Introduction:

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 which 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 antibodies should be performed to confirm the diagnosis of lupus nephritis. The nDNA test kit using the substrate Crithidia luciliae that contains native dsDNA provides a simple technique for detection of antibodies to dsDNA.[4, 5, 6]

Principles:

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 utilizes the giant mitochondrion kinetoplast of the non-pathogenic hemoflagellate 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 labeled with FITC to visualize the reaction. Good correlation of the results has 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 C. 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 immuno-chemical problems due to the presence of single stranded DNA (ssDNA), which may produce false positive.[8, 12] A minimal requirement for anti-dsDNA tests is a negative reaction with antibodies directed to the nucleoprotein of dsDNA, 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, C. luciliae kinetoplast, which does not contain nucleoprotein or ssDNA and is free of false positive.

Materials Provided:

Storage & Stability of Components:

1. FITC Conjugate No. 1501/1500L (3.0 ml) or No. 1533L/1532L (5.0 ml) is to be stored at 2-8 °C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label.
2. The antigen slides of Crithidia luciliae antigen must be stored at 2-8°C or lower upon receipt.
3. nDNA positive control No. 6202L (1.0 ml) should be stored at 2-8°C upon receipt. The control is stable at this temperature until expiration date on the vial label.
4. Universal negative control No. 1000L (1.0 ml) should be stored at 2-8°C or lower upon receipt. The conjugate is stable at this temperature until expiration date on the vial label.
5. Buffer Pack No. 1601 - Phosphate Buffered Saline is stable at room temperature storage as indicated on label. 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 No. 1610 is stable when stored at 2-8° C. Check label for specific expiration date.

Note: All kit components are available separately. Please see the current SCIMEDX Corporation Catalog for more details.

Additional Materials Required but not Provided:

Test tubes and rack or microtiter system
Disposable pipettes

Staining Dish and Slide Forceps
Moisture Chamber
Volumetric Flask (500 ml)
Distilled H_20
Fluorescence Microscope
Paper Towels - lint free

Reagent Preparation:

1. Buffer Pack No. 1601. 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 temperatures, addition of a preservative such as 0.095% sodium azide is strongly recommended.

Test Instruction:

Screening: dilute test serums 1:10 in PBS.
Titration: set up doubling dilutions of serum starting at 1:10, 1:20, 1:40. 1:80, 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 30 µl) 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 19-24°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 (20-30 µl) 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 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 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.

Titer Interpretation:
The titer is the highest dilution of the patient's serum showing a weak 1+ fluorescence of the kinetoplast. A positive reaction at 1:10 and above is significant.

Limitations of Procedure:

1. No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.
2. Additional confirmatory tests for SLE include ANA, complement levels, kidney biopsy, and skin biopsy.[2]
3. Drug induced SLE can give a positive reaction.[9]
4. 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]
5. The C. luciliae assay does not show a good correlation with the activity of renal disease in patients on Immunosuppressive therapy.[11]

Precautions:

1. All human components have been tested by radioimmunoassay for (HB_sA_g) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HB_sA_g 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.

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.

BIBLIOGRAPHY:

1. Tan, E.M., Antinuclear Antibodies: Significance of Biochemical Specificities, Chapter 17, pp. 337-349 in The Immunopathology of the Skin, 2nd Edition, Beutner, E.H., Chorzelski, T.P. and Bean, S.F. John Wiley and Sons, 1979.
2. Sontheimer, R.D. and Gilliam, J.N. An Immunofluorescence Assay for Double Stranded DNA Antibodies Using the Crithidia Luciliae Kinetoplast as a Double Stranded DNA Substrate, J Lab Clin Med, Vol. 91, No. 4, 550-558, 1978.
3. Greenwald, O.A., Peeble, C.L. and Nakamura, R.M. Laboratory Tests for Antinuclear Antibody (ANA) in Rheumatic Diseases. Laboratory Medicine, Vol. 9, No. 4, pp. 1927, 1978.
4. Aarden, L.A., De Groot, E.R. and Feltkamp, T.E.W. Immunology of DNA 111 Crithidia Luciliae: A Simple Substrate for the Detection of Anti-dsDNA with the Immunofluorescence Technique, Ann NY Acad Sci 254-505, 1975.
5. Stingl, G. Meingasser, J.G., Swelty, P. and Knapp, W. An Immunofluorescence Procedure for the Demonstration of antibodies to Native, Double Stranded DNA and of Circulation DNA-Anti-DNA Complexes. Clin Immunopath, Vol. , 131-140, 1976.
6. Crowe, W. and Kushner, I. An Immunofluorescent Method Using Crithidia Luciliae to Detect Antibodies to Double Stranded DNA, Arthritis Rheum. Vol. 20 No. 811, 1977.
7. Chubick, A., Sentheimer, R.D., Gilliam, J.N. and Ziff, M. An Appraisal of Tests for Native DNA Antibodies in Connective Tissue Diseases - Clinical Usefulness of Crithidia Luciliae Assay, Annals of Interm Med, Vol. 89:186-192, 1978.
8. Locker, J.D., Medof, M.E., Bennett, R.M. and Sukhupunyaraska. Characterization of DNA Used to Assay Sera for Anti-DNA Antibodies: Determination of the Specificities of Anti-DNA Antibodies in SLE and Non-SLE Rheumatic Disease States, Jour Immunol. Vol. 118, No. 694, 1977.
9. Alarcon-Segovia, D. and Fishbein, E. Patterns of Antinuclear Antibodies and Lupus Activating Drugs, Jour Rhem. Vol. 2, No. 2, 1975.
10. Decker, J.L. Steinberg, A.D. Reinersten, J.L., Plotz, P.H., Balow, J.E. and Kippel, J.H. Systemic Lupus Erythematosus: Evolving Concepts, Annals Intern Med. Vol. 97:587-604, 1979.
11. Slater, N.G.P., Cameron, J.S. and Lessof, M.A. The Crithidia Luciliae Kinetoplast Immunofluorescence Test in Systemic Lupus Erythematosus, Clin Exp Immunol, Vol. 25: 480-486. 1976.
12. Whiteside, T.L., Dison, J.A. Clinical Usefulness of the Crithidia Luciliae Test for Antibodies to Native DNA, A.J.C.P. Vol. 72, No. 5, 1979.

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Printed in U.S.A. Rev. D 10/23/06

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