Evidence Review for Centers for Disease Control and Prevention Guidance Development on Laboratory Testing to Detect Treponema pallidum Infection (Syphilis)

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Journal Title: CLINICAL INFECTIOUS DISEASES

Volume: Volume 71, Number Suppl 1

Publisher: OXFORD UNIV PRESS INC | 2020-07-01, Pages S1-S3

Type of Work: Article | Final Publisher PDF

Publisher DOI: 10.1093/cid/ciaa348

Permanent URL: https://pid.emory.edu/ark:/25593/vzsfz

Final published version: http://dx.doi.org/10.1093/cid/ciaa348

Clinical Infectious Diseases

SUPPLEMENT ARTICLE

Evidence Review for Centers for Disease Control and Prevention Guidance Development on Laboratory Testing to Detect *Treponema pallidum* Infection (Syphilis)

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The articles in this supplement address key questions on syphilis diagnostics, provide reference tables of test performances, and discuss optimal specimens and knowledge gaps. Laboratory-developed genetic direct detection tests could be most useful at the point of care and add to the currently available serologic methods of nontreponemal and treponemal tests.

Keywords. syphilis diagnostics; *Treponema pallidum* direct detection; serology; systematic review.

"Syphilis strikes back" is the title of a recent Centers for Disease Control and Prevention (CDC) sexually transmitted disease (STD) awareness campaign [1], highlighting the resurgence of syphilis despite its historic nadir in 2000 and 2001. Rates of primary and secondary syphilis in the United States (US) have been increasing continuously in the last 2 decades, from their historic low at 2.1 cases per 100 000 population in these years, to 10.8 such cases in 2018 [2]. This was equivalent to 35 063 primary and secondary cases. In 2018, there were also 1306 congenital syphilis cases in the US, a 185% increase since 2014 alone [2]. Laboratory detection of *Treponema pallidum*, either directly or indirectly, plays an important role in syphilis diagnosis in the appropriate clinical context, as timely and accurate diagnosis with prompt treatment and partner management can contribute to public health prevention efforts. Currently, syphilis diagnosis relies on clinical evaluation, prior history, and interpretation of laboratory-based detection of antibodies, which may not always distinguish between current and past infection. The current laboratory workforce has not necessarily been comprehensively trained on best practices for direct or serologic syphilis testing due to the declining cases in the last century. During a CDC consultation entitled "Syphilis Summit" in 2016, experts reemphasized a need for laboratory diagnostic guidance, targeted to present-day laboratorians and developed with realistic expectations for available equipment and reagents [3]. Such guidance would update and integrate previously issued CDC comments on the topic [4, 5], similar to CDC guidance for the laboratory-based detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* [6]. The need for guidance was subsequently affirmed as an actionable item in the "CDC Call to Action: Let's Work Together to Stem the Tide of Rising Syphilis in the United States" [3, 7]. As a result, CDC initiated an effort to develop such guidance. The current supplement in *Clinical Infectious Diseases* marks an important milestone on this path.

Currently, all syphilis laboratory testing is a supplement to clinical evaluation and diagnosis, for which clinical guidance exists elsewhere (ie, 2015 CDC STD treatment guidelines [8]). This supplement to *Clinical Infectious Diseases* provides reviews of the laboratory tests that are used to support the clinical diagnosis of syphilis. CDC developed key questions to guide literature and other evidence searches of test performance and optimal specimen types of existing laboratory tests. The key questions were:

- What are the performance characteristics for each direct detection test for *T. pallidum*, and what are the optimal specimen types for each test?
- What options are available for *T. pallidum* molecular epidemiology?
- What is the sensitivity and specificity of the nontreponemal and treponemal tests currently approved by the US Food and Drug Administration (FDA) for the diagnosis of syphilis (by stage)?
- What considerations need to be accounted for when choosing a traditional vs reverse algorithm for diagnosis of syphilis?
- What is the sensitivity and specificity of the point-of-care treponemal antibody tests currently approved by the FDA for the diagnosis of syphilis?

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Clinical Infectious Diseases® 2020;71(S1):S1–3

DOI: 10.1093/cid/ciaa348

The literature searches were performed by CDC laboratory experts. CDC partnered with the Association of Public Health Laboratories (APHL) to engage subject matter experts (SMEs) who assisted in the review of the collected evidence. The syphilis SMEs gathered for an in-person meeting in November 2017 to present and discuss their findings, which were summarized by APHL [9]. The current special supplement is a collection of the presentations that document the experts' review methods, analyses, syntheses, and conclusions, which CDC will further use when developing syphilis laboratory guidance.

A Direct Detection Assay for *Treponema pallidum*

This supplement highlights important developments. A direct detection test for active *T. pallidum* infection is urgently needed to identify this infection from clinical specimens. Ideally, this would be a highly sensitive and specific *T. pallidum*–directed DNA, RNA, or protein-based assay for blood or other easily accessible fluids (eg, oral fluid) for all stages of syphilis. Most useful would be a single-step test that could be adapted to a rapid format so it could be performed with minimal laboratory equipment at the point of care. The natural history of *T. pallidum* infection presents a challenge due to the low and transient bacterial burden in accessible anatomic sites depending on stage of infection, and the organism's ability to persist in the nervous system. Another substantial obstacle is the previous unavailability of an in vitro culture system to provide ample material for test evaluation; however, studies on a sustainable culture method are promising [10]. This would allow full exploration of *T. pallidum* genetics and suitable, highly expressed genes or antigens. Nevertheless, the current reviews discuss promising emerging direct genetic detection tests. In the US, they are currently only available in a laboratory-developed format using genetic material from primary syphilis lesions. Theel et al present a performance summary for nucleic acid amplification tests (NAATs) for *T. pallidum* lipoprotein tpp47 and DNA polymerase polA gene targets [11]. They document encouraging NAAT performance data with a sensitivity of 75%–95% in primary lesions [11]. This compares well with the reported 73%–100% sensitivity of direct fluorescence antibody and dark field microscopy testing [11]. Further research and development could enhance test performance of NAAT assays, explore utilization of a range of specimen types, and allow adaptation to simple test formats especially at the point of care. However, as summarized by Theel et al [11], the current status of evidence suggests that the tests are still insensitive in blood, with some hopeful signs that they could already be used for neonatal blood or serum as an adjunct method to support diagnosis of congenital disease. These developments highlight the need for these tests and for developers to engage toward approval by the FDA. Subsequent commercialization, hopefully with next-generation improvements and expandable for other syphilis stages, could broaden demand and accessibility, and ultimately could became the future of syphilis laboratory testing.

Serologic Syphilis Testing

Many factors are important in determining the optimal use of serologic tests, including test performance and test sequence selection (ie, traditional vs reverse sequence algorithm), but also commercial availability or practicality of implementation. Currently, the majority of serologic tests detect host treponemal and nontreponemal antibodies. There have been recent advances in increasing automation for laboratory-based testing formats. There have also been recent advances in rapid test formats, which could be performed at the point of care.

All SME reviewers of treponemal and nontreponemal serology assays noted challenges. The main challenge was lack of a clearly defined gold standard in the literature (eg, clinical diagnosis, polymerase chain reaction testing, dark field microscopy, serology, or a combination). In addition, a need for further research, particularly using well-characterized clinical specimens from all stages of syphilis, was noted to overcome limitations in test interpretation. Individual serologic tests can have substantial limitations for detecting current infection. For nontreponemal tests, difficulties in result interpretation, particularly for low titers in minimally reactive cases, were noted. There is a lack of consistency if different tests are used in longitudinal patient testing due to results and titers differing depending on the assay type performed. Other challenges were that some tests are no longer available due to lack of profitability for manufacturers and lack of costly equipment maintenance for rare usage. Some tests required workforce activities that have been eliminated for cost- and time-saving measures or because training is no longer routinely provided. Another issue is increasing awareness of repetitive motion injuries among laboratory personnel, leading to a preference for newer, automatable test formats.

Tuddenham et al [12] describe the literature evaluation of 4 nontreponemal tests, focusing on the 2 main tests, Venereal Disease Research Laboratory and rapid plasma reagin, for which test performance was summarized during all stages of syphilis. Park et al summarize the literature on 16 treponemal assays [13] and provide reference tables with testing formats, manufacturer, FDA clearance status, and test sensitivity and specificity. Among the manual tests, the authors find that the *Treponema pallidum* particle agglutination test has superior sensitivity compared to other manual treponemal tests; however, not enough evidence exists for a similar conclusion on any particular immunoassay [13].

Traditional Versus Reverse Algorithm

Traditional or reverse algorithm selection and consideration of testing volume, cost, and expected prevalence in the tested

population are discussed by Ortiz et al [14]. Syphilis screening using the reverse algorithm has increased recently, driven by automation in laboratories with high testing volume [15]. Previous correspondence [4, 5] regarding the reverse algorithm focused on the interpretation and resolution of discordance between treponemal and nontreponemal tests in the algorithms. Ortiz et al synthesize the issues and discuss the acceptability of both algorithms [15]. Recent FDA clearance of automated nontreponemal or combined (treponemal/nontreponemal) platforms will continue to expand our experience with test sequence selection.

Rapid Point-of-Care Syphilis Treponemal Antibody Tests

Lastly, Bristow et al [14] present the evidence regarding performance of the Syphilis Health Check, which was the only FDA-cleared rapid syphilis test at the time of literature reviews and of the expert consultations. This test is a rapid qualitative test for the detection of human treponemal antibodies. The article underscores the need for US availability of and data acquisition on additional rapid tests, including nontreponemal and combination tests (ie, treponemal/nontreponemal or syphilis/human immunodeficiency virus tests).

In summary, the existing literature on syphilis laboratory testing is expanding. There is cumulative evidence that genetic direct detection tests could be an additional diagnostic test to our currently available serologic laboratory diagnostic methods. These literature reviews update the data on test performance, identify current knowledge gaps, and highlight the need for innovation.

Notes

Acknowledgments. The authors thank the members of the Centers for Disease Control and Prevention (CDC) Sexually Transmitted Disease Laboratory Group; the external experts who developed or reviewed the key questions or supported the systematic literature reviews; and our colleagues Drs Ina Park and Lara Pereira for insightful review of this manuscript.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC.

Supplement sponsorship. This article appears as part of the supplement National Coalition of STD Directors, sponsored by the CDC.

Potential conflicts of interest. The authors: No reported conflicts of interest. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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