Principle & Practice Issues of Anticoagulation Laboratory Testing

Oral anticoagulant: warfarin
Vitamin K antagonist
Interfere carboxylation of F II, VII, IX, X
Therapeutic range: optimal dose that prevent thrombosis without bleeding complication
Dose response differs from one person to another (from 0.25 to 5 mg/day)
Monitoring is critical: PT is commonly used

Prothrombin Time (PT): Principle

Warfarin

Thromboplastin
Ca++

Ⅻ
XI
Ⅸ
Ⅷ
VII
Va
Ⅱ
Ⅰ
F ⅠBRIN

Total testing process

Preanalytical
Analytical
Postanalytical

Proper order & test selection
Patient preparation
Identification
Specimen collection
Analysis
Report
Transportation

Error in each process
Preanalytical
Analytical
Postanalytical

68%
13%
18%

Pre-analytical phase

- Type of anticoagulant concentration
  - 3.2% V.S. 3.8% sodium citrate
- Ratio blood:anticoagulant
  - Under filling
  - High hematocrit
- Blood Collection Technique: tissue factor
- Order of tube

Underfilling

- Underfilling causes prolonged of PT and APTT.
  - Minimal fill volume for 3.2% sodium citrate
    - 60% for PT, 70% for APTT
  - Minimal fill volume for 3.8% sodium citrate
    - 80% for PT, 90% for APTT

High hematocrit

- For hematocrit > 55%, it is recommended to modify the volume of anticoagulant.

\[ C = (1.85 \times 10^{-3})(100 - Hct)V_{\text{Blood}} \]

where \( C \) is the volume of citrate remaining in the tube, 
\( Hct \) is the hematocrit of the patient, and 
\( V_{\text{Blood}} \) is the volume of blood to be added. (If a 5-mL tube is used, the volume is 4.5 mL.)

Order of Blood Collection

- The tube for hemostasis may be the first tube if drawn by well-trained operator
- Otherwise, draw secondly, but never follow any other anticoagulant tube or tube containing activator.
Analysis Phase: ปัญหาของ PT

- Tissue thromboplastin = tissue factor + phospholipid
- Vary markedly in their responsive to anticoagulant effect of warfarin
- Depend on tissue of origin (human, rabbit) & method of preparation
- Many reporting system: sec, ratio, % activity
- PT result cannot be interchangeable

WHO established international reference preparation (IRP)
- 1977: 67/40, human brain extract added adsorbed bovine plasma (combined reagent)
- 1984: BCT/253, human brain extract, (plain reagent)
- 1996: rTF/95, human recombinant, plain
- 2005: new candidate for rabbit brain thromboplastin
  : OBT/79, bovine, combined
  : RBT/90, rabbit, plain
- 2008: new candidate for rTF/95 replacement

Calibration system proposed by Kirkwood (Thromb Haemostas1983; 49:23844)
- Fresh plasma from 60 patients receiving stabilized dose of warfarin and 20 healthy volunteer
- A linear relationship exists between log of PT obtained with IRP and test thromboplastin

\[
Y_{\log INR} = a \log PT ratio + b
\]
Antilog \(Y_{\log INR}\) = a Antilog \(X_{\log PT ratio}\)

\[
INR = PT ratio^a
\]
\[
a = slope = ISI
\]
\[
INR = International Normalized Ratio
\]
\[
ISI = International Sensitivity Index
\]

Convert PT ratio observed with local thromboplastin into International Normalized Ratio (INR)
\[
INR = \frac{PT patient^{ISI}}{Normal PT^{ISI}}
\]
INR: PT ratio one would have obtained if the WHO IRP had been used to perform PT on the sample
ปัญหาของ INR

- Inter-laboratory variation
- ISI is calibrated by manufacturer by manual method
- Automated machine is widely used
- ISI of thromboplastin is modified by instrument

Proposed solution

1. Instrument-reagent lot specific ISI
2. Mean normal prothrombin time (MNPT) of each own lab
3. Selection of sensitive thromboplastin with ISI < 1.7

Mean normal PT

- Geometric mean of prothrombin time
  จากการทดสอบในเพลีส.pa крови 20 ราย
  ฉล. ควรหาค่า mean ใหม่ทุกครั้งที่มีการเปลี่ยน lot

Analytic Phase : Quality Assurance

- To ensure the reliability of lab testing & reporting
- External quality assessment (EQA) : accuracy
- Internal quality control (IQC) : precision

Analytic Phase : IQC

- Internal quality control (IQC)
  - To ensure day-to-day lab consistency
  - To establish a series of procedures performing consistently over a period of time
  - To perform normal & abnormal control at least

Percentage of Laboratories that use “Geometric Mean” (Thailand NEQAS for Blood Coagulation)

<table>
<thead>
<tr>
<th>Month</th>
<th>33/97</th>
<th>85/131</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec 2005</td>
<td>33%</td>
<td>65%</td>
</tr>
<tr>
<td>Sep 2008</td>
<td>32/97</td>
<td>33%</td>
</tr>
</tbody>
</table>
Analytic Phase: EQA

- **External quality assessment (EQA)**
  - To test competency of individual lab
  - To identify degree of agreement between one lab and the others by sending unknown specimens to its members and compare the results
  - To identify poor lab performance, unreliable reagents and method

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<table>
<thead>
<tr>
<th>Test</th>
<th>Pretreatment Time</th>
<th>APTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Number</td>
<td>0237</td>
<td>0237</td>
</tr>
<tr>
<td>Sample Type</td>
<td>FVIII deficiency</td>
<td>VWD</td>
</tr>
<tr>
<td>Your Reagent</td>
<td>Date-Binding Thromboplastin</td>
<td>Date-Binding Activator F8</td>
</tr>
<tr>
<td>Your Result</td>
<td>18.4</td>
<td>28</td>
</tr>
<tr>
<td>Your Units</td>
<td>s/ps</td>
<td>s/ps</td>
</tr>
<tr>
<td>Your normal range</td>
<td>10-15</td>
<td>20-30</td>
</tr>
<tr>
<td>Your prothrombin ratio</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Participants in your group</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Reagent Specific Median</td>
<td>1.94</td>
<td>1.5</td>
</tr>
<tr>
<td>% Deviation</td>
<td>1.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Overall Patient</td>
<td>791</td>
<td>791</td>
</tr>
<tr>
<td>Overall median</td>
<td>1.71</td>
<td>1.87</td>
</tr>
<tr>
<td>% Deviation</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Overall survey report</td>
<td>Standard</td>
<td>Standard</td>
</tr>
</tbody>
</table>

- **Data from Thailand NEQAS**

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**WHO INTERNATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME FOR BLOOD COAGULATION**

Dr. Wachira Wongwisan
Department of Clinical Pathology
Bangkok 10310
Thailand

**Participant No. 88/TH**

**Your Results On Survey 54**

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**Thai National External Quality Assessment Scheme for Blood Coagulation**

**Data from Thailand NEQAS**

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**CV: Normal control**

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**Sample 2/05**

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**Data from Thailand NEQAS**
**Post analytical phase**

- Critical value INR > 5
- Patients whose INR is elevated to 5.0 can be treated by appropriate dosage reduction.
- Risk of bleeding increasing noticeably when the INR reaches 4.0 and even more sharply when the INR is higher than 5.0
- Immediately report to clinician


**Warfarin**

Patients with bleeding or whose INR is elevated to greater than 5.0 may require more rapid reversal with vitamin K1 treatment.


**Post analytical phase**

- The INR system is not valid for comparison of patients with liver impairment because different reagents do not give the same INR for the same sample.


**Post analytical phase**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innovin</td>
<td>Thromboplastin C</td>
</tr>
<tr>
<td>Warfarin</td>
<td>2.63</td>
</tr>
<tr>
<td>Liver disease</td>
<td>1.88</td>
</tr>
</tbody>
</table>


**Portable PT- INR**

*Portable PT- INR*
### Traditional PT-INR
- Labor intensive
- Time consuming (0.5 - 2 hr.)
- Multiple venepuncture
- Delay in initiating timely dosage adjustment
- Patient dissatisfaction
- High cost for good quality control
  - Geometric mean
  - Regular internal quality control
  - External quality assessment

### Portable PT-INR
- 1990, testing at point of care
- Simple sample collection, finger stick
- Measure clotting time mediated by thromboplastin
- Converted by microprocessor to PT-INR
- Much faster turn around time (< 5 min)
- Easy to use, clinic or home use
- Increase patient satisfaction and improve outcome (*Zucker ML Point of Care 2007; 6: 223-6*)

### Application of portable PT-INR
- Patient self-testing (PST)
  - Patients test their own PT-INR
  - Call in results to the physician’s office
  - Warfarin dose adjusted by physician
- Patient self management (PSM)
  - Patients are trained and allowed to manage their own warfarin therapy
  - Physicians provide certain dosing parameter and guidance to patients

### Advantage of PST/PSM
- Ability to perform more frequent testing
- Better anticoagulant control
- Improved timeliness
- Positive impact on patient empowerment
- Better compliance

### Awareness of PST/PSM
- Readiness of physicians
- Need special procedure and training
- Process-management by qualified provider
- Carefully patient selection
- Indicate only in patients willing and able to
  - Perform the testing
  - Follow dosing instruction
  - Properly trained

### Siriraj’s experiences
- Demand POCT INR meter from clinicians
- More convenience, safety and compliance
- Electrochemical detection
  - Human recombinant tissue factor
  - On-board integrated system control
  - Insensitive to heparin
**Standardization**

- Master lot calibration: against IRP (tTF/95 and CRM149S) by WHO reference method
- 4 sites: mean ISI 1.01 (n=372), CV = 2% (July 2004)
- Production lot calibration: against master lot using 10 normal and 30 patients

**Evaluation study**

- Performance evaluation study (*June 2005*)
  - 4 sites in Netherlands (2), Spain, USA
  - 297 patients enrolled
  - 8 meters at each site (total 32)
  - Good agreement (r = 0.97 - 0.99, CV = 2 - 4.2%)
- User study (*May 2005*)
  - 4 sites in Germany, Austria, and Denmark
  - 75 patients enrolled

**Siriraj’s experiences**

- Evaluation POC meter at Siriraj Hosp. (*July 2006*)
- Precision study
  - Liquid QC material
  - Within run 35 times \( \%CV 1.07 \)
- Accuracy study
  - 39 patients receiving warfarin therapy
  - Permission from research in human ethic
  - One drop (10 ul) of capillary whole blood
  - Compare with traditional PT-INR

**Summary**

- Warfarin therapy needs closed lab monitoring
- Traditional PT-INR needs good quality control for all testing process
  - Pre-analytic phase
  - Analytic phase
  - Post-analytic phase
- Portable PT-INR is available, but high cost, pre-analytic phase is still very critical