Infectious Disease
Serology and Virology

Infectious Disease Serology

Serological Tests Available from the TDH Laboratory

Specimen Collection

Syphilis Serology

Rabies Virus

Virus Culture
Introduction

Diagnostic and immune status serologic assays are performed for various viral, rickettsial, bacterial, fungal, chlamydial and mycoplasmal agents. The assay methods vary depending upon the specific agent for which testing is requested. For specific agents and assay methods refer to Chart V - 1 SEROLOGICAL TESTS AVAILABLE FROM TDH LABORATORY.

Serological testing for infectious agents that are not performed by the Tennessee Department of Health (TDH) Laboratory may be available at the Centers for Disease Control and Prevention (CDC). Consult with the appropriate section at the Nashville laboratory before submitting specimens for testing. According to CDC's guidelines, all specimens submitted to the CDC must come through the state laboratory or receive the state laboratory's approval for direct shipment from the provider to the CDC.

Specimen Acceptance Policy

**HIV-1** -- Serological testing for HIV-1 is available only in support of counseling and testing sites established by the TDH Sexually Transmitted Diseases/HIV (STD/HIV) Control Program.

**Other agents** -- serological testing is available to all public and private health care providers.

**Type of Specimen Required**

**Immunity Screening** -- Immunity screening for rubella is intended for prenatal and family planning patients. Immunity screening for measles and mumps is not routinely available. Arrangements may be made with the TDH Laboratory to perform this screening on a case-by-case basis. A single, whole clotted blood or serum is required for rubella, measles, or mumps immunity screening.

**Diagnostic Testing** -- As a rule, acute and convalescent sera must be submitted for serological testing. The acute serum should be collected as soon after the onset of illness as possible. For the majority of the serological testing offered by the TDH Laboratory, the convalescent serum should be collected 14 days from the time the acute specimen was collected. In most cases, the laboratory requests that the acute and convalescent sera be submitted at the same time. For those agents for which IgM is available, submit the acute specimen when it is collected. See Chart V - 1 SEROLOGICAL TESTS AVAILABLE FROM THE TDH LABORATORY.
Infectious Disease Serology (Continued)

Chart V - 1
Serological Tests Available from the TDH Laboratory

Testing for infectious agents not listed in this chart may be available at the CDC. Consult with the TDH Laboratory concerning testing not listed.

<table>
<thead>
<tr>
<th>Agent or Disease Suspected</th>
<th>Specimen Needed</th>
<th>Test Method</th>
<th>Normal Reference Range</th>
<th>Turn Around Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern Equine encephalitis virus</td>
<td>Acute and convalescent(14 days) sera</td>
<td>IFA IgG, IFA IgM</td>
<td>&lt;1:16, &lt;1:16</td>
<td>5</td>
</tr>
<tr>
<td>Ehrlichia chaffeensis</td>
<td>Acute and convalescent(28 days) sera</td>
<td>IFA, IgG</td>
<td>&lt;1:128</td>
<td>5</td>
</tr>
<tr>
<td>Human immunodeficiency virus Type 1 (HIV-1)</td>
<td>Whole, clotted blood or serum</td>
<td>Screening - EIA, Confirmation - WB</td>
<td>Non-Reactive, Non-Reactive</td>
<td>7</td>
</tr>
<tr>
<td>LaCrosse (California encephalitis group) virus</td>
<td>Acute and convalescent(14 days) sera</td>
<td>IFA IgG, IFA IgM</td>
<td>&lt;1:16, &lt;1:16</td>
<td>5</td>
</tr>
<tr>
<td>Legionella pneumophiliae (Type 1-specific)</td>
<td>Acute and convalescent(28 days) sera</td>
<td>IFA, IgG</td>
<td>&lt;1:128</td>
<td>5</td>
</tr>
<tr>
<td>Measles virus (Rubeola)</td>
<td>Immunity Screening -- Whole clotted blood or serum</td>
<td>EIA (IgG)</td>
<td>Positive (Immune)</td>
<td>5</td>
</tr>
<tr>
<td>Measles virus (Rubeola)</td>
<td>Diagnostic -- Acute and convalescent (14 days) sera</td>
<td>EIA (IgG), EIA (IgM)</td>
<td>Negative, Negative</td>
<td>1</td>
</tr>
<tr>
<td>Mumps virus</td>
<td>Immunity Screening -- Whole clotted blood or serum</td>
<td>EIA (IgG)</td>
<td>Positive (Immune)</td>
<td>5</td>
</tr>
<tr>
<td>Mumps virus</td>
<td>Diagnostic -- Acute and convalescent (14 days) sera</td>
<td>EIA (IgG)</td>
<td>Negative</td>
<td>1</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>Acute and convalescent(14 days) sera</td>
<td>EIA IgM, EIA IgG</td>
<td>Negative, Negative</td>
<td>5</td>
</tr>
<tr>
<td>Q Fever (Coxiella burnetii) Phases 1 and 2</td>
<td>Acute and convalescent(28 days) sera</td>
<td>IFA, IgG</td>
<td>&lt;1:256</td>
<td>5</td>
</tr>
<tr>
<td>Rocky Mountain Spotted Fever (Rickettsia rickettsii)</td>
<td>Acute and convalescent(28 days) sera</td>
<td>IFA, IgG</td>
<td>&lt;1:128</td>
<td>5</td>
</tr>
<tr>
<td>Rubella virus</td>
<td>Immunity Screening -- Whole clotted blood or serum</td>
<td>EIA (IgG)</td>
<td>Positive (Immune)</td>
<td>5</td>
</tr>
</tbody>
</table>
### Infectious Disease Serology (Continued)

#### Chart V - 1 (continued)
Serological Tests Available from the TDH Laboratory

<table>
<thead>
<tr>
<th>Agent or Disease Suspected</th>
<th>Specimen Needed</th>
<th>Test Method</th>
<th>Normal Reference Range</th>
<th>Turn Around Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Louis encephalitis virus</td>
<td>Acute and convalescent (14 days) sera</td>
<td>IFA IgG, IFA IgM</td>
<td>&lt;1:16, &lt;1:16</td>
<td>5</td>
</tr>
<tr>
<td>Typhus (<em>Rickettsia typhi</em>)</td>
<td>Acute and convalescent (28 days) sera</td>
<td>IFA, IgG</td>
<td>&lt;1:128</td>
<td>5</td>
</tr>
<tr>
<td>West Nile Virus (WNV)</td>
<td>Acute and convalescent (14 days) sera or CSF</td>
<td>EIA IgG, EIA IgM</td>
<td>Negative, Negative</td>
<td>5</td>
</tr>
<tr>
<td>Western Equine encephalitis virus</td>
<td>Acute and convalescent (14 days) sera</td>
<td>IFA IgG, IFA IgM</td>
<td>&lt;1:16, &lt;1:16</td>
<td>5</td>
</tr>
</tbody>
</table>

**Abbreviations**
- **EIA**: Enzyme Immunoassay
- **WB**: Western Blot
- **IFA**: Indirect Fluorescent Antibody
- **IgG**: Class Immunoglobulin
- **IgM**: Class Immunoglobulin
- **Quant**: Quantitation, Quantitated

1 The normal reference range as stated in this table is for a single serum.
2 Turn-around time is the number of working days from receipt of the specimen by the testing laboratory until the laboratory sends a report of test results.
3 An EIA procedure is performed at the Knoxville, Jackson and Nashville laboratories to screen serum specimens for antibody to HIV-1. The WB procedure is performed at the Nashville laboratory as a confirmatory test for those specimens found repeatedly reactive for HIV-1 antibody by the EIA procedure. The Knoxville and Jackson laboratories forward specimens for the WB procedure to the Nashville laboratory. Testing is available only to the TDH STD/HIV Control Program's counseling and testing sites.
4 Prior approval required before specimen submission.
Infectious Disease Serology (Continued)

Specimen Collection

Blood
1. Collect an acute serum as soon after the onset of the illness as possible. A convalescent serum should be collected 14 days after the collection of the acute serum. Exceptions to this general rule of collection of specimens are noted in Chart V - 1 SEROLOGICAL TESTS AVAILABLE FROM TDH LABORATORY.

2. Draw at least 5 to 7 ml of blood into a red-top vacuum tube allowing the tube to fill completely. Allow the tube to stand at room temperature to ensure complete clotting of blood. Blood should not be taken for 1 hour after a meal to avoid chylous serum.

3. Store the specimen in a refrigerator until it is sent to the laboratory. If a sample of serum is to be sent to the laboratory, separate the serum from the blood clot by centrifuging the whole clotted blood at 1,500 to 2,000 rpm at room temperature for 10 minutes. Pipette the serum into a new red-top vacuum tube or a sterile plastic screw-capped vial. A minimum of 1 ml of serum should be sent to the laboratory for testing.

4. Acute serum that is held until the collection of a convalescent serum should be separated from the blood clot and stored frozen until collection of the convalescent serum. Acute serum will not be tested routinely unless the TDH Laboratory offers testing for the IgM class of antibody for the analytic testing requested. Convalescent specimens may be run as stand alone specimens in limited situations. Consultation with the supervisor of the Serology Unit is required before the convalescent serum will be tested singly.

Spinal Fluid
Prior arrangement must be made with the TDH Laboratory before cerebrospinal fluid (CSF) specimens are submitted for serologic testing. The VDRL test for syphilis is routinely performed on CSF. The EIA test for West Nile Virus (WNV) IgM is performed on CSF seasonally.

Specimen Identification
1. Use the appropriate form for the test requested:

   Rubella  Rubella Form PH-1917
   HIV-1  HIV-1 Serology Form PH-3173
   Other non-syphilis serology  Immunoserology Form PH-1589

Complete all the information on the form. Include pertinent clinical information with each specimen. Be specific about why the specimen is being submitted to the laboratory.

For rubella, measles (rubeola) and mumps, indicate whether the specimen is for diagnosis of a current infection or for immunity screening and if the patient is a prenatal or family planning patient.

For HIV-1 serological testing, include the information as prescribed by the TDH STD/HIV Control Program.
Infectious Disease Serology ( Continued )

2. Using indelible ink, label each specimen with the patient's first and last name and the date of collection. Attach the tear strip number from the test request form to the specimen and secure it with transparent tape. Those providers submitting electronic test requests should affix a label produced by Laboratory Order Entry (LOE) to the associated specimen. Unlabeled specimens or specimens containing information that does not exactly match the information on the accompanying test request form or electronic record will not be tested.

Shipment of Specimens

1. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines of your current carrier or shipping method.

2. Affix the mailing label (PH-0838), return address and other labeling required by pertinent regulations to the outer container.


<table>
<thead>
<tr>
<th>Location and address for items shipped by UPS, Federal Express and carriers other than the US Postal Service</th>
<th>Postal address for non-specimen mail</th>
<th>Postal address for specimen mail US Postal Service</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tennessee Department of Health Laboratory Services 630 Hart Lane Nashville, TN 37216-2006</td>
<td>Tennessee Department of Health Laboratory Services 630 Hart Lane Nashville, TN 37243</td>
<td>Tennessee Department of Health Laboratory Services P.O. Box 305130 Nashville, TN 37230-5130</td>
</tr>
</tbody>
</table>

4. Use first-class postage on packages shipped via US Postal Service (USPS).

Reporting Procedure and Interpretation

An interpretation of the results is given with each report. For specimens sent to the CDC, the CDC will provide interpretation of test results.

Final Reporting

The results of all specimen requests are reported to the provider who submitted the specimen.

If the result of the specimen is positive for a notifiable disease, this result is also reported to the TDH Communicable and Environmental Disease Services and to the health department in the patient's county of residence.
Criteria for Unacceptable Specimens

1. The specimen is not properly identified with required information.
2. The patient identifier on the specimen does not exactly match the identifier on the test request form or electronic record.
3. The specimen is broken or leaked in transit.
4. The specimen is extensively hemolyzed, lipemic (chylous), extremely turbid, or grossly contaminated with bacteria.
5. Whole, clotted blood was collected more than 7 days prior to receipt by the laboratory and serum not separated from the clot.
6. The quantity of the specimen received is not sufficient to allow accurate completion of test(s) requested. (QNS-Quantity Not Sufficient).
7. An acute serum specimen was submitted a month ago. A convalescent serum specimen has not been received.
8. The convalescent serum was collected sooner than 10 days from the date of collection of the acute serum. (The provider will be notified and asked to provide a more appropriately timed convalescent serum.)
9. No test request form or electronic record was received with the specimen or no specimen was received with a test request form or electronic record.
## Rubella Form PH-1917

### FRONT

**RUBELLA SEROLOGY**

<table>
<thead>
<tr>
<th>Lab No.</th>
<th>A335477</th>
</tr>
</thead>
</table>

**PURPOSE OF SPECIMEN**

- IMMUNITY SCREENING
- FAMOUS PLANNING
- MENTAL
- EXPOSURE/DIAGNOSIS
- DATE OF EXPOSURE
- DATE OF COLLECTION
- CONVULSIVE

**CLINICAL INFORMATION**

EXAMINATION RESULTS: * (SEE REVERSE SIDE)

- IMMUNITY
- NO IMMUNITY
- EQUIVOCAL (SEE REVERSE SIDE)
- UNSATISFACTORY

EXPOSURE/DIAGNOSIS: REPORT OF RESULTS IN LETTER FORM

ENZYME IMMUNOASSAY (EIA) TEST FOR RUBELLA ANTIBODY

IMMUNITY SCREENING: A single serum test and reported as immunity, no immunity, or equivocal based on the following criteria:

- **NO IMMUNITY:** Immune Status Ratio (ISR) less than or equal to 0.90
- **EQUIVOCAL:** Immune Status Histo (ISH) greater than 0.90 but less than 1.10
- **IMMUNITY:** Immune Status Ratio (ISR) greater than or equal to 1.10

*Serum producing equivocal results for 2 of the 3 tests performed on it are reported as equivocal. Another serum should be submitted for testing with the test performed by Laboratory Services, or the new specimen may be submitted to another laboratory offering different test methodology.

EXPOSURE/DIAGNOSIS: Report of test results and interpretation of results are submitted to provider of specimen in letter form and are not reported via this form.

### BACK
HIV-1 Serology Form PH-3173

FRONT

HIV-1 SEROLOGY

DATE REPORTED: LAB RECEIPT DATE/TIME: SPECIMEN CONTROL NO.

COLLECTION DATE: SPECIMEN NO.

TYPE OF SPECIMEN: [ ] SERUM [ ] PLASMA RISK FACTOR(S):

[ ] FOLLOW UP TEST

DATE OF PREVIOUS TEST

TEST RESULTS

NEW-REACTIVE FOR HIV ANTIBODY BY EIA
NON-REACTIVE FOR HIV ANTIBODY BY EIA
REACTIVE FOR HIV ANTIBODY BY WESTERN BLOT
NON-REACTIVE FOR HIV ANTIBODY BY WESTERN BLOT
INDETERMINATE RESULTS FOR HIV ANTIBODY BY WESTERN BLOT

NEGATIVE FOR HIV ANTIBODY
POSITIVE FOR HIV ANTIBODY
INDETERMINATE RESULTS FOR HIV ANTIBODY (SEE BACK OF FORM)

INCOMPATIBLE

EXAMINED BY:

RDA-1160

HIV-1 SEROLOGY

Serum or plasma are the only acceptable specimens for HIV-1 antibody testing.

Perform HIV-1 EIA test at least 6 months after potential exposure and interpret results consistent with local guidelines for HIV-1 antibody results.

Interpretation of test results is based on package insert instructions for the commercial EIA procedure used and on current ASTPHLD/IDC recommendations for the Western Blot procedure.

EIA = Enzyme Immunoassay (Screening Test)
Western Blot (Supplementary Test)

TESTING LABORATORY LOCATION CODES

J = JACKSON BRANCH LAB, 295 SUMMAR DRIVE, JACKSON, TN - DR. ORETHYN E. WALKER, PHD, HOLDE, DIRECTOR
K = KNOXVILLE BRANCH LAB, 1622 TERRY HALL, KNOXVILLE, TN - DR. DAVID L. SMALLERY, PHD, MSS, BCLD, DIRECTOR
N = NASHVILLE REFERENCE LAB, 135 HART LANE, NASHVILLE, TN - DR. DAVID L. SMALLERY, PHD, MSS, BCLD, DIRECTOR

BACK
Infectious Disease Serology (Continued)

Immunoserology Form PH-1589

http://health.state.tn.us/Lab/index.htm  Section 5-10
Introduction

Syphilis is a disease caused by infection with the spirochete Treponema pallidum. Serological tests greatly aid in the diagnosis of syphilis. Serologic assays used to screen patients for syphilis are non-treponemal tests. The non-treponemal test performed by the Tennessee Department of Health (TDH) Laboratory is the Rapid Plasma Reagin test (RPR). Quantitative RPR results may be used to monitor therapy for T. pallidum infections.

Confirmation of reactive RPR screening test results is obtained with specific treponemal tests for syphilis. The Treponema pallidum-Particle Agglutination test (TP-PA) is the TDH Laboratory's primary confirmatory test for T. pallidum-specific antibody. Suspected biologically false-positive results sometimes produced in the RPR test may be investigated with a TP-PA test. The Fluorescent Treponemal Antibody-Absorption-Double Stain Test (FTA-ABS-DS) also detects T. pallidum-specific antibody. It is available in limited circumstances. The TP-PA and FTA-ABS-DS are not screening procedures and are only performed when required for proper patient management.

The Venereal Disease Research Laboratory (VDRL) test is a non-treponemal test used to test cerebrospinal fluids (CSF). Positive test results are quantitated to aid in monitoring therapy for neurosyphilis. The RPR, TP-PA and FTA-ABS-DS tests are not performed on CSF.

Specimen Acceptance Policy

The TDH Laboratory performs serological procedures for syphilis in support of:

- The state prenatal law.
- The TDH Sexually Transmitted Disease Control Program.
- The private medical community for which the state laboratories serve as reference laboratories.
- Other State agencies for which the TDH Laboratory has contracted or agreed to perform tests.

Testing for syphilis, non-treponemal and treponemal-specific, is available to all health care providers.

Tennessee does not require premarital testing for syphilis.

Syphilis screening tests will be performed for persons who intend to be married in a state requiring premarital syphilis testing. The TDH Laboratory will send appropriate premarital forms for the state in which the wedding will be performed with the results of the laboratory tests. Other states may not accept premarital syphilis testing performed by laboratories other than state public health laboratories such as the TDH Laboratory.

Type of Specimen Required

For the tests performed at the TDH Laboratory, the specimen required and the application of the test refer to Chart V - 2 SEROLOGICAL TESTS FOR SYPHILIS.
**Syphilis Serology (Continued)**

### Chart V - 2
**Serological Tests for Syphilis**

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen Required</th>
<th>Application of Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-treponemal Tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPR</td>
<td>Whole, clotted blood, serum, or plasma *</td>
<td>Screening (for example, prenatal or STD clinics), monitoring treatment. Performed at Nashville, Knoxville and Jackson Labs.</td>
</tr>
<tr>
<td>VDRL</td>
<td>Cerebrospinal fluid</td>
<td>Congenital syphilis, central nervous system involvement (neurosyphilis). Performed only at Nashville Lab.</td>
</tr>
<tr>
<td><strong>Treponemal Antibody Tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP-PA</td>
<td>Whole, clotted blood or serum</td>
<td>Detection of false-positive RPR results, monitoring of infants for possible congenital syphilis. Performed at Nashville, Knoxville and Jackson Labs.</td>
</tr>
<tr>
<td><strong>Tests</strong></td>
<td>FTA-ABS-DS</td>
<td>To aid in diagnosis of suspected primary syphilis when the RPR is reactive and the TP-PA test is non-reactive. Performed only at Nashville Lab.</td>
</tr>
</tbody>
</table>

* Plasma can be tested with the RPR test, but plasma is not the preferred specimen. Serum is preferred because it is required for subsequent treponemal antibody tests that may need to be performed after the RPR test is completed. Also, plasma must be tested within 48 hours of collection or the risk of false RPR results is greatly increased.

** Treponemal antibody tests will not routinely be performed on specimens that produce negative results on the screening test (RPR). An exception is that the TP-PA will be performed at the provider's request on specimens that may produce negative RPR results but are from patients (birth to 15-months-old) who may have congenital syphilis.

### Specimen Collection

Draw only one syphilis serology blood tube on each patient, even for those requiring a confirmatory test. Additional tubes are unnecessary for two tests and add to the risk of identification errors.

**WHOLE, CLOTTED BLOOD OR SERUM**

1. Using a 5 to 7 ml red-top vacuum tube, draw enough blood to completely fill the tube. Allow the tube to stand at room temperature to ensure complete clotting of blood. Blood should not be taken for 1 hour after a meal to avoid chylous serum.
Syphilis Serology (Continued)

2. Store the specimen in a refrigerator (2 to 8°C) until it is sent to the laboratory. If serum is to be sent, separate the serum from the blood clot by centrifuging the whole, clotted blood at 1,500 to 2,000 rpm’s at room temperature for 10 minutes. Pipette the serum into a new red-top vacuum tube or plastic screw-capped vial. Submit at least 2 ml of serum.

PLASMA

Plasma is not a recommended specimen for syphilis testing. It may be submitted (1 to 2 ml) for the screening procedure for syphilis (RPR), but is not a suitable specimen for subsequent TP-PA or the FTA-ABS-DS procedures. Plasma must be tested within 48 hours from the time of collection to produce reliable RPR results.

CEREBROSPINAL FLUID (CSF)

Submit 1 to 2 ml of CSF in a sterile, plastic screw-capped vial.

Specimen Identification

1. **Complete all information on the Syphilis Serology Form PH-1578.** Mark the test requested and include pertinent clinical information with each specimen.

2. Label each specimen with the patient’s name and the collection date. Attach the control number on the tear strip to the specimen and secure it with transparent tape. Those providers submitting electronic test requests should affix a label produced by Laboratory Order Entry (LOE) to the associated specimen. Unlabeled specimens or specimens containing information that does not exactly match the information on the accompanying test request form or electronic record will not be tested.

3. When requesting a TP-PA test, the results of an RPR or other screening procedure must be indicated. Previous TP-PA results must be indicated when requesting the FTA-ABS-DS test.

Shipment of Specimens

1. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines of your current carrier or shipping method.

2. Affix the mailing label (PH-0838), return address and other labeling required by pertinent regulations to the outer container.

3. Use first-class postage on packages shipped via US Postal Service (USPS).

4. Ship blood or serum specimens for the RPR or TP-PA to the TDH Laboratory in Jackson, Knoxville, or Nashville. Ship blood or serum specimens for the FTA-ABS-DS test or cerebrospinal fluid specimens for the VDRL test to the Laboratory in Nashville.
Syphilis Serology (Continued)

Interpretation of Laboratory Results

**Screening (RPR)**
Normal: Non-reactive
Abnormal: Reactive

**Confirmatory (TP-PA, FTA-ABS-DS)**
Normal: Non-reactive,
Abnormal: Reactive
Positive reactions will occur within 10 to 90 days following exposure or 7 to 10 days after onset of primary lesion.

Biological false-positive RPR results may occur. Possible causes for biological false-positive RPR results:
- Narcotic addiction
- Hepatitis
- Aging
- Leprosy
- Terminal malignancy
- Pregnancy
- Viral diseases, e.g., chickenpox, measles, infectious mononucleosis, pneumonia, etc.
- Rheumatoid arthritis
- Malaria
- Systemic lupus erythematosus

Reporting Procedure and Interpretation

Results of the non-treponemal tests for syphilis performed on serum or plasma are available within 1 working day after receipt of the specimen. Results of the TP-PA tests are available within 3 working days. The FTA-ABS-DS results are available within 7 working days. Results of the VDRL test on cerebrospinal fluids are available within 7 working days after receipt of the specimen.

<table>
<thead>
<tr>
<th>Results of Tests for Syphilis are Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
</tr>
<tr>
<td>(The RPR and VDRL are quantitated.</td>
</tr>
<tr>
<td>These results are reported as dils.)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Non-reactive</td>
</tr>
</tbody>
</table>

The results of all specimen requests are reported to the provider who submitted the specimen. In addition, the TDH Sexually Transmitted disease (STD) control, the regional STD control representative and the health department in the county where the patient lives are sent reports on positive specimens.

For a premarital test, a premarital certificate for the specific state will be sent to the provider with the laboratory results. **Tennessee does not require a premarital syphilis test.**
Syphilis Serology (Continued)
Criteria for Unacceptable Specimens

1. The specimen is not properly identified with required information.
2. The patient identifier on the specimen does not match that on test request form or electronic record.
3. The specimen is broken or leaked in transit.
4. The specimen is extensively hemolyzed, lipemic (chylous), extremely turbid, or grossly contaminated with bacteria.
5. Whole, clotted blood collected more than 7 days prior to receipt by the laboratory.
6. Plasma collected more than 48 hours prior to receipt by the laboratory.
7. The quantity of the specimen received is not sufficient to allow accurate completion of test requested. (QNS-Quantity Not Sufficient.)
8. Cerebrospinal fluid (CSF) shows evidence of contamination with blood or microbial growth.
9. No test request form or electronic record was received with the specimen, or no specimen was received with the test request form or electronic record.

Syphilis Serology Form PH-1578
FRONT

INSTRUCTIONS
1. Use T3 x 100 mm size vacutainer type tube with no additive for submitting syphilis serology specimen. If serum is sent, send the serum in a vacutainer type tube. At least 5 ml of clotted blood or 2 ml of serum should be submitted.
2. Disturb the specimen control number from the form and attach it to the specimen tube and print the patients name on the specimen tube.
3. Completely fill out the shaded portions of the form.
4. The VDLR test will be used to test CSF.
5. The TP-PA and/or FTA-ABS tests will not be used as screening procedures and will be performed only on serum for which a screening procedure has been performed. The TP-PA test is this laboratory's primary test for confirmation of syphilis antibodies and will be performed before the use of the FTA-ABS test is considered.
6. In the absence of historical or clinical evidence of treponemal infection, this test result should be considered equivocal. A second specimen should be submitted for serology testing.

TESTING LABORATORY LOCATION CODES
J = JACKSON BRANCH LAB, 205 SUMMIT DRIVE, JACKSON, TN - DR. CHRISTINE E. WALKER, PHD, HLD, DIRECTOR
K = KNOXVILLE BRANCH LAB, 1500 CHEROKEE TRAIL, KNOXVILLE, TN - DR. DAVID L. SMALLEY, PHD, MBS, BCLD, DIRECTOR
N = NASHVILLE REFERENCE LAB, 603 9TH LANE, NASHVILLE, TN - DR. DAVID L. SMALLEY, PHD, MBS, BCLD, DIRECTOR

BACK
Introduction

The direct fluorescent antibody procedure to detect rabies virus in brain material is performed at the Tennessee Department of Health Laboratories (TDH) in Jackson, Nashville, and Knoxville. This testing service is available to state rabies control personnel, veterinarians licensed in the state of Tennessee and any health care provider licensed by and practicing in Tennessee. The mouse inoculation procedure to detect rabies virus is available for limited use at the Nashville laboratory.

Specimen Acceptance Policy

Only animals that have potentially exposed a person, household pet, or livestock to rabies or animals of interest to rabies control personnel should be submitted. Exposure is defined as a bite or contamination of scratches, abrasions, open wounds, or mucous membranes with infectious saliva.

Dogs and cats are the only animals that should be kept alive and held 10 days for observation following a bite. Observation is of value because the period of time the virus can be excreted in the saliva prior to onset of signs can be predicted. It is known that dogs and cats can excrete rabies virus up to five days prior to onset of signs. The ten-day observation period for dogs and cats is twice that predicted time, allowing a margin of safety. If a dog or cat shows no clinical signs of rabies after 10 days of observation, it is safe to assume that the animal was not shedding rabies virus at the time of the bite. Conversely, if a dog or cat exhibits signs of rabies, it should be tested. Euthanize the animal and submit only the head to the laboratory for testing.

Unlike dogs and cats, the period of time that rabies virus can be excreted in the saliva of wild carnivores (such as foxes, skunks, raccoons, bobcats, etc.) before the animal shows signs of rabies cannot be predicted; therefore, an animal in this group should not be held for observation following a bite. If testing criteria have been met, these animals should be caught and euthanized immediately. Only the head should be sent to the laboratory for rabies virus detection. Do not destroy the brain.

Bats to be tested should be caught, euthanized, and the entire animal should be submitted to the laboratory for rabies virus detection. Do not destroy the brain.

Specimen Collection

Brain tissue is examined for the detection of rabies infection in animals. Therefore, only the animal's head should be submitted for diagnostic purposes. For bats, the entire DEAD animal should be submitted. Animals should be euthanized in a manner that will not destroy the brain. The animal's neck should be severed at the midpoint between the base of the skull and the shoulders. Specimens must not be frozen, fixed in formalin, or shipped on dry ice.
Rabies Virus (Continued)

Specimen Identification

1. Complete all the information on Rabies Form PH-1584.

2. Label the outside of the specimen container with the type of animal (dog, cat, cow, etc.) and the tear strip number from the accompanying Rabies Form PH-1584. Secure the label with transparent tape.

Shipment of Specimen

1. Packing and shipping specimens to the state public health laboratory requires personnel trained in current regulations. Follow the shipping guidelines of your current carrier or shipping method.

2. Affix the mailing label (PH-0838), return address and other labeling required by pertinent regulations to the outer container.

3. Ship the specimen by the fastest means possible to the TDH Laboratory in Jackson, Knoxville, or Nashville. Transport by the provider’s personal courier is preferred, but shipment by commercial couriers is acceptable, if permitted.

4. It is against US postal regulations to send this type of specimen through the mail.

Reporting Procedure and Interpretation

Positive rabies test results will be reported immediately by telephone to the State Rabies Control Officer or his representative. Unsatisfactory specimens will also be reported to the State Rabies Control Officer or his representative. All test results will be mailed to the provider. The health department of the county in which the animal specimen was obtained will be sent a report of all positive results via the State Rabies Control Officer or his representative. Results are available within 24 to 36 hours after receipt of the specimen except for those specimens received on Friday afternoon.

Results of Fluorescent Rabies Antibody Tests Are Reported

- Rabies virus found by FRA (Fluorescent Rabies Antibody)
- Rabies virus not found by FRA (Fluorescent Rabies Antibody)

The mouse inoculation test, if needed, may take as long as 28 days before a result is available.

Results of Mouse Inoculation Tests Are Reported

- Rabies virus found by mouse inoculation.
- Rabies virus not found by mouse inoculation.
Criteria for Unacceptable Specimens

1. The specimen is received without an accompanying Rabies Request Form, PH-1584.
2. The brain material has deteriorated or decayed to the extent that anatomical features of the brain are no longer distinguishable.
3. The brain has been mutilated to the extent that anatomical features (areas) of the brain can not be distinguished.
4. There is no brain evident in the head submitted.
5. The specimen is fixed in formalin.

NOTE: Frozen specimens are not necessarily unacceptable. Freezing and thawing of brain tissue is very damaging to the tissue. The acceptability of a frozen specimen will be decided once the specimen is thawed. Submitting a frozen specimen usually causes at least a one day delay for rabies testing.

Rabies Form PH-1584
FRONT
Introduction
The Virology Unit is responsible for culturing and identifying viruses in clinical specimens. Virus culture provides a mechanism for the detection and identification of many human viruses that cause a wide variety of common illnesses. Specimens for culture of human viruses will be accepted from both public and private health care providers.

Virus culturing and identification that is not performed at the Tennessee Department of Health (TDH) Laboratory may be available at the Centers for Disease Control and Prevention (CDC) in Atlanta. Consult with the Virology Unit prior to submitting specimens for testing for viruses other than those listed in Chart V-3. According to CDC's guidelines, all specimens submitted to the CDC must come through the state laboratory or receive the state laboratory's approval for direct shipment from the provider to the CDC.

Specimen Collection
Collect specimens for virus isolation during the early, acute, febrile phase of illness. Specimens collected more than one week after onset of symptoms usually do not yield live viruses. The source of the specimen collected must be carefully matched with the virus suspected. The virus isolation services available at the TDH Laboratory and the specimen of choice for each virus is described in Chart V - 3 VIRUSES FOR WHICH ROUTINE CULTURING IS AVAILABLE.

Chart V - 3
Viruses for Which Routine Culturing is Available
Consult with the TDH Laboratory for additional tests
Which may be available at the CDC.

<table>
<thead>
<tr>
<th>Virus</th>
<th>TEST METHOD</th>
<th>SPECIMEN SOURCE/TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus (Types 1-41)</td>
<td>Cell culture</td>
<td>Throat washing or swab, nasopharyngeal wash or swab, conjunctival swab, feces, urine</td>
</tr>
<tr>
<td></td>
<td>FA Neutralization</td>
<td></td>
</tr>
<tr>
<td>Coxsackie virus (A &amp; B)</td>
<td>Cell culture</td>
<td>Throat swab, feces, CSF, pericardial fluid</td>
</tr>
<tr>
<td></td>
<td>Neutralization</td>
<td></td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Cell culture</td>
<td>Urine, throat swab, buffy coat, lung tissue, lung aspirate</td>
</tr>
<tr>
<td></td>
<td>FA</td>
<td></td>
</tr>
<tr>
<td>Echovirus (Types 1-33)</td>
<td>Cell culture</td>
<td>Throat swab, feces, CSF, pericardial fluid</td>
</tr>
<tr>
<td></td>
<td>Neutralization</td>
<td></td>
</tr>
<tr>
<td>Enterovirus (Types 68-71)</td>
<td>Cell culture</td>
<td>Throat swab, feces, CSF, pericardial fluid, vesicle scraping</td>
</tr>
<tr>
<td></td>
<td>Neutralization</td>
<td></td>
</tr>
<tr>
<td>Herpes simplex virus (Types 1 &amp; 2)</td>
<td>Cell culture</td>
<td>Vesicle scraping, brain biopsy, conjunctival swab, urogenital swab</td>
</tr>
<tr>
<td></td>
<td>FA</td>
<td></td>
</tr>
<tr>
<td>Influenza virus (A &amp; B)</td>
<td>Cell culture</td>
<td>Throat washing or swab, nasopharyngeal washing or swab</td>
</tr>
<tr>
<td></td>
<td>FA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PCR with consultation</td>
<td></td>
</tr>
</tbody>
</table>

Virus Culture
(615) 262-6350
## Virus Culture (Cont.d)

<table>
<thead>
<tr>
<th>Virus</th>
<th>TEST METHOD</th>
<th>SPECIMEN SOURCE/ TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles virus (Rubeola)</td>
<td>Cell culture, FA</td>
<td>Throat washing or swab, nasopharyngeal washing or swab, conjunctival secretions</td>
</tr>
<tr>
<td>Mumps virus</td>
<td>Cell culture, HAdI, FA</td>
<td>Throat washing or swab, urine, CSF, buccal swab</td>
</tr>
<tr>
<td>Parainfluenza virus (Types 1, 2, &amp; 3)</td>
<td>Cell culture, FA, HAdI</td>
<td>Throat washing or swab, nasopharyngeal washing or swab</td>
</tr>
<tr>
<td>Poliovirus (Types 1, 2, &amp; 3)</td>
<td>Cell culture, Neutralization</td>
<td>Throat washing or swab, feces, nasopharyngeal washing or swab, rectal swab</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>Cell culture, FA</td>
<td>Nasopharyngeal washing or swab</td>
</tr>
<tr>
<td>Rubella virus</td>
<td>Cell culture, FA</td>
<td>Nasopharyngeal washing or swab, CSF, urine</td>
</tr>
<tr>
<td>Varicella-zoster virus (Chickenpox-shingles)</td>
<td>DFA Requires consultation with a Public Health Investigation Team (PHIT)</td>
<td>Vesicle scraping, swabbing</td>
</tr>
</tbody>
</table>

**ABBREVIATIONS**

- FA - Fluorescent antibody
- DFA - Direct Fluorescent Antibody
- HAdI - Hemadsorption inhibition
- HA - Hemagglutination
- EIA - Enzyme Immunoassay

Collect the specimen aseptically and place it in one of the following environments immediately.

1. Refrigerate at 2 to 8°C if the specimen will be delivered to the laboratory within 48 hours.
2. If receipt of the specimen by the laboratory will be longer than 48 hours from the time of collection, freeze the specimen at -70°C or at the lowest temperature possible and ship to remain frozen during transport.

**NOTE: DO NOT FREEZE THE FOLLOWING (Ship with “cold packs” artificial refrigerant):**

- Specimens for isolation of respiratory syncytial virus (RSV).
- Specimens for isolation of cytomegalovirus (CMV).
- Blood specimens for virus isolation.

**Autopsy or Biopsy**: Collect fresh, unfixed tissue from the probable sites involved using a separate sterile instrument for each sample. Place each specimen into a separate small sterile vial of virus transport medium.

Consult with the Virology Unit prior to submitting autopsy or biopsy specimens for virus isolation.

**Cerebrospinal Fluid (CSF)**: Aseptically collect 2 to 3 ml of CSF and transfer it to a sterile vial.

**Feces**: Place a piece of feces 4 to 8 grams (about the size of a grape) into a sterile container.
Virus Culture (Continued)

**Rectal Swab:** Generally, rectal swabs are less satisfactory than feces for the isolation of viruses. If used, obtain a rectal swab by inserting a dry dacron swab at least 5 cm into the anal orifice, rotating the plastic or aluminum wire shaft and then withdrawing it. Some fecal material must be visible on the swab. Break or cut the shaft only enough so that the swab will fit into the viral transport medium container.

**Throat Swab:** Vigorously rub both tonsils and the posterior wall of the pharynx with a dry, sterile dacron swab. The swab should not touch the tongue or buccal mucosa. Break or cut the shaft only enough so that the swab will fit into the viral transport medium container.

**Throat Washing:** The patient should gargle with approximately 8 ml of suitable washing fluid (such as, tryptose phosphate broth, Hank's balanced salt solution, veal infusion broth, or sucrose-phosphate buffer). Collect the fluid in a sterile container with a leak-proof top.

**Nasal Swab:** Insert a dry dacron/ polyester (not alginate) swab into the nostril parallel to the palate and leave in place for a few seconds. Slowly withdraw it with a rotating motion. Obtain specimens from both nostrils with the same swab. Break off the tip of the swab into a tube containing approximately 1.5 ml of viral transport medium.

**Nasal Washing:** Place the patient in a comfortable position with the head slightly tilted backward. Advise him to keep the pharynx closed by saying “K” while the washing fluid is applied to the nostril. With a transfer pipette, apply 1 to 1.5 ml of washing fluid to one nostril at a time. Ask the patient to tilt his head forward and let the washing fluid flow into a sterile beaker or petri dish. Repeat the process alternately with both nostrils until approximately 8 ml of the washing fluid has been used. Transfer the washings from the sterile catch container (the sterile beaker or petri dish) to a sterile container with a leak-proof top for transport to the laboratory.

**Nasopharyngeal Swab:** Take nasal and throat swabs as described above and place into the same vial of transport medium.

**Nasopharyngeal Washing:** Take nasal and throat washings as described above and place into the same vial of transport medium.

**Urine:** Collect clean-catch urine, preferably the first voided morning urine, in a sterile container.

**Vesicle:** Using a sterile instrument, open the fluid-filled vesicle. Using firm pressure, absorb the fluid with a sterile dacron swab and scrape the perimeter of the lesion obtaining cellular material on the swab tip. Avoid causing excessive bleeding. Break off the swab tip into a vial of virus transport medium.

**Tissue Culture Isolates:** The Virology Unit provides reference services for other virology laboratories throughout Tennessee. Viral isolates should be observed microscopically at the initial laboratory until 50% or more of the available cell sheet is exhibiting viral cytopathic effect (CPE). Once the cell sheet is exhibiting 50% CPE, send a tube of the infected cell culture (frozen or unfrozen) to the TDH Nashville Laboratory for identification and/or typing of the virus. If the specimen is to be transported at ambient temperature, the tube of infected cell culture should be filled with a cell-culture-maintenance medium. If the specimen is to be frozen for transport, no more than 1 ml of maintenance medium should be in the tube. Indicate the type of cell culture and the number of times the virus was passed through culture on the specimen tube. Indicate the suspected virus on the test request form.
Virus Culture (Continued)

Specimen Identification

1. Complete all the information on the Virology Form PH-1579. Include pertinent clinical information with each specimen.

2. Using indelible ink, label each specimen with the patient's first and last name and the date of collection. Attach the tear strip number from the test request form to the specimen and secure it with transparent tape. Those providers submitting electronic test requests should affix a label produced by Laboratory Order Entry (LOE) to the associated specimen. Unlabeled specimens or specimens containing information that does not exactly match the information on the accompanying test request form or electronic record will not be tested.

Shipment of Specimens

1. Specimens that are to be hand-carried to the laboratory within 48 hours of collection of the specimen should be packed and submitted in accordance with shipping requirements for Category B substances if the specimen is a clinical specimen. If a viral isolate is being submitted, it should be shipped as a Category A substance regardless of the means of transport. Specimens which will arrive at the testing laboratory within 48 hours of collection need not be frozen but may be submitted with enough artificial refrigerant to keep the specimen cold during transport.

2. Specimens that will arrive at the testing laboratory more than 48 hours after collection should be frozen as soon after collection as possible. Pack them with enough dry ice to last 48 hours longer than the expected time required for transport of the specimen to the laboratory. Pack specimens so that direct contact with the dry ice is prevented. Ship /transport specimens in accordance with all pertinent regulations.

Do not freeze specimens for isolation of respiratory syncytial virus (RSV) or cytomegalovirus (CMV) or blood specimens. Ship these specimens refrigerated.

3. Affix the mailing label (PH-0838), return address and other labeling required by pertinent regulations to the outer container.

3. Ship the specimen to the TDH Laboratory in Nashville.

Location and Mailing Addresses

<table>
<thead>
<tr>
<th>Location and address for items shipped by UPS, Federal Express and carriers other than the US Postal Service</th>
<th>Postal address for non-specimen mail</th>
<th>Postal address for specimen mail US Postal Service</th>
</tr>
</thead>
</table>
| Tennessee Department of Health Laboratory Services  
630 Hart Lane  
Nashville, TN  37216-2006 | Tennessee Department of Health Laboratory Services  
630 Hart Lane  
Nashville, TN  37243-1404 | Tennessee Department of Health Laboratory Services  
P.O. Box 305130  
Nashville, TN  37230-5130 |
Reporting Procedures and Interpretation

<table>
<thead>
<tr>
<th>Reporting of Results for Virus Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for _________________________</td>
</tr>
<tr>
<td>The name of the virus isolated.</td>
</tr>
<tr>
<td>(The type is included if appropriate.)</td>
</tr>
<tr>
<td>No virus isolated.</td>
</tr>
</tbody>
</table>

Turn-around time for negative cultures varies from 1 to 4 weeks. Cultures yielding virus isolates may require more time for identification of the virus depending upon the isolate involved. Failure to isolate a virus may be the result of a number of factors, including improperly collected specimens, specimens collected at a period in the disease when the patient is not shedding virus, improperly transported specimens, or a lack of sensitivity in the system being used for isolation. Failure to isolate virus should not rule out the virus as a cause of the clinical illness. Conversely, since people may asymptptomatically carry a variety of viruses, viruses may be isolated which are unrelated to the current clinical illness.
Virus Culture (Continued)

The results of all specimen requests are reported to the provider who submitted the specimen. In addition the TDH Communicable and Environmental Disease Services and the health department in the county where the patient lives are sent reports on all positive results.

Criteria for Unacceptable Specimens

1. The specimen is not properly identified with the required information.
2. The patient identifiers on the specimen do not exactly match those on the test request form or the electronic record.
3. The specimen is broken or leaked in transit.
4. The specimen is inappropriate for virus isolation.
5. The quantity of specimen received is not sufficient to perform the requested testing. (QNS - Quantity Not Sufficient.)
6. The specimen is received in a compromising condition (i.e., warm, delayed in transit) situation.

Virology Form PH-1579
FRONT

NOTE: Virology Form has no printing on back

http://health.state.tn.us/Lab/index.htm Section 5-24