

INTENDED USE

The ZEUS IFA Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test System is designed for the qualitative determination of antibodies to *Treponema 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 non-treponemal tests such as the RPR 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 RPR 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 RPR 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 patient's 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).

Expected Serological Findings in Untreated Syphilis (7)			
Phase	Latent Period	RPR	FTA-ABS
Primary Stage	2 - 6 weeks	Reactive	Reactive
Secondary Stage	9 - 12 weeks	Reactive (High Titers)	Reactive
Early Latent Stage	6 months - 2 years	Reactive (Decreasing Titers)	Reactive
Late Stage	10 - 40 years	Approximately 50% Reactive	Reactive

PRINCIPLE OF THE ASSAY

The ZEUS IFA FTA-ABS Test System is a modification of the standard FTA-ABS assay designed to confirm positive non-treponemal screen reagin tests for syphilis. The ZEUS IFA 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. 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).

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. Sorbent contains Thimerosal as a preservative (0.02% w/v).**

• • •		1. <i>Treponema pallidum</i> Substrate Slides: contain fixed <i>T. pallidum</i> (Nichols strain) substrate (antigen) standardized to produce optimum reactivity. Ten, 10-well Slides with desiccant pouch.
CONJ		2. Conjugate: Goat anti-human immunoglobulin labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA. One, 3.5mL, amber-capped, bottle. Ready to use.
CONTROL	+	3. Reactive Control (Human Serum): Will produce positive apple-green staining. One, 1.0mL, red-capped, vial. Ready to use. The 1+ Minimally Reactive Control is a PBS dilution of this Reactive Control. See step 3 of the Assay Procedure for details.
CONTROL	-	4. Non-Specific Control (Human Serum): Will produce no specific Treponemal staining. One, 1.0mL, green-capped, vial. Ready to use.
DIL	SPE	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.
BUF	PBS	6. Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters.
MNTMED		7. Mounting Media (Buffered Glycerol): Two, 3.0mL, white-capped, dropper tipped vials.

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.

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.

4. The Controls are **potentially bio-hazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Bio-safety 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": current edition; and OSHA's Standard for Bloodborne Pathogens (20).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay.** Return unused reagents to their original containers immediately and follow storage requirements.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate.
7. The Conjugate, and Controls contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide. This preservative may be toxic if ingested.
8. Dilution or adulteration of these reagents may generate erroneous results.
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause erroneous results.
12. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
13. Avoid splashing or generation of aerosols.
14. Do not expose reagents to strong light during storage or incubation.
15. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
16. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.:10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
17. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
18. Do not apply pressure to slide envelope. This may damage the substrate.
19. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
20. Unopened/opened 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. Do not freeze.
21. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.
22. **PRECAUTION FOR POSSIBLE CROSS-CONTAMINATION:**
 - a. Due to the close proximity of the test areas on the ZEUS 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 orange ZEUS Slides are designed to facilitate recognition of cross-contamination.
 - b. 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.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, 13 x 100mm or comparable.
4. Test tube racks.
5. Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing Slides between incubation steps.
6. Cover slips, 24 x 60mm, thickness No. 1.
7. Distilled or deionized water.
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.

The following filter systems, or their equivalent, have been found to be satisfactory for routine use with transmitted or incident light darkfield assemblies:

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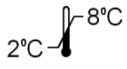
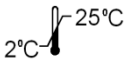
Transmitted Light			
Light Source: Mercury Vapor 200W or 50W			
Excitation Filter	Barrier Filter	Red Suppression Filter	
KP490	K510 or K530	BG38	
BG12	K510 or K530	BG38	
FITC	K520	BG38	
Light Source: Tungsten – Halogen 100W			
KP490	K510 or K530	BG38	

Incident Light			
Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	Dichroic Mirror	Barrier Filter	Red Suppression Filter
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten – Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (16, 17). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. 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. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (19).

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.

ASSAY 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. 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.**
3. Dilute the Reactive and Non-Specific Controls 1:5 in both PBS and Sorbent (e.g.: 50µL of serum + 200µL of Sorbent or PBS). 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.
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. 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.
6. 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. The staggered positioning of the test wells minimizes possible cross contamination (see Precautions Section).
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 distilled water as in step 8, and air dry. Slides must be completely dry before proceeding.
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.
14. 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.
15. 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.
16. Check non-reactive smears by using white light, darkfield illumination in order to verify the presence of treponemes, or alternatively, consider the ZEUS IFA FTA-ABS Double Stain Test System.
17. 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.

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+ Minimally Reactive Control at a dilution other than that recommended by the manufacturer. In such cases it may be advisable to employ the use of secondary standards.

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

NOTES:

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.
3. Additional Controls may be tested according to the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
4. 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

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+ 1+ <1+	Reactive (R) Reactive Minimal (RM)* 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.

LIMITATIONS OF THE ASSAY

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 (13 - 15), not as a screening procedure.

EXPECTED RESULTS

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

PERFORMANCE CHARACTERISTICS**1. 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 ZEUS IFA 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.

2. Clinical Studies:

The ZEUS IFA FTA-ABS Test System was tested in parallel with the standard FTA-ABS procedure in three independent double blind studies (see below):

A. Study One

	ZEUS IFA FTA-ABS Test System	Standard FTA-ABS Test System
Reactive	71	67
Borderline	0	0
Non-Reactive	12	16

Based on the above study, the ZEUS IFA 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 1+ reactive by the ZEUS IFA FTA-ABS Test System method.

B. Study Two and Three

Comparative studies of the ZEUS IFA FTA-ABS Test System and standard FTA-ABS procedure on 50 RPR positive FTA-ABS low level reactive, and 50 RPR positive FTA-ABS non-reactive serum specimens:

	ZEUS IFA FTA-ABS Test System	Standard FTA-ABS Test System
Laboratory A:		
Reactive	45	45
Borderline	3	4
Non-reactive	52	51
Laboratory B:		
Reactive	40	43
Borderline	0	0
Non-reactive	60	57

Based on the above studies, Laboratory A showed 99% agreement between the standard and the ZEUS IFA FTA-ABS Test System. This single discrepancy involved a borderline result on the standard FTA-ABS that was reported as non-reactive with the ZEUS IFA FTA-ABS Test System. 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 ZEUS IFA FTA-ABS Test System, and two specimens that were non-reactive with the standard FTA-ABS and reactive with the ZEUS IFA FTA-ABS Test System.

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