JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1992, p. 561–563
0095-1137/92/030561-03$02.00/0

Vol. 30, No. 3

Modified Indirect Hemagglutination Test for Detection of Treponemal Antibodies in Finger-Prick Blood

J. L. BACKHOUSE,[1]* M. H. LEE,[1] S. I. NESTEROFF,[1] B. J. HUDSON,[2] AND P. A. HAMILTON[3]

Institute of Clinical Pathology and Medical Research, Westmead, New South Wales,[1] and Royal North Shore Hospital, St. Leonards, New South Wales,[2] Australia, and Gaubin Lutheran Hospital, Kar Kar Island, Madang Province, Papua New Guinea[3]

Received 5 September 1991/Accepted 11 December 1991

A modified indirect hemagglutination test for the detection of treponemal antibodies was developed for use with finger-prick blood. By using paired serum and absorbed finger-prick blood from 58 patients from an area previously endemic for yaws and 12 patients without yaws, the modified hemagglutination test was compared with a hemagglutination test for *Treponema pallidum* and the fluorescent treponemal antibody absorption test. The modified hemagglutination test showed 100% specificity and an overall agreement of 96.5% with the hemagglutination test for *T. pallidum* and 94.8% with the fluorescent treponemal antibody test. The modified hemagglutination test appears to be a simple and economical test that is suitable for use in large epidemiological surveys for yaws.

In the past, the clinical diagnosis of yaws has been accepted as an accurate assessment of the prevalence of the disease in areas of high endemicity. Since the mass treatment campaigns of the 1950s and 1960s, the clinical manifestations of the disease have changed (7), and in many areas, even those which were previously of high endemicity for yaws, rural health workers are now unable to give an accurate diagnosis. This problem with clinical diagnosis is even greater in areas of lower endemicity.

In these instances, seroepidemiological surveys will give a more accurate estimate of the prevalence of yaws, detecting incubating, atypical, and latent or asymptomatic yaws. This will assist in determining the most effective control program and will facilitate surveillance. An associated clinical assessment is always necessary, because serological tests alone cannot distinguish between yaws and venereal syphilis.

In mass seroepidemiological surveys it is essential that large numbers of people be examined both conveniently and economically. However, the associated problems are numerous: patient compliance, difficulty with venipuncture in small children, and storage and transport of specimens often over long distances in the heat of the tropics to central testing laboratories. Because yaws has been reported to be increasing again, particularly in parts of Africa (8) and the western Pacific region (2), simpler, more cost-effective methods for evaluation of the prevalence of the disease have become an urgent necessity.

The use of finger-prick blood collected onto absorbent paper has been described for the fluorescent treponemal antibody absorption (FTA-ABS) test (3) and the microhemagglutination assay (5) as easy and economical methods for screening large numbers of patients.

In this study, the results obtained by a modified *Treponema pallidum* hemagglutination technique (TPHA-el) with finger-prick blood are compared with the results obtained by the Venereal Disease Research Laboratory (VDRL) slide test, a *T. pallidum* hemagglutination (TPHA) test, and the FTA-ABS test on serum from the same patients at the same time as the blood was collected by finger prick.

* Corresponding author.

MATERIALS AND METHODS

Patients. A total of 270 children and adults from seven villages located in the southern part of Kar Kar Island, Madang Province, Papua New Guinea, were examined for clinical evidence of yaws. Included in this group were 200 students from a primary school. Eight of these students had suspicious lesions, so blood samples and finger-prick blood were collected. Another 50 paired specimens (a pair consisting of blood collected by both venipuncture and finger prick from each patient) were collected from family groups who presented at Gaubin Hospital seeking treatment for suspected yaws. All 58 paired specimens were classified, according to the clinical appearance of the patients, as follows: group 1, clinical yaws (19 patients); group II, lesions not consistent with yaws (15 patients); and group III, asymptomatic (24 patients). In addition, a control group of similar paired samples was collected from 12 patients presenting at a sexually transmitted disease clinic in Sydney, New South Wales, Australia.

Collection and storage of specimens. Blood samples were collected by venipuncture, and serum was stored at 4°C for 48 h and was then packaged and transported by air to the Institute of Clinical Pathology and Medical Research, Westmead Hospital, Sydney.

The finger-prick blood was collected aseptically onto cards (7 by 11 cm; Schleicher and Schuell no. 903 specimen collection paper) and was allowed to air dry. The cards were stacked "head to end" and sealed in plastic bags for transportation to the Institute of Clinical Pathology and Medical Research, where they were stored at room temperature (25°C) for 2 months.

The stability of the finger-prick blood was examined by repeat TPHA-el tests performed on 42 of the original finger-prick blood samples after storage for an additional 8 months at room temperature (25°C).

Serological tests. All serum samples were tested by the VDRL and FTA-ABS tests by using standard techniques (6). The TPHA test, using chicken erythrocytes sensitized with *T. pallidum*, was performed with Serodia-TP kits (Fujirebio Inc., Tokyo, Japan), in accordance with the instructions of the manufacturer.

TABLE 1. Clinical and serological results from 58 patients examined for yaws

Clinical lesion (group)	Age group[a]	Total	No. of patients with the indicated results[b]								
			VDRL			TPHA		TPHA-el		FTA-ABS	
			Titer ≥8	Titer <8	N	R	N	R	N	R	N
Yaws (I)	C	17	12	2	3	15	2	15	2	15	2
	A	2	2			2		2		2	
Other (II)	C	11	3	3	5	7	4	7	4	8	3
	A	4	1		3	4		3	1	4	
None (III)	C	7	2		5	4	3	4	3	4	3
	A	17	1	4	12	15	2	14	3	15	2
Total		58	21	9	28	47	11	45	13	48	10

[a] C, children, under age 15 years; A, adults, ages 15 years and above.
[b] N, nonreactive; R, reactive.

The TPHA-el test on the finger-prick blood eluate was performed by using reagents from the Serodia-TP kit. Two 5-mm-diameter discs punched from the blood-stained area of the card were placed in 125 μl of absorbing diluent in the first well of a microhemagglutination plate. The blood from the discs was allowed to elute overnight at room temperature. After elution the two discs were removed from the well with tweezers, the eluate was squeezed out, and the discs were discarded. The eluate was equivalent to a 1 in 20 dilution of serum. This was initially determined by adjusting the volume of the diluent in which the discs were eluted until equivalent test results were obtained from both serum and eluate. Blood eluate (25 μl) was placed in corresponding wells 2 and 3 of the plate. A final dilution of 1/80 was achieved in each well by the addition of 75 μl of the unsensitized cell suspension to well 2 and 75 μl of the sensitized cell suspension to well 3. The plate was shaken, allowed to stand, and read after 2 h.

Readings of the TPHA and TPHA-el tests were based on the intensities of the hemagglutination patterns. A reading of 3+ to 1+ was recorded and reported as reactive. Sera giving a reading of ± or a reading of negative were reported as nonreactive.

RESULTS

In this study the clinical findings for 70 patients were compared with the test results obtained with paired serum and absorbed finger-prick blood. Table 1 shows the clinical and serological test results for 58 patients examined for yaws. In this study, a high-titer VDRL (titer, ≥8) test result together with the presence of specific treponemal antibodies was taken as an indication of active yaws.

In group I, a VDRL test titer of ≥8 confirmed the clinical diagnosis of active yaws in 14 of 19 (73.7%) patients overall and 12 of 17 (70.6%) children. The VDRL test was reactive in 16 patients (84.2%). All the specific tests, TPHA, TPHA-el, and FTA-ABS, showed 100% agreement, with 17 (89.5%) being reactive.

In group II, 3 of 11 (27.3%) children and 1 of 4 (25%) adults had serological evidence of active yaws, a total of 4 of 15 (26.7%) patients, and the VDRL test was reactive with sera from 7 (46.7%) patients. Of the 15 patients in this group, sera from 10 of them were reactive in the TPHA, TPHA-el, and FTA-ABS tests. Serum from one patient gave a reactive FTA-ABS test and nonreactive TPHA and TPHA-el tests. Serum from one other patient gave a reactive FTA-ABS test, a reactive TPHA test with a minimal level of reactivity (1+), and a nonreactive TPHA-el test.

TABLE 2. Effect of storage time on agreement between the level of reactivities of the TPHA and TPHA-el tests[a]

TPHA test result	No. of patients with the indicated TPHA-el result at:							
	2 mo				10 mo			
	R (3+)	R (2+)	R (1+)	N	R (3+)	R (2+)	R (1+)	N
R (3+)	25	2			20	2	3	2
R (2+)	1	4					2	3
R (1+)			3	2				5
N				5		1		4
Total	26	6	3	7	20	3	5	14

[a] R, reactive; N, nonreactive.

Of the 24 asymptomatic patients in group III, 3 (12.5%) showed serological evidence of active yaws (VDRL titer, ≥8), 2 of 7 (28.6%) children and 1 of 17 (5.9%) adults. The VDRL test was reactive with sera from 7 (29.2%) patients, the TPHA and FTA-ABS tests were reactive with sera from 19 (79.2%) patients, and the TPHA-el test was reactive with sera from 18 (75.0%) patients. Serum from one patient gave reactive TPHA (1+ level of reactivity) and FTA-ABS tests but a nonreactive TPHA-el test.

The specificity of the TPHA-el test, on the basis of the reactivities of the tests performed with sera from the 12 patients without yaws who presented at the sexually transmitted disease clinic, was 100%, showing agreement with the nonreactive results of the TPHA and FTA-ABS tests.

Table 2 shows the effect of prolonged storage on the TPHA-el test results on finger-prick blood. The agreement between the TPHA-el and TPHA tests after 2 months of storage was 40 of 42 (92.5%) samples, and after 10 months it was 31 of 42 (73.8%) samples. The level of reactivity in the TPHA-el test dropped in 13 tests; of these, 8 became nonreactive. Serum from one patient (Table 1, group II) gave a reactive FTA-ABS test and nonreactive TPHA and TPHA-el tests after 2 months of storage and a reactive TPHA-el test (2+ level) after 10 months of storage. Technical error appears to be the most likely explanation.

DISCUSSION

The TPHA test has been shown to be satisfactory for the serodiagnosis of yaws when it is compared with the FTA-ABS and TPI tests (1). In this comparative study the TPHA-el test showed an agreement of 96.5% with the TPHA test and 94.8% with the FTA-ABS test.

With sera from patients with clinical yaws, the TPHA-el test showed 100% agreement with the TPHA and FTA-ABS tests. There were two seronegative patients in this group, both of whom were children; one presented with mild angular stomatitis and the other presented with a solitary leg ulcer. It is possible that these lesions were not early yaws but the results of other infections.

In patients with lesions not characteristic of yaws (and when there is no access to dark-field microscopy) and also in those patients who are asymptomatic, the diagnosis of yaws relies on the results of specific treponemal tests. In this category (groups II and III), the TPHA-el test showed an agreement of 94.7% with the TPHA test and 92.3% with the FTA-ABS test.

Of the 58 patients examined for yaws, 35 were children

(under 15 years of age) and 23 were adults. Serologically confirmed active yaws was found in 17 (48.6%) children and 4 (17.4%) adults. In the children there was 100% agreement between the TPHA-el and TPHA tests and 97.1% agreement with the FTA-ABS test. One child in group II with a reactive FTA-ABS test presented only with a solitary crusted lesion on the leg. This may have been early yaws (4); however, reactive serology by other tests would normally be expected. In the adults, the TPHA-el test gave 91.3% agreement with the TPHA and FTA-ABS tests. Two adults gave a nonreactive TPHA-el test, a minimum level of reactivity (1+) in the TPHA test, and a reactive FTA-ABS test; these results are consistent with "old yaws" with fading reactivity, with the TPHA-el test possibly being less sensitive in this category.

Prolonged storage of the absorbed finger-prick blood reduced the reactivity of the TPHA-el test. However, the test had an acceptable level of reactivity, in agreement with the TPHA test, within a 2-month time frame from specimen collection to testing. Although temperatures in excess of 25°C may be expected in the tropics, it is envisaged that storage times would be much less than the 2-month period in this study. It is possible that the finger-prick blood specimen that gave a nonreactive TPHA-el test result and corresponding reactive (1+) TPHA test results may have given a reactive TPHA-el test result if the storage time had been less.

The costs of the TPHA-el test compared with those of the TPHA and FTA-ABS tests done with serum or the FTA-ABS test done with finger-prick blood shows that the TPHA-el test is the most economical. Costs of sample collection are halved, as are reagent costs, when the cost of the TPHA test is compared with that of the FTA-ABS test.

The TPHA-el test is simple and the results are obtained quickly and are easy to read, reducing the need for sample testing at one central laboratory. It is well suited for use in small provincial laboratories. The FTA-ABS test, by comparison, requires skilled technicians and expensive equipment which must have regular maintenance.

In conclusion, the TPHA-el test fulfills all requirements for large-scale seroepidemiological surveys for the prevalence of yaws. It is economical and easy to perform, with acceptable levels of specificity and sensitivity when compared with those of the TPHA and FTA-ABS tests.

REFERENCES

1. **Garner, M. F., J. L. Backhouse, G. Daskalopolous, and J. L. Walsh.** 1972. *Treponema pallidum* haemagglutination test for yaws. Br. J. Vener. Dis. **48**:479–482.
2. **Geizer, I.** 1986. Yaws in the Western Pacific Region: an overview. Southeast Asian J. Trop. Med. Public Health **17**(Suppl.): 8–13.
3. **Hopkins, D. R.** 1977. Fluorescent treponemal antibody absorption (FTA-ABS) tests using blood samples collected on filter paper. Am. J. Trop. Med. Hyg. **26**:188–189.
4. **Martin, P. M. V., J. P. Gonzales, M. H. Martin, M. C. Georges-Courbot, M. J. Palisson, and A. J. Georges.** 1988. Clinical aspects and usefulness of indirect absorbed immunofluorescence for diagnosis of yaws in Central Africa. J. Clin. Microbiol. **26**:2432–2433.
5. **Perine, P. L., J. W. Nelson, J. O. Lewis, S. Liska, E. F. Hunter, S. A. Larsen, V. K. Agadji, F. Kofi, J. A. Ofori, M. R. Tam, and M. A. Lovett.** 1985. New technologies for use in the surveillance and control of yaws. Rev. Infect. Dis. **7**(Suppl. 2):295–299.
6. **U.S. Public Health Service.** 1969. Manual of tests for syphilis. U.S. Public Health Service publication no. 411. U.S. Public Health Service, Washington, D.C.
7. **Vorst, F. A.** 1985. Clinical diagnosis and changing manifestations of treponemal infection. Rev. Infect. Dis. **7**(Suppl. 2):327–331.
8. **Widy-Wirsky, R.** 1985. Surveillance and control of resurgent yaws in the African region. Rev. Infect. Dis. **7**(Suppl. 2):227–236.