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Improving the Detection and Surveillance of Sexually Transmitted Infections in Public Health Laboratories

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Improving the Detection and Surveillance of Sexually Transmitted Infections
in Public Health Laboratories

A dissertation in partial satisfaction of the requirements for the Doctor of Philosophy in Environmental Health Sciences

by

Sean A. Buono

2017
ABSTRACT OF THE DISSERTATION

Improving the Detection and Surveillance of Sexually Transmitted Infections
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by

Sean A. Buono

Doctor of Philosophy in Environmental Health Sciences
University of California, Los Angeles, 2017

Professor Hilary Godwin, Co-Chair
Richard J. Jackson, Co-Chair

The overarching goal of the work described herein is to investigate strategies for improving the
detection and surveillance of multidrug-resistant *Neisseria gonorrhoeae* (MDRNG) and syphilis,
two important sexually transmitted infections. First, we conducted a critical review of the literature
to ascertain whether improvements could be made in the way that *Neisseria gonorrhoeae* is
screened and treated. We reviewed the existing literature on the global distribution of MDRNG,
current public health surveillance practices, and new methods for detection and molecular
characterization of *N. gonorrhoeae*. Based on a synthesis of this information, we recommended
that clinical samples should be screened using rapid molecular testing for known antimicrobial
susceptibility markers to enable the treatment paradigm for *N. gonorrhoeae* to be shifted towards
individualized treatment. Next, we conducted studies to provide guidance to public health
laboratories to enable them to choose Syphilis screening assays and algorithms that are both cost
effective and meet public health goals. First, we compared the performance, cost, and ease of use
for three automated treponemal assays: Phoenix Biotech Trep-Sure Syphilis Total Antibody EIA, Siemens Advia Centaur Syphilis, and the DiaSorin Liaison Treponema assay. We found that each of the automated assays performed comparably to the current standard treponemal assay, *Treponema pallidum* particle agglutination (TP-PA). We also investigated the economic impact of the Los Angeles County Public Health Laboratories (LACPHL) switching from the traditional syphilis-screening algorithm that they currently use to a reverse screening algorithm using laboratory and surveillance data collected during 2015. We found that the reverse algorithm would cost less to implement than the traditional algorithm and could potentially identify more cases of syphilis. Further, modest cost increases for follow-up investigations would be offset by laboratory cost savings. Our modeling methods could be used to determine whether the reverse algorithm is cost-effective for other laboratories. Together, these studies underscore the importance of using evidence-based approaches to determine when public health laboratories should implement new technological advances.
The dissertation of Sean A. Buono is approved.

Patrick Allard

Marjan Javanbakht

Richard J. Jackson, Committee Co-Chair

Hilary Godwin, Committee Co-Chair

University of California, Los Angeles

2017
DEDICATION

It takes a village to raise an independent thinker.

My dissertation is dedicated to my parents, teachers, mentors, friends, and family that raised me over the years.

Thank you for your love and support.
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EDUCATION

2008.................. B.S., Biological Sciences, University of California, Irvine

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• Presented a poster on “A Comparison of Three Treponemal Assays” at the APHL Annual Meeting & Tenth Government Environmental Laboratory Conference (Albuquerque, NM, June 6-9, 2016)

• Presented a poster on “Surveillance of Carbapenem-resistant Enterobacteriaceae in Los Angeles, CA” at the APHL Annual Meeting & Tenth Government Environmental Laboratory Conference (Albuquerque, June 6-9, 2016)

• Presented departmental seminar on “Impact Assessment on Syphilis Testing in Los Angeles, CA” in the Department of Environmental Health Sciences, University of California Los Angeles (Los Angeles, April 14, 2016)

• Presented a lecture on “The Bacterial Pathogenesis of Neisseria gonorrhoeae” for MIMG 106 (Bacterial Pathogenesis course), Department of Microbiology, Immunology and Molecular Genetics, University of California Los Angeles (Los Angeles, June 4, 2015)

• Presented departmental seminar on “Stemming the Tide of Drug Resistant Neisseria gonorrhoeae” in the Department of Environmental Health Sciences, University of California Los Angeles (Los Angeles, February 5, 2015)
CHAPTER 1

Introduction and Overview of the Organization of the Thesis

INTRODUCTION

Public health laboratories provide critical services including infectious disease surveillance, reference testing, bioterrorism response, and applied research for their communities.\(^1\) Although they are mandated to provide these vital services, public health laboratories are plagued with daily challenges such as shrinking budgets, an aging workforce, competition with other labs, and an ever-changing technological landscape. Compounding these challenges are newly emerging and re-emerging infectious diseases which continue to threaten vulnerable populations globally. In particular, re-emerging sexually transmitted infections (STIs) pose a great risk to vulnerable populations by causing serious morbidity and mortality, in addition to social stigma.\(^2\) Specifically, re-emerging STIs pose a challenge to public health laboratories that do not adjust their testing strategies to address changes in the microbiology or epidemiology of these pathogens. Two STIs that pose particular challenges to public health laboratories are gonorrhea and syphilis, both of which are increasing in incidence in many urban centers. The practical challenges related to detection and surveillance of both gonorrhea and syphilis may be a contributing factor to their increasing incidence in the population.

CLINICAL PRESENTATION AND EPIDEMIOLOGY OF GONORRHEA

*Gonorrhea* is a sexually transmitted disease that can colonize and invade columnar and transitional epithelial cells in the genitourinary tract, rectum, and throat. In males, gonorrhea
presents as acute urethritis with a thick, purulent discharge 2-7 days after exposure.\textsuperscript{3} Gonorhea is often asymptomatic in women, but may present as mucopurulent cervicitis with abnormal vaginal discharge and bleeding after intercourse.\textsuperscript{3} Pharyngeal and anorectal infections are not uncommon in females or homosexual males, but are often asymptomatic.\textsuperscript{3} Untreated gonococcal infections can lead to severe medical complications. Conjunctivitis occurs in newborns and rarely in adults, with resultant blindness if not rapidly or adequately treated.\textsuperscript{3} Disseminated gonococcal infections may cause pelvic inflammatory disease and sterility in women.\textsuperscript{3} In men, gonorrhea can cause swelling of the epididimis and testes causing epidydimitis and epidydymo-orchitis.\textsuperscript{3} Further, \textit{N. gonorrhoeae} can disseminate into the bones and joints from the bloodstream causing arthritis or, in rare cases, septicemia.\textsuperscript{3}

Gonorrhea infections are increasing globally and locally. The World Health Organization (WHO) estimates 78 million new gonococcal infections every year.\textsuperscript{4} In 2015, gonorrhea was the second most commonly reported notifiable disease in the United States with a rate of 124 gonorrhea cases per 100,000 population.\textsuperscript{5} The rate of reported gonorrhea infections increased 12.8\% since 2014 and 19.9\% since 2011.\textsuperscript{5} California has one of the highest rates of gonorrhea in the United States (140 per 100,000) with most cases concentrated in Los Angeles County (150 per 100,000).\textsuperscript{5} In 2015, the rate of reported gonorrhea infections were highest in males age 20-29 and females 15-24.\textsuperscript{5} Gonorrhea disproportionately affects people of color. In 2015, the rate of reported gonorrhea infections was highest among African Americans (425 per 100,000), Native Americans (193 per 100,000), Pacific Islanders (123 per 100,000), and Hispanics (81 per 100,000), versus whites (44.2 per 100,000) and Asians (22.9 per 100,000).\textsuperscript{5}
**BIOLOGY AND PATHOGENESIS OF NEISSERIA GONORRHOEAE**

*Neisseria gonorrhoeae*, the causative agent of gonorrhea, is a fastidious bacterium. *N. gonorrhoeae* belongs to class β-proteobacteria, order Neisseriales, family *Neisseriaceae*, genus *Neisseria*. Members of the genus *Neisseria* are oxidase-positive, gram-negative coccoid or rod-shaped bacteria that frequently occur in pairs or short chains (commonly called diplococci). *Neisseria* inhabit the mucous membranes of warm-blooded hosts, and pathogenic species (*N. gonorrhoeae* in particular) are more nutritionally demanding. *Neisseria* are capnophilic organisms (i.e., their growth is stimulated by carbon dioxide and humidity). *Neisseria* can be grown in vitro on enriched media (e.g., chocolate agar) or selective media (e.g., Modified Thayer-Martin agar) when incubated at 35-37°C, but does not grow well in liquid culture. *Neisseria* are fastidious organisms, intolerant to inhibitors or chemicals that destabilize the outer membrane of the cell. *N. gonorrhoeae* cells (commonly called gonococci) tend to lyse spontaneously as soon as their growth ceases. Addition of cations such as Mg\(^{2+}\) or mercuric ions has been shown to prevent or delay autolysis, however long term survival in broth culture or transport media has not been proven.

Despite its fastidious nature, *N. gonorrhoeae* is quite a successful pathogen. *N. gonorrhoeae* undergoes phase variation to change the expression of adhesion pili, the structures used to attach to epithelial cells. The *N. gonorrhoeae* genome contains one copy of the *pilE* gene and 20 copies of the *pilS* gene, allowing for greater genetic diversity of the antigenic pili structures. Similarly, *N. gonorrhoeae* contains 8-13 copies of *opa* and *opc* genes, allowing for antigenic variation of outer membrane proteins that facilitate intimate adherence to host cells. Antigenic variation of the lipooligosaccharide, the surface lipid sugar molecule responsible for inflammation and epithelial cell invasion, occurs by slipped-strand misrepair and sialylation during...
different stages of infection.\textsuperscript{13, 15} In addition, polyplody of gonococcal cell units contributes to a high frequency of antigenic variation.\textsuperscript{14} Antigenic and phase variation allow \textit{N. gonorrhoeae} to evade the adaptive immune response, thus no vaccine is currently available for gonorrhea prevention.

Gonococcal infections resist clearance by the innate immune response by two different mechanisms. Once ingested by host neutrophils, \textit{N. gonorrhoeae} survives oxidative burst in the phagolysosome through expression of superoxide dismutase, catalase, and cytochrome C peroxidase.\textsuperscript{16} Sialylated lipooligosaccharide increases the stability of opacity and porin surface proteins by inhibiting the activity of cathepsin G, a protease found in the phagolysosome of neutrophils.\textsuperscript{15, 16} Further, the expression of \textit{mtrCDE} and \textit{farAB} efflux pumps assists in excreting toxic macromolecules from the gonococcal cell unit.\textsuperscript{13, 16} In this sense, \textit{N. gonorrhoeae} has adopted several mechanisms to withstand attack by the innate immune system.

\textit{N. gonorrhoeae} uses five different mechanisms of iron acquisition to overcome nutritional immunity in the host. \textit{N. gonorrhoeae} uses four different receptors that bind and remove iron from human hemoglobin, hemoglobin-haptoglobin complexes, lactoferrin, and transferrin.\textsuperscript{17} The transferrin binding protein (encoded by \textit{tbpAB}) and lactoferrin binding protein (encoded by \textit{lbpAB}) are extremely important for gonococcal survival in vivo.\textsuperscript{17} Under iron-replete conditions, \textit{N. gonorrhoeae} also produces bacteriocin (a siderophore) to sequester iron in the infected host.\textsuperscript{13, 17} These virulence factors are listed in Table 1.1.

\textbf{ANTIMICROBIAL DRUG RESISTANCE IN \textit{N. GONORRHOEAE}}

One of the primary challenges associated with detection and surveillance of gonorrhea is that the gonococcus is now multidrug resistant as a result of evolutionary selection pressure. Since
the discovery of penicillin by Alexander Flemming in 1929, antibiotics have been used in medicine to treat various bacterial infections like gonorrhea. Antibiotics such as penicillin are selectively toxic to bacteria, though in varying degrees depending on whether the bacteria are gram positive or gram negative.\textsuperscript{18} We know now that different antibiotic classes are more or less toxic to bacteria depending on the target organism’s proteome, cell wall structure, and genome.\textsuperscript{19} Soon after the introduction of penicillin in the 1940s, patients became infected with penicillin-resistant bacteria that could transfer penicillin-resistance genes to other bacteria on mobile genetic elements called plasmids.\textsuperscript{20} Stuart B. Levy argued that half of the “drug resistance equation” encompasses the evolutionary selective pressure imposed by antibiotic use which, in turn, affects expression of drug-resistance genes in the microbial host.\textsuperscript{20} Proof of this concept was demonstrated when drug-resistant \textit{Escherichia coli} were isolated from chickens that ate antibiotic-supplemented animal feed.\textsuperscript{21} Low doses of antibiotics in the microbial host select against flora or pathogens that are susceptible to the antibiotic while creating a selective advantage for drug-resistant bacteria.

As a result of selection pressure, \textit{N. gonorrhoeae} has evolved several different mechanisms of drug resistance. \textit{N. gonorrhoeae} acquired the plasmid that encodes \(\beta\)-lactamase, the enzyme that degrades penicillin, from other penicillinase-producing bacteria through conjugation.\textsuperscript{22} Similarly, tetracycline resistance resulted from the acquisition of plasmids that encode the \textit{tetA} antiporter and \textit{tetR} ribosome-protection protein.\textsuperscript{23} Acquisition of these plasmids occurs because gonococci readily exchange genetic information with other bacteria.\textsuperscript{24} Acquisition of plasmids is not the only means of drug resistance in \textit{N. gonorrhoeae}. High-level tetracycline resistance may result from a single point mutation (V57M) in the \textit{rspJ} gene encoding ribosomal protein S10.\textsuperscript{23} Recently, Magnus Unemo and colleagues reported an \textit{N. gonorrhoeae} strain with high-level spectinomycin resistance resulting from a deletion of codon 27 (valine) and a K28E alteration in
the ribosomal protein 5S. Accumulation of chromosomal mutations in gyrA (S91F, D95N or D95G) and parC (S88P and E91G) confer resistance to fluoroquinolones. The combination of mutations in the mtrR efflux pump repressor protein and the 23s rRNA confer high-level resistance to azythromycin. Recently, gonococci with a mosaic-like structure of the penicillin binding protein (penA mosaic) have been associated with reduced susceptibility to oral cepahlosporins, the last class of antibiotics available to treat gonorrhea. The emergence of N. gonorrhoeae with reduced susceptibility to oral cepahlosporins has raised concerns for the waning efficacy of antibiotics and impending dawn of untreatable gonorrhea.

DETECTION AND SURVEILLANCE OF GONORRHEA AT THE END OF THE ANTIBIOTIC ERA

In 2013, the CDC named drug-resistant N. gonorrhoeae as one of the top three most urgent bacterial threats that are becoming resistant to antibiotics. The growing rate of antibiotic resistance and the high variability of susceptible strains make methods for rapidly characterizing antibiotic resistance a priority for effective management of gonococcal infections. Failure to characterize a multidrug-resistant strain can lead to a higher rate of treatment failures. Treatment failures are particularly concerning for men who have sex with men (MSM), because inflammation caused by N. gonorrhoeae facilitates the transmission of HIV. To date, the response to these challenges from the public health community has been to recommend treatment for all cases of confirmed N. gonorrhoeae with the latest antibiotic available (e.g., ceftriaxone). Since no new antibiotics exist as treatment alternatives, we need to change our disease control response to gain more utility out of the antibiotics that are currently available.
Management of drug-resistant bacterial infections depends on timely laboratory data and enumeration of the bacterium’s drug resistance profile. Currently, the gold standard for *N. gonorrhoeae* antimicrobial susceptibility testing (AST) is agar dilution, a method in which bacterial suspensions are incubated with varying concentrations of antibiotics and subsequently plated on agar to determine the minimum inhibitory concentration (MIC).\textsuperscript{35, 36} Although agar dilution is quantitative and definitive, this method is labor intensive and only performed by reference laboratories. An alternative method is the epsilometer test (E-test) in which a strip with varying concentrations of antibiotic is placed on a lawn of gonococci; the resulting zone of inhibition can be used to interpret MIC.\textsuperscript{37} Although E-test is much easier to perform than agar dilution, interpretation of the test is highly subjective and diffusion of the antibiotic into the agar matrix varies depending on the size and chemical structure of the antibiotic.\textsuperscript{37} Finally, few laboratories currently perform *N. gonorrhoeae* AST since most clinical and public health laboratories have switched to molecular detection methods that do not allow recovery of viable *N. gonorrhoeae* cells.\textsuperscript{24, 38, 39}

**Chapter 2** provides a critical review of the literature to ascertain whether improvements could be made to the *N. gonorrhoeae* screening and treatment practices that would help to stem the tide of drug resistance in this organism. The review addresses three questions about the state of gonorrhea surveillance and testing in the global context: What are the challenges associated with individualized drug susceptibility testing? What are the implications of nucleic acid amplification testing? What is the recommended strategy to stem the tide of drug-resistant *N. gonorrhoeae*?
Like gonorrhea, syphilis is a re-emerging STI with important ramifications when left untreated. However, the clinical presentation and biology of syphilis infections are quite different. Syphilis is an acute and chronic treponemal disease characterized by a primary lesion, a secondary eruption involving skin and mucous membranes, long periods of latency, and late lesions of the bone, skin, viscera, central nervous system (CNS), and cardiovascular system. The primary chancre usually appears 3 weeks after infection as an indurated, painless ulcer with exudate at the site of initial invasion; infection may occur without a clinically evident chancre (e.g., in the rectum or on the cervix). Regional lymph nodes may be enlarged, but are rarely tender. Next, invasion of the bloodstream occurs carrying the syphilis bacterium to every tissue in the body. After 4-6 weeks, the primary chancre begins to involute and a generalized secondary eruption of a symmetrical maculopapular rash occurs on the skin, palms, and soles with associated lymphadenopathy. Disruption of the infected host’s mucosa and skin during the primary and secondary stage of syphilis can help facilitate the transmission of HIV. The secondary infection resolves spontaneously within 12 weeks and all untreated infections will become latent for weeks to years. One-third of untreated latent infections will progress to tertiary syphilis. Neurosyphilis (syphilitic meningitis) may occur at any time during the secondary or latent stages of infection and is exacerbated by HIV infection. Tertiary syphilis may also manifest as the formation of gummas in the skin, bone, viscera, aorta (cardiovascular syphilis) and mucosal surfaces. Tertiary syphilis ultimately results in impaired health, disability, disfigurement, or death. Congenital syphilis infections occur with high frequency in pregnant women with untreated early syphilis infections. Syphilis frequently causes abortion or stillbirth and may cause infant
death in up to 80% of congenital infections through preterm delivery or from generalized systemic
disease.3,5,41,44

Although incidence rates fell dramatically in the second half of the 20th century, national
and local rates of syphilis have increased in recent years. Incidence rates of primary and secondary
syphilis fell dramatically since the late 1940s after the introduction of penicillin.45 Moreover, the
widespread use of penicillin has decreased the frequency of late manifestations.3 Since 2001,
national and local rates of syphilis have increased, seemingly confined to epidemics among
homosexual males.45 The WHO estimates 6 million people are infected with syphilis every year
with an estimated 300,000 fetal and neonatal deaths.4 In 2001 the rate of primary and secondary
syphilis in the United States was 2.1 cases per 100,000, the lowest rate since syphilis reporting
began in 1941.5 Unfortunately, the rate of syphilis has increased every year since 2001 and was
reported to be 7.5 cases per 100,000 population in 2015 (a 19% increase).5 During this time, the
rise of syphilis was primarily among MSM, however during 2013-2015 the rate of syphilis
increased 27.3% in women.5 The rate of syphilis infection in California is nearly twice the national
average (12.6 per 100,000) with most cases concentrated in Los Angeles County (43.8 per
100,000).5 As was the case with gonorrhea, syphilis disproportionately affects people of color. In
2015, the rate of reported syphilis infections was highest among African Americans (21.4 per
100,000), Pacific Islanders (10.4 per 100,000), Hispanics (9.1 per 100,000), and Native Americans
(5.6 per 100,000) versus Whites (4.1 per 100,000) and Asians (3.0 per 100,000).5 The underlying
causes for increasing rates of syphilis are still unknown.
Treponema pallidum, the causative agent of syphilis, is one of five bacteria that belong to the genus Treponema (order Spirochetales, family Spirochetaceae). All three subspecies of T. pallidum are human pathogens: T. pallidum subsp. pallidum (venereal syphilis), T. pallidum subsp. pertenue (yaws), and T. pallidum subsp. endemicum (endemic syphilis). Members of the Treponema genus (commonly called treponemes) are microaerophilic gram-negative spirochetes, bacteria that are coiled into regular helices. Treponema are motile bacteria that possess an endoflagellum, exhibiting rapid rotation about the axis (also called “corkscrew motility”) and flexing visible by darkfield or phase-contrast microscopy. Treponemes are fragile organisms that cannot be grown in vitro or stained by conventional methods (i.e., Gram stain). Cultivation of T. pallidum is only possible in an animal model.

T. pallidum is known as the “stealth pathogen” since the cellular surface of this organism is antigenically inert. T. pallidum lacks lipopolysaccharide, the cause of classic inflammatory responses in the host, commonly found among gram-negative bacteria. Unlike other gram-negative bacteria or spirochetes, the outer membrane of T. pallidum contains very few integral and outer-membrane proteins. Further, T. pallidum evades the host’s immune response by antigenic variation of surface antigens such as TrpK. For these reasons, previous attempts to develop a vaccine for syphilis have been unsuccessful.

Before the onset of clinical manifestations, T. pallidum begins to multiply locally at the site of infection and disseminate through blood vessels and lymphatics. Approximately 2-4 weeks after infection, the host produces IgM and IgG antibodies specific for T. pallidum surface lipoproteins called treponemal antibodies. Treponemal antibody titers typically last for the lifetime of the infected host. Host cells and blood vessels damaged by T. pallidum release lipoidal
material during the initial stage of infection, stimulating the production of anti-lipoidal IgM and IgG antibodies known as reagin.\textsuperscript{41, 50, 51} Reagin, or nontreponemal antibodies, are usually produced 4-6 weeks after infection and titers decrease following resolution of the secondary stage of syphilis or treatment.\textsuperscript{50, 51}

**CHANGING PARADIGMS IN THE LABORATORY DETECTION OF SYPHILIS**

Serology is the mainstay for syphilis detection since *T. pallidum* cannot be grown in vitro and the sensitivity of molecular assays (e.g., polymerase chain reaction, PCR) decrease sharply after the secondary stage of infection.\textsuperscript{41, 51} Most laboratories use nontreponemal antibody tests to screen patients for syphilis. Nontreponemal tests such as Rapid Plasma Reagin (RPR) or the Venereal Disease Research Laboratory (VDRL) test use cardiolipin, lecithin, and cholesterol antigens to agglutinate reagin from patient serum.\textsuperscript{41} Nontreponemal tests are mainly reactive during early syphilis (primary, secondary, and early latent stages), affording high sensitivity for detecting current infections.\textsuperscript{41} In addition, nontreponemal tests are semi-quantitative and can be used to monitor treatment.\textsuperscript{41} However, reagin antibodies are produced as a result of other infections (e.g., lyme disease) or auto-immune disorders (e.g., lupus) and may cross-react even if the patient is not currently infected with *T. pallidum*.\textsuperscript{41, 51} Further, nontreponemal tests are subject to the prozone effect in which patients with a high reagin titer may test negative.\textsuperscript{41, 51}

In a traditional syphilis-screening algorithm, reactive nontreponemal tests are confirmed with a treponemal antibody test.\textsuperscript{52} Some treponemal tests such as *Treponema pallidum* particle agglutination (TP-PA) detect treponemal antibodies using particles sensitized with treponemal antigens.\textsuperscript{41} Other treponemal tests such as fluorescent treponemal antibody absorption (FTA-Abs) use fixed *T. pallidum* cells for indirect fluorescent antibody microscopy.\textsuperscript{41} The high specificity of
treponemal assays makes them ideal confirmatory tests for all stages of syphilis infection. However, qualitative treponemal immunoassays are interpreted subjectively and can be labor intensive.

Recently, enzyme immunoassays (EIA) and chemiluminescence immunoassays (CIA) have been developed for the detection of treponemal antibodies. Both EIA and CIA platforms use recombinant *T. pallidum* lipoproteins (e.g., Tp15, Tp17, or Tp47) to form antigen-antibody complexes detectable by enzyme-substrate interactions or chemiluminescence reactions. Treponemal immunoassays are marketed as highly sensitive and specific syphilis screening tests and are amenable to automation. Increasingly, laboratories are switching their testing algorithms to screen with an automated treponemal immunoassay and reflex test with a nontreponemal assay. The U.S. Centers for Disease Control and Prevention recommends the traditional screening algorithm because more false positives are detected by reverse algorithm screening in low-prevalence populations. However, previous reports in the literature have suggested that reverse algorithm is more sensitive for detecting syphilis at all stages of infection (Table 1.2).

In the case of syphilis, a key question for public health laboratories surrounds how changes in the approach to detection of syphilis might affect treatment outcomes at a population level. One question that has arisen is whether some of the increased incidence of syphilis could be due to limitations in the sensitivity and specificity of the traditional screening algorithm. In Chapter 3, we compare the performance (diagnostic sensitivity & specificity, percent agreement, and turnaround time) of three high-throughput syphilis serology assays: Phoenix Biotech Trep-Sure Syphilis Total Antibody EIA, Siemens Advia Centaur Syphilis CIA, and the DiaSorin Liaison Treponema CIA. Additionally, we compare the traditional syphilis-screening algorithm with a reverse algorithm in which any of the three high-throughput assays were used as a screening test.
In Chapter 4, we present a cost-effectiveness model to compare the laboratory costs and outcomes that would be obtained using either the traditional or reverse syphilis-screening algorithms in Los Angeles County. The model was tested using data from the Los Angeles County Department of Public Health and the results from the method comparison study reported in Chapter 3. Further, results from a survey of 34 California Public Health Laboratory Directors were used to determine how the results from Los Angeles County could be generalized to other public health jurisdictions across the state.

Although mostly unrecognized, the work of public health laboratories profoundly affects the communities they serve. Public Health Laboratory Directors and stakeholders can use the mixed-methods approaches discussed here to make decisions that balance cost with adapting to the dynamic nature of re-emerging STIs. Updating antimicrobial susceptibility testing and molecular detection methods can help laboratories respond to multidrug resistant *N. gonorrhoeae* in a shorter amount of time and augment current surveillance practices. Implementing a reverse syphilis-screening algorithm can potentially identify clusters of cases missed by traditional algorithm screening while reducing public health laboratory costs. These approaches have the potential to decrease the prevalence of these re-emerging STIs in our communities.
Table 1.1. Summary of key virulence factors found in *N. gonorrhoeae* and their functions.

<table>
<thead>
<tr>
<th><em>N. gonorrhoeae</em> Gene</th>
<th>Gene Product</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>pilE / pilS</td>
<td>Pillin Protein</td>
<td>Attachment to host epithelial cells</td>
</tr>
<tr>
<td>opa / opc</td>
<td>Opacity Protein</td>
<td>Intimate attachment to host epithelial cells</td>
</tr>
<tr>
<td>porAB</td>
<td>Porin Protein</td>
<td>Anion specific porin for reducing PMN oxidative burst</td>
</tr>
<tr>
<td>lgtABCDE</td>
<td>Lipooligosaccharide</td>
<td>Adhesion and invasion of host epithelial cells, inhibit cathepsin G</td>
</tr>
<tr>
<td>sodB</td>
<td>Superoxide dismutase</td>
<td>Reduction of reactive oxygen species in the PMN phagolysosome</td>
</tr>
<tr>
<td>katA</td>
<td>Catalase</td>
<td>Reduction of reactive oxygen species in the PMN phagolysosome</td>
</tr>
<tr>
<td>ccp</td>
<td>Cytochrome C Peroxidase</td>
<td>Reduction of reactive oxygen species in the PMN phagolysosome</td>
</tr>
<tr>
<td>mtrCDE</td>
<td>Efflux Pump</td>
<td>Excretion of toxic macromolecules from the gonococcal cell</td>
</tr>
<tr>
<td>farAB</td>
<td>Efflux Pump</td>
<td>Excretion of toxic macromolecules from the gonococcal cell</td>
</tr>
<tr>
<td>lpbAB</td>
<td>Lactoferrin Binding Protein</td>
<td>Surface receptor, binds Fe$^{2+}$ from lactoferrin</td>
</tr>
<tr>
<td>tbpAB</td>
<td>Transferrin Binding Protein</td>
<td>Surface receptor, binds Fe$^{2+}$ from transferrin</td>
</tr>
<tr>
<td>hmbR</td>
<td>Hemoglobin Binding Protein</td>
<td>Surface receptor, binds Fe$^{2+}$ from haemoglobin (Hb)</td>
</tr>
<tr>
<td>hpuAB</td>
<td>Hemoglobin-Haptoglobin Uptake Receptor</td>
<td>Surface receptor, binds Fe$^{2+}$ from Hb-Hap complex</td>
</tr>
<tr>
<td>fetA</td>
<td>Bacteriocin</td>
<td>Siderophore for iron sequestration</td>
</tr>
</tbody>
</table>
Table 1.2. A comparison of serodiagnosis of known positive samples for three syphilis-screening algorithms at different stages of syphilis.56

<table>
<thead>
<tr>
<th>Stage of Syphilis</th>
<th>Percentage of Positive Serodiagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Traditional Algorithm</td>
</tr>
<tr>
<td>Primary</td>
<td>75%</td>
</tr>
<tr>
<td>Secondary</td>
<td>99%</td>
</tr>
<tr>
<td>Early Latent</td>
<td>78%</td>
</tr>
<tr>
<td>Late Latent</td>
<td>73%</td>
</tr>
<tr>
<td>Tertiary</td>
<td>68%</td>
</tr>
</tbody>
</table>
REFERENCES:


CHAPTER 2

Stemming the Tide of Drug Resistant *Neisseria gonorrhoeae*: The Need for an Individualized Approach to Treatment

(This chapter was published in the *Journal of Antimicrobial Chemotherapy*, 2015, 70: 374-381)

ABSTRACT

Drug-resistant *Neisseria gonorrhoeae* poses a significant public health challenge. In recent years, gonococci resistant to first and second-line antibiotics have spread worldwide and new strains have developed that are increasingly resistant to third-generation cephalosporins, which are currently our last line of available treatments. Given the timeline required to develop new drugs or an effective vaccine for *N. gonorrhoeae*, a top priority is to use the drugs that we have available as effectively as possible.

Currently, gonorrhea clinical management worldwide is based upon treatment guidelines informed by international gonococcal antimicrobial susceptibility/surveillance programs. This approach, although currently the most practical, is subject to a number of limitations since surveillance data inherently provide population-level information. As a result, basing treatment guidelines on these data can result in prescription of more aggressive or broader treatments than may be needed for a specific individual and hence inadvertently contribute to the development and spread of resistance to existing drugs.

Clearly, methods are needed that would provide patient-specific drug susceptibility information in a timeframe that would allow clinicians to prescribe individualized treatment
regimens for gonorrhea. Fortunately, in recent years, there have been a number of advances in the development of rapid methods for characterizing both genotype and drug resistance phenotypes of \textit{N. gonorrhoeae} strains. Here, we review these advances and propose additional studies that would help facilitate a transition toward an individualized treatment approach for gonorrhea.

\textbf{INTRODUCTION}

New strains of multidrug resistant (MDR) \textit{Neisseria gonorrhoeae} have emerged globally over the span of the past 10 years. The prevalence of gonococci that resist both first and second-line antibiotics has increased dramatically in recent years.\textsuperscript{3} Treatment failures and \textit{N. gonorrhoeae} strains with reduced susceptibility to antibiotics are being reported across Asia, Europe, and now North America (\textbf{Figure 2.1}). These reports reveal an alarming waning efficacy of third-generation cephalosporins, the last line of antibiotics available to treat gonorrhea infections.\textsuperscript{1,2} Under the selection pressure imposed by using broad-spectrum antibiotics, horizontal gene transfer from other \textit{Neisseria} species, especially those that live in the oropharynx, enables \textit{N. gonorrhoeae} to acquire new drug resistance mechanisms;\textsuperscript{4} the \textit{penA} mosaic, which has a high positive predictive value for cephalosporin resistance, is one such example.\textsuperscript{2,5} This bacterium’s ability to acquire and sustain chromosomally-mediated forms of drug resistance poses a unique public health challenge to the control of HIV as well. Treatment failures in gonorrhea increase the risk of HIV transmission in populations heavily impacted by gonorrhea infection, such as men who have sex with men (MSM).\textsuperscript{6,7}

To keep physicians apprised of the latest trends in drug resistance in gonorrhea, the European Centre for Disease Prevention and Control (ECDC) reports surveillance data describing trends in changing antimicrobial break points by country as part of the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP).\textsuperscript{8} In the UK, gonococcal surveillance and
treatment recommendations appear in the annual report from the Gonococcal Resistance to Antibiotics Surveillance Programme (GRASP).\(^9\) Similarly in the USA, the Centers for Disease Control and Prevention (CDC) issues treatment guidelines based on data collected from the Gonococcal Isolate Surveillance Project (GISP).\(^9\) The CDC revises treatment guidelines when treatment failures are observed and a high prevalence of resistant or multiply resistant strains are reported in the surveyed population.\(^10\) The WHO recommends that only drugs with an expected efficacy of 95% and above should be used as first line therapy to treat gonorrhea.\(^11\) The current USA treatment guidelines recommend combination therapy with ceftriaxone and azithromycin or doxycycline.\(^10\) ECDC has published comparable treatment recommendations as informed by Euro-GASP.\(^8\) These guidelines encourage clinicians to treat all gonorrhea cases very broadly by prescribing these antibiotics even where little or no drug resistance has been reported. As a result, even those gonococcal infections that would be treated effectively with other drugs like amoxicillin would receive the broadest treatment available.\(^12\) Historically, this has been problematic because \textit{N. gonorrhoeae} drug resistance has quickly followed the introduction of new antibiotics (Figure 2.2). In general, widespread use of an antibiotic decreases its efficacy over time by promoting drug resistance through selective pressure.\(^13\) Conversely, decreasing the use of an antibiotic reduces the prevalence of drug resistance to that particular antibiotic over time.\(^14\)

We would be able to slow the development of resistance to extended spectrum cephalosporins (ESCs) if each patient were treated with the narrowest, but still effective, treatment for his/her own infection. To be able to implement that approach, however, clinicians would need to know the drug susceptibility or resistance phenotype for their patients’ infections in real-time, or near real-time, so that these data could inform their prescription choices. This approach, which
has been previously shown to decrease turnaround time and reduce antibiotic usage,\textsuperscript{15} could extend the utility of the drugs we currently have to treat gonorrhea infections.

**WHAT ARE THE CHALLENGES ASSOCIATED WITH INDIVIDUALIZED DRUG SUSCEPTIBILITY TESTING?**

Ideally, real-time assays or near real-time tests would best inform the clinician of the patient’s gonorrhea drug phenotype while the patient is still in the clinic. Although we have reliable molecular methods for detecting \textit{N. gonorrhoeae}, there is no commercially available molecular method to characterize \textit{N. gonorrhoeae} drug susceptibility. Moreover, little has been done to improve and speed up cell culture based antimicrobial susceptibility testing (AST). Agar dilution is the current gold standard for performing AST on \textit{N. gonorrhoeae} as recommended by the U.S. CDC and CLSI.\textsuperscript{16,17} Despite the reliability of the data it produces, agar dilution is time consuming and labor intensive, which poses a problem for smaller microbiology or hospital labs. Other AST cell-culture based methods such as the Epsillometer test (E-test) and disc diffusion still take 24 hours at the minimum to generate data and must be performed by experienced lab technologists or microbiologists to ensure comparable results to the gold standard. Broth microdilution methods for AST of \textit{N. gonorrhoeae} have yet to be robust enough for clinical application. However, recent studies have shown progress toward developing a media that supports the growth of this fastidious bacterium.\textsuperscript{18-21} Without additional enhancement to the existing AST methods for gonorrhea, few improvements can be made in drug susceptibility testing and surveillance response times.

Furthermore, most clinical laboratories do not receive clinical specimens allowing for the routine performance of AST on \textit{N. gonorrhoeae}. The introduction of molecular diagnostics for the detection of gonorrhea has extensively replaced culture and isolation methods.\textsuperscript{22} Instead,
gonorrhea surveillance in the USA is conducted by the CDC in concert with a network of clinicians and public health laboratories through GISP. GISP reference laboratories characterize the drug susceptibilities of each isolate collected from sentinel sites across the USA using the agar dilution method.\textsuperscript{23} Performing this assay on aggregated isolates submitted by participating public health laboratories in most cases holds back results for submitted cultures for 3-6 months. This lag time in reporting AST values back to local public health authorities means that it is impractical to use individual GISP results to optimize treatment choices for specific patients. Instead, the goal of GISP is to provide population level information. The CDC reports the aggregate GISP data annually, showing the current trends in gonorrhea drug susceptibility and demographics in populations across the USA.\textsuperscript{24} These results are used to modify treatment guidelines and recommendations in the Morbidity and Mortality Weekly Report (MMWR).\textsuperscript{10} Similarly, the GRASP surveillance data and BASHH guidelines for gonorrhea testing in the UK are reported annually.\textsuperscript{9, 10} Further, the ECDC reports their surveillance data annually and describes GC resistance rates in Europe.\textsuperscript{8}

Because these gonorrhea surveillance programs provide data on the population level, sampling biases can confound observed trends.\textsuperscript{8} Rates of drug resistance in gonorrhea are higher in MSM than in men who have sex with women (MSW) due to different behavior in these sub-populations.\textsuperscript{7, 25, 26} Although some overlap occurs between sexual networks, \textit{N. gonorrhoeae} strains are more or less conserved among MSM compared to MSW.\textsuperscript{27, 28} Depending on the percentage of MSM obtaining services at sentinel sites, there may be oversampling showing an overall higher incidence of drug resistant gonorrhea.\textsuperscript{9} A recent study traced the spread of the \textit{penA} mosaic largely through an MSM sexual network across the USA.\textsuperscript{29} With the inclusion of samples taken from MSM patients the GISP program successfully identified emerging \textit{N. gonorrhoeae} with
reduced susceptibility to cephalosporins in the USA. This sampling design favors the sensitive and early detection of MDR *N. gonorrhoeae* strains circulating in the population. However, the current sampling strategy may not reflect the actual distribution of gonorrhea with reduced susceptibility to cephalosporins in the overall population. The ECDC has recognized this form of sampling bias as a limitation to these surveillance strategies. By encouraging clinicians to treat patients based upon the initial incidence of cephalosporin resistant *N. gonorrhoeae* circulating in the general population rather than tailoring treatment to that specific patient, we decrease the utility of our already limited antibiotic arsenal.

**IMPLICATIONS OF NUCLEIC ACID AMPLIFICATION TESTING**

Ideally, drug susceptibility information would be gathered for individual patients when they get tested for gonorrhea. However, gonorrhea AST relies on cell culture based methods, such as agar dilution or disk diffusion, which most laboratories no longer perform. Although primarily used by international surveillance programs and for legal purposes, cell culture is not the main method used by laboratories to detect gonorrhea infections. In the USA and the UK, public health and clinical microbiology laboratories have moved from routinely performing culture-based gonorrhea testing to using a variety of nucleic acid based assays: more recently nucleic acid amplification tests (NAATs), especially where there are high volumes of patient samples (Figure 2.3). These NAATs have replaced cell culture due to their high degrees of sensitivity and specificity, which can detect asymptomatic infections during routine patient screening. In addition to these benefits, NAATs have been designed as multiplex assays; many NAAT tests can detect *Chlamydia trachomatis* in addition to *N. gonorrhoeae* in some combination assays. Moreover, NAATs have the capability to detect gonorrhea at different body sites from which it
would be difficult to isolate pure *N. gonorrhoeae* cultures. Conversely, NAATs have their own set of limitations. NAATs are susceptible to false positive results due to the high amount of genetic exchange among commensal *Neisseria* species in pharyngeal samples. Complicating matters, some researchers have reported false negatives by competitive inhibition, or competition between multiple targets for a finite number of reagents, in multiplex NAAT assays for detecting *N. gonorrhoeae* and *C. trachomatis*.

The commercial application and practical utility for PCR-based NAATs that can detect both organism and resistance genes has been demonstrated; for example, Cepheid has developed a GeneXpert assay to quantify infection with *Mycobacterium tuberculosis* and to detect rifampicin resistance genes in primary sputum samples in both low prevalence and high prevalence populations. A validation study of the Cepheid assay, published in the *New England Journal of Medicine*, reported sensitive detection of *M. tuberculosis* and rifampicin resistance in less than 2 hours in samples from high prevalence areas. The decreased turnaround time and improved sensitivity of the *M. tuberculosis*-rifampicin combo assay demonstrates how a NAAT test can be used to quickly and effectively identify drug resistant organisms over traditional culture-based methods. Given the limitations previously described for cell culture, the sensitivity and specificity for NAAT technologies has great potential for moving toward guided treatment for gonorrhea.

Presently, no NAAT platforms that provide drug resistance information for gonorrhea are commercially available. Several PCR-based NAATs have been developed to detect resistance markers in *N. gonorrhoeae*. PCR-based assays have been validated to detect the *gyrA* and *parC* mutations associated with reduced susceptibility to fluoroquinolones as well as the 23s rRNA and *mtrR* mutations associated with elevated MICs to Azithromycin. The previously described mosaic *penA* has been widely associated with decreased susceptibility to cephalosporins,
making these alleles a major target for developing and refining nucleic acid amplification tests to specifically detect cephalosporin resistance.\textsuperscript{49-53} With this approach, NAATs would enable clinicians to treat more specifically to the needs of the patient by detecting common drug resistance markers.\textsuperscript{31, 54} Designing new NAATs for identifying drug resistance markers in clinical samples would be easy to implement in hospital and clinical labs that already rely on NAATs for clinical diagnosis.

While the prospect of using NAATs to obtain antibiotic resistance information in near real time shows promise, the rapid evolution of gonorrhea remains an ongoing challenge. Gonococci easily acquire new plasmid and chromosomally-mediated resistance genes to counteract new antibiotics. Multiplex PCR and other NAATs can be used to detect known drug resistance markers, but will not detect novel ones.\textsuperscript{40} The recent discovery of the mosaic \textit{penA} gene demonstrates that not all of the drug resistance mechanisms employed by \textit{Neisseria spp.} have been clearly defined. For example, the variability between \textit{penA} mosaic alleles can lead to different MICs, requiring genomic sequencing to identify those alleles that contribute to cephalosporin resistance.\textsuperscript{5} Moreover, most \textit{penA} mosaics originate in commensal \textit{Neisseria}, making pharyngeal detection of drug-resistant gonorrhea more problematic.\textsuperscript{5} However, NAAT testing can be employed to perform molecular surveillance for well-known drug resistance markers in the population. Speers et al. demonstrated that NAATs could be used to determine local drug resistance patterns in Western Australia, resulting in evidence-based treatment guidelines for patients in that area.\textsuperscript{12} Employing molecular surveillance for gonorrhea drug resistance markers can elucidate these patterns across the globe, enabling local communities to generate evidence-based treatment guidelines.

Although the Western Australia case study demonstrates that NAATs can be used to develop evidence-based treatment guidelines for local jurisdictions, this example highlights why
NAATs cannot be the entire solution. NAATs can be used to reliably detect the TEM-1 and TetM plasmids, conferring penicillin and tetracycline resistance respectively, as well as mutations in the \( gyrA \), \( parC \), and \( mtrR \) genes using PCR-based methods. However, NAATs are less reliable for detecting cephalosporin resistance and azithromycin resistance because of the high sequence variability in the \( penA \) and the 23s rRNA alleles in gonorrhea. Until we can design reliable NAATs to characterize these genetic determinants for cephalosporin and azithromycin resistance, we should expand our culture-based surveillance system and enhance with molecular surveillance. Using this approach, health jurisdictions can better identify the resistance patterns within their communities while informing local treatment guidelines with molecular and culture-based data. This requires isolation of live samples from patients, which can be problematic for laboratories that have abandoned cell culture in order to run NAATs exclusively. However, the presented approach is paramount to understanding the changing landscape of \( N. \) gonorrhoeae drug resistance across the globe.

**RECOMMENDATIONS**

Based on the presented challenges of current drug susceptibility testing and the implications of NAAT testing for gonorrhea, new strategies are needed to detect and treat gonorrhea infections while maintaining an active surveillance program to detect new resistance patterns. Adding drug resistance marker detection markers to current NAAT gonorrhea detection platforms could fundamentally alter patient management from surveillance-based treatment guidelines to individualized treatment based on timely laboratory data. Switching the paradigm to an individualized treatment strategy would lower the evolutionary selection pressure on last line antibiotics while simultaneously gaining greater longevity from our older antibiotics. Patients
without MDR gonorrhea could be treated with combination therapy using these older antibiotics so they don’t develop resistance to our last line drugs. This is particularly important for gonorrhea because many forms of gonorrhea drug resistance are chromosomally-mediated and well maintained despite discontinuation of older antibiotics. Since these chromosomally mediated forms of resistance persist, it is important not to use discontinued antibiotics such as ciprofloxacin in monotherapy to treat uncomplicated gonorrhea: rather, to use older antibiotics in combinations that would delay the development of resistance. Speers et al. demonstrated that combination therapy using multiple drugs (amoxicillin, azithromycin, and probenicid) paired with molecular surveillance for penicillinase producing *N. gonorrhoeae* (PPNG) was effective at delaying the emergence of PPNG in Western Australia. Combination therapies customized to the individual patients’ circumstances would slow *N. gonorrhoeae*’s ability to develop and maintain drug resistance towards a singular antibiotic or antibiotic class. We argue that there is a need for localized molecular surveillance to aid in identifying endemic drug resistance patterns to better inform treatment approaches. However, until commercial NAATs can rapidly and reliably detect molecular resistance markers for individual patients we must supplement this data with culture-based AST.

To keep up with the evolution of this organism, we must develop and validate new drug susceptibility testing methods for characterizing gonococci in a shorter amount of time. As discussed previously, research groups have worked toward broth microdilution for cultivation and AST of *N. gonorrhoeae*. Introducing an effective broth microdilution AST system for gonorrhea would decrease the amount of time required to detect drug resistant isolates while simultaneously reducing required materials, storage space, and labor. Furthermore, broth microdilution has the potential for establishing high-throughput screening for antibiotic resistance.
A high-throughput screening system would greatly enhance current gonorrhea surveillance across the globe by reducing the lag time for generating results and allowing for larger data sets to be collected and tested. Until such a system has been validated and implemented, combining the current international surveillance systems with improving NAATs and molecular surveillance for use in laboratories is our best option for stemming the tide of drug-resistant gonorrhea.
Figure 2.1. Gonorrhea global prevalence and reported drug resistance. This map shows the WHO estimated prevalence of gonorrhea globally using a red color gradient. Countries that have reported *N. gonorrhoeae* strains with reduced susceptibility to azithromycin are identified with a white circle (○). Countries that have reported *N. gonorrhoeae* strains with reduced susceptibility to cephalosporins are shown with a black circle (●). Countries that have reported *N. gonorrhoeae* strains with reduced susceptibility to both azithromycin and cephalosporins are shown with a half-black and half-white circle (■). Fluoroquinolone resistance has been documented and is well distributed globally, but was not included in this map to preserve figure clarity. Countries shaded with a diagonal striped pattern (e.g. Greenland, Mongolia, etc.) were not included in the WHO prevalence data. Countries without circles have not yet reported strains of *N. gonorrhoeae* with reduced susceptibility to azithromycin or cephalosporins.³, ⁷, ⁴⁷, ⁵⁷-⁸⁶
Figure 2.2. Timeline of gonorrhea antibiotic treatments and treatment guidelines. This timeline shows when antibiotics like penicillin, spectinomycin, tetracycline, fluoroquinolones, and cephalosporins were used to treat gonorrhea and when drug resistance was first reported for each of these antibiotics. We observe a pattern that drug resistance quickly follows the widespread use of antibiotics. The WHO, ECDC and US CDC changed the corresponding treatment guidelines based on documented *N. gonorrhoeae* strains with reduced susceptibility to those antibiotics. However, changing the treatment protocol imposes new selection pressure on the bacterium, resulting in natural selection favoring the persistence of *N. gonorrhoeae* strains with reduced susceptibility to the current antibiotic treatment.10-26, 50, 87-93
Figure 2.3. 2013 CAP survey results for gonorrhea NAAT testing. This chart illustrates the percentage of different NAAT platforms used to detect *N. gonorrhoeae* in the 2013 College of American Pathologists (CAP) Proficiency Survey. Nine-hundred and five (905) CLIA certified laboratories participated in this proficiency survey. Most laboratories performed TMA using the Aptima Combo 2 Assay (378) followed by SDA using the BD ProbeTek/Viper XTR (222) and PCR using the Roche COBAS Amplicor platform (125). The other CLIA laboratories that participated reported using the BD Viper XTR with extracted samples (79), the Abbott m2000 (30), Commercial Kits (18), the Qiagen Digene Hybrid Capture 2 [hc2] assay (16), the Roche Amplicor (10), in-house Laboratory Developed Methods (10) and other manufacturers identified by less than 10 participating laboratories (15).
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ABSTRACT

We compared the performance and ease of use for three high-throughput treponemal immunoassays: Phoenix Biotech Trep-Sure Total Antibody EIA, Siemens ADVIA® Centaur Syphilis Assay, and DiaSorin LIAISON® Treponema Assay. One thousand serum samples submitted for routine screening were used in this study. Each assay demonstrated comparable sensitivity, specificity, and percent agreement (98-100%) compared with Treponema pallidum particle agglutination (TP-PA). Thus, treponemal immunoassays are an acceptable alternative for syphilis screening or confirmatory testing. Batch sizes and technologist active time varied between each treponemal immunoassay; the chemiluminescence platforms offered significantly greater batch-ability (random access vs. fixed batch sizes) in less time. When we compared the results obtained using a reverse algorithm approach to those obtained using a traditional algorithm, we found that the reverse algorithm identified 38 additional seropositive individuals that were not detected using the traditional algorithm. Clinical evaluation was useful for resolving cases with discordant serology.
INTRODUCTION

Syphilis rates have increased nationwide in the United States since the year 2000, despite intensive disease control efforts and widespread availability of effective treatments.\textsuperscript{1,2} Most cases of primary and secondary syphilis are reported in men who have sex with men (MSM), but rates have increased in both men and women in every region of the United States.\textsuperscript{2} Untreated syphilis infections may result in cardiovascular involvement, congenital infections in pregnant women, or the development of neurosyphilis. HIV patients, in particular, are at an increased risk of developing neurosyphilis.\textsuperscript{3-5} Accurate, rapid, and early diagnosis of syphilis is important for patient management and disease control.\textsuperscript{6}

Laboratory diagnosis of syphilis relies on serological screening methods based on the host’s immune response to \textit{Treponema pallidum}, the causative agent of syphilis.\textsuperscript{1, 7, 8} The traditional testing algorithm involves screening patient serum with a non-treponemal assay (e.g., rapid plasma reagin [RPR]) followed by confirmation of positives using a treponemal assay (e.g., \textit{Treponema pallidum} particle agglutination [TP-PA]). Increasingly, laboratories are adopting a reverse algorithm in which patient samples are screened by an automated treponemal assay (e.g., enzyme immunoassay [EIA] or chemiluminescence immunoassay [CIA]) and positive samples are reflexed to a non-treponemal assay.\textsuperscript{1, 7, 9, 10} Many laboratories switched to the reverse algorithm since treponemal immunoassays offer improved sensitivity and specificity, remove subjectivity in test interpretation, and reduce time to results as well as manual labor costs when automated.\textsuperscript{7, 8, 11, 12} However, variations in treponemal assay performance in low or high prevalence populations as well as difficulties in interpreting RPR-discordant test results underscore the need to verify the performance of these high-throughput treponemal screening tests.\textsuperscript{8, 12, 13} The purpose of this study was to compare the performance of three commercially available treponemal immunoassays and
evaluate their utility as either a confirmatory treponemal test or as a screening test in a reverse algorithm.

MATERIALS AND METHODS

Study Population

One thousand serum samples submitted to the Los Angeles County Public Health Laboratories (LACPHL) for routine syphilis screening were used in this study. Each serum sample came from an individual patient in Los Angeles County. Residents of Pasadena and Long Beach were excluded because they are served by independent public health departments. Samples were collected from an amalgam of sites including STD clinics, hospitals, and community outreach programs. Approximately 60% of the study samples were collected from MSM community outreach programs.

Serological Testing

Samples were tested prospectively during one week following the CDC recommended traditional algorithm. All samples were initially tested by qualitative and quantitative RPR (Arlington Scientific Inc., Springville, UT) and TP-PA (Fujirebio Diagnostics Inc., Japan). Samples were further characterized using the ADVIA® Centaur Syphilis chemiluminometric immunoassay (CS-CIA, Siemens, Germany), LIAISON® Treponema chemiluminescence immunoassay (LT-CIA, DiaSorin Inc., Stillwater, MN) and the Trep-Sure Total Antibody Enzyme Immunoassay (TS-EIA, Trinity Biotech, Ireland). CS-CIA is a direct sandwich chemiluminometric immunoassay that qualitatively detects total (IgM and/or IgG) antibodies against T. pallidum using recombinant TpN15 and TpN17 antigens. The results of the CS-CIA are
calculated as index values and reported as negative (<0.9), equivocal (0.9 to 1.1), or positive (≥1.1). LT-CIA is a qualitative chemiluminescent immunoassay that detects total (IgM and/or IgG) antibodies directed against *T. pallidum* using recombinant TpN17 antigens. The results of the LT-CIA are calculated as index values and reported as negative (<0.9), equivocal (0.9 to 1.10), or positive (≥1.1). TS-EIA is a qualitative enzyme immunoassay that detects total (IgM and/or IgG) antibodies against *T. pallidum* using proprietary recombinant antigens. The results of the TS-EIA are calculated as index values and reported as negative (<0.8), equivocal (0.8 to 1.2), or positive (≥1.2). Fluorescent treponemal antibody absorption (FTA-Abs, Zeus Scientific, Branchburg, NJ) was performed as needed to resolve disagreements between assays. All equivocal, indeterminate, or invalid test results were repeated once on the same test with a different operator and the second test result was used for data analysis. All assays were performed according to the manufacturer’s instructions.

**Immunoassay Operation**

Each assay was performed as recommended by the manufacturer’s instructions. LT-CIA was automated on the Liaison XL instrument and CS-CIA was automated on the Centaur XP instrument. TS-EIA was performed manually with wash steps automated on the ELx50 microplate washer and absorbance values read on the ELx800 microplate reader (Bio Tek, Winooski, VT). The assay time was recorded for each assay step and summed to yield total time to result (sum of total time for all pre-analytical, analytical, and post-analytical steps) and total active time (total time to result minus the incubation time). Maximum batch size for TP-PA and TS-EIA were defined as the maximum number of samples and required controls that could be run on one 96-
well plate. The maximum batch size for CS-CIA and LT-CIA was capped at 120 samples since both assay platforms allowed for continuous sample loading.

**Data Analysis**

Statistical analysis was conducted using Stata/IC version 14. Treponemal immunoassay qualitative results (positive, negative, or equivocal) were compared with TP-PA, our laboratory’s treponemal test of record, to measure accuracy (diagnostic sensitivity, specificity, and overall agreement). TP-PA indeterminate and invalid test results were encoded as “equivocal” for all statistical analyses and accuracy calculations. Traditional and reverse screening algorithms were compared using the qualitative results for all assays performed in this study. These algorithms are illustrated in Figure 3.1. Surveillance records from the Los Angeles County STD Control Program were queried if a sample tested negative by RPR and positive by one or more treponemal assays in order to determine whether or not a patient had been previously infected with syphilis. The Los Angeles County Department of Public Health Institutional Review Board approved this study.

**RESULTS**

**Immunooassay Performance Compared with TP-PA**

The performance parameters for each treponemal immunoassay are summarized in Table 3.1. Statistical analysis revealed no significant differences in agreement between each treponemal immunoassay and TP-PA. Twenty discordant results were observed between the three different treponemal assays and TP-PA (presented in Table 3.2). Nine of the discordant samples are ones for which TP-PA yielded an “invalid” result (which typically reflects nonspecific particle agglutination). In eight of these cases, the results were negative from all of the other assays
including RPR and FTA-Abs. In addition, five samples yielded “equivocal” test results in the TS-EIA. Four samples with at least one equivocal treponemal test were characterized as seropositive by FTA-Abs. The index values for the positive treponemal tests on these four samples were low, possibly because these four samples could have had a low antibody titer. Two of these samples were RPR-positive whereas the other two were RPR-negative.

**Time to Result, Technologist Active Time, and Batch Size**

CS-CIA yielded the shortest time to result (TTR) of 59 minutes for 118 samples and 2 controls. Similarly, LT-CIA had a short TTR of 1 hour and 15 minutes for 118 samples and 2 controls. Each CIA required 30 minutes of technologist active time (TAT) when testing a batch of 120 samples, or a total 0.25 minutes of active time per test. TS-EIA yielded a TTR at 3 hours and 27 minutes when manually testing 88 samples and 8 controls in each 96-well plate. TS-EIA required 1 hour and 27 minutes of TAT when testing 96 samples, or a total of 0.91 minutes of active time per test. TP-PA had a TTR of 2 hours and 42 minutes when testing 22 samples and 2 controls on a 96-well plate. TP-PA required 42 minutes of TAT when testing a full 96-well plate, or a total of 1.75 minutes of active time per test. TP-PA required almost twice as much active time per test than the TS-EIA. These results are summarized in Table 3.3.

**Algorithm Comparison**

Reverse algorithm screening resulted in 151 samples being categorized as seropositive whereas traditional algorithm screening categorized 113 samples as seropositive. Each of the 38 discordant samples repeatedly tested RPR-negative and TP-PA/CIA/EIA-positive. Surveillance records for these individuals revealed that 26 of the RPR-discordant samples came from patients
whom had a previous history of syphilis, 11 samples came from patients whom did not have a previous history of syphilis, and 1 sample came from a patient whom was previously characterized as a biological false positive. Seventeen of the samples from individuals with a previous history of syphilis tested positive by FTA-Abs while the remaining 9 samples could not be tested due to insufficient sample volume. Seven of the 11 samples with no previous history of syphilis tested positive by FTA-Abs while the remaining 4 samples had tested negative by FTA-Abs.

DISCUSSION

Immunoassay index values were useful in interpreting the serology of samples that tested equivocal on at least one treponemal immunoassay. In each case where the sample tested equivocal on one treponemal immunoassay, the index values of the other immunoassays were close to the positive cutoff, possibly as a result of low antibody titers. Samples that tested invalid or indeterminate on TP-PA usually tested negative by all other treponemal immunoassays with index values far below the negative cutoff. In this sense, these treponemal immunoassays are less subjective than TP-PA and could be useful both for laboratories currently using the traditional algorithm as well as laboratories wanting to switch to a reverse algorithm. The differences we observed could be attributed to different antigens used in each treponemal assay.

As reported previously, clinical evaluation was important for resolving discrepancies between non-treponemal and treponemal assays in a reverse algorithm. Of the 38 RPR-discordant patients, 26 patients with a known history of syphilis and positive treponemal antibody serology would not require further investigation unless they displayed clinical signs or symptoms of infection. However, the 11 seropositive and RPR-negative patients we observed would require a follow-up investigation to establish whether or not they had been infected recently or previously.
Depending on the patients’ clinical manifestations or sexual history, some of these test results could lead to unnecessary supplemental testing or therapy. Alternatively, some of these test results could identify early primary or late cases of syphilis previously missed by traditional algorithm screening since treponemal antibodies may appear earlier during the course of infection than nontreponemal antibodies. Along these lines, the 7 FTA-positive samples with no history of syphilis may have been early/acute syphilis infections missed by traditional algorithm screening. Alternatively, these samples may have come from individuals who had previously been treated for syphilis (or whose infections had spontaneously resolved) or who had untreated late-stage (latent or tertiary) syphilis with RPR sero-reversion. Some of these patients may also have tested positive previously at another clinic or hospital outside of Los Angeles County; in this case, their prior test results would not be available in their case files. Without following up directly with these patients or collecting convalescent sera, we cannot definitively say whether or not these discordant samples were seropositive from a previous infection or had an early primary syphilis infection, a limitation of our study.

The 4 FTA-negative samples with no previous history of syphilis may have been EIA/CIA false positives. However, it seems unlikely that these samples were falsely positive since they tested positive on all three treponemal immunoassays and TP-PA. One limitation of our study was that we could not perform analytical sensitivity on these four samples because the sample volumes were too low. H. Jost and colleagues observed that FTA-Abs demonstrated lower analytical sensitivity than TP-PA, LT-CIA, or TS-EIA. The lower analytical sensitivity of FTA-Abs compared with CS-CIA, LT-CIA and TS-EIA could explain why these samples would have a negative FTA-Abs result and a positive result on all other treponemal assays. Alternatively, these
patients could have had low treponemal antibody titers caused by sero-reversion from a past infection.\textsuperscript{18}

The high performance we observed for each treponemal immunoassay could be attributed to the high prevalence of syphilis in our patient population. Investigators reported in previous studies that reverse algorithm screening with treponemal immunoassays in low-prevalence populations detected a high number of false positives.\textsuperscript{19, 20} The disagreements we observed between treponemal immunoassays and TP-PA represented 0.2% of all test results in this study and were not statistically significant. However, disagreements between treponemal assays may arise if the second treponemal assay is less sensitive than the first treponemal assay, invariably casting doubt as to whether or not the first result was a false positive.\textsuperscript{17} For laboratories interested in adopting a reverse algorithm, we recommend choosing screening and confirmatory treponemal assays with similar levels of analytical sensitivity. Doing so may avoid the need to perform additional confirmatory test.

One limitation in our comparison of the time required for each of the assays is that TS-EIA was performed manually herein. An automated ELISA instrument (e.g., DSX, Dynex Technologies) could be used to automate sample loading, washing, and development steps to reduce TAT as well as the risk of developing repetitive motion injuries. However, the total incubation time of 1 hour and 45 minutes places TS-EIA automation squarely behind CS-CIA and LT-CIA with respect to TTR. In contrast, smaller laboratories could benefit from running TS-EIA if they cannot afford to make the capital investment in a CIA instrument but already have an automated ELISA instrument.
CONCLUSION

Currently, the LACPHL screens approximately 250-300 blood samples daily for syphilis using the traditional screening algorithm. Three skilled technologists perform RPR to balance TTR with the high weekly sample volume. If the LACPHL adopted the reverse algorithm using any one of the treponemal immunoassays evaluated in this study, only one technologist would be required to perform syphilis serology, effectively freeing two technologists to perform other duties. Consequently, the weekly laboratory positivity rate of syphilis would increase from 11.3% to 15.1%. Since most public health laboratories do not receive clinical data upon receipt of specimens, the burden of resolving RPR-discordant serology falls to epidemiological investigators to stage the infection and either initiate follow-up investigation or close the case. 19 Considering the high prevalence of syphilis in Los Angeles County, as well as the high sensitivity and specificity of the treponemal assays evaluated in this study, the reverse algorithm is a viable alternative to the traditional approach.14, 20 Laboratories should consult with their local STD control program in addition to considering their current sample volumes, workflows, and syphilis positivity rates before implementing reverse algorithm screening.
Figure 3.1. A diagram of the syphilis screening algorithms evaluated. [Left] The traditional screening algorithm recommended by CDC and currently implemented at the LACPHL. [Right] The reverse screening algorithm recommended by the Association of Public Health Laboratories (APHL) and CDC.
Table 3.1. Performance parameters for the three treponemal immunoassays studied herein compared to *Treponema pallidum* particle agglutination (TP-PA).

<table>
<thead>
<tr>
<th>Assay and Result</th>
<th>TP-PA Result (No. of Samples)</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>Overall % Agreement (95% CI)</th>
<th>( \kappa ) (95% CI)</th>
<th>( \chi^2 ) P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-CIA POS</td>
<td>149</td>
<td>98.7</td>
<td>99.8</td>
<td>97.9</td>
<td>0.94</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CS-CIA NEG</td>
<td>2</td>
<td>(95.3 – 99.8)</td>
<td>(99.1 – 100)</td>
<td>(96.0 - 99.8)</td>
<td>(0.91-0.97)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CS-CIA EQ</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS-CIA POS</td>
<td>150</td>
<td>98.7</td>
<td>100</td>
<td>98.0</td>
<td>0.94</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LT-CIA NEG</td>
<td>2</td>
<td>(95.36 – 99.84)</td>
<td>(99.56 – 100)</td>
<td>(96.11 – 99.93)</td>
<td>(0.91-0.97)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LT-CIA EQ</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS-EIA NEG</td>
<td>147</td>
<td>97.4</td>
<td>100</td>
<td>98.4</td>
<td>0.92</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TS-EIA EQ</td>
<td>4</td>
<td>(93.4 – 99.3)</td>
<td>(99.6 – 100)</td>
<td>(96.4 – 100)</td>
<td>(0.89-0.95)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Note.* The three treponemal assays studied are: ADVIA<sup>®</sup> Centaur Syphilis chemiluminometric immunoassay (CS-CIA), LIAISON<sup>®</sup> Treponema chemiluminescence immunoassay (LT-CIA), and Trep-Sure Total Antibody Enzyme Immunoassay (TS-EIA). The numbers of positive (POS), negative (NEG), and equivocal (EQ) results are reported for each assay; sensitivity, specificity, overall percent agreement, Cohen’s Kappa (\( \kappa \)), and Chi-squared (\( \chi^2 \)) P-values are reported with 95% confidence intervals (CI’s).
Table 3.2. Comparison of Results Obtained from Each of the Essays for Samples that Yielded Discordant Treponemal Assay Results.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>RPR (RPR Titer)</th>
<th>TP-PA</th>
<th>CS-CIA (Index Value)</th>
<th>LT-CIA (Index Value)</th>
<th>TS-EIA (Index Value)</th>
<th>FTA-Abs (Index Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>286</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>EQ (0.960)</td>
<td>NEG</td>
</tr>
<tr>
<td>288</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>EQ (0.866)</td>
<td>NEG</td>
</tr>
<tr>
<td>667</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>EQ (0.965)</td>
<td>NEG</td>
</tr>
<tr>
<td>674</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>EQ (0.541)</td>
<td>NEG</td>
</tr>
<tr>
<td>675</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>EQ (0.178)</td>
<td>NEG</td>
</tr>
<tr>
<td>953</td>
<td>POS (1:2)</td>
<td>POS</td>
<td>POS (1.64)</td>
<td>POS (4.62)</td>
<td>EQ (0.354)</td>
<td>POS (1+)</td>
</tr>
<tr>
<td>82</td>
<td>NEG</td>
<td>INVALID</td>
<td>POS (6.60)</td>
<td>POS (5.42)</td>
<td>EQ (0.708)</td>
<td>POS (1+)</td>
</tr>
<tr>
<td>35</td>
<td>NEG</td>
<td>INVALID</td>
<td>NEG (0.12)</td>
<td>NEG (0.1)</td>
<td>NEG (0.205)</td>
<td>NEG</td>
</tr>
<tr>
<td>152</td>
<td>NEG</td>
<td>INDETERMINATE</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>NEG (0.113)</td>
<td>NEG</td>
</tr>
<tr>
<td>299</td>
<td>NEG</td>
<td>INVALID</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>NEG (0.067)</td>
<td>NEG</td>
</tr>
<tr>
<td>364</td>
<td>NEG</td>
<td>INVALID</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>NEG (0.096)</td>
<td>NEG</td>
</tr>
<tr>
<td>388</td>
<td>NEG</td>
<td>INDETERMINATE</td>
<td>NEG (0.61)</td>
<td>NEG (0.1)</td>
<td>NEG (0.676)</td>
<td>NEG</td>
</tr>
<tr>
<td>513</td>
<td>NEG</td>
<td>INVALID</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>NEG (0.099)</td>
<td>NEG</td>
</tr>
<tr>
<td>659</td>
<td>NEG</td>
<td>INVALID</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>NEG (0.12)</td>
<td>NEG</td>
</tr>
<tr>
<td>699</td>
<td>NEG</td>
<td>INVALID</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>NEG (0.118)</td>
<td>NEG</td>
</tr>
<tr>
<td>757</td>
<td>NEG</td>
<td>INVALID</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>NEG (0.095)</td>
<td>NEG</td>
</tr>
<tr>
<td>940</td>
<td>NEG</td>
<td>INVALID</td>
<td>NEG (0.1)</td>
<td>NEG (0.1)</td>
<td>NEG (0.047)</td>
<td>NEG</td>
</tr>
<tr>
<td>721</td>
<td>NEG</td>
<td>INDETERMINATE</td>
<td>POS (5.48)</td>
<td>EQ (0.98)</td>
<td>POS (&gt;3.0)</td>
<td>POS (1+)</td>
</tr>
<tr>
<td>443</td>
<td>NEG</td>
<td>NEG</td>
<td>EQ (0.97)</td>
<td>POS (2.83)</td>
<td>NEG (0.334)</td>
<td>POS (1+)</td>
</tr>
<tr>
<td>946</td>
<td>POS (1:4)</td>
<td>POS</td>
<td>EQ (0.97)</td>
<td>POS (2.83)</td>
<td>NEG (0.334)</td>
<td>POS (1+)</td>
</tr>
</tbody>
</table>

Note. POS indicates a positive test result; NEG indicates a negative test result, and EQ indicates an equivocal result. Values provided in parentheses represent RPR endpoint titer or immunoassay index values for positive and equivocal results, where an RPR titer of 1:1 means reactive undiluted and an immunoassay index value greater than 1.1 means the sample is positive.
Table 3.3. Comparison of Turnaround Time for Treponemal Assays Studied Herein

<table>
<thead>
<tr>
<th>Method</th>
<th>Maximum No. Samples per Batch</th>
<th>No. Controls per Batch</th>
<th>Total Time to Result (hr : min)</th>
<th>Total Active Time (hr : min)</th>
<th>Active Time per Test (min/test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP-PA</td>
<td>22</td>
<td>2</td>
<td>2:42</td>
<td>0:42</td>
<td>1.75</td>
</tr>
<tr>
<td>CS-CIA</td>
<td>120</td>
<td>2</td>
<td>0:59</td>
<td>0:30</td>
<td>0.25</td>
</tr>
<tr>
<td>LT-CIA</td>
<td>120</td>
<td>2</td>
<td>1:15</td>
<td>0:30</td>
<td>0.25</td>
</tr>
<tr>
<td>TS-EIA</td>
<td>88</td>
<td>8</td>
<td>3:27</td>
<td>1:27</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Note. The three treponemal assays studied are: ADVIA® Centaur Syphilis chemiluminometric immunoassay (CS-CIA), LIAISON® Treponema chemiluminescence immunoassay (LT-CIA), and Trep-Sure Total Antibody Enzyme Immunoassay (TS-EIA).
REFERENCES


CHAPTER 4

Economic Assessment of a Reverse Syphilis Screening Algorithm in a High Prevalence Population

(A manuscript prepared for submission to Sexually Transmitted Diseases)

ABSTRACT

Background: More laboratories are adopting reverse algorithms that use automated treponemal immunoassays to screen for syphilis. We compared direct costs and downstream consequences of reverse algorithm syphilis screening using laboratory and surveillance data from a local public health department.

Methods: We created a decision analysis model to estimate the cost-effectiveness of switching from a traditional algorithm (Nontreponemal screening) to a reverse algorithm (Treponemal screening). We also surveyed 34 California public health laboratories for syphilis testing practices, workload volumes, and positivity rates.

Results: For a cohort of 57,065 patients in 2015, the estimated total costs (both laboratory and the STD Control Program) were $2,314,763 (traditional) and $2,376,557 (reverse). Reverse algorithm screening was estimated to detect an additional 901 cases of syphilis. Cost of follow-up, positivity rates, and frequency of repeat infections most affected the cost-effectiveness of reverse algorithm.

Conclusions: Although costs were lower for the laboratory, detecting additional syphilis cases increased costs to the STD Control Program. Using the reverse algorithm would have been slightly more expensive for Los Angeles County in 2015, but identified more syphilis cases.
INTRODUCTION

Syphilis infection rates have reached their highest levels since the mid-1990s. Initially, the increasing rate of syphilis infection was attributed primarily to men who have sex with men (MSM), but in 2013-2014 the rate of syphilis infection also increased among heterosexual men and women.\textsuperscript{1, 2} The laboratory detection of syphilis can be challenging since the causative agent \textit{Treponema pallidum} cannot be grown in culture and most public health and clinical laboratories rely on serological tests, for which no gold standard exists.\textsuperscript{3} The increasing incidence of syphilis warrants reevaluation of serological screening approaches since diagnosis, treatment, and follow-up depend on reliable laboratory testing.

There are two basic serological approaches for syphilis screening: traditional or reverse algorithm.\textsuperscript{4, 5} In the traditional algorithm, which is currently the most commonly used approach, patient samples are screened with a nontreponemal assay (e.g., Rapid Plasma Reagin or RPR) and screen-positive samples are confirmed by a treponemal assay (e.g., \textit{Treponema pallidum} Particle Agglutination or TP-PA).\textsuperscript{6, 7} The traditional algorithm generally offers good sensitivity and specificity for identifying primary and secondary syphilis infections, but is subject to several limitations including lower throughput, subjective test interpretation, and low sensitivity for early/late-latent syphilis infections.\textsuperscript{6, 8} By contrast, in the reverse algorithm patient samples are first screened with a treponemal immunoassay (e.g., enzyme immunoassay [EIA] or chemiluminescence immunoassay [CIA]) followed by a non-treponemal assay.\textsuperscript{4-6, 9} Treponemal immunoassays are more amenable to automation and offer objective test interpretation with improved sensitivity and specificity for detecting early/late-latent syphilis.\textsuperscript{4, 6, 8} As a result, the reverse algorithm can pick up cases that might be missed using the traditional algorithm approach. However, since treponemal antibodies persist long after the initial infection, some of the cases that
are picked up by reverse algorithm screening procedures may correspond to patients with a past history of syphilis who may no longer have active disease. Therefore, it is important to know the patient’s history when interpreting syphilis tests.

The algorithm used by laboratories for syphilis screening has important implications for disease control efforts. The U.S. Centers for Disease Control and Prevention (CDC) recommends that local health jurisdictions follow-up with persons newly diagnosed with syphilis to verify treatment and to identify, test, and treat any sexual contacts of the index case. These activities, herein referred to as field services, have been shown to reduce reinfection rates and slow the spread of STIs like syphilis by identifying and treating clusters of infected patients in conserved sexual networks. Changing to a reverse algorithm approach would likely mean that the number of cases identified should increase and more field services would need to be provided. Laboratories should offer a conventional algorithm in addition to a reverse algorithm so clinicians can order the appropriate nontreponemal test for patients with a past history of syphilis.

To know whether or not switching to a reverse algorithm makes sense for a particular laboratory, the implications of that decision for both laboratory costs and field services must be well understood. Previous reports have suggested that the reverse algorithm may not be cost-effective, particularly in low-prevalence settings, because use of the reverse algorithm may result in unnecessary treatment or detect too many biological false positives. Other studies have suggested that the reverse algorithm is cost effective, especially for high volume laboratories. Clearly, further investigation into which specific conditions increase the cost-effectiveness of the reverse algorithm is warranted, particularly in high prevalence settings. Here, we developed a decision-analysis model to compare the cost-effectiveness of traditional and reverse algorithms and used this model to explore how variations in sample volume and prevalence of syphilis in the
surrounding community impact the threshold for cost-effectiveness. By using observed data for Los Angeles County as inputs for the model, we were also able to provide concrete estimates of the impacts of switching to reverse algorithm on field services on an STD Control Program serving a high prevalence population.

MATERIALS AND METHODS

Methods for Cost-Effectiveness Analysis

We created a decision analysis model to compare the cost-effectiveness between the traditional algorithm and reverse algorithm for the Los Angeles County Public Health Department. Patient samples were collected at STD clinics, hospitals, and community outreach programs from various locations around Los Angeles County, excluding residents of Pasadena and Long Beach who are served by independent public health departments. Approximately 60% of the patient samples were collected through MSM community outreach programs. Following Owusu-Edusei et al., we categorized patients by their serostatus into three mutually exclusive categories: uninfected, currently infected, or previously infected. We assumed that previously infected patients had been adequately treated and were not currently infected.

A schematic of the traditional and reverse algorithms is illustrated in Figure 4.1. Following the traditional algorithm used by the Los Angeles County Public Health Laboratories (LACPHL, Downey CA), all patient samples were screened by RPR (Arlington Scientific Inc., Springville UT). Positive qualitative RPR samples were further tested by quantitative RPR and confirmed using TP-PA (Fujirebio, Malvern PA). Patients that were previously infected with syphilis were not tested by TP-PA because of persisting treponemal antibodies after the initial infection. We compared the traditional algorithm to a reverse algorithm in which all patient samples with no
known history of syphilis (uninfected or recently infected) were screened with the LIAISON® Treponema assay (DiaSorin Inc. Stillwater MN) followed by reflex testing with RPR. The LIAISON® Treponema assay is a qualitative chemiluminescent immunoassay (CIA) that detects total (IgM and/or IgG) antibodies directed against *T. pallidum*. We assumed that TP-PA would only be performed on currently infected patients that test negative by RPR (e.g., recently exposed or early/late-latent syphilis infections) and that previously infected patients would be screened only with RPR.

Model probabilities for the traditional and reverse algorithms were based on an unpublished method comparison study that evaluated reverse algorithm screening at the LACPHL during 1 week of routine testing. We assumed a screening test positivity rate of 14.4% for the traditional algorithm, of which 2.6% were previously infected and 11.8% were currently infected. We assumed a 15.1% positivity rate for the reverse algorithm, of which 12.5% would be detected by CIA (current infections) and 2.6% would be detected by RPR (previously infected). We assumed 90.4% of CIA positive patients would test positive by reflex RPR for the reverse algorithm; the remaining 9.6% of RPR-negative, CIA positive patients would be tested by TP-PA. TP-PA positivity rates were assumed to be 95.8% and 91.5% for the traditional and reverse algorithms, respectively. We assumed 43.4% of all syphilis cases identified would provide the contact information for at least one sexual contact based on data provided by the Los Angeles County STD Control Program. Seropositive patients were given an effectiveness score of 1 to represent initiation of field services, including diagnosis, staging, and treatment. An effectiveness score of 2 was given to seropositive patients for which at least one sexual contact and/or cluster contact was identified through field services. We assumed 75% of previously infected patients that test positive by RPR were being tested to monitor efficacy of treatment and were given an
effectiveness score of 0. We assumed 5% of previously infected patients that test positive by RPR were relapse infections and were given an effectiveness score of 1 or 2 depending on the probability of eliciting one sexual contact through follow-up investigation. The LACPHL provided the current list price for RPR and TP-PA whereas the CIA cost was calculated as the sum of reagent and labor costs per batch (expressed in US Dollars). The STD Control Program provided the cost of follow-up for a seropositive patient, including the total cost of conducting a public health investigation, the cost of a return visit to the clinic, and treatment with Benzanthine penicillin G. All input variables, ranges, costs, and sources used in the decision analysis model are listed in Table 4.1.

We estimated and compared the total laboratory screening costs, the total number of individuals identified for field services, and overall cost-effectiveness ratio for each algorithm using a Monte Carlo simulation with a sample size of 57,065 (the total number of screening tests performed by the LACPHL in 2015) with 1000 randomized trials (iterations). Monte Carlo simulations were performed to estimate the cost-effectiveness of each algorithm from the perspective of the STD Control Program and from the perspective of the Public Health Laboratory. We calculated the incremental cost-effectiveness ratio (ICER) using the following equation: ICER = (C_{Reverse} − C_{Traditional}) ÷ (E_{Reverse} − E_{Traditional}) where C represents the average cost per test and E represents the effectiveness score. We then conducted sensitivity analyses on all variables in the model using ranges presented in Table 4.1 to estimate costs (or savings) and effectiveness between the traditional and reverse algorithms when individual variables in the model were changed. Variable ranges for sensitivity analyses were informed by laboratory and epidemiological surveillance data collected in 2013 and 2015.\textsuperscript{18, 19} We used TreeAge Pro Suite version 2016.
(TreeAge Software Inc., Williamstown MA) to construct the decision tree, conduct Monte Carlo simulations and sensitivity analyses, and to illustrate data.

**Methods for Survey of Public Health Laboratories**

In February of 2016, an online voluntary 15-item questionnaire was submitted to all members of the California Association of Public Health Laboratory Directors (CAPHLD) to ascertain the syphilis screening practices at public health laboratories across the state. The questions on the survey were intended to record how many laboratories are performing traditional or reverse algorithm, syphilis workload, positivity rates, and which types of serological assays are performed across the state. The survey participants included 34 local city or county public health laboratory directors and senior microbiologists serving thirty-seven public health jurisdictions. Most of the participants of the survey were public health laboratory directors. Some multiple-choice questions included the opportunity to choose “Other (please specify)” so the participant could write in a response. Answers of “Other” were maintained and were not reclassified during analysis. Survey responses were collected online (www.surveymonkey.com) until June 2016.

**RESULTS**

**Cost-Effectiveness Analysis**

We estimated the cost of traditional algorithm screening to be $2,314,763 with an average cost of $41 per patient. We projected that the total cost after switching to the reverse algorithm would have been $2,376,557 with an average cost of $42 per patient. The reverse algorithm had a slightly higher average effectiveness score (0.18) than the traditional algorithm (0.16), resulting in an additional 901 syphilis cases detected. The reverse algorithm had a lower cost-effectiveness
ratio (231.38) than the traditional algorithm (253.5). The ICER for the reverse algorithm was 54.5. Results of the Monte Carlo simulations suggest that the reverse algorithm is more expensive but more effective than the traditional algorithm. Two distinct distributions for each syphilis-screening algorithm (illustrated in Figure 4.2) were observed, although overlap between the two algorithms suggests scenarios in which the reverse algorithm is more cost-effective than the traditional algorithm.

We estimated the laboratory cost of traditional algorithm screening to be $241,147 with an average cost of $6 per patient. We projected that the total laboratory cost after switching to the reverse algorithm would have been $205,819 with an average cost of $4 per patient. The reverse algorithm had a lower cost-effectiveness ratio (23.5) than the traditional algorithm (40.2). Laboratory ICER was not calculated since the reverse algorithm cost less than the traditional algorithm. These data are summarized in Table 4.2.

One-Way Sensitivity Analyses

To investigate the thresholds at which the reverse algorithm would be less expensive than the traditional algorithm, we performed one-way sensitivity analysis in which the cost-effectiveness of each algorithm was calculated for a range of values for one variable when all other variables are held constant. Seven variables had the biggest effect on the decision analysis model and their thresholds for cost-effectiveness are described here (data presented in Table 4.3). The variable that had the highest impact on our model was the cost of follow-up with reverse algorithm being more cost-effective a cost-effectiveness threshold of $140.15 (Figure 4.3). The probability of a positive screening test (RPR for traditional and CIA for reverse) was the next highest contributor of uncertainty in the model. The reverse algorithm was less expensive if the probability
of a positive CIA was less than 12% or if the probability of a positive RPR screening test was greater than 12%. The traditional algorithm strictly dominated (i.e. was less expensive than) reverse algorithm for all probabilities of identifying a sexual contact or previous syphilis infections (Figure 4.4). The cost of CIA contributed more uncertainty to the model than the cost of RPR. The reverse algorithm was less expensive if the cost of RPR was greater than $6 or if the cost of CIA was less than $1.77.

Two Way Sensitivity Analyses

We conducted two-way sensitivity analyses to investigate how related variables could affect the cost effectiveness of the model. The reverse algorithm was less expensive than the traditional algorithm as long as the CIA test cost was $3.90 lower than the cost of RPR (Figure 4.5). We observed that the cost-effectiveness of the traditional and reverse algorithms had a near linear relationship with respect to screening test positivity rate (Figure 4.6). The reverse algorithm was less expensive than the traditional algorithm as long as the CIA positivity rate was lower than the RPR positivity rate. Conversely, the traditional algorithm was less expensive than the reverse algorithm when the CIA positivity rate was higher than the RPR positivity rate. Two-way sensitivity analysis of the probability of a previous syphilis infection and RPR test cost revealed that the reverse algorithm was only more cost effective when the cost of RPR exceeds $5.60 and the probability of a previous infection was less than 42% (Figure 4.7).

Public Health Laboratory Survey

Of the 34 California Public Health Laboratories (PHLs) surveyed, 76% \((n = 26)\) completed the survey, 11% \((n = 4)\) partially completed the survey, and 11% \((n = 4)\) did not respond. Of those
who responded, 52.9% \( (n = 18) \) reported using a traditional algorithm (screen with a non-treponemal test), 14.7% \( (n = 5) \) reported using a reverse algorithm (screen with a treponemal test), and 20% \( (n = 7) \) reported they no longer perform syphilis serology. Of those who responded, 86% \( (n = 19) \) reported using RPR, 9% \( (n = 2) \) reported using the Venereal Disease Research Laboratory (VDRL) assay, and 36% \( (n = 8) \) reported using either RPR or VDRL as their non-treponemal screening test. Of the reverse algorithm PHLs, 80% \( (n = 4) \) reported using an EIA and 20% \( (n = 1) \) reported using a CIA as their treponemal screening test.

Each of the reverse algorithm PHLs reported a monthly sample volume between 100-500 blood samples a month, which was not significantly higher than the traditional algorithm PHLs that provided their monthly blood sample volumes. The main reason that the reverse algorithm PHLs cited for adopting a reverse algorithm was low reagent and personnel costs for traditional algorithm screening. PHL laboratory directors commented that they were less inclined to invest the capital cost for automating syphilis serology because they had low monthly sample volumes (less than 1,000 blood samples each month). Low prevalence of syphilis (0-5%) was also cited commonly as a main reason for not adopting the reverse algorithm. One outlier was the San Francisco PHL, which serves a high prevalence population (8%) and tests approximately 2,300 samples each month and reported a high re-infection rate as the biggest barrier to adopting the reverse algorithm.

**DISCUSSION**

Although most laboratories performing syphilis serology continue to use a traditional screening algorithm, larger-volume laboratories are quickly adopting the reverse algorithm in order to improve workflow through automating syphilis screening. From the perspective of the
public health system, changes in syphilis detection methods by laboratories serving high syphilis prevalence jurisdictions affect the number of STI investigations conducted by the local health department. We sought out to investigate whether or not the reverse algorithm was cost-effective in a high-prevalence population from the perspective of the public health department as well as the perspective of the laboratory.

From the perspective of the LACPHL, our model suggests that the reverse algorithm is more cost effective than the traditional algorithm. Assuming Los Angeles County continues to screen the same number of patients each year, reverse algorithm screening would save approximately $40,000 in laboratory costs annually. These numbers do not reflect additional savings such as reducing the number of repetitive motion injuries associated with manual test procedures. Further, our model suggested that there would be no tradeoff between cost and effectiveness in identifying syphilis cases. These findings are due, in part, to the high prevalence of syphilis circulating in Los Angeles County and thus high positive predictive value of the assays used for syphilis screening.

Although less costly for the laboratory, additional seropositive patients detected through a reverse screening algorithm would increase field services costs to the STD Control Program. Thus, the reverse algorithm is more expensive from the perspective of the STD Control Program. The $40,000 in laboratory savings is not enough to offset the cost of follow-up investigations, return visits and treatment for the additional 901 cases identified through reverse algorithm screening. Previous reports on the cost-effectiveness of the reverse algorithm raised the concern of overtreatment, particularly with respect to false-positive or previously treated cases. Indeed, this concern may be a barrier for adopting the reverse algorithm for some health jurisdictions since resources are often limited. However, overtreatment of seropositive patients with a previous
history of syphilis can be avoided if the laboratory knows the patient’s history or if the laboratory offers a “nontreponemal-only” option for screening patients with a previous history of syphilis. This approach would reap the maximum cost-savings of the reverse algorithm while keeping overtreatment to a minimum.

The data presented in this study reflect the impacts of switching to a reverse algorithm for Los Angeles County, and may be applicable to other health jurisdictions with a high prevalence of syphilis. One of the strengths of this study was the enumeration of cost-effectiveness thresholds through sensitivity analyses, which may be used to inform other laboratories or health jurisdictions that are evaluating the reverse algorithm. Our survey respondents reported that low workload and capital costs for new instrumentation were the two most important barriers to adopting a reverse algorithm. Indeed, laboratories are less likely to pay a capital cost to automate a low-volume test; thus the reverse algorithm may not be cost-effective for low volume laboratories. Such capital costs can be overcome if the laboratory enters a reagent-rental agreement for new instrumentation.

One of the strengths of this study was using percentages based on laboratory and surveillance data collected in Los Angeles County. Percentages extrapolated from the method comparison study offered a realistic preview of how reverse algorithm testing could be implemented at the Los Angeles County Public Health Laboratories. Our study relied on epidemiological data from the STD Control Program for percentages of previous infections and contacts identified through follow-up investigations. Thus, our findings are contingent upon good recordkeeping and reporting practices.

Laboratories considering a reverse syphilis-screening algorithm should review and include in their decision making process the following information: syphilis prevalence, specimen workload, cost of specific syphilis serology test, technologist salaries and benefits, laboratory
overhead costs, specific field services practices, follow-up costs (investigation, clinic visit, and treatment) and inclusion criteria. Laboratories should also consult with STI epidemiologists or infectious disease control committee to coordinate training for physicians and other clients prior to implementation of a reverse algorithm.

CONCLUSIONS

Early detection and treatment of syphilis depends on cost-effective laboratory testing strategies. Without a gold standard for syphilis detection, laboratories must choose the serological screening algorithm that balances laboratory costs with detecting as many active cases of syphilis as possible. Using the Los Angeles County as a case study, we evaluated the cost-effectiveness between the reverse and traditional syphilis screening algorithms from the perspective of the public health system. Our model suggests that switching to a reverse syphilis-screening algorithm would be more cost-effective than the traditional syphilis-screening algorithm. Laboratory decision makers should carefully consider the implications for disease management in addition to laboratory and downstream public health costs when deciding to switch to using a reverse algorithm for syphilis detection.
A schematic of (A) traditional and (B) reverse algorithm screening procedures used in the decision analysis model. RPR indicates Rapid Plasma Reagin, TP-PA indicates *Treponema pallidum* particle agglutination, and CIA indicates chemiluminescence immunoassay. Patients with a previous history of syphilis were screened only with RPR. All seropositive patients with no previous history of syphilis infection or a relapse were reported to the STD Control Program for follow-up. Sexual contacts were identified through follow-up investigations from less than half of the syphilis cases reported in 2015.
Table 4.1. Description, probability values, and sources for all variables used in the decision analysis model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value (Percentage or Cost in USD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA RPR Positivity Rate*</td>
<td>11.8%</td>
<td>1-40%</td>
</tr>
<tr>
<td>TA RPR Negativity Rate*</td>
<td>85.6%</td>
<td>-</td>
</tr>
<tr>
<td>TA RPR Known Treated Cases*</td>
<td>2.6%</td>
<td>1-100%</td>
</tr>
<tr>
<td>TA TP-PA Positivity Rate*</td>
<td>95.8%</td>
<td>80-100%</td>
</tr>
<tr>
<td>TA TP-PA Negativity Rate*</td>
<td>4.2%</td>
<td>-</td>
</tr>
<tr>
<td>RA CIA Positivity Rate*</td>
<td>12.5%</td>
<td>1-40%</td>
</tr>
<tr>
<td>RA CIA Negativity Rate*</td>
<td>84.9%</td>
<td>-</td>
</tr>
<tr>
<td>RA RPR Known Treated Cases*</td>
<td>2.6%</td>
<td>1-100%</td>
</tr>
<tr>
<td>RA RPR Positivity Rate*</td>
<td>90.4%</td>
<td>1-40%</td>
</tr>
<tr>
<td>RA RPR Negativity Rate*</td>
<td>9.6%</td>
<td>-</td>
</tr>
<tr>
<td>RA TP-PA Positivity Rate*</td>
<td>91.5%</td>
<td>80-100%</td>
</tr>
<tr>
<td>RA TP-PA Negativity Rate*</td>
<td>8.3%</td>
<td>-</td>
</tr>
<tr>
<td>Sexual Contacts Identified†</td>
<td>43.4%</td>
<td>25-75%</td>
</tr>
<tr>
<td>Treated Case RPR Positive†</td>
<td>75%</td>
<td>1-90%</td>
</tr>
<tr>
<td>Relapse Infections†</td>
<td>5%</td>
<td>1-25%</td>
</tr>
<tr>
<td>Cost of Qualitative RPR‡</td>
<td>$4.46</td>
<td>$1-10</td>
</tr>
<tr>
<td>Cost of Quantitative RPR‡</td>
<td>$4.46</td>
<td>$1-10</td>
</tr>
<tr>
<td>Cost of TP-PA‡</td>
<td>$11.48</td>
<td>$1-18</td>
</tr>
<tr>
<td>Cost of CIA</td>
<td>$2.87</td>
<td>$1-10</td>
</tr>
<tr>
<td>Cost of Follow-Up§</td>
<td>$208.32</td>
<td>$85-335</td>
</tr>
</tbody>
</table>

Note. TA denotes traditional algorithm and RA denotes reverse algorithm. Ranges were programmed into the model to allow for sensitivity analyses. (*) Denotes percentages based on method comparison study.18 (†) Denotes percentages based on data from the STD Control Program.19 (‡) Denotes costs provided by the Los Angeles County Public Health Laboratories. (§) Denotes cost includes the sum of follow-up, treatment, and return visit costs.
Table 4.2. Summary of a Monte Carlo simulation approximating the cost of syphilis screening in 2015 by either the traditional or reverse algorithms.

<table>
<thead>
<tr>
<th>Screening Algorithm</th>
<th>Mean Cost per Algorithm (USD)</th>
<th>Cost Standard Deviation</th>
<th>Effectiveness Score</th>
<th>Effectiveness Standard Deviation</th>
<th>CE Ratio</th>
<th>Total Algorithm Screening Cost (US Dollars)</th>
<th>No. Cases Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional (STD Control)</td>
<td>$40.56</td>
<td>3.32</td>
<td>0.16</td>
<td>0.02</td>
<td>253.5</td>
<td>$2,314,762.65</td>
<td>9,350</td>
</tr>
<tr>
<td>Traditional (Lab Only)</td>
<td>$6.43</td>
<td>0.16</td>
<td>0.16</td>
<td>0.02</td>
<td>40.2</td>
<td>$241,146.57</td>
<td>9,350</td>
</tr>
<tr>
<td>Reverse (STD Control)</td>
<td>$41.65</td>
<td>3.39</td>
<td>0.18</td>
<td>0.02</td>
<td>231.8</td>
<td>$2,376,557.27</td>
<td>10,251</td>
</tr>
<tr>
<td>Reverse (Lab Only)</td>
<td>$4.23</td>
<td>0.11</td>
<td>0.18</td>
<td>0.02</td>
<td>23.5</td>
<td>$205,818.95</td>
<td>10,251</td>
</tr>
</tbody>
</table>
Figure 4.2. Plot of the Monte Carlo simulations of cost-effectiveness for the traditional (▲) and reverse (●) algorithms. The estimated cost per test (in USD) is plotted on the y-axis and the estimated effectiveness (i.e., number of patients brought to treatment) is plotted on the x-axis. Each dot represents one iteration (n=1000) of the Monte Carlo Simulation.
Table 4.3. One-way sensitivity analysis results for each variable in the cost-effectiveness model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range</th>
<th>Traditional Algorithm</th>
<th>Reverse Algorithm</th>
<th>CE Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cost (USD)</td>
<td>Effectiveness</td>
<td>Cost (USD)</td>
</tr>
<tr>
<td>RPR Positivity Rate</td>
<td>1-40%</td>
<td>$8-247</td>
<td>0.02-1.10</td>
<td>$42</td>
</tr>
<tr>
<td>TP-PA Positivity Rate</td>
<td>50-100%</td>
<td>$24-42</td>
<td>0.09-0.17</td>
<td>$42</td>
</tr>
<tr>
<td>CIA Positivity Rate</td>
<td>1-40%</td>
<td>$41</td>
<td>0.16</td>
<td>$6-248</td>
</tr>
<tr>
<td>Previous History of Syphilis</td>
<td>1-100%</td>
<td>$40-53</td>
<td>0.16-0.21</td>
<td>$41-56</td>
</tr>
<tr>
<td>Sexual Contacts Identified</td>
<td>25-75%</td>
<td>$30-53</td>
<td>0.12-0.22</td>
<td>$31-55</td>
</tr>
<tr>
<td>Cost of Follow-Up Investigation</td>
<td>$85-335</td>
<td>$20-61</td>
<td>0.16</td>
<td>$19-64</td>
</tr>
<tr>
<td>Cost of RPR</td>
<td>$1-10</td>
<td>$37-47</td>
<td>0.16</td>
<td>$41-43</td>
</tr>
<tr>
<td>Cost of TP-PA</td>
<td>$1-18</td>
<td>$39-41</td>
<td>0.16</td>
<td>$41-42</td>
</tr>
<tr>
<td>Cost of CIA</td>
<td>$1-10</td>
<td>$41</td>
<td>0.16</td>
<td>$40-49</td>
</tr>
</tbody>
</table>

Note. Cost and effectiveness was calculated for each variable for a range of values at ten intervals. Cost and effectiveness values are listed as ranges from low (left) to high (right). Cost-effectiveness (CE) thresholds denote the values at which the reverse algorithm is more cost effective than the traditional algorithm. (*) Indicates cost values that decrease as the variable percentage increases.
Figure 4.3. One-way sensitivity analysis illustrating the cost-effectiveness threshold of the traditional (△) and reverse (○) algorithms as a function of the cost of follow-up investigations. Costs are expressed in USD. The reverse algorithm is more cost effective as long as the follow-up cost is less than $140.15.
Figure 4.4. One-way sensitivity analysis illustrating the cost-effectiveness of the traditional (▲) and reverse (●) algorithms as a function of the probability of having a previous syphilis infection (x-axis). Costs are expressed in USD. The traditional algorithm strictly dominates (i.e., is more cost effective) the reverse algorithm for all probabilities of a previous syphilis infection.
Figure 4.5. Two-way sensitivity analysis of the total cost of RPR (x-axis) and the total cost of CIA (y-axis). Costs are expressed in USD. Red indicates conditions under which the traditional algorithm is less expensive and blue indicates conditions under which the reverse algorithm is less expensive. The reverse algorithm is more cost effective when the RPR test cost is greater than $3.58 and the CIA test cost is less than $6.50.
Figure 4.6. Two-way sensitivity analysis of the probability of a positive RPR (x-axis) and probability of a positive CIA (y-axis). Costs are expressed in USD. Red indicates conditions under which the traditional algorithm is less expensive and blue indicates conditions under which the reverse algorithm is less expensive. The reverse algorithm dominates (i.e., is more cost effective) as long as the CIA positivity rate is lower than the RPR positivity rate.
Figure 4.7. Two-way sensitivity analysis of the probability of a previous syphilis infection (y-axis) and RPR test cost (y-axis). Costs are expressed in USD. Red indicates conditions under which the traditional algorithm is less expensive and blue indicates conditions under which the reverse algorithm is less expensive. The reverse algorithm is more cost effective when the cost of RPR exceeds $5.64 and the probability of a previous infection is less than 42%.
REFERENCES


CHAPTER 5

Overarching Conclusions and Recommendations for Future Studies

INTRODUCTION

Public health laboratories are under constant pressure to adjust their testing strategies in order to control the spread of infectious diseases in the communities they serve. The goal of the work discussed in this dissertation was to evaluate the practical challenges related to the detection and surveillance of gonorrhea and syphilis, two important re-emerging STIs. In Chapter 2, we discussed how the global dissemination of drug resistance and the emergence of multidrug resistant Neisseria gonorrhoeae have imposed a need for individualized testing and treatment strategies for gonorrhea. To help facilitate the transition to a more effective approach to surveillance of N. gonorrhoeae, we proposed the following approaches:

- Adding drug resistance marker detection to current and new nucleic acid amplification tests (NAATs) to generate drug susceptibility profiles to assist in treatment decisions.
- Developing and validating new drug susceptibility tests for characterizing gonococci in a shorter amount of time.

In Chapter 3 and Chapter 4, we evaluated the performance and cost-effectiveness of high-throughput serological screening tests and screening algorithms for syphilis to understand how these different approaches affect our ability to detect syphilis in the population. We found that a reverse sequence syphilis-screening algorithm using a high-throughput treponemal assay as the screening test could detect the same cases of syphilis as the traditional algorithm for a lower average cost per test. However, the reverse algorithm also detected additional syphilis cases, some
of which were footprints of previous syphilis infections and would not require additional testing or follow-up. However, some of the additional syphilis cases detected by reverse algorithm screening procedures were possibly early infections that would not have been detected by a traditional screening algorithm and would require follow-up. To further investigate the differences between these algorithms, we proposed the following approaches:

- Determine the analytical sensitivity of high-throughput treponemal immunoassays with respect to early syphilis and early/late latent syphilis patients.
- Chart review of additional syphilis cases following implementation of a reverse algorithm.
- Evaluation of Automated RPR System for a Reverse or Traditional Syphilis Screening Algorithm.

TOWARDS A MORE EFFECTIVE APPROACH TO SURVEILLANCE OF NEISSERIA GONORRHOEAE

Molecular detection of drug resistance markers should be used to enhance antimicrobial stewardship to stem the tide of multidrug-resistant Neisseria gonorrhoeae

In order to stem the tide of multidrug-resistant Neisseria gonorrhoeae (MDRNG), we proposed adding drug resistance markers to current and new NAATs and point of care tests to generate drug susceptibility profiles for the bacterium. Adding these markers to standard N. gonorrhoeae screening tests would allow clinicians to make more appropriate therapeutic decisions based on drug susceptibility profiles generated by a rapid, molecular test. For example, knowing that the genotype for a sample did not include the gyrA mutation would inform the clinician that ciprofloxacin could be prescribed instead of ceftriaxone, thereby improving
antibiotic stewardship for both antibiotics.\textsuperscript{1} Similar approaches have already been developed and implemented for other important pathogens such as carbapenem-resistant enterobacteiraceae (CRE). Hill et al. discussed how the Verigene Blood Culture Gram-Negative (BC-GN) microarray has been used to detect gram-negative bacilli and drug-resistance markers from positive blood cultures and that using this assay would allow for more effective and timely medical decisions to be made for patient care.\textsuperscript{2} Similarly, Neuner et al. demonstrated that the using the Verigene Blood Culture Gram-Positive (BC-GP) microarray with an antimicrobial stewardship intervention improved time to de-escalation to optimal antibiotic therapy in patients with gram-positive blood cultures.\textsuperscript{3} Future studies should evaluate which drug-resistance markers would provide the greatest clinical utility for the molecular detection of drug resistance in \textit{N. gonorrhoeae}.

**Whole-genome sequencing of multidrug-resistant \textit{N. gonorrhoeae} would enhance molecular surveillance**

Since the publication of our critical review, next-generation sequencing (NGS) methods have become more commonplace in clinical and public health laboratories and will likely replace current sequence typing methods such as pulsed-field gel electrophoresis (PFGE) or multi-locus sequence typing (MLST) in the near future.\textsuperscript{4} Gonorrhea surveillance could be greatly improved if sentinel or regional laboratories participating in the Gonococcal Isolate Surveillance Project (GISP) performed NGS on all \textit{N. gonorrhoeae} isolates to identify prevalent strains and drug resistance patterns circulating in different US population centers. Moreover, NGS data paired with MIC data from phenotype testing would enhance the ability of GISP laboratories to detect novel drug resistance markers and determine whether they are chromosomally mediated or carried on a plasmid.\textsuperscript{4} Previous investigations have shown that multiple genes affect antimicrobial resistance
in *N. gonorrhoeae* and minimum inhibitory concentrations (MICs) may be affected by an accumulation of such mutations.\(^5\) Tobiason et al. demonstrated that *N. gonorrhoeae* cells contain 4-12 copies of the bacterial genome which may contribute to antigenic variation.\(^6\) Another potential application for NGS with respect to *N. gonorrhoeae* is using Contig Ploidy and Allele Dosage Estimation (ConPADE) to investigate how gonococcal polyploidy affects MIC phenotypes.\(^7\)

**Developing new antimicrobial susceptibility testing (AST) methods for characterizing gonococci in a shorter amount of time is a high priority**

In *Chapter 2*, we argued that introducing an effective broth microdilution system would decrease the amount of time required to test gonorrhea isolates for drug resistance in vitro. This research could help to decrease the labor and turnaround times for performing AST on *N. gonorrhoeae* isolates as well as expand our ability to discover new antibiotics that may not diffuse well into an agar medium. A logical first step would be to compare the growth of *N. gonorrhoeae* strains in different liquid culture media previously described in the literature (e.g., Fastidious Broth, GW Broth, GC Broth)\(^8\)-\(^11\) and to determine the variability of the MIC for an antibiotic between each broth recipe and agar dilution. Alternatively, growth studies using fluorescence *in situ* hybridization (PNA-FISH) in liquid culture could be used as a replacement for traditional broth microdilution approaches.\(^12\) For instance, FISH has used to detect *Helicobacter pylori* in the environment and has also been applied to detect clarithromycin-resistant *H. pylori* in culture.\(^13\) A priority would be to develop methods to confirm the growth of *N. gonorrhoeae* in the presence of antibiotics in liquid culture using FISH or FISH in conjunction with WGS to demonstrate which genotypes contribute to drug-resistance phenotypes.
OPTIMIZING THE EFFICIENCY OF SYPHILIS SURVEILANCE

Correlation of Treponemal Immunoassay Indices to Antibody Titer at Different Stages of Syphilis Infection

In Chapter 3, we discussed the use of treponemal immunoassays used as screening tests for syphilis serology. We observed a few cases of disagreement between three different treponemal immunoassays, *Treponema pallidum* Particle Agglutination (TP-PA), and fluorescent treponemal antibody absorption (FTA-Abs). Castro et al. reported differences in the analytical level of agreement among treponemal assays and attributed these differences to new assay configurations that increased the number of binding sites for anti-treponemal IgG and IgM. Such disagreements between treponemal assays in a reverse algorithm present a dilemma in interpreting inconclusive syphilis serology for physicians, particularly when the confirmatory treponemal assay has a lower analytical sensitivity than the treponemal screening assay.

Similarly, disagreements between treponemal assays could be caused by low antibody titers for patients that have been treated for previous syphilis infections. Recently, Berry and Loeffelholz argued that confirmatory testing with TP-PA was unnecessary for specimens with high antibody indices on a treponemal IgG multiplex flow immunoassay (MFI). Indeed, we reported in Chapter 3 low treponemal antibody indices for each trep-discordant specimen. Low IgM/IgG titers caused by early infection or latent infection/sero-reversion could affect the sensitivity of these treponemal screening assays. Presently, few studies have been conducted that demonstrate a direct correlation between antibody titer and the stage of syphilis infection. Here, we propose a follow-up study that evaluates the sensitivity of treponemal immunoassays for
patients at different stages of syphilis infection. Such a study could provide insight into the correlation between antibody titer and stage of syphilis infection.

Chart Review of RPR-Discordant Seropositive Cases Detected Using A Reverse Syphilis Screening Algorithm

We reported that the reverse syphilis-screening algorithm we evaluated in Chapter 4 was more cost-effective than the traditional algorithm and was projected to detect an additional 899 cases of syphilis each year. It is unknown whether these additional cases of syphilis are truly new infections or they are footprints of past infection since the reverse algorithm has not been implemented at the Los Angeles County Public Health Laboratories and chart review for these patients was not available. Goswami et al. reported that reverse algorithm screening for a small, high-risk community largely detected previous syphilis infections. However, patients with a known history of syphilis infection would be excluded reverse algorithm screening and would only be tested with a nontreponemal test.

Presently, the characteristics of patients with discordant syphilis serology detected by reverse algorithm screening procedures in a high-prevalence setting are unknown. A follow-up study should be conducted to describe these seropositive patient populations who test negative on nontreponemal assays to elucidate how many are recently infected (early infection, early latent, or late latent) versus how many are merely footprints of past infection (treated or spontaneously resolved). Further, it should be investigated whether treponemal immunoassay indices can be used to differentiate between these groups of patients. Such a follow-up study could provide valuable insight for laboratories that are considering implementing or have already implemented the reverse algorithm.
REFERENCES

1. Low N, Unemo M. Molecular tests for the detection of antimicrobial resistant Neisseria gonorrhoeae: when, where, and how to use? *Current Opinion in Infectious Diseases* 2016; 29: 45-51.


APPENDIX A

Supporting Information for CHAPTER 4
Table A.1. A summary of the 2015 syphilis screening laboratory and epidemiology data. A total of 57,065 samples were tested in 2015 using a traditional syphilis-screening algorithm (RPR first, TP-PA second).

<table>
<thead>
<tr>
<th>Category</th>
<th>No. Samples Tested</th>
<th>Sample Size per Year</th>
<th>Model %</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR Positive</td>
<td>57,065</td>
<td>4,125</td>
<td>7.23</td>
</tr>
<tr>
<td>RPR Negative</td>
<td>57,065</td>
<td>51,689</td>
<td>90.58</td>
</tr>
<tr>
<td>RPR Treated Cases (Total)</td>
<td>57,065</td>
<td>1,251</td>
<td>2.19</td>
</tr>
<tr>
<td>RPR Treated (Follow-Up)</td>
<td>1,251</td>
<td>939</td>
<td>75.0</td>
</tr>
<tr>
<td>RPR Treated (Relapse)</td>
<td>1,251</td>
<td>63</td>
<td>5.0</td>
</tr>
<tr>
<td>TP-PA Positive (No History)</td>
<td>5376</td>
<td>2,445</td>
<td>45.48</td>
</tr>
<tr>
<td>TP-PA Negative</td>
<td>5376</td>
<td>1,680</td>
<td>31.25</td>
</tr>
</tbody>
</table>

*Note.* RPR-positive patients with a previous history of syphilis (Treated Cases) were not tested with TP-PA since Treponemal antibodies persist after treatment. Seropositive patients with no history of syphilis were reported to the STD Control Program for field services. 1,061 additional cases of syphilis were identified through field services. These data were used to adjust probability ranges for sensitivity analysis.
Table A.2. Summary of results from the method comparison study.

<table>
<thead>
<tr>
<th>Category</th>
<th>Sample Size per Week</th>
<th>Estimated No. Patients per year</th>
<th>No. Samples Tested</th>
<th>Model Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA RPR Positive</td>
<td>118</td>
<td>6,136</td>
<td>52,000</td>
<td>11.8</td>
</tr>
<tr>
<td>TA RPR Negative</td>
<td>856</td>
<td>44,512</td>
<td>52,000</td>
<td>85.6</td>
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<tr>
<td>TA RPR Treated Cases</td>
<td>26</td>
<td>1,325</td>
<td>52,000</td>
<td>2.54</td>
</tr>
<tr>
<td>TA TP-PA Positive</td>
<td>113</td>
<td>5,876</td>
<td>6,136</td>
<td>95.76</td>
</tr>
<tr>
<td>TA TP-PA Negative</td>
<td>5</td>
<td>260</td>
<td>6,136</td>
<td>4.24</td>
</tr>
<tr>
<td>RA CIA Positive</td>
<td>125</td>
<td>6,500</td>
<td>52,000</td>
<td>12.5</td>
</tr>
<tr>
<td>RA CIA Negative</td>
<td>849</td>
<td>44,148</td>
<td>52,000</td>
<td>84.9</td>
</tr>
<tr>
<td>RA RPR Treated Cases</td>
<td>26</td>
<td>1,352</td>
<td>52,000</td>
<td>2.37</td>
</tr>
<tr>
<td>RA RPR Positive</td>
<td>113</td>
<td>5,876</td>
<td>6,500</td>
<td>90.4</td>
</tr>
<tr>
<td>RA RPR Negative</td>
<td>12</td>
<td>624</td>
<td>6,500</td>
<td>9.6</td>
</tr>
<tr>
<td>RA TP-PA Positive</td>
<td>11</td>
<td>572</td>
<td>625</td>
<td>91.52</td>
</tr>
<tr>
<td>RA TP-PA Negative</td>
<td>1</td>
<td>52</td>
<td>625</td>
<td>8.32</td>
</tr>
</tbody>
</table>

Note. A total of 1,000 samples were collected consecutively during one week of syphilis screening and tested by the reverse algorithm (CIA first, RPR second, TP-PA third). Model percentages were extrapolated from the values observed in the method comparison study and assumed to remain constant for 52 weeks (annual sample size of 52,000 patients). Patients with a previous history of syphilis (treated cases) were screened only with RPR. The percentage of additional cases identified through field services was assumed to be the same as the traditional algorithm. These data were used as the main percentages for the decision analysis model.
Figure A.1. A tornado diagram of one-way sensitivity analyses for variables demonstrating high potential effect on the decision analysis model. Expected values (EVs) of the reverse algorithm average test cost are displayed on the horizontal axis. Each bar represents a one-way sensitivity analysis for one variable; wider bars indicate larger potential effect on the expected value generated by the model. Variables are ordered by having the highest potential effect (top) to lowest potential effect (bottom).
Figure A.2. One-way sensitivity analysis illustrating the cost-effectiveness threshold of the traditional (red) and reverse (blue) algorithms as a function of CIA test cost. The reverse algorithm is more cost effective as long as the follow-up cost is less than $1.77.
Figure A.3. One-way sensitivity analysis illustrating the cost-effectiveness of the traditional (red) and reverse (blue) algorithms as a function of TP-PA test cost. The traditional algorithm strictly dominates (i.e., is less expensive) the reverse algorithm for all cost values of TP-PA.
Figure A.4. One-way sensitivity analysis illustrating the cost-effectiveness of the traditional (red) and reverse (blue) algorithms as a function of the probability of identifying a sexual contact. The traditional algorithm strictly dominates (i.e., is less expensive) the reverse algorithm for all probabilities of identifying a sexual contact.
Figure A.5. One-way sensitivity analysis illustrating the cost-effectiveness threshold of the traditional (red) and reverse (blue) algorithms as a function of CIA test cost. The reverse algorithm is more cost effective as long as the follow-up cost is less than $1.77.
Figure A.6. One-way sensitivity analysis illustrating the cost-effectiveness threshold of the traditional (red) and reverse (blue) algorithms as a function of the probability of a positive RPR screening test. The reverse algorithm is more cost effective as long as the probability of a positive RPR screening test is greater than 12%.
Figure A.7. One-way sensitivity analysis illustrating the cost-effectiveness threshold of the traditional (red) and reverse (blue) algorithms as a function of the probability of a positive TP-PA. The reverse algorithm is more cost effective as long as the probability of a positive TP-PA is greater than 98%.