JOURNAL OF CLINICAL MICROBIOLOGY, July 1995, p. 1875–1878 Vol. 33, No. 7
0095-1137/95/$04.00+0

Evaluation of Immunoglobulin G Enzyme Immunoassay for Serodiagnosis of Yaws

J. L. BACKHOUSE[1]* AND B. J. HUDSON[2]

Department of Clinical Microbiology, Westmead Hospital, Westmead New South Wales 2145,[1] and Department of Microbiology, Royal North Shore Hospital, St. Leonards New South Wales 2065,[2] Australia

Received 29 November 1994/Returned for modification 12 January 1995/Accepted 4 April 1995

A commercially available enzyme immunoassay (EIA), the Captia Syphilis-G immunoglobulin G (IgG) EIA, for the detection of IgG antibodies to *Treponema pallidum* was evaluated for use as a screening test for yaws (*Treponema pallidum* subsp. *pertenue*). The IgG EIA was compared with the fluorescent treponemal antibody absorption (FTA-ABS) test. All sera were also examined by the *T. pallidum* hemagglutination test and the Venereal Disease Research Laboratory test. Serum samples from 271 subjects (23 control serum samples from an area nonendemic for yaws, 58 control serum samples from an area endemic for yaws, and 190 serum samples from yaws patients and contacts) were investigated. The overall agreement between the IgG EIA and the FTA-ABS test was 90%, the sensitivity was 99%, and the specificity was 70.2%. The specificity fell as the endemicity of the disease increased: from 94.4% in the nonendemic area controls to 86.4% in the endemic area controls and to 52.3% in the yaws contacts. There was no difference in specificity between children and adults within each of the three groups. Fifteen children with clinical yaws were monitored for 9 months after treatment. The level of treponemal antibody fell consistently in 9 of the 15 children as measured by the antibody index (ratio of absorbance of the test serum to the mean absorbance of the low-titer-positive controls). Reinfection was seen in three children, with the antibody index rising with the Venereal Disease Research Laboratory test titer. The Captia Syphilis-G test is a sensitive assay for the detection of treponemal antibodies in yaws patients. However, the apparent low specificity of the test in the yaws endemic area limits its use as a screening test for yaws.

Despite the initial success of the mass treatment campaigns against yaws of the early 1950s to 1960s, over the last decade it has become obvious that the prevalence of yaws is again on the increase (12). Associated with this increase, the need for seroepidemiological surveys has become more apparent, because the manifestations of yaws in areas which were previously endemic have changed.

The lesions present as attenuated yaws, which is characterized by lesions that are few in number and of short duration. However, periods of latency with a tendency to relapse still occur. Yaws lesions may now be less florid than in former times, but they are still infectious, and destruction of tissue and bone still occurs. The ability of the current generation of rural health workers to make an accurate clinical diagnosis is now complicated by the change in the clinical manifestations of the lesions and suggests that the disease is now underreported. Therefore, a disease that should be eradicable will persist until adequate case detection, treatment, and surveillance are implemented. Improved recognition by education is necessary, as is a surveillance program with appropriate field survey methods (7). Serology therefore assumes importance when clinical detection is difficult in milder but still infectious cases and for detection of active latent yaws.

In screening for syphilis, three different procedures are currently in use, utilizing either the nontreponemal or treponemal tests or a combination of both. The nontreponemal tests such as the Venereal Disease Research Laboratory (VDRL) test (Behringwerke AG, Marburg, Germany) and the rapid plasma reagin card test have been in use for the longest period (9, 17). The *Treponema pallidum* hemagglutination (TPHA) test is also widely used because its sensitivity and specificity have been reported to be higher than those of the nontreponemal tests (15) and comparable to those of the fluorescent treponemal antibody absorption (FTA-ABS) test (11). Other work suggested that the TPHA test was less sensitive than the VDRL test in early syphilis yet more sensitive in late syphilis and that a screening combination of the VDRL and TPHA tests would be effective in all stages of the disease (22). Positive results in one or both tests are confirmed by the FTA-ABS test, which is sensitive in all stages of syphilis but unsuitable for screening purposes (8).

The pathogenic treponemes of venereal syphilis and yaws, *Treponema pallidum* subsp. *pallidum* and *Treponema pallidum* subsp. *pertenue*, are indistinguishable both morphologically and serologically; hence, the screening tests used for the diagnosis of syphilis have been evaluated and are used for the diagnosis of yaws (3, 4). However, none of these tests lend themselves to automation, and the results are read subjectively. Recent work in screening for yaws has been directed to low cost and ease of transport with finger-prick blood samples (1, 16).

An alternative test now available is the immunoglobulin G (IgG) enzyme immunoassay (EIA), which is suitable for automation and allows for the rapid, objective screening of large numbers of serum samples. Evaluations of commercially available EIA kits for the detection of IgG antitreponemal antibody to *T. pallidum* subsp. *pallidum* have been reported previously (2, 6, 10, 13, 18, 23). One of these is the Captia Syphilis-G test (Mercia Diagnostics, Guildford, England) for the detection of IgG antibodies to treponemal infection. The Captia Syphilis-G test has been reported to have an overall sensitivity ranging from 98.3 to 100% and a specificity of greater than 99% in screening for syphilis (6, 10, 13, 23).

In this study, the performance of the Captia Syphilis-G test as a screening test for yaws is compared with those of the

* Corresponding author. Phone: (02) 633-6232. Fax: (02) 893-8659.

TABLE 1. Patterns of test results for serum samples from yaws patients and control subjects and mean AIs for each group

Mean AI	Total no. of serum samples	No. of subjects (no. of serum samples)[a] with given test result						Test result[b]			
		Yaws patients and contacts		Controls from:							
				Yaws endemic area		Yaws nonendemic area					
		Adults (67)	Children (123)	Adults (36)	Children (22)	Adults (17)	Children (6)	VDRL	TPHA	IgG EIA	FTA-ABS
2.98	96	14	68	4	9		1	+	+	+	+
1.92	81	32	26	15	5	3		−	+	+	+
1.87	4		2		2			+	−	+	+
1.20	4	1	2		1			−	−	+	+
0.52	2		1			1		−	−	−	+
0.63	1				1			−	+	−	−
1.50	24	9	12	2		1		−	−	+	−
0.97	1				1			+	−	+	−
0.65	4	1		2	1			+	−	−	−
0.62	54	10	12	13	2	12	5	−	−	−	−

[a] Total number of serum samples, 271.
[b] +, positive; −, negative.

VDRL and the TPHA tests, with the FTA-ABS test as a reference.

MATERIALS AND METHODS

A total of 271 serum samples from Madang Province, Papua New Guinea, were included in the study and stored at −20°C prior to testing. A nonendemic area yaws control group of 23 serum samples was obtained from Madang Hospital, Madang. With the exception of one child, all were residents of villages on the mainland of Madang Province. Yaws was eliminated from this area in the mass treatment campaigns of the late 1950s, and the incidence of syphilis is low in the province (personal observation by B.J.H. [formerly Specialist Physician, Madang Hospital]). All adults were examined clinically, and a personal history was taken; no evidence of yaws or syphilis or past history of genital ulceration was recorded. Likewise, there was no evidence of yaws in the six children in this group. The remaining 248 serum samples were from residents of Karkar Island, which is 64 km off the coastal town of Madang. Yaws is endemic on Karkar Island; however, syphilis has not been reported in the residents. On Karkar Island, the presence of yaws tends to be focal in distribution and therefore village orientated, because visits between villages are uncommon. A control group of 58 serum samples were supplied by Gaubin Hospital, the main hospital on Karkar Island. The serum samples were from subjects with no clinical evidence of yaws and from villages with no recent reports of the disease. The remaining 190 serum samples were from children with clinical evidence of yaws and members of their families. Dark-field microscopy was performed with samples from 19 children from this group. Fifteen of the children with clinical yaws and a positive dark-field microscopy result were monitored at 1 month and 3 months and then at 3-month intervals for 9 months posttreatment.

All sera were examined by the VDRL test and the FTA-ABS test (Behring IgG conjugate) with standard techniques (19); the Serodia-TP (TPHA) test (Fujirebio, Tokyo, Japan), a microhemagglutination assay, was performed according to the manufacturer's instructions.

The Captia Syphilis-G assay. The Captia Syphilis-G test, an indirect IgG EIA, was performed according to the manufacturer's instructions. The kit contains microtitration plates with wells coated with a sonicate of *T. pallidum*; high-positive, low-positive, and negative control sera; dilution buffer; wash buffer; tracer complex; and substrate. Sera were prepared for the assay by dilution to 1:20 in dilution buffer; 100 μl of the diluted sera was then added to the appropriate wells of the microtitration plate, and the plate was incubated at 37°C for 1 h. After incubation, the wells were aspirated and washed five times with the buffer solution. One hundred microliters of tracer complex was then added to each well, and the plate was incubated at 37°C for 1 h. The aspiration-wash cycle was repeated, 100 μl of substrate was added to each well, and then the plate was incubated at room temperature for 30 min. The reaction was stopped by the addition of 25 μl of 2 M sulfuric acid to each well, and the plate was gently agitated. The A_{450} was read with a Dynatech 7000 plate reader blanked on air.

An assay run was considered valid if the absorbance of the negative control was less than or equal to 0.25, the absorbance of the high-positive control was equal to or greater than 0.8, and the (mean) absorbance of the low-positive control was equal to or greater than 1.5 times the absorbance of the low-positive control and less than or equal to 0.5 times the absorbance of the high-positive control. Sera with absorbance values within 10% of the low-positive mean were retested, and a result holding within this range was recorded as positive. The absorbance of each specimen was expressed as an antibody index (AI [the ratio of the absorbance of the test serum to the mean absorbance of the low-positive controls]) as follows: negative, equal to or less than 0.9; equivocal, between 0.9 and 1.1; and positive, equal to or greater than 1.1.

Statistical analysis. Comparisons of proportions were performed by chi-square analysis or Fisher's exact test; *P* values were determined with two-tailed tests. Sensitivity and specificity were calculated by standard methods.

RESULTS

A total of 271 serum samples were examined by four serological methods as shown in Table 1. The sensitivity and specificity of the VDRL and TPHA tests and IgG EIA were calculated with the FTA-ABS test as the standard.

The overall levels of agreement between the FTA-ABS test and the other tests were as follows: 90.0% (244 of 271) with the IgG EIA, 95.9% (260 of 271) with the TPHA test, and 66.1% (179 of 271) with the VDRL test. For FTA-ABS test-positive sera, the sensitivity of the IgG EIA was 98.9% (185 of 187), that of the TPHA test was 94.7% (177 of 187), and that of the VDRL test was 53.5% (100 of 187). The sensitivities of the IgG EIA were comparable for the three groups, and there was no difference between adults and children within the groups. Two serum samples were negative in the IgG EIA and positive in the FTA-ABS test; one was from an adult male in the nonendemic yaws control group, who presented with arthritis; the other was from an 11-year-old female classified with possible clinical yaws. Both serum samples gave minimally reactive immunofluorescence in the FTA-ABS test. Another eight serum samples were positive in both the FTA-ABS test and IgG EIA and negative in the TPHA test; of these, four had a low-positive VDRL titer.

The overall specificity of the IgG EIA compared with the 84 serum samples negative in the FTA-ABS test was 70.2% (59 of 84), compared with 98.8% (83 of 84) for the TPHA test and 94.0% (79 of 84) for the VDRL test. The specificities of the IgG EIA were 94.4% (17 of 18) for the nonendemic area control group and 86.4% (19 of 22) for the endemic area control group, falling to 52.3% (23 of 44) for the yaws contacts. The specificity was comparable for the control groups ($P = 0.5$). Each of the control groups had statistically significantly higher specificity than the yaws contact group (nonendemic area controls versus yaws contacts, $P = 0.002$; endemic area controls versus yaws contacts, $P = 0.007$). There was no significant difference in the level of specificity between adults and children within each of the three groups.

The average AI of the 58 serum samples negative in the FTA-ABS test, TPHA test, and IgG EIA was 0.63, and that of

TABLE 2. Comparison between the AI of the IgG EIA and the VDRL test titer after treatment

Patient no.	IgG EIA AI at mo:					VDRL titer at mo:				
	0	1	3	6	9	0	1	3	6	9
1	2.47	1.99	1.95	1.85	1.78	8	4	0	0	0
2	1.67	2.40	1.67	1.76	1.71	W[a]	2	0	0	0
3	4.35	4.18	4.05	3.95	3.74	64	16	8	2	W
4	1.50	2.89	2.88	I[b]	3.37	8	8	W	I	4
5	3.58	2.49	2.44	I	2.37	16	8	W	I	0
6	2.74	1.47	1.00	I	1.76	4	2	0	I	0
7	3.00	2.74	2.52	2.32	2.20	32	8	1	0	0
8	2.98	3.74	3.36	2.39	I	1	4	2	W	0
9	4.37	1.86	3.94	3.61	3.75	32	16	4	2	2
10	4.19	2.75	3.66	I	3.07	32	8	1	I	0
11	2.76	2.76	I	2.23	2.07	8	4	I	0	0
12	2.54	I	2.11	1.74	1.70	16	I	0	0	0
13	3.78	I	3.05	I	2.58	16	8	1	1	0
14	3.71	3.19	2.29	3.63	3.08	4	2	0	4	4
15	2.09	I	3.74	0.68	3.79	16	I	4	W	0

[a] W, weakly reactive.
[b] I, insufficient serum.

the 177 specimens positive in the three tests was 2.50. In the negative treponemal sera, the mean AI rose from 0.54 in the nonendemic yaws area group to 0.58 in the yaws control group to 0.72 in the yaws group. In positive treponemal sera, the mean AIs in VDRL test-negative, -weakly reactive, and -positive sera were 1.92, 2.23, and 3.08, respectively. The average AI in the TPHA test-negative and FTA-ABS test- and IgG EIA-positive sera was 1.52, and that in the 25 serum samples positive in the IgG EIA and negative in the FTA-ABS and TPHA tests was 1.37.

Response to treatment. Table 2 compares the AI of the IgG EIA and the VDRL test titer results for 15 children who were monitored for 9 months posttreatment. The FTA-ABS and the TPHA tests remained positive for all patients over this period.

In nine patients, the VDRL test titer fell after treatment, and the test was negative by 3 to 9 months. This fall was mirrored by a consistent fall in AI of the IgG EIA in six patients. In two patients, the AIs rose at 6 and 9 months, respectively, with no clinical evidence of reinfection. The AI showed an erratic increase over the 9 months in patient 15, who received treatment at 1, 3, and 9 months after the initial treatment for a chronic ulceropapilloma. The drop in VDRL test titer was slower in one patient, and the patient's serum was still reactive at 9 months; again, the fall was matched by an overall decrease in AI.

Patient 9 had no clinical evidence of reinfection, which could explain the rise in AI at 9 months and the persistence of the low VDRL test titer. The progression of the AI for patient 2 was erratic, although the rise at 1 month posttreatment mirrored that of the VDRL test; there was no record of further treatment. There was insufficient serum remaining from these subjects to repeat the IgG EIA run, and therefore a technical error in the initial dilution step could not be excluded as the reason for the discrepancies in the AI.

Serological evidence of reinfection was seen in three patients, as indicated by a fourfold rise in VDRL test titer at 9, 1, and 6 months, respectively. This rise was accompanied by a clinical lesion in patients 4 and 14. Patient 8 received treatment at 2 months after the initial treatment for a persistent papilloma. In all three patients, the AI rose, mirroring the VDRL test titer, and then fell again after retreatment.

The fall in AI compared with the time taken for the VDRL test to become negative ranged between 1.74 and 0.43, taking from 6 to 9 months, and in all cases, AI continued to fall after the VDRL test had become negative. In the three patients with reinfection, indicated by a fourfold rise in VDRL test titer within a 3-month period, the rise in AI ranged between 0.53 and 1.03 and was more rapid than the fall in AI after treatment.

DISCUSSION

In this study, the overall level of agreement between the Captia Syphilis-G IgG EIA and the FTA-ABS test of 90.0% was significantly lower than those reported for another commercially available EIA, Bio-EnzaBead, of 95.7 and 96.3% (2, 18).

By definition, a screening test must have a high level of sensitivity, and the Captia Syphilis-G test meets this requirement. The test gave an overall sensitivity of 98.9%, which is comparable to the result from a selected clinical series of serum samples from patients with both treated and untreated syphilis (98.3%) (10) and the results of three studies testing consecutive samples (98.4, 100, and 100%) (6, 13, 23). Previous studies of other screening tests with yaws sera have shown the rapid plasma reagin test to have a sensitivity of 83.5% (3) and the TPHA test to have sensitivities of 94.3 and 97% (4, 14).

In 1975, Veldkamp and Vissner reported a correlation between false-positive IgG EIA and FTA-ABS test results (20). In this study, eight serum samples were positive in the Captia Syphilis-G and FTA-ABS tests and negative in the TPHA test. However, it is more probable that the seven children in this group had early yaws. Four children had clinical yaws, which was confirmed by dark-field microscopy performed on serum samples from two patients, and three children aged 5, 6, and 14 years in the yaws control group had levels of immunofluorescence from 2+ to 4+ in the FTA-ABS test; two children also had a positive VDRL test. It is also possible that the one adult in this category who was from the yaws contact group had past yaws with residual levels of treponemal antibody rather than giving false-positive results in both tests. These results suggest that the test is as sensitive as the FTA-ABS test in early yaws, in contrast with the lower sensitivity of 82% reported for the Captia Syphilis-G test in primary syphilis when both the TPHA and FTA-ABS tests were positive (10).

The low overall specificity of 70.2% of the Captia Syphilis-G test compared with the specificity of the FTA-ABS test in this study was unexpected. In comparison, the specificity of the TPHA test was 98.8%, and in an earlier study of yaws from the same region, its specificity was 82.5% (4). In screening for syphilis, the Captia Syphilis-G test has an excellent specificity of >99% (7, 13, 23). An acceptable false-positive rate of 1.5% in patients attending a genitourinary clinic and 0.8% in antenatal patients (17a) was reported, whereas in screening for yaws, the overall rate of false-positive reactions in the test was 29.8%.

It is apparent that the specificity of the Captia Syphilis-G assay must be considered with regard to the prevalence of yaws. When compared with the FTA-ABS and the TPHA tests, the specificity decreased as the endemicity of the disease increased. The Captia Syphilis-G test alone was positive in 1 of 18 nonendemic area control group patients, 2 of 22 yaws endemic area controls, and 22 of 44 of the yaws contacts. It is difficult to explain the fall in specificity from the nonendemic and endemic area control groups to that of the yaws contacts.

The progressive increase in both false-positive reactors and AI in negative sera from the control group to the contacts of the yaws patients leads to speculation that the IgG EIA may be detecting a minimal level of IgG anti-treponemal antibody.

The majority of adults from Karkar Island would have received treatment for yaws in the mass treatment campaign in late 1978 when 92 to 95% of the population was treated (21), and it is conceivable that the FTA-ABS test had faded with time and that the IgG EIA was detecting low levels of treponemal antibody.

Similarly, the children with ages ranging from 4 to 15 years (mean, 10.4 years) may have received treatment for yaws in the past or penicillin for other infections, because they were from villages in reasonable proximity to Gaubin Hospital. All children had clinical lesions in the "possible" category of early infectious yaws, and early detection of infection by the IgG EIA cannot be entirely excluded.

This rationale does not explain the difference in the number of false positives between the control groups and the yaws contact group, particularly the low incidence of false-positive IgG EIAs in the adults from the yaws endemic area control group. A serological survey in 1969 found the prevalence of yaws on Karkar Island to be 67.5% (5); therefore, if the IgG EIA was detecting residual levels of treponemal antibody, a higher incidence of positive reactions could be expected in this group.

The mean AIs in yaws sera for VDRL test-positive and -negative results were 2.98 and 1.92, respectively. This compares favorably with AIs of 3.3 and 1.77 in VDRL test-positive and -negative sera, respectively, in syphilitic patients (23).

Comparison with a predetermined mean AI of the Captia Syphilis-G test may be of value, both as an indicator of level of activity of the disease and in follow-up surveys after treatment as an indicator of a successful response to treatment or of relapse or reinfection, thus performing a diagnostic role similar to that of the nontreponemal test titer.

This approach was applied to the 15 subjects who were monitored for 9 months after treatment. Use of the mean AI for VDRL test-positive subjects to assess the initial activity of the disease gave 6 of 11 with VDRL titers of ≥ 8 and 2 of 4 with titers of <8.

The VDRL became negative after treatment in 11 subjects, whereas the AI at the same time, although lower than the pretreatment AI for each subject, remained elevated above the mean AI of the VDRL test-negative yaws serum samples in 8 subjects. In posttreatment follow-up serology, a fall in VDRL titer to negative is indicative of an adequate treatment response. Because the AI was above that of the mean VDRL test-negative yaws AI in 73% of the subjects when the VDRL test had already become negative, we conclude that the VDRL test remains the most useful monitor of success of treatment and probably of activity of yaws infection.

In a successful eradication campaign, adequate surveillance is of prime importance, initially to determine the extent of the problem and later to monitor the response to treatment in resurveys. A treponemal test makes no distinction between current or past infection. In areas where atypical lesions are present and in latent yaws, a nontreponemal test titer of ≥ 8 is a useful guide to the activity of the disease. The sensitivity of the Captia Syphilis-G test is comparable to those of both the TPHA and FTA-ABS tests for use as a screening test. The VDRL titer would still appear to be a better measure of disease activity and treatment success than the Captia Syphilis-G test.

The Captia Syphilis-G test has excellent sensitivity in yaws sera. However, its use as a screening test will be limited until it is established whether a positive result indicates past or subclinical infection or a false-positive reaction, suggesting a low level of specificity in a yaws endemic area.

ACKNOWLEDGMENTS

We thank Mercia Diagnostics, Guilford, Surry, United Kingdom, for providing the reagents for this evaluation, Baxter Diagnostics Pty. Ltd., Archerfield, Queensland, Australia, for coordinating the supply of the kits, Karen Byth for statistical analysis, and G. L. Gilbert for reviewing the paper.

REFERENCES

1. **Backhouse, J. L., M. H. Lee, S. I. Nesteroff, B. J. Hudson, and P. A. Hamilton.** 1992. Modified indirect hemagglutination test for detection of treponemal antibodies in finger-prick blood. J. Clin. Microbiol. **30:**561–563.
2. **Burdash, N. M., K. K. Hinds, J. A. Finnerty, and J. P. Manos.** 1987. Evaluation of the Syphilis Bio-EnzaBead assay for detection of treponemal antibody. J. Clin. Microbiol. **25:**808–811.
3. **Garner, M. F., and J. L. Backhouse.** 1972. The rapid plasma reagin (circle) card test in yaws. P. N. G. Med. J. **5:**171–173.
4. **Garner, M. F., J. L. Backhouse, G. Daskolopolous, and J. L. Walsh.** 1972. *Treponema pallidum* haemagglutination test for yaws. Comparison with the TPI and FTA-ABS tests. Br. J. Vener. Dis. **48:**479–482.
5. **Garner, M. F., R. W. Hornabrook, and J. L. Backhouse.** 1972. Prevalence of yaws on Karkar Island, New Guinea. Br. J. Vener. Dis. **48:**350–355.
6. **Hooper, N. E., D. C. Malloy, and S. Passen.** 1994. Evaluation of a *Treponema pallidum* enzyme immunoassay as a screening test for syphilis. Clin Diagn. Lab. Immunol. **1:**477–481.
7. **Hudson, B. J., J. L. Backhouse, and E. G. Tsharke.** 1990. Management of yaws in Papua New Guinea. P. N. G. Med. J. **33:**59–65.
8. **Larsen, S. A., C. E. Farshy, B. J. Pender, M. R. Adams, D. E. Pettit, and E. A. Hambie.** 1986. Staining intensities in the fluorescent treponemal antibody-absorption (FTA-ABS) test: association with the diagnosis of syphilis. Sex. Transm. Dis. **13:**221–227.
9. **Larsen, S. A., E. F. Hunter, and E. T. Creighton.** 1990. Syphilis, p. 927–934. *In* K. K. Holmes, P. A. Mardh, P. F. Sparling, P. J. Weisner, W. Cates, Jr., S. M. Lemon, and W. E. Stamm (ed.). Sexually transmitted diseases, 2nd ed. McGraw-Hill, New York.
10. **Lefevre, J.-C., M.-A. Bertrand, and R. Bauriaud.** 1990. Evaluation of the Captia enzyme immunoassays for detection of immunoglobulins G and M to *Treponema pallidum* in syphilis. J. Clin. Microbiol. **28:**1704–1707.
11. **Luger, A., and I. Spendlingwimmer.** 1973. Appraisal of the Treponema pallidum haemagglutination test. Br. J. Vener. Dis. **49:**181–182.
12. **Meheus, A., and G. M. Antal.** 1992. The endemic treponematoses: not yet eradicated. World Health Stat. Q. **45:**228–237.
13. **Nayar, R., and J. M. Compas.** 1993. Evaluation of the DCL Syphilis-G Enzyme immunoassay test kit for the serologic diagnosis of syphilis. Am. J. Clin. Pathol. **99:**282–285.
14. **Noordhoek, G. T., H. J. H. Engelkens, J. Judanarso, J. van der Stek, G. N. Aelbers, J. J. A. van der Sluis, J. D. A. van Embden, and E. Stolz.** 1991. Yaws in West Sumatra, Indonesia: clinical manifestations, serological findings and characterisation of new treponema isolates by DNA probes. Eur. J. Clin. Microbiol. Infect. Dis. **10:**12–19.
15. **O'Neill, P., R. W. Warner, and C. S. Nicol.** 1973. *Treponema pallidum* haemagglutination assay in the routine serodiagnosis of treponemal disease. Br. J. Vener. Dis. **49:**427–431.
16. **Perine, P. L., J. W. Nelson, J. O. Lewis, S. Liska, E. F. Hunter, S. A. Larsen, V. K. Agadzi, 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.
17. **Reed, E. L.** 1965. The rapid plasma reagin (circle) card test for syphilis as a routine screening procedure. Public Health Lab. **23:**96–103.

17a. **Ross, J., A. Moyes, H. Young, and A. McMillan.** 1991. An analysis of false positive reactions occurring with the Captia Syph G EIA. Genitourin. Med. **67:**408–410.

18. **Stevens, R. W., and M. E. Schmitt.** 1985. Evaluation of an enzyme-linked immunosorbent assay for treponemal antibody. J. Clin. Microbiol. **21:**399–402.
19. **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.
20. **Veldkamp, J., and A. M. Vissner.** 1975. Application of the enzyme-linked immunosorbent assay (ELISA) for the sero-diagnosis of syphilis. Br. J. Vener. Dis. **51:**227–231.
21. **Winyard, G. P. A.** 1980. The investigation and management of an out-break of yaws in Papua New Guinea. Paper 71, submitted to the Faculty of Community Medicine, University of London.
22. **Young, H., C. Henrichsen, and D. H. H. Robertson.** 1974. *Treponema pallidum* haemagglutination test as a screening procedure for the diagnosis of syphilis. Br. J. Vener. Dis. **50:**341–346.
23. **Young, H. A., A. Moyes, A. McMillan, and D. H. H. Robertson.** 1989. Screening for treponemal infection by a new enzyme immunoassay. Genitourin. Med. **65:**72–78.