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Performance of Rapid Syphilis Tests in Venous and Fingerstick Whole Blood Specimens

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Objective: **Rapid syphilis screening could facilitate case-identification during U.S. outbreaks.**

Goal: **The goal of this study was to determine the performance of 3 rapid syphilis tests in whole blood specimens in the laboratory and in patients at a sexually transmitted disease (STD) clinic.**

Study: **We tested whole blood samples from STD clinic patients with 3 rapid tests and compared results with the serum treponemal pallidum particle agglutination (TP-PA) test. We evaluated the best performing of the 3 rapid tests on fingerstick specimens from STD clinic patients.**

Results: **The Abbott Determine TP (n = 127) had the highest sensitivity (88%; 95% confidence interval [CI], 81–96%) and lowest rate of indeterminate tests (0.8%), followed by Guardian Biosciences One Step (n = 116) (sensitivity 72%; 95% CI, 60–84%; indeterminate 6.5%), and Phoenix Biotech Trep-Strip IV (n = 71) (sensitivity 70%; 95% CI, 54–85%; indeterminate 30.3%). All 3 tests were 100% specific. The Abbott Determine TP showed excellent performance on fingerstick specimens (n = 99), exhibiting 100% sensitivity (95% CI, 93–100%), 100% specificity, and 2.9% indeterminate.**

Conclusions: **The Abbott Determine TP test was an easy and accurate test that could facilitate rapid detection of syphilis in at-risk patients.**

AFTER A 10-YEAR DECLINE in reported cases of early syphilis, the number of cases in San Francisco increased from 71 in 2000 to 494 in 2002.[1] Similar outbreaks have recently been reported in Chicago, New York, Los Angeles, Boston, Miami, and Seattle.[2–7] A particular concern for public health departments is the impact that the increase in primary syphilis has had on increased human immunodeficiency virus (HIV) transmission.[8] The marked rise in reported cases of syphilis over the past 2 years warrants evaluation of new methods to rapidly detect persons with infection and to improve disease control efforts.

Important considerations of effective screening tests include price, invasiveness, time to result, complexity, and manpower. The current diagnostic tests for syphilis, most notably the Venereal Disease Research Laboratories (VDRL), rapid plasma reagin (RPR), and treponema-pallidum particle agglutination (TP-PA) tests must be performed in the laboratory by trained technologists. The nontreponemal tests alone (RPR and VDRL) range in price from approximately $5 to 16 per sample.[9] All 3 require a blood draw through venous puncture, specimen transport from clinic to laboratory, and appropriate laboratory equipment. Only the stat RPR is a relatively rapid diagnostic test with a turnover time of approximately 20 minutes, whereas both the VDRL and TP-PA tests require at least a 24-hour turnaround time from specimen collection to result.

Although more than 20 rapid treponemal antibody tests are currently being manufactured for use outside of the United States, few have been evaluated in published studies. One study by the Sexually Transmitted Diseases Diagnostics Initiative branch of the World Health Organization evaluated 6 rapid treponemal syphilis tests on 789 samples of sera, 399 of which were positive by the TP-PA, and documented a range of sensitivities from 84.5% to 97.7% and specificities ranging from 92.8% to 98.0%.[10] A second study evaluated the Abbott Determine TP rapid test with 291 serum samples and reported 100% sensitivity and 97% specificity when compared with the RPR.[11]

Although this research documented the strong performance of these tests on serum specimens, it stopped short of evaluating the tests on whole blood from fingerstick specimens, an essential analysis for the evaluation of field-based screening tests. The goals of our study included evaluating the performance of 3 rapid treponemal antibody tests on whole blood venous samples in the laboratory and then assessment of the best performing test with whole blood fingerstick specimens from sexually transmitted disease (STD) clinic patients. The aim of the study was to identify a minimally invasive, rapid, whole blood syphilis test that functions without need for additional laboratory equipment. By meeting these standards, the test could potentially be incorporated into screening or diagnostic programs in the field, at community-based venues, and at health centers that lack laboratory facilities or personnel.

Materials and Methods

We selected 3 rapid tests for evaluation: Abbott Determine Syphilis TP (Abbott Laboratories, Abbott Park, IL), Phoenix Biotech Trep-Strip IV (Phoenix Bio-Tech Corp., Mississauga, Ontario), and Guardian Biosciences One Step (Testmedica Diagnostics, Guardian Biosciences, San Jose, CA). Each of these 3 tests cost less than $5 per test, can be operated without laboratory equipment, and provide results within 15 minutes of blood collection (Table 1). We compared the results of the rapid tests with

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TABLE 1. Summary of Test Characteristics for 3 Rapid Syphilis Kits

	Abbott Determine Syphilis TP		Phoenix Biotech Trep-Strip IV		Guardian One Step	
Buffer required	Yes		Yes		No	
Materials not Included, but required	Laboratory Micropipetter, Tips	Fingerstick Heparinized Cap. Tubes. Lancet	Laboratory Micropipetter, Tips	Fingerstick Heparinized Cap. Tubes. Lancet	Laboratory Micropipetter, Tips	Fingerstick Heparinized Cap. Tubes. Lancet
Test time	15 min		10 min		10 min	
Price/test	$2.00		$2.00		$4.80	

serum TP-PA results as the gold standard for detection of treponemal antibodies.

Patient Enrollment

The study sample was selected from patients attending the San Francisco City Clinic who underwent routine blood drawing for VDRL screening. Patients over the age of 18 who were sexually active were chosen. To evaluate both the sensitivity and specificity of the kits, we aimed to enroll approximately equal numbers of patients who tested positive or negative by the gold standard TP-PA test. To achieve these sample characteristics, we alternated between asymptomatic patients with no history of syphilis and those who we predicted would likely be TP-PA-reactive. The latter group included patients who manifested clinical signs of current syphilis infection (primary or secondary), reported past infection, or had a history of syphilis documented in clinic records. With a sample size of 60 positive TP-PA and 60 negative TP-PA patients and predicted test sensitivities of 90%, we would restrict our 95% confidence intervals (CIs) around our sensitivity estimate to approximately $\pm$ 7%.

Whole Blood Collection for Laboratory Testing

For each patient agreeing to enroll in the study, an extra 2 cc of blood was drawn into an EDTA-containing Vacutainer tube to prevent clotting. Specimens were stored at 4°C until they were tested with each of the 3 kits within 8 hours of specimen collection.

Two laboratory personnel read and recorded test results. We repeated rapid test results that could not be classified as positive or negative. We recorded a second unreadable result as inconclusive. We classified opposite readings between the 2 laboratory personnel as discrepant. We calculated the fraction of tests that were indeterminate (either inconclusive or discrepant) as a marker for the frequency of test failure. We compared results of the tests with the TP-PA as the gold standard.

Test Protocols

Abbott Determine Syphilis TP in the Laboratory. We obtained Abbott Determine Syphilis TP tests (List No. 7D24–33, Abbott Laboratories, South Pasadena, CA) and accompanying buffer (List No. 7D22–11) from a collaborating laboratory in Lima, Peru. We performed tests in accordance with the manufacturer's instructions for whole blood samples. All volumetric measurements were performed with micropipettes.

Abbott Determine Syphilis TP Fingerstick. In addition to the routine blood draw for diagnostic purposes, we obtained whole blood from consenting patients by fingerstick puncture of the middle, ring, or index finger. Before the puncture, we cleansed the wound site with an alcohol swab and dried the site. With the use of a lancet (Tenderlett; International Technidyne Corp., Edison, NJ), we pricked the lateral tip of the finger. We began using the Abbott Determine TP kit with fingerstick whole blood without capillary tubes as a pilot test. The strips showed poor sensitivity using this method (11 of 17 TP-PA-positive samples, 64% sensitivity). We continued the study with the use of capillary tubes for the collection and transfer of whole blood onto the test strips. After discarding the first drop of blood with a sterile gauze pad, we milked blood from the finger and collected blood into heparinized capillary tubes (Fisher Scientific, Atlanta, GA) until approximately half of the tube (approximately 40 μL) was filled. We transferred blood from the capillary tube to the test pad through capillary action. One minute after application of the blood, we added 1 drop of Chase Buffer. We read the tests a minimum of 15 minutes after application of the buffer.

Guardian Biosciences One Step in the Laboratory. We conducted the tests according to the test distributor's protocol for whole blood samples (Testmedica Diagnostics, Guardian Biosciences, San Jose, CA).

Phoenix Biotech Trep-Strip IV Intravenously in the Laboratory. We ordered Phoenix tests and accompanying buffer directly from the manufacturer (Phoenix Bio-Tech Corp., Mississauga, Ontario, Canada). We conducted tests according to the manufacturer's protocol for whole blood samples.

Treponemal Pallidum Particle Agglutination. We tested sera from participating patients with the Serodia TP-PA test (Fujirebio, Inc., Fairfield, NJ) according to the manufacturer's instructions.

The University of California, San Francisco Committee for Human Research, approved this protocol.

Results

Table 2 summarizes the results of the 3 rapid tests performed in the laboratory and the fingerstick whole blood specimens collected at the STD clinic. Included is an evaluation of test failure by the percentage of indeterminate tests (%ID) from each kit. The sensitivity of the Abbott Determine TP whole blood assay in the laboratory was 88% (95% CI, 80–96%); on whole blood fingerstick specimens, the sensitivity was 100% (95% CI, 93–100%). In the laboratory, 0.8% were indeterminate, whereas on fingerstick specimens, 2.9% were indeterminate. The sensitivity of the Phoenix Biotech Trep-Strip IV on venous whole blood was 69% (95% CI, 54–85%) with 30.3% indeterminate tests. The sensitivity of the Guardian Biosciences One Step on venous whole blood was 72% (95% CI, 60–84%) with 6.5% indeterminate tests.

TABLE 2 Results from the Rapid Syphilis Tests Conducted on Whole Blood Venous Specimens in the Laboratory and on Fingerstick Specimens in the Clinic as Compared to the Serodia TP-PA Test as the Gold Standard

Venipuncture Whole Blood Laboratory			Venipuncture Whole Blood Laboratory			Venipuncture Whole Blood Laboratory			Fingerstick Whole Blood Clinic		
	TP-PA			TP-PA			TP-PA			TP-PA	
Determine TP	+	-	Trep-Strep IV	+	-	One Step	+	-	Determine TP	+	-
+	60	0	+	23	0	+	41	0	+	52	0
-	8	59	-	10	38	-	16	59	-	0	47

Conclusions

Of the 3 rapid tests evaluated on whole blood in the laboratory, the Abbott Determine Syphilis TP test had the highest sensitivity (88%) and lowest fraction of indeterminate tests 0.8% (0 inconclusive and 1 discrepant reading). The Guardian One Step test had the next highest sensitivity (72%) and moderate readability, with 6.5% of the tests giving indeterminate results (2 inconclusive and 6 discordant readings). The Phoenix Trep-Strep IV had the lowest sensitivity (70%) and highest fraction of indeterminate tests (30.3%, 23 inconclusive and 8 discrepant readings).

There were challenges to reading all 3 tests. Most notably, proper light is required to detect the lines corresponding to a positive result. Both the Guardian and Abbott tests occasionally produced faint bars in the test window. The inconsistent interpretation of these faint bars caused discrepant readings. Interpreting the Phoenix test was difficult as a result of permanent markings in the test window that were often indistinguishable from a possible test line indicating a positive result.

Overall, we found the performance of the rapid tests to be poorer on whole blood venous samples when compared with previous research with serum samples from the World Health Organization study or from other investigators. We hypothesize that the lower antibody concentration was responsible for this discrepancy. The presence of plasma, red cells, and the Chase Buffer, which is added in a 1:1 ratio, dilute antibodies normally present in serum. The lower antibody concentration could decrease the sensitivity of the test by diminishing the strength of the antigen–antibody interaction that is responsible for producing a positive test. Another discrepancy between our whole blood assay and the serum testing was the presence of EDTA in the tubes with which we collected whole blood. It is possible that the anticoagulant interfered with the antibody–antigen reaction.

With a sensitivity of nearly 90% and fewer indeterminate results than the other 2 rapid syphilis tests, we selected the Abbott Determine TP test for the clinic-based fingerstick specimen evaluation. When tested with fingerstick blood, the Abbott Determine TP performed better than the 3 whole blood evaluations performed in the laboratory, including the Abbott Determine TP assay. Possible reasons for the unexpected superior performance of these tests include the small sample size, the use of heparinized tubes for collection, and the elimination of extra steps, including collection into EDTA tubes and storage at 4°C. Because of the documented accuracy of this test, we propose that it could be useful for the diagnosis of syphilis in field settings and sites where timely results can change management.

Currently, the definitive diagnosis of syphilis is based on positive darkfield microscopy from a primary or secondary mucocutaneous lesion. In the absence of such a lesion, the recommended standard of care is to use a nontreponemal antibody serologic screening test (RPR or VDRL). Positive nontreponemal tests are then confirmed with a treponemal antibody tests (TP-PA or fluorescent treponemal antibody–absorption [FTA-ABS]).[12]

Recent data have called into question the current diagnostic protocol of using nontreponemal antibody assays as screening tests for syphilis. One study reported that 6 of 26 (23%) serum samples from patients at an STD clinic with syphilis confirmed by the treponemal TP-PA were falsely negative by the nontreponemal VDRL (77% sensitivity).[13] Ballard and others documented an RPR sensitivity of 69% compared with an FTA-ABS sensitivity of 87% in a population of patients with genital ulcer disease with confirmed primary syphilis by multiplex polymerase chain reaction assay. Despite previous studies documenting higher nontreponemal antibody titers in some patients coinfected with HIV,[14] the Ballard study reported a decrease in RPR sensitivity to 57% in patients who were coinfected with HIV.[15] Another group has documented 50% sensitivity for the VDRL test as compared with 100% with TP-PA among patients infected with the HIV who had confirmed primary syphilis by darkfield microscopy.[16] Furthermore, there is evidence of significantly higher rates of biologic false-positive nontreponemal tests in patients coinfected with HIV.[17] These studies question the effectiveness and clinical rationale of using nontreponemal antibody tests as syphilis screening tests, especially in high-risk populations with a high prevalence of HIV.

Although historically considered a confirmatory test, treponemal antibody tests could also be effective screening tests. Treponemal tests have comparable, if not superior, performance in terms of sensitivity and specificity in primary, secondary, late, and latent syphilis.[18] Because treponemal assays will remain positive in most individuals with prior syphilis infection, it is important to obtain a comprehensive medical history with a focus on sexual history before performing treponemal testing. These tests are not applicable in patients who report a history of syphilis. However, because of their accurate performance at each stage of syphilis, treponemal tests are particularly useful in supporting a clinical diagnosis, confirming an RPR-positive result, or considering presumptive treatment.

Previous work suggests that these rapid treponemal tests could be just as sensitive as nontreponemal tests to detect recent infection. Lien and others sought to document the accuracy of these tests in patients with syphilis by using the RPR nontreponemal test as a gold standard.[1] They reported a sensitivity rating of 100% for the Abbott Determine TP test. However, because their study used serum samples and a nontreponemal antibody for comparison, it was uncertain how this test would perform in whole blood specimens from patients with syphilis. The current study evaluated the Abbott Determine TP with whole blood and documented similar results when compared with the TP-PA treponemal test.

Unfortunately, the treponemal tests currently available for use in the United States are relatively expensive, take a long time to perform, and require trained laboratory personnel and specialized equipment. Consequently, there exists a need for a rapid, minimally invasive syphilis test that could be effectively incorporated into more extensive public health screenings. A test that meets

these requirements could help public health departments enhance case detection and expand service delivery measures outlined in the Centers for Disease Control and Prevention (CDC) plan to eliminate syphilis.

In addition to price and convenience, a third benefit of rapid screening tests is minimization of losses to follow up. The CDC has recently published data detailing how rapid HIV tests could reduce the number of people tested for HIV who do not return for results or treatment.[19] Before the U.S. Food and Drug Administration clearance of HIV rapid tests in 2002, obtaining results for syphilis and HIV tests required at least 24 hours and a return appointment. In 2000, approximately 31% of people tested for HIV in the United States did not return for their results. Although there are important differences between syphilis and HIV in terms of manifestations and treatment, there are also important similarities related to their diagnoses, including sites that offer testing for the 2 diseases, patient populations at risk, and the social stigma associated with diagnosis. The implementation of a rapid test for syphilis could help reduce losses to follow up in much the same way that a rapid test for HIV could accomplish this goal.

The major limitation of the current study includes a relatively small sample size, resulting in wide estimates of the precision of test performance. Further research with larger samples sizes would help to confirm our current results.

Although larger sample sizes are needed to corroborate our findings, this study suggests that the Abbott Determine TP rapid treponemal antibody test could fulfill the need for an inexpensive, noninvasive, rapid screening test for syphilis. In this evaluation, we have documented its consistent performance compared with the serum TP-PA. As a result of its accuracy, price, and ease of use, it could be an effective test to help public health departments respond to the current nationwide epidemic. Future evaluations should include the use of the Abbott Determine TP test in community-based and field-level syphilis screening efforts.

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