Test Instructions

Enzyme Immunoassay for the Detection of Antinuclear Antibodies

Catalogue-No.: TC 60001

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

Never mix reagents from different test kit lots.

Store reagents at 2-8°C.

1. Clinical Use

Antinuclear antibodies (ANA) are autoantibodies with different specificities against antigens of the cell nucleus. The detection is important for diagnosis of collagenosis especially of systemic lupus erythematosus (SLE) and the mixed connective tissue disease (MCTD) which is strongly associated with it, and also of other rheumatic diseases. In SLE patients and MCTD patients ANA are detectable at almost 100 %. From this follows, that the absence of these antibodies practically excludes a diagnosis of these. A positive detection of ANA is on the other hand not a proof for these diseases because ANA are detectable in other diseases, too.

2. Principle of the Test

The test is based on the covalent binding of HeLa cell nuclei, carefully isolated by density gradient centrifugation, to the solid phase of microtiter strips and subsequent binding of ANA from patient serum. The bound antibodies are detected with an peroxidase-labeled secondary antibody that is directed against human IgG, IgM and IgA.
After the addition of substrate solution, a color stain develops and its intensity is proportional to the concentration and/or the avidity of the detected autoantibodies.

3. Test characteristics

The specifity of the enzyme immuno assay (EIA) was examined by comparison of 282 sera with an indirect immune fluorescence test, an Immunoblotting test and an EIA using recombinant antigens. Interestingly none of the methods used for comparison can be considered absolute "correctly". It was shown for example, that the EIA using recombinant antigens to determine ANA gave rise to wrongly negative results in 1.4% and wrongly positive results in 0.4% of all samples.
The EIA on hand has a high specifity and sensitivity, caused primarily by the specific interaction of ANA with nuclei as its natural target unlike to EIA using an ENA cocktail instead.
The assay allows the effective and economical determination of larger numbers of samples under standardized conditions. It delivers quantitative and objective results, making it possible to determine of different classes of immunoglobulins.
The coefficient of intra and inter variation for the assay was determined with 4.14% and 4.9% respectively.

4. Materials Provided

Item	Quantity
- coated microtiter strips (1x8), breakable	12 strips
- standards, ready to use, **1:** 31.25 U/ml **2:** 62.5 U/ml **3:** 125 U/ml **4:** 250 U/ml **5:** 500 U/ml (all standards contain sodium azide)	750 µl per vial 1 vial each
- negative control serum, ready to use, (contains sodium azide)	1 vial 1 ml
- positive control serum, ready to use, (contains sodium azide)	1 vial 1 ml
- washing buffer concentrate (10x), (contains thimerosal)	1 bottle 50 ml
- sample buffer concentrate (5x), (contains thimerosal)	1 bottle 22 ml
- conjugate buffer, ready to use (contains thimerosal)	1 bottle 20 ml
- peroxidase conjugate concentrate (anti-human IgG/A/M), (100x),	1 vial 200 µl
- peroxidase substrate solution (TMB), ready to use,	1 bottle 12 ml
- stopping solution, H_2SO_4, ready to use,	1 bottle 12 ml

5. Preparation of Reagents

Allow kit to reach room temperature !

5.1. Preparation of Washing Buffer

If any salt has been cristallized inside the bottle, it must be resolved before use. Dilute 1 unit washing buffer concentrate with 9 units distilled water. The diluted buffer is stable for 6 weeks stored at 2 - 8 °C.

5.2. Preparation of Sample Buffer

If any salt has been cristallized inside the bottle, it must be resolved before use. Dilute 1 unit sample buffer concentrate with 4 units distilled water. The diluted buffer is stable for 6 weeks stored at 2 - 8 °C.

5.3. Preparation of Standards

The standards are ready to use.

5.4. Preparation of Control Sera

The control sera are ready to use.

5.5. Preparation of Sera

Use freshly collected serum samples or freeze samples at – 20 °C until analysis. Allow the samples to reach room temperature (30 min). Dilute samples 1 : 100 with sample buffer (10 µl sample to 1 ml buffer).

5.6. Preparation of Conjugate

The amount of conjugate dilution required daily, is to be prepared freshly. Do not use polystyrene tubes to prepare the conjugate dilution. Dilute conjugate 1:100 with dilution buffer (for 1 plate: 100 µl conjugate with 10 ml buffer, for 2 strips: 20 µl conjugate with 2 ml buffer). Remaining solution should be disposed of.

5.7. Preparation of the Substrate

The TMB-substrate solution is ready to use. The opened substrate bottle should be closed carefully. Store substrate solution at 2– 8 °C protected from light.

5.8. Microtiter Strips

The strips are ready to use. Unused strips should be stored in the lockable original bag at 2 - 8 °C.

5.9. Stopping Solution

H_2SO_4 (caution !)

6. Test Procedure

- ***Pipette 100 µl serum dilution*** or (undiluted) standards and control sera into each well, for blanks use ready to use sample buffer instead of serum dilution, seal wells with adhesive foil
- ***Incubate for 1 hour*** at room temperature (RT)
- ***Rinse the wells 3 x*** with min. 200 µl washing buffer per well
- ***Pipette 100 µl of conjugate dilution*** into each well, seal wells with adhesive foil
- ***Incubate for 30 minutes*** **at RT**
- ***Rinse the wells 3 x*** with min. 200 µl washing buffer per well
- ***Pipette 100 µl substrate solution*** into each well.
- ***Incubate for 10 min*** at RT in the dark. At room temperatures above 25 °C the substrate incubation could be shortened, but should never fall short of 5 minutes.
- ***Pipette 100 µl stopping solution*** per well.
- ***Measure at 450 nm*** within the next 30 min after stopping

7. Interpretation of Results

Calibrate measured absorbances against concentrations/units of standards (31.25 U/ml, 62.5 U/ml, 125 U/ml, 250 U/ml, 500 U/ml) in semi log. Determine the units of the examined samples from the standard curve directly.
Results in the range of 40 (cut off value) - 55 U/ml are borderline or slightly positive. Results above 55 U/ml are considered positive.
To prove the functionality of the test, the value determined for the positive control serum is to be expected within the range labeled on the vial. The result of the negative control has to be lower than the cut off value of the testkit.

Precautions

For in vitro diagnostic use only.

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.

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