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 Treponema. The current procedure is described in the 1990 Manual of Tests for Syphilis (24).

SUMMARY

Treponema pallidum, the etiological agent of syphilis, produces in the host two types of antibodies: 1) nonspecific antibodies (reagins), which react with lipid antigens and 2) treponemal antibodies, which react with T. pallidum and closely related spirochetes.

Detection of reagan can be successfully accomplished by use of various commercially available tests. Unfortunately, acute or chronic infections such as malaria, syphilis, infectious mononucleosis, and upper respiratory diseases as well as collagen and immunological diseases such as a rheumatoid arthritis and lupus erythematosis can produce false positive reagan tests.

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

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

PRINCIPLE OF THE TEST

The VIRGO fluorescent antibody assay utilizes the indirect method of fluorescent antibody staining, first described by Weller and Coons in 1954 (25). The procedure is carried out in two basic reaction steps. In step one, the human serum (if any) is allowed to react with the antigenic substrate. Antigen, 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, no antigen-antibody complex will form. After reaction, no complex is formed and all the serum components are washed away in the rinse step. The second step involves adding a fluorescent labeled anti-human antibody to the antigen wells. If the specific antigen-antibody complex is present, the fluorescein labeled antibody will attach to the antigen 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 Antigen Substrate Specific Antibody Antigen-antibody Complex

Step 2 Fluorescent Labeled Antihuman Antibody Antigen Antibody Fluorescein Labeled Fluorescence

INTERPRETATION OF SAMPLE RESULTS

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 of a Reactor from contamination. If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit.

REAGENT PREPARATION

All human blood components of the kit have been tested by approved FDA components from other sources or from different master lots, except as noted. These dilutions are prepared by placing 0.1 mL of the diluent in a test vial and adding 0.025 mL of the control to give a 1:5 dilution. Dilutions are thoroughly mixed. (See Quality Control Section).

4. Preparation of 1.5 dilutions of test samples: To appropriately marked tubes, add 0.1 mL of diluent. Next add 0.025 mL of previously heated (but now cooled) sample. Mix by pipetting.

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).

7. Incubate in a humified chamber at 37°C for 24 h. If the slides are briefly in a light stream of PBS, do not direct the stream into the vials.

8. Rinse the slides thoroughly for 7 minutes in a staining dish of PBS. Change the buffer and wash with an additional 8 minutes. Handle slides gently. Gentle agitation of the buffer is necessary for efficient slide washing.

9. Blot the printed mask of the slide with the blotters provided. Do not allow the wells to dry before the addition of the control reagents.

10. Place a small drop of Buffered Glycerol in each well and cover with a coverslip. The test slides are read immediately at a magnification of 200X-500X. Alternatively, the slides may be read 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

Reactive "++" to "++++" Moderate to Strong

1st or 2nd "+" Equivalent to Minimally Reactive "+" control

0 or 1st "−" Positive, but not useful in diagnosis

Non-specifically reactive, or antigen is present

INTENSIVE CRITICAL: Treponemae appear to be “mote eaten” or to have "specks" of fluorescence throughout their length.

INTERPRETATION OF SAMPLE RESULTS

REPORTING SYSTEM FOR FTA-ABS Test

Initial Test Reading Repeat Test Reading Report

4+ Reactive Reactive

3+ Reactive Reactive

2+ Reactive Reactive

1+ Reactive Reactive

1− Reactive Reactive

Nonreactive Nonreactive

No reagents used for control

Bead fluorescent

Nonreactive

Any fluorescent observed*
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 results.

6. If frozen antigen slides are thawed and refrozen, the treponemes will be inactivated or lost and the tests will be unsatisfactory.

7. If a serum is bacterially contaminated or is excessively hemolyzed, the test results will be invalid.

8. If the FTA-ABS test slides are not stored according to procedures, determination of the antigen may take place.

9. If a precipitate is observed in the conjugate, nonspecific staining may be observed.

10. If the atrypical 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 the FTA-ABS reactive sera by Juran, et al. [2] demonstrated only 23% of the sera obtained from latent late syphilis cases exhibited this reactivity. The reported relative resistance of IgM antibody to inactivation may be as high as 70%, as well as the presence of rheumatoid factor in the sera of FTA-ABS reactive patients has made it the role of the antibody in the detection of syphilis infections impossible.

Wilson and Rodin [3] have found that virtually all sera obtained from untreated early or late latent syphilis 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 inactivation may be as high as 70%, as well as the presence of rheumatoid factor in the sera of FTA-ABS reactive patients has made it the role of the antibody in the detection of syphilis infections impossible.

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) rheumatoid arthritis; (2) lupus erythematosus; (3) heroin addiction; and (4) leproma.

SPECIFIC PERFORMANCE CHARACTERISTICS

Summary of Clinical Triad Data Obtained at Two Independent Laboratories - March/April 1977

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>50 Samples</th>
<th>FTA-ABS (Standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>Nonreactive</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Hemagen FTA-ABS Kit</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>Laboratory F. 50 Samples</td>
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<td>21</td>
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<tr>
<td>Other commercial kit</td>
<td>17</td>
<td>21</td>
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BIBLIOGRAPHY


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 56°C for 30 minutes. Allow same to cool.

↓ Dilute samples and control with sorbent or PBS.

↓ Incubate at 23 ± 2°C for 15 minutes while shaking.

↓ Cover wells with patient samples or controls as described.

↓ Incubate slides in a humidified chamber at 37 ± 2°C 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 ± 2°C 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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