EQUALIS

Calibrators and control materials for
P—Prothrombin time (INR) according to Owren

CERTIFICATE OF ANALYSIS

IVD

REF	LOT	
002 001	40	INR-kalibrator, låg
002 002	41	INR-kalibrator, hög
002 003	42	INR-kontroll

IVD

CAL

2025-04

2 °C 8 °C

Instructions for calibration

Calibrate the measuring system at least once per year or *i*) when changing reagent lot, *ii*) after instrument adjustments that may affect the clotting time, *iii*) when results deviate >2 SD in two consecutive rounds of an external quality assessment scheme, or *iv*) according to local instructions.

The materials are intended for two-point calibration according to a special Excel form “Calibration form, lot 40-42” that is available from Equalis upon request. REF 002 001 and REF 002 002 are used as calibrators, and REF 002 003 is used as a control. Input data are clotting times from repeated measurements of the calibrators. Output data are the ISI-value (International Sensitivity Index) and the normal clotting time.

Calibration should be performed on well-maintained laboratory instruments, and the stability of the reagent and the buffer should be checked.

Instructions for use

Allow the bottle to reach room temperature. Carefully tap the bottle with your finger or against a table so that all the powder is collected at the bottom of the bottle. Open the bottle and add 1.00 mL deionised water of room temperature. Put the stopper back. Keep the bottle in an upright position and carefully rotate the bottle between the palms of your hands about 10 times. Tilt the bottle upside down so that the inner walls come into contact with the liquid and carefully rotate until all powder is dissolved. The reconstituted solution is light yellow and somewhat opaque. Allow the sample to condition for at least 15 minutes before commencing the measurement.

Perform the measurement, as for normal plasma samples, within 6 hours after reconstitution.

When measured, the value of the control (REF 002 003) should be within 2.46 ± 0.15 (LOT 42).

Warnings and precautions

Upon receipt, carefully check that each glass bottle is free from cracks and that the label is attached and fully readable. Defective materials must not be used and should immediately be returned to Equalis.

The materials have been tested and found negative for HBs antigen, HIV antibodies, and HCV antibodies. They are, however, to be treated as potentially infectious, and shall be handled according to appropriate safety routines for products derived from human blood, which includes safe disposal of waste.

The products are intended for *in vitro* use only.

Description of materials

The three materials are derived from citrate anti-coagulated lyophilized human plasma from Swedish donors and are supplied in silanised glass bottles sealed with a rubber stopper and an outer plastic screw cap. Frozen (-70°C) plasma was used for REF 002 001. Plasma, with lowered concentration of K vitamin dependent factors through addition of barium sulphate when the plasma was fresh, was used for REF 002 002 and REF 002 003. The plasma was frozen after the addition of barium sulphate and thawed before production. The materials are stabilised but the additives do not affect the measurand. The plasma was prepared by centrifugation and the platelet concentration is $<20 \cdot 10^9$/L.

Intended use

The materials should be used for calibration and control of measurements of prothrombin time (PT) according to Owren *[Owren et al 1951, Owren 1959]* where the results refer to tissue factor-induced relative time (INR). The certified values are valid only for procedures that comply with the following criteria: *i*) Citrate plasma (0.105, 0.109 or 0.129 mol/L) is diluted 1+20 with buffer and reagent. *ii*) The reagent contains thromboplastin from rabbit brain, and factor V and fibrinogen from bovine plasma. The final concentration of factor V is 5–30 % of the activity of normal plasma and the final concentration of fibrinogen is 0.2–2 g/L. *iii*) Reaction conditions: temperature 35–39ºC, pH 6–9, calcium ion activity 1.2–6.0 mmol/L, and sodium chloride concentration 80–160 mmol/L.

Intended users

The intended users are laboratory personnel in a laboratory environment.

Assignment of INR-values

The assignment of values has been performed mainly according to the previously described procedure for assignment of INR-values to calibrators for Owren-type prothrombin-time assays *[Hillarp et al 2004, Lindahl et al 2004]*.

The relationship between PT% with Owren-methods and PT (INR) with Quick-methods has been shown to be *PT(INR)=(1/PT%+0,018)/0,028.* This relationship was used to assign INR-values to dilution series of normal plasma at each one of six laboratories, who measured the clotting time with documented measurement procedures. These INR-values and the corresponding clotting time were used to construct calibration curves. Materials from LOT 40, 41, and 42 were then measured as samples at each laboratory, and the clotting times were recalculated to INR using the calibration curve. The mean of all measurements at the expert laboratories is the assigned INR-value for each LOT.

Metrological traceability

The certified values are traceable to an internationally agreed reference measurement procedure (WHO's manual tilt tube technique) and the reference thromboplastin WHO IRP 67/40, through RBT/90.

References

Hillarp A, Egberg N, Nordin G, Stigendal L, Fagerberg I, Lindahl TL. Local INR calibration of the Owren type prothrombin assay greatly improves the intra- and interlaboratory variation. Thromb Haemost 2004; 91:300-307.

Lindahl TL, Egberg N, Hillarp A, Ødegaard OR, Edlund B, Svensson J, Sandset PM, Rånby M. INR calibration of Owren-type prothrombin time based on the relationship between PT% and INR utilizing normal plasma samples. Thromb Haemost 2004; 91:1223-1231.

Owren PA, Aas K. The control of Dicumarol therapy and the quantitative determination of prothrombin and proconvertin. Scand J Clin Invest 1951; 3:201-208.

Owren, PA. Thrombotest. A new method for controlling anticoagulant therapy. Lancet 1959; ii: 754-758.

Certified values

REF	LOT	Product name	Type of material	PT (INR) [1]	U[2] (INR)
002 001	40	INR-kalibrator, låg	INR-calibrator, low	1.07	0.10
002 002	41	INR-kalibrator, hög	INR-calibrator, high	3.01	0.18
002 003	42	INR-kontroll	INR-control	2.46	0.14

[1] NPU01685; P—Coagulation, tissue factor-induced; relative time (actual/norm; INR; IRP 67/40; proc.)

[2] The stated uncertainty is an expanded uncertainty *U* obtained by multiplying the combined standard uncertainty u_c with a coverage factor k=2, which corresponds to a level of confidence of approximately 95 %. The estimation of u_c takes into account the uncertainty of the assignment of values to the current materials, and the uncertainty when using the WHO reference procedure and the reference thromboplastin RBT/90 to assign values to other materials.

Stability and homogeneity

Unopened glass bottles can be transported at room temperature. The materials are stable at least until the stated expiry date when stored in their unopened original glass bottles at 2−8ºC in a dry environment protected from light.

The long-term stability has been estimated by repeated measurement of samples from previous lots for up to 10 years after the original assignment of INR-values to those lots. The change in INR-values is negligible until the expiration date of the products.

The manufacturer has performed homogeneity analysis of the materials, by PT-measurements and weight control.

Declaration of conformity

Equalis AB declares that the products covered by this certificate comply with relevant requirements of the Swedish legislation SFS 1993:584 and directive 98/79/EC implemented through the regulation LVFS 2001:7, as well as requirements of quality and documentation set by Equalis.

Equalis AB
Sweden

Helene Sténhoff

Managing Director, Equalis

Further information is available from Equalis

If during the use of the calibrator or as a result of its use, a serious incident has occurred, please report it immediately to Equalis and/or your local distributor as well as to your national authority.

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Revision

Previous version 2023-05-02. Current version: Changed name from Certificate of analysis to Instructions for use, added that well-maintained instruments should be used and that stability of reagent and buffer should be checked, changed stability at reconstitution from 2 to 6 hours, changed the platelet concentration from $<30 \cdot 10^9$/L till $<20 \cdot 10^9$/L, and changed the name of Equalis Managing Director.

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