

Test Instructions

Enzyme Immunoassay for the Detection of IgM, IgG and IgA Rheumatoid Factors (Screening Test)

Catalogue-No.: TC 60000

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

Reagents from different test kit lots must not be mixed.

1. Clinical Use

Rheumatoid factors are autoantibodies which are directed against the Fc-part of the IgG molecule. They are the most frequent autoantibodies in man.

Rheumatoid factors can belong to all classes of immunoglobulins. In practice importance has only been attached to rheumatoid factors of IgM type because only such rheumatoid factors can be detected with hitherto existing tests like latex agglutination or hemagglutination tests.

Rheumatoid factors of IgM type are found in about 75% to 90% of patients with rheumatoid arthritis depending on the sensitivity of the test. There exists a certain correlation between the presence of IgM-rheumatoid factors and the activity of the disease. But in about 10% to 25% of patients with rheumatoid arthritis rheumatoid factors are not detectable (seronegative rheumatoid arthritis), so that a negative test result does not exclude a manifest rheumatoid arthritis.

Apart from that in patients with a freshly established rheumatoid arthritis the synthesis of rheumatoid factors is retarded (for 4 weeks and more).

Depending on the method used in about 19% to 88% of patients with rheumatoid arthritis rheumatoid factors of IgA type can be found. The diagnostic significance of IgA-rheumatoid factors has not yet been clarified unequivocally. An increased concentration of IgA-rheumatoid factors has been found in primary Sjogren's syndrome (70%) and in vasculitis. Rheumatoid factors of IgA type may be responsible for the development of certain forms of nephropathy.

Rheumatoid factors of IgG type can be found mainly in patients with a chronic polyarthritis and they are always associated in such cases with the presence of IgM-rheumatoid factors. Their diagnostic relevance should be looked at critically, because the existing methods for the specific and quantitative determination of IgG-rheumatoid factors are problematic.

If rheumatoid factors are detectable in a patient this finding does not point to an existing rheumatoid arthritis because rheumatoid factors are also found in other diseases.

2. Principle of the Test

The test is based on the covalent immobilisation of rabbit IgG to a chemically activated microtiter plate (patent pending) and subsequent binding of the rheumatoid factors from serum.

For the detection of rheumatoid factors bound in this way a peroxidase-labelled antibody is used which is directed against human IgG, IgM and IgA. After addition of a substrate solution, a color stain develops, the intensity of which is proportional to the concentration of the rheumatoid factors.

3. Material Provided

- coated microtiter strips (1 x 8), breakable	12 strips
- standards; ready to use,	1 vial each à 750 µl
1: 12.5 U/ml	
2: 25 U/ml	
3: 50 U/ml	
4: 100 U/ml	
5: 200 U/ml (contain sodium azide)	
- 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 sodium azide)	1 bottle 22 ml
- conjugate buffer ready to use, (contains thimerosal)	1 bottle 20 ml
- peroxidase conjugate, anti-human Ig(G,A,M), concentrate (100x),	1 vial 200 µl
- peroxidase substrate, (TMB), ready to use,	1 bottle 12 ml
- stopping solution, H ₂ SO ₄ , ready to use,	1 bottle 12 ml

4. Preparation of Reagents

Allow the kit to reach room temperature!

4.1 Preparation of Washing Buffer

Should any of the salts be crystallized they should be resolved before use. Dilute 1 unit washing buffer concentrate with 9 units distilled water. The ready to use buffer is stable for 6 weeks stored at 2 - 8 °C.

4.2 Preparation of Sample Buffer

Dilute 1 unit sample buffer concentrate with 4 units distilled water. Should any of the salts be crystallized they should be returned to solution before use. The ready to use buffer is stable for 6 weeks stored at 2 - 8 °C.

4.3 Preparation of Standards and Control Sera

The standard solutions and control sera are ready to use.

4.4 Preparation of Sera

Use serum samples freshly collected or freeze the samples at minus 20 °C. Do not use samples, which are repeatedly thawed and re-frozen. Do not use serum samples inactivated by heat treatment at 56 °C.

Allow sera to reach room temperature (30 min). Dilute sera 1:100 with sample diluent buffer (10 µl serum with 1.0 ml buffer).

4.5 Preparation of Conjugate

The daily required amount of conjugate solution should be prepared freshly. Do not use polystyrene tubes to prepare the conjugate dilution. Dilute conjugate 1:100 with conjugate diluent 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.

4.6 Preparation of the Substrate

The TMB-substrate solution is ready to use. Used substrate bottle should be closed carefully. Store substrate solution at 4 - 8 °C protected from light.

4.7 Microtiter Strips

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

4.8 Stopping Solution

H₂SO₄ (caution!)

5. Test Procedure

1. Pipette **100 µl sample dilution** resp. (undiluted) standards and control sera into each well, for blanks use dilution buffer instead of sample dilution, seal wells with adhesive foil

2. **Incubate for 1 hour** at room temperature (RT)

3. **Rinse off the wells 3 x** with min. 200 µl washing buffer per well

4. Pipette **100 µl** of anti-human-Ig(G,A,M)- conjugate dilution into each well

5. **Incubate for 30 minutes** at RT

6. **Rinse off the wells 3 x** with min. 200 µl washing buffer per well

7. Pipette **100 µl substrate solution** per well.

8. **Incubate for 10 minutes** at RT in the dark. At a room temperature higher than 25 °C the substrate incubation time should be shortened. The minimum substrate incubation time must be 5 minutes.

9. Pipette **100 µl stopping reagent** per well.

10. Measure at **450 nm** within the next 30 min after stopping

6. Interpretation of Results

Calibrate measured absorbance against concentrations/units of standards (12.5 U/ml, 25U/ml, 50 U/ml, 100 U/ml, 200 U/ml), (linear or semilog). Read the units of the examined sera from the standard curve directly. Results above 40 U/ml (cut off value) are considered positive.

To prove the test function the value of the corresponding positive control serum has to be within the range (see label on the vial). The negative control result has to be lower than the cut off value of the test.

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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