

# Serodiagnosis of Syphilis in the Recombinant Era: Reversal of Fortune

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(See the article by Park et al, on pages 1297–304.)

*Treponema pallidum*, the spirochete that causes syphilis, cannot be cultured. As a result, syphilis is usually diagnosed by tracking the immunologic footprints of its etiologic agent. Serodiagnosis of syphilis requires the detection of 2 distinct types of antibodies, nontreponemal and treponemal [1]. Nontreponemal antibodies, measured by the reactive rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL) tests, are directed against lipoidal antigens of the host and probably the organism; they rise during active infection and often decline following treatment. Their primary usefulness is as a biomarker of disease activity. Treponemal antibodies, detected by the fluorescent treponemal antibody absorbed (FTA-ABS) and *Treponema pallidum* particle agglutination (TP-PA) tests, are directed against *T. pallidum* proteins; they rise early in the course of infection and usually remain detectable for life, even after successful treatment. Neither test should be used alone. “Biologic false positive” nontreponemal tests are associated with

various medical conditions unrelated to syphilis; nontreponemal test reactivity, therefore, must be confirmed by treponemal testing. Conventional treponemal tests use whole organisms and may be falsely reactive because of cross-reacting serum antibodies that in most cases are thought to derive from commensal microorganisms [2]. In addition, a reactive treponemal test cannot distinguish active from inactive infection. Traditionally, serodiagnosis of syphilis has been performed using an algorithm in which sera are screened for nontreponemal antibodies and reactivity is confirmed by testing for treponemal antibodies [1, 3]. The traditional sequence, long recommended by the Centers for Disease Control and Prevention (CDC) [3], has performed well in identifying syphilis patients with active disease and who are most infectious. Along with serologic test results, a patient’s clinical history and physical examination are used to confirm the diagnosis and guide management [3].

The advent of recombinant DNA technology in the early 1980s fostered optimism that serodiagnostic assays using cloned *T. pallidum* antigens would overcome the shortcomings of conventional treponemal tests [4]. The antigens ultimately selected for diagnostic use were thought to be specific for *T. pallidum* and often were formatted as solid-phase immunoassays, a newly available platform. Over the years, a number of enzyme and

chemiluminescence immunoassays (EIAs and CIAs, respectively) have become commercially available [1]. In addition to their analytic sensitivity, these assays have the additional advantages of being automatable and generating a spectrophotometric or luminescent signal that can be stored electronically. To reduce the time and labor required for syphilis screening, many laboratories have adopted reverse-sequence screening in which sera are tested first by a treponemal EIA/CIA followed by reflexive nontreponemal testing of specimens with reactivity above a defined cutoff [5].

Using the reverse-sequence algorithm, discordant (ie, EIA/CIA-reactive, RPR-nonreactive) results would be expected in patients with early primary, latent, or treated syphilis, many of whom do not have nontreponemal antibodies. However, 2 recent studies published in *Morbidity and Mortality Weekly Reports (MMWR)* provide strong evidence that EIAs/CIAs used as screening tests have additional unforeseen performance problems [6, 7]. A 2008 study that assessed reverse-sequence testing in 4 New York City laboratories found that 56% of 6548 EIA-reactive serum specimens were discordant [7]. Approximately 17% of the discordant sera that underwent confirmatory treponemal testing with either a TP-PA or FTA-ABS test were nonreactive, suggesting that the EIA results were false-positives. This study also

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made apparent the burden created for health departments and clinicians who must assess large numbers of patients who would not have been identified using the traditional sequence. A follow-up study published in *MMWR* earlier this year assessed reverse-sequence testing in 6 laboratories in California, Illinois, and New York and included populations at low and high risk for syphilis [6]. The 57% rate of discordance among 4834 EIA/CIA-reactive serum specimens in this analysis was similar to that in the 2008 *MMWR*. Of importance, the rate of unconfirmed EIA/CIA reactivity was higher in the low-risk population than in the high-risk population (41% vs 14%, respectively), further suggesting problems with specificity when EIAs/CIAs are used for initial screening. Although neither *MMWR* report endorsed reverse-sequence testing, they made 2 recommendations regarding its use: (1) EIA/CIA-reactive sera should undergo reflex testing with a nontreponemal test for confirmation and identification of active disease, and (2) discordant sera should be tested reflexively with a conventional treponemal test to confirm EIA/CIA reactivity. The more recent *MMWR* report recommended that the TP-PA test be used exclusively as the confirmatory treponemal test because the FTA-ABS is less sensitive and specific, is inherently subjective, and requires expensive instrumentation [8, 9]. It is ironic that the new-generation serodiagnostic assays resulting from recombinant DNA technology require confirmation by the conventional treponemal tests they were developed to supplant. Consistent with the 2010 Sexually Transmitted Disease Treatment Guidelines, the *MMWR* authors concluded that patients with EIA/CIA-reactive, RPR-nonreactive, and TP-PA nonreactive sera are unlikely to have syphilis and that further evaluation or treatment is not indicated unless primary syphilis is suspected [3, 6].

A report by Park and colleagues [10] in this issue of the *Journal* provides additional insight into the interpretation of discordant serologies identified with

reverse-sequence testing. In this study of 255 discordant sera (CIA+/RPR-) in a low-prevalence population screened for syphilis, patients whose sera had reactive TP-PA results were more likely to have high-risk behaviors, a history of treated syphilis, or higher optical density cutoff index (ODI) values. The correlation between ODI value and TP-PA test reactivity suggests that reporting ODI values might provide useful information to guide clinical management and warrants further study. Another key finding of the Park et al study is that 28% (7/25) of untreated patients with CIA+/RPR-/TP-PA- sera had nonreactive CIAs within 12 months of the initial test. Their data add to the growing body of evidence suggesting caution when interpreting discordant syphilis serologies, particularly in low-risk persons, and underscore the need for confirmatory TP-PA testing.

The study by Park and colleagues serves as a valuable reminder that the problem of discordance is not just one of analytic sensitivity, but also of specificity [10]. In the absence of a gold standard serodiagnostic test, clinical and demographic information is essential in interpreting syphilis serologies and in comparing individual assays. Along with the *MMWR* reports [6, 7], the Park et al study further emphasizes the need for a research agenda to clarify the utility and caveats of reverse-sequence testing. The source of false-positive EIA/CIA results must be determined, with characterization of antigen-binding patterns in discordant sera that are unconfirmed with TP-PA testing. The performance of commercially available treponemal tests should be compared head-to-head using sera from patients whose risk behaviors, clinical histories, and outcomes are known; in these studies, it is essential to include sera from pregnant and human immunodeficiency virus-infected patients. In addition, an assessment of long-term outcomes of untreated individuals with discordant serologies and nonreactive confirmatory treponemal tests will provide data to support evidence-based recommendations

for patient management. Although the CDC continues to recommend the traditional algorithm [3, 6], it recognizes that reverse-sequence testing is here to stay.

## Notes

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