

Consultation on Laboratory Diagnosis of Syphilis

Meeting Summary Report



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Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
APHL	Association of Public Health Laboratories
ART	Antiretroviral Therapy
BFP	Biological False Positive
CDC	Centers for Disease Control and Prevention
CIA	Chemiluminescent Immunoassay
CLIA	Clinical Laboratory Improvement Amendments
CSF	Cerebrospinal Fluid
DFA	Direct Fluorescent Antibody
DFM	Dark-field Microscopy
EIA	Enzyme-linked Immunoassay
FDA	Food and Drug Administration
FTA-ABS	Fluorescent Treponemal Antibody Absorption
HIV	Human Immunodeficiency Virus
IHC	Immunohistochemistry
NAAT	Nucleic Acid Amplification Test
MBIA	Microbead Immunoassay
MHA-TP	Microhemagglutination Assay for <i>Treponema pallidum</i>
PHL	Public Health Laboratory
PrEP	Pre-exposure Prophylaxis
PWID	Persons who inject drugs
QC	Quality Control
RPR	Rapid Plasma Reagin
STD	Sexually Transmitted Disease
SME	Subject Matter Expert
TPHA	<i>Treponema pallidum</i> Hemagglutination
TP-PA	<i>Treponema pallidum</i> Particle Agglutination
TRUST	Toluidine Red Unheated Serum Test
USR	Unheated Serum Reagin
VDRL	Venereal Disease Research Laboratory

Executive Summary

Syphilis rates in the United States are at the highest they have been in more than 20 years. Not every community is affected equally, but rates in every region, most age groups and nearly all race/ethnicities have risen.¹ Additionally, there is a shifting landscape for diagnostic testing for syphilis, including automated nontreponemal assays, wider usage of enzyme immunoassays to identify treponemal antibodies, and even a single FDA cleared, CLIA-waived rapid test. These newer technologies aim to serve different needs and have also resulted in shifts in testing from traditional algorithms where a nontreponemal test is followed by a treponemal test, to those where the algorithm is reversed such that a treponemal test is performed first, followed by a nontreponemal assay. These changes provide for potential opportunities for more rapid and accurate diagnosis, but also create confusion. Lastly, diagnosis of syphilis infection relies on both laboratory results and clinical evaluation of signs, symptoms and prior exposure history.

With this as the backdrop, the Centers for Disease Control and Prevention's (CDC's) Division of Sexually Transmitted Disease Prevention partnered with the Association of Public Health Laboratories (APHL) to create a document for laboratorians that describes the best practices for laboratory diagnosis of syphilis in the United States. A structured external review process was established, culminating in a two-day consultation, November 28-29, 2017, at the Emory Conference Center Hotel, Atlanta, GA.

Summarized Identified Best Practices

For more complete details on best practices from each methodology please refer to the specific sections below.

Direct Detection

1. Neither DFA nor DFM are routinely maintained by clinical or public health laboratories, partially due to lack of standardized or quality control reagents and can be considered obsolete for routine diagnostic purposes, especially in low prevalence settings.
2. NAAT for detection of *T. pallidum* DNA performed on swabs from ulcer or lesion exudate can be used to establish a diagnosis of primary or secondary syphilis or congenital syphilis. However, a negative result by NAAT cannot be used to exclude infection.
3. NAAT performed on whole blood or blood fractions (e.g. serum, plasma, buffy coat, etc.) is not recommended due to low sensitivity in all stages of syphilis in adults except for congenital syphilis
4. NAAT for detection of *T. pallidum* in CSF from seropositive patients with neurologic symptoms may be considered to support the diagnosis of neurosyphilis. A negative NAAT result should not be used to exclude infection.
5. In cases of suspected congenital syphilis infection, NAAT for *T. pallidum* may be considered as an adjunct test to confirm infection in amniotic fluid, CSF or blood. A negative NAAT result in any of these specimen types should not be used to exclude congenital syphilis infection
6. Recommendations cannot be made for use of direct detection methods on ocular fluid or tissue from gummas or other tertiary syphilis lesions due to limited available literature on these disease states.
7. For examination of tissue sections, IHC, using avidin-biotin peroxidase complex chemistry, offers improved sensitivity and specificity over silver stains and is preferred for detection of *T. pallidum* in tissue sections. Silver staining for *T. pallidum* is not recommended as it is prone to staining artifacts and is non-specific

Direct Detection: Molecular Epidemiology

1. Screening of samples for *T. pallidum* DNA using NAATs targeting *poIA*, *tpp47*, or *bmp* should be considered prior to proceeding to typing.
2. Molecular typing of *T. pallidum* is most frequently successful when performed on ulcer or mucocutaneous lesion exudate from patients with primary or secondary syphilis.
3. Molecular typing based on *arp/tpr* PCR and RFLP analysis may be considered.

Serologic Methods: Nontreponemal

1. In general, serum RPR appears to be more sensitive than serum VDRL at detecting nontreponemal antibodies, independent of syphilis stage. Based on more limited data the RPR also appears to be more specific than the VDRL.
2. Serum RPR and VDRL are about 58-78% sensitive for diagnosis of primary syphilis. Serum RPR and VDRL have high sensitivity (nearly 100%) for diagnosis of secondary syphilis. Based on a small number of studies of mixed quality, sensitivity of VDRL for early latent syphilis ranges from 82-100%. Based on a small number of studies of mixed quality, the sensitivity of RPR and VDRL ranges from 64-77% for diagnosing late latent syphilis. Based on only two poor quality studies, sensitivity of serum VDRL for tertiary syphilis is 47-64%.
3. There are limited data available comparing automated and non-automated nontreponemal tests and only two automated assays are FDA cleared. Based on limited data, (including some unpublished manufacturer's data submitted to the FDA) the automated nontreponemal tests appear to have reasonable performance as compared to the non-automated tests.
4. Serum RPR and VDRL titers should not be used interchangeably to manage patients.
5. Neurosyphilis diagnosis is challenging. Different definitions for neurosyphilis across studies, heterogeneity of gold standards used, and inclusion of a mixture of symptomatic and asymptomatic patients were discussed as limitations. Based on current data there are no recommendations for the use of one assay over the other in the diagnosis of neurosyphilis, though limited data suggest that CSF RPR may be less sensitive than CSF VDRL.
6. The sensitivity of CSF VDRL in ocular syphilis is <50%. Limited data suggest that the sensitivity of CSF VDRL is poor (<10%) in otosyphilis.
7. The prevalence of false negative results from nontreponemal syphilis tests is rare (<0.85% of those tested).
8. In most large studies, the overall prevalence of biological false positives in general populations tested for syphilis is $\leq 1.5\%$.

Serologic Methods: Treponemal

1. Sensitivity:
 - a. Manual Tests: Among manual treponemal tests (MHA-TP, FTA-ABS, TP-PA), TP-PA should be considered the preferred test over FTA-ABS given the subjective nature of FTA-ABS interpretation, lack of quality control (QC) reagents and the need for microbiologist experience for the FTA-ABS. MHA-TP should be considered obsolete as it is no longer commercially available in the US.
 - b. Treponemal immunoassays: Among the treponemal immunoassays there are little published data directly comparing performance of the immunoassays by stage or comparing performance versus manual treponemal tests by stage. There are insufficient data to recommend a particular treponemal immunoassay for diagnosis of syphilis.
2. Specificity: The specificity of manual treponemal tests is similar, however TP-PA is the preferred among the manual assays (rationale under recommendation 1). More comparative specificity data is needed for all of the FDA-cleared immunoassays. Data are insufficient to recommend a particular immunoassay based on currently available specificity data.
3. Neurosyphilis: Based on limited performance data for the CSF TP-PA, either the CSF FTA-ABS or TP-PA may be used to support a diagnosis of neurosyphilis.

Serologic Methods: Special Populations

1. There are insufficient data to suggest that performance characteristics for treponemal or nontreponemal tests are significantly different in persons infected with HIV or in pregnant women. Nor are there sufficient data on the performance of the tests used to diagnose congenital syphilis. If there were sufficient data, considerations for algorithm usage in special populations should be addressed.

Serologic Methods: Algorithms

1. Laboratories should take into account their patient population and syphilis risk when considering a traditional or reverse sequence screening algorithm.
2. Decisions based on cost should take into account total costs at both the laboratory level and the system level.
3. The test results report should include a brief description of the method(s) and algorithm used.

Point-of-Care Tests

1. There are insufficient data to make a recommendation regarding the utilization of the FDA-cleared rapid syphilis test at this time in a laboratory or POC setting.
2. Persons that are evaluating the assay are encouraged to publish their findings in a peer-reviewed journal to generate sufficient data for evaluation of performance and utility.
3. For those sites that are currently using the rapid test in a POC setting, it is strongly recommend that a good quality assurance program that includes appropriate training, competency assessments, proficiency testing, and quality controls are in place to ensure accurate results. The public health laboratory in your jurisdiction should be consulted when setting up a rapid testing program. Any information that can be communicated regarding quality assurance, including training, competency assessments, proficiency testing, and quality control and appropriate use would be helpful.

Summarized Research/Diagnostic Development Needs

Direct Detection

1. Additional data are needed on the most appropriate specimen types, and standardized collection and preservation methods for direct detection
2. Additional data are needed on performance characteristics of IHC in different stages of syphilis (e.g., sensitivity in primary lesions, specificity in biopsy sections from genital tracts, etc.).
3. Additional data are needed on utility of ear lobe scraping vs. pricking for collection of capillary blood as an alternative specimen source for patients without lesions.
4. Additional data are needed on performance characteristics of molecular assays in immunosuppressed populations (Limited data on HIV/AIDS positive patients show no difference)
5. Commercial laboratories which offer molecular testing for *T. pallidum* are encouraged to provide information and data on the performance characteristics of their assays. This data should be shared with the consumers (on lab website) and ideally published in some format for peer-reviewed evaluation.
6. Development of a FDA cleared rapid direct detection method with a CLIA waiver for use in point-of-care settings for genital ulcer and/or mucocutaneous lesions are needed.
7. Development of FDA cleared laboratory-based molecular assays for direct detection of *T. pallidum* is needed.
8. Data are needed on the performance and utility of molecular methods for detection/monitoring of *T. pallidum* antibiotic resistance markers.
9. Understanding the role of metagenomic sequencing approaches for identification and typing of *T. pallidum* from different specimen sources.
10. What is the role of molecular typing for monitoring antibiotic resistant strains of *T. pallidum* susceptibility information?

Serology

1. Improve the definition of the performance characteristics of nontreponemal tests, particularly in the late stages of syphilis.
2. Published data are needed on automated nontreponemal tests, based on clinically well characterized sera.

3. Data are needed to better define the performance characteristics of nontreponemal tests in neurosyphilis. (e.g., if CSF nontreponemal tests are nonreactive is it neurosyphilis?)
4. Additional data are needed to gain a better understanding of the relationship between disease activity and nontreponemal antibody titers.
5. Performance data are needed for the immunoassays using clinically characterized specimens.
6. Additional data are needed on the performance of treponemal tests in latent syphilis based on the CDC case definitions for latent syphilis.
7. Additional data are needed on the comparative performance of the CSF TP NAAT (commercially available), CSF FTA-ABS, CSF TP-PA and treponemal CIA/EIA in CSF.
8. Performance data are needed for the immunoassays (serum) in neurosyphilis including evaluation of Captia IgM only assay and where it might fit into testing algorithms.
9. Define serologic window periods using modern treponemal and nontreponemal tests.
10. Additional data are needed directly comparing test performance in general populations to pregnant populations and HIV infected populations including:
 - a. Development of improved tests or methods to diagnose congenital infection, especially for asymptomatic neonates.
 - b. Additional data are needed to establish factors associated with seroreversion, false positive and false negative results in general populations as compared to special populations.
11. Additional data are needed to directly compare laboratory costs for the traditional and reverse algorithms to include instrument purchase (or lease), reagents, labor, and volume.
12. Additional data are needed on performance of both the traditional and reverse algorithms.

Point-of-Care Tests

1. Additional data are needed to compare the FDA cleared syphilis rapid test against and with laboratory-based assays performance in the traditional and reverse algorithms.
2. Additional data will be needed to compare the FDA cleared rapid syphilis tests against each other (once available).
3. Additional data are needed to evaluate the role of rapid syphilis POC tests in linkage to care, and performance in special patient populations with low follow-up as compared to laboratory-based testing currently being performed.
4. Additional data are needed to evaluate the utility of a rapid syphilis POC test for the management of patients with genital ulcer disease (GUD) and/or rash of unknown etiology.
5. Additional data are needed to understand the best setting and application for a syphilis POC test.

Summarized Tools/Resources Needed

Direct Detection

1. Develop standardized process for evaluation of laboratory developed molecular assays for detection of *T. pallidum*.
2. Availability of well characterized primary lesion material through a specimen bank for clinical laboratories (and PHLs) to use to train and validate testing methods in-house as well as to make available to diagnostic manufacturers to develop assays for FDA clearance.
3. Survey US PHLs, STD Clinics, emergency departments and commercial laboratories to determine testing practices for direct detection.

Serology

1. Evidence based guidelines are needed for prozone titrations.
2. Improved criteria and/or diagnostics for neurosyphilis (as well as otic and ocular syphilis).
3. Harmonization of criteria for evaluating performance of treponemal and non treponemal tests, in particular characterization of false positives.
4. Creation or resurrection of the CDC Syphilis Serum Bank with validated specimens, characterized by disease stage using standardized criteria, including sero-negative, darkfield-positive primary syphilis.*
5. Need for a common reference standard or predicate device against which new serologic assays should be measured.
6. Need for a well characterized (clinically) syphilis biorepository including serum, amniotic fluid, placenta, CSF, ulcer scrapings, autopsy specimens, etc. from the general population and special populations to evaluate diagnostic assays.*
7. Development of maternal and fetal/neonatal combination diagnostic algorithms.
8. Develop a cost analysis tool to assist facilities in making decisions regarding choice of algorithm.

* A CDC Syphilis Serum/Specimen Repository was mentioned multiple times throughout the two-day meeting.

Process Summary

Review Process

Beginning in March 2017, CDC and APHL began planning the external review and consultation to develop the best practices document. Key subject matter experts (SMEs) were identified that helped define the key questions and associated search terms for a comprehensive literature search (Appendix 1). For each key question one to two external reviewers were selected and a CDC assistant from the DSTDP Laboratory Branch was assigned to assist (Appendix A). The external reviewer(s) directed the literature review and the CDC assistant(s) worked with the CDC librarian to conduct the search using multiple databases, and provide the necessary abstract lists and electronic files of the manuscripts to the reviewers. The external reviewers were asked to review peer-reviewed data with a focus on recent and relevant data, high-quality data sources, and diagnostic tests that are FDA cleared and/or cleared and currently available in the US or are intended for use in the US. The external reviewer(s) compiled tables of evidence containing all manuscripts that were deemed appropriate to answer the assigned key question. Reviewers also provided their summary interpretations and impressions, which were all provided to all attendees of the consultation.

Consultation

The participants at the consultation were selected to ensure that the meeting allowed for a comprehensive review and input from expert scientists. Attendees included representatives from public health laboratories, clinical laboratories, large commercial laboratories, clinicians, STD programs, staff from the Office of the Director within the National Center for HIV, Viral Hepatitis, STD, and Tuberculosis Prevention (NCHHSTP), DSTDP: Office of Director, Laboratory Reference and Research Branch, Epidemiology and Statistics Branch, Program Development and Quality Branch, Surveillance and Data Management Branch, National Institute of Allergy and Infectious Disease (NIAID) and Food and Drug Administration (FDA) (Appendix 2). Each table at the consultation had representatives from these diverse backgrounds to ensure comprehensive discussion and input.

Attendees were provided with the electronic versions of the tables of evidence approximately one month before the consultation and with printed copies at the consultation. The goal of the consultation was for all attendees to listen to the external reviewer present their review and recommendations for their assigned key question. Attendees were also expected to provide feedback on the findings to generate refined best practices/recommendations for each key question. To ensure each key question was evaluated appropriately the external reviewer conveyed their findings in a 20-30 minute presentation covering the literature search, the major findings and gaps that need to be addressed. After each presentation attendees could ask clarifying questions to the reviewer before each table was asked to discuss the finds and recommendations for 30 minutes followed by reporting back their top 3-5 identified issues, comments, concerns, changes or additions. All details of the report-back were captured by the onsite facilitators and provided to the external reviewers at the end of the first day. During the second day, the external reviewers delivered 10-minute presentations that refined their original findings based on the input from the group the previous day. At the end of the consultation a refined list of best practices/recommendations and research needs were identified.

Report

This document summarizes the findings by the external reviewers combined with the input from the attendees at the meeting. This report is structured by topic and then key question. For each key question that was examined, background and summary information is provided followed by the collective recommendations, the identified research/diagnostic development needs to fully address the question and any tools or resources that would be useful to the field. The recommendations contained within this document represent those of the external reviewers, attendees at the meeting, and APHL. Recommendations contained within this document do not represent recommendations from the Centers for Disease Control and Prevention nor the Division of Sexually Transmitted Disease Prevention.

This is the final meeting report. APHL published a draft summary report open for public comments for six weeks to ensure that everyone that attended the meeting had an opportunity to provide any additional comments before the meeting report was finalized. All submitted comments were reviewed. Comments relevant to the accuracy of the summary meeting report were addressed by APHL and incorporated into this report as needed. Comments about findings in the report were collected and shared with our partners at CDC.

Topic 1: Direct Detection Methods

Key Question 1A: What are the performance characteristics for each direct detection test for *T. pallidum* and what are the optimal specimen types for each test?

Literature review by: Elitza Theel, supported by Allan Pillay

Presented by: Elitza Theel

Background

There are limited options available for direct detection of *T. pallidum*, particularly in the realm of FDA cleared diagnostic assays. Additionally, the clinical manifestations following infection with *T. pallidum* can manifest in a wide variety of ways, and therefore, direct detection methods should be capable of detecting *T. pallidum* from multiple different specimen sources. In addition, some direct detection methods that used to be more widely available are no longer routinely performed (e.g., dark-field microscopy (DFM), direct fluorescence antibody (DFA), whereas others are not widely available (e.g., nucleic acid amplification testing (NAAT). Given these challenges a review of the literature allowed us to draw some conclusions regarding the clinical utility of both traditional and recently developed direct detection methods. These findings are presented in the summary below.

Summary

The performance characteristics of each direct detection method vary depending on assay design (e.g., gene target for NAAT, type of antibody for DFA or immunohistochemistry (IHC), etc.), specimen type, sampling method (e.g., scraping vs. swabbing of secondary skin lesions), stage of disease and duration of therapy prior to sampling. Studies remain limited regarding performance characteristics of DFM, DFA, IHC and silver staining of ulcer or lesion exudate and tissue biopsies. Performance of molecular detection assays for *T. pallidum* varied by specimen source and stage of infection. There was general agreement on the following best practices.

Recommendations

1. Neither DFA nor DFM are routinely maintained by clinical or public health laboratories, partially due to lack of standardized or quality control reagents and can be considered obsolete for routine diagnostic purposes, especially in low prevalence settings.
 - a. Direct Fluorescent Antibody (DFA) using H9-1 mAb provides increased specificity over Dark-field Microscopy (DFM) for direct visualization in ulcer/exudate material with similar sensitivity.²⁻⁴
 - i. DFA sensitivity/specificity ranges from ulcer or lesion exudate: 73%-100%/95%-100%
 - ii. DFM sensitivity/specificity ranges from ulcer or lesion exudate: 58%-98%/45%-100%
 - b. DFM performed appropriately, within 20 minutes of sample collection and by trained staff that have maintained proficiency in the test, can be a useful diagnostic tool in high-prevalence settings. Laboratories in regions with a high incidence of syphilis that are currently performing DFM, with adequate quality controls, may continue to use this test. However, DFM should not be promoted to laboratories not already performing this assay.
 - i. Laboratories already performing DFM can obtain cryopreserved treponemes from the Laboratory Reference and Research Branch (LRRB) in DSTDP for quality control and training purposes. Requests should be sent to stdlaboratoryspecimen@cdc.gov
2. NAAT for detection of *T. pallidum* DNA performed on swabs from ulcer or lesion exudate can be used to establish a diagnosis of primary or secondary syphilis or congenital syphilis. However a negative result by NAAT cannot be used to exclude infection.⁵⁻¹³
 - a. Performance characteristics for assays with different targets (*tpp47* or *poIA*) were comparable.
 - i. Sensitivity of *tpp47* NAAT from exudate/swab: 60%-95%
 - ii. Sensitivity of *poIA* NAAT from exudate/swab: 73%-93%

- b. There are no FDA cleared or standard NAAT methods available for *T. pallidum*. Currently, a single reference laboratory offers a laboratory developed NAAT for *T. pallidum*, however the performance characteristics of this assay have not been published. Additionally, current NAATs in both the clinical and research arenas are on open systems, making quality control more challenging.
3. NAAT performed on whole blood or blood fractions (e.g. serum, plasma, buffy coat, etc.) is not recommended due to low sensitivity (range between targets: 12%-55%) in all stages of syphilis in adults except for congenital syphilis.^{10,12,14-17}
4. NAAT for detection of *T. pallidum* in CSF from seropositive patients with neurologic symptoms may be considered to support the diagnosis of neurosyphilis. A negative NAAT result should not be used to exclude infection.¹⁸⁻²⁰
5. In cases of suspected congenital syphilis infection, NAAT for *T. pallidum* may be considered as an adjunct test to confirm infection in amniotic fluid, CSF or blood. A negative NAAT result in any of these specimen types should not be used to exclude congenital syphilis infection.²¹⁻²⁵
6. Recommendations cannot be made for use of direct detection methods on ocular fluid or tissue from gummas or other tertiary syphilis lesions due to limited available literature on these disease states.
7. For examination of tissue sections, IHC, using avidin-biotin peroxidase complex chemistry, offers improved sensitivity and specificity over silver stains and is preferred for detection of *T. pallidum* in tissue sections. Silver staining for *T. pallidum* is not recommended as it is prone to staining artifacts and is non-specific.²⁶⁻²⁸

Research/Diagnostic Development Needs

1. Additional data are needed on the most appropriate specimen types, and standardized collection and preservation methods for direct detection
 - a. Specimen types such as lesion swabs and ear lobe scrapings are mentioned but no further information about the actual collection methods or storage recommendations
 - b. There is no definition of what metrics a good quality specimen should meet
 - c. There are no data on best swab types, transport media, storage temperatures.
 - d. For each of the different direct detection methods what is the preferred specimen type, fresh vs frozen, or formalin-fixed for IHC?
2. Additional data are needed on performance characteristics of IHC in different stages of syphilis (e.g., sensitivity in primary lesions, specificity in biopsy sections from genital tracts, etc.).
3. Additional data are needed on utility of ear lobe scraping versus pricking for collection of capillary blood as an alternative specimen source for patients without lesions.
4. Additional data are needed on performance characteristics of molecular assays in immunosuppressed populations (Limited data on HIV/AIDS positive patients show no difference)
5. Commercial laboratories which offer molecular testing for *T. pallidum* are encouraged to provide information and data on the performance characteristics of their assays. This data should be shared with the consumers (on lab website) and ideally published in some format for peer-reviewed evaluation.
6. Development of a FDA cleared rapid direct detection method with a CLIA waiver for use in point-of-care settings for genital ulcer and/or mucocutaneous lesions.
7. Development of FDA cleared laboratory-based molecular assays for direct detection of *T. pallidum*.
8. Data are needed on the performance and utility of molecular methods for detection and monitoring of *T. pallidum* antibiotic resistance markers.

Tools/Resources Needed

1. Develop standardized process for evaluation of laboratory developed molecular assays for detection of *T. pallidum*.

2. Availability of well characterized primary lesion material through a specimen bank for clinical laboratories (and PHLs) to use to train and validate testing methods in-house as well as to make available to diagnostic manufacturers to develop assays for FDA clearance.
3. Survey US PHLs, STD Clinics, emergency departments and commercial laboratories to determine testing practices for direct detection.

Key Question 1B: What options are available for molecular epidemiology and what should be considered for specimen collection/preservation?

Literature review by: Elitza Theel, supported by Allan Pillay

Presented by: Elitza Theel

Background

Understanding *T. pallidum* strain prevalence is important from a public health and epidemiologic perspective to understand outbreaks, strain virulence, and evaluate efficacy of intervention strategies to name a few. Additionally, strain information is useful to monitor geographic and temporal spread. However, there is limited to no clinical relevance of *T. pallidum* strain typing for the diagnosis or treatment of syphilis. Even though this testing is performed for surveillance purposes, it is still important to understand performance characteristics and to use the best specimen source possible.

Summary

Methods for subtyping *T. pallidum* are based on NAAT/sequencing of specific target regions, largely based on work by Pillay, A. et. al.²⁹ Subtyping is not recommended for routine clinical diagnostic purposes and is primarily used for epidemiologic purposes. There was general agreement on the following best practices.

Recommendations

1. Screening of samples for *T. pallidum* DNA using NAATs targeting *poIA*, *tpp47*, or *bmp* should be considered prior to proceeding to typing.²⁹
2. Molecular typing of *T. pallidum* is most frequently successful when performed on ulcer or mucocutaneous lesion exudate from patients with primary or secondary syphilis.²⁹⁻³³
 - a. *T. pallidum* typing using whole blood, plasma, serum, buffy coat or CSF specimens are associated with low success rates.
3. Molecular typing based on *arp*/*tpr* PCR and RFLP analysis may be considered.
 - a. Additional strain typing information may be achieved through sequencing of the *T. pallidum* *tp0548* or *tp0279* (*rpsA*) genes.^{34,35}

Research/Diagnostic Development Needs

1. Understanding the role of metagenomic sequencing approaches for identification and typing of *T. pallidum* from different specimen sources.
 - o This method is in the early stages of development and the impact on *T. pallidum* strain typing is currently unknown.
2. What is the role of molecular typing for monitoring antibiotic resistant strains of *T. pallidum* susceptibility information?

Topic 2: Serologic Methods

Key Question 2A: What are the performance characteristics, stratified by the stage of syphilis, for nontreponemal serologic tests?

Literature review by: Susan Tuddenham and Khalil Ghanem, supported by Samantha Katz and Yetunde Fakile
Presented by: Susan Tuddenham

Background

Nontreponemal tests detect antibodies to nonspecific antigens (primarily cardiolipin) and are used in combination with treponemal tests to help diagnose infection with *T. pallidum*. Published studies were reviewed to determine the sensitivity and specificity of nontreponemal tests in serum from the various stages of syphilis and the performance characteristics of CSF nontreponemal tests in the diagnosis of neurosyphilis, ocular syphilis and otic syphilis. The primary tests in current use are Rapid Plasma Reagin (RPR) and Venereal Disease Research Laboratory (VDRL) and to a lesser extent, Tolidine Red Unheated Serum Test (TRUST) and Unheated Serum Reagin (USR). Additionally, there are FDA cleared automated nontreponemal assays. Nontreponemal tests are also used to monitor treatment responses and outcomes, however performance characteristics in this context were not covered in this review.

Summary

Many studies reviewed were retrospective and had small sample sizes, they were also limited by lack of clearly documented clinical staging and well defined gold standards. There were no published papers examining the performance characteristics of the two existing FDA-cleared automated nontreponemal assays. With these limitations, there was general agreement on the following best practices.

Recommendations

1. In general, serum RPR appears to be more sensitive than serum VDRL at detecting nontreponemal antibodies, independent of syphilis stage.³⁶⁻⁴⁰ Based on more limited data the RPR also appears to be more specific than the VDRL.³⁶⁻³⁸
 - a. Comments were made during the consultation by attendees regarding the ease of use of RPR as a consideration for recommending RPR over VDRL.
2. Serum RPR and VDRL are about 62-78% sensitive for diagnosis of primary syphilis.⁴¹⁻⁵³ Serum RPR and VDRL have high sensitivity (nearly 100%) for diagnosis of secondary syphilis.^{41-43,46-48,50,54-57} Based on a small number of studies of mixed quality, sensitivity of VDRL for early latent syphilis ranges from 82-100%.^{55,57-59} Based on a small number of studies of mixed quality, the sensitivity of RPR and VDRL ranges from 61-75% for diagnosing late latent syphilis.^{55,58-60} Based on only 2 studies, the sensitivity of serum VDRL for tertiary syphilis is 47-64%.^{58,61}
3. There are limited data available comparing automated and non-automated nontreponemal tests and only two automated assays are FDA cleared (as of November 27, 2017). Based on limited data, (including some unpublished manufacturer's data submitted to the FDA) the automated nontreponemal tests appear to have reasonable performance as compared to the non-automated tests.⁶²⁻⁶⁹
4. Serum RPR and VDRL titers should not be used interchangeably to manage patients.⁴⁰
5. Neurosyphilis diagnosis is challenging. Different definitions for neurosyphilis across studies, heterogeneity of gold standards used, and inclusion of a mixture of symptomatic and asymptomatic patients were discussed as limitations. Based on current data there are no recommendations for the use of one assay over the other in the diagnosis of neurosyphilis, though limited data suggest that CSF RPR may be less sensitive than CSF VDRL.
 - a. CSF VDRL is 49-87% sensitive and 74-100% specific for diagnosing neurosyphilis.⁷⁰⁻⁷³
 - b. CSF RPR is 51-82% sensitive, and 82-100% specific for diagnosing neurosyphilis.^{70,71,73}

- c. Due to significant heterogeneity in case definitions and limited data, it is not possible to give definitive information on performance characteristics of CSF non-treponemal tests in symptomatic versus asymptomatic neurosyphilis.
6. The sensitivity of CSF VDRL in ocular syphilis is <50%.^{74-91,91} Limited data suggest that the sensitivity of CSF VDRL is poor (<10%) in otosyphilis.^{92,93}
 7. The prevalence of false negative results from nontreponemal syphilis tests is rare (<0.85% of those tested).^{94,95}
 - a. The prozone reaction can occur during any stage of syphilis but is more common in primary and secondary syphilis; neurosyphilis and pregnancy may increase the risk of the prozone reaction.⁹⁴
 - b. A third of prozone reactions may occur when titers are $\leq 1:16$.⁹⁴
 - c. False negatives may be more common if sera are centrifuged at colder temperatures (e.g. 4 degrees centigrade vs. 27 degrees centigrade).⁹⁶
 8. In most large studies, the overall prevalence of biological false positives (BFP) in general populations tested for syphilis is $\leq 1.5\%$.^{52,97-102}
 - a. Several factors are associated with BFPs including older age, certain autoimmune diseases, leprosy, YAWS and HIV infection.^{101,103-110} More limited data exist on the association of malaria, hepatitis B infection, hepatitis C infection, and persons who inject drugs (PWID) with increased risk of BFPs.^{103,111-115} The data for the association of BFPs and pregnancy are conflicting.^{37,95,116}
 - b. The data do not suggest a significant impact of vaccines on BFPs.^{117,118}
 - c. In general, BFPs tend to occur when the nontreponemal titers are low – usually $\leq 1:8$ ^{102,116} (But, there is clear documentation of exceptions where BFPs occur with higher nontreponemal titers; in general, those titers were usually $\leq 1:64$).^{111,112,119}
 - d. The duration of BFPs may depend on their underlying etiology. BFPs in most cases tend to revert to nonreactive. A small study suggests that the majority serorevert within approximately 10 weeks.¹²⁰ In another small study of patients with narcotics addiction, the average duration of the BFP was 25 months.¹¹²

Research/Diagnostic Development Needs

1. Better define the performance characteristics of nontreponemal tests, particularly in the late stages of syphilis.
2. Published data are needed on automated nontreponemal tests, based on clinically well characterized sera.
 - a. Are titers interchangeable with manual tests?
 - b. What recommendations should be made about maintaining manual testing proficiency?
3. Data are needed to better define the performance characteristics of nontreponemal tests in neurosyphilis. (e.g., if CSF nontreponemal tests are nonreactive is it neurosyphilis?)
4. Additional data are needed to gain a better understanding of the relationship between disease activity and nontreponemal antibody titers.

Tools/Resources Needed

1. Evidence based guidelines are needed for prozone titrations.
2. Improved criteria and/or diagnostics for neurosyphilis (as well as otic and ocular syphilis).

Key Question 2B: What are the performance characteristics, stratified by the stage of syphilis, for treponemal serologic tests?

Literature Review By: Ina Park, supported by Yetunde Fakile and Lara Pereira

Presented by: Ina Park

Background

Treponemal tests detect antibodies specific to *Treponema pallidum* which typically persist for life following infection. Treponemal tests are qualitative and are used in conjunction with nontreponemal tests and clinical signs and symptoms to diagnose infection. There are manual tests such as the *Treponema Pallidum* Particle Agglutination Assay (TP-PA), Fluorescent Treponemal Antibody Absorption (FTA-ABS), Microhemagglutination assay for *Treponema pallidum* antibodies (MHA-TP) and *Treponema Pallidum* Hemagglutination (TPHA). Additionally there are automated immunoassays including enzyme (EIA), chemiluminescent (CIA) and microbead (MBIA) based methods. Published studies were reviewed to determine the performance of treponemal tests in serum from the various stages of syphilis and the performance characteristics of CSF treponemal tests in the diagnosis of neurosyphilis.

Summary

Comparison of performance characteristics was limited to mostly manual treponemal tests because few studies of the automated immunoassays included clinically characterized specimens, stratified by stage. Most studies were retrospective and used reactive serology as part of the inclusion criteria which would bias sensitivity estimates towards 100%, particularly for primary syphilis. Studies utilizing previously banked specimens (both CDC and commercial serum banks) were included, but the quality of staging/characterization of these specimens could not be assessed. For primary syphilis it was unclear whether banked specimens included dark field positive-seronegative cases or just cases with reactive nontreponemal and/or treponemal serology. For latent syphilis most studies combined early and late latent into a single category defined as “combined latent syphilis” and only one study used the same case definition currently used in the United States. Some studies included prior treated cases and untreated (current) syphilis cases. The evaluation focused on untreated or current syphilis because the time between treatment and specimen collection was not clear. There was general agreement on the following best practices.

Recommendations

1. Sensitivity:

- a. Manual Tests: Among manual treponemal tests (MHA-TP, FTA-ABS, TP-PA), TP-PA should be considered the preferred test over FTA-ABS given the subjective nature of FTA-ABS interpretation, lack of quality control (QC) reagents and the need for microbiologist experience for the FTA-ABS. MHA-TP should be considered obsolete as it no longer commercially available.
 - i. MHA-TP is less sensitive for primary syphilis (53-88.6%) than FTA-ABS (78-100%) or TP-PA (86-100%).^{43-45,121-131}
 - ii. Based on limited data, FTA-ABS may be less sensitive than TP-PA in primary and secondary syphilis.¹³²
 - iii. For secondary syphilis, sensitivity of TP-PA was 100%. FTA-ABS ranged from 95-100%,¹³² and MHA-TP was 90-100%.^{43,121-130,133}
- b. Treponemal Immunoassays: Among the treponemal immunoassays there are little published data directly comparing performance of the immunoassays by stage or comparing performance versus manual treponemal tests by stage. Data published in peer reviewed journals are insufficient to recommend a particular treponemal immunoassay for diagnosis of syphilis. Additional data from diagnostic assays that have received FDA clearance can be obtained in the 510K Decision Summary and should be considered for more robust analysis.
 - i. Based on a single study, sensitivity of TrepSure EIA in primary syphilis was poor (54.8%, 39.5%-67.8%).¹³⁴ Captia EIA was 82.3-100% sensitive for primary syphilis, but sample sizes were small (6-13 cases).¹³⁵⁻¹³⁷ In a single study comparing Captia EIA to FTA-ABS, both were 100% sensitive.¹³⁵

- ii. Based on limited data, Captia EIA and LIAISON CIA were 100% sensitive for secondary syphilis.¹³⁵⁻¹³⁸
2. Latent Syphilis: Only one study used a similar case definition to the CDC STD Treatment Guidelines.^{132,139} Other studies used a two-year cut-off for early latent disease. Studies that combined early/late latent were not included. More performance data are needed in early versus late latent syphilis, particularly for immunoassays in order to make a recommendation. Among manual assays, TP-PA is preferred due to practical considerations (see rationale under recommendation 1 for Key Question 2B)
 - a. Among the manual assays, sensitivity is similar among FTA-ABS, TP-PA, MHA-TP for diagnosis of early latent syphilis (94.4-100%).^{124,128,130,132,135}
 - b. In one comparative study, sensitivity of FTA-ABS was lower in late latent disease (84.5%, 73.1-91.6%) compared to TPPA (91.4%, 81.4-96.3%).¹³² Sensitivity of TP-PA, MHA-TP and FTA-ABS for late latent disease in other studies was 97-100%.^{124,130,135}
 - c. Among the treponemal immunoassays, Captia EIA was 100% sensitive for untreated early latent syphilis, and 91.7%-100% sensitive for untreated late latent syphilis.¹³⁵⁻¹³⁷ Data for other treponemal immunoassays stratified by early/late latent were not available.
 3. Specificity: The specificity of manual treponemal tests is similar, however TP-PA is the preferred among the manual assays (rationale under recommendation 1 for Key Question 2B). More comparative specificity data is needed for all of the FDA-cleared immunoassays. Data are insufficient to recommend a particular immunoassay based on currently available specificity data.
 - a. The specificity of manual treponemal tests is similar: MHA-TP (98.5-99.7%), FTA-ABS (92-100%), TP-PA (96-100%).^{43,44,121-130,133}
 - b. In a single comparative study, specificity of Architect CIA was 90.5%, while TP-PA was 100%,⁹⁴ otherwise specificity of Architect CIA ranged from 98.4-100%.¹⁴⁰⁻¹⁴³ Specificity of the LIAISON CIA was 100%.^{138,140} Specificity of the Captia EIA was 98.2-100%.¹³⁵⁻¹³⁷
 4. Neurosyphilis: Based on limited performance data for the CSF TP-PA, either the CSF FTA-ABS or CSF TP-PA may be used to support the diagnosis of neurosyphilis.
 - a. In studies of patients with definitive neurosyphilis (reactive CSF VDRL) sensitivity of CSF FTA-ABS is 90.9-100%.¹⁴⁴⁻¹⁴⁶
 - b. Among those with presumptive neurosyphilis where diagnosis is made based on reactive serology, other abnormal CSF indices, clinical signs/symptoms), sensitivity ranged widely (22.2-100%).¹⁴⁷ However, in two of the largest studies, sensitivity of CSF FTA-ABS was 100%.^{148,149}
 - c. Based on limited data, the CSF TP-PA appears to have similar sensitivity to CSF FTA-ABS in studies with a mixed population of patients with definitive/presumptive neurosyphilis, ranging from 83.3-100%.¹⁵⁰⁻¹⁵²
 - d. A negative CSF treponemal test needs to be evaluated within the context of the clinical scenario and syphilis prevalence.
 - i. In a population where the prevalence of syphilis is low, a negative CSF treponemal test would be expected to have almost 100% negative predictive value.
 - ii. In the typical clinical scenario where patients being tested have a high pre-test probability of neurosyphilis (patients with syphilis and neurologic symptoms), a negative CSF treponemal test would not necessarily rule out neurosyphilis.

Research/Diagnostic Development Needs

1. Performance data are needed for the immunoassays using clinically characterized specimens.
 - a. Many assays currently in use had no data of this kind. This is particularly an issue with early primary and late latent disease.

2. Additional data are needed on the performance of treponemal tests in latent syphilis based on the CDC case definitions for latent syphilis.
3. Additional data are needed on the comparative performance of the CSF TP NAAT (commercially available), CSF FTA-ABS, CSF TP-PA and treponemal CIA/EIA in CSF.
4. Performance data are needed for the immunoassays (serum) in neurosyphilis including evaluation of Captia IgM-only assay and where it might fit into testing algorithms.
5. Define serologic windows using modern treponemal and nontreponemal tests.
6. Published data on performance of laboratory developed tests, particularly from large commercial laboratories as outlined in K1A research/diagnostic needs.

Tools/Resources Needed

1. Harmonization of criteria for evaluating performance of treponemal and nontreponemal tests, in particular characterization of false positives.
2. Creation or resurrection of the CDC Syphilis Serum Bank with validated specimens, characterized by stage using standardized criteria, including sero-negative, darkfield-positive primary syphilis.
3. Need for a common reference standard or predicate device against which new assays should be measured.

Key Question 2C: Do laboratory tests perform differently when applied to special populations such as HIV positive individuals and/or pregnant women? What tests should be used in cases of congenital syphilis?

Literature Review By: Jeanne Sheffield and Ahizechukwu Eke, supported by Yetunde Fakile and Lara Pereira

Presented by: Jeanne Sheffield

Background

The previous two key questions were structured to evaluate the overall performance of nontreponemal and treponemal tests as well as stratify how they perform at various stages of syphilis disease. An additional consideration is how these tests perform in special circumstances, including those of altered immune status. Since these tests are based on detection of antibodies, immunologic changes within the infected host could also impact the ability to diagnose disease within that population. In particular, three challenging populations are HIV positive individuals, pregnant women and infants exposed to or with congenital infections. Screening and treatment of special populations are a component of the STD Treatment Guidelines.¹³⁹

Summary

Performance data on laboratory tests in special populations is extremely limited. For evaluation of laboratory test performance in pregnancy, most papers do not include direct comparisons to non-pregnant populations. Of note, most syphilis laboratory testing is performed for screening which impacts performance characteristics. Extrapolation to the performance in non-pregnant populations suggests potentially higher false positive rates with immunoassays and discordant tests remains an issue if using a treponemal test followed by a nontreponemal test. Congenital syphilis diagnosis remains very difficult with the currently available tests due to maternal IgG crossing the placenta. Overall, HIV infected individuals may have higher biologic false positive non-treponemal tests and higher false negative treponemal tests. There was general agreement on the following best practices.

Recommendations

There are insufficient data to suggest that performance characteristics for treponemal or nontreponemal tests is significantly different in persons infected with HIV or in pregnant women. Nor are there sufficient data on the performance of the tests used to diagnose congenital syphilis. If there were data, considerations for algorithm usage in special populations should be addressed.

Supporting Information

1. Pregnancy:¹⁵³⁻¹⁵⁶

- a. False positive nontreponemal test results may occur so confirmation with a treponemal test is mandatory.
 - i. Prozone reaction may be more common in pregnant women. Sample dilution should be performed if clinical suspicion of syphilis.
 - b. RPR should be the nontreponemal test for serum (consistency is vital).
 - c. The reverse sequence testing algorithm, if used for pregnancy, should be performed in a timely manner, preferably with all the results of all tests included on a single report. Interpretation of the results should be integrated into the report.
2. Congenital Syphilis:¹⁵⁷⁻¹⁶³
- a. Testing of the infant should be performed on neonatal blood, not umbilical cord blood.
 - b. If a paired maternal and neonatal nontreponemal test is performed, both should be performed in the same lab using the same nontreponemal test
 - c. Identification of spirochetes in tissue and fluid by DFM is useful if positive, but not sensitive.
 - d. Placental pathology should not be used for diagnosis alone though it is a useful adjunct to the diagnosis of congenital syphilis
3. HIV-positive Persons:^{119,164-169}
- a. Some published studies have reported higher rates of biologic false positive results in HIV positive populations compared to HIV non-infected populations while others have reported no difference.
 - b. Prozone reaction may be more common in HIV infected persons. Sample dilution should be performed if clinical suspicion of syphilis.
 - c. Serologic response after adequate treatment may differ in HIV infected patients including slower responses, varying titers and seroreversion.

Research/Diagnostic Development Needs

1. Additional data are needed directly comparing test performance in general populations to pregnant populations and HIV infected populations including:
 - a. Matched syphilis stages
 - b. Matched co-morbidities (CD4 cell count, antiretroviral therapy, pre-exposure prophylaxis)
 - c. Response to treatment (nontreponemal titers)
 - d. Biologic false positive results (nontreponemal tests)
2. Development of improved tests or methods to diagnose congenital infection, especially for asymptomatic neonates.
3. Additional data are needed to establish factors associated with seroreversion, false positive and false negative results in general populations as compared to special populations.

Tools/Resources Needed

1. Well-characterized (clinically) syphilis biorepository including serum, amniotic fluid, placenta, CSF, ulcer scrapings, autopsy specimens, etc. from the general population and special populations to evaluate diagnostic assays.
2. Development of maternal and fetal/neonatal combination diagnostic algorithms.

Key Question 2D: What considerations (i.e. diagnostic and cost-effective implications) should be taken into account when screening for syphilis using either the traditional and reverse algorithm?

Literature Review By: Daniel Ortiz and Michael Loeffelholz, supported by Mayur Shukla

Presented by: Daniel Ortiz

Background

Laboratories use a combination of treponemal and nontreponemal antibody tests to screen for and confirm syphilis infections by using one of two serologic testing algorithms—the traditional algorithm or the reverse algorithm. Data from several studies suggest that approximately 80% of laboratories in the US are utilizing the traditional algorithm and 20% are utilizing the reverse algorithm.¹⁷⁰ The traditional syphilis algorithm starts with a nontreponemal test, with positive samples being reflexed to a treponemal test. In 2009, an expert panel was convened to discuss syphilis diagnostics and formalized the reverse algorithm,¹⁷¹ which starts with a treponemal screening test with positive samples reflexed to a nontreponemal test and discordant samples resolved with a second treponemal test. Laboratories are choosing to use both algorithms for a variety of reasons. We evaluated published studies to provide laboratories guidance regarding the advantages and disadvantages of the two algorithms.

Summary

There are limited data on the performance of syphilis testing algorithms as a whole and even less about cost and efficiency. As more laboratories face pressures due to increasing volume and are trying to keep budgets down they need to be aware of the implications of the testing algorithm that is utilized. The laboratory should be in communication with clients/stakeholders when making decisions on the testing algorithm. Information such as patient population and syphilis risk are important factors for choosing the best algorithm to meet the needs of all stakeholders. Additionally, independent of which algorithm is used, all the results should be reported together with a final algorithm interpretation.

Recommendations

1. Laboratories should take into account their patient population and syphilis risk when considering a traditional or reverse sequence screening algorithm.
2. Decisions based on cost should take into account total costs at both the laboratory level and the system level.
3. The test results report should include a brief description of the method(s) and algorithm used.

Supporting Information

Traditional Algorithm

1. Sensitivity
 - a. Based on laboratory data, the diagnostic sensitivity of nontreponemal assays can vary with high discordance rate between the RPR and VDRL.^{38,172}
 - b. Nontreponemal assays generate more false-negative results due to decreased sensitivity of RPR and the VDRL assay during primary and possibly latent syphilis although it can be difficult to differentiate latent from past treated syphilis based on results of treponemal tests alone.^{45,60,129,138,173–179}
 - c. The prozone reaction can also cause false negative RPR results, usually during secondary syphilis. Prozone reaction can occur in approximately 1% of all RPR reactive patients.^{94,180,181}
 - d. However, another study found the RPR to be 23% more sensitive than a treponemal enzyme immunoassay (EIA) at detecting primary cases.¹³⁴
2. Automation
 - a. There are two FDA cleared automated nontreponemal assay currently available (as of November 28, 2017) but no peer reviewed studies were available for review.

3. Cost

- a. One publication that examined the costs between the two algorithms concluded that the traditional algorithm was more cost-effective in low and high prevalence settings, as long as the cost of treponemal test remained at \$9.50 or higher. They also concluded that the traditional algorithm is more cost-effective than the reverse algorithm due to a lower number of follow-ups and less overtreatment.¹⁸²
- b. This finding contrasts with another publication that concluded that the reverse algorithm will identify more syphilis cases and thus generate cost savings to the health-care system.¹⁸³

Reverse Algorithm

1. Sensitivity

- a. Among FDA cleared treponemal assays, all have similar sensitivity and specificity.^{38,131,140,184-189}
 - i. However, three different groups have reported that only 59% - 75% of positives by one treponemal chemiluminescent immunoassay (CIA) are confirmed by TP-PA.^{94,190-192} However, in another manuscript they were only able to confirm 72% of samples positive with a treponemal CIA with TP-PA.¹⁹³
- b. Treponemal screening assays are at least as sensitive as nontreponemal assays.^{45,60,129,138,173-177,184,194,195}
 - i. However, two other publications have found the VDRL and the RPR to be more sensitive than a treponemal EIA.^{60,134}
- c. Analytical sensitivity of treponemal assays varies.^{186,196}
 - i. This does not affect diagnostic sensitivity when most cases are latent, but could affect diagnostic sensitivity during primary syphilis.

2. Automation

- a. There are 9 FDA cleared automated treponemal assays with multiple published, peer-reviewed studies.

3. Overall Performance

- a. Screening with a treponemal assay will identify more cases, usually presumed past treated cases of syphilis.^{173,175,197}
- b. However, treponemal screening assays detect active and treated syphilis, which results in more confirmatory testing.^{198,199}
- c. Strength of signal or signal/cut off ratio could potentially be used (off-label) in lieu of confirmatory testing to reduce unnecessary procedures or laboratory costs.^{84,176,190,193,200-203}
- d. Treponemal confirmatory test discordance could be attributed to the different analytical sensitivities of the confirmatory tests.^{186,196,203}

4. Cost

- a. One manuscript concludes the reverse algorithm is more cost effective since it will generate more correct diagnoses, which will lead to less health-service cost.¹⁸³
 - i. However, a second paper found that the reverse algorithm was slightly more expensive than traditional algorithm.²⁰⁴

Research/Diagnostic Development Needs

1. Additional data are needed to directly compare laboratory costs for both algorithms to include instrument purchase (or lease), reagents, labor, and volume.
2. Additional data are needed on performance of both algorithms.

- a. In populations with low, medium, high prevalence rates.
- b. On persons with well characterized clinical disease or condition (i.e. HIV, pregnancy).

Tools/Resources Needed

1. Develop a cost analysis tool to assist facilities in making decisions regarding choice of algorithm.

Topic 3: Point-of-Care Tests

Key Question 3A: What serologic based point-of-care (POC) tests are available to support a syphilis diagnosis, including single syphilis POC tests and combination syphilis/HIV and nontreponemal/treponemal POC tests, and what are the performance characteristics?

Key Question 3B: What laboratory considerations should be made concerning the use of point-of-care tests?

Literature Review By: Anthony Tran, supported by Cheng Chen

Presented by: Anthony Tran

Background

The use of point-of-care (POC) tests to support syphilis serologic diagnosis could facilitate faster linkage to treatment and could expedite partner tracing and notification. While several syphilis POC tests are available on the global market, including HIV, syphilis (treponemal and nontreponemal) and a number of tests that detect both HIV and syphilis or treponemal and nontreponemal antibodies, there is only one FDA cleared rapid test for detection of treponemal antibodies in whole blood, plasma or serum using a lateral flow immunochromatographic design. In 2014, the FDA granted a waiver under CLIA for use on fingerstick whole blood in CLIA-waived settings (nonclinical). With rates of syphilis on the rise and the heightened interest in rapidly identifying syphilis infections, understanding the performance and utility of this rapid and point-of-care test both in the field and the laboratory is necessary.

Summary

There is a paucity of data available for evaluation of the FDA cleared rapid POC treponemal assay.²⁰⁵⁻²⁰⁷ Additional test performance data, including data used for the 510K application to FDA should be evaluated. Available data from the domestic use of rapid POC tests, that are not yet FDA cleared, could be of utility to further understand what considerations should be made concerning the use of rapid syphilis POC tests.

Recommendations

1. There are insufficient data to make a recommendation regarding the utilization of the FDA cleared rapid syphilis test at this time in a laboratory or POC setting.
2. Laboratories that are evaluating the assay are encouraged to publish their findings in a peer-reviewed journal to generate sufficient data for evaluation of performance and utility.
3. For those sites that are currently using the rapid test in a POC setting, it is strongly recommended that a good quality assurance program that includes appropriate training, competency assessments, proficiency testing, and quality controls are in place to ensure accurate results. The public health laboratory in your jurisdiction should be consulted when setting up a rapid testing program. Any information that can be communicated regarding quality assurance, including training, competency assessments, proficiency testing, and quality control and appropriate use would be helpful.

Research/Diagnostic Development Needs

1. Additional data are needed to compare the FDA cleared syphilis rapid test against and with laboratory-based assays performance in the traditional and reverse algorithms.
 - a. Evaluate the cost-benefit for algorithms that include a rapid and/or POC test.

2. Additional data will be needed to compare the FDA cleared rapid syphilis tests against each other (once available).
3. Additional data are needed to evaluate the role of rapid syphilis POC tests in linkage to care, and performance in special patient populations with low follow-up as compared to laboratory-based testing currently being performed.
4. Additional data are needed to evaluate the utility of a rapid syphilis POC test for the management of patients with genital ulcer disease (GUD) and/or rash of unknown etiology.
5. Additional data are needed to understand the best setting and application for a syphilis POC test.
 - a. Can it be used for screening in a reverse algorithm?
 - b. Can it be used for surveillance purposes?
 - c. Can it be used as a supplemental test?
 - d. What are the minimum performance characteristics of a syphilis POC test to ensure that it could be used for screening and/or supplemental test in a syphilis diagnostic algorithm?
 - e. Comparing rapid testing programs with an established quality assurance program to those without.

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Appendix A. External Review Summary

Direct Detection of <i>Treponema pallidum</i>			
	Key Question	External SME	CDC Assistant
K1A	What are the performance characteristics of each direct detection test for <i>T. pallidum</i> and what are the optimal specimen types for each test? [Dark-field microscopy, Direct fluorescent antibody, NAAT, Immunohistochemical or silver staining of tissue]	Elitza Theel	Allan Pillay
K1B	What options are available for molecular epidemiology and what should be considered for specimen collection/preservation?		

Serology			
	Key Question	External SME	CDC Assistant
K2A	What are the performance characteristics, stratified by the stage of syphilis, for nontreponemal serologic tests?	Susan Tuddenham & Khalil Ghanem	Samantha Katz & Yetunde Fakile
K2B	What are the performance characteristics, stratified by the stage of syphilis, for treponemal serologic tests?	Ina Park	Yetunde Fakile & Lara Pereira
K2C	Do laboratory tests perform differently when applied to special populations such as HIV positive individuals and/or pregnant women? What tests should be used in cases of suspected congenital syphilis?	Jeanne Sheffield & Ahizechukwu Eke	Lara Pereira & Yetunde Fakile
K2D	What considerations (i.e. diagnostic and cost-effective implications) should be taken into account when screening for syphilis using either the traditional or reverse algorithm?	Daniel Ortiz & Michael Loeffelholz	Mayur Shukla

Point of Care Tests			
	Key Question	External SME	CDC Assistant
K3A	What serologic based point of care (POC) tests are available to support a syphilis diagnosis, including single syphilis POC tests and combination syphilis/HIV and nontreponemal/treponemal POC tests and what are the performance characteristics?	Anthony Tran	Cheng Chen
K3B	What laboratory considerations should be made concerning the use of point of care tests?		

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