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It Is Time To Use Treponema-Specific Antibody Screening Tests for Diagnosis of Syphilis

Assays that detect treponema-specific antibodies, which are either automated or can be done as point-of-care tests, have been developed, some of which are FDA approved. These assays have the advantage of being easily performed and demonstrate high sensitivity, both key features of an infectious disease screening test. As a result, many high-volume clinical laboratories have begun to offer a reverse syphilis testing algorithm where a treponema-specific test is used for screening, followed by a nontreponemal test (i.e., rapid plasma reagin [RPR]) to assess disease activity and treatment status. Concerns about physicians being able to understand and apply this new testing algorithm have been expressed (8). In this point-counterpoint, Michael Loeffelholz of the University of Texas Medical Branch at Galveston explains why his laboratory has adopted this reverse algorithmic approach. Matthew Binnicker of the Mayo Clinic, Rochester, MN, explains why the reverse algorithm may not be suitable for all clinical laboratories and every clinical situation.

POINT

Several expert committees and organizations now either recommend or include the option for the use of treponema-specific assays to screen for syphilis. In this approach, a reactive treponemal screening assay is followed by a quantitative nontreponemal assay to diagnose active disease and to monitor response to treatment (i.e., the "reverse algorithm"). This algorithm also consists of a second and different treponemal assay that is used either to confirm all reactive screening results or only to resolve discordant screening and nontreponemal assay results (Fig. 1). The Association of Public Health Laboratories (http://www.aphl.org/aphlprograms/infectious/std/Documents/LaboratoryGuidelinesTreponemapallidumMeetingReport.pdf, last accessed 11 September 2011), the United Kingdom Health Protection Agency (6), and the International Union Against Sexually Transmitted Infections (7) all endorse or encourage the use of a reverse algorithm that begins with a treponemal immunoassay. The United States Centers for Disease Control and Prevention (CDC) continues to recommend the traditional algorithm (3). However, in that report, the CDC acknowledges the use of treponemal immunoassays as screening assays and provides recommendations for laboratories that choose this reverse algorithm approach.

The traditional approach to the diagnosis of syphilis begins with a nontreponemal assay, either the venereal disease research laboratory (VDRL) test or, more commonly, the RPR test that detect anticardiolipin antibodies (Fig. 1). Since these antibodies are not specific for syphilis, reactive nontreponemal assay results must be confirmed with an assay that detects antibodies produced against *T. pallidum*. Traditional treponemal assays used in this algorithm are fluorescent treponemal antibody absorption (FTA-ABS), microhemagglutination for *T. pallidum*, *T. pallidum* hemagglutination (TPHA), and TPPA. In 1975, Veldkamp and Visser described for the first time the use of a laboratory-developed enzyme immunoassay (EIA) for the diagnosis of syphilis and suggested that it could serve as a first-line screening assay based on its high sensitivity (16). Another early, laboratory-developed EIA using whole-cell sonicates of *T. pallidum* was more sensitive than VDRL. As a result, additional cases of secondary and late syphilis were detected using the treponema-specific EIA (13). In 1989, Young et al. described the performance of a commercial EIA that detected anti-*T. pallidum* IgG (19). Against a panel of over 1,200 specimens, EIA sensitivity and specificity were comparable to those of VDRL. In a subsequent publication in 1992, perhaps the first published in-depth discussion of a treponemal assay-based testing algorithm, the same group proposed that EIA replace VDRL as the screening test (18). During the 1990s, a number of EIAs became commercially available (5, 14, 15). Since 2000, the options have expanded to include chemiluminescence immunoassay (CLIA) (10) and multiplex flow immunoassay (MFI) (2).

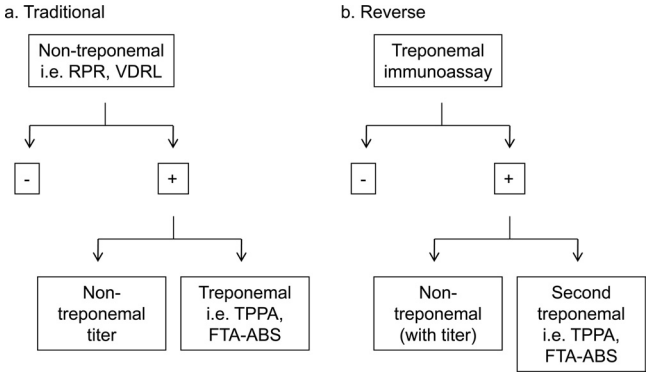

FIG 1 Syphilis testing algorithms.

Recommendations that include the use of treponemal immunoassays that detect either IgG or IgG and IgM (total antibody) as the single screening test are based primarily on their excellent sensitivity and specificity but also on the ability to automate testing on high-throughput instrumentation. I will address each of these attributes and discuss my own experience with the change from the traditional to the reverse algorithm for the diagnosis of syphilis. While IgM-based immunoassays are available to diagnose acute primary and congenital infections and for follow-up, I

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TABLE 1 Sensitivity of treponemal immunoassays and nontreponemal assays

Sample category	% Sensitivity of: IA[a]	VDRL	RPR	Reference
Consecutive, unselected	100		99.1	14
	98.4	98.4		19
Characterized				
Untreated primary syphilis	84.8–97.0			5
	57.1, 100[b]		85.7	11
	77.3	77.3		13
	82	73		18
Untreated secondary syphilis	97.4–100			5
	100, 100[b]		96.8	11
	100	100		13
Untreated latent and late syphilis	94.7–100			5
	100, 100[b]		100	11
	90.5	52.4		13
Treated syphilis	97.1–100			5
	96.1, 98.7[b]			11
	95.8			13

[a] IA, immunoassay. All methods are EIAs, except where noted otherwise.
[b] CLIA used.

will not discuss these, as they are generally inappropriate for routine screening purposes.

Immunoassays are at least as sensitive as RPR. With the possible exception of early primary syphilis, published data overwhelmingly show that treponemal immunoassays are at least as sensitive as nontreponemal assays for the diagnosis of syphilis (Table 1). Pope et al. tested 75 FTA-ABS-positive serum samples from patients with various stages of syphilis (13); 67 (89%) were positive by EIA, and 58 (77%) were positive by VDRL. Among untreated cases (all disease categories), VDRL sensitivity was 71%. Among cases of primary syphilis, EIA sensitivity was 75% and VDRL sensitivity (untreated cases only) was 77%. Excluding cases of primary syphilis, EIA sensitivity increased to 96%, whereas VDRL was essentially unchanged at 78%. Cole et al. evaluated the performance of 10 EIAs based on the testing of 114 specimens from syphilitic patients (5). Nontreponemal assays were not evaluated. Among secondary and latent cases of syphilis, the sensitivities of the EIAs ranged from 96 to 100%. Among primary cases, the sensitivities of nine EIAs ranged from 93 to 98%, while the sensitivity of one kit was 88%. Marangoni et al. evaluated the performance of commercial CLIA and EIA kits (11). Among 54 cases of untreated syphilis, the sensitivities of CLIA, EIA, and RPR were 100, 94, and 96%, respectively. Among seven untreated primary syphilis cases, the sensitivities of CLIA, EIA, and RPR were 100, 57, and 86%, respectively. Among 77 cases of treated latent syphilis, the sensitivities of CLIA and EIA were 99 and 96%, respectively. Binnicker et al. evaluated the performance of several EIAs and an MFI and showed comparable sensitivities of 96 to 97% versus FTA-ABS and 99 to 100% versus a consensus defined as a majority of treponemal tests in agreement (2). Of 94 specimens positive by consensus, RPR was positive for 66 (70%). However, due to the absence of clinical data, it was not possible to determine if any specimens were from treated patients. Finally, Mishra et al. showed that confirmed positivity rates increased significantly (likely due to the detection of additional latent syphilis infections) after changing their screening method from RPR to treponemal EIA (12). Collectively, these data show that the overall sensitivity of immunoassays is at least as good as that of nontreponemal assays but that it is decreased during primary syphilis. Indeed, a seronegativity window of up to 4 weeks during early primary syphilis is recognized (8). It should be pointed out that nontreponemal assays also suffer from lower sensitivity during primary syphilis (8). This limitation of syphilis screening assays in general, but immunoassays in particular (for the purpose of this debate), can be effectively mitigated by attaching the following (or similar) comment to all negative immunoassay screening results: "Cannot exclude incubating or early syphilis. Submit a second sample if clinically indicated." An important consideration for laboratories considering an EIA-based algorithm is the variable performance of commercial EIAs. Schmidt et al. evaluated eight EIAs for the detection of either IgG or total antibodies in patients with primary syphilis. The sensitivities of these commercial assays ranged from 23 to 77%, compared to 44% for VDRL (15). Marangoni et al. reported CLIA and EIA sensitivities of 100 and 57%, respectively, for primary syphilis (11). With regard to secondary, latent, and late syphilis, most studies show superior sensitivity of treponemal immunoassays over nontreponemal assays. This is due in part to the prozone reaction, which specifically affects nontreponemal assays. The prozone reaction occurs in agglutination or precipitation tests when an excess of antibody forms small complexes, preventing visible agglutination. By late syphilis, the sensitivity of nontreponemal assays declines to 71 to 73% (8). In my opinion, the lower sensitivity of nontreponemal assays during latent and late syphilis may be a greater threat to infected individuals and to public health than false-negative immunoassay results during early primary syphilis, because latent or late disease is more likely to remain unsuspected than recent exposures.

Immunoassays are more specific than RPR. The use of treponemal immunoassays to screen for syphilis eliminates biological false positives due to the presence of anticardiolipin antibodies from other diseases. A number of studies have shown that immunoassays have fewer false positives than nontreponemal assays (10, 11, 13). Head-to-head comparisons showed specificities of 98 to >99% for immunoassays and consistently slightly lower specificities of 98 to 99% for RPR (11). Excellent specificity notwithstanding, any test will have a poor positive predictive value when performed on populations with extremely low disease prevalence. For this reason, reverse algorithm guidelines recommend that all specimens positive by a treponemal immunoassay also be tested by a nontreponemal assay and/or a second, different treponemal assay such as TPPA or TPHA (Fig. 1) (6, 7). In a direct comparison of reverse and traditional algorithms, Binnicker et al. reported a higher false-reactivity rate by reverse screening (1). However, all false-reactive IgG results were negative by RPR and TPPA and the overall results would have been interpreted as negative at the completion of the testing algorithm. In its entirety, the reverse algorithm is likely to have excellent positive predictive value, although additional studies are necessary to confirm this.

Immunoassays can be automated, reducing labor costs and increasing throughput. A significant advantage of immunoassays is that they can be automated, significantly reducing labor and increasing sample throughput compared to other syphilis tests (2,

14). This is an important attribute of immunoassays, as labor is usually the single greatest direct cost to the laboratory, and directors are frequently faced with the challenge to do more (i.e., testing) with less (i.e., staff). While automation of syphilis screening would have the greatest impact on laboratories with high sample volumes, even laboratories with lower volumes could appreciate the benefit if they were able to combine multiple immunoassays with a single instrument. A recent study that included all costs (including testing, treatment, follow-up, etc.) demonstrated that a reverse algorithm consisting of EIA screening followed by immunoblot assay confirmation was more cost-effective than a traditional algorithm (4).

University of Texas Medical Branch experience with the change from the traditional to the reverse algorithm. We changed our syphilis screening assay from RPR to the Bioplex IgG MFI (Bio-Rad, Hercules, CA) in December 2010, performing approximately 110 IgG tests each day, 7 days a week (ca. 3,250/month). All syphilis testing is performed during a single shift, and IgG testing is completed by noon. Overall, approximately 96% of specimens are nonreactive by MFI, requiring no further testing. When the IgG test is reactive, RPR titer determination and TPPA are performed and reported in the afternoon. Yen-Lieberman et al. reported that a positive RPR result was sufficient to verify syphilis (17). However, we found that a weakly reactive RPR titer of 1:1 was not always sufficient to verify syphilis in specimens reactive by IgG MFI, while specimens with RPR titers of $\geq$1:2 were always confirmed positive by TPPA (9). We and others have also found that the Bioplex MFI strength of signal (antibody index) predicts the TPPA result (9, 17) and could potentially be utilized to eliminate unnecessary TPPAs (a Bioplex antibody index of $\geq$8 was 99% predictive of a positive TPPA [9]). Our experience has shown that, as recommended by Mishra et al. (12), implementation of the reverse algorithm requires communication regarding interpretation of results.

In conclusion, consistent with recommendations (6, 7), laboratories should consider implementing the reverse algorithm for diagnosis of syphilis. This approach is more sensitive than the traditional algorithm; detecting cases of secondary, latent, and late syphilis missed by nontreponemal screening assays. Rare false-negative immunoassay results during early primary syphilis can be mitigated by attaching appropriate comments to negative test results. By subjecting specimens with positive immunoassay results to an RPR titer determination and a second treponemal assay to verify infection status, the reverse algorithm has nearly 100% specificity. Finally, automation of the immunoassay reduces laboratory labor costs and may also reduce the overall cost burden associated with the detection, treatment, and follow-up of syphilis.

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COUNTERPOINT

At our institution, we follow the reverse screening algorithm for syphilis testing, partly due to the high volume of samples (>21,000 serum samples in 2010) submitted for syphilis serology each year. Therefore, I agree with many of the points presented by Loeffelholz on the utility of reverse screening. However, the implementation of this testing algorithm has created a substantial amount of confusion and anxiety among healthcare providers and patients, especially when the results of treponemal screening (e.g., EIA) and RPR are discordant (e.g., EIA reactive, RPR nonreactive). Although reverse screening possesses several advantages (e.g., automation, objective interpretation of results, increased sensitivity) over traditional testing, the data discussed here suggest

that it is premature to recommend that all clinical laboratories screen for syphilis using a treponemal assay.

The Centers for Disease Control recommends screening with a nontreponemal test. A recent communication from the CDC indicated that screening for syphilis with a nontreponemal test, such as RPR, is still recommended (2). Due to the lower overall specificity of nontreponemal tests, samples testing reactive by RPR should be tested by a treponemal assay (e.g., TPPA) for confirmation. This recommendation is based on years of experience with the traditional testing algorithm, as well as a number of studies suggesting that the results of RPR screening may show a higher degree of correlation with disease activity than the reverse testing algorithm (2, 3, 5). This is due to the fact that treponemal tests (e.g., EIA) are not able to distinguish between active disease and past, successfully treated, syphilis (8). In contrast, nontreponemal tests, such as RPR, generally become negative following treatment and therefore, can be used to monitor a patient's response to therapy (2, 7).

Reverse screening may increase the rate of patients with screen-reactive, and potentially falsely reactive, results. In 2011, the CDC published a landmark study in which syphilis serology results ($n = 140{,}176$) from five clinical laboratories using reverse screening between 2006 and 2010 were reviewed (2). Three of these laboratories served areas with a low incidence of syphilis, while the remaining two labs served a high-prevalence population. Among the 140,176 samples screened by EIA or chemiluminescence immunoassays (CLIA), 4,834 (3.4%) were reactive by the treponemal test. Of the 4,834 EIA/CLIA-reactive samples, 2,734 (56.7%) were nonreactive by RPR, of which 866 (31.6%) were also nonreactive by a second treponemal test (e.g., TPPA or FTA-ABS), suggesting falsely reactive EIA/CLIA results. Interestingly, the percentage of discordant (EIA/CLIA-reactive, RPR-nonreactive) serum samples was higher in the low-prevalence population than in the high-prevalence population (60.6 versus 50.6%). It was also observed that the percentage of discordant serum samples testing nonreactive by TPPA or FTA-ABS was greater in the low-prevalence population than in the high-prevalence group (40.8% versus 14.4%) (2). These data suggest that reverse screening may identify a higher number of patients with falsely reactive results than the traditional algorithm, especially when used in areas with low disease prevalence.

In a similar study, Park et al. (6) analyzed the results of 21,623 serum samples screened by a treponema-based CLIA between August and October 2007. Among the 21,623 samples tested, 439 (2%) were reactive by CLIA and 255/439 (58%) were subsequently found to be nonreactive by RPR. Furthermore, this study demonstrated that 71/255 (28%) discordant (CLIA-reactive, RPR-nonreactive) serum samples were negative by TPPA, suggesting a falsely reactive CLIA result. Interestingly, this rate of probable, falsely reactive EIA/CLIA screening results is similar to that reported in the CDC study (31.6%) (2). Together, these studies raise concerns that the use of the reverse algorithm, particularly in low-prevalence populations, may increase the rate of patients with a reactive screening test and the number of falsely reactive screening results. It is clear that an increased rate of falsely reactive screening results is problematic; however, it should also be emphasized that an overall increase in the rate of reactive screening results (whether truly or falsely reactive) may also have important implications, as this may lead to more patient follow-ups, unnecessary treatment, and patient anxiety regarding the results. An important limitation of the studies addressing the performance of the reverse algorithm is that parallel screening by RPR was not performed, and therefore, it is impossible to conclude whether reverse screening yields a higher false-reactive rate than the traditional algorithm.

To address this limitation, our laboratory performed a direct comparison of the reverse and traditional screening algorithms using serum samples collected from 1,000 patients residing in a population with a low prevalence of syphilis (Olmsted County, MN). The findings, which are reported in this issue of the *Journal of Clinical Microbiology*, indicate that the traditional algorithm showed a false reactivity rate of 0.0% (0/1000), compared to the 0.6% (6/1000) obtained by reverse screening ($P = 0.03$). All patients testing reactive by RPR ($n = 4$) were confirmed to be positive by TPPA, and the results of RPR correlated with disease and treatment status in all cases. In contrast, the results of reverse screening were reactive for six patients who subsequently tested negative by TPPA and had no clinical or treatment history of syphilis. The nonreactive TPPA results of these six patients are highly suggestive of falsely reactive screening results obtained by the reverse algorithm.

Reverse screening may result in increased cost, more patient follow-ups, and overtreatment. As the number of laboratories performing reverse screening has increased, there has been significant interest in better defining the economic and clinical impact of this testing algorithm. A significant limitation of implementing reverse screening in small clinical laboratories is the presumed higher cost of this testing algorithm. Our laboratory recently reviewed the list-fee reagent cost for four treponemal assays that are commonly used for screening purposes (1). The costs ranged from ~$3.00/patient for the Trep-Sure and Trep-Chek IgG EIAs (Phoenix Biotech, Jamestown, NY) and $9.00/patient for the BioPlex IgG multiplex flow immunoassay (Bio-Rad, Hercules, CA) to as high as $18.75/patient for the Trep-ID EIA (Phoenix Biotech). This is compared to a list-fee reagent cost of only $0.51/patient for RPR (BD, Franklin Lakes, NJ). Importantly, these costs do not account for technologist time or instrumentation that may be needed to automate testing. To further assess the effects of treponemal screening, Owusu-Edusei et al. (5) constructed a decision analysis model to approximate and compare the clinical effects (e.g., patient follow-ups, treatment, etc.) and costs of the traditional and reverse screening algorithms. The authors analyzed a simulated cohort of 200,000 patients (prevalence of 0.5% current infections, 5% previous infections) and estimated that the use of reverse screening would result in (i) the treatment of 118 more cases (986 versus 868), (ii) a substantially higher number of patient follow-ups (11,450 versus 3,756), and (iii) overtreatment (964 versus 38) compared to traditional testing. Furthermore, their cost analyses suggested that the net costs of reverse screening was higher than that of the traditional algorithm ($1.6 million versus $1.4 million), with an estimated cost-effectiveness ratio of $1,671 for reverse screening to $1,621 for traditional testing (5).

Traditional screening is appropriate for low-volume clinical laboratories. There are several other factors that support the continued use of traditional screening, particularly in small clinical laboratories. First, nontreponemal assays (e.g., RPR) are relatively easy to perform, allowing these methods to be validated and implemented in hospitals and small clinical laboratories. Second, initial testing with RPR may allow for a more rapid screening method compared to the reverse algorithm, especially when a low number of samples are tested each day. Finally, screening with

RPR does not require expensive instrumentation (as is commonly the case for reverse screening), making the traditional algorithm a cost-effective approach for most small clinical laboratories.

Despite recent advances in technology and instrumentation, the diagnosis of syphilis remains a challenging endeavor. The reverse screening algorithm offers several significant advantages, including an objective interpretation of screening results, the potential to automate testing, and enhanced sensitivity for the identification of patients with late/latent or early syphilis (4). However, there are a number of important limitations to reverse screening (e.g., higher cost, increased detection of patients with reactive screening results) that will likely prevent the universal implementation of this testing algorithm. The traditional approach (RPR screening) has a proven track record and is suitable for most low-volume clinical laboratories. When the reverse screening algorithm is used, it is critical that samples with discordant results (e.g., EIA reactive, RPR nonreactive) be tested by a second treponemal assay (e.g., TPPA) to assist in the interpretation of results (2). Furthermore, healthcare providers must carefully review each patient's clinical and treatment history to discern the accuracy of test results and the appropriate clinical management.

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I have no conflicts of interest to declare.

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SUMMARY

Points of agreement:

- Reverse screening has several important advantages, including an objective interpretation of screening results, the potential to automate testing, and enhanced sensitivity for the identification of patients with late/latent or early syphilis.
- Treponeme-specific enzyme-linked immunosorbent assays cannot differentiate between active disease and previous infection. This may lead to more discordant results between the screening test and RPR. Such discordant specimens need to be tested by a second treponemal test such as the *Treponema pallidum* particle agglutination (TPPA) test (see Fig. 1).
- Nontreponemal test results generally become negative following treatment, so reactive results by the traditional algorithm are much less likely in previously infected patients.

Points requiring further study:

- Because parallel studies have not been done, it is unclear if the traditional RPR screening test or the treponema-specific screening test is likely to result in more false-positive results in a population with a high prevalence of syphilis.
- The value of the reverse algorithm in pregnant women and HIV-positive individuals has not been firmly established and requires greater study.
- The cost-effectiveness of the reverse algorithm has not been proven. Does its increased sensitivity in late/latent syphilis and primary syphilis outweigh its impact on the cost of care of patients who have false-positive screening tests because of previous infections?

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