INSTRUCTIONS FOR USE

C-Reactive Protein (CRP) Latex Test

Catalog No. 52030-50, 52030-100

Intended use

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

Summary and Explanation of the Test

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 CRP1, various immunoprecipitation techniques have been applied for its detection. The C-Reactive Protein (CRP) Latex 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.

Test principle

The test kit employes anti-human CRP antibodies immobilized onto latex particles. Antibody bound particles are combined with patient serum samples to detect the presence of CRP. CRP levels are regulated by the patient in response to chronic or acute inflammation caused by a variety of sources. The test kit contains 50 or 100 qualitative tests worth of latex beads coated with anti-human CRP antibodies. The test procedure has a single rection step performed by adding the CRP Latex suspension directly to the specimen on the Black test card. This mixture is incubated for two minutes by manual (hand held) rocking technique to facilitate agglutination of latex particles in the presence of CRP. Reactions are read visually and reported differently for qualitative and semi-quantitative assays. Qualitative assays are reported as a binary positive or negative result for the agglutination of the CRP Latex suspension. Semi-quantitative results are reported as an approximate titer value being the inverse of the lowest dilution providing a positive agglutination result.

Contents of the Kit 52030-50

Component	Spec.	Component Description	Qty
CRP Latex	2mL	Latex particle coated with anti-human CRP antibody	1
CRP Positive Control[1]	0.5mL	Stabilized Human Sera	1
Latex Negative Control[1]	0.5mL	Stabilized Human Sera	1
Glycine Buffer 20X	2mL	Concentrated Glycine Buffer	1
Black Card	Each	Low absorption coated test card	9
0.05mL blunt cut pipet	Each	0.05mL volumetric dispensing pipet	50

Note: Exchange of components from different lots is not allowed. Store all reagents at 2–8°C; do not freeze.

Contents of the Kit 52030-100

Component	Spec.	Component Description	Qty
CRP Latex	4mL	Latex particle coated with anti-human CRP antibody	1
CRP Positive Control[1]	1mL	Stabilized Human Sera	1
Latex Negative Control[1]	1mL	Stabilized Human Sera	1
Glycine Buffer 20X	2mL	Concentrated Glycine Buffer	1
Black Card	Each	Low absorption coated test card	17
0.05mL blunt cut pipet	Each	0.05mL volumetric dispensing pipet	100

Note: Exchange of components from different lots is not allowed. Store all reagents at 2–8°C; do not freeze.

WARNING – Human Sera

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. However, as no known test method can offer complete assurance that hepatitis B virus, HIV Virus, or other infectious agents are absent, all human serum products and patient specimens should be handled in accordance with good laboratory practices.

Additional Materials and Equipment (not supplied with the test kit)

- Timer
- Tube - minimum volume 500ul
- Micropipettes and associated tips
- Physiological saline

Precautions

Strict adherence to assay instructions is required. Any variation in pipetting technique, incubation time or temperature, and use of reagents beyond the expiry date can cause differences and can affect the results. 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.

- The kit/reagents should not be used beyond the expiration date on the kit label.
- To avoid cross contamination, disposable pipette tips should be used.
- All reagents and samples should be prepared before starting the procedures.
- If the kit will be used multiple times, ensure the reagents are sealed fully and store components in original kit box at recommended storage temperature.
- Positive and negative controls should be included in each run of the assay.
- Modifications to the kit components or procedures may provide false results

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.

Test Procedure

Reagent Preparation

- All reagents must be brought to room temperature and mixed thoroughly prior to use.
- All specimens must be brought to room temperature and mixed thoroughly prior to use.
- Tightly close unused kit reagents and store at 2–8°C in original kit packaging.
- Do not interchange components of different lots. Do not use reagents beyond the expiration date indicated on labels.
- Dilute Glycine Buffer 20X, 1:20 with water.

Sample Preparation

- All specimens must be brought to room temperature and mixed thoroughly prior to use.
- 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. Avoid repeated freezing and thawing of samples.

Assay Procedure Qualitative

1. Bring all test reagents and serum specimens to room temperature. Gently shake the CRP latex vial to re-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 the black card by hand for two (2) minutes. Observe the internal area of each reaction oval for macroscopic clumping using an indirect oblique light source.

Assay Procedure Semi-Quantitative

1. For each test serum to be titrated, set up at least six tubes 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 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 six 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 Assay Procedure 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

Borderline positive results 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): Neat specimen = 6, 1:2 = 12, 1:4 =24, 1:8 = 48

Quality Control

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.

Limitation of the Test

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, hemolytic 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 adults3 and normal children4. 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/L5 while the CRP content of normal serum is less than 12 mg/L6. The KSL 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 titer 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 KSL and Behring Latex Tests) was found to contain 6.6 mg/L of CRP by the Behring nephelometry method.

References

1. MacCleod, C.M., & O.T.Avery: J. Exper.Med. 73, 191, 1950.
2. Singer, J.M., & C.M. Plotz: Am.J.Med., 21, 888, 1956.
3. Scherffarth, F.: M. Perez-Miranda & H. Goetz: Blut 20, 296, 1970.
4. Saxtad, J., L.A. Nilsson & L.A. Hanson: Acta Paediat. Scand. 59, 25, 1970.
5. Wood, H.F. & M.McCarty: J. Clin.Invest. 30, 616, 1951.
6. Nilsson, L.A., Acta Path.Microbiol.Scand. 73, 129, 1968.

Symbol Key


Consult Instructions for Use


Manufacturer


Temperature Limit


Date of Manufacture


Use by Date

Batch Code / Lot Number

Catalogue / Part Number

In Vitro Diagnostic Use


Number of Tests

1.800.960.1080
55 Amherst Villa Road
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www.kslbiomedical.com

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