Objectives

- Compute and report the PT/INR
  - Computing the ISI
  - Local INR validation
  - Local ISI calibration
- Determine the heparin therapeutic range
- Assay PTT for factor level sensitivity
- Validate a new reagent lot
- Compute clinical efficacy sensitivity and specificity
- Perform a ROC analysis

Prothrombin Time

- Armand Quick 1935
  - Rabbit brain tissue thromboplastin
  - Calcium phosphate
- Coumadin FDA-cleared 1952
  - First use of PT for Coumadin monitoring in dogs in 1945
- Reported in seconds with normal control
  - Plasma collected in potassium oxalate
  - Refined as prothrombin time ratio: $\frac{PT_{\text{patient}}}{PT_{\text{control}}}$
- Reagent variations adversely affected Rx

1980: PT Ratio Based on MRI

$$PTR = PT_{\text{patient}} + PT_{\text{MRI}}$$

$PT_{\text{MRI}} = \text{antilog} (\sum \log PT + n)$

Where...

- PTR = PT ratio
- MRI = mean of RI
- PT = prothrombin time (protime)
- RI = reference interval (normal range)
- $\sum \log PT = \text{sum of the logs of each normal PT}$
- $n = \text{number of normal PTs analyzed to calculate RI}$

Weakness of the PTR

<table>
<thead>
<tr>
<th>ISI</th>
<th>PT</th>
<th>PTR</th>
<th>INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>3–42 s</td>
<td>2–3</td>
<td>2–3</td>
</tr>
<tr>
<td>2.4</td>
<td>17.5–20 s</td>
<td>1.5–1.7</td>
<td></td>
</tr>
</tbody>
</table>

Therapeutic range narrows as ISI rises.
International Normalized Ratio (INR)

The result that would have been obtained using...

- The manual (tilt-tube) technique and...
- WHO human brain international reference thromboplastin preparation IRP 67/40, the ISI of 1.0

INR = PTR\^ISI

Where:
- INR = international normalized ratio
- ISI = international sensitivity index
- PTR = prothrombin time ratio using MNPT


International Sensitivity Index (ISI)

- Manufacturers compute thromboplastin ISI to correlate to the IRP; ‘truth.’
- For each reagent lot...
  - 20 normal & 60 Coumadin plasmas tested using...
  - 2° IRP keyed to primary IRP
  - Manual tilt-tube technique
  - Multiple expert laboratories
- Manufacturers provide instrument-specific ISIs
  - Multiple instruments: mechanical and optical


ISTH Recommendation

- Choose responsive thromboplastin; ISI near 1.0
  - Recombinant or affinity purified thromboplastins
  - Sensitive to factor deficiency
  - Responsive to therapy
  - Reproducible
- Calculate “local ISI” calibration with ref plasmas


Local ISI Calibration

WHO Expert Committee on Biological Standardisation

- Perform PTs on 4–5 reference plasmas
  - Plasmas with PTs assigned using a “well-defined” thromboplastin on a “well-defined instrument”
  - If reference plasmas unavailable, use 100 pt specimens
  - Graph ref PTs as Y, local PTs as X on log-log plot
  - Compute slope
  - Multiply ref ISI X slope to assign current ISI
  - Use same approach for lot-to-lot comparisons


IL Validators and Calibrators

- HemosIL INR Validate
  - Set of 3 certified Coumadin plasmas with INR 1.6–5.
  - Assay every six months to meet CAP requirements
  - Use only with HemosIL RecombiPlasTin or PT Fibrinogen HS Plus
- HemosIL ISI Calibrate
  - Use only if INR Validate results exceed limits
  - Set of 4 certified known PT/INR plasmas with INR 0.9–5
  - Assay and enter PT results into ISIWeb (“Easy”-web)
  - Record computed ISI and enter into instrument circuitry
Coag QA: Why are we Different?

**THE FRTSMA FACTOR**
Your Interactive Hemostasis Resource

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### Coagulometer ISI Derivation
Rapid Method Using PT/INR Line


### Regression Comparing Thromboplastin Lots Using Patient Plasma


### Efficacy of Local Calibration

Proportion of plasmas that deviate < 10% from certified values before correction (BC, without the PT/INR Line) and after with the PT/INR Line using 3–5 calibrant plasmas per set. Plasmas were selected at random (R), from clusters with varying INR ranges (C), or from clusters that included a normal plasma (C + 1N).


### Coumadin Therapeutic Window


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**The UNFRACTIONATED HEPARIN**
Crude Extract of Porcine Mucosa

- Unbranched sulfated mucopolysaccharide glycosaminoglycan

**Active Pentasaccharide**


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fritsmafactor.com
**Action of Unfractionated Heparin**

UFH activates antithrombin (AT) to bind thrombin (IIa), or Xa.

- IIa:Xa = 4:1

**Monitoring UFH Therapy**

**Standard Schedule**

- Perform “baseline” PTT to rule out factor deficiency, inhibitors, lupus anticoagulant
  - 1–3% have baseline PTT > upper limit of RI: alternative?
- Initiate therapy: bolus + continuous infusion
- At least 4–6 h after bolus, but not >24 h, collect & perform second PTT
- Adjust dose to PTT therapeutic range
  - Lab-published range: ex vivo curve
  - Never use 1.5–2.5 x mean of normal range

**Chromogenic Anti-Xa Heparin Assay**

- Intensity at 405 nm is inversely proportional to patient heparin concentration

**UFH Rx Range Using the PTT**

**The ‘Brill-Edwards’ Curve**

- Collect 20–30 specimens from pts on UFH
  - No Coumadin, PT normal
  - No more than 10% repeat specimens from single patient
  - Representative demographics for race, sex, age
- Collect 10 normals
- Assay PTT and chromogenic anti-Xa
- Graph paired results
- Select PTT limits in seconds that equals 0.3–0.7 chromogenic Xa heparin units

**Heparin Therapeutic Range**

Data courtesy of the University of Alabama at Birmingham Special Coagulation Laboratory

- PTT prolonged by heparin, lupus anticoagulant, XI, XII, IX, V, II, Fg deficiencies
- PF3: Prolonged
- PF4: Prolonged
- Platelet count: Prolonged
- Platelet aggregation: Prolonged
- Thrombin time: Prolonged
- Activated partial thromboplastin time: Prolonged

**Fritsma MG, in Keohane EM, Smith LJ, Walenga JM. Rodak’s Hematology, Clinical Principles and Applications, 2015**
Coag QA: Why are we Different?

4-28-15

fritsmafactor.com

PTT/Anti-Xa Data, Three Routine Days

Data courtesy of the University of Alabama at Birmingham Special Coagulation Laboratory

Limitations of PTT in UFH Monitoring

- Antithrombin deficiency or consumption renders PTT non-responsive, "heparin resistance"
- Lupus anticoagulant, present in 1-3% of unselected individuals, prolongs baseline PTT
- Coagulopathy prolongs PTT
- Coagulation factor inhibitor prolongs PTT
- Elevated FVIII renders PTT insensitive to heparin
- Reagent variations require recalibration to the anti-Xa heparin assay, new target ranges with each lot


Partial Thromboplastin Time Factor VIII & IX Sensitivity

- Prepare series of plasmas of known activity levels
  - For instance, 100, 80, 60, 40, 20, <1%
  - Dilute factor deficient plasmas with normal control plasmas
  - Or retain measured patient plasmas
- Record PTT results in seconds versus factor level...


Lot to Lot: ACL Units (ACUs)

Lot to Lot: ACL Units (ACUs)

For Audience Response

- What would you do about these lot-to-lot results?
  1. The low level of the new reagent is off by only 1, just accept the new lot
  2. The low level of the new reagent is off by only 1, just repeat validation
  3. The new reagent is far enough out you will have to compute a new reference range
  4. The new reagent is far enough out you should just reject it and require a new lot from the manufacturer

Lot to Lot: ACL Units (ACUs)

Variance Limit 10%; Systematic Error?

Initial run (Unacceptable)

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Old Kit ACUs</th>
<th>New Kit ACUs</th>
<th>% Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low specimen</td>
<td>7</td>
<td>6</td>
<td>-14%</td>
</tr>
<tr>
<td>Mid-low specimen (Mix)</td>
<td>12</td>
<td>12</td>
<td>0%</td>
</tr>
<tr>
<td>Middle specimen (Mix)</td>
<td>20.5</td>
<td>19.4</td>
<td>-5%</td>
</tr>
<tr>
<td>Mid-high Specimen (Mix)</td>
<td>31</td>
<td>27</td>
<td>-20%</td>
</tr>
<tr>
<td>High specimen</td>
<td>48</td>
<td>48</td>
<td>0%</td>
</tr>
<tr>
<td>Low control (from kit)</td>
<td>9</td>
<td>11</td>
<td>+8%</td>
</tr>
<tr>
<td>Middle control (from kit)</td>
<td>22</td>
<td>24</td>
<td>+8%</td>
</tr>
<tr>
<td>High control (from kit)</td>
<td>48</td>
<td>49</td>
<td>+2%</td>
</tr>
</tbody>
</table>

Second run (Acceptable)

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Old Kit ACUs</th>
<th>New Kit ACUs</th>
<th>% Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low specimen</td>
<td>7</td>
<td>6.5</td>
<td>-11%</td>
</tr>
<tr>
<td>Mid-low specimen (Mix)</td>
<td>12</td>
<td>12</td>
<td>0%</td>
</tr>
<tr>
<td>Middle specimen (Mix)</td>
<td>20.5</td>
<td>19.4</td>
<td>-5%</td>
</tr>
<tr>
<td>Mid-high Specimen (Mix)</td>
<td>31</td>
<td>29</td>
<td>-10%</td>
</tr>
<tr>
<td>High specimen</td>
<td>48</td>
<td>48</td>
<td>0%</td>
</tr>
<tr>
<td>Low control (from kit)</td>
<td>9</td>
<td>11</td>
<td>+8%</td>
</tr>
<tr>
<td>Middle control (from kit)</td>
<td>22</td>
<td>24</td>
<td>+8%</td>
</tr>
<tr>
<td>High control (from kit)</td>
<td>48</td>
<td>49</td>
<td>+2%</td>
</tr>
</tbody>
</table>
Coag QA: Why are we Different?

For Audience Response

- What would you do about the repeated lot-to-lot results?
  1. The low level of the new reagent is off by only 0.5, just accept the new lot
  2. The low level of the new reagent is off by only 0.5, just repeat the validation again
  3. The new reagent is far enough out you will have to compute a new reference range
  4. The new reagent is far enough out you should just reject it and require a new lot from the manufacturer
Coag QA: Why are we Different?

Clinical Efficacy: Frequency Distribution

**Reality: Reset Limit to 60%**

- Assay Value
- Frequency
- 60%
- TN
- No Disease
- TP
- Disease

**Clinical Efficacy: Frequency Distribution

Reality: Raise Limit to 90%**

- Assay Value
- Frequency
- 90%
- FP
- False Pos
- No Disease
- TP
- True Pos

Comparing Methods for Clinical Efficacy

- Assay ≥30 specimens
  - Include low, mid-range, high levels

<table>
<thead>
<tr>
<th>Disease or Condition:</th>
<th>Absent</th>
<th>Present</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Or Reference Test:</td>
<td>Normal</td>
<td>Abnormal</td>
<td></td>
</tr>
<tr>
<td>New Test Normal</td>
<td>True Neg</td>
<td>False Neg</td>
<td>Sum</td>
</tr>
<tr>
<td>New Test Abnormal</td>
<td>False Pos</td>
<td>True Pos</td>
<td>Sum</td>
</tr>
<tr>
<td>Total</td>
<td>Sum</td>
<td>Sum</td>
<td>Grand Sum</td>
</tr>
</tbody>
</table>


False Positive, False Negative

- **True positive**: assay result correctly identifies those with a disease or condition
- **False positive**: assay result incorrectly identifies disease or condition where none is present (false alarm)
- **True negative**: assay correctly identifies those without a disease or condition
- **False negative**: assay result incorrectly rules out disease or condition where it is present (miss)


Clinical Efficacy Example Using 200 Specimens

<table>
<thead>
<tr>
<th></th>
<th>No Disease</th>
<th>Disease</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Normal</td>
<td>92</td>
<td>10</td>
<td>102</td>
</tr>
<tr>
<td>Test Abnormal</td>
<td>8</td>
<td>90</td>
<td>98</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>

- False negatives (misses): 10/200 = 5%
- False positives (false alarms): 8/200 = 4%
Clinical Sensitivity
The likelihood that an assay will identify all subjects who have a disease or condition

\[
\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}} \times 100\%
\]

\[
\text{Sensitivity} = \frac{90}{90 + 10} \times 100\% = 90\%
\]

Clinical Sensitivity
• The proportion of subjects who tested positive out of all positive subjects tested
• The probability the test is positive given that the subject has the disease or condition
• The higher the sensitivity, the fewer cases that go undetected
• However, the higher the sensitivity, the higher the false positive rate

Detection Rate for Clopidogrel P2Y12 Receptor Blockade

Determine % sensitivity: \( TP \div (TP+FN) \times 100 \)

Detection rates of P2Y12-receptor blockade by clopidogrel

<table>
<thead>
<tr>
<th>Method</th>
<th>PFA P2Y 3.2%</th>
<th>PFA P2Y 3.8%</th>
<th>VN P2Y12</th>
<th>LTA 20 µM ADP</th>
<th>WBA 5 µM ADP</th>
<th>WBA 10 µM ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>59%</td>
<td>95%</td>
<td>60%</td>
<td>88%</td>
<td>89%</td>
<td>72%</td>
</tr>
<tr>
<td>Notes</td>
<td>PFA/P2Y: Siemens PFA 100/200 P2Y12 cartridge; VN: Accumetrics VerifyNow P2Y12 cartridge; LTA: light transmittance aggregometry; WBA: whole blood aggregometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Concordance with PFA P2Y 3.2% (94.3%)

<table>
<thead>
<tr>
<th>Method</th>
<th>VN P2Y12</th>
<th>WBA 5 µM ADP</th>
<th>WBA 10 µM ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordance</td>
<td>71%</td>
<td>64%</td>
<td>69%</td>
</tr>
<tr>
<td>Notes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Concordance with VN P2Y12 (90.0%)

<table>
<thead>
<tr>
<th>Method</th>
<th>WBA 5 µM ADP</th>
<th>WBA 10 µM ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordance</td>
<td>88%</td>
<td>89%</td>
</tr>
<tr>
<td>Notes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Effect of Prevalence on The Number of True and False Positives

<table>
<thead>
<tr>
<th>Total counted: 10,000</th>
<th>Prevalence</th>
<th>Disease</th>
<th>True Positives</th>
<th>False Positives</th>
<th>TF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selected sample</td>
<td>20.00%</td>
<td>2000</td>
<td>1800.00</td>
<td>200.00</td>
<td>1960.00</td>
</tr>
<tr>
<td>Unselected but common</td>
<td>1.00%</td>
<td>100</td>
<td>99.00</td>
<td>1.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Unselected uncommon</td>
<td>0.10%</td>
<td>10</td>
<td>9.90</td>
<td>0.10</td>
<td>10.00</td>
</tr>
<tr>
<td>Unselected rare</td>
<td>0.01%</td>
<td>1</td>
<td>0.99</td>
<td>0.01</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* False positive rate is 2.0%; 2% of subjects without disease are classified as positive for the disease by the assay
* True positive rate is 98% of subjects with disease are correctly classified

At a prevalence of 1/10,000, an assay with a 2% false positive rate identifies 200 false positive results for every true positive result

Clinical Specificity
The likelihood a test will identify all the subjects who do not have the disease or condition

\[
\text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}} \times 100\% \]

\[
\text{Specificity} = \frac{92}{92 + 8} \times 100\% = 92\%
\]
Clinical Specificity

• The proportion of subjects who tested negative of all negative subjects tested
• The probability the test is negative given the subject is not sick
• The higher the specificity, the fewer healthy subjects are identified as having the disease

Predictive Value of a Positive Test

The likelihood that a positive test identifies a disease or condition

\[
\text{Positive Predictive Value} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Positives}} \times 100\%
\]

Positive Predictive Value = $\frac{90}{90 + 8} \times 100\%$

Positive Predictive Value = 92%


Predictive Value of a Negative Test

The likelihood that a negative test confirms the absence of a disease or condition

\[
\text{Negative Predictive Value} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Negatives}} \times 100\%
\]

Negative Predictive Value = $\frac{92}{92 + 10} \times 100\%$

Negative Predictive Value = 90%

"You can only predict things after they have happened." Eugene Ionesco

ROC Analysis

• Receiver operating characteristic analysis
• A graph of the true positive rate versus the false positive rate in a binary system as its discrimination threshold (limit, "cutoff") is incrementally varied
• Assay quality is assessed as area under the curve (AUC)

Acceptable Assay

\[
\text{AUC} = 0.85
\]

A Mediocre Assay

\[
\text{AUC} = 0.70
\]
A Worthless Assay

<table>
<thead>
<tr>
<th>Cutoff</th>
<th>TP Rate</th>
<th>FP Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>70%</td>
<td>0.05</td>
<td>0.30</td>
</tr>
<tr>
<td>71%</td>
<td>0.10</td>
<td>0.35</td>
</tr>
<tr>
<td>72%</td>
<td>0.15</td>
<td>0.40</td>
</tr>
<tr>
<td>73%</td>
<td>0.20</td>
<td>0.45</td>
</tr>
<tr>
<td>74%</td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>75%</td>
<td>0.30</td>
<td>0.55</td>
</tr>
<tr>
<td>76%</td>
<td>0.35</td>
<td>0.60</td>
</tr>
<tr>
<td>77%</td>
<td>0.40</td>
<td>0.65</td>
</tr>
<tr>
<td>78%</td>
<td>0.45</td>
<td>0.70</td>
</tr>
<tr>
<td>79%</td>
<td>0.50</td>
<td>0.75</td>
</tr>
<tr>
<td>80%</td>
<td>0.55</td>
<td>0.80</td>
</tr>
</tbody>
</table>

AUC ~0.50

The End