IVD

CE

MediRox

Instructions for Use [EN]

MRX PT Owren

REF K5026

For *In vitro* Diagnostic Use.

1 Intended use

Clotting test for quantitative determination of the prothrombin time (PT) in citrated human plasma according to the Owren's method. Can be used to monitor warfarin therapy and to evaluate the function of the extrinsic pathway of the coagulation cascade. Intended to be used by professional laboratory personnel using coagulation analysers.

2 Background and principle of method

Prothrombin time determination using MRX PT Owren is dependent on the activity of vitamin K-dependent coagulation factors, FII, FVII and FX. Owren's PT method is based on stimulation of the extrinsic coagulation pathway via activation of FVII by thromboplastin in the presence of calcium. The activated complex (FVIIa-tissue factor) activates FX. Subsequently, FXa, in complex with FV, activates prothrombin to thrombin leading to the conversion of fibrinogen to fibrin, detected as a clot.
In patients treated with vitamin K-antagonists, the activity of vitamin K-dependent coagulation factors will be lower and result in a prolonged PT.

When analysing PT using MRX PT Owren, sample diluted in MRX PT Buffer and reagent are mixed, and the clotting time is measured. The final dilution of sample is 1:21. The reagent is enriched with FV and fibrinogen from bovine plasma (deficient of FII, FVII and FX), making it insensitive to variations in patient FV and fibrinogen levels. Also, due to the 1:21 sample dilution, the Owren PT method is relatively insensitive to interferences.

3 Components

MRX PT Owren consists of:
10 × 10 mL lyophilised rabbit brain thromboplastin, bovine plasma, preservatives, and stabilisers.

4 Warnings and precautions

Wear suitable clothing for protection. Avoid contact with skin and eyes. Do not empty into drains. Waste must be disposed of in accordance with local regulations.

MRX PT Owren contains sodium azide (less than 0.1%) to prevent microbial growth; use proper disposal procedures.

MRX PT Owren contains Bovine Serum Albumin and bovine plasma. The animals were approved by veterinarians by ante- and post-mortem inspections.
However, as no method can offer complete assurance, this material should be handled as potentially infectious.

If MRX PT Owren is reconstituted according to 1X method, precipitation can occur if the reagent, water, and calcium chloride used for reconstitution are colder than 15 °C. Do not use the reagent for analysis if precipitation occurs.

5 Preparation

MRX PT Owren should be reconstituted and allowed to mature for 24 hours prior to use (see section 6 Storage and stability).
The reagent can be reconstituted according to two methods, 1X or 2X method. In the 1X method, calcium chloride is added to the MRX PT Owren reagent at the time of reconstitution while calcium chloride is added separately during analysis in the 2X method.
If the reagent is kept in the instrument for several days, it is important to mix the reagent at least daily before use. Continuous stirring is not necessary.

5.1 Reconstitution according to 1X method

- **Note:** See warnings and precautions.
- Allow the reagent, deionised water, and calcium chloride solution to reach room temperature (15 - 25 °C).
- Add 5 mL deionised water (e.g. MRX Laboratory Water, K5036) to the reagent vial.
- Keep the reagent for 10 - 15 minutes at room temperature (15 - 25 °C). It is important to mix

gently by swirling at the beginning, in the middle, and at the end of this period. The reagent will dissolve into a slightly opaque colourless liquid.

- After 10 - 15 minutes, add 5 mL 25 mM calcium chloride (e.g. MRX Calcium Chloride, K5049/K5050/K5051) to the vial with reconstituted reagent. Mix the reagent by swirling or inverting several times.
- Allow the reconstituted reagent to mature 24h at 15 - 25 °C before use.

5.2 Reconstitution according to 2X method

- Allow the reagent and deionised water to reach room temperature (15 - 25 °C).
- Add 5 mL deionised water (e.g. MRX Laboratory Water, K5036) to the reagent vial.
- Keep the reagent for 10 - 15 minutes at room temperature (15 - 25 °C). It is important to mix gently by swirling at the beginning, in the middle and at the end of this period. The reagent will dissolve into a slightly opaque colourless liquid.
- Allow the reconstituted reagent to mature 24 h at 2 - 8 °C before use.

6 Storage and stability

Store at 2 - 8 °C. After reconstitution, stable for 4 days (1X method) or 6 days (2X method) after the first 24 h (Figure 1).

Important: MRX PT Owren reconstituted with the 1X method must be stored at 15 - 25 °C during the initial 24 h before storage in 2 - 8 °C while MRX PT Owren reconstituted with the 2X method should be stored at 2 - 8 °C (Figure 1).

Stability data is valid provided that reconstituted reagent is stored closed in the original vial and that no contamination occurs.

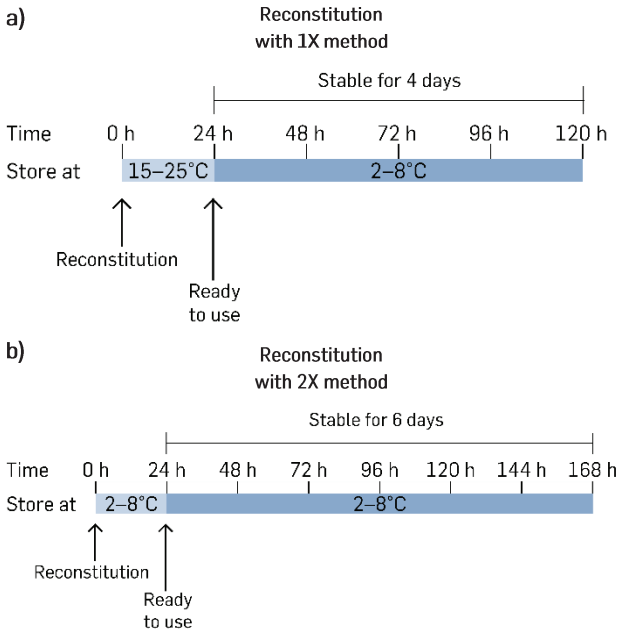

Figure 1. Stability and storage temperatures for MRX PT Owren reconstituted with the 1X (**a**) and 2X (**b**) methods.

7 Specimen collection and preparation

Venous blood is collected in 3.2% sodium citrate at a ratio of 9 parts blood to 1 part anticoagulant (1:10 ratio). The ratio is critical. Trauma or stasis during blood sampling should be avoided. Blood should not be collected through a heparin lock or other heparinised line. Inverse immediately after sampling. The presence of any clots in a specimen is a cause for rejection. Centrifuge to produce platelet-poor plasma and use for analysis. Refer to CLSI guideline H21-A5 for further instructions on specimen collection, handling, and storage.[1]

8 Procedure

8.1 Calibration

Each lot of MRX PT Owren must be calibrated prior to determination of INR in patients' plasma samples. During calibration, reagent lot and instrument specific values for normal clotting time (NCT) and International Sensitivity Index (ISI) required for INR determination (see Section 11 Results) are established. Calibration must be performed for each method (1X and 2X methods). It is recommended to perform a new calibration if a new lot of MRX PT Buffer is implemented.

Local ISI calibration can be performed using lyophilised calibrant plasmas,[2,3] such as the Calibration kit provided by Equalis. Refer to instructions provided by the calibrator supplier for calibration procedure.

Document ID: IFU-4620 Version: 2.0 Issue date: 2022-04-04

It is also possible to determine lot and instrument specific mean normal prothrombin time (MNPT) and ISI using guidelines provided by WHO.[4]

8.2 Automated instruments

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

8.3 Semi-automated and manual instruments

Analysis with reagent reconstituted according to 1X method

- Make sure that the final volume in the cuvette is within instrument specification.
- Predilute plasma samples by mixing 100 µL plasma sample with 600 µL MRX PT Buffer (K5037/K5038/K5061) and pre-heat the diluted plasma sample to 37 °C.
- Pre-heat MRX PT Owren to 37 °C.
- Start the reaction by mixing 100 µL diluted plasma sample with 200 µL MRX PT Owren. Incubate at 37 °C and start recording the time.
- Record the clotting time in seconds.

Analysis with reagent reconstituted according to 2X method

- Make sure that the final volume in the cuvette is within instrument specification.
- Predilute plasma samples by mixing 100 µL plasma sample with 600 µL MRX PT Buffer (K5037/K5038/K5061) and pre-heat the diluted plasma sample to 37 °C.
- Pre-heat MRX PT Owren and 25 mM calcium chloride (e.g. MRX Calcium Chloride) to 37 °C.
- Mix 100 µL diluted plasma sample and 100 µL MRX PT Owren.
- Start the reaction by addition of 100 µL 25 mM calcium chloride. Incubate at 37 °C and start recording the time.
- Record the clotting time in seconds.

9 Material required but not provided

Coagulation analyser, cuvettes, pipettes, and the following:

Calibrator	REF
Refer to section 8.1	-

Control material	REF
MRX Routine Normal Control	K5039
MRX Routine Abnormal Control	K5040

Solutions	REF
MRX PT Buffer	K5037 K5038 K5061
Calcium chloride (25 mM) e.g. MRX Calcium Chloride	K5049 K5050 K5051
Deionised water for reconstitution e.g. MRX Laboratory Water	K5036

10 Quality control

To maintain consistent assay results, it is recommended that control plasmas are assayed at regular intervals. MRX Routine Controls (K5039/K5040) are recommended for MRX PT Owren. Each laboratory should establish a control range to determine the allowable variation in the day-to-day performance of the test, as well as appropriate intervals for analysing controls in accordance with good laboratory practice.

11 Results

Results are reported in International Normalised Ratio (INR). INR is calculated by dividing the obtained clotting time by the normal clotting time or mean normal PT (see Section 8.1 Calibration) raised to the lot specific ISI value for the reagent and measurement system according to the formula below.[5,6]

$$INR = (\text{patient's PT} / \text{control PT})^{ISI}$$

Patient's PT= The patient's PT in seconds.
Control PT = The normal PT time (NCT or MNPT) in seconds. Lot specific value for the reagent and instrument combination determined during calibration.
ISI = Lot specific value for the reagent and instrument combination determined during calibration.

Note: The ISI and NCT/MNPT values are specific for each lot of reagent, each reagent/instrument combination, and reconstitution method (1X or 2X methods).

12 Expected values

Expected values for non-affected population are INR 0.9 - 1.2[7] while expected values for affected population (e.g. patients treated with vitamin K-antagonists) are INR 2.0 - 3.5.[8]

Document ID: IFU-4620 Version: 2.0 Issue date: 2022-04-04

13 Limitations and interfering substances

Results may be affected by insufficient blood sampling with a shifted ratio of sodium citrate to patient plasma. The PT results may also be affected by heparin. MRX PT Owren is affected by direct/novel oral anticoagulants (DOACs/NOACs). Therefore, analysis of DOAC containing plasma samples is not recommended using MRX PT Owren.

MRX PT Owren is insensitive to the following substances on Sysmex CS-2100i:

Interfering substance	Tolerance
Bilirubin	Up to 40 mg/dL
Haemoglobin	Up to 1000 mg/dL
Triglycerides	Up to 1000 mg/dL
Unfractionated heparin	Up to 50 U/dL

Each laboratory is recommended to determine its own sensitivity to heparins.

14 Analytical performance characteristics

The following performance data was obtained with a Sysmex CS-2100i instrument. Performance will depend on the instrument used.

Precision:

Sample	Mean INR	Repeatability CV
Level 1	1.0	0.8%
Level 2	3.0	1.1%

15 Reporting of incidents

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

16 Additional Information

A paper copy of these Instructions for Use is available on request. Contact your local distributor.
The instrument-specific application sheet is available from your local distributor.

17 References

1. CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline – Fifth Edition. CLSI document H21-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
2. LINDAHL, Tomas L., et al. INR calibration of Owren-type prothrombin time based on the relationship between PT% and INR utilizing normal plasma samples. *Thrombosis and haemostasis*, 2004, 91.06: 1223-1231.
3. HILLARP, Andreas, et al. Local INR calibration of the Owren type prothrombin assay greatly improves the intra-and interlaboratory variation. *Thrombosis and haemostasis*, 2004, 91.02: 300-307.
4. WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION. MEETING; WORLD HEALTH ORGANIZATION; WORLD HEALTH ORGANIZATION. EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION. *Who Expert Committee on Biological Standardization: Sixty-second Report*. World Health Organization, 2013.
5. LOELINGER, E. A. ICSH/ICTH recommendation for reporting prothrombin time in oral anticoagulant control. *Thromb Haemostas*, 1985, 53: 155-156.
6. WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION, et al. *WHO Expert Committee on Biological Standardization: forty-eighth report*. World Health Organization, 1999.
7. ZIERK, Jakob, et al. Data mining of reference intervals for coagulation screening tests in adult patients. *Clinica Chimica Acta*, 2019, 499: 108-114.
8. HIRSH, Jack, et al. Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range. *Chest*, 1998, 114.5: 445S-469S.

18 Definition of symbols

	Manufacturer	 nordicbiomarker.com/IFU	Consult electronic instructions for use
	CE mark		Use-by date
	In vitro diagnostic medical device		Temperature limit
	Catalogue number		Biological risks
	Batch code		Contains biological material of animal origin

19 Revision history

Version	Changes to previous version
2.0	K5061 has changed its name to MRX PT Buffer. Added languages.