Flow Cytometry in Translational and Clinical Science—Gap Analysis

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Presentation Overview

1. Translational and Clinical Science
2. Best Practices to Get to High Confidence Data
3. Contracting Gaps
4. Expanding Gaps and Concern
Translational and Clinical Science
Translational and Clinical Science

Pathway to accelerate the progression of scientific advances from the bench-to-the-bedside

Basic Science

Drug Development

Clinical Research

Clinical Practice

Image: VLitwin
Stakeholders

Academic centers

Technology vendors

Diagnostics industry

Private foundations

Patient advocacy groups

BioPharma

FDA/NIST

Scientific societies

NIH
Translational Science – The Good News

Provides scientists with a path to ensure that their work will have impact

Abundant opportunities in translational science

 Routinely applied to the drug development process

- To increase the success rate of bringing new therapies to patients
- To decrease the timelines and costs of developing new therapies
- To allow for more informed decision making along the drug development pathway
- To build therapeutic potential and drug labeling claims
Translational Science – The Bad News

Outcomes are disappointing

  Translational processes need to be scientifically backed up by robust methods

- Francis Collins, Science Translational Medicine 2011, 3:1

- The Case for Standards in Life Science Research

- Putting Translational Science on to a Global Stage
  Nature Reviews Drug Discovery 2016, 15:217

- What does it mean when cancer findings can’t be reproduced?
  Richard Harris, NPR January 18, 2017
The application of robust analytical method validation will, without question, lead to more success in the translational space.
Best Practices to Get to High Confidence Data Contracting Gaps
Sources of Variability

Reducing Sources of Variability

1. Flow Cytometry Method Validation
2. Instrument Standardization
3. Reference Material

Figure from Maecker et al, Nature Immunology 11:975
Contracting Gaps
Method Validation
Flow Cytometry Method Validation

• No Official Guidance from Regulatory Agencies
  - Currently!
  - But we are getting much closer

• New CLSI Guideline in preparation
  - H62- Validation of Assays Performed by Flow Cytometry

• Impact
  - Regulatory agencies often recognize CLSI guidelines
Target Audience

- Research laboratories (academic and non-academic)
- Clinical Laboratories
- Reagent/Instrument Manufactures
- Drug discovery, development, and manufacturing
- Regulatory Agencies
H62 Document Writing Committee

- CAP representation
- FDA representation
- NIST representation
- AAPS representation
- ICCS and ESCCA representation
- Members from USA, Canada, UK, Germany, Switzerland
- Members from biopharmaceutical, CRO, clinical laboratories, reagent/instrument manufacturers, regulatory agencies
The Dream Team

**Leadership**
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Teri Oldaker, Vice Chair
Raul Louzoa, Secretary
Dave Sterry, CLSI Standards Director

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Document Outline

• Scope -- Recommendations and practical instructions

• Quality System Essentials

✓ Fit for Purpose / Iterative Approach

✓ Instrument Qualification, Setup, and Standardization

✓ Assay Development and Optimization

✓ Assay Validation

• Examination Phase

• Post-Examination Phase
Contracting Gaps
Instrument Standardization
Instrument Standardization

- **Goal of instrument standardization**
  - Reproducibly set gains (PMT voltages) to achieve equivalent fluorescence measurements (MFIs)
    - Experiment to experiment
    - Instrument to instrument
    - Lab to Lab
    - Platform to platform
  - Accurately measure / assign fluorescence spillover values which are used for fluorescence compensation
  - Maintain consistent longitudinal fluorescence measurements

- **Inter-instrument variation**
  - Major source of variability
    - Within the same lab
    - Between experiments
    - Multicenter clinical trials
Recent Advances

• New instrumentation
  - Built-in, automated processes for setup and between instrument standardization

• Existing instruments
  - Processes for reducing between instrument/platform variability
    o Peer reviewed publications
    o Vendor derived process

• Automated algorithms for compensation

• Fluorescence beads for compensation
Conclusions

• Inter-instrument variability is reduced when hard dyed beads are used for standardization

• Inter-instrument variability is FURTHER reduced when instruments are standardized with covalently linked fluorochrome beads

• Hard dyed beads are not optimal for monitoring between instrument variability

• Covalently linked fluorochrome beads or comp beads are better for monitoring between instrument variability
Gaps

• Processes for standardization are complex, expensive, time consuming.
  - Opportunity to streamline the process with add-on software tools.

• We can’t all trade in our instruments for the newer ones.
Contracting Gaps
Reference Material
Importance of Reference Material

• The lack of cellular reference material contributes to the challenges in validating flow cytometric methods

• Cellular reference material would facilitate the validation of analytical accuracy

• Cellular reference material is a critical part of overall quality monitoring
  ✓ Instrument performance qualification
  ✓ Daily run acceptance criteria
  ✓ Inter-assay variation
  ✓ Inter-instrument variation
  ✓ Inter-analyst variation
  ✓ Inter-laboratory variation
  ✓ Longitudinal assay performance
  ✓ Longitudinal instrument monitoring
Available Reference Material

Preserved Whole Blood

• Pros
  - Good overall matrix control
  - Good evaluating reagent lots
  - Many subsets are detectable

• Cons
  - Established ranges from the manufacture are only for the major lymphocyte subsets
    - Very broad
    - Not useful for accuracy
    - No ranges for “off-label” cell types
  - High and Low QC material usually calibrated to CD4 T cell counts
    - Values of other subsets in the High and Low QC may be the same
    - Values in the Low Level may be higher than the High Level
  - Relatively short shelf-life
    - Continuously assessing mean values in new lots
    - Several lots of material are used in longitudinal studies
  - Loss of resolution of labile markers
  - Decreased resolution of dim markers
Available Reference Material

Lyophilized Lymphocytes

• Pros
  - Long shelf-life
  - Good control for assays using PBMC
  - Good evaluating reagent lots

• Cons
  - Not a good matrix control for whole blood assays
  - Limited to lymphocyte assays
  - May or may not have established ranges
Advances with Reference Material

• Dried leucocytes
  - 1 year shelf-life
Advances with Reference Material

• Lyophilized PBMC
  - Customized preparations

• Novel materials
  - Slingshot Biosciences
  - Polymer droplets that can mimic the physical/optical cell type
  - FlowCytes™ WBC Cell Mimics
  - Conceivably imbedded appropriate antigens in the polymer
Remaining Gaps Reference Material

• Leukemia/lymphoma controls
  - We need them!
  - Useful for the validation of leukemia/lymphoma diagnostic panels
  - Critical for MRD panel validations
Expanding Gaps
### Bench vs Bedside in Flow Cytometry

#### Bench

- **Constituents**
  - Basic research
  - Clinical research
  - Drug development
  - Biotech
  - Instrument and reagent vendors
- **Funding**
  - Grants
  - Investments
  - Internal

#### Bedside

- **Constituents**
  - Local hospital
  - University Medical Centers
  - Reference Labs
- **Funding / Reimbursement**
  - Fee for service
  - Medicare
  - Insurance agencies
Flow Cytometry Reimbursement

- Continued cuts from Centers for Medicare and Medicaid (CMS) for reimbursement for flow cytometry services for Medicare patients
  - Physician Fee Schedule
  - Clinical Laboratory Fee Schedule
- Medicare rates influence private insurance reimbursement rates

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J. Hussong. ICCS Advocacy Committee Chair
Dimensionality

Bench

• Cytof
• >20 Flow cytometry
• 12-18 Flow cytometry

Bedside

• FDA approved/ CE marked Instruments
  - 4-10 color
• FDA approved/ CE marked leukemia panels
  - 5-8 colors
Validation

**Bench**

- Extensive Validation
  - Manufacturers
  - Biopharma
    - GLP/Toxicology
    - Clinical Testing
      - Exploratory
      - Primary/Secondary endpoint
      - Enrollment criteria
      - Complementary diagnostic

- Maybe no validation
  - Research environment
  - Non-regulated biopharma (drug discovery)

**Bedside**

- ???
- No official guidance
  - Not clear what’s needed and when
  - Wide range of intended-use of data
- Lack of staff/time
- Gap of understanding of validation principals and value-added
Conclusions

• “It is the best of times, it is the of worst times”

• “It takes a village”
  - Even greater collaboration between bench and bedside scientist is required
    - Education
    - Resource (information sharing)
    - Application Tools from Vendors
  - We need to make sure that the innovation from the Bench makes it to the Bedside

• Resource Gaps
  - Bench- Greater funding is needed to fuel innovation
  - Bedside- Better resources for patient care and treatment
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