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Reactivity of Microhemagglutination, Fluorescent Treponemal Antibody Absorption, Venereal Disease Research Laboratory, and Rapid Plasma Reagin Tests in Primary Syphilis

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Seroreactivity of sera from 109 patients with first-infection primary syphilis was 98.2% in the fluorescent treponemal antibody absorption test, 92.7% in the rapid plasma reagin 18-mm circle card test, 72.5% in the microhemagglutination test (MHA-TP), and 72.5% in the Venereal Disease Research Laboratory test. Seroreactivity of sera from 18 patients with primary syphilis with documented previous infection(s) was 100% in the fluorescent treponemal antibody absorption test, the rapid plasma reagin 18-mm circle card test, and the MHA-TP test and 88.9% in the Venereal Disease Research Laboratory test. The MHA-TP test failed to confirm reactivity in 13 of 79 sera which were reactive in the Venereal Disease Research Laboratory test and in 24 of 101 sera which were reactive in the rapid plasma reagin 18-mm circle card test. Testing another production lot of MHA-TP reagents resulted in even poorer correlation. The reactivity of the MHA-TP test in primary syphilis appeared to vary with the sensitivity of the production lot of reagents.

The control of syphilis depends on prompt and accurate diagnosis of all stages of the disease. Although primary syphilis is highly infectious, symptoms may be minimal. Diagnosis by dark-field microscopy is not always possible; therefore, serological tests are of prime importance in the diagnosis of primary syphilis. Nontreponemal (cardiolipin) procedures are used to screen sera for reactivity, whereas treponemal tests, such as the fluorescent treponemal antibody absorption (FTA-ABS) test and the more recently developed microhemagglutination (MHA-TP) test, are used to confirm that the reactivity of nontreponemal methods is specific for syphilis.

In a previous study (4), the reactivity of the Venereal Disease Research Laboratory (VDRL) nontreponemal test was compared with that of the FTA-ABS and MHA-TP tests in primary syphilis. The MHA-TP test appeared to be a reasonable substitute for the FTA-ABS test, since a high degree of reactivity was observed. Furthermore, the MHA-TP test confirmed the reactivity in 97% of VDRL-reactive sera. Sera for which the MHA-TP test failed to confirm reactivity were all weakly reactive in the VDRL test. It appeared that the MHA-TP test could be relied upon to confirm most weakly reactive and all VDRL-reactive sera from primary syphilitics. The relationship between the quantitative rapid plasma reagin 18-mm circle card (RPR) test and the MHA-TP test should be determined, because the RPR is also a widely used screening test. Since sera from the previous study (4) were no longer available, a new study was initiated to evaluate the reactivity of the VDRL, RPR, MHA-TP, and FTA-ABS tests in primary syphilis.

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MATERIALS AND METHODS

Selection of sera. Sera were saved from patients with a positive dark-field examination. Patients who had primary lesions but were developing secondary manifestations were considered secondary cases. Sera from patients with dark-field-positive, secondary syphilis were eliminated from the study. Medical records were reviewed to classify patients as having first-infection or reinfection primary syphilis; a negative patient history or previous serological test was used to differentiate first-infection from reinfection syphilis.

Storage of sera. The VDRL test was performed on fresh, heat-inactivated (56°C, 30 min) serum. Serum was stored at 4°C until the RPR, MHA-TP, and FTA-ABS tests were performed.

Syphilis serological tests. The VDRL, RPR, and FTA-ABS tests were performed as previously described (11). The MHA-TP and quantitative RPR tests

TABLE 1. Reactivity of four serological tests with sera from 109 patients with first-infection primary syphilis

Test	No. (%) of patients with sera that were:	
	Reactive	Nonreactive
FTA-ABS	107 (98.2)	2 (1.8)
RPR	101 (92.7)	8 (7.3)
MHA-TP	79 (72.5)	30 (27.5)
VDRL	79 (72.5)[a]	30 (27.5)

[a] A total of 9 patients (8.3%) had sera that were weakly reactive.

were performed according to the instructions of the manufacturers. The VDRL antigen was prepared by the Texas Department of Health Laboratory, Austin. RPR test kits were purchased from Hynson, Wescott, and Dunning, Inc., Baltimore, Md. Reactive, minimally reactive, and nonreactive serum controls provided with the kits were tested with each group of sera. MHA-TP test kits were obtained from Ames Co., Elkhart, Ind. Positive and negative control sera were included with each group of sera. Reactivity within 1 dilution of the expected titer of the positive control serum and lack of hemagglutination with unsensitized cells and with the negative control serum were required to establish test validity. FTA-ABS antigen, sorbent, fluorescent-antibody conjugate, and control sera were purchased from Beckman Instruments, Inc., Fullerton, Calif. New lots of reagents were evaluated against 25 sera with a known range of reactivity. Test sera that showed 1+ fluorescence in the original test and in the repeat test were considered reactive. Sera that exhibited borderline fluorescence were omitted from the study because of the equivocal nature of this reaction.

RESULTS

The performances of the four serological tests for syphilis in documented cases of first-infection primary syphilis are shown in Table 1. The FTA-ABS test was the most sensitive test for the detection of antibodies in primary syphilis. The RPR test exhibited greater sensitivity than did either the MHA-TP or the VDRL test. Retesting primary syphilitic sera with a second lot of MHA-TP reagents resulted in considerable variation in sensitivity from the first lot (Table 2).

The ability of the FTA-ABS and MHA-TP tests to confirm first-infection primary syphilis in VDRL-reactive sera is shown in Fig. 1. Sera apparently were collected so early in the disease from 30 patients that the VDRL test was nonreactive. The seroreactivity of sera from these 30 patients in the FTA-ABS and MHA-TP tests was 93 and 50%, respectively. Sera that developed any degree of reactivity in the VDRL test showed uniform reactivity in the FTA-ABS test. The data indicate that consistent confirmation of VDRL reactivity by the MHA-TP test was not attained until a VDRL titer of 1:32 or greater developed.

The correlation of RPR reactivity with FTA-ABS and MHA-TP reactivity is shown in Fig. 2. Sera from only eight patients failed to react in the RPR test. Six of the eight RPR-negative sera reacted in the FTA-ABS test. All RPR-reactive sera were reactive in the FTA-ABS test. Although the MHA-TP test detected antibodies in 50% of the RPR-nonreactive sera, the test was deficient in the detection of antibodies in RPR-reactive sera. The MHA-TP test confirmed the presence of treponemal antibodies in low titer (RPR titer, 1:1) sera in only 33% of the cases. The results shown in Fig. 2 indicate that MHA-TP reactivity was certain only in sera with an RPR titer of 1:64 or greater. A straight line rather than a point-to-point display was used in Fig. 1 and 2, since relatively small numbers of tests were reflected in each.

The reactivity of sera from 109 patients with first-infection primary syphilis in the VDRL and RPR tests is shown in Fig. 3. The line in Fig. 3 denotes a slope of 1, which would be obtained if two tests were to agree perfectly. The RPR test exhibited more sensitivity than did the VDRL test in sera with low titers. The majority of the sera (16 of 30) that were nonreactive in the VDRL test exhibited a titer of 1:1 in the RPR test. The reactivity of the two tests was comparable in sera with a VDRL titer of 1:8 or greater. Sera from eight patients were nonreactive in both tests.

Reinfection with *Treponema pallidum* occurs and may significantly affect the seroactivity of the serological tests for syphilis (Table 3). The reactivity of all four tests in reinfection syphilis was significantly higher than the reactivity in first-infection syphilis. All of the methods test-

TABLE 2. Reactivity of two production lots of MHA-TP reagents

Stage of syphilis	No. of patients	No. (%) of patients with sera reactive with:	
		Lot 1 reagent (lot no. 0118010)	Lot 2 reagent (lot no. 1111120)
Primary	61	43 (70.5)	31 (50.8)
Reinfection primary	9	9 (100)	8 (88.9)
Secondary	11	11 (100)	11 (100)

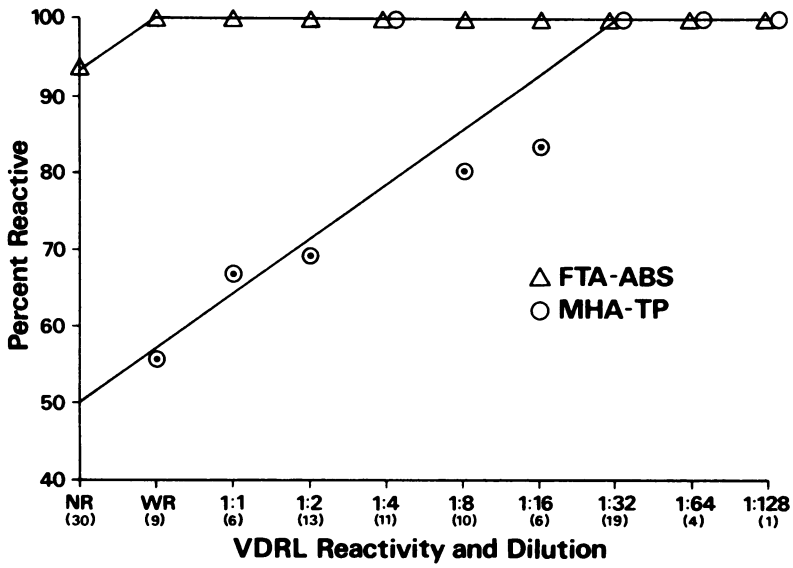

FIG. 1. Correlation of VDRL titers with FTA-ABS and MHA-TP reactivity in 109 patients with first-infection primary syphilis. NR, Nonreactive; WR, weakly reactive. Numbers in parentheses indicate the number of patients in each reaction category.

ed, with the exception of the VDRL test, were reactive with the 18 sera tested.

DISCUSSION

The FTA-ABS test is the most sensitive and specific test available to confirm the presence of antibodies in syphilitic serum. The lower cost and ease of performance make the MHA-TP assays for treponemal antibodies popular. MHA-TP assays have been proven to be as sensitive as the FTA-ABS test for all stages of syphilis other than the primary stage (14). There is disagreement over the reactivity of MHA-TP tests in primary syphilis. Several investigators reported a lower sensitivity for MHA-TP tests than for nontreponemal tests in primary syphilis

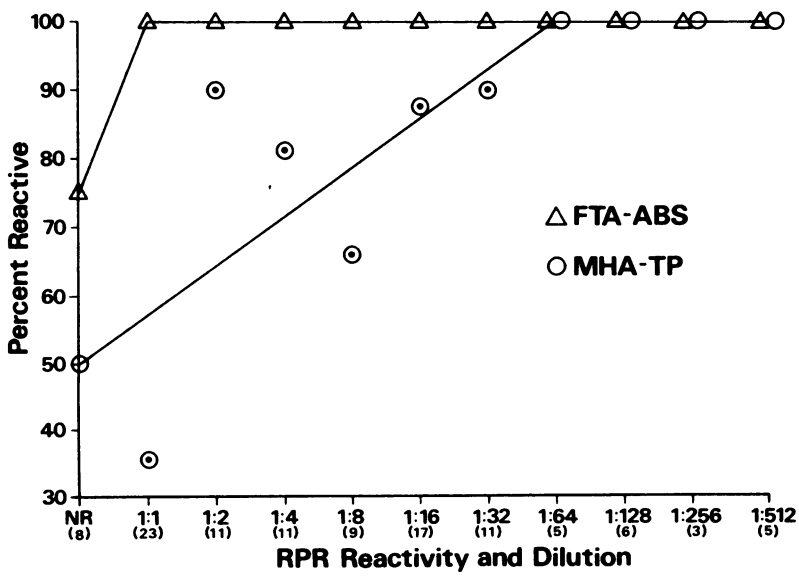

FIG. 2. Correlation of RPR titers with FTA-ABS and MHA-TP reactivity in 109 patients with first-infection primary syphilis. NR, Nonreactive. Numbers in parentheses indicate the number of patients in each reaction category.

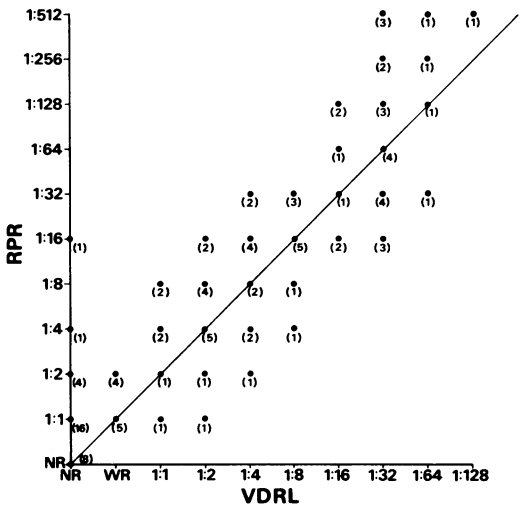

FIG. 3. Comparison of RPR and VDRL reactivity in 109 patients with first-infection primary syphilis. NR, Nonreactive; WR, weakly reactive. Numbers in parentheses indicate the number of patients in each reaction category.

TABLE 3. Reactivity of four serological tests with sera from 18 patients with reinfection primary syphilis

Test	No. (%) of patients with sera that were:	
	Reactive	Nonreactive
FTA-ABS	18 (100)	0 (0)
RPR	18 (100)	0 (0)
MHA-TP	18 (100)	0 (0)
VDRL	16 (88.9)[a]	2 (11.1)

[a] Only 1 patient (5.5%) had serum that was weakly reactive.

(3, 7, 8, 13, 14, 16), whereas others reported a higher sensitivity (1, 15). Luger and Spendlingwimmer (9) reported that MHA-TP test reactivity was greater than the FTA-ABS test reactivity in 21 cases of primary syphilis.

The reactivity of the MHA-TP test in the present study conflicts with previous results reported in our laboratory (4). One could speculate that the disagreement in MHA-TP reactivities may be due to differences in patient populations. Fiumara (6) reported that sera from patients with reinfection syphilis exhibited greater seroreactivity than did sera from patients with first-infection syphilis. It is unlikely that reinfection primary syphilis would represent enough cases in any of the studies to influence significantly MHA-TP reactivity. Inclusion of the results for the 18 reinfection syphilis cases we observed during the course of this study would not change the fact that the MHA-TP test failed to react with sera that were from patients with first-infection syphilis and that had significant VDRL or RPR test titers. The variability of lots observed in this study suggests that the reported disagreements over the reactivity of the MHA-TP test in primary syphilis may be due to the variable sensitivity of different lots of reagents.

The failure of some lots of MHA-TP reagents to detect antibodies in first-infection syphilis is puzzling. Proper reactivity with the high-titer serum control should relate the ability of sensitized erythrocytes to detect antibodies to *T. pallidum*. Perhaps a different immunoglobulin is produced in early syphilis. Atwood and Miller (2) found immunoglobulin M (IgM) antibodies in the serum of most patients with primary syphilis. O'Neal and Nichol (10) found that IgM antibodies specific for treponemes disappeared within 2 years after treatment but that IgG persisted for more than 20 years. The MHA-TP test reacts consistently when IgG antibodies would be expected to be present, i.e., in reinfection primary, secondary, latent, late, and treated syphilis. Taliafero et al. (15) found that avidity of IgM and IgG antibodies for sheep erythrocytes increased with increasing antibody titer. Perhaps less avid treponeme-specific IgM or early IgG antibodies are difficult to detect with MHA-TP tests. Reports by some investigators (1, 4, 9) of high reactivity indicate that some lots of MHA-TP reagents can detect early antibodies to syphilis. The inclusion of low-titer primary syphilitic sera in premarket tests and quality control procedures might ensure the production of a test with consistently high reactivity in primary syphilis.

The high degree of reactivity of the RPR test in primary syphilis was somewhat surprising. Reed (12) suggested that the RPR test was more sensitive than the VDRL test in detecting antibodies to syphilis. Dyckman et al. (5) reported comparable reactivity of the RPR and VDRL tests (64.9 and 63.1%, respectively) with sera from 111 patients with primary syphilis. Wentworth et al. (16) found that 87.1% of 31 sera from patients with primary syphilis were reactive in the RPR test.

Differences in reader interpretations of reactivity could account for the variable reports. Our results, however, suggest that test interpretation is not the reason for the greater sensitivity of the RPR test. One would expect uniformly higher RPR titers, regardless of the VDRL titer, if RPR sensitivity were due to reader error. Perhaps lot-to-lot variations occur in the RPR test, accounting for reports of variable reactivity. It is difficult to obtain an accurate history from patients with respect to the time of lesion appearance. It

may be possible that sera obtained from patients in the early stages of primary syphilis may have antibodies that are more readily detectable by some tests than by others. This may account for the increased sensitivity of the RPR test.

It can be seen that antibodies to first-infection primary syphilis are more difficult to detect than are antibodies to the later stages of syphilis. Reactivity with serum from an early primary case should be one of the standards against which the efficiency of serological tests for syphilis are established. The inclusion of primary syphilis serum as a test control also is indicated.

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