DyonMed S.A.
Point of Care Solutions

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ISO 9001:2008 (No. 010110003)| ISO 13485:2003 (No 01210005)
by TÜV Austria Hellas

DyonCRP®
(*C-reactive protein*)

No. Of Cat.: DNDL101

Rapid test for semi-quantitative detection of C-reactive protein in sample of whole blood, fingerstick blood, serum or plasma

SUMMARY

DyonCRP® (*C-reactive protein*) is a rapid, non-invasive, highly accurate and easy to perform qualitative test that allows a health professional to detect semiquantitative C-reactive protein in a sample of whole blood serum or plasma.

The acute phase protein (CRP, C-reactive protein) is a major indicator for the diagnosis of inflammation or tissue necrosis. The composition of CRP is made in the liver and induced by proinflammatory cytokines such as interleukin-6. The CRP reaches the site of inflammation through the plasma and contributes actively to endogenous immune response. Systemic inflammation accompanied by a drastic increase in the CRP concentration in blood. In recent years, the detection of CRP on point of emergency care patient (point of care) has been successfully incorporated in the daily practice of the health professional (outpatient, emergency medicine, pathology practice, Melbye & Stocks, 2006.)

The measurement of CRP levels has now become necessary in clinical practice and differentiates, not only in emergencies and chronic conditions that require multidisciplinary approaches (Windgassen et al, 2011). Besides the internal medicine, the use of CRP in cardiology extends, oncology surgery, orthopedics and rheumatology (Felson et al,. 2011). CRP is also considered necessary to infectious diseases and pediatrics (Cals et al, 2009), while also contributing and helping to reduce irrational use of antibiotics (Cals et al, 2010). Finally, in the gastroenterology CRP is a key indicator of Differential between inflammatory bowel diseases, and therapeutic monitoring (Langhorst et al, 2008; Henriksen et al, 2011; Ricanek et al, 2011).

The plasma levels of CRP rise 6-8 hours after a bacterial infection or a trauma, not reaching a plateau after approximately 48 hours after infection. The level of CRP decreased rapidly after regression or cessation of root cause, while the CRP has a half life of 48 hours. Normally, the degree of inflammation and inflammation affects the activity increasing CRP. Values between 10 and 40 mg / l may be found during inflammation such as in patient's local bacterial infections, abscesses, minor injuries, often in malignant tumors, viral infections, etc. In severe inflammatory diseases that require immediate medical attention, the CRP values can reach up to 100 mg / l. Values above 100 mg / l may be found during sepsis, burns or surgeries (Macy et al, 1997.)

PRINCIPLE OF THE METHOD

The test strip **DyonCRP®** with the tip immersed in the sample , which is diluted in buffer. The sample migrates along the test strip from the bottom to the tip thereof. If the sample contains CRP, it bounds to the first antibody anti -CRP, which is connected to red labelled colloidal gold. The red labelled CRP- antibody complex now migrates with the sample along the membrane, wherein the second anti -CRP antibody is immobilized in lines of different concentrations. The labelled CRP-antibody complex is fixed by the immobilized antibodies of the membrane, resulting in the formation of red lines. The number of lines depends on the concentration of CRP in the sample. The more CRP is in the sample, the more red lines will appear. An additional red control line will appear at the end of the membrane, internal control review, stating the correct procedure. The formation of the control line is independent of the concentration of CRP in the sample. Failed to display the red control line means that the result is invalid. In this case the test should be repeated.

STORAGE AND STABILITY

Store the kit at 2-30 ° C. If the kit was stored in the refrigerator, it should be transferred to room temperature before proceeding with the examination. Under these conditions, the kit is stable until the expiration date printed on the package.

PACKAGE CONTENTS

Each kit contains the following:
- 10 test strips (dipsticks) individually wrapped
- 10 tubes solution for 20 tests
- 1 rack for tubes solution
- 10 capillaries
- 10-pin scarification
- 1 package manual in English.

SAMPLE COLLECTION AND PREPARATION

1. Before use, make sure all the ingredients have come to room temperature. Cold buffer or any concentrated liquid in test strip can lead to invalid results.
2. Using a sterile tip, get a drop of blood (10 μl) from the tip of a finger.
3. Tap the supplied in the kit capillary tube in the punctured finger and observe the blood is transferred to a capillary until it reaches the maximum (about 10 μl) Dilute the collected blood directly (step 4) to avoid clotting.
4. Put the capillary, as is, with blood, in the tube of step 1 containing the dilution solution.
5. Close the lid and shake the tube vigorously for ~ 10 seconds, so that the blood is released from the capillary so that the sample and the diluting solution can be mixed completely.
6. Leave the diluted sample to stand in special racks for ~ 1 min.
7. The final sample obtained can be controlled directly or until after 8 h.

Note: the above steps are the same regardless of whether the presence of blood is taken EDTA, citrate or heparin.

TEST PROCEDURE-INTERPRETATION OF RESULTS

Prior to testing, ensure that the final sample and the plastic package with test cassette have come to room temperature. The test cassette must be opened only shortly before the examination, since the method is sensitive to moisture.

1. Open the foil pouch and remove the test strip at the end of which is printed the word «CRP». Avoid diligently to touch the white response area.
2. Open the tube with the diluted specimen (step 7) and dip the test strip into the liquid backwards. Make sure that the test region immersed only to the point "MAX". Avoid splash reaction area with wet or immerse it deep into liquid.
3. Allow the test strip in the sample for about 15 seconds or until a pink liquid forehead appears to move in reaction area.
4. Remove the test strip and place it on a flat, non-absorbent surface. Alternatively, the test strip can be left in the vial. Start the timer.
5. Wait for colored lines. Interpret the result just after 5 minutes. (Interpretation of the test results for a time longer than 5 minutes will give values greater than the actual)

Positive Results = at least two colored lines.
The test result is positive **DyonCRP®** for CRP where the control line (C) and at least one of the lines T1, T2 and T3 are simultaneously visible. The appearance of the effect of the test lines (T1, T2 and T3) depends on the concentration of CRP in the sample. The higher the concentration of CRP in the sample the more lines are visible, as follows:

• When the red line the result of the examination (T1) and the red control line (C) are also visible (albeit to varying degrees): the sample contains at least 10 mg / l CRP. The greater the concentration of CRP, the more enhanced will be the color intensity of T1.
• When redrawn result of the examination (T1) and (T2) and the red control line (C) is also visible (albeit to varying degrees): the sample contains at least 40 mg / l CRP. In these conditions, the color intensity of T1 is greater than that of T2.
• When all the red lines result of the consideration T1, T2 and T3 and the red control line (C) is also visible (albeit to varying degrees): the sample contains at least 80 mg / l CRP. Accordingly, the colour intensity of T2 is greater than that of T3.

The formation of the test line, which depends on the concentration of CRP, illustrated in the diagram below. Note that the number of lines of test results increases with increasing concentration of CRP. The colour intensity of the lines is weak in appearance, but later increased with increasing concentrations of CRP.

Note that the three lines have different colour intensity. For the range of concentrations shown in the diagram the colour intensity of the lowest line is the strongest, and the middle, and especially the upper line of the test results have clearly lighter colour intensity of the lowest line.

The colour of the control line is independent of the concentration of CRP or that of linear results of the test presenting always bright red colour.

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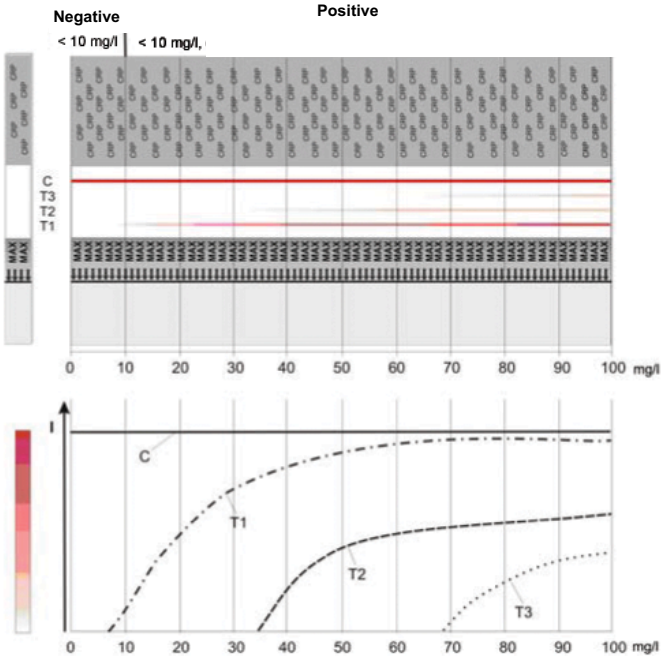

Negative Result = only visible line C.
The test is negative if (1) colored line appears in the Control Zone (C).

Invalid = No colored line in the Control Zone (C)
The test is invalid if no matching row in the Control Zone (C), even if a colored line has appeared in the test zone (T). If this happens reread the instructions and repeat the test using a new device or contact Dyonmed SA.

PRECAUTIONS

Precautions regarding the collection, handling, storage and disposal of samples and used kit components. Handle all specimens as if they contain infectious agents. Take precautions against microbiological hazards throughout the process. Apply standard procedures for proper disposal of specimens. Operation is not affected by known environmental factors other than temperature.

RESTRICTIONS

1. The assay **DyonCRP®** should be used for the qualitative detection of human CRP. CRP is not a specific marker for certain diseases. As in all in vitro diagnostic tests the result should never be interpreted by itself, but also in relation to other clinical findings. Often the increase of CRP precedes the actual symptoms, so the time frame should be taken into account. 2. The idiosyncratic variation CRP values are rather high. Normally, the values > 10 mg / l must be considered abnormally elevated. 3. The test can be used to assess cardiovascular risk because they are not sensitive enough for it. 4. The characteristics are valid only if the contents of the package are in good condition. 5. **DyonCRP®** device is for single use only. In the event that a dissolved sample should be retested, use another device. 6. The test **DyonCRP®** is exclusively for health care professionals.

QUALITY CONTROL

Internal Quality Control: The colour line appears in the Zone (C) is an internal control procedure. Confirm correct specimen volume and correct technical process. A clean background is the internal negative control. If the test works correctly in the Control Zone background should be white to light pink and it should not be difficult to interpret the result. External Quality Control: Each facility or health practitioner who uses the test should develop its own guidelines for testing and certification.

FUNCTIONAL CHARACTERISTICS

1. Calibration: The assay was calibrated on the basis of internationally standard model 85/506 of the World Health Organization. The detection limit of the test strip **DyonCRP®** is calibrated in such a way that a concentration of 10 mg / l in insoluble samples leads to a positive red line after dilution with the supplied solvent. If the undiluted sample contains more than 40 mg / l CRP, appears a second red line. Concentrations more than 80 mg / l CRP lead to emergence of a third red line. The ranges of concentration (concentration ranges) could be confirmed compared with a reference test quantitatively.
2. Comparative Study of Operation. **DyonCRP®** was compared to a commercially available semi-quantitative detection kit for CRP concentration range from 0 to 200 μg / ml. In each CRP concentration was triple analysis with both methods (78 analyzes total). The results showed greater accuracy of 99.9% **DyonCRP®** assay compared to the reference method.
3. Study Operation in clinic (SOC): **DyonCRP®** was tested in 3 different SOC using samples negative, weak positive, moderately positive and strongly positive. Each sample was tested at levels of five replicates over 5 days. The results showed 100% accuracy with the expected values.
4. Precision study: three workshops took samples of whole blood by double blind procedure in which added purified CRP. 6 samples were prepared with concentrations of 10, 30, 40, 70, 80 and 100 μg / ml CRP. Five replicates of each concentration were tested in each laboratory with a total test 30 tests per lab. All samples also were tested by the test **DyonCRP®**. The results showed 100% agreement with the expected values.
5. Interference studies. **A**. The following substances possibly inserted proved not intervene in the calculation of the CRP assay by rapid **DyonCRP®** as the indicated concentrations: biotin (200 ng / mL), bilirubin (10 mg / dL), hemoglobin (200 mg / dL), cholesterol (800 mg / dL), triglycerides (1250 mg / dL) in vitro **B**. Control of the following drugs at therapeutic levels showed no interference in the calculation of CRP: adenine, atropine, dopamine, oxazepam, albumin (bovine), caffeine, erythromycin, oxytetracycline, allopurinol, kaptoprili, gentasic acid, propranolol, ambroxol, chloramphenicol, isoprotenolol, Theophylline, ampicillin sinarizin, isosorbide dinitrate, L-thyroxine, ascorbic acid, cyclophosphamide, nifedipine, urea, atenolol, cyclosporin, nystatin, uric acid.
6. Prozone. Prozone phenomenon was not presented in CRP concentrations up to 2,000 μg / ml.

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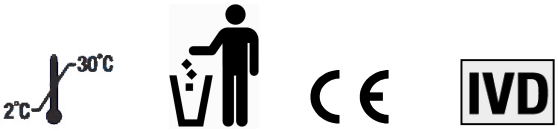

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