NORDIC BIOMARKER

MRX Protein C

A chromogenic assay for the analysis of Protein C activity

REF K5020 CE

1. Intended use

MRX Protein C is a chromogenic test for quantitative determination of Protein C activity in citrated human plasma as an aid in diagnosis of Protein C deficiency. MRX Protein C is intended to be used by professional laboratory personnel using automated coagulation analyzers measuring at 405 nm.

2. Summary and principle

Protein C is a vitamin K dependent glycoprotein with anticoagulant properties[1,2]. When activated by thrombin, activated Protein C (APC) regulates the coagulation process by inactivating factors Va and VIIIa. This disrupts the tenase- and prothrombinase complexes, which inhibits thrombin activation[3,4,5].

Protein C deficiencies, hereditary and/or acquired, increases the risk for developing thromboembolic disease[6]. There are two different types of Protein C deficiency; type I refers to reduced biosynthesis whereas type II is characterized by a decrease in functional activity[7].

MRX Protein C measures Protein C activity, thus covering both types of Protein C deficiency. The principle involves activation of all functional Protein C present in a plasma sample by a serine protease extracted from the venom of Agkistrodon contortrix. After incubation, a chromogenic substrate which is readily hydrolysed by APC is added. The pNA released is proportional to the Protein C level in the sample, which can be measured at 405 nm.

3. Composition

Protein C Activator: 3 x 2.5 mL Protein C activator in a buffer containing preservatives and stabilizers.

Protein C Substrate: 3 x 2.5 mL chromogenic substrate for Protein C in a solution containing preservatives and stabilizers.

4. Warnings and precautions

For in vitro diagnostic use. Handling by professional laboratory personnel only. The results should be used together with other clinical and diagnostic information for forming a diagnosis and for patient management.

The activator and substrate contain sodium azide (< 0.1 %) and 2-methylisothiazol-3(2H)one (< 0.0015 %) to prevent microbial growth; use proper disposal procedures.

The activator contains Bovine Serum Albumin (< 1.0 %) for stability. The animals received ante- and post-mortem inspections under veterinarian's supervision and were apparently free from infectious and contagious diseases. However, as no method can offer complete assurance that infectious agents are absent, this material should be handled as any potentially infectious material.

EUH208: Contains 2-methylisothiazol-3(2H)one. May produce an allergic reaction.

EUH210: Safety data sheet available on request.

5. Reagent preparation, storage and stability

Protein C Activator: Ready to use. Store at 2-8 °C. Do not freeze. Swirl the vial gently a few times before each day it is used, to ensure a homogenous suspension. After opening, stable for 2 months at 2-8 °C in original vial.

Protein C Substrate: Ready to use. Store at 2-8 °C. Do not freeze. Swirl the vial gently a few times before each day it is used, to ensure a homogenous suspension. After opening, stable for 2 months at 2-8 °C in original vial. Discard if the substrate solution appears yellow.

Note: Protein C substrate is light-sensitive and should be stored in the dark.

6. Procedure

For each instrument, refer to its operator's manual and to the instrument-specific MRX Protein C application sheet.

7. Specimen collection and preparation

Citrated plasma is used for testing. Nine volumes of freshly drawn venous blood are immediately added into and mixed with one volume of 3.2 % buffered tri-sodium citrate solution. Centrifuge at 3000 x g for 10 minutes and extract the supernatant. Refer to CLSI Approved Guideline H21-A5 for more detailed instructions on specimen collection, handling and storage.

8. Material required but not provided

MRX Protein C Calibrator (Part # K5023): contains human plasma with a Protein C concentration assigned against WHO 2nd International Standard (NIBSC code 02/342).

Instrument/analyzer capable of chromogenic detection at 405 nm. Pipettes and high quality de-ionized water. Protein C control plasmas, e.g. MRX Specialty Normal Control (Part # K5057) and MRX Specialty Abnormal Control (Part # K5058). Phosphate buffered saline (PBS) for sample dilution.

9. Quality control

To maintain consistent assay results, it is recommended that control plasmas are assayed at regular intervals. Each laboratory should establish a control range to determine the allowable variation in the day to day performance of the test. Recalibration is suggested when control materials are not within the acceptable range or if a new lot of reagent is used.

10. Results

The results are reported in % Protein C, where 100 % corresponds to 1 IU/mL Protein C of the WHO 2nd International Standard (NIBSC code 02/342). Refer to the instrument's operator's manual and instrument-specific application sheet for details.

11. Limitations

Warfarin and aprotinin causes inhibition of activated Protein C, thus, it is expected to observe low Protein C activity in warfarin/aprotinin treated patients.

MRX Protein C is insensitive to the following substances on Sysmex CS-series instruments: hemoglobin (up to 200 mg/dL), conjugated bilirubin (up to 20 mg/dL), unconjugated bilirubin (up to 20 mg/dL) triglycerides (up to 350 mg/dL) and heparin (up to 2 U/mL).

Turbid or opalescent plasma may cause erratic results and should be interpreted with caution; dilute the sample and re-assay.

12. Expected values

Protein C activity is expressed in %, where 100 % corresponds to 1 IU/mL of the WHO International Standard (NIBSC 02/342).

Normal Protein C levels are typically in the range 70 – 150 %, where the levels for males are somewhat higher than for females[8].

Due to variation in results between populations, each laboratory should set its own reference range.

13. Performance characteristics

The performance will depend on the instrument used. Refer to the instrument-specific MRX Protein C application sheet for details. As an example, the performance data below was obtained with a Sysmex CS-series instrument.

MRX Protein C on Sysmex CS-series instruments has a reportable range of 8-186 % Protein C activity. Precision: CV is 1.4 % for a sample with 103 % Protein C activity and 2.8 % for a sample with 18 % Protein C activity.

When compared to another chromogenic assay, MRX Protein C correlates as follows:

y (Hemos IL Protein C) = 0.96 x (MRX Protein C); $R^2 = 0.99$

14. Reporting of incidents

Any serious incidents that has occurred in relation to this device shall be reported to Nordic Biomarker as well as the national competent authority in which the user is established.

References

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