MediRox

CE

Revision: 2020-02-26
Changes: Storage conditions and stability: Stability for reconstituted 1X reagent stored at 2-8 °C changed from 7 days to 5 days. Updated information regarding use of reagent for same day and for several days.
Reconstitution: Changed information regarding precipitation; do not use reagent if precipitation occurs.
Background and principle and calibration: General update, information about PT% removed
Performance characteristics: precision changed to CS-2100i data
References: updated
Supersedes: 2019-01-14

MRX Owren's PT
Art.-Nr. GHI131-4, GHI131-10, GHI131-10SI, GHI131-20

English

INTENDED USE

MRX Owren's PT is intended for determination of prothrombin complex activity and provides information about the activity of the vitamin K-dependent coagulation factors FII, FVII and FX.

FOR IN VITRO DIAGNOSTIC USE.

BACKGROUND AND PRINCIPLE OF METHOD

MRX Owren's PT reagent is used for determining prothrombin complex activity, prothrombin time (PT), in plasma, citrated blood and capillary blood from patients administered vitamin K-antagonists and for detecting coagulation factor deficiency in the extrinsic pathway. MRX Owren's PT method is dependent on the activity of vitamin K-dependent coagulation factors, FII, FVII and FX. In patients, treated with vitamin K-antagonists, the activity of the vitamin K-dependent coagulation factors will be inhibited and result in a prolonged PT. A PT method is based on activation of the extrinsic pathway of FVII by thromboplastin, in the presence of $CaCl_2$. 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.

When analysing PT, sample 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 (deficent 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 method is relatively insensitive to heparin. This insensitiveness for heparin can be further enhanced by using a sample dilution buffer with polybrene.

The international recommendations of reporting PT is in international normalized ratio (INR) and refers to the international sensitivity index (ISI) of thromboplastins[1,2]. The INR is the quotient between the clotting time for a sample and the clotting time for normal plasma samples (MNPT) raised to the method's ISI value.

A reference interval of PT INR ≤1.2 and a treatment interval for vitamin K-antagonists (VKA) of PT INR 2-3 are commonly used[3].

- INR = (patient PT/MNPT)^{ISI}
- ISI = lot specific value for the reagent and measurment system.
- MNPT = lot specific value for the reagent and measurement system.

Each laboratory should determine the laboratory specific MNPT and ISI due to variation in instruments and the reagent set-up. The ISI and MNPT values are specific for each lot of reagent and each reagent/instrument combination.

PRODUCT DESCRIPTION

MRX Owren's PT: Lyophilized reagent consisting of thromboplastin from rabbit, bovine plasma, preservatives and stabilizers.

Available in the following packaging:

GHI131-4	10 x 4 mL
GHI131-10	10 x 10 mL
GHI131-10SI	10 x 10 mL
GHI131-20	10 x 20 mL

PRECAUTIONS

Only for *in vitro* diagnostic use. The product contains sodium azid (<0.1%) to prevent bacterial growth. MRX Owren's PT reagent should be handled by trained laboratory personnel only. Wear suitable clothing for protection. Avoid eye and skin contact. Waste is disposed of according to local regulations. Do not empty into drains. Detailed information can be found in the Material Safety Data Sheet.

MATERIAL REQURIED BUT NOT INCLUDED

25 mM $CaCl_2$ (GHI155).

PT Buffer (MRX150) or PT Buffer with polybrene (MRX152) or Owren's Buffer (GHI150) or Owren's Buffer with polybrene (GHI152).

Diluent (GHI154) or CLSI CLRW type water or equivalent[4].

RECONSTITUTION

MRX Owren's PT reagent can be reconstituted according to two methods, 1X or 2X method.

The difference between 1X and 2X method is that for 1X method $CaCl_2$ is added to the reconstituted MRX Owren's PT reagent and for 2X method $CaCl_2$ is added separately during analysis. The 1X method can often gain a higher throughput on automatic instruments, but requires higher demands on system cleanness as a $CaCl_2$ activated reagent will be more sensitive to contamination. Thus, a PT reagent inactivated due to the absence of $CaCl_2$ (2X method), is less sensitive to contamination.

If MRX Owren's PT is reconstituted according to 1X method, precipitation can occur if the reagent, water and $CaCl_2$ used for reconstitution are colder than 15 °C.

Do not use the reagent for analysis if precipitation occurs.

To avoid precipitation, it is important that the water and $CaCl_2$ used for reagent reconstitution are room-tempered (15-25 °C).

Reconstitution of reagent according to 1X method:

Allow the reagent to reach room temperature (15-25 °C).

Add room-tempered Diluent (GHI154) or CLSI CLRW type water or equivalent[4] to the reagent vial, according to the table below.

	GHI131-4	GHI131-10 GHI131-10SI	GHI131-20
GHI154 Diluent or CLSI CLRW type water or equivalent	2 mL	5 mL	10 mL

Keep the reagent for 10-15 minutes at room-temperature, 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 room-tempered 25 mM $CaCl_2$ (GHI155) to the vial with reconstituted reagent, according to the table below.

	GHI131-4	GHI131-10 GHI131-10SI	GHI131-20
GHI155 25 mM $CaCl_2$	2 mL	5 mL	10 mL

Mix the reagent by swirling or inverting several times.

If the reagent is kept in the instrument for several days it is important to mix the reagent at least daily prior use. Continuous stirring is not necessary.

See section storage conditions and stability for further information.

Reconstitution of reagent according to 2X method:

Allow the reagent to reach room temperature (15-25 °C).

Add room-tempered Diluent (GHI154) or CLSI CLRW type water or equivalent[4] to the reagent vial, according to the table below.

	GHI131-4	GHI131-10 GHI131-10SI	GHI131-20
GHI154 Diluent or CLSI CLRW type water or equivalent	2 mL	5 mL	10 mL

Keep the reagent for 10-15 minutes at room temperature, 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. $CaCl_2$ is added separately, (by the instrument) during analysis.

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.

See section storage conditions and stability for further information.

MediRox

CE

Revision: 2020-02-26
Changes: Storage conditions and stability: Stability for reconstituted 1X reagent stored at 2-8 °C changed from 7 days to 5 days. Updated information regarding use of reagent for same day and for several days.
Reconstitution: Changed information regarding precipitation; do not use reagent if precipitation occurs.
Background and principle and calibration: General update, information about PT% removed
Performance characteristics: precision changed to CS-2100i data
References: updated
Supersedes: 2019-01-14

MRX Owren's PT
Art.-Nr. GHI131-4, GHI131-10, GHI131-10SI, GHI131-20

English

STORAGE CONDITIONS AND STABILITY

Unopened reagent stored at 2-8 °C is stable until the expiration date shown on the vial.

Use of reagent same day as reconstitution:

1X method: Keep the reconstituted reagent at room temperature (15-25 °C) for 2 hours before use, the reagent is then stable for 6 hours at 2-8 °C and 15-25 °C.

2X method: Keep the reconstituted reagent at room temperature (15-25 °C) for 1 hour before use, the reagent is then stable for 6 hours at 2-8 °C and 15-25 °C.

Use of reagent over several days:

If the reagent is intended to be used over several days reconstitute the reagent 24 hours before use.

Storage temperature during the first 24 hours:

1X method: 15-25 °C

2X method: 15-25 °C for continued storage at 15-25 °C and 2-8 °C for contiuned storage at 2-8 °C.

Stability after reconstitution, in closed original vial

Reconstitution method	Storage temperature	Stability (days)
1X	2-8 °C	5
2X	2-8 °C	7
1X	15-25 °C	5
2X	15-25 °C	5

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection, handling and storage is carried out in accordance with CLSI guideline H21-A5 Vol. 28 No.5[5]. Insufficient filling and presence of a clot in a specimen is a cause for rejection.

PROCEDURE

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

PROCEDURE MANUAL INSTRUMENTS

Predilutions for plasma, citrate- and capillary blood

- 100 µl plasma +
  600 µl PT Buffer or Owren's Buffer
- 50 µl capillary blood +
  200 µl PT Buffer or Owren's Buffer
- 50 µl citrated blood (1+9) +
  170 µl PT Buffer or Owren's Buffer

Procedure plasma samples

Analysis with reagent recontituted according to 1X method

- 100 µl plasma diluted 1:7, pre-heated to 37 °C
- 200 µl MRX Owren's PT, pre-heated to 37 °C

Analysis with reagent reconstituted according to 2X method

- 100 µl plasma diluted 1:7, pre-heated to 37 °C
- 100 µl MRX Owren's PT reagent, pre-heated to 37 °C
- Mix plasma and MRX Owren's PT reagent
- Add 100 µl 25 mM $CaCl_2$ pre-heated to 37 °C

For both methods the following is applicable: mix immediately and let react at 37 °C. The clotting time is the time period from the last addition (of reagent for the 1X method and $CaCl_2$ for the 2X method) until a clot is formed.

CALIBRATION

The measurement system can be calibrated according to the WHO guidelines[6] or by using lyophilized calibrant plasmas[7,8].

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 two or three level controls from MediRox. Each laboratory is recommended to set up an internal quality control program.

- 3-Level controls MRX170-MRX183 or
- 2-Level controls GHI162-GHI170

LIMITATIONS AND INTERFERING SUBSTANCES

The MRX Owren's PT results may be affected by insufficient blood sampling with a shifted ratio of sodium citrate to patient plasma and blood sampling in EDTA instead of sodium citrate. The PT results may also be affected by heparin and the vitamin K levels.

The MRX Owren's PT is not affected by the following substances in concentrations up to:

Heparin UFH*	0.5 IU/mL
Triglycerides	10 g/L
Bilirubin	0.5 g/L
Haemoglobin	10 g/L

*Unfractionated Heparin using PT buffer (MRX150)/Owren's buffer (GHI150).

PT buffer/Owren's buffer with polybrene (MRX152/GHI152) increase the insensitivity to heparin and the reagent is not affected by unfractionated heparin up to 1 IU/mL. Each laboratory is recommended to determine its own sensitivity to heparins.

PERFORMANCE CHARACTERISTICS

Precison

The precision analysis was performed according to CLSI guideline EP05-A3[9] (20 days, 2 runs per day, 2 replicates per run) using normal control plasma and abnormal control plasma. The analyses were performed on Sysmex CS-2100i using PT Buffer MRX150.

	Repeatability (CV%)	Intra-device (CV%)
Normal control plasma	0.7	1.1
Abnormal control plasma	1.2	1.7

REFERENCES

1. Loeliger E.A.ICSH/ICTH Recommendations for reporting prothrombin time in oral anticoagulant control Thromb Haemost, 54 (1985), pp. 155-156
2. WHO Expert Committee on Biological Standardization Forty-eighth report. WHO Technical Report Series, No. 889
   World Health Organization, Geneva (1999) pp. 64–93
3. Theodorsson E. and Berggren Söderlund M. (red). Laurells Klinisk Kemi i praktisk medicin. Studentlitteratur 2018. ISBN: 978-91-44-11974-8
4. Clinical and Laboratory Standards Institute. Preparation and Testing of Reagent Water in the Clinical Laboratory, Fourth Edition, CLSI Document C3-A4; Vol. 26 No. 22.
5. CLSI Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays 5th Ed. CLSI document H21-A5 Vol. 28 No.5
6. WHO Expert Committee on Biological Standardization. Sixty-second report. WHO Technical Report Series, No. 979
   World Health Organization, Geneva (2013) pp. 271-316
7. Lindahl T.L et al. INR calibration of Owren-type prothrombin time based on the relationship between PT% and INR utilizing normal plasma samples. Thromb Haemost. 2004 91:1223-31.
8. Hillarp A. et al. Local INR calibration of the Owren type prothrombin assay greatly improves the intra- and interlaboratory variation. A three-year follow-up from the Swedish national external quality assessment scheme. Thromb Haemost. 2004 91:300-7.
9. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guidline – Third Edition. EP05 – A3. Wayne, PA: Clinical and Laboratory Standard Institute; 2014.