ALPHADIA IFA ANA (RL) IgG Assay

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

RAT

CAT # AD ARL48	48 TESTS
CAT # AD ARL96	96 TESTS
CAT # AD ARL144	144 TESTS

MOUSE

CAT # AD AOL48	48 TESTS
CAT # AD AOL96	96 TESTS
CAT# AD AOL144	144 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

Antinuclear antibody (ANA) tests are commonly performed on sera from patients with various connective tissue diseases, particularly in systemic lupus erythematosus (SLE), for diagnostic evidence, prognostic significance and management of therapy. The highest titers of ANA are found in active SLE and the presence of these antibodies is the second most common manifestations of SLE. Immunofluorescence is the test of choice for screening for the presence of ANA since it detects 95-100% of active SLE cases. The presence of ANA has been well documented in different disease states as well as in healthy relatives of SLE patients. The incidence of positive ANA varies with each disease (see Table 1). Rat or mouse liver is utilized for ANA detection in this test system.

PRINCIPLES

ANA antibodies are not organ or species specific. The primary test reaction involves circulating antinuclear antibodies present in the patient's serum which attach to their homologous nuclear antigens. This occurs during the incubation period while the serum covers the antigen surface. A rinsing period is followed by a secondary reaction. The reagent used in the secondary reaction is a fluorescein labelled anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope to visually identify various morphological patterns of nuclear fluorescence.

The clinical significance of the various nuclear immunofluorescence patterns is useful in evaluating patients for the presence of one of the connective tissue diseases. The homogeneous pattern is the most common pattern and is associated with SLE. The peripheral pattern confirms a clinical diagnosis of SLE. True speckled nuclear fluorescence is seen in Scleroderma, Raynaud's disease, Rheumatoid Arthritis and Sjogren's syndrome. Nucleolar fluorescence is seen mainly in Scleroderma and Sjogren's syndrome.

Various drugs have been reported to induce or activate SLE. Patients on these drugs often demonstrate varying levels of ANA in their serum, see Table II.

MATERIALS PROVIDED

Storage and stability of components

1. FITC Conjugate N° CGER2 - 3 ml with Evans blue counterstain is to be stored at 2-8°C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label. This reagent contains antibodies which will react with the human IgG, IgM and IgA immunoglobulin classes.

2. The Antigen slides of rat or mouse liver sections must be stored at 2-8°C or lower upon receipt. Check label for specific expiration date.

3. ANA Positive control - 1 ml should be stored at 2-8°C upon receipt. Check label for specific expiration date.

4. Universal negative control N° NC05 - 1 ml should be stored at 2-8°C or lower upon receipt. Check label for specific expiration date.

5. Buffer pack N°PBS1 - Phosphate Buffer Saline is stable at room temperature storage for 5 years. The reconstituted buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

6. Mounting medium N° TMM3 - 3ml is stable when stored at 2-8°C. Check label for specific expiration date.

NOTE : All kit components are available separately.

Additional materials required but not provided

Test tubes and rack or microtiter system
Disposable pipettes
Staining dish and slide forceps
Moisture chamber
Distilled water
Fluorescence microscope
Paper towels

Reagent preparation

Buffer pack.

Rehydrate buffer with 1 liter of sterile distilled water.

SPECIMEN COLLECTION

Serological specimens should be collected under 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 3 to 6 months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperature, addition of a preservative such as 0.01% (thimerosal) or 0.095% sodium azide is strongly recommended.

TEST INSTRUCTION

Screening : dilute test serums 1:20 in PBS.
Titration : set up doubling dilutions of serum starting at 1:20 (i.e 1:20, 1:40, 1:80, 1:160...)

1. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.

2. Place a drop of diluted serum (20-30µl) and controls over the antigen wells.

3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 24°C).

4. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly on to the well.
5. Wash in PBS for five minutes. Repeat using fresh PBS.

6. Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up wells to blotter holes. Place slide on top of blotter. Do not allow tissue to dry. Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer.

7. Deliver 1 drop (25-30µl) of conjugate per antigen well. Repeat steps 3-6.

8.Place 4-5 drops of mounting medium on slide.

9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope. Note : To maintain fluorescence, store mounted slide in a moisture chamber placed in the dark refrigerator.

QUALITY CONTROL
1. Positive and negative serum controls must be included in each day's testing to confirm reproductibility, sensitivity and specificity of the test procedure.

2. The negative serum control should result in little (+) or no fluorescence. If this control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.

3. The positive serum control should result in bright 3+ to 4+ fluorescence. If this control shows little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.

4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

RESULTS

The slide should be examined under 400x high dry or oil immersion objective at a final magnification of 1000x. A positive result is observed as one of four basic staining patterns seen individually or in various combinations. The characteristic patterns are best seen when viewed using high dry objectives. The positive control demonstrates a homogeneous ANA pattern.

1. Homogeneous (Diffuse)
An even, finely diffuse fluorescence of the entire nucleus is seen.
2. Peripheral (Rim, Shaggy)
The nuclear membrane is more intensely fluorescent than the central area.
3. Speckled
The nuclei show numerous small « specks » of fluorescence throughout the nucleus.
4. Nucleolar
The nucleoli are uniformly stained and appear as 1 to 5 large spherical areas of fluorescence scattered throughout the nucleus.

Pattern Interpretation :

The nuclear immunofluorescence patterns found in SLE can be of prognostic significance.

Homogeneous
High titer anti-DNA antibodies suggest SLE with probable renal involvement and is associated with an intermediate prognosis.

Peripheral
Confirms clinical diagnosis of SLE. Renal involvement, confirmed by anti-DNA tests is associated with an intermediate prognosis.

Speckled
Large and small speckles seen in benign SLE and associated with good prognosis.

Nuclear
High titers are associated with Sjogrenôs syndrome and Scleroderma.

LIMITATIONS OF PROCEDURE
1. No diagnosis should be based upon a single ANA test result, since various host factors must be taken into consideration.

2. Among these host factors are age and sex. There is an increasing significance in positive ANA results in both males and females as age increases. Normal females between 20-60 have a 7% incidence of ANA : normal males, a 4% incidence. Normal males and females over 80 years of age have a 50% incidence of ANA.

3. Various medications including antibiotics, tranquilizers, aspirin and birth control pills can induce a lupus like condition resulting in high ANA titers, see Table II. Drug-induced Lupus generally goes into a sustained clinical remission following removal of the triggering medication.

4. Various autoimmune processes induce positive ANA tests.
5. Further evidence for a diagnosis of SLE is provided by low complement levels, particularly C1,C3 and C4.

6. ANA tests may not agree with LE Prep tests or with latex tests.

7. Presence of antibodies to double stranted native DNA is diagnostic for SLE.

8. Management of therapy should be based not only on positive serologic tests for SLE, but should include the presence of active clinical disease.

9. Elderly patients with SLE have a better prognosis and their clinical symptoms differ substantially from those seen in younger patients.

10.Although the predominant class of antinuclear antibodies (ANA) is Immunoglobulin G, the presence of immunoglobulin E may be of pathogenic importance in SLE.

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 absence of HBsAg or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to insure valid results.
5. For in vitro diagnostic use.
6. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
7. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.

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