Vanadium (V)	7440-62-2
Zinc (Zn)	7440-66-6

^aCerium has been included as method analyte for correction of potential interelement spectral interference.

^bThis method is *not* suitable for the determination of silica in solids.

1.2 For reference where this method is approved for use in compliance monitoring programs [e.g., Clean Water Act (NPDES) or Safe Drinking Water Act (SDWA)] consult both the appropriate sections of the Code of Federal Regulation (40 CFR Part 136 Table 1B for NPDES, and Part 141 § 141.23 for drinking water), and the latest **Federal Register** announcements.

1.3 ICP-AES can be used to determine dissolved analytes in aqueous samples after suitable filtration and acid preservation. To reduce potential interferences, dissolved solids should be <0.2% (w/v) (Section 4.2).

1.4 With the exception of silver, where this method is approved for the determination of certain metal and metalloids contaminants in drinking water, samples may be analyzed directly by pneumatic nebulization without acid digestion if the sample has been properly preserved with acid and has turbidity of <1 NTU at the time of analysis. This total recoverable determination procedure is referred to as “direct analysis”. However, in the determination of some primary drinking water metal contaminants, preconcentration of the sample may be required prior to analysis in order to meet drinking water acceptance performance criteria (Sections 11.2.2 through 11.2.7).

1.5 For the determination of total recoverable analytes in aqueous and solid samples a digestion/extraction is required prior to analysis when the elements are not in solution (e.g., soils, sludges, sediments and aqueous samples that may contain particulate and suspended solids). Aqueous samples containing suspended or particulate material 1% (w/v) should be extracted as a solid type sample.

1.6 When determining boron and silica in aqueous samples, only plastic, PTFE or quartz labware should be used from time of sample collection to completion of analysis. For accurate determination of boron in solid samples only quartz or PTFE beakers should be used during acid extraction with immediate transfer of an extract aliquot to a plastic centrifuge tube following dilution of the extract to volume. When possible, borosilicate glass should be avoided to prevent contamination of these analytes.

1.7 Silver is only slightly soluble in the presence of chloride unless there is a sufficient chloride concentration to form the soluble chloride complex. Therefore, low recoveries of silver may occur in samples, fortified sample matrices and even fortified blanks if determined as a dissolved analyte or by “direct analysis” where the sample has not been processed using the total recoverable mixed acid digestion. For this reason it is recommended that samples be digested prior to the determination of silver.

STATE OF TENNESSEE



NPDES PERMIT

No. TN00.....

Authorization to discharge under the
National Pollutant Discharge Elimination System (NPDES)

Issued By

**STATE OF TENNESSEE
DEPARTMENT OF ENVIRONMENT AND CONSERVATION
DIVISION OF WATER RESOURCES
William R. Snodgrass - Tennessee Tower
312 Rosa L. Parks Avenue, 11th Floor
Nashville, Tennessee 37243-1102**

Under authority of the Tennessee Water Quality Control Act of 1977 (T.C.A. 69-3-101 et seq.) and the delegation of authority from the United States Environmental Protection Agency under the Federal Water Pollution Control Act, as amended by the Clean Water Act of 1977 (33 U.S.C. 1251, et seq.)

Discharger:

is authorized to discharge:

from a facility located:

to receiving waters named:

in accordance with effluent limitations, monitoring requirements and other conditions set forth herein.

This permit shall become effective on:

This permit shall expire on:

Issuance date:

for Tisha Calabrese Benton
Director

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TN0056332PMT.DOC

Parameter	Qualifier Value	Unit	Sample Type	Frequency	Statistical Base
Nitrogen, Ammonia total (as N)	<=	mg/L	Composite	Three Per Week	Weekly Average
Description : External Outfall, Number : 001, Monitoring : Percent Removal, Season : All Year					
CBOD, 5-day, 20 C, % removal	>=	%	Calculated	Three Per Week	Daily Minimum
CBOD, 5-day, 20 C, % removal	>=	%	Calculated	Three Per Week	Monthly Average Minimum
TSS, % removal	>=	%	Calculated	Three Per Week	Monthly Average Minimum
TSS, % removal	>=	%	Calculated	Three Per Week	Daily Minimum

Parameter	Qualifier Value	Unit	Sample Type	Frequency	Statistical Base
Description : External Outfall, Number : 001, Monitoring : Raw Sewage Influent, Season : All Year					
CBOD, 5-day, 20 C	Report	mg/L	Composite	Three Per Week	Monthly Average
CBOD, 5-day, 20 C	Report	mg/L	Composite	Three Per Week	Daily Maximum
Flow	Report	Mgal/d	Continuous	Daily	Daily Maximum
Flow	Report	Mgal/d	Continuous	Daily	Monthly Average
Total Suspended Solids (TSS)	Report	mg/L	Composite	Three Per Week	Monthly Average
Total Suspended Solids (TSS)	Report	mg/L	Composite	Three Per Week	Daily Maximum

Parameter	Qualifier Value	Unit	Sample Type	Frequency	Statistical Base
Description : External Outfall, Number : 001, Monitoring : Wet Weather, Season : All Year					
Bypass of Treatment	Report	occur/mo	Occurrences	Continuous	Monthly Total
Overflow use, occurrences	Report	occur/mo	Occurrences	Continuous	Monthly Total

Note: The permittee shall achieve 85% removal of CBOD₅ and TSS on a monthly average basis. The permittee shall report all instances of overflow and/or bypasses. See Part 2.3.3.a for the definition of overflow and Part 1.3.5.1 for reporting requirements.

Note: Unless elsewhere specified, summer months are May through October; winter months are November through April.

Note: See Part 1.2.3 for test procedures.

Note: See Part 3.4 for biomonitoring test and reporting requirements. See next page for percent removal calculations.

Total Suspended Solids (TSS)	<=	34	mg/L	Composite	Three Per Week	Daily Maximum
pH	>=	6	SU	Grab	Five Per Week	Minimum
pH	<=	9	SU	Grab	Five Per Week	Maximum

Description : External Outfall, Number : 001, Monitoring : Effluent Gross, Season : Summer

<u>Parameter</u>	<u>Qualifier Value</u>	<u>Unit</u>	<u>Sample Type</u>	<u>Frequency</u>	<u>Statistical Base</u>
CBOD, 5-day, 20 C	<=	11.2	Composite	Three Per Week	Monthly Average
CBOD, 5-day, 20 C	<=	20	Composite	Three Per Week	Daily Maximum
CBOD, 5-day, 20 C	<=	15	Composite	Three Per Week	Weekly Average
CBOD, 5-day, 20 C	<=	375	Composite	Three Per Week	Monthly Average
CBOD, 5-day, 20 C	<=	500	Composite	Three Per Week	Weekly Average
Nitrogen, Ammonia total (as N)	<=	52.5	Composite	Three Per Week	Weekly Average
Nitrogen, Ammonia total (as N)	<=	2	Composite	Three Per Week	Daily Maximum
Nitrogen, Ammonia total (as N)	<=	1	Composite	Three Per Week	Monthly Average
Nitrogen, Ammonia total (as N)	<=	1.6	Composite	Three Per Week	Weekly Average
Nitrogen, Ammonia total (as N)	<=	35	Composite	Three Per Week	Monthly Average
Nitrogen, total (as N)	Report	-	Composite	Twice Per Month	Daily Maximum
Nitrogen, total (as N)	<=	4	Composite	Twice Per Month	Monthly Average
Phosphorus, total (as P)	Report	-	Composite	Twice Per Month	Daily Maximum
Phosphorus, total (as P)	<=	3	Composite	Twice Per Month	Monthly Average

Description : External Outfall, Number : 001, Monitoring : Effluent Gross, Season : Winter

<u>Parameter</u>	<u>Qualifier Value</u>	<u>Unit</u>	<u>Sample Type</u>	<u>Frequency</u>	<u>Statistical Base</u>
CBOD, 5-day, 20 C	<=	18.7	Composite	Three Per Week	Monthly Average
CBOD, 5-day, 20 C	<=	750	Composite	Three Per Week	Weekly Average
CBOD, 5-day, 20 C	<=	625	Composite	Three Per Week	Monthly Average
CBOD, 5-day, 20 C	<=	22.5	Composite	Three Per Week	Weekly Average
CBOD, 5-day, 20 C	<=	30	Composite	Three Per Week	Daily Maximum
Nitrogen, Ammonia total (as N)	<=	1.9	Composite	Three Per Week	Monthly Average
Nitrogen, Ammonia total (as N)	<=	65	Composite	Three Per Week	Monthly Average
Nitrogen, Ammonia total (as N)	<=	4	Composite	Three Per Week	Daily Maximum
Nitrogen, Ammonia total (as N)	<=	95	Composite	Three Per Week	Weekly Average

1.0. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

1.1. NUMERIC AND NARRATIVE EFFLUENT LIMITATIONS

The City of Limestone is authorized to discharge treated domestic wastewater from Outfall 001 to the Big Limestone Creek at mile 3.8. Discharge 001 consists of municipal wastewater from a treatment facility with a design capacity of .005 MGD. Discharge 001 shall be limited and monitored by the permittee as specified below:

Notes: The permittee shall achieve % removal of CBOD₅ and TSS on a monthly average basis. The permittee shall report all instances of overflow and/or bypasses. See Part 2.3.3.a for the definition of overflow and Part 1.3.5.1 for reporting requirements.

Unless elsewhere specified, summer months are May through October; winter months are November through April.

See Part 1.2.3 for test procedures.

See Part 3.4 for biomonitoring test and reporting requirements. See next page for percent removal calculations.

See Part 1.2.3 for test procedures.

Total residual chlorine (TRC) monitoring shall be applicable when chlorine, bromine, or any other oxidants are added. The acceptable methods for analysis of TRC are any methods specified in Title 40 CFR, Part 136 as amended. The method detection level (MDL) for TRC shall not exceed 0.05 mg/l unless the permittee demonstrates that its MDL is higher. The permittee shall retain the documentation that justifies the higher MDL and have it available for review upon request. In cases where the permit limit is less than the MDL, the reporting of TRC at less than the MDL shall be interpreted to constitute compliance with the permit.

The wastewater discharge must be disinfected to the extent that viable coliform organisms are effectively eliminated. The concentration of the *E. coli* group after disinfection shall not exceed 126 cfu per 100 ml as the geometric mean calculated on the actual number of samples collected and tested for *E. coli* within the required reporting period. The permittee may collect more samples than specified as the monitoring frequency. Samples may not be collected at intervals of less than 12 hours. For the purpose of determining the geometric mean, individual samples having an *E. coli* group concentration of less than one (1) per 100 ml shall be considered as having a concentration of one (1) per 100 ml. In addition, the concentration of the *E. coli* group in any individual sample shall not exceed a specified maximum amount. A maximum daily limit of 487 colonies per 100 ml applies to lakes and exceptional Tennessee waters. A maximum daily limit of 941 colonies per 100 ml applies to all other recreational waters.

There shall be no distinctly visible floating scum, oil or other matter contained in the wastewater discharge. The wastewater discharge must not cause an objectionable color contrast in the receiving stream.

The wastewater discharge shall not contain pollutants in quantities that will be hazardous or otherwise detrimental to humans, livestock, wildlife, plant life, or fish and aquatic life in the receiving stream.

Sludge or any other material removed by any treatment works must be disposed of in a manner that prevents its entrance into or pollution of any surface or subsurface waters. Additionally, the disposal of such sludge or other material must be in compliance with the Tennessee Solid Waste Disposal Act, TCA 68-31-101 et seq. and the Tennessee Hazardous Waste Management Act, TCA 68-46-101 et seq.

Nothing in this permit authorizes take for the purposes of a facility's compliance with the Endangered Species Act. (40 C.F.R. 125.98(b)(1))

For the purpose of evaluating compliance with the permit limits established herein, where certain limits are below the State of Tennessee published required detection levels (RDLs) for any given effluent characteristics, the results of analyses below the RDL shall be reported as Below Detection Level (BDL), unless in specific cases other detection limits are demonstrated to be the best achievable because of the particular nature of the wastewater being analyzed.

For CBOD₅ and TSS, the treatment facility shall demonstrate a minimum of % removal efficiency on a monthly average basis. This is calculated by determining an average of all daily influent concentrations and comparing this to an average of all daily effluent concentrations. The formula for this calculation is as follows:

$$\left[1 - \frac{\text{average of daily effluent concentration}}{\text{average of daily influent concentration}} \right] \times 100\% = \% \text{ removal}$$

The treatment facility will also demonstrate % minimum removal of the CBOD₅ and TSS based upon each daily composite sample. The formula for this calculation is as follows:

$$\left[1 - \frac{\text{daily effluent concentration}}{\text{daily influent concentration}} \right] \times 100\% = \% \text{ removal}$$

1.2. MONITORING PROCEDURES

1.2.1. Representative Sampling

Appropriate flow measurement devices and methods consistent with accepted scientific practices shall be selected and used to insure the accuracy and reliability of measurements of the volume of monitored discharges. The devices shall be installed, calibrated and maintained to insure that the accuracy of the measurements is consistent with accepted capability of that type of device. Devices selected shall be capable of measuring flows with a maximum deviation of less than plus or minus

10% from the true discharge rates throughout the range of expected discharge volumes.

Samples and measurements taken in compliance with the monitoring requirements specified above shall be representative of the volume and nature of the monitored discharge, and shall be taken at the following location(s):

Influent samples must be collected prior to mixing with any other wastewater being returned to the head of the plant, such as sludge return. Those systems with more than one influent line must collect samples from each and proportion the results by the flow from each line.

Effluent samples must be representative of the wastewater being discharged and collected prior to mixing with any other discharge or the receiving stream. This can be a different point for different parameters, but must be after all treatment for that parameter or all expected change:

- a. The chlorine residual must be measured after the chlorine contact chamber and any dechlorination. It may be to the advantage of the permittee to measure at the end of any long outfall lines.
- b. Samples for *E. coli* can be collected at any point between disinfection and the actual discharge.
- c. The dissolved oxygen can drop in the outfall line; therefore, D.O. measurements are required at the discharge end of outfall lines greater than one mile long. Systems with outfall lines less than one mile may measure dissolved oxygen as the wastewater leaves the treatment facility. For systems with dechlorination, dissolved oxygen must be measured after this step and as close to the end of the outfall line as possible.
- d. Total suspended solids and settleable solids can be collected at any point after the final clarifier.
- e. Biomonitoring tests (if required) shall be conducted on final effluent.

1.2.2. Sampling Frequency

Where the permit requires sampling and monitoring of a particular effluent characteristic(s) at a frequency of less than once per day or daily, the permittee is precluded from marking the "No Discharge" block on the Discharge Monitoring Report if there has been any discharge from that particular outfall during the period which coincides with the required monitoring frequency; i.e. if the required monitoring frequency is once per month or 1/month, the monitoring period is one month, and if the discharge occurs during only one day in that period then the permittee must sample on that day and report the results of analyses accordingly.

1.2.3. Test Procedures

- a. Test procedures for the analysis of pollutants shall conform to regulations published pursuant to Section 304 (h) of the Clean Water Act (the "Act"), as amended, under which such procedures may be required.
- b. Unless otherwise noted in the permit, all pollutant parameters shall be determined according to methods prescribed in Title 40, CFR, Part 136, as amended, promulgated pursuant to Section 304 (h) of the Act.
- c. Composite samples must be proportioned by flow at time of sampling. Aliquots may be collected manually or automatically. The sample aliquots must be maintained at ≤ 6 degrees Celsius during the compositing period.
- d. In instances where permit limits established through implementation of applicable water criteria are below analytical capabilities, compliance with those limits will be determined using the detection limits described in the TN Rules, Chapter 0400-40-03-.05(8).
- e. All sampling for total mercury at the municipal wastewater plant (application, pretreatment, etc.) shall use Methods 1631, 245.7 or any additional method in 40 CFR 136 with a maximum detection limit of 5 ng/L.

1.2.4. Recording of Results

For each measurement or sample taken pursuant to the requirements of this permit, the permittee shall record the following information:

- a. The exact place, date and time of sampling;
- b. The exact person(s) collecting samples;
- c. The dates and times the analyses were performed;
- d. The person(s) or laboratory who performed the analyses;
- e. The analytical techniques or methods used, and;
- f. The results of all required analyses.

1.2.5. Records Retention

All records and information resulting from the monitoring activities required by this permit including all records of analyses performed and calibration and maintenance of instrumentation shall be retained for a minimum of three (3) years, or longer, if requested by the Division of Water Resources.

1.3. REPORTING

1.3.1. Monitoring Results

Monitoring results shall be recorded monthly and submitted monthly using Discharge Monitoring Report (DMR) forms supplied by the Division of Water Resources. Submittals shall be postmarked no later than 15 days after the completion of the reporting period. A completed DMR with an original signature shall be submitted to the following address:

**STATE OF TENNESSEE
DEPARTMENT OF ENVIRONMENT AND CONSERVATION
DIVISION OF WATER RESOURCES
COMPLIANCE & ENFORCEMENT SECTION
William R. Snodgrass - Tennessee Tower
312 Rosa L. Parks Avenue, 11th Floor
Nashville, Tennessee 37243-1102**

A copy of the completed and signed DMR shall be mailed to the Johnson City Environmental Field Office (EFO) at the following address:

**STATE OF TENNESSEE
DEPARTMENT OF ENVIRONMENT AND CONSERVATION
DIVISION OF WATER RESOURCES
Johnson City Environmental Field Office
2305 Silverdale Road
Johnson City, Tennessee 37601**

A copy should be retained for the permittee's files. In addition, any communication regarding compliance with the conditions of this permit must be sent to the two offices listed above.

The first DMR is due on the 15th of the month following permit effectiveness.

DMRs and any other information or report must be signed and certified by a responsible corporate officer as defined in 40 CFR 122.22, a general partner or proprietor, or a principal municipal executive officer or ranking elected official, or his duly authorized representative. Such authorization must be submitted in writing and must explain the duties and responsibilities of the authorized representative.

The electronic submission of DMR data will be accepted only if formally approved beforehand by the division. For purposes of determining compliance with this permit, data approved by the division to be submitted electronically is legally equivalent to data submitted on signed and certified DMR forms.

1.3.2. Additional Monitoring by Permittee

If the permittee monitors any pollutant specifically limited by this permit more frequently than required at the location(s) designated, using approved analytical methods as specified herein, the results of such monitoring shall be included in the

calculation and reporting of the values required in the DMR form. Such increased frequency shall also be indicated on the form.

1.3.3. Falsifying Results and/or Reports

Knowingly making any false statement on any report required by this permit or falsifying any result may result in the imposition of criminal penalties as provided for in Section 309 of the Federal Water Pollution Control Act, as amended, and in Section 69-3-115 of the Tennessee Water Quality Control Act.

1.3.4. Monthly Report of Operation

Monthly operational reports shall be submitted on standard forms to the appropriate Division of Water Resources Environmental Field Office in Jackson, Nashville, Chattanooga, Columbia, Cookeville, Memphis, Johnson City, or Knoxville. Reports shall be submitted by the 15th day of the month following data collection.

1.3.5. Bypass and Overflow Reporting

1.3.5.1. Report Requirements

A summary report of known or suspected instances of overflows in the collection system or bypass of wastewater treatment facilities shall accompany the Discharge Monitoring Report. The report must contain the date and duration of the instances of overflow and/or bypassing and the estimated quantity of wastewater released and/or bypassed.

The report must also detail activities undertaken during the reporting period to (1) determine if overflow is occurring in the collection system, (2) correct those known or suspected overflow points and (3) prevent future or possible overflows and any resulting bypassing at the treatment facility.

On the DMR, the permittee must report the number of sanitary sewer overflows, dry-weather overflows and in-plant bypasses separately. Three lines must be used on the DMR form, one for sanitary sewer overflows, one for dry-weather overflows and one for in-plant bypasses.

1.3.5.2. Anticipated Bypass Notification

If, because of unavoidable maintenance or construction, the permittee has need to create an in-plant bypass which would cause an effluent violation, the permittee must notify the division as soon as possible, but in any case, no later than 10 days prior to the date of the bypass.

1.3.6. Reporting Less Than Detection

A permit limit may be less than the accepted detection level. If the samples are below the detection level, then report "BDL" or "NODI =B" on the DMRs. The permittee must use the correct detection levels in all analytical testing required in the permit. The required detection levels are listed in the Rules of the Department of

Environment and Conservation, Division of Water Resources, Chapter 0400-40-03-.05(8).

For example, if the limit is 0.02 mg/l with a detection level of 0.05 mg/l and detection is shown; 0.05 mg/l must be reported. In contrast, if nothing is detected reporting "BDL" or "NODI =B" is acceptable.

1.4. COMPLIANCE WITH SECTION 208

The limits and conditions in this permit shall require compliance with an area-wide waste treatment plan (208 Water Quality Management Plan) where such approved plan is applicable.

1.5. REOPENER CLAUSE

This permit shall be modified, or alternatively revoked and reissued, to comply with any applicable effluent standard or limitation issued or approved under Sections 301(b)(2)(C) and (D), 307(a)(2) and 405(d)(2)(D) of the Clean Water Act, as amended, if the effluent standard, limitation or sludge disposal requirement so issued or approved:

- a. Contains different conditions or is otherwise more stringent than any condition in the permit; or
- b. Controls any pollutant or disposal method not addressed in the permit.

The permit as modified or reissued under this paragraph shall also contain any other requirements of the Act then applicable.

1.6. SCHEDULE OF COMPLIANCE

Full compliance and operational levels shall be attained from the effective date of this permit.

2.0. GENERAL PERMIT REQUIREMENTS

2.1. GENERAL PROVISIONS

2.1.1. Duty to Reapply

Permittee is not authorized to discharge after the expiration date of this permit. In order to receive authorization to discharge beyond the expiration date, the permittee shall submit such information and forms as are required to the Director of the Division of Water Resources (the "director") no later than 180 days prior to the expiration date. Such forms shall be properly signed and certified.

2.1.2. Right of Entry

The permittee shall allow the director, the Regional Administrator of the U.S. Environmental Protection Agency, or their authorized representatives, upon the presentation of credentials:

- a. To enter upon the permittee's premises where an effluent source is located or where records are required to be kept under the terms and conditions of this permit, and at reasonable times to copy these records;
- b. To inspect at reasonable times any monitoring equipment or method or any collection, treatment, pollution management, or discharge facilities required under this permit; and
- c. To sample at reasonable times any discharge of pollutants.

2.1.3. Availability of Reports

Except for data determined to be confidential under Section 308 of the Federal Water Pollution Control Act, as amended, all reports prepared in accordance with the terms of this permit shall be available for public inspection at the offices of the Division of Water Resources. As required by the Federal Act, effluent data shall not be considered confidential.

2.1.4. Proper Operation and Maintenance

- a. The permittee shall at all times properly operate and maintain all facilities and systems (and related appurtenances) for collection and treatment which are installed or used by the permittee to achieve compliance with the terms and conditions of this permit. Proper operation and maintenance also includes adequate laboratory and process controls and appropriate quality assurance procedures. This provision requires the operation of backup or auxiliary facilities or similar systems, which are installed by a permittee only when the operation is necessary to achieve compliance with the conditions of the permit. Backup continuous pH and flow monitoring equipment are not required.
- b. Dilution water shall not be added to comply with effluent requirements to achieve BCT, BPT, BAT and or other technology based effluent limitations such as those in State of Tennessee Rule 0400-40-05-.09.

2.1.5. Treatment Facility Failure (Industrial Sources)

The permittee, in order to maintain compliance with this permit, shall control production, all discharges, or both, upon reduction, loss, or failure of the treatment facility, until the facility is restored or an alternative method of treatment is provided. This requirement applies in such situations as the reduction, loss, or failure of the primary source of power.

2.1.6. Property Rights

The issuance of this permit does not convey any property rights in either real or personal property, or any exclusive privileges, nor does it authorize any injury to private property or any invasion of personal rights, nor any infringement of federal, state, or local laws or regulations.

2.1.7. Severability

The provisions of this permit are severable. If any provision of this permit due to any circumstance, is held invalid, then the application of such provision to other circumstances and to the remainder of this permit shall not be affected thereby.

2.1.8. Other Information

If the permittee becomes aware of failure to submit any relevant facts in a permit application, or of submission of incorrect information in a permit application or in any report to the director, then the permittee shall promptly submit such facts or information.

2.2. CHANGES AFFECTING THE PERMIT

2.2.1. Planned Changes

The permittee shall give notice to the director as soon as possible of any planned physical alterations or additions to the permitted facility. Notice is required only when:

- a. The alteration or addition to a permitted facility may meet one of the criteria for determining whether a facility is a new source in 40 CFR 122.29(b); or
- b. The alteration or addition could significantly change the nature or increase the quantity of pollutants discharged. This notification applies to pollutants, which are subject neither to effluent limitations in the permit, nor to notification requirements under 40 CFR 122.42(a)(1).

2.2.2. Permit Modification, Revocation, or Termination

- a. This permit may be modified, revoked and reissued, or terminated for cause as described in 40 CFR 122.62 and 122.64, Federal Register, Volume 49, No. 188 (Wednesday, September 26, 1984), as amended.
- b. The permittee shall furnish to the director, within a reasonable time, any information which the director may request to determine whether cause exists for modifying, revoking and reissuing, or terminating this permit, or to determine compliance with this permit. The permittee shall also furnish to the director, upon request, copies of records required to be kept by this permit.
- c. If any applicable effluent standard or prohibition (including any schedule of compliance specified in such effluent standard or prohibition) is established for any toxic pollutant under Section 307(a) of the Federal Water Pollution Control Act, as amended, the director shall modify or revoke and reissue the permit to conform to the prohibition or to the effluent standard, providing that the effluent standard is more stringent than the limitation in the permit on the toxic pollutant. The permittee shall comply with these effluent standards or prohibitions within the time provided in the regulations that establish these standards or prohibitions, even if the permit has not yet been modified or revoked and reissued to incorporate the requirement.
- d. The filing of a request by the permittee for a modification, revocation, reissuance, termination, or notification of planned changes or anticipated noncompliance does not halt any permit condition.

2.2.3. Change of Ownership

This permit may be transferred to another party (provided there are neither modifications to the facility or its operations, nor any other changes which might affect the permit limits and conditions contained in the permit) by the permittee if:

- a. The permittee notifies the director of the proposed transfer at least 30 days in advance of the proposed transfer date;
- b. The notice includes a written agreement between the existing and new permittees containing a specified date for transfer of permit responsibility, coverage, and liability between them; and
- c. The director, within 30 days, does not notify the current permittee and the new permittee of his intent to modify, revoke or reissue, or terminate the permit and to require that a new application be filed rather than agreeing to the transfer of the permit.

Pursuant to the requirements of 40 CFR 122.61, concerning transfer of ownership, the permittee must provide the following information to the division in their formal notice of intent to transfer ownership: 1) the NPDES permit number of the subject permit; 2) the effective date of the proposed transfer; 3) the name and address of the transferor; 4) the name and address of the transferee; 5) the names of the responsible parties for both the transferor and transferee; 6) a statement that the transferee assumes responsibility for the subject NPDES permit; 7) a statement that the transferor relinquishes responsibility for the subject NPDES permit; 8) the signatures of the responsible parties for both the transferor and transferee pursuant to the requirements of 40 CFR 122.22(a), "Signatories to permit applications"; and, 9) a statement regarding any proposed modifications to the facility, its operations, or any other changes which might affect the permit limits and conditions contained in the permit.

2.2.4. Change of Mailing Address

The permittee shall promptly provide to the director written notice of any change of mailing address. In the absence of such notice the original address of the permittee will be assumed to be correct.

2.3. NONCOMPLIANCE

2.3.1. Effect of Noncompliance

All discharges shall be consistent with the terms and conditions of this permit. Any permit noncompliance constitutes a violation of applicable state and federal laws and is grounds for enforcement action, permit termination, permit modification, or denial of permit reissuance.

2.3.2. Reporting of Noncompliance

a. 24-Hour Reporting

In the case of any noncompliance which could cause a threat to public drinking supplies, or any other discharge which could constitute a threat to human health or the environment, the required notice of non-compliance shall be provided to

the Division of Water Resources in the appropriate Environmental Field Office within 24-hours from the time the permittee becomes aware of the circumstances. (The Environmental Field Office should be contacted for names and phone numbers of environmental response team).

A written submission must be provided within five days of the time the permittee becomes aware of the circumstances unless the director on a case-by-case basis waives this requirement. The permittee shall provide the director with the following information:

- i. A description of the discharge and cause of noncompliance;
 - ii. The period of noncompliance, including exact dates and times or, if not corrected, the anticipated time the noncompliance is expected to continue; and
 - iii. The steps being taken to reduce, eliminate, and prevent recurrence of the noncomplying discharge.
- b. Scheduled Reporting

For instances of noncompliance which are not reported under subparagraph 2.3.2.a above, the permittee shall report the noncompliance on the Discharge Monitoring Report. The report shall contain all information concerning the steps taken, or planned, to reduce, eliminate, and prevent recurrence of the violation and the anticipated time the violation is expected to continue.

2.3.3. Overflow

- a. "**Overflow**" means any release of sewage from any portion of the collection, transmission, or treatment system other than through permitted outfalls.
- b. Overflows are prohibited.
- c. The permittee shall operate the collection system so as to avoid overflows.
- d. No new or additional flows shall be added upstream of any point in the collection system, which experiences chronic overflows (greater than 5 events per year) or would otherwise overload any portion of the system. Unless there is specific enforcement action to the contrary, the permittee is relieved of this requirement after: 1) an authorized representative of the Commissioner of the Department of Environment and Conservation has approved an engineering report and construction plans and specifications prepared in accordance with accepted engineering practices for correction of the problem; 2) the correction work is underway; and 3) the cumulative, peak-design, flows potentially added from new connections and line extensions upstream of any chronic overflow point are less than or proportional to the amount of inflow and infiltration removal documented upstream of that point. The inflow and infiltration reduction must be measured by the permittee using practices that are customary in the environmental engineering field and reported in an attachment to a Monthly Operating Report

submitted to the local TDEC Environmental Field Office. The data measurement period shall be sufficient to account for seasonal rainfall patterns and seasonal groundwater table elevations.

- e. In the event that more than 5 overflows have occurred from a single point in the collection system for reasons that may not warrant the self-imposed moratorium or completion of the actions identified in this paragraph, the permittee may request a meeting with the Division of Water Resources EFO staff to petition for a waiver based on mitigating evidence.

2.3.4. Upset

- a. "**Upset**" means an exceptional incident in which there is unintentional and temporary noncompliance with technology-based effluent limitations because of factors beyond the reasonable control of the permittee. An upset does not include noncompliance to the extent caused by operational error, improperly designed treatment facilities, inadequate treatment facilities, lack of preventive maintenance, or careless or improper operation.
- b. An upset shall constitute an affirmative defense to an action brought for noncompliance with such technology-based permit effluent limitations if the permittee demonstrates, through properly signed, contemporaneous operating logs, or other relevant evidence that:
 - i. An upset occurred and that the permittee can identify the cause(s) of the upset;
 - ii. The permitted facility was at the time being operated in a prudent and workman-like manner and in compliance with proper operation and maintenance procedures;
 - iii. The permittee submitted information required under "Reporting of Noncompliance" within 24-hours of becoming aware of the upset (if this information is provided orally, a written submission must be provided within five days); and
 - iv. The permittee complied with any remedial measures required under "Adverse Impact."

2.3.5. Adverse Impact

The permittee shall take all reasonable steps to minimize any adverse impact to the waters of Tennessee resulting from noncompliance with this permit, including such accelerated or additional monitoring as necessary to determine the nature and impact of the noncomplying discharge. It shall not be a defense for the permittee in an enforcement action that it would have been necessary to halt or reduce the permitted activity in order to maintain compliance with the conditions of this permit.



Common Deficiencies Found in the Wastewater Treatment Plant Laboratory

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This handout has been prepared based on deficiencies found while performing Performance Audit Inspections (PAI) at wastewater treatment plants for the Tennessee Department of Environment and Conservation in the Division of Water Resources. Suggestions have been made in an effort to assist the operator of a wastewater treatment plant in performing his laboratory duties thus improving the self-monitoring program of the facility.

Every wastewater treatment plant has its own distinctive characteristics and needs. Many of the observations and suggestions provided may not apply to every facility.

Quality assurance and quality control requirements can be found in the 40CFR part 136.7 (promulgated May, 2012), method update rule (MUR). Guidance documents may be found on the Fleming Training Center web page. <http://www.tn.gov/environment/article/wr-ftc-waste-water-information>

The following are problems associated with records and reports:

1. Failure to keep permits and records at the wastewater treatment plant.
(NPDES Permit Part 1, 2.1.3 & 1.2.5)
All permits and records should be kept at the wastewater treatment plant. In most cases this is a requirement in the permits.
2. Failure to keep records for three years. **(NPDES Permit Part 1, 1.2.5)**
All records and information resulting from the monitoring activities required by the permits are to be retained for a minimum of three (3) years, or longer, if requested by the Division of Water Resources. This includes all worksheets and scrap pieces of paper where calculations for analyses are performed and calibration and maintenance of instrumentation.
3. Uncertainty of what to do with records past the three (3)-year period.
If this Division has not given instructions to retain them past the three (3)-year period then, the facility can either destroy or archive the records.
4. Inadequate or no worksheets on which to record data. Refer to permit.
(NPDES Permit Part 1, 1.2.4)
5. Illegible and unorganized data recorded on worksheets.
(NPDES Permit Part 1, 2.1.3)
All work should be written in a manner where inspectors are able to interpret the data. Only the EPA approved methods may be used to perform the required analyses.
6. Failure to document calibration and maintenance of equipment.
(NPDES Permit Part 1, 1.2.5)
Documentation of calibration and maintenance of equipment should also be maintained.

7. Failure to monitor parameters at the required frequencies. **(NPDES Permit Part 1, 1.2.2)**
 Parameters should be monitored at their required frequencies (according to the permit). Analyses should not be locked into specific days during the week. These days should be changed to ascertain any potential problems that may occur during an entire week.

8. Inability to understand what should be recorded on Monthly Operational Reports (MORs).
 All columns on an MOR should be filled out to the best of the operator's ability.
(NPDES Permit Part 1, 1.3.1)

9. Uncertainty of whether a computer-generated MOR is permissible or not.
 Should an operator want to submit his data on computer-generated MORs, he should use the same format as the State-provided MORs. The operator should provide both the MOR and the computer-generated MOR to the field office for approval.
(NPDES Permit Part 1, 1.3.1)

The initial documentation, even if hand-written, becomes the permanent record and must be maintained. Storage of data and other information generated on computer is acceptable but a very rigorous tracking system with passwords and control level for data must be in place. Also, all records must be backed up on routine schedule and second disc or tape stored at a secure second location. These all have to be in place before electronic data is acceptable.

10. Failure to fill out DMRs correctly.
 The Discharge Monitoring Reports (DMRs) are not being filled out correctly, due to a misleading feature of the pre-printed DMR form. The headings near the top of the sheet labeled. **(NPDES Permit Part 1, 1.3.1)**

| AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS |

These have caused some misunderstanding as to the information required. The sampling intervals and units typed in the stippled gray portion to the right of each parameter listed should be followed, even where there is a conflict with the pre-printed heading. For example, the entry for "Solids, Total Suspended, Effluent Gross Value", in the fourth column to the right, the monthly average value should be entered because "MO AVG." is typed in the stippled gray area. (The pre-printed column heading "MINIMUM" should be ignored.)

11. Failure to calculate weekly averages correctly.
 Weekly averages should be reported by averaging the data in each week (Sunday through Saturday) of the month, then reporting the maximum value of those averages. If the week is not complete by the end of one month, then that week's data should be reported with the month in which the week ends.



12. Failure to submit DMRs and MORs in a timely manner.

Discharge Monitoring Reports (DMRs) and Monthly Operational Reports (MORs) frequently arrive late. DMRs should be postmarked no later than 15 days after the

completion of the reporting period. (MORs) are to be submitted, in the field office, by the 15th day of the month following data collection. **(NPDES Permit Part 1, 1.3.1)**

13. Failure to have a Quality Control program. **(NPDES Permit Part 1, 1.2.3)**

A Quality Control (QC) program should be initiated by each facility. An acceptable QC program would incorporate both quality control (precise and accurate data, e.g. duplicates) and quality assurance (QA) (How closely the data agrees. Statistics are commonly used.). Blanks are used to indicate contamination, duplicates measure precision, and spike samples measure accuracy. Duplicates should be analyzed in accordance with the method of analyses and 40CFR part 136.7 (May, 2012).

Also, there is a QA section at the beginning of each part of Standard Methods for the Examination of Water and Wastewater (SM) e.g. 1020, 2020, 3020, 4020, 5020, 6020, etc.

14. Failure to have an SOP. **(NPDES Permit Part 1, 2.1.4 and 40CFR part 136.7)**

Each facility should also have its own Standard Operations Procedure (SOP). An SOP would provide what is typically done at the laboratory and how operations are accomplished. This would include collection of samples to the actual method used for each laboratory analyses. An SOP should be available upon request and provide reference to EPA approved methods of analyses (e.g. SM and Hach methods).

15. Use of correction fluid to cover transcription errors.

It is unacceptable to use correction fluid to correct record keeping errors. Errors should be crossed out with a single line, initialed and the correct information entered.



THE FOLLOWING ARE PROBLEMS ASSOCIATED WITH THE LABORATORY.

General Laboratory Considerations

1. Failure to follow approved laboratory procedures. **(NPDES Permit Part 1, 1.2.3)**

The **40 CFR Part 136** provides the approved test procedures for the analysis of pollutants under the **Clean Water Act**.

Other equivalent, acceptable and approved laboratory procedures are



available (e.g. Hach Company products. See their EPA compliant methods for WW (wastewater) on the Hach website <http://www.hach.com/epa>). Any QA/QC must meet the requirements set forth in the 40CFR part 136.7. Not complying will make the self-monitoring data questionable and in some cases invalid; thus, resulting in violations.

2. Improperly marking containers.

If samples from more than one wastewater treatment plant are analyzed, containers should be marked distinctly to avoid any confusion between samples.

3. Using expired chemicals in analyses.

Chemicals should not be used beyond the expiration date. Doing so will place sample data in jeopardy of not being legally defensible. Purchase according to need. It won't be a bargain if the reagents expire before they are opened.

Uncertainty of when reagents have expired.

Mark the date received on the bottles and/or contact the manufacturer regarding expiration dates for the reagents.

4. Uncertainty on whether to purchase prepared reagents or prepare in-house reagents.

Some reagents such as sodium thiosulfate, 10%, used for preservation and chlorine removal for bacteriological sample collection, can be prepared at the plant with minimal cost. Although prepared reagents tend to be costly they also can save valuable time.

5. Failure to properly label in-house prepared reagents.

The in-house prepared reagents should be labeled with date, time, initials, and possible health hazards. A log of the reagents prepared should be kept. They should also be in compliance with OSHA regulations 29 CFR 1910.1200(f)(1)

6. Storing food and drink in ovens or refrigerators where samples are kept.

Food and drink should not be stored in refrigerators or ovens that also contain chemicals and wastewater samples. Chemicals should be kept away from foods, as it is an unnecessary safety hazard. Some chemicals can be absorbed in food.

7. Failure to document dates, times and initials for collection and analyses.

(NPDES Permit Part 1, 1.2.4)

The dates and times that the analyses were performed should be documented as should the name of the person who collected and analyzed the samples. This is especially important when transporting samples from one facility to another for analysis. The method of analyses should also be documented. This can either be referenced to the SOP or written directly on the daily worksheet. If the method can be found in SM, the method number should be followed by the SM committee's approval date. This can be found in the footnote in introductory section A of the method (e.g. 4500-NH₃ D-1997).



8. Failure to adequately clean glassware.

Glassware should be washed thoroughly in a tub of soapy water (or dish washer) using phosphate-free detergent (preferably lab grade), rinsed well with tap water and a final rinse with distilled water. Just rinsing the glassware is not considered cleaning.

Think ... representative sample for the lab 24 hours.

9. Failure to maintain a log for equipment. **(NPDES Permit Part 1, 2.1.5)**

A log should also be kept to record the temperatures of the ovens, water bath and incubator. It's advantageous to place the log near the equipment being monitored, but can also be recorded on the daily worksheet. This log should include the date, time, temperature and the initials of whomever is doing the checking.

**The following are problems frequently found with the Settleable Solids (SS) analysis.
(SM 2540 F-1997)**

Failure to follow EPA approved methodology.

Too frequently, deviation of the SS methodology has been found. The appropriate methodology for SS requires that the sample be shaken well and be poured into an Imhoff cone; be allowed to settle for 45 minutes, and then very slowly stirred (one revolution) to dislodge material adhering to the sides. Do NOT stir fast. The solids in the sample should then be allowed to settle for another 15 minutes then be read to the nearest 0.1 mL/L mark.

Laboratories typically have either the glass or the plastic cones. The lowest marking on the glass cone is the 0.1 mL/L mark. The plastic cones have a screw cap at the tip and don't provide a reliable a reading that low. If the lowest marking is the 0.5 mL/L and the solids level is below this, the one would report <0.5 mL/L. You can't record < 0.1 mL/L if the lowest mark is 0.5 mL/L. If you happen to have a glass cone and a plastic cone, use the glass for the final and the plastic for the raw. Otherwise, you must properly record what you see.

**The following are problems frequently found with Dissolved Oxygen (DO) analysis.
(SM 4500-O G-2001 and Hach method 10360 for LDO)**

1. Failure to collect DO sample properly.

When collecting the sample be sure to collect the sample with a BOD bottle using the stopper. Fill the BOD bottle to near the top and then stopper immediately to avoid any further contact with the air. This assures that no air bubbles are caught between the stopper and the sample.

2. Failure to properly calibrate the DO meter.

Air Calibration for the meter is acceptable by the EPA. If the air calibration method is used, the membranes must be kept fresh. Change the membrane at a minimum of once per



month. If air bubbles are visible under the membrane, the membrane must be changed. Follow manufacturer instructions for proper maintenance and calibration.

Winkler method is also acceptable by the EPA and is considered the better of the two methods used to calibrate the DO meter. Three bottles should be filled with distilled water. The first and third bottles should be titrated as required by the EPA approved method to determine the DO. The second bottle is used to adjust the setting on the meter to the averaged DO value found by titration of the other two bottles.

3. Failure to standardize the titrant.

If the titrant, 0.0375 N sodium thiosulfate is used in the Winkler method, then it must be standardized daily according to the approved methodology.

Another option is to titrate with a 0.03750N phenylarsine oxide (PAO) solution. This can be purchased pre-standardized. PAO solutions are stable. No further standardization would be necessary.

Even the luminescent dissolved oxygen (LDO) probe must be calibrated properly. The cover for the probe typically replaced yearly.

Currently, the barometric pressure and room temperature should be recorded at the same time that the DO meter is calibrated. The standard (or theoretical) DO value obtained from these readings should also be recorded. This can be found on the USGS website. <http://water.usgs.gov/software/DOTABLES/> . A table can be tailored to one's specific needs on this website. This standard value and the value used to set the meter must lie within 10% of each other

4. Failure to store DO probes properly.

DO probes can be stored in a BOD bottle containing at least 1 inch of water. Refer to the operations manual for the DO or LDO probe and meter or contact the YSI service center for assistance regarding proper maintenance of the equipment. Keep this bottle clean.

The following are problems frequently found with the Biochemical Oxygen Demand/Carbonaceous Biochemical Oxygen Demand (BOD/CBOD) analysis (SM 5210 B-2001)

1. Uncertainty of when to seed the sample. (5210B.4.d, 5.d. & 6.d.)

If an effluent composite sample is collected prior to disinfection, no seeding is required for BOD. However, seeding is required for the CBOD method since the nitrification inhibitor must be added to the sample.

Seeding is required for both influent and effluent samples for CBOD analysis. *“Comparing Apples to Apples”* as the saying goes.



If an effluent composite sample is collected following disinfection, the sample must be checked for any residual chlorine (if not UV disinfected) prior to setting up the CBOD samples. The samples then must be seeded.

2. Failure to adequately dechlorinate a sample. (5210B.4.b.2.)

Just allowing the samples to sit for a short period of time in order to dissipate any residual chlorine is not as effective as chemical de-chlorination. Besides, the samples must be set up and analyzed within two hours of collection. Chemical dechlorination also eliminates the chlorine in a shorter period of time. Failure to properly dechlorinate would result in artificially low results.

3. Failure to allow the samples to come to room temperature before making dilutions. (5210B.4.b.4.)

Since composite samples are kept at 6°C, or below, the samples should be warmed to $20 \pm 3^\circ\text{C}$ (comparable to 68°F) before making dilutions. This may be accomplished by allowing the samples to come to room temperature (approximately 68°F) or by setting the sample bottles in a warm water bath.

4. Initial DO values do not meet the method criteria. (5210B.8.b.)

Samples with initial DO's that are greater than 9.0 mg/l at 20°C (e.g. stream samples) are considered supersaturated with oxygen. These samples may be vigorously shaken or aerated with clean, compressed air to bring down to saturation, less than or equal to 9.0 mg/l. (5210 B 4.b.4.) The samples should be brought to room temperature and the initial DO ≤ 9.0 mg/L before setting up dilutions and analyzed within two hours of collection.

5. Introducing contamination into the sample.

Cross-contamination, which can produce inaccurately high results, can be avoided by properly rinsing the graduated cylinders between measuring sample volumes. Specific graduated cylinders can be delegated for each influent, effluent and stream sample. Clean, Clean, Clean!!!

6. Failure to adequately stir/shake the sample prior to making dilutions.

Sample should be stirred or shaken thoroughly to obtain a representative sample.

7. Failure to set up the appropriate amount of dilutions. 5210B.5.c.

At least three dilutions should be set up for both influent and effluent samples appropriate QA/QC measures.

8. Uncertainty of the purpose of the glucose-glutamic acid (GGA) check. 5210B.6.b.

To check the quality of the seed material and analytical technique, it is recommended that a glucose-glutamic acid check be set up with each set of samples. A typical value of 198 mg/L \pm 30.5 mg/L has been found for the GGA concentration. 164 \pm 30.7 mg/L (nitrification inhibitor added) is recommended for GGA concentration in CBOD analysis.



A low value could be indicative of a toxic affect caused from trace metals in the dilution water (e.g. copper). It could also indicate that the seed material concentration is weak (relatively inactive).

A high value could be indicative of several factors e.g. contamination from improper rinsing of glassware after cleansing, imprecise measurement of standards, or too much seed material added to GGA.

Should the measurement fall outside the previously given range, the tests should be reported and flagged as to the findings. The problem should then be investigated in order to determine and eliminate the source.

9. Failure to follow the BOD criteria for reporting results. 5210B.6.a, 7. and 8.b.

Follow the BOD criteria for reporting the results. EPA approved methodology states that the initial DO must be less than or equal to 9.0 mg/l. The samples must deplete at least 2.0 mg/L DO and must leave 1.0 mg/L final DO. Results from dilutions that do not meet these criteria are considered invalid and should be discarded.

The blank oxygen depletion should be 0.2 mg/L or less as required by EPA approved methodology. Otherwise, an investigation should be initiated to determine the cause. Check for soap residue due to improper rinsing. The results still can be reported but must be marked that the blank is outside the guidelines. The blank oxygen depletion is NOT subtracted from the sample depletion. (5210 B. 7.a.3.)

Be wary of the existence of toxicity trending.

10. Uncertainty of which nitrification inhibitor to use.

There are two nitrification inhibitors available for use in the CBOD analysis method, **2-Chloro-6 (Trichloromethyl) Pyridine** and **Nitrification Inhibitor, Formula 2533™**. The biggest difference between the two is that Formula 2533™ is more soluble than the other, thereby yielding better results.

The dispenser cap for the nitrification inhibitor is well worth the money.

11. Failure to properly monitor the temperature of composite samplers. (40CFR part 136, table II)

The temperature of the composite samplers are not generally monitored or maintained at the required temperature of $\leq 6^{\circ}\text{C}$. During the collection of composite samples, the temperature of the samplers must maintain temperatures at $\leq 6^{\circ}\text{C}$ or below (but above freezing 0°C).



12. Failure to properly monitor the temperature of the BOD incubator. (5210 B. 5.h.)

The BOD incubator temperature must be maintained at $20^{\circ} \pm 1^{\circ}\text{C}$. A log should be kept for each piece of equipment to record the date, time, temperature, and the initials of whoever was checking it. It is just as easy to record the temperature on the daily worksheet.

Check and record the temperature using a *calibrated* thermometer placed in water inside the incubator. Do not use the digital thermometer found on top of the incubator.

**The following are problems frequently found with the pH analysis.
(SM 4500-H B.-2000, revised 2011)**

1. Failure to compensate for temperature.

Since pH is temperature dependent, an automatic temperature compensator (ATC) probe must be used or the temperature must be measured and manually set on the instrument. This temperature of the samples should be recorded.

2. Failure to properly store the pH electrode.

Store electrodes according to manufacturer instructions! Orion states using pH 7 buffer with 1 gram of KCl (potassium chloride).

3. Using expired pH standards.

Do not use expired pH standards. Plan to buy what can be used before the expiration date.

4. Failure to properly calibrate the meter.

A two-point calibration is required. A two-point calibration brackets the normal pH range found at the plant. It provides a line on which the best possible data point for the sample can be found. If the sample falls outside the calibrated range, the instrument should be recalibrated.

If the probe that you use has the capability of performing a 3-point calibration, follow manufacturer procedures. This is a great way to ensure that the samples will be bracketed by the calibration buffers.

5. Failure to use fresh standard solutions for calibration of the pH meter.

Prepare fresh standard solutions for each daily use for calibration. If stock bottles are used, be sure to cap the bottle after pouring out an amount used for standardizing.

Never pour used reagent back into the stock bottle. This is a big source of contamination. This method could jeopardize the integrity of the sample, which is directly related to the calibration of the meter.

6. Improper calibration procedure used in calibrating the pH meter.

Start by adjusting the intercept knob with a pH 7 buffer (the isopotential point). The slope or calibrate function on the meter should be set with a second buffer. This buffer depends



on the desired range, either above pH 7 (e.g. pH 10) for the higher pH range, or below pH 7 (e.g. pH 4) for the lower pH range.

There are pH meters that provide a 3-point calibration allowing the use of pH buffers 7, 4 and 10. Follow the manufacturer directions.

7. Failure to rinse the electrode prior to reading the sample.
Be sure to rinse the electrode well after standardizing the meter and prior to reading the pH of the sample. Pat the probe dry with a soft cloth or tissue to remove the liquid. This minimizes cross-contamination.
8. Failure to stir the sample.
Stirring the sample at a rate of about one revolution/second is necessary in obtaining a quick and accurate reading.
9. Failure to run the sample immediately after collection.
Don't collect the pH sample until the meter has been calibrated. The analysis of the samples should be run immediately. 40CFR part 136, table II states that the holding time is within 15 minutes of collection.
10. Failure to have a backup probe.
An extra pH probe should be available in the event that the current probe malfunctions.
11. Uncertainty of how to maintain an electrode.
The electrolyte should be added to the new electrode, through a filler hole in the side, when it is ready for use. After the electrolyte has been added, it should not be used for at least one hour. Be sure to rinse off any excess electrolyte and pat dry the electrode. Do not rub the electrode vigorously because it could create a static charge in the probe. This will assure that none of the electrolyte is introduced into the sample.

Be sure to take the filler-hole cap off when taking readings for quicker response times. This cap may be replaced when not in use to prevent evaporation of the electrolyte.

The following are problems frequently found with the Total Suspended Solids (TSS) analysis. (SM 2540 D-1997)

1. Failure to adequately shake the sample.
The biggest problem with the TSS is that operators are not shaking the sample well enough prior to analysis. This is necessary to obtain a representative sample.
2. Uncertainty of why results are too **high**. (When comparing results to another lab's results.)
Frequently, sample volumes are too high for the amount of solids in the sample. This requires longer filtration periods for elimination of the liquid. Prolonged filtration times



may produce high results due to excessive solids on the clogged filter. By reducing the sample size, more satisfactory results should be obtained.

A constant weight must be demonstrated prior to reporting the results. This is to ensure that all the moisture has been eliminated. If this is not accomplished then erroneously high results may be obtained. The re-drying, cooling, desiccating and weighing cycle is

required unless shown otherwise. The weight change must be less than 4% of the previous weight or 0.5 mg, whichever is less. (2540 D.3.c.)

The drying oven should be kept clean to minimize contamination of samples.

3. Uncertainty of why results are too **low**. (When comparing results to another lab's results.)

Solids can be lost under the filter paper when pouring in the sample. Wetting the filter paper with distilled water prior to pouring the sample will lessen the possibility of the solids being lost under the filter rather than collected on the filter.

Filtered samples should not be placed directly on the oven rack. The filters could stick to the rack and lose fibers, which could change the weight of the sample. They could also gain contaminants or spill the filter contents. Aluminum weighing dishes are recommended.

4. Lack of good laboratory technique.

Do not use fingers to pick up crucible or filter. The oil on your fingers adds weight. Use tongs instead.

5. Uncertainty of when to change the desiccant.

Make sure that the color indicator desiccant is changed when the color turns to pink.

An option would be to obtain the non-color indicator and mix it with the color indicator desiccant. This would incorporate cost with performance.

6. Failure to properly monitor the temperature of the drying oven.

The oven should be maintained at a temperature between 103 - 105°C at all times.

7. Failure to perform quality control procedures (SM 2020I and II tables)

A duplicate should be performed once every 20th sample.

A distilled water blank should be analyzed with every 20th sample for quality assurance.



The following are problems frequently found with the Total Chlorine Residual analysis. (Hach method 8167)

1. Failure to calibrate the instrument prior to use (or check the calibration for Hach Pocket Colorimeters).

The Instrument calibration should be checked prior to each use. Chlorine or KMnO_4 standards may be used. There are several companies that manufacture pre-made standards. The chlorine standards are typically added to samples that have already been analyzed (standard addition method). See Hach method of analysis.

2. Uncertainty of the proper method to use.

The permit limit must be considered when deciding the appropriate method to use. The low limits presently being given require the lowest possible detection limits that a method can provide. DPD, electrode and amperometric methods are currently the methods that provide the lowest detection limits available. Current permits state the following:

“Total residual chlorine (TRC) monitoring shall be applicable when chlorine, bromine, or any other oxidants are added. The acceptable methods for analysis of TRC are any methods specified in Title 40 CFR, Part 136 as amended. The method detection level (MDL) for TRC shall not exceed 0.05 mg/l unless the permittee demonstrates that its MDL is higher. The permittee shall retain the documentation that justifies the higher MDL and have it available for review upon request. In cases where the permit limit is less than the MDL, the reporting of TRC at less than the MDL shall be interpreted to constitute compliance with the permit.”

Iron, manganese, nitrates and organic mercaptans can interfere with DPD readings giving a false positive for chlorine. Some facilities are unable to use the colorimeter due to these interferences.

The amperometric method requires greater skill than the colorimetric method. However, less interference is found.

3. Uncertainty of what the lowest reportable value should be.

The amperometric method requires greater skill than the colorimetric method but there are fewer problems with these interferences.

The current instrument detection limit is 0.05 mg/L. Many permit limits lie below this value. Many operators have been reporting <0.1 mg/L. This was the old detection limit and should not be used anymore. If the value in the permit limit is within the capability of the instrument, it is preferable that the operators report the actual value that is obtained.

However, MUR now requires that the method detection limit (MDL) must now be determined by the laboratory. However, follow permit requirements until the permit language changes.



**The following are problems frequently found with the Ammonia as Nitrogen (NH₃-N) analysis.
(SM 4500-NH₃ – 1997)**

1. Failure to properly preserve samples. (4500-NH₃ A.3.)

If samples are to be analyzed with within 24 hours of collection, refrigerate until removed for analysis. If the analysis cannot be performed within this time period, the samples must be preserved by acidifying to pH and store refrigerated to $\leq 6^{\circ}\text{C}$ for 28 days (40CFR part 136, table II).

Oh, don't forget that this table can also provide guidance in which to store samples should a problem arises at the facility and the analysis cannot be performed.

2. Failure to adequately shake the sample.

Thoroughly shaking the sample is necessary to obtain a more representative sampling.

3. Failure to distill samples.

The analytical method should be consulted regarding the need for distillation. If this method is unclear, distillation is required. Distillation is required to remove impurities that would interfere with the analyses and produce erroneous results. (40CFR part 136 table 1B, footnote 6) It requires distillation for all methods unless comparability has been shown. "Manual Distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary; however, manual distillation will be required to resolve any controversies."

Comparable is defined as <20 RPD (relative percent difference). Since the likelihood of the distilled and undistilled samples will meet this requirement, it recommended that a sample be distilled and compared with the undistilled sample, once per year.

If distillation is required, guidance can be found in the 40CFR part 136 table 1B, footnote 6.

Did you say Ammonia-free water? Hopefully, someone has found something out there to assist in this matter. Personally, I've had difficulty coming up with something. The EPA 350.2 method provides guidance

EPA Method 350.2

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6.0 Reagents

6.1 Distilled water should be free of ammonia. Such water is best prepared by passage through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin.

Regeneration of the column should be carried out according to the manufacturer's instructions.

NOTE 1: All solutions must be made with ammonia-free water.



Remember, not only does the air that we breathe have oxygen; it also has nitrogen which is a component of ammonia. So, when you distill the water minimize the exposure to the air as it is distilled off.

4. Failure to properly calibrate the instrument.

Instrument calibration is required prior to running the analysis. At least two standards should be used to bracket the sample. If the sample falls outside this range, then the sample can be diluted or new standards prepared.

The following are problems frequently found with Fecal Coliform analysis. Some of this information may also apply to the *E.coli* analyses (Hach m-coli Blue method of analyses - comparable to EPA 1603)

1. Failure to use the approved method.

Only use approved methods outlined in the 40CFR part 136 table 1A. Don't get this confused with total Coliform.

2. Failure to use a microscope with the appropriate magnification.

A microscope with magnification of 10X to 15X is required to obtain a valid colony count. This magnification is necessary to determine the presence of small colonies in a sample that might otherwise be missed.

3. Failure to monitor the temperature of the water bath.

Maintain the temperature of the water bath at a uniform and constant temperature of $44.5 \pm 0.2^{\circ}\text{C}$ at all times. *E.coli* incubator temperature should be maintained at $35 \pm 0.5^{\circ}\text{C}$.

The thermometer must be graduated in 1/10 of $^{\circ}\text{C}$ which is needed for the $\pm 0.2^{\circ}\text{C}$ and **kept clean.**

4. Failure to follow proper sterilization procedures.

0.1 mL of a 10% sodium thiosulfate solution should be added to each sample bottle prior to sterilization. Do not use the preservative N-10 (0.1005-0.0995) sodium thiosulfate. This is potentially harmful to the fecal coliform culture since this is stronger than the 10% (approximately 0.03N) solution stated in the EPA approved procedures.

The sterilization procedure should be done for 15 - 20 minutes at 121°C (250°F).

If you have to use a pressure cooker as an autoclave, do not close the petcock until steam comes out of the ports. When the sterilization period is complete, turn steam supply off; allow glassware to slowly cool before removing. The pressure cooker is only to be used as a **temporary** method of sterilization. The Autoclave is preferable.



5. Failure to properly collect the sample.
While collecting the sample in the sterilized bottle, care must be taken to eliminate other sources of contamination (e.g. unsterilized dippers used to pour the sample into the sterilized sample bottles).
6. Uncertainty whether to use distilled water in place of buffered dilution water.
Do **NOT** use distilled water in place of the buffered dilution water. EPA approved methodology requires the use of buffered dilution water in the procedures. The method shows you how to prepare this.
7. Failure to prepare the appropriate number of dilutions.
Many operators are preparing only one dilution. Prepare three dilutions of the sample in order to obtain a 20 - 60-colony count. Do the same to obtain 20 - 80-colony counts for *E.coli*.
8. Performing analysis with faulty equipment.
Avoid using a leaking filter apparatus. This could give erroneously low results.
9. Failure to perform quality control ("Quick Reference for *E.coli* Analysis)
http://www.tn.gov/assets/entities/environment/attachments/wwt_quick_reference_e_coli.pdf
It is recommended that a positive control sample be set up at least once per month as a quality control check (e.g. 1 mL of effluent prior to chlorination).

Perform duplicate samples at least once every 10 samples.
10. Failure to perform analyses due to high flow conditions.
Sampling during high flow events occasionally has been avoided by the operator due to frequently obtaining TNTC (Too Numerous To Count) data. (This is a violation of the permit since representative sampling is required at a specified frequency.) At several plants this occurrence is the norm rather than the exception. Operators who have worked at these plants for a number of years can probably guess as to the appropriate dilutions that could be used to obtain 20 - 60 colony counts. It's better to indicate what actually happens at the plants rather than indicate no problems. These problems would lend proof that there is a need for plant renovations.
11. Failure to report data correctly.
There are specific guidelines that should be followed when reporting data. TNTC is not to be reported on the MOR. These guidelines are provided upon request or may be found in *EPA Microbiological Methods for Monitoring the Environment Water and Wastes*, EPA-600/8-78-017, December 1978.



The following are problems frequently found with the *E.coli* analysis.

1. Failure to use the methods approved for wastewater.

In the March 2007 40 CFR part 136 the Colilert, Colilert 18 and mColiBlue 24 were approved for *E. coli* analyses. Well, there are others listed as well. But, these particular methods have fought long and hard for “approval”.

2. Failure to use appropriate temperature.

Follow instructions. Don't assume *E. coli* can be used at the same temperature as fecal coliform or visa versa.

3. Failure to count colonies in the appropriate range.

The appropriate colony count range for Hach's m-Coli Blue method is 20 - 80. You are counting the blue colonies only and not the total colonies.

4. The “Quick Reference Guide to *E.coli* Analysis” can be found on the FTC website.

<http://www.tn.gov/environment/article/wr-ftc-waste-water-information>

The following are problems frequently found with the IDEXX Colilert analysis.

1. Failure to monitor the incubator temperature at $35 \pm 0.5^{\circ}\text{C}$. The thermometer must be graduated in 1/10 of $^{\circ}\text{C}$

2. Failure to have a color comparator available to confirm fluorescent blue wells. Yellow wells indicate the presence of total coliforms. The yellow wells that fluoresce under UV light indicate the presence of *Ecoli*.

3. Failure to have the correct Quanta Tray available for use in expected high count flows.

Trays come in 51-well and 97-well sizes. The 51-well tray allows MPN counts up to 200 per 100 mL of sample. The 97-well tray allows MPN for up to 2419 per 100 mL of sample.

4. Failure to properly dispose of used Quanti trays.

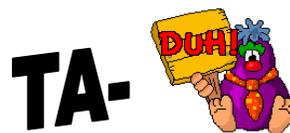
Samples, reference materials and equipment known or suspected to have viable bacteria attached or contained must be sterilized prior to disposal.

5. Failure to use **sterile water** for sample dilution.

Do not use the sterile dilution water that is used in the fecal coliform or m-coli Blue analyses.

6. Failure to perform quality assurance controls.

Purchase Quanti-Cult control samples (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* cultures) to check the quality control of the method.



Section 4
Dissolved Oxygen



Dissolved Oxygen and Temperature



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Definition

- Dissolved Oxygen
 - "DO" for short
 - measurement of the amount of oxygen dissolved in a unit volume of water
 - indicator of usefulness of water for a specific application



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Hold Times

- 15 minutes - note time of collection & time of analysis!



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Applications

- Useful for maintaining a stream fit for swimming, fishing and/or as a source of potable water...
- DO level must be kept high (in English - permits have minimum limits; DO levels must be kept above this limit)
- Low DO levels = can have harmful effects on receiving waters; causes suffocation of fish & promotes growth of harmful bacteria.



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Applications

- Sewage treatment:
- Specialized bacteria (also known as activated sludge) is added to solids in waste water treatment plants
- optimum level of DO necessary for process
- too low DO = bacteria die & decompose
- too high DO = process becomes costly

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Theory

- Amount of Oxygen that a given volume of water can hold is a function of:
 - The pressure the atmospheric oxygen is exerting at the air-water interface....
 - The temperature of the water.
 - The amount of other substances dissolved in the water.

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Theory

- The higher the atmospheric pressure and the lower the temperature and conductivity, the more oxygen can be dissolved in the water.

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Theory

<http://water.usgs.gov/software/DOTABLES/>

Temp °C	Solubility of oxygen, in milliliters of mercury																				
Temp °C	795	790	785	780	775	770	765	760	755	750	745	740	735	730	725	720	715	710	705	700	
15.0	10.5	10.4	10.3	10.2	10.1	10.1	10.0	9.9	9.8	9.7	9.7	9.6	9.5	9.5	9.4	9.3	9.3	9.2	9.1	9.0	9.0
15.5	10.4	10.3	10.2	10.1	10.0	10.0	9.9	9.8	9.7	9.6	9.6	9.5	9.4	9.4	9.3	9.2	9.2	9.1	9.0	8.9	8.9
16.0	10.2	10.1	10.0	9.9	9.8	9.8	9.7	9.6	9.5	9.4	9.4	9.3	9.2	9.2	9.1	9.0	8.9	8.8	8.7	8.7	8.6
16.5	10.1	10.0	9.9	9.8	9.7	9.7	9.6	9.5	9.4	9.3	9.3	9.2	9.1	9.1	9.0	8.9	8.8	8.7	8.6	8.5	8.5
17.0	10.0	9.9	9.8	9.7	9.6	9.6	9.5	9.4	9.3	9.2	9.2	9.1	9.0	8.9	8.8	8.7	8.6	8.5	8.4	8.4	8.3
17.5	9.9	9.8	9.7	9.6	9.5	9.5	9.4	9.3	9.2	9.1	9.1	9.0	8.9	8.8	8.7	8.6	8.5	8.4	8.3	8.3	8.2
18.0	9.8	9.7	9.6	9.5	9.4	9.4	9.3	9.2	9.1	9.0	9.0	8.9	8.8	8.7	8.6	8.5	8.4	8.3	8.2	8.2	8.1
18.5	9.7	9.6	9.5	9.4	9.3	9.3	9.2	9.1	9.0	8.9	8.9	8.8	8.7	8.6	8.5	8.4	8.3	8.2	8.1	8.1	8.0
19.0	9.6	9.5	9.4	9.3	9.2	9.2	9.1	9.0	8.9	8.8	8.8	8.7	8.6	8.5	8.4	8.3	8.2	8.1	8.0	8.0	7.9
19.5	9.5	9.4	9.3	9.2	9.1	9.1	9.0	8.9	8.8	8.7	8.7	8.6	8.5	8.4	8.3	8.2	8.1	8.0	7.9	7.9	7.8
20.0	9.4	9.3	9.2	9.1	9.0	9.0	8.9	8.8	8.7	8.6	8.6	8.5	8.4	8.3	8.2	8.1	8.0	7.9	7.8	7.8	7.7
20.5	9.3	9.2	9.1	9.0	8.9	8.9	8.8	8.7	8.6	8.5	8.5	8.4	8.3	8.2	8.1	8.0	7.9	7.8	7.7	7.7	7.6
21.0	9.2	9.1	9.0	8.9	8.8	8.8	8.7	8.6	8.5	8.4	8.4	8.3	8.2	8.1	8.0	7.9	7.8	7.7	7.6	7.6	7.5
21.5	9.1	9.0	8.9	8.8	8.7	8.7	8.6	8.5	8.4	8.3	8.3	8.2	8.1	8.0	7.9	7.8	7.7	7.6	7.5	7.5	7.4
22.0	9.0	8.9	8.8	8.7	8.6	8.6	8.5	8.4	8.3	8.2	8.2	8.1	8.0	7.9	7.8	7.7	7.6	7.5	7.4	7.4	7.3
22.5	8.9	8.8	8.7	8.6	8.5	8.5	8.4	8.3	8.2	8.1	8.1	8.0	7.9	7.8	7.7	7.6	7.5	7.4	7.3	7.3	7.2
23.0	8.8	8.7	8.6	8.5	8.4	8.4	8.3	8.2	8.1	8.0	8.0	7.9	7.8	7.7	7.6	7.5	7.4	7.3	7.2	7.2	7.1
23.5	8.7	8.6	8.5	8.4	8.3	8.3	8.2	8.1	8.0	7.9	7.9	7.8	7.7	7.6	7.5	7.4	7.3	7.2	7.1	7.1	7.0
24.0	8.6	8.5	8.4	8.3	8.2	8.2	8.1	8.0	7.9	7.8	7.8	7.7	7.6	7.5	7.4	7.3	7.2	7.1	7.0	7.0	6.9
24.5	8.5	8.4	8.3	8.2	8.1	8.1	8.0	7.9	7.8	7.7	7.7	7.6	7.5	7.4	7.3	7.2	7.1	7.0	6.9	6.9	6.8
25.0	8.4	8.3	8.2	8.1	8.0	8.0	7.9	7.8	7.7	7.6	7.6	7.5	7.4	7.3	7.2	7.1	7.0	6.9	6.8	6.8	6.7
25.5	8.3	8.2	8.1	8.0	7.9	7.9	7.8	7.7	7.6	7.5	7.5	7.4	7.3	7.2	7.1	7.0	6.9	6.8	6.7	6.7	6.6
26.0	8.2	8.1	8.0	7.9	7.8	7.8	7.7	7.6	7.5	7.4	7.4	7.3	7.2	7.1	7.0	6.9	6.8	6.7	6.6	6.6	6.5
26.5	8.1	8.0	7.9	7.8	7.7	7.7	7.6	7.5	7.4	7.3	7.3	7.2	7.1	7.0	6.9	6.8	6.7	6.6	6.5	6.5	6.4
27.0	8.0	7.9	7.8	7.7	7.6	7.6	7.5	7.4	7.3	7.2	7.2	7.1	7.0	6.9	6.8	6.7	6.6	6.5	6.4	6.4	6.3
27.5	7.9	7.8	7.7	7.6	7.5	7.5	7.4	7.3	7.2	7.1	7.1	7.0	6.9	6.8	6.7	6.6	6.5	6.4	6.3	6.3	6.2
28.0	7.8	7.7	7.6	7.5	7.4	7.4	7.3	7.2	7.1	7.0	7.0	6.9	6.8	6.7	6.6	6.5	6.4	6.3	6.2	6.2	6.1
28.5	7.7	7.6	7.5	7.4	7.3	7.3	7.2	7.1	7.0	6.9	6.9	6.8	6.7	6.6	6.5	6.4	6.3	6.2	6.1	6.1	6.0
29.0	7.6	7.5	7.4	7.3	7.2	7.2	7.1	7.0	6.9	6.8	6.8	6.7	6.6	6.5	6.4	6.3	6.2	6.1	6.0	6.0	5.9
29.5	7.5	7.4	7.3	7.2	7.1	7.1	7.0	6.9	6.8	6.7	6.7	6.6	6.5	6.4	6.3	6.2	6.1	6.0	5.9	5.9	5.8

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Common Conversions w/ BP

- Uncorrecting pressure readings
- EXAMPLE - The local airport provides you with a "corrected" barometric pressure of 29.65 inches of Hg.
- To UNcorrect this measurement:
- NOTE:** Pressure drops by 26 millimeters (mm, about 1 inch) for every 1000 feet above sea level. $26 \div 1000 = 0.026$.
- That's why during the process, we multiply the altitude in feet by 0.026.

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Common Conversions w/ BP

- Determine the altitude (in feet) of your facility/lab (you can use the altitude of the city/town/village).
 - Blueprints
 - Internet
 - GPS

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Common Conversions w/ BP

- Determine the correction factor (CF):

$$CF = [760 - (\text{Altitude} \times 0.026)] \div 760$$

$$= [760 - (543 \times 0.026)] \div 760$$

$$= [760 - 14.1] \div 760$$

$$= 745.9 \div 760 = \mathbf{0.9795}$$

Therefore, true uncorrected barometric pressure = $29.65 \times 0.9795 = 29.04$
- EXAMPLE - The "corrected" BP was given as 29.65 inches of Hg.
- FTC elevation = 543 ft

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Common Conversions w/ BP

- Convert inches of mercury (Hg) to mm of mercury (Hg):

$$\text{inches of Hg} \times 25.4 = \text{mm of Hg}$$

Therefore $29.04 \times 25.4 = 737.6$

Your uncorrected BP is either 29.04 inches of Hg or 737.6 mm of Hg.

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BP Conversion Example

- Example: Weather.com reports a stable 29.88* in of Hg. We are at 547 ft and our water sample is at 21°C.
 - CF = $[760 - (\text{Altitude} \times 0.026)] \div 760$
 $= 745.8 \div 760 = \mathbf{0.98}$
 - Therefore, **true uncorrected** barometric pressure = $29.88 \times \mathbf{0.98} = 29.32$ in of Hg
 - Or $29.32 \times 25.4 = 744.7$ mm of Hg

*Weather.com reports values at sea level, this needs to be corrected for your elevation.

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BP Conversion Example

- Find % Saturation Calibration Value:
 - $\frac{(0.21)(745)(100)}{160} = \frac{15,645}{160} = 97.8\%$
- Find Concentration Calibration Value:
 - At 21°C, the oxygen solubility table reports 8.915 mg/L
 - $(8.915)(.978) = 8.72$ mg/L

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BP Conversion Example

- The pressure of oxygen at sea level is **160 mm of Hg** because oxygen is about 21% of the earth's atmosphere **and** 21% of 760 (average sea level barometric pressure) is about 160 mm of Hg.
- https://www.fondriest.com/pdf/ysi_do_handbook.pdf

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BP Conversion Example

- Find % Saturation Calibration Value:
 - $\frac{(0.21)(745)(100)}{160} = \frac{15,645}{160} = 97.8\%$
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 - At 21°C, the oxygen solubility table reports 8.915 mg/L
 - $(8.915)(.978) = 8.72$ mg/L

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BP Conversion Example

Easy way - Direct Read from Barometer & Thermometer to determine DO from USGS DO table



Barometric Pressure (BP) -uncorrected Temperature -C USGS DO Table (mm Hg)

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Effect of Partial Pressure of Oxygen on Dissolved O₂

- Water in contact with air will absorb air (O₂)
- until the pressure at air-water interface is equal...
- said to be saturated - about 5 to 10 parts of oxygen to one million parts of water



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Effect of Temperature on Dissolved Oxygen

- Pot of boiling water...
 - bubbles form on bottom & sides of pot...
 - number & size of bubbles increase with temperature....
 - These are air bubbles that have been dissolved in water.



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Two Types of Measurement

- Electrode
- Winkler titration

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Electrodes

- An electrode system where DO reacts at the cathode producing a measurable electrochemical effect.
- Effect can be galvanic (ability to conduct an electrical current), polarographic (electrochemical), or potentiometric (measurement of voltages).

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Electrodes

- Electrode system separated from the sample stream by semi-permeable membrane, which permits DO in sample to pass through to the electrode system, but prevents passage of liquids & ionic fluids.
- Most units are temperature compensated (thermistor or resistance thermometer).
- Another type of probe does not use semi-permeable membrane; system consists of a reference electrode & thallium measuring electrode.

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Electrodes

- O₂ concentration determined by measuring voltage potential when DO comes in contact with the thallium electrode.
- Thallous-ion concentration is proportional to the dissolved oxygen in the sample.

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Dissolved Oxygen

- Electrochemical method.
- Two metal electrodes in contact with electrolyte and separated from test solution by gas permeable membrane and a constant voltage created.
- Oxygen diffuses through membrane and is reduced at the cathode by the voltage.
- This process produces a current flow, which is detected by the meter, and is proportional to the partial pressure of oxygen.

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● ● ● Dissolved Oxygen

- Meter calibrated in saturated air
- Winkler test may be used as a QC check
- Correction for altitude or barometric pressure
- Membrane checked and changed regularly
- Temperature calibration data verified

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● ● ● Dissolved Oxygen Analysis

- Analyze immediately
maximum holding time = fifteen min
- Preservation = none
- Sample should be measured insitu
- May be collected as a grab sample with minimal aeration into a BOD bottle with a glass stopper

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● ● ● Taking D.O. Readings



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● ● ● Common Deficiencies

- Samples for dissolved oxygen were collected in a bucket and then poured into the BOD bottle
- The D.O. probe was immersed in the water during calibration
- The D.O. probe had a water droplet on the end during calibration
- There was an air bubble under the membrane on the probe
- The meter was air calibrated by placing the probe on the counter

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● ● ● Luminescent Dissolved Oxygen



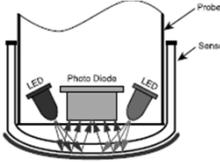
- No membrane
- No electrolyte to foul or poison
- Won't affect readings
- Accurate & stable readings

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Luminescent Dissolved Oxygen

- The sensor is coated with a luminescent material, called luminophore, which is excited by blue light from an internal LED.



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Luminescent Dissolved Oxygen

- As the luminescent material relaxes it emits red light, and this luminescence is proportional to the dissolved oxygen present.
- The luminescence is measured both in terms of its maximum intensity and its decay time.
- An internal red LED provides a reference measurement before every reading to ensure that the sensor's accuracy is maintained.

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Winkler Titration

- Winkler method (azide modification)
 - SM 4500-OC
 - Na₂SO₄ is to be standardized quarterly
 - Winkler test must be run within 8 hours of adding the reagents*, due to instability of solution once reagents are added. (*MgSO₄ & alkaline-iodine-azide solution)

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Winkler

- Titrimetric wet chemistry test that measures the amount of oxygen present based on conversion of oxygen to iodine



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Winkler

- Titrate with 0.025M Sodium Thiosulfate
 - Use a burette with 0.5 mL increments
- Titrate until a pale straw color



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DO QA/QC

Parameter	Method	DOC	MDL	LRB	LFB	LFM / LFMID	Dup	ICAL / CCV	Control Charts	Corrective Action	QC Acceptance	Batch Size	*QC Frequency
Oxygen, dissolved	SM4500-O G - 2001 Hach Method 10360 Oct. 2011	X					X	X, Calibrate meter daily & verify with air-saturated water		X	X	20	Depends on Permit
		X					X		X	X	20	Depends on Permit	

DO Hach Method 10360, LDO Measurement Oct. 2011

- DOC
- Dup
- ICAL/CCV
- Corrective Action
- QC Acceptance
- Batch Size
- QC Frequency



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DO Hach Method 10360, LDO Measurement Oct. 2011

- Demonstration of Capability
 - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
 - Hach Method 10360 9.2.1 – Prepare and measure four samples of air-saturated water according to section 7.2.
 - 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
 - 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately 20 ± 3°C.
 - 7.2.3 – With a steady gentle stream of filtered air (≈ 10-40 mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Hach Method 10360 continued
 - 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature (20 ± 3°C) for 30 minutes and note the barometric pressure [uncorrected] of the laboratory during preparation. The barometric pressure reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.
 - 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
 - 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman..
- Real people language – prepare dilution water that is air-saturated and analyze four bottles and compare to the theoretical dissolved oxygen concentration.

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Barometric Pressure (mm Hg)

Temp	570	580	590	600	610	620	630	640	650	660	670	680	690	700	710	720	730	740	750	760
0	10.95	11.14	11.33	11.53	11.72	11.91	12.11	12.3	12.49	12.69	12.88	13.07	13.27	13.46	13.65	13.85	14.04	14.23	14.43	14.62
1	10.64	10.83	11.02	11.21	11.39	11.58	11.77	11.96	12.15	12.34	12.52	12.71	12.9	13.09	13.28	13.46	13.65	13.84	14.03	14.22
2	10.35	10.53	10.72	10.91	11.08	11.27	11.45	11.63	11.82	12	12.18	12.37	12.55	12.73	12.91	13.1	13.28	13.46	13.65	13.83
3	10.07	10.25	10.43	10.61	10.79	10.96	11.14	11.32	11.5	11.68	11.86	12.03	12.21	12.39	12.57	12.75	12.93	13.11	13.28	13.46
4	9.81	9.98	10.15	10.33	10.5	10.68	10.85	11.02	11.2	11.37	11.54	11.72	11.89	12.07	12.24	12.41	12.59	12.76	12.93	13.11
5	9.55	9.72	9.89	10.06	10.23	10.4	10.57	10.74	10.91	11.08	11.25	11.42	11.59	11.75	11.92	12.09	12.26	12.43	12.6	12.77
6	9.31	9.47	9.64	9.8	9.97	10.14	10.31	10.47	10.63	10.8	10.96	11.13	11.29	11.46	11.62	11.79	11.95	12.12	12.28	12.45
7	9.08	9.24	9.4	9.56	9.72	9.88	10.04	10.2	10.37	10.53	10.69	10.85	11.01	11.17	11.33	11.49	11.66	11.82	11.98	12.14
8	8.85	9.01	9.17	9.33	9.48	9.64	9.8	9.95	10.11	10.27	10.43	10.58	10.74	10.9	11.06	11.21	11.37	11.53	11.69	11.84
9	8.64	8.79	8.95	9.11	9.25	9.41	9.56	9.71	9.87	10.02	10.18	10.33	10.48	10.64	10.79	10.94	11.1	11.25	11.41	11.56
10	8.43	8.58	8.73	8.88	9.03	9.18	9.33	9.49	9.64	9.79	9.94	10.09	10.24	10.39	10.54	10.69	10.84	10.99	11.14	11.29
11	8.24	8.38	8.53	8.68	8.82	8.97	9.12	9.26	9.41	9.56	9.71	9.85	10	10.15	10.29	10.44	10.59	10.73	10.88	11.03
12	8.05	8.19	8.33	8.48	8.62	8.77	8.91	9.05	9.2	9.34	9.48	9.63	9.77	9.91	10.06	10.2	10.35	10.49	10.63	10.78
13	7.86	8.01	8.15	8.29	8.43	8.57	8.71	8.85	8.99	9.13	9.27	9.41	9.55	9.69	9.83	9.97	10.11	10.26	10.4	10.54
14	7.69	7.83	7.97	8.1	8.24	8.38	8.52	8.65	8.79	8.93	9.07	9.2	9.34	9.48	9.62	9.76	9.89	10.03	10.17	10.31
15	7.52	7.66	7.79	7.93	8.06	8.2	8.33	8.47	8.6	8.74	8.87	9	9.14	9.27	9.41	9.54	9.68	9.81	9.95	10.08
16	7.36	7.49	7.62	7.76	7.89	8.02	8.15	8.28	8.42	8.55	8.68	8.81	8.95	9.08	9.21	9.34	9.47	9.61	9.74	9.87
17	7.2	7.33	7.46	7.59	7.72	7.85	7.98	8.11	8.24	8.37	8.5	8.63	8.76	8.89	9.02	9.15	9.28	9.41	9.54	9.66
18	7.05	7.18	7.31	7.43	7.56	7.69	7.81	7.94	8.07	8.2	8.32	8.45	8.58	8.7	8.83	8.96	9.09	9.21	9.34	9.47
19	6.91	7.03	7.16	7.28	7.41	7.53	7.66	7.78	7.9	8.03	8.15	8.28	8.4	8.53	8.65	8.78	8.9	9.03	9.15	9.28
20	6.77	6.89	7.01	7.13	7.26	7.38	7.5	7.62	7.75	7.87	7.99	8.11	8.24	8.36	8.48	8.6	8.73	8.85	8.97	9.09
21	6.63	6.75	6.87	6.99	7.11	7.23	7.35	7.47	7.59	7.71	7.83	7.95	8.07	8.19	8.31	8.43	8.55	8.67	8.79	8.92
22	6.5	6.62	6.74	6.85	6.97	7.09	7.21	7.33	7.45	7.56	7.68	7.8	7.92	8.04	8.15	8.27	8.39	8.51	8.63	8.74
23	6.37	6.49	6.61	6.72	6.84	6.95	7.07	7.19	7.3	7.42	7.53	7.65	7.77	7.88	8	8.11	8.23	8.35	8.46	8.58
24	6.25	6.36	6.48	6.59	6.71	6.82	6.94	7.05	7.16	7.28	7.39	7.51	7.62	7.73	7.85	7.96	8.08	8.19	8.3	8.42
25	6.13	6.24	6.36	6.47	6.58	6.69	6.81	6.92	7.03	7.14	7.25	7.37	7.48	7.59	7.7	7.81	7.93	8.04	8.15	8.26
26	6.02	6.13	6.24	6.35	6.46	6.57	6.68	6.79	6.9	7.01	7.12	7.23	7.34	7.45	7.56	7.67	7.78	7.89	8	8.11
27	5.9	6.01	6.12	6.23	6.34	6.45	6.56	6.67	6.77	6.88	6.99	7.1	7.21	7.32	7.43	7.54	7.64	7.75	7.86	7.97
28	5.8	5.9	6.01	6.12	6.23	6.34	6.45	6.56	6.67	6.77	6.88	6.99	7.1	7.21	7.32	7.43	7.54	7.65	7.76	7.87
29	5.69	5.8	5.9	6.01	6.12	6.23	6.34	6.45	6.56	6.67	6.78	6.89	7.0	7.11	7.22	7.33	7.44	7.55	7.66	7.77
30	5.59	5.69	5.79	5.9	6	6.11	6.22	6.33	6.44	6.55	6.66	6.77	6.88	6.99	7.1	7.21	7.32	7.43	7.54	7.65

Can be made at <http://water.usgs.gov/software/DOTABLES/>
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DO Hach Method 10360, LDO Measurement Oct. 2011

- Calibration
 - 7.1.1 – Add approximately 1 inch (2.54 cm) of reagent water to a clean BOD bottle and stopper).
 - 7.1.2 – Shake vigorously for ~ 10 seconds.
 - 7.1.3 – Allow for the BOD bottle and its contents to equilibrate to room temperature. Room temperature should be approximately 20 ± 3°C.
 - 7.1.4 – The stopper may now be removed from the BOD bottle and the LBOD probe inserted for calibration purposes.
- Real people language – calibrate daily by following manufacturer's instructions.

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Duplicate
 - Real people language – analyze 2 samples for DO, grab sample in a bucket and dip probe twice to get two readings
 - Target value is to get close to the first value and have a small RPD
 - **2014 Update – For reporting purposes, all duplicates should be reported according to your permit limits. If your permit sets a minimum, then the minimum value should be reported even if falls outside your permit limit.**

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Continuing Calibration Verification (CCV) - **daily**
 - 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
 - 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately $20 \pm 3^\circ\text{C}$.
 - 7.2.3 – With a steady gentle stream of filtered air ($\approx 10\text{-}40$ mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.
 - 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature ($20 \pm 3^\circ\text{C}$) for 30 minutes and note the barometric pressure [uncorrected] of the laboratory during preparation. The barometric pressure reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Continuing Calibration Verification (CCV) continued
 - 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
 - 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman...
 - 9.3.1 – Upon air calibration, prepare a calibration verification standard with each analytical batch of 20 samples or less in an 8 hour period.
 - 9.4.3 – Initially and at the end of each analytical batch of samples, analyze a dilution water sample that is air-saturated

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Continuing Calibration Verification (CCV) continued
 - Real people language – prepare dilution water that is air-saturated and analyze bottles and compare to the theoretical dissolved oxygen concentration (± 0.2 mg/L) after daily samples or with each batch of 20 samples or less in an 8-hour period.
- Batch Size
 - 9.3.1 – ... with each analytical batch of 20 samples or less in an 8 hour period.

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DO SM4500-O G – 2001 Membrane Electrode Method

- DOC
- Dup
- ICAL/CCV
- Corrective Action
- QC Acceptance
- Batch Size
- QC Frequency



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DO SM4500-O G – 2001 Membrane Electrode Method

- Demonstration of Capability
 - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
 - Follow Hach Method 10360 9.2.1 – Prepare and measure four samples of air-saturated water according to section 7.2.
 - 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
 - 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately $20 \pm 3^\circ\text{C}$.
 - 7.2.3 – With a steady gentle stream of filtered air ($\approx 10\text{-}40$ mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.

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DO SM4500-O G – 2001 Membrane Electrode Method

- Hach Method 10360 continued
 - 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature ($20 \pm 3^\circ\text{C}$) for 30 minutes and note the barometric pressure [uncorrected] of the laboratory during preparation. The barometric pressure reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.
 - 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
 - 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman...
 - Real people language – prepare dilution water that is air-saturated and analyze four bottles and compare to the theoretical dissolved oxygen concentration.

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Temp. (deg C)	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
0	10.95	11.14	11.33	11.53	11.72	11.91	12.11	12.29	12.49	12.68	12.87	13.07	13.27	13.46	13.65	13.85	14.04	14.23	14.43	14.62
1	10.64	10.83	11.02	11.21	11.39	11.58	11.77	11.96	12.15	12.34	12.52	12.71	12.9	13.09	13.28	13.46	13.65	13.84	14.03	14.22
2	10.35	10.53	10.72	10.9	11.08	11.27	11.45	11.63	11.82	12	12.18	12.37	12.55	12.73	12.91	13.1	13.28	13.46	13.65	13.83
3	10.07	10.25	10.43	10.61	10.79	10.96	11.14	11.32	11.5	11.68	11.86	12.03	12.21	12.39	12.57	12.75	12.93	13.11	13.28	13.46
4	9.81	9.98	10.15	10.33	10.5	10.68	10.85	11.02	11.2	11.37	11.54	11.72	11.89	12.07	12.24	12.41	12.59	12.76	12.93	13.11
5	9.55	9.72	9.89	10.06	10.23	10.4	10.57	10.74	10.91	11.08	11.25	11.42	11.59	11.75	11.92	12.09	12.26	12.43	12.6	12.77
6	9.31	9.47	9.64	9.8	9.97	10.14	10.31	10.47	10.63	10.8	10.96	11.13	11.29	11.46	11.62	11.79	11.95	12.12	12.28	12.45
7	9.08	9.24	9.4	9.56	9.72	9.88	10.04	10.2	10.37	10.53	10.69	10.85	11.01	11.17	11.33	11.49	11.65	11.82	11.98	12.14
8	8.85	9.01	9.17	9.33	9.48	9.64	9.8	9.95	10.11	10.27	10.43	10.58	10.74	10.9	11.06	11.21	11.37	11.53	11.69	11.84
9	8.64	8.79	8.95	9.1	9.25	9.41	9.56	9.71	9.87	10.02	10.18	10.33	10.48	10.64	10.79	10.94	11.1	11.25	11.41	11.56
10	8.43	8.58	8.73	8.88	9.03	9.18	9.33	9.49	9.64	9.79	9.94	10.09	10.24	10.39	10.54	10.69	10.84	10.99	11.14	11.29
11	8.24	8.38	8.53	8.68	8.82	8.97	9.12	9.26	9.41	9.56	9.71	9.85	10.01	10.15	10.29	10.44	10.59	10.73	10.88	11.03
12	8.05	8.19	8.33	8.48	8.62	8.77	8.91	9.05	9.2	9.34	9.48	9.63	9.77	9.91	10.06	10.2	10.35	10.49	10.63	10.78
13	7.86	8.01	8.15	8.29	8.43	8.57	8.71	8.85	8.99	9.13	9.27	9.41	9.55	9.69	9.83	9.97	10.11	10.26	10.4	10.54
14	7.69	7.83	7.97	8.11	8.24	8.38	8.52	8.65	8.79	8.93	9.07	9.2	9.34	9.48	9.62	9.76	9.89	10.03	10.17	10.31
15	7.52	7.66	7.79	7.93	8.06	8.2	8.33	8.47	8.6	8.74	8.87	9	9.14	9.27	9.41	9.54	9.68	9.81	9.95	10.08
16	7.36	7.49	7.62	7.76	7.89	8.02	8.15	8.28	8.42	8.55	8.68	8.81	8.95	9.08	9.21	9.34	9.47	9.61	9.74	9.87
17	7.2	7.33	7.46	7.59	7.72	7.85	7.98	8.11	8.24	8.37	8.5	8.63	8.76	8.89	9.02	9.15	9.28	9.41	9.54	9.66
18	7.05	7.18	7.31	7.44	7.56	7.69	7.81	7.94	8.07	8.2	8.32	8.45	8.58	8.7	8.83	8.96	9.09	9.21	9.34	9.47
19	6.91	7.03	7.16	7.28	7.41	7.53	7.66	7.78	7.9	8.03	8.15	8.28	8.4	8.53	8.65	8.78	8.9	9.03	9.15	9.28
20	6.77	6.89	7.01	7.13	7.26	7.38	7.5	7.62	7.75	7.87	7.99	8.11	8.24	8.36	8.48	8.6	8.73	8.85	8.97	9.09
21	6.63	6.75	6.87	6.99	7.11	7.23	7.35	7.47	7.59	7.71	7.83	7.95	8.07	8.19	8.31	8.43	8.55	8.67	8.79	8.91
22	6.5	6.62	6.74	6.85	6.97	7.09	7.21	7.33	7.45	7.56	7.68	7.8	7.92	8.04	8.15	8.27	8.39	8.51	8.63	8.74
23	6.37	6.49	6.61	6.72	6.84	6.95	7.07	7.19	7.3	7.42	7.53	7.65	7.77	7.88	8	8.11	8.23	8.35	8.46	8.58
24	6.25	6.36	6.48	6.59	6.71	6.82	6.94	7.05	7.16	7.28	7.39	7.51	7.62	7.73	7.85	7.96	8.08	8.19	8.3	8.42
25	6.13	6.24	6.35	6.47	6.58	6.69	6.81	6.92	7.03	7.14	7.25	7.37	7.48	7.59	7.7	7.81	7.93	8.04	8.15	8.26
26	6.02	6.13	6.24	6.35	6.46	6.57	6.68	6.79	6.9	7.01	7.12	7.23	7.34	7.45	7.56	7.67	7.78	7.89	8	8.11
27	5.9	6.01	6.12	6.23	6.34	6.45	6.56	6.67	6.77	6.88	6.99	7.1	7.21	7.32	7.43	7.53	7.64	7.75	7.86	7.97
28	5.8	5.9	6.01	6.12	6.23	6.33	6.44	6.54	6.65	6.76	6.87	6.97	7.08	7.19	7.29	7.4	7.51	7.61	7.72	7.83
29	5.69	5.8	5.9	6.01	6.11	6.22	6.32	6.43	6.53	6.64	6.74	6.85	6.95	7.06	7.16	7.27	7.38	7.48	7.59	7.69
30	5.59	5.69	5.79	5.9	6	6.11	6.21	6.31	6.42	6.52	6.63	6.73	6.83	6.94	7.04	7.14	7.25	7.35	7.46	7.56

Can be made at <http://water.usgs.gov/software/DOTABLES/>

DO SM4500-O G – 2001 Membrane Electrode Method

- Calibration
 - Calibrate daily (day of) by following manufacturer's instructions
 - Using barometric pressure is best
- Duplicates of the sample
 - Run on a 5% basis, one for every 20 samples
 - Calculate %RPD, ≤ 20%
 - **2014 Update – For reporting purposes, all duplicates should be reported according to your permit limits. If your permit sets a minimum, then the minimum value should be reported even if falls outside your permit limit.**

DO SM4500-O G – 2001 Membrane Electrode Method

- Continuing Calibration Verification (CCV) – daily
 - 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
 - 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately 20 ± 3°C.
 - 7.2.3 – With a steady gentle stream of filtered air (≈ 10-40 mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.
 - 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature (20 ± 3°C) for 30 minutes and note the barometric pressure of the laboratory during preparation. The barometric pressure reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.

DO SM4500-O G – 2001 Membrane Electrode Method

- Continuing Calibration Verification (CCV) continued
 - 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
 - 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman...
 - 9.3.1 – Upon air calibration, prepare a calibration verification standard with each analytical batch of 20 samples or less in an 8 hour period.
 - 9.4.3 – Initially and at the end of each analytical batch of samples, analyze a dilution water sample that is air-saturated
 - Real people language – prepare dilution water that is air-saturated and analyze bottles and compare to the theoretical dissolved oxygen concentration.

Temperature

- NIST (National Institute of Standards & Technology) traceable thermometer
- Scale marked in 0.1 °C
- Calibrate annually by checking against a NIST certified thermometer
- Corrections can be made up to ± 4 °C

Temperature

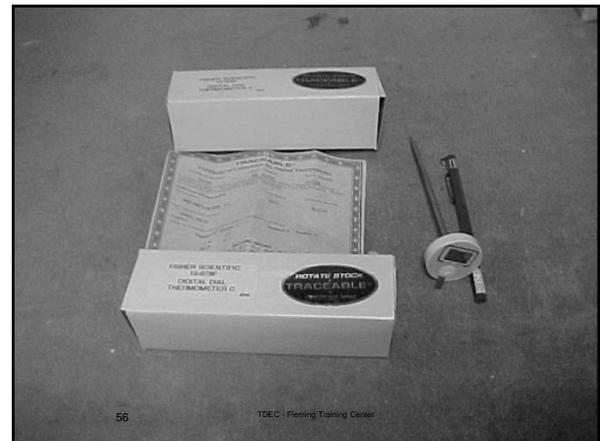
- Clean the probe end with deionized water
- Swirl the thermometer in the sample and allow at least one minute to equilibrate
- Suspend the thermometer away from the sides and bottom of container
- Record readings to nearest 0.5 °C
- May be measured insitu with probe if verified against NIST traceable thermometer

● ● ● Temperature Conversion

$$^{\circ}\text{F} = (9/5 ^{\circ}\text{C}) + 32$$

$$^{\circ}\text{C} = 5/9 (^{\circ}\text{F} - 32)$$

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● ● ● Temperature QA/QC

Parameter	Method	DOC	MDL	LRB	LFM	LFM / LFMID	Dup	ICAL / CCV	Control Charts	Corrective Action	QC Acceptance	Batch Size	QC Frequency
Temperature	SM2550 B - 2000							X, verify against NIST thermometer		X			Annually

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● ● ● Temperature SM2550 B – 2000 Thermometric Measurement

- ICAL
 - Have thermometers verified **annually** by an NIST thermometer
- Corrective Action
- QC Frequency

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Hach Method 10360
Luminescence Measurement of Dissolved Oxygen in Water and Wastewater and
for Use in the Determination of BOD₅ and cBOD₅
Revision 1.2
October 2011

1. Scope and Application

- 1.1 This method is for the measurement of dissolved oxygen (DO) in surface and ground water, municipal and industrial wastewater, and for use in Biochemical Oxygen Demand₅ (BOD₅) and carbonaceous Biochemical Oxygen Demand₅ (cBOD₅) determination.
- 1.2 The method may be used as a replacement for the modified Winkler and membrane electrode procedures for the measurement of DO in wastewater treatment processes such as aeration and biological nutrient basins, effluent outfalls, receiving water, and water for BOD₅ and cBOD₅ determination.
- 1.3 The method is for use in the United States Environmental Protection Agency's (EPA's) survey and monitoring programs for the measurement of DO and for the determination of BOD₅ and cBOD₅ under the Clean Water Act.
- 1.4 This method is capable of measuring DO in the range of 0.20 to 20 mg/L.
- 1.5 This method is restricted to luminescence probe technologies where calibration is performed by single-point water-saturated air (100% saturation).

2. Summary of Method

- 2.1 This luminescence-based sensor procedure measures the light emission characteristics from a luminescence-based reaction that takes place at the sensor-water interface. A light emitting diode (LED) provides incident light required to excite the luminophore substrate. In the presence of dissolved oxygen the reaction is suppressed. The resulting dynamic lifetime of the excited luminophore is evaluated and equated to DO concentration.

3. Interferences

- 3.1 There are no known agents that interfere with luminescence DO detection and quantification with this method.

4. Safety

- 4.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of any chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 17.5-17.6.

5. Equipment for the Measurement of Dissolved Oxygen

- 5.1 BOD bottle 300-mL with stoppers and plastic caps (Hach # 62016 and 241906, or equivalent).
- 5.2 Magnetic Stirring plate (optional)

- 5.3 Magnetic stirring device (optional)
- 5.4 Pipette, serological, 1-mL (Hach Catalog Number 919002, or equivalent)
- 5.5 Pipette, serological, 5-mL (Hach Catalog Number 53237, or equivalent)
- 5.6 Pipette, serological, 10-mL (Hach Catalog Number (53238, or equivalent)
- 5.7 Pipette Filler (Hach Catalog Number 1218900, or equivalent)
- 5.8 Meter and LBOD Probe (Hach Catalog Number 8508500) for DO measurement in BOD bottles, or equivalent as defined in Section 1.5 of this method)
- 5.9 Meter and LDO Probe (Hach Catalog Number 8505200 or 8506300) for DO measurement in open containers and water bodies, or equivalent as defined in Section 1.5 of this method)
- 5.10 Dispenser Cap, for Nitrification Inhibitor (Hach Catalog Number 4590)
- 5.11 Temperature controlled environment for BOD bottle incubation, $20 \pm 1^{\circ}$ C.

6. REAGENTS

- 6.1 Phosphate Buffer Solution - 1.7 g AR grade Ammonium Chloride, 8.5 g Potassium Phosphate Monobasic, 17.7 g Sodium Phosphate Dibasic, 21.7 g Potassium Phosphate Dibasic diluted to 1000 mL with deionized water, APHA, pH 7.2, (Hach Catalog Number 43149, or equivalent)
- 6.2 Calcium Chloride Solution - 27.5 g AR grade Calcium Chloride diluted to 1000 mL with deionized water, APHA, for BOD (Hach Catalog Number 42849, or equivalent)
- 6.3 Ferric Chloride Solution - 0.25 g Ferric Chloride diluted to 1000 mL with deionized water, APHA, for BOD (Hach Catalog Number 42953, or equivalent)
- 6.4 Glucose-glutamic Acid - Standard Solution, Voluette™ Ampoule, 300-mg/L, 150 mg glucose and 150 mg glutamic Acid to 1000 mL in deionized water, 10 mL (Hach Catalog Number 1486510, or equivalent) or, ezGGA Ampoules, 450 mg/L, 225 mg/L Glucose and 225 mg/L Glutamic Acid, (Hach Catalog Number 25144-20, or equivalent)
- 6.5 Magnesium Sulfate Solution - 22.5 g Magnesium Sulfate diluted to 1000 mL with deionized water, APHA, (Hach Catalog Number 43094, or equivalent)
- 6.6 Nitrification Inhibitor - (Hach Catalog Number 253334)
- 6.7 Potassium Iodide Solution (100 g AR grade Potassium Iodide diluted to 1000 mL with deionized water) (Hach Catalog Number 1228949, or equivalent)
- 6.8 Sodium Thiosulfate Solution – 0.025 N (Hach Catalog Number 35253, or equivalent)
- 6.9 Sodium Hydroxide Solution - 1 N (Hach Catalog Number 104532, or equivalent)
- 6.10 Sodium Hydroxide Pellets - ACS (Hach Catalog Number 18734, or equivalent)
- 6.11 Starch Indicator - 5.5 g AR grade Starch, and 1.25 g AR grade Salicylic Acid diluted to 1000 mL with deionized water (Hach Catalog Number 34932, or equivalent)
- 6.12 Sulfuric Acid Solution - 0.020 N (Hach Catalog Number 104532, or equivalent)
- 6.13 Sulfuric Acid Solution - 1.000 N (Hach Catalog Number 127053, or equivalent)

Note: The Phosphate Buffer Solution should be refrigerated to decrease the rate of biological growth.

7. Standards for Calibration

7.1 Initial LDO/LBOD Probe Calibration

7.1.1 Add approximately 1 inch (2.54 cm) of reagent water to a clean BOD bottle and stopper.

7.1.2 Shake vigorously for ~ 10 seconds.

7.1.3 Allow for the BOD bottle and its contents to equilibrate to room temperature. Room temperature should be approximately $20 \pm 3^{\circ}\text{C}$.

7.1.4 The stopper may now be removed from the BOD bottle and the LBOD probe inserted for calibration purposes.

7.1.5 The luminescence technology for measuring dissolved oxygen is a superior technique from that of Winkler titration and membrane potentiometric measurement and has no interferences associated with the oxygen detection process. Therefore, for calibration and measurement purposes, do not adjust the calibration luminescence measurement to that of Winkler or membrane measurement readings.

Note: Section 7.1 is a suggested procedure for the preparation of water-saturated air. Other procedures for the preparation of water-saturated air may be used that are equally effective.

7.2 Calibration Verification, Initial Precision and Recovery, and On-going Precision and Recovery

7.2.1 Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle.

7.2.2 Allow the water to equilibrate to room temperature. Room temperature should be approximately $20 \pm 3^{\circ}\text{C}$.

7.2.3 With a steady gentle stream of filtered air ($\approx 10 - 40$ mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.

7.2.4 At the completion of aeration, let water re-equilibrate to room temperature ($20 \pm 3^{\circ}\text{C}$) for 30 minutes and note the barometric pressure of the laboratory during preparation. The barometric pressure reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.

7.2.5 Transfer the aerated water to a BOD bottle until overflowing and stopper.

7.2.6 Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman referenced in Section 17.2 of this method.

Note: Section 7.2 is a suggested procedure for the preparation of air-saturated water. Other procedures for the preparation of air-saturated water may be used that are equally effective.

8. Sample Collection Preservation and Storage

8.1 Samples should be collected in polyethylene, fluoropolymer, polypropylene, or glass containers.

8.2 Immediately upon collection, cool samples to $\leq 6^{\circ}\text{C}$.

- 8.3 Prepare samples within 48 hours of collection and preservation.
- 8.4 See Title 40 of the Code of Federal Regulations Part 136.3, Table II for further information regarding required sample collection containers, preservation techniques and holding times for collection of water for measurement of DO and for the determination of BOD5 and cBOD5.

9. Quality Control

9.1 It is recommended that each laboratory that uses this method be required to operate a formal quality assurance program (Reference 17.1). The minimum requirements of this program consist of an initial demonstration of laboratory capability and ongoing analyses of laboratory prepared water standards as a test of continued performance to assess accuracy and precision. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 9.2.

9.1.2 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control. These procedures are described in Sections 9.3 and 9.4, respectively.

9.1.3 Accompanying QC for the determination of DO is required per analytical batch. An analytical batch is a set of samples processed during a contiguous 8-hour period, not to exceed 20 samples. Each analytical batch should be accompanied by a calibration verification and ongoing precision and recovery sample, resulting in a minimum of three analyses (1 CV, 1 sample, and 1 OPR). Perform additional CV and OPR for each batch that exceeds 20 samples.

9.2 Initial Demonstration of Laboratory Capability

9.2.1 Initial precision and recovery (IPR) - To establish the ability to generate acceptable precision and accuracy for the measurement of DO in water, the analyst shall perform the following operations:

9.2.1.1 Prepare and measure four samples of the IPR standard (Section 7.2) according to the procedure beginning in Section 11.

9.2.1.2 Using the results of the set of four analyses, compute the average percent recovery (\bar{X}) and the standard deviation of the percent recovery (s) for DO. Use the following equation for calculation of the standard deviation of the percent recovery:

$$s = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

where:

n = Number of samples

x = Concentration in each sample

s = standard deviation of the percent recovery

9.2.1.3 Compare s and \bar{X} with the corresponding limits for initial precision and recovery in Section 18, Table 4. If s and \bar{X} meet the acceptance criteria, system performance is

acceptable and analysis of samples may begin. If, however, s exceeds the precision limit or X falls outside the range for recovery, system performance is unacceptable. In this event correct the problem, and repeat the test.

9.3 Calibration Verification

9.3.1 Upon air calibration, prepare a calibration verification standard (Section 7.2) with each analytical batch of 20 samples or less in an 8 hour period. Analyze according to the procedure beginning in Section 11 and compare the recovery results to those in Section 18, Table 4.

9.4 Ongoing Calibration and Precision and Recovery

9.4.1 To demonstrate that the analysis system is in control, and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations

9.4.2 Prepare a precision and recovery standard (Section 7.2) with each analytical batch according to the procedure beginning in Section 11.

9.4.3 Initially, and at the end of each analytical batch of samples, analyze a precision and recovery standard and compare the concentration recovery with the limits for ongoing precision and recovery in Tables 4 and 5. If the recovery is in the range specified, measurement process is in control and analysis of samples may proceed. If, however, the recovery is not in the specified range, the analytical process is not in control. In this event, correct the problem, recalibrate and verify the calibration and reanalyze analytical batch, repeating the ongoing precision and recovery test.

9.4.4 The laboratory should add results that pass the specification in Tables 4 and 5 to IPR and previous OPR data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of the percent recovery (sr). Express the accuracy as a recovery interval from $R - 2sr$ to $R + 2sr$. For example, if $R = 95\%$ and $sr = 5\%$, the accuracy is 85% to 105%.

9.5 Depending upon specific program requirements, field replicates may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9.6 Glucose-Glutamic Acid Seed Strength Check

9.6.1 Many factors can influence the BOD analysis (toxicity from sample matrix, contaminated dilution water, poor quality seed, etc. In order to insure sufficient seeding in the BOD test, a glucose-glutamic acid check is performed in parallel with each day's BOD₅ and cBOD₅ test samples. Well prepared dilution water and an active seed will produce a BOD₅ of 198 ± 30 mg/L BOD (Reference 17.7).

9.6.1.1 Prepare in triplicate a 300-mL BOD bottle with 3.0 mL of the 300 mg/L Standard Solution of GGA (Section 6.4) or 1 ampoule (2.0 mL) of ezGGA (Section 6.4) with each day of samples prepared for BOD determination.

9.6.1.2 When using ezGGA ampoules, place the ezGGA ampoule in the ampoule breaker (provided with ezGGA ampoules) and rinse the assembly with reagent water. Hold the ampoule and breaker over the rim of the BOD bottle, break and allow the ampoule to fall into the BOD bottle. Leave the ampoule in the BOD bottle during the incubation period and reading of DO.

GGA Standard Solution

$$\frac{3.0 \text{ mL} \times 0.300 \text{ mg/mL GGA}}{300 \text{ mL final volume}} \times 1000 \text{ mL/L} = 3.0 \text{ mg/L GGA per bottle}$$

ezGGA Ampoule

$$\frac{1 \text{ ampoule (2.0 mL)} \times 0.450 \text{ mg/mL GGA}}{300 \text{ mL final volume}} \times 1000 \text{ mL/L} = 3.0 \text{ mg/L GGA per bottle}$$

9.6.1.2 Add seed at three different volumes (typically 4 mL, 6 mL, and 8 mL,) to the GGA bottles. Other volumes may be required, depending on the strength of the seed being used.

9.6.1.3 Bring to volume with dilution water and analyze as described in Sections 12.9 and 12.10.

Note: GGA BOD₅ recovery results outside of 198 ± 30 mg/L should be investigated as to causation. If toxicity of dilution water has been ruled out as a probable cause for low recovery, it is likely that the seed is of low activity or poor quality. Either increase the seed amount or use a seed of higher quality. High GGA recoveries are generally due to incorrect amount of GGA Standard Solution.

10. Calibration and Standardization

10.1 Because of the possible diversity of future LDO instrument hardware and, no detailed operating conditions are provided. The analyst is advised to follow the recommended operating conditions provided by the manufacturer. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions satisfy the analytical requirements of this method and to maintain quality control data verifying instrument performance and analytical results.

10.2 Water-saturated air (Section 7.1) is used for instrument calibration.

10.3 Calibration verification (Section 7.2) is performed with air-saturated water prior to any DO sample measurements to the method specifications in Section 14.

11. Procedure for Measuring DO in Grab Samples, Outfalls, and Open Water Bodies

11.1 Instrument Setup

11.1.1 Follow the instrument manufacturer's instructions for instrument setup (Hach Document DOC022.53.80021 for Hach LDO IntelliCal™ Rugged and Standard Probes, Hach Document Number DOC022.53.80116 for LBOD probes, and Hach Catalog Number 5790018 for Hach LDO process probes.

Note: Manufacturer's instructions are only for instrument set and use. These instructions do not preclude the calibration and performance requirements of this method.

11.2 Measurement of DO

11.2.1 For samples in an open vessel, container, or water body, place the LDO probe into the water sample to be measured and stir gently with probe or add a stir bar. Do not put the probe on the bottom or sides of the container. Stir the sample at a moderate rate or put the probe in flowing conditions. Read sample. The display will show "Stabilizing" and a progress bar as the probe stabilizes in the sample. The display will show the lock icon when the reading stabilizes.

- 11.3.1 For BOD₅ and CBOD₅ prepared samples, insert the LBOD probe into the BOD bottle for DO determination. Insure that there are no air bubbles that may have collected around the probe or sensor. Turn on the stir paddle and read sample. The display will show "Stabilizing" and a progress bar as the probe stabilizes in the sample. The display will show the lock icon when the reading stabilizes.

12. Procedure for the Preparation and Determination of BOD₅ and cBOD₅ Samples

- 12.1 The BOD test is a 5-day test. Follow all steps carefully to make sure that the test does not have to be repeated.
- 12.2 The dilution water for this test must be fully air-saturated immediately before use and determined to not have an oxygen demand or any toxins. When incubated for 5 days at $20 \pm 1^\circ \text{C}$, the dissolved oxygen concentration in the dilution water must not change by more than 0.2 mg/L. Air-saturation is a function of water temperature and laboratory barometric pressure. Use an oxygen saturation table such as in Section 17.2 of this method to insure full air-saturation of dilution water.
- 12.3 Distilled Water Preparation
- 12.3.1 The distilled water must be prepared very carefully to make sure that no source of oxygen demand or toxins are added. The water that is used to prepare the dilution water must be of very high quality. The water must not have any organic compounds or any toxic compounds such as chlorine, copper, and mercury at a concentration level that would interfere would the BOD seed and inhibit microbiological growth of organisms.
- 12.3.2 For best results, use an alkaline permanganate distillation for preparing dilution water. Resin in ionization cartridges will occasionally release organic materials that have an oxygen demand.
- 12.3.3 Store the distilled water in clean jugs at a temperature of $20 \pm 3^\circ \text{C}$. Fill the containers to about $\frac{3}{4}$ full and shake the jugs to saturate the water with air. Alternatively, saturate the water with air as described in Section 7.2.1. A small aquarium pump or air compressor can be used to saturate the water with air. Insure that the air is filtered and that the air filter does not grow bacteria.
- 12.4 Dilution Water Preparation
- 12.4.1 Using the distilled water prepared above in Section 12.3, select a BOD nutrient buffer pillow from the BOD nutrient buffer pillows in Table 1.
- 12.4.2 Add the contents of the BOD Nutrient Buffer Pillow to the distilled water in a jug with ample headspace. Cap the jug and shake vigorously for one minute to dissolve the nutrients and to saturate the water with air.
- 12.4.3 Alternatively, prepare the dilution water by adding 1 mL each of the following solutions per liter of distilled water prepared in Section 12.4:
- Phosphate Buffer Solution – 1.7 g AR grade Ammonium Chloride, 8.5 g Potassium Phosphate Mono Basic, 17.7 g Sodium Phosphate Dibasic, 21.7 g Potassium Phosphate Dibasic to 1000 mL with deionized water, APHA, pH 7.2, (Hach Catalog Number 43149, or equivalent)
- Calcium Chloride Solution - 27.5 g AR grade Calcium Chloride to 1000 mL with deionized water, APHA, for BOD (Hach Catalog Number 42849, or equivalent)

Ferric Chloride Solution - 0.25 g Ferric Chloride to 1000 mL deionized water, APHA, for BOD (Hach Catalog Number (42953, or equivalent)

- 12.5.4 Cap the jug and shake vigorously for one minute to dissolve the nutrients and to saturate the water with air.

Note: Dilution water should be prepared immediately before use unless it can be demonstrated that the dilution water blank has no DO depletion greater than 0.2 mg/L.

12.5 Seed Preparation

- 12.6.1 Use raw sewage or other reliable sources for the bacterial seed that will yield 198 ± 30 mg/L BOD with the GGA check sample in Section 9.6. Potential seed sources include wastewater influent, primary effluent, soil, and domestic sewage.
- 12.6.2 Allow raw sewage to stand undisturbed at $20 \pm 3^\circ\text{C}$ for 24 to 36 hours before use.
- 12.6.3 When seeding samples with raw sewage, always pipette from the upper portion of the sewage.

12.7 Sample Size Selection Guide

- 12.7.1 Make an estimate of the sample volumes that are necessary for the test. At least 2.0 mg/L of DO should be consumed during the test and at least 1.0 mg/L of un-depleted DO should remain in the bottle.
- 12.7.2 Samples such as raw sewage will have a high BOD. Small sample volumes must be used because large samples will deplete all of the oxygen in the sample. Samples with a low BOD must use larger sample volumes to insure that adequate oxygen is depleted to give accurate results.
- 12.7.3 Refer to the Minimum Sample Volume in Table 2 to select the minimum sample volume. For example, if a sewage sample is estimated to contain 300 mg/L BOD, the minimum sample volume is 2 mL. For sewage effluent with an estimated BOD of 40 mg/L, the minimum sample volume is 15 mL.
- 12.7.4 Refer to the Maximum Sample Volume in Table 3 to select the maximum sample volume. At 1000 in elevation, with an estimated BOD₅ of 300 mg/L, the largest sample volume is 8 mL. For a BOD of 40 mg/L, the maximum volume of sample is 60 mL.

12.8 Sample Matrix Pretreatment

- 12.8.1 Determine the pH of each sample at a sample temperature of $20 \pm 3^\circ\text{C}$, prior to BOD sample preparation. For samples that have pH of less than 6 or greater than 8, adjust the pH accordingly with a solution of sulfuric acid (H₂SO₄) or sodium hydroxide (NaOH). Strength of pH adjustment solution should be at a concentration that does not dilute the sample by greater than 0.5 percent. Always seed samples that have been pH adjusted
- 12.8.2 For sample matrices that contain residual chlorine, de-chlorinate with a solution of Sodium Thiosulfate (Na₂S₂O₃).
- 12.8.2.1 Measure 100 mL of sample into a 250 mL Erlenmeyer flask. Using a 10-mL serological pipette and pipette filler, add 10 mL of 0.020 N Sulfuric Acid Standard Solution and 10 mL of Potassium Iodide Solution, 100-g/L, to the flask.
- 12.8.2.2 Add three full droppers of Starch Indicator Solution and swirl to mix.

12.8.2.3 Fill a 25-mL burette with 0.025 N Sodium Thiosulfate Standard Solution and titrate the sample from dark blue to colorless.

12.8.2.4 Calculate the amount of 0.025 N Sodium Thiosulfate Solution to add to the sample:

$$\text{mL } 0.025 \text{ N Sodium Thiosulfate required} = \text{mL titrant used} \times \text{volume of remaining sample divided by } 100$$

12.8.2.5 Add the required amount of 0.025 N Sodium Sulfate Standard Solution to the sample. Mix thoroughly and wait 10 to 20 minutes before performing the BOD test.

Note: Samples should be brought to a temperature of $20 \pm 3^{\circ}$ C. prior to making dilutions.

12.9 Sample Preparation

12.9.1 Select the sample volume as described in Section 12.7. Select a minimum of three different volumes for each sample.

12.9.1.1 If the minimum sample volume is 3 mL or more, determine the DO concentration in the undiluted sample; this determination can be omitted when analyzing sewage and settled effluents known to have dissolved oxygen content near 0 mg/L.

12.9.2 Stir the sample gently with a pipette. Use the pipette to add the determined sample volumes to the BOD bottles.

12.9.3 Add the appropriate seed to the individual BOD bottles as described in Section 12.6.

12.9.3.1 Separately, with each batch of BOD samples, prepare a seed sample with dilution water. Measure the BOD of the seed for subtraction from the sample BOD.

12.9.3.2 A seed that has a BOD₅ of 200 mg/L (a typical range for domestic sewage) will typically deplete at least 0.6 mg/L DO when added at a rate of 3 mL/L of dilution water.

12.9.4 If the test is for cBOD₅, add two portions of Nitrification Inhibitor (approximately 0.16 g) to each bottle. The oxidation of nitrogen-based compounds will be prevented.

12.9.5 Fill each bottle to just below the lip with dilution water.

12.9.5.1 Allow the dilution water to flow down the sides of the bottle to prevent air bubbles from becoming entrapped in the bottle.

12.9.6 Fill an additional BOD bottle with only dilution water. This will be the dilution water blank.

12.9.7 Stopper the bottles carefully to prevent air bubbles from becoming entrapped.

12.9.7.1 Tightly twist the stopper and invert the bottles several times to mix.

Note: The sample preparation procedures in Section 12.9 are designed for a BOD sample analysis volume of 300 mL. A 60-mL sample preparation volume may also be used.

12.10 Sample Analysis

12.10.1 Measure the initial dissolved oxygen concentration in each bottle with the LBOD probe within 30 minutes of sample preparation.

12.10.2 After the initial DO measurement, stopper the bottles carefully to prevent air bubbles from becoming entrapped.

12.10.2.1 Add dilution water to the lip of each BOD bottle to make a water seal.

12.10.3 Place a plastic cap over the lip of each bottle and incubate at $20 \pm 1^\circ\text{C}$ for five days.

12.10.4 After 5 days, measure the remaining dissolved oxygen concentration in each bottle with the LBOD probe.

12.10.4.1 At least 1.0 mg/L DO should have remained in each bottle.

12.10.4.2 Discard results of samples where the DO is depleted below 1.0 mg/L.

13. BOD and cBOD Calculations

13.1 When Dilution Water Not Seed (generally influent and primary treated influent to treatment)

$$\text{BOD}_5 \text{ or cBOD}_5, \text{ mg/L} = \frac{D_1 - D_2}{P}$$

where:

BOD₅ or cBOD₅ = BOD value from the 5-day test

D₁ = DO of diluted sample immediately after preparation, in mg/L

D₂ = DO of diluted sample after 5 day incubation at $20 \pm 1^\circ\text{C}$, in mg/L

P = Decimal volumetric fraction of sample used

13.2 When Dilution Water Requires Seed

$$\text{BOD}_5 \text{ or cBOD}_5, \text{ mg/L} = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

as defined above plus:

B₁ = DO of seed control before incubation, in mg/L

B₂ = DO of seed control after incubation, in mg/L

f = ratio of seed in diluted sample to seed in seed control (% seed in diluted sample/%seed in seed control) or, if seed material is added directly to sample or to seed-control bottles:

f = (volume of seed in diluted sample/volume of seed in seed control)

13.3 Averaged Results

13.3.1 Averaged results from different dilutions are acceptable if more than one sample dilution meets all of the following criteria:

13.3.1.1 The remaining un-depleted DO is at least 1 mg/L.

13.3.1.2 The final DO value is at least 2 mg/L lower than the initial prepared sample DO

13.3.1.3 There is no evidence of toxicity at higher sample concentrations

14. Method Performance for Dissolved Oxygen in Reference Water and GGA BOD₅ Recovery

Acceptance Criterion	Section	Limit
Initial DO Accuracy in Reagent Water	9.2.1	95% to 105%
Initial Precision in Reagent Water	9.2.1	2.1%
On-going DO Accuracy	9.4.1	95% to 105%

15. Pollution Prevention

- 15.1 There are no standards or reagents used in this method that when properly disposed of, pose any threat to the environment.

16. Waste Management

- 16.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect air, water, and land by minimizing and control all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.
- 16.2 For further information on waste management, consult "The Waste Management manual for Laboratory Personnel", and Less is Better: Laboratory Chemical Management for Waste Reduction", both available from the American Society's Department of Government relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

17. References

- 17.1 Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA, EMSL-CI, Cincinnati, OH 45268, EPA-600-4-79-019, March 1979.
- 17.2 Hitchmen, M.L. (1978) *Chemical analysis*. Vol. 49. *Measurement of Dissolved Oxygen*. Wiley and sons, New York.
- 17.3 Title 40, Code of Federal Regulations (40 CFR), Part 136.
- 17.4 Protocol for EPA Approval of New Methods for Organic and Inorganic Analytes in Wastewater and Drinking Water (EPA-821-B-98-003, March 1999).
- 17.5 "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976)
- 17.6 "Safety in Academic Chemistry Laboratories," American Chemical Society, Committee on Chemical Safety, 3rd Edition, 1979.
- 17.7 "Standard Methods for the Examination of Water and Wastewater", 20th Edition; American Public health Association: 1015 Fifteenth Street, NW, Washington, D.C. 2005, 1998; Method 5210B.

18. Tables

18.1 Nutrient Buffer Preparation Options

Table 1 - BOD Nutrient Buffer Pillows

Volume of Dilution Water to Prepare	Hach BOD Nutrient Pillow Catalog Number
300 mL) add pillow to each BOD Bottle	1416066
3 liters	1486166
4 liters	2436466
6 liters	1486266
19 liters	1486398

Note: Hach BOD Nutrient Pillows are formulated with the same reagents adjusted for volume preparation as in Sections 6.1 through 6.3.

18.2 Sample Volume Selection Guides

Table 2 - Minimum Sample Volume Selection Guide

Sample Type	Estimated BOD mg/L	Minimum Sample Volume (mL)
Strong Waste	600	1
Raw and Settled Sewage	300	2
	200	3
	150	4
	100	6
	75	8
	60	10
Oxidized Effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted River Water	6	100
	4	200
	2	300

Table 3 – Maximum Sample Volume Selection Guide

BOD at Sea Level (mg/L)	BOD at 1000 ft Elevation (mg/L)	BOD at 5000 feet Elevation (mg/L)	Maximum Sample Volume (mL)
615	595	508	4
492	476	406	5
410	397	339	6
304	294	251	8
246	238	203	10
205	198	169	12
164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

Note: Samples with higher concentrations of BOD should be pre-diluted.

18.3 Performance Criteria

Table 4 - Initial Precision and Recovery Method Performance

IPR Range	IPR DO Conc. (mg/L)	97.5% Lower Limit of Recovery (%)	97.5% Upper Limit of Recovery (%)	95% Upper Limit of Precision (%)
High	7.22 – 9.23	95.8	104.8	1.26

Table 5 - Calibration Verification Performance

CV DO Concentration	Average % Recovery	% Standard Deviation	% Relative Standard Deviation
7.22 mg/L – 9.23 mg/L	100.1	2.5	2.5

19. Glossary of Definitions and Purposes

The definitions and purposes are specified to this method but have been conformed to common usage as much as possible.

19.1 Units of Weight and Measure and their Abbreviations

19.1.1 Symbols
°C degrees Celsius

19.1.2 Alphabetical characters
mg/L milligram per liter

19.2 Definitions, acronyms, and abbreviations

19.2.1 LDO[®] - Luminescence dissolved oxygen

19.2.2 LBOD[®] - Luminescence biochemical oxygen demand

19.2.3 BOD - Biochemical oxygen demand

19.2.4 BOD₅ - Biochemical oxygen demand, 5-day test

19.2.5 cBOD₅ - Carboneous biochemical oxygen demand, 5-day test

19.2.6 DO: Dissolved oxygen

19.2.75 CV: Calibration verification

19.2.8 IPR: Initial precision and recovery

19.2.9 OPR: On-going precision and recovery

Oxygen, Dissolved

Direct Measurement Method¹

Method 10360**0.1 to 20.0 mg/L (or 1 to 200% saturation) O₂****LDO probe****Scope and application:** For water, wastewater and process water applications.

¹ Luminescent dissolved oxygen is USEPA approved for dissolved oxygen measurements in wastewater treatment process (e.g., aeration and biological nutrient basins, effluent outfalls and receiving water). Refer to USEPA 40CFR Part 136.3.



Test preparation

Instrument-specific information

This procedure is applicable to the meters and probes that are shown in [Table 1](#). Procedures for other meters and probes can be different.

Table 1 Instrument-specific information

Meter	Probe
HQ30d portable single input, multi-parameter HQ40d portable dual input, multi-parameter HQ430d benchtop single input, multi-parameter HQ440d benchtop dual input, multi-parameter	IntelliCAL LDO101 LDO

Before starting

Refer to the meter documentation for meter settings and operation. Refer to probe documentation for probe preparation, maintenance and storage information.

Prepare the probe before initial use. Refer to probe documentation.

When an IntelliCAL™ probe is connected to an HQd meter, the meter automatically identifies the measurement parameter and is prepared for use.

The IntelliCAL LDO101 probes automatically adjust for barometric pressure, elevation and temperature.

Do not touch the probe cap with a hand, fingers or any surface that can scratch the cap.

Prepare the probe before initial use. Refer to probe documentation.

Condition the probe before use. To condition the probe, put the probe in 100 mL of tap water for 30 minutes before use.

For probes that are continuously in aqueous solutions, condition the sensor cap for 72 hours.

Calibrate the probe before initial use. Refer to [Calibration](#) on page 3.

Salinity affects the concentration of dissolved oxygen in the sample. To correct for salinity effects, refer to the probe documentation.

Analyze the samples immediately. The samples cannot be preserved for later analysis.

Stir the samples at a slow and constant rate to prevent the formation of a vortex.

Air bubbles under the sensor tip can cause slow response or measurement errors. To remove the bubbles, carefully shake the probe.

For rugged electrodes, it may be necessary to remove the shroud before measurement and calibration.

Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.

Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

Description	Quantity
BOD bottle, 300 mL or Erlenmeyer flask, 250 mL	1
Beaker (laboratory test)	1

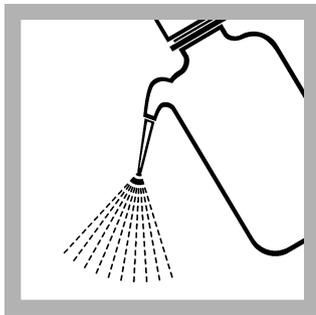
Refer to [Consumables and replacement items](#) on page 4 for order information.

Sample collection

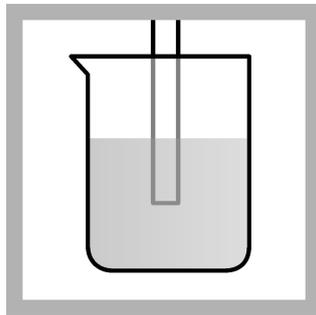
The main consideration with sample collection is to prevent contamination of the sample with atmospheric oxygen.

- Analyze the samples immediately. The samples cannot be preserved for later analysis.
- Analyze the samples at the collection site if possible.
- Do not introduce air into the sample.

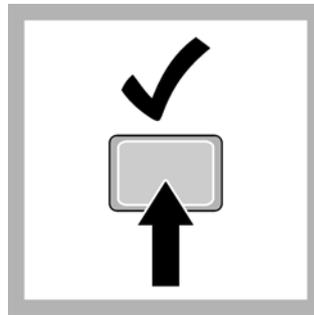
Test procedure



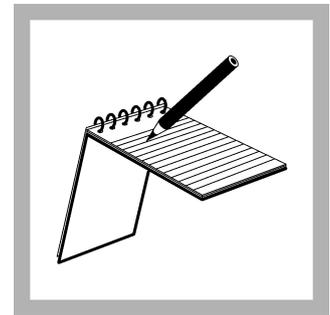
1. Rinse the probe with deionized water. Dry the probe with a lint-free cloth.



2. Laboratory test: Put the probe in a beaker that contains the solution. Do not let the probe touch the stir bar, bottom or sides of the container. Remove the air bubbles from under the probe tip. Stir the sample at a slow to moderate rate.
Field test: Put the probe in the sample. Move the probe up and down to remove bubbles from the probe tip.



3. Push **Read**. A progress bar is shown. When the measurement is stable, the lock icon is shown.

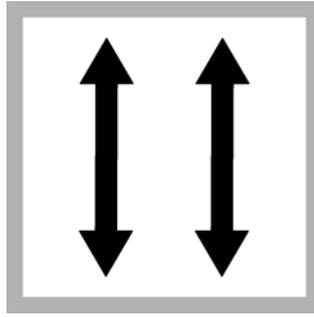


4. When the value is stable, store or record the mV value and the temperature value.

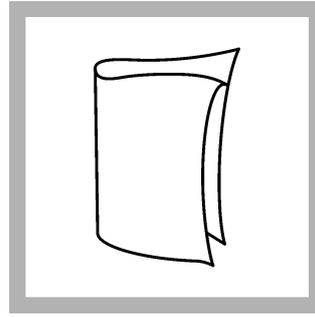
Calibration



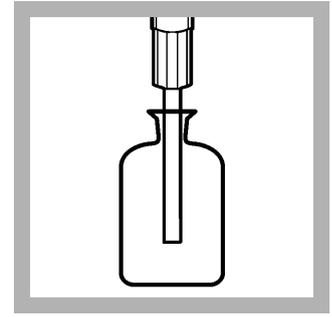
1. Add a small amount of water (approximately 1 cm) to the bottom of narrow-neck bottle, such as a BOD bottle.



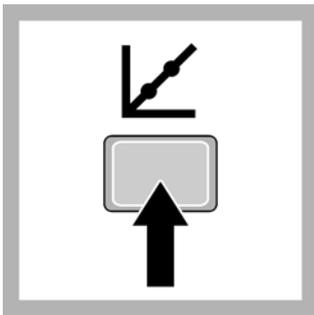
2. Insert a stopper and shake the bottle vigorously for several minutes.



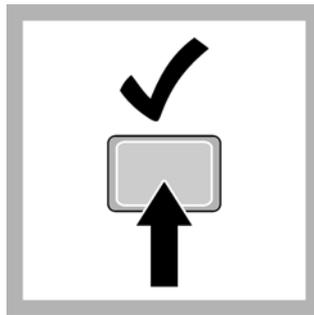
3. If the probe cap is wet, carefully dry the probe cap with a soft cloth.



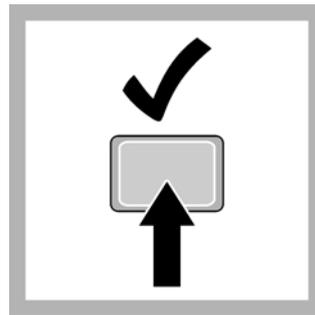
4. Remove the stopper. Put the probe in the bottle.



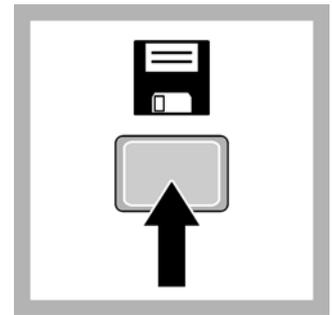
5. Push **Calibrate**.



6. Push **Read**. A progress bar is shown. When the measurement is stable, the lock icon is shown.



7. Push **Done**. The calibration summary shows. The slope value is the comparison between the latest calibration and the factory calibration shown as a percentage.



8. Push **Store** to accept the calibration.

Clean the probe

Clean the probe when:

- Drifting/inaccurate readings occur as a result of contamination on the sensing element or incorrect storage conditions.
- Slow response time occurs as a result of contamination on the sensing element.

For general contamination, complete the steps that follow.

1. Rinse the probe with deionized water. Blot dry with a lint-free cloth.
2. If harsh contaminants are attached to the probe, polish the probe tip with a soft cloth or cotton swab to remove the contaminants.
3. Soak the probe in deionized water for 1 minute.

Summary of method

The oxygen sensor is a clear, oxygen impermeable hard substrate. An oxygen-sensitive luminescent dye and a scattering agent is on the substrate. A final overlay of dark pigment is added to keep stray light out of the measurement cell. The luminescent dye gives a red light when exposed to blue light. The scattering agent distributes the light in the sensor matrix and contributes to the opacity of the sensor. Pulses from a red LED are used as an internal reference. The duration of the luminescence is proportional to the concentration of dissolved oxygen in the sample.

Consumables and replacement items

HQd meters and probes

Description	Unit	Item no.
HQ30d portable single input, multi-parameter meter	each	HQ30D53000000
HQ40d portable dual input, multi-parameter meter	each	HQ40D53000000
HQ430d benchtop single input, multi-parameter meter	each	HQ430D
HQ440d benchtop dual input, multi-parameter meter	each	HQ440D
IntelliCAL™ LDO101 luminescent/optical dissolved oxygen (LDO) probe, 1 m cable	each	LDO10101
IntelliCAL™ LDO101 luminescent/optical dissolved oxygen (LDO) probe, 3 m cable	each	LDO10103
IntelliCAL™ LDO101 luminescent/optical dissolved oxygen (LDO) probe, 5 m cable	each	LDO10105
IntelliCAL™ LDO101 luminescent/optical dissolved oxygen (LDO) probe, 10 m cable	each	LDO10110
IntelliCAL™ LDO101 luminescent/optical dissolved oxygen (LDO) probe, 15 m cable	each	LDO10115
IntelliCAL™ LDO101 luminescent/optical dissolved oxygen (LDO) probe, 30 m cable	each	LDO10130

Accessories

Description	Unit	Item no.
BOD bottle with glass stopper, 300 mL	each	62100
BOD bottle with glass stopper, 300 mL	6/pkg	62106
BOD bottle with glass stopper, 300 mL	24 pkg	62124
Beaker, polypropylene, 50-mL, low form	each	108041
Flask, Erlenmeyer, 250-mL	each	50546
Probe clips, color-coded, for IntelliCAL probes	50/pkg	5818400
Probe, depth markers, rugged LDO probe only	10/pkg	5828610
Probe stand, universal	each	8508850



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
 In the U.S.A. – Call toll-free 800-227-4224
 Outside the U.S.A. – Contact the HACH office or distributor serving you.
 On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
 WORLD HEADQUARTERS
 Telephone: (970) 669-3050
 FAX: (970) 669-2932

Section 5 Biochemical Oxygen Demand





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BIOCHEMICAL OXYGEN DEMAND

BOD

1

OUTLINE

- Introduction to BOD
 - What is BOD?
 - Why is BOD Important?
 - BOD Calculations



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OUTLINE

- Laboratory
 - Set up BOD Bottles
 - Measure Initial DO
- How is BOD Run?



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WHAT IS BOD?

- Biochemical Oxygen Demand is the amount of oxygen, expressed in mg/L or parts per million (ppm), that bacteria take from the water when they oxidize organic matter.

(Hach, Clifford; R. Klein; C. Gibbs. Introduction to Biochemical Oxygen Demand. Hach Company, 1997.)

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WHAT IS BOD?

- BOD is a measure of organic strength of the wastewater:
 - Changes in dissolved oxygen concentration are used as an indirect measure of organic content.

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HISTORY OF BOD

- The Royal Commission on River Pollution, which was established in 1865 and the formation of the Royal Commission on Sewage Disposal in 1898 led to the selection in 1908 of BOD₅ as the definitive test for organic pollution of rivers.
- Five days was chosen as an appropriate test period because this is supposedly the longest time that river water takes to travel from source to the ocean in the U.K.

Source: http://en.wikipedia.org/wiki/Biochemical_oxygen_demand

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HISTORY OF BOD

- o In 1912, the commission also set a standard of 20 mg/L BOD₅ as the maximum concentration permitted in sewage works discharging to rivers, provided that there was at least an 8:1 dilution available at dry weather flow.
- o This was contained in the famous 20:30 (BOD:Suspended Solids) + full nitrification standard that was used as a yardstick in the U.K. up to the 1970s for sewage works effluent quality.

Source: http://en.wikipedia.org/wiki/Biochemical_oxygen_demand

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HISTORY OF BOD

- o The United States includes BOD effluent limitations in its secondary treatment regulations.
- o Secondary sewage treatment is generally expected to remove 85 percent of the BOD measured in sewage and produce effluent BOD concentrations with a 30-day average of less than 30 mg/L and a 7-day average of less than 45 mg/L.

Source: http://en.wikipedia.org/wiki/Biochemical_oxygen_demand

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WHAT IS BOD?

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WHAT IS BOD?

- o All three must be present in order to have BOD.

, Food, and O₂

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WHAT IS BOD?

Food - Organic material (carbon), exerts carbonaceous oxygen demand (cBOD)

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WHAT IS BOD?

Food - Reduced forms of nitrogen, exert nitrogenous oxygen demand

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WHAT IS BOD?

Carbonaceous
Oxygen
Demand (cBOD) + Nitrogenous
Oxygen
Demand = BOD

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WHAT IS BOD?

- o Dissolved oxygen levels are monitored before and after an incubation period, to determine the amount of oxygen depletion.

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WHAT IS BOD?

- o Incubation conditions:
 - Temperature – $20 \pm 1^\circ\text{C}$
 - Time – 5 days
 - In the dark



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WHY IS BOD IMPORTANT?

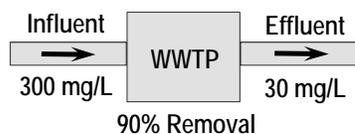
- o Wastewater treatment plants (WWTP) aim to remove BOD from the influent (raw) water.
 - BOD can be used for process control and to measure the efficiency of a WWTP.

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WHY IS BOD IMPORTANT?

- o BOD Removal Efficiency
 - BOD of effluent versus influent

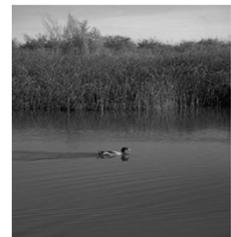


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WHY IS BOD IMPORTANT?

- o BOD measurements help in monitoring the effect of effluent on the dissolved oxygen concentration of the receiving water body.
- o BOD may be regulated by permit requirements.



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BOD TEST REQUIREMENTS

- o Multiple sample dilutions
- o Dilution water blank
- o Seed control
- o Seed blank
- o Glucose/Glutamic Acid Standard
- o Duplicates

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SAMPLE VOLUME DILUTION ESTIMATION

- o Industrial wastes = 0.1 - 1.0 %
- o Raw\settled sewage = 1.0 - 5.0 %
- o Oxidized effluent = 5.0 - 25 %
- o Polluted river water = 25 - 100 %

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HOW IS BOD MEASURED?**Dilution Method****BODTrak**

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(Not EPA Approved for Permit Compliance)

BOD CALCULATIONS

- o Using the dilution method, three values must be known in order to calculate BOD:
 - Initial DO
 - Final DO
 - Volume of sample

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BOD DILUTION METHOD

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BOD – DILUTION METHOD

- o Glassware
- o Preparing dilution water
- o Seeding dilution water
- o Sampling and sample handling
- o Determining range and sample volumes
- o Obtaining data points
- o Running Standards

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GLASSWARE

- Glassware must be extremely clean!
 - Have a set of glassware dedicated to BOD testing.
 - Clean glassware thoroughly before each use
 - Clean with a dilute bleach solution
 - Rinse at least 3x with DI water
 - Clean with 1:1 sulfuric acid
 - Rinse at least 3x with DI water
 - Allow to air dry

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DILUTION WATER

- Source water
- Reagents
- Method criteria
- Failure factors
- Other considerations

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DILUTION WATER SOURCE WATER

- 22nd Ed. Standard Methods 5210B 4.C
 - Serves to check the quality of unseeded dilution water and the cleanliness of bottles
 - May be distilled, tap or receiving stream
 - As long as they meet depletion of < 0.2 mg/L
 - Free of heavy metals
 - Ex: copper

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DILUTION WATER REAGENTS

- Use clean glassware, tubing (medical grade) and bottles
- Distilled water should be used in preparing reagents in advance (sterilized is preferred)
- Discard if any sign of precipitation or biological growth
- Commercial reagents are acceptable

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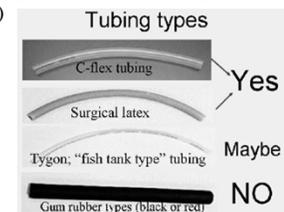
DILUTION WATER METHOD CRITERIA

- Preferably run two blanks, beginning and ending of sample set
- Dilution water blanks must meet quality control limits, < 0.2 mg/L DO (preferably < 0.1 mg/L)
 - Otherwise discard and prepare fresh solution
- No seed or nitrification inhibitor is added for dilution water blank
- Run one nitrification inhibitor blank
- Total of three blanks
 - One dilution water blank at beginning
 - One nitrification blank at beginning
 - One dilution water blank at end

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DILUTION WATER FAILURE FACTORS

- Check out this site for more information: <http://dnr.wi.gov/regulations/labcert/BODDH2O.html>
- Tubing constructed of oxygen-demand leaching material (consider medical grades)



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DILUTION WATER FAILURE FACTORS

- Slime growth in delivery tubing
- Poor water quality/improperly maintained system
- Poorly cleaned bottles or dilution water storage container
- Improperly calibrated probe

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DILUTION WATER FAILURE FACTORS

- Poor quality air for aeration.
- Deionizer systems can leach organics and grow bacteria.
 - Must be cleaned on a regular basis.
 - Try reverse osmosis (RO)/polisher combination systems that will produce ASTM Type 1 water.

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DILUTION WATER OTHER CONSIDERATIONS

- Store bought distilled water
 - What are the containers made of?
 - QA/QC of water? What's in it?
- If it ain't broke, don't fix it.
 - If the water that you purchased works satisfactorily and shows good QA/QC, continue using it.
- Use an all glass still, avoid metal stills.

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PREPARING DILUTION WATER

- Store distilled water in a BOD incubator until the temperature reaches 20°C.
- Water is then saturated with oxygen and is ready for use.



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PREPARING DILUTION WATER



Add buffer
and nutrients

- After adding buffer and nutrients, shake the bottle vigorously to ensure saturation
- Use immediately!
 - Storage of prepared dilution water for more than 24 hours after adding nutrients, minerals and buffer is not recommended unless dilution water blanks consistently meet quality control limits

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SEEDING DILUTION WATER



- Add seed to the dilution water (if needed)
- Seed can be added to:
 - Bulk dilution water preparation (good)
 - Individual bottles (better)

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SEEDING DILUTION WATER

- Seed may be necessary when testing:
 - Oxidized effluents
 - Toxic effluents
 - Samples with insufficient microorganisms
 - ***cBOD samples***

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SEEDING DILUTION WATER

- Sources of seed:
 - Settled domestic wastewater
 - Effluent from primary clarifiers
 - Diluted mixed liquor from an aeration basin
 - Undisinfected effluent
 - Receiving water from below the point of discharge
 - Purchased BOD seed
- When effluent of mixed liquor from a biological treatment process is used as a seed source, inhibition of nitrification is recommended

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SEEDING DILUTION WATER

- How much seed should be added?
 - Seed should contribute an oxygen demand ranging from 0.6-1.0 mg/L.
 - The amount of seed added should be adjusted from this range to that required to provide glucose-glutamic acid (GGA) check results of 198 ± 30.5 mg/L
 - For example, if 1 mL of seed is needed to achieve 198 ± 30.5 mg/L, then use 1 mL in each BOD bottle receiving the test wastewater

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SEEDING DILUTION WATER

- How is BOD of the seed determined?
 - Run seed control to determine the BOD of the seed.
 - Seed controls are run as if they were samples
 - Bottles containing dilution water and specific volumes of seed

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SEED CONTROL FACTOR

- Divide the DO depletion by the volume of seed in mL for each seed control bottle having a 2.0 mg/L depletion and greater than 1.0 mg/L minimum residual DO and average the results.

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SAMPLING AND SAMPLE HANDLING

- Sampling Considerations
 - Preferable to sample BEFORE any disinfection
 - If sampling after any disinfection, samples MUST be seeded

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SAMPLING AND SAMPLE HANDLING

- o Sample Pre-Treatment
 - Composite samples kept at 1-6 °C
 - Recommended Hold Time =
 - o Analysis must be run within 2 hours of collection (grab or end of 24-hour compositing period)
 - o Refrigerate if unable to do so, ≤6° C
 - o 48-hour holding time (40 CFR 136, Table II) @ ≤ 6°C
 - Sample Temperature (20 ± 3 °C)
 - Sample (Dilution) pH (between 6.0-8.0)
 - Check residual chlorine
 - o If present, (1) quench chlorine, (2) seed samples
 - Samples Supersaturated? (DO > 9 mg/L at 20 °C)
 - o Warm; shake or aerate to remove O₂

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DETERMINING RANGE AND SAMPLE VOLUME

- o Run a series of sample dilutions, at least 3 dilutions
 - Want 2 mg/L oxygen depletion in the first dilution
 - o Minimum sample volume
 - Want 1 mg/L oxygen remaining in the last dilution
 - o Maximum sample volume
 - Bring sample to 20 ± 3°C before checking initial DO

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DETERMINING RANGE AND SAMPLE VOLUME

- o Fill bottles to top without adding bubbles
- o If > 67% (200 mL) sample after dilution, add nutrients, mineral and buffer solutions directly to the sample at a rate of 1 mL/L (0.3 mL/300-mL bottle) or commercial prepared product
- o Add NI to partially filled sample bottle for cBOD.
 - Seeding is required
- o After preparing dilution, measure initial DO within 30 minutes

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DETERMINING RANGE AND SAMPLE VOLUME

First Dilution

2mg/L oxygen demand

Last Dilution

1mg/L oxygen remaining

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DETERMINING RANGE AND SAMPLE VOLUME

- o Selection of sample volumes used in the test depends on two factors:
 - Type of sample
 - Elevation

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DETERMINING RANGE AND SAMPLE VOLUME

Table 2 Determining Minimum Sample Volume

Sample Type	Estimated BOD mg/L	mL of Sample*
Strong Trade Waste	600	1
Raw and Settled Sewage	300	2
	200	3
	150	4
	120	5
	100	6
	75	8
Oxidized Effluents	60	10
	50	12
	40	15
	30	20
Polluted River Waters	20	30
	10	60
	6	100
	4	200
	2	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

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DETERMINING RANGE AND SAMPLE VOLUME

Table 3 Determining Maximum Sample Volume

Estimated BOD at			mL of Sample*
sea level	1000 ft.	5000 ft.	
2460	2380	2032	1
1230	1189	1016	2
820	793	677	3
615	595	508	4
492	476	406	5
410	397	339	6
304	294	251	8
246	238	203	10
205	198	169	12
164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

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DETERMINING RANGE AND SAMPLE VOLUME

o If our sample is approximately 300mg/L BOD, what should the minimum and maximum sample volumes be?

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DETERMINING RANGE AND SAMPLE VOLUME

Sample Type	Estimated BOD mg/L	mL of Sample*
Strong Trade Waste	600	1
Raw and Settled Sewage	300	2
	200	3
	150	4
	120	5
	100	6
	75	8
	60	10
Oxidized Effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted River Waters	6	100
	4	200
	2	300

Estimated BOD at			mL of Sample*
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164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

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DETERMINING RANGE AND SAMPLE VOLUME

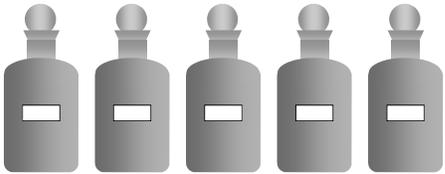
o If our sample is approximately 300mg/L BOD, what should the minimum and maximum sample volumes be?

- Minimum volume = 2mL
- Maximum volume = 8mL

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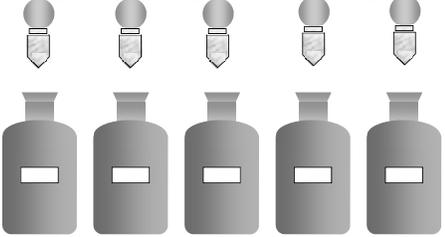
DETERMINING RANGE AND SAMPLE VOLUME



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DETERMINING RANGE AND SAMPLE VOLUME

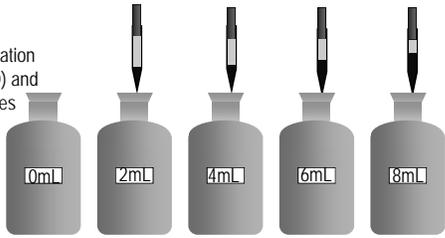


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DETERMINING RANGE AND SAMPLE VOLUME

Can add nitrification inhibitor (CBOD) and seed after bottles are $\frac{2}{3}$ full



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DETERMINING RANGE AND SAMPLE VOLUME

- o Nitrification:
 - Nitrosomonas + $\text{NH}_3 + \text{O}_2 \rightarrow \text{NO}_2^-$
 - Nitrobacter + $\text{NO}_2^- + \text{O}_2 \rightarrow \text{NO}_3^-$
 - o Nitrogenous demand observed if these microbiologically mediated reactions occur.

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DETERMINING RANGE AND SAMPLE VOLUME

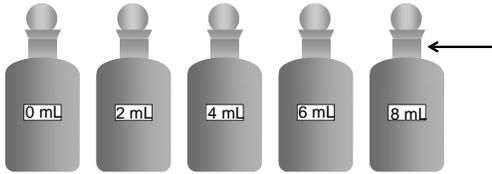
- o Nitrification inhibitor
 - Prevents Nitrosomonas from oxidizing ammonia to nitrite, preventing nitrogenous oxygen demand in the sample (CBOD measurement).
 - TCMP – 2-chloro-6-(trichloromethyl)pyridine

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DETERMINING RANGE AND SAMPLE VOLUME

- o Fill bottles past the neck with dilution water and invert to mix (no air bubbles).



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OBTAINING DATA POINTS

- o Dissolved oxygen concentration must be measured prior to incubation
 - Winkler titration
 - o Duplicate bottles must be prepared
 - Dissolved oxygen meter

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OBTAINING DATA POINTS

- o Measure DO
- o Prior to measurement, prepare probe by:
 - Polarizing
 - Polishing
 - Calibrating
 - o Water saturated air or versus Winkler titration

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CALIBRATION

- o Winkler titration - best; most accurate
 - Relies on chemistry
- o Probe: Air-saturated water
 - Reagent water at 20°C shaken/aerated to saturate
 - Maximum DO at 20°C ~ 9.00 mg/L
 - Meter result shouldn't vary greatly from the saturation point
 - Correct for pressure and/or altitude differences

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CALIBRATION

- o Probe: Water-saturated air (most common)
 - Air-calibration chamber → calibrate at sample temperature.
 - Minimizes errors caused by temperature differences.
 - Keep interior of the chamber just moist -- not filled with water.
 - Typical for probes
 - Probe is stored in a constant humidity environment
 - Container should be sealed somehow (to maintain constant humidity)

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OBTAINING DATA POINTS



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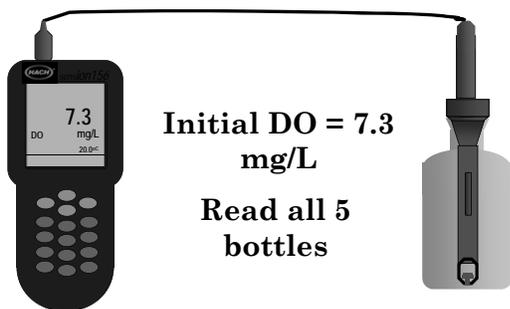
OBTAINING DATA POINTS



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OBTAINING DATA POINTS



**Initial DO = 7.3
mg/L**

**Read all 5
bottles**

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OBTAINING DATA POINTS

- o After measuring DO, replace any lost volume in the bottle with dilution water.
- o Replace stopper – watch for air bubbles!

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OBTAINING DATA POINTS

- Fill area in around stopper with dilution water.
- Cover with plastic BOD bottle cap.

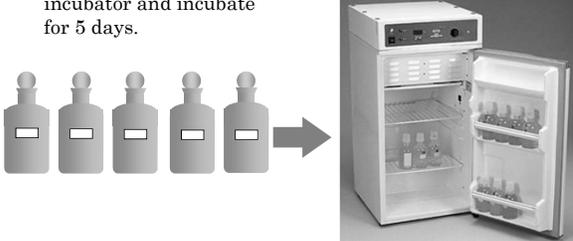


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OBTAINING DATA POINTS

- Transfer samples to 20°C incubator and incubate for 5 days.

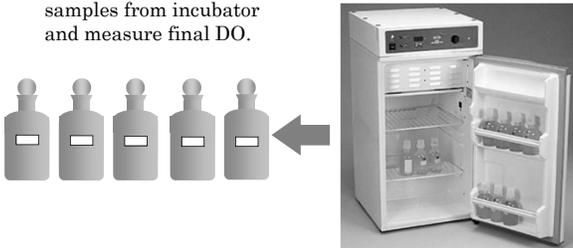


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OBTAINING DATA POINTS

- After 5 days, remove samples from incubator and measure final DO.



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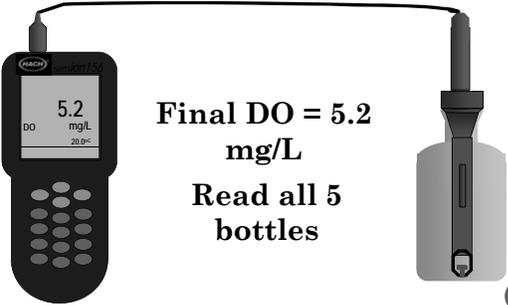
OBTAINING DATA POINTS



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OBTAINING DATA POINTS



**Final DO = 5.2
mg/L**

**Read all 5
bottles**

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OBTAINING DATA

- Plug data into equation:

$$BOD_5, \text{ mg/L} = \frac{(\text{Initial DO} - \text{Final DO})}{P}$$

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OBTAINING DATA

- o Plug data into equation:

$$P = 2/300 = 0.00667$$

$$\text{BOD}_5, \text{ mg/L} = \frac{(7.3 - 5.2)}{0.00667}$$

$$\text{BOD}_5, \text{ mg/L} = 315 \text{ mg/L}$$

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OBTAINING DATA

- o If bottles were seeded:

$$\text{BOD}_5, \text{ mg/L} = \frac{(D_1 - D_2) - (B_1 - B_2) f}{P}$$

D = DO of sample

B = DO of seed

P = Sample volume/300

F = Ratio of seed in sample to seed in control

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OBTAINING DATA

Seeding Calculations					
Bottle	DO _i	DO _f	Depletion	ml_seed	DO depletion per mL seed
Seed 1	8.5	0.3	X 8.2	30	---
Seed 2	8.4	1.6	6.8	÷ 20	= 0.34
Seed 3	8.4	4.3	4.1	÷ 10	= 0.41

Avg the seed controls that meet depletion criteria
 $(0.34 + 0.41) \div 2 = 0.375 \text{ mg/L DO depletion per mL seed}$

So, 2 mL undiluted seed is added to each sample bottle.
 $0.375 \times 2 = 0.75 \text{ mg/L}$

Therefore, 0.75 mg/L is subtracted from the depletion of each BOD depletion to obtain BOD result.

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BOD SOLIDS HANDLING BIAS

- o Homogeneous
 - Mix sample while removing aliquot
- o Use wide bore pipette
- o Pipette as fast as possible to prevent loss of solids
- o Pipette each sample dilution separately

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BOD QC CHECKS

- o Sample depletion criteria
 - Depletion should be greater than 2.0 mg/L
 - Final DO should be > 1.0 mg/L
- o Seed control should also meet sample depletion criteria
 - Seed contribution should be between 0.6 and 1.0 mg/L
 - Blank's DO depletion < 0.2 mg/l
 - GGA range approx. $198 \pm 30.5 \text{ mg/L}$

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BOD QUALITY CONTROL

- o Solubility of oxygen in water at 20°C is 9.2 mg/L
- o Super saturation = initial DO > 9.0 mg/L
- o Average all dilutions that meet QC criteria
- o Suspect toxicity if smallest sample concentration yields greatest uptake
- o What if no samples meet criteria?
 - Report qualified results

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COMMON SOURCES OF ERROR

- o Not adjusting pH to within 6.0 – 8.0
 - Adjustment not required if effluent is between 6.0-8.0
 - Otherwise, adjust sample temperature to $20 \pm 3^\circ\text{C}$, then adjust pH to 7.0 to 7.2 (H_2SO_4 or NaOH)
 - If pH is adjusted, samples must be seeded
- o Improper calibration of DO meter
- o Incubation temperatures not constant
- o Initial DOs above saturation

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COMMON SOURCES OF ERROR

- o Depletion criteria not met
 - Not depleting 2.0 mg/L
 - Final DO <1.0 mg/L
- o Subtracting blanks
- o Not seeding when required
- o Seed strength not constant
- o Not analyzing GGA samples
- o Not evaluating for toxicity
- o Improper calculations
- o Water quality issues

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RUNNING STANDARDS

- o Be careful purchasing GGA from Hach, **some** are not the same concentration as specified in Standard Methods
 - Standard Methods - 150 mg/L each GGA
 - Hach BOD Standard – 300mg/L each GGA (divide by 2)
 - Hach ezGGA – gives 198 mg/L BOD results

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RUNNING STANDARDS

- o The concentration of standard does NOT equal BOD.
 - 150mg/L each = 198 ± 30.5 mg/L BOD
 - 300mg/L each = 396 ± 61 mg/L BOD

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BOD – TAKE HOME MESSAGES

- o BOD is an indirect measure of organic content.
- o BOD is measured by oxidizing organics using microorganisms (under specific conditions) and directly measuring the amount of oxygen consumed in the process.

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QA/QC STEPS

Parameter	Method	DOC	MDL	Method Blank	IFPB	LFM / LFMD	Dup	ICAL / CCV	Charts	Control	Corrective Action	QC Acceptance	Batch Size	*QC Frequency
BOD ₅ / CBOD ₅	SM5210 B - 2001	X		X	X		X	X Calibrate meter daily	X	X	X	X	20	Depends on Permit

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BOD5/cBOD5 SM5210 B – 2001 & HACH METHOD 10360

- o DOC
- o LRB
- o LFB
- o Dup
- o ICAL/CCV
- o Control Charts
- o Corrective Action
- o QC Acceptance
- o Batch Size
- o QC Frequency



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BOD5/cBOD5 SM5210 B – 2001 & HACH METHOD 10360

- o Minimum DO depletion (including seed bottles) of 2.0 mg/l
- o Minimum residual DO of at least 1.0 mg/l
- o Dilution water quality check (nutrient, mineral, buffer) must not be more than 0.2 mg/l (0.1 is preferred)
- o Seed control of three dilutions. Smallest to give at least 2.0 mg/l depletion and the largest to at least 1.0 mg/l residual....

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BOD5/cBOD5 SM5210 B – 2001 & HACH METHOD 10360

- o Demonstration of Capability (DOC)
 - Run a laboratory-fortified blank (LFB) at least four times and compare to the limits listed in the method
 - Real people language: Each operator running this test needs to analyze 4 samples of GGA at a concentration of 198±30.5 mg/L
 - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
 - Recommend backup analyst do this once a year.

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BOD5/cBOD5 SM5210 B – 2001 & HACH METHOD 10360

- o Method Blanks
 - Real people language: analyze dilution water
 - o Preferably one at the beginning and one at end
 - o 2014 Update – Removed – If you run cBOD5 results, then also add one Nitrification Inhibitor (NI) (10 mg/L)
 - Run on daily (day of)
 - Target value is less than 0.20 mg/L (preferably less than 0.10 mg/L)

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BOD5/cBOD5 SM5210 B – 2001 & HACH METHOD 10360

- o Laboratory Fortified Blank
 - Real people language: analyze a Glucose/Glutamic Acid (GGA) standard at a concentration of 198±30.5 mg/L
 - Run on a 5% basis, one for every 20 samples
 - **2014 Update** - If permit requires cBOD, add nitrification inhibitor (NI) to one GGA bottle once/quarter (or more often if the Lot # of NI changes), which should be equal to 164 ±30.7 mg/L

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BOD5/cBOD5 SM5210 B – 2001 & HACH METHOD 10360

- o Duplicate
 - Analyze 2 samples for BOD or CBOD
 - o Example, if you run 6, 9 and 12 mL on your raw/influent sample, run a second 9 mL sample.
 - o You would end up with a total of 4 bottles for your raw/influent sample
 - Run on a 5% basis, one for every 20 samples
 - Calculate %RPD, ≤ 20%
 - **2014 Update - For reporting purposes, average results that meet method criteria.**

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BOD5/cBOD5 SM5210 B – 2001 & HACH METHOD 10360

- Initial Calibration (ICV)
 - Calibrate daily (day of) by following manufacturer's instructions
 - Using barometric pressure is best
- Continuing Calibration (CCV)
 - Prepare dilution water that is air-saturated and analyze bottles and compare to the theoretical dissolved oxygen concentration (± 0.2 mg/L).
 - Same as DO CCV if using a different probe



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BOD5/cBOD5 SM5210 B – 2001 & HACH METHOD 10360

- Corrective Action - 1020 B.5., B.8., & B.15.
- 5210 B.7.b. – Identify results in the test reports when any of the following quality control parameters is not met:
 - Dilution water exceeds 0.20 mg/L (5210B.6c)
 - Glucose-glutamic acid check falls outside of acceptable limits (5210B.6b)
 - Test replicates show more than 30% difference between high and low values
 - Seed control samples do not meet the above criteria in all dilutions (5210B.6d) or
 - Minimum DO is less than 1.0 mg/L (5210B.7a3)

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BOD5/cBOD5 SM5210 B – 2001 & HACH METHOD 10360

- QC Acceptance Criteria
 - Blanks < 0.20 mg/L
 - GGA = 198 ± 30.5 mg/L (if running cBOD, add NI to one bottle once/quarter or more often if NI Lot# changes, and it should = 164 ± 30.7 mg/L)
 - RPD $< 20\%$
 - Minimum of three dilutions for each sample, at least one sample must have valid data with at least 2.0 mg/L depletion and a residual of 1.0 mg/L

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3. Calculations

a. LFM recovery:

$$\frac{(C_s \times f) - C}{S} \times 100 = \% \text{ Recovery LFM or LFMD}$$

where:

- C_s = LFM concentration determined experimentally,
 f = spike dilution correction,
 C = concentration of sample before spiking, and
 S = concentration of spike.

NOTE: f should be more than 0.95. Spiking that dilutes a sample by more than 5% changes the matrix significantly. Ideally, keep f above 0.99 (equivalent to 1% dilution of sample due to spike addition) so f can be ignored and the equation simplified to eliminate f .

b. LFB recovery:

$$\frac{C_b}{I} \times 100 = \% \text{ Recovery LFB}$$

where:

- C_b = LFB concentration determined experimentally, and

5210 BIOCHEMICAL OXYGEN DEMAND (BOD)*

5210 A. Introduction

1. General Discussion

The biochemical oxygen demand (BOD) determination is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of wastewaters, effluents, and polluted waters. The test has its widest application in measuring waste loadings to treatment plants and in evaluating the BOD-removal efficiency of such treatment systems. The test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron. It also may measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. The seeding and dilution procedures provide an estimate of the BOD at pH 6.5 to 7.5.

Measurements of oxygen consumed in a 5-d test period (5-d BOD or BOD₅, 5210B), oxygen consumed after 60 to 90 d of incubation (ultimate BOD or UBOD, 5210C), and continuous

* Approved by Standard Methods Committee, 2001. Editorial revisions, 2011. Joint Task Group: 21st Edition—James C. Young (chair), George T. Bowman, Sabry M. Kamhawy, Terry G. Mills, Marlene Patillo, Ray C. Whittemore.

I = initial concentration of analytes added to LFB.

c. Relative percent difference:

$$\left[\frac{|LFM - LFMD|}{\left(\frac{LFM + LFMD}{2} \right)} \right] \times 100 = \%RPD$$

or

$$\left[\frac{|D_1 - D_2|}{\left(\frac{D_1 + D_2}{2} \right)} \right] \times 100 = \%RPD$$

where:

- LFM = concentration determined for LFM,
 $LFMD$ = concentration determined for LFMD,
 D_1 = concentration determined for first duplicate, and
 D_2 = concentration determined for second duplicate.

4. References

1. MEIER, P.C. & E.E. Zünd. 2000. *Statistical Methods in Analytical Chemistry*, 2nd ed. Wiley Interscience, New York, N.Y.
2. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Definition and procedure for the determination of the method detection limit, rev. 1.11, 40 CFR Part 136, Appendix B. *Fed. Reg.* 5:23703.

oxygen uptake (respirometric method, 5210D) are described here. Many other variations of oxygen demand measurements exist, including using shorter and longer incubation periods and tests to determine rates of oxygen uptake. Alternative seeding, dilution, and incubation conditions can be chosen to mimic receiving-water conditions, thereby providing an estimate of the environmental effects of wastewaters and effluents.

The UBOD measures the oxygen required for the total degradation of organic material (ultimate carbonaceous demand) and/or the oxygen to oxidize reduced nitrogen compounds (ultimate nitrogenous demand). UBOD values and appropriate kinetic descriptions are needed in water quality modeling studies such as UBOD:BOD₅ ratios for relating stream assimilative capacity to regulatory requirements; definition of river, estuary, or lake deoxygenation kinetics; and instream ultimate carbonaceous BOD (UCBOD) values for model calibration.

2. Carbonaceous Versus Nitrogenous BOD

A number of factors, for example, soluble versus particulate organics, settleable and floatable solids, oxidation of reduced iron and sulfur compounds, or lack of mixing may affect the accuracy and precision of BOD measurements. Presently, there

is no way to include adjustments or corrections to account for the effect of these factors.

Oxidation of reduced forms of nitrogen, such as ammonia and organic nitrogen, can be mediated by microorganisms and exert nitrogenous demand. Nitrogenous demand historically has been considered an interference in the determination of BOD, and the inclusion of ammonia in the dilution water contributes an external source of nitrogenous demand. The interference from nitrogenous demand can now be prevented by an inhibitory chemical. If an inhibiting chemical is not used, the oxygen demand measured is the sum of carbonaceous and nitrogenous demands.

Measurements that include nitrogenous demand generally are not useful for assessing the oxygen demand associated with organic material. Nitrogenous demand can be estimated directly from ammonia nitrogen (Section 4500-NH₃); and carbonaceous demand can be estimated by subtracting the theoretical equivalent of the nitrite and nitrate produced in uninhibited test results. However, this method is cumbersome and is subject to considerable error. Chem-

5210 B. 5-Day BOD Test

1. General Discussion

The method consists of filling with diluted and seeded sample, to overflowing, an airtight bottle of specified size and incubating it at the specified temperature for 5 d. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between initial and final DO. Because the initial DO is determined shortly after the dilution is made, all oxygen uptake occurring after this measurement is included in the BOD measurement.

For sampling and storage procedures, see 5210B.4a.

2. Apparatus

a. *Incubation bottles*: Use glass bottles having 60 mL or greater capacity (300-mL bottles having a ground-glass stopper and a flared mouth are preferred). Clean bottles with a detergent, rinse thoroughly, and drain before use.

b. *Air incubator or water bath*, thermostatically controlled at 20 ± 1°C. Exclude all light to prevent possibility of photosynthetic production of DO.

3. Reagents

Prepare reagents in advance but discard if there is any sign of precipitation or biological growth in the stock bottles. Commercial equivalents of these reagents are acceptable and different stock concentrations may be used if doses are adjusted proportionally. Use reagent grade or better for all chemicals and use distilled or equivalent water, preferably sterilized, for making all solutions.

a. *Phosphate buffer solution*: Dissolve 8.5 g KH₂PO₄, 21.75 g K₂HPO₄, 33.4 g Na₂HPO₄·7H₂O, and 1.7 g NH₄Cl in about 500 mL distilled water and dilute to 1 L. The pH should be 7.2 without further adjustment. Alternatively, dissolve 42.5 g

ical inhibition of nitrogenous demand provides a more direct and more reliable measure of carbonaceous demand.

The extent of oxidation of nitrogenous compounds during the 5-d incubation period depends on the concentration and type of microorganisms capable of carrying out this oxidation. Such organisms usually are not present in raw or settled primary sewage in sufficient numbers to oxidize sufficient quantities of reduced nitrogen forms in the 5-d BOD test. Many biological treatment plant effluents contain sufficient numbers of nitrifying organisms to cause nitrification in BOD tests. Because oxidation of nitrogenous compounds can occur in such samples, inhibition of nitrification as directed in 5210B.5e) is recommended for samples of secondary effluent, for samples seeded with secondary effluent, and for samples of polluted waters.

3. Reference

1. YOUNG, J.C. 1973. Chemical methods for nitrification control. *J. Water Pollut. Control Fed.* 45:637.

KH₂PO₄ and 1.7 g NH₄Cl in about 700 mL distilled water. Adjust pH to 7.2 with 30% NaOH and dilute to 1 L.

b. *Magnesium sulfate solution*: Dissolve 22.5 g MgSO₄·7H₂O in distilled water and dilute to 1 L.

c. *Calcium chloride solution*: Dissolve 27.5 g CaCl₂ in distilled water and dilute to 1 L.

d. *Ferric chloride solution*: Dissolve 0.25 g FeCl₃·6H₂O in distilled water and dilute to 1 L.

e. *Acid and alkali solutions, 1N*, for neutralization of caustic or acidic waste samples.

1) Acid—Slowly and while stirring, add 28 mL conc sulfuric acid to distilled water. Dilute to 1 L.

2) Alkali—Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1 L.

f. *Sodium sulfite solution*: Dissolve 1.575 g Na₂SO₃ in 1000 mL distilled water. This solution is not stable; prepare daily.

g. *Nitrification inhibitor*:

1) 2-chloro-6-(trichloromethyl) pyridine—Use pure TCMP or commercial preparations.*

2) Allylthiourea (ATU) solution—Dissolve 2.0 g allylthiourea (C₄H₈N₂S) in about 500 mL water and dilute to 1 L. Store at 4°C. The solution is stable for not more than 2 weeks.

h. *Glucose-glutamic acid solution*: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 L. Prepare fresh immediately before use unless solution is maintained in a sterile condition. Store all glucose-glutamic acid mixtures at 4°C or lower. Commercial preparations may be used but concentrations may vary.

i. *Ammonium chloride solution*: Dissolve 1.15 g NH₄Cl in about 500 mL distilled water, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

* Nitrification Inhibitor Formula 2533 (2% TCMP on sodium sulfate), Hach Co., Loveland, CO, or equivalent

j. **Source water for preparing BOD dilution water:** Use demineralized, distilled, tap, or natural water for making sample dilutions (see 5210B.4c).

4. Preparatory Procedures

a. **Sampling and storage:** Samples for BOD analysis may degrade significantly during storage between collection and analysis, resulting in low BOD values.

1) **Grab samples**—If analysis is begun within 2 h of collection, cold storage is unnecessary. If analysis is not started within 2 h of sample collection, keep sample at or below 4°C from the time of collection. Begin analysis within 6 h of collection; when this is not possible because the sampling site is distant from the laboratory, store at or below 4°C and report length and temperature of storage with the results. In no case start analysis more than 24 h after grab sample collection. When samples are to be used for regulatory purposes make every effort to deliver samples for analysis within 6 h of collection.

2) **Composite samples**—Keep samples at or below 4°C during compositing. Limit compositing period to 24 h. Use the same criteria as for storage of grab samples, starting the measurement of holding time from end of compositing period. State storage time and conditions as part of the results.

b. **Sample preparation and pretreatment:**

1) **All samples**—Check pH; if it is not between 6.0 and 8.0, adjust sample temperature to $20 \pm 3^\circ\text{C}$, then adjust pH to 7.0 to 7.2 using a solution of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) of such strength that the quantity of reagent does not dilute the sample by more than 0.5%. Exceptions may be justified with natural waters when the BOD is to be measured at in-situ pH values. The pH of dilution water should not be affected by the lowest sample dilution. Always seed samples that have been pH adjusted.

2) **Samples containing residual chlorine compounds**—If possible, avoid samples containing residual chlorine by sampling ahead of chlorination processes. If residual chlorine is present, dechlorinate sample. In some samples chlorine will dissipate within 1 to 2 h of standing in the light. This dissipation often occurs during sample transport and handling. For samples in which chlorine residual does not dissipate in a reasonably short time, destroy chlorine residual by adding Na_2SO_3 solution. Determine required volume of Na_2SO_3 solution on a 100- to 1000-mL portion of neutralized sample by adding 10 mL 1 + 1 acetic acid or 1 + 50 H_2SO_4 , 10 mL potassium iodide (KI) solution (10 g /100 mL) per 1000 mL sample and titrating with Na_2SO_3 solution to the starch-iodine end point for residual. Add to neutralized sample the proportional volume of Na_2SO_3 solution determined by the above test, mix, and after 10 to 20 min check sample for residual chlorine. (NOTE: Excess Na_2SO_3 exerts an oxygen demand and reacts slowly with certain organic chloramine compounds that may be present in chlorinated samples.) Do not test chlorinated/dechlorinated samples without seeding.

3) **Samples containing other toxic substances**—Certain industrial wastes, for example, plating wastes, contain toxic metals. Such samples often require special study and treatment.

4) **Samples supersaturated with DO**—Samples containing DO concentration above saturation at 20°C may be encountered in cold waters or in water where photosynthesis occurs. To prevent loss of oxygen during incubation of such samples, reduce DO to saturation by bringing sample to about $20 \pm 3^\circ\text{C}$ in partially

filled bottle while agitating by vigorous shaking or by aerating with clean, filtered compressed air.

5) **Samples containing hydrogen peroxide**—Hydrogen peroxide remaining in samples from some industrial bleaching processes such as those used at paper mills and textile plants can cause supersaturated oxygen levels in samples collected for BOD testing. Mix such samples vigorously in open containers for sufficient time to allow the hydrogen peroxide to dissipate before setting up BOD tests. Check adequacy of peroxide removal by observing dissolved oxygen concentrations over time during mixing or by using peroxide-specific test strips. Mixing times can vary from 1 to 2 h depending on the amount of hydrogen peroxide present. The peroxide reaction can be considered complete when the DO no longer increases during a 30-min period without mixing.

c. **Selection and storage of source water for BOD sample dilution:** Obtain water from suitable source—distilled, tap, or receiving water. Make sure the water is free of heavy metals, specifically copper, and toxic substances, such as chlorine, that can interfere with BOD measurements. Protect source water quality by using clean glassware, tubing, and bottles. Deionized water often contains sufficient amounts of organics and microorganisms to cause failure of the dilution water quality control check (5210B.6c). Source water may be stored before use as long as the prepared dilution water (5210B.5a) meets quality control criteria in the dilution water blank (5210B.6c). Such storage may improve the quality of some source waters but may allow biological growth to cause deterioration in others. Storage of prepared dilution water (5210B.5h) for more than 24 h after adding nutrients, minerals, and buffer is not recommended unless dilution water blanks consistently meet quality control limits. Discard stored source water if the dilution water blank shows more than 0.20 mg/L DO depletion in 5 d (see 5210B.6c).

d. **Preparation of seed suspension:** It is necessary to have present in each BOD bottle a population of microorganisms capable of oxidizing the biodegradable organic matter in the sample. Domestic wastewater, unchlorinated or otherwise undischarged effluents from biological wastewater treatment plants, and surface waters receiving wastewater discharges usually contain satisfactory microbial populations. Some samples (for example, some untreated industrial wastes, disinfected wastes, high-temperature wastes, wastes having pH values less than 6 or greater than 8, or wastes stored more than 6 h after collection) do not contain a sufficient microbial population. Seed such samples by adding a population of suitable microorganisms. The preferred seed is obtained from a biological treatment system processing the waste. In this case, use supernatant from settled domestic wastewater, effluent from primary clarifiers, diluted mixed liquor from an aeration basin, undischarged effluent, or receiving water from below the point of discharge. When effluent or mixed liquor from a biological treatment process is used as a seed source, inhibition of nitrification is recommended. Do not use seed from effluents that have been disinfected by chlorine or other means. Commercial seed sources may be used but are more likely to be unadapted to the wastewater constituents. Do not filter seed sources; filtering removes the seed microorganisms.

When acclimated seed sources are not available, develop an acclimated seed in the laboratory by continuously aerating a sample of settled domestic wastewater and adding small daily

increments of sample from the waste in question. Use a soil suspension, activated sludge, or a commercial seed preparation to obtain the initial microbial population. Determine the existence of a satisfactory population by testing the performance of the seed in BOD tests on the sample. BOD values that increase with time of adaptation to a steady high value indicate successful seed acclimation.

5. Testing Procedure

a. **Preparation of dilution water:** Transfer desired working volume of source water (5210B.4c) to a suitably sized bottle (glass is preferred). Check to ensure that the dissolved oxygen concentration is at least 7.5 mg/L before using water for BOD tests. If not, add DO by shaking bottle or by aerating with organic-free filtered air. Alternatively, store the water in cotton-plugged bottles long enough for the DO concentration to approach saturation. Add 1 mL each of phosphate buffer, MgSO_4 , CaCl_2 , and FeCl_3 solution/L to prepared source water (5210B.4c). Mix thoroughly and bring temperature to $20 \pm 3^\circ\text{C}$. Prepare dilution water immediately before use unless dilution water blanks (5210B.6c) show that the water is acceptable after longer storage times. If the dilution water blanks show a DO depletion greater than 0.20 mg/L, obtain a satisfactory water by improving purification or use water from another source. Do not add oxidizing agents or expose dilution water to ultraviolet light in attempts to bring the dilution blank into range.

b. **Sample temperature adjustment:** Bring samples to $20 \pm 3^\circ\text{C}$ before making dilutions.

c. **Preparation of dilutions:** Using the dilution water prepared as in ¶ a above, make at least three dilutions of prepared sample estimated to produce a residual DO of at least 1.0 mg/L and a DO uptake of at least 2.0 mg/L after a 5-d incubation. Five dilutions are recommended if experience with a particular sample does not produce at least three bottles having acceptable minimum DO depletions and residual limits (5210B.6a). A more rapid analysis, such as COD (Section 5220), may be correlated approximately with BOD and serve as a guide in selecting dilutions. In the absence of prior knowledge, use the following percentages of wastewater when preparing dilutions: 0.01 to 1.0% for strong industrial wastes, 1 to 5% for raw and settled wastewater, 5 to 25% for biologically treated effluent, and 25 to 100% for polluted river waters. The number of bottles to be prepared for each dilution depends on the DO technique and the number of replicates desired. Prepare dilutions in volumetric containers (Class A glass or equivalent) and then transfer to BOD bottles or prepare directly in BOD bottles. Either dilution method can be combined with any DO measurement technique.

1) **Dilutions prepared in volumetric containers**—Using a wide-tipped pipet, add desired amount of prepared sample to individual volumetric cylinders or flasks. Mix the sample well immediately before pipetting to avoid loss of solids by settling. For dilutions greater than 1:100 make a primary dilution before making final dilution in the bottle. Fill cylinders or flasks at least two-thirds full of dilution water without entraining air. Add appropriate amounts of seed suspension (¶ d below) and nitrification inhibitor (¶ e below). Dilute to final level with dilution water (¶ a above). Mix well but avoid entraining air. Siphon mixed dilution into a suitable number of BOD bottles, taking care not to let solids settle in the cylinder or flask during transfer.

2) **Dilutions prepared directly in BOD bottles**—Using a wide-tip volumetric pipet, add the desired sample volume to individual BOD bottles. Fill each BOD bottle approximately two-thirds full with dilution water. Add appropriate amounts of seed suspension (¶ d below) and nitrification inhibitor (¶ e below) to the individual BOD bottles. When a bottle contains more than 67% of the sample after dilution, nutrients may be limited in the diluted sample and subsequently reduce biological activity. In such samples, add the nutrient, mineral, and buffer solutions (5210B.3a-e) directly to diluted sample at a rate of 1 mL/L (0.30 mL/300-mL bottle) or use commercially prepared solutions designed to dose the appropriate bottle size.

d. **Addition of seed suspension:** If seeding is used, add seed suspensions to the dilution vessels or to individual BOD bottles before final dilution as described in ¶ c above. Do not add seed directly to wastewater samples if they contain materials that are toxic before dilution. Generally, 1 to 3 mL of settled raw wastewater or primary effluent or 1 to 2 mL of a 1:10 dilution of mixed liquor/300-mL bottle will provide a suitable amount of microorganisms. Do not filter seed suspension before use. Agitate the seed suspension during transfer to ensure that the same quantity of microorganisms is added to each BOD bottle. Always record the exact volume of seed suspension added to each bottle. The DO uptake attributable to the seed added to each bottle generally should be between 0.6 and 1.0 mg/L, but the amount of seed added should be adjusted from this range to that required to provide glucose-glutamic acid (GGA) check results of 198 ± 30.5 mg/L. For example, if 1 mL of seed suspension is required to achieve 198 ± 30.5 mg/L BOD in the glucose-glutamic acid check, then use 1 mL in each BOD bottle receiving the test wastewater. **How much seed to add to GGA**

e. **Addition of nitrification inhibitor:** Samples that may require nitrification inhibition¹ include, but are not limited to, biologically treated effluents, samples seeded with biologically treated effluents, and river waters. Note the use of nitrogen inhibition and the chemical used when reporting results. (NOTE: TCMP is the preferred nitrification inhibitor but requires handling and transfer in a solid form. Allylthiourea is not always effective in inhibiting nitrification within the 5-d incubation period and concentrations above 2 mg/L may cause increases in carbonaceous BOD measurements. ATU concentrations above 2 mg/L also can adversely affect the azide modification of the iodometric method.) Seed all samples to which nitrification inhibitor has been added. The amount of seed should be consistent with that required to achieve GGA test results in the range of 198 ± 30.5 mg/L (5210B.6b).

1) **Nitrification inhibition using 2-chloro-6-(trichloromethyl)pyridine (TCMP)**—Add 10 mg TCMP/L to diluted sample or 3 mg TCMP to each 300-mL bottle or sample dilution vessel, or proportional amounts to other sized bottles, after initial sample dilution but before final filling of the bottles with dilution water. Do not add TCMP to BOD bottles before they are at least two-thirds filled with diluted sample. (NOTE: TCMP dissolves slowly and can float on top of the sample if not mixed well). Some commercial TCMP formulations are not 100% TCMP; adjust dosage appropriately.

2) **Nitrification inhibition using allylthiourea (ATU)**—Add 1 mL ATU solution [5210B.3g2)]/L diluted sample or 0.3 mL/300-mL test bottle or sample dilution vessel. Do not add ATU to

BOD bottles until they are at least two-thirds filled with diluted sample.

f. Sealing of bottles: Complete filling of each bottle by adding enough dilution water that insertion of the stopper leaves no bubbles in the bottle. Mix the sample by turning the bottle manually several times unless a DO probe having a stirrer is used immediately to measure initial DO concentration. As a precaution against drawing air into the dilution bottle during incubation, use a water seal. Obtain satisfactory water seals by inverting bottles in a water bath or by adding water to the flared mouth of special BOD bottles. Place a paper or plastic cup or foil cap over flared mouth of bottle to reduce evaporation of the water seal during incubation.

g. Determination of initial DO: Use the azide modification of the iodometric method (Section 4500-O.C) or the membrane electrode method (Section 4500-O.G) to determine initial DO on all sample dilutions, dilution water blanks, and, where appropriate, seed controls. Replace any displaced contents with sufficient diluted sample or dilution water to fill the bottle, stopper all bottles tightly, and water seal before beginning incubation. After preparing dilution, measure initial DO within 30 min. If the membrane electrode method is used, take care to eliminate drift in calibration between initial and final DO readings. If the azide modification of the titrimetric iodometric method is used, prepare an extra bottle for initial DO determination for each sample dilution.

h. Sample incubation: Incubate at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ the stoppered and sealed BOD bottles containing desired dilutions (¶ a above), seed controls (5210B.6d), dilution water blanks (5210B.6c), and glucose-glutamic acid checks (5210B.6b). Exclude light to avoid growth of algae in the bottles during incubation.

i. Determination of final DO: After 5 d \pm 6 h of incubation, determine DO in all sample dilutions, and in all blanks and checks as in 5210B.6b-d, using the azide modification of the titrimetric method or the membrane electrode method.

6. Quality Control Checks

The quality control practices considered to be an integral part of each method are summarized in Table 5020.I.

a. Minimum residual DO and minimum DO depletion: Only bottles, including seed controls, giving a minimum DO depletion of 2.0 mg/L and a residual DO of at least 1.0 mg/L after 5 d of incubation are considered to produce valid data, because at least 2.0 mg oxygen/uptake L is required to give a meaningful measure of oxygen uptake and at least 1.0 mg/L must remain throughout the test to ensure that insufficient DO does not affect the rate of oxidation of waste constituents. Exceptions occur for reporting purposes only when the depletions for tests using undiluted samples in all bottles fall below 2.0 mg/L and when the residual DO in all dilutions is less than 1.0 mg/L (5210B.7). When using membrane electrodes for measuring DO, make frequent calibration checks to ensure accurate DO readings (Section 4500-O.G.3a).

b. Glucose-glutamic acid check: The glucose-glutamic acid check is the primary basis for establishing accuracy and precision of the BOD test and is the principal measure of seed quality and set-up procedure. Together with each batch of samples, check seed effectiveness and analytical technique by using pro-

cedures in 5210B.5 to make BOD measurements on an equal weight mixture of glucose and glutamic acid as follows: Add sufficient amounts of standard glucose-glutamic acid solution (5210B.3h) to give 3.0 mg glucose/L and 3.0 mg glutamic acid/L in each of three test bottles (20 mL GGA solution/L seeded dilution water, or 6.0 mL/300-mL bottle). Commercial solutions may contain other glucose-glutamic acid concentrations; adjust doses accordingly. Add nitrification inhibitor if seed is obtained from a source that is nitrifying. Evaluate data as described in 5210B.8, Precision and Bias. The resulting average BOD for the three bottles, after correction for dilution and seeding, must fall into the range of 198 ± 30.5 mg/L. If the average value falls outside this range, evaluate the cause and make appropriate corrections. Consistently high values can indicate the use of too much seed suspension, contaminated dilution water, or the occurrence of nitrification; consistently low values can indicate poor seed quality or quantity or the presence of a toxic material. If low values persist, prepare a new mixture of glucose and glutamic acid and check the sources of dilution water and source of seed.

c. Dilution water quality check: With each batch of samples incubate one or more bottles of dilution water that contains nutrient, mineral, and buffer solutions but no seed or nitrification inhibitor. This dilution water blank serves as a check on quality of unseeded dilution water and cleanliness of incubation bottles. Determine initial and final DO as in 5210B.5g and i. The DO uptake in 5 d must not be more than 0.20 mg/L and preferably not more than 0.10 mg/L, before making seed corrections. If the dilution water blank exceeds 0.20 mg/L, discard all data for tests using this dilution water or clearly identify such samples in data records.

d. Seed control: Determine BOD of the seed suspension as for any other sample. This is the *seed control*. Ideally, make three dilutions of seed such that the smallest quantity gives at least 2.0 mg/L DO depletion and the largest quantity results in at least 1.0 mg/L DO residual after 5 d of incubation. Determine the DO uptake per milliliter of seed added to each bottle using either the slope method or the ratio method. For the slope method, plot DO depletion in milligrams per liter versus milliliters of seed for all seed control bottles having a 2.0 mg/L depletion and 1.0 minimum residual DO. The plot should present a straight line for which the slope indicates DO depletion per milliliter of seed. The DO-axis intercept is oxygen depletion caused by the dilution water and should be less than 0.20 mg/L (see ¶ c above). For the ratio method, divide the DO depletion by the volume of seed in milliliters for each seed control bottle having a 2.0 mg/L depletion and greater than 1.0 mg/L minimum residual DO and average the results. Seed dilutions showing widely varying depletions per milliliter of seed ($\pm 30\%$) suggest the presence of toxic substances or large particulates in the seed suspension. In this case, check or change the seed source.

7. Data Analysis and Reporting

a. Calculations:

1) For each test bottle having 2.0 mg/L minimum DO depletion and at least 1.0 mg/L residual DO, calculate BOD as follows:

$$\text{BOD}_5, \text{ mg/L} = \frac{(D_1 - D_2) - (S)V_s}{P}$$

where:

D_1 = DO of diluted sample immediately after preparation, mg/L,
 D_2 = DO of diluted sample after 5 d incubation at 20°C , mg/L,
 S = oxygen uptake of seed, Δ DO/mL seed suspension added per bottle (5210B.6d) ($S = 0$ if samples are not seeded),
 V_s = volume of seed in the respective test bottle, mL, and
 P = decimal volumetric fraction of sample used; $1/P$ = dilution factor.

2) If DO depletion is less than 2.0 mg/L and sample concentration is 100% (no dilution except for seed, nutrient, mineral, and buffer solutions), actual seed-corrected, DO depletion may be reported as the BOD even if it is less than 2.0 mg/L.

3) When all dilutions result in a residual DO < 1.0 , select the bottle having the lowest DO concentration (greatest dilution) and report:

$$\text{BOD, mg/L} > \frac{(D_1 - D_2) - (S)V_s}{P}$$

In the above calculations, do not make corrections for DO uptake by the dilution water blank during incubation. This correction is unnecessary if dilution water meets the blank criteria stipulated in 5210B.6c. If the dilution water does not meet these criteria, proper corrections are difficult; do not record results or, as a minimum, mark them as not meeting quality control criteria.

b. Reporting: Average the test results for all qualified bottles within each dilution series. Report the result as BOD_5 if nitrification is not inhibited. Report results as CBOD_5 if nitrification is inhibited. Samples showing large differences between the computed BOD for different dilutions, for example, greater than 30%, may indicate the presence of a toxic substance or analytical problems. When the effect becomes repetitive, investigate to identify the cause. Identify results in the test reports when any of the following quality control parameters is not met:

- Dilution water blank exceeds 0.20 mg/L (5210B.6c),
- Glucose-glutamic acid check falls outside acceptable limits (5210B.6b),
- Test replicates show more than 30% difference between high and low values,
- Seed control samples do not meet the above criteria in all dilutions (5210B.6d), or
- Minimum DO is less than 1.0 mg/L [5210B.7a3].

8. Precision and Bias

There is no measurement for establishing bias of the BOD procedure. The glucose-glutamic acid check prescribed in 5210B.6b is intended to be a reference point for evaluation of dilution water quality, seed effectiveness, and analytical technique. Single-laboratory tests using a 300-mg/L mixed glucose-glutamic acid solution provided the following results:

Number of months:	14
Number of triplicates:	421
Average monthly recovery:	204 mg/L
Average monthly standard deviation:	10.4 mg/L

In a series of interlaboratory studies,² each involving 2 to 112 laboratories (and as many analysts and seed sources), 5-d BOD measurements were made on synthetic water samples containing a 1:1 mixture of glucose and glutamic acid in the total concentration range of 3.3 to 231 mg/L. The regression equations for mean value, X , and standard deviation, S , from these studies were:

$$X = 0.658 (\text{added concentration, mg/L}) + 0.280 \text{ mg/L}$$

$$S = 0.100 (\text{added concentration, mg/L}) + 0.547 \text{ mg/L}$$

For the 300-mg/L mixed primary standard, the average 5-d BOD would be 198 mg/L with a standard deviation of 30.5 mg/L. When nitrification inhibitors are used, GGA test results falling outside the 198 ± 30.5 control limit quite often indicate use of incorrect amounts of seed. Adjust amount of seed added to the GGA test to achieve results falling within this range.

a. Control limits: Because of many factors affecting BOD tests in multilaboratory studies and the resulting extreme variability in test results, one standard deviation, as determined by interlaboratory tests, is recommended as a control limit for individual laboratories. Alternatively, each laboratory may establish its control limits by performing a minimum of 25 glucose-glutamic acid checks (5210B.6b) over a period of several weeks or months and calculating the mean and standard deviation. Use the mean ± 3 standard deviations as the control limit for future glucose-glutamic acid checks. Compare calculated control limits to the single-laboratory tests presented above and to interlaboratory results. If the glucose-glutamic acid test results are outside the range of 198 ± 30.5 , re-evaluate the control limits and investigate source of the problem. If measured BOD for a glucose-glutamic acid check is outside the accepted control limit range, reject tests made with that seed and dilution water or identify such tests clearly in all data records and reports.

b. Working range and detection limit: The working range is equal to the difference between the maximum initial DO (7 to 9 mg/L) and minimum DO residual of 1 mg/L corrected for seed, and multiplied by the dilution factor.

Detection limits are established by the minimum DO depletion and minimum DO residuals as follows:

- The lower detection limit for unseeded samples that require dilution ($S = 0$; $P < 1.0$) is 2 mg/L multiplied by the dilution factor as established by the requirement for a minimum DO depletion of 2 mg/L.
- The lower limit for seeded samples that require dilution ($S > 0$; $P < 1.0$) is approximately 1 mg/L as established by the minimum depletion of 2.0 mg/L minus the maximum seed correction, which should be less than about 1 mg/L.
- The lower limit for unseeded samples that require no dilution ($S = 0$; $P = 1.0$) is equal to the detection limit of the DO measurement method (~ 0.1 mg/L).
- The lower detection limit for seeded samples that require no dilution ($S > 0$; $P = 1.0$) is 0 mg/L, as established by the difference between the sample DO depletion and the seed correction.

9. References

1. YOUNG, J.C. 1973. Chemical methods for nitrification control. *J. Water Pollut. Control Fed.* 45:637.
2. U.S. ENVIRONMENTAL PROTECTION AGENCY, OFFICE OF RESEARCH AND DEVELOPMENT. 1986. Method-by-Method Statistics from Water Pol-



Sample Dilutions Criteria

REQUIREMENTS FOR VALID BOD₅ RESULTS

Blank depletion must be ≤ 0.2 mg/L DO

Initial DO must be ≤ 9.0 mg/L

Samples must deplete at least 2.0 mg/L DO

Samples must have at least 1.0 mg/L DO remaining
at the end of the incubation period



Seeding Calculations

DO
depletion
per mL

<u>Bottle</u>	<u>DO_i</u>	<u>DO_f</u>	<u>Depletion</u>	<u>mL seed</u>	<u>seed</u>
Seed 1	8.5	0.3	X 8.2	30	-----
Seed 2	8.4	1.6	÷ 6.8	20	= 0.34
Seed 3	8.4	4.3	÷ 4.1	10	= 0.41

Avg the seed controls that meet depletion criteria
 $(0.34 + 0.41) \div 2 = 0.375$ mg/L DO depletion per mL seed

So, 2 mL undiluted seed is added to each sample
 bottle. $0.375 \times 2 = 0.75$ mg/L

Therefore, 0.75 mg/L is subtracted from the depletion of
 each BOD depletion to obtain BOD result.

BOD (CBOD) (5210 B-2001)

Power point presentation

Setup teams (2 people per team)

Check incubator temperature ($20 \pm 1^\circ\text{C}$)

Nitrification inhibitor for CBOD samples (Hach #253335)

Add to all samples except dilution water for CBODs

Prepare deionized water (4L per team)

Set up BOD bottles (13-16 bottles)

2 dilution water blanks

2 GGA standards

3 seed controls

3-4 influent sample dilutions (including duplicate)

3-4 effluent sample dilutions (including duplicate)

Prepare dilution water (5210 B-2001 5.a.)

Clean bottle for dilution water (3L capacity)

**BOD Nutrient Buffer Powder Pillows, 3L,
Hach #1486166 (1 batch per team with a little left over)**

Dilution water for influent samples per team

1200mL

Dilution water for effluent samples per team

600mL

BOD Nutrient Buffer Pillows, 300 mL, Hach #1416066

*Used for 300mL sample (any dilution greater than 200mL) (5210 B-2001
5.c.2.)*

Obtain influent, effluent and mixed liquor (for seed material if not using freeze-dried seed) samples from STP**Preparation of samples (5210 B-2001 4.b.1.)*****Check pH (if not between 6.0 and 8.0, then)***

Adjust temperature to $20 \pm 3^\circ\text{C}$

Adjust pH to 7.0 to 7.2 using sulfuric acid and/or sodium hydroxide

Treat for total residual chlorine (5210 B-2001 4.b.2.)***Supersaturation (5210 B-2001 4.b.4.) initial DO <9.0 mg/L before setting up dilutions******Dilutions (5210 B-2001 5.c.)***

Influent (50mL per team)

3.0mL

6.0mL

9.0mL

duplicate

Effluent (1000mL per team)

75mL

150mL

300mL

duplicate

Set up seed controls (5210 B-2001 6.d.) Can use freeze-dried seed material; settled raw wastewater or primary effluent or ~1-2mL of 1:10 dilution of mixed liquor per 300mL bottle

25.0mL for mixed liquor dilution

30.0mL

35mL

Set up GGA (glucose glutamic acid) standard

2 bottles 6mL GGA (according to instructions)

Adding amount of seed material, same as samples

Set up 2 bottles of dilution water one at the beginning and one at the end of the samples

CBOD / BOD WORKSHEET

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Incubator Temperature °C					

Location Collected: _____ **Initial Date:** _____ **After Cl₂:** _____ **Sample Seeded:** _____
Sample Type: _____ **Final Date:** _____ **Before Cl₂:** **Time Sample Collected:** _____

	INFLUENT (RAW)			EFFLUENT			UP STREAM			DOWN STREAM			BLANKS	
	DUP			DUP			DUP			DUP			300ml	300ml
mL. used OR % Concentration (P)													100%	100%
Bottle Number														
Initial DO (D ₁)														
(-) Final DO (D ₂)														
Oxygen Demand (D ₁ - D ₂)														
(-) Depletion due to seed (B ₁ - B ₂)f														
Dilution Factor (1/P) Vol/300mL = P														
5-Day BOD (mg/L)														

Avg. mg/L

For unseeded samples: $BOD_5, \text{mg/L} = \frac{(D_1 - D_2)}{P}$

For seeded samples: $BOD_5, \text{mg/L} = \frac{(D_1 - D_2) - (B_1 - B_2) f}{P}$

Add 4mL of settled primary clarifier effluent or settled raw sewage for each liter of seeded dilution water. Manufactured seed material is also available.

Seed Control				
mL. used OR % Concentration (P)				
Initial DO (B ₁)				
Final DO (B ₂)				
Oxygen Demand				

Seed Dilution Water

(Oxygen Demand Calculation)

Source of seed material: _____

Initial Date: _____ Final Date: _____

% Concentration Used: _____

If seed material is added directly to sample or to seed control

$f \text{ Value: } f = \frac{(\text{volume of seed in diluted sample})}{(\text{volume of seed in seed control})}$

Glucose-Glutamic Acid (GGA) Test(18th edition Standard Methods 5210B)

Intended use for evaluation of dilution water quality, seed effectiveness, and analytical technique

Use 6 mL of BOD Standard and ___ mL of seed (typically 2 mL)

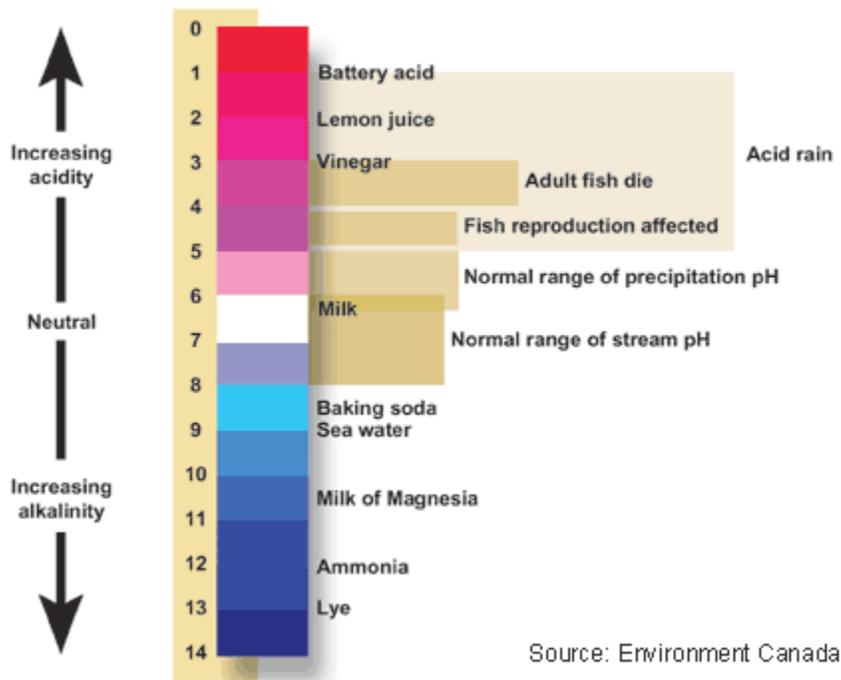
	GGA std			
mL. GGA used	6	6		
mL seed	2	2		
Bottle Number				
Initial DO (D ₁)				
(-) Final DO (D ₂)				
Oxygen Demand (depletion) (D ₁ - D ₂)				
(-) Depletion due to seed (B ₁ - B ₂)f				
Net depletion due to GGA (D ₁ - D ₂) - (B ₁ - B ₂)f				
Dilution Factor (300/6)	50	50		
5-Day BOD (mg/L)				

GGA std BOD values should lie within the range of 198 ± 30.5 mg/L. (167.5-228.5 mg/L)

1. Weak seed usually causes LOW GGA results.
2. Some sewage seeds are relatively inactive and yield LOW GGA results.
3. Soap contamination typically yields HIGH GGA results.
4. Distilled water contaminated with copper yields LOW GGA results.
5. Too much seed usually causes HIGH GGA results
6. BOD bottles that are not properly rinsed is indicated by HIGH GGA results and would also result in high blank depletion

EXAMPLE WORKSHEET

Section 6 pH





pH

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pH

- ▶ One of the most important and frequently used tests in water chemistry
- ▶ A measure of the intensity of the acidic or alkaline character of a solution
- ▶ Logarithmic scale of ionic activity 0 to 14 s.u.
- ▶ pH values cannot be averaged

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pH Measurement

- ▶ pH is typically measured with a meter and probe
- ▶ This is an electrochemical method of analysis



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pH Theory

- ▶ Acid
 - ▶ increases the hydrogen ion (H⁺) concentration in a solution
- ▶ Base
 - ▶ increases the hydroxide ion (OH⁻) concentration in a solution

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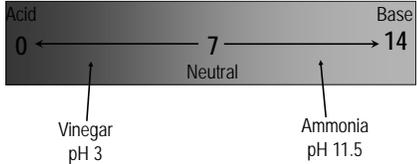
pH Theory

- ▶ pH is defined as the negative log of the molar hydrogen ion concentration in aqueous solution

$$\text{pH} = -\log [\text{H}^+]$$

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pH Scale



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pH Scale

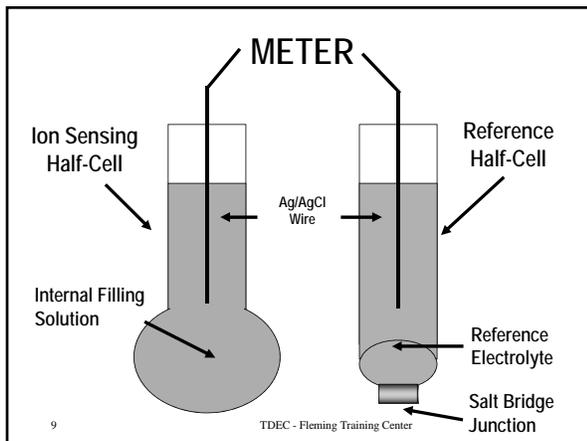
- ▶ pH is a negative logarithmic function
- ▶ Each decrease in pH unit = 10X increase in acidity
 - ▶ Solution at pH4 is 10X more acidic than solution at pH5
 - ▶ Solution at pH 4 is 100X more acidic than pH6 solution

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How Does a pH Probe Work?

- ▶ Probe measures hydrogen ion concentration
 - ▶ Two electrodes in probe:
 - a) sensing half-cell,
 - b) reference half-cell

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Reference Half-Cell

- ▶ Dispenses reference solution which completes circuit for meter

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Sensing Half-Cell

pH 7 Solution

H⁺ conc. the same both inside and outside glass bulb

*No potential develops

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Sensing Half-Cell

pH 7 Solution

H⁺ conc. the same both inside and outside glass bulb

*No potential develops

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Sensing Half-Cell

pH 4 Solution
 H^+ conc. 1000x greater outside glass bulb
 *Potential develops

Hydrogen ion concentration fixed at pH 7

1000H⁺
1000H⁺
1000H⁺
1000H⁺
1000H⁺
1000H⁺
1000H⁺
1000H⁺
1000H⁺
1000H⁺

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Sensing Half-Cell

pH 4 Solution
 H^+ conc. 1000x greater outside glass bulb
 *Potential develops

180mV

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Sensing Half-Cell

pH 10 Solution
 H^+ conc. 1000x greater inside glass bulb
 *Potential develops

Hydrogen ion concentration fixed at pH 7

H⁺
H⁺
H⁺
H⁺
H⁺
H⁺
H⁺
H⁺
H⁺
H⁺

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Sensing Half-Cell

pH 10 Solution
 H^+ conc. 1000x greater inside glass bulb
 *Potential develops

-180mV

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Calibration

▶ A calibration curve allows the meter to convert a measured millivolt potential into a pH reading.

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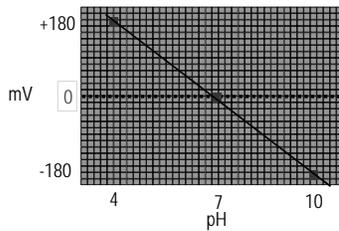
Calibration

▶ The optimal slope for pH is -58 ± 3 mV/decade.

What does this mean?

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Calibration



The mV values from the previous demos (4, 7, and 10 buffers) are plotted on a graph of mV versus pH. A line is drawn between the points and the slope determined. Since slope is rise over run, it will be in units of mV per pH unit, or mV per decade (since the difference between pH units is 10X).

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Calibration

- ▶ -180mV difference measured between pH4 and pH7
- ▶ pH4 to pH7 (3 pH units) is 1000x concentration change
- ▶ Decade = 10-fold concentration change
= 1pH unit
- ▶ $-180/3 = -60 \approx -58\text{mV/decade}$

▶ 20

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Importance of pH control

- ▶ Ammonia toxicity is influenced by pH
- ▶ pH plays an important role in the solubility of metal salts
- ▶ pH affects the rate at which chlorine reacts to form chloramines (which are less effective disinfectants)

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pH Sampling

- ▶ Holding time = 15 minutes
- ▶ Preservation = none
- ▶ Sample container = glass or plastic
- ▶ Grab sample
- ▶ Continuous monitoring possible

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pH Meter Calibration

- ▶ Follow manufacturers instructions
- ▶ Use fresh buffers (4, 7, & 10 s.u.)
- ▶ Stir buffers and samples at the same speed without a vortex
- ▶ Rinse and blot dry electrodes between samples and buffers
- ▶ Accurate and reproducible to within 0.1 s.u.

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pH Meter Calibration (cont.)

- ▶ Start with pH = 7.0 buffer (usually)
- ▶ Second buffer 3 s.u. different that brackets expected sample pH (4 or 10)
- ▶ Immerse in a third buffer - reading should be within 0.1 s.u.
- ▶ If response is accurate - read and record previous buffers as samples (pH and temperature)

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Common Deficiencies

- ▶ The pH meter was calibrated using one buffer or expired buffers
- ▶ The continuous pH meter was not calibrated on a regular basis
- ▶ Buffers were left open and being reused for a week

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Maintenance

- ▶ New probe
- ▶ Calibration
- ▶ Measurement/Storage
- ▶ Troubleshooting
- ▶ Cleaning



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New Probe

- ▶ Condition new pH probe in pH 7 buffer for approximately 30 minutes before initial use
- ▶ Probes must also be conditioned after cleaning or if it had been stored dry as long term storage

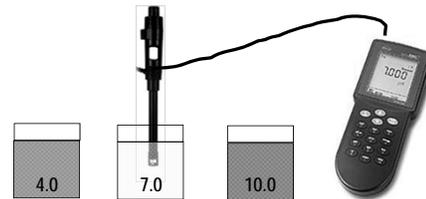


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Calibrate

- ▶ Calibrate pH meters daily using two or three buffer solutions



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Measurement

- ▶ Place probe into sample, stir, and wait for readings to stabilize
- ▶ Rinse and **blot** dry between measurements
- ▶ Storage between measurements
 - ▶ As suggested by probe manufacturer
 - ▶ pH electrode storage solution (*best*)

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Troubleshooting

- ▶ mV reading in pH 7 buffer
 - ▶ Should read 0 ± 30 mV in pH 7 buffer
- ▶ Response time
 - ▶ May require cleaning if slow in buffered solution
- ▶ Slope
 - ▶ Optimal slope is -58 ± 3 mV/decade

▶ 30

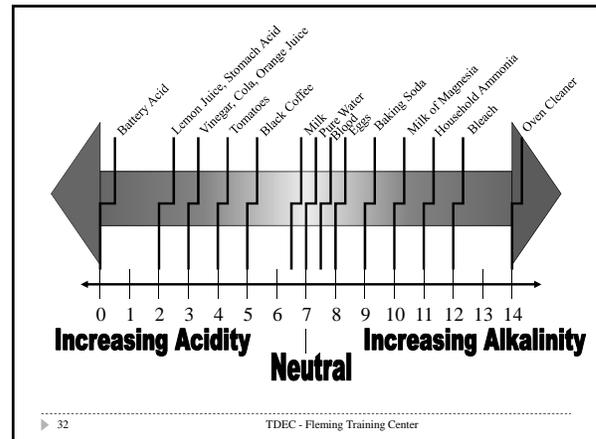
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Cleaning

- ▶ Slow response may indicate need for cleaning
 - ▶ Alternate soaking in dilute hydrochloric acid and dilute sodium hydroxide
 - ▶ Rinse with deionized water
 - ▶ Condition in pH 7 buffer before use
 - ▶ Filler hole button was not removed
- ▶ Read probe manual for cleaning method recommended by manufacturer

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pH SM4500-H⁺ B – 2000 Electrometric Method

- ▶ DOC
- ▶ Dup
- ▶ ICAL/CCV
- ▶ Corrective Action
- ▶ QC Acceptance
- ▶ Batch Size
- ▶ QC Frequency



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pH SM4500-H⁺ B – 2000 Electrometric Measurement

- ▶ Read to 1/10th units only, 0.0 s.u.
- ▶ Demonstration of Capability (DOC)
 - ▶ Run buffer at least four times and compare to the limits listed in the method
 - ▶ Real people language: each operator running this test need to calibrate and analyze 4 buffers at a pH of 7
 - ▶ Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
 - ▶ Recommend backup analyst do this once a year.

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pH SM4500-H⁺ B – 2000 Electrometric Measurement

- ▶ Initial Calibration
 - ▶ Calibrate per manufactures instructions with fresh buffers daily (day of).
 - ▶ **2014 Update – Analyze a 7 buffer solution as a sample after calibration and before samples to verify initial calibration (ICV), should be within ± 0.2 s.u.**
- ▶ Calibration Verification
 - ▶ Read 7 buffer after analyzing samples daily



▶ 35

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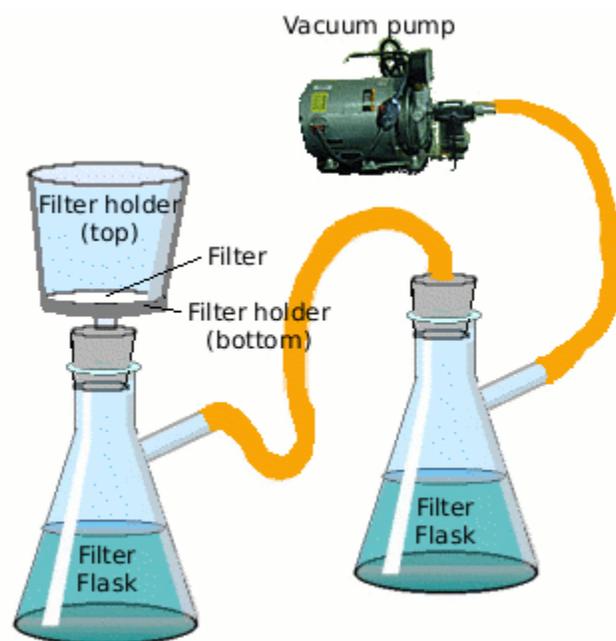
pH SM4500-H⁺ B – 2000 Electrometric Measurement

- ▶ Duplicates of the sample
 - ▶ Run on a 5% basis, one for every 20 samples
 - ▶ Within ± 0.2 s.u.
 - ▶ **2014 Update – For reporting purposes, all duplicates should be reported according to your permit limits. If your permit sets a minimum or maximum limit such as pH, then the minimum or maximum value should be reported even if falls outside your permit limit.**

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Section 7 Solids





Analysis of Solids in Wastewater

Wastewater Laboratory Class



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Why do I care about Solids?

- Major function of STP is reduction organic loading of wastewater for safe discharge to receiving stream
 - Biological treatment: monitored through BOD and COD (Demands)
 - Sedimentation: monitored through total suspended solids (TSS)

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Wastewater Solids Removal

- Most suspended solids are organic
- Oxygen Demand
- Serve as refuge for harmful bacteria
- Unsightly appearance

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Solids Testing

- Why should I care about solids tests?
 - Assessment of compliance
 - Control of Biological and Physical treatment process
 - Poor data = Poor decisions

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Definitions

- Total Solids
 - All residue left after drying
- Dissolved Solids
 - The portion of TS which pass through a 2.0um filter
- Suspended Solids
 - The portion retained on the 2.0um filter
- Fixed Solids
 - The portion of TS, DS, TSS which remains after ignition at 550°C
- Volatile Solids
 - The portion which burned away at 550°C

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Standard Methods 2540B-G

- Sources of error
 - Sampling
 - Sub sampling
 - Measuring
 - Filter/Bowl preparation
 - Filter/Bowl handling
- Remedies
 - MIX WELL
 - Measure quickly

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Standard Methods 2540B-G

- Temperatures
 - Each Method has a specified drying Temp.
 - Minimize opening desiccator
 - Weigh quickly, dry samples attract moisture
- Rinse Water
 - Type III, distilled or deionized water

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Standard Methods 2540B-G

- Sampling
 - Glass or plastic containers, watch for particles adhering to container walls especially plastic.
- Begin test ASAP is preferred.
- Sampling Holding
 - Preserve at 4°C to minimize bio. decomp.
 - Hold <24 hours preferred
 - Never hold over 7 days

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Method Choice

- Methods B-F
 - Potable, surface, saline, domestic and industrial wastewater up to 20,000mg/L
- Method G
 - Solid and Semisolid samples >2%
 - Biosolids, per 40 CFR 503

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Total Solids 2540-B

- Temperature 103-105°C
- Calculations based on sample volume

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Dissolved Solids 2540-C

- Temperature 180°C
- Non-regulatory at 103-105°C

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Total Suspended Solids 2540-D

- Temperature 103-105°C
- Interferences
 - Limit sample size to 200mg of residue, small filter
 - Avoid prolonged filtration times >5-7 min.
- Glass fiber filter 22-125mm diameter.
 - Whatman 934AH 1.5um
 - Gelman A/E 1.0um
 - Millipore AP40
 - E-D Scientific Grade 161

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TSS SM2540 D – 1997
Dried at 103-105°C



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TSS SM2540 D – 1997
Dried at 103-105°C

- Filter preparation:
 - Pre-wash approved glass fiber filter by rinsing three times with 20mL of deionized (DI) water
 - Dry in weighing pans at 103 – 105° C for 1 hour and cool in desiccator
 - Record the initial weight of the filter & pan after drying
 - Repeat this cycle of drying, desiccating and weighing until a constant weight has been reached

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TSS SM2540 D – 1997
Dried at 103-105°C

- Sample Analysis:
 - Choose a sample volume to yield between 2.5 - 200 mg dried residue
 - Assemble filtering apparatus and filter
 - Wet filter with small amount of distilled water to seal before applying the sample
 - Sample must be well mixed before applying to filter
 - Filter sample

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TSS SM2540 D – 1997
Dried at 103-105°C

- Sample Analysis – continued:
 - Wash filter and apparatus three times with 10 mL of DI water
 - Transfer filter into support pans
 - Dry in oven at 103-105 ° C for at least 1 hr and cool in desiccator
 - Weigh filter until a constant weight is obtained

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TSS SM2540 D – 1997
Dried at 103-105°C

- Fats, Oil, and Grease
 - May interfere due to difficulty in drying to a constant weight in time range.
- Duplicates should weigh within 5% of their average weight
- Weigh to a constant weight, within 4% or 0.0005g, whichever is less with at least a second drying/cooling/weighing step.

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Total Suspended Solids Analysis
Procedure to Omit Re-drying/Re-cooling/Re-weighing

How to acquire acceptable results for the total suspended solids comparability data:

- The maximum holding time for a total suspended solids sample prior to analysis is 7 days if stored at temperatures of 6°C and below (not 0°C). (40CFR part 136, Table JJ)
- EPA recommends that 4-7 different samples, in duplicate, be collected and analyzed for this procedure in order to prove that the step for "reheating, recooling, and reweighing" is unnecessary. "Different" could mean samples collected 4-7 consecutive days or 4-7 samples run in one day. These 4-7 samples are dried *overnight* at 103-105°C.
- The next morning, the filters are removed from the oven, allowed to cool in the desiccator and weighed.
- The samples are then returned to the drying oven for one hour, recooled and reweighed.
- The resulting data should be examined to determine if the difference between the overnight values and the redried values are less than 4% or 0.5 mg, whichever is less. If so, the redrying step may be omitted for a normal set of samples.
- This procedure excludes atypical samples. (i.e. high fat, oil and grease samples).
- The operator may choose not to perform this study and continue to follow the procedure for redrying/recooling/reweighing as stated the method (Std Methods, 2540 D).

The study should be re-evaluated at least once per year or whenever a change in sample characteristics occurs and kept on file at the treatment plant.

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TSS SM2540 D – 1997 Dried at 103-105°C

- Uses
 - Influent and effluent regulatory tests
 - Mixed Liquor Suspended Solids MLSS
 - Return Activated Sludge RAS
 - Clarifier Core Suspended Solids
 - Stream samples
 - Some digester solids tests

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TSS SM2540 D – 1997 Dried at 103-105°C

Sample	Common Range, mg/L
Influent	150 – 400
Primary Effluent	60 – 150
Secondary Effluent	10 – 60
Tertiary Effluent	0.0 – 3.0
MLSS	1,000 – 5,000
RAS	2,000 – 12,000

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TSS Calculations

- TSS in mg/L is equal to the amount of residue retained on the filter in mg per liter of sample
- Formula: $TSS\ mg/L = \frac{(A - B) \times 1,000,000}{\text{sample volume mL}}$
- Where:
 - A = wt. of filter + dried residue (g)
 - B = initial wt. of filter (g)
 - 1,000,000 = conversion factor (1 g = 1000 mg & 1L = 1000 mL)

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TSS Calculations

- Wt. of filter + residue = A = 1.0215 g
- Wt. of filter = B = 1.0160 g
- Sample volume = 200
- $Mg/L = \frac{(1.0215 - 1.0160)(1,000,000)}{200}$
 $= \frac{(0.0055)(1,000,000)}{200}$
 $= \frac{5500}{200}$
 $= 27.5\ mg/L$

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TSS Calculations

- **ppm** is an abbreviation of **parts per million**. **ppm** is a value that represents the part of a whole number in units of 1/1000000.
- $1\ ppm = 1\ mg/l = 1\ ug/ml = 1000\ ug/L$
- **Parts** can be any measure, pints, gallons, or a drop of water (grape juice, antifreeze, etc.). The size of the sample doesn't matter. It's the **RATIO** of the tested parts to the total number of parts that's important.
- Now think about a milligram. It is 1/1000th of a gram, making it 1/1,000,000th of a kilogram. **Put another way, a liter of water weighs 1,000,000 milligrams.** One million milligrams... see where this is going?

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TSS Calculations

- Let's say you're given 4000 mg/L of suspended solids and you want to determine the % of solids. This is measurement is the same as 4000 ppm (parts per million).
- Divide the ppm by 1,000,000 then multiply by 100 to get %.

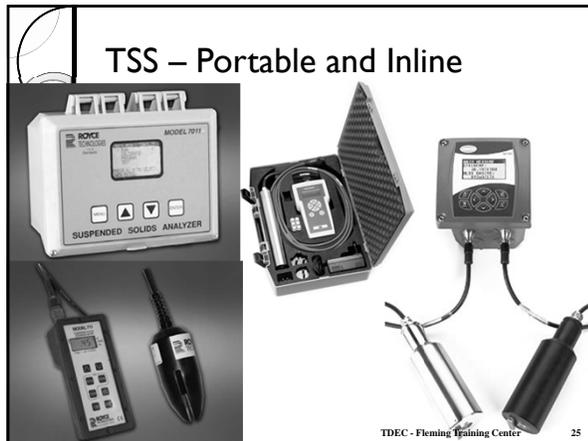
$$\frac{4,000}{1,000,000} = \frac{4}{1000}$$

$$= 0.004 \times 100$$

$$= 0.4\%$$

- Alternatively, divide the % value by 100 and multiply by 1,000,000 to get **ppm**.

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TSS SM2540 D – 1997 Dried at 103-105°C

- DOC • Corrective Action
- LRB • QC Acceptance
- LFB • Batch Size
- Dup • QC Frequency
- ICAL

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TSS SM2540 D – 1997 Dried at 103-105°C

- Demonstration of Capability (DOC)
 - Run a laboratory-fortified blank (LFB) at least four times and compare to the limits listed in the method
 - Real people language: each operator running this test need to analyze 4 samples of an Total Suspended Solids Standards
 - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
 - Recommend backup analyst do this once a year.

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TSS SM2540 D – 1997 Dried at 103-105°C

- Blanks
 - Filter 100 mL of deionized/distilled water through a pre-washed/pre-dried/pre-weighed filter with each batch of 20 or fewer samples
 - Run on a 5% basis, one for every 20 samples
 - **2014 Update – Should be less than 2.5 mg/L**
- Laboratory Fortified Blank
 - Real people language: analyze a TSS standard that can be prepared from recipe (next slide) or bought premade
 - Run on a 5% basis, one for every 20 samples

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TSS SM2540 D – 1997 Dried at 103-105°C

- To prepare TSS check samples from dry reference material:
 - Dry the reference material* in the desiccator
 - On an analytical balance, weigh 0.1000 gram of the dry powder, put it in a 1000 mL volumetric flask, bring it to the mark with distilled or deionized water and shake well until well suspended.
 - Measure 100 mL and process as usual for environmental samples.
 - A difference of 10 mg should be obtained.
 - Calculation: $\frac{(A - B) (1000)}{\text{Vol. used}} = \frac{(10 \text{ mg}) (1000)}{100 \text{ mL}} = 100 \text{ mg/L}$

*Example of material available from Fisher

- Celite 545 Filter Aid (Powder), Fisher Chemical, 500 gram bottle – Cat#C212-500

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TSS SM2540 D – 1997 Dried at 103-105°C

- Standard Methods 2020.B.2.a
 - Check instrument balances daily
- Standard Methods 9020.B.4.b
 - Service balances annually or more often as conditions change or problems occur
 - Check balances routinely, preferably daily before use, with at least two working weights that bracket the normal usage range (e.g. ANSI/ASTM Class 1 or NIST Class S accompanied by appropriate certificate) for accuracy, precision and linearity.
 - Record results along with date and technicians initials
 - Recertify reference weights as specified in the certificate of calibration or at least every 5 years.

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TSS SM2540 D – 1997

Dried at 103-105oC

- Initial Calibration
 - Check balances daily (day of) with at least 2 working weights that bracket the normal usage range and record results on bench sheet or separate log book
- Duplicates
 - Run on a 10% basis, one for every 10 samples
 - Calculate %RPD
 - Target value should be close to the first value and have a small RPD (less than 15%)
 - **2014 Update – For reporting purposes, average sample and duplicate.**

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TSS SM2540 D – 1997

Dried at 103-105oC

- **2014 Update** - Create and maintain control charts if you have 20-30 data points within 90 days.
 - If you do not meet the above criteria, follow QC Acceptance Criteria below.
 - Blanks < 2.5 mg/L
 - LFB ± 15%
 - RPD < 15%

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SS SM2540 F – 1997

Settleable Solids

- Important process control test for rates and volume of sedimentation
- Report as mL/L
 - Minimum Detection Limit 0.1mL/L
 - Adjust for water layers if present
- Basin control: Imhoff cone
 - Sample volume: 1 L
 - Solids settle 45 min
 - Gently stir sides
 - Solids settle another 15 minutes
 - Read after a total of 60 min

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SS SM2540 F – 1997

Settleable Solids

Sample	Common Range, mL/L
Influent	8 – 20
Primary Effluent	0.1 – 3
Secondary Effluent	0.1 – 0.5

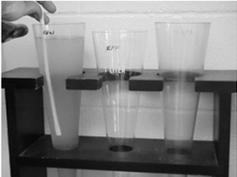


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SS SM2540 F – 1997

Settleable Solids

- Dup
- Corrective Action
- Batch Size
- QC Frequency



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SS SM2540 F – 1997 Settleable Solids

- Duplicates
 - For example, pour up 1000 mL of effluent into Imhoff then pour up another 1000 mL of effluent in another Imhoff. Wait 45 min, stir, wait 15 min, read. Figure RPD for both samples.
 - Calculate RPD, (less than 20%)
 - Run on a 5% basis (see batch size for more information).
 - **2014 Update – For reporting purposes, average sample and duplicate.**



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SS SM2540 F – 1997 Settleable Solids

- **2014 Update** - QC Acceptance Criteria below.
 - RPD < 20%
 - Reporting Limit = lowest graduation mark on Imhoff cone

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Total Solids 2540-G

- For solid and semisolid materials
 - Required or Biosolids per 40 CFR 503
- Calculations based of wet and dry weight.

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Fixed and Volatile 2540-E

- An additional step of the Total, Suspended, or Dissolved Solids test.
- Sample is ignited at 550°C for 1 hr.
- Fixed Solids or Ash remains
- Volatile Solids were burned away.

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Balance Operation

- Key to quality solids tests
- Care of Balance
 - Vibration, heat, sunlight
 - Dust, dirt, moisture
- Calibration
 - Outside contractor
 - In house, "S" class weights

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2540 D. Total Suspended Solids Dried at 103–105°C

1. General Discussion

a. Principle: A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105°C. The increase in weight of the filter represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, it may be necessary to increase the diameter of the filter or decrease the sample volume. To obtain an estimate of total suspended solids, calculate the difference between total dissolved solids and total solids.

b. Interferences: See 2540A.2 and 2540B.1b. Exclude large floating particles or submerged agglomerates of nonhomogeneous materials from the sample if it is determined that their inclusion is not representative. Because excessive residue on the filter may form a water-entrapping crust, limit the sample size to that yielding no more than 200 mg residue. For samples high in dissolved solids thoroughly wash the filter to ensure removal of dissolved material. Prolonged filtration times resulting from filter clogging may produce high results owing to increased colloidal materials captured on the clogged filter.

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Tables 2020:I and II.

2. Apparatus

Apparatus listed in 2540B.2 and C.2 is required, except for evaporating dishes, steam bath, and 180°C drying oven. In addition:

Aluminum weighing dishes.

3. Procedure

a. Preparation of glass-fiber filter disk: If pre-prepared glass fiber filter disks are used, eliminate this step. Insert disk with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with three successive 20-mL portions of reagent-grade water. Continue suction to remove all traces of water, turn vacuum off, and discard washings. Remove filter from filtration apparatus and transfer to an inert aluminum weighing dish. If a Gooch crucible is used, remove crucible and filter combination. Dry in an oven at 103 to 105°C for 1 h. If volatile solids are to be measured, ignite at 550°C for 15 min in a muffle furnace. Cool in desiccator to balance temperature and weigh. Repeat cycle of drying or igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less. Store in desiccator until needed.

b. Selection of filter and sample sizes: Choose sample volume to yield between 2.5 and 200 mg dried residue. If volume filtered fails to meet minimum yield, increase sample volume up to 1 L. If complete filtration takes more than 10 min, increase filter diameter or decrease sample volume.

c. Sample analysis: Assemble filtering apparatus and filter and begin suction. Wet filter with a small volume of reagent-grade water to seat it. Stir sample with a magnetic stirrer at a speed to shear larger particles, if practical, to obtain a more uniform (preferably homogeneous) particle size. Centrifugal force may separate particles by size and density, resulting in poor precision when point of sample withdrawal is varied. While stirring, pipet a measured volume onto the seated glass-fiber filter. For homogeneous samples, pipet from the approximate midpoint of container but not in vortex. Choose a point both middepth and midway between wall and vortex. Wash filter with three successive 10-mL volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 min after filtration is complete. Samples with high dissolved solids may require additional washings. Carefully remove filter from filtration apparatus and transfer to an aluminum weighing dish as a support. Alternatively, remove the crucible and filter combination from the crucible adapter if a Gooch crucible is used. Dry for at least 1 h at 103 to 105°C in an oven, cool in a desiccator to balance temperature, and weigh. Repeat the cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 0.5 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight. If volatile solids are to be determined, treat the residue according to 2540E.

4. Calculation

$$\text{mg total suspended solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

where:

A = weight of filter + dried residue, mg, and
B = weight of filter, mg.

5. Precision

The standard deviation was 5.2 mg/L (coefficient of variation 33%) at 15 mg/L, 24 mg/L (10%) at 242 mg/L, and 13 mg/L (0.76%) at 1707 mg/L in studies by two analysts of four sets of 10 determinations each.

Single-laboratory duplicate analyses of 50 samples of water and wastewater were made with a standard deviation of differences of 2.8 mg/L.

6. Bibliography

- DEGEN, J. & F.E. NUSSBERGER. 1956. Notes on the determination of suspended solids. *Sewage Ind. Wastes* 28:237.
- CHANIN, G., E.H. CHOW, R.B. ALEXANDER & J. POWERS. 1958. Use of glass fiber filter medium in the suspended solids determination. *Sewage Ind. Wastes* 30:1062.
- NUSBAUM, I. 1958. New method for determination of suspended solids. *Sewage Ind. Wastes* 30:1066.

TSS Exercise

Total _____ Solids
SM ____ D-2011

1. A sample that is _____ is filtered through a weighed standard glass-fiber filter.
2. The residue retained on the filter is _____ to a constant weight at _____ to _____°C.
3. Exclude large floating _____ or submerged agglomerates of nonhomogeneous _____ from the sample if their inclusion is not _____.
4. Because _____ on the filter may form a water-_____ crust, limit the _____ to that yielding no more than _____ residue.
5. For samples _____ in _____ thoroughly _____ the filter to ensure removal of dissolved material.
6. *Preparation of _____ filter disk:*
7. If _____ glass fiber filter disks are used, eliminate this step.
8. Insert disk with _____ up in filtration apparatus.
9. Apply vacuum and wash disk with _____ portions of _____ water.
10. Continue suction to remove _____ of water, turn vacuum off, and _____ washings.
11. Remove filter from _____ apparatus and transfer to an _____ weighing dish.
12. Dry in an oven at 103 to 105°C for _____.
13. If _____ solids are to be measure, ignite at _____ for 15 min in a _____.
14. Cool in _____ to balance temperature and _____.
15. _____ cycle of _____ or igniting, _____, _____, and _____ until a _____ weight is obtained or until weight change is _____ of the previous weighing or _____, whichever is _____.
16. Store in _____ until needed.
17. *Selection of filter and _____:*
18. Choose sample _____ to yield between 2.5 and 200 _____ dried residue.
19. If volume filtered fails to meet _____ yield, _____ sample volume up to _____.
20. If _____ takes more than _____. increase filter diameter or _____ sample volume.
21. *Sample _____:*
22. Assemble filtering apparatus and filter and being _____.
23. _____ filter with a small _____ of _____ water to _____ it.

24. Stir sample with magnetic stirrer at a speed to shear larger particles, _____, to obtain a more uniform (_____) particle size.
25. _____ force may separate particles by _____, resulting in _____ when point of sample withdrawal is varied.
26. While _____, pipet a measured volume onto the seated glass-fiber filter.
27. For homogeneous samples, pipet from approximate _____ of container but ____ in _____.
28. Choose a point both _____ and _____ between wall and vortex.
29. Wash filter with _____ successive _____ volumes of reagent-grade water, allow _____ drainage between washings, and _____ suction for about ____ after filtration is _____.
30. Samples with _____ dissolved solids may require additional _____.
31. Carefully remove filter from filtration apparatus and _____ to an aluminum weighing dish as a _____.
32. Dry for _____ at 103 to 105°C in an oven, cool in a desiccator to balance temperature, and weight.
33. _____ the cycle of drying, cooling, desiccating, and weight until a constant weight is obtained or until the weight change is less than ____ of the previous weight or _____ whichever is less.
34. Analyze _____ of all samples in _____.
35. _____ determinations should agree within ____ of their _____ weight.

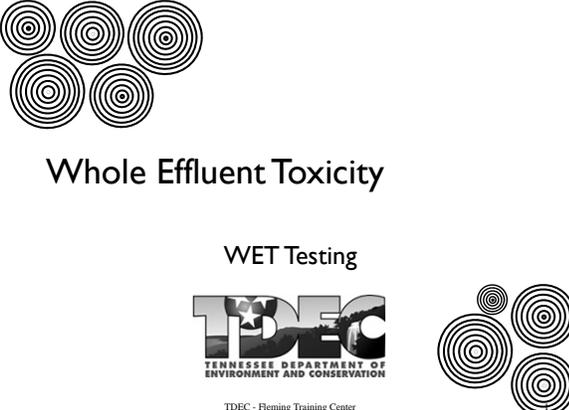
TSS Sample Worksheet

Sample	Dish Number	Filter Weight, g (B)	Sample Volume, mL (C)	Filter + dried sample, g (A)	Filter + dried sample, g (2nd)	TSS = <u>(A-B)(1,000,000)</u> C
Blank			100			
Effluent			100			
Raw			25			
Mixed Liquor			5			
Dup Raw			25			
Blank			100			

Section 8
WET Testing

Whole Effluent Toxicity



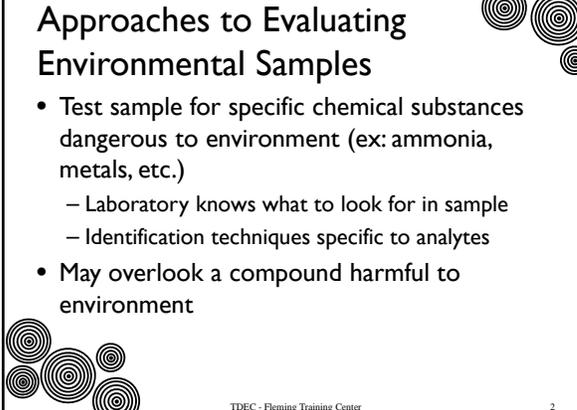


Whole Effluent Toxicity

WET Testing



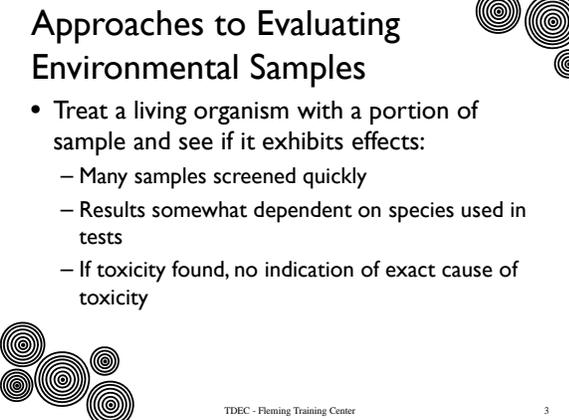
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Approaches to Evaluating Environmental Samples

- Test sample for specific chemical substances dangerous to environment (ex: ammonia, metals, etc.)
 - Laboratory knows what to look for in sample
 - Identification techniques specific to analytes
- May overlook a compound harmful to environment

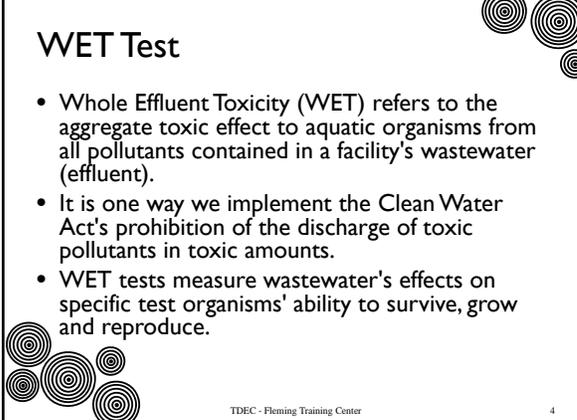
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Approaches to Evaluating Environmental Samples

- Treat a living organism with a portion of sample and see if it exhibits effects:
 - Many samples screened quickly
 - Results somewhat dependent on species used in tests
 - If toxicity found, no indication of exact cause of toxicity

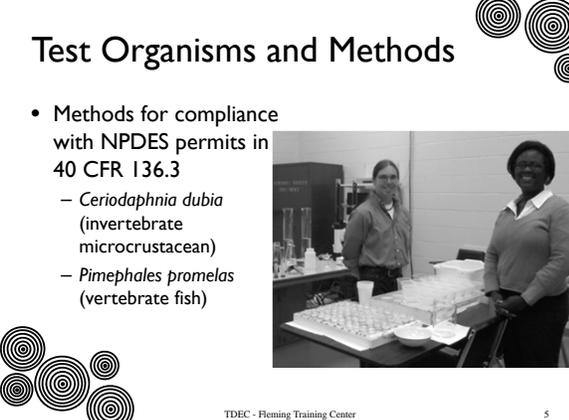
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WET Test

- Whole Effluent Toxicity (WET) refers to the aggregate toxic effect to aquatic organisms from all pollutants contained in a facility's wastewater (effluent).
- It is one way we implement the Clean Water Act's prohibition of the discharge of toxic pollutants in toxic amounts.
- WET tests measure wastewater's effects on specific test organisms' ability to survive, grow and reproduce.

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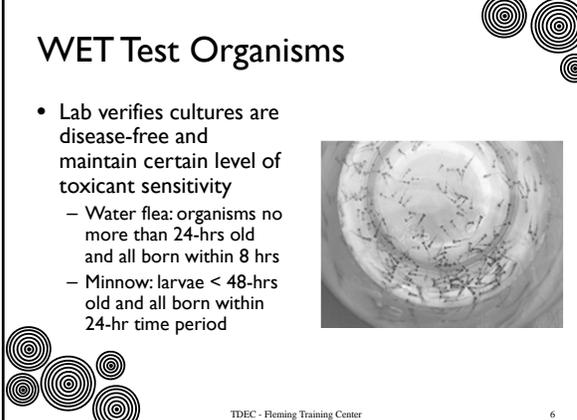


Test Organisms and Methods

- Methods for compliance with NPDES permits in 40 CFR 136.3
 - *Ceriodaphnia dubia* (invertebrate microcrustacean)
 - *Pimephales promelas* (vertebrate fish)

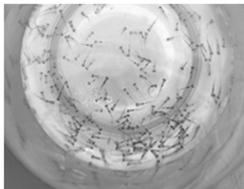


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WET Test Organisms

- Lab verifies cultures are disease-free and maintain certain level of toxicant sensitivity
 - Water flea: organisms no more than 24-hrs old and all born within 8 hrs
 - Minnow: larvae < 48-hrs old and all born within 24-hr time period



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Effluent Sampling

- Collected as composite over 24 hrs
- Chilled to 4°C during and after sampling
- Hold time 36 hrs from last aliquot collection until beginning of testing
- Sample size: 4L or 1 gal “cubitainer”

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Biomonitoring Requirements, Chronic

- NPDES Permit 3.4
 - The permittee shall conduct a 3-Brood *Ceriodaphnia dubia* Survival and Reproduction Test and a 7-day Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test on samples on final effluent...
 - The measured endpoint for toxicity will be the inhibition concentration causing 25% reduction in survival, reproduction and growth (IC_{25}) of the test organisms.

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Chronic Sample Preparation

- Effluent alkalinity, hardness, conductivity, residual chlorine, pH, etc. determined at lab
- Chronic tests conducted by filtering sample through 60- μ m plankton net
- Example: Undiluted sample (100%), 50%, 25%, 12.5%, and 6.25 (or less for no-effect concentration) are tested

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Chronic – Water Flea Tests

- Test chamber: 30-50 mL plastic cups, each with 15 mL and 1 organism
- Minimum 10 replicates for each concentration for total of 50 test organisms and 10 controls
- Reference toxicant often run concurrently (sodium chloride) for quality control
- Food added to each chamber
- **Incubate at 25°C; 16 hrs light
8 hrs dark**



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Chronic – Water Flea Tests

- Next day, surviving creatures and offspring are counted and recorded
- Living test organisms transferred to clean test chamber
- Food is added and organisms are incubated
- Test continues 6-8 days
- Total number surviving organisms for each test are tallied with number of broods and total offspring for each adult

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Chronic – Minnow Testing

- Test chambers are 500-1000mL glass beakers containing 250mL test solution
- Each beaker contains 10-15 embryo fish
- Usually 4 replicates for each concentration
- Reference toxicant (copper sulfate) often run for quality control
- Food added to each chamber
- **Incubate at 25°C; 16 hrs light/8 hrs dark**

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Chronic – Minnow Testing

- Next day, dead fish are removed
- Test chamber cleaned of debris with siphon
- Fresh test solution added to bring level back to original volume
- Food is added; test chamber put in incubator

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Chronic – Minnow Testing

- Test continues for 7 days
- Surviving fish for each replicate tallied
- Growth determined by placing all surviving fish for each replicate in tared weigh boat, drying at 60°C for 24 hrs or 100 °C for 6 hrs
- Average growth reported to nearest 0.001 mg

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Quality Assurance

- Control organisms: survival \geq 80%
- *Ceriodaphnia*
 - At least 60% of control adults must have produced three broods by 7 \pm 1 days
 - Surviving control adults must produce at least 15 offspring
- Fathead minnows
 - Average dry weight of controls at 7 days must be 0.25 mg per fish or greater
 - If minimum control requirements not met, tests are invalid and must be repeated

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Interpretation of Chronic Results

- Data processed and plotted to determine:
 - NOEC- maximum concentration of effluent that gives no statistical difference from controls
 - LOEC- lowest concentration that produces a statistically significant difference between controls and test samples
 - IC_{25/50}- toxicant concentration that produces 25 or 50% reduction in effect

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Biomonitoring Requirements, Acute

- NPDES Permit 3.5
 - The permittee shall conduct a 48-hour static acute toxicity test on two test species on samples of final effluent...
 - The test species to be used are Water Fleas (*Ceriodaphnia dubia*) and Fathead Minnows (*Pimephales promelas*).
 - The measured endpoint for toxicity will be the concentration causing 50% lethality (LC50) of the test organisms.
 - The LC50 shall be determined based on a 50% lethality as compared to controls, and as derived from linear interpolation.

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Acute Bioassay

- Conducted with *Pimephelas promelas* and *Ceriodaphnia dubia*
- Determine “end of pipe” conditions
- Effects in 100% effluent
- Last 48-96 hrs
- Objective: determine effluent concentration that causes 50% lethality during short term exposure

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Interpretation of Acute Results

- If result of a WET test on the effluent is at or near the permit limit , you have a violation
- Retest conducted
- If toxic effect consistently found, a Toxicity Reduction Evaluation (TRE) is conducted

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Toxicity Reduction Evaluation (TRE)

- Systematic evaluation of effluent
- Determine sources of toxicity and how to control toxicity
- May include chemical screening, process reviews, evaluation of plant performance, and toxicity identification evaluation (TIE)

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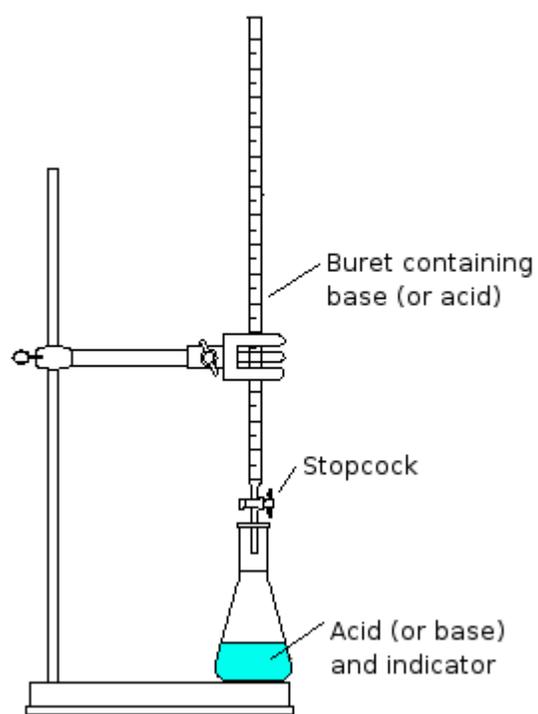
Toxicity Identification Evaluation (TIE)

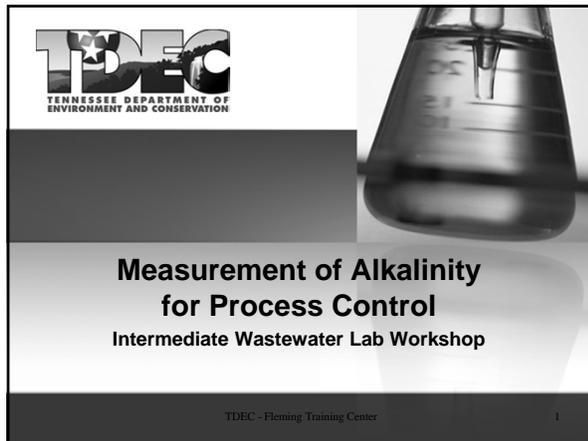
- Effluent samples are manipulated to remove suspect chemicals
- Samples re-tested to see if toxicity remains
- If successful, provides clue to source of toxicity

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Section 9 Alkalinity





Introduction

- Alkalinity is a general measure of the ionic characteristics of water.
- Others: pH, redox potential, hardness, and conductivity.
- Not normally a compliance-monitoring requirement.

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2

Alkalinity

- Defined as the measurement of a water's capacity to neutralize an acid
- An acid releases H^+
- The alkalinity in the water will absorb H^+
- Most common ions that add alkalinity are OH^- , CO_3^- , HCO_3^-

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3

Importance in Wastewater Treatment

- Chemical and biological treatment systems
- Biological nutrient removal
- Anaerobic digestion control
- Ammonia removal by air stripping

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Activated Sludge Alkalinity

- Essential to process control
- Insufficient alkalinity:
 - Reduces organism activity
 - May result in low effluent pH
 - May result in extremely high chlorine demand in disinfection process

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Alkalinity Determination

- Titration against a standard acid:
 - Color change of standard indicator
 - pH meter
- Results expressed as total alkalinity, mg/L as calcium carbonate
- Buret Titration Method, SM 2320 B

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Alkalinity Determination

- Measured by determining the amount of acid needed to drop the pH of a sample to a certain endpoint
 - Phenolphthalein alkalinity is measured by titrating to a pH of 8.3
 - Total alkalinity is measured by titrating to a pH of 4.5

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pH End Points

Sample Composition	End Point pH	
	Total Alkalinity	Phenolphthalein Alkalinity
Alk ~ 30 mg/L	pH 4.9	pH 8.3
Alk ~ 150 mg/L	pH 4.6	pH 8.3
Alk ~ 500 mg/L	pH 4.3	pH 8.3
Silicates or Phosphates present	pH 4.5	pH 8.3
Industrial Waste or Complex System	pH 4.5	pH 8.3
Routine or Automated Process	pH 4.5	pH 8.3

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Alkalinity

- Alkalinity caused by OH^- is called
 - *hydroxyl alkalinity*
- Alkalinity caused by CO_3^- is called
 - *carbonate alkalinity*
- Alkalinity caused by HCO_3^- is called
 - *bicarbonate alkalinity*
- The combined effect of all three types is called
 - *total alkalinity*

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Apparatus

- Buret and stand
- Beaker, 250 mL
- Stir plate
- Stir bar



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Sampling and Storage

- Collect samples in clean plastic or glass bottles
- Avoid excessive agitation or prolonged exposure to air
- Analyze as soon as possible
 - May be stored for 24 hrs at 4°C
- Warm to room temperature before analysis.

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Interferences

- Highly colored or turbid samples may mask the color change at the end point.
 - Use a pH meter for these samples.
- Chlorine may interfere with indicators.
 - Add one drop 0.1N sodium thiosulfate to eliminate this interference.

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Alkalinity

DOC316.53.01151

USEPA¹ Buret Titration Method² (0 to 5,000 mg/L as CaCO₃)

Method 8221
Buret Titration

Scope and Application: For water, wastewater and seawater

¹ USEPA Accepted

² Adapted from *Standard Methods for the Examination of Water and Wastewater, 2320 B*



Test preparation

Before starting the test:

Read the entire procedure before starting the test.

A pH meter is required for NPDES reporting and is recommended for best results.

Substitute six drops of Phenolphthalein Indicator Solution for the Phenolphthalein Indicator Powder Pillow if necessary

Substitute six drops of Bromcresol Green-Methyl Red Indicator Solution for the Bromcresol Green-Methyl Red Powder Pillow if necessary.

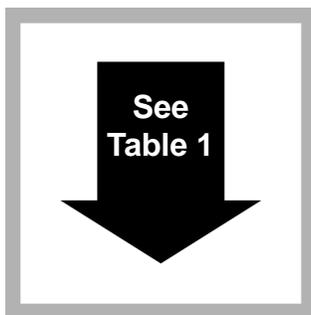
Results in mg/L as CaCO₃ ÷ 17.12 = grains per gallon

Collect the following items:

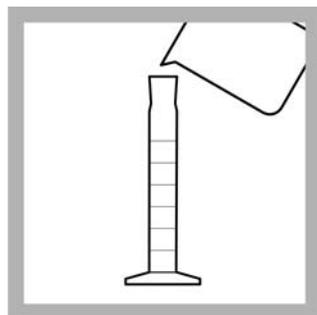
Description	Quantity
Bromcresol Green-Methyl Red indicator powder pillow	1
Phenolphthalein indicator powder pillow	1
Sulfuric acid standard solution, 0.020 N	varies ¹
Buret clamp, double	1
Buret, Class A, 25-mL	1
Graduated cylinder	varies ¹
Erlenmeyer flask, 250-mL	1
Funnel, Micro	1
Support Stand	1

¹ See *Consumables and replacement items* on page 6.

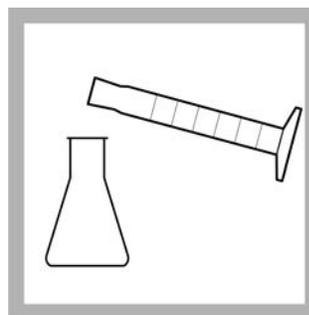
Buret titration (Method 8221)



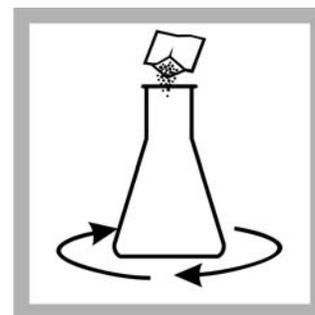
1. Select a sample volume from the *Sample volume selection for expected concentration* table that corresponds to the expected alkalinity concentration in mg/L as calcium carbonate (CaCO_3).



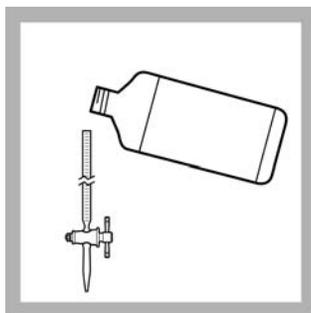
2. Use a graduated cylinder or pipet to measure the sample volume.



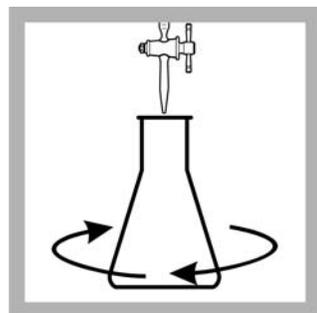
3. Transfer the sample into a 250-mL Erlenmeyer flask. Dilute to about 50-mL with deionized water if necessary.



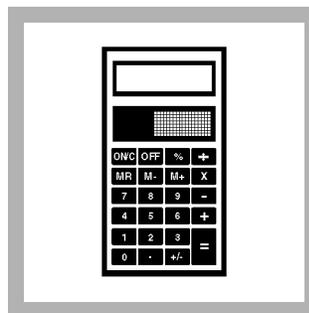
4. Add the contents of one Phenolphthalein Indicator Powder Pillow. Swirl to mix. (Omit this step when using a pH meter.)



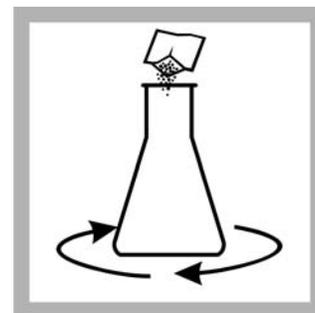
5. Fill a 25-mL buret to the zero mark with 0.020 N Sulfuric Acid standard solution.



6. While swirling the flask, titrate the sample until the solution color changes from pink to colorless (pH 8.3).
If the solution is colorless before titrating with sulfuric acid, the phenolphthalein alkalinity is zero.



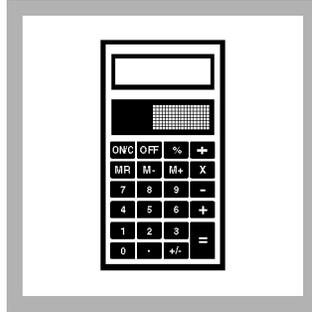
7. Calculate:
 $\text{mL Titrant} \times \text{multiplier used} = \text{mg/L phenolphthalein alkalinity as CaCO}_3$.



8. Add the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow to the titrated sample. Swirl to mix.
Do not add indicator if a pH meter is used.
Specific sample composition may require titration to a specific pH (see the *Alkalinity relationship table*).

Buret titration (Method 8221)

9. Continue the titration until a light pink end point is reached.



10. Calculate:
 $\text{mL Titrant} \times \text{multiplier}$
 used = mg/L total alkalinity
 as CaCO_3 .

Table 1 Sample volume selection for expected concentration

Range (mg/L as CaCO_3)	Sample Volume (mL)	Sulfuric Acid	Multiplier
0–500	50	20353	20
400–1000	25	20353	40
1000–2500	10	20353	100
2000–5000	5	20353	200

The end points in the *Alkalinity endpoints* table are recommended for determining total alkalinity in water samples of various compositions and alkalinity concentrations.

Table 2 Alkalinity endpoints

Sample composition	End point pH	
	Total Alkalinity	Phenolphthalein Alkalinity
Alkalinity about 30 mg/L	pH 4.9	pH 8.3
Alkalinity about 150 mg/L	pH 4.6	pH 8.3
Alkalinity about 500 mg/L	pH 4.3	pH 8.3
Silicates or phosphates present	pH 4.5	pH 8.3
Industrial wastes or complex system	pH 4.5	pH 8.3
Routine or Automated Analyses	pH 4.5	pH 8.3

Total alkalinity primarily includes hydroxide, carbonate, and bicarbonate alkalinities. The concentration of these types in a sample may be determined when the phenolphthalein and total alkalinities are known (*Alkalinity relationship* table).

Table 3 Alkalinity relationship

Row	Result of Titration	Hydroxide Alkalinity Equals:	Carbonate Alkalinity Equals:	Bicarbonate Alkalinity Equals:
1	Phenolphthalein Alkalinity equal to 0	0	0	Total Alkalinity
2	Phenolphthalein Alkalinity equal to Total Alkalinity	Total Alkalinity	0	0
3	Phenolphthalein Alkalinity less than one-half of Total Alkalinity	0	Phenolphthalein Alkalinity times 2	Total Alkalinity minus two times Phenolphthalein Alkalinity
4	Phenolphthalein Alkalinity equal to one-half of Total Alkalinity	0	Total Alkalinity	0
5	Phenolphthalein Alkalinity greater than one-half of Total Alkalinity	2 times Phenolphthalein Alkalinity minus Total Alkalinity	2 times the difference between Total and Phenolphthalein Alkalinity	0

Use the *Alkalinity relationship* table with the following procedure:

1. Does the phenolphthalein alkalinity equal zero? If yes, use Row 1.
2. Does the phenolphthalein alkalinity equal total alkalinity? If yes, use Row 2.
3. Divide the total alkalinity by 2 to calculate one-half the total alkalinity.
4. Select Row 3, 4 or 5 based on comparing the result of step c (one-half total alkalinity) with the phenolphthalein alkalinity.
5. Perform the required calculations if any.
6. Check your results. The sum of the three alkalinity types will equal the total alkalinity.

Example:

A sample has 170 mg/L as CaCO₃ phenolphthalein alkalinity and 250 mg/L as CaCO₃ total alkalinity. What is the concentration of hydroxide, carbonate, and bicarbonate alkalinities?

- a. The phenolphthalein alkalinity does not equal zero but 170 mg/L.
- b. The phenolphthalein alkalinity does not equal total alkalinity (170 mg/L vs. 250 mg/L).
- c. One-half of the total alkalinity equals 125 mg/L.
- d. Because the phenolphthalein alkalinity of 170 mg/L is greater than one-half the total alkalinity of 125 mg/L, select Row 5.

The hydroxide alkalinity is equal to:

$$2 \times 170 = 340$$

$$340 - 250 = 90 \text{ mg/L hydroxide alkalinity}$$

The carbonate alkalinity is equal to:

$$250 - 170 = 80$$

$$80 \times 2 = 160 \text{ mg/L carbonate alkalinity}$$

The bicarbonate alkalinity is equal to zero mg/L.

Check:

90 mg/L hydroxide alkalinity + 160 mg/L carbonate alkalinity + 0 mg/L bicarbonate alkalinity = 250 mg/L

The answer is correct.

The sum of each type equals the total alkalinity (250 mg/L).

Interferences

Chlorine at levels above 3.5 mg/L cause a yellow-brown color upon the addition of the Bromcresol Green-Methyl Red Indicator Powder Pillow. Residual chlorine interference with the indicator may be removed by adding a drop of 0.1 N Sodium Thiosulfate Standard Solution* before adding the indicator.

Highly colored or turbid samples may mask the color change at the end point. Use a pH meter for these samples, titrating to pH 8.3 for phenolphthalein alkalinity and the appropriate pH (see the *Alkalinity endpoints* table) for total alkalinity.

Sampling and storage

Collect samples in plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation and prolonged exposure to air. Samples should be analyzed as soon as possible after collection but can be stored at least 24 hours by cooling to 4 °C (39 °F) or below. Warm to room temperature before analyzing.

Accuracy check

End point confirmation

- To accurately determine the phenolphthalein alkalinity end point, mix the contents of one Phenolphthalein Indicator Powder Pillow and the contents of one pH 8.3 Buffer Powder Pillow with 50 mL of deionized water in a 250-mL Erlenmeyer flask. The resulting color is the end point.
- To accurately determine the total alkalinity end point, mix the contents of one pH 4.5 Buffer Powder Pillow and the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow with 50 mL of deionized water in a 250-mL Erlenmeyer flask. Titrate to a light pink color change.

Standard additions method (Sample Spike)

Perform the standard additions method check as follows:

1. Break the top off an Alkalinity Voluette® Ampule Standard Solution, 0.500 N.
2. Use the TenSette Pipet* to add 0.1 mL of standard to the sample titrated in step 6 or step 9. Resume titration back to the same end point. Record the volume of titrant needed.
3. Repeat, using two more additions of 0.1 mL. Titrate to the end point after each addition.
4. The mL of titrant required should increase by 2.5 mL for each 0.1 mL increment of standard added.

Summary of method

Alkalinity is expressed as P (phenolphthalein) alkalinity or as T (total) alkalinity. Both types are determined by titration with a Sulfuric Acid Standard Solution to an end point evidenced by the color change of an indicator solution or determined with a pH meter. The P alkalinity is determined by titration to a pH of 8.3 and registers the total hydroxide and one half the carbonate present. The T alkalinity is determined by titration to a pH of 4.5. The total alkalinity includes all carbonate, bicarbonate and hydroxide alkalinity. Alternatively, total alkalinity end points may be determined by using a pH meter and titrating to the specific pH required for the sample composition.

* See *Consumables and replacement items* on page 6.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Catalog number
Bromcresol Green-Methyl Red Indicator Powder Pillows	1 pillow	100/pkg	94399
Phenolphthalein Indicator Powder Pillows	1 pillow	100/pkg	94299
Sulfuric Acid Standard Solution, 0.020 N	varies	1 L	20353

Required apparatus

Description	Quantity/test	Unit	Catalog number
Buret Clamp, double	1	each	32800
Buret, Class A, 25-mL	1	each	2636540
Select one or more based on sample volume:			
Cylinder, graduated, 5-mL	—	each	50837
Cylinder, graduated, 10-mL	—	each	50838
Cylinder, graduated, 25-mL	—	each	50840
Cylinder, graduated, 50-mL	—	each	50841
Flask, Erlenmeyer, 250-mL	1	each	50546
Pipet, volumetric, Class A, 5-mL,	—	each	1451537
Pipet, volumetric, Class A, 10-mL	—	each	1451537
Pipet Filler, Safety Bulb	—	each	1465100
Ampule Breaker	—	each	2196800
Funnel, Micro	1	each	2584335
Support Stand	1	each	56300

Required standards

Description	Unit	Catalog number
Alkalinity Standard Solution, Voluette® Ampules, 0.500 N, 10-mL	16/pkg	1427810
Buffer Powder Pillows, pH 4.5	25/pkg	89568
Buffer Powder Pillows, pH 8.3	25/pkg	89868
Water, deionized	4 L	27256

Optional items

Description	Unit	Catalog number
Sodium Thiosulfate Standard Solution, 0.1 N	—	32332
TenSette Pipet, 0.1–1.0 mL	—	1970001
Tips for Tensette Pipet	50/pkg	2185696



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
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 Outside the U.S.A. – Contact the HACH office or distributor serving you.
 On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
 WORLD HEADQUARTERS
 Telephone: (970) 669-3050
 FAX: (970) 669-2932

	Influent	Effluent
	mL sample used =	mL sample used =
Bench 1	total mL to pH 4.5 =	total mL to pink =
Bench 2	total mL to pink =	total mL to pH 4.5 =
Bench 3	total mL to pH 4.5 =	total mL to pink =
Bench 4	total mL to pink =	total mL to pH 4.5 =
Bench 5a	total mL to pH 4.5 =	total mL to pink =
Bench 5b	total mL to pink =	total mL to pH 4.5 =

Influent Alkalinity - Titrate to pH 4.5 or pink color

$$\text{Alkalinity} = \frac{(\text{mL titrant used to reach pH of 4.5})(0.02 \text{ N H}_2\text{SO}_4)(50,000)}{\text{mL sample}}$$

$$\text{Alkalinity} = \frac{(\text{_____})(0.02 \text{ N H}_2\text{SO}_4)(50,000)}{\text{_____ mL sample}} =$$

Effluent Alkalinity - Titrate to pH 4.5 or pink color

$$\text{Alkalinity} = \frac{(\text{total mL titrant used to reach pH of 4.5})(0.02 \text{ N H}_2\text{SO}_4)(50,000)}{\text{mL sample}}$$

$$\text{Alkalinity} = \frac{(\text{_____})(0.02 \text{ N H}_2\text{SO}_4)(50,000)}{\text{_____ mL sample}} =$$

Orion Alkalinity Method =

Section 10 Chemical Oxygen Demand



1

Chemical Oxygen Demand (COD)

Approved Methods Under the Clean Water Act (NPDES)

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2

Why Test For COD??

- To measure the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant.

3

Sample Collection

- Plastic or glass bottles
- Bottles must be thoroughly cleaned
- Representative sample (100 mL)
- Composite sample preserve at 4°C
- Analyze as soon as possible (same day) OR
- Preserve with H₂SO₄ to pH<2 and cool to 4° C
- Holding time is 28 days after preservation

4

Chemical Oxygen Demand vs. Biochemical Oxygen Demand

- COD is a good estimate of the first-stage oxygen demand for most municipal wastewaters.
- COD measures the strength of the organic waste that is too toxic for BOD.
- COD results are quicker.
- COD results can be used to estimate BOD.

5

COD vs BOD (Disadvantages)

- Does not measure the rate of biodegradability.
- Cannot be used to predict the effects of an effluent on the DO in receiving waters OR
- Treatment of a particular wastewater by biological processes.



Watertown, TN

6

Summary of Method

- The method oxidizes organic substances in the wastewater sample using:
 - Potassium dichromate in 50% sulfuric acid
 - Silver sulfate (catalyst)
 - Mercuric sulfate (remove interferences)
 - Sample heated or reflux for 2 hours

7

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Selection of Method

- **Titrimetric (blue-green to reddish):**
 - EPA Method 410.1, 410.2 & 410.3
 - SM 5220C (18th, 19th, & 20th editions)
- **Colorimetric (manual or automated):**
 - EPA Method 410.4
 - SM 5220D
 - Hach Method 8000

8

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Laboratory Apparatus

- **Titrimetric Method:**
 - Reflux ground-glass apparatus
 - Heat source
 - Buret
- **Colorimetric Method:**
 - Digestion tubes
 - Hot block (150°C)
 - Spectrometer 420nm and/or 600 nm



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Calculations

- **Titrimetric**
 - COD, mg/L = $\frac{(A-B)N \times 8000}{S}$
 - A = mL of titrant for blank
 - B = mL of titrant for sample
 - N = Normality of titrant
 - S = mL of sample used

10

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Calculations (continued)

- **Colorimetric:**
 - Direct reading in mg/L from instrument
 - If different sample volumes used then,
COD, mg/L = $\frac{(\text{mg O}_2 \text{ in final vol.})(1000)}{\text{mL sample}}$

11

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Quality Assurance/Quality Control

- **Titrimetric Method EPA 410.2 & 410.3:**
 - DI Blank
 - Chloride check
 - Standardize Titrant
 - $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$
- **Hach Method 8000:**
 - Pre-programmed calibration curve
 - Accuracy Check Standard (potassium acid phthalate)
 - DI Blank

12

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Colorimetric Methods - QA/QC

- **EPA Method 410.4/SM5220D**
 - **Initial Demonstration of Performance:**
 - Linear Calibration Range (LCR)
 - Quality Control Sample (QCS)
 - Method Detection Limit (MDL)

13

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Colorimetric Methods - QA/QC

- EPA Method 410.4/SM5220D
 - Laboratory Performance:
 - Laboratory Reagent Blank (LRB)
 - Laboratory Fortified Blank (LFB)
 - Instrument Performance Check (IPC)
 - Analyte Recovery & Data Quality:
 - Matrix Spike, Duplicate & Reference Standard

14

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COD Audit Checklist

- Sample collection & preservation
- Holding time
- Approved Method
- Heat regulated devices checked for accuracy
- Apparatus e.g. ground-glass used (titrimetric)
- Glassware cleaned properly
- QC samples analyzed
- Results reported correctly

Oxygen Demand, Chemical

DOC316.53.01099

USEPA¹ Reactor Digestion Method²

Method 8000

0.7 to 40.0³ mg/L COD; 3 to 150 mg/L COD;
20 to 1500 mg/L COD; 200 to 15,000 mg/L COD

Scope and Application: For water, wastewater; digestion is required

¹ Ranges 3 to 150 mg/L COD and 20 to 1500 mg/L COD are USEPA approved for wastewater analyses (Standard Method 5220 D), *Federal Register*, April 21, 1980, 45(78), 26811-26812.

² Jirka, A.M.; Carter, M.J., *Analytical Chemistry*, 1975, 47(8), 1397

³ The ULR range is not available on the DR 2700 or the DR/2400.



Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test

Table 1 Instrument-specific information

Instrument	Light shield	Adapter
DR 5000	—	—
DR 2800	LZV646	—
DR 2700	LZV646	—
DR/2500	—	—
DR/2400	—	5945700

Before starting the test:

DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.

DR 2700 and DR/2400: Ultra low range (0.7 to 40.0 mg/L) is not available.

Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidentally misused. Please read all warnings and associated MSDS sheets.

Run one blank with each set of samples. Run all tests (the samples and the blank) with the same lot of vials. The lot number appears on the container label. See *Blanks for colorimetric determination*.

Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Be prepared to wash spills with running water.

Wear appropriate eye protection and clothing for adequate user protection. If contact occurs, flush the affected area with running water. Review and follow reagent MSDS safety instructions carefully.

If high chloride samples are being tested, refer to the Alternate reagents section.

Collect the following items:

Description	Quantity
Beaker, 250-mL	1
Blender	1
COD Digestion Reagent vials	varies
DRB200 Reactor	1
Light Shield or adapter (see <i>Instrument-specific information</i>)	1
Magnetic stirrer and stir bar	1
Opaque shipping container for storage of unused, light-sensitive reagent vials	varies
Pipet, TenSette®, 0.1 to 1.0 mL, with tips (for 200–15,000 mg/L range)	1
Pipet, volumetric, 2.00 mL	2
Pipet Filler, safety bulb	1
Test Tube Rack	2

See *Consumables and replacement items* for reorder information.

Reactor digestion procedure

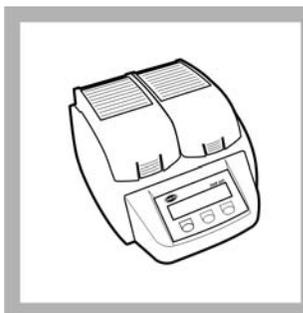


1. Homogenize 100 mL of sample for 30 seconds in a blender. For samples containing large amounts of solids, increase the homogenization time.

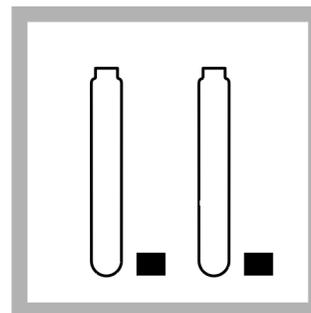
If the sample does not contain suspended solids, omit steps 1 and 2.



2. For the 200–15,000 mg/L range or to improve accuracy and reproducibility of the other ranges, pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate.

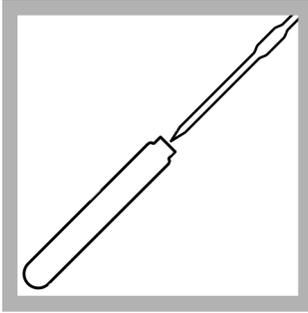


3. Turn on the DRB200 Reactor. Preheat to 150 °C.
See the DRB200 User Manual for selecting pre-programmed temperature applications.



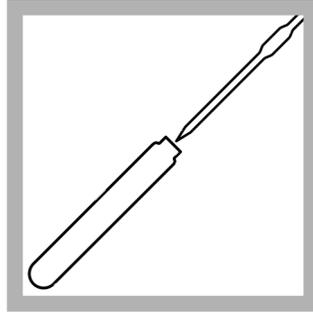
4. Remove the caps from two COD Digestion Reagent Vials. (Be sure to use vials for the appropriate range.)

Reactor digestion procedure (continued)



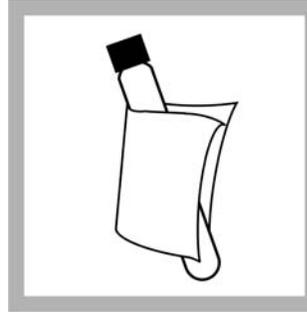
5. Prepared Sample:
Hold one vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of sample to the vial.

For the 200–15,000 mg/L vials: Use a TenSette® Pipet to add 0.20 mL of sample to the vial.

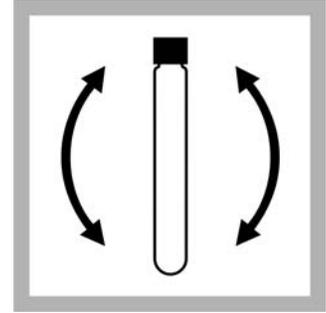


6. Blank Preparation:
Hold a second vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of deionized water to the vial.

For the 200–15,000 mg/L vials: Use a TenSette Pipet to add 0.20 mL of sample to the vial.



7. Cap the vials tightly. Rinse them with water and wipe with a clean paper towel.



8. Hold the vials by the cap over a sink. Invert gently several times to mix. The sample vials become very hot during mixing.

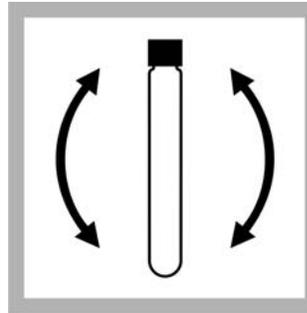
Insert the vials in the preheated DRB200 Reactor. Close the protective lid.



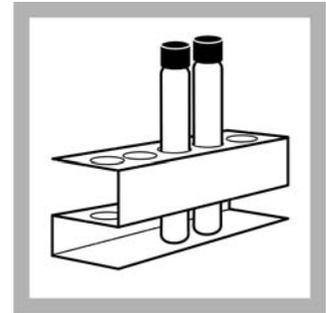
9. Heat the vials for two hours.



10. Turn the reactor off. Before removing the vials, wait about 20 minutes for the vials to cool to 120 °C or less.



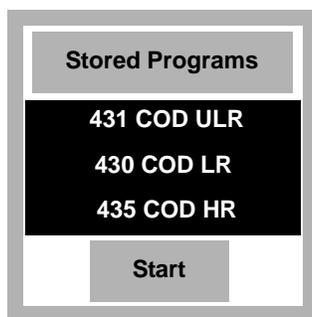
11. Invert each vial several times while still warm.



12. Place the vials into a rack and cool to room temperature.

Proceed to *Colorimetric determination*.

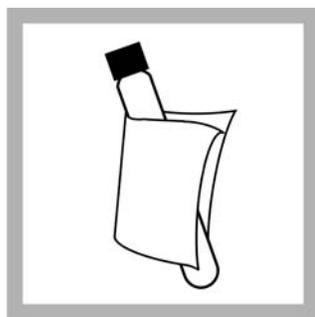
Colorimetric determination



1. Select the test.

Insert an adapter or light shield if required (see *Instrument-specific information*).

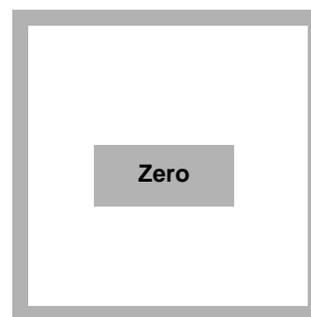
Refer to the user manual for orientation.



2. Clean the outside of the vials with a damp towel followed by a dry one.



3. Insert the blank into the 16-mm cell holder.



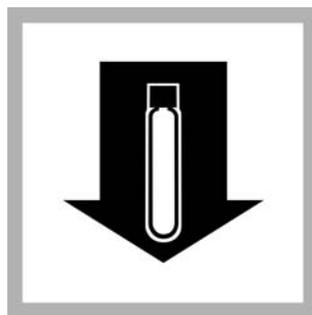
4. **ZERO** the instrument.

The display will show:

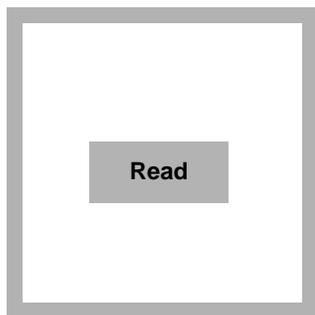
0 mg/L COD

or

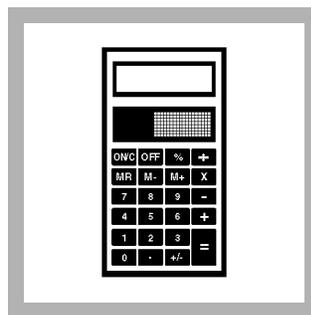
0.0 mg/L COD



5. Insert the sample vial into the 16-mm cell holder.



6. **READ** the results in mg/L COD.



7. If using High Range Plus COD Digestion Reagent Vials, multiply the result by 10.

For most accurate results with samples near 1500 or 15,000 mg/L COD, repeat the analysis with a diluted sample.

Blanks for colorimetric determination

The blank may be used repeatedly for measurements using the same lot of vials. Store the blank in the dark.

1. Monitor decomposition by measuring the absorbance at the appropriate wavelength. Refer to the *Range-specific test wavelengths* table.
2. Zero the instrument in the absorbance mode. Use a vial containing 5 mL of deionized water and measure the absorbance of the blank. Record the value.
3. Prepare a new blank when the absorbance has changed by about 0.01 absorbance units.

Interferences

Chloride is the primary interference when determining COD concentration. Each COD vial contains mercuric sulfate that will eliminate chloride interference up to the level specified in *Column 1* of the *Interfering substances* table. Dilute samples with higher chloride concentrations. Dilute the sample enough to reduce the chloride concentration to the level given in *Column 2*.

Note: For best results, use the low range and ultra-low range test for samples with high chloride concentrations (approaching maximum concentration) and low COD concentrations.

If sample dilution will cause the COD concentration to be too low for accurate determination, add 0.50 g of mercuric sulfate (HgSO_4) to each COD vial before the sample is added.

The additional mercuric sulfate will raise the maximum chloride concentration allowable to the level given in *Column 3*.

Table 2 Interfering substances

Vial range	Column 1 (Maximum chloride concentration)	Column 2 (Suggested chloride concentration for diluted samples)	Column 3 (Maximum chloride concentration with mercuric sulfate)
Ultra Low Range ¹ (0.7–40.0 mg/L)	2000	1000	N/A
Low Range (3–150 mg/L)	2000	1000	8000
High Range (20–1500 mg/L)	2000	1000	4000
High Range Plus (200–15,000 mg/L)	20,000	10,000	40,000

¹ Ultra Low Range is not available on the DR 2700 or the DR/2400.

Sample collection, preservation and storage

- Collect samples in glass bottles.
- Use plastic bottles only if they are known to be free of organic contamination.
- Test biologically active samples as soon as possible.
- Homogenize samples containing solids to assure representative samples.
- Samples treated with sulfuric acid* to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C can be stored up to 28 days.
- Correct the test results for volume additions.

* See *Optional reagents and apparatus*.

Accuracy check

Standard solution method

Note: Refer to the instrument user manual for specific software navigation instructions.

Required for accuracy check:

- Potassium acid phthalate (KHP), dried overnight at 120 °C
- Deionized water, organic free
- Class A volumetric flasks
- Class A volumetric pipet

0.7 to 40.0 mg/L range

1. Prepare a 30-mg/L COD standard solution as follows:
 - a. Dissolve 850 mg of dried KHP in 1000-mL of organic-free deionized water to make a 1000-mg/L standard.
 - b. Pipet 3.00 mL of the 1000 mg/L standard into a 100-mL volumetric flask.
 - c. Dilute to volume with deionized water and mix well.
2. Use 2 mL of the 30 mg/L COD solution in place of the sample. Follow the *Colorimetric determination* test procedure. The result should be 30 mg/L. Refer to the *Standard adjust* instructions in this procedure to adjust the curve with the reading obtained from the standard.

3 to 150 mg/L range

1. Prepare a 100-mg/L COD standard solution as follows:
 - a. Dissolve 85 mg of dried KHP in 1000-mL of organic-free deionized water to make a 1000-mg/L standard.
 - b. Pipet 10 mL of the 1000 mg/L standard into a 100-mL volumetric flask.
 - c. Dilute to volume with deionized water and mix well.
2. Use 2 mL of the 100-mg/L solution in place of the sample. Follow the *Colorimetric determination* test procedure. The result should be 100 mg/L. Refer to the *Standard adjust* instructions in this procedure to adjust the curve with the reading obtained from the standard

20 to 1500 mg/L range

1. Prepare a 500-mg/L COD standard solution as follows:
 - a. Dissolve 425 mg of dried KHP in 1000-mL of organic-free deionized water to make a 500-mg/L standard.
 - b. Mix well.
2. Use 2 mL of the 500 mg/L COD solution in place of the sample. Follow the *Colorimetric determination* test procedure. The result should be 500 mg/L. Refer to the *Standard adjust* instructions in this procedure to adjust the curve with the reading obtained from the standard.

Note: Alternately, use 2 mL of 300 mg/L, 800 or 1000 mg/L COD standards for accuracy check.

200 to 15,000 mg/L range

1. Prepare a 10,000-mg/L COD standard solution as follows:
 - a. Dissolve 8.500 g of dried KHP in 1000-mL of organic-free deionized water.
2. Use 0.2 mL of the 10,000 mg/L COD solution in place of the sample. Follow the *Colorimetric determination* test procedure. The result should be 10,000 mg/L (after multiplying by 10). Refer to the *Standard adjust* instructions in this procedure to adjust the curve with the reading obtained from the standard.

Standard adjust

1. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADJUST
DR 2800	OPTIONS>MORE>STANDARD ADJUST
DR 2700	OPTIONS>MORE>STANDARD ADJUST
DR/2500	OPTIONS>STANDARD ADJUST
DR/2400	OPTIONS>STANDARD ADJUST

2. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Alternate reagents

Mercury-free COD2 Reagents can provide a mercury-free testing option for non-reporting purposes. For process control applications, COD2 Reagents will eliminate mercury waste and save on disposal costs. These reagents are fully compatible with test procedures and calibration curves programmed into the spectrophotometer. Determine chloride and ammonia for accurate results.

Important Note: *COD2 reagents are not approved for USEPA reporting purposes. Because COD2 reagents do not contain mercury as a masking agent, they exhibit a positive interference from chloride. Request a copy of the COD Reagent Vial Information Brochure, Lit. No. 1356, for more information about specific applications.*

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
430 (Range, 3–150 mg/L)	DR 5000	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 8000 mg/L COD (High Range Plus)	77–83 mg/L COD	3 mg/L COD
	DR 2800		77–83 mg/L COD	3 mg/L COD
	DR 2700		77–83 mg/L COD	3 mg/L COD
	DR/2500	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 10,000 mg/L COD (High Range Plus)	77.6–82.4 mg/L COD	3 mg/L COD
	DR/2400		77.6–82.4 mg/L COD	3 mg/L COD
431 (Range, 0.5–40.0 mg/L)	DR 5000	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 8000 mg/L COD (High Range Plus)	28.8–31.2 mg/L COD	0.5 mg/L COD
	DR 2800		28.8–31.2 mg/L COD	0.5 mg/L COD
431 (Range, 0.7–40.0 mg/L)	DR/2500	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 10,000 mg/L COD (High Range Plus)	29.0–31.0 mg/L COD	0.7 mg/L COD
435 (Range, 20–1500 mg/L)	DR 5000	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 8000 mg/L COD (High Range Plus)	785–815 mg/L COD	23 mg/L COD
	DR 2800		785–815 mg/L COD	23 mg/L COD
	DR 2700		785–815 mg/L COD	23 mg/L COD
	DR/2500	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 10,000 mg/L COD (High Range Plus)	778–822 mg/L COD	20 mg/L COD
	DR/2400		778–822 mg/L COD	20 mg/L COD
435 (Range, 200–15,000 mg/L)	DR 5000	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 8000 mg/L COD (High Range Plus)	7850–8150 mg/L COD	230 mg/L COD
	DR 2800		7850–8150 mg/L COD	230 mg/L COD
	DR 2700		7850–8150 mg/L COD	230 mg/L COD
	DR/2500	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 10,000 mg/L COD (High Range Plus)	9780–10,220 mg/L COD	200 mg/L COD
	DR/2400		9780–10,220 mg/L COD	200 mg/L COD

Summary of method

The results in mg/L COD are defined as the milligrams of O₂ consumed per liter of sample under the conditions of this procedure. The sample is heated for two hours with sulfuric acid and a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion (Cr₂O₇²⁻) to green chromic ion (Cr³⁺).

When the 0.7–40.0 or the 3–150 mg/L colorimetric method is used, the amount of Cr⁶⁺ remaining is determined. When the 20–1500 mg/L or 200–15,000 mg/L colorimetric method is used, the amount of Cr³⁺ produced is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences.

Test results are measured at the wavelengths specified in the *Range-specific test wavelengths* table.

Table 3 Range-specific test wavelengths

Range in mg/L COD	Wavelength
0.7 to 40.0 mg/L ¹	350 nm
3 to 150 mg/L	420 nm
20 to 1500	620 nm
2000 to 15,000 mg/L	620 nm

¹ Not available on the DR/2400 or the DR 2700

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Select the appropriate COD Digestion Reagent Vial:			
Ultra Low Range, 0.7 to 40 mg/L COD	1–2 vials	25/pkg	2415825
Low Range, 3 to 150 mg/L COD	1–2 vials	25/pkg	2125825
High Range, 20 to 1500 mg/L COD	1–2 vials	25/pkg	2125925
High Range Plus, 200 to 15,000 mg/L COD	1–2 vials	25/pkg	2415925
Water, deionized	varies	4 L	27256

Alternate reagents¹

Description	Quantity/Test	Unit	Catalog number
Select the appropriate COD Digestion Reagent Vial:			
COD2, Low Range, 0 to 150 mg/L COD	1–2 vials	25/pkg	2565025
COD2, High Range, 0 to 1500 mg/L COD	1–2 vials	25/pkg	2565125
COD2, High Range, 0 to 1500 mg/L COD	1–2 vials	150/pkg	2565115
COD2, High Range Plus, 0 to 15,000 mg/L COD	1–2 vials	25/pkg	2834325
COD Digestion Reagent Vials, 3 to 150 mg/L COD	—	150/pkg	2125815
COD Digestion Reagent Vials, 200 to 1500 mg/L COD	—	150/pkg	2125915

¹ These reagents are not approved for USEPA reporting purposes. Request a copy of the COD Reagent Vial Information Brochure, Lit. No. 1356, for more information about specific applications.

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Blender, 2-speed, 120 VAC	1	each	2616100
Blender, 2-speed, 240 VAC	1	each	2616102
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Pipet Filler, safety bulb	1	each	1465100
Pipet, Volumetric, Class A, 2.00 mL	1	each	1451536

Recommended standards and apparatus

Description	Unit	Catalog number
Beaker, 250-mL	each	50046H
COD Standard Solution, 300-mg/L	200 mL	1218629
COD Standard Solution, 300-mg/L	500mL	1218649
COD Standard Solution, 800-mg/L	200 mL	2672629
COD Standard Solution, 1000-mg/L	200 mL	2253929
Oxygen Demand Standard (BOD, COD, TOC), 10-mL ampules	16/pkg	2833510
Pipet, TenSette®, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Potassium Acid Phthalate, ACS	500 g	31534
Stir Bar	each	2095352
Stirrer, Electromagnetic, 120 VAC, with electrode stand	each	4530001
Stirrer, Electromagnetic, 230 VAC, with electrode stand	each	4530002
Test Tube Rack	each	1864100
Disposable wipes	70/pkg	2096900

Optional reagents and apparatus

Description	Unit	Catalog number
Balance, analytical, 80 g capacity, 115 VAC	each	2936701
COD Digestion Reagent Vials, ULR 0.7-40.0 mg/L	150/pkg	2415815
COD Digestion Reagent Vials, HR plus, 200-25,000 mg/L	150/pkg	2415915
Flask, volumetric, 1000 mL Class A	each	1457453
Flask, volumetric, 100-mL Class A	each	1457442
Mercuric Sulfate, 28 g	—	191520
Pipet, volumetric, 3-mL, Class A	each	1451503
Pipet, volumetric, 10-mL, Class A	each	1451538
Sulfuric Acid, 500 mL	—	97949
Wastewater Influent Standard for mixed parameters NH ₃ -N, NO ₃ -N, PO ₄ , COD, SO ₄ , TOC	500mL	2833149
Wastewater Effluent Standard, for mixed parameters NH ₃ -N, NO ₃ -N, PO ₄ , COD, SO ₄ , TOC	500mL	2833249
Weighing Paper, 76x76 mm	500/pkg	1473800
Finger cots	2/pkg	1464702
Gloves, chemical resistant 9-9 ½" ¹	1 pair	2410104
Safety goggles, vented	each	2550700
EZ COD™ Recycling Service with 5-gal ² bucket	each	2895405

¹ Other sizes are available² 20 and 50 gal are available.



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:

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Outside the U.S.A. – Contact the **HACH** office or distributor serving you.

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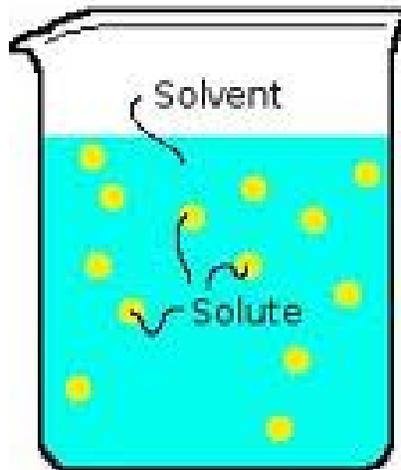
FAX: (970) 669-2932

COD Laboratory

Samples: Blanks (deionized water)
 Screened raw influent
 Oxidation ditch effluent – settle supernatant
 Plant effluent
 COD Standard

<u>Sample</u>	<u>Vial No.</u>	<u>COD, mg/L</u>
Blank – High Range	_____	_____
Standard - _____	_____	_____
Screened Influent	_____	_____
Oxidation Ditch Effluent	_____	_____
Plant Effluent	_____	_____
Duplicate	_____	_____
Blank – Low Range	_____	_____

Section 11 Solutions



1

Solution Chemistry

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2

Solutions

- Basically, an even mixture of two or more chemicals
- A solution consist of two parts:
 - Solute
 - Solvent
- The solute part of the solution is dissolved in the solvent
- The most common solvent is water

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3

Concentration

- The measure of a solution that describes the amount of solute in the solvent
- Listed below are expressions for concentration:
 - milligrams per liter (mg/L)
 - grains per gallon
 - percent strength
 - molarity (M)
 - normality (N)

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4

Milligrams per Liter and Grains per Gallon

- These express weight per volume
- mg/L is the most commonly accepted measurement in water and wastewater industry
- 1ppm (part per million) is equivalent to 1 mg/L

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5

Percent Strength

- % Strength = $\frac{\text{weight of solute}}{\text{weight of solution}}$
- Weight of Solution = Weight of solute + weight of solvent

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Molarity

- Molarity = moles of solute / liters of solution
- Mole - ~~Any of various small, insectivorous, burrowing mammals with thickset bodies bearing silky light brown to dark-gray fur, rudimentary eyes, tough muzzles, and strong forefeet for digging and usually living underground.~~ Total weight / molecular weight
- Mole – Individual atoms have extremely minute masses. Chemists use a fundamental unit called a *mole*. The mole is the amount of substance of a system which contains as many elementary particles as there are atoms in 0.012 kg of carbon -12 (¹²C).

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Normality

- *Normal solutions* are solutions which have a specific number of equivalent masses of the acid or base dissolved in the solution per liter. A 1 N solution (a 1 normal solution) contains 1 equivalent mass per liter, a 2 N solution contains 2 equivalent masses per liter, and so on.
- Normality = $\frac{\text{number of equivalent weights of solute}}{\text{liter of solution}}$
- Number of equivalent weights = $\frac{\text{total weight of solute}}{\text{equivalent weight}}$
- Equivalent weight = $\frac{\text{molecular weight}}{\text{number of positive charges}}$

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Molarity and Normality

- How many grams of Na_2CO_3 would it take to make 1 L of a 1 molar and a 1 normal solution?
- Step 1 - Determine molecular weight (mass of the molecule):
 - Na (2 moles of Na atoms) = $22.99 \text{ amu} \times 2 = 45.98 \text{ amu}$
 - C (1 mole of C atom) = $12.01 \text{ amu} \times 1 = 12.01 \text{ amu}$
 - O (3 moles of O atom) = $16.00 \text{ amu} \times 3 = 48.00 \text{ amu}$
 - Molecular weight (grams/mole) = 105.99 amu
 - (This means 1 mole of Na_2CO_3 = 105.99 grams)
- $\text{Na}_2\text{CO}_3 \rightarrow \text{Na}^+ + \text{Na}^+ + \text{CO}_3^{2-}$

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Molarity

$\text{g} = 1 \text{ mole} \times \text{g/mole}$
molecular weight

- Step 2 - Calculate the grams required
 - grams required = (# moles required)(g/mole)
 - grams required = (1 mole)(105.99 g/mole)
 - grams required = 105.99 g
- Step 3 - Calculate molarity of the solution
 - Molarity = $\frac{\# \text{ moles}}{\text{volume of solvent}}$
 - Molarity = $\frac{1 \text{ mole}}{1 \text{ liter of water}}$
 - Molarity = 1M
- Every 105.99 grams of Na_2CO_3 in 1L of water gives a 1M solution

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Molarity Shortcut

Na = $22.99 \text{ amu} \times 2 = 45.98 \text{ amu}$
 C = $12.01 \text{ amu} \times 1 = 12.01 \text{ amu}$
 O = $16.00 \text{ amu} \times 3 = 48.00 \text{ amu}$
 Molecular weight = 105.99 amu

$\text{Na}_2\text{CO}_3 \rightarrow \text{Na}^+ + \text{Na}^+ + \text{CO}_3^{2-}$

- Grams required =
 (Molarity needed)(molecular wt)(L sol'n)
 $\frac{\text{mole/liter}}{\text{grams/mole}} \times \frac{\text{grams/mole}}{\text{liter}}$
 = (1M)(105.99)(1 L)
 = 105.99 g for a 1 molar solution

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Normality

$\text{Na}_2\text{CO}_3 \rightarrow \text{Na}^+ + \text{Na}^+ + \text{CO}_3^{2-}$

- How many grams of Na_2CO_3 would it take to make 1 L of a 1 normal solution?
- Step 2 - Calculate the equivalent weight for Na_2CO_3
 - Equivalent weight = $\frac{\text{molecular weight}}{\# \text{ of (+) charges}}$
 - Equivalent weight = $\frac{105.99}{2}$
 - Equivalent weight = 53 grams

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Normality

- Step 2b - Calculate the number of equivalents
 - #Equivalent weights = $\frac{\text{total weight}}{\text{equivalent weights}}$
 - #Equivalent weights = $\frac{105.99 \text{ g}}{53 \text{ g/equivalent}}$
 - #Equivalents = 2

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Normality

- OR Step 2c - Calculate the grams required
 - g required = (#equivalents)(g/equivalents)
 - g required = (2 equivalent wts)(53g/equivalents)
 - g required = 106

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Normality

- Step 3 - Calculate Normality
 - Normality = $\frac{\text{\# of equivalents}}{\text{volume of solvent}}$
 - Normality = $\frac{2 \text{ equivalents}}{1 \text{ L of water}}$
 - Normality = 2N
 - Every 105.99 grams of Na_2CO_3 in 1L of water gives a 2N solution
 - to make a 1N solution, cut the grams in half

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Normality Shortcut

Na = 22.99 amu x 2 = 45.98 amu
 C = 12.01 amu x 1 = 12.01 amu
 O = 16.00 amu x 3 = 48.00 amu
 Molecular weight = 105.99 amu

$\text{Na}_2\text{CO}_3 \rightarrow \text{Na}^+ + \text{Na}^+ + \text{CO}_3^{2-}$

- Grams required =

$$= \frac{(\text{Normality needed})(\text{molecular weight})(\text{L sol'n})}{\text{\# of positive charges}}$$

$$= \frac{(1 \text{ N})(105.99)(1 \text{ L})}{2}$$

$$= 53 \text{ g for a 1N solution of } \text{Na}_2\text{CO}_3$$

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Dilutions

- Weakening a stock solution
- Start with a stock solution, add an amount to the volumetric flask, and fill to mark
- Can use the following calculation with any expression of concentration or normality

Use this equation to make calculation:

$$C_1V_1 = C_2V_2$$

$$N_1V_1 = N_2V_2$$

1 means stock or initial concentration or volume

2 is the concentration or volume you end up with

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Dilution Examples

- Operator has 12% bleach on hand and wants to make 25 gallons of 8%.

$$C_1V_1 = C_2V_2$$

$$(0.12)(V_1) = (0.08)(25 \text{ gal})$$

$$V_1 = \frac{(0.08)(25 \text{ gal})}{0.12}$$

$$V_1 = 16.7 \text{ gals}$$

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Dilution Examples

- Operator has 100 mL of 5N sulfuric acid on hand and wants to make 1N. How much water should be added?

$$N_1V_1 = N_2V_2$$

$$(5\text{N})(100 \text{ mL}) = (1\text{N})(V_2)$$

$$\frac{(5\text{N})(100 \text{ mL})}{1 \text{ N}} = (V_2)$$

$$500 \text{ mL} = V_2$$

500 mL is final volume, you started with 100 mLs and added 400 mL to get a final volume of 500 mL

Intermediate Wastewater Laboratory Workshop Laboratory Solutions

1. A laboratory solution is made using 52 milligrams of sodium chloride (NaCl) dissolved in 1-liter volumetric flask filled to the mark. What is the mg/L concentration of the solution?
2. If 33 pounds of a chemical is added to 148 pounds of water, what is the % strength by weight?
3. You are given 100 mL of 2.8N HCl. How many mL of water should be added to make 0.4N HCl?
4. 250 mL of 3N NaOH is diluted to 1000 mL. What is the new normality of the solution?

13. An operator needs to make 1-liter of a 1N and a 1M solution of sodium hydroxide (NaOH). How many grams would be needed for each?
14. An operator needs to make $\frac{1}{2}$ -liter of a 5N and a 5M solution of ferric sulfate $\text{Fe}_2(\text{SO}_4)_3$. How many grams would be needed for each?

Answers

- | | | |
|--------------------|--------------------|---------------------|
| 1. 52 mg/L | 8. 50 mL | 13. 40 g for 1M |
| 2. 18.2% | 9. 2.25N | 40 g for 1N |
| 3. 600 mL to add | 10. 292 mL to add | 14. 999.78 g for 5M |
| 4. 0.75N | 11. 40 mL | 166.7 g for 5N |
| 5. 5N | 12. 84.01 g for 1M | |
| 6. 525.5 mL to add | 84.01 g for 1N | |
| 7. 66.67 mL | | |

Common Valences

1+

Ammonium, NH_4^+
 Cuprous, Cu^+
 Hydrogen, H^+
 Hydronium, H_3O^+
 Potassium, K^+
 Silver, Ag^+
 Sodium, Na^+

2+

Barium, Ba^{2+}
 Calcium, Ca^{2+}
 Cupric, Cu^{2+}
 Ferrous, Fe^{2+}
 Lead, Pb^{2+}
 Magnesium, Mg^{2+}
 Mercuric, Hg^{2+}
 Nickel, Ni^{2+}
 Zinc, Zn^{2+}

3+

Aluminum, Al^{3+}
 Chromic, Cr^{3+}
 Ferric, Fe^{3+}

1-

Acetate, $\text{C}_2\text{H}_3\text{O}_2^-$
 Bicarbonate, HCO_3^-
 Bromide, Br^-
 Chlorate, ClO_3^-
 Chloride, Cl^-
 Hydroxide, OH^-
 Iodide, I^-
 Nitrate, NO_3^-
 Nitrite, NO_2^-
 Bisulfate, HSO_4^-

2-

Carbonate, CO_3^{2-}
 Chromate, CrO_4^{2-}
 Peroxide, O_2^{2-}
 Sulfate, SO_4^{2-}
 Sulfide, S^{2-}
 Sulfite, SO_3^{2-}

3-

Phosphate, PO_4^{3-}

Periodic Table of the Elements

Period																																				
	Group 1		Group 2												Group 13		Group 14		Group 15		Group 16		Group 17		Group 18											
1	1.00794 H 1														4.00260 He 2																					
2	6.941 Li 3		9.01218 Be 4												10.81 B 5		12.011 C 6		14.0067 N 7		15.9994 O 8		18.998403 F 9		20.179 Ne 10											
3	22.98977 Na 11		24.305 Mg 12												26.98154 Al 13		28.0855 Si 14		30.97376 P 15		32.06 S 16		35.453 Cl 17		39.948 Ar 18											
4	39.0983 K 19		40.08 Ca 20		44.9559 Sc 21		47.88 Ti 22		50.9415 V 23		51.996 Cr 24		54.9380 Mn 25		55.847 Fe 26		58.9332 Co 27		58.69 Ni 28		63.546 Cu 29		65.39 Zn 30		69.72 Ga 31		72.59 Ge 32		74.9216 As 33		78.96 Se 34		79.904 Br 35		83.80 Kr 36	
5	85.4678 Rb 37		87.62 Sr 38		88.9059 Y 39		91.224 Zr 40		92.9064 Nb 41		95.94 Mo 42		(98) Tc 43		101.07 Ru 44		102.906 Rh 45		106.42 Pd 46		107.868 Ag 47		112.41 Cd 48		114.82 In 49		118.71 Sn 50		121.75 Sb 51		127.60 Te 52		126.905 I 53		131.29 Xe 54	
6	132.905 Cs 55		137.33 Ba 56		138.906 La 57		178.49 Hf 72		180.948 Ta 73		183.85 W 74		186.207 Re 75		190.2 Os 76		192.22 Ir 77		195.08 Pt 78		196.967 Au 79		200.59 Hg 80		204.383 Tl 81		207.2 Pb 82		208.980 Bi 83		(209) Po 84		(210) At 85		(222) Rn 86	
7	(223) Fr 87		226.025 Ra 88		227.028 Ac 89		(261) Rf 104		(262) Db 105		(263) Sg 106		(264) Bh 107		(265) Hs 108		(268) Mt 109		(269) Uun 110		(272) Uuu 111		(277) Uub 112				(285) Uuq 114									

KEY

Atomic Mass → 12.011 ← Selected Oxidation States

Symbol → **C**

Atomic Number → 6

Electron Configuration → 2-4

Relative atomic masses are based on ¹²C = 12.000

Note: Mass numbers in parentheses are mass numbers of the most stable or common isotope.

**Denotes the presence of (2-8-) for elements 72 and above

*The systematic names and symbols for elements of atomic numbers above 109 will be used until the approval of trivial names by IUPAC.

140.12 Ce 58	140.908 Pr 59	144.24 Nd 60	(145) Pm 61	150.36 Sm 62	151.96 Eu 63	157.25 Gd 64	158.925 Tb 65	162.50 Dy 66	164.930 Ho 67	167.26 Er 68	168.934 Tm 69	173.04 Yb 70	174.967 Lu 71
232.038 Th 90	231.036 Pa 91	238.029 U 92	237.048 Np 93	(244) Pu 94	(243) Am 95	(247) Cm 96	(247) Bk 97	(251) Cf 98	(252) Es 99	(257) Fm 100	(258) Md 101	(259) No 102	(260) Lr 103

Intermediate Wastewater Laboratory Workshop Laboratory Solutions

1. A laboratory solution is made using 52 milligrams of sodium chloride (NaCl) dissolved in 1-liter volumetric flask filled to the mark. What is the mg/L concentration of the solution?

$$\frac{52 \text{ mg}}{1 \text{ L}} = \boxed{52 \text{ mg/L}}$$

2. If 33 pounds of a chemical is added to 148 pounds of water, what is the % strength by weight?

$$\% \text{ strength} = \frac{\text{weight of chemical, lbs}}{\text{wt. of water} + \text{wt. of chem.}} \times 100$$

$$= \frac{33}{33+148} \times 100 = \frac{33}{181} \times 100 = \boxed{18.2\%}$$

3. You are given 100 mL of 2.8 N HCl. How many mL of water should be added to make 0.4 N HCl?

$$(C_1)(V_1) = (C_2)(V_2)$$

$$(2.8)(100) = (0.4)(V_2)$$

$$\frac{280}{0.4} = 700 \text{ mL} = V_2$$

$$700 - 100 = \boxed{600 \text{ mL}}$$

4. 250 mL of 3 N NaOH is diluted to 1000 mL . What is the new normality of the solution?

$$(250)(3) = (C_2)(1000)$$

$$\frac{750}{1000} = C_2 = \boxed{0.75 \text{ N}}$$

5. V_1 C_1 V_2
500 mL of 10N NaOH is diluted to 1 liter. What is the new normality of the solution? $1L = 1000\text{ mL}$

$$(500)(10) = (C_2)(1000)$$

$$\frac{5000}{1000} = C_2 = \boxed{5\text{ N}}$$

6. You are given V_1 C_1 C_2
20 mL of 30N HCl. How many mL of water should be added to make 1.1N HCl?

$$(20)(30) = (V_2)(1.1)$$

$$\frac{600}{1.1} = V_2 = 545\text{ mL}$$

$$545 - 20 = \boxed{525\text{ mL}}$$

7. An operator needs a 0.1N solution in order to conduct an analysis. The operator has a 1.5N solution on hand. How much (mL) of the 1.5N solution is needed to make 1L of 0.1N solution?

$$C_1 \quad V_2 \quad C_2$$

$$(V_1)(1.5) = (1000)(0.1)$$

$$V_1 = \frac{100}{1.5} = \boxed{66.7\text{ mL}}$$

8. An operator needs a 0.1N solution in order to conduct an analysis. The operator has 2.0N solution on hand. How many milliliters of the 2.0N solution is needed to make one liter of 0.1N solution?

$$C_1 \quad V_2 \quad C_2$$

$$(2.0)(V_1) = (1000)(0.1)$$

$$V_1 = \frac{100}{2.0} = \boxed{50\text{ mL}}$$

9. V_1 C_1 V_2
450 mL of 5N NaOH is diluted to 1L. What is the new normality of the solution?

$$(450)(5) = (1000)(C_2)$$

$$\frac{2250}{1000} = C_2 = \boxed{2.25\text{ N}}$$

10. You are given 8 mL of $15\text{N H}_2\text{SO}_4$. How much water (in mL) should be added to make $0.4\text{N H}_2\text{SO}_4$?

$$V_1 C_1 = V_2 C_2$$

$$(8)(15) = (0.4)(V_2)$$

$$\frac{120}{0.4} = V_2 = 300 \text{ mL}$$

$$300 - 8 = \boxed{292 \text{ mL}}$$

11. An operator needs a 0.2N solution in order to conduct analysis. The operator has 2.5N solution on hand. How many mL of the 2.5N solution is needed to make one-half liter of 0.2N solution?

$$V_1 C_1 = V_2 C_2$$

$$(V_1)(2.5) = (500)(0.2)$$

$$V_1 = \frac{100}{2.5} = \boxed{40 \text{ mL}}$$

12. An operator needs to make 1-liter of a 1N and a 1M solution of sodium bicarbonate (NaHCO_3). How many grams would be needed for each? (Hint: bicarbonate = HCO_3^-)

$$\begin{array}{r} \text{Na} \rightarrow 22.98977 \times 1 = 22.99 \\ \text{H} \rightarrow 1.00794 \times 1 = 1.01 \\ \text{C} \rightarrow 12.0111 \times 1 = 12.01 \\ \text{O} \rightarrow 15.9994 \times 3 = 48.00 \\ \hline 84.01 \end{array} \quad \text{Na}^+ \text{HCO}_3^-$$

$$\begin{aligned} \text{Molarity} \rightarrow \text{grams needed} &= (\text{molarity needed}) (\text{molecular wt.}) (\text{L sol'n}) \\ &= (1\text{M})(84)(1\text{L}) = \boxed{84 \text{ g}} \end{aligned}$$

$$\begin{aligned} \text{Normality} \rightarrow \text{grams needed} &= \frac{(\text{normality needed}) (\text{molecular wt.}) (\text{L sol'n})}{\# \text{ of positives}} \\ &= \frac{(1\text{N})(84)(1\text{L})}{1} = \boxed{84 \text{ g}} \end{aligned}$$

13. An operator needs to make 1-liter of a 1N and a 1M solution of sodium hydroxide (NaOH). How many grams would be needed for each?

$$\begin{array}{r} \text{Na} \rightarrow 22.98977 \\ \text{O} \rightarrow 15.9994 \\ \text{H} \rightarrow 1.00794 \\ \hline 39.99711 \end{array} \quad \text{Na}^+ \text{OH}^-$$

$$\text{molarity} = (1\text{M})(40)(1\text{L}) = \boxed{40\text{g}}$$

$$\text{normality} = \frac{(1\text{N})(40)(1\text{L})}{1} = \boxed{40\text{g}}$$

14. An operator needs to make ½ -liter of a 5N and a 5M solution of ferric sulfate $\text{Fe}_2(\text{SO}_4)_3$. How many grams would be needed for each?

$$\begin{array}{r} \text{Fe} \rightarrow 55.847 \times 2 = 111.694 \\ \text{S} \rightarrow 32.06 \times 3 = 96.18 \\ \text{O} \rightarrow 15.9994 \times 12 = 191.9928 \\ \hline 399.8668 \end{array} \quad \text{Fe}_2^{-3} (\text{SO}_4)_3^{-2}$$

$$\text{molarity} = (5\text{M})(399.8668)(0.5\text{L}) = \boxed{999.7\text{g}}$$

$$\text{normality} = \frac{(5\text{N})(399.8668)(0.5\text{L})}{6} = \boxed{166.6\text{g}}$$

Answers

- | | | |
|--------------------|--------------------|---------------------|
| 1. 52 mg/L | 8. 50 mL | 13. 40 g for 1M |
| 2. 18.2% | 9. 2.25N | 40 g for 1N |
| 3. 600 mL to add | 10. 292 mL to add | 14. 999.78 g for 5M |
| 4. 0.75N | 11. 40 mL | 166.7 g for 5N |
| 5. 5N | 12. 84.01 g for 1M | |
| 6. 525.5 mL to add | 84.01 g for 1N | |
| 7. 66.67 mL | | |