Letters to the Editor

The Impact of Analytical Sensitivity on Screening Algorithms for Syphilis

To the Editor:

Assays for syphilis serology can be broadly divided into 2 categories; treponemal tests that detect antibodies against the infectious agent Treponema pallidum, and nontreponemal tests that measure antibodies against nonspecific antigens, such as cardiolipin. Historically, syphilis diagnosis has used a nontreponemal assay such as rapid plasma reagin (RPR) for screening, followed by a treponemal assay to confirm syphilis infection in screen-positive patients. With the increased availability of automated assays for treponemal antibodies, however, many laboratories have shifted to a “treponemal-first” algorithm for syphilis testing. The merits of the 2 approaches have been debated in the literature in terms of clinical utility and cost-effectiveness (1, 2).

The main difference between the 2 approaches is the identification of treponemal-positive, RPR-negative patients when a treponemal-first algorithm is used (3). Discriminating between latent syphilis infection and a false-positive screening test result can be challenging for patients without a clear history of prior disease. In such cases, the CDC has recommended retesting samples with a second treponemal assay to confirm the presence of antitreponemal antibodies (4).

For such an algorithm to be valid, the analytical sensitivity of the confirmatory treponemal test must be at least equivalent to the screening assay. We performed an analytical sensitivity analysis of 5 syphilis assays, including 2 automated immunoassays [BioPlex 2200 Syphilis IgG (Bio-Rad Laboratories) and LIAISON Treponema (DiaSorin)], 2 microplate methods [Trep-Sure™ (Phoenix Bio-Tech) and CAPTIA™ Syphilis-G (Trinity Biotech)], and a manual agglutination assay [SERODIA TP-PA (Fujirebio)]. We identified a set of 10 well-characterized clinical samples that were strongly positive for treponemal antibodies and that represented a range of clinical presentations (4 with active disease, 6 with past infection) and RPR status. Each sample was then serially diluted with equivalent volumes (i.e., 2-fold dilution) of normal serum to produce samples with gradually decreasing amounts of treponemal antibody. Replicates of each dilution were tested with the recommended protocols for each manufacturer’s assay, and results were classified as positive or negative according to the manufacturer-recommended cutoffs.

All 5 assays yielded strongly positive results for undiluted samples, and results remained positive to a dilution of at least 1 volume of sample in 10 volumes of serum. Owing to the wide range of clinical presentations, however, the initial amount of treponemal antibody present in the sample varied greatly between patients. To facilitate interpatient comparisons, we normalized the data by defining an “index sample” for each patient, i.e., the most dilute sample that gave positive results in all 5 assays.

We then calculated the number of additional dilutions required to convert to a negative result beyond the index sample. The mean number of dilutions (x) required for each assay to reach a negative result is indicated in the inset. Pairwise one-way ANOVA was performed for each pair of tests, with the following combinations achieving statistical significance: TREP vs LIA, P = 0.02; TPPA vs TREP, P = 0.02; TPPA vs BIOP, LIA, and CAPT: all P values < 0.001. BIOP, BioPlex 2200 Syphilis IgG assay (Bio-Rad); LIA, LIAISON Treponema assay (DiaSorin); TREP, Trep-Sure assay (Phoenix Bio-Tech); CAPT, CAPTIA Syphilis-G assay (Trinity Biotech); TPPA, SERODIA TP-PA assay (Fujirebio).
dilutions. These results were reflected in results for individual samples, which showed that the Trep-Sure and TP-PA assays were the last 2 assays to give positive results in 8 of the 10 samples tested. We observed no associations between analytical sensitivity and sex, age, or RPR status for any of the 5 assays.

Following the approach outlined above, we have demonstrated differences in analytical sensitivity for 5 commonly used assays for treponemal antibodies. Although conclusions are somewhat limited by the small sample size, such differences could potentially have been produced by a combination of assay-specific cutoffs and enhanced sensitivity in assays that detect IgM antibodies. Regardless of the cause, this information has implications for the design and interpretation of syphilis-testing algorithms. The CDC and others have recommended TP-PA assays for confirmation, and the high analytical sensitivity of this assay suggests that it would be a suitable choice in a wide range of situations. Many other assay combinations could also be effective, as long as the confirmatory test has a sensitivity equivalent to or greater than that of the screening assay. We recommend exercising caution in situations in which a highly sensitive assay (such as TP-PA or Trep-Sure) is used for the initial screen. In such cases, the possibility of a false-negative result exists if a less sensitive assay is used for confirmatory testing, particularly when the initial screening result is weakly positive. We suggest that interpretive comments for such results reflect this possibility and potentially recommend follow-up testing by alternative methods.

For laboratories that use a “treponemal-first” algorithm for syphilis testing, the evaluation of a treponemal-positive, RPR-negative patient is a clinically important task. The data in this study indicate that the relative analytical sensitivities of the assays used for screening and confirmation can influence clinical interpretations of the results. We recommend that laboratories consider this feature of treponemal assays when designing reflex panels for syphilis testing.

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References


WanMing Zhang
Belinda Yen-Lieberman
Cathy Means
Rob Kreller
Joan Waletzky
Thomas M. Daly*

Section of Immunopathology
Pathology and Laboratory Medicine Institute
Cleveland Clinic
Cleveland, OH

* Address correspondence to this author at:
Clinical Pathology, LL3
Pathology and Laboratory Medicine Institute
Cleveland Clinic
9500 Euclid Ave.
Cleveland, OH 44195
Fax 216-444-4414
E-mail dalyt@ccf.org

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