

Rapid immunochromatographic test for the detection of elevated levels of CRP from whole blood samples in case prediction of coronary events.

INTRODUCTION

C-reactive protein (CRP) is an acute phase protein. The concentration of CRP rapidly increases in whole blood after onset of an infection or tissue damage. In viral infections the CRP value remains low (below 10 mg/ml) or is only moderately elevated. Change in the pathological process is reflected with great sensitivity in the CRP value. Determination of CRP levels is widely used in the differential diagnosis and follow-up of the following diseases: septicaemia, meningitis, pneumonia, epiglottitis, enteritis, septic arthritis, upper urinary tract infection, pelvic inflammatory diseases, appendicitis, rheumatic diseases and infection following and premature rupture of membranes.

Additionally recent laboratory and clinical findings have demonstrated that atherosclerosis is not simply a disease of lipid deposits and that chronic inflammation plays an important role in the inception and progression of the atherosclerotic lesion. Current knowledge suggests that CRP may be an indicator of plaque stability. CRP's prognostic utility in patients with acute coronary syndromes and its ability to predict future coronary events in apparently healthy men and women have been demonstrated.

TEST PRINCIPLE

The Biocard™ hsCRP test is based on immunochromatography. The reaction takes place in a nitrocellulose membrane. A CRP-specific monoclonal antibody has been applied to the membrane to form test reaction zone. The other antibody is bound to coloured gold

particles to form the label, which is applied on the filter.

CRP in the sample reacts with the label antibody. In the test zone the particles with CRP are captured by another anti-CRP-antibody and a coloured test line is formed. The rest of the particles will be captured by the second stationary antibody zone, thus forming the control line. A control line is always formed when the test is working properly.

Testing is performed by adding 3 drops (appr. 110 µl) of diluted whole blood sample into the round sample well of the Biocard™ testing device. The sample flows through the membrane in 15 minutes. The result can then be read with the AMI Predictor Reader Unit.

Intensity of the test line depends on the concentration of CRP in the sample.

Sensitivity and specificity of the test are high and false negative results due to an excess of CRP have not been demonstrated. The test device is stored at room temperature and has a long shelf life.

MATERIALS PROVIDED

BIOCARD™ hsCRP
REF 3-003-000

- 10 pcs disposable Biocard™ hsCRP test devices
 - 10 pcs disposable pipettes
 - 10 pcs sample dilution buffer vials
 - 10 pcs disposable end-to-end 10 µl capillaries (Hep)
 - 10 pcs disposable lancettes
- Instructions for use

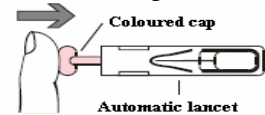
Materials needed but not provided with the kit: timer.

SAMPLE COLLECTION AND STORAGE

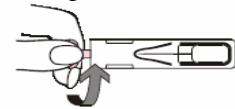
Whole blood and serum diluted in the sample dilution buffer can be used as a sample for Biocard™ hsCRP test. Samples shall be stored refrigerated (+2...+8 °C). For a longer storage serum samples should be frozen at -20°C. The diluted samples shall be used during the same working day.

TEST PROCEDURE

1. Press slowly the coloured cap of the automatic lancet until it clicks into the casing.

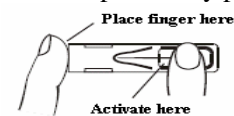


2. After the audible click, twist off the coloured cap.



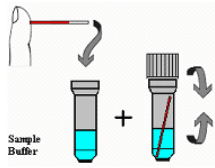
3. Gently massage the fingertip and clean it with the alcohol-soaked swab. Let the finger dry.

4. Press the round opening of the automatic lancet firmly against the cleaned fingertip, and activate it with the button. The puncture is practically painless.

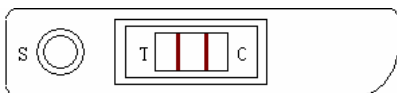
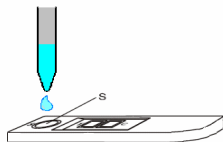


5. Press a drop of blood out of your fingertip. Open the plastic vessel and remove with caution the glass capillary. Hold one end of the glass capillary horizontally in the drop of blood until it has completely filled.

- Place the filled glass capillary in the tube containing buffer and close the tube firmly with the cap. Shake the tube several times until blood from the capillary is mixed completely with the buffer.



- Open the buffer tube and take a few drops of diluted sample with the pipette. Hold the pipette containing the diluted blood sample vertically over the round sample well (S) and drop 3 drops in it. After applying the drops, do not touch the test card for 2 minutes. The test result can be read after 15 minutes with the AMI Predictor by locating test cassette to Reading unit and selecting "Read test result".
- Another possibility is to carefully locate test cassette after dropping the sample to the sample well to Ami Predictor reader unit and start "Run test" function. Reader unit will measure the concentration automatically after 15 minutes.



Read test result with Ami Predictor Reader Unit. Follow the instruction of the Reader Unit. Intensities of the test and control lines varied as a result of CRP concentration.

INTERPRETATION OF THE RESULTS

The AMI Predictor quantifies exactly the concentration of CRP. The usual way to give CRP results is $\mu\text{g/ml}$. However because of our AMI Reader's character we have to give results as ng/ml .

Following values can be used for predicting the risk for coronary events:

CRP CONCENTRATION <2000 ng/ml (same as $<2\mu\text{g/ml}$)

CRP concentration is at a normal level. There is no evidence of risk of coronary events based to this test result.

CRP CONCENTRATION 2000-10000 ng/ml (same as 2-10 $\mu\text{g/ml}$)

CRP concentration is elevated. CRP levels between 2-10 $\mu\text{g/ml}$ indicate an increased likelihood of progression to myocardial infarction. There are however also some other things which should be ruled out before making the decision. For example viral infection and sunburn can cause slightly elevated CRP levels.

CRP CONCENTRATION >10000 ng/ml (same as $>10\mu\text{g/ml}$)

This must likely mean acute bacterial infections.

It is not possible to evaluate the likelihood of progression to myocardial infarction with this result.

STABILITY AND STORAGE

Store the test devices at ambient temperature ($+2\dots+27^\circ\text{C}$). The shelf life of BiocardTM hsCRP test is indicated of the pouches and the kit.

PRECAUTIONS AND LIMITATIONS

If instructions for use are not carefully followed, false results may appear.

BIOCARDTM hsCRP tests shall be used only for *in vitro* detection of

CRP in whole blood samples or serum samples according to the instructions of use.

General laboratory procedures and precautions shall be followed in handling and disposal of samples and used testing material.

Do not reuse BIOCARDTM hsCRP test units. Do not use expired tests or tests from a lot not showing proper performance when tested with the controls. Do not use a test unit from a pouch, which has been damaged during storage.

Note: When assessing the final diagnosis, instead of relying solely on the test result given by BiocardTM hsCRP test, it is necessary to take into consideration all patient information, which may have an influence on the diagnosis.

References:

A.Niskanen, M.Saramäki, E.Schwandt. Myeloperoxidase (MPO) and hs-CRP as predictive factors for myocardial infarctions CLI, 2006, Vol 30, Issue 7. p. 38-39.

J. Mackay, G.Mensah. Atlas of heart Disease and stroke. WHO Nonserial Publications 2004

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