PRINCIPLE OF THE METHOD
Serum anti-nDNA antibodies bind to the corresponding antigen present in Crithidia luciliae. The resulting antigen-antibody complexes are detected by means of a fluorescein labeled anti-human immunoglobulin, and visualized with the aid of a fluorescence microscope.1

CONTENTS

COMPOSITION

A. Slides: Crithidia luciliae fixed on each well.
B. PBS (10x): Sodium phosphate 112.5 mmol/L, potassium phosphate 30 mmol/L, sodium chloride 1.15 mol/L, sodium azide 0.95 g/L, pH 7.2.
C+. nDNA Positive Control: Human serum containing anti-native DNA antibodies (nDNA), sodium azide 0.95 g/L.
C-. Negative Control: Human serum, sodium azide 0.95 g/L.
D. IgG FITC/Evans: Goat anti-human IgG conjugated with fluorescein isothiocyanate (FITC), Evans blue 0.01 g/L, sodium azide 0.95 g/L.
E. Mounting Medium: Mowiol 12%, Glycerol 30%, Tris 20mmol/L sodium azide 0.95 g/L.
F. Blotting Paper.

Human sera used in the preparation of the positive and negative controls have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for HBs antigen. However, the controls should be handled cautiously as potentially infectious.

STORAGE
Store at 2-8 °C.

REAGENT PREPARATION
PBS: Dilute the Reagent B 1/10 with distilled water. Stable for 1 week at 2-8 °C. All other reagents provided are ready to use.

ADDITIONAL EQUIPMENT
- Mois chamber
- Wash tray
- Coverslips 24 x 60 mm
- Fluorescence microscope equipped with a 495 nm excitation filter and a 525 nm emission filter for FITC visualization.

SAMPLES
Serum or plasma collected by standard procedures. Stable for 1 week at 2-8 °C. Dilute samples 1/10 in PBS (see Reagent Preparation) before assay.

PROCEDURE
1. Bring the reagents and samples to room temperature.
2. Place 1 drop (25 µL) of the diluted sample or Control on each slide well (A), making sure that it is completely covered (Note 1).
3. Incubate the slide for 30 minutes at room temperature (15-30 °C) into a moist chamber.
4. Drain sample drops off by gently tapping the inclined slide. Avoid cross-contamination of the sera.
5. Rinse gently the slide with PBS (see Reagent Preparation) (Note 2).
6. Wash thoroughly the slide by immersing in a washing tray filled with PBS for 5 minutes. Change PBS and repeat wash.
7. Carefully dry off the slides by using the blotting paper provided. Keep the cells preparation moist along the procedure.
8. Place 1 drop of Reagent D on each well. Incubate the slide for 30 minutes at room temperature (15-30 °C) into a moist chamber.
9. Wash (step 6) and dry (step 7).
10. Place several drops of Reagent E on the slide and cover with a coverslip avoiding the formation of air bubbles.

READING
Examine the slide using the fluorescence microscope (250-400x). For best results, the slides should be read immediately. Select reading fields in the area between the center area and the edge area, with uniform spacing between cells. Fluorescent intensity in the center end edge areas is not representative of the slide preparation.

Crithidia luciliae is a hemoflagellate with a modified mitochondrion called kinetoplast containing double stranded DNA (nDNA), not associated to histones, which apparently lacks any other nuclear antigen. The kinetoplast is a rounded organelle smaller than the nucleus and located between this and the basal body.2,3,4

Observation of specific fluorescent staining of the kinetoplasts at the recommended dilution should be considered as a positive result. Nuclei, basal body or flagellum might give fluorescent staining but only kinetoplast fluorescence must be scored.

Positive sera may be titered. The serum titer is defined as the highest dilution showing a positive result.
QUALITY CONTROL
Positive Control (C+) and Negative Control (C-) provided with kits ORG 871 should be tested together with the patients samples, in order to verify the assay performance.
Positive Control (C+) should give the above described specific staining.
Negative Control (C-) should not give any specific staining.
Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

ASSAY CHARACTERISTICS
IgG FITC/Evans is calibrated against the WHO International Standard for FITC labeled sheep anti-human IgG for the demonstration of antibodies in human serum.

DIAGNOSTIC CHARACTERISTICS
The Crithidia luciliae immunofluorescence test for anti-nDNA antibodies has a high diagnostic specificity, but a fairly high diagnostic sensitivity for Systemic Lupus Erythematosus (SLE). They are the most frequently detected autoantibodies associated to SLE: 95% in SLE patients with renal involvement, 50 - 70% in SLE patients without renal involvement and 40% in patients with inactive SLE. Anti-nDNA antibodies are rarely found in healthy individuals (5).
ORGENTEC's anti-nDNA antibodies kit was used to test 80 sera from SLE patients as well as healthy donors. The results are described as follows:

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>POS</th>
<th>NEG</th>
<th>Sens.</th>
<th>Spec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE (systemic lupus erythematosus)</td>
<td>43</td>
<td>29</td>
<td>14</td>
<td>67%</td>
<td>100%</td>
</tr>
<tr>
<td>SLE without renal involvement</td>
<td>25</td>
<td>15</td>
<td>10</td>
<td>80%</td>
<td>100%</td>
</tr>
<tr>
<td>SLE with renal involvement</td>
<td>18</td>
<td>14</td>
<td>4</td>
<td>75%</td>
<td>100%</td>
</tr>
<tr>
<td>Other autoimmune diseases</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy controls</td>
<td>17</td>
<td>0</td>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES
1. Avoid touching the cells fixed into the wells along the procedure.
2. Use a squeeze bottle or a pipette to wash the slides, avoiding cross-contamination among the adjacent samples.

BIBLIOGRAPHY
2. Aarden LA, de Groot ER und Feltkamp TEW. Immunology of DNA III. Crithidia luciliae, a simple substrate for the determination of anti-dsDNA with the immunofluorescence technique. Ann NY Acad Sci 1975; 254: 505-515.

January 2011