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 >2SD in two consecutive rounds of an external quality assessment scheme, or iv) according to local instructions.

The materials are primarily intended for two-point calibration according to a special Excel form “INR-calibration form” that is available at www.equalis.se. REF 002 001 and 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.

For instruments applying three-point calibration, REF 002 003 can also be used as a calibrator.

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 on 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 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 2 hours after reconstitution.

When measured, the value of the control sample should be within 2.37±0.44 (LOT 27).

Warnings and precautions

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

The materials has 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.

The products are intended for in vitro use only.
Description of materials

The three materials are manufactured by MediRox AB, Nyköping (Sweden). The materials are derived from citrate anticoagulated 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. Fresh plasma, with lowered concentration of K vitamin dependent factors (through addition of barium sulphate), was used for REF 002 002 and 002 003. The materials are stabilised but the additives do not affect the measurand. The plasma was prepared by centrifugation, and the platelet concentration is <5·10^9/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 35–39 ºC, pH 6–9, calcium ion activity 1.2–6.0 mmol/L, and sodium chloride concentration 80–160 mmol/L.

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 prothrombine-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 \( PT(INR) = \frac{1}{PT% + 0.018} / 0.028 \). This relationship was used to assign INR-values to dilution series of normal plasma at each one of six expert laboratories, who measured the clotting time with documented measurement procedures. These INR-values and the corresponding clotting time was used to construct calibration curves. Materials from LOT 25, 26, and 27 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.

The measurements were performed at Karolinska University Hospital (Stockholm), Linköping University (co-ordinator), Malmö University Hospital, Sahlgrenska University Hospital (Gothenburg) and Örebro University Hospital.

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


Certified values

<table>
<thead>
<tr>
<th>REF</th>
<th>LOT</th>
<th>Product name</th>
<th>Type of material</th>
<th>PT (INR)</th>
<th>U² (INR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>002 001</td>
<td>25</td>
<td>INR-kalibrator, låg</td>
<td>INR-calibrator, low</td>
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<td>0,08</td>
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<tr>
<td>002 002</td>
<td>26</td>
<td>INR-kalibrator, hög</td>
<td>INR-calibrator, high</td>
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<td>002 003</td>
<td>27</td>
<td>INR-kontroll</td>
<td>INR-control</td>
<td>2,37</td>
<td>0,45</td>
</tr>
</tbody>
</table>

¹ NPU 01685; 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 - 8 °C in a dry environment protected from light.

The long-term stability was estimated by repeated measurement of samples from previous lots up to 65 months 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.

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