CE

ANTI-nDNA ANTIBODIES (nDNA)

COD 44825 24 tests	COD 44818 60 tests	COD 44817 120 tests
COD 44820 10 x 6 tests	COD 44819 10 x 12 tests	
STORE AT 2-8ºC		
Reagents for the qualitative determination of anti-nDNA antibodies Only for *in vitro* use in the clinical laboratory		

ANTI-nDNA ANTIBODIES (nDNA)

Indirect Immunofluorescence
CRITHIDIA LUCILIAE

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

	COD 44825	COD 44818	COD 44817
A. Slides	4 x 6 tests	10 x 6 tests	10 x 12 tests
B. PBS (10x)	1 x 100 mL	1 x 100 mL	1 x 100 mL
C+. nDNA Positive Control	1 x 0.3 mL	1 x 0.3 mL	2 x 0.3 mL
C-. Negative Control	1 x 0.3 mL	1 x 0.3 mL	2 x 0.3 mL
D. IgG FITC/Evans	1 x 3 mL	1 x 3 mL	2 x 3 mL
E. Mounting Medium	1 x 3 mL	1 x 3 mL	1 x 3 mL
F. Blotting Paper	1 x 10	1 x 10	1 x 10

	COD 44820	COD 44819
A. Slides	10 x 6 tests	10 x 12 tests

COMPOSITION

A. Slides: Crithidia luciliae fixed on each well.

B. PBS (10x): Sodium phosphate, potassium phosphate, sodium chloride, 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, sodium azide 0.95 g/L.

E. Mounting Medium: Mowiol, glycerol, Tris, 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 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contamination is prevented during their use.

Indications of deterioration:

– Liquid components: Presence of particulate material, turbidity.

– Slides: rips in the sealing bag, macroscopic defects on the cell preparation like scratches or cells peeling off.

AUXILIARY REAGENTS

B. PBS (10x).

D. IgG FITC/Evans, conjugate with Evans blue counterstain.

E. Mounting Medium.

C+. nDNA Positive Control.

C–. Negative Control.

REAGENT PREPARATION

PBS: Dilute Reagent B 1/10 with distilled water. Stable 1 month at 2-8ºC if stored well closed at the recommended temperature and if care is taken to prevent contamination during its use.

All other reagents are provided ready to use.

ADDITIONAL EQUIPMENT

– Moist 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 2-8ºC.

Dilute samples 1/10 in PBS (see Reagent Preparation) before assay.

For titration of positive samples, make two-fold serial dilutions starting from 1/10 in PBS.

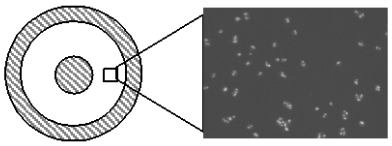
PROCEDURE

1. Bring the reagents and samples to room temperature.
2. Place 1 drop (25 µL) of the diluted sample or controls on each slide well (A), making sure that it is completely covered (Note 1).
3. Incubate the slide for 30 minutes at room temperature (15-30ºC) into a moist chamber.
4. Drain sample drops off by gently tapping the inclinated 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 (15-30ºC) 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 indicated in the picture, 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 cod 44825, cod 44817 and cod 44818 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.

Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

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].

BioSystems anti-nDNA antibodies kit was used to test 80 sera from SLE patients as well as healthy donors. The results are described as follows

	N	POS	NEG	Sens.	Spec.
SLE *(systemic lupus eritematosus)*	43	29	14	**67%**	**100%**
SLE without renal involvement	*25*	*15*	*10*	*60%*	*100%*
SLE with renal involvement	*18*	*14*	*4*	*78%*	*100%*
Other autoimmune diseases	20	0	20		
Healthy controls	17	0	17		

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.

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