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ABBREVIATIONS

APHL  Association of Public Health Laboratories
CDC  Centers for Disease Control and Prevention
CIA  Chemiluminescent immunoassay
CMIA  Chemiluminescent microparticle immunoassay
CSF  Cerebrospinal fluid
EIA  Enzyme-linked immunoassay
FDA  Food and Drug Administration
FTA-ABS  Fluorescent treponemal antibody absorption
MBIA  Microbead immunoassay
RPR  Rapid plasma reagin
STD  Sexually transmitted disease
TP-PA  Treponema pallidum particle agglutination
TRUST  Toluidine red unheated serum test
USR  Unheated serum reagin
VDRL  Venereal Disease Research Laboratory

DEFINITIONS

**Biological false positive:** A positive reaction on a nontreponemal test that is not due to an infection with *T. pallidum*

**End point titer:** Measurement of antibodies (i.e. nontreponemal) through serial dilution of the patient specimen to determine the highest dilution at which a reactive result (agglutination/flocculation) is still produced. Also known as complete titer.

**Prozone effect:** A false negative or false minimally reactive result due to high concentrations of antibody and/or antigen, which prevents the formation of immune complexes necessary to visualize flocculation/agglutination. To detect or rule-out the prozone effect, consider testing the sample neat and then diluted. Prozone is also known as the hook effect.

**Serofast:** Sustained nontreponemal titers that decline less than four-fold (or two dilutions) 6-12 months after treatment but fail to completely serorevert and continue to persist after treatment.

**Inadequate serologic response:** Sustained nontreponemal titers that do not decline by at least four-fold following treatment.
INTRODUCTION

Syphilis rates in the United States are at the highest they have been in more than 20 years. Diagnosis of a syphilis infection still relies on both clinical evaluation and one of two multitest laboratory testing algorithms to indicate current or past infection with the causative agent, *Treponema pallidum*. Definitive laboratory diagnosis has always been challenging due to both the wide array of clinical manifestations and the lack of a single optimal test.\(^5\) *T. pallidum*, similar to other spirochetes, is difficult to culture. Very few laboratories have successfully cultured *T. pallidum*, but there are some published studies and resources available if a laboratory is interested in pursuing this method for research purposes.\(^2,3\) Scientists must therefore use alternative methods to detect and identify *T. pallidum* and clinical evaluation still plays a critical role in determining a case of syphilis.\(^4\)

To maximize public health impact, accurate and timely diagnostic testing should be combined with clear diagnostic result reporting and expedited linkage to medical care in an effort to provide timely treatment and services for infected persons. Laboratory reports should indicate each test that was performed, the result of each test and the overall laboratory interpretation.

The *Suggested Reporting Language for Syphilis Serology Testing* (2015)\(^5\) was originally developed by the Association of Public Health Laboratories (APHL) Sexually Transmitted Diseases Subcommittee to provide guidance to laboratories performing the two most common syphilis serology testing algorithms—the *traditional* and *reverse*. This document is intended to clarify complex testing outcomes and guide laboratory reporting of test results to both providers and health department surveillance programs. The suggested reporting language presented may require adjustments to meet individual facility or jurisdiction requirements, but major deviations should be considered carefully because misinterpretation of syphilis serology testing algorithms may have serious therapeutic implications.

The use of standardized language when reporting laboratory results is particularly important for testing that involves multitest algorithms. The traditional and reverse testing algorithms involve a series of tests which can be performed by more than one laboratory to determine the presence or absence of syphilis infection. Lack of clarity in results reporting can lead to incomplete testing, misinterpretation of results by health care providers, unnecessary additional testing, delays in care for infected persons and inaccurate estimates of disease burden.

RATIONALE FOR DOCUMENT UPDATE

This document has been updated as new information became available and/or changes related to the availability of US Food and Drug Administration (FDA)-cleared assays for the detection of antibodies to *T. pallidum* and detection of nontreponemal antibodies elevated in the presence of a syphilis infection. This update to the *Suggested Reporting Language for Syphilis Serology Testing* supersedes the previous version and updates the terminology used throughout the document to align with available FDA-cleared assays and forthcoming CDC publications. Since 2015, several assays received FDA clearance including three automated nontreponemal assays (Gold Standard Diagnostics AIX 1000\(^6\), Arlington Scientific ASI Evolution\(^8\) and Bio-Rad BioPlex2200 Syphilis Total).

Additionally, a large portion of the background section which covered the history and landscape of diagnostic testing for syphilis has been removed. There are many resources available that cover the broader context of diagnostic testing for syphilis such as the National STD Curriculum syphilis content, CDC’s webpage on syphilis testing, APHL’s Overview of Syphilis Diagnosis modules and the Manual of Tests for Syphilis (ISBN: 978-0875531748).

DIAGNOSTIC TESTS FOR *TREPOCMEA PALLIDUM*

Treponemal assays that detect the pathogen itself or antibodies to the pathogen have also evolved over the course of the 20th and now 21st centuries.\(^4,6\) This document will focus on serologic treponemal assays which detect antibodies to *T. pallidum*. There are several tests that detect treponemal antibodies. Many of these tests are immunoassays with varying methods of detection, such as enzyme immunoassays (EIA), chemiluminescent (microparticle) immunoassays (CIA, CMIA) and microbead immunoassays (MBIA). In addition to immunoassays there are also particle agglutination
tests, fluorescent absorption methods and rapid methods (Table 1). These methods may detect IgG, IgM, or both IgG and IgM antibodies produced against *T. pallidum*.4,6–8 Methods such as cerebrospinal fluid fluorescent treponemal antibody absorption (CSF FTA-ABS) and CSF *Treponema pallidum* particle agglutination (CSF TP-PA) may be used on CSF in suspected cases of neurosyphilis.

Nontreponemal assays detect the immune response to the release of cardiolipin, cholesterol and lecithin, which are elevated in numerous chronic conditions and infections including syphilis.9 If the nontreponemal assay is reactive, the serum or plasma specimen is serially diluted two-fold to determine the endpoint titer. Automated nontreponemal assays have limited ranges of on-instrument titers, however, end-point titers must be determined and reported even when using these methods as they are essential for monitoring treatment. While the majority of nontreponemal assays are performed on serum, CSF may also be tested in suspected cases of neurosyphilis, ocular syphilis and congenital syphilis by VDRL.

**Table 1: Serologic Methods for Syphilis Diagnosis**

<table>
<thead>
<tr>
<th>Method</th>
<th>Manual vs. Automated</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nontreponemal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venereal Disease Research Laboratory (VDRL)a</td>
<td>Manual</td>
<td></td>
</tr>
<tr>
<td>Rapid plasma reagin (RPR)</td>
<td>Manual or Automated</td>
<td></td>
</tr>
<tr>
<td>Toluidine red unheated serum test (TRUST)</td>
<td>Manual</td>
<td></td>
</tr>
<tr>
<td>Unheated serum reagin (USR)</td>
<td>Manual</td>
<td></td>
</tr>
<tr>
<td><strong>Treponemal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescent treponemal antibody absorption (FTA-ABS)a</td>
<td>Manual</td>
<td>IgM/IgG</td>
</tr>
<tr>
<td><em>Treponema pallidum</em> particle agglutination (TP-PA) assay</td>
<td>Manual</td>
<td>IgM/IgG</td>
</tr>
<tr>
<td>Line immunoassay (LIA)</td>
<td>Manual</td>
<td>IgG</td>
</tr>
<tr>
<td>Enzyme-linked immunoassay (EIA)</td>
<td>Manual/Automated</td>
<td>IgG or IgM/IgG</td>
</tr>
<tr>
<td>Chemiluminescent immunoassay/Chemiluminescent microparticle immunoassay (CIA/CMIA)</td>
<td>Automated</td>
<td>IgG or IgM/IgG</td>
</tr>
<tr>
<td>Microbead immunoassay (MBIA)</td>
<td>Automated</td>
<td>IgG or IgM/IgG</td>
</tr>
<tr>
<td>Rapid antibody test</td>
<td>Manual</td>
<td>IgG</td>
</tr>
</tbody>
</table>

a. These methods may be performed on cerebrospinal fluid (CSF) to support a diagnosis of neurosyphilis. For further information on diagnosis of neurosyphilis please refer to the *Consultation on Laboratory Diagnosis of Syphilis*9 and the CDC STD Treatment Guidelines.11

**ALGORITHMS**

Laboratories use a combination of both nontreponemal and treponemal antibody tests to screen and confirm syphilis infections as outlined in one of the two serologic testing algorithms—the traditional algorithm or reverse algorithm. There are many factors to consider when deciding on a suitable testing strategy for a given jurisdiction and each algorithm has its own strengths and weaknesses.

The traditional syphilis algorithm (Figure 1), begins with a nontreponemal test, followed by a treponemal test and has the advantage of familiarity and cost. This strategy has been in use for many years. The laboratorians, epidemiologists, clinicians and researchers alike are accustomed to the results and its interpretation.

The reverse algorithm (Figure 2), also referred to as the reverse sequence algorithm, begins with a treponemal method followed by a nontreponemal test. This approach is more frequently used by laboratories with high testing volumes. The reverse algorithm will identify past infections and has the potential to detect infections earlier than the traditional algorithm, but additional studies are needed to support or refute these findings.

The selection of the most appropriate testing algorithm for a given laboratory or jurisdiction will need to evaluate several
factors such as:

- Prevalence (which indirectly affects positive and negative predictive values)
- Testing volume and throughput
- Labor needs
- Sensitivity
- Specificity
- Turnaround time
- Cost.\textsuperscript{17–21}

The interpretation of results obtained by both testing algorithms must take into account patient symptoms and clinical history. Determination of current infections requires thorough clinical examination and further evaluation of exposure history.

**Traditional Syphilis Serology Testing Algorithm: Description of Test Methods and Suggested Interpretation**

The traditional algorithm (Figure 1) begins with a qualitative nontreponemal assay such as an RPR. If the nontreponemal assay is reactive, the patient specimen is serially diluted two-fold to determine the end point antibody titer. The results are utilized for clinical management of the patient and to help determine efficacy of treatment. The second step in the algorithm is to confirm the presence of *T. pallidum*-specific antibodies by performing a treponemal assay. Refer to Table 2 for interpretation of results.

**Figure 1: Traditional Syphilis Serology Testing Algorithm**

```
\begin{tikzpicture}[node distance=2cm]
    \node (start) {Nontreponemal (e.g., RPR or VDRL)};
    \node (reactive) [below of=start, fill=red] {Reactive\textsuperscript{a}};
    \node (treponemal) [below of=reactive, fill=cyan] {Treponemal (e.g., TP-PA)};
    \node (reactive2) [below of=treponemal, fill=red] {Reactive};
    \node (nonreactive) [below of=treponemal, fill=gray] {Nonreactive};
    \node (consistent) [below of=reactive2, fill=gray] {Consistent with past or current (potentially early) syphilis\textsuperscript{b}};
    \node (falsepositive) [below of=reactive2, fill=gray] {Syphilis unlikely; biological false positive possible};
    \node (noevidence) [below of=nonreactive, fill=gray] {No laboratory evidence of syphilis};

    \draw[->] (start) -- (reactive);
    \draw[->] (reactive) -- (treponemal);
    \draw[->] (treponemal) -- (reactive2);
    \draw[->] (treponemal) -- (falsepositive);
    \draw[->] (treponemal) -- (noevidence);
    \draw[->] (reactive2) -- (consistent);
    \draw[->] (reactive2) -- (falsepositive);
    \draw[->] (nonreactive) -- (falsepositive);
    \draw[->] (nonreactive) -- (noevidence);

\end{tikzpicture}
```

\textbf{a.} Perform a quantitative nontreponemal test to determine the end-point titer. \textbf{b.} Clinical correlations, including past titer(s), is necessary to determine whether the infection is past, current or potentially early.
<table>
<thead>
<tr>
<th>Test Outcomes</th>
<th>Test Sequence</th>
<th>Interpretation for Laboratory Report</th>
<th>Further Actions&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Step 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nontreponemal Assay (Qualitative)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Nonreactive</td>
<td>No laboratory evidence of syphilis</td>
</tr>
<tr>
<td></td>
<td>Nontreponemal Assay (Quantitative)</td>
<td>Not Indicated</td>
<td></td>
</tr>
<tr>
<td>Nontreponemal Assay</td>
<td>Step 2</td>
<td>Treponemal Assay</td>
<td></td>
</tr>
<tr>
<td>Nonreactive</td>
<td>Nonreactive</td>
<td>Treponemal antibodies detected. Syphilis unlikely; biological false positive possible&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Clinical evaluation should be performed to identify current signs and symptoms or past history of infection or treatment. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.</td>
</tr>
<tr>
<td>Weakly Reactive&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Weakly Reactive&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Nonreactive</td>
<td></td>
</tr>
<tr>
<td>Weakly Reactive&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Weakly Reactive&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Reactive</td>
<td>Treponemal antibodies detected. Consistent with past or current (potential early) syphilis</td>
</tr>
<tr>
<td>Reactive</td>
<td>Reactive at ≥ 1:1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Nonreactive</td>
<td>Nontreponemal antibodies detected. Syphilis unlikely; biological false positive possible</td>
</tr>
<tr>
<td>Reactive</td>
<td>Reactive at ≥ 1:1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Reactive</td>
<td>Treponemal and nontreponemal antibodies detected. Consistent with past or current (potential early) syphilis</td>
</tr>
</tbody>
</table>

**Special Circumstances:** Not recommended in algorithm, for use if both tests are ordered by provider.

<table>
<thead>
<tr>
<th>Test Outcomes</th>
<th>Test Sequence</th>
<th>Interpretation for Laboratory Report</th>
<th>Further Actions&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonreactive</td>
<td>Not Indicated</td>
<td>Nonreactive</td>
<td>No laboratory evidence of syphilis</td>
</tr>
<tr>
<td>Nonreactive</td>
<td>Not Indicated</td>
<td>Reactive</td>
<td>Treponemal antibodies detected. Consistent with past or current (potential early) syphilis</td>
</tr>
</tbody>
</table>

<sup>a</sup> This table is for testing and reporting of serum specimens only. <sup>b</sup> If the result in nonreactive or weakly reactive, consideration should be given to the possibility of the prozone effect. <sup>c</sup> Comments under “Further Action” can be included as language in the laboratory report or can be used as guidance for laboratorians to discuss test results with health care providers. <sup>d</sup> A weakly reactive result is a reportable result from VDRL and RPR. <sup>e</sup> Refer to package insert for specific reporting language, for certain methods a 1:1 titer may be reported as minimally reactive in certain circumstances. <sup>f</sup> For a summary of factors associated with biological false positives review Topic 2, Recommendation 8 of APHL Consultation on Laboratory Diagnosis of Syphilis, Meeting Summary Report.<sup>9</sup>
Reverse Syphilis Serology Testing Algorithm: Description of Test Methods and Suggested Interpretation

The first step in the reverse algorithm (Figure 2) is a treponemal assay. If the treponemal assay is reactive, the specimen is then reflexed to a qualitative nontreponemal assay. If the qualitative nontreponemal assay is reactive, the patient specimen is serially diluted two-fold to determine the end point antibody titer. If the qualitative nontreponemal assay is nonreactive, a second orthogonal treponemal assay should be incorporated to aid in the resolution of the syphilis status of the patient. For interpretation of the results please see Table 3.

Figure 2: Reverse Syphilis Serology Testing Algorithm

- Treponemal (e.g., EIA, CIA, CMIA or MBIA)
  - Reactive
  - Nontreponemal (e.g., RPR or VDRL)
    - Reactive
      - Consistent with current or past syphilis
    - Nonreactive
      - Treponemal
        - Reactive
          - Consistent with current or past syphilis (potentially early)
        - Nonreactive
          - Inconclusive for syphilis
          - No laboratory evidence of syphilis

a. Perform a quantitative nontreponemal test to determine the end-point titer. b. The second treponemal test should utilize a unique platform and/or antigen, different than the first treponemal test, commonly a TP-PA is used at this step. Other publications have tables comparing platforms and antigens in treponemal tests. c. Clinical correlation, including past titer(s), is necessary to determine whether the infection is past, current or potentially early. d. This result could represent an early infection if the first treponemal immunoassay is more sensitive OR false positivity from the first treponemal test.
Table 3: Guidance for Reporting Results from the Reverse Syphilis Serology Testing Algorithm performed on Serum

<table>
<thead>
<tr>
<th>Test Outcomes</th>
<th>Test Sequence</th>
<th>Interpretation for Laboratory Report</th>
<th>Further Actionsc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Step 1</td>
<td>Step 2</td>
<td>Step 3</td>
</tr>
<tr>
<td>Nonreactive</td>
<td>Not Indicated</td>
<td>Not Indicated</td>
<td>No laboratory evidence of syphilis</td>
</tr>
<tr>
<td>Reactive</td>
<td>Nonreactive</td>
<td>Nonreactive</td>
<td>Treponemal antibodies not confirmed. Inconclusive for syphilis; potential early syphilis, possible false positive</td>
</tr>
<tr>
<td>Reactive</td>
<td>Nonreactive</td>
<td>Reactive</td>
<td>Treponemal antibodies detected. Consistent with past or current (potential early) syphilis</td>
</tr>
<tr>
<td>Reactive</td>
<td>Reactive at ≥ 1:1d</td>
<td>Not Indicated</td>
<td>Treponemal and nontreponemal antibodies detected. Consistent with current or past syphilis.</td>
</tr>
</tbody>
</table>

Special Circumstances: Not recommended in algorithm, for use if both tests are ordered by provider.

| Nonreactive | Reactive at ≥ 1:1d | Nonreactive | Nontreponemal antibodies detected. Syphilis unlikely; biological false positive possible.a | Clinical evaluation should be performed to identify current signs and symptoms or past history of infection. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm. |

---
a. This table is for testing and reporting of serum specimens only. b. If the result in nonreactive or weakly reactive, consideration should be given to the possibility of the prozone effect. c. Comments under “Further Action” can be included as language in the laboratory report or can be used as guidance for laboratorians to discuss test results with health care providers. d. Refer to package insert for specific reporting language, for certain methods a 1:1 titer may be reported as minimally reactive in certain circumstances. e. For a summary of factors associated with biological false positives review Topic 2, Recommendation 8 of APHL Consultation on Laboratory Diagnosis of Syphilis, Meeting Summary Report.
GUIDANCE ON REPORTING TEST RESULTS TO HEALTHCARE PROVIDERS

Healthcare providers are likely to be more familiar with reporting and interpretation of syphilis laboratory testing results from a traditional algorithm due to its extensive and long-term use. Laboratories that change algorithms or test methods within the algorithm should ensure that clinicians ordering and receiving results are educated on how the updated test or algorithm could change the interpretation of results. APHL recommends that all reports from laboratories using either algorithm should include interpretative comments as well as the results from all tests used in the algorithm and when appropriate, recommendations for follow-up and additional testing. Suggested further actions are included in Tables 2 and 3 to guide submitters on appropriate next steps following testing. Healthcare providers should consult the current STD Treatment Guidelines for further detailed information. APHL also recommends including a statement on the laboratory report indicating that the laboratory results should be interpreted in the context of all clinically relevant information. If the healthcare provider is interested in further assistance in interpreting the laboratory results in the context of the clinical information, there is a Clinical Consultation Service available for clinical decision support and is advisory in nature.

The following are some general guidelines to follow when reporting syphilis laboratory test results to healthcare providers:

- Laboratories should specify the assay that was used (e.g., VDRL, RPR, TRUST, EIA, CIA, CMIA, MBIA, TP-PA, etc.) and the results of each assay.
- Laboratories should report all results together with the interpretation to the extent possible. If the entire recommended testing algorithm is not completed and preliminary reports are released to the provider, the laboratory should indicate what test(s) are pending, any additional tests that are necessary to establish the laboratory diagnosis, and request any additional specimens required to complete testing.
  - However, in situations where the patient might benefit, laboratories may report the results of each test in the algorithm as it becomes available, without waiting for the final interpretation. In this case, the final report should include all final results and the interpretation. Example scenarios might include: a screening test is performed in-house but the reflex testing is referred to an outside reference laboratory, supplemental testing is batched thus delaying the report of results; or if an expectant mother is delivering without prior testing or is at high risk for syphilis.
- The diagnosis of syphilis infection has implications for increased risk of infection with other sexually transmitted diseases—particularly HIV—and healthcare providers should concurrently screen for HIV and other STDs.

GUIDANCE ON LABORATORY REPORTING FOR SURVEILLANCE

All states, the District of Columbia, US territories and dependent areas require that laboratories report test results indicative of syphilis infection to the appropriate surveillance program. Department regulations may differ, so follow the requirements of your jurisdiction. The following reporting principles will facilitate accurate case reporting related to the syphilis testing algorithms:

- If the interpretation of the results from either the traditional or reverse serologic algorithm does not indicate a syphilis infection (i.e., no laboratory evidence of syphilis infection), it should not be reported to surveillance.
- If the interpretation of the results from either the traditional or reverse serologic algorithm is consistent with a syphilis infection, the laboratory should report to the health department:
  - The overall result or conclusion of the algorithm, AND
  - Results from all tests (including nonreactive/negative results) performed as part of the testing algorithm, preferably using the corresponding LOINC (Logical Observation Identifiers and Codes).
- If the interpretation of the results from either the traditional or reverse serologic algorithm was not completed (a test may have been referred to another laboratory), or the overall interpretation was inconclusive (indicating additional testing may be necessary, or clinical judgment and patient history is required for interpretation), the laboratory should follow jurisdictional requirements for reporting incomplete or inconclusive results.
REFERENCES


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3. Centers for Disease Control and Prevention. Treponema pallidum Requests by Commercial Companies. Available at: https://www.cdc.gov/std/syphilis/lab/treponema-pallidum-requests.htm


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The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public’s health in the US and globally. APHL's member laboratories protect the public’s health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

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Office of Surveillance, Epidemiology and Laboratory Services (OSELS)
National Center for HIV, Viral Hepatitis, STDs and TB Prevention (PS)
National Center for Zoonotic, Vector-borne, and Enteric Diseases (CK)
National Center for Environmental Health (NCEH)
Coordinating Office for Terrorism Preparedness and Emergency Response (CTPER)