INTENDED USE

The VIRGO® FTA-ABS IgG indirect fluorescent antibody (IFA) test is intended for the detection and titration of *Treponema pallidum* IgG antibodies in human sera. The test procedure is based on the method developed by Hunter, *et al.,* to remove nonspecific reactions by absorbing test samples with nonpathogenic treponemes. The current procedure is described in the 1990 Manual of Tests for Syphilis.[1]

SUMMARY

Treponema pallidum, the etiological agent of syphilis, produces in the host two types of antibodies: 1) nontreponemal antibodies (regain), which react with lipid antigens and 2) treponemal antibodies, which react with *T. pallidum* and closely related strains.[2]

Detection of regain can be successfully accomplished by use of various commercially available tests. Unfortunately, acute or chronic infections such as malaria, leprosy, infectious mononucleosis and upper respiratory diseases as well as collagen and immunological diseases such a rheumatoid arthritis and lupus erythematosus can produce false positive regain tests.[1,3-12]

Tests for syphilis employing treponemal antigens are of most value in testing sera from patients presenting diagnostic problems. Such individuals most frequently have reactive regain tests in the absence of clinical or historical evidence of syphilis, or may have nonreactive regain tests and clinical signs of late syphilis. A reactive treponemal test is considered good evidence of past or present syphilitic infection, providing that other treponematoses can be ruled out.

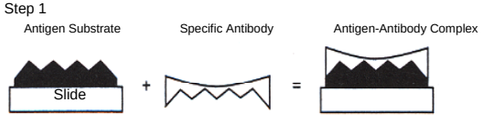
The FTA-ABS kit manufactured by Hemagen Diagnostics, Inc., provides all the necessary reagents for the rapid determination of antibody to *Treponema pallidum* in human sera. Antigenic substrate, control sera, FITC conjugate, sorbent, buffer, coverslip mounting media and an instructional insert are included in the kit.

PRINCIPLE OF THE TEST

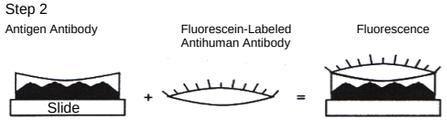
The VIRGO fluorescent antibody assays utilize the indirect method of fluorescent antibody staining, first described by Weller and Coons in 1954.[16] The procedure is carried out in two basic reaction steps. In step one, the human serum to be tested is brought into contact with the antigenic substrate. Antibody, if present in the test serum, will attach to the antigen, forming an antigen-antibody complex. If the serum being tested does not contain antibody for this particular antigen, no complex is formed and all the serum components are washed away in the rinse step. The second step involves adding a fluorescein labeled anti-human antibody to the test wells. If the specific antigen-antibody complex is formed in step one, the fluorescein labeled antibody will attach to the antibody moiety of the complex in step two. A positive reaction, bright apple-green fluorescence, can be seen with the aid of a fluorescence microscope.

Principle of Indirect Fluorescent Antibody Testing

Step 1



Step 2



CONTENTS OF THE KIT

902300	902301	Test Kit Product Number
60 Tests	**200 Tests**	**Number of Tests per Kit**
15 x 4 well	**25 x 8 well**	**Slides:** Fixed *Treponema pallidum* (Nichols Strain)
1 (10 mL)	**2 (10 mL)**	**Vial(s) Sorbent** (liquid): A concentrated, standardized extract of a Reiter Treponeme culture. Sorbent removes the nonspecific antibody for treponemes in human serum without significantly reducing the specific antibodies to *T. pallidum.*
1	**1**	**Vial FTA-ABS Reactive Control:** Lyophilized human serum
1	**1**	**Vial FTA-ABS Nonspecific Control:** Lyophilized human serum
1	**2**	**Vial(s) FITC Conjugate:** Liquid, inactivated rat anti-human IgG (heavy & light chains)
1	**2**	**Vial(s) FTA-ABS Conjugate Diluent** contains 2% Tween-80
2	**4**	***Packages Powdered Phosphate Buffer:** (PBS) pH 7.4 ± 0.2
1	**1**	***Vial(s) (2 mL) Buffered Glycerol**
2	**3**	***Packages (5 each) Blotters**

*These components may be interchanged between different master lots. Additional supplies are available from Hemagen Diagnostics, Inc.

MATERIALS REQUIRED BUT NOT SUPPLIED

Test tubes and racks for making dilutions

Pipettes for preparing dilutions

Coverslips, 22 x 50 mm., No. 1 thickness

Humidified chamber

Magnetic stir plate (optional)

Staining dish and slide-holder rack

Fluorescence microscope. Refer to manufacturer's instruction manual for the filter system that gives optimum results for FITC (Maximum excitation wavelength = 490 nm. Mean emission wavelength = 520 nm.)

PRECAUTIONS

1. HANDLE ALL ASSAY SPECIMENS, SLIDES, REACTIVE AND NON-SPECIFIC CONTROLS AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS.

   All human blood components of the kit have been tested by approved FDA methods and found to be negative for both hepatitis B surface antigen (HBsAg) and for antibodies to human immunodeficiency virus type 1. Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, specimens and kit reagents should be handled at the Biosafety Level 2, as recommended for any potentially infectious human serum or blood specimen.[17,18]
2. The antigenic substrates are fixed in methanol.
3. Do not pipette by mouth.
4. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
5. All materials used in this assay, including reagents, samples and wiping materials should be disposed of in a manner that will inactivate infectious agents.

HANLDING PRECAUTIONS

1. **For *In Vitro* Diagnostic Use.** No U.S. Standard or Potency.
2. Do not use the kit or individual reagents beyond their labeled expiration dates.
3. The components of this kit have been tested as a unit. **Do not** interchange components from other sources or from different master lots, except as noted.
4. Protect the conjugate from prolonged exposure to light.

REAGENT STORAGE AND STABILITY

1. ***T. pallidum* Antigen Slides** – store at 2-8°C
2. **Reactive Control, Nonspecific Control, Sorbent and Conjugate** – store lyophilized reagents and sorbent at 2-8°C. **After rehydration**, Reactive Control, Nonspecific Control and FITC Conjugate should be made up in aliquots and stored at -20°C or colder if not used within one week.
3. **Powdered PBS, Diluent and Buffered Glycerol** can be stored at 2-30°C if desired. The test kit can be used through the expiration date on the outer box label.

NOTE: Precautions were taken in the manufacture of this product to protect the reagents from contamination. After reconstitution, care should be exercised to protect the reagents in this kit from contamination. If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit.

REAGENT PREPARATION

Allow reagents and slides to reach room temperature 15 to 30 minutes before use.

1. **Slides and Glycerol:** Ready to use.
2. **PBS:** Dissolve contents of one package in 1 liter of distilled or deionized water. Seal container to prevent contamination or evaporation.
3. **Controls:** Dehydrate the Reactive and Nonspecific Controls with 1.0 mL of distilled or deionized water. Aliquot for storage at -20°C if not used within one week.
4. **Conjugate:** Dehydrate with 2.0 mL of FTA-ABS Conjugate Diluent (supplied). Aliquot for storage at -20°C or colder if not used within one week.

SPECIMEN COLLECTION AND HANDLING

1. Serum samples may be stored at room temperature for up to 24 hours. For longer term storage, they may be stored at 2-8°C (for up to three days), or frozen at -20°C or colder. Place at 37°C only until the samples are thawed. Remove and mix thoroughly before use. Self-defrosting freezers are not recommended. Avoid multiple free-thaw cycles.[19]
2. Optimal performance of the VIRGO FTA-ABS IFA depends upon the use of fresh serum samples. Specimens should be collected aseptically. Early separation from the clot prevents hemolysis of serum.[20] No anticoagulants or preservatives should be added.
3. For best results, another sample should be drawn if bacteriological contamination or lipids are present. If another sample cannot be obtained, filtering (0.45µ) or centrifugation (approximately 3000 x G for 10 minutes) is required.
4. Occasionally, the specimen may contain certain proteolytic enzymes which attack and digest the substrate. This is especially true of specimens contaminated with microorganisms. Such specimens may be heated to 56°C for 30 minutes. If this fails to reduce the enzymatic activity, another sample should be obtained from the patient.

TEST PROCEDURE

Specimens may contain infectious agents and should be handled accordingly.

For optimal results, DO NOT allow substrate wells to dry out while performing the test.

1. **Remove the slides and required reagents from the refrigerator** and allow them to reach room temperature (15 to 30 minutes).
2. **Heat all sera (control serum aliquots and test samples) for 30 minutes at 56ºC in a water bath prior to testing**. Sera previously heated and stored in aliquots should be heated to 56°C for at least 10 minutes before reuse.[12] Allow the heated sera to cool to room temperature before proceeding to next step.
3. **Seven controls will be required for each group of samples being tested.** The following table indicates the dilution for each control:

Controls	Dilution
1. Reactive Control	1:5 in PBS
2. Reactive Control	1: 5 in Sorbent
3. Nonspecific Control	1:5 in PBS
4. Nonspecific Control	1:5 in Sorbent
5. Minimally Reactive Control to Yield a 1+ Fluorescent Reaction	See FTA-ABS Dilution Notice
6. Nonspecific Fluorescence Controls	Undiluted PBS
7. Nonspecific Fluorescence Controls	Undiluted Sorbent

   These dilutions are prepared by placing 0.1 mL of the diluent in a test tube and adding 0.025 mL of the control to give a 1:5 dilution. Dilutions must be thoroughly mixed. (See Quality Control Section).
4. **Preparation of 1:5 dilutions of test samples:** To appropriately marked tubes, add 0.1 mL of sorbent. Next add 0.025 mL of previously heated (but now cooled) sample. Mix by pipetting. Incubate at room temperature for 15 minutes. During incubation, mix by shaking the test tube rack a few times.
5. **Remove the slides from the pouch** just before use and label, keeping in mind that seven controls must be run each time the test is performed.
6. **Cover each well with one sample, either a control serum dilution or a dilution of a patient serum specimen (see Step #3 above).** (~ 10 – 20 µL per well).
7. **Incubate in a humidified chamber at 37 ± 2ºC for 30 minutes.**
8. **Rinse the slides briefly** in a light stream of PBS. Do not direct the stream into the wells.
9. **Rinse the slides thoroughly for 7 minutes** in a staining dish of PBS. **Change the buffer and wash for an additional 8 minutes.** Handle slides gently. Gentle agitation of the buffer is necessary for efficient slide washing.
10. **Blot the painted mask of the slide** with the blotters provided. **Do not allow the wells to dry before conjugate addition.**
11. **Cover each well with one drop (~10 µL) of FITC Conjugate**.
12. **Incubate in a humidified chamber at 37 ± 2ºC for 30 minutes.** Protect from intense light.
13. **Repeat steps 8 and 9. Blot the painted mask of the slide** with the blotters provided. **Do not allow the wells to dry before the addition of conjugate.**
14. **Place a small drop of Buffered Glycerol in each well** and cover with a coverslip.
4. For best results, **the slides should be read immediately at a magnification of 200-500X.** Alternatively, the slides may be read within 24 hours. However, they should be stored at 2-8°C in the dark, and sealed to prevent the mounting fluid from drying.

CRITERIA FOR GRADING FLUORESCENCE INTENSITY

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
Atypical	Varied: treponemes appear to be "moth eaten" or to have "beads" of fluorescence throughout their length

INTERPRETATION OF SAMPLE RESULTS

Reporting System for FTA-ABS Test

Initial Test Reading	Repeat Test Reading	Report
4+		Reactive
3+		Reactive
2+		Reactive
1+[a]	>1+	Reactive
1+	1+	Reactive Minimal[b]
1+	<1+	Nonreactive
<1+		Nonreactive
N		Nonreactive
Bead fluorescence		Atypical fluorescence observed[c]

[a]Retest all specimens that initially give 1+ intensity of fluorescence.

[b]In the absence of historical or clinical evidence of treponemal infection, this test result should be considered equivocal. A second specimen should be obtained 1-2 weeks after the initial specimen and submitted to the laboratory for serologic testing.

[c]Beaded fluorescence has been observed in serum from patients with active systemic lupus erthematosus and from patients with other autoimmune diseases.

QUALITY CONTROL

1. The FTA-ABS Reactive Control provided with each test kit should be assayed in each test at the dilutions stated on the kit enclosure to provide 4+ and 1+ reading standards. The levels of fluorescence intensity corresponding to these reading standards are described in the insert on Criteria for Grading Fluorescence Intensity.
2. Each lot of Reactive Control must be titrated to an endpoint dilution. The endpoint titer must be within ± one two-fold serial dilution of the Reactive Control titer reported in the VIRGO FTA-ABS Dilution Notice. If the results obtained are out of range, the test is invalid and should be repeated.
3. If a gradual decrease in the reactive control is observed over a period of time, this may indicate deterioration of control serums, reagents, or the fluorescent light source.
4. Quality Control results were obtained on a Nikon® microscope equipped for epiilliumination with a 50W HBO mercury ARC lamp, B filter system for FITC and a 40X dry objective (NA 0.65). Differences in endpoint reactivity and fluorescence intensity may be affected by the type and condition of fluorescence equipment used (see Microscope Specifications at the end of the package insert).

The following table outlines expected control results:[a]

Control		Reaction
Reactive Control:	1:5 PBS dilution	R 4+
	1:5 sorbent dilution	R (3+ to 4+)
Minimally Reactive Control[b]	1+ control dilution	R 1+
Nonspecific serum controls:	1:5 PBS dilution	R (2+ to 4+)
	1:5 sorbent dilution	N - ±
Nonspecific staining controls:	Undiluted PBS	N
	Undiluted Antigen	N

Key: R = Reactive N = Nonreactive

[a]Test runs in which these control results are not obtained are considered unsatisfactory and should not be reported.

[b]This is a dilution in PBS of the Reactive Control serum, which will give the minimal degree of fluorescence (1+) considered reactive.

LIMITATIONS OF THE PROCEDURE

1. The FTA-ABS test is not intended for routine use or as a screening procedure. Its greatest value is to distinguish true-positive nontreponemal results from false-positive results and to establish the diagnosis of late latent or late syphilis.
2. Problems arise when this test is used as a screening procedure, because approximately 1% of the general population will be false positive.22-24
3. Although false-positive results in the FTA-ABS test are often transient and the cause is unknown, a definite association has been made between false positive FTA-ABS results and the diagnosis of systemic, discoid, and drug-induced varieties of lupus erthematosus.25-29
4. Unexplained FTA-ABS reactive results may also occur in elderly patients.

The articles by Hunter[30] and Miller[31] should be consulted for a discussion of the FTA-ABS test procedure and limitations.

SOURCES OF ERROR

1. If serum from one well is allowed to run onto another well, then serum from a person without syphilis may appear reactive.[6,5]
2. If the FTA-ABS test is used as a screening procedure rather than to confirm the reactive results of a nontreponemal test or as a specific diagnostic test in patients with signs or symptoms suggestive of late syphilis, the positive predictive value of the FTA-ABS test is decreased.
3. If the microscope is not properly aligned, the intensity and quality of fluorescence will be affected.
4. If reagents become bacterially contaminated, the antibody may be reduced and the results may be invalid.
5. If reagent storage instructions are not followed, the reagents will not produce satisfactory control results.
6. If frozen antigen slides are thawed and refrozen, the treponemes will be difficult to see and the test results will be unsatisfactory.
7. If a serum is bacterially contaminated or is excessively hemolyzed, the tests results will be invalid.
8. If antigen slides are not stored according to procedures, deterioration of the antigen may take place.
9. If a precipitate is observed in the conjugate, nonspecific staining may be observed.
10. If the atypical staining pattern of beaded fluorescence is not recognized, these specimens may be incorrectly reported as reactive.
11. If FTA-ABS test slides are read on a microscope equipped with incident illumination, the nonreactive wells must be examined by darkfield examination for the presence of treponemes.

GENERAL PERFORMANCE CHARACTERISTICS

The role of antibody class to the detection of antibody to *T. pallidum* is not clear. The evaluation of FTA-ABS reactive sera by Julian, *et al.*[33] demonstrated only IgG reactivity. In addition, the investigation of IgG conjugate by Hunter, *et al.*[34] has shown that these reagents possess predominantly IgG activity; the level of anti-light chain activity was insufficient to be recognized following adjustment to the working dilution using established procedures.

Wilkson and Rodin[35] have found that virtually all sera obtained from untreated early or early latent syphilitic patients possess FTA-ABS IgM antibody; in contrast only 23% of the sera obtained from latent late syphilis cases exhibited this reactivity. The reported relative resistance of IgM antibody to sorbent[36] as well as the prevalence of rheumatoid factor in the sera of FTA-ABS IgM reactive patients has made the role of this antibody in the detection of syphilis infections uncertain.

The FTA-ABS test is recognized as the most acceptable method for confirming the presence of antibody to *T. pallidum,* but false positives do occur and have been demonstrated in patients with the following conditions: 1)pregnancy; 2) lupus erthematosus; 3) heroin addiction; and 4) leprosy.

SPECIFIC PERFORMANCE CHARACTERISTICS

Summary of Clinical Trial Data Obtained at Two Independent Laboratories – March/April 1977*

Laboratory #1. 30 Samples

FTA-ABS (Standard)

Reactive	Borderline	Nonreactive
17	2	11

Hemagen FTA-ABS Kit

Reactive	Borderline	Nonreactive
17	3	10

Laboratory #2. 60 Samples

Other commercial kit

Reactive	Borderline	Nonreactive
17	0	43

Hemagen FTA-ABS Kit

Reactive	Borderline	Nonreactive
18	0	42

*These results were obtained prior to the issuance of the new 1990 guidelines for interpretation by the CDC.

EXPECTED VALUES[37]

Stage	Time After Exposure (infection)	Darkfield	Usual Laboratory Findings STS	Treponemal Tests
Primary (appearance of lesion at site of contact)	Approx. 3 weeks (10-90 days)	Positive until lesion heals		Reactive (FTA-ABS)
	Approx. 4 weeks	Satellite lymph nodes remain positive	Reactive (titers increasing)	
Secondary (lesions of skin and mucous membranes)	Approx 12 weeks (6 weeks to 6 months)	Positive (easy to find only in moist lesions)	Reactive (titers only)	Reactive (FTA-ABS)
Latent "Early"	6 months to 2 years	No visible lesions	Reactive with declining titers	Reactive (FTA-ABS)
Latent "Late"	Over 2 years			
Late	Over 4 years		As high as 50% may be nonreactive	Reactive (FTA-ABS)
Prenatal and Congenital	*In utero* infection occurs usually 4th month	Cord blood may be positive	Reactive at birth or few weeks later	Reactive at birth

MICROSCOPE SPECIFICATIONS

Comparable filter systems are shown below:

Transmitted Light Fluorescence:

Light Source	Exciter Filter	Barrier Filter
Mercury Vapor	**KP490 + BG38**	**KP510, K530**
200W	**(4 mm.) or**	
50W	**BG12 (4 mm.)**	
	+ BG38 (4 mm.)	
Tungsten	KP490 + BG38	KP510, K515, K530
Halogen	(4 mm.)	
50W		
100W		

Incident Light Fluorescence:

Light Source	Exciter Filter	Dichroic Beam Splitting Mirror	Barrier Filter
Mercury Vapor	**KP500 + BG38**	**TK-510**	**K510**
200W	**(4 mm.) or BG23 (4 mm.) for stronger red suppression.**		**K520**
100W			
50W	**Edgefilter 450 nm., 480 nm. For narrow band excitation, suppression of tissue auto-fluorescence**		
Tungsten	**KP500 + BG38**	**TK510**	**K510**
Halogen	**(4 mm.)**		**K515**
50W			**K530**
100W			

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SUMMARY OF VIRGO FTA-ABS

IMPORTANT: **It is recommended that one be familiar with the detailed procedure in the package insert before using this summary.**

Heat inactive serum samples by heating at 56ºC for 30 minutes. Allow same to cool.

↓

Dilute samples and control with sorbent or PBS.

↓

Incubate at 23 ± 2ºC for 15 minutes while shaking.

↓

Cover wells with patient samples or controls as described.

↓

Incubate slides in a humidified chamber at 37 ± 2ºC for 30 minutes.

↓

Rinse slides briefly with PBS. Wash for 15 minutes, changing buffer 1X. Blot slides.

↓

Cover each well with FITC Conjugate.

↓

Incubate slides in a humidified chamber at 37 ± 2ºC for 30 minutes.

↓

Repeat wash step described above.

↓

Place a small drop of Buffered Glycerol on each well and cover with a coverslip.

↓

Read slides immediately at 200-500X magnification on fluorescence microscope.

Fluorescent Treponemal Antibody-Absorption/ FTA-ABS IgG IFA

Immunofluorescence Test Kit for the Detection of Antibody to *Treponema pallidum* In Human Sera

FOR *IN VITRO* DIAGNOSTIC USE

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Columbia, Maryland 21045
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