

# An Evaluation of the Relative Sensitivities of the Venereal Disease Research Laboratory Test and the *Treponema pallidum* Particle Agglutination Test Among Patients Diagnosed With Primary Syphilis

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**Background:** Because definitive methods for diagnosing primary syphilis are limited, it is important to optimize the sensitivity of serodiagnosis.

**Objective:** To determine the most sensitive testing approach to the diagnosis of primary syphilis, using the commonly available serologic tests: the Venereal Disease Research Laboratory (VDRL) test and the *Treponema pallidum* particle agglutination (TP-PA) test.

**Methods:** Sensitivities of 2 serologic testing strategies for primary syphilis were compared among 106 darkfield-confirmed cases treated in San Francisco from January 2002 through December 2004.

**Results:** The sensitivity of the diagnostic strategy using VDRL confirmed by TP-PA was 71% (95% CI, 61%–79%). Substituting Rapid Plasma Reagin test for VDRL in a subset of 51 patients produced the same sensitivity (71%; 95% CI, 56%–83%). The sensitivity of TP-PA as the first-line diagnostic test was 86% (95% CI, 78%–92%). The sensitivity of the former approach was significantly lower among HIV-positive patients, compared with HIV-negative patients (55% vs. 77%,  $P = 0.05$ ).

**Conclusions:** The TP-PA test as the first-line diagnostic test yielded higher sensitivity for primary syphilis than did the use of the currently recommended strategy.

THE CLINICAL PRESENTATION OF primary syphilis typically includes an anogenital ulcer, often associated with enlarged regional lymph nodes.<sup>1</sup> Lesions of primary syphilis are highly infectious, making prompt, accurate diagnosis and treatment crucial to interrupt the cycle of transmission. This is of particular concern in the context of increasing rates of syphilis in the United States. Rates of infectious syphilis (primary and secondary stages) decreased greatly between 1990 and 2000, reaching an all-time low since reporting had begun in 1941. Rates then increased each year from 2000 to 2005, from 2.1 to 3.0 cases per 100,000 population.<sup>2</sup>

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Outbreaks among men who have sex with men, many of whom were coinfecting with HIV, have been reported in several US cities, including San Francisco, CA.<sup>3</sup>

Serologic tests for syphilis constitute the mainstay of syphilis diagnosis in the United States. They are widely available, inexpensive, and familiar to most practicing clinicians. Nontreponemal tests detect antilipoidal antibodies in serum or cerebral spinal fluid and can be used to quantify an antibody response by testing incremental dilutions of sera.<sup>4</sup> Examples include the Venereal Disease Research Laboratory (VDRL) test and the rapid plasma reagin (RPR) test. The nontreponemal tests lack specificity, and sensitivity is reported to be compromised in primary syphilis and late in the course of infection.<sup>4,5</sup> The treponemal tests detect antibodies specific to *Treponema pallidum*, the causative agent of syphilis. Examples include the Serodia *Treponema pallidum* particle agglutination (TP-PA) test (Fujirebio, Malvern, PA), the fluorescent treponemal antibody-absorbed test, and the Captia Syphilis-G enzyme immunoassay (Trinity Biotech, Jamestown, NY).

Because the appearance of treponemal antibodies generally precedes that of the nontreponemal antibodies, treponemal tests would be expected to be the more sensitive tests early in the course of infection. According to the Centers for Disease Control and Prevention, sensitivities of the fluorescent treponemal antibody and TP-PA tests are 84% and 88%, respectively, whereas the sensitivities of the VDRL and RPR tests are 78% and 86%, respectively.<sup>5</sup> Although the treponemal tests are more specific than the nontreponemal tests, making them useful confirmatory tests, their use is limited in patients who have previously been infected with syphilis because the result usually remains positive for life.<sup>4</sup>

In the United States, the nontreponemal tests are recommended for screening asymptomatic patients and as first-line diagnostic tests in patients with suspicious symptoms; positive nontreponemal results are then confirmed using a treponemal test.<sup>4</sup> Outside the United States, treponemal tests are recommended as screening and first-line diagnostics,<sup>6,7</sup> and some large US laboratories use

treponemal tests for screening.<sup>8</sup> Point-of-care serologic tests, although not cleared by the US Food and Drug Administration, are also under investigation to facilitate on-site testing, diagnosis, and prompt treatment.<sup>9–11</sup>

The diagnosis of primary syphilis is particularly challenging. Identification of *T. pallidum* by dark field (DF) microscopy or direct fluorescent antibody staining is considered to give a definitive diagnosis. Neither test is widely available, and both require skilled personnel to perform the test and interpret the findings.<sup>4</sup> DF microscopy must be performed immediately using a fresh specimen collected from the lesion to evaluate the morphology and motility of any spirochetes that are present. DF is classified under the Clinical Laboratory Improvement Amendments regulations as a nonwaived, moderate complexity test. Reagents for direct fluorescent antibody staining have been unavailable or difficult to obtain in the United States for several years. A polymerase chain reaction test has been developed,<sup>12</sup> but is not cleared by the Food and Drug Administration for clinical use. Unfortunately, the sensitivities of all serologic tests are low (<90%) in primary syphilis, when antibody response may still be developing.<sup>1,5</sup>

A new testing approach to the diagnosis of primary syphilis that optimizes serologic diagnostic capabilities that are readily available in the United States could aid in the management and control of the current syphilis outbreaks, especially because many patients are diagnosed in the private sector where DF microscopy is not available. This evaluation compared the sensitivities of 2 testing strategies using VDRL and TP-PA to determine the best use of serologic tests in the evaluation of patients with genital ulcers suspected to be primary syphilis. Using DF-confirmed cases of primary syphilis, we sought to determine whether performing a treponemal test, specifically TP-PA, would detect more cases than the current recommended testing strategy of performing a nontreponemal test confirmed by treponemal test. This work was undertaken as part of routine public health program evaluation and clinical quality improvement activities. Standard precautions were taken to protect patient confidentiality.

### Methods

This study was a cross-sectional analysis of the sensitivities of serologic tests for syphilis in the diagnosis of primary syphilis. Study cases were selected from among patients diagnosed with primary syphilis at the San Francisco STD specialty clinic. Cases were identified with the use of the electronic medical record database maintained by the San Francisco STD Prevention and Control Services. Cases included DF-confirmed primary syphilis diagnoses reported from January 1, 2002 through December 31, 2004. Exclusion criteria consisted of a prior history of syphilis (because serologic tests for syphilis, particularly the treponemal tests, often remain positive for life) and lack of either a nontreponemal or treponemal test on the day of diagnosis.

In all cases, DF microscopy was performed at the STD clinic on-site laboratory by clinicians who are experienced in DF technique. Serum specimens were collected at the STD clinic site, and the VDRL and TP-PA tests were performed by the San Francisco Department of Public Health Laboratory according to manufacturer's directions. Individual chart review was performed if both VDRL and TP-PA results were not recorded in the database. For a subset of cases, qualitative RPR card tests were performed at the STD clinic laboratory by trained staff. Case demographic variables and HIV status were abstracted from the medical record database.

Two diagnostic strategies were considered<sup>1</sup>: VDRL with positive results confirmed by TP-PA; and<sup>2</sup> TP-PA alone. Positive TP-PA findings were reflexed to VDRL for a quantitated result to use in assessing serologic activity of syphilis and for monitoring the effectiveness of treatment. Using DF as the gold standard for true positivity, we calculated the sensitivities of the 2 testing strategies. To assess the potential effect of using RPR as the nontreponemal test, a subset of cases with RPR results also were analyzed. In addition, the sensitivities of the diagnostic strategies were calculated for HIV-negative and -positive cases. Data were analyzed using Statistical Analysis System.<sup>13</sup> Exact methods were used for calculating *P* values, comparing proportions, and calculating 95% confidence limits.

### Results

Two hundred fifteen primary syphilis cases were diagnosed at the STD specialty clinic of the San Francisco Health Department from January 1, 2002 through December 31, 2004. One hundred fifty-three had been diagnosed by a positive DF test. Of these, 19 cases were excluded because of a prior history of syphilis, and 28 were excluded because either the VDRL test or the TP-PA test had not been performed on the day of diagnosis. Our final study group, therefore, consisted of 106 cases, all of whom had a DF-positive genital or anal lesion, and VDRL and TP-PA serologies performed on the day of diagnosis. Compared with the study group, those cases not included in the analysis were similar in terms of gender, sexual orientation, age, and race/ethnicity. However, a greater proportion of nonstudy cases were HIV-infected: 51% compared with 31% of study cases (*P* = 0.007).

Of the 106 study cases, 77 had a reactive VDRL test and of those, 75 were confirmed with a positive TP-PA. Thus the sensitivity of the strategy requiring both tests to be serially positive was 71% (Table 1). In contrast, 91 cases had a reactive TP-PA for a sensitivity of 86%. Test concordance between VDRL and TP-PA was 83% (88/106). In 13 (12%) cases, both tests were negative.

Qualitative RPR results from the day of diagnosis were available for 51 of the study cases. Of the 37 (73%) cases that were positive by RPR, 36 were positive by TP-PA. A testing strategy that relied on RPR with confirmation by TP-PA would have a

TABLE 1. Sensitivity of Serologic Testing Strategies in Detecting Dark-Field-Confirmed Primary Syphilis

Testing Strategy	Total (N = 106)		HIV-Negative (N = 65)		HIV-Positive (N = 29)		<i>P</i> *
	No. Cases Diagnosed	Sensitivity (95% CI)	No. Cases Diagnosed	Sensitivity (95% CI)	No. Cases Diagnosed	Sensitivity (95% CI)	
VDRL confirmed by TP-PA	75	70.8% (61.1, 79.2)	50	76.9% (64.8, 86.5)	16	55.2% (35.7, 73.6)	0.05
TP-PA as first-line test	91	85.9% (77.7, 91.9)	57	87.7% (77.2, 94.5)	24	82.8% (64.2, 94.2)	0.53

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VDRL indicates Venereal Disease Research Laboratory; TP-PA, *Treponema pallidum* particle agglutination; CI, confidence interval.

\*HIV negative compared to HIV positive.

sensitivity of 71% (36/51) with a 95% CI of 56% to 83%. Among this subset of 51 cases, no difference was seen in the sensitivity of the strategy using VDRL confirmed by TP-PA.

The sensitivities of testing strategies were compared between HIV-positive cases and HIV-negative cases (Table 1). The strategy using VDRL confirmed by TP-PA performed less well among HIV-positive patients compared with HIV-negative patients (55% vs. 77%,  $P = 0.05$ ). However, TP-PA performed similarly among HIV-positive and HIV-negative cases (83% vs. 88%).

### Discussion

Testing with TP-PA yielded the highest sensitivity (86%) for these patients with DF-confirmed primary syphilis. The currently recommended serial testing strategy that relies on a positive nontreponemal test confirmed by treponemal test would have detected only 71% of cases. This testing strategy appears to be less sensitive because it depends on a reactive result of a diagnostic test with limited sensitivity in primary syphilis. The biologic reasons for this difference in test performance are unclear and deserve further investigation. The RPR has been reported to be somewhat more sensitive than the VDRL in primary syphilis (86% vs. 78%)<sup>5</sup>; however, in this study, the 2 tests performed equally.

Although the sensitivities of the individual nontreponemal and treponemal tests in this study were similar to previously reports, a diagnostic strategy employing routine use of a treponemal test as the first-line diagnostic test (followed by a reflexive nontreponemal titer) would maximize the sensitivity of serologic tests when primary syphilis is suspected. This may be particularly helpful in settings where DF is not available. Using the best possible serologic diagnostic strategy in these settings is important given the current syphilis outbreaks in which a high proportion of patients are men who have sex with men who are HIV-infected.<sup>3</sup> Syphilis increases the transmission risk of HIV,<sup>14</sup> and the clinical course of syphilis may be more severe in HIV-coinfected patients.<sup>15,16</sup>

No testing strategy was 100% sensitive; 12% of DF-confirmed cases were missed by both VDRL and TP-PA. In addition, results of serologic tests are not immediately available in most clinical settings. These factors underscore the importance of treating presumptively for primary syphilis based on the patient's risk factors and physical findings. The recommended treatment for primary syphilis continues to be benzathine penicillin G, 2.4 million units intramuscularly in a single dose.<sup>17</sup>

This study has a number of limitations. First, the DF test is prone to errors of interpretation, such that some of the study cases may have been diagnosed on the basis of a false positive DF result. Second, about one-third of the study cases were HIV-infected, which may not be representative of other populations diagnosed with primary syphilis. If HIV serostatus affects the performance of these tests, our overall findings may not be generalizable to other at-risk populations. Third, in populations with high rates of treated syphilis, using TP-PA as the first-line diagnostic test may not give considerable added benefit, because the treponemal tests usually remain positive for life. However, in our population, only 12% had previously been diagnosed with syphilis. Fourth, the specificities of the testing strategies evaluated and the potential for overdiagnosis when using the proposed strategy cannot be determined from our data.

The large number of syphilis cases seen in San Francisco over the past several years provided an ideal opportunity to study the tests commonly used to diagnose primary syphilis. This study analyzed a current practice approach using test results obtained from actual clinic procedures, not a study protocol. The recommended change in diagnostic approach involves switching to a treponemal test, such as the TP-PA, as the first-line diagnostic test. This change could be easily implemented because these tests are already available and in general use; no change in test technology would be required. The nontreponemal titer should always be obtained reflexively for assessing the response to treatment. Although better diagnostics are still needed, this diagnostic approach would yield a modest improvement over the current practice in diagnosing primary syphilis.

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