Standardization of Platelet Function Testing
CLSI Guideline H58-P
Platelet Function Testing by Aggregometry

Marlies Ledford-Kraemer
MBA, BS, MT(ASCP)SH
CLOT-ED, Inc

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^{o}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 170*g* 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 10^9/L
  - For PRP platelet counts in excess of 400 x 10^9/L, recommended target PRP count, subsequent to dilution with autologous PPP, is 200-250 x 10^9/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 x 10^9/L are diluted with equal parts preservative-free sterile saline immediately prior to testing
  - Platelet counts less than 100 x 10^9/L are tested undiluted
- Agonists
  - **ADP**: use at 5-20 µM
  - **Arachidonic Acid**: use at 0.5-1.0 mM
  - **Collagen**: use at 1-5 µg/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 *rpms* 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

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- Moffat KA, Ledford-Kraemer MR, Nichols WL, Hayward CP. Variability in clinical laboratory practice in testing for disorders of platelet function: results of two surveys of the North American Specialized Coagulation Laboratory Association. Thromb Haemost 2005;93:549-53.
- Zhou L, Schmaier AH. Platelet aggregation testing in platelet-rich plasma: description of procedures with the aim to develop standards in the field. Am J Clin Path 2005;123:172-83.
- Zucker MB. Platelet aggregation measured by the photometric method. Methods Enzymol 1989;169:117-33.
- Ingerman-Wojenski CM, Silver MJ. A quick method for screening platelet dysfunctions using the whole blood lumi-aggregometer. Thromb Haemost 1984;51:154-6.
- Kundu SK, Heilmann EJ, Sio R, Garcia C, Ostgaard RA. Characterization of an in vitro platelet function analyzer - PFA-100. Clin Appl Thromb Hemost 1996;2:241-49.

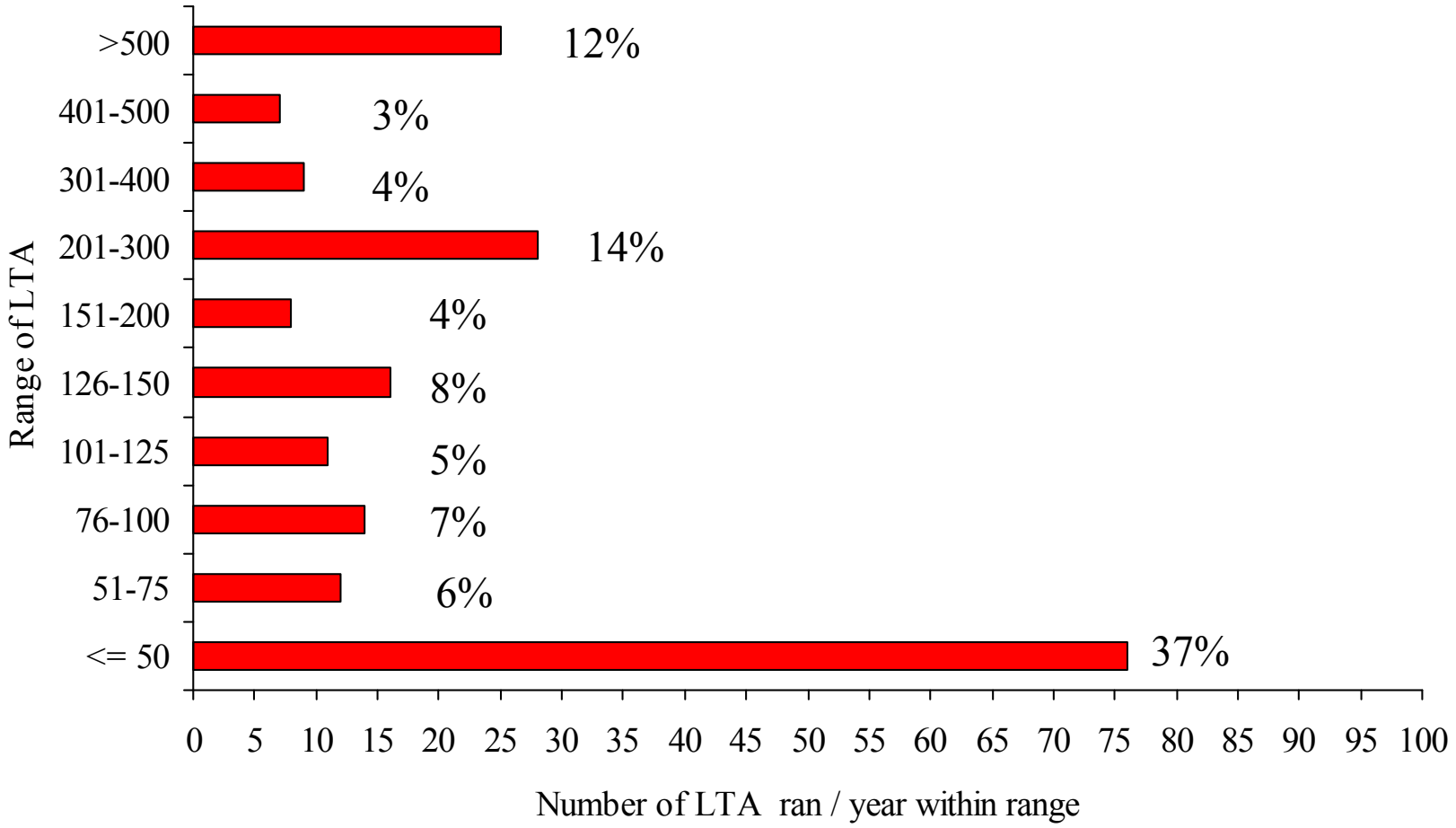
ISTH 2007 Survey
Clinical Laboratory Responses

- Clinical laboratory responses = 245
- NASCOLA respondents = 43 (18% of total)

Aggregation tests performed annually

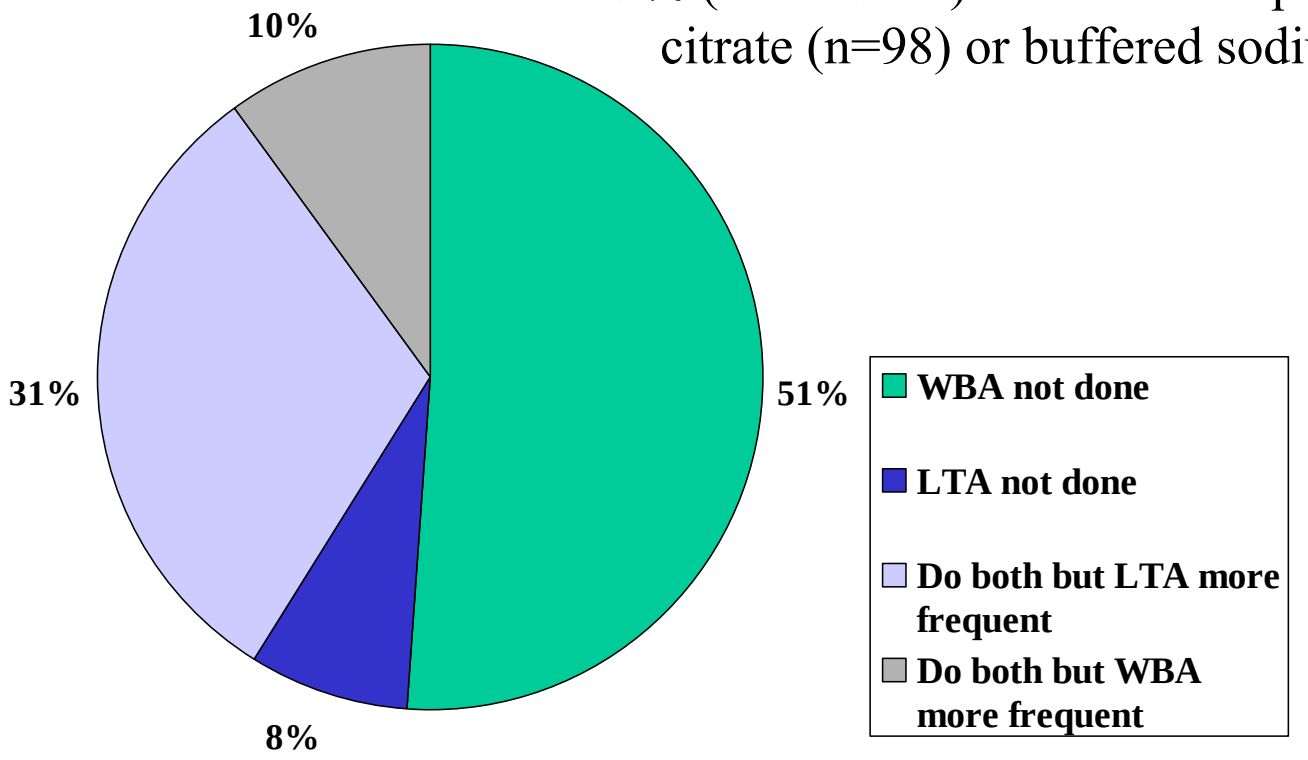
range: 3 to 5000 tests

(n=206 labs)



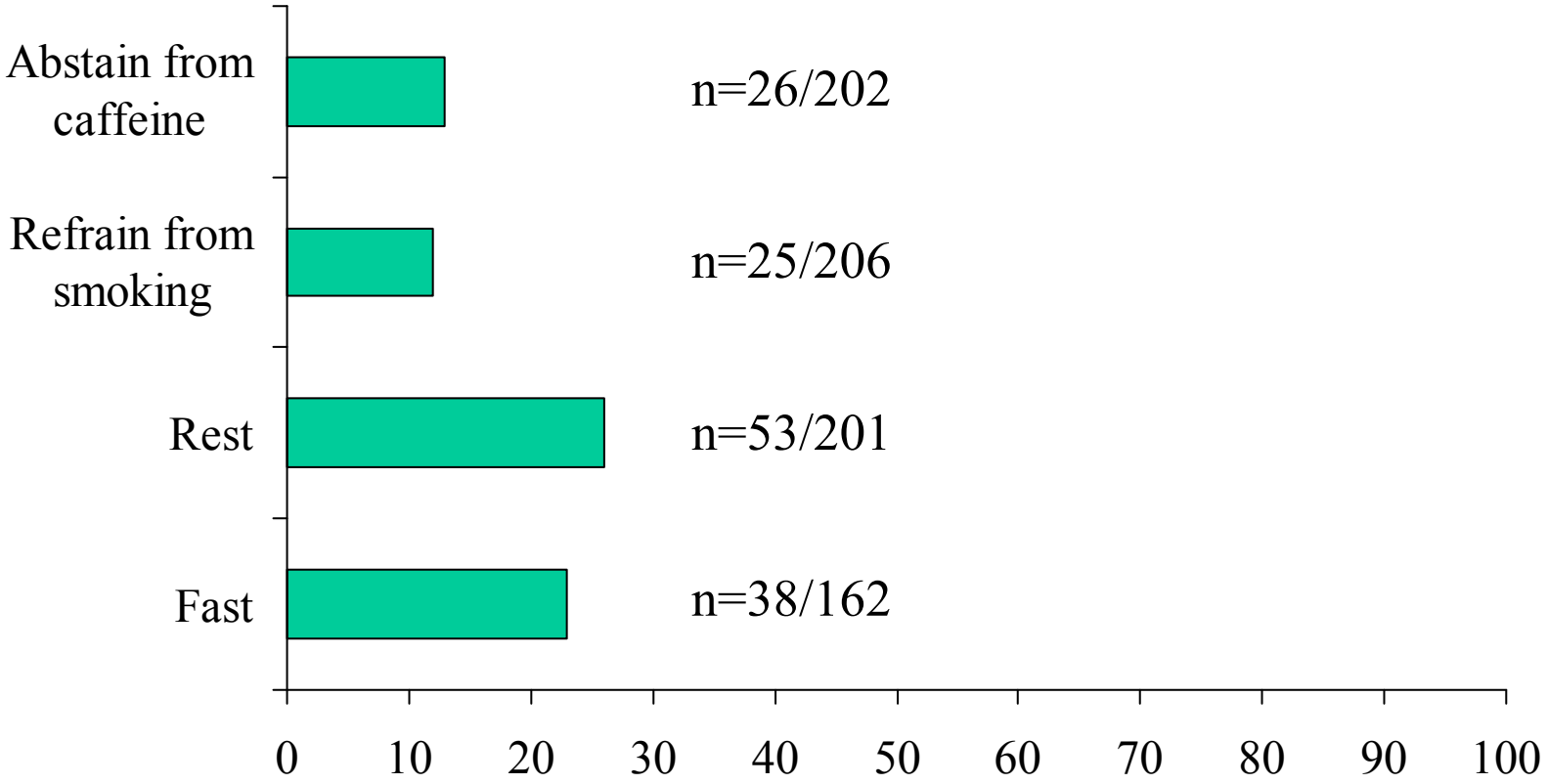
Type of Aggregometry n=121 responses

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

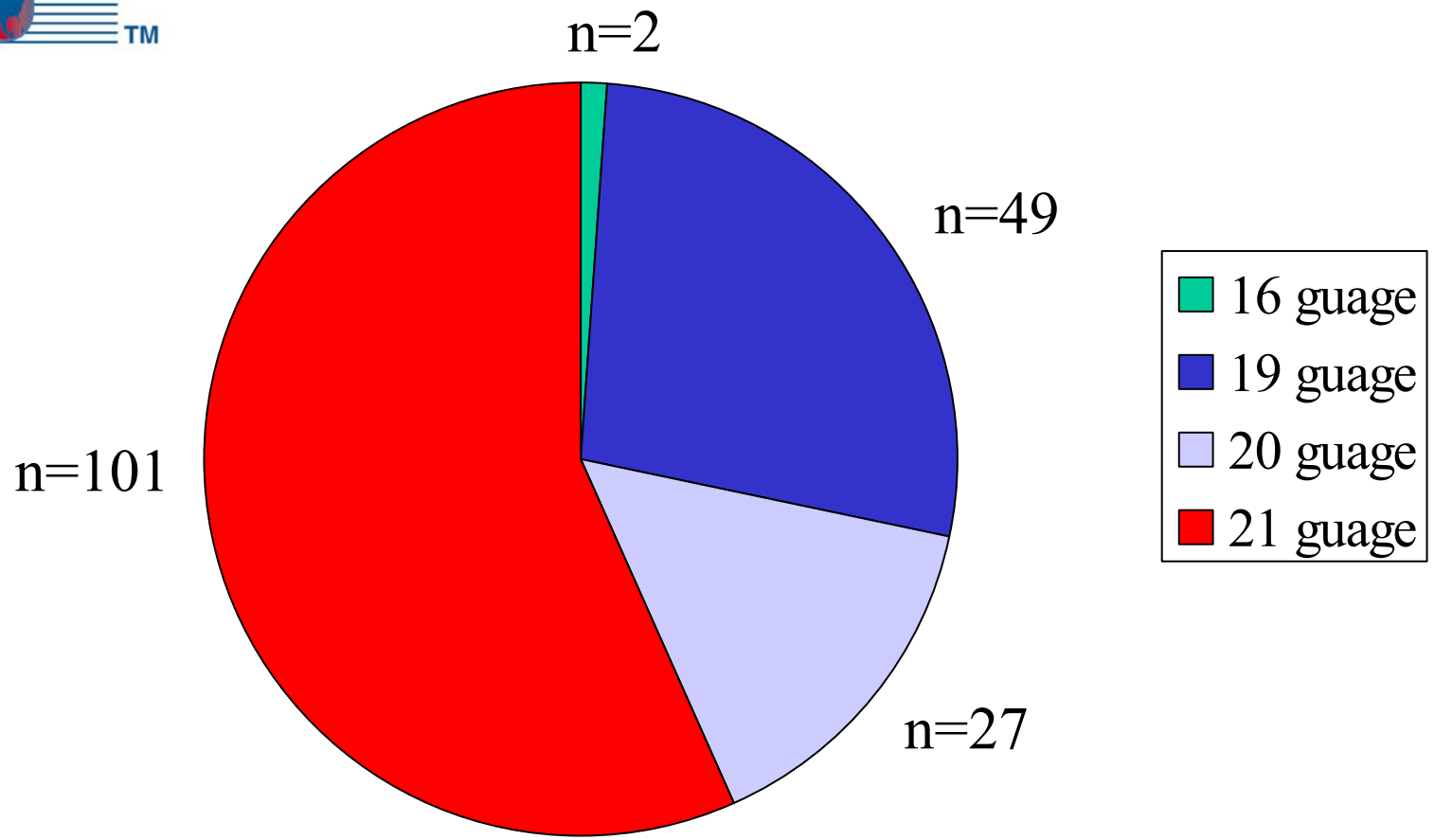


Pre-analytical Variables

Patients are required to……

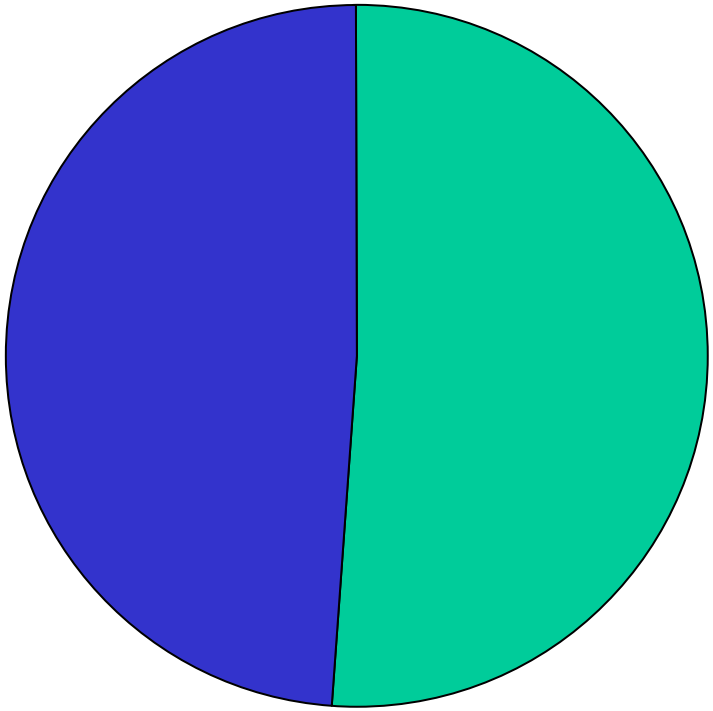


Needles Used For Sample Collection (n=179)

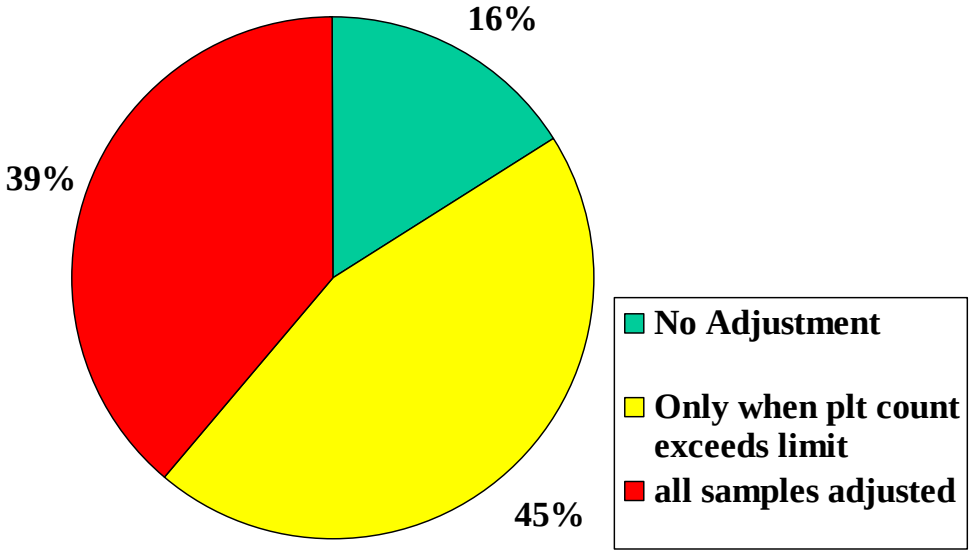


- 32% (n=79/245) used a butterfly needle

Study a normal control in parallel with the patient

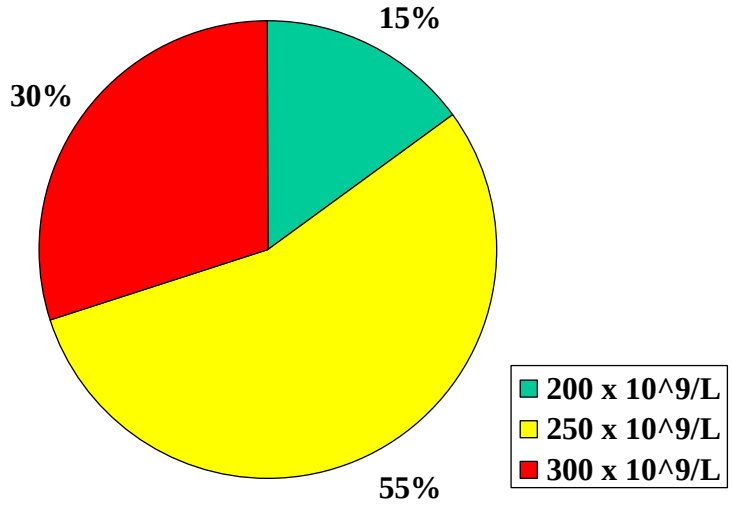


PRP Count Adjustment
n=173 responses

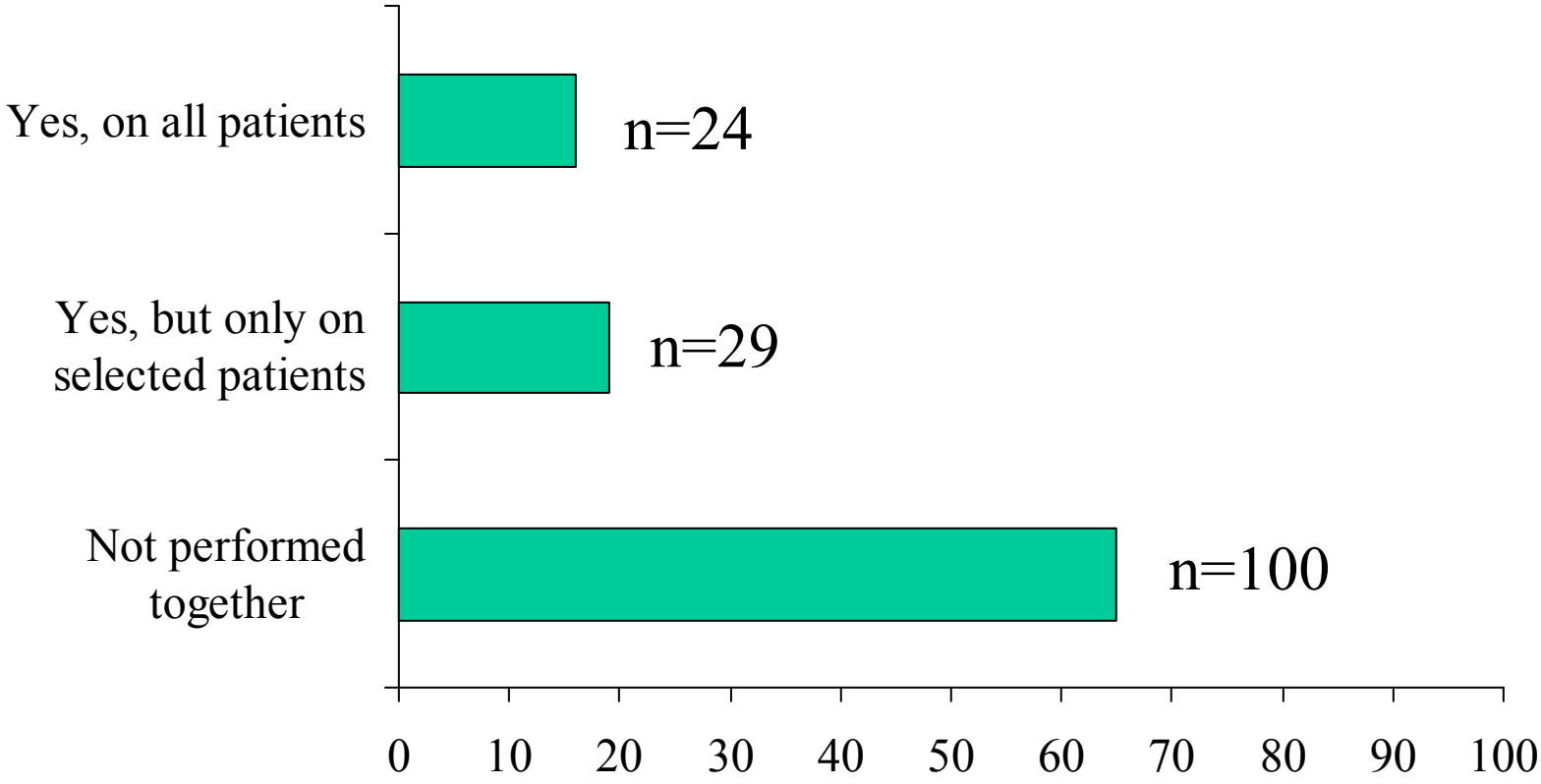


149 adjust with PRP

Value of Adjusted Counts
n=119 responses

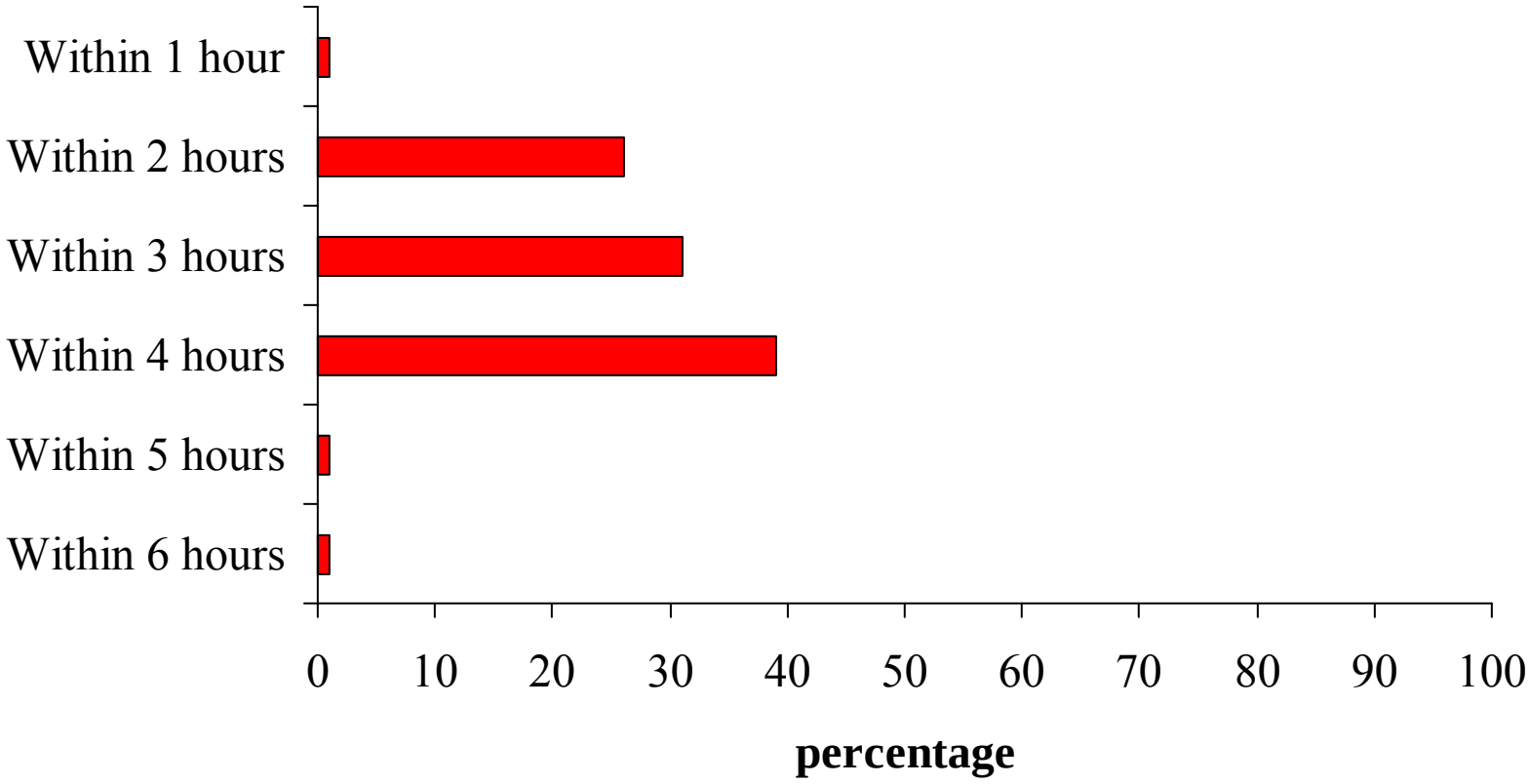


Aggregation and Secretion Studies Performed Together?
n=153 responses



Maximal Time Allowed for Completing LTA

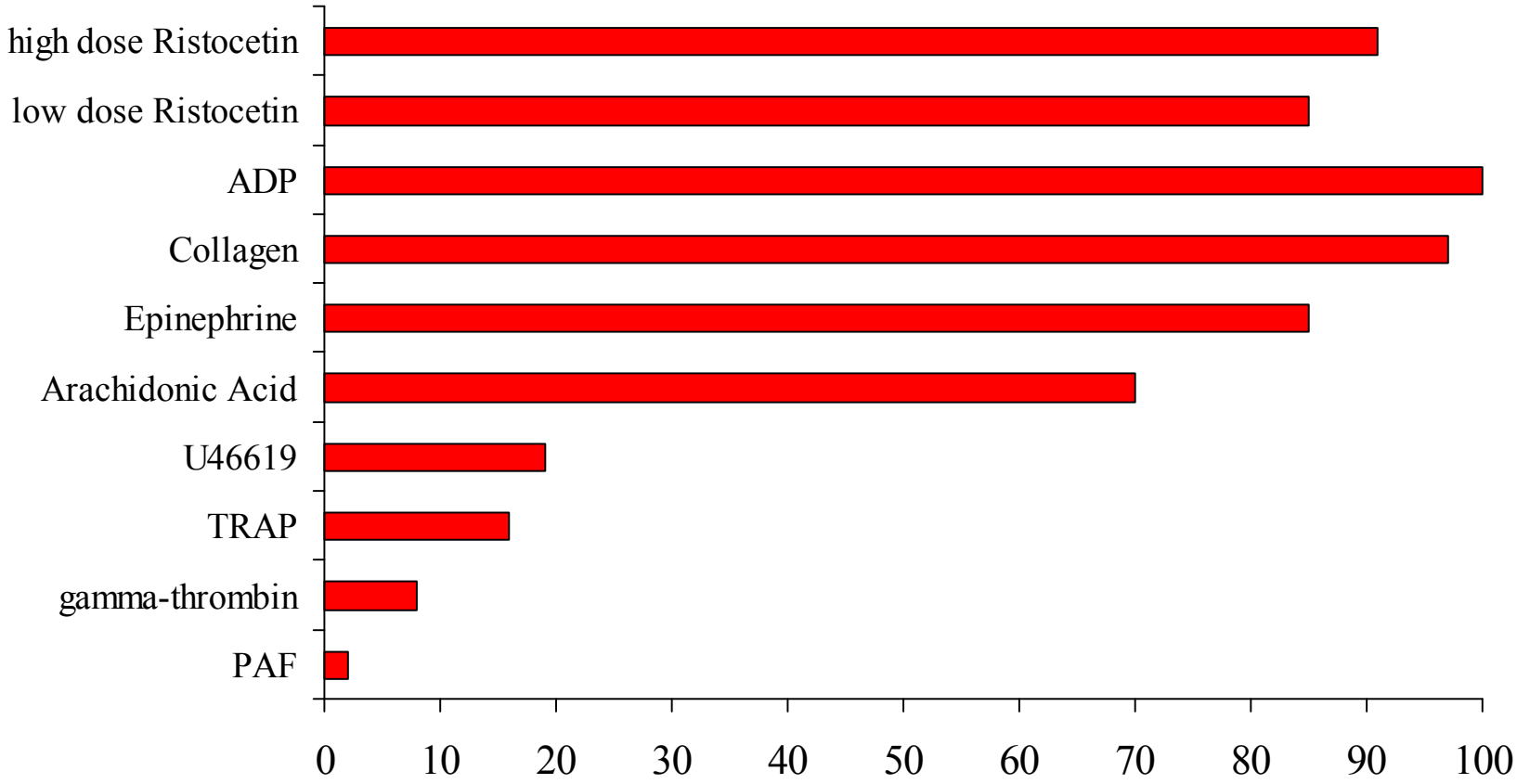
n=140 responses



Common LTA Agonists

at least one concentration

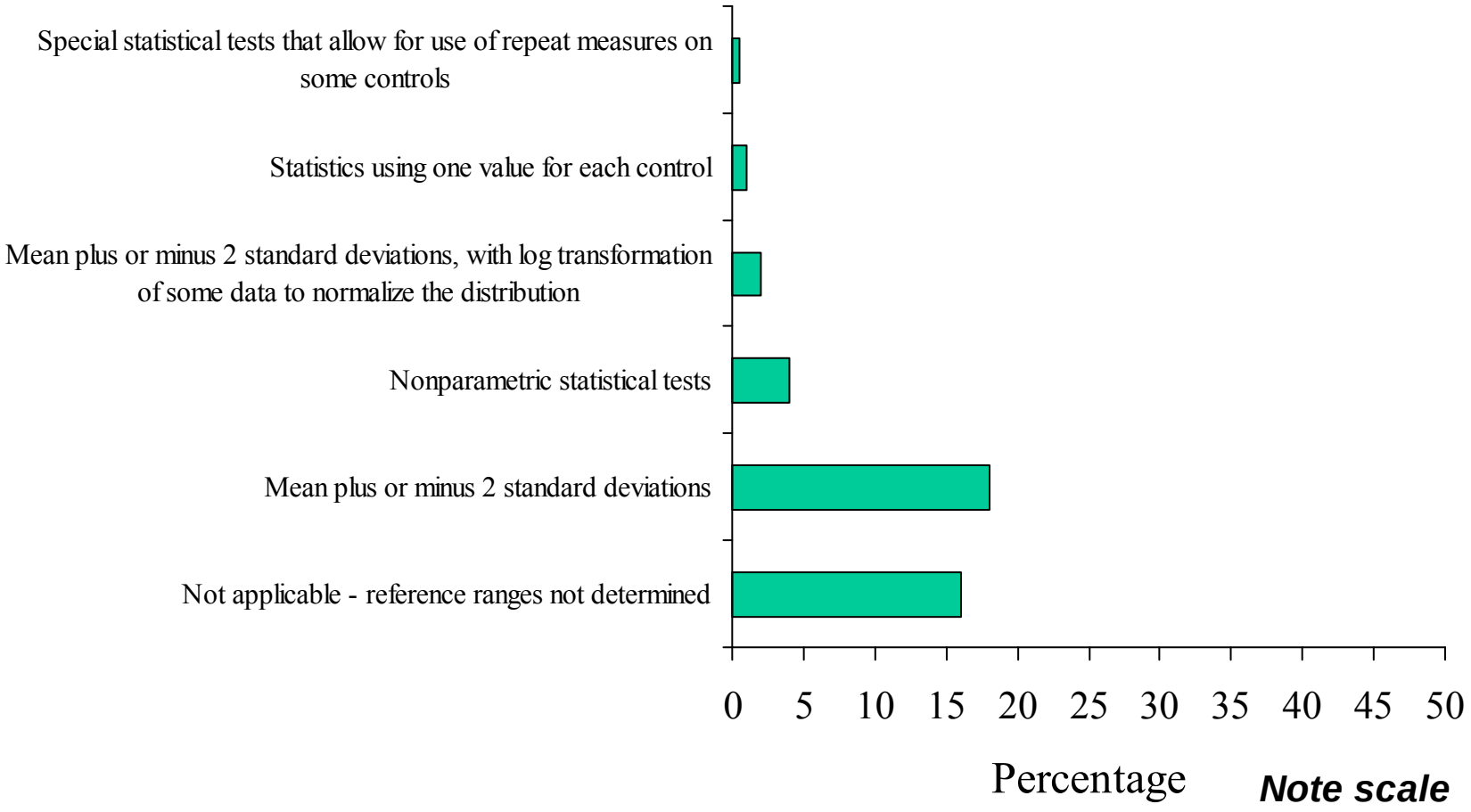
n=149 responses



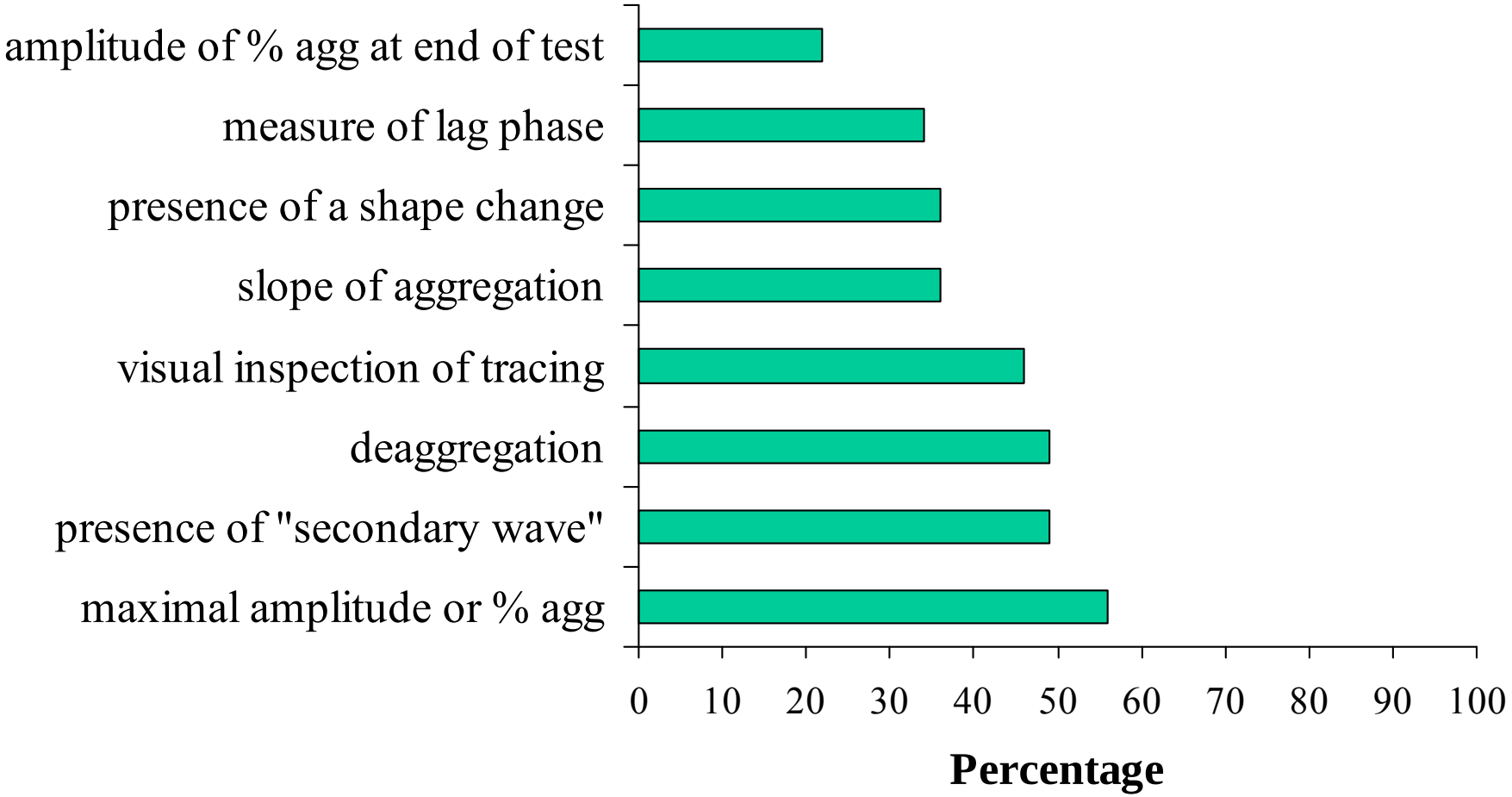
Times for Monitoring LTA Responses

Agonist (n)	Range (minutes)	Most common
ADP	3 – 10	5 minutes
Epinephrine	3 - 10	
Collagen	3 - 10	
TRAP	3 - 10	5 minutes (n=6) 10 minutes (n=6)
PAF	3 – 10	10 minutes
U46619	3 – 10	5 minutes
Arachidonic Acid	3 – 10	5 minutes
Gamma-thrombin	3 - 10	10 minutes
Ristocetin	3 – 10	5 minutes

Statistical Approach for Determining LTA RI



Parameters Evaluated for LTA



Parameters Included in Report