New CLSI Coagulation Guidelines: 2009 Update

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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

CLSI Document H21-A5

- **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)*

CLSI Document H47-A2

- **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*

CLSI Document H58-A

- **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*

CLSI Document H51

- **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*

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- **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

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- **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**

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- **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

CLSI Document H47-A2

- **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

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- 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

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- **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

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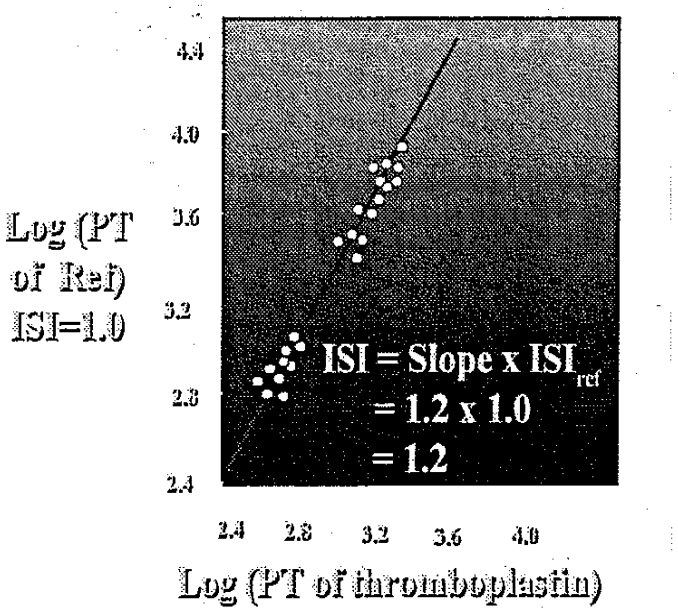
- **Alternative calibration system proposed for patients with chronic liver disease***
  - Analogous to current INR system VKA
  - Measurement of paired liver disease/normal PT values against IRP and thromboplastin to be calibrated; slope is used to determine ISI_{Liver}



J Thromb Haemost 2008;6:243-248

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- **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**

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- **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

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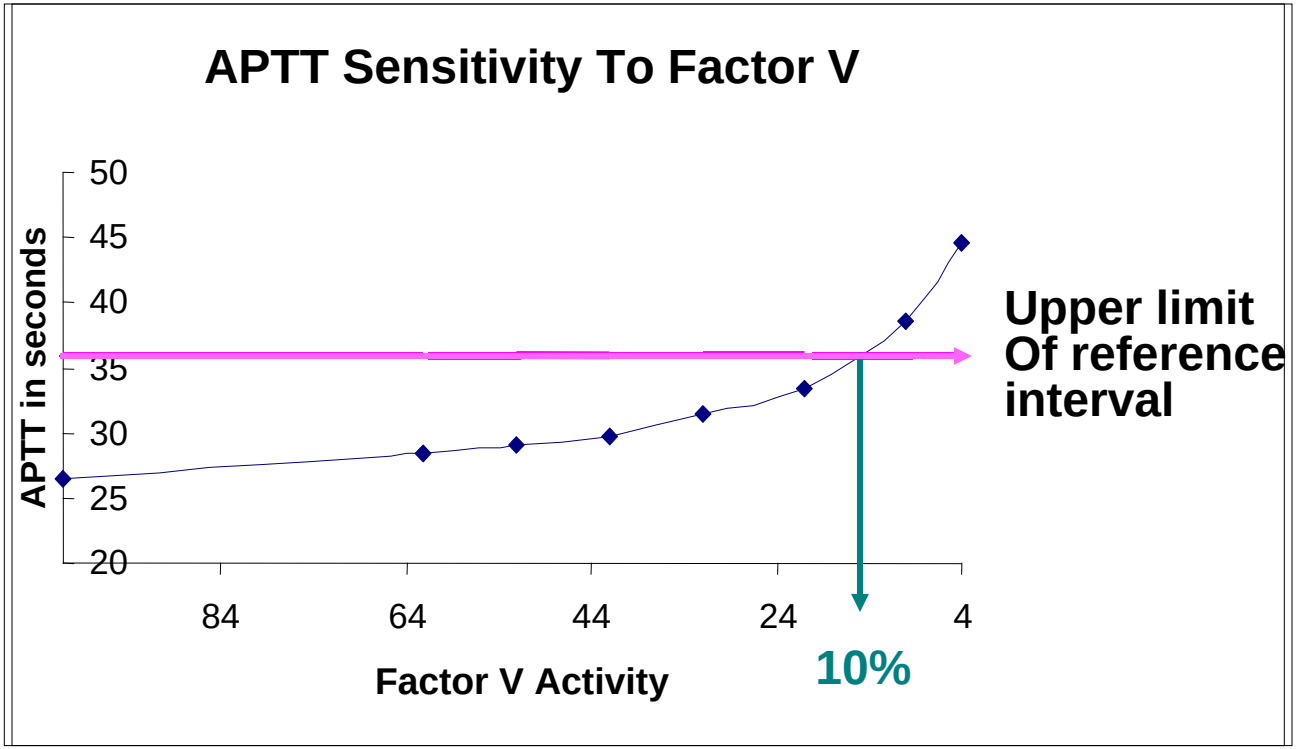

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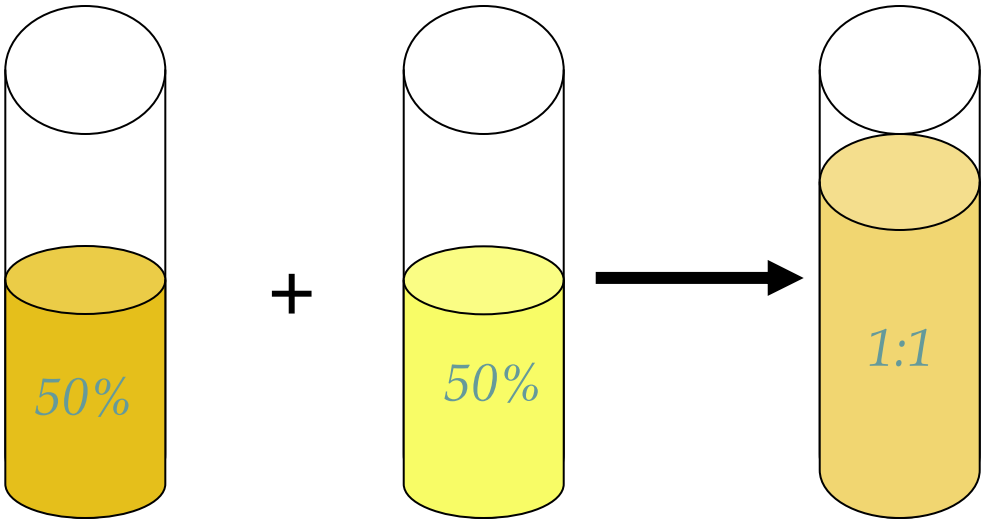
- **APTT and PT Mixing Studies**
  - PT mixing studies rarely needed
    - Most prolonged PTs are related to factor deficiencies, LA may prolong PT depending on thromboplastin/PL used
  - Normal pooled plasma – platelet free, ~100% factor levels, negative for LA
  - Incubated mix needed when there is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

Mixing Studies (APTT or PT)

- Normal Plasma Mixing Study



Patient
↑ APTT
or PT

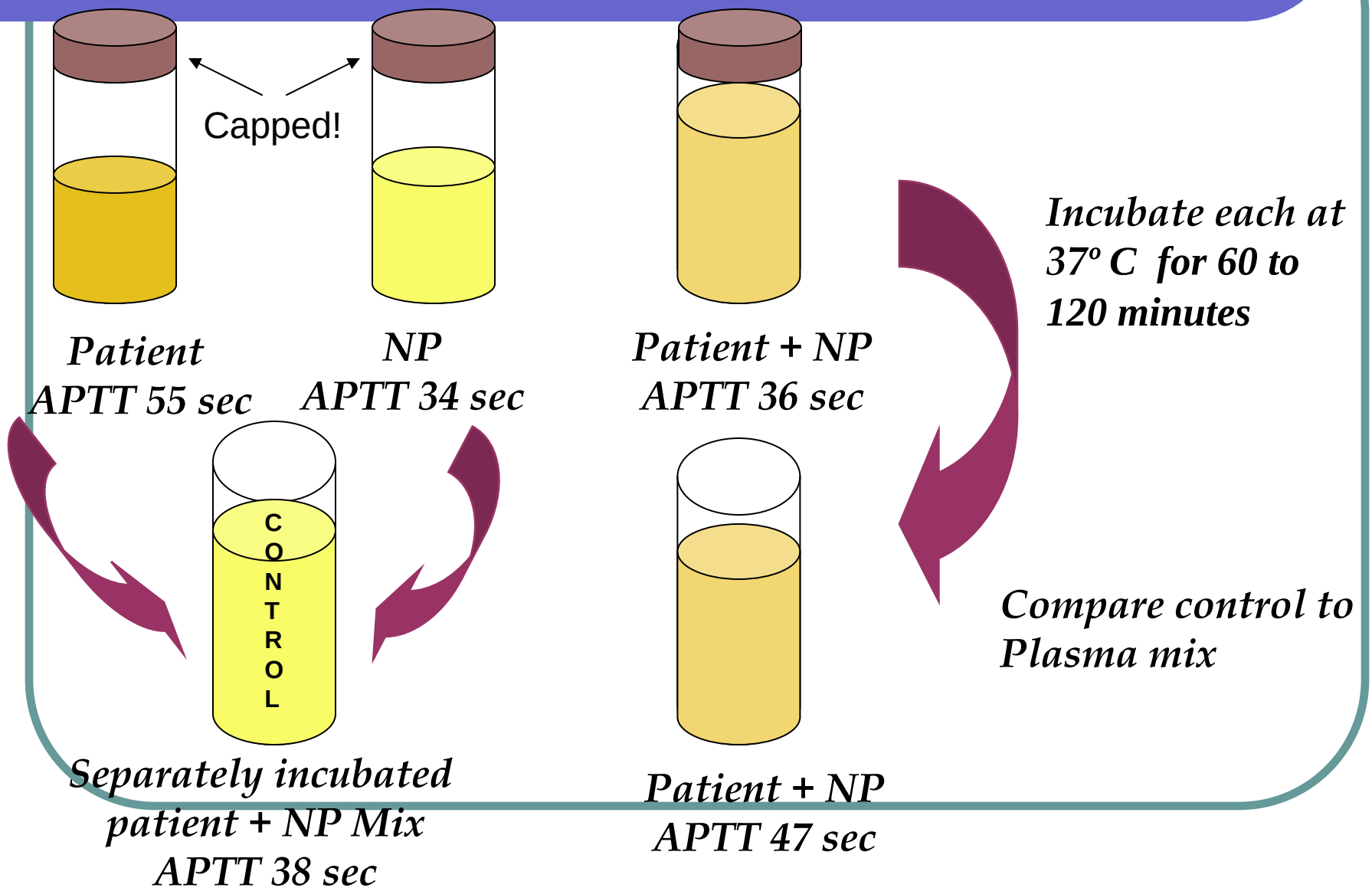
Normal
Pooled
Plasma

Perform
APTT or
PT on
Mixture

Results of APTT or PT

1. Corrects
2. Fails to Correct

Incubated Mixing Study: Method



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- **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

A = Clot Time of Patient
B = Clot Time 1:1 Mix
C = Clot Time of NPP

$$\text{Index} = \frac{B - C}{A} \times 100$$

High index: inhibitor
Low index: factor deficiency*

*** Index cut-off must be established by each laboratory**

CLSI Document H58-A

- ***New*** guideline on platelet function testing by aggregometry

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

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- **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 <u>AND</u> reports of "resistance" to these therapies

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- **Platelet Fn Testing by Aggregometry**
  - 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

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- **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

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- **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

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- **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

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- **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

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- **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

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- **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

CLSI Overview

- **Thank you for you attention!**