New CLSI Coagulation Guidelines: 2009 Update

Dorothy M. (Adcock) Funk MD
Medical Director, Esoterix Coagulation
MAYO/NASCOLA Quality Conference
April 2009
Disclosures

None
Program Objectives

- Bring awareness of recently published coagulation focused CLSI guidelines and those guidelines currently being drafted
- Highlight the important features of two recently published guidelines
CLSI: Coagulation-focused Guidelines

- **Four guidelines recently published**
  - Pre-analytical variables
  - Coagulometer evaluation protocol
  - PT/APTT testing
    - PT/INR calibration – published 2005
  - Platelet function testing

- **Two guidelines currently in process**
  - Quantitative D-dimer
  - Von Willebrand Factor Antigen and Activity
Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline – Fifth Edition

Dorothy M. (Adcock) Funk MD,
Daniel M. Hoefner MT, PhD
Kandice Kottke-Marchant MD, PhD
Richard A. Marlar, PhD
Diane I. Szamosi, MA, MT, SH
David J. Warunek, PhD, MBA
CLSI Document H57-A

Protocol for the Evaluation, Validation, and Implementation of Coagulometers; Approved Guideline

- Chris Gardiner, FIBMS, MSC, PhD
- Dorothy M. (Adcock) Funk MD
- Leonthena R. Carrington, MBA, MT(ASCP)
- Kandice Kottke-Marchant MD, PhD
- Richard A. Marlar, PhD
- David L. McGlasson, MS, CLS/NCA, H(ASCP)
- Kathleen Fisher Trumbull, MS MT(ASCP)
- Joseph L. Wheeler, BS
- Robert L. Biddle, MBA, MT(ASCP), CLS
- Christine Daniele, MT(ASCP)
One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline – Second Edition

Richard A. Marlar, PhD
Janet Cook, MT(ASCP)
Marilyn Johnston, ART
Stephen Kitchen, FIBMS, PhD
Samuel J. Machin, MB, ChB, FRCPath
Diane Shafer, MT(ASCP)
Laura Worfolk, PhD
Platelet Function Testing by Aggregometry; Approved Guideline

Douglas J. Christie, PhD, FAHA
Leonthena R. Carrington, MBA, MT(ASCP)
Eli Cohen, PhD
Paul Harrison, PhD, MRCPath
Thomas S. Kickler, MD
Marlies Ledford-Kraemer, MBA, BS, MT(ASCP)SH
Kandice Kottke Marchant, MD, PhD
Alvin H. Schmaier, MD
Melanie McCabe White
Thrity Avari, MS
Barbara A. DeBiase
Margaret L. Rand, PhD
Assays of von Willebrand Factor Antigen and Ristocetin Cofactor Activity

- Dorothy (Adcock) Funk, MD
- Stephen Duff, MBA
- Emmanuel Favaloro, BSc(Hons) PhD
- Connie Miller, PhD
- William Nichols, MD
- Robert Gosselin, CLS
- Kathleen Trumbull, MS, MT(ASCP)
- Juergen Patzke, Dr. Rev. Nat
CLSI Document H59

- Quantitative D-Dimer with Emphasis on the Evaluation of Venous Thromboembolic Disease

- John Olson, MD PhD
- Dorothy (Adcock) Funk, MD
- Valerie Ginyard, BSMT (ASCP)
- Kethleen Trumbull, MS MT (ASCP)
- Elizabeth Van Cott, MD
- Thomas Wissel, PhD
- Marc Grimaux
New Coagulometer Protocol

- Provides recommendations on selection, evaluation, implementation and validation of laboratory coagulation instruments (not POC)
  - Pre-acquisition assessment
    - Provides lists of instrument application, analytical performance, IT, customer support characteristics
  - Implementation and validation
    - Testing for Precision, Accuracy, Comparability, Carryover, QC, Calibration, Data Handling and Interface
CLSI Document H47-A2

- Guideline **significantly** up-dated
- Major Additions:
  - Prothrombin Time
    - Local PT/INR validation and calibration
    - Reporting INR in patients with liver disease
  - **Determining factor sensitivity**
  - PT and APTT mixing studies
Validation of INR and local calibration of PT/INR system

- FDA cleared validation and/or calibration plasmas are not yet available
- Information also available in H54
The INR and liver disease

- INR is validated only for patients on vitamin K antagonist therapy (warfarin)
- Quantity and quality of factor deficiency differs between liver disease and vitamin K def/antagonism*
  - Factor levels for any given INR differ between liver disease and vitamin K antagonist therapy
    - Not just impaired carboxylation but impaired synthesis
      - Certain thromboplastins are more sensitive to PIVKA proteins (rabbit brain based)
    - FV and fibrinogen levels lower in liver disease
  - Liver disease - impaired clearance activated factors
MELD (model for end-stage liver disease) score used to assess severity of liver disease, specifically used to prioritize patients for liver transplantation

- Mathematical score based on bilirubin, creatinine, and PT expressed as INR
- PT/INR when used for patients with cirrhosis demonstrates considerable inter-laboratory variation
The INR and liver disease

Studies have shown that the range of PT/INR results for different thromboplastins in patient with liver disease can show up to 47% difference* and this can vary MELD scores by 39%

- Range of INR spanned from 2.3 to 4.3 in liver disease
- Range of INR in warfarin treated patients 3.0 to 3.6 or 16%

*Hepatology 1996;24:1392-4
Alternative calibration system proposed for patients with chronic liver disease*

- Analogous to current INR system VKA
- Measurement of paired liver disease/normal PT values against IRP and thromboplastin to be calibrated; slope is used to determine $ISI_{Liver}$

• INR system is validated only for patients receiving AVK
• Agreement in INR between thromboplastins in patients with liver disease, may be no worse than agreement when using other methods of reporting
• Reporting of only INR may be acceptable for all patient groups
Factor Sensitivity (Responsiveness)

- Level of factor activity at which the APTT (or PT) test result rises above the upper limit of the established reference interval
- Dependent on reagent, normal pooled plasma and factor deficient plasma used should be between 30 to 45%
- Begin with ~100% factor activity and perform multiple dilutions into factor deficient plasma
- Calculate expected % factor activity based on dilution and beginning factor activity
- Perform APTT and PT on each dilution
- Factor level where APTT or PT becomes abnormal determines responsiveness
APTT Sensitivity To Factor V

APTT in seconds

Factor V Activity

Upper limit Of reference interval

10%
• **APTT and PT Mixing Studies**
  
  - PT mixing studies rarely needed
    - Most prolonged PTs are related to factor deficiencies, LA may prolong PT depending on thromboplastin/PL used
  
  - Normal pooled plasma – platelet free, ~100% factor levels, negative for LA
  
  - Incubated mix needed when there is correction of immediate NPP mix
Mixing Studies (APTT or PT)

- **Normal Plasma Mixing Study**

  - Patient $\uparrow$ APTT or PT
  - Normal Pooled Plasma
  - Perform APTT or PT on Mixture

  **Results of APTT or PT**
  1. Corrects
  2. Fails to Correct
Incubated Mixing Study: Method

Incubate each at 37°C for 60 to 120 minutes

Compare control to Plasma mix

Patient
APTT 55 sec

NP
APTT 34 sec

Patient + NP
APTT 36 sec

Patient + NP
APTT 47 sec

Separately incubated patient + NP Mix
APTT 38 sec

Capped!
Mixing Studies – Definition of Correction

- Correction in relation to APTT or PT normal reference interval
  - Upper limit of 2 SD or 3 SD
  - Upper limit + 5 seconds

- Correction in relation to normal pool
  - Normal pool + 5 seconds
  - Normal pool plus 10%

- Rosner index
  \[ \text{Index} = \frac{B - C}{A} \times 100 \]
  - High index: inhibitor
  - Low index: factor deficiency*

* Index cut-off must be established by each laboratory
New guideline on platelet function testing by aggregometry

- Light Transmission Aggregometry
- Impedance Aggregometry
- Flow and Shear Devices (POC)
  - PFA
  - Cone and Plate analyzer
CLSI Document H58-A

- Platelet Fn Testing by Aggregometry
  - Pre-analytical conditions
    - Patient preparation
    - Specimen collection
    - Sample processing
  - Specimen Testing
  - Quality control

No previous standardization document
Surge in anti-platelet drug therapies AND reports of “resistance” to these therapies
Patient and family history should be obtained
Knowledge of medication and dietary history
Patient conditions: fasting, rested, abstain from smoking immediately prior to testing
  - No drugs that affect platelet function 14 days
    - Unless specifically testing for drug effect
  - Adequate platelet count for method of testing
Sample Collection for Aggregometry

- Evacuated tube or syringe acceptable
  - Needle gauge between 19 and 21
  - Winged collection set is acceptable
- Discard tube not needed
- With syringe draw, syringe must be removed before adding sample to tube with anticoagulant
Platelet Fn Testing by Aggregometry

Anticoagulant

- 3.2% sodium citrate preferred, 9:1 ratio
  - Under-filled tubes or patient with an elevated hematocrit have blunted response to agonists due to reduced availability of calcium ions
- ACD-A acceptable for PRP aggregation of flow cytometric analysis of platelets
- EDTA, heparin, ACD not acceptable
Platelet Fn Testing by Aggregometry

- Specimen transport
  - Room temperature avoiding temperature extremes
  - Avoid use of pneumatic tube and/or traumatic handling, maintain tubes in up-right position ideally
  - Transport rapidly, allowing sufficient time for testing
    - Testing generally needs to be completed within three to four hours
- Maintain specimens capped to preserve pH
Agonists Used for Testing

- ADP, collagen, epinephrine, arachidodnic acid and low dose/high dose ristocetin are standard
  - Little consensus on concentrations that should be used; concentrations for PRP and whole blood vary
- ATP release can be measured simultaneously with aggregation using luciferin/luciferase reagent causing generation of light read against an ATP standard curve
Quality Control for Platelet Aggregometry

- Reagent quality control is emphasized
  - Abnormal patient results should have verification that reagents are functioning properly by testing normal platelets
    - Normal platelet control is recommended with each aggregometry
  - New reagent shipments should be tested in duplicate against normal platelets as well as previous lot of reagent
Reference Interval Determination

- Minimum of 20 normal subjects
  - Not pregnant, males and females, no medications or hematologic disorders
  - Test cohort over time (inter-assay precision)
  - Test 10 samples in duplicate (intra-assay precision)
- Pediatric range not required
New CLSI D-dimer and VWF Guidelines

- Should be available for consensus review early 2010
- Input from the coagulation community is important and vital!
  - All comments are reviewed and written replies published with the documents
Thank you for your attention!