Evaluation of Two Immunoblot Assays and a Western Blot Assay for the Detection of Antisyphilis Immunoglobulin G Antibodies

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In the present study, two immunoglobulin G (IgG) immunoblot assays and one IgG Western blot assay were compared to the rapid plasma reagin test (RPR), the fluorescent treponemal antibody absorption test (FTA-ABS), and the Treponema pallidum particle agglutination assay (TP-PA). The agreement levels of the Viramed, Virotech, and MarDx assays were 97.0%, 96.4%, and 99.4%, and the agreements of samples inconclusive by FTA-ABS and resolved by TP-PA were 91.7%, 83.3%, and 69.4%, respectively.

Syphilis, a disease caused by Treponema pallidum, is transmitted congenitally or through sexual intercourse (8–9). Non-treponema-based tests such as the rapid plasma reagent test (RPR) are used to detect syphilis infection (6, 9–10). These tests may produce false-positive results in pregnant women and patients with infections (3, 5–6, 9, 11). An algorithm has been developed for the serological diagnosis of syphilis which includes a non-treponema-based screening test and a treponema-based confirmatory assay (1–2, 7, 11). Traditional confirmatory assays include the fluorescent treponemal antibody absorption test (FTA-ABS) and the T. pallidum particle agglutination assay (TP-PA) (9).

Western blot-based assays to detect immunoglobulin G (IgG) antibodies may prove useful, especially in cases where the FTA-ABS is inconclusive. In the present study, results of two immunoblot assays and one Western blot assay were compared to FTA-ABS/TP-PA and RPR results, as well as to each other.

***Human sera.*** A total of 200 human serum samples sent to Associated Regional and University Pathologists (ARUP) laboratories for syphilis testing were collected. Procedures were followed in accordance with the ethical standards established by the University of Utah in accordance with the Helsinki Declaration of 1975. All patient samples were deidentified according to the University of Utah Institutional Review Board protocol (no. 7275) to meet the Health Information Portability and Accountability Act guidelines. Specimens were stored at −20°C until testing and then stored at 2 to 8°C.

***Non-treponema-based testing.*** All 200 samples were tested by RPR according to the manufacturer’s protocol (Arlington Scientific, Inc., Springville, UT).

***Treponema-based testing.*** One hundred forty-two samples were tested by FTA-ABS (Inverness Medical, Waltham, MA), and 32 inconclusive samples were further tested by TP-PA (Fujirebio, Malvern, PA). Both assays were performed according to the manufacturers’ protocols. The 32 inconclusive FTA-ABS samples were included to reflect the high percentage of inconclusive FTA-ABS samples sent to our reference laboratory from primary screening laboratories.

**Syphilis blot testing.** All 200 samples were tested using two immunoblot assays and one Western blot assay, the Treponema ViraBlot test kit IgG (Viralab Inc., Oceanside, CA), the Treponema pallidum IgG line immunoblot (Genzyme Virotech GmbH, Rüsselsheim, Germany), and the T. pallidum IgG Marblot strip test system (MarDx Diagnostics, Inc., Carlsbad, CA). Each assay was performed according to the manufacturer’s protocol.

***Statistical analysis.*** To determine overall agreement, sensitivity, specificity, and 95% confidence intervals (CI), two-by-two contingency table analysis with Yates-corrected chi-square testing was used (4). Equivocal results were excluded from the calculations. Samples that disagreed were repeated on each test. Receiver operating characteristic (ROC) curves were analyzed using MedCalc version 10.1.3.0 (MedCalc Software, Mariakerke, Belgium).

Of the 200 samples used in this study, 142 were tested by treponema-based assays and RPR and 58 were tested exclusively by RPR. Samples were considered positive if they tested positive in the FTA-ABS assay or the TP-PA assay. Samples that were inconclusive according to the FTA-ABS assay were resolved by the TP-PA assay.

For the ViraBlot assay, the overall agreement, sensitivity, and specificity were 97.0%, 95.5% (95% CI, 90.4 to 97.9) and 97.8% (95% CI, 95.2 to 99.0%), respectively (Table 1), with no equivocal results. The Virotech assay had overall agreement, sensitivity, and specificity values of 96.4%, 90.0% (95% CI, 84.7 to 91.4%), and 99.2% (95% CI, 96.8 to 99.9%), respectively, with five (2.5%) equivocal results. The MarDx assay had overall agreement, sensitivity, and specificity values of 99.4%, 98.2% (95% CI, 94.3 to 98.2%), and 100.0% (95% CI, 98.2 to 100.0%), respectively, with 25 (12.5%) equivocal results.

To determine if the manufacturers’ cutoff criteria were optimal, ROC curves were generated. The ViraBlot assay produced an ROC curve with an area under the curve (AUC) of 0.988 (P < 0.0001). The optimal cutoff criterion for maximum sensitivity and specificity matched the manufacturer’s protocol. For the Virotech assay, an ROC curve with an AUC of 0.987 (P < 0.0001) was produced. This ROC curve indicated that by...
The Treponema ViraBlot test kit IgG had overall agreement, sensitivity, and specificity values of 97.0%, 95.5% (95% CI, 90.4 to 97.9), and 97.8% (95% CI, 95.2 to 99.0%), respectively. The ROC curve indicated that by reducing the cutoff criterion by one band, the number of equivocal results would decrease from 10 to 4 with a significant decrease in sensitivity and specificity values of 99.4%, 98.2% (95% CI, 94.3 to 98.2%), and 100.0% (95% CI, 98.2 to 100.0%), respectively.

Although FTA-ABS testing offers high sensitivity and specificity, the subjectivity of the test results in a high number of equivocal results. This would reduce the number of false-negative results. The Marblot assay produced an ROC curve with an AUC of 0.988. The ROC curve indicated that by reducing the cutoff criterion by one, the number of equivocal results would decrease from 25 to 14 without significantly decreasing sensitivity or specificity. However, this is still an unacceptably high number of equivocal samples.

All three assays had high accuracy (96.4 to 99.4%); however, they varied greatly in the number of equivocal results. The large number of equivocal results generated by both the Marblot and Virotech assays limits their utility. Table 2 illustrates that the ViraBlot assay performed the best when resolving inconclusive FTA-ABS/TP-PA results, as well as minimize the interpretation. This allowed easier interpretation and reduced the number of equivocal results.

Western blot and immunoblot assays offer additional, accurate treponemal tests that can supplement the current syphilis testing algorithm. As an esoteric reference laboratory, our laboratory often receives specimens for FTA-ABS testing that were previously inconclusive by FTA-ABS at another laboratory. Our data indicate that the ViraBlot assay would be the best choice of blot assay to use to resolve these inconclusive FTA-ABS results.

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