CLSI Guidelines for INR Validation and Local Calibration: 2010 Update

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IBTO Reference Coagulation Lab
H47-A2 : PT/APTT testing

H54-A : INR validation and local PT/INR calibration- published 2005
•PT Principle:

The prothrombin time (PT) is the most frequently performed coagulation assay.

It is commonly used to monitor antivitamin K (AVK) therapy.

The responsiveness of commercial PT reagents (thromboplastins) to reduced coagulation factor activities varies significantly, resulting in a wide range of PT results on identical patient samples.
**Thromboplastin**

**Tissue Factor** *(Specific Protein for initiating extrinsic system of Coagulation)* +

**Phospholipid** *(necessary for surface assembly of the coagulation complexes)* +

**Calcium ions** *(for correct orientation and binding of complexes)*

Tissue extraction  
Tissue Culture  
Genetic technology

Human-Derived  
Animal Derived  
Genetic technologies
Prothrombin Time

LABORATORY TECHNIQUES IN THROMBOSIS – A MANUAL

WHO International Reference Materials for Thromboplastins

1st International Reference Preparation
human, combined 67/40 (1976)

1st IRP, bovine, combined
68/434 (1978)

1st IRP, rabbit, plain
70/178 (1978)

2nd IRP, bovine, combined,
OBT/79 (1983)

2nd IRP, human, plain
BCT/253 (1983)

2nd IRP, rabbit, plain
RBT/79 (1982)

3rd ISI, recombinant
human, plain
rTF/95 (1996)

3rd IRP rabbit, plain
RBT/90 (1995)
In 1983 for correction of this variation in responsiveness of reagents, WHO introduced \textbf{INR} system of reporting PT results

\begin{equation}
\text{INR} = (\text{PT Ratio})\text{ISI}
\end{equation}

\text{INR} is a calculated value dependent on \textbf{International Sensitivity Index}.

\textbf{ISI} : Responsiveness of the PT reagent. Determined by comparing the commercial PT reagent to a WHO standard.
Vertical lines represent therapeutic range in terms of prothrombin ratios, for any given ISI.
**International sensitivity index (ISI)**

Patient samples have **shorter clotting times** when **less responsive reagents are used** and **longer CT** with **more responsive reagents**.
Limitations of INR system

It generally works very well and is the method of choice for lab monitoring of AVK therapy

BUT: **Not applying INR/ISI system** or **Incorrect ISI** may severely compromise management of patients on AVK therapy.
International normalized ratio (INR)

It is a common and fixed misconception that for an individual patient’s plasma sample the INR will be identical with different thromboplastins.
<table>
<thead>
<tr>
<th>Causes of variation in INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Different <em>sodium citrate</em> concentration for ISI determination compared to that used locally</td>
</tr>
<tr>
<td>2- Incorrect determination of <strong>MNPT</strong></td>
</tr>
<tr>
<td>3- Incorrect <strong>ISI</strong> value applied locally</td>
</tr>
<tr>
<td>4- Others</td>
</tr>
</tbody>
</table>
Optimizing Current PT/INR Test Systems

• Prior to Local Verification or Calibration

• PT Citrate concentrations of 0.129 mol (3.8%) should not be used for PT tests.

• Mean Normal Prothrombin Time
  Geometric mean of the prothrombin times of the healthy adult population calculated from at least 20 fresh samples from healthy individuals, including those of both sexes

\[
\text{INR} = \left( \frac{\text{PT Ratio}}{\text{ISI}} \right)
\]

\[
\text{PT ratio} = \frac{\text{PT Patient}}{\text{LMNPT}}
\]
Description of the Geometric Mean (GM)

The GM is calculated by the following equation:

\[ GM = \sqrt[n]{X_1 \cdot X_2 \cdot X_3 \cdots X_n} \]

Taking the logarithm of both sides yields:

\[ \log(GM) = \frac{\log(X_1) + \log(X_2) + \log(X_3) + \cdots + \log(X_n)}{n}. \]

Taking the antilog of both sides yields:

\[ GM = \text{antilog}\left\{\frac{\log(X_1) + \log(X_2) + \log(X_3) + \cdots + \log(X_n)}{n}\right\}. \]
ISI value assigned by the Manufacturer

• **Generic**:  
  An ISI determined for a thromboplastin that is not instrument specific. Thromboplastins with a generic ISI should never be used clinically unless validated locally.

• **Instrument Specific/Instrument-Model Specific/Method-of-clot-detection-specific**:  
  The ISI difference between different brands (manufacturers) of photo-optical or mechanical instruments can be important.
  **Example**: a human placental thromboplastin can have an ISI of 1.20 for one brand of photo-optical instrument, but an ISI of 1.00 for another brand.
Verification of INR Results

For laboratories using generic ISIs, verification is mandatory and local ISI calibration is strongly recommended.

Verification is recommended for all laboratories using instrument-specific ISIs.
Protocol for Verification Procedure

• Should use lyophilized or fresh frozen certified plasmas

• A minimum of three certified plasmas should be used with a range of INRs between 1.5 and 4.5

• Should be run in duplicate over at least a two-day period

• The difference between within-day and between-day duplicates should not exceed 10%.
Protocol for Verification Procedure

The INRs of the certified plasmas should be determined using the laboratory’s routine thromboplastin with the manufacturer- assigned ISI value (if appropriate) and routine instrument(s)

- True value + (15% multiplied by the true value); and
- True value – (15% multiplied by the true value).

The INR of the certified plasmas using the local system should compare to the value assigned to the certified plasmas by ±15%.
Frequency of Verification

• Verification is **mandatory** if a reagent with a **generic ISI** is put into place and **is highly recommended** with the use of a thromboplastin with an **instrument-specific ISI**

• Verification should take place with any change in:
  - reagent,
  - reagent lot number,
  - instrument, or
  - following a major instrument repair

Major changes in quality control or major discrepancies in external quality programs

• **At a minimum**, if no major changes occur, the verification should occur at least **once per year**
If Verification Fails

Clinical results *cannot be reported* until the discrepancy is addressed

local PT/INR *calibration* should be performed
Local System Calibration

1-Determination of Local ISI

2-Establishing Direct INR (Locally)
Local System Calibration
(calculating Local ISI)

A modification of the WHO method for ISI determination

• No. of certified plasmas: 6 AVK plasmas (20 artificially depleted plasmas)
  2 Normal plasmas (7 normal plasmas)
Local System Calibration (calculating Local ISI)

A modification of the WHO method for ISI determination

- PT of the certified plasma determined using local thromboplastin/instrument combination
- The PT value is plotted against the assigned PT value of the certified plasma
- The ISI = Slope of the orthogonal regression line
Local System Calibration (Direct INR)

- No. of certified plasmas: 3 AVK plasmas
  1 Normal pooled plasma
<table>
<thead>
<tr>
<th>Test</th>
<th>PT/INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample no.</td>
<td>09/04</td>
</tr>
<tr>
<td>Your reagent</td>
<td>STA Neoplastin CI Plus</td>
</tr>
<tr>
<td>Your INR result</td>
<td>3.9</td>
</tr>
<tr>
<td>Reference group using your reagent</td>
<td></td>
</tr>
<tr>
<td>number of labs.</td>
<td>-</td>
</tr>
<tr>
<td>median INR</td>
<td>-</td>
</tr>
<tr>
<td>Your % deviation</td>
<td>-</td>
</tr>
<tr>
<td>Overall reference group results</td>
<td></td>
</tr>
<tr>
<td>number of labs.</td>
<td>435</td>
</tr>
<tr>
<td>median INR</td>
<td>3.19</td>
</tr>
<tr>
<td>Your % deviation</td>
<td>22.3</td>
</tr>
<tr>
<td>Your results are assessed against*</td>
<td>overall</td>
</tr>
<tr>
<td>Your performance</td>
<td>Outwith consensus</td>
</tr>
<tr>
<td>Your previous % deviation (Survey 44)</td>
<td>30.9</td>
</tr>
<tr>
<td>Your interpretation</td>
<td>Overdosed</td>
</tr>
<tr>
<td>Reference group interpretation (%)</td>
<td></td>
</tr>
<tr>
<td>Underdosed</td>
<td>19.6</td>
</tr>
<tr>
<td>Adequate</td>
<td>72.4</td>
</tr>
<tr>
<td>Overdosed</td>
<td>8.3</td>
</tr>
</tbody>
</table>
### WHO IEQAS

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<tr>
<th>Test</th>
<th>PT/INR</th>
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<tbody>
<tr>
<td>Sample no.</td>
<td>16/04</td>
</tr>
<tr>
<td>Your reagent</td>
<td>STA Neoplastin CI Plus</td>
</tr>
<tr>
<td>Your INR result</td>
<td>4.4</td>
</tr>
<tr>
<td>Reference group using your reagent</td>
<td></td>
</tr>
<tr>
<td>number of labs.</td>
<td>5</td>
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<tr>
<td>median INR</td>
<td>4.1</td>
</tr>
<tr>
<td>Your % deviation</td>
<td>7.3</td>
</tr>
<tr>
<td>Overall reference group results</td>
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<tr>
<td>number of labs.</td>
<td>401</td>
</tr>
<tr>
<td>median INR</td>
<td>4.39</td>
</tr>
<tr>
<td>Your % deviation</td>
<td>0.2</td>
</tr>
<tr>
<td>Your results are assessed against*</td>
<td>Overall</td>
</tr>
<tr>
<td>Your performance</td>
<td>Within consensus</td>
</tr>
<tr>
<td>Your previous % deviation (Survey 46)</td>
<td>22.3</td>
</tr>
<tr>
<td>Your interpretation</td>
<td>Overdosed</td>
</tr>
<tr>
<td>Reference group interpretation (%)</td>
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</tr>
<tr>
<td>Underdosed</td>
<td>0.5</td>
</tr>
<tr>
<td>Adequate</td>
<td>3.5</td>
</tr>
<tr>
<td>Overdosed</td>
<td>96.0</td>
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</tbody>
</table>
Thank you for your attention!