**INTENDED USE**

The VIRGO Anti-DNA indirect fluorescent antibody (IFA) test is intended for the detection and titration of anti-native DNA antibodies in human sera.

**SUMMARY**

Systemic Lupus Erythematosus (SLE) is a well-known chronic inflammatory illness whose clinical manifestations range from localized skin lesions to a destructive systemic disorder without cutaneous changes. SLE is characterized by remissions and exacerbations; with distinct immunologic abnormalities, most notably, the presence of antinuclear antibodies (ANA).

In SLE these antibodies form with nuclear DNA in tissue sections of human or animal origin. A more specific test for anti-DNA utilizes Crithidia luciliae, a tachyzoan as an antigenic substrate. C. luciliae possesses a giant mitochondrion, the kinetoplast, in which double stranded (ds) (native DNA or nDNA) is concentrated. This nDNA lacks the histone-like antigens found in association with native antigen substrate nuclei.

Detection of DNA by IFA has been shown to correlate well with the binding of DNA in the Farr assay. IFA however has the advantages of being rapid, reproducible and easily performed.

The Anti-DNA test manufactured by Hemagen Diagnostics, Inc., provides all the necessary equipment to permit the performance of an indirect fluorescent antibody titer to native DNA in human sera. Antigenic substrate, control sera, FITC conjugate, buffer, cover slips and an instruction manual 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 Welser and Coons in 1954. 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 fluorescent labeled antibody to test wells. If the specific antigen-antibody complex is formed in step one, the fluorescent labeled antibody will attach to the complex in step two. A positive reaction, bright apple-green fluorescence, can be seen with the aid of a fluorescence microscope.

**Preparation of Indirect Fluorescent Antibody Testing**

**TEST PROCEDURE**

1. The sera is titered by serial two-fold dilutions to determine the dilution at which the serum will just react with the fixed antigen.

2. Predilute the test reagents.

3. Stain the slides for a maximum of 30 minutes.

4. Wash the slides and cover with a coverslip.

**NOTES:**

- This is a negative result.
- Correct identification of staining patterns necessitates knowledge of the relative sites of the intracellular organelles. The kinetoplast is smaller than the nucleus, and located posterior to it.

**INTERPRETATION OF SAMPLE RESULTS**

**SUMMARY**

Systemic Lupus Erythematosus (SLE) is a well-known chronic inflammatory illness whose clinical manifestations range from localized skin lesions to a destructive systemic disorder without cutaneous changes. SLE is characterized by remissions and exacerbations; with distinct immunologic abnormalities, most notably, the presence of antinuclear antibodies (ANA). The VIRGO anti-DNA IFA is a diagnostic tool used for measuring the antibody response in SLE patients.

**TEST PROCEDURE**

1. The endpoint is the highest dilution showing a 1+ intensity of the kinetoplast.

2. A strongly positive result may be seen in the absence of a fluorescing kinetoplast.

3. Correct identification of staining patterns necessitates knowledge of the relative sites of the intracellular organelles. The kinetoplast is smaller than the nucleus, and located posterior to it.

**INTERPRETATION OF SAMPLE RESULTS**

**SUMMARY**

The Anti-DNA test, using C. luciliae as a substrate, has been shown to be a useful diagnostic aid in separating SLE from other ANA positive sera. Anti-DNA titers in SLE sera may be used as a sole criterion for diagnosis, all clinical and laboratory data must be taken into account.

2 Occasionally, some SLE patients undergoing steroid treatments may show negative anti-DNA titers. These sera, when tested using the modified Crithidia luciliae filter system that gives optimum results for FITC (Maximum excitation 490nm, Maximum emission wavelength 520nm).

3. Correct identification of staining patterns necessitates knowledge of the relative sites of the intracellular organelles. The kinetoplast is smaller than the nucleus, and located posterior to it.

**LIMITATIONS OF THE PROCEDURE**

1. The control sera are representative of positive and negative reactions. At the 1:10 screening dilution, the Positive Control represents a strong (3-4+) reaction. If the fluorescence intensity of the Positive Control is less than the acceptable range, the test is invalid and should be repeated.

2. Each kit of Positive Control must be titrated to an end-point reaction. The endpoint titr will be maintained in one or two-fold serial dilution of the Positive Control IFA 1+ Dilution Kit. If the reaction is not visible, the test is invalid and should be repeated.

3. At the screening dilution, the Negative Control should not display apple-green fluorescence. If apple-green fluorescence is observed, the test is invalid and should be repeated.

4. Quality Control results were obtained on a Nikon microscope equipped for epillumination with a 50W HBO mercury arc lamp. B filter system for FITC. Differences in endpoint reactions and fluorescence intensity may be affected by the type and condition of fluorescence equipment used (see Microscope Specifications at the end of the instructions).

**TEST RESULTS IN SLE AND OTHER DISEASES**

Test Samples provided by a clinical rheumatology department in the metropolitan Washington, D.C. area.

Those SLE patients that show negative binding are either in natural or immunotheraphy induced remission.


Antibody/
Anti-\(n\)DNA IgG IFA

Immunofluorescence Test Kit
for the Detection of
Anti-Native DNA IgG Antibodies

FOR IN VITRO DIAGNOSTIC USE

Hemagen Diagnostics, Inc.
VIRGO® Products Division
Columbia, Maryland 21045
Phone: (800) 436-2436
(443) 367-5500
Web Site: www.hemagen.com