

**FLUORESCENT TREPONEMAL ANTIBODY-
ABSORPTION DOUBLE STAINING (FTA-ABS DS)
TEST**

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FLUORESCENT TREPONEMAL ANTIBODY-ABSORPTION DOUBLE STAINING (FTA-ABS DS) TEST

TEST PRINCIPLES

The fluorescent treponemal antibody-absorption double staining (FTA-ABS DS) test is an indirect fluorescent antibody technique used as a confirmatory test for syphilis. The test was developed specifically for performing the FTA-ABS test¹⁻⁶ with microscopes using incident illumination. The FTA-ABS DS requires that the microscope be equipped with filters for reading rhodamine and fluorescein excitation. 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 coats the treponeme. Class-specific tetramethylrhodamine isothiocyanate (TMRITC)-labeled antihuman immunoglobulin G (IgG) is then added; this reagent combines with the patient's antibodies which are adhering to *T. pallidum* and results in a visible test reaction (fluorescing treponemes) when examined by fluorescence microscopy with the rhodamine filter in place. Finally, a counterstain, fluorescein isothiocyanate (FITC)-labeled anti-treponemal globulin, is added to locate *T. pallidum* when the slide is examined with the FITC filter.

Principles of Incident Light Fluorescent Microscopy

Transmitted light fluorescent microscopy focuses the exciting light through the bottom of the slide onto the specimen. The opposite is true of incident light fluorescent microscopy where the exciting light is directed downward onto the specimen through the cover slip (Figure 13:1). The exciting light is directed away from the observer which is optimal and also scatters less before striking the specimen which can result in very intense emission if a fluorescent dye is present. The eyepiece and the light source, although mounted at 90 ° to each other, share a common dichroic beam splitting mirror with its surface oriented at 45 ° to the light source, objective and eyepiece. The mirror is designed to reflect certain shorter wavelengths of light (excitation light) down onto the specimen through the objective while allowing longer wavelength light emitted by any excited fluorochromes to pass directly back through the mirror into the eyepiece. Most exciting wavelengths reflected by the specimen and slide do **not** pass through to the observer but instead are bounced back into the light source, reversing their original right angle path. However, some exciting light reflected by the specimen will penetrate the mirror. A suppression filter is positioned above the mirror to block this light from the observer, insuring a black background.

A different mirror and barrier filter is required for different fluorochromes. A TK580 dichroic beam splitting mirror and K580 suppression filter are used to view the TMRITC conjugated to the detecting antibody used in the FTA-ABS-DS test. A TK510 beam splitter and a K515 barrier filter are used to view the FITC-anti-*T. pallidum* immunoglobulin, used in the FTA-ABS-DS test as a counterstain to ensure that there is antigen on the slide.

Figure 13:1.

The exciter filter placed between the lamp and the dichroic splitting mirror allows only exciting wavelengths to reach the specimen. This arrangement is more efficient for screening out of unwanted background color downstream because the spectrum to be suppressed is made much narrower before striking the slide. Two combined KP490 filters provide excellent excitation for FITC, passing a narrow band of light centered on the excitation maximum (490 nm).

Replacing the KP490 filter with a KP560 permits passage of an appropriate band for TMRITC excitation (absorption maximum 555 nm, emission maximum 582 nm). Both exciters require protection from heat (BG22) and removal of far red light (BG38) which they both allow to pass and which may result in an undesirable background. The exciting band passing through the KP560 is narrowed further by cutting off the lower end of the band with a K530 edge filter. These lower wavelengths can interfere with emitted light. The BG36 filter may also be used in this excitation assemblage for TMRITC emission, if a mercury lamp is used, to dampen a bright line at 578 nm. The basic principle remains one of complementary excitation/emission systems, one system allowing passage of light that the other does not.

Unlike the situation with transmitted darkfield fluorescence, the objectives are often selected for the largest numerical aperture possible. The requirements of a darkfield condenser are not a factor here and a large aperture combined with a small intense lamp arc (as in the HBO100) avoids loss of light energy as the beam passes through the objective to the slide. However, this light can prove to be too intense for readings comparable to the FTA-ABS transmitted light fluorescent system. The KP490 allows more light through than the BG12, more than is necessary for an FITC counterstain. This intensity is dampened by doubling up on the KP490 filter. If the light for rhodamine excitation proves to be too intense it can be controlled with an iris diaphragm inserted into the oil objective and/or an extra BG36 filter included in the excitation group.

The FTA-ABS-DS test was designed around the fluorescent incident light system and has the basic advantage that the optic system need not be refocused or realigned in any way when the operator switches from verifying presence of antigen to verification of fluorescence specific for the presence of antibody. Also, reactive, non-specific and 1+ control slide preparations can be switched out by simply rotating the objective in and out of the optical path, thus permitting identical conditions for controlled comparisons.

SPECIMEN COLLECTION AND HANDLING

Specimen

1. Avoid accidental infection when collecting and processing samples by observing universal precautions (Chapter 2).
2. Serum and spinal fluid are the most appropriate samples for the FTA-ABS DS test; however, the use of spinal fluid has received somewhat limited evaluation.^{7,8}
3. An acceptable serum specimen should not contain particulate matter that would interfere with staining and 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 will lead to invalid results.⁷
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 the specimen should be tested. If a test result is still desired by the ordering physician, then the condition of the sample must be stated on the report, and any limitations in test result interpretation noted.⁹

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

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 (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 specimens are not tested within 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 $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thawing of specimens. Specimens must be at room temperature (23 - 29°C; 73 - 85°F) at the time of testing.
7. If serum specimens 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.¹⁰

Spinal Fluid

1. Centrifuge the specimen at room temperature at 1,000 to 1,200 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 specimen will not be tested within 5 days, freeze the specimen at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thawing of specimens.
3. Do not heat CSF specimens.
4. If CSF specimens 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.¹⁰

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. **Class specific TMRITC-labeled antihuman IgG.** Antihuman IgG is prepared by ion-exchange chromatography of immune goat antiserum to the Fc fragment of pooled normal human serum IgG and labeled with TMRITC according to recommended procedures¹¹ (**Scimedx (BioDx), Denville, NJ; Zeus Scientific, Inc., Raritan, NJ**).
3. **FITC-labeled anti-treponemal globulin.** Anti-treponemal globulin is a fractionated human serum obtained from a patient with clinical evidence of secondary syphilis or a fractionated

rabbit anti-treponemal serum; the IgG fraction labeled with FITC by standard procedures¹¹ (**Becton-Dickinson, Microbiology Systems, Cockeysville, MD; Scimedx (BioDx), Denville, NJ; Zeus Scientific, Inc., Raritan, NJ**).

4. **Sorbent.**¹² 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**).
5. **Reactive control serum.** A pool of human serum obtained from syphilitic donors whose serum is 4+ reactive; the pools are 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**).
6. **Nonspecific control serum.** 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**).
7. **Oil.** A low fluorescence, nondrying, immersion oil, type A (Cat.# 1248, **R. P. Cargille, Inc., Cedar Grove, NJ**).
8. **Acetone.** ACS reagent grade.

Prepared

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

Prepare by the following formulation:

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

Adjust to pH 7.2 ± 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 pH 7.2 PBS to nine parts glycerine (reagent grade).
4. **Distilled water.** Sterilize appropriate volumes for 15 minutes, 121°C at 15 pounds pressure.

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 HB0-50, HB0-200 or Xenon XB0-150;
6X 5A Tungsten
 - b. Oculars 10X
 - c. Objective 40X/1.30 oil, 63X/1.30 oil, 100X/1.25 oil
 - d. Filters **TMRITC** **FITC**

BG38	BG38
BG36	K480
KP560	KP490 (2 filters)
K530	TK510
TK580	K515
K590	

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

Microscopes should be adjusted and in satisfactory working condition before testing the serum specimen 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 fluorescent treponemal antibody-absorption double staining (FTA-ABS DS) test should be conducted in parallel with a standard reagent to verify that the new reagent is of standard reactivity. Parallel testing should be performed on at least two days, with different serum samples of graded reactivity, for each test period.

Record the results of all check testing.

Reagent Preparation

1. *T. pallidum* antigen smears
 - a. Wipe slides with clean gauze to remove dust particles.
 - b. Rehydrate antigen according to manufacturer's instructions. Opened vials stored at 2 - 8°C, are stable for 1 week.
 - c. 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 dispersed (single organisms rather than clumps of treponemes) before making slides for the FTA-ABS test.
 - d. 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 for at least 15 minutes.
 - e. 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.

2. Sorbent
 - a. 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.

3. Rhodamine labeled antihuman IgG globulin (Conjugate)
 - a. 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; store at 2° - 8°C.
 - b. 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

- c. 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 DS procedure described below.
- d. Include a nonspecific staining control with each conjugate dilution.
- e. Simultaneously, set up a previously satisfactory conjugate at its titer 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.
- f. Read slides in the following order:
 - 1) Examine the 3 control slides to ensure that reagents and testing conditions are satisfactory.
 - 2) Examine the slides with new conjugate; start with the lowest dilution of conjugate. Record readings as 1+, 2+, 3+ or 4+.
 - 3) When the 4+ endpoint dilution is obtained, go back two dilutions to determine whether intensity is the same on both dilutions.
- g. 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 13:1).
- h. The new conjugate should not stain nonspecifically at three doubling dilutions below the working titer of the conjugate.
- I. Dispense conjugate for storage as directed by the manufacturer in not less than 0.3 ml quantities and store at -20°C or lower. 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.
- j. Verify the titer of the conjugate after 3 or more days of storage in the freezer.

Table 13:1 Titration of Rhodamine-Labeled Conjugate

	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 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+	±

* 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).

4. Titration of the FITC-labeled anti-treponemal globulin
 - a. Prepare twofold dilutions of conjugate in PBS containing 2% Tween 80.
 - b. Place 30 µl of each dilution on *T. pallidum* antigen smears.
 - c. Incubate slides in moist chamber at 35° - 37°C for 20 minutes.
 - d. Rinse and mount slides as described in the FTA-ABS DS test procedures.
 - e. Determine the 4+ endpoint dilution and use this as the working dilution for the counterstain.

5. Control sera
 - a. Rehydrate according to the manufacturer's directions.
 - b. Aliquot in 0.25 ml amounts, and store at -20°C for as long as acceptable reactivity is obtained.

Criteria of Acceptability

1. *T. pallidum* Antigen

- a. Enough organisms should remain on slides after staining that tests may be read without difficulty.
 - b. The antigen should not contain background material that stains to the extent that it interferes with the reading of the test.
 - c. No significant change in the number or the appearance of organisms should occur on antigen smears stored as described by the manufacturer.
 - d. The antigen should not stain nonspecifically with the antihuman conjugate.
 - e. The antigen should not stain with FITC-labeled antirabbit globulin. Because the antigen is extracted from infected rabbit tissue, rabbit anti-*T. pallidum* immunoglobulin may block antigenic sites if the treponemes have not been thoroughly washed during antigen preparation. In such cases the antigen would appear to stain nonspecifically with some antihuman conjugates.
 - f. The antigen should stain with FITC-labeled anti- *T. pallidum* conjugate.
 - g. Reportable test results on controls and individual sera should be comparable to those obtained with a previously satisfactory antigen.
2. FTA-ABS DS test sorbent
- a. The new sorbent should remove nonspecific reactivity of the nonspecific control serum.
 - b. The new sorbent may reduce the intensity of staining slightly, but should not reduce the intensity of fluorescence of the reactive (4+) control serum to 3+.
 - c. The nonspecific staining control serum sample should be nonreactive with the new sorbent.
 - d. Test results on controls and individual sera should be comparable to those obtained with a previously satisfactory reference sorbent.
 - e. The sorbent should be usable when rehydrated to the indicated volume on the label or according to accompanying directions.
 - f. Sorbent should not contain treponemes or microbial contaminants.
 - g. Reagent should be clear.
3. Conjugate: Rhodamine-labeled antihuman IgG:

- a. A satisfactory conjugate should not stain a reference antigen nonspecifically at three doubling dilutions below the working titer of the conjugate.
 - b. Reagents should be clear when rehydrated. If not, centrifuge at 500 x g for 10 minutes before testing.
 - c. Test results on controls and individual sera should be comparable to those obtained with reference conjugate.
 - d. Most manufacturers designate on the label the working titer of the conjugate, which 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 fluorescence microscope assembly available.
 - e. The conjugate should be certified by the manufacturer to not stain human IgA or IgM at two doubling dilutions below the working titer. Affinity purified TMRITC labeled antihuman IgG (Fc) specific antibody is recommended.
4. Conjugate: Fluorescein-labeled anti-treponemal globulin
- a. The conjugate should stain *T. pallidum* with 3+ to 4+ intensity when used at the working dilution recommended by the manufacturer.
 - b. Reagent should be clear and sparkling before use. If precipitation occurs when rehydrated, centrifuge at 500 x g for 10 minutes to clarify the reagent. If reagent does not clear with centrifugation, do not use.
5. Control Serum Samples
- a. 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.
 - b. The minimally reactive (1+) control, prepared according to manufacturer's directions, must be comparable in degree or intensity of fluorescence to that of the reference minimally reactive (1+) control.
 - c. Nonspecific control serum, diluted 1:5 in PBS, must display 2+ or greater reactivity.
 - d. Nonspecific control serum, diluted 1:5 in sorbent, must be nonreactive.
6. FTA-ABS DS test kits

- a. For the multiple unit product, the lot or control number would permit tracing the identity of the individual units.
- b. All components must function as claimed by the producer and must meet the criteria of acceptability for individual reagents.
- c. Comparable results with clinical specimens must be obtained when compared with reference FTA-ABS DS test reagents in the standard FTA-ABS DS 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 DS test procedure.
 - a. Antigen: Test five reactive (1+ - 4+) and five nonreactive serum samples.
 - b. Sorbent: Same as a; however, at least one of the five nonreactive serum samples should display $\geq 2+$ 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: Use five reactive (1+ - 4+) and five nonreactive serum samples to perform side by side test comparisons with either a kit previously found to be satisfactory or with reference reagents.
6. Review test results and determine whether the new reagent meets the criteria of acceptability.

Daily Controls

1. Prepare the following controls for each test run (see Table 13:2).
 - 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 μ l of reactive control serum into a tube containing 200 μ l of PBS; mix well.
 - 2) Absorbed: Transfer 50 μ l of reactive control serum into a tube containing 200 μ l 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.
 - c. Nonspecific control serum: a nonsyphilitic serum showing $\geq 2+$ fluorescence in the unabsorbed test and essentially no fluorescence (- to \pm) in the absorbed test.
 - 1) Unabsorbed: Transfer 50 μ l of nonspecific control serum into a tube containing 200 μ l of PBS; mix well.
 - 2) Absorbed: Transfer 50 μ l nonspecific control serum into a tube containing 200 μ l of sorbent; mix well.
 - d. Controls for nonspecific staining by conjugate:
 - 1) Antigen smear overlaid with 30 μ l of PBS in place of the serum.
 - 2) Antigen smear overlaid with 30 μ 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 μ l of sorbent into a test tube for each test serum.

Table 13:2. Control Pattern for the FTA-ABS DS Test*

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

5. Add 50 µl of the heated test serum 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 13:2), 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 TMRITC-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. Dilute FITC-labeled anti-treponemal 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 moist chamber and incubate at 35° - 37°C for 20 minutes.
15. Repeat step 10.
16. Mount slides immediately by placing a small drop of mounting medium on each smear and applying a cover glass.
17. Place slides in a darkened room and read within 4 hours.

READING AND REPORTING RESULTS

1. Place a small drop of oil on the top of the coverslip and focus the microscope with the 40X oil immersion objective using the FITC filter. Switch to the 100X oil objective, and use the fine adjustment knob to bring the field into focus. Usually, if the stage is not lowered when changing slides, only minor adjustment between smears is needed.
2. Read FITC fluorescence first to verify the presence of treponemes on the smear, and then read rhodamine (test) fluorescence.
3. Using the minimally reactive (1+) control slide as the reading standard, record the intensity of fluorescence of the treponemes in the test specimen as shown in Table 13:3 and report the results according to the system shown in Table 13:4.

Table 13:3. Recording Intensity of Fluorescence

Reading	Intensity of fluorescence
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 fluorescence
Atypical**	Varied: treponemes appear to be Amoth eaten@ or to have Abeads@ of fluorescence throughout their length.

* Retest all specimens that initially give a 1+ intensity of fluorescence.

Table 13:4. Reporting System for FTA-ABS DS 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 procedure¹³ 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 ¹⁴ AND RANGES

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}}$$

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 13:5. Performance of the FTA-ABS DS Test in Untreated Syphilis*

Stage	Sensitivity	Range	Specificity	Range
Primary	80	69 - 90		
Secondary	100			

Latent	100		
Nonsyphilis		98	97 - 100
* results of CDC studies expressed in percent.			

INTERPRETATION OF RESULTS

The FTA-ABS DS test is not intended for routine use or as a screening procedure. Its greatest value is to distinguish true-positive nontreponemal results from false-positive results and to establish the diagnosis of late latent or late syphilis. A reactive FTA-ABS DS test result confirms the reactivity of the nontreponemal test used as the initial test for syphilis. A reactive FTA-ABS DS 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. Time for reading slides may be delayed beyond 4 hours. Slides should be protected from light and stored at 2° - 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. 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 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. Be careful in handling and washing slides 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.¹⁵
2. If multicircle slides are used and serum from one well is allowed to run onto another well, then serum from a person without syphilis may appear reactive.¹⁶

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 DS 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 DS 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 be observed.
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.

TEST LIMITATIONS

1. Problems arise when this test is used as a screening procedure, because serum from approximately 1% of the general population will give false-positive reactions.¹⁷⁻²⁰ 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 MHA-TP result.
3. Although false-positive results in the FTA-ABS DS test are often transient and the cause is unknown, a definite association has been made between false-positive FTA-ABS DS results and the diagnosis of systemic, discoid, and drug-induced varieties of lupus erythematosus.^{13, 21-23}
4. The FTA-ABS DS test may be reactive in persons from areas where yaws or pinta was, or is, endemic.
5. Unexplained FTA-ABS DS reactive results may also occur in elderly patients.

PROCEDURAL NOTES FOR FTA-ABS DS CSF

The acceptability of cerebrospinal fluid specimens for use in the FTA-ABS test has been evaluated in previous studies.^{7,8} While the VDRL-CSF test is still the only recommended test for detecting neurosyphilis, the FTA-ABS DS test is highly sensitive and specific. A nonreactive FTA-ABS DS CSF test result can be used to rule out neurosyphilis (Table 13:6); however the significance of a reactive test is unknown.^{7,8, 24}

Procedure

Perform the FTA-ABS DS CSF test as described under the Procedure for the FTA-ABS DS test. Controls are identical to those for the serum test.

Reading and Reporting Results

Results are read as described for the FTA-ABS DS test Tables 13:3 and 13:4. In addition the following statement should accompany the FTA-ABS DS CSF test report: AThe significance of a reactive FTA-ABS DS CSF test is unknown. The CSF from persons treated in the secondary or latent stage of syphilis and without of signs neurosyphilis may be reactive. A nonreactive result in the FTA-ABS DS CSF tests suggests the absence of neurosyphilis.@

Calculations¹⁴ and Ranges

Table 13:6. Performance of the of Treponemal and Nontreponemal Tests in the diagnosis of Neurosyphilis⁸

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.^{7,8,24}
2. Spinal fluid samples that visually appear to be free of serum or blood, still may have undetectable amounts of blood in them which could give erroneous reactive results due to serum immunoglobulins.⁷

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