

Suggested Reporting Language for Syphilis Serology Testing



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Background

Diagnosis of syphilis infections has historically utilized a combination of clinical indicators and both non-treponemal and treponemal assays to indicate the presence of the causative agent, *Treponema pallidum*. The use of various combinations of serological assays in testing algorithms has led to reporting language that can vary within and between laboratories with respect to terminology, technical verbiage, and overall test interpretation. This could cause confusion or clinical misinterpretation and negatively impact or delay proper patient management decisions. The Association for Public Health Laboratories (APHL) Sexually Transmitted Diseases (STD) Subcommittee and associated workgroup including subject matter experts from public health laboratories developed this document through consensus. The document was created to provide suggested reporting language to aid laboratory professionals, clinicians, healthcare workers, epidemiologists and program staff in the interpretation of the two most commonly used syphilis serologic testing algorithms. The reporting guidance in this document is only suggestive and may need to be adapted or modified depending on factors such as jurisdictional requirements or advances in diagnostic technology.

Introduction

Syphilis was first documented as a disease process in the 1500s but it took until 1905 for the spirochete *T. pallidum* to be isolated by Shaudin and Hoffman and was subsequently confirmed as the causative agent of syphilis in 1912.¹⁻³ Definitive laboratory diagnosis has always been challenging due to both the wide array of clinical manifestations and the lack of a single optimal test.⁴ *T. pallidum*, similar to other spirochetes, is nearly impossible to culture, eliminating the gold standard method for laboratory diagnosis from the list of options available to laboratorians. Scientists have therefore had to utilize alternative methods to detect and identify *T. pallidum*. Diagnosis for hundreds of years was entirely clinically based and clinical evaluations still play a critical role in identifying cases of syphilis today.⁵

Once the spirochete was identified, laboratorians were quick to find new methods to detect the pathogen. In 1906, Landsteiner and Mucha introduced the use of dark field microscopy as a method to directly detect the presence of *Treponema pallidum* in a chancre lesion.⁶ August Wasserman subsequently developed the first serologic test for syphilis.^{3,5,7} Throughout the 1900s both treponemal and non-treponemal assays were developed and improved upon. The current non-treponemal assays are non-specific for syphilis.³ They are based on detecting the body's antibody response to the release of cardiolipin, which is elevated in numerous chronic conditions and infections including syphilis.⁸ If the non-treponemal assay is reactive, the serum or plasma specimen is diluted two-fold to an endpoint, to determine the titer of the antibody present. The current methodologies include the Venereal Disease Research Laboratory (VDRL), Rapid Plasma Reagin (RPR) and Tolidine Red Unheated Serum Test (TRUST).^{3,5,9,10} The VDRL assay, developed in 1946¹¹ remains the only assay FDA-approved for testing cerebrospinal fluid and has otherwise widely been replaced by the RPR assay. The RPR assay, developed in 1957¹² is a modification of VDRL that is visualized by the naked eye with the assistance of charcoal particles rather than microscopically as with the VDRL test. The TRUST assay is also based on a modification of the VDRL assay and is procedurally similar to the RPR assay.¹³

Treponemal assays, that either directly detect the pathogen or antibodies to the pathogen, have also evolved over the course of the 20th century and now 21st century.^{3,5} While rare, direct detection of *T. pallidum* is still utilized in some jurisdictions that are performing dark-field microscopy. Other direct detection methods include polymerase chain reaction (PCR) and direct fluorescent antibody (DFA) assays.^{3,9,10}

There are also several tests that detect treponemal antibodies including *Treponema pallidum*-particle agglutination (TP-PA) assay,¹⁴ Fluorescent Treponemal Antibody-Absorption (FTA-ABS) assay,¹⁵ immunoassays, and rapid point-of-care tests such as the Syphilis Health Check™ CLIA waived assay. With the widespread introduction of immunoassays in the 1980s,³ syphilis testing was adapted to this platform, which allowed for higher throughput and provided objective results. There are now several versions of immunoassays depending on the method of detection; enzyme immunoassays (EIA), chemiluminescent immunoassays (CIA), and microbead immunoassays (MIA). These immunoassays may detect IgG, IgM or both IgG and IgM antibodies produced against *Treponema pallidum*.^{3,5,10}

Laboratories use a combination of these treponemal and non-treponemal antibody tests to screen for and confirm syphilis infections by using one of two serologic testing algorithms—the traditional algorithm or the reverse algorithm. There are many factors to consider when deciding on what testing strategy will be most suitable for a given jurisdiction and each algorithm has its strengths and weaknesses. The traditional syphilis algorithm, consisting of a non-treponemal test, followed by a treponemal test, has the advantage of familiarity and cost. This strategy has been in use for many years so laboratorians, epidemiologists, clinicians and researchers alike are accustomed to the results and how to interpret them.

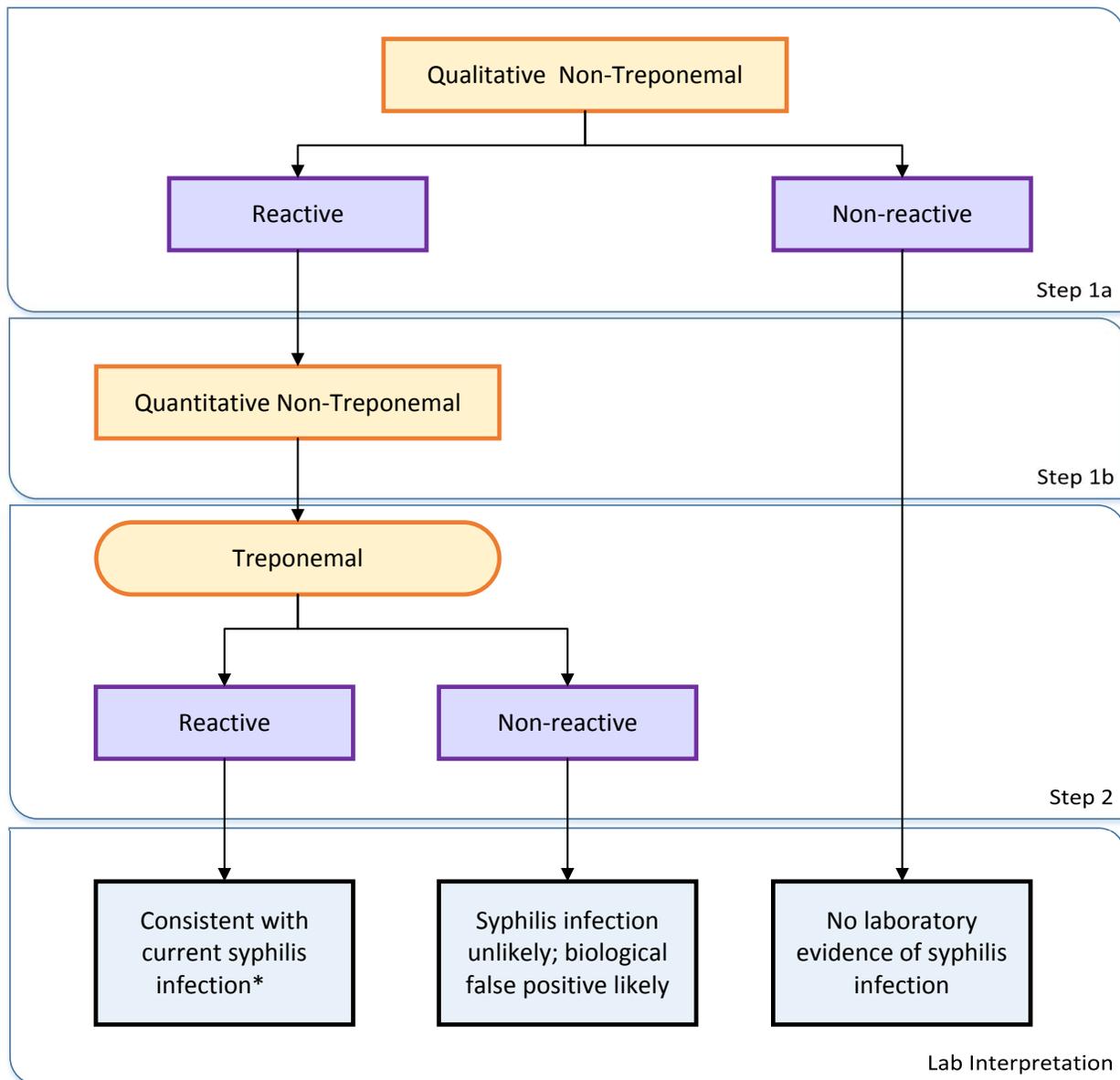
In 2009, an expert panel was convened to discuss syphilis diagnostics and formalized the “reverse” algorithm,¹⁶ which starts with a treponemal screening test followed by a non-treponemal test. This approach might be more attractive to laboratories that have high testing volumes and where the manual labor involved with non-treponemal tests is no longer appropriate for laboratory workflow and staffing needs. The reverse algorithm will identify past infections previously undetected with the traditional algorithm, and has the potential to detect early infections but more studies are needed to support or refute this. The selection of the testing algorithm used in a facility needs to take into consideration factors such as prevalence, which indirectly affects positive and negative predictive values, as well as testing volume and throughput, labor needs, sensitivity, specificity, turnaround time and cost considerations.¹⁷⁻²¹

The interpretation of both algorithms can be difficult to understand. Determination of current infections must be accompanied by a thorough clinical examination and evaluation of exposure history. It is recommended that the laboratory consider inclusion of the standard reporting language below, as appropriate, but laboratories must also remain in compliance with their regulatory requirements. This language emphasizes clear and concise interpretation of results, regardless of assay or target. The following reporting language was developed to clarify and guide how to report test results to clinicians or submitting agencies as well as recommendations for follow-up testing.

Description of Syphilis Traditional Algorithm, Test Methods and Suggested Interpretation

The traditional algorithm (Figure 1) begins with a non-treponemal assay and reflexes to a treponemal assay. If the non-treponemal assay is reactive, the serum or plasma specimen is diluted two-fold to an endpoint to determine the titer of the antibody present. This is utilized for clinical management of the patient to help determine efficacy of treatment. To confirm that the antibody that is present is due to *T. pallidum*, a reflex treponemal-specific confirmation assay is performed. For interpretation of the results and more detailed information please see Table 1.

Figure 1: Traditional Syphilis Serology Testing Algorithm



*If titer <1:4 consider these values associated with possible serofast condition.²² Serofast is used to refer to those persons with early syphilis with non-treponemal titers that neither increase nor decrease 4-fold after treatment.^{2,3}

Table 1: Suggested Guidance for Reporting Results from the Traditional Syphilis Serologic Testing Algorithm^a

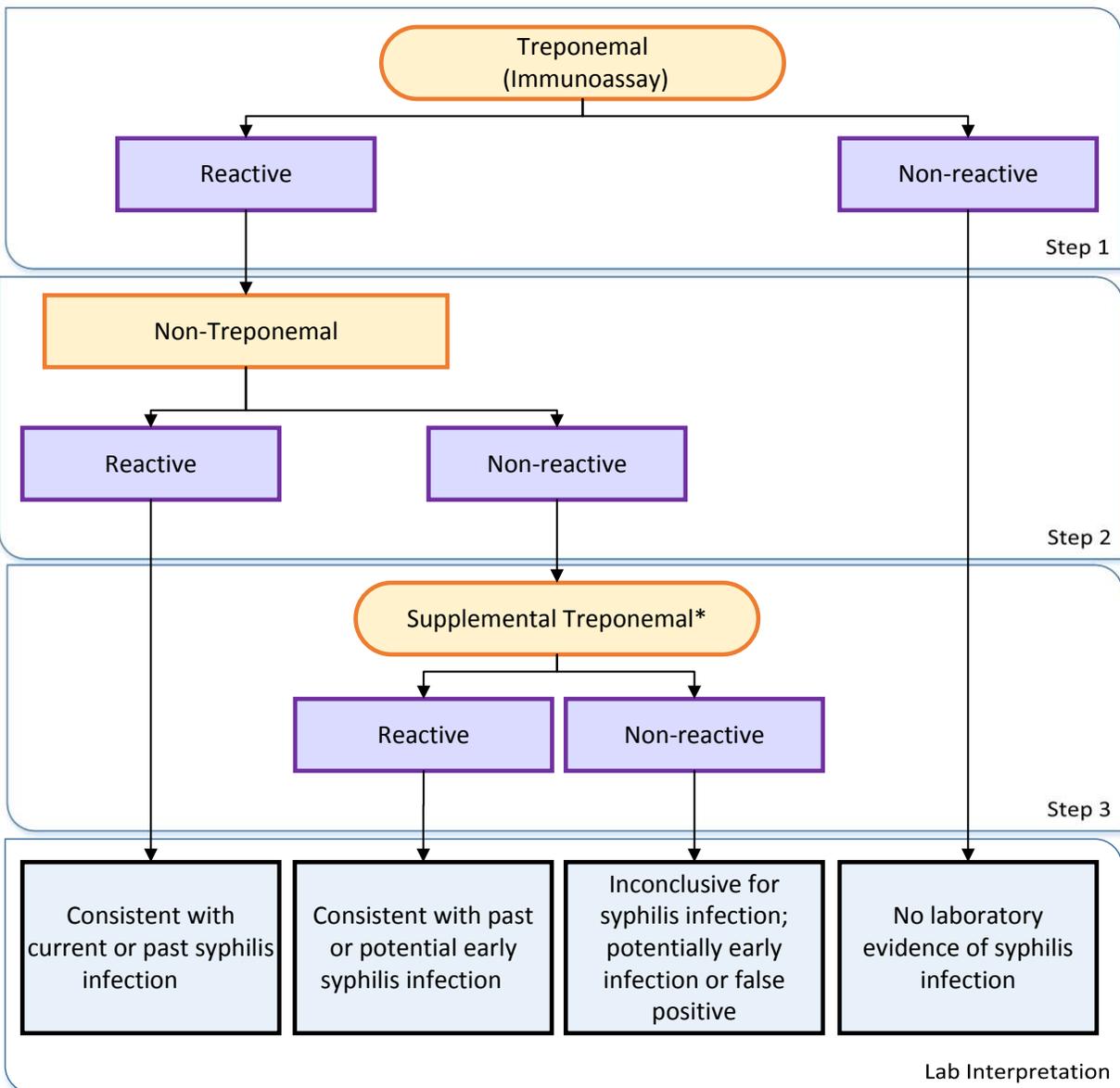
Test Outcomes	Test Sequence			Interpretation for Laboratory Report	Further Actions ^b
	Step 1a	Step 1b	Step 2		
	Qualitative Non-Treponemal Assay (For Example: RPR)	Quantitative Non-Treponemal Assay (For Example: RPR)	Treponemal Assay (For Example: TP-PA)		
Non-reactive	Not Indicated	Not Indicated	No laboratory evidence of syphilis infection	If recent exposure is suspected, re-draw sample in 2-4 weeks and repeat algorithm.	
Weakly Reactive ^c	Weakly Reactive, prozone ^d has been ruled out	Non-reactive	Syphilis infection unlikely	Clinical evaluation should be performed to identify signs, symptoms or past history of infection. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.	
Weakly Reactive	Weakly Reactive, prozone has been ruled out	Reactive	Treponemal antibodies detected. Consistent with past or potential early syphilis infection	Clinical evaluation should be performed to identify signs, symptoms or past history of infection. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.	
Reactive	Reactive at 1:1, 1:2, or 1:4	Non-reactive	Non-treponemal antibodies detected. Syphilis infection unlikely; biologic false positive likely	Clinical evaluation should be performed to identify signs, symptoms or past history of infection. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.	
Reactive	Reactive at 1:1, 1:2, or 1:4	Reactive	Treponemal and non-treponemal antibodies detected. Consistent with current or past syphilis infection, or due to sero-fast ^e condition	Clinical evaluation should be performed to identify signs, symptoms or past history of infection.	
Reactive	Reactive \geq 1:8	Non-reactive	Non-treponemal antibodies detected. Inconclusive for syphilis infection; biologic false positive likely	Clinical evaluation should be performed to identify signs, symptoms or past history of infection. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.	
Reactive	Reactive \geq 1:8	Reactive	Treponemal and non-treponemal antibodies detected. Consistent with current syphilis infection	Clinical evaluation should be performed to identify signs, symptoms or past history of infection.	
Special Circumstances: Not recommended in algorithm, for use if both tests are ordered by provider.					
Non-reactive	Not Indicated	Non-reactive	No laboratory evidence of syphilis infection	Sample can be reported as nonreactive for syphilis. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.	
Non-reactive	Not Indicated	Reactive	Treponemal antibodies detected. Consistent with past or potential early syphilis infection	Clinical evaluation should be performed to identify signs, symptoms or past history of infection. If past history of treatment reported, no further management is needed unless recent exposure suspected. If no past history of treatment, follow guidelines for treatment of latent syphilis infection. ²⁴ If recent exposure is suspected, re-draw sample in 2-4 weeks and repeat algorithm.	

a-This table is for testing and reporting of serum specimens only. b-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. c-A weakly reactive result is only obtained when testing with VDRL, not RPR. d-The zone in which no agglutination occurs due to antibody excess. This phenomenon can be corrected by diluting the specimen and re-testing. e-An unchanged VDRL of 1:2 or less and RPR of 1:4 or less is considered sero-fast.²²

Description of Syphilis Reverse Algorithm, Test Methods and Suggested Interpretation

The reverse algorithm (Figure 2) begins with a treponemal assay, reflexes to a non-treponemal assay and may also incorporate a second treponemal assay. If the treponemal screening assay is reactive, the serum or plasma specimen is reflexed to a qualitative non-treponemal assay, which is quantitated if reactive. If the qualitative non-treponemal assay is non-reactive, a second treponemal assay may be incorporated to aid in the resolution of the syphilis status of the patient. For interpretation of the results and more detailed information please see Table 2.

Figure 2: Reverse Syphilis Serology Testing Algorithm



*The supplemental treponemal test should utilize a unique platform and or antigen, different than the first treponemal test. Other publications have tables comparing platforms and antigens in treponemal tests.⁵

Table 2: Suggested Guidance for Reporting Results from the Reverse Syphilis Serologic Testing Algorithm^a

Test Outcomes	Test Sequence			Interpretation for Laboratory Report	Further Actions ^b
	Step 1	Step 2	Step 3		
	Treponemal Assay (For Example: Immunoassay)	Non-Treponemal Assay (For Example: RPR)	Treponemal Assay (For Example: TP-PA)		
Non-reactive	Not Indicated	Not Indicated	No laboratory evidence of syphilis infection	If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.	
Reactive	Non-reactive	Non-reactive	Treponemal antibodies not confirmed. Inconclusive for syphilis infection; potentially early infection, false positive likely.	Clinical evaluation should be performed to identify signs, symptoms or past history of infection. If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.	
Reactive	Non-reactive	Reactive	Treponemal antibodies detected. Consistent with past or potential early syphilis infection.	Clinical evaluation should be performed to identify signs, symptoms or past history of infection. If past history of treatment reported, no further management is needed unless recent exposure suspected. If no past history of treatment, follow guidelines for treatment of latent syphilis infection. ²⁴ If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.	
Reactive	Reactive \geq 1:1	Not indicated	Treponemal and non-treponemal antibodies detected. Consistent current or past with syphilis infection.	Clinical evaluation should be performed to identify signs, symptoms or past history of infection.	
Special Circumstances: Not recommended in algorithm, for use if both tests are ordered by provider.					
Non-Reactive	Reactive \geq 1:1	Non-Reactive	Non-treponemal antibodies detected. Unlikely to be a syphilis infection; biological false positive likely.	Clinical evaluation should be performed to identify signs, 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- 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.

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. We also recommend that reports from laboratories using either the traditional or reverse 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 Table 1 and 2 to guide submitters on appropriate next steps following testing. Healthcare providers should also consult the STD Treatment Guidelines for further detailed information.²⁴ It is recommended to include a statement on the laboratory report indicating that the laboratory results should be interpreted in the context of all clinically relevant information.

Below are some general guidelines to follow when reporting syphilis laboratory testing results to healthcare providers:

- Laboratories should specify the assay that was used (e.g., VDRL, RPR, EIA, CIA, MIA, TP-PA, etc.) and the results of each assay.
- In situations where persons might benefit, laboratories can report the results of each test in the algorithm as it becomes available, without waiting for the final interpretation. This might be the case when a screening test is performed in-house, but the reflex testing is referred to an outside reference laboratory or when 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.
- If the entire recommended testing algorithm is not completed, 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.
- 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 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 surveillance program. Department regulations may differ; therefore, 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:

- o The overall result or conclusion of the algorithm, AND
- o Results from all tests (including non-reactive/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 serology 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 local requirements for reporting incomplete or inconclusive results.

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