

Expected values

Establishing «normal values» for rheumatoid factor is difficult since its presence has little diagnostic value if it is not accompanied by signs that suggest the existence of rheumatoid arthritis.

In at least 70-80% of patients with rheumatoid arthritis the existence of rheumatoid factor can be proved through significant titers. The higher titers are usually related with the most serious cases of rheumatoid arthritis. The remaining patients are considered seronegative having rheumatoid factor titers falling in the normal range or not detectable by conventional tests.

In approximately 3-5% of the normal population there can be found rheumatoid factor at low titers. This proportion increases with the age of the population being studied.^{3,4}

Performance characteristics

Sure-Vue® RF was evaluated by comparison with a commercially available latex test (samples 1:6 diluted). A total of 192 serum samples from hospital patients were tested following the qualitative test. This study demonstrated a 91.7% agreement between the tests. The discrepant results were subsequently re-analyzed by means of a quantitative latex enhanced turbidimetric method being their titers between 10 and 30 IU/mL.

In a second study the **Sure-Vue® RF** was compared to another commercially available latex test (undiluted samples). A total of 223 serum samples were tested including random patients samples from a hospital and stored samples previously known to be positive for rheumatoid factor. This study demonstrated a 96.0% agreement between both tests. The discrepant sera exhibited borderline results.

A panel of 10 positive serum samples was tested on three consecutive days using the semiquantitative test. The results of the study indicate that **Sure-Vue® RF** in-house reproducibility (within one dilution) was 100%.

References

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Sure-Vue® RF

Rapid test for the qualitative and semiquantitative determination of rheumatoid factor in serum by agglutination of latex particles on slide. Measurement of rheumatoid factor may aid in the diagnosis of rheumatoid arthritis.

Summary

The term rheumatoid factor evolved from the observations of Waaler¹ and Rose², who noted that serum from a high proportion of patients with rheumatoid arthritis agglutinated sheep erythrocytes sensitized with specific rabbit antibodies. Rheumatoid factor is now defined as a group of closely related antibodies specific to antigenic determinants on the Fc fragments of human or animal immunoglobulin G. They commonly belong to the three major classes of immunoglobulins.^{3,4}

A variety of tests are now available for the determination of rheumatoid factor being agglutination of human gammaglobulin coated latex particles one of the most widely used.^{3,5} A positive latex test, indicating the existence of rheumatoid factor, may be decisive for the diagnosis of rheumatoid arthritis in patients with inflammatory arthritis.

Principle

The **Sure-Vue® RF** reagent is a suspension of polystyrene latex particles of uniform size coated with human gammaglobulin. Latex particles allow visual observation of the antigen-antibody reaction. If the reaction takes place, due to the presence of rheumatoid factor in the serum, the latex suspension changes its uniform appearance and a clear agglutination becomes evident. This change occurs because the rheumatoid factor present in the serum reacts with the IgG coated to the latex particles, starting the formation of a web between them.

When you mix the latex reagent with the serum, if the serum contains approximately more than 10 IU/mL of rheumatoid factor, a clear agglutination will appear.

Results are expressed in International Units per mL (IU/mL) based on the WHO International Reference Preparation of Rheumatoid Arthritis Serum.⁶

Reagents

- a) **Latex reagent:**
Suspension of human IgG coated polystyrene latex particles in a buffer. Contains sodium azide 0.1%.
- b) **Positive control:**
Diluted human serum containing more than 10 IU/mL of rheumatoid factor. Ready to use. Contains sodium azide 0.1%.
- c) **Negative control:**
Diluted human serum containing less than 10 IU/mL of rheumatoid factor. Ready to use. Contains sodium azide 0.1%.

Precautions

Sure-Vue® RF is intended for IN VITRO diagnostic use.

The 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.

Each donor unit used in the preparation of the controls of this kit was tested by an FDA approved method for the presence of HIV 1/2 and HCV antibodies as well as for hepatitis B surface antigen and found to be negative.

WARNING: POTENTIALLY BIOHAZARDOUS MATERIAL.

Because no test method can offer complete assurance that HIV 1/2, HCV, hepatitis B virus, or other infectious agents are absent, the controls of this kit and serum samples should be handled carefully following procedures recommended for biohazardous material.⁷

Storage

The reagents will remain stable through the expiration date shown on the label, if stored between 2 and 8°C. Do not freeze. The reagents can be damaged by improper handling, especially temperature extremes. Checking with the positive and negative controls provided will permit detection of reagents deterioration.

The reagents should not be used after the expiration date shown on the label.

The latex reagent, once shaken, must be uniform without visible clumping. When stored a slight sedimentation may occur and should be considered normal.

Do not use reagents if they become contaminated.

The reagent dropper dispenses drops of 50 µL ± 10%. The dropper must be held perpendicular to the slide surface and a single drop allowed to fall. Do not use another dropper without previously checking the volume of the drop.

Available packaging

- Kit 50 tests, Cat. No. 23 038003.
Contains: 1 x 2.5 mL reagent, 1 x 1.0 mL positive control, 1 x 1.0 mL negative control and 9 disposable slides with 6 sections each.
- Kit 100 tests, Cat. No. 23 038002.
Contains: 2 x 2.5 mL reagent, 1 x 1.5 mL positive control, 1 x 1.5 mL negative control and 18 disposable slides with 6 sections each.

Material required but not provided

- Normal saline (0.9% NaCl, only for semiquantitative test).
- Automatic pipettes.
- Disposable stirrers.
- Rotator.
- Timer.

Sample collection

Use fresh serum collected by centrifuging clotted blood. If the test cannot be performed on the same day, the serum may be stored between 2 and 8°C for no longer than 8 days after collection. For longer storage, store samples frozen (-20°C).

It is not necessary to inactivate the serum.

As in all serological tests, hemolytic, lipemic or turbid sera may cause incorrect results and should not be used.

Do not use plasma.

Procedure

PREVIOUS MANIPULATIONS

Control of the latex reagent:

- Before performing a set of determinations it is advisable to check the latex reagent with each of the controls, positive and negative, included in the kit.
- Both controls should be used following the steps outlined in the QUALITATIVE TEST.
- The reaction between the positive control and the reagent should show a clear agglutination, different from the uniform appearance of the negative control. If no agglutination takes place, the test should be repeated, and the kit discarded if there is no positive reaction.

QUALITATIVE TEST

- Allow reagents and samples to reach room temperature (20 to 30°C).
- Gently shake the reagent vial to disperse and suspend the latex particles in the buffer solution. Vigorous shaking should be avoided.
- Place 50 µL of the serum onto one section of the disposable slide.
- Place one drop of reagent next to the drop of serum.
- Mix both drops with a stirrer covering the whole surface of the slide section.
- Gently rotate the slide for 2 minutes manually or on a rotary shaker set at 80-100 rpm.
- Look for the presence or absence of agglutination after the aforementioned period of time.

Interpretation of the results

The presence of agglutination indicates a content of rheumatoid factor in the serum equal to or greater than 10 IU/mL.

The absence of agglutination indicates a content of rheumatoid factor in the serum of less than 10 IU/mL.

POSITIVE REACTIONS:

- 3+ Large clumping with clear background.
- 2+ Moderate clumping with fluid slightly opaque in background.
- 1+ Small clumping with opaque fluid in background.

NEGATIVE REACTIONS:

No visible clumping, uniform suspension.

SEMIQUANTITATIVE TEST

Allow reagents and samples to reach room temperature (20 to 30°C).

Preparation of two-fold serial dilutions of the serum on the slide (see the descriptive diagram for the technique):

- Place 50 µL of normal saline on slide sections 2 through 6.
- Using an automatic pipette, place 50 µL of the serum onto slide section 1 and 50 µL directly into the drop of normal saline on slide section 2.
- Using the same pipette take in and release several times the mixture made on section 2 and transfer 50 µL of the mixture to section 3. Repeat in this manner serially through section 6, discarding 50 µL from section 6.

Section	1	2	3	4	5	6
Saline µL	-	50	50	50	50	50
Serum µL	50	50	-	-	-	-
Mix and transfer µL		└─ 50 ─┘	└─ 50 ─┘	└─ 50 ─┘	└─ 50 ─┘	└─ 50 ─┘ →
Dilution	1:1	1:2	1:4	1:8	1:16	1:32
IU/mL	10	20	40	80	160	320

- Gently shake the reagent vial and add one drop of reagent to each section.
- Mix both drops using a stirrer covering the whole surface of the slide section.
- Gently rotate the slide for 2 minutes manually or on a rotary shaker set at 80-100 rpm.
- Look for the presence or absence of agglutination after the aforementioned period of time.

Interpretation of the results

The approximate titer will correspond to the highest serum dilution that still presents a clearly visible agglutination (see diagram).

Limitations of the procedure

- Reading of the results after more than 2 minutes may give false positive results.
- The strength of agglutination is not necessarily indicative of relative rheumatoid factor concentration.
- Since increased levels of rheumatoid factor may accompany certain acute immune responses such as infectious mononucleosis, certain diseases such as sarcoidosis, systemic lupus erythematosus and Sjögren's syndrome and may also be found in a considerable percentage of elderly individuals, the interpretation of the clinical significance of a positive test result must be made with caution. Less commonly, a positive test may result in situations where chronic inflammatory disease is suspected, such as bacterial endocarditis, tuberculosis, leprosy, etc.^{3,5}
- Certain patients with rheumatoid arthritis may show negative results for rheumatoid factor.^{3,4}