FTA-ABS (Syphilis) Titrable IFA Kit

Cat # 351010-T

PRINCIPLE
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 DAI. 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).

SPECIMEN
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 2-8ºC for no longer than 5 days. If delay in testing is anticipated, store test sera at -20ºC or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results.
EQUIPMENT AND MATERIALS

Equipment:
1. Water bath adjusted to 56°C.
2. 35 to 37°C incubator.
3. Serological pipette. Preferably a pipetting device for delivery of 0.01mL.
4. Timer.
5. Test tubes and test tube racks.
7. Staining dish.
8. Moist chamber.
9. Coverslips, 24 x 60mm, thickness No. 1.
10. Distilled water
11. Properly equipped fluorescence microscope.

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

### TRANSMitted LIGHT

**Light Source: Mercury vapor 200W or 50W**

<table>
<thead>
<tr>
<th>Excitation Filter</th>
<th>Barrier Filter</th>
<th>Red Suppression Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>KP490</td>
<td>KG10 or KG30</td>
<td>KG38</td>
</tr>
<tr>
<td>BG12</td>
<td>KG10 or KG30</td>
<td>KG38</td>
</tr>
<tr>
<td>FITC</td>
<td>KG20</td>
<td>KG38</td>
</tr>
<tr>
<td><strong>Light Source: Tungsten – Halogen 100W</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KG490</td>
<td>KG10 or KG30</td>
<td>KG38</td>
</tr>
</tbody>
</table>

### INCIDENT LIGHT

**Light Source: Mercury vapor 200, 100, 50W**

<table>
<thead>
<tr>
<th>Excitation Filter</th>
<th>Dichroic Mirror</th>
<th>Barrier Filter</th>
<th>Red Suppression Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>KG500</td>
<td>TK510</td>
<td>KG10 or KG30</td>
<td>KG38</td>
</tr>
<tr>
<td>FITC</td>
<td>TK510</td>
<td>KG10 or KG30</td>
<td>KG38</td>
</tr>
<tr>
<td><strong>Light Source: Tungsten – Halogen 50 and 100W</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KG500</td>
<td>TK510</td>
<td>KG10 or KG30</td>
<td>KG38</td>
</tr>
<tr>
<td>FITC</td>
<td>TK510</td>
<td>KG10 or KG30</td>
<td>KG38</td>
</tr>
</tbody>
</table>

### Materials

**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.

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.

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.

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.

**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.

2. Buffered glycerol (mounting media), pH 7.2 ± 0.1.

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

**STORAGE REQUIREMENTS**

1. Substrate slides containing *T. pallidium*: 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.

**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.
### Expected Control Readings

<table>
<thead>
<tr>
<th>Reactive Control Serum</th>
<th>Non-specific Control Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 1:5 in Buffered Saline</td>
<td>R (4+)</td>
</tr>
<tr>
<td>2. 1:5 in Sorbent</td>
<td>R (3+ to 4+)</td>
</tr>
<tr>
<td>3. Minimally Reactive Control Serum, PBS Dilution</td>
<td>1+</td>
</tr>
<tr>
<td>4. 1:5 In Buffered Saline</td>
<td>R (2+)</td>
</tr>
<tr>
<td>5. 1:5 in Sorbent</td>
<td>N</td>
</tr>
</tbody>
</table>

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.

### PROCEDURE – STEPWISE

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 non-specific 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. Wash slides in a staining dish for 10 minutes with two changes of PBS and gently agitate slides by dipping them in and out of the PBS at least ten times.

9. Rinse slides for about 5 to 10 seconds in a gentle stream of distilled water as in step 7, and air dry. Slides must be dry before adding conjugate.

10. Place a small drop (0.01mL) of conjugate on each well and incubate in a moist chamber at 35-37°C for 30 minutes.

11. Repeat steps 7, 8, and 9.

12. Place a small amount (4-5 drops) of mounting media between the two rows of offset wells and coverslip.

13. Read slides in the dark with a properly assembled fluorescence microscope. Slides should be examined immediately. If a delay is necessary, place slides in a darkened room and read within four hours.

14. Study each well microscopically with a high dry objective. A combination BG12 excitation filter (not > 3mm thickness), plus an OG1 barrier filter, or their equivalent, have been found to be satisfactory for routine use.

15. Check non-reactive smears by using white light, dark field illumination in order to verify the presence of treponemes, or alternatively, consider the DAI FTA-ABS Double Stain test system.

16. Using the minimally reactive control well as the reading standard, record the intensity of fluorescence of the treponemes in all control and patient unknown wells according to the control pattern chart below.

**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).

### CALCULATIONS/REPORTING RESULTS

#### INTERPRETATION OF RESULTS

<table>
<thead>
<tr>
<th>Reading</th>
<th>Intensity of Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>2+ to 4+</td>
<td>Moderate to strong</td>
</tr>
<tr>
<td>1+</td>
<td>Equivalent to Minimally Reactive (1+) Control*</td>
</tr>
<tr>
<td>± to &lt; 1+</td>
<td>Visible staining, but less than 1+</td>
</tr>
<tr>
<td>-</td>
<td>None or vaguely visible, but without distinct fluorescence</td>
</tr>
</tbody>
</table>

* Retest all specimens with the intensity of fluorescence of (1+)

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**Guide for Reading FTA-ABS Test Reading and Reporting Results**

<table>
<thead>
<tr>
<th>Initial Test Reading</th>
<th>Repeat Test Reading</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+, 3+, 2+</td>
<td>Reactive (R)</td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>&gt;1+</td>
<td>Reactive (R)</td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>Reactive Minimal (RM)*</td>
</tr>
<tr>
<td></td>
<td>&lt;1+</td>
<td>Non- Reactive (NR)</td>
</tr>
<tr>
<td>&lt;1+</td>
<td></td>
<td>Non-Reactive (NR)</td>
</tr>
<tr>
<td>N or ±</td>
<td></td>
<td>Non Reactive (NR)</td>
</tr>
</tbody>
</table>

- In the absence of historical or clinical evidence of treponemal infection, this test result should be considered equivocal. A second specimen should be submitted for serologic testing.
PROCEDURE NOTES
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 vigorous 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.

LIMITATIONS OF THE PROCEDURE
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.

REFERENCES
1. Hunter EF, Deacon WE, and Meyer PE: An improved FTA test for syphilis, the absorption


FTA-ABS
(Syphilis) Titratable IFA TEST SYSTEM

Cat # 351010-T

Titration Procedure for FITC labeled Anti human Globulin for use in FTA-ABS Test

INTEDED USE
The Diagnostic Automation, Inc. FTA-ABS IFA test system is designed for the qualitative determination of antibodies of 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.

REAGENT
Anti-human globulin labeled with FITC. Reconstitute with 1.0mL sterile distilled water and use as directed below. Frozen are stable for 6 months.

If the lot number of the new lot of conjugate received is the same as the lot currently in use in your laboratory, it is not necessary to titer the conjugate. Simply run the new bottle in parallel with the old to determine that the titer or working dilution is the same.

a. Prepare test dilutions of AHG diluted in PBS. (NOTE: If substrate slides other than DAI are employed, prepare PBS containing 2% Tween-80.) Include the titer indicated on the label.

Prepare higher or lower dilutions if necessary.

b. For the purpose of controlling reagents and test conditions, prepare a sample of the reference conjugate (lot currently in use) diluted to its working dilution in PBS. (NOTE: If substrate slides other than DAI are employed, prepare PBS containing 2% Tween-80.)

c. Prepare Reactive and Minimally Reactive Control Serums in accordance with the FTA-ABS technique.

1. Prepared the Reactive (4+) control serum by diluting an aliquot of the FTA-ABS reactive control serum to 1:5 in PBS. Prepare the minimally reactive 1+ control serum by diluting an aliquot of the FTA-ABS reactive control serum in PBS to the dilution on the reactive control serum vial.

d. Test each conjugate dilution and at the same time, test the reference conjugate at its working dilution in accordance with the FTA-ABS technique on antigen smears treated with .01mL of reactive (4+) control serum, on antigen smears treated with 0.1mL of PBS (nonspecific staining control).

e. Read the slides in the following order:

2. Read the control wells of the slides to ensure that reagents and testing conditions are satisfactory.

3. Read of slides with the new conjugate starting with the lowest dilution. Record reading in pluses.

f. The endpoint of the titration is the highest dilution giving maximum (4+) fluorescence with the reactive control serum. The working dilution of the new conjugate is one doubling dilution below the endpoint. In the following example, the dilution determined for the working dilution is 1:40. This working dilution should give an acceptable (1+) reading with minimally reactive control serum.
The new conjugate should be show nonspecific staining at three doubling dilutions below its working dilution. In the example, the conjugate would meet this criterion since there is no staining with the 1:5 dilution on the nonspecific staining control. There should not be excessive filming at the working dilution.

**EXAMPLE:**

<table>
<thead>
<tr>
<th>Titration of New Conjugate</th>
<th>Nonspecific Staining Control</th>
<th>Reactive (4+) Control Serum (1:5 in PBS)</th>
<th>Minimally Reactive (1+) Control Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Conjugate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titer 1:400</td>
<td>-</td>
<td>4+</td>
<td>1+</td>
</tr>
<tr>
<td>New Conjugate Titration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:5</td>
<td>-</td>
<td>4+</td>
<td>2+</td>
</tr>
<tr>
<td>1:10</td>
<td>-</td>
<td>4+</td>
<td>1+ to 2+</td>
</tr>
<tr>
<td>1:20</td>
<td>-</td>
<td>4+</td>
<td>1+</td>
</tr>
<tr>
<td>1:40</td>
<td>-</td>
<td>4+</td>
<td>1+</td>
</tr>
<tr>
<td>1:80</td>
<td>-</td>
<td>4+</td>
<td>&lt;1+</td>
</tr>
<tr>
<td>1:160</td>
<td>-</td>
<td>3+</td>
<td>(1+)</td>
</tr>
</tbody>
</table>

Following the completion of the above steps, and obtaining the expected values, this reagent may be used in the performance of the DAI FTA-ABS test. Please refer to the appropriate product insert.

**STORAGE CONDITIONS**

- Dilute the remainder of the reconstituted AHG 1:2 with PBS. Store 0.3mL aliquots at -20°C or below.
- Frozen aliquots are stable for six months or until the expiration date printed on the vial, whichever comes first.
- An aliquot of conjugate is thawed the day it is used and diluted to its working dilution (allowing for the 1:2 dilution prior to freezing).
- The first time that a frozen aliquot of new conjugate is thawed, it should be run in parallel with the reference conjugate in order to verify the equivalent function of the two frozen conjugates at their working dilutions.

<table>
<thead>
<tr>
<th>Date Adopted</th>
<th>Reference No.</th>
</tr>
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<tbody>
<tr>
<td>2004-09-22</td>
<td>DA-FTA-ABS (Syphilis) Titrable-IFA-2009</td>
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