JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1993, p. 102–106
0095-1137/93/010102-05$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 31, No. 1

Comparison of the Fluorescent Treponemal Antibody Absorption (FTA-ABS) Test with the FTA-ABS Double Staining Test for Detection of Antitreponemal Immunoglobulin M in the 19S Fraction of Human Serum

GÉRARD OZANNE,[1]* MARIE-ALIX D'HALEWYN,[1] AND SANDRA A. LARSEN[2]

Laboratoire de Santé Publique du Québec, 20045 Chemin Ste-Marie, Ste-Anne-de-Bellevue, Québec, Canada, H9X 3R5,[1] and Treponemal Pathogenesis and Immunobiology Branch, Division of Sexually Transmitted Diseases Laboratory Research, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333[2]

Received 11 June 1992/Accepted 1 October 1992

A widely used immunoglobulin M (IgM) detection assay for the diagnosis of neonatal congenital syphilis is the fluorescent treponemal antibody absorption test used with fractionated serum (FTA-ABS 19S IgM test). Reading the results of the FTA-ABS test is more cumbersome than reading those of the FTA-ABS double staining (FTA-ABS-DS) test, a confirmatory test for specific IgG. To verify that the FTA-ABS-DS test used with an anti-human IgM conjugate could detect specific IgM in fractionated serum samples (FTA-ABS-DS 19S IgM test), 164 fractionated (QUIK-SEP IgM Isolation System; ISOLAB, Inc., Akron, Ohio) serum specimens from infected neonates or adults or from IgG-seronegative subjects were tested by both techniques. The sensitivity limits of the two tests were assessed with reactive serum samples diluted to an endpoint titer. Samples nonreactive by the FTA-ABS 19S IgM test (n = 74) were either nonreactive (n = 65), minimally reactive (n = 5), or reactive (n = 4) by the FTA-ABS-DS 19S IgM test. Samples minimally reactive by the FTA-ABS 19S IgM test (n = 32) were minimally reactive (n = 1) or reactive (n = 31) by the double staining test. All samples reactive by the FTA-ABS 19S IgM test (n = 58) were also reactive by the FTA-ABS-DS 19S IgM test. There was a directly proportional linear relationship (r = 0.9794) between titers obtained by both tests. FTA-ABS-DS 19S IgM titers were constantly equal to or higher than FTA-ABS 19S IgM titers. Fluorescence intensity reading repeatability was 91.4% for the FTA-ABS-DS 19S IgM test and 81.7% for the FTA-ABS 19S IgM test (P = 0.015). Because the more easily read FTA-ABS-DS 19S IgM test is at least as sensitive as, if not more sensitive than, the FTA-ABS 19S IgM test, it is a good alternative to the latter test for the detection of specific IgM in human fractionated sera for those using fluorescence microscopes with incident light.

Congenital syphilis is an important (1) and growing (2) public health problem. Several serologic tests for the detection of human immunoglobulin M (IgM) directed against *Treponema pallidum* have been described for the immunodiagnosis of congenital syphilis in the neonate (1, 6, 8, 10), yet none of these tests has the status of a standard method. Among them, the fluorescent treponemal antibody absorption (FTA-ABS) test (5) used with a fluorochrome-labeled anti-human IgM on fractionated sera (FTA-ABS 19S IgM test) (1) is the recommended method for the detection of specific IgM. The FTA-ABS 19S IgM test has been reported to be both sensitive and specific (6). However, reading the slides is cumbersome, because locating treponemal antigens on a slide and focusing must be done using dark-field microscopy before the detection of human IgM-linked treponemes can be carried out by fluorescence microscopy.

The FTA-ABS double staining (FTA-ABS-DS) test is designed for fluorescence microscopes with incident illumination (4). Tetramethyl-rhodamine isothiocyanate (TMRITC)-labeled anti-human IgG is used to detect the presence of antitreponemal human IgG, whereas fluorescein isothiocyanate (FITC)-labeled antitreponemal antibody is used as a counterstain. All of the reading is carried out by using fluorescence microscopy with two sets of fluorescence filters, one for FITC (antigen localization and focus) and one for TMRITC (detection of human IgG-linked treponemes). By eliminating the need for dark-field microscopy, the focusing is less complicated and thus the overall reading time is shorter with the FTA-ABS-DS test than with the FTA-ABS test.

At the Laboratoire de Santé Publique du Québec, the FTA-ABS 19S IgM test is carried out routinely for the serodiagnosis of congenital syphilis. The standard FTA-ABS-DS test with an IgG conjugate is carried out routinely for the confirmation of reactive nontreponemal test results. Detection of antitreponemal IgM in fractionated serum using both tests with an anti-IgM conjugate was compared to determine whether the more easily read FTA-ABS-DS test was an acceptable alternative to the FTA-ABS test. To our knowledge, the FTA-ABS-DS test has not been previously evaluated, other than in our laboratories, for the detection of specific IgM in fractionated serum (FTA-ABS-DS 19S IgM test). Our results indicated that the FTA-ABS-DS 19S IgM test is at least as sensitive as, if not more sensitive than, the FTA-ABS 19S IgM test in detecting specific IgM in fractionated sera. Also, the FTA-ABS-DS 19S IgM test has a significantly higher fluorescence intensity reading repeatability and is easier to perform than the FTA-ABS 19S IgM test.

* Corresponding author.

TABLE 1. Study groups[a]

Group no.	Subjects		No. of serum samples	Serologic result (no. of subjects):			
	No.	Type		TRUST	MHA-TP	FTA-ABS-DS (IgG)	FTA-ABS 19S IgM
1	22	Nn/Ad	22	R	R	R	Rm (6), R (16)
2	23	Nn	23	R	R	R	NR
3	12	Nn	12	NR	NR	NR	NR
4[b]	24	Ad	82	R	R[c]	R[c]	??
5	25	Ad	25	NR	NR	NR[d]	??

[a] Ad, adult subject; Nn, neonate; NR, nonreactive; R, reactive; Rm, minimally reactive; ??, IgM antitreponemal antibody status unknown at the beginning of the study; MHA-TP, microhemagglutination assay for *T. pallidum*.

[b] Group 4 included 24 series of sequential bleedings, each series showing at least a fourfold increase or decrease in TRUST titers, suggesting recent or active infection and thus the likelihood of IgM antibodies; at least one specimen in each series showed a TRUST titer of ≥1:256.

[c] Reactive by MHA-TP and/or FTA-ABS-DS (IgG) test.

[d] Nonreactive by FTA-ABS-DS (IgG) test when carried out.

MATERIALS AND METHODS

Serum samples. A panel of 164 selected serum specimens received at the Laboratoire de Santé Publique du Québec for syphilis serology was used. Previous to this study, they had been tested using the toluidine red unheated serum test (TRUST) (11) and the microhemagglutination assay for *T. pallidum* (MHA-TP) (Seratek kit; Miles, Elkhart, Ind.) and/or the FTA-ABS-DS (IgG) test (Zeus Scientific, Inc., Branchburg, N.J.). Also, all serum samples from neonates and some samples from adults had been tested with the FTA-ABS 19S IgM test (12) before this study. The five study groups used in this study are described in Table 1.

Serum fractionation. Serum samples were fractionated by using the QUIK-SEP IgM Isolation System (ISOLAB, Inc., Akron, Ohio) following the manufacturer's instructions; fractions were kept frozen (−20°C) until they were tested.

Immunofluorescence. *T. pallidum* (Nichols strain) antigen slides and sorbent from the same manufacturer (Zeus Scientific, Inc.) were used for both the FTA-ABS and FTA-ABS-DS tests. The FTA-ABS test was carried out following the Centers for Disease Control's instructions (12) with FITC anti-human IgM antibody conjugate (Jackson Immuno Research [BIO/CAN Scientific], Mississauga, Ontario, Canada). The FTA-ABS-DS test system from Zeus was used following the manufacturer's instructions (13) with TMRITC anti-human IgM antibody conjugate (Jackson Immuno Research) and FITC antitreponemal antibody conjugate (Zeus Scientific, Inc.) (working dilution, 1:10). Both IgM conjugates were IgG anti-human IgM Fc5μ fragment-specific sera isolated from goat antiserum by immunoaffinity chromatography; neither reacted with human IgG, IgA, or the light chains common to most human Igs (product specifications; Jackson Immuno Research). An optimal anti-human IgM conjugate working dilution of 1:60 for both conjugates was determined by box titration with a panel of two serum specimens reactive in the FTA-ABS 19S IgM test (3+ and 1+) and one serum specimen nonreactive in the FTA-ABS 19S IgM test. This panel had given stable results (reactivities and titers) over a period of 3 years. Readings were carried out with a Dialux microscope (Leitz) equipped with an HBO 50 lamp, an I2/3 filter (FITC), and an M2 filter (TMRITC); 10× oculars and 63/0.85 EF objectives were used for the FTA-ABS test; 6.3× oculars and 100/1.25 oil EF objectives were used for the FTA-ABS-DS test. The intensities of both FITC (FTA-ABS 19S IgM) and TMRITC (FTA-ABS-DS 19S IgM) staining were graded by the same person for a given sample, on a scale of <1+ to 4+ or reported as negative (no fluorescence) (6).

Design. All serum samples were tested by both IgM detection techniques carried out simultaneously for a given sample. For each technique and each sample, three successive fluorescence intensity readings were carried out by three different persons. The order of the readers was permuted regularly. Each first-run intensity reading was classified as nonreactive (no fluorescence), repeat (intensity of <1+ or 1+), or reactive (intensity of ≥2+). These three results (one for each reader) were recorded as triplets, e.g., repeat-repeat-nonreactive. The mean of the fluorescence intensity readings was recorded. All samples with two or more individual readings interpreted as "repeat" were retested. Final interpretations followed Centers for Disease Control guidelines (6). Samples giving a mean reading of ≥2+ by either of the two techniques were serially diluted twofold in phosphate-buffered saline (pH 7.2) and then retested by the two techniques to obtain their titers (the last dilution giving a 1+ intensity reading).

The ease of use of each technique was evaluated by the following criteria: type of equipment required, number and simplicity of the steps, time to process one slide (10 wells), ease of reading, and potential causes of errors.

Data analysis. Data were stored in a dBase data file (dBase IV; Ashton Tate, Torrance, Calif.) and processed by using Reflex 2.0 software (Borland International, Scotts Valley, Calif.). Statistical analyses were performed by using True Epistat 4.0 (Epistat Services, Richardson, Tex.). Reading repeatability was evaluated by using tridimensional contingency tables to determine the number of concordant triplets of readings for each technique. The numbers obtained for each technique were compared by a chi-square test. The final interpreted results from both tests were compared by using two-dimensional contingency tables analyzed by a McNemar's test. Each study group was analyzed individually, and then all serum specimens were regrouped for final analysis. FTA-ABS-DS 19S IgM and FTA-ABS 19S IgM titers were compared by using regression analysis.

RESULTS AND DISCUSSION

Comparison of the ease of use. Staining of slides was easy to perform with both techniques. Staining of FTA-ABS-DS 19S IgM slides required more time (30 min) and reagents (one additional conjugate) than that of FTA-ABS 19S IgM slides. No special equipment was required for reading, other than a fluorescence microscope with incident illumination and two sets of fluorescence filters (TMRITC and FITC) for the FTA-ABS-DS test and one set of fluorescence filters (FITC) and a dark-field condenser for the FTA-ABS test. FTA-ABS-DS 19S IgM slides were easier to read than

TABLE 2. Reading repeatability for the two techniques

Results for reading no.[a]:			No. of serum samples for each type of triplet:	
1	2	3	FTA-ABS 19S IgM	FTA-ABS-DS 19S IgM
NR	NR	NR	64 134[b]	55 150[b]
REP	REP	REP	26	9
R	R	R	44	89
NR	NR	REP	0	1
NR	REP	NR	2	0
NR	REP	REP	0	2
REP	R	REP	5	1
REP	REP	R	1	3
REP	NR	REP	0	1
REP	NR	NR	0	1[c]
R	R	REP	7[c]	3[c]
R	REP	R	3	2
R	REP	REP	12[c]	0

[a] Successive readings of the same stained sample carried out by three different technicians. NR, nonreactive; REP, repeat; R, reactive.

[b] Total number of serum samples which gave three concordant readings within a given triplet.

[c] Number of serum samples which showed a decrease in fluorescence intensity from reading 1 to reading 3 for a given triplet.

FTA-ABS 19S IgM slides, since all of the steps of the reading were carried out using fluorescence microscopy instead of dark-field and fluorescence microscopy. No atypical staining was observed with either of the techniques. The average reading time for one slide, by a well-trained technician, was four times longer with the FTA-ABS 19S IgM test (8 min) than with the FTA-ABS-DS 19S IgM test (2 min). As expected (9), fluorescence fading was slower when TMRITC (double staining) was used than when FITC (recommended method) was used. When more than five slides were processed in one run, the FTA-ABS-DS 19S IgM test was more efficient to use than the FTA-ABS 19S IgM test, because the shorter reading time for FTA-ABS-DS 19S IgM slides compensated for the longer staining process. Overall, the FTA-ABS-DS 19S IgM test was easier to use than the FTA-ABS 19S IgM test.

Reading repeatability. By the FTA-ABS 19S IgM test, 134 serum samples gave concordant first-run triplet results (Table 2). The 30 remaining triplets had two of three concordant results; 19 of 30 (63%) showed decreases in fluorescence intensity from reading 1 to reading 3. By the FTA-ABS-DS 19S IgM test, 150 serum specimens gave concordant triplets (Table 2). The 14 remaining triplets had two of three concordant results; 4 of 14 (28%) showed decreases in fluorescence intensity from reading 1 to reading 3. The percentage of concordant triplets was significantly higher for the FTA-ABS-DS 19S IgM test (150 of 164; 91.4%) than for the single staining technique (134 of 164; 81.7%) ($P = 0.015$).

No serum sample gave a triplet that included both nonreactive and reactive results (Table 2), indicating that clearly contradictory results between readings of the same stained sample by different technicians is unlikely with either technique.

The number of FITC-stained specimens (63%) showing a decrease in fluorescence intensity from readings 1 to 3 being greater than that of TMRITC-stained ones (28%) may explain the difference in reading repeatability between the techniques. This difference cannot be attributed to the sequence of readers, since readers were permuted regularly. Since fluorescein fluorescence fades more rapidly (half-life, 30 s) than rhodamine fluorescence (half-life, 120 s) (9), the faster fading in the FTA-ABS 19S IgM test is probably due to the anti-IgM-linked fluorochrome used (fluorescein). Also, it took more time to read an FTA-ABS 19S IgM slide than it did to read an FTA-ABS-DS 19S IgM slide, suggesting that photodecomposition (3) might have been more extensive with the FTA-ABS 19S IgM test, which resulted in decreased fluorescence intensity.

TABLE 3. Distribution of results obtained by FTA-ABS 19S IgM and FTA-ABS-DS 19S IgM tests when sera from group 4 were tested

Result by FTA-ABS 19S IgM test	No. of serum samples with the following result by the FTA-ABS-DS 19S IgM test:		
	Nonreactive	Minimally reactive	Reactive
Nonreactive	5	5	4
Minimally reactive	0	1	25
Reactive	0	0	42

Reproducibility of test results. Forty-eight serum samples initially reactive by either the FTA-ABS 19S IgM test or the FTA-ABS-DS 19S IgM test were reactive when retested by a different technician using the same technique, giving 100% reproducibility for both techniques.

Agreement between the two techniques for specific IgM detection. All serum samples from groups 2, 3, and 5 were nonreactive by both IgM detection techniques. In group 1, 6 samples were minimally reactive and 16 samples were reactive by the FTA-ABS 19S IgM test; all of them were reactive by the FTA-ABS-DS 19S IgM test. Agreement between the two techniques for serum samples from group 4 is shown in Table 3. Overall agreement between the two techniques is shown in Table 4. Fewer specimens were nonreactive when the FTA-ABS-DS 19S IgM test was used (65 of 164) than when the FTA-ABS 19S IgM test was used (74 of 164) ($P = 0.0539$).

Two of these study groups can be used to assess the specificities of the techniques. Neonates in group 3 were seronegative by an anticardiolipin test (TRUST) and two confirmatory techniques (Table 1), indicating that they were unlikely to have congenital syphilis (1). Adults in group 5 showed no serologic evidence of recent or past syphilis (seronegative in screening and confirmatory tests) (Table 1). Even though early primary IgG-negative infection (6) cannot be ruled out for these 37 subjects, none of them were IgM positive by either of the two techniques. These results indicate that these techniques seem to be equally specific.

Because fluorescence fading might have caused the FTA-ABS 19S IgM results to be underscored when the mean of

TABLE 4. Distribution of all results obtained by FTA-ABS 19S IgM and FTA-ABS-DS 19S IgM tests

Result by FTA-ABS 19S IgM test	No. of serum samples with the following result by the FTA-ABS-DS 19S IgM test:		
	Nonreactive	Minimally reactive	Reactive
Nonreactive	65	5	4
Minimally reactive	0	1	31
Reactive	0	0	58

TABLE 5. Descriptions of FTA-ABS 19S IgM nonreactive, FTA-ABS-DS 19S IgM minimally reactive, or reactive serum samples

Subject no.	Date (mo/day/yr)[a]	Titer by TRUST	IgM result by[b]:	
			FTA-ABS	FTA-ABS-DS
1	5/18/89	256	Rm	R
	2/8/90	64	NR	→ R
2	1/12/89	256	NR	→ Rm
	1/17/89	1,024	NR	→ Rm
3	4/27/89	256	R	R
	11/15/89	8	NR	→ R
4	12/12/89	1,024	Rm	R
	10/2/90	128	NR	→ Rm
5	12/15/89	32	NR	→ R
	2/7/90	128	R	R
	7/18/90	8	NR	→ Rm
	2/22/91	8	NR	→ R
6	2/1/90	1,024	R	R
	8/8/90	16	NR	→ Rm

[a] Dates of phlebotomies.
[b] →, FTA-ABS-DS 19S IgM discordant result; Rm, minimally reactive; NR, nonreactive; R, reactive.

the three consecutive readings was considered, we evaluated the agreement only between the first readings for each technique. The agreement between final interpreted results (124 of 164; 75.6%) (Table 4) was not different from the agreement between the two techniques' first readings (127 of 164; 77.4%) (P = 0.6963), indicating that the discordant results were not due to the higher fading of FITC causing overall underscoring for some samples.

Erroneous FTA-ABS test results, due to problems with reagents or slides, have been reported (7). In our study, the same slide lots and commercial reagents, except the conjugates, were used for both techniques and the techniques were run simultaneously, so it is unlikely that the reagents might be responsible for the discordant results. Many of the FTA-ABS problems associated with proper microscope focusing with dark-field microscopy are eliminated with the FTA-ABS-DS test (4). We noted that when illumination, the dark-field condenser height, and the quantity of oil used were not optimized before the FTA-ABS 19S IgM reading, some fluorescence could be lost. Thus, the discordant results might be due to underscoring of FTA-ABS 19S IgM slides due to unnoticed, slight misadjustment of the microscope during the reading.

Descriptions of the nine serum specimens nonreactive by the FTA-ABS 19S IgM test that were either minimally reactive or reactive by the FTA-ABS-DS 19S IgM test, along with the data for the previous or next serum sample from the same subject, are given in Table 5. Infection in these subjects reactive by TRUST was confirmed by a reactive result by the MHA-TP. The significant change in TRUST titers suggested recent or active infection and thus the likelihood that antitreponemal IgM would be present. Most of these subjects had been or became reactive by the FTA-ABS 19S IgM test while they were monitored (subjects 1, 3, 4, 5, and 6). One subject (subject 6) had documented reinfection. On the basis of these results and the fact that the FTA-ABS-DS 19S IgM test seems to be specific on the basis of results from groups 3 and 5, it is unlikely that these nine FTA-ABS-DS 19S IgM results were false-reactive results.

TABLE 6. Examples of persistence of antitreponemal IgM

Subject no.	Date (mo/day/yr)[a]	TRUST titer	IgM titer:	
			FTA-ABS	FTA-ABS-DS
1	11/6/89	512	1:16	1:32
	12/8/89	128	1:8	1:32
	3/1/90	64	1:4	1:8
	6/7/90	16	1:2	1:8
2	6/5/90	512	1:256	1:256
	6/21/90	256	1:128	1:256
	8/22/90	64	1:64	1:256
	11/15/90	16	1:16	1:32

[a] Dates of phlebotomies.

Antitreponemal IgM persistence. Some adult subjects remained reactive for antitreponemal IgM for several months by the two techniques, with higher titers for the FTA-ABS-DS 19S IgM test (Table 6). Since the TRUST titers were decreasing during the same period, it suggests that the IgM detected was not due to reinfection but was residual IgM from the original infection. Thus, the detection of specific IgM may not be useful for confirming that a syphilis infection in adult subjects is recent, since specific IgM seems to persist for at least several months following infection.

Comparison of the IgM titers. When results from the comparative endpoint titration study of half of the reactive serum samples (n = 48) had been analyzed, it was evident that the FTA-ABS-DS 19S IgM test gave titers consistently equal to or higher than those of the FTA-ABS 19S IgM technique, and thus comparative titration was stopped. There was a directly proportional (slope, 0.7825) linear relationship (r = 0.9794) between titers obtained by both techniques (Fig. 1). The fact that the y intercept of the regression line is at 2.0089 combined with a clear positive slope shows that FTA-ABS-DS 19S IgM titers were higher than FTA-ABS 19S IgM titers. This was especially evident for low FTA-ABS 19S IgM titers (<1:32).

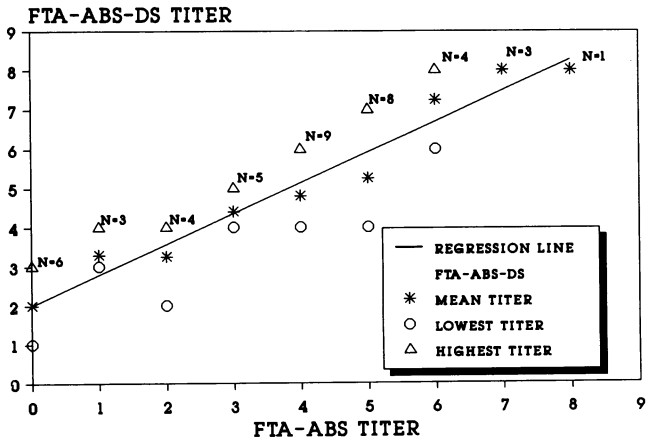

FIG. 1. Relationship between FTA-ABS 19S IgM and FTA-ABS-DS 19S IgM titers of 48 titrated serum specimens. Titers are expressed as the $\log_2$ dilution reciprocal. FTA-ABS-DS 19S IgM mean titers are geometric means. N, number of serum specimens for each FTA-ABS 19S IgM dilution.

In conclusion, the FTA-ABS-DS 19S IgM test is at least as sensitive as, if not more sensitive than, the FTA-ABS 19S IgM test. It has a higher reading repeatability and is easier to read than the FTA-ABS 19S IgM test. Therefore, the FTA-ABS-DS 19S IgM test represents a good alternative to the FTA-ABS 19S IgM test for the detection of specific IgM in fractionated sera for the serodiagnosis of congenital syphilis in the neonate for those using fluorescence microscopes with incident light.

ACKNOWLEDGMENTS

We thank Lise Leblanc, Micheline Pelletier, and Claire Sauvé from the Laboratoire de Santé Publique du Québec and Martha Fears and Charles Parham from the Centers for Disease Control for their excellent technical assistance during this work. We also thank the Diagnostics Division of Miles Canada and Zeus Scientific, Inc., for providing us with the FTA-ABS-DS kits necessary for this study. Also, many thanks to Johanne Lefebvre, André Ste-Marie, Luc Massicotte, Donald Murphy, and Guy St-Germain for their critical reviews of the manuscript.

REFERENCES

1. **Centers for Disease Control.** 1988. Guidelines for the prevention and control of congenital syphilis. Morbid. Mortal. Weekly Rep. **37**(Suppl. 1):1–13.
2. **Centers for Disease Control.** 1989. Congenital syphilis–New York City, 1986-1988. Morbid. Mortal. Weekly Rep. **38**:825–829.
3. **Cherry, W. B.** 1970. Fluorescence emission with special reference to standardization in immunofluorescence. *In* E. J. Holborow, W. D. Brighton, G. Sander, and C. E. D. Taylor (ed.), Standardization in immunofluorescence. Blackwell Scientific Publications, Oxford.
4. **Hunter, E. F.** 1990. Fluorescent treponemal antibody-absorption double staining (FTA-ABS-DS) test, p. 141–152. *In* S. A. Larsen, E. F. Hunter, and S. J. Kraus (ed.), A manual of tests for syphilis. American Public Health Association, Washington, D.C.
5. **Hunter, E. F., W. E. Deacon, and P. E. Meyer.** 1964. An improved FTA test for syphilis, the absorption procedure (FTA-ABS). Publ. Health Rep. **107**:410–412.
6. **Larsen, S. A.** 1989. Syphilis. Clin. Lab. Med. **9**:545–557.
7. **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.
8. **Lewis, L. L., L. H. Taber, and R. E. Baughn.** 1990. Evaluation of immunoglobulin M Western blot analysis in the diagnosis of congenital syphilis. J. Clin. Microbiol. **28**:296–302.
9. **Nairn, R. C., and J. R. Marrack.** 1969. Fluorescent protein tracing, p. 91. E. and S. Livingstone, Ltd., Edinburgh.
10. **Pedersen, N. S., J. Pedersen Sheller, A. V. Ratnam, and S. K. Hira.** 1989. Enzyme-linked immunosorbent assays for detection of immunoglobulin M to nontreponemal and treponemal antigens for the diagnosis of congenital syphilis. J. Clin. Microbiol. **27**:1835–1840.
11. **Pettit, D. E., S. A. Larsen, P. S. Harbec, J. C. Feeley, C. E. Parham, D. D. Cruce, E. A. Hambie, and M. W. Perryman.** 1983. Toluidine red unheated serum test, a nontreponemal test for syphilis. J. Clin. Microbiol. **18**:1141–1145.
12. **U.S. Department of Health and Human Services.** 1987. Reference reagents for the fluorescent treponemal antibody-absorption (FTA-ABS) and the FTA-ABS double staining (FTA-ABS-DS) procedures. Monograph B72.
13. **Zeus Scientific, Inc.** 1987. Monograph ZS7001-1M-587.