CE

ANTI-NUCLEAR ANTIBODIES HEp-2 (ANA-HEp-2)

COD 44108 24 tests	COD 44508 60 tests	COD 44509 120 tests
COD 44546 10 x 6 tests	COD 44547 10 x 12 tests	
STORE AT 2-8ºC		
Reagents for the qualitative determination of anti-nuclear antibodies Only for *in vitro* use in the clinical laboratory		

ANTINUCLEAR ANTIBODIES HEp-2 (ANA-HEp-2)

Indirect Immunofluorescence
HEp-2 CELLS

PRINCIPLE OF THE METHOD

Serum anti-nuclear antibodies (ANA) bind to the corresponding antigens present in HEp-2 cells. 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 44108	COD 44508	COD 44509
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+.	ANA-Ho Positve 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 4	1 x 10	1 x 10

		COD 44546	COD 44547
A.	Slides	10 x 6 tests	10 x 12 tests

COMPOSITION

A. **Slides**: HEp-2 cells cultured on each well.

B. **PBS (10x):** Sodium phosphate, potassium phosphate, sodium chloride, sodium azide 0.95 g/L, pH 7.2.

C+. **ANA-Ho Positive Control**: Human serum containing anti-nuclear antibodies (ANA) homogeneous pattern, 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 culture like scratches or monolayer peeling off.

AUXILIARY REAGENTS

B. **PBS (10x).**

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

E. **Mounting Medium.**

C+. **ANA-Ho Positive Control.**

C+. **ANA-Sp Positive Control**: Human serum containing anti-nuclear antibodies (ANA) speckled pattern, sodium azide 0.95 g/L.

C+. **ANA-Nu Positive Control**: Human serum containing anti-nuclear antibodies (ANA) nucleolar pattern, sodium azide 0.95 g/L.

C+. **ANA-Ce Positive Control**: Human serum containing anti-nuclear antibodies (ANA) centromere pattern, sodium azide 0.95 g/L.

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 7 days at 2-8ºC.

Dilute samples 1/80 in PBS (see Reagent Preparation) before the assay.

For titration of positive samples, make two-fold serial dilutions starting from 1/160 in PBS.

However, each laboratory should establish its own sample dilution based on the population characteristics.

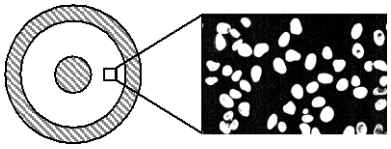
PROCEDURE

1. Bring the reagents and samples to room temperature.
2. Place 1 drop (25 µL) of the diluted sample or control on each slide (A) well, 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 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 substrate 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. Select fields with uniform spacing between cells and uniform nuclei brightness. Fluorescent intensity in the center end edge areas is not representative of the slide preparation.



well selected field

Observation of specific fluorescent staining, described as follows at the recommended dilution, should be considered as a positive result.

ANA: There are different nuclear and cytoplasmatic fluorescent staining patterns that are considered as a positive result for the ANA test. Different staining patterns can coexist in the same serum. Patterns may be modified with the dilution of the serum. The main antinuclear patterns are described below, following the ICAP nomenclature (International Consensus on ANA Patterns)[2]:

AC-1 Nuclear homogeneous: Homogeneous and regular fluorescence across all nucleoplasm. The nucleoli maybe stained or not stained depending on cell substrate. Mitotic cells (metaphase, anaphase, and telophase) have the chromatin mass intensely stained in a homogeneous hyaline fashion.

AC-3 Centromere: Discrete coarse speckles (40-80/cell) scattered in interphase cells and aligned at the chromatin mass on mitotic cells. e.g. anti-CENP B

AC-4 Nuclear fine speckled Fine tiny speckles across all nucleoplasm. The nucleoli may be stained or not stained. Mitotic cells (metaphase, anaphase, and telophase) have the chromatin mass not stained. e.g. anti-SS-A/Ro, anti-SS-B/La

AC-5 Nuclear large/coarse speckled: Coarse speckles across all nucleoplasm. The nucleoli may be stained or not stained. Mitotic cells (metaphase, anaphase, and telophase) have the chromatin mass not stained. e.g. anti-Sm, anti-U1 RNP

AC-8 Homogeneous nucleolar: Diffuse fluorescence of the entire nucleolus, while the metaphase plate shows no staining. e.g. anti-PM-Scl, anti-Th/To.

AC-9 Clumpy nucleolar: Irregular staining of the nucleoli and Cajal bodies with a peri-chromosomal staining at the metaphase plates. e.g. anti-fibrillarin

AC-21 Cytoplasmatic reticular/AMA: Coarse granular filamentous staining extending throughout the cytoplasm. e.g. anti-mitochondrial antibodies

Positive sera may be tittered. The serum titer is defined as the highest dilution showing a positive result.

When none of the above specific stainings are observed, the result should be considered negative for these autoantibodies.

QUALITY CONTROL

Positive Control (C+) and Negative Control (C–) provided with kits cod 44108, cod 44508 and cod 44509 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

The IgG FITC/Evans conjugate is calibrated versus the International Standard of the WHO for sheep anti-human immunoglobulins conjugated with FITC.

The specificity of the ANA-Ho Positive Control has been verified against the AF/CDC1 reference serum from the Centers for Disease Control.

DIAGNOSTIC CHARACTERISTICS

ANA: Sensitivity of antinuclear antibodies determination is higher than 95% for systemic lupus erythematosus, although specificity is fairly low[3].

- **Nuclear homogeneous (AC-1)**: Indicative of systemic lupus erythematosus.
- **Nuclear speckled (AC4, AC-5)**: Highly related to systemic lupus erythematosus, mixed connective tissue disease, Sjögren's syndrome, polymyositis or scleroderma.
- **Nucleolar (AC-8, AC-9)**: In approximately 50-70% of the patients with overlapping scleroderma and polymyositis/dermatomyositis syndromes. They are found in up to 33% of patients with systemic scleroderma, specially those with renal complications[3].
- **Centromere (AC-3)**: In patients with systemic sclerosis, especially in a cutaneous limited form of the disease (80%). Occasionally, in some other connective diseases[4].
- **Mitochondrial (AC-21):** Common in patients with primary biliary cirrhosis, systemic sclerosis, and rare in other systemic autoimmune rheumatic diseases.

BioSystems antinuclear antibodies kit was used to test 140 sera from a variety of autoimmune disease patients (systemic lupus erythematosus, Sjögren syndrome, scleroderma, CREST syndrome, dermatopolymyositis, rheumatoid arthritis, autoimmune hepatitis and primary billiary cirrhosis), as well as healthy donors. The results showed a diagnostic sensitivity and specificity for all the autoimmune diseases of 98,3% and 93%, respectively.

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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3. Hollingsworth PN et al. Antinuclear antibodies. In: James B. Peter and Yehuda Schoenfeld eds. Autoantibodies. Elsevier, 1996.
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