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). 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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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 nonreactive test strip showing single, control line.

REFERENCES