

INTEX[®]-CRP Card Test

Instructions for use

Immunoassay for the quantitative determination of CRP (C-reactive protein) in human blood

A. General information

1. Introduction

The C-reactive protein (CRP) is an acute-phase protein that is produced in the liver in response to stimulation by cytokines (IL-6, IL-1, TNF alpha, interferon gamma). It binds to surface molecules of microorganisms (bacteria, fungi, parasites), causes agglutination and precipitation and activates the complement system via the classical pathway. CRP binds to lymphocytes with Fc-receptors and can cause thrombocyte aggregation.

Measuring CRP levels is an established method for monitoring an inflammation reaction. The normal concentration of CRP in human blood is < 6 mg/L. Within 6-48 hours upon initiation of an inflammation the blood CRP level rises. This rise precedes the clinical symptoms and the increase of the blood sedimentation rate.

The determination of CRP is – largely – independent of factors which affect the blood sedimentation rate and the number of leukocytes. The kinetic of CRP is not affected by its physiological function. The momentary plasma level therefore is an exclusive indicator of the synthesis rate and thus the inflammation reaction. CRP measurement is beneficial in relation to the measuring of the blood sedimentation speed, particularly when working with children, since the needed blood quantity is smaller, the test duration is shorter and the response to therapy can be observed more readily.

2. Clinical relevance

The concentration of CRP in blood is a very sensitive indicator for inflammation activity. Due to the short reaction time and short half-life (20-30 hours), a change in inflammation reaction is indicated rapidly. Thereby, the effects of different inflammations will sum up.

The individual, genetically determined CRP level (base value) shows only small fluctuations in healthy individuals. An increased CRP level is therefore almost always coupled to pathological changes and therefore is very important for the diagnosis and the control of the chosen therapy.

Increased concentrations (6-100 mg/L) are found with acute diseases, e.g. with local bacterial infections, bacterial superinfections, viral infections as well as with rheumatoid disorders and cardiac infarcts. Also, an uncomplicated post operational process will be accompanied by a brief increase in CRP concentration. The CRP rise correlates with the state of a disease and with the quantity of the tissue affected by an inflammation.

Values over 100 mg/L indicate a high inflammatory activity and appear for example with sepsis, heavy infections and tumors. Levels above 300 mg/L are considered as excessive and can even indicate a possibly life-threatening situation.

CRP for some particular diseases:

Upper respiratory tract: Bronchitis, flu and infections of the upper respiratory tract are the most frequent infections. CRP often makes it possible to distinguish between viral and bacterial inflammations. High CRP values (> 25 mg/L) indicate a bacterial inflammation (e.g. pneumonia); whereas a small rise in CRP level may indicate a viral infection.

Infectious complications during pregnancy: During pregnancy, the base value of the CRP generally increases; up to over 10 mg/L towards the end of pregnancy. If the CRP level does not decrease after birth, this strongly indicates infectious complications. A rise of the CRP level during pregnancy beyond the increased base value can indicate intrauterine infections or a premature blister jump.

Meningitis: A limit of approx. 20 mg/L helps to distinguish between bacterial and viral meningitis. For young people and adults this limit shifts to 50 mg/L.

Inflammatory intestine disease: With the help of the CRP we can differentiate between organic intestine diseases and irritable colon. For Crohn`s disease the CRP level correlates well with the activity of the disease.

Rheumatoid arthritis: Patients with rheumatoid arthritis show increased CRP levels as a function of the disease process (up to over 200 mg/L). These correlate well with the clinical symptoms. However, the dynamics of the change of the inflammation activity is considerably smaller than with infections.

Cardiovascular diseases: Due to cell death during acute myocardial infarction, the CRP level increases. This rise depends on the extent of the cell damage. It has been shown that an increased CRP base value can predict the risk of a cardiovascular disease and of cerebral apoplexy.

For some particular diseases there is only a small increase in CRP level, despite an active inflammation process together with tissue damage. This is the case for e.g. systemic lupus erythematoses, ulcerative colitis, leukemia, sclerodermatitis and dermatomyositis. These are, however, well-defined diseases, where the diagnostic power of the CRP is not reduced. If a patient with such a disease suffers from an additional infection, the CRP level will increase as expected.

CRP in healthy individuals: When interpreting CRP values it has to be considered that the base level is influenced by different patient characteristics. The following factors have to be taken into account:

Age	The base values for children are lower than for adults.
Smoking	Adults can have a 10-fold higher CRP median.
Nutrition	Severe metabolic conditions can lead to a weak acute-phase reaction.
Physical activity	Depending on the condition, CRP rises after physical activity.
Pregnancy	During pregnancy, the CRP value continuously increases up to 20 mg/L at time of delivery.
Pretesting Treatment	Antibiotics and steroids influence the acute-phase reaction and thus the CRP level.

3. Test principle

The INTEX[®]-CRP Card Test quantitative is an immunochemical rapid test for the determination of the CRP concentration in human blood. The CRP contained in the sample (diluted capillary blood, heparin blood or EDTA blood) binds to an anti-CRP antibody, which is immobilized to a permeable membrane in the test field. Subsequently a colloidal gold-coupled monoclonal anti-human-CRP antibody is added to the test field which binds to the CRP on the membrane. After a washing step the quantity of the bound colloidal gold can be determined by the resulting colour. The intensity of the colour is proportional to the content of CRP in the sample. The INTEX[®]-CRP Card Test quantitative is evaluated by using the INTEX[®] CRP-Densitometer.

4. Range of measurement

The INTEX[®]-CRP Card Test quantitative is read by the INTEX[®] CRP-Densitometer. The INTEX[®] CRP-Densitometer can read the specific CRP in the range of **8-200 mg/L**.

5. Literature

Husebekk A, Hansson L-O: C Reactive Protein in Clinical Practice, ed 2. Oslo, Nycopharma AS, 1999.

Claus DR, Osmand AP, Gewurz H: Radioimmunoassay of human C-reactive protein and levels in normal sera. J Lab Clin Med 1976;87:120-128.

Wood WG, Ludemann J, Mitusch R, Heinrich J, Maass R, Frick U: Evaluation of a sensitive immunoluminometric assay for the determination of C-reactive protein (CRP) in serum and plasma and the establishment of reference ranges for different groups of subjects. Clin Lab 2000;46:3-4.

Pepys MB: The acute phase response and C-reactive protein. In Weatherall DJ, Ledingham JGC, Warrell DA (eds): Oxford Textbook of Medicine, ed 2. Oxford, Oxford University Press, 1995, pp. 1527-1533.

B. Usage information

6. Technical data

Specimen:	Capillary blood, EDTA blood or heparin blood
Sample volume:	5 µL
Duration:	5 - 10 min
Concentration range:	8 - 200 mg/L
Order number:	CRPQ3520 / CRPQ3550

7. Kit contents

- 20 / 50 pouches with test cards
- 20 / 50 glass capillaries (5 µL)
- 20 / 50 tubes with blood dilution buffer (1 mL each)

- 1 tube with washing buffer (1.5 / 3.75 mL)
- 1 glass vessel with gold conjugate (1.2 / 2.3 mL)
- 1 / 2 tube with positive control (0.9 mL)
- 1 instruction sheet

8. Storage and stability

The INTEX[®]-CRP Card Test quantitative should be stored at 2–8°C and can be used until the expiry date printed on the kit. Reagents in each kit are matched for best performance and therefore should not be interchanged with reagents from other packages.

9. Additionally needed material

- INTEX[®]-CRP Densitometer
- Micropipette for 20 µL volume and pipette tips
- Rack for blood dilution buffer tubes
- Disposable gloves

10. Sample collection

The INTEX[®]-CRP Card Test quantitative is realized using human blood, which is obtained through the usual procedures for medical practices. Only a volume of 5 µL is required for the test. Samples in coagulation-inhibited vessels can be stored up to 5 days in the refrigerator at 2-8°.

11. Assay procedure

Preparatory steps

① Preparation of the test

Bring test cards (one for each sample), liquid reagents and sample to room temperature (18-30°C). Gently mix the gold conjugate.

Open the pouch, take out the test card and mark it with a waterproof pen (e.g. patient identification, sample number). Do not touch the sample well. If several tests are performed in parallel, do not forget to label the blood dilution buffer tubes as well.

② Blood dilution

The samples are diluted 1:200 as follows: Take up the blood with the glass capillary (end-to-end volume 5 µL) and transfer the whole capillary into the blood dilution buffer tube. Close the tube and mix thoroughly. The solution should be clear (complete lysis) before applying to the test field.

③ Immunological reaction

From the diluted sample, 40 µL are applied to the sample well in two steps: With a micropipette, take up 20 µL and slowly apply it to the sample well. Wait until the liquid has been soaked into the well and apply another 20 µL. Avoid air bubbles on the membrane.

④ Detection

Take up 20 µL of gold conjugate solution, apply it to the sample well and wait until it is completely soaked in. Then apply another 20 µL.

⑤ Washing

Apply 20 µL of washing buffer. Wait until the liquid has been soaked into the well and apply another 20 µL.

Positive Control

The positive control can be used to test the quality of the test cards. The application has to be carried out as indicated above. Instead of the analytical sample 2x 20 µL of positive control can be added to the test card. There is enough positive control for two test cards.

12. Evaluation of the INTEX[®] -CRP Card Test quantitative

After the last washing step, the result can be read. The CRP test is positive when the sample well clearly shows a red brown color. Samples with a concentration less than 8 mg/L only show a slightly rose color. The color intensity of the sample well is readable with the INTEX[®] -CRP Densitometer, so you can prove a concentration range of **8-200 mg/L** of specific CRP. It is important, that you measure the CRP-value within 5 minutes after the last washing.









IMPORTANT:



Read the result with the INTEX[®] -CRP Densitometer within 5 minutes after the last washing. Over time, the coloration may change and later readings are invalid.

13. Interpretation of the results

A positive result (>8 mg/L) indicates an inflammatory reaction (see above). However, the CRP level has always to be interpreted together with the clinical symptoms. The measurement of CRP concentration cannot replace medical examination. It is a means to help finding the right diagnosis.

14. Symbols

	Article number		For single use only
	Lot number		Expiry date
	Storage		Content
	Only for in vitro diagnostics		Instruction sheet

INTEX[®] -CRP Card Test quantitative		
	20 test cards	CRPQ3520
	50 test cards	CRPQ3550



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Diagnostika

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