

Cenogenics

R. F. Latex Test Set

INTENDED USE

Rapid latex agglutination test for the qualitative screening and semi-quantitative determination of Rheumatoid Factor (anti-gamma globulin).

SUMMARY AND EXPLANATION

Rheumatoid arthritis is a chronic systemic disease of unknown etiology. It is frequently characterized by swelling and pain in the joints and by inflammatory and degenerative processes involving cartilage, synovial membrane or muscle tissue. The disease is widespread in the United States and throughout the world, and is found in all age groups. Most typically, its onset is in young adults in their thirties and forties. While no specific cure has yet been found, early therapy is of great value in halting or minimizing irreversible damage to the joints. For this reason prompt diagnosis is of great importance.

A characteristic of rheumatoid arthritis is the presence in the blood and in synovial fluid of a reactive group of proteins called Rheumatoid Factor.^{1,2} These are macroglobulins having a molecular weight of 1 million. In the opinion of many investigators, the Rheumatoid Factors are antibodies directed against "altered" human gamma globulins.^{4,5,6} The Rheumatoid Factors are found in 70-100% of cases of definite rheumatoid arthritis depending on the test procedure used to detect them. Because of this widespread incidence of RF, its demonstration is a useful laboratory criterion for the diagnosis of suspected rheumatoid arthritis. By comparison the occurrence of RF in osteoarthritis or rheumatic fever is less than 2 and 3% respectively. It should be noted that incidence of RF have been reported in a variety of nonrheumatic diseases such as pulmonary tuberculosis, bacterial endocarditis and syphilis as well as others. A significant incidence of RF in the aged has also been observed.

Since the discovery of RF, there have been many techniques developed to identify and quantitate these factors. The most generally useful techniques have been agglutination procedures employing polystyrene particles coated with a layer of adsorbed human gamma globulin.⁷ The RF present in a test serum reacts with the coating material causing a visible agglutination of the inert latex particles. It is this reaction which is the basis of the CENOGENICS' RF TEST.

CENOGENICS' RF TEST SET for the detection of Rheumatoid Factors rapidly and accurately identifies the presence of RF, one of the criteria for the diagnosis of rheumatoid arthritis.

In the presence of Rheumatoid Factor positive antiserum, CENOGENICS' latex-globulin RF reagent can be used to demonstrate agglutination both qualitatively and quantitatively.

PRINCIPLES OF THE PROCEDURE

The principle of the test is an immunologic reaction between the Rheumatoid Factor (RF), a macromolecular molecule globulin found in serum and the corresponding IgG coated onto finely dispersed polystyrene latex particles.

REAGENTS

RF LATEX REAGENT: a suspension of polystyrene latex particles in glycine-saline buffer, pH 8.4 ± 0.2 . The latex particles are coated with human IgG.

GLYCINE-SALINE BUFFER (20X) CONCENTRATE, pH 8.4 ± 0.2 , is to be diluted 1:20 with distilled water.

POSITIVE CONTROL SERUM: a stabilized human serum containing rheumatoid factors reactive with the latex reagent.

NEGATIVE CONTROL SERUM: a stabilized human serum nonreactive with the latex reagent.

Note: All reagents are preserved with sodium azide (1mg/ml).

WARNING

For in vitro diagnostic use.

STORAGE CONDITIONS

Store at 2° — 8° C.

STABILITY

Expiration date is specified on the label. Biological indication of product instability is evidenced by inappropriate reaction of the latex reagent with the corresponding positive and negative controls.

SPECIMENS

Collection and preparation:

The test should be performed on serum. Specimen can be drawn by venipuncture or convenient fingertip method. Plasma should not be used because fibrinogen may cause nonspecific agglutination of the latex particles.

Interfering substances:

Heavy bacterial contamination may cause positive agglutination. Markedly lipemic sera should not be tested because of the possibility of nonspecific reactions.

Storage conditions:

Fresh specimens should be used. If testing is delayed, specimens should be refrigerated (or frozen where applicable).

PROCEDURE

Materials supplied with the LATEX TEST SET:

1. RF Latex Reagent
2. Glycine-Saline Buffer
3. Positive Control Serum
4. Negative Control Serum
5. 6-Well Glass Slide
6. Applicator Sticks
7. Disposable Pipettes

Materials required but not provided:

1. Test Tubes (for dilution)
2. Serological Pipettes
3. Laboratory Timer
4. Laboratory Rotator (optional)

METHOD I (SCREENING)

1. Bring all reagents and serum samples to room temperature.
2. Prepare a 1:20 dilution of the serum to be tested using the diluted glycine-saline buffer (1 + 19).
3. Shake the RF reagent gently, expel contents of the dropper and refill. Mix one drop (approx. 0.05 ml) with one drop of the diluted serum on glass slide with applicator stick.
4. Continue to mix for one minute with rotator or by hand. Observe for macroscopic clumping using the indirect oblique light source.
5. Positive and negative controls should be run with each series of test sera. The controls supplied by CENOGENICS are to be used exactly as outlined in steps 1 thru 4 above without further dilution.

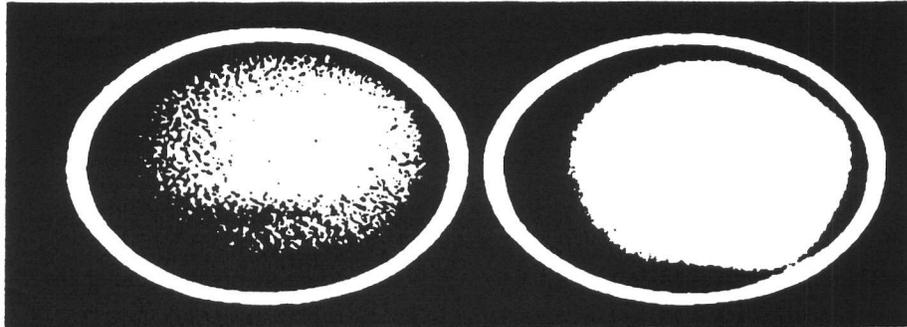
6. The reaction of the test serum is compared to the positive and negative control sera.

QUALITY CONTROL PROCEDURE

A positive control will produce within one minute coarse agglutinated flocs against a clear background.

A negative control will produce no agglutination. It should be used as a basis for comparison. The relative degree of smoothness of the reagent itself should be considered and incorporated in reading the results.

If the indicated results, using the positive and negative controls are not obtained, the RF Latex Kit should not be used.



Coarse Agglutination =
Positive

No Agglutination =
Negative

RESULTS

An agglutination of the latex particle suspension is a positive result.

A weakly reactive serum produces a very fine granulation or partial clumping. The results should be read at one minute because nonspecific reactions may occur after this period.

Sera that are positive in the screening test should be retested in the titration test to provide verification for borderline interpretations.

METHOD II (QUANTITATIVE TUBE TEST)

1. Allow all reagents to come to room temperature.
2. Place eleven (12 x 75 mm) test tubes into a rack and label 1 through 11. Pipet 1.9 ml of diluted glycine-saline buffer (1:20) into tube #1, 1.0 ml buffer into tubes 2-9, and 0.8 ml buffer into tubes 10 and 11.
3. Pipet 0.1 ml of patient serum into tube #1. Mix and transfer 1.0 ml from tube #1 into tube #2. Continue this procedure through tube #9 and discard 1.0 ml from tube #9.
4. Tubes 10 - 11 are controls. Pipet 0.2 ml of the RF Positive Control into tube #10 and 0.2 ml of the RF Negative Control into tube #11.
5. The dilutions are as follows:

Tube No.	Dilutions
1	1:20
2	1:40
3	1:80
4	1:160
5	1:320
6	1:640
7	1:1280
8	1:2560
9	1:5120
10	Positive Control
11	Negative Control

6. Into each tube (1-11) add one drop of RF Latex Reagent using the disposable pipet provided.
7. Shake the tubes and incubate for 15 minutes in a 37°C water bath.
8. Centrifuge the tubes at approximately 1000 RCF for 2 minutes.
9. Gently shake the tubes (manually) until the sediment is dislodged from the bottom. Gently tilt the tubes back and forth until an even suspension is obtained.

10. Examine each tube for macroscopic agglutination utilizing oblique illumination.

RESULTS

1. The titer at which agglutination can still be observed is a measure of the Rheumatoid Factor activity. If a secondary standard of the International Reference Preparation of Rheumatoid Arthritis Serum is used, the results can be expressed in IU/ml, using the following equation:

$$\text{IU/ml of sample} = \frac{\text{IU/ml/standard} \times \text{titer of sample}}{\text{titer of standard}}$$

2. A titer of 80 or greater is considered a positive reaction.
3. A titer of 20 or 40 is considered a weakly positive reaction.

If there is agglutination in only a single tube other than 1:20 dilution, it is considered to be a negative result.

QUALITY CONTROL FOR QUANTITATIVE TUBE TEST

1. A negative control tube (#11) should form a smooth suspension. If it shows clumping, the test is invalid and must be repeated.
2. The positive control tube (#10) should show visible agglutination. If no clumping is observed, the test is invalid and must be repeated.

LIMITATIONS OF PROCEDURE

The results obtained by using the qualitative slide test versus the quantitative tube test are not directly comparable due to the fact that they are two different test conditions.

The slide test provides a quick screening procedure, while the tube dilution may be more valuable clinically since it has the ability to provide quantitative information.

EXPECTED VALUES AND SPECIFIC PERFORMANCE CHARACTERISTICS

The clinical significance of RF determination consist in differentiating between rheumatoid arthritis, in which the rheumatoid factor has been demonstrated in the serum of approximately 80% of the cases examined and rheumatic fever in which the rheumatoid factor is almost always absent.⁸ The RF is more frequently positive in active processes of greater duration than in diseases which are less active or are still in early stages.

It is occasionally found in the serum of patients with polyarthritis nodosa, systemic lupus erythematosus and a variety of chronic inflammatory illnesses such as tuberculosis, leprosy, syphilis and bacterial endocarditis. Sera tested from these related diseases showed positive reactions in approximately 6% of tested cases.

Approximately 3.5% of known rheumatoid patients do not react in the screening test, on the other hand, 2% of sera from apparently healthy individuals gave RF reaction.

BIBLIOGRAPHY

1. Waaler, E., Acta Path, Microb. Scand. 17, 1&2, 1940.
2. Rose, H.M., Ragan C., Pierce, E. & Lipman, M.O., Proc. Soc. Exptl. Viol. Med., 68, 1, 1948.
3. Waller, M., C.R.C. Reviews in Medical Sci., June 1971, P. 173.
4. Bandilla, K.L. and McDuffie, F.C., Arthritis Rheum., 12, 74, 1969.
5. Hannestad, K., Clin. Exp. Immunol., 3, 671, 1968.
6. Epskin, W., Johsen, A. & Ragan C., Proc. Soc. Exptl. Biol-Med, 91, 235, 1956.
7. Lane, J.J. Jr. and Decker, J.L., JAMA 173, 982, 1960.
8. Muller W., The Serology of Rheumatoid Arthritis; Berlin-Goettlingen-Heidelberg, 1962, P. 97.

CENOGENICS CORPORATION
Morganville, N.J. 07751

Printed in U.S.A.
PG-0324-02