Centers for Disease Control

William L. Roper, M.D., M.P.H.
Director

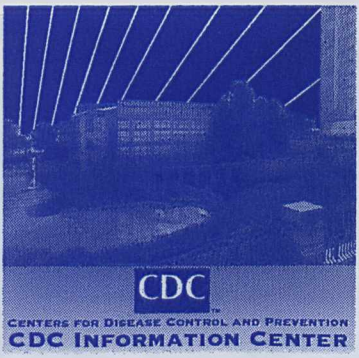
National Center for
Infectious Diseases

James M. Hughes, M.D.
Acting Director

Division of Sexually Transmitted
Diseases Laboratory Research

Stephen A. Morse, Ph.D.
Director

Treponemal Patho
Immunology Branc



Produced October 1991
Program Resources, Nati

The FTA-ABS 19S IgM Test for Congenital Syphilis: A Recommended Method

Table of Contents

Fluorescent Treponemal Antibody-Absorption 19S Immunoglobulin M (FTA-ABS 19S IgM) Test

Test Principles

The fluorescent treponemal antibody-19S immunoglobulin M (FTA-ABS 19S IgM) test is an indirect fluorescent antibody technique (1-3). The FTA-ABS 19S IgM test differs from the original FTA-ABS IgM test (4) in that the serum is fractionated by affinity chromatography to eliminate interference caused by rheumatoid factor and competition of IgM with IgG antibody (5). The IgM fraction of the patient's serum, diluted 1:5 in sorbent (an extract from cultures of *Treponema phagedenis*, Reiter treponeme), is layered on a microscope slide to which *T. pallidum* subspecies *pallidum* has been fixed. If the fractionated serum contains IgM antibody, it will coat the treponeme. Next, fluorescein isothiocyanate (FITC)-labeled, class-specific mu chain anti-human immunoglobulin is added; this combines with the patient's antibodies, which are adhering to *T. pallidum*, and results in a visible test reaction when examined by fluorescence microscopy.

Equipment

1. Incubator, 35°C to 37°C
2. Water bath, adjustable to 56°C
3. Centrifuge
4. Safety pipetting devices
5. Micropipettors delivering 10 µl to 200 µl
6. Loop, bacteriological, standard, 2 mm, 26 gauge, platinum
7. Bibulous paper
8. Slide board with moist chamber and paper towels
9. Staining dishes, glass or plastic, with removable slide carriers

10. Microscope slides, 2 x 3 inches, with frosted end, 1-mm thick, with two etched circles (1 cm inside diameter)
11. Coverslips, no. 1, 22-mm square
12. Test tubes (12 x 75 mm) and holders
13. Discard containers and disinfectants
14. Disposable latex gloves, safety glasses, and protective clothing
15. Fluorescence microscope equipment:

a.	Lamps	HBO-50, HBO-200, or Xenon XBO-150; 6V 5A Tungsten
b.	Oculars	10 X
c.	Objective	10X, 40X (Fluorite)
d.	Filters	BG-12 or KP490 K515 or K530
e.	Condenser	Darkfield D1.20-1.40

16. Mixer: Vortex Jr. or equivalent

Reagents

To Purchase

1. ***Treponema pallidum* antigen.** A suspension of *T. pallidum* (Nichols strain) extracted from rabbit testicular tissue and washed in phosphate-buffered saline (PBS) to remove rabbit globulin.
2. **Fluorescein isothiocyanate (FITC)-labeled, class-specific, mu chain anti-human globulin.** Prepared by ion-exchange chromatography of immune rabbit antiserum to the IgM fraction of pooled normal human serum, absorbed to remove cross-reacting antibody, and labeled with FITC according to recommended procedures (6). Checking the specificity of the conjugate for human mu chain is the responsibility of the laboratory performing the test. IgG fractions from the Isolab fractionation (see below) may be collected and, if sufficiently concentrated and free of IgM, may be used to evaluate the specificity of the anti-IgM conjugate.

3. **Sorbent** (7). Prepared from cultures of nonpathogenic Reiter treponemes, usually with no preservative added. Frequently dispensed in 5-ml amounts and freeze-dried; however, also sold in liquid state.
4. **Reactive control serum.** A pool of human serum samples obtained from syphilitic donors that are 4+ reactive when tested in the FTA-ABS 19S IgM test. The samples are dispensed and freeze-dried. Use to prepare the 4+ serum controls and the Minimally Reactive 1+ control. The 1+ control demonstrates the minimal degree of fluorescence reported as reactive and is used as a reading standard.
5. **Nonspecific control serum.** A serum pool obtained from nonsyphilitic individuals. No preservative is added. This control may demonstrate nonspecific reactivity at a 1:5 dilution in PBS and essentially no staining when diluted 1:5 in sorbent.
6. **Oil.** A low fluorescence, nondrying, immersion oil, type A, Cargille no. 1248.
7. **Acetone.** ACS reagent grade.
8. **Isolab IgM/IgG Systems II column, Quik Sep* IgM or equivalent separation method.** Commercially available kits contain IgM isolation columns with IgG wash buffer and IgM elution buffer.

To Prepare

1. **Phosphate-buffered saline (PBS).** Prepare in distilled water, and store large volumes in Pyrex (or equivalent) or polyethylene bottles.

   Use the following formulation:

NaCl	7.65 g
Na_2HPO_4	0.724 g
KH_2PO_4	0.21 g
Distilled H_2O	1000 ml

   Determine the pH and adjust to pH 7.2 ± 0.1 with 1N NaOH.

*The use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control, Public Health Serivce, or the U.S. Department of Health and Human Services.

Table 1. Titration of Conjugate

Conjugate dilutions	Nonspecific staining control (PBS)	Reactive (4+) control serum (1:5 in PBS)	Reactive (1+) control serum
Reference conjugate dilution 1:400	-	4+	1+
New conjugate			
1:12.5	<1+	4+	3+
1:25	-	4+	3+
1:50	-	4+	2+
1:100	-	4+	2+
1:200	-	4+	1+*
1:400	-	4+	<1+
1:800	-	3+	±

The dilution selected for the working titer is 1:200, one doubling dilution below the 4+ endpoint (1:400), 1+ staining with the 1+ control dilution (1:200), and no nonspecific staining for three doubling dilutions below the working dilution (1:25).

2. **Titration of anti-human IgM globulin** (Table 1)
   a. Rehydrate conjugate according to directions. If you observe cloudiness, centrifuge at 500 x g for 10 minutes. Divide into small portions and store at -20°C. Do not refreeze thawed conjugate but store at 2°C to 8°C.
   b. Prepare 4+ and 1+ dilutions of the Reactive control serum to determine conjugate working dilution.
   c. Run the control pattern with a reference or a known conjugate.
3. **Sorbent.** Rehydrate freeze-dried material with sterile distilled water or according to manufacturer's direction. The rehydrated sorbent may be stored at 2°C to 8°C or at -20°C and is usable as long as acceptable reactivity is obtained and the product is not contaminated.

4. **Control sera**
   a. Rehydrate according to manufacturer's directions.
   b. Divide into 0.25-ml portions, and store them at -20°C for as long as acceptable reactivity is obtained.
   c. Prepare the following controls for each test run (Table 2).
      1) Reactive (R) 4+ control serum: a syphilitic serum specimen showing 4+ fluorescence in the unabsorbed test and only slightly reduced fluorescence in the absorbed test.
         a) Unabsorbed: Transfer 50 µl of Reactive control serum into a tube containing 200 µl of PBS; mix well.
         b) Absorbed: Transfer 50 µl of Reactive serum into a tube containing 200 µl of sorbent; mix well.
      2) Minimally reactive (MR) control serum: This is a dilution in PBS of the Reactive control serum, which will give the minimal degree of fluorescence (1+) considered reactive.
      3) Nonspecific (NS) control serum: a nonsyphilitic serum specimen showing no fluorescence (-) in the absorbed test.
         a) Unabsorbed: Transfer 50 µl of Nonspecific control serum into a tube containing 200 µl of PBS; mix well.
         b) Absorbed: Transfer 50 µl of Nonspecific control serum into a tube containing 200 µl of sorbent; mix well.
      4) Controls for nonspecific staining by conjugate:
         a) Antigen smear overlaid with 30 µl of PBS in place of the serum.
         b) Antigen smear overlaid with 30 µl of sorbent in place of the serum.

Preparing Test Samples

1. Obtain infant's blood by heel-stick procedure or venous collection method. Collect into tube without anticoagulant.

Table 2. Control Pattern for the FTA-ABS 19S IgM Test*

Reactive control:	a. 1:5 PBS dilution	R4+
	b. 1:5 sorbent dilution	R(4+-3+)
Minimally Reactive:	1+ control dilution	R1+
Nonspecific serum controls:	a. 1:5 PBS dilution	R(1+)
	b. 1:5 sorbent dilution	N-±
Nonspecific staining controls:	a. Antigen, PBS, conjugate	N
	b. Antigen, sorbent, conjugate	N

* Test runs in which these control results are not obtained are considered unsatisfactory and should not be reported.

2. Use cord blood only if infant's serum is unobtainable. Use of cord blood may lead to erroneous results if specimen is improperly collected or contaminated with mother's blood.
3. The proper collection procedure for cord blood is the following:
   a. Don gloves, gown, and safety glasses.
   b. Clean the outside of the umbilicus, double-clamp it, and make a fresh cut.
   c. Collect blood in a tube with an opening large enough that blood does not run down the outside of the tube.
   d. Decontaminate the outside of the tube with household bleach or disinfectant on gauze if necessary, and place a stopper in the tube.
4. Observe recommended safety precautions for handling human serum or blood products (8).
5. Separate serum from cellular constituents by centrifugation at 1000-1200 x g for 10 ± 5 minutes. Transfer serum to test or storage tubes.
6. Store serum for fractionation at 2°C to 8°C for no longer than a week. Freeze at -20°C for longer storage.

7. Using an Isolab IgM/IgG Systems II column, Quik Sep IgM, or equivalent separation method, fractionate serum according to manufacturer's directions. Record volume of serum fractionated.
8. Heat fraction at 56°C for 30 minutes.

Performing the Test

1. Identify previously prepared antigen slides by numbering the frosted end.
2. Number each tube and slide to correspond to the test fraction and the control serum to be tested.
3. Prepare Reactive (4+), Minimally Reactive (1+), and Nonspecific control serum dilutions in sorbent or PBS, according to the directions.
4. Pipette 200 µl of sorbent into a test tube for each test fraction.
5. Add 50 µl of the heated test fraction to the appropriate tube and mix eight times.
6. Cover the appropriate antigen smears with 30 µl of the Reactive (4+), Minimally Reactive (1+), and Nonspecific control serum dilutions.
7. Cover the appropriate antigen smears with 30 µl of the PBS and 30 µl of the sorbent for Nonspecific staining controls a and b (Table 2), respectively.
8. Cover the appropriate antigen smears with 30 µl of the test fraction dilutions.
9. Prevent evaporation by placing slides in a moist chamber and incubate them at 35°C to 37°C for 30 minutes.
10. Follow this rinsing procedure:
    a. Place slides in slide carriers and rinse for 5 seconds with running PBS.
    b. Place slides in a staining dish containing PBS for 5 minutes.
    c. Agitate slides by dipping them in and out of the PBS at least 30 times.
    d. Using fresh PBS, repeat steps b and c.
    e. Rinse slides for 5 seconds in running distilled water and gently blot with bibulous paper.

Table 4. Reporting System for FTA-ABS 19S IgM Test

Initial test reading	Repeat test reading	Report
4+		Reactive
3+		Reactive
2+		Reactive
1+	>1+	Reactive
	1+	Reactive Minimal*
	<1+	Nonreactive
<1+		Nonreactive
N		Nonreactive

*In the absence of historical or clinical evidence of treponemal infection, this test result should be considered equivocal. A second specimen should be obtained 1-2 weeks after the initial specimen and submitted to the laboratory for serologic testing.

FTA-ABS 19S IgM Double-Staining (DS) Test

Microscope Equipment

FTA-ABS 19S IgM DS with incident illumination

Lamp:	HBO-50, HBO-100, or HBO-200	
Oculars:	6X, 8X, or 10X	
Objectives:	4OX/1.30 oil, 63X oil, 100X/1.25 oil	
Filters:	**FITC**	**TMRITC**
	BG38	BG38
	K480	BG36
	2KP490	KP560
	TK510	K530
	K515	TK580
		K590

Method

Follow the FTA-ABS 19S IgM procedure through step 10. Then proceed with the following steps:

11. Dilute TMRITC-labeled anti-human IgM globulin to its working titer in PBS containing 2% Tween 80, and place approximately 30 µl of the diluted conjugate on each smear.
12. Repeat steps 9 and 10.
13. Dilute FITC-labeled antitreponemal globulin to its working titer in PBS containing 2% Tween 80, and place approximately 30 µl of the diluted conjugate on each smear.
14. Place slides in a moist chamber and incubate them at 35°C to 37°C for 20 minutes.
15. Repeat step 10 of the standard procedure.
16. Mount slides immediately by placing a small drop of mounting medium on each smear and applying a cover glass.
17. Examine slides as soon as possible. If a delay in reading is necessary, place slides in a darkened room and read within 4 hours.
18. Locate and focus treponemes with the FITC filter system. After the treponemes have been located, dial in the TMRITC filters to read specific fluorescence.
19. Read test results as in step 16 of the FTA-ABS 19S IgM test procedure.

Evaluation of IgM Fractionations

To determine the purity of fractionation, test fraction on duplicate antigen slides with class-specific anti-human IgG on one set of slides and class-specific anti-human IgM conjugate on the other set.

1. Include appropriate controls as described in Performing the Test, steps 3-8, for the anti-human IgG slides and the anti-human IgM slides.
2. Dilute IgM fraction 1:2 to 1:16 in PBS.
3. Cover appropriate antigen smears with 30 µl of the undiluted fraction.
4. Cover appropriate antigen smears with 30 µl of the fraction dilutions.

5. Prevent evaporation by placing slides in a moist chamber and incubate at 35°C to 37°C for 30 minutes.
6. Rinse as described under Performing the Test, 10a-10e.
7. Dilute FITC-labeled, class-specific anti-human IgG to the appropriate dilution, and add 30 µl to each smear of one set of slides.
8. Dilute FITC-labeled, class-specific anti-human IgM to the appropriate dilution, and add 30 µl to each smear of the second set of slides.
9. Repeat steps 12-16 under Performing the Test.
10. IgM fractions should not stain with the anti-human IgG conjugate. Samples grossly contaminated (3-4+ staining) with IgG* should be concentrated and refractionated or fractionation can be repeated using a smaller volume of serum in the column.

Interpretation of Results

Report results of the FTA-ABS 19S IgM test as described in Table 4. If controls do not give results as shown in Table 2, repeat tests. Do not report test results on patient's serum when unsatisfactory control results are obtained. Until the FTA-ABS 19S IgM test obtains the Standard Status, add the following disclaimer to the report:

The FTA-ABS 19S IgM is considered an Investigational Status test; thus the results obtained should not be used as the sole criterion for the confirmed diagnosis of the presence or absence of congenital syphilis. According to the CDC Surveillance Case Definition (9,10), a presumptive case of congenital syphilis is:

"A. Any infant whose mother had untreated or inadequately treated syphilis at delivery, regardless of findings in the infant; or

B. Any infant or child who has a reactive treponemal test for syphilis and one of the following:

*More likely to occur with high-titered serum.

1. Evidence of congenital syphilis on physical examination or on long bone x-ray
2. Reactive cerebrospinal fluid (CSF) VDRL
3. Elevated CSF cell count or protein (without other cause)
4. Reactive test for FTA-ABS 19S-IgM antibody."

Test Limitations

1. The FTA-ABS 19S IgM test, as the FTA-ABS IgM test, is not proposed for use in testing adults for syphilis nor to follow treatment. It is proposed as a test to aid in the detection of syphilis in the neonate.
2. Problems arise when the FITC-labeled anti-human IgM conjugate is not a class-specific reagent, that is, when it detects IgA and IgG antibody.
3. The FTA-ABS 19S IgM test is proposed to eliminate the problem with IgM anti-IgG reactivity (rheumatoid factor) by separating or fractionating the serum. The IgM fraction used for testing should be tested for IgG to determine if fractionation was satisfactory.
4. The FTA-ABS 19S IgM test is proposed to reduce and determine the number of false-negative results (11) which have been previously reported when testing whole serum.

Acceptable Variations

1. Time for reading slides may be delayed beyond 4 hours. Slides should be protected from light and stored at 2°C to 8°C. However, for the run to be valid when reading is delayed, the ***complete control pattern must be clearly satisfactory*** when the slides are read.
2. Conjugates that have been filter-sterilized and contain a preservative, such as sodium azide, to prevent bacterial contamination may be stored at 2°C to 8°C. Any precipitate or cloudiness should be removed by centrifugation as described under Preparing Test Reagents.

3. Slides may be held 30 minutes to 1 hour in the PBS rinse step, should the test be interrupted. Control slides that do not meet the pattern invalidate the run.
4. Multicircle slides may be used rather than the two-circle slide. Add only 10-µl volumes to antigen smears. Handle and wash slides carefully to prevent serum runover.
5. With accurate micropipettors, the 1:5 test dilution may be prepared by pipetting 25 µl of serum into 100-µl volumes of diluent.

Sources of Error

1. If reagent evaluation procedures are not strictly followed, results will be unreliable.
2. If multicircle slides are used and the serum fraction from one well is allowed to run onto another well, serum from a person without syphilis may appear reactive (12).
3. If microscope slides are not clean, the test will be difficult to read, and the results may be unreliable.
4. If the microscope is not properly aligned and the control pattern is not obtained, the test results are invalid.
5. If reagents become bacterially contaminated, the antibody may be reduced and the results may be invalid.
6. If reagent storage instructions are not followed, the reagents will not produce satisfactory control results.
7. If frozen antigen slides are thawed and refrozen, the treponemes will be difficult to see, and the test results will be unsatisfactory.
8. If antigen slides are not dried and stored according to the procedure, or if too much volume is placed on the slide, the antigen may wash off. Generally, one loop of antigen is sufficient for two 1-cm circles.
9. If too many smears are fixed in a given volume of acetone, the background staining may be increased.
10. If rehydrated antigen does not adhere to the slides, too few treponemes may be observed.
11. If a precipitate is observed in conjugate preparation, nonspecific staining may be observed.

References

1. Müller, F., Oelerich, S. 1978. A modified technique of the IgM-FTA—19S test for the demonstration of competitively inhibited antibodies in syphilis. Aryth. Lab. 14:386-391.
2. Schmidt, B., Boltzmann, L. 1979. The 19S-IgM-FTA-ABS test in the serum diagnosis of syphilis. WHO/VDT/RES 79.362.
3. Müller, F., Lindenschmidt, E.G. 1981. Comparative studies of the 19S (IgM)-TPHA and the SPHA for the demonstration of specific 19S (IgM) class antibodies in untreated and treated syphilis. WHO/VDT/RES 81.368.
4. Scotti, A.T., Logan, L. 1968. A specific IgM antibody test in neonatal congenital syphilis. J. Pediat. 73:242-243.
5. Reimer, C.B., Black, C.M., Phillips, D.J., et al. 1975. The specificity of fetal IgM: antibody or anti-antibody. Ann. N.Y. Acad. Sci. 254:77-93.
6. McKinney, R., Thacker, L., Herbert, G.A. 1976. Conjugation methods in immunofluorescence. J. Dent. Res. 55:A38-44.
7. Stout, G.W., Kellogg, D.S., Jr., Falcone, V.H., McGrew, B.E., Lewis, J.S. 1967. Preparation and standardization of the sorbent used in the fluorescent treponemal antibody-absorption (FTA-ABS) test. Health Lab. Sci. 4:5-8.
8. Centers for Disease Control and National Institutes for Health. 1985. Biosafety in microbiological and biomedical laboratories. Washington, D.C., Government Printing Office.
9. Zenker, P.N., Berman, S.M. 1990. Congenital syphilis: reporting and reality. Am. J. Public Health 80:271-272.
10. Larsen, S.A., Zenker, P.N. 1990. Congenital syphilis: past, present, and future. Clin. Microbiol. Newslett. 12:23, 181-182.
11. Kaufman, R.E., Olansky, B.A., Wiesner, P.J. 1974. The FTA-ABS (IgM) test for neonatal congenital syphilis: a critical review. J. Am. Ven. Dis. Assoc. 1:79-84.
12. Hunter, E.F., Adams, M.R., Orrison, L.H., et al. 1979. Problems affecting performance of the fluorescent treponemal antibody-absorption test for syphilis. J. Clin. Microbiol. 9:163-166.