

Data Processing and Quality Assurance in a Kinetic Enzyme Assay

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Abstract

As a not-for-profit research organization with limited in-house programming resources, we have developed processes by which we are able to apply stringent quality control and quality assurance practices using commercially available software and have applied the practices to a kinetic screening environment. We required that the results from each plate achieve the specific control criteria described in this presentation to be classified as valid. Criteria required include metrics such as median and standard deviation of control values within a range consistent with historical data for the screen. Z values above 0.5 and also consistent with historical data, and plate section ratios that indicate a random distribution of compounds and thus across each primary screen plate. As the kinetic dimension of this assay indicates, an additional layer of complexity is introduced as each well of data is measured 10 times over 160 seconds. It is necessary to incorporate this information into our established QC criteria. The example provided will detail the process by which we evaluated data for a screen of 200,000 compounds from the NIH Small Molecule Repository in an assay of a bacterial HMG-CoA reductase.

Introduction

The expanding role of high-throughput screening (HTS) has created a tremendous amount of data for both probe and drug development areas. The potential that high-throughput technologies bring to basic science is changing the standards by which data is considered. The National Institute of Health Roadmap Initiative provides many examples. The amount of data being generated and published has grown exponentially. In order to achieve the benefits that such a vast quantity of data can provide, the scientific community also bears the responsibility of assuring that the data being considered is of the highest possible quality. In trusting the available data, there is the potential for increased costs associated with time spent on analysis and follow-up decisions made based on poorly curated data. As the scope of projects and the size of experiments expand, there is an increasing need to assure quality. Among the areas where this can be accomplished are process tracking, plate and control quality tracking, and statistical analysis of each screening campaign. Without the luxury of an on-site programming staff, we have incorporated commercial software packages into our workflow to address each of these areas. We have used UniFlow LIMS system by UniConnect as our in-lab process tracking tool, IDBS' ActivityBase XE for plate quality tracking based on both intra- and inter-plate controls in addition to various visualization tools, and SAS as our primary statistical analysis package.

Each of the aforementioned tools were used as part of our routine in a recent screening campaign conducted by the Southern Research Molecular Library Center, a participant in the National Institute of Health Roadmap Initiative, to search for useful probes for bacterial HMG-CoA reductase. The kinetic assay was screened against a library of approximately 200,000 compounds from BioFocus/DPI, the Molecular Libraries Small Molecule Repository (MLSMR). The kinetic aspect of this assay provided an additional layer of quality observation beyond the requirements for a single endpoint assay.

Materials and Methods

Purified recombinant *Enterococcus faecalis* HMG-CoA reductase was provided by Drs. Cynthia Stauffacher and Victor Rodwell at Purdue University. Compounds in DMSO were dispensed at 25 nL into 384-well plates for a resulting 10 μM test concentration. Fifteen μL of HMG-CoA reductase reagent mix which included coenzyme A, NADP, and mevalonate in assay buffer was added to each well of the previously compound dosed plates. The reaction was initiated with the addition of 10 μL of HMG-CoA reductase diluted in assay buffer. The final reaction conditions were 2 mM coenzyme A, 4 mM NADP, 4 mM mevalonate, and 15 μg/ml HMG-CoA reductase diluted in assay buffer (100 mM Tris-HCl (pH 8), 100 mM KCl, 2% DMSO and 0.1% Tween 20). The test plate was immediately transferred to a Perkin Elmer Envision microplate reader and absorbance was measured at 340 nm every 16 seconds for 160 seconds. Each plate had 64 control wells in the four outside columns with 32 containing the complete reaction mixture with carrier control (full reaction) and 32 in which the mevalonate had been left out (background).

Every step of the process was electronically logged via barcodes scanned into our UniFlow LIMS system using a workflow designed for this assay (Figure 1). The LIMS system alerts the scientist to any deviation from the established protocol. This includes requiring that the screener has read the current protocol, has met all safety training requirements such as annual testing, is using the correct reagents in the correct order including confirmation that no expired reagents are allowed. The LIMS system is also used to be sure all equipment used in the process has passed calibration and QC checks within the established timeframe.

Figure 1: UniFlow LIMS process tracking

Figure 2: Historical Controls

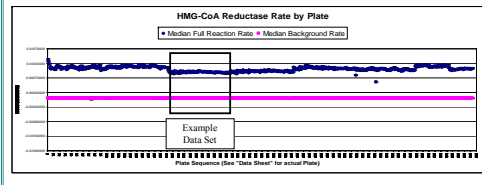


Figure 3: Intra-Plate comparisons

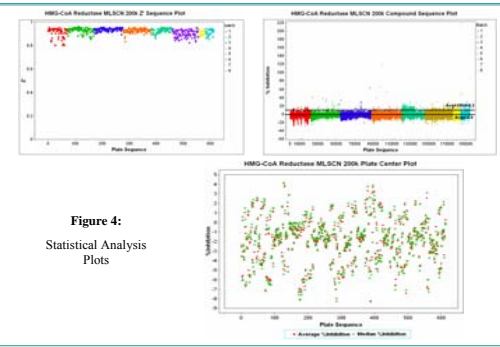


Figure 4: Statistical Analysis Plots

Materials and Methods

Plate associations are then transferred from the UniFlow LIMS to ActivityBase for accurate compound tracking. Kinetic data files from the readers are captured and recorded for each of the 10 time points, followed by calculations of slope for each well's reaction. The average, median and standard deviation for both positive (background reaction) and negative (full reaction) are calculated for both the slope and the first kinetic read (OD1). These values are queried and plotted in an internally available Excel document of "Historical Controls" to assess whether the values are consistent throughout the screening campaign (Figure 2). In addition to requiring values observed in control wells to meet specific criteria within the plate as seen in the Z values and throughout the screen as seen in the Historical Control document, each plate is divided into various sections and ratios are calculated and returned to assess consistent liquid handling in compound wells. If the compound library used is randomly distributed across wells and plates, indicating that "hits" should not cluster in any particular area or in any specific group of plates. To visualize inter-plate compound distribution, we evaluate the number of hits per plate; for intra-plate compound distribution we calculated medians, standard deviations and ratios for each plate comparing top to bottom, left to right, inner to outer, quadrant 1 to quadrant 4 and quadrant 2 to quadrant 3 (Figure 3). This QC data is presented with each batch of data. If a particular plate area exceeds the ratio by >15% the plate is highlighted for further review and possible repeat screening.

Statistical analyses of the data are routinely performed to assure the quality and reproducibility of the assay results and statistical report showing the histogram, scatter plot, Bland-Altman plot, and sequence plot, minimum significant difference, test of significance, Pearson's and Spearman's correlations, and linear regression analysis generated using SAS and JMP (Figure 4).

Immediately following the successful completion of an HTS assay, the validated results are exported in various formats to facilitate analyses and formatted appropriately and uploaded to PubChem (Bioassay 1066). To date our Center has processed and uploaded 2.6 million data points to PubChem from a total of 74 unique primary and dose-response HTS and HCS assays.

Results and Conclusions

By using the UniFlow LIMS for process tracking many potential problems were avoided and a tangible record of events was created. Calculating and documenting the average, median and standard deviation for both positive (background reaction) and negative (full reaction) are calculated for both the slope and the first kinetic read (OD1). These values are queried and plotted in an internally available Excel document of "Historical Controls" to assess whether the values are consistent throughout the screening campaign. Acceptable values for background OD1 fell between 0.087 and 0.1, with expected slope between -0.00004 and 0.00003. Acceptable values for the full reaction OD1 fell between 0.231 and 0.386, with expected slope between 0.00056 and 0.00133. Z values for the screen ranged from 0.8 to 0.91. A predetermined cutoff of 20% was used to determine that no single plate had >3 hits. These data in conjunction with calculated ratios allowed us to identify, reject and retest plates that contained questionable data.

Statistical analyses illustrated a cutoff of ~9.5 % Inhibition could be considered as active, based on a median ¹/inhibition for all experimental compounds [-1.89] plus 3 times the standard deviation [3.78] identifying 792 hits (0.4% hit rate) for clustering and structural analysis.

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References

- Rasmussen, L, Maddox, CB, Harten, B, White, EL, A successful LIMS implementation: Case Study at Southern Research Institute. *JALA* 6 (2007) 384-390.
- Assay Guidance Manual Version 5.0*, 2008, Eli Lilly and Company and NIH Chemical Genomics Center. Available online at: http://www.ncgc.nih.gov/guidance/manual_toe.html (last accessed 2008 28 Mar).