

(e.g. Latex Tests). The presence of RF (usually >20 IU/ml) may lead to false positive results.

EXPECTED VALUES

Normal adult levels of CRP are reported to be less than 12 mg/L. Trace levels of CRP had been reported in the sera of apparently healthy adults³ and normal children⁴. The CRP level can increase significantly (>10 fold) above 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/L⁵ while the CRP content of normal serum is less than 12 mg/L⁶.

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.

<u>Expected Result</u>	<u>Pulse CRP Test</u>	<u>Behring CRP Test</u>
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

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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 antibodies (CRP) in serum.

SUMMARY AND PRINCIPLES

CRP usually appears in the sera of patients 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 a number of 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 CRP¹, 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 1957². 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:** Contains polystyrene latex particles coated with anti-human CRP in a stabilized buffer with less than 0.1% sodium azide as preservative.
- CRP Positive Control:** Human Serum that contains more than 6mg/L CRP and less than 0.1% sodium azide as preservative.
- CRP Negative Control:** Human serum that has been diluted and stabilized with buffer and contain less than 0.1% sodium azide as preservative.
- Glycine-saline Buffer:** To be diluted 1:20 with distilled water.
- (20X) Concentrate**
- Disposable pipettes and test slides.**

Additional Items Required:

Physiological saline, serological pipettes, 12 x 75 mm test tubes and timing device.

STORAGE & STABILITY

When not in use, store reagents and controls at 2 - 8 degree Celsius. DO NOT FREEZE. Prior to use, allow reagents and controls to warm up to room temperature. Expiration date is specified on the kit label and on each vial. Biological indication of product instability is evidenced by inappropriate reaction of the latex reagent with the corresponding positive and negative control sera.

PRECAUTIONS

This product is for In Vitro Diagnostic Use Only.

Each donor unit used in the preparation of this product has been tested by an FDA approved method and found 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.
2. Gently shake the CRP latex vial to disperse and suspend latex particles.
3. Positive and negative controls should be tested with each series of test.
4. Using the disposable pipette provided, place one drop of test serum onto a circle on the slide. Use a separate disposable pipette for each test serum. Important: The Pulse CRP Latex Reagent must be agitated well for about 10 seconds prior to using on each day's testing. Do not use a vortex mixer. Deliver one drop of CRP Latex to each circle that contains specimens on the slide. Spread the resulting mixture by using the paddle end of the pipette. Do not use the same paddle end to mix each test serum or control as this will cause cross-contamination.
5. Gently tilt and rotate slide by hand for two (2) minutes.
6. Observe for macroscopic clumping using the indirect oblique light source.
7. Compare the reaction of the test serum to the CRP positive and negative control sera.

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.

5. Repeat steps 3 to 7 as given in Method I (Qualitative).

RESULTS

Qualitative

Positive Result: Agglutination

Negative Result: Smooth milky suspension

Since negative results may be caused by antigen excess, the test should be repeated using a diluted serum sample in case prozone effect is suspected.

Semi-Quantitative

Sera that are positive in the screening test should be retested in the titration test to provide verification for borderline interpretations. The greatest dilution of test sample showing agglutination is considered the endpoint. Multiplication of the dilution factor by 6 mg/L will yield the approximate level of CRP present. The following table is only shown as an example for the determination of CRP concentration in specimen. Actual specimen will have CRP concentrations higher or lower than the levels indicated in this table.

<u>DILUTION</u>	<u>CONCENTRATION (mg/L)</u>
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

1. The results of this test SHOULD NOT be used as a single diagnostic tool to make a clinical diagnosis. Instead, the test results must be evaluated together with other clinical findings and observed symptoms to aid in the final diagnosis.
2. This test is designed to be performed by hand rotation. The use of a mechanical rotator could yield false positive/negative results.
3. The strength of the agglutination reaction is not indicative of the CRP concentration.
4. Weak reactions may occur with slightly elevated or markedly elevated concentrations.
5. A prozone phenomena (antigen excess) may cause false negatives. It is advisable, therefore, to check all negative sera by retesting at a 1:10 dilution. Reaction times longer than specified may produce apparent false reactions due to a drying effect.
6. Strongly lipemic or contaminated sera can cause false positive reactions.
7. Only serum should be used in this test.
8. Specimens containing Rheumatoid factor (RF) should not be used. The presence of RF can be confirmed by using commercially available RF Tests