Stanbio
RaPET® CRP
Procedure No. 1130

CE

Latex agglutination slide test for the Qualitative and Semi-quantitative determination of C-Reactive Protein in non-diluted serum

Intended Use

RaPET® CRP is intended for the qualitative and the semi-quantitative detection of C-Reactive Protein (CRP) in human serum. The latex slide test is intended to be used as an aid in evaluation of the amount of injury to body tissues.

Summary and Principle

C-Reactive Protein was first described by Tillet and Francis in 1930. They concluded that sera of patients suffering from acute infection precipitated with a non-proteic pneumococcus extract called C polysaccharide in the presence of calcium ions.[1] The protein which caused this reaction was therefore called C-Reactive Protein. All acute inflammatory processes (infectious and non-infectious) result in a rise in serum C-Reactive Protein (CRP) as a nonspecific phenomenon.[2] While the CRP concentration is generally below 6 mg/L in the sera of healthy adults, in a number of disease states these values are often exceeded within 4 to 8 hours after an acute event and reach levels of about 20 to 500 mg/L[3].

Since an elevated CRP level is always associated with pathological changes, determination of CRP is of great value in diagnosis, treatment, and monitoring of inflammatory conditions.[4,5,6] CRP is a more sensitive and reliable indicator of inflammatory processes than the erythrocyte sedimentation rate (ESR) and the leukocyte count. The serum CRP concentration increase occurs faster than that of the ESR and, when the condition subsides, CRP falls very quickly, reaching normal levels several days before the ESR normalizes.

Various immunoprecipitation methods have been developed for its detection of CRP since the discovery that rabbits form agglutinating antibodies against CRP.[7]

The procedure presented is essentially that of Singer and Platz[8], and has the advantages of simplicity and prompt results. The principle of the test involves an immunologic reaction between CRP (as an antigen) and the corresponding antibody affixed to the surface of latex particles. Mixing the latex reagent with the serum sample, causes a clear agglutination if the sample contains > 6mg/L of CRP. Results are expressed in mg/L of C-Reactive Protein based on the WHO International Standard for Human CRP.

Reagents

CRP Latex Reagent, Ref. No. 1131 (White Cap)
Suspension of 1% polystyrene latex particles coated with <2% Goat IgG anti-human CRP in a saline buffer.

CRP Positive Control, Ref. No. 1132 (Red Cap)
Stabilized human serum, containing more than 6 mg/L of C-Reactive Protein.

Negative Control, Ref. No. 1192 (Green Cap)
Stabilized human serum, containing less than 6 mg/L of C-Reactive Protein.

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 at 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 glycine-saline 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

Materials Required (Not Provided)
Timer Rotator (optional)
Test Tubes & Rack (Semi-quantitative test only)
Serological Pipettes (Semi-quantitative test only)

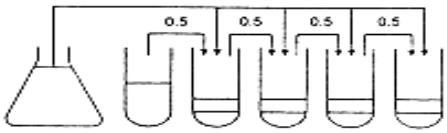
Procedure

A. Qualitative Method

1. Bring all 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 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. Tilt the slide back and forth for 2 minutes manually so that the mixture rotates slowly inside the cells or place the slide on a automatic rotator 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 CRP concentration using a dilution series. The patient sample is diluted with the Glycine-Saline Buffer as shown:



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

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

Quality Control

A positive control and a negative control should be included in each test series for 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 CRP concentration greater than 6 mg/L in the sample. Those samples that do not show agglutination contain CRP concentrations less than 6 mg/L.

B. Semi-Quantitative Test Results

The highest sample dilution which still shows distinct agglutination is reported. The CRP content of the patient sample is taken from the following table:

Dilution of Agglutination	CRP Concentration - mg/L (± 20%)
1:2	≥ 12
1:4	≥ 24
1:8	≥ 48
1:16	≥ 96

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 CRP concentration in the sample. In the qualitative test procedure, weak reactions may occur with markedly elevated concentrations. Agglutination reactions are weaker between 200 and 400 mg/L than lower concentrations but no false negatives were found up to 400 mg/L. When CRP concentrations>400mg/L are expected, the sample should be diluted and retested. 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.

Expected Values

By the literature, healthy adults usually have CRP concentrations of less than 5mg/L.[9] Since CRP is an unspecific indicator for various disease processes and reference ranges are subject to many influence parameters, which may differ due to investigational groups, every laboratory should establish the relevant upper limit of their reference range. By the data generated by Stanbio for correlation in the below study, of 60 patient samples tested, 36 patients were found positive with values >6 mg/L and24 patients were found negative with values <6 mg/L.[10]

Performance Characteristics[10]

Relative Sensitivity: The performance of RaPET® CRP was compared with a competitor's test and determined to be 100% in agreement. Ranges measured were 6 mg/L to 420 mg/L.

Relative Specificity: The assay is specific for CRP. A 100% agreement was determined between the RaPET® CRP and a competitor's test. In an interference study, three concentrations of RF (755.5, 377.8 and 23.6 IU/mL) were added in CRP free, 6 mg/L and 21 mg/L CRP spiked solutions. None of these concentrations of CRP interfered with the assay.

Reproducibility: In a within run precision study, two serum controls were assayed 20 times on both the RaPET® CRP and a competitor's test. This study demonstrated a 100% agreement between these two tests.

Linearity: In a linearity study, a standard of 250 mg/L was serially diluted. Standards of 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9 mg/L and a negative were tested. All results were positive excluding 3.9 mg/L and the negative sample which gave negative results.

Prozone: Concentrations of standard as high as 250 mg/L 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 32 run in duplicate ranged from 6 to 256 mg/L.

Sample	Correlation Coefficient	Sample Size	Regression Equation	Total Error
Serum	0.964	32	Y = 0.93X + 0.36	1.03

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

References

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10. Stanbio Laboratory Data

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