INTENDED USE
The CRP Test is a rapid latex agglutination test for the qualitative and semi-quantitative determination of C-reactive protein.

PRINCIPLE OF THE TEST
C-reactive protein usually appears in the sera of patients in the acute stages of a number of inflammatory conditions - most bacterial and some viral infections; acute rheumatic fever with or without carditis; rheumatoid arthritis and most other collagen diseases, and in a number of other conditions characterized by inflammation. C-reactive protein can usually be demonstrated in cases of acute myocardial infarction and in several types of malignancies particularly those that are metastatic.

Since the discovery that rabbits form precipitating antibodies against CRP, various immunoprecipitation techniques have been applied for its detection. The CRP is based on the latex-agglutination method introduced by Singer et al., in 1957. The CRP technique has the advantage of rapid performance in comparison to other tests for the detection of CRP. The results are readable after 2 minutes reaction time.

6. Dispensing/Mixing Pipettes:
5. 6-cell Glass Slide
3. Positive Control Serum, stabilized human serum containing CRP as an antigen.

When serum is drawn from a patient and tested at appropriate time intervals, changes in the levels of C-reactive protein can be used as an index of recovery. The use of C-reactive Protein Test to measure the effectiveness of therapy is of great clinical significance, particularly in the management of patients with acute rheumatic fever.

The principle of the test is an immunologic reaction between CRP as an antigen and the corresponding antibody coated on the surface of biologically inert latex particles.

REAGENT AND MATERIALS PROVIDED
1. CRP Reagent, is a suspension of polystyrene latex particles in glycine-saline buffer pH: 8.6 ± 0.2. Latex particles are coated with monospecific anti-human CRP, produced in laboratory animals.
2. Buffer Solution 20X Concentrate, pH: 8.6 ±0.2. to be diluted 1:20 with distilled water.
3. Positive Control Serum, stabilized human serum containing CRP as an antigen.
4. Negative Control Serum, stabilized human serum, nonreactive with the latex reagent.
5. 6-cell Glass Slide
6. Dispensing/Mixing Pipettes:

MATERIALS REQUIRED, BUT NOT PROVIDED
Test Tubes (for dilution)
Pipettes (serological)
Lab rotator or applicator sticks for mixing
Laboratory Timer

WARNING
The CRP Test is designed for in vitro diagnostic use only.

STORAGE AND STABILITY
Store at 2°C - 8°C. Do not freeze.
Expiration date is specified on the kit label. Biological indication of product instability is evidenced by inappropriate reaction of the latex reagent with the corresponding positive and negative sera.

SPECIMEN COLLECTION AND HANDLING
Specimen can be drawn by venipuncture or convenient finger-tip method, after complete clotting retraction, the serum is separated for testing.

Interfering Substances:
Strongly lipemic sera and/or bacterial contamination may cause false positive agglutination.

Storage Conditions
The serum specimen should be stored refrigerated. If testing is to be prolonged in excess of 24 hours, serum should be frozen. Bacterial contamination may cause protein denaturation.

PROCEDURE A. SCREENING
1. Bring all reagents and serum samples to room temperature.
2. Shake the CRP test reagent gently, expel the contents of dropper and refill, then place one drop (approx. 0.05 ml) onto glass slide. Using dispensing pipette provided, add one drop of the undiluted patient serum onto the glass slide, and mix both together with the paddle end of the pipette.
3. Continue to mix for about 2 minutes with rotator or by hand and observe for macroscopic clumping using the indirect oblique light source.
4. Positive and negative controls should be run with each series of test sera. The controls supplied are to be used exactly as outlined in steps 1 thru 3 above except they are used without further dilution and, because they are supplied with a dropper-tip, no pipette is needed to dispense.
5. The reaction of the test serum is compared to the CRP positive and negative control sera.

QUALITY CONTROL PROCEDURE
A positive control will produce, usually within 1 minute, course agglutinated foci against a clear background. A negative control will produce, usually no agglutination. It should be used for a basis for comparison. The relative degree of smoothness of the CRP reagent itself should be considered and incorporated in reading the results.

If the indicated results using the positive and negative controls are not obtained, the CRP test kit should not be used.

RESULTS
An agglutination of the latex particle suspension is a positive result. Since negative results may be caused by antigen excess, the test should be repeated using a diluted serum.

PROCEDURE B. SEMI-QUANTITATIVE
The CRP KIT is also suitable for titration purposes.
1. Prepare a 1:20 dilution of concentrated Glycine-Saline Buffer (1+19)
2. Serum to be titrated is serially diluted in diluted glycine-saline buffer and going out 5 or more tubes. (1:2, 1:4, 1:8, 1:16 and 1:32 or more)
3. Place one drop of negative and positive controls on slide. (Do not attempt to dilute the CRP positive control serum for comparative or other purposes as no correlation exists between actual titer of the control and titer of unknown sera.)
4. Place one drop of each dilution individually in successive rings and proceed as in screening methodology.

RESULTS
To get mg%, multiply highest dilution of positive result dilution by 0.65.
mg% = 0.65 x dilution
e.g. the highest dilution of positive result is 1:32
mg% = 0.65 x 32
= 20.8 mg%.

LIMITATIONS OF THE PROCEDURE
The strength of the agglutination reaction is not indicative of the CRP concentration. Weak reaction may occur with slightly elevated or markedly elevated concentrations. 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 (4 minutes) may produce apparent false reactions due to a drying effect. Strongly lipemic or contaminated sera can cause false positive reactions.

EXPECTED VALUES
Normal adult levels of C-reactive protein (CRP) are reported to be less than 1.2 mg/100ml when they can be detected. Recent refined techniques, however, have shown the routine appearance of trace amounts of the protein in the sera of apparently normal children and healthy adults.

SPECIFIC PERFORMANCE CHARACTERISTICS
In comparing CRP tests, it must be remembered that the different techniques vary in sensitivity. The latex agglutination technique is more sensitive than precipitation in capillary tubes or in agar gel and give positive results at lower CRP concentrations. Reference 6,8,10.

For this reason the latex agglutination test usually gives a higher percentage of positive results than the other methods. Expressed in absolute terms, the amount of C-reactive protein in serum from patients with strongly positive CRP reactions is given by different workers as 33mg/100ml11 14mg/100ml12, while the content of normal serum is less than 1.2 mg/100ml13.

PRECAUTIONS
All human blood components used to prepare controls have been tested for Hepatitis B surface antigen (HbAg) and HTLV-III antibodies by an FDA approved procedure and found to be non-reactive. No known test method for HB Ag or HTLV-III antibodies offers total assurance that a human derived product will not transmit hepatitis or HTLV-III virus. The user is therefore cautioned to handle reagents as if being capable of transmitting these diseases.

The reagents in each kit are matched. Reagents from different kits must not be interchanged or pooled. If the kit does not yield expected results when controls are tested, the kit should be discarded. Mix the reagents well before use. Use clean equipment. Traces of detergent or dried reactants on the test slide may adversely affect test performance and results.

WARNING: The components of the test kit contain azide. Sodium azide may react with lead and copper pluming to form explosive metal azides. Upon disposal, flush lines with a large volume of water to prevent azide build up.

BIBLIOGRAPHY

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