

LDTs in Flow Cytometry: ICSH/ICCS Guidelines for Validation of Fluorescent Cell-based Diagnostic Testing

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Hematologic Malignancies, Silver Springs, MD

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Agenda for Discussion

- **Flow Cytometry in Clinical Laboratories**
- **Recent Regulatory Landscape**
- **Our Challenges**
- **ICSH/ICCS Initiative to develop LDT Guidelines**
- **Pathway ahead for Guidelines**

Flow Cytometry in Clinical Laboratories

- Used for more than 30+ years
- Variety of applications
 - Simple enumeration assays (CD4, CD34)
 - Complex pattern recognition (Leukemia)
- Most are LDT
- Numerous consensus documents and standards.
 - Published in peer reviewed journals
- Established EQA surveys.

Cell-based fluorescent assays in clinical FCM

FDA cleared	LY	PMNs	MN	RBC	Pts	Prog
T Cell Subsets	✓					
CD34 Stem Cell Counts						✓
FMH by anti-HbF or anti-RhD				✓		

Cell-based fluorescent assays in Flow

LDT, but CLSI or ICSH recommended method	LY	PMNs	MN	RBC	Pts	Prog
Leukemia/ Lymphoma/ MDS evaluations	✓	✓	✓	✓	✓	✓
PNH screen		✓	✓	✓		
Reticulocytes, including IRF				✓		
Chronic Granulomatous Disease and other genetic causes of PMN dysfunction		✓	✓			
Hereditary Spherocytosis and related defects (EMA test)				✓		
Immunoplatelet count (CD61, CD42, CD41)					✓	
Genetic causes of bleeding or thrombo-cytopenia					✓	

Cell-based fluorescent assays in Flow

LDT	LY	PMNs	MN	RBC	Pts	Prog
Infection/sepsis		✓	✓			✓
Anti-PMN titer (ANCA)		✓				
HLA-B27	✓					
Hematoflow immuno-differential kit	✓	✓	✓			✓
HIT assay					✓	
Genetic immunodeficiency Assays	✓					
Allogenic transfusion detection				✓		

Challenges to Flow FDA clearance

- Evolving Technology
 - 4 color → 5/6 color → 8 color → > 10 colors
- Consensus for Leukemia, Lymphoma, and MDS diagnosis
 - Partial at best
 - Apprentice nature of medical training
- Cost prohibitive
 - Annual sales of < \$1,000,000

Challenges to Flow FDA clearance

- Interpretative nature of data analysis
 - Unlike
 - simple chemistry analyte
 - nucleic acid test positive/negative
 - Like
 - H & E or IHC in histopathology
 - the slide/plots do not provide the diagnosis, the interpreter does!
- No existing “predicate devices”
 - requires expertise by regulatory reviewers
 - requires guidelines appropriate to the technology

ASR Rule Impact to Constituents

This has impacted the ability of labs to provide patient access to critical and innovative tests leading to the perception that regulatory protection has impeded providing quality care to U.S. patients.

- ASR Cocktails
 - No longer available for purchase
 - Labs must create their own
 - Reduced quality
 - Clinical flow labs cannot purchase cocktails as ASR, therefore are required to manufacture them themselves – often of reduced quality
 - Manufacturers are reluctant to sell antibodies to clinical labs if not ASR; clinical labs are restricted in access to reagents
- Clinical lab resources are primarily dedicated to testing, not manufacturing, but forced do to so

ASR Rule Impact to Constituents

- Cell-based LDT Validations
 - No clear guidelines
 - Challenges due to lack of available samples
- Information restrictions
 - Manufacturers cannot provide guidance on validation
 - Manufacturers cannot provide or instructions for reagent use
 - Clinical labs are obstructed from access to information
- Manufacturers are reluctant to sell non-FDA cleared instruments to clinical labs, clinical labs are obstructed from new technology

Current Related References and Guidelines

- **International Clinical Cytometry Society (ICCS) consensus guidelines:**
 - Borowitz et al: Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry. 78B:211-230, 2010.
 - Davis BH et al. US-Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: Medical indications. Cytometry (Communications in Clinical Cytometry) 30:249-263, 1997 .
 - Davis BH et al: 2006 Bethesda International Consensus Recommendations on the Flow Cytometric Immunophenotypic Analysis of Hematolymphoid Neoplasia: Medical Indications. Cytometry, 72B:S5-S13, 2007
 - Wood BL et al: 2006 Bethesda International Consensus recommendations on the immunophenotypic analysis of hematolymphoid neoplasia by flow cytometry: Optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia 72B: S14-S22, 2007
- **CLSI Document guidelines for flow cytometry, some co-authored with ICSH:**
 - H42-A2, Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline—Second Edition, 2007
 - H43-A2, Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition, 2007
 - H44-A2, Methods for Reticulocyte Counting (Automated Blood Cell Counters, Flow Cytometry, and Supravital Dyes); Approved Guideline—Second Edition, 2003
 - H52-A, Fetal Red Cell Detection; Approved Guideline, 2001 (A2 to be released within next 3-6 months)
 - I/LA24-A, Fluorescence Calibration and Quantitative Measurement of Fluorescence Intensity; Approved Guideline, 2004
 - I/LA26-A, Performance of Single Cell Immune Response Assays; Approved Guideline, 2004
 - I/LA29-A, Detection of HLA-Specific Alloantibody by Flow Cytometry and Solid Phase Assays; Approved Guideline, 2008

Cell-Based Validation Challenges

- Relative-quantitative: Uses calibration, such as beads, but lacks actual quantitative standard or reference material. Numeric data is reported.
 - Examples: CD34 counts; Neutrophil CD64 up-regulation for infection/sepsis
- Quasi-quantitative: Does not use calibration standard, but has a continuous response. Numeric data is reported.
 - Examples: Immunogenicity assays, Zap-70 in CLL, DNA content and cell cycle analysis, phenotypic and functional biomarker assays, receptor occupancy assays
- Qualitative: Lacks proportionality to the amount of analyte. Categorical data is reported.
 - Examples: Immunophenotyping assays for leukemia, MDS and lymphoma; immunohistochemical (IHC) assays

Validation Requirements (CLIA, CAP)

- Accuracy
- Precision (Repeatability/Reproducibility)
- Analytic sensitivity (detection limit)
- Analytic specificity (interfering substances)
- Sample types and stability
- Reportable range
- Reference intervals
- Calibration and control materials

Cell-Based Validation Challenges

- Accuracy: “Truth” as compared to reference method, clinical endpoint or predicate device.
 - Cannot compare to a stable standard
 - No archived samples to test
 - Sample stability is restrictive
 - PT Survey material (not always available for LDTs)
 - Split sample with another laboratory running same test same method
 - Compare with another method with documented clinical equivalency
 - Compare results with clinical diagnosis; only if serves as diagnostic test for a unique clinical entity.
 - May require one or more of these approaches.

Cell-Based Validation Challenges

- Precision/Reproducibility: the closeness of agreement between results of successive measurements of the same measure under **same** and **changed** conditions.
 - Samples have limited stability for prolonged reproducibility
 - Different days (independent experiments same day)
 - Different technicians
 - Different instruments
- **What impacts precision in flow cytometry?**
 - The frequency of the target population
 - The total number of events acquired
 - Stability of cell viability

Cell-Based Validation Challenges

- Analytic Specificity: Specificity of reagents is defined by how well the reagents recognize the correct target cells without “non-specific binding”.
 - The justification for cell subset phenotype must be provided in the form of a published reference or laboratory validation
 - The gating strategies must be verified to establish the cell subset of interest is included and other cellular populations are excluded.
 - The specificity of the antibodies must be verified.
- **What impacts specificity in flow cytometry?**
 - The markers used to define the cellular population of interest;
 - Gating strategy defining the target (or control) population;
 - The specificity of the monoclonal antibodies and other reagents.
 - Cellular viability; blockers of non-specific binding

Cell-Based Validation Challenges

- Analytic Sensitivity: The ability to distinguish signal from background as well as small genetic variations in a given cell population for expression level of a marker or multiple markers.
 - The ability to detect minimum staining intensity above non-specific or negative staining

- **What impacts sensitivity in flow cytometry?**

- Number of events acquired
- Gating strategy and “purity”
- Signal to noise ratio for each antibody
- Instrument sensitivity
- Cell viability and blockers of non-specific binding
- It is not always relevant or necessary to assess the LLOD and LLOQ for flow cytometric methods
- For assays designed to measure antigen levels, cellular depletion or rare event detection it is necessary to establish the LLOD and LLOQ. (MRD, receptor occupancy, antigen quantitation)

Cell-Based Validation Challenges

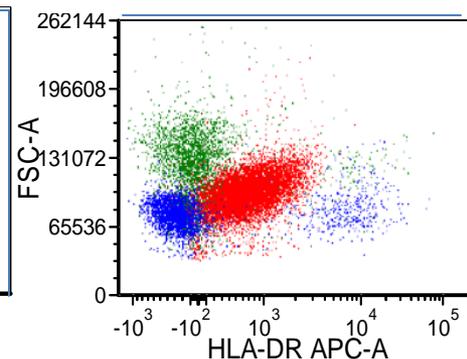
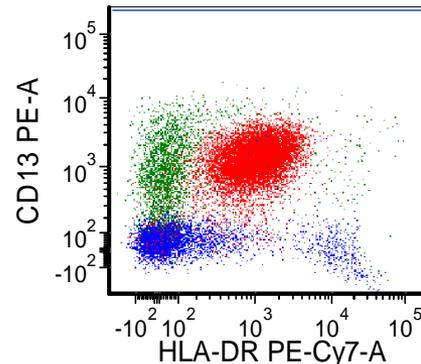
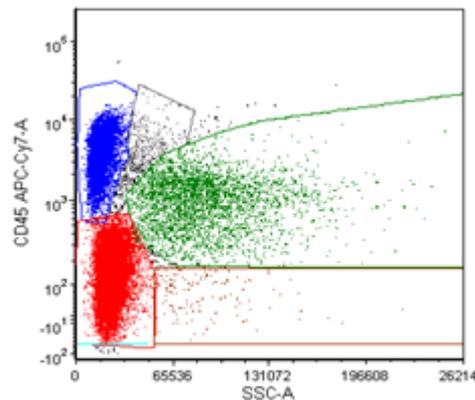
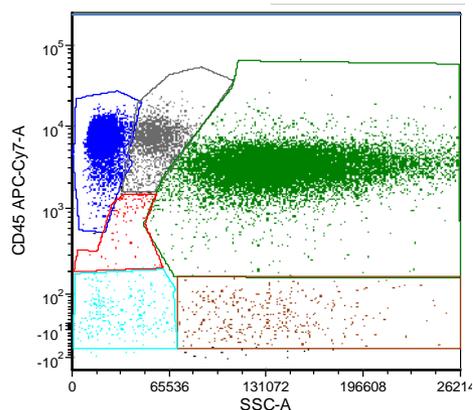
- **Stability**: Lack of variability (consistency or reproducibility) in the measured analyte relative to time from sample collection to analysis, storage/analysis temperature and conditions, blood collection (anticoagulant) procedures.
- **What impacts stability in flow cytometry?**
 - Time
 - Temperature
 - Anticoagulant
 - Cocktail stability
 - Time between stain and acquisition

Cell-Based Validation Challenges

- Reportable Range/Reference
 - Reference Ranges are only applicable for semi- and quasi-quantitative assays.
 - Sample availability may be limited
 - A reference range may need to be disease specific
 - In general the CLSI C28-A3 is applicable
 - FCM for Hematologic malignancies is considered qualitative, no reference range is needed, interpretation a function experience – medical or pathological interpretation.
 - Is an abnormal population present?
 - Immunophenotypic description
 - No clinical action is taken on enumeration, just diagnosis

Cell-Based Validation Challenges

- Calibration and Control materials
 - Simple relative and quasi-quantitative assays
 - Commercially available multi-level controls are available for some assays
 - Complex qualitative assays
 - In hematologic malignancies, every sample contains all or most normal cell populations that can be used as internal positive and negative controls for each sample.



The Regulatory Climate is Changing

- Increased uncertainty about LDT regulations and oversight.
- Manufacturers concern about cell based 510K and PMA requirements
- Requests for guidance from our constituents
- We decided to be proactive and develop a guidance document

ICSH Working Group: Guidelines for validation of cell based fluorescence IVD assays

- **Co-chaired by Brent Wood (President, International Clinical Cytometry Society; Professor of Pathology, Univ of Washington, USA), David Barnett (UK NEQAS, UK), and Teri Oldaker (Secretary/Treasurer, ICCS; Genoptix, USA), Bruce H. Davis (Treasurer, ICSH; Past-Chair, CLSI Hematology Committee; Trillium Diagnostics, USA)**
 - Panel of 36 international experts, 10 observers of corporate sponsors met in Dedham, Maine in March, 2011, followed by internet exchange of information and document drafts
 - Membership represents multiple countries (USA, UK, France, Spain, Canada, Germany, S Korea, China, Japan, The Netherlands, Australia) and stake holders (EuroFlow, Pharma clinical trials, FDA, flow cytometry diagnostic companies, national and international reference labs, etc).
 - Experience in the development and/or standardization of cell-based fluorescence assays required as inclusion of “expert” to working group.

ICSH Working Group: Guidelines for validation of cell based fluorescence IVD assays

- **Timeline:**

- Draft completed for circulation and comment from members of ICSH, ICCS, ESCCA, EuroFlow, ISAC, FDA, CAP, Industry representatives, others – July 1, 2012
- Final Draft submitted to ICSH and ICCS for review and approval – Fall 2012

- **Final Deliverable: Published guideline in Cytometry B on Guideline for Validation of Fluorescent Cell Based Assays**
➔ **Special Issue Co-Edited by G. Marti & MC Bene, May 2013**

ICSH/ICCS Cell-based Fluorescence LDT Guideline: Experts

- Bruce H Davis MD, Amar Dasgupta MD, Steven Kussick MD, Jin Han MD PhD, Annalee Estrellado, Patrick O'Neil
- Shabnam Tanqri MD, Horacio Vall PhD, David Kaplan MD, Bob Hoffman PhD, Norman Purvis PhD, Anna Porwit MD PhD, Ben Hunsberger, T. Vincent Shankey PhD
- Brent Wood MD PhD, Dragan Jevremovic MD, Marie C Béné PharmSciD PhD, Ming Yan, Patrick Jacobs, Virginia Litwin PhD
- David Barnett PhD, Raul Louza, Peter Gambell, Jitakski De MD, Teri Oldaker, Curtis Hanson MD

ICSH/ICCS

Cell-based Fluorescence LDT Guideline:

- Pre-analytical Considerations

 - Sample storage, stability, transport

 - Cell counts, viability and use of morphology as needed

- 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

 - Resulting categories, data and sample storage

 - Internal and external quality assurance

Value of Cell Based Assays

- Cell-based assays are here to stay (and will increase) They are clearly and inherently different than chemistry assays, requiring a modified approach.
- Flow Cytometry for Hematologic Malignancies is multifaceted, requiring an integrated, medically and scientifically based, approach.
- Expertise is required at the technical, scientific and the interpretive level both for practice and regulation

Value of Cell Based Assays

- ICSH and ICCS have developed broad, expert driven guidelines to address the uniqueness of cell based assay validations.
- We respectfully ask FDA to consider this ICSH/ICCS guidance document as the basis for any guidelines for evaluation of flow cytometry diagnostic assays.