Stanbio RaPET® RF Procedure No. 1155

Latex agglutination slide test for the Qualitative and Semi-quantitative determination of Rheumatoid Factor in non-diluted serum

Intended Use

RaPET® RF is intended for the qualitative and semi-quantitative detection of rhematoid factor in human serum. The latex slide test is intended to be used as an aid in the diagnosis of rheumatoid arthritis.

Summary and Principle

Rheumatoid factors (RF) are antibodies which are directed against the changed tertiary structure of the IgG Fc fragment[1,2]. They occur in all immunoglobulin classes; the RF isotypes are designated IgM, IgG and IgA rheumatoid factors. Testing for RF has a high diagnostic value as their exclusion or detection can support or place doubt on a tentative diagnosis made on the basis of case history data and clinical findings. A seropositive rheumatoid arthritis generally has a more unfavorable prognosis than the seronegative form.

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 latex particles coated with a layer of absorbed human gamma globulin.[3] The RF present in a test specimen reacts with the coated material causing a visible agglutination of inert latex particles. It is this reaction which is the basis of the Stanbio RaPET® RF test. Utilizing our ***Ra***pid ***P***article ***E***nhanced ***T***echnology (RaPET®), test serum found positive for RF develops an immunologic reaction due to its macromolecular globulins reacting with the corresponding IgG coated polystyrene latex particles.

Reagents

RF Latex Reagent, Ref. No. 1156 (White Cap)
Suspension of 1% polystyrene latex particles coated with human gamma-gobulin in a saline buffer.

RF Positive Control, Ref. No. 1157 (Red Cap)
Stabilized human serum, containing more than 20 IU/mL (formulated to be 25 IU/mL) of Rheumatoid Factor.

Negative Control, Ref. No. 1192 (Green Cap)
Stabilized human serum, free of Rheumatoid Factor.

Glycine-Saline Buffer (20X) Concentrate, Ref. No. 1191
Solution of glycine and sodium chloride, pH 8.2 ± 0.1.

Warnings and Precautions: For In Vitro Diagnostic Use.
The controls used in this kit have been tested by an FDA-approved method and found non-reactive for the presence of HBsAg and antibody to HIV. While these methods are highly accurate, no test can offer complete assurance that infectious agents are absent. This material, as well as all patient samples, should be handled as though capable of transmitting infectious disease. The United States Food and Drug Administration recommends such samples be handled as Center for Disease Control's Biosafety Level 2.

Reagents in this kit contain sodium azide as a preservative. Sodium azide has been reported to form lead or copper azide in laboratory plumbing which may explode on percussion. Flush drains with water thoroughly after disposing of fluids containing sodium azide.

Reagent Preparation and Stability

Reagents are stable when stored at 2-8°C until the expiration date shown on their respective labels. Prepare Glycine-Saline Buffer by adding the Concentrate (5mL) to a 100mL volumetric flask and diluting to the 100mL mark with distilled or deionized water. Store prepared reagent at 2-8°C for 12 months from date of preparation.**Do Not Freeze!**

Specimen Collection and Preparation

It is recommended that serum only be used. Samples must have clotted completely and contain no particulates nor traces of fibrin after thawing. Do not heat inactivate test sera or controls. Avoid repeated freeze/thawing of specimens.

Sample Stability: Serum specimens are reportedly stable up to 8 days at 2-8°C and for up to 3 months if they are frozen (at or below -25°C) within 24 hours after venipuncture.

Interfering Substances: In all serological tests, hemolytic, lipemic or turbid sera may cause incorrect results and should not be used. Use only a clean, dry slide washed in mild detergent and rinsed thoroughly with distilled water.

Materials Provided

Plastic Slide - 6 cell
Disposable Pipette/Mixers

Material Required (Not Provided)

Timer Rotator (optional)
Test Tubes & Rack (Semi-quantitative test only)
Serological Pipettes (Semi-quantitative test only)

Procedure

A. Qualitative Method

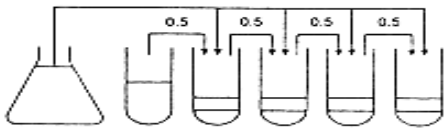
1. Bring reagents and patient samples to room temperature.
2. Gently shake the reagent vial to disperse and suspend the latex particles. **Do Not Shake Vigorously!**
3. Using a dispensing pipette provided, add one drop (40µL) of the non-diluted patient sample(s) onto a separate cell(s) on the slide. Also place one (1) drop of the positive and negative control on separate

cells of the test slide.
4. Gently mix the contents of the latex reagent including the contents of the glass dropper. Fill the dropper with the well-mixed latex suspension and place one (1) drop next to the drop of serum sample(s) on each of the separate cells in use.
5. Mix both drops with the mixer provided, covering the whole surface of each of the cell(s).
6. Gently rotate the slide for 2 minutes manually or on a rotary shaker set at 80 - 100 rpm.
7. After the 2 minutes, examine each cell(s) for the absence or presence of agglutination.

B. Semi-Quantitative Method

1. This reaction can be used to estimate the RF concentration using a dilution series. The patient sample is diluted with the diluted Glycine-Saline Buffer as shown:

Dispense 0.5mL into each tube



Buffer | Patient Serum | 1:2 | 1:4 | 1:8 | 1:16

Thereafter, each is tested with the latex as described under Procedure section "A. Qualitative Method."

Quality Control

A positive control and a negative control should be included in each test seriesfor both qualitative and semi-quantitative methods. A positive control will produce a coarse agglutination against a clear background, while a negative control will produce a smooth, homogeneous suspension.

Results

A. Qualitative Method

Agglutination identifies a RF concentration greater than 20 IU/mL in the sample. Samples that do not show agglutination contain RF concentrations less than 20 IU/mL.

B. Semi-Quantitative Test Results

The titer of the serum is the reciprocal of the highest dilution which exhibits a positive reaction.
An estimate of the RF concentration in the specimen can be expressed in IU/mL by using the following equation:

IU/mL of specimen = IU/mL control × specimen titer

Limitations

Reaction times greater than 2 minutes can lead to false positive results (due to drying effect). Very lipemic sera can also cause non-specific reactions.
Strength of agglutination is not indicative of the RF concentration in the sample.
Highly lipemic sera may cause unspecific reactions.

Expected Values

The clinical significance of RF determination consists 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.[4] Rheumatoid Factor is occasionally found in the serum of patients with polyarteritis nodosa, systemic lupus erythematosis and a variety of chronic inflammatory illnesses such as tuberculosis, leprosy, syphillis and bacterial endocaritis. Approximately 3.5% of known rheumatoid patients do not react in the screening test. Additionally, 2% of the sera from healthy individuals gave positive RF reactionsin the screening test. It is recommended that each laboratory establish ranges of expected values since differences exist between laboratories and local population.

Performance Characteristics[5]

Relative Sensitivity: The performance of RaPET® RF was compared with a competitor's test and determined to be 100% in agreement. Ranges measured were 15.6 IU/mL to 2105 IU/mL.
Relative Specificity: The assay is specific for Rheumatoid factor. A 100% agreement was determined between the RaPET® RF and a competitor's test. In an interference study, three concentrations of CRP (5, 10 and 25 mg/dL) were added in RF free, 20 IU/mL and 900 IU/mL RF spiked solutions. None of these concentrations of CRP interfered in the assay.
Reproducibility: In a within run precision study, two serum controls were assayed 20 times on both the RaPET® RF and a competitor's test. This study demonstrated a 100% agreement between these tests.
Linearity: In a linearity study, a standard of 2105 IU/mL was serially diluted. Standards of 2105, 1000, 500, 250, 125, 62.5, 31.2, 20, 15.6 and 7.8 IU/mL were tested. All results were positive excluding 15.6 and 7.8 IU/mL.
Prozone: Concentration of standard as high as 2105 IU/mL produced positive results.
Correlation Study: (Semi-quantitative) A comparison study was performed between the semi-quantitative procedure described (Y) and a similar established technique (X). Serum samples of 31 run in duplicate ranged from 20 to 640 IU/mL.

Sample	Correlation Coefficient	Sample Size	Regression Equation	Total Error
Serum	0.998	31	Y=1.003X - 2.82	8.69

(Qualitative) A comparison study was performed between the RaPET® RF qualitative procedure and a competitor's test and determined to be 100% in agreement.

References

1. Kunkel, H.G., Williams, R.C.: Ann. Rev. Med. 15:37, 1964
2. Winchester, R.J.: Ann. NY. Acad. Sci. 256:73, 1975
3. Lane, J.J. Jr. and Deckar, J.L., JAMA 173, 982, 1960.
4. Muller W., The Serology of Rheumatoid Arthritis; Berlin-Goettingen-Heidelberg, 1962, P. 97.
5. Stanbio Laboratory Data

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