Syphilis Testing Using the Reverse Algorithm at the State of Connecticut Public Health Laboratory (CT SPHL)

Until recently, the CT SPHL provided syphilis testing following a traditional algorithm.[1,2] Testing involved screening specimens with a nontreponemal assay – the Venereal Disease Research Laboratory (VDRL) test, followed by a confirmatory treponemal specific assay – the *Treponema pallidum* particle agglutination (TP-PA) test.

Starting in November 2023, the CT SPHL implementated a reverse syphilis testing algorithm[1,2] which starts with an assay that detects specific IgM and IgG antibodies to *Treponema pallidum*. The advantages of a reverse algorithm can include detection of early infection (before nontreponemal antibodies can be detected), latent infection (after nontreponemal antibodies have disappeared), automated workflow and objective results reporting[3,4].

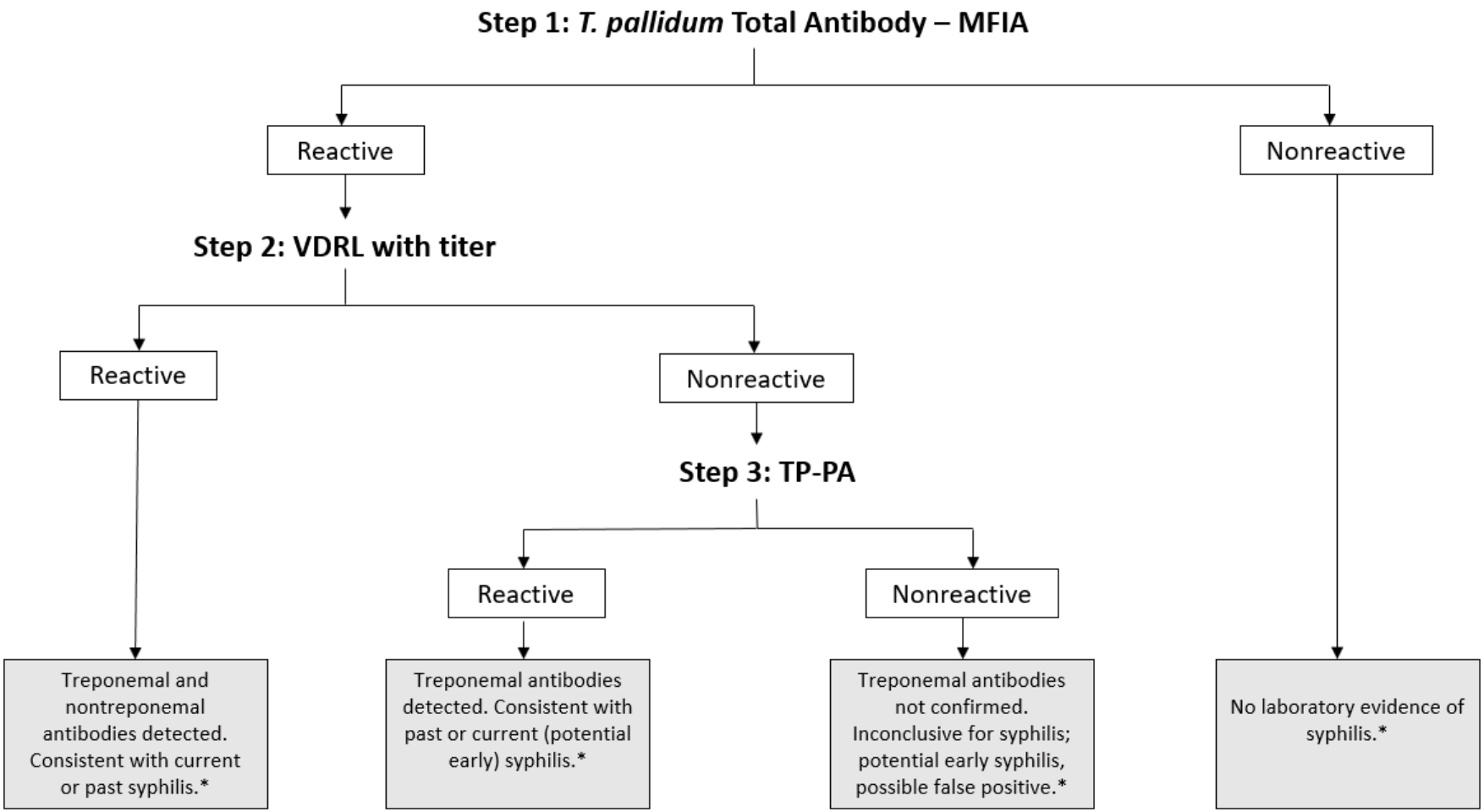
Reverse Syphilis Testing Algorithm (Serum) – Assays at the CT SPHL

Step 1: Specimens are screened/tested with a **Syphilis Total Antibody-MFIA**, a multiplex flow immunoassay intended for the qualitative detection of total (IgG/IgM) antibodies to Treponema pallidum in human serum. The assay utilized at the CT SPHL is the BioPlex 2200 Syphilis Total Assay[5].

Step 2: Specimens reactive by the MFIA screen are reflexively tested by the **Venereal Disease Research Laboratory (VDRL) assay**, a nontreponemal test for the detection of reagin, an antibody-like substance, by the qualitative and quantitative slide flocculation tests[6,7]. 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[1]. Specimens reactive by the VDRL assay are tested to an endpoint titer.

Step 3: Specimens nonreactive by the VDRL assay are reflexively tested by a second treponemal specific test, the ***Treponemal pallidum* particle agglutination (TP-PA) assay**[8].

Reverse Syphilis Test Algorithm at CT SPHL (Serum)



*APHL. Suggested Reporting Language for Syphilis Serological Testing. Second Edition, August 2020.

For test result interpretation and further actions please refer to the Association of Public Health Laboratories' "Suggesting Reporting Language for Syphilis Serological Testing, August 2020" https://www.aphl.org/programs/infectious_disease/std/Documents/ID-2020Aug-Syphilis-Reporting-Language.pdf

Table 3: Guidance for Reporting Results from the Reverse Syphilis Serology Testing Algorithm performed on Serum[a]

Test Outcomes	Test Sequence			Interpretation for Laboratory Report	Further Actions[c]
	Step 1	Step 2	Step 3		
	Treponemal Assay	Nontreponemal Assay (Quantitative)[b]	Treponemal Assay		
	Nonreactive	Not Indicated	Not Indicated	No laboratory evidence of syphilis	If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm.
	Reactive	Nonreactive	Nonreactive	Treponemal antibodies not confirmed. Inconclusive for syphilis; potential early syphilis, possible false positive	If recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm. If clinical suspicion is low no further evaluation is necessary.
	Reactive	Nonreactive	Reactive	Treponemal antibodies detected. Consistent with past or current (potential early) syphilis	Clinical evaluation should be performed to identify current signs and symptoms or past history of infection. If past history of treatment reported, no further management is needed unless symptomatic or recent exposure suspected. If no symptoms or past history of treatment, and if recent exposure is suspected, redraw sample in 2-4 weeks and repeat algorithm. If results repeat consult with clinician or use the Clinical Consultation Service.
	Reactive	Reactive at ≥ 1:1[d]	Not Indicated	Treponemal and nontreponemal antibodies detected. Consistent with current or past syphilis.	Clinical evaluation should be performed to identify current signs and symptoms or past history of infection.
	Special Circumstances: Not recommended in algorithm, for use if both tests are ordered by provider.				
	Nonreactive	Reactive at ≥1:1[d]	Nonreactive	Nontreponemal antibodies detected. Syphilis unlikely; biological false positive possible.[e]	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.[9]

References

1. Association of Public Health Laboratories. Suggested Reporting Language for Syphilis Serological Testing. August 2020. Available at: https://www.aphl.org/programs/infectious_disease/std/Documents/ID-2020Aug-Syphilis-Reporting-Language.pdf
2. Centers for Disease Control and Prevention. Sexually Transmitted Infections Treatment Guidelines, 2021. Available at: https://www.cdc.gov/std/treatment-guidelines/STI-Guidelines-2021.pdf
3. Rhea-McManus J, Aguanno J. Syphilis testing: Reverse to move forward. MLO-ONLINE.COM. March 2023. Available at: https://www.mlo-online.com/continuing-education/article/21293765/syphilis-testing-reverse-to-move-forward
4. Satyaptura F, Hendry S, Braddick M, Sivabalan P, Norton R. The Laboratory Diagnosis of Syphilis. *J Clin Microbiol*. 2021;20;59(10).
5. Bio-Rad Laboratories, Inc. BioPlex 2200 Syphilis Total & RPR Instructions for Use. Ref. 12000650. 665-0562E October 2020.
6. Becton, Dickinson and Company. BD VDRL Antigen & BD Difco VDRL Test Control Serum Set Package Insert. 8085886(04). July 2015.
7. Arlington Scientific Inc. ASI VDRL Antigen Test Package Insert. 6004-950 09/09. 2009.
8. Fujirebio Diagnostics, Inc. SERODIA®-TP-*PA* Product Insert. Part No.: 093131.00 Rev 002. 08/2017.