



ImmuGlo™
**Anti-native DNA (*n*DNA)
Antibody Test**
Crithidia luciliae Substrate
For *in vitro* Diagnostic Use

PRODUCT INSERT

Catalog No. 1106
Catalog No. 1106-2

48 Determination
96 Determination

INTENDED USE

An indirect immunofluorescence antibody test for the detection and quantitation of antibodies to native (double stranded) deoxyribonucleic acid (*n*DNA) in human serum.

SUMMARY AND EXPLANATION

Antibodies to *n*DNA are specific for systemic lupus erythematosus (SLE) and rarely occur in patients with rheumatoid arthritis, scleroderma or other autoimmune disorders¹. The frequency and titer of these antibodies fluctuate with disease activity and tend to disappear upon immunosuppressive treatment and during remission. There is a good correlation between the disease activity and anti-*n*DNA antibody levels²⁻⁷.

The two most commonly employed methods for detecting anti-*n*DNA antibodies are radioimmunoassay and immunofluorescence. The specificity and sensitivity of the *Crithidia luciliae* immunofluorescent method are comparable or even better than radioimmunoassay⁸⁻¹⁴. The indirect immunofluorescent test using *Crithidia luciliae* as the antigenic substrate is a simple and specific method for detecting anti-*n*DNA antibodies. *Crithidia luciliae* contains a kinetoplast, an organelle consisting of compact circular DNA which reacts bright apple-green when the test specimen is positive.

For technical assistance please contact:



IMMCO Diagnostics, Inc.
60 Pineview Drive
Buffalo, NY 14228-2120
Telephone: (716) 691-0091
Fax: (716) 691-0466
Toll Free USA/Canada: 1-800-537-TEST
E-Mail: info@immcodiagnostics.com

or your local product distributor

PRINCIPLES OF PROCEDURE

In the indirect immunofluorescence method used in this kit, patients' sera are incubated on smears of *Crithidia luciliae* to allow binding of antibodies to the substrate. Any antibodies not bound are removed by rinsing. Bound antibodies of the IgG class are detected by incubation of the substrate with fluorescein-labeled, anti-human IgG conjugate. When observed under a fluorescence microscope equipped with appropriate filters, positive reactions appear as apple green fluorescence of the kinetoplast with or without associated nuclear staining^{15,16}. The titer, which is the reciprocal of the highest dilution giving a positive reaction, is determined by testing serial dilutions¹⁷.

REAGENTS

Storage and Preparation

Store all reagents at 2°-8°C. Ready for use after equilibration to room temperature.

Precautions

For *in vitro* Diagnostic Use. All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. All human serum specimens and human derived products should be treated as potentially hazardous, regardless of their origin. Follow good laboratory practices in storing, dispensing, and disposing of these materials¹⁸.

WARNING - Sodium azide (NaN₃) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this insert to ensure valid results. Do not interchange kit components with those from sources other than the same catalog number from IMMCO DIAGNOSTICS. Do not use beyond expiration date.

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REFERENCES

1. Feltkamp TEW (Ed). The significance of the determination of anti-DNA and DNA/anti-DNA complexes. Scand J Rheumatol Suppl 11: 7-64, 1975.
2. Davis P, Perry JS and Russell AS. Correlation between levels of DNA antibodies and clinical disease activity in systemic lupus erythematosus. Ann Rheum Dis 36: 157-159, 1977.
3. Tan EM, Schur PH, Carr RI and Kunkel HG. Deoxyribonucleic acid (DNA) and antibodies to DNA in serum of patients with systemic lupus erythematosus. J Clin Invest 45: 1732-1740, 1966.
4. Tan EM. Autoantibodies to nuclear antigens (ANA): Their immunobiology and medicine. Adv Immunol 33: 167-240, 1982.
5. Pemin H, Halberg P and Christiansen E. Antibodies against double-stranded DNA in patients with connective tissue diseases. Acta Med Scand 203: 61-65, 1978.
6. Edmonds JP, Johnson GD, Ansell BM and Holoborow EJ. The value of tests for antibodies to DNA in monitoring the clinical course of SLE. Clin Exp Immunol 22: 9-15, 1975.
7. Isenberg D and Shoenfeld Y. The origin and significance of anti-DNA antibodies. Immunology Today 8: 279-282, 1987.
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9. Crowe W and Kushner I. An immunofluorescent method using *Crithidia luciliae* to detect antibodies to double-stranded DNA. Arth Rheum 20: 811-814, 1977.
10. Kumar V, Beutner EH and Chorzelski TP. Autoimmunity and the Skin. In "Concepts Immunopathology", Cruse JM and Lewis RE Jr., Eds, Karger NT, 318-383, 1985.
11. Somerfield SD, Roberts MW and Booth RJ. Double-stranded DNA antibodies: a comparison of four methods of detection. J Clin Pathol 34: 1032-1035, 1981.
12. Stingl G, Meingassner JG, Swelty P and Knapp W. An immunofluorescence procedure for the demonstration of antibodies to native, double stranded DNA and of circulating DNA-anti-DNA complexes. Clin Immunol Immunopathol 6: 131-140, 1986.
13. Whiteside TL and Dixon JA. Clinical usefulness of the *Crithidia luciliae* test for antibodies to native DNA. Am J Clin Pathol 72: 829- 835, 1979.
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Materials Provided

- | | | |
|-------------------|--|--|
| 6 x | 8-well Substrate Slides, | <i>Catalog No. 1106</i> |
| 12 x | 8-well Substrate Slides, | <i>Catalog No. 1106-2</i> |
| 1 x 0.5 ml | nDNA Positive Control. | Human serum containing antibodies to nDNA*. |
| 1 x 0.5 ml | Negative Control. | Human serum, exhibiting no measurable reactivity with the procedure*. |
| 1 x 5.0 ml | Goat anti-human IgG FITC Conjugate, | heavy and light chain specific. Ready to use. Protect from light* . |
| 1 x 60 ml | Buffered Diluent. | Ready to use*. |
| 2 x 1 | Phosphate Buffered Saline. | Dissolve each vial to 1 liter. |
| 1 x 5.0 ml | Mounting Medium. | Do not freeze* . |
| 1 x 12 | Coverslips | |

*CAUTION - Contains <0.1% NaN₃

Materials Required but not Provided

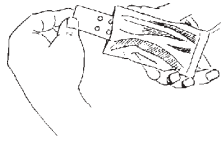
- Fluorescence microscope
- Micropipette or Pasteur pipette
- Serological pipettes
- Staining dish (e.g. Coplin jar)
- Small test tubes (e.g. 13 x 75 mm) and test tube rack
- Distilled or deionized water
- 1 liter container
- Wash bottle
- Absorbent paper towels
- Incubation chamber

Only serum specimens should be used for this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of this test and should not be used. Store specimens at 2°- 8°C for no longer than one week. For longer storage, serum should be frozen at -20°C. Avoid repeated freezing and thawing of samples.

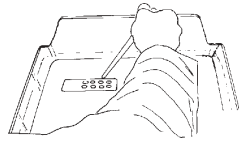
PROCEDURE

Test Method

The indirect immunofluorescence staining procedure is illustrated in the following figures:



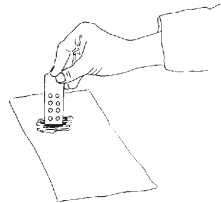
1. Let pouch equilibrate to room temperature, then remove slide(s) from pouch.



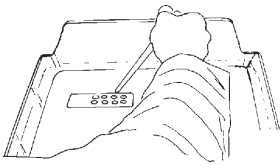
2. Place slide(s) into moisture chamber and add samples and controls. Cover and incubate 30 minutes.



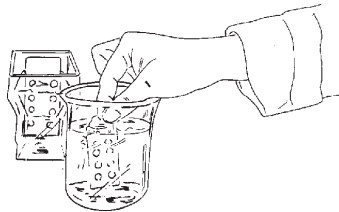
3. Rinse along the midline of the slide(s). Avoid hitting substrate with buffer stream.



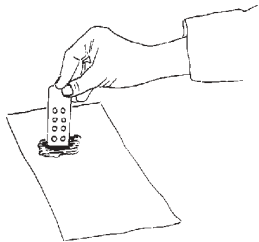
4. Blot edge of slide(s) on absorbent paper. Proceed immediately with next step.



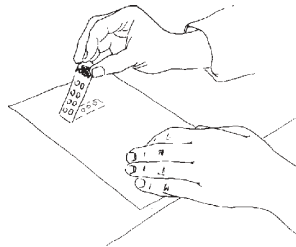
5. Apply Conjugate to each well. Cover and incubate 30 minutes.



6. Dip slide(s) into beaker with PBS. Wash 10 minutes in staining dish.



7. Blot edge of slide(s) on absorbent paper. Proceed immediately with next step.



8. Mount cover slip and read under fluorescent microscope.

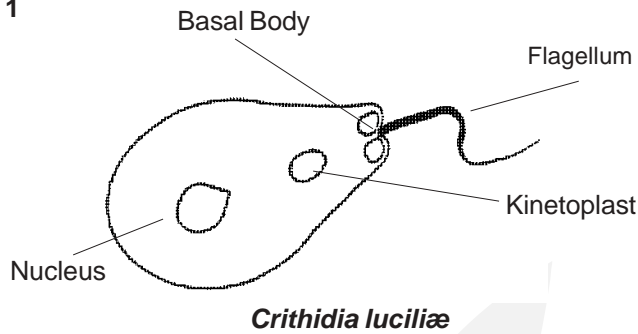
PERFORMANCE CHARACTERISTICS

The ImmuGlo™ *n*DNA Antibody test was compared with another commercially available fluorescent antibody test using *C. luciliae* as a substrate. The comparison included 106 serum samples from normal subjects as well as from patients with the diagnosis of SLE, scleroderma, or rheumatoid arthritis. Sera were tested according to the procedure and screening dilution recommended by the manufacturer. These yielded comparable results as summarized in the following table:

Comparison of Kits using *C. luciliae* Substrate for the Detection of Antibodies to *n*DNA

Clinical Condition	n	IMMCO		Other	
		Positive	% Positive	Positive	% Positive
SLE	28	19	68	13	46
Scleroderma	23	0	0	0	0
Rheumatoid Arthritis	8	0	0	0	0
Normal Controls	106	0	0	0	0

Figure 1



LIMITATIONS OF THE PROCEDURE

In some cases, sera positive for kinetoplast staining may either be very weak or negative at the initial screening dilution prozone phenomenon. In such doubtful cases the sera should be screened at higher dilutions and, if positive, antibody titers determined.

In rare instances false positive reactions may be observed. These may be due to the presence of high levels of lipoproteins or other proteins which bind to DNA^{19,20}.

The goat anti-human IgG FITC Conjugate supplied in this kit is primarily heavy chain specific but has some light chain activity. It reacts primarily with IgG class autoantibodies, but may, to a lesser degree, react with light chains of other classes such as IgM.

The clinician should consider the results of all positive indirect immunofluorescence tests along with the results of other laboratory tests and the clinical condition of the patient when making a diagnosis.

EXPECTED VALUES

As seen in the following table, anti-*n*DNA antibodies are not detected (titer <10) in sera from normal subjects or patients with scleroderma or rheumatoid arthritis. Anti-*n*DNA antibodies occur (titer ≥10) in over one half of patients with SLE.

Incidence of Antibodies to *n*DNA in Various Collagen-Vascular Disorders as detected by Indirect Immunofluorescence on *C. luciliae*

Clinical Condition	No. Tested	No. Positive	% Positive
SLE	28	19	68
Scleroderma	23	0	0
Rheumatoid Arthritis	8	0	0
Normal Controls	106	0	0

A. Screening:

- Step 1.** Dilute each patient serum 1:10 and with the buffered diluent provided (0.1 ml serum + 0.9 ml diluent).
Do not dilute Positive or Negative Controls. Save the undiluted sera to determine antibody titers if screening tests are found to be positive.
- Step 2** Allow pouches containing substrate slides to equilibrate to room temperature for **10-15 minutes**. Carefully remove the slides without touching the substrate.
- Step 3** Label the slides and place them in an incubation chamber lined with paper towels moistened with water to prevent drying.
- Step 4** Invert dropper vial and gently squeeze to apply **1 drop** (approximately 50 µl) of the **Negative Control** to well #1. Similarly apply **1 drop of Positive Control** to well #2. Avoid overfilling the wells.
- Step 5** Using a micropipette or Pasteur pipette, apply **1 drop** of patient's diluted serum (approximately 50 µl) to the other wells. Avoid overfilling the wells.
- Step 6** Place the lid on the incubation chamber and incubate slides **30 minutes** at room temperature.
- Step 7** Remove a slide from the incubation chamber and rinse gently with approximately 10 ml of PBS using either a pipette or a wash bottle. Direct PBS along the midline of the slide. **CAUTION: Avoid buffer stream hitting the substrate. Wash gently but thoroughly. To prevent the slide from drying, proceed immediately with Step 8 while the slide is still wet.**
- Step 8** Blot the edge of the slide on the paper towel to remove excess PBS. Place the slide in the incubation chamber. Immediately invert the **Conjugate** dropper vial and gently squeeze to apply **1 drop** (approximately 50 µl) to each well.
- Step 9.** Repeat **Steps 7 and 8** for each slide.
- Step 10** Replace the lid on the incubation chamber. Incubate **30 minutes** at room temperature.
- Step 11** Remove a slide from the chamber. Hold the slide at the frosted end and dip the slide in a beaker containing PBS to remove excess conjugate. Place the slide in a staining dish filled with PBS for **10 minutes**. If desired, 2-3 drops of Evans blue counterstain may be added to the final wash. Repeat for the remaining slides. **NOTE:** Improper washing may lead to increased background fluorescence.

Step 12 Remove a slide from the staining dish. Blot the edge of the slide on a paper towel to remove excess PBS. **To prevent slide from drying, proceed immediately with Step 13 while the slide is still wet.**

Step 13 Mount the coverslip. Place **3 drops** of the Mounting Medium evenly spaced on a coverslip and invert the slide onto the coverslip. To remove any air bubbles gently apply pressure along the edge of the coverslip. Avoid any movement of the coverslip.

Step 14 Repeat **Steps 12 and 13** for each slide.

Step 15 Examine for specific fluorescence under a fluorescence microscope at a magnification of **200x** or greater. The presence of the anti-fading agent in the mounting medium allows extended viewing of a field without appreciable loss of staining intensity.

Slides may be read as soon as prepared. However, because of the presence of anti-fading agent in the mounting medium, no significant loss of staining intensity occurs if reading is delayed. Slides should be stored in the dark at 2°- 8°C.

B: End Point Determination (Titration)

A serum positive in the screening test may be further tested following **Steps 5 through 13** to determine the titer. Each test run should include the Positive and Negative controls. Make serial two-fold dilutions starting at **1:10** (see below). Using one slide, a serum may be tested at dilutions ranging from 1:10 to 1:320. If positive at a 1:320 dilution, the titer is reported as greater or equal to 320. Alternatively, additional slides may be used to obtain endpoints for those sera still positive at a 1:320 dilution. The reciprocal of the highest dilution producing a positive reaction is the titer.

Preparation of Serial Dilutions

Number eight tubes 1 through 6. Add 0.9 ml of buffered diluent to tube 1 and 0.2 ml to tubes 2 through 6. Pipette 0.1 ml of undiluted serum to tube 1 and mix thoroughly. Transfer 0.2 ml from tube 1 to tube 2 and mix thoroughly. Continue transferring 0.2 ml from one tube to the next after mixing to yield the dilutions depicted as follows:

Tubes	1	2	3	4	5	6
Serum	0.1 ml					
	+					
Buffered Diluent	0.9 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Transfer		0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Final dilution	1:10	1:20	1:40	1:80	1:160	1:320 etc.

Quality Control

Both a positive and negative control serum should be included with each test run. The negative control should show no fluorescence of the kinetoplast, whereas the positive control should have 2+ or greater staining intensity of this structure.

If expected results are not obtained, the run should be repeated. If inadequate results continue to occur with the controls, these may be due to:

- Contamination as a result of improper storage or handling. If signs of contamination such as turbidity are seen, discard and use another control.
- Problems with the optical system of the fluorescence microscope. These may include: improper alignment, use of the bulb beyond the expected performance life, etc.
- Allowing the slide to dry during the procedure.

RESULTS

The results of the tests for anti-*n*DNA antibodies should be reported as negative (<10), positive, (greater or equal to 320) or alternatively positive with titer.

Read only fields which contain well separated *C. luciliae*. Observe for specific staining of the kinetoplast (see figure 1). Staining of the nucleus or the polar body should not be interpreted as a positive DNA antibody test. Absence of specific staining of the kinetoplast is considered negative for antibodies to *n*DNA.