Standardization Requirements for Clinical Trials

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Covance Central Laboratory Services
Founder and Co-Chair, AAPS Flow Cytometry Action Program Committee
Presentation Overview

Challenges of Using Flow Cytometry in Global Clinical Trials

Reducing Sources of Variability

Monitoring
Challenges of Using Flow Cytometry in Global Clinical Trials

- Limited Specimen Stability
  Requires Regional Sample Processing

- Global Standardization

- Advanced, High-complexity Technology
  Requires Highly Skilled Staff

- Lead Time for Assay Development and Validation
Reducing Sources of Variability
Sources of Variability

DESIGN PROCESS TO REDUCE SOURCES VARIABILITY AT EVERY STEP IN THE PROCESS

1. Pre-analytical
2. Instrumentation
3. Analytical
4. Post-analytical
Take Home Message

Sweat the Small Stuff

- It's all small stuff
- The devil is truly in the details
Pre-Analytical
Pre-Analytical

Specimen Collection

Transportation

Stability Validation

References


Instrumentation
2. Instrumentation

- Validation
- Daily Setup
- Inter-instrument Standardization
Validation

PROVIDES ASSURANCE THAT THE DATA GENERATED ON THE INSTRUMENT ARE RELIABLE AND PRECISE

- Performance Qualification (PQ)
- Installation Qualification (IQ)
- Operational Qualification (OQ)
Instrument Validation

Planning
- Identify Flow Cytometer
- Assess Compliance Needs
- Allocate Resources
- Assemble Validation Team

Testing
- Draft SOPs and Validation Plan
- Define User and Functional Specifications
- Perform Compliance/Risk Assessment
- Testing Protocols (IQ/OQ/PQ)

Implementation
- Execute IQ/OQ
- Execute PQ
- IQ/OQ/PQ Report
- Finalize SOPs
- Release System for Production
- Train Users

Manage Life Cycle
- Maintain Validated State
- Change Control
- System Retirement

References
Litwin V, Green C. 2013.

Installation and Operational Qualification (IQ/OQ)

TO DEMONSTRATE THE INSTRUMENT AND THE ASSOCIATED SOFTWARE ARE INSTALLED AND FUNCTIONING PER MANUFACTURER’S SPECIFICATION AND USER’S REQUIREMENT

**Instrument Vendor**
- Provides instrument hardware and associated software manuals
- IQ/OQ packages

**Validation Team**
- Oversees the process
- Ensures the proper documentation to meet regulatory requirements
Testing Components of IQ and OQ

Typical IQ Parameters Include:

- Environment (e.g., space requirements)
- Utilities (temperature and electrical requirements, hardware and software)

Typical OQ Parameters Include:

- Software functionality
- Optical Precision
- Automated sample acquisition
- System alerts

Testing can be performed by vendor, qualified internal staff, or contracted external consultants.
Performance Qualification (PQ)

Depends on the Intended Use

Testing Should be Performed by Qualified Staff with Expertise

PQ Typically Includes:

- Basic Instrument Performance Qualification
  - Integrated QC Applications
  - Linearity and Sensitivity
- Extended Performance Qualification
  - Inter-instrument and Inter-laboratory Comparability
  - Longitudinal Performance
Daily Setup

Instrument Cleaning

Daily Performance Checks and Monitoring
Instrument Cleaning and PM

Make sure the instrument is clean and well maintained.

Make sure that all air is purged from the sheath filters, bubble filter, flow cell, and lines.

Follow the vendor recommendations and instrument user’s manual.

If you are not cleaning, you are setting yourself up for failure. You will not have a good clean baseline to monitor.
Performance Checks and Monitoring

Critically important process to ensure high quality, reproducible data

Ensures that all lasers, detectors and the fluidics system are functioning correctly

Performed using fluorescent beads

- Designed to ensure optimal optical alignment
- Check fluidics performance
- Adjust laser delay

Tracking instrument performance on a day-by-day basis
Performance Checks and Monitoring

Universal Process

- http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm334772.htm
  - Cyto-Calc
  - 1X Rainbow Beads
  - Unstained Comp beads
  - QSCB

Instrument-specific Systems

- BD Biosciences
  - Calibration Setup and Tracking (CS&T) beads
- Beckman-Coulter
  - Autostartup Scheduler
  - Flow-Check™ and Flow-Set™ beads
Example of a baseline and daily performance report from BD FACSCanto II, which provide up to 30 metrics for analyzing the performance of the cytometer.
Daily Setup

Best Practices

• Warm up the instrument for at least 30 minutes (or manufacturer’s recommend) before running beads
  • Gage time for the laser that takes the longest
• Perform a bubble filter purge and degas before running the daily beads.
  • Bubbles look like beads and cells
  • So anything we can do to mitigate bubbles is a best practice
• Better to spend a little extra time on the front end and have everything pass versus hours of troubleshooting
Daily Setup

Prepare a Standard Operating Procedure (SOP)

• User-friendly SOP
• Follow CAP/CLSI Guidelines
• Don’t re-write the Users’ Manual
  • *Just reference the appropriate section*
• Do describe what to do and when to do it
• Do include warning signs and corrective action steps
  • *Escalation tree*
• Do include “At-a-Glance” sheets / flow chart(s) in the Appendix
• Follow the SOP
Inter-Instrument Standardization

- Standardized PMT Settings
- Standardized Compensation
- Standardized Controlled Templates-Acquisition and Analysis
- Restricted User Preferences
Instrument-to-Instrument Standardization

Step 1:

- Create a standard configuration that can be exported, shared, and imported across cytometers

Step 2:

- Cytometer Performance and Setup
- Characterize all instruments using setup beads
Instrument-to-Instrument Standardization

Step 3:

- Create a “virtual” instrument using the baseline data from all instruments.
- The linearity and relative SD of electronic noise (rSDEN) values are used to define the “virtual” predicate instrument.

An example of the baseline report for the blue laser

<table>
<thead>
<tr>
<th>Laser</th>
<th>Detector</th>
<th>Parameter</th>
<th>Linearity Min Channel</th>
<th>Linearity Max Channel</th>
<th>Slope</th>
<th>Intercept</th>
<th>Electronic Noise Robust SD</th>
<th>Qr</th>
<th>Br</th>
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<tbody>
<tr>
<td>Blue</td>
<td>D</td>
<td>FITC</td>
<td>197</td>
<td>174901</td>
<td>7.5100</td>
<td>-15.94</td>
<td>18.11</td>
<td>0.0842</td>
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<tr>
<td>Blue</td>
<td>C</td>
<td>PE</td>
<td>177</td>
<td>157054</td>
<td>7.4772</td>
<td>-15.40</td>
<td>20.38</td>
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<tr>
<td>Blue</td>
<td>A</td>
<td>PE-Cy7</td>
<td>146</td>
<td>153291</td>
<td>7.4885</td>
<td>-16.42</td>
<td>19.21</td>
<td>0.0078</td>
<td>6.98</td>
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</table>

<table>
<thead>
<tr>
<th>Detectors</th>
<th>rSDen Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>530/30 (488)-A</td>
<td>16.2</td>
</tr>
<tr>
<td>585/42 (488)-A</td>
<td>18.5</td>
</tr>
<tr>
<td>670 LP (488)-A</td>
<td>19</td>
</tr>
<tr>
<td>780/60 (488)-A</td>
<td>19</td>
</tr>
<tr>
<td>660/20 (633)-A</td>
<td>14.5</td>
</tr>
<tr>
<td>780/60 (633)-A</td>
<td>15</td>
</tr>
<tr>
<td>450/50 (405)-A</td>
<td>14.5</td>
</tr>
<tr>
<td>510/50 (405)-A</td>
<td>16</td>
</tr>
</tbody>
</table>

Sensitivity\(_{relative}\) = $\sqrt{\frac{Q_r}{B_r}}$
Step 4:

- Create an Application Settings from the “virtual” predicate instrument
- Standardized Assay PMT Settings
  - $2.5 \times \text{rSDEN}$
  - *Way to discriminate the negative from the dim, and not have more than 15% of the negative events be due to the Electronic Noise*
  - *Ensures that electronic noise does not interfere with the measurements at the low end of the scale*
  - *Allows for a much larger dynamic range for the positive cells*
Instrument-to-Instrument Standardization

Step 5:

- Verify that the brightest populations are within the linear dynamic range
- *Stain fresh blood sample with a bright marker*
Step 6:

- Establish ASTV for fluorescence with CS&T beads

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Bright</td>
<td>19,547</td>
<td>26,366</td>
<td>56,013</td>
<td>44,958</td>
<td>40,875</td>
<td>75,470</td>
<td>15,401</td>
<td>42,950</td>
</tr>
</tbody>
</table>

ASTV Basic Covance CST lot: 26808

COVANCE SOLUTIONS MADE REAL™
Four Standardized Instruments

Lab 1:
- Instrument 1
- Instrument 2

Lab 2:
- Instrument 3
- Instrument 4
Instrument-to-Instrument Standardization

**Initial Setup:**

1. Define Standard
2. Compile Baseline Report For Predicate Values
3. Create ASTV Template On Single Instrument
4. Standardize Other Instruments With ASTV Target Values
5. Save Application Settings

**Daily Workflow:**

1. Run CS&T Performance Check
2. Open Specific Experiment And Apply AS
3. Apply Compensation
4. Run Samples
Standardized Daily Workflow

Single Standard Configuration

Controlled Acquisition Templates

- Less manual intervention
- Time savings/Less error prone/Greater consistency
- Information within template
  - Parameter labels (mAb-fluorochrome)/key words/Acquisition Criteria/Analysis Template/Threshold
- Updated Application Settings (PMT voltages) applied to the template

Restricted User Preferences

- Best option for labs with multiple techs, multiple instruments, numerous different panels performed daily, many locations

Application Settings

- Updated Application Settings (PMT voltages) applied to the template
- CST daily
- ASTV adjusted based on daily setup
- Compensation
References, Standardization


4. Becton Dickinson Technical Note Standardizing Application Setup Across Multiple Flow Cytometers Using BD FACSDiva™ Version 6 Software. Ellen Meinelt, Mervi Reunanen, Mark Edinger, Maria Jaimes, Alan Stall, Dennis Sasaki, Joe Trotter


3. Analytical

- Assay Optimization and Validation
- Global Method SOP
- Staff Training
- Same Reagents
- Same QC Material
Assay Development & Validation Approach

Fluorochrome-antigen Pairing Must Follow Current Best Practices

Properly Optimized Panels will Increase Data Quality and Performance

Fit-for-Purpose Method Validation

Validated Assay
Fit-for-Purpose Method Validation

**INITIAL VALIDATION**

- The 1st Laboratory To Validate The Assay
  - Specificity (Assay Development Phase)
  - Intra-assay Imprecision
  - Inter-assay Imprecision
  - Robustness/Inter-analyst Variability
  - Robustness/Inter-instrument Variability
  - Lower Limit Of Quantitation (LLOQ)
  - Limit Of Detection (LOD)
  - Stability
  - (Reference Intervals)
  - (Intra- And Inter-subject Variability)
  - (Disease State Samples)

**TECHNOLOGY TRANSFER VALIDATION**

- The Next Laboratory To Validate The Assay
  - Intra-assay Imprecision
  - Inter-assay Imprecision
  - Robustness/Inter-analyst Variability
  - Robustness/Inter-instrument Variability
  - Sample Correlation
References, Assay Development and Validation


Sample Processing

**Global Method SOP**

- Technology Transfer Validation to all sites
- Staff Training Documentation, CAP process

**Reagents**

- Same Reagents
- Same QC Material

**Global Acquisition Template**

- Described and Identified in Global Method SOP
- Controlled
- Associated With Instrument-to-instrument Standardization Process
- Instrument Settings, PMT Voltages
- Compensation
Post-Analytical
Data Analysis

Global Analysis Templates

- Described and Identified in Global Method SOP
- Controlled

Centralized Gating

- For high complexity panels
- A primary gater and a backup
- Ph.D./Delegate review of all gated data

Specimen Receipt At Lab → Processed And Acquired Same Day → Data Storage On Internal Secured Server → Data Analysis At Expert Site → Ph.D. Review And Approval → Data Reporting From Initial Lab
Monitoring
Instrument Performance
Performance Checks / Monitoring-BD

DAILY MONITORING

Voltages

• Look for trends don’t wait until the change is 50 volts
• If you are close to a 50v change, call service before you fail
• 20-30 volt change, call service, watch closely

Optical Background (Br)

• Should not be increasing
• If increasing try a long clean
• Look at Br in FITC and PE channels
• Excess debris will contribute

Fluorescence Detection Efficiency (Qr)

• Should not be decreasing
Performance Checks / Monitoring-BD

MONTHLY REVIEW LEVY JENNINGS

Monitor Key Performance Metrics

Compare to Previous

Look for Trends

• Trends may not be visible over a 30- or 60-day period
• May need to review over a longer time period

Plot Averages per Month to Track Long Term
Voltages

- **Increase**
  - More voltage is required to achieve CST targets
  - Usually requires a service call

- **Trending up**
  - Laser may need re-alignment
  - Where is the trending?
    - Look at all the channels
    - Is the trending on one laser or all?
  - Blue laser all channels—
    - Thresholding and laser delays off the blue laser
  - One detector only
    - Likely a PMT or filter
Br (Relative Optical Background)

- **Increase**
  - Indicates background going up
  - Do not worry about value, worry about the trend
  - Dirty flow cell, dirty tubing
  - Do a deep clean

Qr (Relative Fluorochrome-specific Detection Efficiency)

- **Decrease**
  - Indicates optical efficiency going down
  - Could be a degradation in the filter

rCV (Robust Coefficient Of Variation)

- **Bright bead %rCV’s approaching 6**
  - Indicates alignment issue
Additional Performance Monitoring

Record MFI

Monitor

• Linearity in each detector
  • $r^2$, intercept, and slope values
• rCV% for selected peaks
• Resolution
  • Visual inspection
  • Resolution of negative and dim peaks should be constant

Consider a Pilot Run

• Establish data driven criteria

CYTO-CAL CALIBRATION PARTICLES
(PERFETTO ET AL, NATURE PROTOCOLS 7:2076, 2012)
Laser 488: Peak 5 Median Fluorescence

530 / 30 (FITC)

% CV : 10.8

% CV = (SD ÷ Mean) × 100

585 / 42 (PE)

% CV : 3.4

670 LP (PerCP, PerCP-Cy5.5)

% CV : 6.4

48

780 / 60 (PE-Cy7, 7AAD)

% CV : 6.8

wrong settings applied

Grand Mean ± 2SD

under investigation

wrong settings applied

Wrong settings applied

wrong settings applied

wrong settings applied

under investigation
Assay Performance
Data Monitoring

Proficiency Testing

• External
  • CAP
• Internal
  • Twice Yearly Sample Exchange

QC Monitoring

• Daily
  • Results must be within established ranges prior to the release of patient results.
  • BioRad Unity™ Real Time• 2.0 Statistical Package, Westgard Statistical Process Control (SPC) Rules, and Westgard Advisor™
• Monthly
• Reviewed for trending
• Global comparison
## CAP Proficiency Testing -- TBNK

### 2012 FL-B CAP Survey

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Specimen Number</th>
<th>Inter-laboratory %CV</th>
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<tbody>
<tr>
<td>NK Lymph CD16+/56+/CD3</td>
<td>FL-07</td>
<td>5.00</td>
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<tr>
<td></td>
<td>FL-08</td>
<td>5.16</td>
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<tr>
<td></td>
<td>FL-09</td>
<td>6.43</td>
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<tr>
<td>CD3/CD4+ T Lymph Absolute</td>
<td>FL-07</td>
<td>16.37</td>
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<td></td>
<td>FL-08</td>
<td>4.56</td>
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<td></td>
<td>FL-09</td>
<td>4.82</td>
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<td>CD3/CD8+ T Lymph Absolute</td>
<td>FL-07</td>
<td>13.38</td>
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<td></td>
<td>FL-08</td>
<td>6.66</td>
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<td>FL-09</td>
<td>6.38</td>
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<tr>
<td>CD3+ T Lymph Absolute</td>
<td>FL-07</td>
<td>9.79</td>
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<td></td>
<td>FL-09</td>
<td>3.99</td>
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<td>CD18+ B Lymph Absolute</td>
<td>FL-07</td>
<td>10.72</td>
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<td></td>
<td>FL-08</td>
<td>8.95</td>
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<td></td>
<td>FL-09</td>
<td>10.16</td>
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<tr>
<td>CD16+/56+/CD3- MK Lymph</td>
<td>FL-07</td>
<td>14.87</td>
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<td>FL-08</td>
<td>7.16</td>
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<td></td>
<td>FL-09</td>
<td>10.05</td>
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</table>
## Global Cross Correlation -- MRB

### CD19+ Global Split Testing – Absolute Count (cells/μL)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Indy</th>
<th>Geneva</th>
<th>% Diff</th>
<th>Mean</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>157.500</td>
<td>208.200</td>
<td>32.190</td>
<td>182.850</td>
<td>35.850</td>
<td>19.606</td>
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<tr>
<td>S3</td>
<td>180.100</td>
<td>196.600</td>
<td>9.162</td>
<td>188.350</td>
<td>11.667</td>
<td>6.194</td>
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<tr>
<td>S4</td>
<td>92.000</td>
<td>63.700</td>
<td>30.761</td>
<td>77.850</td>
<td>20.011</td>
<td>25.705</td>
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<tr>
<td>S5</td>
<td>263.500</td>
<td>244.700</td>
<td>7.135</td>
<td>254.100</td>
<td>13.294</td>
<td>5.232</td>
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<tr>
<td><strong>Grand Mean</strong></td>
<td><strong>17.039</strong></td>
<td><strong>209.870</strong></td>
<td><strong>18.993</strong></td>
<td><strong>12.164</strong></td>
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### CD20+ Global Split Testing – Absolute Count (cells/μL)

<table>
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<tr>
<th>Sample ID</th>
<th>Indy</th>
<th>Geneva</th>
<th>% Diff</th>
<th>Mean</th>
<th>SD</th>
<th>%CV</th>
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</thead>
<tbody>
<tr>
<td>S1</td>
<td>155.500</td>
<td>207.700</td>
<td>33.569</td>
<td>181.600</td>
<td>36.911</td>
<td>20.325</td>
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<td>S2</td>
<td>334.900</td>
<td>354.500</td>
<td>5.852</td>
<td>344.700</td>
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<tr>
<td>S3</td>
<td>177.600</td>
<td>193.000</td>
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<td>185.300</td>
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<td>S4</td>
<td>89.100</td>
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<td>75.750</td>
<td>18.880</td>
<td>24.924</td>
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<tr>
<td>S5</td>
<td>261.200</td>
<td>246.800</td>
<td>5.513</td>
<td>254.000</td>
<td>10.182</td>
<td>4.009</td>
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<tr>
<td><strong>Grand Mean</strong></td>
<td><strong>16.714</strong></td>
<td><strong>208.270</strong></td>
<td><strong>18.144</strong></td>
<td><strong>11.831</strong></td>
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## Global QC Performance

<table>
<thead>
<tr>
<th>Population</th>
<th>Level 1 QC</th>
<th></th>
<th>Level 2 QC</th>
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<tbody>
<tr>
<td></td>
<td>Global Mean %CV</td>
<td>N</td>
<td>Global Mean %CV</td>
<td>N</td>
</tr>
<tr>
<td>CD3⁺%</td>
<td>2.46 (1.48 to 2.82)</td>
<td>346</td>
<td>1.56 (1.00 to 1.79)</td>
<td>346</td>
</tr>
<tr>
<td>CD3⁺,CD4⁺%</td>
<td>5.55 (4.13 to 6.43)</td>
<td>346</td>
<td>2.20 (1.33 to 2.35)</td>
<td>346</td>
</tr>
<tr>
<td>CD3⁺,CD8⁺%</td>
<td>3.03 (2.54 to 3.60)</td>
<td>346</td>
<td>3.91 (2.86 to 4.57)</td>
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</tr>
<tr>
<td>CD19⁺%</td>
<td>4.10 (3.48 to 4.47)</td>
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<td>5.10 (4.27 to 5.73)</td>
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<tr>
<td>CD3⁻, CD16⁺/CD56⁺%</td>
<td>4.97 (4.04 to 6.51)</td>
<td>347</td>
<td>6.04 (4.24 to 7.80)</td>
<td>347</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Population</th>
<th>Level 1 QC</th>
<th></th>
<th>Level 2 QC</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Global Mean %CV</td>
<td>N</td>
<td>Global Mean %CV</td>
<td>N</td>
</tr>
<tr>
<td>CD3⁺ abs</td>
<td>3.97 (3.15 to 4.38)</td>
<td>344</td>
<td>4.18 (3.35 to 5.08)</td>
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<tr>
<td>CD3⁺,CD4⁺ abs</td>
<td>6.27 (5.23 to 7.25)</td>
<td>344</td>
<td>4.32 (3.06 to 5.21)</td>
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<tr>
<td>CD3⁺,CD8⁺ abs</td>
<td>4.98 (3.87 to 5.10)</td>
<td>344</td>
<td>5.43 (4.71 to 6.21)</td>
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<tr>
<td>CD19⁺ abs</td>
<td>5.38 (4.48 to 5.96)</td>
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<td>7.12 (5.30 to 9.02)</td>
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<tr>
<td>CD3⁻, CD16+/CD56+ abs</td>
<td>6.98 (4.62 to 8.61)</td>
<td>347</td>
<td>7.80 (3.45 to 10.72)</td>
<td>347</td>
</tr>
</tbody>
</table>

### TBNK Assay

**October 2011**

Streck CD-Chex Plus QC

- (same lot #)

Nine different Instruments

- 2 in Geneva
- 4 in Indianapolis
- 1 in Shanghai
- 2 in Singapore
Summary of Standardization Requirements for Clinical Trials

The same as for any quality lab but with a greater emphasis on longitudinal aspects

Instrument validation, standardization, and monitoring

Assay optimization, validation, and monitoring