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Received: April 8, 2021. Accepted: June 14, 2021.
Published online: June 30, 2021.
DOI:10.7883/yoken.JJID.2021.164

Advance Publication articles have been accepted by JJID but have not been copyedited or formatted for publication.
Evaluation of The Diagnostic Algorithms for Serodiagnosis of Syphilis

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Running title: Serodiagnosis of syphilis

Keywords: Reverse algorithm, serodiagnosis, syphilis, Treponema pallidum
Summary

We analyzed the performance parameters of the traditional and the reverse algorithms to find out which one is more convenient for serodiagnosis of syphilis. In total, 4789 serum samples were obtained in a cross-sectional study. Venereal Disease Research Laboratory (VDRL), Treponema pallidum Haemagglutination Assay (TPHA) and Chemiluminescent Microparticle Immunoassay (CMIA) tests were performed for every serum sample. In case of discordance between results, the TPHA was applied as a second treponemal test. Overall, 207 patients were serodiagnosed with syphilis. Among 4789 subjects tested, 125 (2.6%) and 206 (4.3%) were positive using the traditional algorithm and the reverse algorithm, respectively. The missed diagnosis rate of the traditional algorithm was 42.5%. The reverse algorithm had higher sensitivity than the traditional algorithm. Sensitivity levels of the traditional and the reverse algorithms were 57.49% and 99.85% respectively. The false positivity of the reverse algorithms was 0.02%.

Introduction

Syphilis is a chronic infection caused by Treponema pallidum subsp. pallidum, is characterized by many diverse clinical manifestations. Symptomatic stage of the disease may be interrupted by very long asymptomatic periods which is called latent syphilis (1, 2). T. pallidum is generally transmitted sexually and also from mother to child or through blood transfusion which makes identification and control of this disease a public health priority (3-5). As T. pallidum cannot be stained with simple laboratory stains or cultured in vitro, some other methods have been developed to identify syphilis in various stages (1). So the mainstay of the laboratory diagnosis of syphilis is the serological detection of specific and nonspecific antibodies against T. pallidum (6, 7). Serologic tests are divided into two categories, treponemal and nontreponemal antibody tests. Syphilis-specific immunoglobulin M and immunoglobulin
G antibodies occur two and four weeks after infection, respectively, appearing earlier than nontreponemal antibodies. With adequate treatment the nontreponemal antibody levels decrease however treponemal antibodies do not disappear mostly (4, 8).

In recent years, despite technological improvements through time, the diagnosis of syphilis is still challenging due to diverse clinical manifestations of disease and multitude of test interpretations. In the absence of a reliable gold standard, different diagnostic algorithms are applied according to local considerations: epidemiological facts, cost-effectiveness, excessive workload (3, 8, 9).

Traditional syphilis screening algorithm starts with a nontreponemal test (e.g. rapid plasma reagin [RPR] test or VDRL test). When the result is positive, a treponemal test (e.g. TPHA or enzyme immunoassay [EIA]) is performed (3, 10). Besides, a reverse algorithm for screening is used by many clinical laboratories as an alternative to the traditional algorithm (6, 8, 9). Reverse syphilis screening algorithm starts with treponemal tests (e.g. enzyme or chemiluminescence immunoassays [EIA/CIA]) and followed by a nontreponemal test when it is positive. In the case of discordant results, TPHA is performed as a second treponemal test.(10-12)

Even though the US Centers for Disease Control and Prevention (CDC) still recommends the traditional screening algorithm, the tendency is toward using reverse screening algorithm in both North America and Europe because of several significant advantages it offers. These advantages are feasibility for automatization bring with it objectivity, and enhanced sensitivity especially for the diagnosis of syphilis in the early and late/latent phase. Using the treponemal test first, will also identify persons who have a positive treponemal test and negative nontreponemal test. Most of these persons will have old treated syphilis, but some may still be infected (7, 13, 14). The objective of this study was to investigate the main differences and
performance parameters of the two diagnostic algorithms for syphilis: the traditional and the reverse.

**Materials and methods**

**Subjects**

We conducted a cross-sectional study on 4789 subjects with results of syphilis serological testing performed at University of Health Sciences, Ankara Training and Research Hospital, between October 2018 and May 2020 to analyze two diagnostic algorithms used for syphilis: traditional algorithm and reverse algorithm. The subjects of this study included outpatients, inpatients, and populations undergoing routine health examinations. These subjects underwent syphilis testing for screening or diagnosis. This study was approved both by the University of Health Sciences and the Ankara Training and Research Hospital Review Board, Ankara, Turkey (No:8).

**Serological tests**

During this period, the syphilis serologic testing for each sample was performed using Immutrep VDRL (Omega Diagnostics, Scotland, United Kingdom), *Treponema pallidum* haemagglutination (TPHA) (Spinreact, S.A.U, Spain), and an automated Architect Syphilis TP (Abbott Diagnostics, Wiesbaden, Germany), which is a CMIA, qualitatively detects both IgM and IgG antibodies to *T. pallidum*, according to the manufacturer’s instructions. For VDRL and TPHA tests, the results expressed as titers. Results of Immutrep VDRL ≥1/2 titer and TPHA ≥1/80 titer were considered positive. For CMIA, all screened sera were determined as positive with S/Co value ≥1.00 and negative with S/Co <1.00 Duplicate tests were excluded. All the serological testing was performed for every serum sample. In our study, FTA-abs IgM/IgG test
(Euroimmun, Germany) was considered as the gold standard test, which was performed in case of discordance between treponemal tests.
**Screening Algorithms**

According to the traditional algorithm, the VDRL test was performed first. If the VDRL test was negative, patients confirmed negative. The CMIA test was performed when the VDRL test was positive. In case of discordance between the test results, the TPHA test was applied as a second treponemal test. When the TPHA test is positive, patients confirmed positive. According to the reverse algorithm, the CMIA test was performed first. If the CMIA test was negative, patients confirmed negative. The VDRL test was performed when the CMIA test was positive. In case of discordance between the test results, the TPHA test was applied as a second treponemal test. When the TPHA test is positive, patients confirmed positive. In case of discordance between treponemal tests, the FTA-Abs IgM/IgG test was considered as gold standard and performed at the Reference Center.

**Statistical Analysis**

IBM SPSS version 26 statistical program was used for statistical evaluation and descriptive information was shown by number and percentage distributions. The χ2 test and z score were used to compare proportions. The percentages of agreement and κ coefficients were calculated to determine the agreements between algorithms. The agreement according to κ values were categorized as almost perfect (>0.90), strong (0.80–0.90), moderate (0.60-0.79), weak (0.40-0.59), minimal (0.21–0.39) and none (0-0.20) (15). The correlation between S/Co values and the TPHA titers was analyzed by the Spearman correlation test. The diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy values were calculated with 95% confidence interval (CI). Optimal cutoff point of the S/Co values for CMIA was identified from analysis of the ROC curve and area under the ROC (AUROC) was calculated. A p value of <0.05 was considered statistically significant.
Results

Comparison of the traditional and reverse algorithms

Of the prospectively collected 4789 sera (one sample per patient), 206 (4.3%) and 125 (2.6%) were positive using reverse screening (CMIA) and traditional screening (VDRL), respectively (Fig. 1). The clinical information for 215 subjects, which have any positive test results, listed in Table 1.

Among 125 VDRL positive samples, 118 (94.4%) were confirmed to be positive by CMIA and were suggestive of syphilis. The seven samples were negative and six (0.13%) of seven were interpreted as biological false positive. One of the seven samples’ VDRL titer was 1/32 and the patient was diagnosed with molluscum contagiosum. That sample was found to be positive by FTA-Abs IgG and suggestive of syphilis. The missed diagnosis rate of the traditional algorithm was 42.5%.

Among 206 CMIA positive samples, 118 (57.3%) samples were confirmed to be positive by VDRL and were suggestive of syphilis. Eighty eight (42.7%) discordant samples were tested with second treponemal test (TPHA) and 82 were confirmed to be positive. Six samples (7.3%) were negative by TPHA. In case of discordance between treponemal tests, the FTA-Abs IgM/IgG test was performed in seven samples (five CMIA positive, TPHA negative and two CMIA negative, TPHA positive). Among six TPHA negative samples, five were tested with FTA-Abs IgM/IgG test (one sample was not sufficient for FTA-Abs). Four of five CMIA positive, TPHA and VDRL negative samples were FTA-Abs positive indicating false negativity of the TPHA test. The remaining one of five sample was FTA-Abs negative which was interpreted as false positivity of the CMIA test. TPHA positive, CMIA and VDRL negative two samples were detected and those samples were also tested with FTA-Abs IgM/IgG test. Two
TPHA positive, CMIA and VDRL negative samples were FTA-Abs positive indicating false negativity of the CMIA test. Overall, 207 patients were diagnosed with syphilis. The sensitivity of the traditional and the reverse algorithm were 57.49% (95% confidence interval [CI], 50.45-64.31%) and 98.55 (95 CI, 95.8-99.7), respectively. Further information is available in Table 2, Fig. 2.

Sensitivity and specificity levels of VDRL, CMIA and TPHA tests were 57.4%, 98.5%, 97.6%; 99.8%, 99.9%, and 100%, respectively. Almost perfect agreement was observed between TPHA and CMIA \([\kappa: 0.980 (95\% \text{ CI}, 0.966-0.993, p<0.001)\)]\]. The false positivity of the traditional and the reverse algorithms were 0.13% and 0.02% respectively. The moderate agreements were found between the traditional and the reverse algorithm \([\kappa: 0.717 (95\% \text{ CI}, 0.657-0.777, p<0.001))\] (Table 3). Percent agreement was 59%, which indicates 41% discordance between diagnostic algorithms. A positive correlation was found between the CMIA S/Co values and the TPHA titers (Spearman correlation coefficient, \(\rho=0.609, p<0.001\)).

High CMIA results are associated with high TPHA/VDRL titers.

The optimal cutoff index (COI) for syphilis screening by CMIA

The AUROC for syphilis screening was 0.986 (95% CI, 0.972-1.000), COI: 3.77 S/Co, p<0.001. Sensitivity: 96.5%, specificity: 100% (Fig. 3). FTA-Abs test positive samples are considered as true positive. According to the FTA-Abs results, ROC analysis is repeated and the AUROC was 0.990 (95% CI, 0.978-1.000), COI: 1.87 S/Co, p<0.001. Sensitivity: 96.6%, specificity: 100%.

**Discussion**

The results of the current study report that the positivity rate of the reverse algorithm was higher than the traditional algorithm. The positive screening rate is 4.3% by the reverse
algorithm compared with 2.6% by the traditional algorithm in our population. The positivity rate was reported as 1.5% and 0.4% by the reverse and the traditional algorithm respectively in a low prevalence population (11). In another study, the positivity rate was reported as 0.8% and 0.1% by the reverse and the traditional algorithm respectively in a low prevalence population (16). In a high prevalence population, Tong et al reported the positivity rate as 11.4% by the reverse algorithm and 8.9% by the traditional algorithm (8). In our study, positivity rates of both algorithms was higher than low prevalence population studies and lower than high prevalence population study of Tong et al. These diversities in positivity rates for syphilis screening may be due to epidemiological factors, as our study population is not a screening population, but mainly consists of symptomatic patients tested for clinical diagnosis of syphilis.

The current study indicated that the missed diagnosis rate of the traditional algorithm was 42.5% (88/207). Due to insufficient clinical information about the patients’ symptoms or the stage of the disease and previous treatment status, we were unable to discriminate treated syphilis from latent syphilis. In a retrospective study in Turkey, 362 subjects underwent syphilis testing for diagnosis, discordance between RPR and TPHA was determined to be 40.7%, which is similar to our study (17). Among 2,749 subjects clinically diagnosed with syphilis, Tong et al. demonstrated the missed diagnosis rate of the traditional algorithm as 24.2%. Further analysis of these subjects show that 7.8% were cases of early syphilis and 58.6% cases of diagnosed with syphilis for the first time (8). Considering possible consequences of untreated syphilis, missed diagnoses caused by the traditional algorithm should be considered as a serious quality problem for a clinical laboratory.

In both low and high prevalence of syphilis, screening with reverse algorithm yields a higher false positivity than traditional screening does (11, 13). According to data reported by
the CDC in 2011, the false positivity rate was 2.9 times greater in the low prevalence population than in the high prevalence population (40.8%, 14.1%, respectively) (13). Park et al. determined that 28% of discordant (CIA positive, RPR negative) serum samples were negative by the second treponemal test (TPPA), were interpreted as false-positive CIA result (18). The false positivity rate of the reverse algorithm was 1.0% and 0.9% in moderate-high prevalence and high prevalence syphilis populations, respectively (8, 19). False positivity rate of the reverse algorithm in our study (0.02%) was dramatically lower than the rates described formerly in the literature. Despite the increased false positivity rate in low prevalence populations, the reverse algorithm is better due to its ability to detect cases of early and late syphilis missed by the traditional algorithm (11, 19). Specificity of screening tests must be high which is crucial to avoid false positivity, especially in low prevalence populations such as blood donors and pregnant women (20, 21).

In the case of discordant results of the syphilis serologic tests, performing the second treponemal test is recommended by the US Centers for Disease Control and Prevention (13). Using a second treponemal test seems to cause increased costs, however it could prevent unnecessary treatment of patients with false-positive serological test results (22). Some correlation between true syphilis infection status determined by confirmatory tests and EIA/CIA S/Co ratios are shown in recent studies (7, 18, 23). Determining a cutoff value for CMIA test is very important to identify potential false positivity and reduce costs (23, 24). In our study, optimal signal cut-off value of CMIA with 100% specificity was 3.77 S/Co. Above 3.77 S/Co, there is no need for confirmatory test.

Although there has been a dramatic increase in the incidence of syphilis in Turkey in recent years, according to local epidemiological data (25), our study is one of the few studies
that evaluate the diagnostic algorithms in syphilis diagnosis in a large Turkish population in the literature.

Our study has some limitations. First, because of insufficient medical records, we could not discriminate between previously treated syphilis and early/latent syphilis. Second, as most of the subjects in our study were men, our population may not represent the general population. Notwithstanding these limitations the current study shows that the reverse algorithm can detect more patients with early/latent syphilis, which leads less patients to be untreated. The CMIA test is found to be more sensitive than the VDRL. Therefore, it was thought that it would be more useful to use the CMIA test with many advantages instead of the VDRL test in screening populations with low syphilis prevalence such as pregnant women, healthcare providers and blood donors.

The key to deciding which algorithm to use lies in being able to distinguish the benefits vs the costs of identifying these persons with only positive treponemal tests. Thus, all the physicians dealing with syphilis be aware of pros and cons of both algorithms.

Acknowledgements

The authors would like to thank Ali Teoman Evren, M.D. for his contributions to language editing.

Conflict of interest

None to declare.
References


Figure Legends

Figure 1. Traditional and Reverse algorithms for syphilis diagnosis.
Abbreviations: VDRL, Venereal Disease Research Laboratory; CMIA, chemiluminescence microparticle immunoassay; TPHA, T. pallidum haemagglutination assay; FTA-Abs, fluorescent treponemal antibody absorption.

Figure 2. Number of samples according to the test results. Positive results indicate as bold.
Abbreviations: VDRL, Venereal Disease Research Laboratory; CMIA, chemiluminescence microparticle immunoassay; TPHA, T. pallidum haemagglutination assay; FTA-Abs, fluorescent treponemal antibody absorption.
*FTA-Abs test did not performed in one sample because of insufficient volume.

Figure 3. Receiver operator characteristic (ROC) curves of the CMIA for syphilis diagnosis.
The AUROC: 0.986 (95% CI, 0.972-1.000), COI: 3.77 S/Co, p<0.001.
Table 1. Clinical information of 215 positive subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (n:215)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Overall 40, median, range 1-88)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>40 (1-86)</td>
</tr>
<tr>
<td>Female</td>
<td>40 (1-88)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>160 (74.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>55 (35.7%)</td>
</tr>
<tr>
<td>Pregnancy status (n:55)</td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>3 (5.5%)</td>
</tr>
<tr>
<td>Not pregnant</td>
<td>52 (94.5%)</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>23 (10.7%)</td>
</tr>
<tr>
<td>Negative</td>
<td>192 (89.3%)</td>
</tr>
<tr>
<td>Reason for testing</td>
<td></td>
</tr>
<tr>
<td>Screening</td>
<td>34 (15.8%)</td>
</tr>
<tr>
<td>Diagnostic</td>
<td>153 (71.2%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>28 (13%)</td>
</tr>
</tbody>
</table>

Abbreviations: HIV, human immunodeficiency virus
Table 2. Comparison of traditional and reverse algorithm’s performance parameters

<table>
<thead>
<tr>
<th>Results</th>
<th>Syphilis Status*</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
<th>Accuracy, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traditional algorithm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>119</td>
<td>6</td>
<td>57.49 (50.45-64.31)</td>
<td>99.87 (99.72-99.95)</td>
<td>95.17 (89.78-97.79)</td>
<td>98.12 (97.81-98.39)</td>
</tr>
<tr>
<td>Negative</td>
<td>88</td>
<td>4575</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse algorithm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>204</td>
<td>1</td>
<td>98.55 (95.8-99.7)</td>
<td>99.98 (99.8-100)</td>
<td>99.51 (96.6-99.9)</td>
<td>99.93 (99.8-99.98)</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>4580</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
One sample did not analyzed because of insufficient volume.

Table 3. Direct comparison of the algorithms

<table>
<thead>
<tr>
<th>Reverse Algorithm</th>
<th>Traditional Algorithm</th>
<th>Total</th>
<th>Agreement (%)</th>
<th>$\kappa$ value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>118</td>
<td>82</td>
<td>200</td>
<td>118/200 (59)</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>4582</td>
<td>4589</td>
<td>4582/4589 (99.8)</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
<td>4664</td>
<td>4789</td>
<td>4700/4789 (98)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval
Figures

Figure 1. Traditional and Reverse algorithms for syphilis diagnosis.
Figure 2. Number of samples according to the test results. Positive results indicate as bold.
Figure 3. Receiver operator characteristic (ROC) curves of the CMIA for syphilis diagnosis. The AUROC: 0.986 (95% CI, 0.972-1.000), COI: 3.77 S/Co, p<0.001.