Using CLSI H62 to Bring Quality to the Clinical Flow Cytometry Laboratory

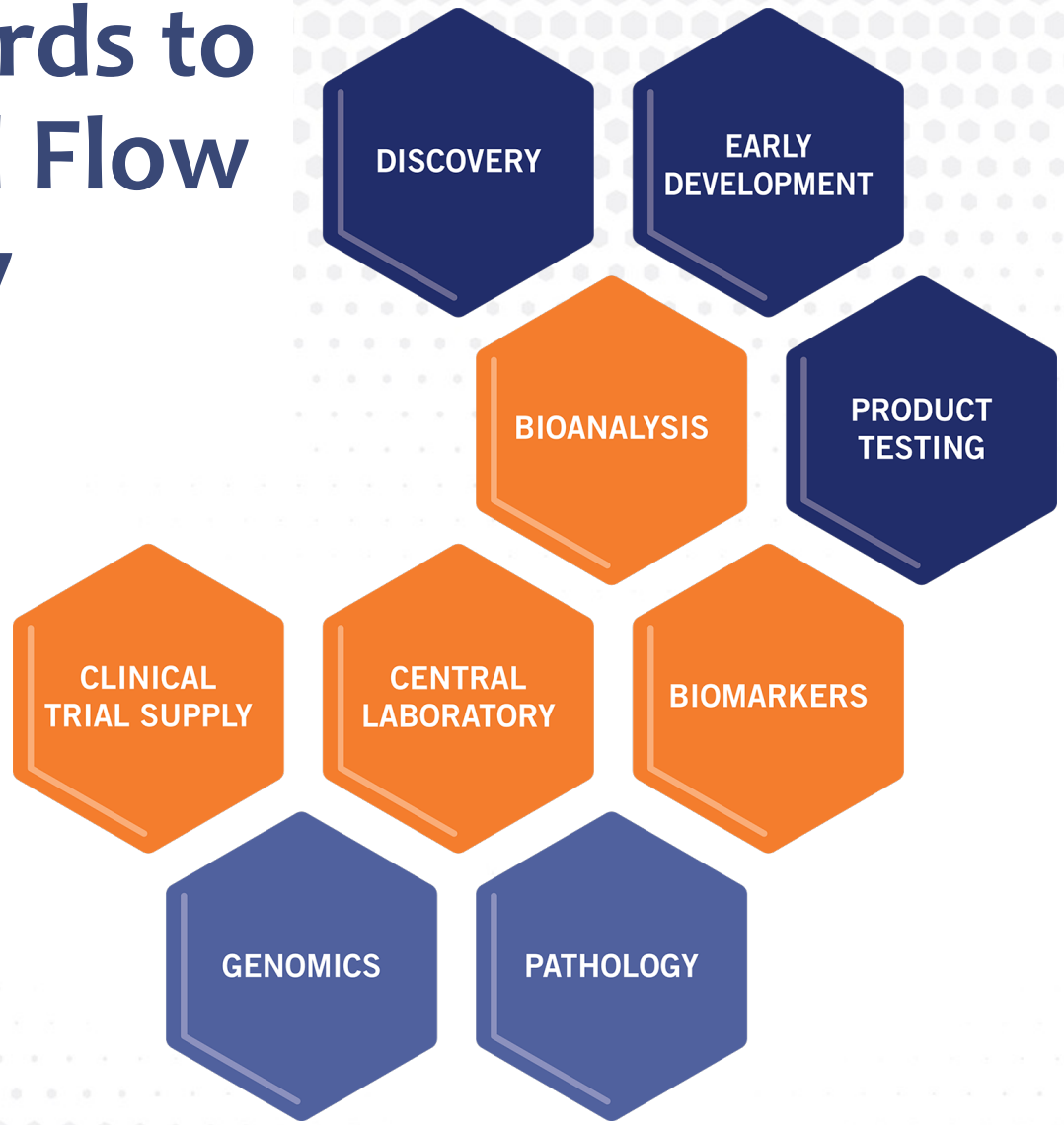
ESCCA 2023
ESCCA-ICCS joint session:
Standardization in Clinical Flow Cytometry:
Design, Validation, and Analysis

Virginia Litwin, Ph.D.
Scientific Affairs

eurofins | BioPharma Services

Utrecht, Netherlands
September 27, 2023

Using Documentary Standards to Bring Quality to the Clinical Flow Cytometry Laboratory



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Documentary Standards

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- Introduction to CLSI
- New CLSI Documents
- CLSI H62 1st Edition
  Validation of Assays Performed by Flow Cytometry

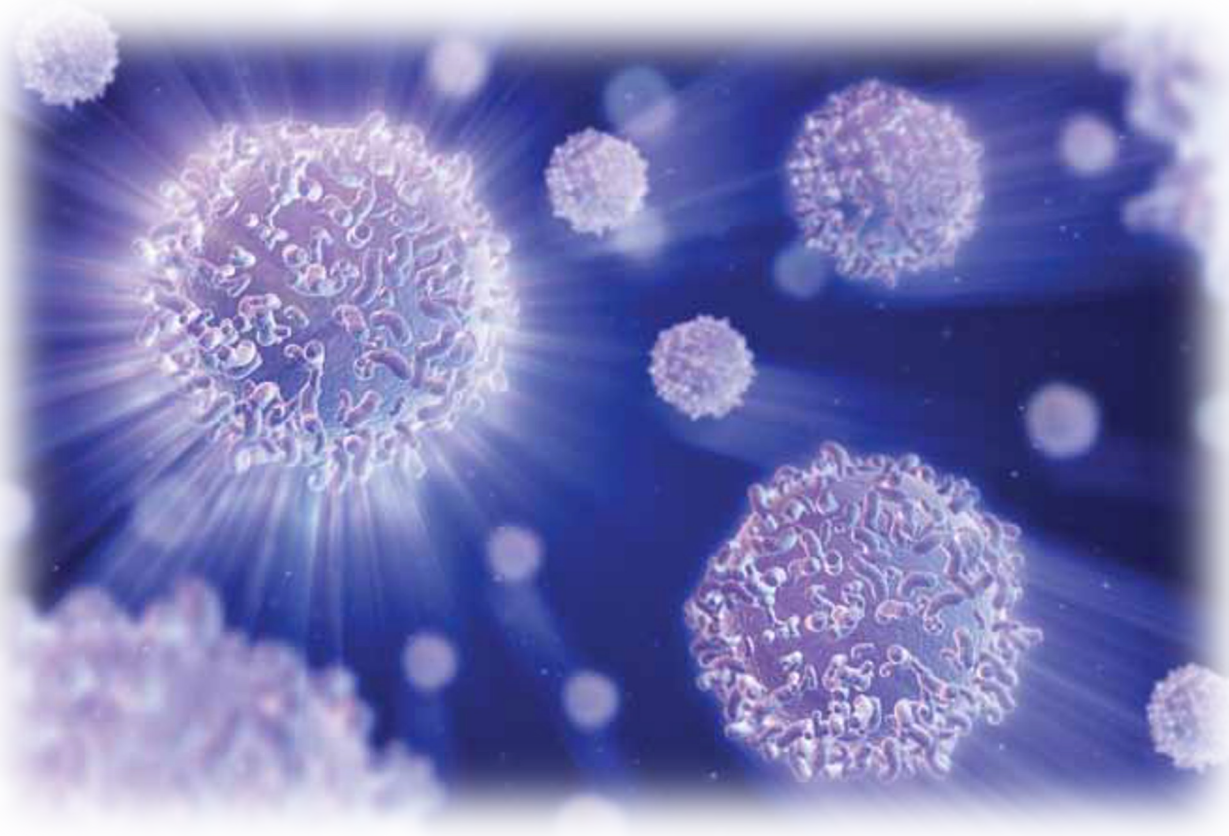


Image from Litwin, et al. Clinical Laboratory News, 12: 8, 2013.

What is CLSI?

- Clinical **AND** Laboratory Standards Institute
- Not-for-profit organization that develops laboratory standards
- Global leaders in setting laboratory standards worldwide
- Recognized by laboratories, accreditors, and government agencies
- Aligned with ISO
- ANSI compliant

ANSI
American National Standards Institute

Governance

- ✓ Establish policies

CLSI Board of Directors

- ✓ Specialty area SME
- ✓ Manage Document Review Process
- ✓ First review from DDC
- ✓ Provide advice to DDC and CC

Consensus Council

Expert Panels for Each Specialty Area

- ✓ Set priorities for projects
- ✓ Ensures procedures were followed
- ✓ Final approval of documents

Document Development Committees, Subcommittees, and Working Groups

- ✓ Develop documents
- ✓ Revise documents

Why CLSI?

- Consensus Documents
- Extensive review process
- Alignment with International Organization for Standardization (ISO)
- Regulatory agencies often recognize CLSI guidelines

Consensus Document Review Process

October 2016

Submit Proposal

CLSI Expert Panel Review
Feedback and Revisions
CLSI Consensus Council Review
Feedback and Revisions

April 2017

CLSI
Authorizes project

Document Development Committee (DDC)
Writes draft document

Kick off meeting October 2017
Draft Completed February 2019
- 165 pages
- 19 figures
- 24 tables
- 118 references
- 6 appendices

Voting
DDC and CLSI Delegates

September 2019

Open Comment Period
General Public
CLSI Expert Panel, CLSI Board of Directors

- 283 requests for document
- 722 comments submitted

DDC
Responds to comments

Commenters
Receives resolutions
Accept or Appeal

- 3 groups submitted appeals
- 17 total appeals comments (2.4%)

DDC
Responds to appeals

CLSI
Final review and vote

October 2021

Document is published

Records and Communications

- General Process for review and documentation
- Comments and resolutions archived
- Email communications re: minor corrections
- Five-year Review Process
  - Conducted by the appropriate Expert Panel
- Technical Questions sent to Expert Panel
  - Responses archived, addressed in the next update
  - Technical Questions
    standard@clsi.org

Review Process for Existing Documents

- Five-year Review Process
  - Conducted by the appropriate Expert Panel
- Assess technical validity
- Consider new information or changes in technology
- Determine whether the existing document is globally applicable
- Consideration for recommending consolidation of related documents
- Recommendation
  - ? Reaffirmation
  - ? Revision
  - ? Withdrawal
  - ? Archive

Expert Panel Hematology, Immunology and Ligand Assays

Flow Cytometry Documents

- H42

  Enumeration of Immunologically Defined Cell Populations by Flow Cytometry

- H43

  Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells

- H52

  Red Blood Cell Diagnostic Testing Using Flow Cytometry

- H62

  Validation of Assays Performed by Flow Cytometry

H42 Enumeration of Immunologically Defined Cell Populations by Flow Cytometry

- 2nd Edition
  - TBNK
  - CD34 Stem Cells

- 3rd Edition Under Revision
- Leadership
  - Chair: Virginia Litwin, Eurofins, Montreal, Canada
  - Vice Chair: Mathew Morrow, UMass Memorial Health, Worcester, Massachusetts

- New Focus

H42 3rd Edition

Chapter 3: Instrumentation

3.1 Types of Instruments

- Photomultiplier tubes (PMT)
- Avalanche photodiode (APD)
- Spectral

3.2 Best Practices for Instrument Setup

- Analog instruments
- Digital instruments
- Spectral

3.3 Instrument Quality Control

- Monitoring
- Instrument drift

Chapter 4: Development and Optimization of Multicolor Immunophenotyping Assays

Chapter 5: Current recommended phenotype

- TBNK
- T-cells and subsets
- B-cells and subsets
- NK cells and subsets
- Monocytes and subsets
- Myeloid derived suppressor cells
- Eosinophils
- Basophils
- Chimeric Antigen Receptor (CAR) cells

H42 3rd Edition

Chapter 7: Troubleshooting TBNK Assays

- Abnormal samples
- Troubleshooting for Proficiency Testing Samples

Chapter 9: Quality Improvement

Implementation of new technologies (instruments, reagents, software) and processes into the laboratory workflow

New Flow Cytometry CLSI Documents

- **CD34 Stem Cells**
- **Leadership**
  - Chair: Robert Sutherland, University of Toronto, Oakville, Ontario, Canada
  - Vice Chair: Ahmad Al-Attar, UMass Memorial Health, Worcester, Massachusetts

New Flow Cytometry CLSI Documents

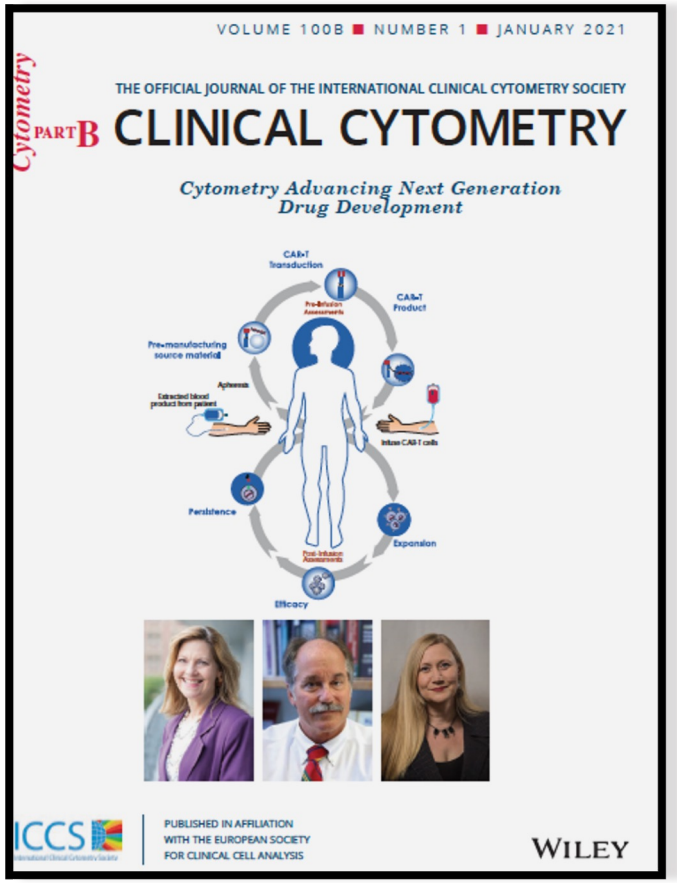
- **Rare Event Detection**
- **Leadership**
  - Chair: Ben Hedley, Princess Margaret Hospital, Ontario, Canada
  - Vice Chair: Ulrike Sommer, Novartis, Basel, Switzerland

ORIGINAL ARTICLE

CLINICAL CYTOMETRY WILEY

High-sensitivity flow cytometric assays: Considerations for design control and analytical validation for identification of Rare events

Ulrike Sommer[1] | Steven Eck[2] | Laura Marszalek[3] | Jennifer J. Stewart[4] | Jolene Bradford[5] | Thomas W. McCloskey[6] | Cherie Green[7] | Alessandra Vitaliti[1] | Teri Oldaker[8] | Virginia Litwin[9]



Free Access https://bit.ly/3iDS5qb

Other Flow Cytometry CLSI Documents

H43 Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Third Edition

- To be revised soon
- Chair: Wolfgang Kern, MLL, Munich, Germany
- Vice Chair: Sindhu Cherian, University of Washington, Seattle, Washington

H52- RBC Diagnostic Testing Using Flow Cytometry – Second Edition

ILA26- Performance of Single Cell Immune Response assays

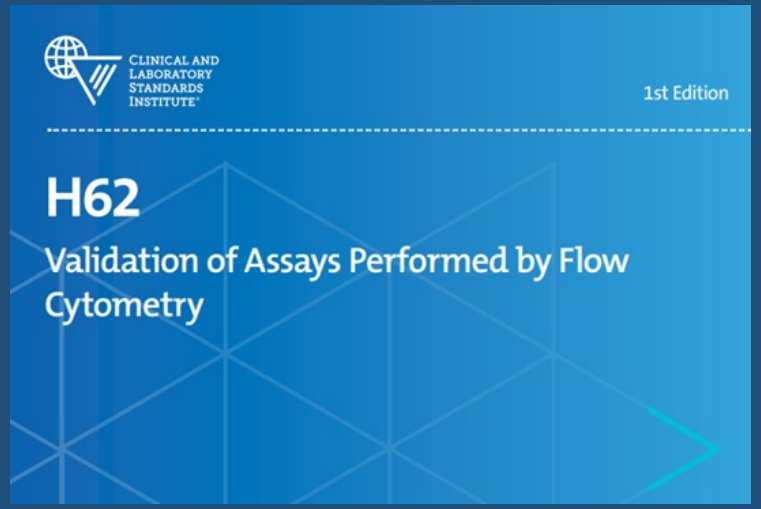
- Intra-cellular cytokine staining
- ELISpot
- CSFE
- To be revised soon, looking for chairs

CLSI H62 1st Document Development Committee

37 Members

Constituencies

- Government (NIST, FDA)
- Industry (Pharma, Manufacturers)
- Professional (Clinical labs, CRO, NIH)



Scientific Societies

- ICCS
- AAPS
- CAP
- ESCCA
- ISAC

Provenance

- Canada
- Germany
- Switzerland
- UK
- USA

CLSI H62 1st Edition

Focus

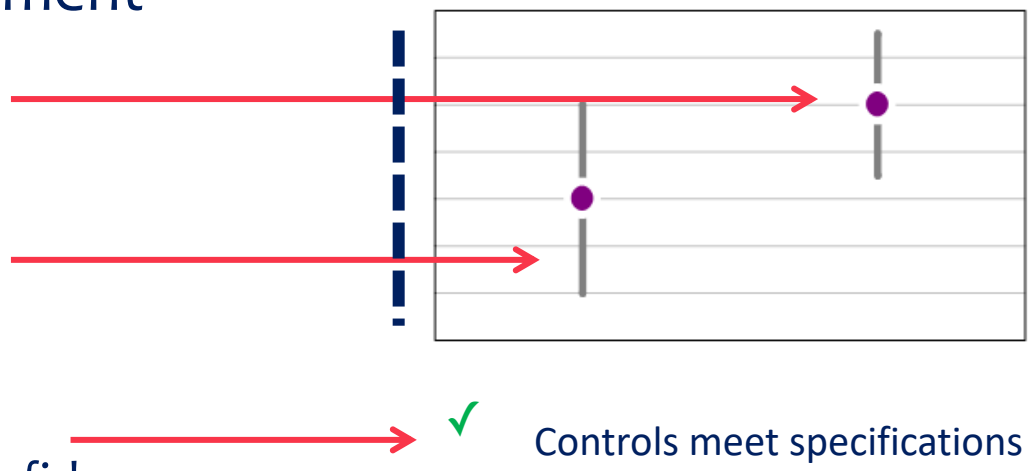
- Comprehensive document
- Covers all aspects of flow cytometry testing
  - From uncrating the instrument
  - To archiving the data
- Target audience
  - Basic Science Laboratories
  - Clinical Laboratories
  - Pharmaceutical and Biotechnology
  - Manufacturers
  - Regulatory Agencies

Why a Flow Specific Document?

- Existing validation guidelines are not fully applicable
  - Soluble analyte vs cell based
- Unique challenges associated with the validation of flow cytometric methods
  - The complexity of cellular analytes (normal and diseased)
  - Highly complex and configurable instrumentation
  - Different software used throughout the process
    - Instrument setup and calibration
    - Sample acquisition
    - Data transfer and storage
    - Data analysis
- The lack of TRUE reference material
- Data are not derived from a calibration curve
- Rapid rate of technological advancement
  - Instrumentation
  - Reagents
  - Software
- Rapid rate of biological discoveries
  - New receptors
  - New cell subsets

Measurement Assurance

- A systematic approach that informs on the comparability and confidences in the result
- Enables data-driven decision making
- Considers the components of the measurement
  - Value
    - it is on a scale; enables compared to other measurements
  - Uncertainty
    - variability in the measurement; statistics
  - Evidence
    - evaluation of the measurement system; confidence



John T. Elliott Jr., NIST
NIST-FDA Flow Cytometry Worshop, 2017

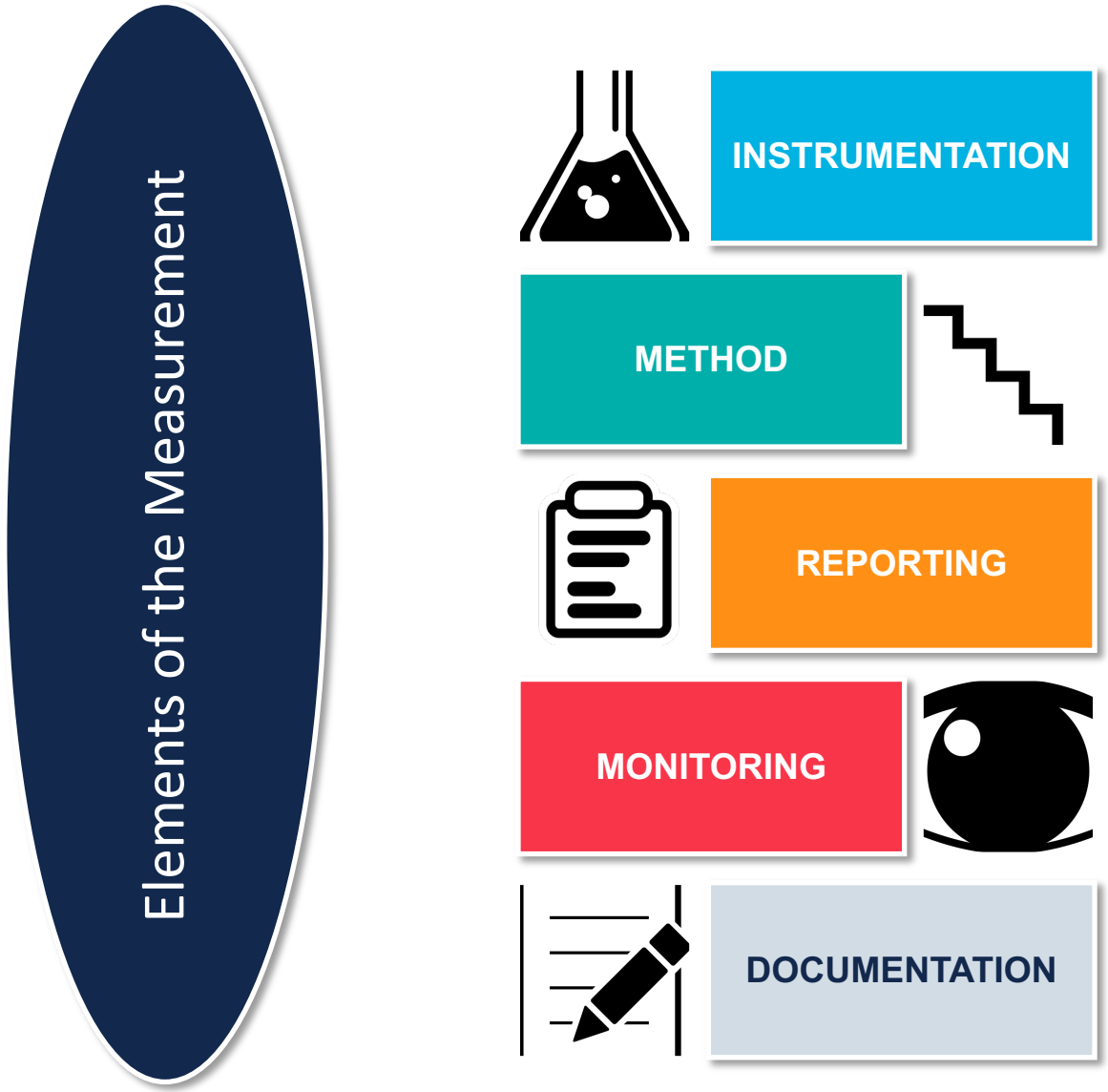
Building Measurement Assurance

- Define different elements that contribute to the measurement
- Define a process for each element
- Define key performance indicators for each elements
- Define key performance specifications (acceptability criteria)
- Define how to maintain and monitor the key performance indicators
  - Manage the life cycle of the process
  - Don't set it and forget it

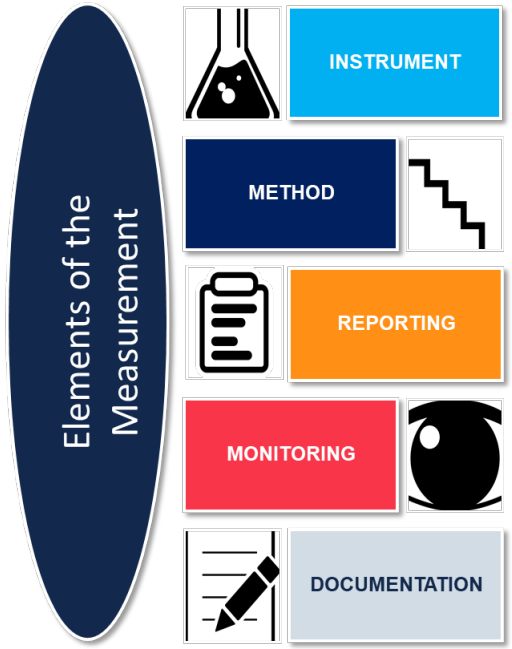
"Treat the assay as a measurement process."

John T. Elliott Jr., NIST
NIST-FDA Flow Cytometry Worshop, 2017

Measurement Assurance in Flow Cytometry



Document Ethos



Chapter 4	Instrument Qualification & Standardization
Chapter 5	Assay Development & Optimization
Chapter 3 & 6	Validation & Verification
Chapter 2 & 7	Monitoring & Quality Control

Webinar Resources

Clinical & Laboratory Standards Institute
https://clsi.org › education › h62-... · Vertaal deze pagina

On Demand - H62 Webinar

18 aug 2022 — The Clinical and Laboratory Standards Institute (**CLSI**) recently released the first edition of **CLSI H62**, Validation of Assays Performed by Flow ...

Xtalks
https://xtalks.com › Webinars · Vertaal deze pagina

Design Control and Validation Aligned with CLSI H62

5 apr 2023 — This **webinar** will address those concerns by introducing the concept Design Control and how its application can facilitate assay development and ...

The Scientist
https://www.the-scientist.com › val... · Vertaal deze pagina

Validating Assays in Flow Cytometry: Learn from the ...

In this **webinar**, Virginia Litwin, Steve Eck, and Nicolas Bailly will discuss key aspects of the Clinical and Laboratory Standards Institute's Guideline **H62**, ...

Closing Remarks

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- Standardization needs standards
- Standards need to come from a solid consensus
- Standards need to be communicated
- Standards need to be used
- Standards need to evolve, be revised

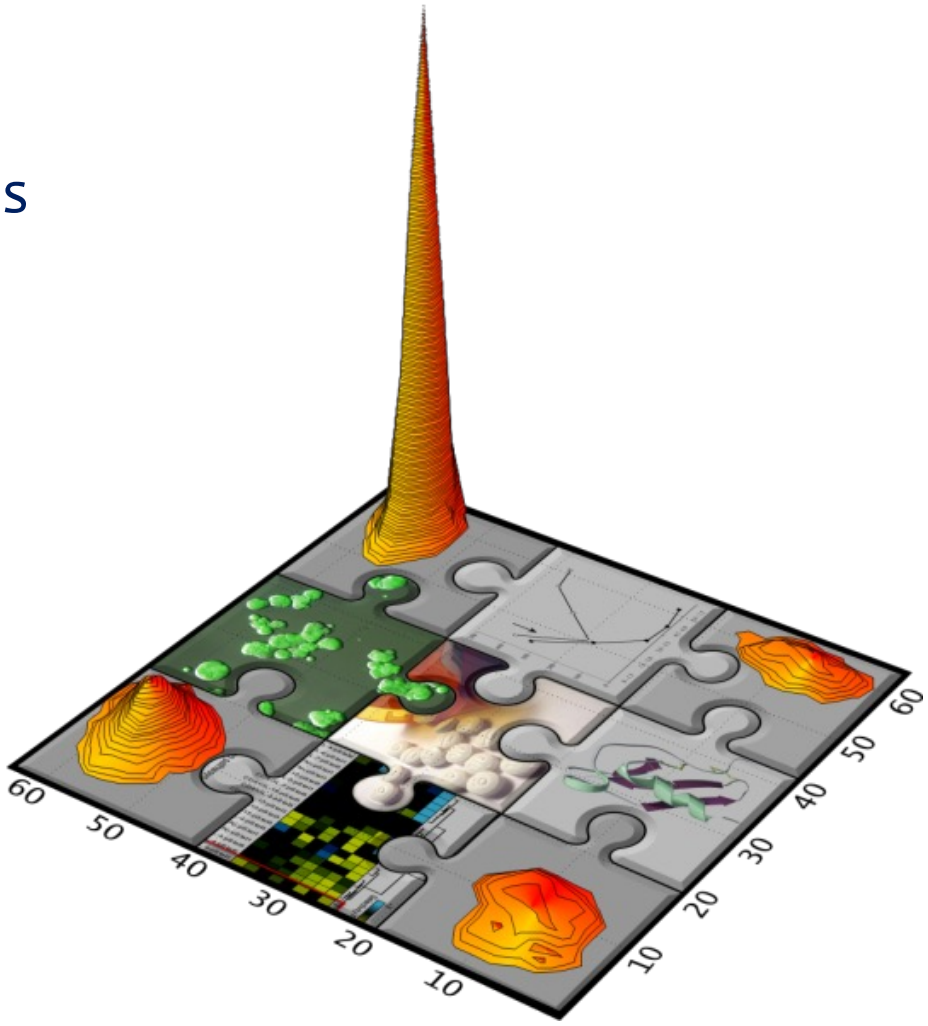

Figure courtesy of Ira Schieren, Columbia University

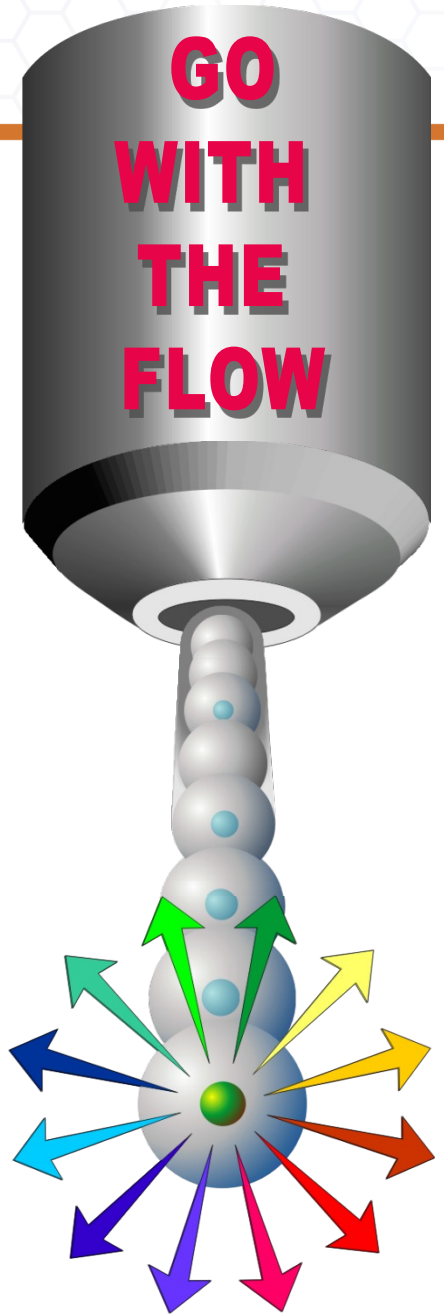

Figure courtesy of Ira Schieren, Columbia University

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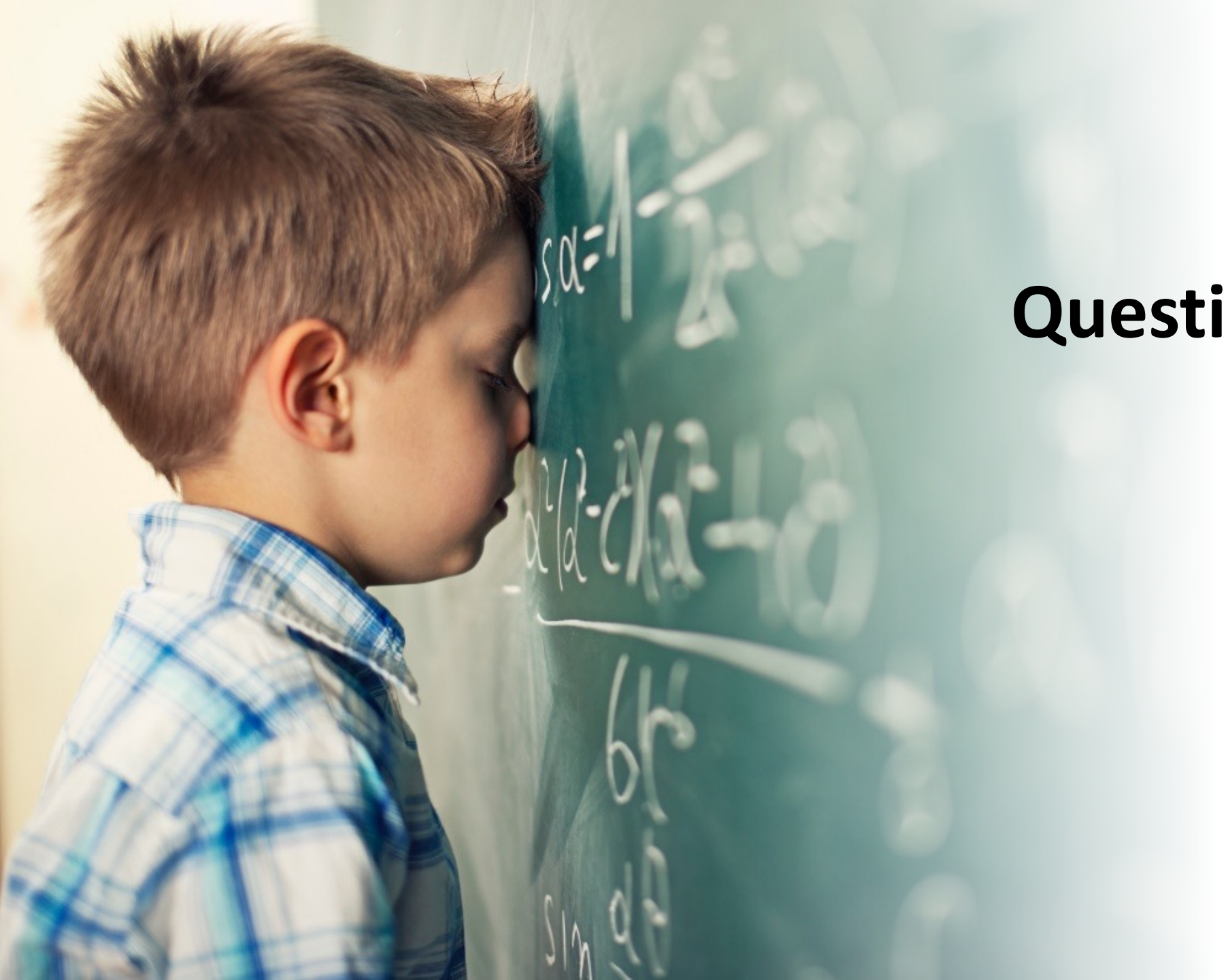

Questions?