**INTENDED USE**

The VIRGO ANA Fluorescent Antibody Assay (kits) are intended for use in the detection of anti-nuclear antibodies in human sera.

**SUMMARY**

Systemic Lupus Erythematosus (SLE) is a syndrome with manifestations that range from a localized skin lesion to a destructive systemic disorder without any curative treatments. The antibody in serum of patients with SLE may be found in the articular, vascular, and central nervous systems, and in the kidneys. The antibody in a patient's serum is the hallmark of SLE.

**INTENDED USE**

1. The viral fluorescent antibody assay utilizes the indirect method of fluorescent antibody staining, first described by Weller and Coons in 1964. The procedure is carried out in two basic reaction steps. In the first step, the human specimen or sera is brought into contact with the antigenic substrate. In the second step, the antigen-antibody complex is formed, and the fluorescent-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.

2. This assay is intended for the detection and titration of anti-nuclear antibodies in serum (IFA) test is intended for the detection and titration of anti-nuclear antibodies in human sera.

**Vial Negative Control:**

Lympholized human serum.

**Vial (s) FITC Conjugate:**

Lympholized human immunoglobulin (IgG) heavy and light chains with counterstain.

**Package Powdered Phosphate Buffer (PBS):**

pH 7.4 ± 0.2

**Vial (2 mL) Buffer Solution**

Bufferstock to dilute PBS.

**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).

Humidified chamber.

**PREPARATION**

1. The control sera are representative of typical positive and negative reactions. They are stored at room temperature for up to 24 hours. For longer term storage, they may be stored at 2°C to 8°C, or frozen at -20°C or colder. Place at 37°C only until the samples are thawed. Remove and mix thoroughly before use. Defrosting freezers are not recommended. Avoid multiple freeze-thaw cycles.

2. The control sera are representative of the fluoroimmunoassay for human IgM and IgA antibodies to human immunodeficiency virus type 1. The control is not used for the detection of HIV-1 or HIV-2.

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1. 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.

2. The screen dilution of sample or control. For quantitative determination, prepare serial two-fold dilutions.

3. Quality Control results were obtained on a Nikon® microscope equipped with a 50W HBO mercury ARC lamp. A 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).

**INTERPRETATION OF SAMPLE RESULTS**

**RESULTS**

**SIGNIFICANCE**

Screening:

- No fluorescence or fluorescence intensity < 1+ at the 1:40 screening dilution.
- Positive by IFA for ANA.
- Titers for a single serum sample.

**LIMITATIONS OF THE PROCEDURE**

1. The VIRGO ANA KB IFA Test Procedure and Interpretation of Test Results must be followed closely to obtain reliable test results.

2. An ANA titer is rarely helpful in distinguishing between various autoimmune connective tissue disease, but titers in the range of 1:1280, particularly with speckled, nuclear or peripheral staining patterns, are consistent with SLE or SCL-100D and mixed connective tissue disease (MCTD). However, patients with SLE or other autoimmune phenomena may show wide variation in ANA titers depending on the clinical state of the disease. Again, no single serological determination should be used as sole criterion for diagnosis; all clinical and laboratory data must be taken into account.

**PERFORMANCE CHARACTERISTICS**

Fluorescent antinuclear antibody titers and patterns in various connective tissue disorders (n=143) as well as a normal control population (n=149) were determined using Hemagen’s human tissue culture substrate. Results are presented in Tables I and II.

<table>
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<th>Light Source</th>
<th>Exciter Filter</th>
<th>Dichroic Beam</th>
<th>Splitting Mirror</th>
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**BIBLIOGRAPHY**


