

Expected values

The presence of C-reactive protein in a human serum or plasma has long been used as a sensitive indicator of an inflammatory or necrotic process. Levels of C-reactive protein increase in a variety of diseases: pulmonary and acute respiratory diseases, acute abdominal diseases, diseases of the kidney and urinary tracts, rheumatic fever and rheumatoid arthritis, cardiovascular diseases, diseases of the gastrointestinal system, metabolic and endocrine disturbances, malignant and benign tumours and a few skin diseases.³ C-reactive protein in concentrations of 0.02-13.5 mg/L has been regularly demonstrated in sera obtained from apparently healthy children and adults of both sexes. C-reactive protein must therefore be considered a normal constituent of the serum. A weak positive correlation was found between C-reactive protein concentration and age. No significant difference in C-reactive protein concentration is demonstrable between men and non-pregnant women. The mean value of C-reactive protein in adults is 0.47 mg/L.²

Performance characteristic

Sure-Vue® CRP was evaluated by comparison with two other commercially available latex tests (samples 1:6 diluted). A total of 204 serum samples from hospital patients were tested following the qualitative test. This study demonstrated a 98.5% agreement with either of the other two tests. The discrepant results were obtained with samples near the limit of sensitivity of the reagents.

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® CRP** in-house reproducibility (within one dilution) was 100%.

References

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Sure-Vue® CRP

Rapid test for the qualitative and semiquantitative determination of C-reactive protein in serum by agglutination of latex particles on slide. Measurement of C-reactive protein aids in the evaluation of the amount of injury to body tissues.

Summary

C-reactive protein was first described in 1930 by Tillet and Francis who showed that the sera of patients suffering from acute infection precipitated with a non-protein pneumococcus extract called C polysaccharide in the presence of calcium ions.¹ The protein which caused this reaction was therefore called C-reactive protein. Later it was shown that C-reactive protein is frequently found in the sera of normal persons in very low concentrations, in most cases not exceeding a level of 6 mg/L.²

However, during the inflammatory process, whether of an infectious or other nature, the titers of C-reactive protein can reach levels that are far above normal values. In these cases C-reactive protein titers increase and decrease more quickly than the red cells sedimentation rate.³⁻⁵

The increase of C-reactive protein occurs in a non specific way in many different kinds of tissular aggression, such as, for example, in infectious states, rheumatic fever, rheumatoid arthritis, myocardial infarct, malignant tumour, abdominal abscess, peritonitis, burns, etc.³⁻⁷ For this reason a high C-reactive protein concentration in serum lacks diagnostic value when the patients illness is not defined.

Nevertheless, it is very useful for following-up and monitoring such illnesses, as well as for the differential diagnosis in certain disease states.

Principle

The **Sure-Vue® CRP** reagent is a suspension of polystyrene latex particles of uniform size coated with the IgG fraction of an anti-human CRP specific serum. Latex particles allow visual observation of the antigen-antibody reaction. If the reaction takes place, due to the presence of C-reactive protein in the serum, the latex suspension changes its uniform appearance and a clear agglutination becomes evident. This change occurs because the C-reactive protein 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 6 mg/L of C-reactive protein, a clear agglutination will appear.

Results are expressed in mg/L of C-reactive protein based on the WHO International Standard for Human C-Reactive Protein.

Reagents

- a) **Latex reagent:**
Suspension of polystyrene latex particles coated with rabbit IgG anti-human CRP in a buffer.
Contains sodium azide 0.1%.
- b) **Positive control:**
Diluted human serum containing more than 6 mg/L of C-reactive protein.
Ready to use.
Contains sodium azide 0.1%.
- c) **Negative control:**
Diluted human serum containing less than 6 mg/L of C-reactive protein.
Ready to use.
Contains sodium azide 0.1%.

Precautions

Sure-Vue® CRP 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.⁸

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 038001.
 Contains: 1 x 2.5 mL reagent, 1 x 1 mL positive control, 1 x 1 mL negative control and 9 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 C-reactive protein in the serum equal to or greater than 6 mg/L.
 The absence of agglutination indicates a content of C-reactive protein in the serum of less than 6 mg/L.

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
mg/L	6	12	24	48	96	192

- 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 C-reactive protein concentration. When C-reactive protein exceeds 200 mg/L, weak reactions may occur due to antigen excess. If concentrations higher than 400 mg/L are suspected, samples should be tested diluted.
- Serum samples containing elevated concentrations of rheumatoid factor may give false positive results. When clinical circumstances suggest that rheumatoid factor may be present it is recommended that the serum be tested for rheumatoid factor.