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See external label



2°C-8°C



Σ= tests



cat.#351010-DS

FTA-ABS Double Stain Test System

Catalog No. 351010-DS

PLEASE READ THIS MATERIAL BEFORE USING THE KIT

INTENDED USE

The Diagnostic Automation FTA-ABS Double Stain test system is designed to confirm positive non-treponemal reagent tests for syphilis and is for *in vitro* diagnostic use. This product is not FDA cleared (approved) for use in testing (i.e., screening) blood or plasma donors.

SIGNIFICANCE AND BACKGROUND

Serological procedures for syphilis are currently divided into two general groups of tests:

1. The non-treponemal antigen reagent screen tests, of which the Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin Card (RPR) procedures are the most frequently employed.
2. The treponemal antigen tests, of which the Fluorescent Treponemal Antibody-Absorption (FTA-ABS), and more recently, the Fluorescent Treponemal Antibody-Absorption Double Stain (FTA-ABS-DS) tests are the most commonly employed confirmatory test procedures (1-9).

Although the nontreponemal tests such as the VDRL procedure provide a relatively simple and reliable means to screen syphilis patients, they also produce a significant number of biologically false positive (BFP) reactions. These reactions are defined as patients whose sera give a positive VDRL reaction (usually weakly reactive or titer less than 1:8), a negative FTA-ABS and no history or physical findings to suggest syphilis (10-11). Consequently, a VDRL positive screen should be confirmed with a more specific test for syphilis such as the FTA-ABS procedure. Biological false positive results may, on occasion, be associated with acute and chronic infections, while up to 20% BFP may be associated with patients with lepromatous leprosy, certain drugs, pregnancy, autoimmune disease such as systemic lupus, and other diseases where hypergammaglobulinemia develops (11-15). Approximately 10% of BFP are attributed to aging alone, particularly in the eighth decade (10). Some patients with chronic BFP may also produce positive FTA-ABS results (11). Most of these reactions are usually borderline. Although the FTA-ABS procedure is more specific, the relatively low incidence of false positive FTA-ABS reactions emphasizes the need to interpret serological results in light of patient's complete history and clinical picture. The FTA-ABS procedure is the method most recommended for confirming positive reagent tests (5,6). When the FTA-ABS test was compared to other procedures, the FTA-ABS test was shown to provide greater sensitivity and clinical correlation, particularly in untreated cases of syphilis (6,11,12).

The FTA-ABS Double Stain (DS) test enables workers performing the test to use microscopes equipped with incident illumination. In the FTA-ABS-DS test, a class specific rhodamine-labeled (TMRITC) anti-human immunoglobulin G (IgG) globulin is used as a primary reagent and a fluorescein labeled (FITC) antitreponemal globulin is used as a counterstain reagent (1-4).

PRINCIPLE

The DAI Scientific Fluorescent Treponemal Antibody-Absorption Double Stain (FTA-ABS-DS) test system is a modification of the standard FTA-ABS test designed to confirm positive non-treponemal screen reagent tests for syphilis. The DAI Scientific FTA-ABS-DS test system employs nonviable *T. pallidum* (Nichols strain) cells as a substrate (antigen). These substrate cells are reacted with specially treated patient sera in the first step (see methods). If treponemal antibodies are present in the patient sera, an antigen-antibody reaction takes place between the substrate cells and the circulating antitreponemal antibodies in the patient sera.

In the second step, antihuman globulin labeled with rhodamine is added to the *T. pallidum* substrate cells. In the third step, antitreponemal FITC-labeled globulin is used as a counterstain reagent.

The substrate cells are then examined with a fluorescence incident illuminator microscope. The FITC selection of filters is used first to read the FITC reaction to determine the presence or absence of treponemes without the use of a darkfield condenser. The intensity of rhodamine staining is graded on a scale of 1+ to 4+, or as negative (no fluorescence).

KIT COMPONENTS

Reagents Provided:

- *Treponema pallidum* substrate slides (#7002): Each well contains fixed *T. pallidum* (Nichols strain) substrate (antigen) standardized to produce optimum reactivity. The slides are ready to use once removed from the freezer and equilibrated to room temperature. Slides should be allowed to reach room temperature before opening the foil package. Use thawed slides the same day. Do not refreeze thawed slides.
- Anti-treponemal globulin labeled with FITC (#7003DS): Available in kit #7001DS or as separate components (#7003D).
- Anti-human globulin labeled with rhodamine (TMRITC) (#7003DSR): Available in kit form #7001DS or as separate components (#7003D).
- Sorbent: This is a standardized product of a Reiter Treponeme culture ready-to-use as it comes from the bottle. Sorbent removes nonspecific human serum antibodies that may interfere with the FTA-ABS-DS test. Sorbent is provided in liquid form (20mL).
- FTA-ABS reactive control: Lyophilized human *T. pallidum* antibody control. Reconstitute with 1.0mL sterile distilled water. The 1+ minimal reactive control is a PBS dilution of this reactive control. (See Step #6 in the Test Procedure Section). Discard remainder of thawed aliquot after use. DO NOT REFREEZE ONCE THAWED.
- FTA-ABS nonspecific control: Lyophilized human nonspecific *T. pallidum* antibody control. Reconstitute with 1.0mL sterile distilled water. Discard remainder of thawed aliquot after use. DO NOT FREEZE ONCE THAWED.

Reagents Not Provided:

Alternative reactive reagents that may be used but are not supplied:

- *T. pallidum* antigen suspension.

Non-reactive Materials:

- Buffered glycerol: pH 7.2 ± 0.2 in a convenient dropper vial. Store at 2-8°C.
- Phosphate-buffered-saline: pH 7.2. Pour contents of each packet into 1 liter of distilled water. Use at room temperature. Store reconstituted buffer at 2-8°C. Store unopened buffer packets at room temperature.

PRECAUTION

1. All sera, antisera, and buffered glycerol contain preservative which may be toxic if ingested.
2. Each donor unit used in the preparation of the controls was found to be negative when tested by an FDA approved method for the presence of HBsAg, and for antibodies to HIV-1, HIV-2, and HCV.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Because no test method can offer complete assurance that human immunodeficiency virus, hepatitis B virus, or other infectious agents are absent, this specimen/reagent should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institute of Health manual "Biosafety in Microbiological and Biomedical Laboratories, 1984, p. 12-16, 3rd edition-1993, and OSHA Standard for Bloodborne Pathogens (22).

3. Do not apply pressure to the slide envelope. This may damage the *T. pallidum* substrate cells.
4. The components of this test system are matched for optimum sensitivity and reproducibility. Reagents from other DAI test kits, and those of other manufacturers or sources should not be interchanged without substantiating the validity of the results obtained therefrom. Follow the directional insert procedures carefully.
5. Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected.
6. Do not freeze and thaw reagents more than once. Repeated freezing and thawing destroys antibody activity. Do not store reagents in self-defrosting freezer.
7. Always run controls with each batch of tests.
8. Do not test serum that is lipemic or contains fibrin. Contaminated sera should not be employed.

PRECAUTIONS FOR POSSIBLE CROSS-CONTAMINATION

Due to the close proximity of the test areas on the DAI multiwell substrate slides, it is possible that test sera, controls, and conjugate may occasionally cross-contaminate from one well to the next. Although cross-contamination should not occur if the test procedure is carefully adhered to, the slides should be examined after each incubation period for possible cross-contamination. The dark blue DAI slides are designed to facilitate recognition of cross-contamination.

A study by CDC (16) has shown that cross-contamination from a well containing a highly reactive serum to a well containing a negative serum, could result in a false positive reaction within 30 seconds. It is therefore imperative that the technologist guard against possible cross-contamination by carefully following the instructions for rinsing the slides.

PRECAUTION IN INTERPRETATION OF RESULTS

DAI has established that following rigorous washing of the multiwell substrate slides, one may occasionally observe a single reactive substrate organism in an otherwise totally negative well. If this should occur the test should be interpreted as non-reactive. In order for a test to be considered positive, a majority of the substrate cells in any test well must be similarly stained.

ADDITIONAL MATERIALS REQUIRED

- Water bath adjusted to 56°C.
- 35°-37°C incubator.

- Serological pipette, preferably a pipetting device for delivery of 10µl.
- Timer.
- Test tubes and test tube racks.
- Volumetric flasks.
- Staining dishes.
- Moist chamber
- Cover slips, 24 x 60mm, thickness No. 1.
- Distilled water.
- Tween-80 if substrate slides other than DAI are employed.
- Properly equipped fluorescence microscope. The below filter combinations or their equivalent have proven satisfactory for routine use with mercury light source only.
- The exciter, dichroic and barrier filter combinations, together with recommended band pass ranges are shown in the table below. Consult your microscope manufacturer for the appropriate filter combinations for your particular microscope.

STORAGE CONDITIONS

- Anti-treponemal globulin labeled with FITC and anti-human globulin labeled with rhodamine (TMRITC): After reconstitution aliquot and store in freezer at -20°C or lower. Frozen aliquots are stable for 6 months. Discard remainder of thawed aliquot after use.
- FTA-ABS reactive control: After reconstitution aliquot and store in freezer at -20°C. Stable for 6 months frozen. Discard remainder of thawed aliquot after use.
- FTA-ABS nonspecific control: After reconstitution aliquot and store in freezer at -20°C. Stable for 6 months frozen. Discard remainder of thawed aliquot after use.
- *Treponema pallidum* substrate slides: Store at -20°C or colder.
- Sorbent: Store at 2-8°C.

NOTE: All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed. Do not use after expiration date.

SPECIMEN COLLECTION

Only freshly drawn and properly refrigerated sera, obtained by approved aseptic venipuncture procedures, should be used in this assay (20,21). No anticoagulants or preservatives should be added. Avoid using hemolytic, lipemic, or bacterial contaminated sera. Sera should be stored at 2-8°C for no longer than 5 days. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results.

TEST PROCEDURE

1. Heat all test sera and controls for 30 minutes in a water bath adjusted to 56°C prior to testing.
2. Reconstitution, storage, and dilutions for FITC and TMRITC reagents are as follows:
 - a. Tap bottle to free powder from neck of bottle and stopper and carefully remove stopper.
 - b. Rehydrate with 1.0mL distilled water.
 - c. If a precipitate is observed in the rehydrated material, centrifuge at approximately 1000 x g for at least 10 minutes and decant.
 - d. Place 0.3mL aliquots into vials or test tubes with stoppers adequate to prevent evaporation during storage.
 - e. Store in the frozen state at -20°C or lower.
 - f. At times that testing is to be performed, remove one frozen aliquot and thaw.
 - g. Use phosphate-buffered-saline as diluent (or PBS containing 2% Tween 80 if substrate slides other than DAI are employed).
 - h. Unused portion of the thawed aliquot should not be refrozen. Discard remainder of thawed aliquot after use.
 - i. Use reagent according to standard FTA-ABS procedures as noted above with properly equipped fluorescence microscope for rhodamine and fluorescein excitation.
 - j. The working titer obtained in this laboratory is printed on the bottle. It is to be considered a guide, not necessarily the actual titer used in your laboratory.
3. PREPARATION OF FTA-ABS-DS ANTIGEN SMEARS (OPTIONAL METHOD)
 - a. Mix antigen suspension well with a disposable pipette and rubber bulb by drawing the suspension into and

expelling it from the pipette at least 10 times to break the treponemal clumps and ensure an even distribution of treponemes. Determine by darkfield examination or by direct FA that treponemes are adequately dispersed before making slides for FTA test.

- b. Smear one loopful of *T. pallidum* antigen evenly within 3 to 4, 1cm circles by using a standard 2mm, 26-gauge, platinum wire loop. Allow to air dry at least 15 minutes.
- c. Fix smears in acetone for 10 minutes and allow to air dry thoroughly. * (Not more than 60 slides should be fixed with 200mL acetone). Store acetone fixed slides at -20°C or below. Do not thaw and refreeze antigen smears. Alternatively, slides may be fixed in 10% methanol for approximately 6 seconds. Methanol fixed slides must be used immediately after fixation.
* Proceed to steps 18-25 under FTA-ABS-DS test on serum to stain treponemes directly with fluorescein labeled anti-treponemal globulin. On stained smears, if treponemes are clumped, mix antigen as in "a" above before making additional antigen slides. If treponemes are evenly dispersed, proceed as in step "c".
4. Identify prepared antigen slides with appropriate serum numbers.
5. Number the tubes to correspond to the sera and control sera being tested, and place them in racks.
6. Prepare reactive (4+), minimally reactive (1+), and non-specific control serum dilutions in sorbent or PBS or both. Dilute the reactive and non-specific controls 1:5 in PBS and in sorbent. Add 200µl of PBS or sorbent to respective test tubes. Then add 50µl of reactive or nonspecific control serum. Prepare the minimal 1+ reactive control directly from the heated reactive control aliquot. The recommended dilution factor is noted on the reactive control bottle. Dilution is made in PBS. Example: 1+ = 1:400, or 1+ = 1 part reactive serum + 399 parts PBS, or 100µl sera + 39.9 mL PBS = 1:400 dilution. This would represent the 1+ minimally reactive control. NOTE: 1+ minimal reactive control may not be stored frozen. It should be made fresh from heated reactive control on each day.
7. Pipette 200µl sorbent into a test tube for each test serum.
8. Add 50µl of the heated test serum to the appropriate tube and mix eight times.
9. Cover the appropriate antigen smears with 10µl of the reactive (4+), minimally reactive (1+), and nonspecific control serum dilutions.
10. Cover the appropriate antigen smears with 10µl of the test serum dilution.
11. Prevent evaporation by placing slides in a moist chamber.
12. Place slides in an incubator at 35°C - 37°C for 30 minutes.
13. Rinsing Procedure:
 - a. Rinse slides briefly with PBS. This is best accomplished by slightly tilting the slide and flooding the multiwell slide with a stream of PBS directed between the top and bottom rows of the slide. Tilt slide in opposite direction and repeat rinse. The staggered positioning of the test wells minimizes possible cross-contamination (see Precautions Section). NOTE: DO NOT AIM THE STREAM OF PBS DIRECTLY ON THE WELLS.
 - b. Place slides in slide carriers and rinse slides with running PBS for approximately 5 seconds.
 - c. Place slides in staining dish and soak for 5 minutes in PBS. Agitate gently (50X).
 - d. Repeat Step c.
 - e. Rinse slides in running distilled water for approximately 5 seconds.
14. Air dry slide to remove all water.
15. Dilute rhodamine (TMRITC) labeled anti-human globulin to its working titer in PBS (or PBS containing 2% Tween 80 if substrate slides other than DAI are employed).
16. Place approximately 10µl of the diluted conjugate on each smear.
17. Repeat steps 11 through 14.
18. Dilute fluorescein-labeled (FITC) anti-treponemal globulin to its working titer in PBS (or PBS containing 2% Tween 80 if substrate slides other than DAI are employed).
19. Place approximately 10µl conjugate on each smear.
20. Repeat step 11.
21. Place slides in an incubator at 35°C - 37°C for 20 minutes.

22. Repeat Steps 13 and 14.
23. Place a small amount (4-5 drops) of mounting media between the two rows of offset wells and coverslip.
24. Examine slides as soon as possible. If a delay in reading is necessary, place slides in a dark room and read within 4 hours.
25. Locate and focus treponemes with the fluorescein (FITC) filter system.
26. After the treponemes have been located, dial in the rhodamine filter to read specific red fluorescence.
27. Using the minimally reactive (1+) control slide as the reading standard, record the intensity of fluorescence according to the chart entitled "Reading and Reporting Results" in this insert.

QUALITY CONTROL

Prepare reactive and nonspecific controls in both PBS buffer and sorbent. Prepare a 1+ minimally reactive control in PBS buffer. PBS buffer and sorbent controls should be run with each assay.

It is recommended that the control slide be read prior to evaluating test results. This will assist in establishing the references required to interpret the test sample. If controls do not appear as described below, test results may be invalid.

NOTE: The type and condition of the microscope used may influence the visual appearance of the image obtained. The 1+ reaction may vary due to the type of microscope employed, the light source, age of the bulb, filter assembly, filter thickness, as well as other parameters. As a result, it may be necessary for laboratories to prepare the 1+ minimal reactive at a dilution other than that recommended by the manufacturer. In such cases it may be advisable to employ the use of secondary standards such as those which may be obtained through the Centers for Disease Control (CDC).

1. If above controls fail to produce the expected reactions, then tests must be repeated.
2. The nonspecific control in PBS is to ensure that this control is working and should therefore demonstrate a 2+ fluorescent staining intensity. The nonspecific control in sorbent ensures that the sorbent is working optimally, and should therefore demonstrate a non-reactive appearance without distinct fluorescence.

The PBS buffer and sorbent are to be placed undiluted in separate wells. The sorbent and PBS controls should demonstrate non-reactive appearance, without distinct fluorescence.

Each of the controls in the FTA-ABS Double Stain test system will demonstrate a 1+ to 2+ fluorescent staining intensity when the FITC filters are employed to read the reactions. The FITC filters are to determine the presence or absence of treponemes.

LIMITATIONS

- The FTA-ABS-DS test is not useful in measuring the effectiveness of therapy.
- Biological false positive may occur at a low frequency.
- The FTA-ABS-DS test should be employed as a confirmatory test for syphilis (17-19), not as a screening procedure.

EXPECTED VALUES

The expected values in normal individuals is nonreactive (N) result.

PERFORMANCE CHARACTERISTICS

The Diagnostic Automation FTA-ABS-DS test system and components produce results that are considered to be equivalent to those obtained with the DAI Scientific FTA-ABS test system.

CLINICAL STUDIES

The FTA-ABS-DS method has been tested in parallel with the standard FTA-ABS procedure and the results are shown in the following 3 tables.

R = REACTIVE B = BORDERLINE N = NON-REACTIVE

There were two discrepancies in the first study. The first discrepancy involved a specimen which was negative with the FTA-ABS-DS method and borderline with the standard FTA-ABS method. The second discrepancy involved a specimen that was interpreted as borderline with the FTA-ABS-DS and as reactive with the standard FTA-ABS method.

R = REACTIVE B = BORDERLINE N = NON-REACTIVE

When testing sera from non-syphilitic in the FTA-ABS-DS method, fewer borderline reactions were observed than with the FTA-ABS method. No reactive observations were observed with the non-

syphilitic sera. When comparing the two methodologies on syphilitic cases, the results were essentially the same, except in primary cases as noted in Table 2.

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Study No. 2 (1)

Table 3. Comparison of the FTA-ABS-DS procedure and the reference FTA-ABS procedure on 265 sera from syphilitic and nonsyphilitic individuals.

Category	FTA-ABS DOUBLE STAIN			FTA-ABS REFERENCE TEST		
	R	B	N	R	B	N
SYPHILITIC						
Primary	2	1	3	6	0	0
Secondary	7	0	0	7	0	0
Latent	11	0	0	11	0	0
Late	2	0	0	2	0	0
Treated Stage	2	0	0	2	0	0
Congenital (Unknown)	1	0	0	1	0	0
POSSIBLE SYPHILIS						
Stage Unknown	11	0	0	11	0	0
NON-SYPHILITIC	0	4	221	2	10	213



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