Cell Product Characterization and release testing: Assay validation, reference standards and a statistical approach

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Required Laboratory and Infrastructure Elements

• Infrastructure that supports Quality
  ➢ Instrumentation, training, SOP

• Established master lots for reference samples and all reagents
  ➢ Developed and used for assay qualification, validation and testing

• Productive relationship with process development and manufacturing groups
**Assay Qualification:** Establishes that an assay will provide meaningful data under the specific conditions used

- No pre-determined performance specifications
- No set guidelines for qualifying assay
- Used to determine method performance capabilities (such as validation parameters)

**Assay Validation:** Establishes the conditions (specifications) to assure that the assay is working appropriately every time it is run

- Specifications established **prior** to validation
- Specifications **must be met** at every run
How do you validate biological assays?

- Biological assays very difficult to validate due to inherent variability
  - Stochastic events
  - Temporal differences in samples intra-patient
  - Genetic variability inter-patient

Establish in a statistically significant manner:

- Specificity
- Accuracy
- Precision (inter- and intra-assay)
- Calibration/standard curve (upper and lower limits of quantification)
- Detection limit
- Robustness

- Understand assay as much as possible
- Establish master stocks for all reagents and reference standards
- Utilize rigorous SOP and apply excellent technical skills
- Expert statistical support
Example Validation Process workflow

EXPLORATORY STUDIES FOR ASSAY

• Define what needs to be measured and what assays need to be developed
• Perform initial evaluations on test sample
# Example release testing for T cell product

<table>
<thead>
<tr>
<th>TEST</th>
<th>DETAILS</th>
<th>TYPE</th>
<th>LAB</th>
<th>FRESH/FROZEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell surface phenotype</td>
<td>%CD3+</td>
<td>Release</td>
<td>in-house</td>
<td>FRESH</td>
</tr>
<tr>
<td>% CAR+</td>
<td>Flow based surface stain</td>
<td>Release</td>
<td>in-house</td>
<td>FRESH</td>
</tr>
<tr>
<td>Antigen-specific cytotoxicity</td>
<td>Flow-based cytotoxicity</td>
<td>Release</td>
<td>in-house</td>
<td>FRESH</td>
</tr>
<tr>
<td>Cytokine Dependence</td>
<td>Restim +/- IL2/IL15, Guava cell count</td>
<td>Release</td>
<td>in-house</td>
<td>FRESH</td>
</tr>
<tr>
<td>Viability</td>
<td>Guava</td>
<td>Release</td>
<td>in-house</td>
<td>FROZEN</td>
</tr>
<tr>
<td>Avg. copies transgene/cell</td>
<td>Q-PCR</td>
<td>Release</td>
<td>in-house</td>
<td>FRESH</td>
</tr>
<tr>
<td>RCL test</td>
<td>Q-PCR</td>
<td>Release</td>
<td>in-house</td>
<td>FRESH</td>
</tr>
<tr>
<td>Sterility (Gram/KOH)</td>
<td>Microbiology</td>
<td>Release</td>
<td>send out</td>
<td>FROZEN</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>Microbiology</td>
<td>Release</td>
<td>send out</td>
<td>FROZEN</td>
</tr>
<tr>
<td>Sterility USP</td>
<td>External CRO</td>
<td>Release</td>
<td>send out</td>
<td>FROZEN</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>External CRO</td>
<td>Release</td>
<td>send out</td>
<td>FROZEN</td>
</tr>
</tbody>
</table>
Comparisons between two lysis curves can be made on the basis of ED_{50} values from log_{2} transformed E:T values.

**Statistical underpinnings of cytotoxicity assay**

Need to have enough data points to model curve
   Need more data points than parameters evaluated- for 4 parameter-based model needs at least 8 data points.
   More data points reduce the variance on the parameter estimates
   More power to determine if two parameters are significantly different

Use ED_{50} (actual or predicted from curve fit)
Use ED_{x} in “sweet part” of curve

Comparisons between two lysis curves can be made on the basis of ED_{50} values from log_{2} transformed E:T values
Establishment of release specifications for T cell products using prediction intervals

Establishment of statistically defined ranges, dependent on number of replicate measurements and SD

*Gaussian distribution required assumption

95% prediction interval: Average +/- (K x S.D.)
K depends on N:
N (K): 3 (4.99); 5 (3.04); 7 (2.62); 10 (2.37); 20 (2.14)

<table>
<thead>
<tr>
<th>RELEASE TEST</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>Average</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface immunoreceptor expression</td>
<td>61.7</td>
<td>66.6</td>
<td>79.0</td>
<td>69.11</td>
<td>8.94</td>
</tr>
<tr>
<td>Surface CD3+</td>
<td>98.5</td>
<td>97.2</td>
<td>97.0</td>
<td>97.6</td>
<td>0.80</td>
</tr>
<tr>
<td>Antigen Specific cytolysis at 6.25:1 E:T</td>
<td>51.5</td>
<td>43.6</td>
<td>48.1</td>
<td>47.7</td>
<td>3.96</td>
</tr>
<tr>
<td>Avg Copy transgene/cell</td>
<td>0.49</td>
<td>0.69</td>
<td>0.87</td>
<td>0.7</td>
<td>0.19</td>
</tr>
<tr>
<td>Avg Copy transgene/cell *</td>
<td>0.79</td>
<td>1.03</td>
<td>1.10</td>
<td>1.0</td>
<td>0.16</td>
</tr>
</tbody>
</table>

* Normalized for surface immunoreceptor-positive
Assay integration
Two mechanisms for assay integration

• **Assay Standardization**
  – Feasible/appropriate in late stage trials, small consortia
    • Requires single SOP, single source of reagents (or documented equivalency)
    • Difficult to implement for early stage trials and translational studies

• **Assay Harmonization**
  – Feasible for early stage studies
    • Laboratory-specific SOP and reagents possible
    • Process identifies variables that impact data
The CIC/CRI Immunoassay proficiency program

Partnership between academia, biotech, pharma and CRO
Close working relationship/partnership with CIP

Establishment of Harmonization panels to Identify:

- Issues and deficiencies of current assay practices
- Sources for assay variability within and among institutions
- Protocol details that optimize assay performance
- Efforts initiated in 2004

To date panels:

- ELISPOT
- Serum
- CFSE proliferation
- Luminex
- ICS
- Multimer
- Gating strategies
Summary Lab performance 30 “quality committed” labs evaluated responses to CMV, Flu, Mart-1

Average: 5.8/9 responses detected
Assay Harmonization improves assay and laboratory performance

Objectives:
1. Decrease variability by implementing Harmonization guidelines
2. Refine harmonization guidelines

Panel 1 outcome

<table>
<thead>
<tr>
<th>Percent of labs missing weak response</th>
<th>50</th>
<th>45</th>
<th>40</th>
<th>35</th>
<th>30</th>
<th>25</th>
<th>20</th>
<th>15</th>
<th>10</th>
<th>5</th>
<th>0</th>
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</thead>
<tbody>
<tr>
<td>Implementation of Harmonization Guidelines</td>
<td>47 (n= 36)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Further Implementation of Harmonization Guidelines</td>
<td></td>
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</tbody>
</table>

(van der Burg, SH, Kalos, M et al SciTM 2011)
Summary/Conclusions

- Cell product assay validation requires considerable infrastructure and commitment to quality operations.

- Reference standards play a critical role in the assay validation process and the development of robust validated assays.

- With appropriate rigor in thought and design, statistical underpinnings can be applied to cell-based bioassays.

- The inherent variability associated with cell-based bioassays has the potential to result in wider than “expected” acceptance ranges for assay performance:
  - Increased replicate testing can mitigate this in part.