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2°C-8°C



Σ=36-60 tests



cat#230596-SP

Immunofluor ANA Hep-2

For qualitative and semiquantitative test of antinuclear antibodies (ANA) in human serum.

36-60 tests

IN VITRO DIAGNOSTIC USE

Method principles

The term “antinuclear antibodies” describes a variety of antibodies which react when together with constituents of the cell nuclei, including DNA, RNA, proteins and ribonucleoproteins. These antibodies are frequently found in patients suffering rheumatic or connective tissue conditions, especially systemic lupus erythematosus (SLE).

Virtually all SLE patients are ANA-positive. In 1982 a sub-committee of the “American College of Rheumatology” reviewed SLE classification criteria, incorporating ANA to the diagnosis. ANA test is an excellent yet unspecific screening for SLE (a negative result virtually discards SLE). Patients suffering other connective tissue diseases, such as rheumatoid arthritis, sclerodermia and dermatomyositis are oftentimes ANA-positive; moreover, low titers of ANA were found in other diseases and in healthy subjects. ANA-positivity may be detected following severe burns or viral infections, as well as in healthy subjects, especially in the elderly.

Therefore, ANA-positive samples should be titrated up to their endpoint and reevaluated through more specific antibody tests, such as anti-DNA (for double-strand anti-DNA antibodies) and ENA (for extractable nuclear antibodies), in order to improve test specificity.

Indirect immunofluorescence is the reference method for ANA. Applicable substrates include tissue sections or different types of cellular lines. Cellular line substrates are known to be more suitable than tissue sections because rapid cell divisions provide clinically significant antigens, including centromere SSA (Ro), Scl-70 and PCNA/Cyclin.

Three other critical factors, apart from the type of substrate, affect ANA test, as: 1) The imprint fixative using in the slide preparation, 2) The fluorescein:protein (F/P) ratio, 3) The specific immunoglobulin subclasses of the conjugate. Some fixatives or combinations of them are known to destroy some nuclear antigens, so their use should be avoided. The sensitivity of the conjugates' unspecific blank images depends on the F/P ratio, whereas conjugate specificity is based on the reactivity of the immunoglobulin subclasses. Virtually all clinically significant autoantibodies present specific IgG subclasses leveled with ANA-specific IgM and IgA, while only IgM and IgA ANA antibodies were found in some healthy blood donors. Thus, IgG specific conjugates are more suitable for diagnosis. The

substrate used in the kit Immunofluor ANA HEP-2 is a cellular line, and the conjugate is highly purified anti-human IgG, with a carefully selected F/P ratio.

The reagents used in this kit are adjusted to detect clinically significant antibodies, including SSA and Scl-70, which cannot be detected by other commercial ANA tests. Furthermore, the specific IgG conjugate discards the physiologic positive results usually resulting from the low titers of specific IgM.

Summary and explanation of the assay

Indirect immunofluorescence technique involves sample incubation in the antigenic substrate and further rinsing of the non-reacting antibodies. The substrate is afterwards incubated along with the fluorescein-labeled specific antigammaglobulin and the unbound reagent is then rinsed. Reading is performed with fluorescence microscope. Antibody-positive samples present an apple-green fluorescence in the cell or nucleous areas which are bound to the antibodies.

Test procedure

1) Buffer preparation

Reconstitute phosphate buffer saline (PBS) up to 1 liter with distilled water. A solution 0.01 M with a pH of 7.2-7.4 is obtained. If necessary adjust with (NaOH 1N or HCL 1N). Store at 2-8°C, in a clean covered container. In case of change of pH, cloudiness, or precipitation, discard.

2) Sample dilution

A) Initial screening: dilute the patients' samples with PBS 1/40 (E.G.: Add 20ml serum to 0.78 ml of PBS).

NOTE: An initial screening dilution 1/20 is suggested for children under 10 years of age.

B) Titration: dilute the positive samples with PBS to obtain graded dilution from the initial one (E.G. 1/80, 1/160,... 1/2560).

3) Samples inoculation

Bring the slides to room temperature without unpacking. Once unpacked, place them in a suitable wet chamber and add one drop of positive and negative control to sites 1 and 2 respectively. Add one drop (50-75µl) of unknown serum 1-40 at the other site (one site per patient.) Incubate the slide for 30 minutes in a wet chamber (plastic or glass flat-bottom container covered with a wet filter paper to keep the right humidity conditions.)

NOTE: Incubation of samples and conjugates may be reduce to 10 minutes each without any sensitivity loss (especially during initial screening.).

IMPORTANT: Avoid drying of the reaction areas over the following steps.

4) Rinsing

Following incubation with serum dilution, rinse with PBS, using plastic dropper or pipette. Bend the slide down and dry some PBS on it, minimizing sample contact between reactive areas. Do not drip PBS straight on the reaction sites, so as not to harm the substrate.

Place the slides in a coplin jar and rinse twice or three times with buffer (5' each time), gently stirring during rinses.

5) Application of the conjugate

Dilute the labeled antigammaglobulin supplied with the kit (suggested dilution: ____) and add 10 µl of Evans blue per ml. of gammaglobulin dilution.

Stop rinsing the slides one at a time, stir them on blotting paper to drain off the excess PBS, drying around the reaction areas (if necessary.) Place all the slides back into the wet chamber.

Immediately apply a drop of conjugate (50-75 µl.) per area, making sure that each zone is thoroughly covered.

Incubate in wet chamber at room temperature for 30'. Protect from excessive light.

6) Rinsing

Repeat step 4.

7) Mounting

Stop rinsing the slides one at a time, stir them on blotting paper to drain off the excess PBS, and add 3-4 drops of mounting medium along the slides.

Gently place the cover on the slide, checking that no bubbles are formed. Drain the excess mounting medium off the preparation edges with blotting paper. Clean the rear side of the slide.

8) Reading

Read the slides in a fluorescence microscope as soon as possible. They should preferably be examined on the same day in which the above procedure is performed. Otherwise, store them in refrigerator at 2-8°C away from light, and read them on the following day. The mounting medium should not dry off between the slide and its cover. If it does, add some additional mounting medium.

Contents of the kit

For 36 tests

- 1- 3 slides with 12 HEp-2 cell reaction areas.
- 2- Antihuman antigamma globulin IgG labeled with fluorescein isothiocyanate (0.15 ml).
- 3- Positive control human serum (1.0 ml). Ready to use.
- 4- Negative control human serum (1.0 ml). Ready to use.
- 5- Phosphate saline buffer (PBS): 2 foil bags. Each container is to be diluted in distilled water to obtain one liter.
- 6- Mounting medium (5.0 ml).
- 7- Evans blue (5.0 ml). Ready to use.
- 8- Slide covers.
- 9- User's guide.

For 60 tests

- 1- 5 slides with 12 HEp-2 cell reaction areas.
- 2- Antihuman antigamma globulin IgG labeled with fluorescein isothiocyanate (0.20 ml).
- 3- Positive control human serum (1.0 ml). Ready to use.
- 4- Negative control human serum (1.0 ml). Ready to use.
- 5- Phosphate saline buffer (PBS): 3 foil bags. Each container is to be diluted in distilled water to obtain one liter.
- 6- Mounting medium (5.0 ml). Ready to use.
- 7- Evans blue (5.0 ml).
- 8- Slide covers.
- 9- User's guide.

Unstability of reconstituted substances

Reconstituted phosphate buffer saline keeps stable for up to 15 days at 2-8°C.

For longer storage periods, add sodium azide 0.1% to prevent contamination.

Required material (not supplied)

- 1- Pasteur pipettes.
- 2- Dropper.
- 3- Immersion oil (optional).
- 4- Accurate pipettes.
- 5- A bowl for rinsing the slides.
- 6- Fluorescence microscope with FITC filter system (maximum stimulation wavelength of 490 nm, mean emission wavelength, 530 nm, excursions to 600-1000).

Warnings and precautions

- 1- Sodium azide 0.1% is used as a preservative. When disposing of reagents, use large amounts of water to prevent waste azide from accumulating in the pipeline. Sodium azide can be poisonous if ingested.
- 2- Control human serum components used in this kit have been found negative for anti-HIV antibodies and hepatitis B surface antigen (HBsAg), but because no diagnosis test can warrant absolute certainty as to the absence of these or other infectious agents, human controls and reagents should be treated as potentially infectious.
- 3- Substitution of components with other not supplied in the system may originate inconsistent results.
- 4- Keep the reaction areas wet throughout the whole technique (avoid any artificial drying techniques, such as air, stove, or heat drying). After rinsing, remove excess humidity with blotting paper; dry only the side outer edges of the slide. Immediately after drying, abundantly spread a properly diluted solution of antigammaglobulin and/or mounting medium, depending on the concerned stage.

Limitations of the procedure

- 1- High titers of ANA suggest connective tissue disease but has no diagnostic status. ANA results should be complemented with other serologic results and with the patient's medical record.
- 2- ANA patterns may be altered if the sample is titrated up to its endpoint. This happens because low antibody titers reach the system sensitivity when a more diluted sample is tested.
- 3- The sensitivity of this method depends upon a variety of external factors, including the type of microscope used, lamp intensity and period, the system of lenses, the system of filters, and the observer.

Storage

Store in refrigerator at 2-8°C.

The kit components are stable up to the expiry date printed in the external and internal labels.

The reagents may be hampered if exposed to light or frozen.

Specimen collection

Treat all blood, plasma and serum samples as though they were capable of transmitting infectious diseases. Antiseptically extract 5-8 ml. of venous blood. Keep the blood at room temperature until separated from serum, to prevent any hemolysis which might hamper test results. Patients should go on an 8-hour fast prior to sample collection.

This test can only be performed on serum. Do not process hemolyzed, contaminated or lipemic samples. Sera may be kept in refrigerator (2-8°C) for up to 72 hours. For longer storage periods, freeze at -20°C. Avoid repeated freezing-unfreezing cycles.

Interpretation of results

Negative reaction

A sample is considered negative if its specific nuclear image is equal or lower than the negative control. Samples may exhibit different degrees of unspecific fluorescence due to the presence of heterophil antibodies or low levels of cytoplasm autoantibodies, such as is the case with the contractile proteins.

Positive reaction

A sample is considered positive if its specific nuclear image is higher than the negative control.

Pattern interpretation

A variety of patterns may be found in nuclear and cytoplasmic images depending on the types and relative amounts of sample autoantibodies, as:

- Homogenous pattern: A solid nuclear taint image, with or without apparent masking of the nucleolus is observed.

Nuclear antigens: ds DNA, ss DNA, histones.

Related diseases: High titers are suggestive of SLE; low titers are suggestive of SLE or other connective tissue diseases.

- Peripheral pattern: A solid taint image is seen around the nuclear membrane, becoming faint towards the nuclear center.

Nuclear antigens: ds DNA, ss DNA, DNP, histones.

Related diseases: High titers are suggestive of SLE; low titers are suggestive of SLE or other connective tissue diseases.

- Speckled pattern: A fine or granulated taint is found at the nuclei, usually lacking fluorescence at the nucleoli.

Nuclear antigens: Sm, RNP, Scl-70, SSA, SSB and other antigen-antibody systems, as yet uncharacterized.

Related diseases: High titers are suggestive of SLE; low titers are suggestive of SLE (Sm antibody), mixed connective tissue disease (RNP antibody), sclerodermia (Scl-70 antibody) or Sjögren's syndrome sicca complex (antibody SSB), low titers are suggestive of other connective tissue diseases

- Nucleolar pattern: A taint image presenting large and thick granulations, usually containing less than 6 granulations per cell, with or without occasional fine granulations, is found in the nuclei.

Nuclear antigens: RNA 4-6S and other unknown nuclear antibodies.

Related diseases: High titers are predominant in sclerodermia and Sjögren's syndrome.

- Centromeric pattern: A slightly granular taint image is observed. Granulations are highly discrete, usually appearing as a multiple of 46.

Nuclear antigens: Chromosomal centromere (kinetochore).

Related diseases: Highly suggestive of CREST, a variant of Progressive Sclerosis Syndrome (PSS). CREST is a kind of PSS with prominent calcinosis, Raynaud's phenomenon, esophagic dysmotility and skin-restricted injury (usually in the fingers and the face), and telangiectasia.

- Mitochondrial pattern: A discretely granulated taint image is found at the cytoplasm and relatively absent from the nuclear area.

Antigens: Various types of mitochondrial antigens.

Related disease: High titers are suggestive of primary biliary cirrhosis.

Patterns should be used with caution in the test of specific antibodies, except in the case of nucleolar and centromeric images, which present distinctly defined antigens and a characteristic pattern. Because many autoantibodies or combinations of them may induce homogenous or speckled images, specific antibody tests, (such as a-DNA or ENA) are recommended in such cases.

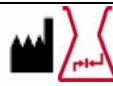
Characteristics of the test

A number of patients presenting connective tissue disease were compared against 200 healthy blood donors. Following are the obtained results:

Group of patients	No.	Positives with Immunofluor ANA HEp-2
SLE	105	105
Drug-induced lupus	24	24
Rheumatoid arthritis	40	28
Sclerodermia	24	18
Dermatomyositis	14	10
Sjögren's syndrome	14	10
Healthy subjects	200	5

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