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Serological diagnosis of syphilis: a comparison of different diagnostic methods

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Abstract

Introduction: Serological tests' limitations in syphilis diagnosis as well as numerous test interpretations mean that patients with discordant serology results can present diagnostic and treatment challenges for clinicians. We analyzed three common diagnostic algorithms for detecting suspected syphilis in high-prevalence populations in Slovenia.
Methods: The prospective study included a total of 437 clinical serum samples from adults throughout Slovenia tested with Rapid Plasma Reagin (RPR), *Treponema pallidum* hemagglutination (TPHA), and an automated chemiluminescence immunoassay (CIA) according to the manufacturer's instructions. In addition to percent agreement, kappa coefficients were calculated as a secondary measure of agreement between the three algorithms.
Results: Overall, of 183 subjects that had seroreactive results, 180 were seroreactive in both the reverse sequence and the European Centre for Disease Prevention and Control (ECDC) algorithm. The traditional algorithm had a missed serodiagnosis rate of 30.0%, the overall percent agreement between the traditional and the reverse algorithm (or the ECDC algorithm) was 87.6%, and the kappa value was 0.733. However, the reverse and ECDC algorithm failed to detect three subjects with positive serodiagnosis determined by additional confirmative treponemal assays.
Conclusions: Our results supported the ECDC algorithm in the serodiagnosis of syphilis in high-prevalence populations and the use of nontreponemal serology to monitor the response to treatment.

Keywords: syphilis, treponema, sexually transmitted infections, serodiagnosis

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Introduction

The presence of clinical signs or medical history with direct detection of the bacterium *Treponema pallidum* subsp. *pallidum* in clinical specimens and/or reactive treponemal and non-treponemal tests is required to diagnose syphilis (1, 2). Currently, there are three common approaches to the serological diagnosis of syphilis. First, the US Centers for Disease Control and Prevention (CDC) recommends a traditional screening algorithm starting with a nontreponemal assay (e.g., the Rapid Plasma Reagin [RPR] or Venereal Disease Research Laboratory [VDRL] test) to identify persons with possible untreated infection; this screening is followed by a confirmative treponemal assay (e.g., *Treponema pallidum* hemagglutination [TPHA], *Treponema pallidum* particle agglutination [TPPA], or the fluorescent treponemal antibody-absorption [FTA-ABS] test). Second, an updated reverse sequence algorithm based on the availability of automatable treponemal immunoassay suggests that samples may be screened using a treponemal assay (e.g., enzyme immunoassay [EIA] or chemiluminescence immunoassay [CIA]), and, if reactive, either a quantitative non-treponemal or a second, different treponemal assay is used to assess disease and treatment status and confirm suspected infection. Third, there is the European Centre for Disease Prevention and Control (ECDC) algorithm, which starts with a primary treponemal screening test followed by a second, different confirmative treponemal assay (1, 3–5). However, due to serological tests' limitations and the lack of a reliable gold standard for syphilis diagnosis as well as numerous test interpretations, patients with discordant serology results can present diagnostic and treatment challenges for clinicians (6, 7). Regardless of the algorithm used, the choice of treponemal-specific assays with incomparable performance properties may introduce the possibility of having uncertainty in the serodiagnosis of syphilis.

The goal of this study was to compare two commercially available total antibody treponemal assays: a conventional TPHA test with an automated CIA run on the random access Siemens Immulite® 2000 analyzer. The study was designed to analyze three different algorithms with the implementation of both treponemal tests for detecting suspected syphilis in high-prevalence populations in Slovenia.

Materials and methods

The prospective study included a total of 437 clinical serum samples from adults with suspected syphilis from hospitals and clinics throughout Slovenia submitted for the first time to routine screening for syphilis to our laboratory from September 2013 to December 2014. The syphilis serologic testing for each sample was performed using a RPR test with antigens containing cardiolipin, lecithin, and cholesterol (bioMérieux, Netherlands), a TPHA test with antigens of the Nichols strain of *T. pallidum* (Randox, UK), and an automated CIA with the recombinant antigen Tp17 (Immulite® 2000 Syphilis Screen Test, Siemens, UK) according to the manufacturer's instructions. Samples with discrepant results between RPR, TPHA, and CIA were further tested with other treponemal assays, the IgG-FTA-ABS test (bioMérieux, France), the 19 S IgM-FTA-ABS test (bioMérieux, France) or IgM-EIA test (Captia™ NMT Syphilis IgM, Trinity Biotech, Ireland), and the IgG-Line Immuno Assay (LIA, INNO-LIA Syphilis Score, Fujirebio, Belgium). The remaining 200 samples of a total of 637 samples tested were

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randomly selected from our serum collections of non-sexually transmitted disease (STD) clinic patients stored as a part of non-STD routine diagnostics and tested with TPHA and CIA to challenge the specificity of the CIA in comparison with the TPHA assay.

Statistical analyses were performed using IBM® SPSS® software, version 21.0 for Windows. In addition to percent agreement, kappa coefficients were calculated as a secondary measure of agreement. The agreement of the results by kappa values is categorized as very good (0.81 to 1.00), good (0.61 to 0.80), moderate (0.41 to 0.60), fair (0.21 to 0.40), or poor (< 0.20) (8).

Results

Following the testing of the 437 serum samples, the results of the CIA were compared to those of the TPHA test. Overall, 180 subjects had TPHA-positive / CIA-positive results, and 247 subjects had TPHA-negative / CIA-negative results; seven subjects were TPHA-negative / CIA-positive, and three subjects were TPHA-positive / CIA-negative. The overall percent agreement and kappa value were 97.7%, κ = 0.953 (95% confidence interval [CI] = 0.924 to 0.982, $p < 0.001$). These data indicated that there was a very good strength of agreement between the TPHA test and the CIA. Using the TPHA test as the standard test, the CIA had 100% sensitivity and 98.8% specificity. In addition, we analyzed the 200 serum samples of the non-STD clinic-adult patients, and the agreement of the CIA compared to the TPHA test was 100%. All 200 serum samples were TPHA-negative / CIA-negative.

Ten samples from subjects with suspected syphilis and the discordant TPHA and CIA results were further tested to assess the possibility of false test results (Table 1). Three sera found to be positive by the TPHA test were found to be negative by all other tests, suggesting three TPHA false positives (Table 1, patients 1–3). Similarly, three sera found to be positive by the CIA were negative by all other tests, suggesting three CIA false positives (Table 1, patients 8–10). The remaining four samples were positive by the CIA and negative by the TPHA test. All four samples were also positive by the IgG-FTA-ABS test, three of them were positive by the IgG-Inno-LIA test, and one sample was indeterminate. A review of each patient's medical record was performed to determine the reason for testing and the final clinical interpretation of results. Three patients (Table 1, patients 4, 5, and 7) were reactive by the CIA, the IgG-FTA-ABS, and the IgG-Inno-LIA, but nonreactive by the RPR and the TPHA. Because these patients were both highly likely to have major epidemiologic risk factors for syphilis and were not previously treated for syphilis, all three were diagnosed with possible latent syphilis and were treated appropriately. One patient (Table 1, patient 6) was reactive by the CIA and had low titre sera determined by the IgG-FTA-ABS, but had an indeterminate IgG-INNO-LIA and nonreactive RPR and TPHA test. The patient was first syphilis-screened as a blood donor and was managed then by a dermatologist. Because the patient had no contact with syphilis as well as no clinical history of any STD, this finding was interpreted as a probably falsely positive serodiagnosis, and the patient was not treated for syphilis. If the TPHA test had been selected as the screening test for the reverse algorithm or the ECDC algorithm, then four samples positive by other treponemal assays would have been missed, possibly resulting in a false serological result.

In this study, we also found four RPR-positive / TPHA-negative / CIA-negative cases that were confirmed to be biological false-positive reactions. All four had false-positive RPR titers less than 8. A review of each patient's medical record revealed that two patients had hepatitis B virus infection, one patient had herpes zoster, and one had a false-positive reaction of unknown cause.

In addition, we further analyzed the agreement between the three syphilis testing algorithms. Of 437 subjects that were tested for syphilis, 180 had reactive results in both the reverse sequence and the ECDC algorithm. Our results indicated that with the traditional algorithm 126 of the 180 subjects would be diagnosed with syphilis; however, 54 subjects that were RPR-negative / TPHA-positive / CIA-positive would not be diagnosed (Fig. 1). The missed serodiagnosis rate was 30.0%. The overall percent agreement between the traditional and reverse algorithm (or the ECDC algorithm) and kappa value were 87.6%, κ = 0.733 (95% CI = 0.669 to 0.797, $p < 0.001$). The direct comparison of the reverse and ECDC algorithm gave an overall percent agreement and kappa value of 100% and κ = 1.000 (95% CI = 1.000 to 1.000, $p < 0.001$). These data indicated that there was a very good strength of agreement between the reverse and ECDC algorithm. However, both, the reverse and ECDC algorithm failed to detect three subjects with positive serodiagnosis determined by additional confirmative treponemal assays. These three cases were screened reactive by the CIA but were not confirmed by the TPHA test. The selection of a second, analytically less-sensitive treponemal test may introduce the possibility of having a false serological result.

Discussion

The laboratory diagnosis of syphilis still relies on nontreponemal and treponemal serologic tests. Decisions on which treponemal test a laboratory should use depend on many factors, including cost, ease of use, suitability for automation, and performance characteristics. A significant advantage of immunoassays is that they can be automated, significantly reducing labor costs and increasing sample throughput compared to other syphilis tests. There are a number of automated treponemal antibody assays evaluated elsewhere, mostly with relatively high sensitivity and specificity (9–12). One of these, the chemiluminescence immunoassay run on the Siemens Immulite® 2000 analyzer, was evaluated in this study. Although

Table 1 | Serologic results for ten serum samples with discrepant results in the TPHA and the Immulite 2000 CIA. Abbreviations: RPR = rapid plasma reagin, TPHA = *Treponema pallidum* hemagglutination, CIA = chemiluminescence immunoassay, FTA-ABS = fluorescent treponemal antibody-absorption, EIA = enzyme immunoassay, LIA = line immunoassay; ID = indeterminate.

Sample	TPHA	Immulite 2000 CIA	RPR	IgG-FTA-ABS	IgM-FTA-ABS	Captia IgM-EIA	IgG-Inno-LIA
1	1:160	Neg	Neg	Neg	Neg	/	Neg (Tp47/Tp17/Tp15/TmpA 0)
2	1:160	Neg	Neg	Neg	Neg	/	Neg (Tp47/Tp17/Tp15/TmpA 0)
3	1:320	Neg	Neg	Neg	Neg	/	Neg (Tp47/Tp17/Tp15/TmpA 0)
4	Neg	Pos	Neg	1:20	Neg	/	Pos (Tp47/Tp17 1+, Tp15/TmpA 0)
5	Neg	Pos	Neg	1:10	Neg	Neg	Pos (Tp47/Tp17 1+, Tp15/TmpA 0)
6	Neg	Pos	Neg	1:20	Neg	Neg	ID (Tp47/Tp15/TmpA 0, Tp17 2+)
7	Neg	Pos	Neg	1:20	Neg	Neg	Pos (Tp17/TmpA 2+, Tp15 0.5+, Tp47 0)
8	Neg	Pos	Neg	Neg	Neg	Neg	Neg (Tp47/Tp15/TmpA neg/Tp17 0.5+)
9	Neg	Pos	Neg	Neg	Neg	Neg	Neg (Tp47/Tp15/TmpA neg/Tp17 0.5+)
10	Neg	Pos	Neg	Neg	Neg	/	Neg (Tp47/Tp17/Tp15/TmpA neg)

our findings showed a very good strength of agreement between the two treponemal assays (i.e., 97.7%; CIA sensitivity and specificity in high-prevalence populations compared to the reference TPHA test were 100% and 98.8%, respectively), there were samples with discordant results that became a focus for further investigation. Based on the results of other treponemal tests (i.e., IgG-FTA-ABS and IgG-LIA) as well as those of the RPR and the treponemal IgM assays to determine the likelihood of past or recent infection, three samples were interpreted as probable false-positive TPHA results and three samples as probable false-positive CIA results, giving each assay a false-reactive rate similar to that which was previously reported elsewhere (less than 1%) (13–15); that is, 3/437 (0.68 %). Although associations of false-positive results of hemagglutination tests with specific conditions were established, the cause in the three subjects was unknown. In contrast, all three subjects with probable false-positive CIA results were intravenous drug users. Despite our finding, more studies are needed to understand the real cause of false-positive EIAs/CIAs.

Four TPHA-negative samples were positive in the CIA. After analysis with other treponemal and nontreponemal tests, and when evaluated together with patients' history of STD, three samples were interpreted as probable false-negative TPHA results, implying that the CIA may have been more sensitive in detecting latent syphilis than the TPHA. However, one sample was interpreted as probable falsely positive serodiagnosis, even though the CIA as well as the low titre IgG-FTA-ABS test were positive. The patient was advised to be followed up due to a possible seroconversion of the TPHA and RPR. The high specificity of the treponemal screening test is crucial to avoid false positive samples, resulting in lower positive predictive values especially in low-prevalence populations, such as blood donors and pregnant women (16, 17). In this study, among 200 samples of non-STD clinic-patients, all samples were TPHA-negative / CIA-negative, suggesting an excellent agreement and specificity of both treponemal assays.

Traditionally, sera submitted for syphilis testing have been screened using a nontreponemal test, such as RPR. This algorithm has demonstrated reliable performance in correlating results with disease status (5, 7, 18, and 19). Screening for syphilis using a treponemal assay detects a higher number of patients with reactive results compared to traditional screening. Our data indicated that the missed serodiagnosis rate of the traditional screening would be 30.0%. Due to the main limitation of our study of not having clinical information on the patient's symptoms/signs or stage of the disease, we were unable to evaluate the diagnostic accuracy of the algorithms compared with clinical diagnosis. Tong et al. demonstrated the missed diagnosis rate of the traditional screening as 24.2% among 2,749 patients diagnosed with syphilis by clinicians (7). These findings supported past work suggesting that reverse screening may detect a higher rate of screen-reactive patients with past untreated and inappropriate treated syphilis or early syphilis (5, 6). Our data demonstrated that the reverse algorithm, in which serum samples were first tested by the automated CIA, facilitated the detection of patients highly likely to have destructive latent disease stages, while offering the specific and objective screening approach.

The screening strategy for syphilis recommended by the ECDC involves a primary treponemal screening test followed by a second confirmatory treponemal test (1). The results obtained from a large cohort (7) as well as our data support the application of the ECDC algorithm for syphilis screening of a high-prevalence populations. The direct comparison of the reverse and ECDC algorithm in our study gave an overall percent agreement and kappa value of 100% and $\kappa = 1.000$, suggesting that a nontreponemal assay is unnecessary for serodiagnosis of syphilis. Once syphilis has been diagnosed, a nontreponemal test is performed to assess disease activity and treatment status. In the cases in which the first treponemal test is positive and the confirmatory treponemal test is negative, then it is inconclusive whether the first screening test is a false positive or is more sensitive. Consequently, it would be advisable for a laboratory to select two treponemal assays with comparable performance to avoid having discrepant results (20). In order to potentially resolve these discrepancies, the results of other treponemal confirmatory

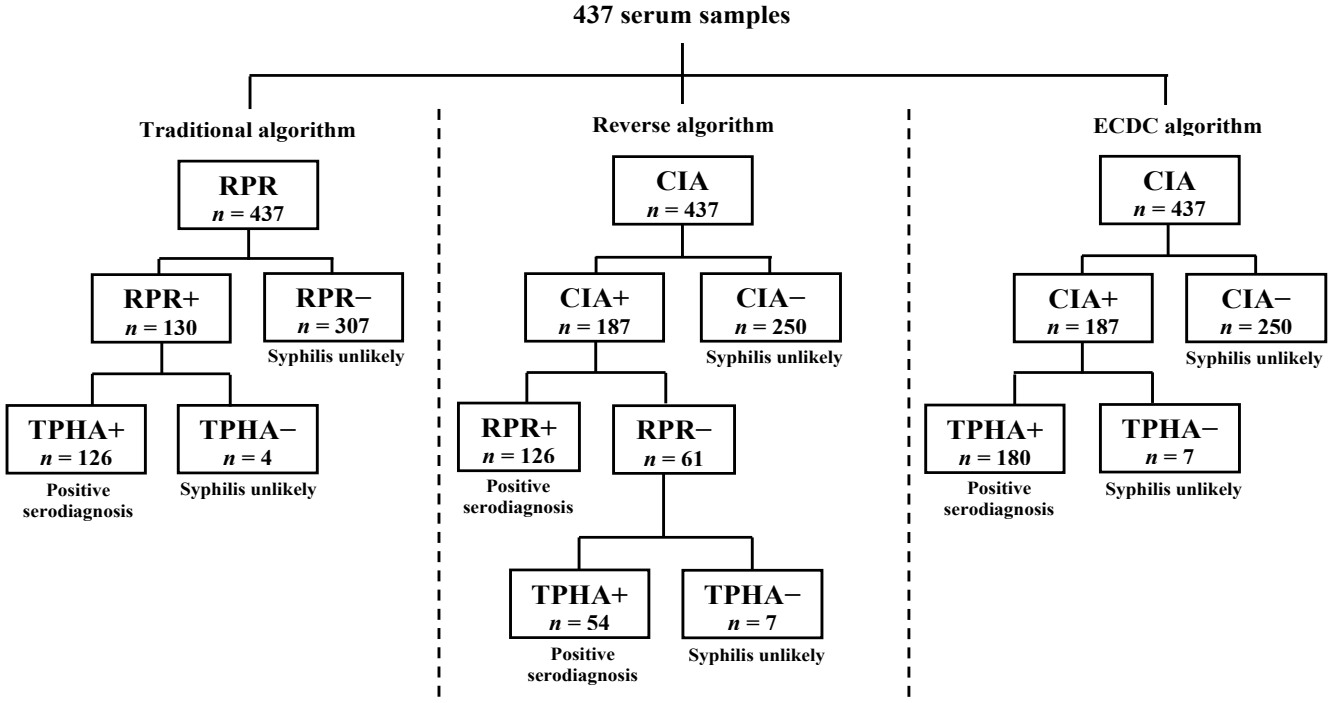

Figure 1 | Various testing algorithms for syphilis serodiagnosis. Abbreviations: RPR = rapid plasma reagin, TPHA = *Treponema pallidum* hemagglutination, CIA = chemiluminescence immunoassay, ECDC = European Centre for Disease Prevention and Control.

tests should be reviewed. In this study, three of a total of 183 syphilis seropositive samples were CIA-positive / TPHA-negative, giving both the reverse and ECDC algorithm a missed serodiagnosis rate of 1.64% that went undetected unless it was further investigated by other treponemal assays.

Our results demonstrate comparable performance among the two treponemal assays evaluated. However, our data suggest that each method has limitations, including the potential for false-positive and false-negative results. In addition, we support the ECDC algorithm in the serodiagnosis of syphilis in high-prevalence populations. Clinicians must still collect other relevant information needed to diagnose and stage patients with suspected syphilis, and they must continue to use nontreponemal serology to monitor response to treatment.

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