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C-Reactive Protein (CRP) Latex Test

INTENDED USE

The PULSE CRP LATEX TEST (PULSE CRP TEST) is intended to be used for the qualitative screening and semi-quantitative determination of C-Reactive Protein (CRP) in serum.

SUMMARY AND PRINCIPLES

CRP usually appears in patient sera in the acute stages of a number of inflammatory conditions such as most bacterial and some viral infections; acute rheumatoid fever with or without carditis; rheumatoid arthritis and most other collagen diseases; and in other conditions characterized by inflammation. CRP is considered to be a sensitive indicator of inflammation. Changes in the serum level of CRP with time from the same patient can be used as an index of recovery. The use of the CRP test to measure the effectiveness of therapy is of great clinical significance in cases such as acute rheumatoid fever. Since the discovery that rabbits form precipitating antibodies against CRP[1], various immunoprecipitation techniques have been applied for its detection. The PULSE CRP TEST is based on the latex-agglutination method introduced by Singer et al in 1957[2]. The principle of this test is based on the immunological reaction between CRP as an antigen and the corresponding antibody coated on the surface of biologically inert latex particles.

MATERIALS SUPPLIED

CRP Latex Reagent: Latex particles with anti-human CRP in buffer with < 0.1% sodium azide.
CRP Positive Control: Human serum (> 6mg/L CRP) with < 0.1% sodium azide.
CRP Negative Control: Human serum with < 0.1% sodium azide.
Glycine-Saline Buffer Concentrate (20X): To be diluted 1:20 with distilled water.
Disposable pipettes and test slides.
Additional Items Required: Serological pipettes, 12 x 75 mm test tubes and timing device.

STORAGE & STABILITY

Store reagents and controls at 2 - 8 degree Celsius. DO NOT FREEZE. Prior to use, warm reagents and controls to room temperature. Product instability is shown by improper reaction between latex reagent with the positive and negative control sera.

PRECAUTIONS

This product is for In Vitro Diagnostic Use Only. Each donor unit used in this product has been tested by an FDA approved method and found to be non-reactive for the presence of HbsAg and antibody to HIV Virus. Because no known test method can offer complete assurance that hepatitis B virus, HIV Virus, or other infectious agents are absent, all human blood-based products should be handled in accordance with good laboratory practices. The preservative sodium azide may react with metal plumbing to form explosive metal oxides. In disposal, flush with a large volume of water to prevent metal azide build up.

SPECIMEN COLLECTION

The test should be performed on fresh serum specimens only. If testing is delayed, specimens should be refrigerated (or frozen where applicable). Bacterial contamination may cause protein denaturation and false positive agglutination.

PROCEDURE

A. Method I (Qualitative)

1. Bring all test reagents and serum specimens to room temperature. Gently shake the CRP latex vial to suspend the latex particles.
2. Place one drop of test serum (with disposable pipette) onto a circle on the slide. Use new pipette for each test serum. Deliver one drop of CRP Latex to each circle that contains specimens or controls. Use paddle end of pipette to spread the mixture on each circle. Do not use the same paddle end to mix each mixture as this will cause cross-contamination.
3. Gently tilt and rotate slide by hand for two (2) minutes. Observe for macroscopic clumping using the indirect oblique light source.

B. Method II (Semi-Quantitative)

1. For each test serum to be titrated, set up a least 6 test tubes (12 x 75 mm) and label 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, etc.
2. To each tube add 0.2 ml of Diluted Glycine-Saline Buffer.
3. To Tube No. 1 add 0.2 ml of undiluted test serum.
4. Serially make two-fold dilutions by mixing contents of Tube No. 1 with pipette and transferring 0.2 ml to Tube No. 2. Repeat serial transfers for each tube. For the 6 tubes, the dilutions range from 1:2 to 1:64. If required, additional serum dilutions can be added. Repeat steps 1 to 3 as given in Method I (Qualitative).

RESULTS

Qualitative

Positive Result: Agglutination **Negative Result:** Smooth milky suspension

Negative results may be caused by antigen excess (caused prozone effect). The test should be repeated with undiluted serum sample in case prozone effect is suspected.

Semi-Quantitative

Positive sera should be retested to provide verification for borderline interpretations. The highest dilution of sample showing agglutination is the endpoint. Multiplication of the dilution factor by 6mg/L will yield the approximate CRP level.

Dilutions/Concentration (mg/L): 1:1 (neat specimen) = 6, 1:2 = 12, 1:4 =24, 1:8 = 48

QUALITY CONTROL PROCEDURE

Positive and Negative controls should be included in each test series. The Positive control should produce visible agglutination; and Negative control should produce no agglutination.

LIMITATIONS OF THE PROCEDURE

The results of this test SHOULD NOT be used as a single diagnostic tool to make a clinical diagnosis. Instead, other clinical findings must be evaluated with other observed symptoms to aid in the final diagnosis. This test is to be performed by hand rotation. The use of a mechanical rotator could yield false positive/negative results. Strength of agglutination in screening test is not indicative of actual CRP titer. Reaction time longer than 2 minutes may produce apparent false positive reactions due to a drying effect. Lipemic, haematic or contaminated sera can cause false positive reactions. Only serum should be used in this test. Specimens containing Rheumatoid factor (RF) should not be used. The presence of RF (usually >20 IU/ml) may lead to false positive results. The presence of RF can be confirmed by using commercially available RF Tests (e.g. Latex Tests).

EXPECTED VALUES

Normal adult CRP levels are reported to be less than 12 mg/ but trace levels of CRP had been reported in the sera of apparently healthy adults[3] and normal children[4]. The CRP level can increase significantly (>10 fold) over the normal values with the onset of a substantial inflammatory stimulus.

PERFORMANCE CHARACTERISTICS

It must be stressed that the latex agglutination technique is more sensitive than precipitation in capillary tubes or in agar gel methods, thus giving positive results at lower CRP levels. The CRP levels in patients with strongly positive CRP reactions had been detected as high as 330 mg/L[5] while the CRP content of normal serum is less than 12 mg/L[6]. The PULSE CRP TEST was compared to another commercial CRP Latex Test and CRP Nephelometry Test, both produced by Behring. A total of 42 specimens were evaluated with the following results. The titres of the specimens were determined by all three methods and showed comparable results.

Expected Result	Pulse CRP Test	Behring CRP Test
Positive	14/14	12*/14
Negative	27+/28	27/28

* The two specimens were found to contain 7.8 and 6.5 mg/L CRP using the Behring nephelometry method.
+ The one negative specimen (determined by both the Pulse and Behring Latex Tests) was found to contain 6.6 mg/L of CRP by the Behring nephelometry method.

BIBLIOGRAPHY

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