MediRox

FOR RESEARCH USE ONLY

Revision: 2020-01-31

MRX PT DOAC Reagent Art. No: MRX1101-4
MRX PT DOAC Reagent Buffer Art. No: MRX1102-4

INTENDED USE

For functional analysis of the extrinsic pathway of the coagulation cascade in patients treated with direct oral anticoagulants (DOACs).

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SUMMARY AND PRINCIPLE

The PT DOAC assay can detect the DOACs apixaban (Eliquis®), rivaroxaban (Xarelto®), edoxaban (Lixiana®) and dabigatran (Pradaxa®) in citrated human plasma. The PT DOAC assay is based on two prothrombin time (PT) assays, a DOAC-sensitive PT assay and a DOAC-insensitive PT assay.

A PT method is based on activation of the extrinsic pathway, of coagulation factor (F) VII by thromboplastin, in presence of calcium. The activated complex (FVIIa-tissue factor) activates FX. FXa, in complex with FV, activates prothrombin (FII) to thrombin leading to the conversion of fibrinogen to fibrin, detected as a clot. The time between the mixing of sample and reagent and the detection of the clot is the PT. A PT result is dependent on the levels of FII, FVII and FX in the plasma sample.

In samples from patients treated with DOACs, the activity of either FXa or FIIa in the DOAC-sensitive assay will be appreciably inhibited. This will result in a prolonged PT, as manifested by an elevated DOAC Normalized Ratio (DNR) for the DOAC-sensitive assay. In contrast, the International Normalized Ratio (INR) is minimally affected by the presence of DOACs in the DOAC-insensitive assay. INR and DNR determined by both the DOAC-sensitive, and the DOAC-insensitive assay will show a difference between the DNR and the INR, for samples containing DOACs, and no difference for DOAC-free samples. The difference can be expressed as an above-unity clot time ratio (CTR). DOAC is detected by a CTR significantly greater than that unity.

PRODUCT DESCRIPTION

The PT DOAC Reagent consists of lyophilised rabbit brain thromboplastin, a plasma fraction of bovine origin, buffer, stabilisers and <0.1% sodium azide as preservative.

The PT DOAC Reagent Buffer (4 mL) consists of buffers, bovine serum albumin and <0.1% sodium azide as a preservative.

- PT DOAC Reagent (MRX1101): 20 x 4 mL
- PT DOAC Reagent Buffer (MRX1102): 20 x 4 mL

PRECAUTIONS

The PT DOAC assay is for research use only and cannot be used for patient diagnosis or treatment. The PT DOAC Reagent and PT DOAC Reagent Buffer consists of material from a bovine origin. All donor animals were sourced from BSE-free herds. The cattle received ante- and post mortem health inspection by a veterinarian, and were tested free from infectious- and contagious agents. As precaution, the material should, however, be treated as potentially infectious. Wear appropriate clothing and avoid contact with skin and eyes. Waste is disposed according to local regulations. Detailed information can be found in the Material Safety Data Sheet.

PREPARATION

Dissolve the content of each PT DOAC Reagent vial in PT DOAC Reagent Buffer by pouring all the buffer in the buffer vial into the reagent vial with lyophilised reagent. Replace the stopper and shake immediately for 15 seconds. Visually inspect the solution to confirm the complete reconstitution of the reagent. Keep the reconstituted reagent at 2-8 °C for 60 minutes and mix prior use.

STORAGE CONDITIONS AND STABILITY

Unopened reagent stored at 2-8 °C is stable until the expiration date shown on the vial. The reconstituted reagent is stable for 4 days when stored in 2-8 °C in the closed original vial.

SPECIMENT COLLECTION AND PREPARATION

It is recommended that specimen collection, handling and storage to be carried out according to the guidelines in CLSI H21-A5 Vol. 28 No.5[1]. The presence of a clot in a specimen is cause for rejection.

INSTRUMENT AND TEST PROCEDURE

The analysis procedure is intended for use on automated coagulation systems.

Procedure, DOAC insensitive assay:

- Allow the reconstituted PT DOAC Reagent to reach 37 °C
- Add 20 µL PT DOAC Sample Diluent to 10 µl plasma sample, mix and allow the mix to reach 37 °C
- Add 180 µL PT DOAC Reagent to the plasma sample pre-mixed with PT DOAC Sample Diluent and immediately start recording the time
- Record the clotting time in seconds

Procedure, DOAC sensitive assay:

- Allow the reconstituted PT DOAC Reagent to reach 37 °C
- Allow 50 µL plasma sample to reach 37 °C
- Add 100 µL PT DOAC Reagent to the plasma and immediately start recording the time
- Record the clotting time in seconds

CALIBRATION AND CALCULATION OF RESULTS - INR, DNR and CTR

DOAC insensitive assay - INR

The lot specific normal clotting time (NCT) and the International Sensitivity Index (ISI) are generated by local calibration of the DOAC insensitive assay using MRX PT DOAC Calibrators (MRX1104).

INR is calculated from the following equation: $INR=(\text{patient PT}/NCT)^{ISI}$

DOAC sensitive assay - DNR

The lot specific normal clotting time (NCT) and the DOAC Sensitivity Index (DSI) are generated by local calibration of the DOAC sensitive assay using MRX PT DOAC Calibrators (MRX1104).

DNR is calculated from the following equation: $DNR=(\text{patient PT}/NCT)^{DSI}$

PT DOAC result – CTR

The PT DOAC result is expressed in a CTR, calculated as follows:

CTR=(DOAC sensitive assay, DNR)/(DOAC insensitive assay, INR)

QUALITY CONTROL

In accordance with good laboratory practice it is necessary to run controls to ensure accuracy and reproducibility of the results. It is recommended to use MRX PT DOAC Controls (MRX1108). Each laboratory is recommended to set up an internal quality control program.

REFERENCE INTERVAL

The reference interval was determined according to the guidelines in CLSI EP28-A3c[2]. The CTR was determined in plasma samples from 120 blood donors, of whom 59 were women (mean age 67 years) and 61 were men (mean age 68 years). Ongoing anticoagulant therapy and a history of thrombosis or haemophilia were exclusion criteria. Analysis were performed using a Sysmex CS2100i instrument. The reference interval was calculated according to the resampling/bootstrap method with 95% CI. This reference interval should be considered as a guideline, each laboratory is recommended to establish its own local reference range for CTR measurements.

PT DOAC	Reference interval
Mean	1.18
2.5 percentile	0.98
97.5 percentile	1.38

LIMITATIONS AND INTERFERING SUBSTANCES

PT DOAC Reagent is insensitive to the following substances: haemoglobin up to 10 g/L, bilirubin up to 500 mg/L and lipids up to 2.5 g/L. The CTR may be affected by substances influensing the extrinsic pathway of the cogulation cascade, for example heparin, warfarin and lupus anticoagulants. The PT DOAC Reagent is insensitve to unfractionated heparin up to 0.1 IU/mL and low molecular weight heparin up to 0.5 IU/mL. In a study on 88 samples with PT (INR) > 2, 10 samples showed a CTR > 1.38 (97.5 percentile of the reference interval).

PRECISION

The precision of the PT DOAC Reagent was determined according to CLSI guideline EP05-A3[3] using the PT DOAC Controls and a plasma pool spiked with a DOAC corresponding to a low CTR value.

Sample	Mean (CTR)	Repeatability (CV %)	Intra-device (CV %)
PT DOAC Control Normal	1.05	1.05	2.05
PT DOAC Control Low	1.59	0.61	2.29
PT DOAC Control High	2.89	0.75	3.45
Low CTR sample	1.12	0.81	1.99

LIMIT OF BLANK (LoB) AND LIMIT OF DETECTION (LoD)

The LoB and LoD for the PT DOAC Reagent were determined according to the guidelines in CLSI EP17-A210[4]. LoB in CTR was 1.12 and LoD in CTR was 1.15.

REFERENCES

1. CLSI. Preparation and Testing of Reagent Water in the Clinical Laboratory, Fourth Edition, CLSI Document C3-A4; Vol. 26 No. 22
2. CLSI. Defining, Establishing, and Verifying Reference Invervals in the Clinical Laboratory; Approved Guideline-Third Edition. EP28-A3c.
3. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guidline – Third Edition. EP05 – A3.
4. CLSI. Evaluation of Detection Cabability for Clinical Laboratory Measurement Procedures; Approved Guidline – Second Edition. EP17 – A2.