C-Reactive Protein
CRP EIA

96 Tests 88 99 74

INTENDED USE
For the quantitative measurement of human C-Reactive Protein (CRP) in serum to aid in the follow up of rheumatic fever, inflammatory processes and other tissue-destructive lesions. For professional use only.

SUMMARY AND EXPLANATION OF THE TEST
C-reactive protein (CRP) has been regarded as an acute-phase reactant in serum. It consists of five single subunits, which noncovalently linked and assembled, as a cyclic pentamer with a molecular weight range from 110,000 to 140,000. CRP has been found to be increased in serum of patients with a wide variety of diseases including infections by gram-positive and gram-negative organisms, acute phase of rheumatoid arthritis, abdominal abscesses, inflammation of the bile duct, myocardial infarction, malignant tumors. CRP may be found in patients with Guillain-Barre syndrome and multiple sclerosis, certain viral infections, tuberculosis, acute infectious hepatitis, many other necrotic and inflammatory diseases burned patients, and after surgical trauma.

CRP is a remarkably consistent indicator of cardiovascular risk. Several prospective studies associate elevated CRP with a 2- to 3-fold increase in risk of a coronary event in apparently healthy populations. CRP also provides predictive data additive to that provided by cholesterol measurements in determining the risk of an acute coronary event.

Although the detection of elevated levels of CRP in the serum is not specific for any particular disease, it is a useful indicator of inflammatory processes. CRP levels rise in serum within hours of the onset of inflammation, reach a peak during the acute stage and decrease with the resolution of inflammation or trauma. The detection of CRP is a more reliable and sensitive indicator of the inflammatory process than the erythrocyte sedimentation rate, which may also be influenced by physiological changes not associated with an inflammatory process.

Current quantification methods including latex agglutination, nephelometry, radial immunodiffusion have the general disadvantage of low sensitivity. ELISA provides the highest sensitivity and specificity.

PRINCIPLE OF THE PROCEDURE
CRP is a microwell enzyme linked immunosorbent assay based on the sandwich principle. The wells are coated with anti-CRP antibodies. During the first incubation, CRP will form a complex on the well. Unbound substances are washed off. Enzyme conjugate is then added to form a sandwich complex in the second incubation. Unbound substances are washed off again. TMB Chromogenic substrate is added to develop a color. The enzymatic reaction is stopped by the addition of acid and the intensity of color developed is read using a microwell reader at 450 nm. The CRP in specimens run concurrently with standards can be determined from the standard curve.

ASSAY PROCEDURE
1. Place the desire number of coated strips into the holder.
2. Dispense 50 µl of standards and 1:100 diluted specimens into appropriate wells.
3. Dispense 50 µl of Sample diluent to each well and incubate for 30 minutes at room temperature.
4. Remove incubation solution and wash three times with distilled water or tap water.
5. Dispense 100µl of Enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove incubation solution and wash three times with distilled water or tap water.
7. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 30 minutes at room temperature.
8. Add 100 µl of 2 N HCL to stop reaction.
9. Read O.D. at 450 nm with a microwell reader.

MATERIALS PROVIDED
1. Microwell Strips:
   - Anti-CRP antibodies coated wells (12 x 8 wells).
2. TBM Chromogenic Substrate:
   - 1 vial (15 ml).
   - 1 vial (12 ml).
4. Sample diluent: Green color.
   - 1 vial (50 ml).
5. Standard set: 0,5,10,25,50,100 ng/ml (1.0 ml/vial)
6. Stop solution: 2 N HCL
   - 1 vial (12 ml)

SPECIMEN COLLECTION AND HANDLING
1. Collect blood specimens and separate the serum.
2. Specimen may be refrigerated at 2 - 8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum samples.

WARNINGS AND PRECAUTIONS
1. Do not pipette by mouth. Do not smoke, eat or drink in the areas in which specimens or kit reagents are handled.
2. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

STORAGE AND STABILITY
1. Store the kit at 2 - 8 °C.
2. Keep microwells sealed in a dry bag with desiccant.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

PREPARATION FOR ASSAY
1. Bring all reagents and specimens to room temperature (20-25°C), and gently swirl to obtain thorough mixing.
2. Have all reagents and samples ready before the start of the assay.
3. Once the test is begun, it must be performed without interruption to get the most reliable and consistent results.

Sample dilution 1:100

ASSAY PROCEDURE

Sample dilution 1:100

Do not dilute Standards

Sample dilution 1:100

5 µl / 500 µl

50 + 50 / 100 / 100

30 / 30 / 30 RT

09/01 H / jg
PROCEDURE NOTE
1. It is important to wash microwells thoroughly and remove the last droplets of water to achieve the best results.
2. Pipette all reagents and samples into the bottom of the wells.
3. Absorbance is a function of time and temperature of incubations. It is recommended to have all reagents and sample caps removed and all needed wells assigned and secured in holder. It will ensure the equal elapsed time for each pipetting without interruption.

CALCULATION OF RESULTS
1. Construct a standard curve by plotting O.D. 450 nm on the y-axis against the concentration of CRP ng/ml on the x-axis linear graph paper or log-log graph paper.
2. Using the O.D. value of each specimen, determine the concentration of CRP from the standard curve.
3. An example of typical results:

<table>
<thead>
<tr>
<th>CRP ng/ml</th>
<th>Net O.D. at 450 nm</th>
<th>Value obtained from standard curve (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0 (Blank 0.113)</td>
</tr>
<tr>
<td>5</td>
<td>0.266</td>
<td>0.249</td>
</tr>
<tr>
<td>10</td>
<td>0.562</td>
<td>0.548</td>
</tr>
<tr>
<td>25</td>
<td>1.276</td>
<td>1.166</td>
</tr>
<tr>
<td>50</td>
<td>1.999</td>
<td>2.179</td>
</tr>
<tr>
<td>100</td>
<td>3.018</td>
<td>3.043</td>
</tr>
<tr>
<td>patient A</td>
<td>0.650</td>
<td>0.661</td>
</tr>
<tr>
<td>patient B</td>
<td>1.490</td>
<td>1.505</td>
</tr>
</tbody>
</table>

EXPECTED VALUES
It is recommended that each laboratory determine its own normal value and abnormal range.
CRP is present in all healthy individuals; in the serum of neonates the range is from 10 to 350 ng/ml and in adults the range is from 68 to 8,200 ng/ml; increase to 500 µg/ml in subjects with inflammation from multiple nonspecific diseases.

PERFORMANCE CHARACTERISTICS
Accuracy (Recovery)

<table>
<thead>
<tr>
<th>Initial ng/ml</th>
<th>Expected (ng/ml)</th>
<th>Observed (ng/ml)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>37.5</td>
<td>40.1</td>
<td>107</td>
</tr>
<tr>
<td>25</td>
<td>62.5</td>
<td>65.6</td>
<td>105</td>
</tr>
<tr>
<td>50</td>
<td>30.0</td>
<td>28.8</td>
<td>96</td>
</tr>
<tr>
<td>50</td>
<td>75.0</td>
<td>81.0</td>
<td>108</td>
</tr>
</tbody>
</table>

Parallelism

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Calculated (ng/ml)</th>
<th>Observed (ng/ml)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 in 4</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3 in 4</td>
<td>75</td>
<td>81</td>
<td>108</td>
</tr>
<tr>
<td>2 in 4</td>
<td>50</td>
<td>48</td>
<td>96</td>
</tr>
<tr>
<td>1 in 4</td>
<td>25</td>
<td>26</td>
<td>104</td>
</tr>
</tbody>
</table>

Precision (Reproducibility)
Inter-assay (n=10) and intra-assay (n=10), coefficient of variation, were evaluated at three different pooled serum samples:

<table>
<thead>
<tr>
<th>N</th>
<th>Inter-assay Pool A</th>
<th>Pool B</th>
<th>Pool</th>
<th>Intra-assay Pool A</th>
<th>Pool B</th>
<th>Pool</th>
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<tbody>
<tr>
<td>10</td>
<td>28.5</td>
<td>65.7</td>
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<td>10</td>
<td>27.4</td>
<td>64.1</td>
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<tr>
<td>82.5</td>
<td>3.0</td>
<td>5.4</td>
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<td>80.9</td>
<td>2.4</td>
<td>4.6</td>
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<td>5.4</td>
<td>10.5</td>
<td>8.2</td>
<td></td>
<td>4.7</td>
<td>8.9</td>
<td>7.2</td>
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<tr>
<td>6.5</td>
<td></td>
<td></td>
<td></td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity (Minimum detectable concentration)
The minimal detectable concentration of CRP is estimated to be 0.4 ng/ml. A minimal detectable concentration is defined as that concentration of CRP which corresponds to the absorbance that is two standard deviations from the mean absorbance of 20 serum samples with zero dose of CRP.

LIMITATIONS
As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory finds have been evaluated.

REFERENCES