An Innovative Alternative Testing Strategy for the Prompt and Accurate Diagnosis of Syphilis in a High Prevalence Setting

Anthony Tran[1], Brian Louie[2], Carmela Madayag[2], Mae Ling Mak[2], Susan Philip[3], Kyle Bernstein[3], Robert Kohn[3], Mark Pandori[2]

[1]University of California, Berkeley, [2]San Francisco Department of Public Health Laboratory, [3]San Francisco Department of Public Health

INTRODUCTION

Traditional serological syphilis testing includes screening with a nontreponemal assay that detects antibodies to lipoidal material released from host cells damaged by *T. pallidum* in the blood. Reactive specimens are confirmed with treponemal tests that detect antibodies specific to *T. pallidum*. It has been suggested that this traditional algorithm be reversed so that screening begins with a treponemal test, such as an immunoassay (IA). If reactive, the IA would be followed by a nontreponemal test for confirmation. This suggestion is due to the ability to automate the screening methodology thus increasing laboratory workflow, alleviating manual labor and potentially decreasing costs. In cases of discordance between the screening treponemal test and the confirmatory nontreponemal test, the CDC suggests that a second treponemal test (such as a TPPA) could be utilized to serve as a supplemental test. We evaluated the performance of this reverse sequence diagnostic algorithm relative to the traditional algorithm and developed a novel testing strategy for a high prevalence setting that would reduce turnaround time (TAT) of diagnosis to one day.

METHODS

Serum specimens were prospectively collected from July 3, 2012 to August 15, 2012 from two STD clinics in San Francisco. In total, 2,350 specimens were included in the study. The traditional diagnostic algorithm of VDRL (BD, Franklin Lakes, NJ) followed by TPPA (Fujirebio Diagnostics, Inc., Malvern, PA) was performed on all specimens and results were reported out according to standard protocols. Remnant serum from all 2,350 specimens were stripped of identifying information and blinded. Each specimen then received a TREP-SURE™ EIA (TS-EIA) (Trinity Biotech, Jamestown, NY) and TPPA test. Results of all three testing methodologies were analyzed to evaluate the performance of both algorithms. Additionally, the CAPTIA Syphilis-IgM Capture EIA (Trinity Biotech, Jamestown, NY) was performed on all discordant TS-EIA and VDRL specimens in the reverse sequence algorithm to determine early and active infection. Finally, the Syphilis Health Check rapid test (SHC-RT) (Trinity Biotech, Jamestown, NY) was performed on a subset of 249 specimens to evaluate its performance as a potential replacement for the TPPA.

The TS-EIA is an antibody sandwich assay that detects both IgM and IgG antibodies to *T. pallidum* in human serum or plasma. Results were provided in the form of qualitative and quantitative outputs. The signal-to-cutoff (S/CO) ratio (or index value) of each result was calculated by dividing the OD value by the mean of the cutoff calibrator controls. These index values determine the qualitative output (positive or negative) of each specimen tested and are proportional to the amount of antibody to *T. pallidum* found in each specimen.

Receiver operating characteristic (ROC) analyses were conducted to examine the relationship between the TS-EIA S/CO ratios and TPPA test results. This analysis was used to evaluate the optimal threshold cut-off point for the assay. The area under the ROC curve (AUROC) was calculated to quantify the discrimination of the EIA in predicting TPPA results.

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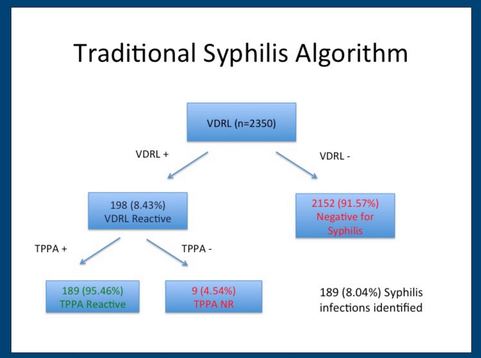

Figure 1. Results from Traditional Syphilis Testing Algorithm

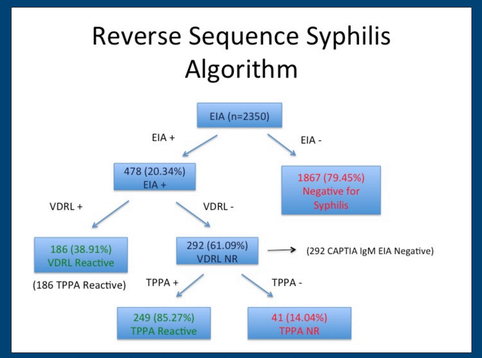

Figure 2. Results from Reverse Sequence Syphilis Testing Algorithm

Table 1. Relationship of TPPA results to corresponding TREP-SURE™ EIA S/CO ratios

	TREP-SURE™ EIA Results	TPPA Results		
S/CO	Positive	Reactive	Nonreactive	Inconclusive
<0.80	0	11 (0.6%)	1854 (99.3%)	2 (0.1%)
0.80-1.20	0	0	5 (100.0%)	0
1.21-3.463	39	3 (7.7%)	35 (89.7%)	1 (2.6%)
≥3.464	439	429 (97.7%)	9 (2.1%)	1 (0.2%)

Note: S/CO ratios <0.80 are considered Negative, 0.80-1.20 is Equivocal and ≥1.21 are Positive.

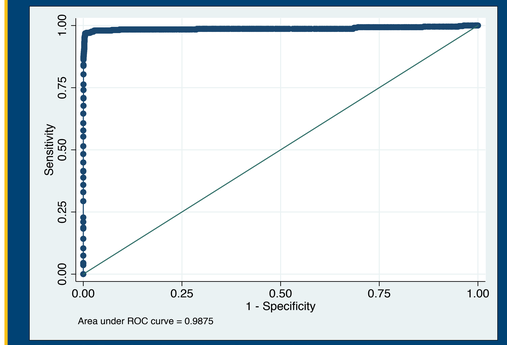

Figure 3. Receiver Operating Characteristic curve demonstrating discrimination of the TS-EIA in predicting TPPA positivity

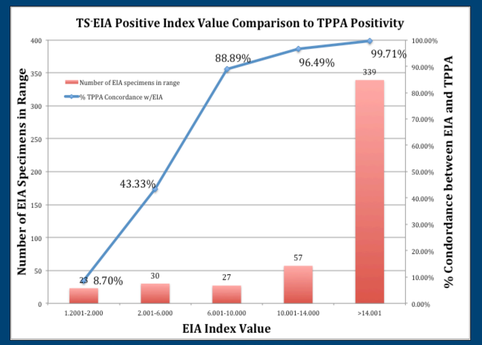

Figure 4. Relationship between TS-EIA S/CO ratios and corresponding positive TPPA results for all positive TS-EIA specimens (n=478)

Table 2. Performance of Syphilis Health Check rapid test against specimens of varying categories

		Syphilis Health Check	
Specimen Type	Number of Specimens	Reactive	% Correct
Primary	11	9	81.8%
Secondary	28	27	96.4%
Biological False Positive	9	1	88.9%
Isolated EIA Positive	44	1	97.7%
Discordant Nontreponemal/Treponemal (VDRL-/TPPA+/TS-EIA+)	50	30	60.0%
Discordant Treponemal[a] (TPPA+/TS-EIA-)	11	4	36.4%

[a]This group includes three primary and one secondary syphilis infection. The HC-RST correctly identified one primary (1 of 3) and one secondary (1 of 1) infection.

Table 3. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the Syphilis Health Check rapid test

		95% CI
Sensitivity	92.1%	85.55%-97.01%
Specificity	98.1%	93.27%-99.71%
Positive Predictive Value	97.8%	92.27%-99.67%
Negative Predictive Value	93.6%	87.32%-97.39%

RESULTS

For the purposes of this study, the traditional syphilis algorithm of a VDRL screen followed by TPPA confirmation was selected as the "gold standard" algorithm. 198 (8.4%) of 2,350 specimens were found reactive by VDRL, of which 189 were reactive on a TPPA, resulting in the detection of 189 infections (Figure 1). When the same specimens were reversed and screened by the TS-EIA, 478 (20.3%) were positive, 186 (38.9%) of which confirmed reactive by VDRL (Figure 2). Of the 292 (61.1%) discordant EIA reactive and VDRL non-reactive specimens, only 249 (85.3%) were reactive by TPPA. Therefore, the reverse sequence algorithm, beginning with a treponemal EIA was able to detect 186 current and active syphilis infections.

Signal-to-cutoff values of all EIA results were examined, revealing a very strong correlation between this value and the probability of reactivity on a TPPA. Through the use of ROC analyses, the threshold S/CO ratio that correlated best with a positive TPPA test result was determined as ≥3.464. Figure 3 illustrates the relationship between TPPA results and TS-EIA S/CO ratios. The AUROC was calculated as 0.9875, which indicates that the TS-EIA has an excellent ability to predict TPPA positivity. Figure 4 demonstrates the high concordance between the two assays at varying ranges of TS-EIA S/CO ratios. With a strong agreement between TS-EIA S/CO ratios and TPPA results established, the number of secondary treponemal tests that could be eliminated in the reverse sequence algorithm were examined. Among discordant specimens (TS-EIA+/VDRL-), 254/292 (87%) possessed TS-EIA results that were above the threshold index value of 3.464. If the necessity to conduct all of these TPPA assays were eliminated, the laboratory would have needed to performed 38 follow-up TPPA tests instead of 292 (87% reduction). Table 1 illustrates the relationship of TS-EIA index values and TPPA results. However, with the drastically reduced amount of TPPA tests that are needed to be performed, batching specimens would be the only economical way to conduct the testing. Next, we examined the feasibility of replacing the TPPA with the SCH-RT as the second treponemal test in the reverse algorithm. The rapid test was able to identify 82% of primary and 96% of secondary syphilis infections (Table 2) and had a sensitivity of 92.1% and specificity of 98.1% (Table 3) vs. the specially selected panel of specimens.

CONCLUSION

Screening with an EIA for syphilis infection in a high prevalence setting resulted in the detection of more seropositive individuals than screening with VDRL, but did not result in the detection of more syphilis cases. An algorithm that begins with an EIA screen may require additional tests for the resolution of discordant cases. When EIA reactive, VDRL non-reactive specimens are detected, the use of TPPA as a reflex test may not provide clear guidance regarding treatment.

A possible solution to the limitations of a reverse algorithm may be to utilize the signal-to-cutoff ratio on the initial screening EIA to predict TPPA reactivity as it would eliminate, in a large number of cases, the need to perform the TPPA test. However, this may result in prolonged TAT due to the need to batch samples for economical reasons. The inclusion of a rapid test, such as the SHC-RT, could significantly reduce the TAT required to report a positive syphilis diagnosis to as little as one day.