DWR–NPDES–SOP–G–11-Quality Assurance for E. Coli Analysis-01012024
Quality Assurance for E. coli Analysis

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1) **EFFECTIVE DATE: 01/01/2024**

2) **SIGNATURES:**

April Grippo (May 30, 2024 16:25 CDT)

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- Thermometers – 9020B.4.a
    - Annually check accuracy of all working temperature-sensing devices…against a certified 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
    - For general purposes, use thermometers graduated in increments of 0.5 °C or less
    - If using liquid-based thermometers to measure temperatures in air incubators and refrigerators, keep thermometer bulb in water or glycerol. When testing temperatures exceed 50 °C (e.g., autoclave spore check functionality), place the thermometer bulb in a glass container filled with sand
    - Ensure thermometer markings are legible and the liquid column or glass case has no break or change. Discard thermometers with illegible graduation marks
- Autoclave – 9020B.4.h
    - For routine use, verify the autoclave temperature weekly by using a maximum registering thermometer (MRT) to confirm that 121 °C has been reached
    - Test monthly for sterilization efficacy at the media's normal sterilization time and temperature using a biological indicator (e.g., commercially available *Geobacillus stearothermophilus* in spore strips, suspensions, or capsules, preferably at a 1 x 106 concentration)
        - Place the indicator in glassware containing a liquid to simulate actual autoclave sterilization performance on media
    - Each quarter, use a calibrated timer or national time signal to check the timing of all three cycles for a media run (≤ 15 min conditioning cycle, 15 min sterilization cycle, and ≥ 15 min exhaust cycle)
    - Keep autoclave clean and free of debris by checking both trap and seals monthly
    - Service autoclaves annually either in-house or through service contracts.
- Refrigerator – 9020B.4.i
    - Maintain temperature at 2-8 °C
    - Every day while in use, check and record temperature (corrected, if necessary), also noting date and observer's initials
    - Clean units annually, or more frequently if needed
- Membrane filtration equipment (if MF procedure is used) – 9020B.4.k
    - Wash and rinse filtration assemblies thoroughly after use, wrap in nontoxic paper or foil, and sterilize
    - UV sterilize or boil funnels between samples
        - If using boiling water, make sure membrane filtration equipment is cool before adding next sample

- Membrane filters and pads (if MF procedure is used) – 9020B.5.i.3
    - When each lot of membranes arrives at the laboratory, record lot number and date received
    - If lot is held for one or more years, carefully check for brittleness and discard lots that appear brittle
- Ultraviolet lamps (if used) – 9020B.4.l
    - When in use, disconnect lamps monthly and clean bulbs with a soft cloth moistened with ethanol
- Incubator – 9020B.4.o
    - During usage periods, check and record calibration-corrected temperature twice daily (morning and afternoon, separated by at least 4 hours) on each shelf in use to ensure temperature consistency throughout unit
    - If using a glass thermometer, submerge bulb and stem in water or glycerin to the immersion mark

Laboratory Supplies

- Glassware – 9020B.5.a
    - pH check – To test clean glassware for alkaline or acid residue, add a few drops of 0.04% bromthymol blue (BTB) or other pH indicator and observe the color reaction
        - If there is no residual, the reaction should be neutral (blue-green for bromthymol blue)
- Dilution water bottles – 9020B.5.c
    - Commercially available bottles prefilled with dilution water are acceptable
    - Check one per lot for pH and volume (99 ± 2mL) and examine bottles for a precipitate
    - Discard by expiration date
    - Before use of each batch or lot, conduct sterility (one bottle per lot or quarter with that same lot number, whichever is more frequent)
        - Sterility Checks – 9020B.9.d
            - Check each new batch (or lot, if commercially prepared) of buffered water for sterility before first use by adding 50 mL of it to 50 mL of a double-strength non-selective broth (e.g. tryptic soy, trypticase soy, or tryptose broth)
            - Alternatively, aseptically pass 100 mL of dilution water through a membrane filter and place filter on nonselective medium
            - Incubate at 35 ± 0.5˚C for 48 hours and observe for growth
            - For membrane filter tests, check the sterility of the entire process by using sterile reagent or dilution water as the sample at the beginning and end of each filtration series of samples and test for growth
- Sample bottles – 9020B.5.d

  - Test for sterility at least one of each presterilized lot purchased from a vendor. If growth occurs, discard entire batch or lot
  - Check one per batch or lot for efficacy of dichlorination agent, accuracy of 100-mL mark (if present), and auto-fluorescence properties (if used for fluorescence testing)
- Multi-well trays and sealers – 9020B.5.e
  - Every month, evaluate the heat sealer's performance by adding one to two drops of a food-color dye to 100 mL deionized water sample, run the multi-well tray through the sealer, and visually check each well for leakage
  - Clean and conduct preventative maintenance on sealer annually, or more frequently if needed
  - When using multi-well trays for growth studies, check one per lot for sterility beforehand by aseptically adding 100 mL of sterile tryptic soy broth or other non-selective medium, sealing, and incubating at 35 ± 0.5 °C for 24 and up to 48h. No growth indicates sterility
  - Real people language – analyze a method blank once per lot (of sterile water, media, bottles, and trays) or once per quarter, whichever is more frequent, to demonstrate sterility
  - As a monthly check of sealer efficiency, perform and document a visual check that trays are properly sealed. If all sample wells are positive for total coliform and sufficient contrast, visually examine the tray cells for leakage and document the check. If insufficient color contrast is present, use food-color dye as previously recommended by method

Method Specific QC Requirements

- Coliforms – Total and E. coli Hach Method 10029 – mColiBlue24®
  - Blank – daily
    - Run at least one membrane filter blank at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter, placing in a petri dish with mColiBlue broth and testing for growth
  - Positive and Negative Controls – Check certified control cultures with each lot of media and petri dishes with pads OR once a quarter, whichever is more frequent
    - Pseudomonas aeruginosa is recommended as a negative control and Escherichia coli as a positive control
  - Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent

- Enzyme Substrate Test SM 9223 B (2016) – Colilert Method
  - Before using each lot of new medium, verify its performance via positive and negative control organisms

- To conduct culture controls, inoculate medium with three control bacteria: E. coli, a total coliform strain other than E. coli (e.g., Enterobacter cloacae), and a noncoliform (see Table 9020:VI)
- An uninoculated negative control should also be analyzed
- Incubate these controls at 35 ± 0.5 °C as indicated above

- In addition, test medium and vessels (bottles, multi-well trays, tubes) to confirm sterility and lack of autofluorescence
- Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent

Reference

Baird, Rodger., and Laura Bridgewater. *Standard Methods for the Examination of Water and Wastewater*. 23rd edition. Washington, D.C.: American Public Health Association, 2017

Revision Number	Date	Brief Summary of Change
0	January 2014	Initial issuance of the Guidance
1	September 2021	Updates to reflect changes in 23rd edition of Standard Methods for the Examination of Water and Wastewater.
2	December 11, 2023	Grammatical and word choice changes, effective date