

Comparison of the Serodia *Treponema pallidum* Particle Agglutination, Captia Syphilis-G, and SpiroTek Reagin II Tests with Standard Test Techniques for Diagnosis of Syphilis

VICTORIA POPE,* MARTHA B. FEARS, WILLIAM E. MORRILL, ARNOLD CASTRO,
AND SUSAN E. KICKERT

*Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases,
Centers for Disease Control and Prevention, Atlanta, Georgia*

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We compared the microhemagglutination assay for *Treponema pallidum* (MHA-TP), a treponemal test, with two other treponemal tests, the Serodia *Treponema pallidum* particle agglutination (TP-PA) assay and the Captia Syphilis-G enzyme immunoassay, using 390 clinical serum samples. We also compared two nontreponemal tests, the rapid plasma Reagin (RPR) card test and the SpiroTek Reagin II test. Agreements of the MHA-TP with the TP-PA test and the Syphilis-G test were 97.4 and 97.7%, respectively. There was 89.2% agreement between the RPR and Reagin II tests. The Reagin II test was more apt to be reactive if the treponemal test was also reactive. We conclude that either the Serodia TP-PA test or the Captia Syphilis-G test is an appropriate substitute for the MHA-TP and that the Spirotek Reagin II test could substitute for the RPR test as a screening test.

The Serodia *Treponema pallidum* particle agglutination (TP-PA) test (Fujirebio America, Inc., Fairfield, N.J.) has been available internationally for the past few years (1), with the Fujirebio microhemagglutination assay for *Treponema pallidum* (MHA-TP) having been phased out in that market. Although we routinely used the MHA-TP for serum samples submitted to the Centers for Disease Control and Prevention (CDC) Syphilis Diagnostic Immunology Laboratory for testing, the test is also no longer available domestically. The Clinical Laboratory Improvement Act of 1988 requires that whenever a new test is placed in use, it must first be validated (2). Therefore, before replacing the MHA-TP, we evaluated two possible replacements: the TP-PA assay and the Captia Syphilis-G enzyme immunoassay (EIA) (Trinity Biotech, Dublin, Ireland).

In addition, there is currently a trend to use automation whenever possible to reduce personnel costs. The automated tests usually used are those in the EIA format. The only nontreponemal test in the EIA format that is currently available is the SpiroTek Reagin II EIA (Organon Teknika, Durham, N.C.). None of the standard nontreponemal tests, the Venereal Disease Research Laboratory (VDRL) test, the unheated serum reagin (USR) test, the rapid plasma Reagin (RPR) 18-mm circle card test (CDC), or the toluidine red unheated serum test (TRUST), is suitable for the current methods of automation.

We tested blinded, unlinked serum samples obtained from the Georgia Department of Human Resources (GDHR) using the MHA-TP and the TP-PA and Syphilis G tests to determine the suitability of the TP-PA and Syphilis-G tests as replacement confirmatory tests for the MHA-TP. We also tested the sera in the RPR and Reagin II tests to determine if the Reagin II test was a viable alternative to the RPR test for routine screening of clinical specimens.

* Corresponding author. Mailing address: Syphilis Diagnostic Immunology Activity, DASTLR/NCID, Mailstop D-13, Atlanta, GA 30333. Phone: (404) 639-3224. Fax: (404) 639-3976. E-mail: vxp1@cdc.gov.

MATERIALS AND METHODS

Serum samples. We obtained 390 serum samples from GDHR. The sera were unlinked from any patient identifiers. Previous results for serum samples were not known at the time of testing. The TP-PA test was evaluated with a panel of characterized serum samples from the CDC syphilis serum bank. This panel consisted of serum samples from 100 persons diagnosed with syphilis, 100 with diseases other than syphilis (DOTS), and 50 who were considered biologic false positives (BFP) in the nontreponemal tests. Of the 100 persons in the DOTS category, 26 were classified as having rheumatic fever, and 17 had other forms of coronary disease. Seven had various neurologic disorders that might be confused with neurosyphilis, four had autoimmune diseases, and the others had a wide variety of disorders ranging from cancer to abdominal pain.

Serologic tests for syphilis. The RPR test (5), MHA-TP (4), Syphilis-G test (7), and Reagin II test (8) were done according to standard techniques. The TP-PA test was done according to manufacturer's directions. Briefly, sample diluent was added to each of four wells in a round-bottom microtiter plate. One hundred microliters was added to the first well, and 25 μ l was added to wells 2 through 4. Next, 25 μ l of serum sample was added to the first well, making this a 1:5 initial dilution of the sample. The contents of the first well were mixed, and 25 μ l was transferred to the second well. This procedure was continued through well 4, with 25 μ l being discarded from the fourth well. Twenty-five microliters of unsensitized particles was added to the third well, the 1:20 dilution, and 25 μ l of sensitized particles was added to the fourth well, the 1:40 dilution of serum. The final serum dilutions were 1:40 for the unsensitized control well and 1:80 for the test well. The contents of the wells were mixed thoroughly using a vibrating shaker. The plates were covered and left at room temperature for 2 h. Reactive and nonreactive controls were included in each run. A sample was considered reactive if a mat of particles covered the bottom of the well. A 1+ reactive sample had a diffuse ring of particles around the periphery of the mat of particles, while a 2+ reactive sample lacked this ring. A sample with a button of particles in the bottom of the well was considered nonreactive.

The fluorescent treponemal antibody absorption (FTA-ABS) double-staining (FTA-ABS DS) test (CDC) (3) was done on DOTS and BFP serum samples that had discrepant results in the TP-PA and RPR tests.

RESULTS

The clinical diagnosis was not known for any of the 390 serum samples obtained from the GDHR; therefore, the sensitivity and specificity of each test could not be determined using these samples. The sensitivity of the TP-PA test, based on the 100 serum samples from patients with documented syphilis, and the specificity, based on the 100 serum samples from the DOTS group and the 50 samples from the BFP group, are given in Table 1. The four samples that were false positive in the DOTS category, which were all from patients with rheu-

TABLE 1. Sensitivity and specificity of the TP-PA test by category of documented serum samples

Diagnosis	No. of serum samples	TP-PA test	
		% Sensitivity	% Specificity
Primary syphilis			
Untreated	9	89	
Treated	15	87	
Secondary syphilis			
Untreated	20	100	
Treated	30	100	
Latent syphilis			
Untreated	6	100	
Treated	20	95	
DOTS	100		96
BFP	50		94

matic fever, were nonreactive in the FTA-ABS DS test. When the 50 specimens from the BFP group were tested in the TP-PA test, 3 were reactive, for a specificity of 94% (Table 1). All three were nonreactive in the FTA-ABS DS test. For two of the three specimens, there was no apparent reason for the false-positive reactivity. However, the third serum sample was from an injecting drug user who had gonorrhea, herpes, and probable treated latent syphilis. Since we could not determine if the reactivity in this sample was due to the drug use or possible old treated syphilis, we excluded this sample. The overall specificity of the TP-PA test for the nonsyphilis group was 96%.

The results of the comparison of the MHA-TP versus the Serodia TP-PA test and the Syphilis-G test for the 390 undocumented serum samples are given in Table 2. There was an overall agreement in the three treponemal tests of 96%, with only 13 of the serum samples not agreeing in the treponemal tests and 2 being inconclusive in the MHA-TP. When any two treponemal tests were compared, the agreement was 97% (range, 96.9 to 97.2%). Of the two serum samples that were inconclusive in the MHA-TP, one was nonreactive in all the other tests and 1 was reactive in the TP-PA and Syphilis-G tests but nonreactive in the FTA-ABS test. Five serum samples were reactive only in the TP-PA test. Two of these five were nonreactive in the FTA-ABS DS test, two were reactive in the FTA-ABS DS test, and one was RPR and FTA-ABS DS test reactive but Reagin II and Syphilis-G test nonreactive. Two specimens were nonreactive only in the TP-PA test and were reactive in all the other tests, including the FTA-ABS DS test. One serum sample was reactive only in the MHA-TP and nonreactive in the FTA-ABS DS test. One serum sample was nonreactive only in the MHA-TP but reactive in the other tests, including the FTA-ABS DS test. Of the four samples that were reactive only in the Syphilis-G test, one was FTA-ABS DS

TABLE 2. Comparison of MHA-TP results with Serodia TP-PA test and Captia Syphilis-G EIA results for 390 serum samples

MHA-TP result	No. of samples with the following result ^a :				
	TP-PA NR, EIA NR	TP-PA NR, EIA Eq	TP-PA NR, EIA R	TP-PA R, EIA NR	TP-PA R, EIA R
Nonreactive	125	1	3	4	1
Reactive	1	0	2	1	250
Inconclusive	1	0	0	0	1

^a NR, nonreactive; R, reactive; Eq, equivocal. No samples were equivocal in the Captia Syphilis-G EIA and reactive in the Serodia TP-PA test.

TABLE 3. Comparison of RPR test results with SpiroTek Reagin II test results for 390 serum samples

RPR test result	No. of samples with the following SpiroTek Reagin II test result:		
	Equivocal	Nonreactive	Reactive
Nonreactive	5	90	26
Reactive	1	10	26

test nonreactive but Reagin II test reactive, two were FTA-ABS DS test reactive (one of these was also reactive in the Reagin II test but not the RPR test), and one was equivocal in the Syphilis-G test and reactive in the Reagin II and FTA-ABS DS tests. One serum sample was nonreactive only in the Syphilis-G test but reactive in all the other tests, including the FTA-ABS DS test.

There was less agreement between the RPR and Reagin II tests, the nontreponemal tests (Table 3). Ten sera were false positive in the RPR test only (all titers, 1:1). Twenty serum samples were Reagin II test false positive; 5 of these were equivocal. Eighteen serum samples were false positive in both the RPR test (11 with a titer of 1:2, 5 with a titer of 1:2, and 2 with a titer of 1:16) and the Reagin II test (1 of which was equivocal and had an RPR test titer of 1:1). Ten sera were false negative in the RPR test but reactive in all the other tests, 1 was false negative in the Reagin II test but reactive in all the other tests, and 2 were false negative in the Reagin II test but reactive in all the other tests. One sample was Reagin II, Syphilis-G, and FTA-ABS DS test reactive but nonreactive in all the other tests, as mentioned above.

We examined what the probable diagnosis would have been for the clinical samples using either the Reagin II or the RPR test as the nontreponemal screening test and the TP-PA test, Syphilis-G test, or MHA-TP as the treponemal confirmatory test. The results are shown in Table 4. Regardless of the treponemal test used as the confirmatory test, if the Reagin II test rather than the RPR test used as the screening test, more patients would have been diagnosed as having syphilis.

DISCUSSION

Very little difference was found among the three treponemal tests. Laboratories that have routinely used the MHA-TP may

TABLE 4. Number of patients who would have been diagnosed as having syphilis, being BFP, or being false negative based only on nontreponemal and treponemal test results

Test	No. of patients with the indicated result in the following test:					
	RPR		NR (missed) ^d	RGN ^a		NR (missed)
	R	BFP ^c		Syphilis	BFP	
MHA-TP	242	27	12	251	33	3
TP-PA	242	27	15	250	34	7
Syphilis-G	242	27	15	252	31	5

^a RGN, Reagin II test.

^b Diagnosed as having syphilis based on reactive nontreponemal and treponemal test results.

^c Diagnosed as BFP based on reactive nontreponemal test result and nonreactive treponemal test result.

^d Diagnosed as not having syphilis based on nonreactive nontreponemal test, but the treponemal test was reactive, indicating either past or present syphilis.

be more comfortable using the TP-PA test. Compared with the MHA-TP, the TP-PA test is a little easier to set up, incubation time is shorter, the results seem to be easier to read because of fewer equivocal reactions, and the test is not subject to heterophile reactions, which were occasionally a problem in the MHA-TP. In the MHA-TP, the procedure stated that sera that had heterophile reactions with unsensitized cells should be removed by titration from both sensitized and unsensitized cells. If the reactivity with sensitized cells was two dilutions higher than that with unsensitized cells, then the test could be reported as reactive. If there was no difference or only one dilution difference between the reactivity with unsensitized cells and that with sensitized cells, then the results had to be reported as inconclusive. Because the TP-PA test uses a gel particle instead of a red blood cell as the carrier for the treponemal antigens, heterophile reactions are eliminated.

The four reactive samples in the DOTS category that were from rheumatic fever patients may indicate that the TP-PA test may show false-positive results in diseases where there is heart muscle damage. There were 27 serum samples from patients with rheumatic fever and 20 from patients with other heart-related problems ranging from the nondescript term coronary problems to atherosclerosis and angina. However, there were none from persons with myocardial infarction. Cardiolipin isolated from beef heart is one of the major components of the VDRL test antigen. Although rheumatic fever is not as common today as it was a generation ago, other diseases and conditions, such as heart attacks, that also damage heart muscle are prevalent. None of the serum in the DOTS category was from persons who had been diagnosed with myocardial infarction.

While agreeing quite well with the MHA-TP, the Syphilis-G test, like other EIA tests, is prone to equivocal results. All tests with equivocal results need to be repeated. If the result is still equivocal, then a second serum sample collected at least 7 days after the first sample should be requested. Testing of this sample will determine whether the initial result was due to low levels of treponemal antibody (repeat test is reactive) or was a false-positive result (repeat test is nonreactive). The Syphilis-G test does have the advantages that it can be automated and the results are objective.

The Reagin II test is the only nontreponemal test in the EIA format. This makes it useful for screening large numbers of serum samples. Although the number of false-positive results appeared to be slightly higher than with the RPR test (10 with the RPR test versus 20 with the Reagin II test), the Reagin II test also appeared to detect 10 more cases of syphilis, based on the treponemal test results. The RPR test has a reported sensitivity of 86% for primary syphilis (6), while the Reagin II test has a sensitivity of 93% (8). The reported specificities of the two tests are 98% for the RPR test (5, 6) and 97% for the Reagin II test (8). The VDRL test is also 98% specific, but it is only 78% sensitive in detecting primary syphilis (5, 6). The slight decrease in specificity in the Reagin II test is offset by the

extra sensitivity, especially with the current federal syphilis elimination directive and the goal of 1,000 or fewer cases of primary and secondary syphilis by the year 2005 (9). Again, serum samples giving equivocal results should be retested, and a second serum sample should be requested if the repeat result is still equivocal.

Both the Reagin II and the Syphilis-G tests are considered provisional status tests. The TP-PA test is considered an investigational status test, mainly because there is a lack of published data. The only standard status tests available are the FTA-ABS and FTA-ABS DS tests. Both tests require many controls and a lot of personnel time, and neither is suitable if large numbers of serum samples need to be tested. The RPR test requires a lot of personnel time if large numbers of sera need to be screened and if the titers of a high percentage of reactive samples need to be determined. Based on practical issues, such as personnel costs and the number of tests to be run, the Syphilis-G and TP-PA tests seem to be appropriate alternatives to the MHA-TP for use as confirmatory tests, and the Reagin II test is appropriate as a screening test. However, the Reagin II test cannot be used to monitor treatment efficacy, since that depends on a fourfold decrease in titer. The RPR test, VDRL test, USR test, or TRUST is needed to determine titers. Laboratory personnel must run any new test in parallel with the test that they have been using to determine comparability (2).

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ERRATUM

Comparison of the Serodia *Treponema pallidum* Particle Agglutination, Captia Syphilis-G, and SpiroTek Reagin II Tests with Standard Test Techniques for Diagnosis of Syphilis

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AND SUSAN E. KICKERT

*Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases,
Centers for Disease Control and Prevention, Atlanta, Georgia*

Volume 38, no. 7, p. 2543–2545, 2000. Page 2544, Table 3, column 4, line 2: “26” should read “258.”