

Rapid immunochromatographic test for the detection of elevated levels of MPO (Myeloperoxidase) from whole blood samples in case prediction of coronary events.

INTRODUCTION

Myeloperoxidase (MPO) has been shown to be potential risk marker for atherosclerosis. Biochemically belonging to the haem peroxidase superfamily, MPO is the most abundant constituent of the azurophilic granules of polymorphonuclear leucocytes (neutrophils, monocytes and some of tissue macrophages). MPO amplifies the oxidative potential of its cosubstrate hydrogen peroxidase, which then generates hypochlorous acid and other oxygen radicals with bactericidal and viricidal properties. When secreted into the extracellular space, MPO appears to be responsible for converting LDL cholesterol to its oxidised form, which then attaches itself to the endothelial plaques of coronary arteries

US studies involving the measurement of MPO levels in 600 patients presenting with chest pain have shown that MPO measurement may be particular beneficial in such patients who have low initial troponin levels. MPO determination made it easier to identify patients who experienced a major adverse cardiac event in the period 30 days to six months after presentation. Without using MPO levels, only 54 % such cases could be identified where as with MPO 85 % were identified. Such studies have been confirmed in another European study. These studies suggest that when the MPO level is above a certain cut off value (350 ng/ml) there is a considerably increased risk of heart attack.

TEST PRINCIPLE

The Biocard™ MPO test is based on immunochromatography. The

reaction takes place in a nitrocellulose membrane. A MPO-specific monoclonal antibody has been applied to the membrane to form 2 test reaction zones. The other antibody is bound to coloured gold particles to form the label, which is applied on the filter.

MPO in the sample reacts with the label antibody. In the test zone the particles with MPO are captured by another anti-MPO-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 MPO in the sample. Sensitivity and specificity of the test are high and false negative results due to an excess of MPO have not been demonstrated. The test devices and dilution buffers are stored at room temperature and has 18 months shelf life.

MATERIALS PROVIDED

BIOCARD MPO

Ref. 3-030-000

- 10 pcs Disposable Biocard™ MPO test devices
- 10 pcs Disposable pipettes
- 10 vials of sample dilution buffer
- 10 disposable end-to-end 10 µl capillaries (Hep)
- 10 disposable lancettes

Instructions for use

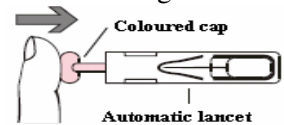
Materials needed but not provided with the kit: timer.

SAMPLE COLLECTION AND STORAGE

Whole blood and serum diluted in the sample dilution buffer can be used as a sample for Biocard MPO 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

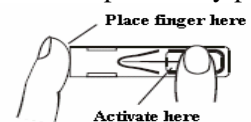
1. Press slowly the coloured cap of the automatic lancet until it clicks into the casing.



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



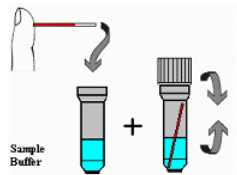
3. Gently massage the fingertip and clean it with the alcohol-soaked swab. Let the finger dry.
4. Press the round opening of the automatic lancet firmly against the cleaned fingertip, and activate it with the button. The puncture is practically painless.



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

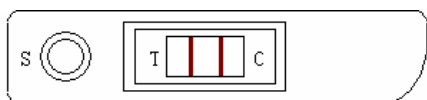
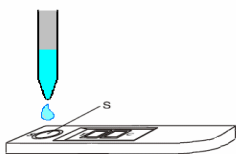
horizontally in the drop of blood until it has completely filled.

- 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.



Intensities of the test and control lines varied as a result of MPO concentration. Results will be compared against internal calibration curve.

INTERPRETATION OF THE RESULTS

The AMI Predictor quantifies exactly the concentration of MPO

from 50 ng/ml level. Below 50 ng/ml levels samples will give negative interpretation.

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

MPO CONCENTRATION <350ng/ml

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

MPO CONCENTRATION >350 ng/ml

MPO concentration is elevated. MPO levels above 350 ng/ml indicate an increased likelihood of progression to myocardial infarction.

STABILITY AND STORAGE

Store the test devices at ambient temperature (+2...+27°C). The shelf life of Biocard™ MPO is indicated of the pouches and the kit.

PRECAUTIONS AND LIMITATIONS

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

BIOCARD™ MPO tests shall be used only for *in vitro* detection of MPO 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 BIOCARD™ MPO 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 Biocard MPO 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-MPO 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
- S. Baldus, C. Heeschen, T. Meinertz, A.M. Zeiher, J.P. Eiserich, T. Munzel, M.L. Simoons, C.W. Hamm Myeloperoxidase serum levels predict risk in patients with aute coronary syndrome. Circulation 2003; 108(12): 9034-9035
- S.J. Nicholls, S.L. Hazen Myeloperoxidase and cardiovascular disease. Arteriocler Thromb Vasc Biol 2005; 25:1102-1111

MANUFACTURER

Ani Biotech Oy
Tiilitie 3,
01720 Vantaa, FINLAND
<http://www.anibiotech.fi>
e-mail: info@anibiotech.fi
tel. +358-20-1557510
fax. +358-20-1557511