

ACCUTEX C-REACTIVE PROTEIN (CRP) LATEX TEST

A qualitative and semiquantitative serological test for the detection of C-Reactive Protein in serum or plasma as an aid in the diagnosis of inflammatory disease.

QUALITATIVE AND SEMIQUANTITATIVE

SUMMARY

C-reactive protein is one of several “acute-phase” proteins in response to inflammation and/or necrosis. Although it derived its name from the fact that it reacted with the C-polysaccharide of pneumococcal cell walls, elevated CRP levels have been associated with virtually all acute bacterial infections, some tumors and various types of tissue destruction such as myocardial infarction and postoperative necrosis. ^(1,2,3,4,5) Most viral infections (infectious mononucleosis and hepatitis are notable exceptions) and many gastrointestinal disorders fail to induce elevations in CRP. ^(3,5) As an indicator of inflammation and/or necrosis, CRP increases soon after the tissue insult and decreases at a faster rate upon recovery than other acute-phase proteins or other indicators of inflammation such as sedimentation rates and leukocyte counts. ^(2,4,6,7,8) Thus, CRP tests can aid the physician in the detection, diagnosis, and prognosis involving tissue damage and inflammation. ^(8,9) CRP tests are often used as an aid in monitoring patients with acute rheumatic fever and rheumatoid arthritis. ⁽²⁾ Quantitative C-reactive protein measurements have been found to provide reliable early indication of postoperative, inflammatory complications if monitored on a daily basis. ⁽⁸⁾ Likewise, sequential samples during therapy of acute bacterial infections and other inflammatory diseases may be used to monitor the effectiveness of the therapy. ^(4,6,10)

Assays for C-reactive protein are the most widely used serological tests for the detection of an inflammatory and/or necrotic process. ⁽¹⁰⁾ Methods of CRP determination include capillary precipitation, Ouchterlony immunodiffusion, radial immunodiffusion, particle counting immunoassay and particle agglutination tests. ^(7,11,13)

PRINCIPLE

The Accutex C-Reactive Protein Test provides a suspension of polystyrene latex particles which have been coated with antibody to human C-reactive protein. The CRP produced in response to inflammation or necrosis binds to the specific antibody coating the latex particles. In specimens having abnormally high levels of CRP, this binding is evident by rapid agglutination of the latex.

STABILITY AND STORAGE

Indications of deterioration: lack of clear agglutination with the Positive Control Serum, agglutination with the Negative Control Serum, any turbidity in the Glycine Diluent or extreme turbidity in either control serum. The reagents in this kit are stable until their expiration date when stored as directed.

However, as with most reagents, they can be damaged by improper handling, especially temperature extremes. Use of the Positive and Negative Control Sera provided will permit detection of reagent deterioration.

Store reagents at 2-8°C when not in use. All other kit components may be stored at room temperature if desired. Do not freeze reagents. Discard any unused control serum when the CRP Latex Reagent is depleted.

SPECIMEN HANDLING

Use fresh serum free from contamination. Collect blood in a clean, dry tube and allow to clot at room temperature for at least 10 minutes before removing serum. If not tested immediately, specimens may be stored at 2-8°C for a maximum of 72 hours. If longer storage is required, the sample may be frozen and tested at a later time. Repeated freezing and thawing should be avoided.

Specimens must be clear and free of particulate matter before testing. If necessary, centrifuge to clarify specimens before testing. Contaminated or grossly hemolyzed specimens should not be used.

Warning: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sink, flush with a large volume of water to prevent azide buildup.

Warning: Human sourced material. Treat as potentially infectious. 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, HCV antibodies 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 using appropriate precautions as detailed in Centers for Disease Control/National Institutes of Health manual “Biosafety in Microbiological and Biomedical Laboratories, 1984.”

PROCEDURE

REAGENTS AND MATERIALS PROVIDED

- 1 CRP Latex Reagent: a suspension of polystyrene latex particles coated with goat antibody to human C-reactive protein in glycine buffer.
 - 1 CRP Positive Control Serum: a human serum pool known to have a positive reaction with the Accutex CRP Latex Reagent.
 - 1 CRP Negative Control Serum: a human serum pool known to have a negative reaction with the Accutex CRP Latex Reagent.
 - Glycine Diluent: 0.1M glycine and 0.15M sodium chloride.
- All four reagents contain 0.1% sodium azide as a preservative.**
- Test Slide
 - 50 Disposable dispensing-spreading pipettes

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Mechanical slide rotator (optional)

The following items are required for the semiquantitative test procedure:

- Serological pipettes or safety pipetting device with disposable tips
- Disposable test tubes (12 x 75 mm, 10 x 75 mm or 13 x 100 mm)
- Test tube rack

PROCEDURE OUTLINE

Note: Allow all reagents and test samples to reach room temperature before starting the test. Thoroughly clean the slide before each use. The slide should be washed with detergent, rinsed thoroughly with water and wiped dry with a lint-free tissue.

A. QUALITATIVE TEST

1. Because of the possibility of prozone, both the undiluted serum and its 1:5 dilution are to be tested. Prepare a 1:5 dilution of each specimen in a clean test tube using the Glycine Diluent provided (e.g., 0.4 ml of diluent plus 0.1 ml of test specimen).
 2. Using the six place oval ring slide provided, place one drop (50 lambda) of the positive control on the first ring and place one drop of the negative control in the second ring. The remaining rings are to be used for patient samples. Place one drop of the undiluted patient sample and one drop of a 1:5 dilution of the same sample in the next oval ring. Repeat for all patient samples.
 3. Gently resuspend the CRP Latex Reagent and add one drop to each position containing sample to be tested. Mix with stirring rod and spread evenly over the entire oval ring.
 4. Rock and rotate the slide in the same motion for three minutes and read immediately under direct light.
- Note: Circulating CRP levels have been shown to closely parallel clinical conditions and indicate the severity of the inflammatory and/or necrotic process.^(8,10) Therefore, test specimens demonstrating agglutination in this Qualitative Test should be tested by the following Semiquantitative Test procedure.

B. SEMIQUANTITATIVE TEST

Dilutions of positive samples should be made in test tubes. For test tube dilutions:

1. Using a clean pipette for each dilution, prepare 2-fold serial dilutions from 1:2 to 1:32 using the Glycine Diluent in clean test tubes as indicated in the following example:

Tube	1	2	3	4	5
Diluent	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Specimen	0.5 ml	-	-	-	-
Mix and Transfer	0.5 ml	0.5 ml	0.5 ml	0.5 ml	
Dilution	1:2	1:4	1:8	1:16	1:32
2. Using each dilution as a separate test specimen, proceed with Steps 3 through 7 of the Semiquantitative Test Procedure. (Include undiluted sample if not tested previously on that day with the same lot of CRP Latex Reagent.)
3. If the highest dilution tested (1:32) is positive, prepare additional 2-fold dilutions and repeat Step 2.

QUALITY CONTROL

CRP Positive and Negative Control Sera should be included in each test series. The Positive Control Serum should produce clear agglutination; the Negative Control Serum should produce no agglutination.

RESULTS

The "upper limit of normal" CRP level is approximately 8ug/ml.⁽¹²⁾ The Accutex CRP Latex Reagent is designed to agglutinate in the presence of levels of CRP in excess of that amount.

A. QUALITATIVE TEST

Agglutination (positive reaction) indicates the level of C-reactive protein is above normal (approximately 1.0mg/dl or greater). Positive test specimens should be subjected to the Semiquantitative Test. The lack of agglutination (negative reaction) indicates the level of C-reactive protein is within the normal range.

B. SEMIQUANTITATIVE TEST

The titer of the C-reactive protein is the reciprocal of the highest dilution which exhibits a positive reaction. The level of CRP in mg/dl is approximately equal to the titer. The actual concentration of CRP has been found to closely parallel clinical condition and is a reliable indicator of the severity of the inflammatory and/or necrotic process.^(8,10) Therefore, repeat testing on samples obtained at intervals of one to several days may be very useful in monitoring a patient's response to therapy.^(4,6,10) Sequential determinations also may be helpful in the detection and monitoring of postoperative complications involving inflammation and/or necrosis.⁽⁸⁾

LIMITATIONS OF TEST PROCEDURES

Although the Accutex Latex Reagent is highly sensitive and specific, a diagnosis of inflammation and/or necrosis should not be made on the basis of a positive test result without the support of patient history and hematological, microbiological or other clinical evidence. Similarly, a negative test result cannot completely rule out an inflammatory and/or necrotic process. Incubation of the test for longer than the recommended time may cause false positive reactions.

Levels of CRP increase during pregnancy beginning late in the first trimester. Abnormal levels may be demonstrated in about 40% of women in the second and third trimester. Up to 80% may be positive at delivery. Also, women on various oral contraceptives, as well as women with intrauterine devices, may have elevated levels of CRP.⁽²⁾ In apparently normal persons age 60 and above the incidence of positive reactions increases. The significance of such CRP elevations is uncertain since many persons in this group may have degenerative, subclinical and/or occult diseases.⁽²⁾

In the Qualitative Test Procedure, weak reactions may occur with undiluted test specimens in cases of extremely elevated CRP levels. For this reason, the 1:5 dilution of the test specimen must always be included as indicated in the procedure. Reactions with the 1:5 dilution may be considerably stronger than with the undiluted test specimen in those rare cases showing a strong prozone.

SPECIFIC PERFORMANCE CHARACTERISTICS

To assure proper performance, each lot of Accutex CRP Latex Reagent has been tested by the procedures described against a panel of human sera of established reactivity levels.

No false positive reactions were observed with the Accutex CRP Latex Test in testing performed on 50 samples from apparently healthy individuals possessing CRP levels below 0.75mg/dl (as determined by radial immunodiffusion).

Similarly, no false negative reactions were observed in qualitative testing performed on 80 hospital patients with various types of inflammatory disease and CRP levels between 1.0 and 35.0mg/dl. Furthermore, the CRP estimates obtained in semiquantitative tests on those 80 samples closely matched the radial immunodiffusion values. This data indicates that both sensitivity and specificity of the Accutex C-Reactive Protein Test are greater than 99%.

In a study on precision, a panel of 10 serum samples with CRP levels from 4.0 to 10.0mg/dl were tested on 10 consecutive days by the Semiquantitative Test method (100 determinations). Only 1 determination gave more than a 2-fold difference from the mean titer for a sample.

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