Flow Cytometry in Translational and Clinical Science—Gap Analysis

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NIST-FDA Flow Cytometry Workshop: Building measurement assurance in flow cytometry

Gaithersburg, MD
October 25, 2017

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Accelerating Precision Medicine

Presentation Overview

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- Translational and Clinical Science
- Best Practices to Get to High Confidence Data Contracting Gaps
- Expanding Gaps and Concern

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Translational and Clinical Science

Translational and Clinical Science

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

Basic Science

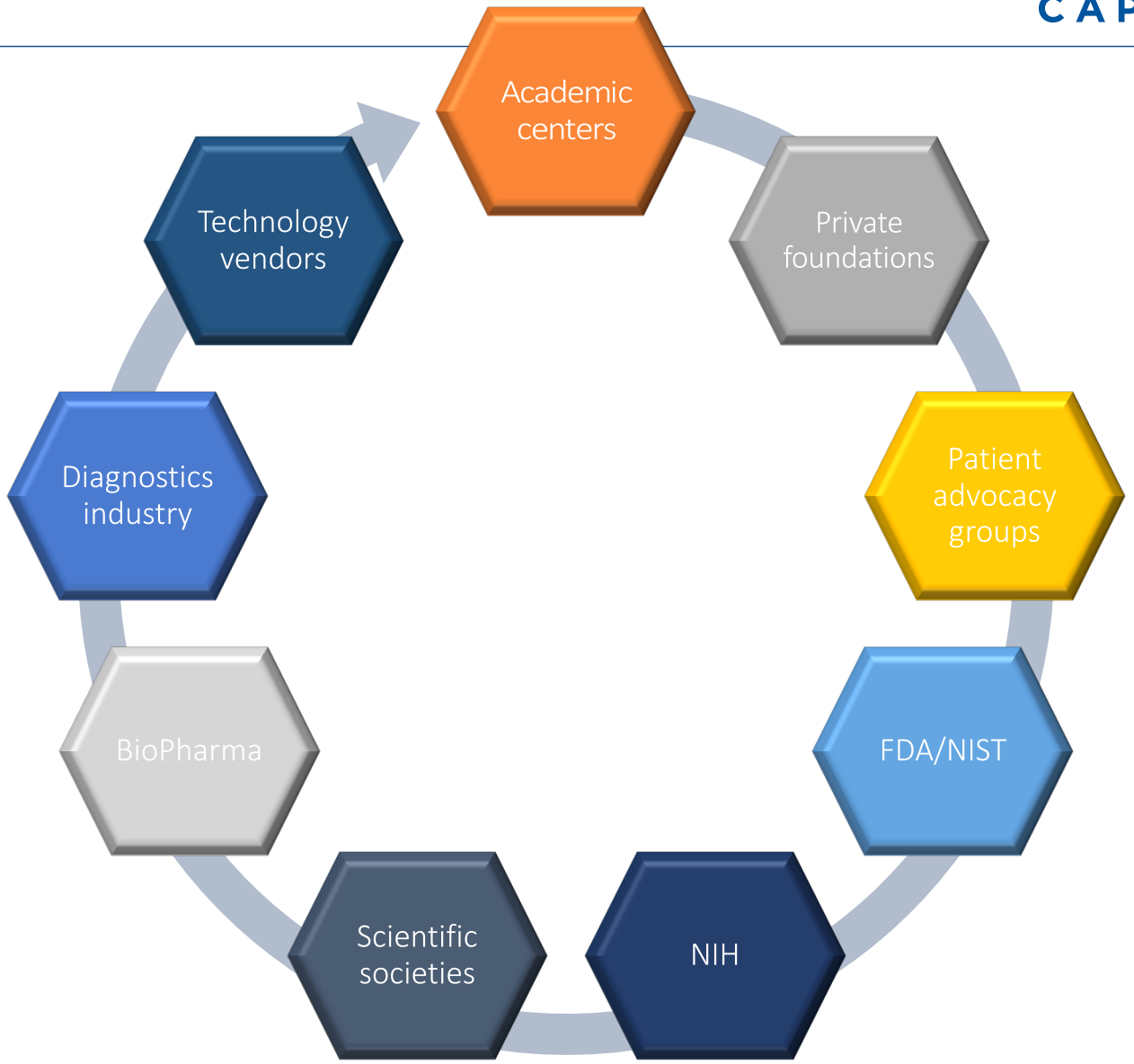
Drug Development

Clinical Research

Clinical Practice

Image: VLitwin

Stakeholders



Translational Science – The Good News

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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

DISCOVERY → PRE-CLINICAL → CLINICAL

- 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

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Outcomes are disappointing

- **Martin Wehling, Journal of Translational Medicine 2008, 6:31**

  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

Translational Science – Opportunities

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Strategy for better outcomes

The application of robust analytical method validation will, without question, lead to more success in the translational space

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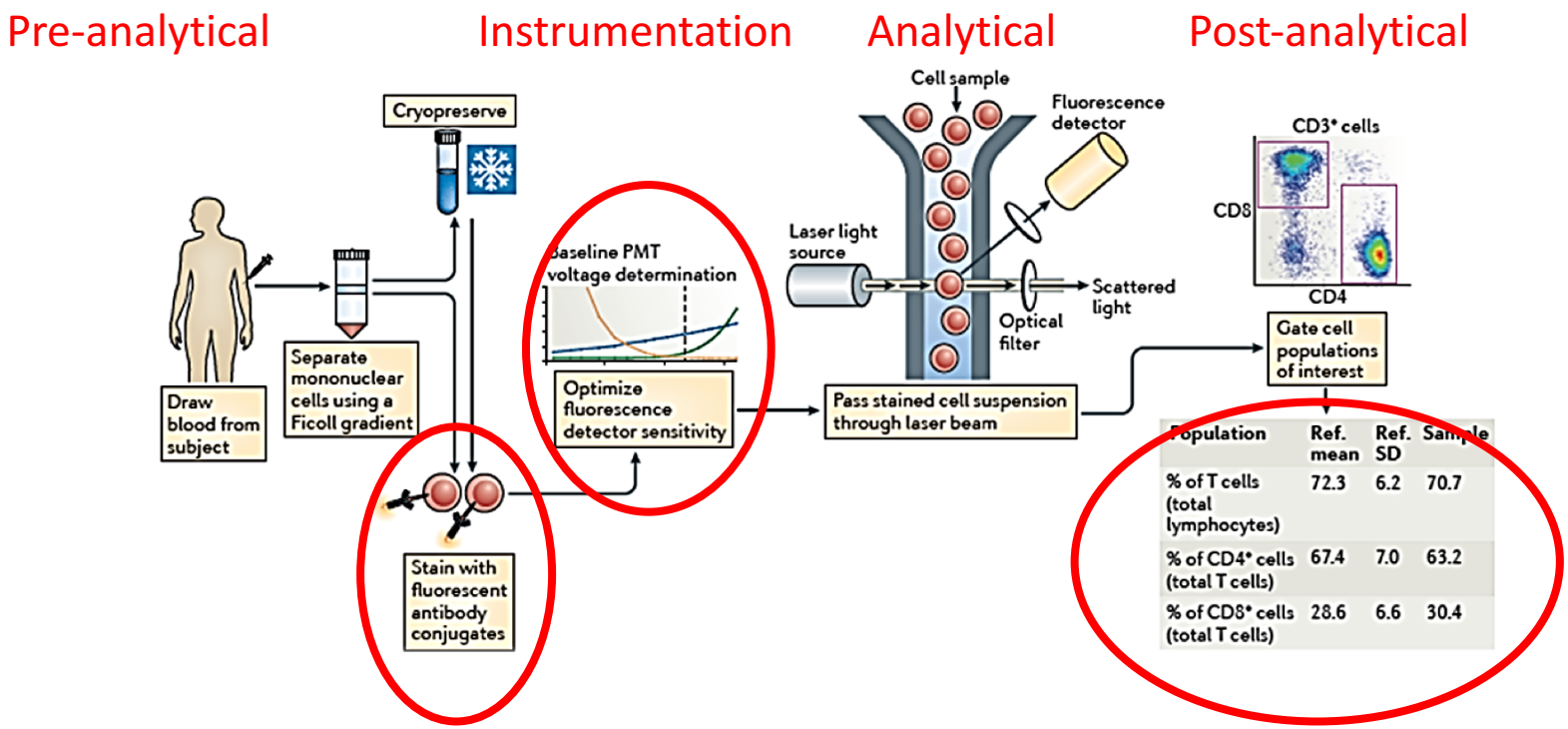
Best Practices to Get to High Confidence Data Contracting Gaps

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

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Contracting Gaps

Method Validation

Flow Cytometry Method Validation

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- 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

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

H62 Document Writing Committee

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- 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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Raul Louzoa, Secretary
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Document Outline

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- 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

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Contracting Gaps

Instrument Standardization

Instrument Standardization

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- 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

Instrument Standardization

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Recent Advances

- New instrumentation
  - Built-in, automated processes for setup and between instrument standardization
- Existing instruments
  - Processes for reducing between instrument/platform variability
    - Peer reviewed publications
    - Vendor derived process
- Automated algorithms for compensation
- Fluorescence beads for compensation

Instrument Standardization

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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

I. Athanasiadou and C. Gonneau. Challenges of flow cytometry for global clinical trials. ESCCA, 2017

Mark Ettinger. A New Method For QUANTITATIVE STANDARDIZATION of Flow Cytometry Instruments. Contract Pharma. 2015

Instrument Standardization

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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

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Contracting Gaps

Reference Material

Importance of Reference Material

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- 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

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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

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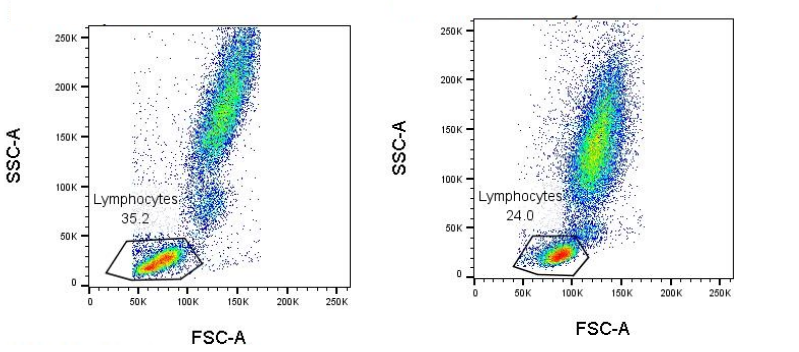
Lyophilized Lymphocytes

- 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

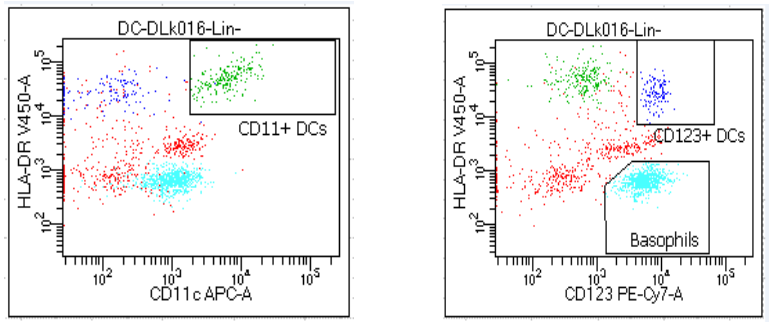
- Dried leucocytes
  - 1 year shelf-life



Fresh Blood

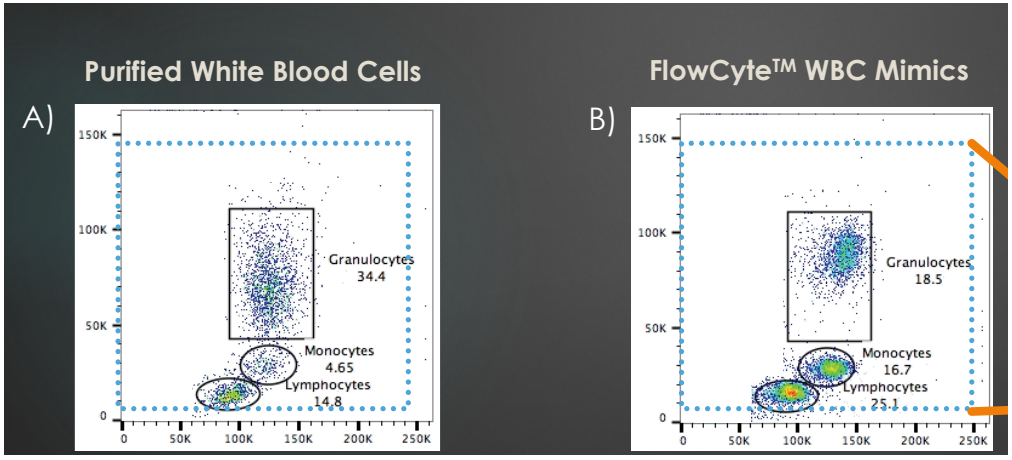
BD Horizon Dri Leukocytes

Dendritic Cells (DCs) and Basophils



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

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- Leukemia/lymphoma controls
  - We need them!
  - Useful for the validation of leukemia/lymphoma diagnositic panels
  - Critical for MRD panel validations

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Expanding Gaps

Expanding Gap

Basic Science

Drug Development

Clinical Research

Clinical Practice

Bench vs Bedside in Flow Cytometry

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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

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- 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

CPT Code	Description	Decrease in Payment 2018 vs 2016
88184	1st marker TC	80%
88185	Additional markers TC	66%
88187	Professional 2-8 markers	66%
88188	Professional 9-15 markers	71%
88189	Professional 16 and greater markers	78%

J. Hussong. ICCS Advocacy Committee Chair

Dimensionality

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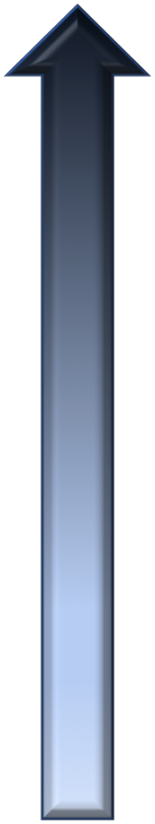
Bench

- 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

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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

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- “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

Acknowledgements

- BD Biosciences
  - Alan Stall
  - Marybeth Sharkey
  - Joan Batchelder
- ICCS
  - Fiona Craig, Mayo Clinical Arizona, ICCS Past President
  - Jerry Hussong, Sonic Healthcare, Chair ICCS Advocacy Committee