Standardization of Platelet Function Testing
CLSI Guideline H58-P

Platelet Function Testing by Aggregometry

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Conflict of Interest

- None to disclose
Clinical and Laboratory Standards Institute (CLSI)

- Global, nonprofit, standards-developing organization that promotes the development and use of voluntary consensus standards and guidelines within the healthcare community
- Based on the principle that consensus is an efficient and cost-effective way to improve patient testing and services
- Committee members for the development of consensus guidelines consist of individuals from industry, academia, and government
Members:
Subcommittee on Platelet Function Testing
(Area Committee on Hematology)

- Douglas Christie, PhD: Dade Behring - Chairman
- Leonthena Carrington, MBA, MT(ASCP): FDA Center for Devices
- Eli Cohen, PhD: Haemoscope Corporation
- Paul Harrison, PhD: Churchill Hospital, Oxford, UK
- Thomas Kickler, MD: Johns Hopkins University
- Marlies Ledford-Kraemer, MBA, MT(ASCP)SH: CLOT-ED, Inc.
- Kandice Kottke-Marchant, MD, PhD: Cleveland Clinic
- Alvin Schmaier, MD: Case Western Reserve University
- Melanie McCabe White: Univ of Tennessee Health Science Cntr
- David Sterry, MT(ASCP): CLSI Staff Liaison
Advisors to Subcommittee

- Kenneth Ault, MD: Maine Medical Center
- Thrity Avari, MS: Chronolog Corporation
- Barbara DeBiase: Sienco, Inc.
- Richard Marlar, PhD: Oklahoma City VA Medical Center
- Margaret Rand, PhD: The Hospital for Sick Children, Toronto
- Ravindra Sarode, MD: UT Southwestern Medical Center
- Jun Teruya, MD: Baylor College of Medicine
- William Trolio: Bio/Data Corporation
Timeline for H58
Proposed Guideline

• **January 2004**: project proposal submitted to CLSI by Dr Christie
  – Rationale: *Currently there are no specific standards or guidelines for clinical laboratory platelet function testing….this proposal is intended to be a first step toward providing for [that] standardization…*

• **August 2005**: project approved
  – Nominations for subcommittee membership submitted and approved

• **May 2006**: first conference call to determine writing assignments

• **October 2006**: two day on-site meeting at CLSI headquarters in Pennsylvania to review first draft of document

• **December 2006-April 2007**: conference calls (5) to reach consensus

• **April 2007**: vote by subcommittee to accept consensus document

• **June 2007**: document submitted to Area Committee on Hematology for review and vote

• **June 30, 2007**: proposed guideline published

• **October 2007**: review delegate comments and consider document revisions
Scope of H58-P Guideline

• Specifies requirements/recommendations for specimen collection, pre-examination considerations, patient preparation, sample processing, testing, and quality control in relation to platelet function testing by aggregometry

• Covers anticoagulants, specimen storage and transport temperatures, sample selection for various methodologies, establishment of reference intervals, result reporting, assay validation, and troubleshooting

• Intended for use by clinicians, hospital and reference laboratorians, manufacturers, and regulatory agencies

• Guideline is not intended for use with global hemostasis, platelet counting, flow cytometry, point-of-care, or research systems

• Guideline does not address therapeutic guidance or interpretation
Guideline Chapters and Writing Assignments

- **Introduction**
  - Paul Harrison
- **Specimen collection and processing**
  - Marlies Ledford-Kraemer
- **Light transmission aggregometry using platelet rich plasma (PRP)**
  - Alvin Schmaier and Melanie White
- **Impedance aggregometry using whole blood**
  - Thrity Avari and Lee Carrington
- **High shear device (platelet function analyzer)**
  - Doug Christie
- **Quality control**
  - Tom Kickler and Barb DeBiase
Recommendations: Pre-examination Issues

- Knowledge of patient medication and dietary history
- Evacuated tube systems, syringes, or butterfly cannulae systems may be used with needle gauge sizes between 19 and 21 (23 gauge size acceptable for pediatrics)
- Anticoagulant is 3.2 % (105–109 mmol/L) trisodium citrate
  - Anticoagulant to blood ratio is 1 part trisodium citrate to 9 parts blood
    - Suggest citrate be adjusted to compensate for hematocrits greater than 45%
- Specimens should be transported at room temperature (20-25°C), hand carried (no pneumatic tubes), and not be exposed to vibration, shaking or agitation
- PRP sample preparation for light transmission aggregometry (LTA)
  - Centrifuge at 170g for 15 minutes at room temperature
  - Store PRP in a capped plastic tube with limited surface area-to-volume ratio
  - Platelet count of platelet poor plasma (PPP) should be less than 10 x 10⁹/L
  - For PRP platelet counts in excess of 400 x 10⁹/L, recommended target PRP count, subsequent to dilution with autologous PPP, is 200-250 x 10⁹/L
  - Maintain PRP at room temperature and use within 4 hours after platelet donation
Recommendations: Light Transmission Aggregometry

- LTA performance requires a standardized cuvette volume for PRP, stirring, standardized light transmittance between 0 to 100%, a run time between 5-10 minutes, and a chart speed of > 2 cm/min
- Agonists
  - **ADP**: use at 0.5-10 μM final concentrations (typical starting concentration = 5μM)
  - **Arachidonic Acid**: use at a single dose between 0.5-1.6 mM
  - **Collagen (type unspecified)**: use at 1-5 μg/ml (typical starting concentration = 2.0 μg/ml)
  - **Epinephrine**: use at a range of 0.5-10 μM (typical starting concentration = 5 μM)
  - **Ristocetin** (ristocetin induced platelet aggregation or RIPA):
    - **High dose**: 0.8 – 1.5 mg/ml
    - **Low dose**: < 0.5 mg/ml
  - **Gamma-thrombin**: use at various concentrations (titrated)
- LTA reporting
  - Percent final aggregation, percent maximum aggregation, slope (initial rate of chart deflection), or in the case of ADP and epinephrine, the minimal concentration needed to induce second wave aggregation
Recommendations:
Impedance Aggregometry

• Whole blood platelet counts between 100 – 1,000 $\times 10^9$/L are diluted with equal parts preservative-free sterile saline immediately prior to testing
  – Platelet counts less than 100 $\times 10^9$/L are tested undiluted
• Agonists
  – **ADP**: use at 5-20 $\mu$M
  – **Arachidonic Acid**: use at 0.5-1.0 mM
  – **Collagen**: use at 1-5 $\mu$g/ml
  – **Epinephrine**: not recommended as an agonist as fewer than 50% of subjects respond
  – **Ristocetin** (RIPA):
    • **High dose**: 1.0 mg/ml
    • **Low dose**: 0.25 mg/ml
• ATP release is quantified by comparing the resulting luminescence values from each test/agonist to a previously tested 2.0 nmole ATP standard
• Reporting impedance aggregation without/with luminescence
  – Amplitude, slope (maximum rate of chart deflection), ATP release, lag time, and area under the curve (AUC).
Recommendations: Quality Control

- Simultaneously test a normal sample at the time a patient or patients is/are tested
- When receiving a new shipment of reagent, determine its reactivity with normal platelets
- Participate in proficiency testing (exchange of samples)
- Reference intervals need to be established for aggregation and secretion responses for each agonist at specific concentrations that are used for patient testing
  - Reference intervals must be reported with patient results
- Measure precision by testing a minimum of ten samples in duplicate and calculating the coefficient of variation of the duplicate pairs
- Determine potency of an agonist by doing dose response to varying concentrations of agonist
- Perform independent temperature measurement using an appropriate probe in a cuvette containing water or saline (and stir bar) inserted in a testing well
- Mechanical stirrers need to have rpm\text{s} validated at recommended intervals
- If a laboratory has more than one platelet aggregometer, reproducibility between instruments and even between channels on the same instrument must be validated
Selected References

ISTH 2007 Survey
Clinical Laboratory Responses

- Clinical laboratory responses = 245
- NASCOLA respondents = 43 (18% of total)
Aggregation tests performed annually
range: 3 to 5000 tests
(n=206 labs)
Type of Aggregometry
n=121 responses

59% (n=145/245) collected samples into 3.2% sodium citrate (n=98) or buffered sodium citrate (n=47)
Pre-analytical Variables

Patients are required to:

- Abstain from caffeine: n=26/202
- Refrain from smoking: n=25/206
- Rest: n=53/201
- Fast: n=38/162
Needles Used For Sample Collection
(n=179)

- 16 guage: n=2
- 19 guage: n=49
- 20 guage: n=27
- 21 guage: n=101

• 32% (n=79/245) used a butterfly needle
Study a normal control in parallel with the patient

No 49%
(n=99 / 204)

Yes 51%
(n=105 / 204)
PRP Count Adjustment
n=173 responses

No Adjustment: 45%
Only when plt count exceeds limit: 16%
All samples adjusted: 39%

Value of Adjusted Counts
n=119 responses

200 x 10^9/L: 55%
250 x 10^9/L: 30%
300 x 10^9/L: 15%

149 adjust with PRP
Aggregation and Secretion Studies Performed Together?
n=153 responses

- **Yes, on all patients**: n=24
- **Yes, but only on selected patients**: n=29
- **Not performed together**: n=100
Maximal Time Allowed for Completing LTA

n=140 responses

- Within 1 hour
- Within 2 hours
- Within 3 hours
- Within 4 hours
- Within 5 hours
- Within 6 hours
Common LTA Agonists at least one concentration
n=149 responses

- high dose Ristocetin
- low dose Ristocetin
- ADP
- Collagen
- Epinephrine
- Arachidonic Acid
- U46619
- TRAP
- gamma-thrombin
- PAF
# Times for Monitoring LTA Responses

<table>
<thead>
<tr>
<th>Agonist (n)</th>
<th>Range (minutes)</th>
<th>Most common</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>3 – 10</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>3 - 10</td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>3 - 10</td>
<td></td>
</tr>
<tr>
<td>TRAP</td>
<td>3 - 10</td>
<td>5 minutes (n=6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 minutes (n=6)</td>
</tr>
<tr>
<td>PAF</td>
<td>3 – 10</td>
<td>10 minutes</td>
</tr>
<tr>
<td>U46619</td>
<td>3 – 10</td>
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</tr>
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<td>Arachidonic Acid</td>
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<td>3 - 10</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Ristocetin</td>
<td>3 – 10</td>
<td>5 minutes</td>
</tr>
</tbody>
</table>
Statistical Approach for Determining LTA RI

- Special statistical tests that allow for use of repeat measures on some controls
- Statistics using one value for each control
- Mean plus or minus 2 standard deviations, with log transformation of some data to normalize the distribution
- Nonparametric statistical tests
- Mean plus or minus 2 standard deviations
- Not applicable - reference ranges not determined

Note scale
Parameters Evaluated for LTA

- amplitude of % agg at end of test
- measure of lag phase
- presence of a shape change
- slope of aggregation
- visual inspection of tracing
- deaggregation
- presence of "secondary wave"
- maximal amplitude or % agg
Parameters Included in Report

- Amplitude of aggregation at the end of the observation
- Presence of a shape change
- Measure of lag phase
- Slope of aggregation
- Visual inspection of the tracing
- Deaggregation
- Presence of "secondary wave"
- Maximal amplitude or % aggregation
- Overall interpretative comment

Percentage