PROTHROMBIN TIME

WHY USE LOW ISI (HIGH SENSITIVITY)
The Prothrombin time is the functional determination of the extrinsic coagulation pathway.

It is a widely used laboratory assay for the detection of inherited or acquired coagulation defects related to the extrinsic pathway.
TERMS

• INR – INTERNATIONAL NORMALIZED RATIO
• WHO – WORLD HEALTH ORGANISATION
• ISI – INTERNATIONAL SENSITIVITY INDEX
• IRP – INTERNATIONAL REFERENCE PREPARATION
INR – INTERNATIONAL NORMALIZED RATIO

Ever wondered why you obtain different results for

• different company PT reagents?
• different lots of the same companies PT reagents?

PT REAGENTS ARE MADE FROM TISSUE FACTORS

BEING BIOLOGICAL PRODUCTS IT EXHIBITS A LOT OF VARIABILITY

WHO expert committee report on Biological Standardization, Report 34
ISI is a correction factor developed to correlate the sensitivity of commercial Thromboplastins to the 1st IRP. (International Reference Preparation).

IRP – A very responsive batch of human brain extract was designated as the 1st International Reference Preparation (IRP).
The ISI of the 1st IRP was 1.0

The INR actually compares the prothrombin ratio measurement to the 1st IRP.

The INR represents the prothrombin time that would have been obtained if the IRP had been used as the reagent.

Responsive PT reagents have lower ISI values.

Analytical precision is improved with reagents with low ISI.

Reagents with low ISI discriminates normal and warfarin treated patients better.
# LOW ISI VS HIGH ISI

<table>
<thead>
<tr>
<th>LOW ISI</th>
<th>HIGH ISI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Called as High sensitivity assay</td>
<td>Called as Low sensitivity assay</td>
</tr>
<tr>
<td>The reagent is highly sensitive to even small changes in the PT</td>
<td>The reagent is NOT sensitive to changes in PT</td>
</tr>
<tr>
<td>Accommodates Factor deficiency</td>
<td>DOES NOT accommodate Factor deficiency</td>
</tr>
<tr>
<td>Higher levels of precision</td>
<td>Poor precision</td>
</tr>
<tr>
<td>Better global commutability with PT assays</td>
<td>Poor Commutability</td>
</tr>
<tr>
<td>Shift towards low ISI assays globally</td>
<td>Shift away from High ISI kits globally</td>
</tr>
</tbody>
</table>
SAMPLES AND THEIR ROLE

MOST OF THE PROBLEMS CAUSED IN PT TESTING IS BECAUSE OF SAMPLING.
SAMPLES AND THEIR ROLE

ANTICOAGULANT

Whole blood should be collected with 3.2% Sodium Citrate As the anticoagulant.

DO NOT USE 3.8% SODIUM CITRATE.

Effect of 3.2% vs 3.8% sodium citrate concentration on routine coagulation testing, Adcock et al., Am J Clin Path 1997 Jan; 107 (1):105-10
SAMPLES AND THEIR ROLE

PREPARATION OF SAMPLES

Collect whole blood 9 volumes with 1 volume of 3.2% Sodium Citrate

Collect samples in a plastic tube

DO NOT USE GLASS VESSELS

Mix whole blood and Sodium Citrate well

Centrifuge at 2500 rpm for 15 minutes.

The tube now has a clear area of Citrated plasma and a sediment plug.

PERFORM TESTS WITHIN 1-2 HOURS AFTER SPINNING

Clinically relevant differences in PT and INR values related to Blood sample Collection in Plastic VS Glass Tubes
Eberhard W. Fiebig et al; Am J Clin Path; 2005; 124(6) 902-909
SAMPLES AND THEIR ROLE

STORAGE OF SAMPLES

- Remove citrated plasma from the centrifuged sediment
- Place the same in a plastic container
- Refrigerate

DO NOT FREEZE. (freezing prolongs clotting time).

- Stored samples must be mixed well before use.

Effect of freezing method and storage at -20°C and -70°C on PT, aPTT and Plasma Fibrinogen levels Sonja Alesci et al; Thrombosis Research; Vol 124, Issue 1, May 2009.
WHO introduced INR in the early 80’s as a means of Standardizing PT results.

\[
\text{INR} = \frac{\text{Patient PT}}{\text{Mean Normal PT}} \times \text{ISI} = \text{(Prothrombin Ratio)}
\]
MEAN NORMAL PT

FACTS

- Each lab must establish Mean Normal PT for the reagent and the instrument used

- Do not use Mean Normal PT derived from one company’s reagent with the other.

Procedures for Validation of INR and Local Calibration of PT/INR systems; Approved Guidelines CLSI; Vol 25 No.23.
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MEAN NORMAL PT

MEASUREMENT

- Collect 10 - 20 fresh samples (apparently normal)
- Measure clotting times for PT using BIOREXFARS PT reagent
- Assuming that the values obtained are:
  14 seconds, 12.1, 11.9, 16.2, 12, 12.5, 17.6, 12.3, 16.2, 17.5.
- Choose 3 samples with the lowest clotting time – 12.1, 11.9, 12 secs
- Pools these samples together.
- Mix them well.
- Measure clotting time for this pool using BIOREXFARS PT Reagent.
- The value obtained is close to 12.0 seconds.
- Label this pool 100% Sample.

A 100% sample denotes that all clotting factors are ok and 100% clotting has occurred.
Preparation of 50% and 25% pools

- 50% pool is prepared by diluting 1 volume of 100% pool with 1 volume of physiological saline.

- 25% pool is prepared by diluting 1 volume of 100% pool with 3 volumes of physiological saline.

- Measure clotting times for the 50% and 25% pools with BIOREXFARS PT reagent.

- Let us assume that the results were 14.8 seconds for 50% and 26.7 seconds for the 25% pool.

Construct a graph on a semi log graph paper using the Clotting times obtained for the 100%, 50% and 25% pools.
MEAN NORMAL PT

Typical graph with 100%, 50% and 25% pools

Typical graph of 3 different company’s PT rgts
PATIENT RESULTS

Let us assume that the Clotting time for a patient sample is 14.1 secs.

ISI VALUE OF THE KIT = 1.22
MEAN NORMAL PT = 12.0 SECS

\[
\text{INR} = \left(\frac{\text{Patient PT}}{\text{Mean Normal PT}}\right)^{\text{ISI}}
\]

\[
\text{INR} = \left(\frac{14.1}{12.0}\right)^{1.22}
\]

\[
(1.17)^{1.22}
\]

\[
\text{INR} = 1.26
\]
### Reading the INR Table

#### Mean Patient (MNP) and Patient Results

<table>
<thead>
<tr>
<th>%</th>
<th>R</th>
<th>INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100.0</td>
<td>1.00</td>
</tr>
<tr>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

#### ISI 1.22

Choose this column to correlate the patient values for INR / Ratio or %.

This row denotes the mean normal PT values derived by running the pooled samples. (eg: 12.0 secs)

Choose the column with the value obtained as the Mean Normal PT with the pooled sample (eg: 12.0 secs).
ISI FOR PT REAGENTS CAN BE SET IN TWO WAYS

- BY assaying known INR values which are traceable to WHO RBT 05.

- BY using reference or traceable samples and assaying them in duplicates and plotting them as logarithms. The ISI is then calculated using the slope of the line that is drawn as a best fit line between these sets of log values.
SETTING ISI FOR PT REAGENTS

BIOREXFARSFARS METHOD FOR SETTING ISI

- BY assaying known INR values which are traceable to WHO RBT 05.

In BIOREXFARSFARS Diagnostics we use the AK Calibrant, which is a commercially available calibration material for setting ISI values.

The advantages of using the AK Calibrant are:

- 4 levels
- Known INR values
- Traceable to WHO RBT 05
USE OF AK CALIBRANT

1. Reconstitute lyophilised material supplied.
2. Ensure homogeneity.
3. Choose the reagent to be ISI assigned.
4. Incubate the PT reagent till it reaches 37°C.
5. Assay all the 4 calibration material with the PT.

Calibration curve drawn from the seconds derived for the lyophilised calibrators.

Point 5 in the next slide.

INR VALUE

ISI VALUE

SECONDS
**HOW IS IT DONE?**

**CALIBRATION CURVE**

1. Note down the seconds after assaying the cal samples.
2. In the graph provided, plot the seconds vs the INR values.
3. Draw a line thru the seconds that have been plotted.
4. The line can be extended to the ISI area of the Y2 axis.
5. Drop a line from the first value (seconds) to the X axis.
6. Construct a line from this point (that meets the x axis) to the Y2 axis.
7. Read the ISI value off this line.
CHECKING THE ISI VALUE?

**ISI VALUE CHECKS**

1. Run controls with known INR values.
2. Note the seconds for these controls.
3. With the MNPT values for the PT Reagent, calculate the INR for the control.
4. Compare the INR values derived from the PT reagent with the prescribed INR values of the control.
5. The deviation should not be more than 10%.
6. Run as many levels as possible, preferably normal and elevated INRs.