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Comparing three treponemal tests for syphilis screening☆

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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 ability to batch (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.

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1. 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 (Centers for Disease Control and Prevention, 2015; Morshed, 2014). 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 (Centers for Disease Control and Prevention, 2015). 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 (Firlag-Burkacka et al., 2016; Mattei et al., 2012; Poliseli et al., 2008). Accurate, rapid, and early diagnosis of syphilis is important for patient management and disease control (Centers for Disease Control and Prevention, 2008).

Laboratory diagnosis of syphilis relies on serological screening methods based on the host's immune response to *Treponema pallidum*, the causative agent of syphilis (Morshed, 2014; Soreng et al., 2014; Zanto, 2010). 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. *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 (Centers for Disease Control and Prevention, 2011; Morshed, 2014; Soreng et al., 2014; Tong et al., 2014). An increasing number of laboratories have 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 (Donkers et al., 2014; Gratzer et al., 2014; Rhoads et al., 2017; Soreng et al., 2014; Zanto, 2010). 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 (Gratzer et al., 2014; Morshed and Singh, 2015; Zanto, 2010). 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.

2. Materials and methods

2.1. 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

☆ Conflicts of Interest: None

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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.

2.2. Serological testing

Samples were tested prospectively during one week following the CDC recommended traditional algorithm (Centers for Disease Control and Prevention, 2011). All samples were initially tested by qualitative and quantitative RPR (Arlington Scientific Inc., Springville, UT) and TP-PA (Fujirebio Diagnostics, Inc., Japan). Remnant samples were further characterized using the ADVIA® Centaur Syphilis assay (CS-CIA, Siemens, Germany), LIAISON® Treponema assay (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 (0.9–1.1), 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 (0.9–1.10), 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 (0.8–1.2), 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.

2.3. 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.

2.4. 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 Fig. 1. We checked surveillance records from the Los Angeles County STD Control Program 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. This study and its protocols were approved by the Los Angeles County Department of Public Health Institutional Review Board.

3. Results

3.1. Immunoassay performance compared with TP-PA

The performance parameters for each treponemal immunoassay are summarized in Table 1. Statistical analysis revealed no significant differences in agreement between each treponemal immunoassay and TP-PA. Twenty discordant results were observed between the 3 different treponemal assays and TP-PA (presented in Table 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

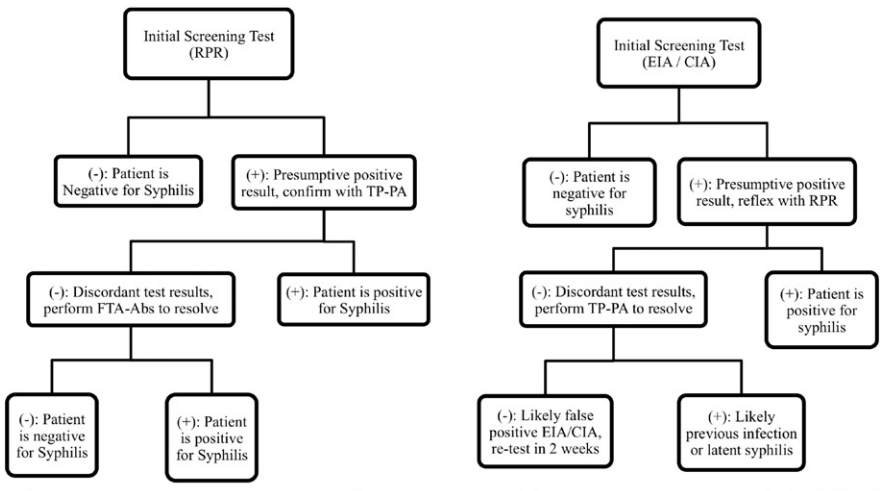

Fig. 1. A diagram of the syphilis screening algorithms evaluated. [Left] The traditional screening algorithm recommended by CDC and currently implemented at the LACPHL. [Right] The reverse screening algorithm recommended by the Association of Public Health Laboratories (APHL) and CDC.