Application of Bioassay Performance Monitoring Process with Statistical Tools

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Introduction

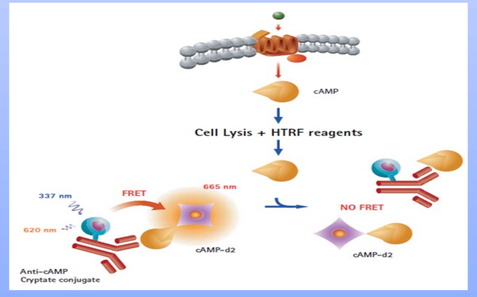
Cisbio's HTRF bioassay is a competitive immunoassay between native cAMP produced by cells stimulated by compound binding to receptors on cell membrane and the cAMP labeled with the dye d2 (Figure 1). The tracer binding is visualized by a McAb anti-cAMP labeled with Cryptate. The specific signal (i.e. energy transfer) is inversely proportional to the concentration of cAMP in standard or sample. The specific signals are calculated based on the formulas of

$$\text{Ratio} = \left(\frac{A_{665nm}}{B_{620nm}}\right) X\,10000$$

$$\text{Delta F\%} = \left(\frac{\text{Standard or Sample Ratio} - \text{Negative Control Ratio}}{\text{Negative Control Ratio}}\right) X100$$

This cell-based potency assay was developed and qualified in Amylin Pharmaceuticals Inc. and transferred to Biocon Limited. The assay performance in Biocon Limited has been monitored and key parameters have been analyzed during trouble shooting.

Figure 1. The Mechanism of HTRF bioassay



Problems in Assay Performance

It was found that assays started generating higher CV for negative controls (Figure 2), standards and samples. The assays also were found having smaller dynamic range (Figure 3). Since the sample acceptance criteria and system suitability are evaluated by parallelism and relative potency recovery between reference standard and sample, the assays were valid. However, the higher variation among replicates with smaller dynamic range of the curve shows that there are fundamental problems either in assay operations or in instrument hardware. The investigation for the root cause was initiated.

Figure 2. Control Charts for CV of Average Readout at 620 nm (whole plate for each data point) and CV of Negative Control Readout at 665 nm (n=8 for each data point)

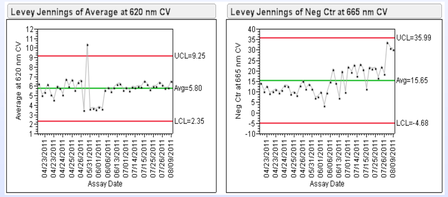
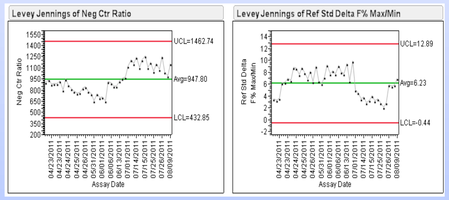


Figure 3. Control Charts for Negative Control Ratio (n=8 for each data point) and Reference Standard Max/Min (n=3 for each data point)



Findings during Trouble Shooting

Although negative control readout at 665 nm was stable, there is a downward trend for the average of whole plate readout at 620 nm (Figure 4, 5). Therefore, the ratios of negative control were increased dramatically since 7/1/11 (Figure 3). The statistical test demonstrates that there is a significant difference for both negative control ratio and reference standard Max/Min between the assays performed from 4/23/11 to 6/15/11 (29 assays) and from 7/1/11 to 8/9/11 (16 assays) (Figure 6). In order to prove the hypothesis that the instrument might be the root cause, the same assay plate was read by two same make and model instruments located in different laboratories (SpectraMax M5e). The results clearly demonstrate that the instrument located in R&D had some hardware issues which caused the problems described above (Figure 7).

Figure 4. Control Charts of Average Readout at 620 nm (whole plate for each data point) and Negative Control Readout (n=8 for each data point) at 665 nm

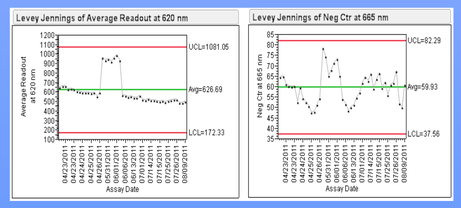


Figure 5. Analysis of Variance for Average Readout at 620 nm (whole plate is one data point. 7 Outliers from a total of 45 assays were excluded from the regression analysis)

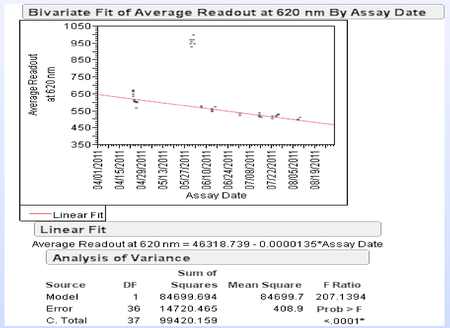


Figure 6. Significant Test of Assays Performed in Two Stages

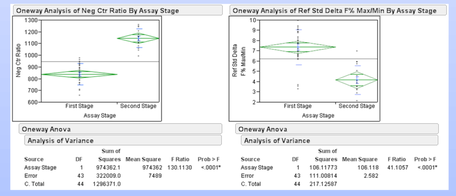
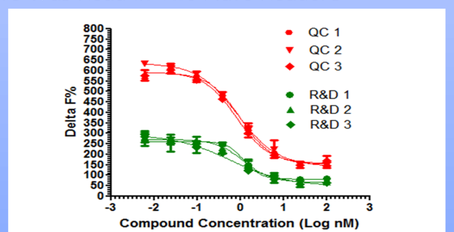


Figure 7. Same Plate Was Read in Two Plate Readers



	R&D 1	QC 1	R&D 2	QC 2	R&D 3	QC 3
IC50	1.226	1.035	1.194	0.8914	0.5961	0.8398

Conclusion

1. Cell-based potency assays are with complexity and variability
2. Assay performance monitoring process is very helpful to find problems and initiate investigation at early stage
3. The statistical program provides powerful tool for trouble shooting
4. The root cause was identified and the problem was solved by replacement of instrument hardware
5. It is suggested to have separate instrument calibration and qualification for HTRF mode