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U.S. Centers for Disease Control (CDC) and Prevention and California Department of Public Health (CDPH) Laboratory Recommendations for Syphilis Testing

Dear Health Care Providers and Laboratory Directors,

CDPH Office of Sexually Transmitted Infections (STIs) and Hepatitis C Virus (HCV) would like to highlight **best practices regarding syphilis testing** to accurately diagnose, manage, and treat syphilis cases in California. Given the complexity of syphilis management, this letter summarizes key points from [CDC Laboratory Recommendations for Syphilis Testing](#) in addition to providing guidance on syphilis case scenarios and follow-up care.

Diagnosing syphilis based on laboratory findings requires interpreting **TWO types of serologic antibody tests**: treponemal and nontreponemal¹.

- **Treponemal** antibody tests ([Table 1](#)) specifically react to *T. pallidum*, the causative agent of syphilis, and generally persist after treatment and cannot be used to distinguish between a current infection or a previously treated infection.
- **Nontreponemal** antibody tests ([Table 2](#)) broadly react to both host and *T. pallidum*, and provide a quantitative measure of infection, often referred to as titers. Nontreponemal tests are important to help determine active infection or reinfection and are used to monitor response to treatment over time.
- **Both a treponemal AND a nontreponemal test are required** to confirm an initial diagnosis of syphilis.
 - The best practice is to order syphilis testing that includes reflex confirmatory testing if the initial test is reactive

¹ The term, “nontreponemal” is a misnomer as assays measure antibodies to lipoidal antigens of **both** host cells and *Treponema pallidum* (causative bacterium of syphilis), with potential false positive results from recent conditions such as infections, vaccinations, or injection drug use, or underlying autoimmune or chronic conditions. Correct terminology, therefore, is “lipoidal antigen tests.” For brevity, this document will use the singular term, nontreponemal.

Diagnosing syphilis requires understanding of the two syphilis screening algorithms: [traditional and reverse sequence](#) (as defined by the order in which treponemal and nontreponemal tests are performed).

- Either the traditional OR the reverse sequence screening algorithm is acceptable for use.
- [Algorithm preference](#) may be based on patient populations served or laboratory resources, including staff, space, costs, and test volume.
- Each algorithm has limitations: more false positives might occur with the reverse sequence algorithm in low-prevalence populations, and the traditional algorithm might be less sensitive for detecting early or late latent syphilis infections.
- Health care providers should be aware of their institution's chosen syphilis screening algorithm to ensure all required tests have been done.

The following are more details on treponemal and nontreponemal tests:

- **Treponemal tests** (Enzyme Immunoassay [EIA], Chemiluminescence Immunoassay [CIA], *T. pallidum* Particle Agglutination [TPPA], Fluorescent Treponemal Antibody-Absorption [FTA-ABS])
 - Ordered as the initial test in the [reverse sequence algorithm](#).
 - Will remain positive in patients with a known history of syphilis even if their infection was previously adequately treated.
 - Newer, automated treponemal immunoassays (e.g., EIA/CIA) perform similarly (except the Trep-Sure EIA, which is not recommended for use).
 - TPPA is the preferred manual treponemal test (vs FTA-ABS).
 - Serologic point-of-care (POC) tests
 - Syphilis Health Check ([Diagnostics Direct](#)) and Dual Path Platform HIV-Syphilis assay ([Chembio Diagnostics](#)) are treponemal tests and currently the only FDA-cleared [CLIA-waived](#) tests to detect syphilis.
 - [First to Know](#) is a treponemal test and is currently the only over-the-counter syphilis test available.
 - Any positive treponemal POC test result requires follow-up laboratory-based nontreponemal testing to confirm the diagnosis of syphilis.
 - Empiric treatment prior to confirmatory testing should be considered for patients with symptoms of primary or secondary syphilis, or patients who are at risk of more severe outcomes (e.g., pregnant patients) or loss to follow-up (e.g., patients with unstable housing).
- **Nontreponemal (lipoidal antigen) tests** (Rapid Plasma Reagin [RPR], Venereal Disease Research Laboratory [VDRL])
 - Ordered as the initial test in the [traditional algorithm](#).

- RPR ≠ VDRL and are not interchangeable due to different test methods and results can vary between laboratories. Ideally using the same test type and laboratory is recommended whenever possible.
 - Quantitatively referred to as titers.
 - Require an endpoint titer (i.e., definitive number without “>” or “<” mathematical symbols).
 - Some automated RPR tests have constrained serum dilution ranges (e.g., 1:4-1:64) and are thus reported as “<1:4” or “>1:64.”
 - In such cases, specimens require a manual RPR test to establish an endpoint titer to accurately monitor treatment response.
 - Laboratory systems should have reflex policies to automate this, and health care providers should contact their laboratories to request endpoint titers and new policies if this occurs.
 - A **day of treatment titer** should ideally be drawn on the first day treatment is started, regardless of when the initial test was performed, to provide an accurate baseline titer.
 - **Monitor titers at intervals following treatment:**
 - **Primary and secondary syphilis**
 - 6 and 12 months;
 - Persons with HIV - 3, 6, 9, 12, and 24 months.
 - **Latent or unknown duration syphilis**
 - 6, 12, and 24 months;
 - Persons with HIV - 6, 12, 18 and 24 months in.
 - See [2021 CDC Treatment Guidelines](#).
 - A **fourfold change in titer** (equivalent to two dilutions) between two results with the same type of tests over time is considered clinically significant. Ideally, follow up titers are performed at the same lab. Examples: a fourfold decrease (e.g., 1:16 to 1:4) can indicate an adequate serologic response to treatment, or “serologic cure,” while a fourfold increase (e.g., 1:8 to 1:32) that is sustained for more than 2 weeks can indicate re-infection in a previously treated individual.
 - **Serofast** is the term used when titers have at least a fourfold decline but fail to revert to nonreactive after adequate treatment and remain persistently reactive; typically these titers are <1:8. See [Syphilis - STI Treatment Guidelines \(cdc.gov\)](#).
- False-negative RPR tests caused by concentrated antibodies (**prozone effect**) are rare (<0.85%). Dilution is not recommended in most cases but should be performed if a patient presents with signs/symptoms of syphilis and a nonreactive nontreponemal test.

Nontreponemal and treponemal tests should be interpreted in the same manner regardless of **pregnancy** or **HIV** status.

Persons with reactive syphilis serologies should be evaluated for signs and symptoms of **neuro/otic/ocular syphilis**; if present, a lumbar puncture with cerebrospinal fluid (CSF) examination may be warranted. Additional details and review of supporting literature on syphilis laboratory testing such as **CSF, direct detection** (e.g., darkfield microscopy, nucleic acid amplification), or **serologic POC tests**, can be found on CDC's official [Laboratory Recommendations for Syphilis Testing](#)

CDPH released updated [Recommendations for Syphilis Screening](#) in October 2024. Additionally, the CDPH STD Control Branch can answer questions regarding syphilis test result interpretations by contacting stdcb@cdph.ca.gov, and the California Prevention Training Center (CAPTC) at the University of California San Francisco has resources for [clinical interpretation of syphilis screening results](#) and provides an [online clinical consultation service](#) for syphilis and other sexually transmitted infections.

As a reminder, per Title 17, California Code of Regulations [2500](#) requires every health care provider to report on a case or suspected case of syphilis, and [2505](#) requires laboratories to report initial findings as well as any subsequent findings to the local health officer for the local health jurisdiction in which the patient resides. Even if laboratory reports are automated, providers are still required to submit **Confidential Morbidity Reports (CMRs)** with additional patient and treatment information. Providing applicable information to the local health jurisdiction is required—see [Healthcare Provider HIPAA Disclosure 2021](#) for additional details. Reports should include the entire set of treponemal and nontreponemal test results.

If you have any questions about this guidance, please reach out to stdcb@cdph.ca.gov.

Sincerely,



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California Department of Public
Health

Table 1. Treponemal Tests

Test Acronym	Test Name (available commercial brands in US)	Mode of Test*	Algorithm (Testing Sequence ^δ)
EIA ^ο	Enzyme ImmunoAssay(s) - (ADVIA Centaur, Bioplex 2200 Syphilis IgG, Diasorin Liaison, Trep-Sure, INNO-LIA) - (Captia Syphilis IgG EIA, Trep-Sure EIA, Zeus Scientific EIA)	Automated (Automated) (Manual)	Reverse (1st)
CIA ^ο	Chemiluminescence ImmunoAssay	Automated	Reverse (1st)
TPPA ^ο	<i>T. pallidum</i> Particle Agglutination	Manual	Reverse (2nd) ^φ
FTA-ABS	Fluorescent Treponemal Antibody-Absorption	Manual	Reverse (2nd) ^φ
MFIA	Multiplex Flow (microbead) ImmunoAssays	Automated	Reverse (1st)
TPHA [≈]	<i>T. pallidum</i> Hemagglutination Assay	Manual	
MHA-TP [≈]	Micro Hemagglutination Assay for <i>T. pallidum</i> antibodies	Manual	
POC	Point of Care (serologic testing) -Syphilis Health Check (Diagnostics Direct) -Dual Path Platform (DPP) HIV-Syphilis Assay (Chembio Diagnostics) - First to Know over-the-counter (NOWDx)	Manual	
DBS	Dried Blood Spot tests	Both	

Table 2. Nontreponemal (lipoidal antigen) Tests

Test Acronym	Test Name	Mode of Test*	Algorithm as Initial Test
RPR ^ο	Rapid Plasma Reagin	Manual	Traditional
VDRL ^ο	Venereal Disease Research Laboratory	Manual	Traditional
TRUST [≈]	Toluidine Red Unheated Serum Test	Manual	
USR [≈]	Unheated Serum Reagin	Manual	

*This column depicts usual performance; however, many tests can be performed both manually and with automation

^δCommon sequence of test performance per [specified algorithm](#)

^οDenotes the most commonly available and performed tests per [specified algorithm](#)

^φIn the reverse sequence algorithm, discordant results should be adjudicated by a second treponemal assay (TPPA preferred)

[≈]Not commonly used or no longer available in US

References:

1. [CDC Laboratory Recommendations for Syphilis Testing, United States, 2024 | MMWR](#)
2. [Syphilis Health Check™ – Diagnostics Direct, LLC \(diagnosticsdirect2u.com\)](#)
3. [DPP® HIV Syphilis USA – Chembio Diagnostics, Inc.](#)
4. [Waived Tests | Laboratory Quality | CDC](#)
5. [Sexually Transmitted Infections Treatment Guidelines, 2021 | MMWR \(cdc.gov\)](#)
6. [Syphilis - STI Treatment Guidelines \(cdc.gov\)](#)
7. [California Department of Public Health \(CDPH\) Updates Syphilis Screening Recommendations](#)
8. [First to Know syphilis test by NOWDiagnostics](#)

Resources:

1. [Clinical Interpretation of Syphilis Screening Algorithms - California PTC](#)
2. [CLIA-Waived Syphilis Point-of-Care Testing Options for Providers](#)
3. [Considerations for the Implementation of Point of Care Tests for Syphilis](#)
4. [STDCCN Ask Your Question](#)
5. [How to Report STDs with the CMR](#)