

Anti-nDNA Antibody Test System

RESULTS

The slide should be examined under 40X high dry or oil immersion objective at a final magnification of 100X. A positive result is seen as a fluorescent circular dot (kinetoplast) which is located against the cytoplasmic membrane in between the nuclear and flagellar ends of the organism. Frequently, a serum will produce both nuclear and kinetoplast fluorescence simultaneously; this reaction will also be a positive. Negative reactions include nuclear fluorescence alone or basal body (polar) fluorescence. Occasionally, non-specific cytoplasmic staining can produce diffuse staining which surround the negative image of the kinetoplast and the nucleus, but this is not read as a positive reaction.

Titre interpretation:

The titre is the highest dilution of the patient's serum showing a weak 1+ fluorescence of the kinetoplast. A positive reaction at 1/5 and above may be significant.

Positive results can be confirmed on the Bio-Diagnostics Anti-dsDNA Quantitative Rainbow ELISA (cat. no. 103-002).

TEST LIMITATIONS

- No diagnosis should be based on a single serologic test since various host factors must be taken into consideration. Additional confirmatory tests for SLE include ANA, complement levels, kidney biopsy and skin biopsy (2).
- Drug induced SLE can give a positive reaction (9).
- The class of circulating antinuclear antibody from patients with lupus nephritis is mostly IgG (in particular, subclasses IgG-1 and IgG-3), the dominant complement fixing class in humans. If only IgM anti-DNA is present, renal disease does not occur. The nDNA test cannot distinguish between these two antibody classes (10).
- The Crithidia luciliae assay does not show a good correlation with the activity of renal disease in patients on immunosuppressive therapy (11).

LITERATURE REFERENCES

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EC REP

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INSTRUCTIONS FOR USE

Anti-nDNA Antibody Test System

Catalogue Number:

K8050 - 50 Tests **K8100** - 100 Tests **K8200** - 200 Tests

Also for: **Crithidia luciliae slides** **L058** - 5 well **L108** - 10 well
 DNA Positive control **C020N**

INTENDED USE

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

SUMMARY AND EXPLANATION

Antinuclear antibodies are present in the blood of patients with certain connective tissue disorders. Systemic Lupus Erythematosus (SLE) patients produce many different types of nuclear antibodies, and those with the specificity for double stranded DNA (dsDNA) have a high correlation with SLE patients (1, 2). Antibodies directed to native dsDNA cannot be detected by standard immunofluorescent antinuclear antibody (ANA) methods which rely on different nuclear fluorescent patterns to determine the type of antibody. Antibodies to DNA that react with both double and single stranded DNA produce the same rim and/or homogeneous patterns (3). Among the various ANA immunofluorescent patterns, the rim pattern confirms a clinical diagnosis of SLE and as many as 33% of these patients have some renal disease. Tests that can unequivocally detect the presence of only native dsDNA antibodies should be performed to confirm the diagnosis of lupus nephritis. The nDNA test kit using the substrate Crithidia luciliae which contains native dsDNA provides a simple technique for detection of antibodies to dsDNA (4, 5, 6).

PRINCIPLE OF THE TEST

Antibodies directed against native dsDNA are not species or organic specific. A useful test for detection of antibodies to native dsDNA is the immunofluorescent technique which utilises the giant mitochondrion kinetoplast of the non-pathogenic haemoflagellate Crithidia luciliae as a substrate for pure dsDNA (2). Standard indirect immunofluorescent techniques are used in the nDNA test, which includes the overlaying of the substrate with patient serum and the use of an anti-human globulin labelled with FITC to visualise the reaction. Good correlation of the results have been found between the immunofluorescent (IF) technique and the radioimmunoassay of Farr. The Crithidia luciliae IF test has been shown to have equivalent sensitivity to the Farr test as well as the millipore filter KBDNA assay (7, 12).

The IFA method offers the following advantages: simplicity, economy, speed and specificity by virtue of the fact that the dsDNA of the Crithidia luciliae kinetoplast, appears to be free of single stranded DNA and histone contamination (2, 12). In contrast, the Farr assay requires expensive materials and equipment and may have immunochemical problems due to the presence of single stranded DNA (ssDNA), which may produce false positives (8, 12). A minimal requirement for anti-dsDNA tests is a negative reaction with antibodies directed to the nucleoprotein of ssDNA, as these antibodies are present in many sera from patients with connective tissue diseases other than SLE. The nDNA test uses a highly specific substrate, Crithidia luciliae kinetoplast, which does not contain nucleoprotein or ssDNA and is free of false positive.

Anti-nDNA Antibody Test System

WARNINGS AND PRECAUTIONS

1. This positive control has been tested and found to be negative or non-reactive for STS, HBsAg, HBV by PCR (NAT), HIV 1/2 antibody, PRP, HIV by PCR (NAT), HCV antibody and HCV PCR (NAT). However, these tests cannot guarantee the absence of infectious agents. All human components should be handled with appropriate care.
2. The conjugate and controls included in the kit contain 0.1% sodium azide or 0.01% thiomersal as preservatives. Although these are at low concentrations, 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	Crithidia luciliae substrate antigen slides (L058: 5 well or L108: 10 well)
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 +	nDNA Positive Control no: C020N/C020N-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.

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	Ready for use
Temperature limitation	In vitro diagnostic medical device	

Anti-nDNA Antibody Test System

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2°C to +8°C. 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.

TEST INSTRUCTIONS

Screening: dilute test serums 1/5 (1 part patient sample to 4 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 1/5 (i.e., 1/5, 1/10, 1/20, 1/40, 1/80, 1/160, 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 18-24°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 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 in the kinetoplast. If this control shows bright kinetoplast 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 of the kinetoplast. 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 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.