ALPHADIA IFA ANTI – Treponema Pallidum IgG Assay

IMMUNOFLUORESCENCE ASSAY
FOR THE DETECTION
OF ANTI - FTA ANTIBODIES IN
HUMAN SERUM

CAT # AD FTA100	100 TESTS
CAT # AD FTA400	400 TESTS

FOR IN VITRO DIAGNOSTIC USE
CONS : 2 - 8°C

ALPHADIA sa/nv
DIAGNOSTIC PRODUCTS
AVENUE VESALE 26 - B1300 WAVRE
BELGIUM
TEL : 32 (0) 10 24 26 49
FAX : 32 (0) 10 24 55 99
contact@alphadia.be

INTRODUCTION

The etiologic agent of syphilis (Treponema pallidium) produces two types of antibodies in the host. One type of antibody response reacts with non-treponemal lipid antigens (reagin) and is the basis for current serologic tests for syphilis (STS). The second type of antibody reacts with treponemal antigens and is used for confirmatory testing purposes.

Various test procedures can be used for screening tests which include the Unheated Serum Reagin (USR), Rapid Plasma Reagin Card test (RPR card), Automated Reagin (AR) and VDRL slide test.

Unfortunately, many acute or chronic infectious diseases, collagen and immunological diseases result in biologic false positive (BFP) reactions. Rheumatoid arthritis, lupus erythematosus pregnancy, heroin addiction, lepromatous leprosy, autoimmune diseases, diseases in which hypergammaglobulinemia develops, all can produce BFP reactions. BFP reactions are generally weakly reactive and titer less than 1:8 in reagin screening tests. The development and modification of FTA-ABS tests for syphilis alleviated some the problems encountered with reagin screening tests. The clinical picture and patient history of BFP reactors consistently suggest some disease other than syphilis. This is generally supported by negative results in the FTA-ABS test.

Confirmatory tests utilizing treponemal antigens are recommended when the etiologic agent is not observed and only a serological diagnosis is possible. The FTA-ABS test should be used as a confirmatory procedure only in conjunction with a positive STS screening result. The FTA-ABS test should not be used as a screening test since the FTA-ABS test does not distinguish a current active syphilitic infection from a previous cured syphilitic infection.

The laboratory findings in untreated syphilis are summarized for the various clinical stages of the disease in Table 1. A reactive FTA-ABS test alone is good evidence of past or present treponemal infection. A reactive FTA-ABS test along with a reactive STS test generally confirms the diagnosis of syphilis when other treponematoses can be ruled out.

Table 1

			Usual laboratory finding	
Stage	Time after exposure (findings)	Darkfield	STS	Treponemal tests
Primary (appearance of lesion at site contact)	-Approx. 4 weeks (17-97 days) -Approx. 4 weeks (17-97 days)	-Positive until lesion heals -Satellite lymph nodes remain positive	-Reactive titers increasing -Reactive (titers increasing 4-6 weeks)	Reactive FTA-ABS Reactive FTA-ABS
Second (lesions of skin and mucous membranes)	Approx. 12 weeks (6 weeks to 6 months)	Positive (easy to find only in moist lesions)	Reactive - titers high	Reactive FTA-ABS
Latent « early »	6 months to 2 years	No visible lesions	Reactive with declining	Reactive FTA-ABS
Latent « late »	Over 2 years	No visible lesions	Reactive with declining	Reactive FTA-ABS
Late	10-40 years		As high as 50% may be non-reactive	Reactive FTA-ABS
Prenatal and congenital	In utero infection occurs usually after 4th month	Cord blood may be positive	Reactive at birth or few weeks later	Reactive at birth

Positive finding include; cerebral spinal fluid, aqueous humor of eyes, synovial fluid and infected organs.

PRINCIPLES

The FTA-ABS reaction detects circulating antibodies against the etiologic agent of syphilis, *Treponema pallidium*. The primary reaction involves antibodies which attach to antigens along the surface and internal structure of the microorganism. This reaction occurs during the incubation step while the serum is diluted 1:5 in sorbent and covers the smears Treponema pallidium. The sorbent is prepared from saprophytic Reiter treponeme culture which contains substances that remove non-specific antibodies to « group treponemal antigens » found in normal individuals ,but does not significantly absorb the antibodies against the virulent treponema in the diseased population.

A rinsing period follows the primary incubation, which removes all unbound serum antibody. A secondary reaction and incubation period then follows. The reagent used in the secondary reaction is a fluorescein labeled anti-human conjugate, which covers the smear. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescence microscope. Florescence intensity of patient serum is recorded relative to control standards which establish the specificity and sensitivity of the test procedure.

The intensity of fluorescence is graded on a scale of 4+ to negative (no fluorescence).

MATERIALS PROVIDED

1. FTA substrate slide (Treponema pallidium) 10 well slides
2. Sorbent : 5 or 25 ml liquid.
3. Nonspecific control serum : liquid
4. Reactive control serum : liquid. See label for 1+ control titer.
5. FITC-labeled anti-human globulin : liquid.
6. Phosphate buffered saline : PBS pH 7.2 ± 0.1
7. Mounting medium : 3 ml pH 7.2 ± 0.1

ADDITIONAL MATERIALS REQUIRED

Water bath set at 56°C.
Test tubes 12x75 mm and rack.
Staining dishes and slide holder.
Distilled water.
Fluorescence microscope.
Moisture chamber.
Bibulous paper.
Pipettes.

STORAGE AND STABILITY

1. FITC labeled antihuman globulin conjugate

Conjugate is stable at 2-8°C, refer to product label for expiration date. Repeated freezing and thawing or prolonged room temperature exposure will deteriorate the reconstituted reagent. The conjugate may be aliquoted into small amounts and frozen once for storage up to 6 months at -20°C.

2. FTA-ABS antigen slides

Must be stored at 2-8°C or lower. Refer to product label for expiration date.Slides are ready to use once they reach room tempearture.

3. FTA-ABS reactive control

Upon receipt, store at 2-8°C. Refer to product label for expiration date. The reactive control is stable when stored at 2-8°C. Refer to titer on vial label for 1+ control.

4. FTA-ABS nonspecific control

Upon receipt, store at 2-8°C. Refer to product label for expiration date. The nonspecific control is stable when stored at 2-8°C.

5. FTA-ABS sorbent

The sorbent is a standardized extraction of Reiter treponeme culture. This sorbent group treponemal antigens is capable of absorbing major non-specific Treponemal antibodies. Store at 2-8°C. Refer to product label for specific expiration date. Sorbent is used undiluted and requires no centrifugation. Care should be taken to prevent contamination.

6. Phosphate buffered saline - PBS
PBS is stable at room temperature storage. Refer to product label for expiration date. The reconstituted buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

7. Mounting medium (pH 7.2)
Upon receipt, store at 2-8°C. Check label for specific expiration date.

SPECIMEN COLLECTION
Serological specimens should be collected using aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analyzed within a few days. Serum may be held for months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thimerosal or 0.095% sodium azide is strongly recommended.

CONJUGATE TITRATION
The reactivity of the conjugate has been established at RTD for the FTA-ABS test, using ALPHADIA components, which are interchangeable with other lots of ALPHADIA components. However, since microscopes can vary in their optical sensitivity and a wide variety of transmitted and epifluorescent microscopes are in use, variation in absolute fluorescence can occur between these microscopes.

The antihuman conjugate is ready for use. The following titration is recommended in order to adjust test reactivity of the FITC-labeled antihuman globulin conjugate when the observed fluorescence of the 1+ and 4+ controls are either over reactive or under reactive as fluorescent test standards. The dilution of the reactive control serum for a 1+ reaction must remain constant as specified on the product label. Any change in ultimate dilution from the specified 1+ dilution will adversely affect the sensitivity of the FTA-ABS test results. The only adjustable parameter in the procedure is through titration of the conjugate (only when necessary) ALPHADIA conjugate is at its optimal staining (fluorescence) dilution which is one doubling action below its endpoint (maximum 4+ titer).

1. Under Reactive Controls :
When the reactive controls are less reactive than previous test runs, the following may be useful in the resolution of this problem. Check the alignment of the microscope and change the light source making any conclusions on the sensitivity of the reagents. Also check filters, objectives and eyepieces for cleanliness, since this will affect the optical sensitivity and resolution of the image. Only 1 coverslips should be used. Coverslips that are too thick affect image brilliance and clarity. Excessive application of mounting medium may cause a problem in clarity of the microscope image.

2. Over Reactivity of 1+ Control :
If the reactive controls are too bright : prepare serial dilutions of the antihuman globulin conjugate in PBS, i.e. 1:2, 1:4, 1:8, 1:16. Check using the reactive control diluted 1:5 in PBS, the 1+ dilution of the reactive control in PBS as specified on the label, and the nonspecific control diluted 1:5 in sorbent.

Select the dilution which enables best visual differentiation of all controls

Example	Desired results	1:2	1:4	1:8	1:16
Reactive 1:5 PBS	= 4+	4+	4+	4+	3+
Reactive 1+ PBS	= 1+	2+	1+	±	±
NS control 1:5 sorbent	= neg ±	1+	±	-	-

In the example above, the 1:4 dilution would be selected.

TEST INSTRUCTIONS

1. Serum preparation : Heat inactivated test sera in a water bath adjusted to 56°C for 30 minutes.
Previously heated sera and control sera should be heated for only 10 minutes at 56°C.

2. Reactive control serum : The heat inactivated reactive control serum is diluted 1:5 in PBS and 1:5 in sorbent by adding 0.2 ml of sorbent or PBS to respective labeled test tubes and adding 0.05 ml of reactive control serum to each respective test tube. Prepare the 1+ reading standard by diluting the heat inactivated reactive control serum to the specified dilution listed on the control vial (i.e.; 1+ = 1:500 is prepared by adding 0.1 ml to 49.9 ml of PBS). Prepare the 1+ dilution fresh for each day's testing. Discard unused portion.

3. Nonspecific control serum : The heat inactivated nonspecific control is diluted 1:5 in PBS and 1:5 in sorbent by adding 0.2 ml of sorbent or PBS to respective labeled test tubes and adding 0.05 ml of nonspecific control serum to each respective test tube. Mix well.

4. Sorbent control : A sorbent control consists of sorbent without any additional test serum or control serum added into a well on the control side.

5. Conjugate control : The conjugate control consists of PBS without any additional test serum or control serum added to a well on the control side.

6. Prepare a 1:5 dilution of all test specimens in sorbent by adding 0.2 ml of sorbent to respective labeled test tubes and then adding 0.05 ml test specimen to each respective test tube. Mix well : sorbent should be mixed well before dilutions are made.

7. Remove slides from refrigerator and allow to reach room temperature. Tear envelope along notched area. Remove slide, being careful not to touch the smears. Identify slides using pencil or pen with water-insoluble ink.

8. Cover the appropriate identified antigen smears with 20-30 µl of all test and control sera.

9. Incubate in a moisture chamber for 30 minutes at 37°C.

10.Rinsing procedure :
a. Gently rinse with a steady stream of PBS between the top and bottom rows on the slide for approximately 5 seconds.
Note : do not aim the stream directly on the wells.
b. Place slides in staining dish with PBS for 5 minutes. Agitate slides frequently.
c. Using fresh PBS, repeat step b.
d. Rinse slides in distilled water for approximately 5 secondes.

11. Allow slides to air dry thoroughly at room temperature (19-26°C) or place in 36-38°C incubator for 10 minutes or until dry. Use bibulous paper and discard paper after each application. Slides must be dry before adding conjugate.

12. Dispense one drop of conjugated reagent (20-30 µl) into each specimen area.

13. Repeat steps 9-11.

14. Coverslip slides using a minimal amount of mounting medium. Place mounting medium between the two rows and coverslip.

15. Examine slides as soon as possible. If a delay in reading is necessary, place slides in a refrigerator in the dark. Read within 4 hours. Store in refrigerator in moisture chamber if slides cannot be read immediately.

RESULTS
The slides should be examined at a magnification of approximately 400x. Verify nonreactive smears by using dartkfield illumination to observe treponemas in the microscope field of view.

Using the 1+ reactive control slides as the reading standard, record the intensity of fluorescence of the treponema according to Table II.

Table II

Control pattern illustration	Reaction
Reactive Control : a. 1:5 PBS dilution b. 1:5 sorbent dilution	R4 + R(3+ - 2+)
Minimally Reactive (1+) R1+	
Non-Specific serum controls : a. 1:5 PBS dilution b. 1:5 sorbent dilution	R(2+ - 4+) N
Non-Specific staining controls : a. Antigen, PBS and conjugate b. Antigen, sorbent and conjugate	N N

Test runs in which these control results are not obtained are considered unsatisfactory and therefore the test results should not be reported.
Read section entitled « conjugate Titration ».

Table III - Reporting System for FTA-ABS Tests

Initial Test Reading	Repeat Test Reading	Report
4+, 3+, 2+		Reactive
1+	> 1+	Reactive
1+		Reactive Minimal
<+		Nonreactive
<1+		Nonreactive
N,±		Nonreactive

Retest all specimens with the intensity fluorescence of 1+.
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 serological testing.

LIMITATIONS OF PROCEDURE
1. A diagnosis should not be based on a single serologic test since various host factors must be taken into consideration.
2. A STS screening test should be performed prior to FTA-ABS testing.
3. False positive FTA-ABS tests have been reported in lupus erythematosus, demonstrating a beaded fluorescent pattern.
4. A positive FTA-ABS test is produced in syphilis and all other treponematoses (e.g.; yaws, pinta, bejel).
5. The FTA-ABS test is not useful in determining the effectiveness of antibiotic therapy.
6. The FTA-ABS test is not a screening test.

QUALITY CONTROL
1. Reactive control diluted 1:5 in PBS, sorbent and to the 1+ minimally reactive control must be run with each day's testing to insure sensitivity of results.
2. Non-specific control diluted 1:5 in PBS and sorbent must be run to insure specificity of results.

PRECAUTIONS
1. All human components have been tested by radioimmunoassay for HBsAg and HTLVIII/LAV by an FDA approved method and found to be negative (not repeatedly reactive). However, this does not assure the abscence of HBsAg or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The 0.095% sodium azide included in the reactive and non-reactive controls is toxic if ingested.
3. The 0.095% sodium azide included in the conjugate is toxic if ingested.

4. Do not use components beyond their expiration dates.
5. Follow the procedural instructions exactly as they appear in this insert to assure valid results.
6. For in vitro diagnostic use.
7. Handle slides by the edges since direct pressure on the antigen walls may damage the antigen.
8. Do not store reagents in « Frost Free » freezers.
9. Do not freeze and thaw reagents more than once.

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