

**FLUORESCENT TREPONEMAL  
ANTIBODY-ABSORPTION (FTA-ABS)  
TEST**

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# FLUORESCENT TREPONEMAL ANTIBODY-ABSORPTION (FTA-ABS) TEST

## TEST PRINCIPLES

The fluorescent treponemal antibody-absorption (FTA-ABS) test<sup>1-3</sup> is an indirect fluorescent antibody technique used as a confirmatory test for syphilis. The patient's serum, which has been 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 patient's serum contains antibody, the antibody will coat the treponeme. Next, fluorescein isothiocyanate (FITC)-labeled antihuman immunoglobulin is added; this combines with the patient's IgG and IgM antibodies that are adhering to *T. pallidum*, and results in a visible test reaction when examined by fluorescence microscopy.

### Principles of Darkfield Transmitted Light Fluorescent Microscopy

The FTA-ABS test uses an FITC labeled anti-human immunoglobulin in a second incubation step which allows antigen to be rendered fluorescent by unlabeled, reactive antisera used in the first incubation. This test uses only one fluorescent dye and the presence of treponemes on the slide must be verified by darkfield microscopy.

If the fluorescent dye (FITC) is excited by near ultraviolet-blue light, it emits longer wavelengths of light in the yellow-green portion of the color spectrum, making visible those immune complexes formed *in situ*.

To observe a fluorescent object under the microscope there must be contrast (a dark background). Because the light source (HBO-200 or HBO-50 mercury arc) covers a spectrum greater than and including both the excitation and emission wavelengths, filters are required to screen out light other than that emitted by the dye (Figure 12:1). It is more efficient and effective to limit the energy to excitation frequencies upstream of the specimen than to try to eliminate even more non-emitted light downstream.

The elimination of lamplight immediately peripheral to the excitation band is accomplished by an excitation filter such as the broad band BG-12 filter or narrow band KP490 and includes a sharp cutoff of any wavelengths falling in the emitting range as well as wavelengths immediately below the excitation area. Excitation filters do allow passage of another band of longer wavelength light which could cause a red background to the observer's eye. Thus, nearer to the light source is a red absorbing filter (BG-38 or BG-14) which passes on a band of blue and green wavelengths to the BG-12, preabsorbing a large portion of undesired red light and heat on one end of the transmitted band and far ultraviolet on the other, narrowing the spectrum to within the capabilities of the BG-12. Even closer to the lamp there must be a protective heat absorbing filter (BG-22 or KG-1).

The darkfield condenser aids the filtering process that must later occur downstream of the specimen by focusing the light coming from the BG-12 at such an oblique angle so that none except that scattered by the specimen can enter the objective. Because of this, downstream filtration of exciting  
Figure 12:1

light need not be as extensive as would be necessary if a brightfield condenser were used. To assure this characteristic the objective should have an aperture 0.05 units less than the condenser (i.e., condenser 1.20, objective 1.15 or less) which can be achieved with a funnel stop or an iris diaphragm.

A K510 (or OG-1, K515 etc.) barrier filter is placed between the objective and the ocular, often on a sliding mount, to intercept both exciting and emitted wavelengths deflected by the specimen, removing the exciting wavelengths from the passed light. Only darkness would be seen if the antigen had not reacted with human sera and then with FITC-labeled anti-human immunoglobulin.

To confirm unstained antigen, the microscope is switched over to the 50-watt tungsten-halogen light source for normal darkfield operation.

**Note.** The accurate centering and vertical focusing of the darkfield condenser light cone on the specimen is the major means for controlling the image background and thereby, the perceived brightness of a fluorescent specimen. The individual microscope should be equipped with an instruction book on centering the lamp, which usually needs only occasional adjustment.

It is preferable to use a cardioid condenser rather than a paraboloid condenser for darkfield fluorescent microscopy. Also, the incandescent arc of the lamp must fill the entrance pupil of the condenser. For this reason the intense small arc of the HBO-100 lamp so suitable for epillumination is not suitable for transmitted darkfield fluorescence. The Xenon 150 lamp may be used (although it doesn't peak at 490 nm), but it is expensive.

The immersion oil fills the space between the top of the condenser and the bottom of the slide and should be bubble free with slide and coverslip thickness correct for brand of microscope in use.

The KP490 interference filter, sometimes used to replace the BG-12 (a dyed glass filter), passes a narrow band of light centered around the FITC excitation maximum of 490 nm. This filter is expensive and sensitive to heat and, thus, is often simply hand mounted directly over the mirror at the base of the scope.

The fluorescent excitation and emission maxima of FITC (490 nm and 517 nm, respectively) are not always targeted for use by a particular filter system because they are so close to each other. Also, the maximum intensity of lamp output may be at a wavelength less than the excitation maximum. Thus excitation may occur at 425 nm and emission as received by the eye could be 520 nm plus.

## **SPECIMEN COLLECTION AND HANDLING**

### **Specimen**

1. Avoid accidental infection from needle sticks or cuts when collecting and processing specimens by observing universal precautions (Chapter 2).
2. Serum is the most appropriate specimen for the FTA-ABS test; however, spinal fluid may be used.<sup>4,5</sup>

3. An acceptable serum specimen should not contain particulate matter that would interfere with reading test results. Specimens that are excessively hemolyzed, grossly contaminated with bacteria, chylous or otherwise extremely turbid are unsatisfactory. A specimen is too hemolyzed for testing when printed matter cannot be read through it.

**Note.** Hemolysis may be caused by transporting blood in freezing or extremely hot weather without proper insulation.

4. An acceptable spinal fluid specimen must be crystal clear. Any visible tinge of blood may lead to invalid results.<sup>4</sup>
5. Not all unsuitable specimens should be discarded or not analyzed. When an unsatisfactory sample is received in the laboratory, notify the requesting physician and discuss whether testing is appropriate for that specimen. If the ordering physician still desires a test result, then the condition of the sample must be stated on the report, and a notation made on any limitation in test result interpretation.<sup>6</sup>

## **Collection**

The procedures for the collection and processing of venous blood and spinal fluid are discussed in Chapter 3.

1. Collect whole blood or cerebrospinal fluid (CSF) into a clean, dry tube without an anticoagulant.
2. Label each specimen with patient identifier and date.

## **Handling**

### **A. Serum**

1. Allow sufficient time (approximately 20 minutes) at room temperature for the specimen to clot.
2. Centrifuge the specimen at room temperature at 1000 to 1200 x g for at least 5 minutes to sediment cellular elements (see Chapter 3).
3. Transfer serum to a clean, dry, labeled tube and heat the serum in a 56°C waterbath for 30 minutes.
4. Remove the serum from the waterbath and examine for debris. Recentrifuge all serum specimens containing particulate debris.
5. Reheat serum at 56°C for 10 minutes if testing is delayed more than 4 hours.
6. Store serum specimens at refrigerator temperature (2° - 8°C) if testing is to be delayed. If a delay of more than 5 days is anticipated before testing, freeze the specimen at ≤-20°C.

Avoid repeated freeze-thawing of specimens. Specimens must be at room temperature (23° - 29°C; 73° - 85°F) when tested.

7. If serum samples are to be shipped to a testing site, specimen containers must be leakproof and placed within a leakproof plastic bag. Paperwork should be submitted in a separate plastic bag, if included with the sample.<sup>7</sup>

#### B. Spinal Fluid

1. Centrifuge the specimen at room temperature at 1000 to 1200 x g for at least 5 minutes and transfer to a clean labeled tube.
2. Store CSF specimens at refrigerator temperature (2° - 8°C). If a delay of more than 5 days is anticipated before testing, freeze the specimen at -20°C. Avoid repeated freeze-thawing of specimens.
3. Do not heat CSF samples.
4. If CSF samples are to be shipped to a testing site, specimen containers must be leakproof and placed within a leakproof plastic bag. Paper work should be submitted in a separate plastic bag, if included with the sample.<sup>10</sup>

## MATERIALS

### Reagents

#### Purchased

1. *Treponema pallidum* antigen. The antigen is a suspension of *T. pallidum* (Nichols strain) extracted from rabbit testicular tissue and washed in phosphate buffered saline (PBS) to remove rabbit globulin (**Becton-Dickinson, Microbiology Systems, Cockeysville, MD; Difco Laboratories, Inc., Detroit, MI; INCSTAR Corp., Stillwater, MN; Pharmacia Diagnostics, Division of Electro-Nucleonics, Fairfield, NJ; Scimedx (BioDx), Denville, NJ; Zeus Scientific, Inc., Raritan, NJ**) Store unopened vials at 2° to 8°C. The unopened antigen is stable until the expiration date.
2. FITC-labeled antihuman immunoglobulin. FITC-labeled antihuman immunoglobulin is prepared by ion-exchange chromatography of immune rabbit anti-serum to the immunoglobulin G (IgG) fragment of pooled normal human serum and labeled with FITC according to recommended procedures<sup>8</sup> (**Zeus Scientific, Inc., Raritan, NJ**).
3. Sorbent.<sup>9</sup> Sorbent is prepared from cultures of nonpathogenic Reiter treponemes, usually with no preservative added. It is frequently dispensed in 5 ml amounts and freeze-dried; however, it is also sold in liquid state (**Becton-Dickinson, Microbiology Systems, Cockeysville, MD; Difco Laboratories, Inc., Detroit, MI; INCSTAR Corp., Stillwater,**

**MN; Pharmacia Diagnostics, Division of Electro-Nucleonics, Fairfield, NJ; Scimedx (BioDx), Denville, NJ; Zeus Scientific, Inc., Raritan, NJ).**

4. Reactive control serum. A pool of human serum is obtained from syphilitic donors that are 4+ reactive; the pooled serum is dispensed and freeze-dried. Use to prepare the 4+ serum controls and the minimally reactive 1+ control. The 1+ control displays the least degree of fluorescence reported as reactive and is used as a reading standard (**Difco Laboratories, Inc., Detroit, MI; INCSTAR Corp., Stillwater, MN; Scimedx (BioDx), Denville, NJ; Zeus Scientific, Inc., Raritan, NJ**).
5. Nonspecific control serum. The nonspecific control serum is a serum pool obtained from individuals without syphilis. No preservative is added. This control shows a  $\geq 2+$  nonspecific reactivity at a 1:5 dilution in PBS and essentially no staining when diluted 1:5 in sorbent (**Difco Laboratories, Inc., Detroit, MI; INCSTAR Corp., Stillwater, MN; Scimedx (BioDx), Denville, NJ; Zeus Scientific, Inc., Raritan, NJ**).
6. Oil. A low fluorescence, nondrying, immersion oil, type A (**Cat.# 1248, R. P. Cargille, Inc., Cedar Grove, NJ**).
7. Acetone. ACS reagent grade.

#### Prepared

1. PBS. Prepare in distilled water and store large volumes in Pyrex (or equivalent) or polyethylene bottles.

Prepare by the following formulation:

NaCl	7.65 g
Na <sub>2</sub> HP0 <sub>4</sub>	0.724 g
KH <sub>2</sub> P0 <sub>4</sub>	0.21 g
Distilled H <sub>2</sub> O	1000 ml

Determine the pH and adjust to pH 7.2  $\forall$ 0.1 with 1N NaOH.

2. 2.0% Tween 80 (polysorbate 80) in PBS. Heat the reagents in a 56°C water bath. To 49 ml of sterile PBS, add 1 ml of Tween 80 by measuring from the bottom of a pipette and rinsing the pipette in the mixture. Adjust to pH 7.2 with 1N NaOH. Discard if a precipitate develops or the pH changes.
3. Mounting medium. Add one part PBS, pH 7.2, to nine parts glycerine (reagent grade).
4. Distilled water. Sterilize appropriate volumes for 15 minutes at 15 pounds pressure and 121°C.

#### Equipment

1. Incubator, 35° - 37°C
2. Waterbath, adjustable to 56°C
3. Centrifuge
4. Safety pipetting devices
5. Micropipettors delivering 10 µl to 200 µl
6. Loop, bacteriological, standard, 2 mm diameter, 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, 1 x 3 inches, with a frosted end, 1-mm thick, with 2 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 XB0-150;  
6X 5A Tungsten
  - b. Oculars 10X
  - 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

### **CALIBRATION**

#### **Pipettors and Tips**

With the pipettors currently available, the measurement of small serum volumes is routine. Most manufacturers include in the specifications of their pipettors the accuracy for frequently used



microliter volumes. Daily use may affect pipettors, making them lose their initial accuracy. The differences in disposable tips from sources other than the manufacturer of the pipettor, is probably the most common error. For budgetary reasons, a less expensive brand of pipette tips may be substituted for those of the manufacturer. Although the less expensive brand may be satisfactory, the laboratory should verify the accuracy and precision of the substitute pipet tips in their test system. Commercial kits to check pipettor accuracy are available. Also, manufacturers provide procedures for checking the accuracy of their equipment. Historically, the gravimetric or spectrophotometric procedures, which use the weight of water or the absorbance of a substance at a given wavelength, have been the most accepted methods used to calibrate pipettors. These procedures should not be used instead of those specified by the manufacturer's.

## **Microscope**

Microscope should be adjusted and in satisfactory working condition before testing the serum sample for antibodies to *T. pallidum*. An FITC quality control slide (**Immuno Concepts, Sacramento, CA**) may be used to standardize fluorescent staining intensity before viewing test specimens.

## **QUALITY CONTROL**

It is the responsibility of the laboratorian to ensure that reagents are of good quality and standard reactivity.

Testing of antigen, sorbent, and conjugate for the FTA-ABS test should be conducted in parallel with testing of a standard reagent to verify that a new reagent is of standard reactivity. Parallel testing should be performed on at least two days, by using different serum samples of graded reactivity for each test period.

Record the results of all check testing.

## **Reagent Preparation**

*T. pallidum* antigen smears

1. Wipe slides with clean gauze to remove dust particles.

**Note.** Clean additional slides by sonic vibration or alcohol wiping if treponemes are not clearly observed after staining initial slide.

2. Rehydrate antigen according to manufacturer's instructions. Opened vials stored at 2° - 8°C, are stable for 1 week.
3. To prepare slides, mix antigen suspension thoroughly on a Vortex Jr. or the equivalent for 10 seconds. Determine by darkfield examination that treponemes are adequately dispensed (single organisms rather than clumps of treponemes) before making slides for the FTA-ABS test.

4. Prepare very thin *T. pallidum* antigen smears within each circle by using a 2-mm wire loop. Place one loop of antigen within the two 1-cm circles. Allow to air dry at least 15 minutes.
5. Fix slides in acetone for 10 minutes and air dry. Fix no more than 60 slides with 200 ml of acetone. Store acetone fixed smears at -20°C. Do not thaw and refreeze smears.

#### Sorbent

Rehydrate freeze-dried material with sterile distilled water or according to manufacturer's direction. The rehydrated sorbent may be stored at 2° - 8°C or at -20°C and is usable as long as acceptable reactivity is obtained and the product is not contaminated.

#### Fluorescein labeled antihuman immunoglobulin (Conjugate)

1. Rehydrate conjugate according to directions. If cloudiness is observed, centrifuge at 500 x g for 10 minutes. Aliquot in small volumes and store at -20°C. Do not refreeze thawed conjugate, but store at 2° - 8°C.
2. Prepare serial doubling dilutions of the new conjugate in PBS pH 7.2, containing 2% tween 80 so that the dilutions include the titer suggested by the manufacturer.

Example: i) 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640

ii) 1:12.5, 1:25, 1:50; 1:100, 1:200, 1:400, 1:800

3. Test each conjugate dilution with the reactive 4+ control serum diluted 1:5 in PBS, and the appropriate minimally reactive 1+ control dilution following the FTA-ABS procedure described below.
4. Include a nonspecific staining control with each conjugate dilution.
5. Simultaneously, set-up a previously satisfactory conjugate at its working dilution with a reactive 4+ control serum, a minimally reactive 1+ control serum, and a nonspecific staining control with PBS to control reagents and test conditions.
6. Read slides in the following order:
  - a. Examine the three control slides to ensure that reagents and testing conditions are satisfactory.
  - b. Examine the slides with new conjugate; start with the lowest dilution of conjugate. Record readings as 1+, 2+, 3+, or 4+.
  - c. When 4+ endpoint dilution is obtained, go back two dilutions to determine whether intensity is the same with both dilutions.

- d. The endpoint of the titration is the highest dilution giving maximum 4+ fluorescence with the reactive serum control and a 1+ reading with the 1+ dilution. The working titer of the new conjugate is one doubling dilution below the endpoint and should be the endpoint of the minimally reactive control (Table 12:1).
- e. The new conjugate should not stain nonspecifically at three doubling dilutions below the working titer of the conjugate.
- f. Dispense conjugate for storage as directed by the manufacturer in not less than 0.3 ml quantities and store at  $\leq -20^{\circ}\text{C}$ . A conjugate with a working dilution of 1:1000 or higher may be diluted 1:10 with sterile PBS containing 0.5% bovine serum albumin and 0.1% sodium azide for freezing. Label 1:10 dilution to avoid confusion with later use.
- g. Verify the titer of the conjugate after 3 or more days of storage in the freezer.

**Table 12:1. Titration of Conjugate**

	<b>Nonspecific staining control (PBS)</b>	<b>Reactive (4+) control serum (1:5 in PBS)</b>	<b>Reactive (1+) control serum</b>
Reference conjugate dilution (1:400)	-	4+	1+
New conjugate dilutions			
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+	$\pm$

\* 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).

### Control Sera

1. Rehydrate according to the manufacturer=s directions.
2. Aliquot in 0.25 ml amounts, and store at  $-20^{\circ}\text{C}$  for as long as acceptable reactivity is obtained.

### Criteria of Acceptability

*T. pallidum* antigen

1. Enough organisms should remain on slides after staining that tests may be read without difficulty.
2. The antigen should not contain background material that stains to the extent that it interferes with the reading of the test.
3. No significant change in the number or the appearance of organisms should occur on antigen smears stored as described by the manufacturer.
4. The antigen should not stain nonspecifically with the antihuman conjugate.
5. The antigen should not stain with FITC-labeled antirabbit globulin.
6. Reportable test results on controls and individual sera should be comparable to those obtained with a previously satisfactory antigen.

#### FTA-ABS test sorbent

1. The new sorbent should remove nonspecific reactivity of the Nonspecific control serum.
2. The new sorbent should not reduce the intensity of fluorescence of the reactive (4+) control serum to less than 3+.
3. The nonspecific staining control serum sample should be nonreactive with the new sorbent.
4. Test results on controls and individual sera should be comparable to those obtained with a previously satisfactory reference sorbent.
5. The sorbent should be usable when rehydrated to the indicated volume on the label or according to accompanying directions.
6. Sorbent should not contain treponemes or microbial contaminants.
7. Reagent should be clear.

#### Fluorescein-labeled antihuman immunoglobulin conjugate

1. A satisfactory conjugate should not stain a reference antigen nonspecifically at three doubling dilutions below the working titer of the conjugate.
2. Reagents should be clear when rehydrated. If not, centrifuge at 500 x g for 10 minutes before testing.
3. Test results on controls and individual sera should be comparable with those obtained with reference conjugate.

4. Most manufacturers designate the working titer of the conjugate on the label; this was determined under the testing conditions and with the equipment in their laboratories. Since conditions and equipment differ from one laboratory to another, it is necessary to titrate and test a new lot of conjugate with the available fluorescence microscope.

#### Control serum samples

1. Reactive (4+) control serum must show strong (4+) fluorescence when diluted 1:5 in PBS, and only slightly reduced fluorescence (4+ to 3+) when diluted 1:5 in sorbent.
2. The minimally reactive (1+) control, prepared according to manufacturer's directions, must be comparable in degree or intensity of fluorescence with that of the reference minimally reactive (1+) control.
3. Nonspecific control serum, diluted 1:5 in PBS, must display  $\geq 2+$  reactivity.
4. Nonspecific control serum, diluted 1:5 in sorbent, must be nonreactive.

#### FTA-ABS test kits

1. For the multiple unit product, recording the lot or control number would permit tracing of the identity of the individual units.
2. All components must function as claimed by the producer and must meet the criteria of acceptability for individual reagents.
3. Comparable results with clinical specimens must be obtained when compared with reference FTA-ABS test reagents in the standard FTA-ABS test procedures.

#### **Evaluation Procedure**

1. Reconstitute the reagents according to manufacturers' directions. Mix well to dispense treponemes evenly in the antigen preparation or to rehydrate other lyophilized reagents completely.
2. Observe gross appearance, i.e., clarity and particulate matter.
3. Control serum, sorbent, and conjugate should be centrifuged at 500 x g before use if cloudiness or particulate matter is observed on rehydration.
4. Aliquot and store reagent according to manufacturer's recommendations.

5. After rehydration and storage, each preparation should be compared with a reference reagent on at least 2 testing days. Examine with test controls and individual sera of graded reactivity according to the FTA-ABS test procedure.
  - a. Antigen: Test five reactive (1+ - 4+) and five nonreactive serum samples.
  - b. Sorbent: Same as a; however, the five nonreactive serum samples should display at least 2+ nonspecific reactivity when diluted 1:5 in PBS.
  - c. Antihuman conjugate: Same as a; however, selection of nonreactive serum with increased immunoglobulin M (IgM) levels may be helpful in choosing the conjugate working dilution.
  - d. FTA-ABS kits: Test all specimens with a, b, and c.
6. Review test results and determine whether the new reagent meets the criteria of acceptability.

### **Daily Quality Control**

1. Prepare the following controls for each test run (see Table 12:2).
  - a. Reactive 4+ control serum: a syphilitic serum showing 4+ fluorescence in the unabsorbed test and only slightly reduced fluorescence in the absorbed test.
    - 1) Unabsorbed: Transfer 50 µl of reactive control serum into a tube containing 200 µl of PBS; mix well.
    - 2) Absorbed: Transfer 50 µl of reactive control serum into a tube containing 200 µl of sorbent; mix well.
  - b. Minimally reactive control serum: This is a dilution in PBS of the reactive control serum, which will give the least degree of fluorescence (1+) considered reactive.

Table 12:2. **Control Pattern for the FTA-ABS Test\***

<b>Controls</b>	<b>Dilution</b>	<b>Reading</b>
Reactive	a. 1:5 PBS dilution b. 1:5 sorbent dilution	R4+ R(4+-3+)
Minimally Reactive:	1 + control dilution	R1+
Nonspecific serum	a. 1:5 PBS dilution b. 1:5 sorbent dilution	R(2+-4+) N-±
Nonspecific staining	a. Antigen, PBS, conjugate b. Antigen, sorbent, conjugate	N N

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

- c. Nonspecific control serum: a nonsyphilitic serum showing  $\geq 2+$  fluorescence in the unabsorbed test and essentially no fluorescence (- to  $\nabla$ ) in the absorbed test.
  - 1) Unabsorbed: Transfer 50  $\mu$ l of nonspecific control serum into a tube containing 200  $\mu$ l of PBS; mix well.
  - 2) Absorbed: Transfer 50  $\mu$ l nonspecific control serum into a tube containing 200  $\mu$ l of sorbent; mix well.
- d. Controls for nonspecific staining by conjugate:
  - 1) Antigen smear overlaid with 30  $\mu$ l of PBS in place of the serum.
  - 2) Antigen smear overlaid with 30  $\mu$ l of sorbent in place of the serum.

### **PROCEDURE**

1. Identify previously prepared antigen slides by numbering the frosted end.
2. Number each tube and slide to correspond to the test serum 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  $\mu$ l of sorbent into a test tube for each test serum.
5. Add 50  $\mu$ l of the heated test serum to the appropriate tube and mix eight times.
6. Cover the appropriate antigen smears with 30  $\mu$ l 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 12:2), respectively.
8. Cover the appropriate antigen smears with 30 µl of the test serum dilutions.
9. Prevent evaporation by placing slides in a moist chamber and incubate at 35° - 37°C for 30 minutes.
10. Rinsing procedure:
  - a. Place slides in slide carriers and rinse 5 seconds with running PBS.
  - b. Place slides in 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.
11. Dilute FITC-labeled antihuman IgG 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. Mount slides immediately by placing a small drop of mounting medium on each smear and applying a cover glass.
14. Place slides in a darkened room and read within 4 hours.
15. Check smear by darkfield microscopy using the tungsten lamp first, to verify the presence of treponemes on the smear, then read FITC (test) fluorescence.

### **READING AND REPORTING RESULTS**

1. Using the minimally reactive (1+) control slide as the reading standard, record the intensity of fluorescence of the treponemes as shown in Table 12:3.

**Table 12:3. Recording Intensity of Fluorescence**

<b>Reading</b>	<b>Intensity of fluorescence</b>
2+ to 4+	Moderate to strong
1+*	Equivalent to Minimally Reactive (1+) control
∇ to <1+	Visible staining but less than (1+)
-	None or vaguely visible but without distinct



Atypical\*\* fluorescence  
 Varied: treponemes appear to be Amoth-eaten@ or to have Aheads@ of fluorescence throughout their length.

- \* Retest all specimens that initially give a 1+ intensity of fluorescence.
- \*\* Atypical or beaded pattern of fluorescence has been described in patients with lupus or other autoimmune diseases.<sup>10</sup>

2. Report results of the FTA-ABS test as described in Table 12:4. If controls do not give results as shown in Table 12:2, repeat tests. Do not report test results on patient=s serum when unsatisfactory control results are obtained.

Table 12:4. Reporting System for FTA-ABS 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
Beaded fluorescence		Atypical fluorescence observed**
* Without 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.		
** Beading fluorescence has been observed in serum from patients with active systemic lupus erythematosus and from patients with other autoimmune diseases. (The deoxyribonucleic acid [DNA] absorption procedure <sup>10</sup> is available upon request when serum samples are submitted through the State Health Department of Laboratories to the Sexually Transmitted Diseases Branch, Division of AIDS, STDs, and Tuberculosis Laboratory Research, CDC, Atlanta, GA 30333.)		

### CALCULATIONS <sup>11</sup> AND RANGES

**Sensitivity**

$$\frac{TP}{TP + FN}$$

**Specificity**

$$\frac{TN}{TN + FP}$$

TP = True Positive, the number of individuals who test reactive that actually have syphilis  
 FN = False Negative, the number of persons who test nonreactive that have syphilis  
 TN = True Negative, the number of persons who test nonreactive that do not have syphilis  
 FP = False Positive, the number of persons who test reactive that do not have syphilis

Table 12:5. Performance of the FTA-ABS Test in Untreated Syphilis

Stage	Sensitivity	Range	Specificity	Range
Primary	84	70 - 100*		
Secondary	100			
Latent	100			
Late	96			
Nonsyphilis			97	94 - 100**

\* range of sensitivity (expressed in %) in CDC studies.  
 \*\* range of specificity (expressed in %) in CDC studies

### INTERPRETATION OF RESULTS

The FTA-ABS test is not intended for routine use or as a screening procedure. Its greatest value is to distinguish true-positive nontreponemal test results from false-positive results and to establish the diagnosis of late latent or late syphilis. A reactive FTA-ABS test result confirms the reactivity of the nontreponemal test used as the initial test for syphilis. A reactive FTA-ABS test result suggests current or past infection with pathogenic treponemes. A reactive FTA-ABS test may also support the diagnosis of late syphilis or long-standing late latent syphilis in the small proportion of such cases in which the nontreponemal test has become nonreactive but infection is suspected because of the clinical presentation or a history consistent with syphilis infection. A nonreactive FTA-ABS test result suggests that the reactive nontreponemal test result is a false-positive reaction.

### ACCEPTABLE VARIATIONS

1. If the slides will not be read within 4 hours of preparation, they should be protected from light and stored at 2° - 8°C.

**Note.** For the run to be valid when reading is delayed, the complete control pattern must be clearly satisfactory when the slides are read.

2. Conjugate that has been filter sterilized and contains a preservative, such as sodium azide, to prevent bacterial contamination may be stored at 2° - 8°C. Any precipitate or cloudiness should be removed by centrifugation as described under Preparing Test Reagents.
3. Slides may be held from 30 minutes to 1 hour in PBS should the test processing be interrupted. A run is invalid if the control slides do not meet the control pattern.

4. Multicircle slides may be used in place of the 2-circle slide. Add only 10  $\mu\text{l}$  volumes to antigen smears. Prevent serum runover when handling and washing slides.
5. With accurate micropipettors, the 1:5 test dilution may be prepared by pipetting 25  $\mu\text{l}$  of serum into 100  $\mu\text{l}$  volumes of diluent.

### **SOURCES OF ERROR**

1. If reagent evaluation procedures are not strictly followed, results will be unreliable.<sup>12</sup>
2. If multicircle slides are used and serum from one circle is allowed to run onto another circle, then serum from a person without syphilis may appear falsely reactive due to cross contamination with an adjacent reactive serum.<sup>13</sup>
3. If microscope slides are not clean, the tests will be difficult to read and the results may be unreliable.

**Note:** Slides may first need to be cleaned by sonic vibration or wiping with alcohol.

4. If the FTA-ABS test is used as a screening procedure rather than to confirm the reactive results of a nontreponemal test or as a specific diagnostic test in patients with signs or symptoms suggesting late syphilis, the positive predictive value of the FTA-ABS test is decreased.
5. If the microscope is not properly aligned and the control pattern is not obtained, the test results are invalid.
6. If reagents become contaminated with bacteria, the antibody may be reduced and the results may be invalid.
7. If reagent storage instructions are not followed, the reagents will not produce satisfactory control results.
8. If frozen antigen slides are thawed and refrozen, the treponemes will be difficult to see and the test results will be unsatisfactory.
9. If a serum is contaminated with bacteria or is excessively hemolyzed, the test results will be invalid.
10. 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.
11. If more than 60 slides are fixed in a 200 ml volume of acetone, the background staining may be increased.
12. If rehydrated antigen does not adhere to the slide, too few treponemes may occur.

13. If a precipitate is observed in conjugate preparation, nonspecific staining may be observed.
14. If the atypical staining pattern of beaded fluorescence is not recognized, these specimens may be incorrectly reported as reactive.
15. If FTA-ABS test slides are read on a microscope equipped with incident illumination, the nonreactive slides must be examined by darkfield examination for the presence of treponemes.

### **TEST LIMITATIONS**

1. Problems arise when the FTA-ABS test is used as a screening procedure, because serum from approximately 1% of the general population will give false-positive results.<sup>14-16</sup> Treponemal tests are more likely than nontreponemal tests to remain reactive in patients who have been successfully treated for syphilis.
2. All treponemal tests tend to remain reactive following treponemal infection; therefore, they should not be used to evaluate response to therapy. Because of the persistence of reactivity, probably for the life of the patient, the treponemal tests are of no value to the clinician in determining relapse or reinfection in a patient who has had a reactive FTA-ABS result.
3. Although false-positive results in the FTA-ABS test are often transient and the cause is unknown, a definite association has been demonstrated between false-positive FTA-ABS results and the diagnosis of systemic, discoid, and drug-induced varieties of lupus erythematosus.<sup>10, 17-20</sup>
4. The FTA-ABS test may be reactive in persons from areas where yaws or pinta was, or is, endemic.
5. Unexplained FTA-ABS reactive results may also occur in elderly patients.

### **PROCEDURAL NOTES FOR FTA-ABS CSF**

The acceptability of cerebrospinal fluid specimens for use in the FTA-ABS test has been evaluated in previous studies.<sup>4, 5</sup> While the VDRL-CSF test is still the only test recommended for detecting neurosyphilis, the FTA-ABS CSF test is highly sensitive and specific for the detection of treponemal antibodies. A nonreactive FTA-ABS CSF may be used to rule out neurosyphilis (Table 12:6). However the significance of a reactive test in the diagnosis of neurosyphilis is unknown, because the reactivity may be due to antibodies remaining from a previously adequately treated case of syphilis or from minute amounts of reactive serum contaminating the CSF.<sup>4, 5, 21</sup>

#### **Procedure for FTA-ABS CSF Test**

Perform the FTA-ABS CSF Test as described under the Procedure for the FTA-ABS test with the following exception: Do not heat spinal fluid before testing. Controls are identical to those for the serum test.

## Reading and Reporting of FTA-ABS CSF Results

Results are read as described for the FTA-ABS test Tables 12:3 and 12:4. In addition the following statement should accompany the FTA-ABS CSF test report: AThe significance of a reactive FTA-ABS CSF test is unknown. The CSF from persons treated in the secondary or latent stage of syphilis and without signs of neurosyphilis may be reactive. A nonreactive result in the FTA-ABS CSF test suggests the absence of neurosyphilis.@

## Calculations<sup>11</sup> and Ranges

Table 12:6. Performance of Treponemal and Nontreponemal Tests in the Diagnosis of Neurosyphilis<sup>5</sup>

Test	Sensitivity	Specificity
FTA-ABS CSF	100	94.2
VDRL -CSF	50	99.8
MHA-TP - CSF	65	99.2
TRUST- CSF	40	85.3
RPR - CSF	40	85.2

### TEST LIMITATIONS

1. The significance of a reactive test result is unknown.<sup>4,5,21</sup>
2. Spinal fluid samples that appear to be free of serum or blood, still may be contaminated and give erroneous reactive results.<sup>4</sup>

### PROCEDURAL NOTES FOR FTA-ABS 19S IgM

The procedure for the FTA-ABS 19S IgM test the diagnosis of congenital syphilis is available upon request from the Bacterial STD Branch, Division of AIDS, STD and TB Laboratory Research, National Center for Infectious Disease, Center for Disease Control and Prevention, Atlanta, GA 30333.

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