TEST LIMITATIONS

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. Both sexes over 80 years of age have a 50% incidence of ANA.
3. Various medications including antibiotics, tranquillisers, aspirin and birth control pills can induce a lupus like condition resulting in high ANA titres. 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 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 stranded 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.

TABLE 1: INCIDENCE OF ANA IN VARIOUS DISORDERS

Disease	% Incidence	Disease	% Incidence
Systemic lupus erythematosus	95-100	Dermatomyositis, polymyositis	10-30
Lupoid hepatitis	95-100	Polyarteritis Nodosa	15-25
Progressive systemic sclerosis (scleroderma)	75-80	Drug associated SLE-like syndrome	-50
Rheumatoid arthritis	25-60	Rheumatic fever	-5
Juvenile arthritis	15-30	Miscellaneous diseases	10-50
Feilty's syndrome	95-100	Generally normal values	-5
Sjögren's syndrome	45-75	Normal old age	-40
Chronic discoid lupus	15-50	Healthy relative of SLE patient	-25

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INSTRUCTIONS FOR USE

Antinuclear (ANA) Antibody Test System

K4805 - 48 Tests **K9605** - 96 Tests
K5005 - 50 Tests **K0005** - 100 Tests

Also for: **Rat Liver slides** **S4101** - 4 well **S8101** - 8 well
S5101 - 5 well **S0101** - 10 well

INTENDED USE

The Bio-Diagnostics Antinuclear Antibody Test System is an immunofluorescent antibody (IFA) test to detect the presence of antinuclear antibodies in human serum.

SUMARY AND EXPLANATION

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 titres 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 utilised for ANA detection in this test system.

PRINCIPLE OF THE TEST

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 whilst 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. True speckled nuclear fluorescence is seen in Scleroderma, Raynaud's disease, Rheumatoid Arthritis, and Sjögren's syndrome. Nucleolar fluorescence is seen mainly in Scleroderma and Sjögren's syndrome.

Various drugs have been reported to induce or activate SLE and patients on these drugs often demonstrate varying levels of ANA in their serum.

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WARNINGS AND PRECAUTIONS

1. All human components in the controls have been screened and found to be negative for STS, HBsAg and antibodies to HIV-1, HIV-2, HCV; found to be negative or non-reactive for STS; found to be negative or non-reactive for HBV by PCR (NAT); tested and found negative for RPR by an FDA registered laboratory. All human components should be handled with appropriate care.
2. The reagents included in the kit contain 0.1% sodium azide or 0.01% Thiomersal as preservatives. Although this is at a low concentration, these reagents should be considered toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to ensure valid results.
5. For *in vitro* diagnostic use only.
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 artefacts.

KIT CONTENTS

SLIDE	Rat liver substrate antigen slides (S4101, S8101, S5101 or S0101)
CONJ **IgG**	FITC Conjugate with Evans Blue Counterstain: J501/J501-5.This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.
CONTROL **+**	ANA homogenous Positive Control no: C001N / C001N-0.5
CONTROL **+**	ANA speckled Positive Control no: C002N / C002N-0.5
CONTROL **+**	ANA nucleolar Positive Control no: C003N / C003N-0.5
CONTROL **-**	Universal Negative Control no: C000N/C000N-0.5
IFA/DFA **PBS**	Buffer Pack no: R002
MM	Mounting Medium no: R005

Note: All kit components are available separately. Please see the Bio-Diagnostics Ltd catalogue for more details.
Also available are Positive controls for Centromere (C018N / C018N-0.5).

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes and rack or microtitre system	Disposable pipettes	Staining Dish and Slide Forceps
Moisture Chamber	Volumetric Flask (500 ml)	Distilled Water
Fluorescence Microscope	Paper Towels – lint free	

REAGENT PREPARATION

1. Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

Manufacturer — Contains sufficient for <n> tests — **RFU** Ready for Use

Temperature limitation — **IVD** In vitro diagnostic medical device

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2ºC to +8ºC. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents.
Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

Anti-Nuclear Antibody Test System

TEST INSTRUCTIONS

Screening: dilute test serums 1/20 (1 part patient sample to 19 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.
Titration: set up doubling dilutions of serum starting at 1/20, (i.e. 1/20, 1/40, 1/80, 1/160, 1/320, etc.).

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 to 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 18-24ºC).
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 onto the well.
5. Wash in PBS for 5 minutes. Repeat using fresh PBS.
6. Carefully dry the back and edges of the slide using a paper towel. **Do not allow tissue to dry**.
7. Deliver 1 drop (20-30µl) 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 a dark refrigerator.

QUALITY CONTROL

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, 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 controls should result in bright 3+ to 4+ fluorescence. If these controls show 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 non-specific 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.

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 immunofluorescent patterns found in SLE can be of prognostic significance.

Peripheral:	Confirms clinical diagnosis of SLE. Renal involvement confirmed by anti-DNA tests (catalogue numbers: K8050/K8100), is associated with an intermediate prognosis.
Homogeneous:	High titre anti-DNA antibodies suggest SLE with probable renal involvement and are associated with an intermediate prognosis.
Speckled:	Large and small speckles seen in benign SLE and associated with good prognosis.
Nucleolar:	High titres are associated with Sjögren's syndrome and Scleroderma.