The Journey Towards Standardization in Validation of Fluorescent Cell-Based Assays

Teri Oldaker, CLS, Ccy
Genoptix Medical Laboratory

Flow Cytometry Workshop ISCT 2015, Las Vegas, NV
Objectives

- Background
- Why standardize?
- Stakeholders
- Key Milestones
- Changing regulatory landscape
- Next steps
Laboratory Tests

- Helps to determine the presence, extent, or absence of disease and monitor the effectiveness of treatment.
- Accuracy and reproducibility are critical as: lab results impact ~70% of medical decisions.
- Regulatory requirements exist for laboratories to establish performance specifications (validate) and monitor over time (QC).
- Historically most laboratory tests were soluble analytes in serum or plasma.
Soluble Analyte Assays

Assays that measures the presence or concentration of macromolecule in serum, plasma or urine. Generally a calibrator (with known concentration) is used to determine the unknown concentration. (example chemistry, immunoassay)
Soluble Analyte Assays

- Historically: ~80% were soluble analyte assays.
- Most are moderate and few high complexity assays (CLIA).
- Generally, IVD/CE cleared and sold by manufacturers as kits or automated systems.
- Available standards and calibrators.
- Established validation guidelines available through Clinical Laboratory Standards Institute (CLSI).
Cell-Based Assays

Assays based on the use and behavior of live cells. Calibrators are rarely available. Require more manipulation than biochemical-based assays and rely on relatively indirect endpoints. (example flow cytometry)
Fluorescent Cell-based Assays

- Emerging technology: Rapidly increasing and are predicted to be ~50% in the next decade.
- No validation guidelines available.
- Established consensus, CLSI “procedural” guidelines and EQA programs.
- Very few IVD/CE cleared. (most are LDTs)
- No standards or assay calibrators...
- Only validation guidelines are CLSI validation guidelines (specific for soluble analytes).
WHY IS THIS IMPORTANT?
Quality Requirements

✓ Our responsibility is quality patient testing
✓ The Customer defines Quality
  ✓ Ordering/treating clinician
  ✓ Principle investigator
  ✓ Hospital/Reference labs
  ✓ Patient
✓ Accurate and consistent test results that are standardized over time regardless of variables.
Why Standardize?

- Meet customer requirements
- Consistency and reliability of results
- Monitoring treatment over time
- Results impact patient care
- Credibility in the field and as a technology
- Reduce waste and rework (thereby cost)
- Improves Clinical Trial Data
How can we achieve Standardization?

- Teaching and Training
- Good Laboratory Practice
- Good Internal Quality Control
- External Quality Assurance
- Appropriate Instrument and Assay Validation
- National/International Guidelines
How can we achieve Standardization?

- Teaching and Training
- Good Laboratory Practice
- Good Internal Quality Control
- External Quality Control
- Appropriate Instrument and Assay Validation
- National/International Guidelines
Assay Evolution

- Clinical utility identified
- Assay quality requirements defined
- Method selection (flow cytometry)
- Optimization*
- Validation*
- Implementation*
- Revalidation*

*Herein lies a problem
Flow Cytometry Assays

- Technology 30+ years
- Variety of applications
- Numerous consensus documents and “procedural” standards
- Established EQA surveys
- Most flow assays are still LDTs – WHY?
Laboratory Developed Tests (LDTs)

- Are tests that are developed, validated and used for in-house pathology and diagnostic purposes.
- Intended to be used by the laboratory entity where they are developed.
- Cannot be sold to other laboratories.
- Are not currently subjected to the FDA approval process. (enforced discretion)
LDT Performance Specifications

- Accuracy
- Precision/Reproducibility
- Analytical Sensitivity
- Analytical Specificity
- Stability
- Reference intervals
- Linearity
Cell-Based Validation Challenges

- No stable reference standards
- Limited sample stability and availability.
- Thousands of events/parameters per test.
- Addresses a variety of clinical conditions which are pleomorphic or patient specific.
Cell-Based Validation Challenges

- Samples contain different populations of cells with different autofluorescence or background fluorescence.
- Linearity: low expression + high expression does not = intermediate expression.
Cell-Based Validation Challenges

- There was a need for fluorescent cell-based validation guidelines.
- Many of the same challenges with laboratories validating LDTs have been encountered by all stakeholders.
A diverse group of key stakeholders have been interested in generating guidance documents.
To promote discussion regarding the proper application of flow cytometry in drug development with an emphasis on establishing best practices regarding assay and instrument validation.
Clinical Laboratory Medicine

Dedicated to promoting excellence in clinical applications of flow cytometry through practice, education, and research. Members include all individuals engaged in the practice of clinical flow cytometry, whether on the bench or at the bedside.

http://www.cytometry.org
Clinical Laboratory Medicine

ICSH

International Council for Standardization in Haematology

✓ Founded as a standardizing committee associated with the European Society of Haematology in 1963.

✓ The ICSH is a not-for-profit organisation that aims to achieve reliable and reproducible results in laboratory analysis in the field of diagnostic haematology.

✓ The ICSH coordinates Working Groups of experts to examine laboratory methods and instruments for haematological analyses, to deliberate on issues of standardization and to stimulate and coordinate scientific work as necessary towards the development of international standardization materials and guidelines.

http://icsh.org
International Society for Advancement of Cytometry

To serve a multidisciplinary community by leading technological innovation, scholarship, and the exchange of knowledge in the quantitative cell sciences.

Our vision is to advance the impact of cytometry in meeting current and emerging challenges in the life, biomedical, and physical sciences.

http://isac-net.org
FDA Mission:

To protect the public health by assuring the safety, efficacy, and security of drugs, biological products, medical devices, food, cosmetics, and radiation emitting products.
Flow IVD Manufacturers

- Technology 30+ years
- Variety of applications
- Numerous consensus documents and standards
- Established EQA surveys
- Most flow assays are LDTs*

*Herein lies a problem
Challenges to Flow IVD Clearance

✓ Challenges due to lack of available samples
✓ No existing predicate devices.
  – Requires expertise by regulatory reviewers
  – Requires guidelines appropriate to the technology
✓ No guidelines for cell-based assay submission.
✓ Reluctance to sell non-FDA cleared instruments to clinical labs. (obstructed from new technology)
✓ Manufacturers cannot sell antibody “cocktails”, lab must cocktail their own (ASR Rule)
KEY MILESTONES
Why

- Usage of biomarker data was impeded by a lack of understanding on how to interpret biomarker data
- Application of existing validation paradigms was not appropriate for biomarker research
- One set of rules should not be applied to all technologies
AAPS - Flow Cytometry APC Papers

Why

- Flow cytometric methods can be more challenging to validate than other technologies
- Analytical issues
  - Cellular analytes
  - Lack of cellular reference material
  - Highly complex reagents
    - mAb, fluorescent tags, tandem dyes
  - Highly complex instrumentation

Research paper
Recommendations for the validation of flow cytometric testing during drug development: I instrumentation
Cherie L. Green, Lynette Brown, Jennifer J. Stewart, Yuanxin Xu, Virginia Litwin, Thomas W. Mc Closkey

Research paper
Recommendations for the validation of flow cytometric testing during drug development: II assays
Denise M. O’Hara, Yuanxin Xu, Zhiyan Liang, Manjula P. Reddy, Dianna Y. Wu, Virginia Litwin
Flow Cytometry APC Key Points

Types of Validations

- FDA /CE IVD methods
- LDT phenotypic biomarker assays
- LDT functional biomarker assays
- Immunogenicity
- Pharmacokinetic assays

Validation Recommendations

- Number of samples
- Number of replicates
- Number of analytical runs
- Acceptance criteria
ICSH/ICCS Workgroup

36 International experts in flow cytometry development/standardization

Controversy and confusion about regulation of LDT in US clinical labs

- No FDA guidelines for LDT
  - Current guidelines designed for clinical chemistry methods
  - Need for flow-specific guidelines
  - Flow-specific guidelines should be prepared by flow experts

Cytometry Part B (Clinical Cytometry) 84B, 2013
http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4957
ICSH/ICCS Guidelines

- Pre-analytical Considerations
- Analytical Performance
  - Optimization/validation of instrument, sample prep, antibody/reagents, compensation and data analysis
- Performance Characteristics
  - Validation samples
  - Detailed criteria to assess required performance specifications
- Post-analytical Considerations
FDA Public Workshop - Clinical Flow Cytometry

WHO

Gerald E. Marti, MD, PhD
Leader in CLL and Flow Cytometry Standardization

WHY

- Address lack of biologic controls in hematologic malignancy testing
- Address minimal residual disease
  - Use as a surrogate marker in clinical trials
  - Discuss consensus methods

http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm334772.htm
Technology/Scientific Sector

2014 Scientific Tutorial

Understanding Method Validation as Applied to Flow Cytometry

- **Current Format:** Streaming
- **Future Format:** WebCourse

http://isac-net.org
Other Benefits

- We collaborated as an industry and united our efforts
- Momentum is growing and we are working together for a common goal.
CHALLENGING LANDSCAPE
NEXT STEPS?
Regulatory Climate for Clinical Assays

- Increased Uncertainty about LDT Regulations and Oversight
- Labs Concern About CLIA *versus* FDA Requirements for LDT Validations
- Manufacturers Concern About Cell Based Approval Requirements
FDA Regulatory Oversight of LDT - 2014

✓ Assay = Device

✓ Defines LDT as an IVD intended for clinical use and designed, manufactured and used with in a single laboratory.

✓ Assays designed by entities owning several labs and developing an assay in one lab and transferring the assay to other labs within the network are NOT LDT.

✓ States that CLIA accreditors only evaluate the lab’s ABILITY to perform the test but does not evaluate the VALIDITY of the test.
Flow Cytometric Devices 2014

- Only addresses IVD, not LDT assays.
- Out-of-date references.
- ICCS advocacy committee response.
- Has been removed from the FDA website.
Next Steps

- **ICCS**
  
  Formed Advocacy Committee
  
  First meeting November 2014

- **CLSI**
  
  Requested CLSI to adopt Special Issue Practice Guidelines into CLSI Flow Cytometry Guidelines

- **FDA**
  
  Requested to adopt the Special Issue Practice Guidelines as FDA guidelines.
Path to Standardization
Summary

- Fluorescent cell-based assays require different validation practices than soluble analyte assays.
- "Recognized" practice guidelines for validation of CBAs will benefit a diverse set of stakeholders.
- Efforts towards standardization in this area have begun and made a big impact in the industry.
- The changing regulatory landscape will require continued collaborative work from all stakeholders.
Questions?