| YALE-NEW HAVEN HOSPITAL | TITLE: **FTA-ABS Indirect Fluorescent Antibody Testing (Zeus-Wamploe)** | | DEPT OF LAB MEDICINE Policy and Procedure Manual |
|---|---|---|---|
| | | | DOCUMENT # IMM 15 |
| | SOFT codes: FTABS | | Page 1 of 8 |
| **WRITTEN BY:** Josephine Annuziata | **EFFECTIVE DATE:** January 24, 1992 | **REVISION:** June 5, 2013 Penny Smith | **SUPERCEDES:** Revision from 8/3/2009 |

## I. Intended Use

The treponemal test is used to confirm a diagnosis of syphilis in those patients demonstrating a positive response in a non treponemal test.

## II. Introduction

Treponemal and non treponemal tests have been used in the diagnosis of syphilis. The non treponemal tests, such as the RPR card and the VDRL, detect reagent, a substance that may be present in serum for syphilitic persons. The treponemal test is used to confirm a diagnosis of syphilis in those patients demonstrating a positive response in a non treponemal test. The FTA-ABS procedure resulted in improvement in sensitivity and specificity over earlier procedures utilizing treponemal antigens.

## III. Method Description

The FTA-ABS test is based upon an indirect fluorescent antibody procedure. Patient samples are diluted in sorbent to remove nonspecific antibodies and the samples are then incubated with Treponema pallidum antigen. If specific antibodies are present in the patient's serum, stable antigen-antibody complexes are formed. The formed complexes bind fluorescein-labeled antihuman immunoglobulin. The resultant positive reaction is observed as apple-green fluorescence of the Treponema pallidum organisms when examined under a fluorescence microscope.

## IV. Specimen Requirements

The test should be performed on serum only (from red top tube). Separate serum by centrifugation, 3000 rpm for 15 minutes. **Serum specimens may be stored at 2-8°C if tested within 24-48 hours**; otherwise, they **should be stored frozen at -20°C or below for up to 6 weeks.** Do not freeze and thaw sera more than once. Allow serum specimens to reach room temperature before testing. Do not perform the assay on grossly hemolyzed specimens.

**Standard Aliquot volume = 500 uL**
**Minimum Aliquot volume = 200 uL**
**Grossly Hemolyzed: Reject**
**Stability: 2 days refrigerated (2-8°C), 6 weeks frozen (-20°C)**

## V. Reagents and Materials Supplied with Kit:

1. SUBSTRATE SLIDES: T. pallidum (Nichol's strain) 10 wells per slide.

2. Conjugate: Goat anti-human immunoglobulin labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA. One, 3.5mL, clear-capped, bottle. Ready to use.

3. Reactive Control (Human Serum): Will produce positive apple-green staining. One, 0.5mL, red-capped, vial. Ready to use. The 1+ Minimally Reactive Control is a PBS dilution of this Reactive Control. See the Assay Procedure for details.

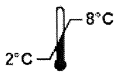
4. Non-Specific Control (Human Serum): Will produce no specific Treponemal staining. One, 0.5mL, green-capped, vial. Ready to use.

5. Sorbent: Standardized product of a Reiter treponeme culture. Sorbent removes non-specific human serum antibodies that may interfere with the FTA-ABS test. One, 20.0mL, green-capped, bottle. Ready to use

6. Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of CLRW. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters. **Good for 1 month.**

7. MOUNTING MEDIUM: Ready for use. Contains phosphate buffered glycerol. Store at 2-8°C. Stable until expiration date.

### STORAGE CONDITIONS

| | |
|---|---|
|   | Unopened Test System. |
| | Mounting Media, Conjugate, Sorbent, Slides, Reactive and Non-Specific Controls. |
| | Rehydrated PBS (Stable for 30 days). |
| | Phosphate-buffered-saline (PBS) Packets. |

### REAGENT NOTES

1. The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS IFA Test Systems, as long as the product

numbers are identical: Sorbent (Product #: FA7006-1), Mounting Media (Product #: FA7009S), and PBS (Product #: 7008S).

2. Test System also contains:
   a. Component Label containing lot specific information inside the Test System box.
   b. A CD containing all ZEUS IFA Product Inserts, providing instructions for use.

## VI. Additional Reagents Required But Not Provided

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, 13 x 75mm
4. Test tube racks.
5. Staining dish
6. Cover slips, 24 x 60mm, thickness No. 1.
7. CLRW
8. Properly equipped fluorescence microscope.
9. 1 Liter Graduated Cylinder.
10. Laboratory timer to monitor incubation steps.
11. Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).
12. Water Bath: 56°C.
13. Incubator: 35 - 37°C.

## VII. Procedure

1. Call a pending list by test code FTABS and make sure all samples are accounted for.
2. 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.**
3. Remove Slides from refrigerated storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove Slides. **Do not apply pressure to flat sides of protective envelope.**
4. Dilute the Reactive and Non-Specific Controls 1:5 in PBS and Sorbent. **Add 100µL of PBS or Sorbent to respective test tubes. Then add 25µL of Reactive or Non-Specific Control serum.** Prepare the 1+ Minimally Reactive Control directly from the heated Reactive Control aliquot. The recommended dilution factor is noted on the Reactive Control vial. Dilution is made in PBS.
   a. Example:
   b. 1+ = 1:400 or 1+ = 1 part reactive serum + 399 parts PBS,
   c. or 100µL sera + 39.9mL PBS = 1:400 dilution.
   d. This would represent the 1+ Minimally Reactive Control.
5. Prepare 1:5 dilutions of all test specimens in Sorbent.
   a. To appropriately labeled tubes, add 200µL of Sorbent.

b. Add 50µL of heat inactivated serum specimen. Mix well.

6. 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 day's testing (see Interpretation of Results section). All dilutions must be thoroughly mixed prior to testing.
7. **Each patient and control is run in duplicate wells.** Add 10µL of diluted test and Control sera to each appropriately identified Substrate Slide well. Include 10µL of Sorbent and 10µL of PBS in their respective wells.
8. Incubate at 35 - 37°C for 30 minutes.
9. 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 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).
10. Wash Slides for two, 5 minute intervals, changing PBS between washes.
11. Rinse Slides for about 5 - 10 seconds in a gentle stream of CLRW as in step 8, and air dry. Slides must be completely dry before adding Conjugate.
12. Place 10µL of Conjugate on each well.
13. Repeat steps 7 - 10.
14. Place a small amount (4 - 5 drops) of Mounting Media between the two rows of offset wells and coverslip.
15. 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.
16. 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.
17. Check non-reactive smears by using white light, darkfield illumination in order to verify the presence of treponemes.
18. Using the 1+ 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.
19. A second technologist will read the slides independently. If a discrepancy is seen a third reader will be used to break the tie.

## VIII. Quality Control

### A. Assay Quality Control

Prepare Reactive and Non-Specific 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.

**Expected Control Readings:**

| | |
|---|---|
| **Reactive Control** | |
| 1:5 in PBS | R (4+) |
| 1:5 in Sorbent | R (3+ to 4+) |
| Minimally Reactive Control, PBS Dilution | 1+ |
| **Non-Specific Control** | |
| 1:5 in PBS | R (2+) |
| 1:5 in Sorbent | N |
| **Control for nonspecific staining by Conjugate** | |
| PBS | N |
| Sorbent | N |

**NOTE:**

1. **If the Controls (above) fail to produce the expected reactions, the test may be invalid and must be repeated.**
2. **The Non-Specific Control in PBS is to ensure that this Control is working, and should therefore demonstrate a 2+ fluorescent staining intensity. The Non-Specific Control in Sorbent ensures that the Sorbent is working optimally, and should therefore demonstrate a non-reactive appearance without distinct fluorescence.**

B. **Quality Control of New Kits**

Each new lot of kit must be pretested by assaying quality control and previously tested Reactive and Non-Reactive patient samples. Results are entered into the Reagent Verification log for supervisor review.

## IX. Interpretation of and Reporting of Results

### A. Interpretation of FTA-ABS Tests

1. Verify the presence of treponemes on each well before reading fluorescent reactions.

2. Using Minimally reactive (1+) control slide as the reading standard, record the intensity of fluorescence of the treponemes according to the description below.

3. **Repeat all specimens with the intensity of fluorescence of (1+).**

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

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

| Initial Test Reading | Repeat Test Reading | Report |
|---|---|---|
| 4+, 3+, 2+ | | Reactive (R) |
| 1+ | >1+<br>1+<br><1+ | Reactive (R)<br>Reactive Minimal (RM)*<br>Non-Reactive (NR) |
| <1+ | | Non-Reactive (NR) |
| N or ± | | Non Reactive (NR) |

*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.

**B. Resulting of FTA-ABS**

1. Results are entered as Non- Reactive, Reactive or Minimal Reactive.
2. The following comment (@FTAB) is added by the Soft LIS system to all Reactive Minimal results@FTAB.

"This result should be considered equivocal if clinical or historical evidence of Treponemal infection is absent. Repeat test suggested."

3. All Reactive and Reactive Minimal results, as well as Non-Reactive results on patients with Reactive VDRL results are reported to the state as Significant Findings. Results are to be entered into the significant finding log book.

## X. Limitations

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

## XI. References


1. Zeus Scientific, Inc. "FTA-ABS IFA Test System", Product Literature, Product Series FA7001 (IVD), 2011

2. Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test. Manual of Tests for Syphilis, 1990, Chapter 12. Ed., Larsen SA, Hunter EF and Krause SJ.


## XII. Appendix

IMM 15-A FTA-ABS Worksheet
IMM15-B FTA-ABS Indirect Fluorescent Antibody Testing (Zeus-Wamploe)- Quick Reference Guide (QRG)

## XIII. History

**Revised 6/5/13 by Penny Smith**

- **Updated procedure section to reflect changes to kit by manufacturer and created a QRG.**
- **Reagents are now ready for use, no longer reconstituted.**
- **Prepared PBS buffer will be saved in the refrigerator and reused until it's 30 day expiration date.**
- **Discrepancies between readers will not be repeated instead a third reader will break the tie.**

# FTA-ABS WORKSHEET

DATE: 1ST READER: 2ND READER:

| | | | | | |
|---|---|---|---|---|---|
| SLIDE 1 | REACTIVE PBS 1 | REACTIVE SORB 2 | NON-SPEC PBS 3 | NON-SPEC SORB 4 | MIN-REACTIVE 5 |
| | 10 | 9 | 8 | 7 | 6 |
| RESULT | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| SLIDE 2 | PBS 1 | 2 | 3 | 4 | 5 |
| | SORBANT 10 | 9 | 8 | 7 | 6 |
| RESULT | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| SLIDE 3 | 1 | 2 | 3 | 4 | 5 |
| | 10 | 9 | 8 | 7 | 6 |
| RESULT | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| SLIDE 4 | 1 | 2 | 3 | 4 | 5 |
| | 10 | 9 | 8 | 7 | 6 |
| RESULT | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| SLIDE 5 | 1 | 2 | 3 | 4 | 5 |
| | 10 | 9 | 8 | 7 | 6 |
| RESULT | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| SLIDE 6 | 1 | 2 | 3 | 4 | 5 |
| | 10 | 9 | 8 | 7 | 6 |
| RESULT | | | | | |

## QRG: FTA-ABS Indirect Fluorescent Antibody Testing (Zeus-Wamploe)

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. Remove Slides from refrigerated storage and allow them to warm to room temperature (20 - 25°C). **Do not apply pressure to flat sides of protective envelope.**

3. Dilute the Reactive and Non-Specific Controls 1:5 in PBS and Sorbent. **Add 100µL of PBS or Sorbent to respective test tubes. Then add 25µL of Reactive or Non-Specific Control serum.** Prepare the 1+ Minimally Reactive Control directly from the heated Reactive Control aliquot. The recommended dilution factor is noted on the Reactive Control vial. Dilution is made in PBS. Example:
    a. 1+ = 1:400 or 1+ = 1 part reactive serum + 399 parts PBS,
    b. or 100µL sera + 39.9mL PBS = 1:400 dilution.
    c. This would represent the 1+ Minimally Reactive Control.

4. Prepare 1:5 dilutions of all test specimens in Sorbent.
    a. To appropriately labeled tubes, add **200µL of Sorbent**.
    b. Add **50µL of heat inactivated serum specimen**. Mix well.

5. A total of seven Controls are required according to CDC recommendations for each day's testing (see Interpretation of Results section). All dilutions must be thoroughly mixed prior to testing.

6. **Each patient and control is run in duplicate wells.** Add 10µL of diluted test and Control sera to each appropriately identified Substrate Slide well. Include 10µL of Sorbent and 10µL of PBS in their respective wells.

7. Incubate at 35 - 37°C for 30 minutes.

8. 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 of the Slide. Tilt Slide in opposite direction and repeat rinse.

9. Wash Slides for two, 5 minute intervals, changing PBS between washes.

10. Rinse Slides for about 5 - 10 seconds in a gentle stream of CLRW as in step 8, and air dry. Slides must be completely dry before adding Conjugate.

11. Place 10µL of Conjugate on each well.

12. Repeat steps 7 - 10.

13. Place a small amount (4 - 5 drops) of Mounting Media between the two rows of offset wells and coverslip.

**Signature Approval for Annual Review**

**Document Author**
Josephine Annunziata
January 24, 1992

**Title: FTA-ABS Indirect Fluorescent Antibody Testing**
**Document #: IMM15**

**Revised June 5, 2013 Penny Smith**

| Name (Print) | Title | Signature | Date of Review | Revision Page and Section # (Use Procedure Review Log to document staff review) | Issue Date for Training if Applicable | Effective Date for Use |
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| TEODORICO LEE | LAB MANAGER | Teodorico Lee | 8/26/09 | Header and signature Page | N/A | 8/3/09 |
| BRIAN SMITH | LAB DIRECTOR | Brian R. Smith MD | 8/26/09 | Header and signature Page | N/A | 8/3/09 |
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| BRIAN SMITH | LAB DIRECTOR | Brian R. Smith MD | 12/27/10 | | | |
| TEODORICO LEE | LAB MANAGER | Teodorico Lee | 1/25/12 | Review | | |
| BRIAN SMITH | LAB DIRECTOR | Brian R. Smith MD | 1/25/12 | | | |
| TEODORICO LEE | LAB MANAGER | Teodorico Lee | 6/5/13 | **-Updated procedure section to reflect changes to kit by manufacturer and created a QRG.<br>-Reagents are now ready for use, no longer reconstituted.<br>-Prepared PBS buffer will be saved in the refrigerator and reused until its 30 day expiration date.<br>-Discrepancies between readers will not be repeated instead a third reader will break the tie.** | | 6/5/13 |
| BRIAN SMITH | LAB DIRECTOR | | 6/5/13 | | | 6/5/13 |
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