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C-REACTIVE PROTEIN (CRP) A LATEX SLIDE TEST

INTENDED USE
C-Reactive Protein (CRP) latex slide test is used for the qualitative and semi-quantitative measurement of C-reactive protein (CRP) in human serum.

INTRODUCTION
C-Reactive Protein (CRP), the classic acute-phase of human serum, is synthesized by hepatocytes. Normally, it is present only in trace amounts in serum, but it can increase by as much as l000-fold in response to injury or infection. The clinical measurement of CRP in serum, therefore, appears to be a valuable screening test for organic disease and a sensitive index of disease activity in inflammatory, infective, and ischemic conditions.[1,2] MacLeod and Avery found that antibody produced against purified CRP provided a more sensitive test than the C-polysaccharide assay.[3] Since that time a number of immunological assays have been devised to measure CRP such as capillary precipitation, double immunodiffusion, and radical immunodiffusion.[4,5]

The CRP reagent kit is based on the principle of the latex agglutination assay described by Singer and Plotz.[6] The major advantage of this method is the rapid three (3) minute reaction time.

PRINCIPLE
The CRP reagent kit is based on an immunological reaction between CRP antisera bound to biologically inert latex particles and CRP in the test specimen. When serum containing greater than 0.8 mg/dl CRP is mixed with the latex reagent, visible agglutination occurs.

REAGENTS
1. *CRP Latex Reagent:* A suspension of uniform polystyrene particles coated with monospecific antihuman CRP (goat) in glycine buffer, pH 8.8 $\pm$ 0.5; reagent sensitivity adjusted to approximately 0.8 mg/dl. **MIX WELL BEFORE USING**.
2. *CRP Positive Control Serum:* A stabilized prediluted human serum containing more than 0.8 mg/dl CRP.
3. *CRP Negative Control Serum:* A stabilized prediluted human serum non-reactive with the test reagent.
4. *Glycine-Saline Buffer (20x):* pH 8.2 ± 0.1: A diluent containing 0.1M glycine and 0.15M NaCl. Dilute glycine-saline buffer before using by adding 1 part glycine-saline buffer solution to 19 parts distilled water. All reagents contain 0.1% (w/v) sodium azide as preservative.

WARNINGS AND PRECAUTIONS
1. Reagents containing sodium azide may combine with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide buildup.
2. For *in vitro* diagnostic use.
3. Positive and negative controls prepared using human sera found negative for hepatitis B surface antigen (HBsAg); however, handle controls as if potentially infectious.

REAGENT STORAGE AND STABILITY
1. Reagents are stable until stated expiration date on bottle label when stored refrigerated (2 - 8°C).
2. **DO NOT FREEZE.**
3. The CRP latex reagent, once shaken must be uniform without visible clumping. When stored refrigerated, slight sedimentation may occur and should be considered normal.
4. Do not use the latex reagent or controls if they become contaminated.

SPECIMEN COLLECTION AND STORAGE
1. Use fresh serum collected by centrifuging clotted blood.
2. If the test cannot be carried out on the same day, the serum may be stored between 2 - 8°C for no longer than 72 hours after collection.
3. For longer periods, the sample must be frozen.
4. As in all serological tests, hemolytic or contaminated serum must not be used.
5. Do not use plasma.

MATERIALS AND REAGENTS PROVIDED
1. CRP Latex Reagent
2. CRP Positive Control
3. CRP Negative Control
4. Glycine-Saline Buffer
5. Reaction Slide
6. Pipette/Stir Sticks

MATERIALS REQUIRED BUT NOT PROVIDED
1. Timer
2. Test tubes
3. Test tube rack

PROCEDURE
QUALITATIVE TEST:
1. Bring reagents and specimens to room temperature before use.
2. Place one drop (50 µl) of the CRP Positive Control on field #1 of the reaction slide. Place one drop (50 µl) of the CRP Negative Control on field #2. The remaining fields are used for test specimens. Using pipettes provided, place one drop of the undiluted specimens on successive fields. Retain pipette/stir sticks for mixing step.
3. Gently resuspend the CRP Latex Reagent and add one drop to each test field. Use pipette/stir stick to spread reaction mixture over the entire test field.
4. Rotate the slide for three (3) minutes and read immediately under direct light.

SEMI-QUANTITATIVE TEST:
1. Set up at least five test tubes: 1:2, 1:4, 1:8, 1:16, etc.
2. Dilute sample according to dilution factor on each test tube with diluted saline solution.
3. Place one drop of each of positive and negative controls onto separate slide fields. Place one drop of each dilution on successive fields of the reaction slides.
4. Gently resuspend the CRP Latex Reagent and add one drop to each test field.
5. Mix well with the flat end of the pipette. Gently rock the slide for three (3) minutes and read immediately under direct light.

6. The titer of the serum is the reciprocal of the highest dilution exhibiting a positive reaction multiplied by the concentration of the positive control.

mg/dL of serum = concentration of assay cutoff control × reciprocal of last dilution showing a positive result

Dilution	Reciprocal	Conc. of serum (mg/dL)
1:2	2	1.6
1:4	4	3.2
1:8	8	6.4
etc.		

QUALITY CONTROL
1. CRP Positive and Negative Control should be included in each test batch.
2. Acceptable performance is indicated when a uniform milky suspension with no agglutination is observed with the CRP Negative Control and agglutination with large aggregates is observed with the CRP Positive Control.

INTERPRETATION
Negative Result: A negative reaction is indicated by a uniform milky suspension with no agglutination as observed with the CRP Negative Control.

Positive Result: A positive reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared to the CRP Negative Control (Figure 1).

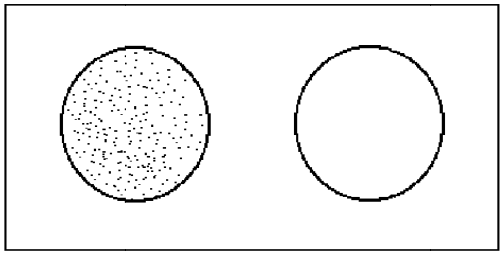

Figure 1.

EXPECTED VALUES
1. CRP in healthy individuals is approximately 0.02-1.35mg/dl. The mean value in adults is 0.047mg/dl.
2. A weak positive correlation was found between CRP and age.
3. It is important to determine the level of CRP for monitoring patient progress. This is due to (1) the concentration of CRP is an index of tissue damage incurred and (2) increasing or decreasing levels of CRP (e.g. daily) indicate the progress of inflammatory process.[7]

LIMITATIONS
1. Reaction time is critical. If reaction time exceeds three (3) minutes, drying of the reaction mixture may cause false positive results.
2. Freezing the CRP Latex Reagent will result in spontaneous agglutination.
3. Intensity of agglutination is not necessarily indicative of relative CRP concentration; therefore, screening reactions should not be graded.
4. A false negative can be attributed to a prozone phenomena (antigen excess). It is recommended, therefore, to check all negative sera by retesting at a 1:10 dilution with glycine buffer.

PERFORMANCE
1. Sensitivity: greater than 0.8 mg/dl
2. Linearity: 25 mg/dl.
3. Comparison:
   A. Qualitative Results: A study performed using CRP Latex Reagent and a commercially available product yielded 100% accuracy.
   B. Semi-quantitative Results: A panel of 32 CRP positive serum samples was assayed (semi-quantitated) on same day. The results of the study indicated that CRP Latex Reagent has 92.9 % precision.

REFERENCES
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