Reduction in Bioassay Variability: Myths and Realities of Automation

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CASSS Bioassays 01 November, 2011
5 years of automation experience in a QC laboratory

Myth: Automation is only worthwhile when you run a high volume of the same assay.

Reality: A good analyst is better than a robot any day.
Why are We Concerned about Variability?

- Bioassays have a reputation for high level of variation
  - Typical %CV reported in the literature is 5 -15% for cell-based assays
  - %CVs of up to 40% have been reported
- Detection of true differences and distinction of groups
  - Confidence in results
    - Support product development
    - Support changes in manufacturing process
    - Product release and stability
- OOS and OOT Investigations
  - Investigation is essential in identifying potential issues, but often ends in a conclusion of “within normal assay variability”
Why are We Concerned about Variability?

- High cost of replicates
  - Variability causes us to not believe the result of one assay and average result of several for the reported result
    - Adds extra time to reporting result
    - Increased cost for not only for running assays, but also review, calculation of and review of mean result

The converse:
- Understanding and controlling variability
  - Provides predictable results
  - May allow result of a single assay to be credible
Sources of Variability

- **Dilutions**
  - Assays are so sensitive that several ten-fold serial dilutions are necessary to reach linear range
  - Each dilution adds an error factor = propagated error
    - 4 10-fold dilutions with 5% negative bias: Dilute sample from 10 mg/mL to 1 µg/mL = 0.000814506/0.001 = 81.5% target or 19.5% bias.

<table>
<thead>
<tr>
<th>Dilution Step</th>
<th>Sample Volume</th>
<th>Concentration (mg/mL)</th>
<th>Final Volume</th>
<th>Final Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.095 mL</td>
<td>10</td>
<td>1 mL</td>
<td>0.95</td>
</tr>
<tr>
<td>2</td>
<td>0.095 mL</td>
<td>0.95</td>
<td>1 mL</td>
<td>0.09025</td>
</tr>
<tr>
<td>3</td>
<td>0.095 mL</td>
<td>0.09025</td>
<td>1 mL</td>
<td>0.00857375</td>
</tr>
<tr>
<td>4</td>
<td>0.095 mL</td>
<td>0.00857375</td>
<td>1 mL</td>
<td>0.000814506</td>
</tr>
</tbody>
</table>

- **Cells**
  - Cells are living entities that respond to their environment (pH change, waste product build up, nutrient depletion, signals from other cells). Their response may also change with time in culture. This intrinsic variability can cause day-to-day variability in bioassay results.
Control of Variability of Dilutions

Automated Liquid Handling Systems

Consistency

- Reduction in error caused by fatigue
- Eliminate bias introduced by analyst

Accuracy

- Infinite ability to adjust actual volume delivered across diluents with varied viscosity
- Decreased potential for technical error

Safety

- Decreased potential for repetitive motion injury

Increased throughput

- One analyst can operate several robots simultaneously
Automation can decrease variability and get labs proficient with a new method quickly.

Example: Cell-based Reporter Gene Assay for a growth factor.

Transferring lab had been running method routinely (approximately 10 assays/month) for 6 years.

Receiving lab had only completed training and readiness assays.
Automated Cell Counting

Triplicate counts for Cell Line 4 using automated cell counter are $8.69 \times 10^5$, $8.79 \times 10^5$ and $8.60 \times 10^5 = 2\%$ variation.

General trend in change in cell number observed by all analysts, but 20\% variation in actual count between analysts.
Automated Cell Counting

- Set size and shape constraints for consistency
- Can count many more cells than analyst (50-100 fields: typically total of 300-1500 cells) for accuracy
- Signal is proportional to cell number, so more cells = a higher signal, but why should this matter in a relative potency assay?
- Non-optimized cell density results in variability – analogy to critical reagent not in excess
Cell Density and Variability

Reporter Gene Bioassay for Growth Factor

Study 1

Effect most evident for 130% potency sample – cell response saturated
Cell Density and Variability

**Reporter Gene Bioassay for Growth Factor Study 2:**

- **Cell Density Titration:** Evaluation of the variance component table shows that 3.5x has less variability than 2x which has less variability than the 1x cell concentration for the 130% potency sample. The 3.5x also has less variability than the 2x and target cell concentration for the 100% and 70% potency samples.

<table>
<thead>
<tr>
<th>Cell Density</th>
<th>Target % Relative Potency</th>
<th>Average % Relative Potency</th>
<th>Total %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>72.3</td>
<td>7.97%</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>103.0</td>
<td>10.55%</td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>140.7</td>
<td>20.18%</td>
<td></td>
</tr>
<tr>
<td>2X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>73.4</td>
<td>10.16%</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>101.1</td>
<td>11.35%</td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>133.8</td>
<td>15.85%</td>
<td></td>
</tr>
<tr>
<td>3.5X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>70.2</td>
<td>7.63%</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>98.3</td>
<td>6.04%</td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>127.6</td>
<td>9.1%</td>
<td></td>
</tr>
</tbody>
</table>
Cell Cycle Variation

Reversible Cell Cycle Block can have Impact to Variability of Results (example of a 5-point parallel line cell proliferation assay)

<table>
<thead>
<tr>
<th>Cell Treatment</th>
<th>Mean % Relative Potency</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>107.7</td>
<td>9.4</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>106.6</td>
<td>5.1*</td>
</tr>
<tr>
<td>DMSO</td>
<td>109.4</td>
<td>13.3</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>97.7</td>
<td>7.6</td>
</tr>
</tbody>
</table>

*lowest background
Cell Surface Receptor Expression Variation

Decreased Receptor Expression with Cell Passage

Optimal Receptor Expression Dependent upon Removal of Growth Factor Prior to Use of Cells in Assay
“Ready-to-Plate” Cells

- Maintenance of cells in culture is a labor intensive process requiring skill and diligence
- The concept of using cells directly from the freezer has been proven for high-throughput screening assays
- Adaptation of this practice to routine bioassay testing is a labor saving convenience and provides a more consistent cell population over time with resultant decreased assay variability
- A large bank of cells at the optimum condition for use in an assay is prepared and cryopreserved
- The same bank of cells is used across testing sites and over time within a site.
Automation alone will not “fix” a method which has not been optimized

Method Performance Run Chart: Automation of Method Prior to Optimization and Control of Cells

Optimized Cell Density
Automated Method “Ready-to-Plate” Cells

Optimized Cell Density
“Ready-to-Plate” Cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>%Relative Potency</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>84</td>
<td>97</td>
<td>8.5</td>
</tr>
<tr>
<td>252</td>
<td>99</td>
<td>8.1</td>
</tr>
<tr>
<td>420</td>
<td>99</td>
<td>4.8</td>
</tr>
<tr>
<td>588</td>
<td>97</td>
<td>8.5</td>
</tr>
<tr>
<td>756</td>
<td>99</td>
<td>8.1</td>
</tr>
<tr>
<td>924</td>
<td>99</td>
<td>4.8</td>
</tr>
<tr>
<td>1092</td>
<td>96</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Avg = 0.9855

<table>
<thead>
<tr>
<th>Sample</th>
<th>%Relative Potency</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>96</td>
<td>7.2</td>
</tr>
<tr>
<td>16</td>
<td>99</td>
<td>4.8</td>
</tr>
<tr>
<td>32</td>
<td>99</td>
<td>4.8</td>
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<tr>
<td>40</td>
<td>99</td>
<td>4.8</td>
</tr>
<tr>
<td>56</td>
<td>99</td>
<td>4.8</td>
</tr>
<tr>
<td>64</td>
<td>99</td>
<td>4.8</td>
</tr>
<tr>
<td>80</td>
<td>99</td>
<td>4.8</td>
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<tr>
<td>96</td>
<td>99</td>
<td>4.8</td>
</tr>
<tr>
<td>104</td>
<td>99</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Avg = 0.9747
Validation Results for Optimized Method Using Automation and “Ready-to-Plate” Cells

Cell Proliferation Assay for a Peptibody

Overall % CV = 2.9 compared to previous validation for this method without automation, which used cultured cells only and fewer robustness features for which the overall % CV was 6.0%
Decreased Variability Revealed Minor Method Faults

Automation

Understanding/Control of Biology (Cells)

Precision

Reduced Variability

Linearity Failure for Precise Data

Change to non-linear format and curve fit

Improved Method

Plate Location Effect

Qualify and Validate Method
Automation Myths and Realities

Myth: Automated Liquid Handling Systems don’t make mistakes
Mystery of low potency results

Degraded air gap was allowing system water to leak into dilution tubes

Reality: Automated Liquid Handling Systems have only as much knowledge and experience as the script programmer
Automation Myths and Realities

Myth: Robots can work un-supervised
   Reality: you may get a lunch break, but otherwise count on having someone in the lab to address the robot’s demands
   Reality: Scrupulous maintenance and calibration checks are essential

Myth: Automation can be installed as a COTS package
   Reality: Careful resource planning is required to avoid frustration and project failure
   Reality: A step-wise implementation plan in the spirit of continuous improvement may provide better results
“Plug and Play”

- Automatic Liquid Handling System Manufacturers may not sell the best plate reader/shaker/plate washer
- 21st Century Bioanalytical Lab subgroup of Ligand Binding Assay Bioanalytical Focus Group has a white paper in process with aim to encourage manufacturers of equipment for automation to collaborate and provide end users with many options
Points for Discussion

- Phase – driven variability expectations/allowance
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Mark DiMartino
Sydney Zaremba
## Plate Location Effect

% Relative Potency: Reference Standard Compared to itself in all plate positions

<table>
<thead>
<tr>
<th>Plate Position</th>
<th>Assay 1</th>
<th>Assay 2</th>
<th>Assay 3</th>
<th>Assay 4</th>
<th>Assay 5</th>
<th>Assay 6</th>
<th>Assay 7</th>
<th>Assay 8</th>
<th>Assay Average</th>
<th>Position Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.96494</td>
<td>0.92747</td>
<td>0.93859</td>
<td>0.93136</td>
<td>0.96597</td>
<td>0.95601</td>
<td>0.93215</td>
<td>0.93791</td>
<td>0.9598</td>
<td>0.946022</td>
</tr>
<tr>
<td>2</td>
<td>0.9398</td>
<td>0.94391</td>
<td>0.96961</td>
<td>0.93433</td>
<td>1.00994</td>
<td>0.98056</td>
<td>0.96283</td>
<td>0.98462</td>
<td>0.94485</td>
<td>0.963383</td>
</tr>
<tr>
<td>3</td>
<td>0.94928</td>
<td>0.95464</td>
<td>0.92213</td>
<td>0.92432</td>
<td>0.95423</td>
<td>0.94328</td>
<td>0.93366</td>
<td>0.95422</td>
<td>0.92342</td>
<td>0.939909</td>
</tr>
<tr>
<td>4</td>
<td>0.97774</td>
<td>0.97406</td>
<td>0.97735</td>
<td>0.97773</td>
<td>1.03759</td>
<td>1.00565</td>
<td>0.98146</td>
<td>0.95553</td>
<td>0.97264</td>
<td>0.984417</td>
</tr>
<tr>
<td>5</td>
<td>0.92995</td>
<td>0.95837</td>
<td>0.93964</td>
<td>0.97413</td>
<td>0.96053</td>
<td>0.97834</td>
<td>0.95405</td>
<td>0.91548</td>
<td>0.93638</td>
<td>0.949652</td>
</tr>
</tbody>
</table>

| Assay Average  | 0.952342 | 0.95169 | 0.949464 | 0.948374 | 0.986562 | 0.972768 | 0.95283 | 0.949552 | 0.947418 |
| Assay Standard Deviation | 0.020142 | 0.018206 | 0.024392 | 0.026834 | 0.036907 | 0.024775 | 0.02174 | 0.026765 | 0.020397 |