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See external label



2°C-8°C



Σ= tests



cat.#351010

FTA-ABS Test System**Catalog No. 351010-T**

PLEASE READ THIS MATERIAL BEFORE USING THE KIT

INTENDED USE

The Diagnostic Automation FTA-ABS IFA test system is designed for the qualitative determination of antibodies to *Treponema pallidum* (*T. pallidum*), and is intended to be used as an aid in the confirmation of syphilis antibodies. 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 reagin screen test of which the Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin Card (RPR) procedures are the most frequently employed; and, 2. The treponemal antigen tests of which the Fluorescent Treponemal Antibody-absorbed (FTA-ABS) is the most commonly employed confirmatory test procedure (1-5).

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 titers less than 1:8), a negative FTA-ABS, and no history or physical findings to suggest syphilis (6,7). 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 (7-11).

Approximately 10% BFP are attributed to aging alone, particularly in the eighth decade (6). Some patients with chronic BFP may also produce positive FTA-ABS results (7). False positive FTA-ABS results have been reported in patients with hypergammaglobulinemia, lupus erythematosus (7-10), and pregnancy (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 the light of the patients complete history and clinical picture. The FTA-ABS procedure is the method most recommended for confirming positive reagin tests (1-5). 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 (2, 7-8).

PRINCIPLE OF THE IFA ASSAY

The Diagnostic Automation Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test System is a modification of the standard FTA-ABS test designed to confirm positive non-treponemal screen reagin tests for syphilis. The Diagnostic Automation FTA-ABS test system employs nonviable *T. pallidum* (Nichols strain) cells as a substrate (antigen). The reaction occurs in two steps:

1. The substrate cells are reacted with specially treated patient sera in the first step. If the treponemal antibodies are present in the patient sera, an antigen-antibody reaction takes place between the substrate cells and the circulating anti-treponemal antibodies in the patient sera.
2. In the second step, goat anti-human immunoglobulin labeled with fluorescein isothiocyanate (FITC) is added to the *T. pallidum* substrate cells. The substrate cells are then examined with a fluorescence microscope. The intensity of staining is graded on a scale of 1+ to 4+ or as negative (no fluorescence).

KIT COMPONENTS**Reactive Reagents:**

1. *Treponema pallidum* substrate slides containing fixed *T. pallidum* (Nichols strain) substrate (antigen) standardized to produce optimum reactivity. Ready to use once equilibrated to room temperature. Slides should be allowed to reach room temperature (20-25°C) before opening the foil pouch. Use slides the same day. Do not refreeze thawed slides. (Product #: 7002).
2. Goat anti-human immunoglobulin labeled with FITC. Reconstitute with 3.0mL sterile distilled water and use as directed. Store

frozen in small aliquots at -20°C or lower. Do not refreeze once thawed. Discard unused aliquots after each days testing. (Product #: 7003).

3. Sorbent: Standardized product of a Reiter treponeme culture. Ready to use. Sorbent removes nonspecific human serum antibodies that may interfere with the FTA-ABS test. (Product #: 7006).
4. FTA-ABS Reactive Control: Lyophilized human *T. pallidum* antibody control. Reconstitute with 1.0mL sterile distilled water. Store frozen in small aliquots at -20°C or lower. Use immediately after thawing. The 1+ minimal reactive control is a PBS dilution of this reactive control (see step 2 under Test Procedure section). Do not refreeze once thawed. (Product #:7004).
5. FTA-ABS Nonspecific Control: Lyophilized human nonspecific *T. pallidum* antibody control. Reconstitute with 1.0mL sterile distilled water. Store frozen in small aliquots at -20°C or lower. Do not refreeze once thawed. (Product #: 7005).

Non-reactive Reagents:

1. Phosphate-buffered-saline (PBS), pH 7.2 ± 0.2: Pour contents of each packet into 1 liter of distilled water. Use at room temperature. (Product #: 7008).
2. Buffered glycerol (mounting media), pH 7.2 ± 0.1. (Product #:7009).

NOTE: All sera, antisera, and buffered glycerol contains preservative, thimerosal, mercury derivative 1:10,000.

PRECAUTION

1. For *In Vitro* Diagnostic Use.
2. All sera, antisera, and buffered glycerol contain preservative which may be toxic if ingested.
3. 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, these specimens/reagents, as well as patient samples 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 Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 1984, p.12-16,3rd edition-1993, and OSHA Standard for Bloodborne Pathogens (18).

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

PRECAUTION FOR POSSIBLE CROSS-CONTAMINATION

Due to the close proximity of the test areas on the DAI multi-well 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 (12) 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.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Water bath adjusted to 56°C.
- 35 to 37°C incubator.
- Serological pipette. Preferably a pipetting device for delivery of 0.01mL.
- Timer.
- Test tubes and test tube racks.
- Volumetric flasks.
- Staining dish.
- Moist chamber.
- Coverslips, 24 x 60mm, thickness No. 1.
- Distilled water.

- Bibulous paper.
- Properly equipped fluorescence microscope.

The following filter combinations have proven satisfactory for routine use with both mercury or halogen light sources:

STORAGE CONDITIONS

1. Substrate slides containing *T. pallidum*: Store at -20°C or lower.
2. Goat anti-human immunoglobulin labeled with FITC: Store at 2-8°C **unreconstituted**. 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.
3. FTA-ABS reactive control: Store at 2-8°C **unreconstituted**. After reconstitution aliquot and store in freezer at -20°C. Stable for 6 months frozen. Discard remainder of thawed aliquot after use.
4. FTA-ABS nonspecific control: Store at 2-8°C **unreconstituted**. After reconstitution aliquot and store in freezer at -20°C. Stable for 6 months frozen. Discard remainder of thawed aliquot after use.
5. Sorbent: Store at 2-8°C.
6. Phosphate-buffered-saline (PBS): Store packets at 2-25°C. Rehydrated PBS is stable for 30 days when stored at 2-8°C.
7. Buffered glycerol: Store at 2-8°C.

NOTE:

- 1) All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date.
- 2) Do not freeze and thaw reagents more than once. Repeated freezing and thawing destroys antibody activity.

SPECIMEN COLLECTION

Only freshly drawn and properly refrigerated sera, obtained by approved aseptic venipuncture procedures, should be used in this assay (16,17). 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. **NOTE:** Previously heated sera should be reheated for at least 10 minutes prior to re-testing.
2. Dilute the reactive and non-specific controls 1:5 in PBS and sorbent. Add 0.2mL of PBS or sorbent to respective test tubes. Then add 0.05mL 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 0.1mL sera + 39.9 mL PBS = 1:400 dilution.

This would represent the 1+ minimally reactive control.
3. Reserve 2 wells on the control slide. One for the sorbent control, the other for the PBS (conjugate) control. A total of seven controls are required according to CDC recommendations for each days testing (see interpretation). All dilutions must be thoroughly mixed prior to testing.
4. Prepare 1:5 dilutions of all test specimens in sorbent.
 - a. To appropriately labeled tubes, add 0.2mL of sorbent.
 - b. Add 0.05mL of heat inactivated serum specimen. Mix well.
5. Add 0.01mL of diluted test and control serums to each appropriately identified substrate slide well. Include 0.01mL of sorbent and 0.01mL of PBS in their respective wells.
6. Place slides in a moist chamber and incubate at 35-37°C for 30 minutes.
7. Rinse slides briefly with PBS. This is best accomplished by slightly tilting the slide and flooding the multi-well slide with a stream of PBS directed between the top and bottom rows on the slide. Tilt slide in opposite direction and repeat rinse. The staggered positioning of the test wells minimizes possible cross contamination. (See Precaution Section).
- 8.

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Expected Control Readings

Reactive Control Serum

- | | |
|---|--------------|
| 1. 1:5 in Buffered Saline | R (4+) |
| 2. 1:5 in Sorbent | R (3+ to 4+) |
| 3. Minimally Reactive Control Serum, PBS dilution | 1+ |

Nonspecific Control Serum

- | | |
|---------------------------|--------|
| 4. 1:5 in Buffered Saline | R (2+) |
| 5. 1:5 in Sorbent | N |

Control for nonspecific staining by Conjugate

- | | | |
|--------------------|---|---|
| 6. Buffered Saline | | N |
| 7. Sorbent | N | |

NOTE:

- 1) If above controls fail to produce the expected reactions, 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.
- 3) Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

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.

INTERPRETATION OF RESULTS

LIMITATIONS

1. The FTA-ABS test is not useful in measuring the effectiveness of therapy.
2. Biological false positives may occur at a low frequency.
3. The FTA-ABS test should be employed as a confirmatory test for syphilis (13-15), not as a screening procedure.

EXPECTED VALUES

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

PERFORMANCE CHARACTERISTICS

Reproducibility:

Inter- and intra-laboratory reproducibility studies were performed over a 10 day period by two independent laboratories. Coded undiluted serum specimens were tested in parallel with the DAI Scientific FTA-ABS test system in a double blind study. The results showed 100% inter- and intra-laboratory reproducibility. These studies were conducted in accordance with the recommended CDC protocol.

Clinical Studies:

The Diagnostic Automation FTA-ABS test system was tested in parallel with the standard FTA-ABS procedure in three independent double blind studies (See below):

Based on the above study, the Diagnostic Automation FTA-ABS test system agreed with the standard FTA-ABS procedure in greater than 95% of the cases. The four discrepancies involved specimens that were reported as non-reactive by the independent laboratory, and less than 1+ reactive by the Diagnostic Automation method.

Study No. 2

Comparative studies of the Diagnostic Automation FTA-ABS test system and standard FTA-ABS procedure on fifty VDRL positive FTA-ABS low level reactive, and fifty VDRL positive FTA-ABS non-reactive serum specimens:

Based on the above studies, laboratory A showed 99% agreement between the standard and DAI Scientific's FTA-ABS procedure. This single discrepancy involved a borderline result on the standard FTA-ABS that was reported as non-reactive with the Diagnostic Automation method.

Laboratory B showed seven discrepancies or 93% agreement between the two procedures. Five of these discrepancies involved specimens that were reactive with the standard FTA-ABS and non-reactive with the Diagnostic Automation method, and two specimens that were non-reactive with the standard FTA-ABS and reactive with the Diagnostic Automation test system.

STUDY 1					
Confirmatory FTA-ABS results of 83 RPR and VDRL Reactive Sera					
		DAI Scientific FTA-ABS		Standard FTA-ABS	
Reactive		71		67	
Borderline		0		0	
Non-Reactive		12		16	



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ISO 13485-2003