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Preliminary Evaluation of an Immunochromatographic Strip Test for Specific *Treponema pallidum* Antibodies

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We evaluated a prototype immunochromatographic strip (ICS) test for qualitative detection of *Treponema pallidum* antibodies in 353 sera from 157 patients. For sera from 43 syphilis patients, the ICSs were reactive, while for sera from 114 patients without syphilis, including 22 with biologically false-positive Rapid Plasma Reagin test results, the ICSs were nonreactive. The ICS test may expand the available options for serological testing for syphilis.

Untreated syphilis causes congenital infections and neurologic illness and potentiates transmission of human immunodeficiency virus. Testing for *Treponema pallidum* antibodies is important in syphilis screening and in confirming the diagnosis of suspected syphilis. Serological testing is usually performed as a two-step procedure (4). Sera are initially screened with a relatively inexpensive, quantitative nontreponemal tests such as the Venereal Disease Research Laboratory or Rapid Plasma Reagin (RPR) test (4). To improve test specificity, sera that are reactive in nontreponemal assays are often tested with a second, confirmatory serological test such as the fluorescent treponemal antibody absorbtion (FTA-ABS) test or the microhemagglutination assay for *T. pallidum* (4). There is a need for reliable, specific, and rapid serological tests for syphilis which can be performed in nonlaboratory settings to guide clinical decision making. The immunochromatographic strip (ICS) test to identify serum antibodies to a recombinant *T. pallidum*-specific antigen is a new test which requires no specialized equipment and a minimum of technical training and which can be performed at room temperature in only 8 min. In this pilot study, we compared the performance of the ICS test to those of the standard RPR and FTA-ABS tests.

Sera and patients. A total of 353 sera from 157 patients were evaluated. Two hundred one sera were collected from 30 patients at the time of syphilis diagnosis and up to 12 months following treatment. Fifteen additional sera from patients with false-positive RPR test results (RPR reactive and FTA-ABS nonreactive) were also evaluated, as were 137 consecutively collected sera from 112 patients that had been screened for syphilis while attending a sexually transmitted disease clinic. For comparison purposes, the Macro-Vue RPR (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and the FTA-ABS indirect fluorescent-antibody tests (Zeus Scientific, Inc., Raritan, N.J.) were performed as described in the manufacturers' instructions (4). Syphilis diagnosis and interpretation of RPR and FTA-ABS test results were performed in accordance with recommended procedures (4). Two hundred sixteen sera had been stored at −70°C, and 137 were tested without freezing within 48 h of collection.

ICS test. The strip used in the ICS test is configured as a 5- by 75-mm strip. For the present study, recombinant 47-kDa antigen capture reagent was applied to nitrocellulose strips laminated to plastic backing and immobilized as a thin horizontal line across the strip. A second line of mouse anti-human immunoglobulin G, which served as a positive control for each test strip, was similarly applied a short distance above the reagent line. Polyester pads were then attached to the top and bottom of the backing. The lower pad was impregnated with colloidal gold signal reagent, and the upper filter paper pad served as a receptacle for excess serum which accumulated after wicking through the nitrocellulose strip by capillary action.

For testing with the ICS, sera at room temperature were vortexed and 65 μl of each serum specimen was transferred to a test tube. ICSs were placed in the tubes so that the sera could be absorbed by the lower pad of the test strip and could wick through the nitrocellulose strip. After 8 min, the strip results were read. A positive reaction was characterized by the appearance of two lines on the cellulose strip, while a negative reaction was characterized by only one visible line (the control) (Fig. 1). All sera were tested in blinded fashion with the RPR, FTA-ABS, and ICS tests. ICS tests were interpreted and classified independently by two observers.

A total of 353 sera collected from 157 patients were included in this pilot study. The first group of specimens consisted of 201 sera from 30 patients with early syphilis collected at the time of diagnosis and therapy and up to 12 months posttreatment. Sera from all 30 patients were reactive in the RPR, FTA-ABS, and ICS tests at the time of syphilis diagnosis. For 171 sera collected from these patients following treatment, the RPR test was negative for 31 and the FTA-ABS test was negative for 2 while the ICS test was positive for 169 of 171 serum specimens.

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FIG. 1. ICS test for specific *T. pallidum* antibodies. Left, reactive test strip showing reactive control (upper) and test (lower) lines; right, nonreactive test strip showing single, control line.

For one patient with early syphilis, the RPR (peak titer, 1:32), FTA-ABS, and ICS tests were all positive for serum collected at the time of diagnosis and for four additional serum specimens collected over 7 months of posttherapy follow-up. Sera collected 9 and 12 months following treatment, however, were nonreactive in all three tests.

All ICS tests were independently interpreted by two observers with 100% agreement. Of the 199 positive ICS test results for sera from known syphilis patients, 34 were weakly positive (i.e., the band intensity on the ICS was faint). All sera that were weakly positive by the ICS test were FTA-ABS test reactive. Eleven sera (33%) with weakly positive ICS test results were RPR nonreactive, while the other 23 sera which gave faint ICS test results were RPR positive with a range of titers from 1:1 to 1:512. Thus, in some cases, sera with high RPR titers were relatively weakly positive by the ICS test (but were nonetheless clearly positive). Conversely, some FTA-ABS-reactive specimens with low titers or negative results for the RPR test gave strongly positive ICS results. No patient serum specimen with a reactive FTA-ABS test result had a negative ICS test result.

The second group of specimens consisted of 15 sera collected from 15 patients with biologically false-positive RPR (RPR-reactive and FTA-ABS-nonreactive) serological tests for syphilis. All sera from these patients were negative by the ICS test.

The third group of specimens consisted of 137 sera collected from 112 patients attending the local Department of Health sexually transmitted disease clinic and who had been screened for syphilis as part of routine care. The FTA-ABS tests were reactive for 13 patient sera (8 of these were RPR nonreactive) and nonreactive for 124 (7 of these were RPR positive [biologically false positive]). Agreement between the ICS and FTA-ABS test results was 100%. Assuming that all 13 patients with reactive FTA-ABS tests had syphilis at the time of serum collection or in the past, the sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) of the ICS tests for this relatively small number of patients were all 100%.

In summary, the ICS test permits the evaluation of serological evidence of syphilis by an assay that could be readily performed in nonlaboratory settings. The ICS test in this pilot study utilized a recombinant 47-kDa antigen that is expressed and made as a complete protein in *Escherichia coli* and that is purified by conventional methods (2). The 47-kDa antigen has also been used in immunoblot assays with other *T. pallidum* recombinants, including the 44.5-, 17-, and 15-kDa antigens (1, 2, 3).

In the present study, we found that the ICS test provides accurate, qualitative detection of antibodies to *T. pallidum*. The test appears to be more sensitive than the RPR test, yielding positive test results for a number of treated syphilis patients who had nonreactive RPR tests but reactive FTA-ABS tests. The ICS test was also nonreactive for 22 patients with biologically false-positive (RPR-reactive and FTA-ABS-nonreactive) sera. The qualitative nature of the test was also evident in that ICS test reactivity did not appear to correlate with RPR titers.

We believe that the ICS test has the potential to contribute to serological screening that is performed as part of syphilis control efforts, particularly in settings where the prevalence of previously treated syphilis is low or where logistical constraints make a test which can give results rapidly and without laboratory equipment outweigh the need to avoid treatment of patients who have had syphilis and have been successfully treated in the past. For instance, the test might be helpful in settings where at-risk patients are not reliably available for subsequent follow-up (i.e., emergency departments or short-term correctional facilities) or where treatment delays might have serious consequences (i.e., for evaluation of pregnant women). Conversely, in some settings where the prevalence of treated syphilis is high or where the response to therapy is being assessed, the relatively sensitive, qualitative nature of the test might be less helpful to clinicians. Further study to examine both the performance and utility of this test is warranted.

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