

Process improvements in cell screening through introduction of PAA Workcells and Data analysis tools

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Introduction

Over the last several years the ASG within Oncology at AstraZeneca has been implementing changes to its cell based secondary screening process to deliver a efficient flexible screening service supporting a diverse oncology pipeline.

Secondary cell screening has moved from 96 well manually cultured cells to 384 well SelectT delivered, or assay ready cryopreserved, cells. We have also implemented acoustic compound dosing in every assay. Screening requests are managed via laboratory management software (Thermo's Nautilus) in which multiple samples or assay requests can be efficiently managed. We have adopted a modular automation approach using mainly workcells custom built by Process Analysis and automation (PAA). One model is designed for acoustic dosing, fixing, and incubation. The second workcell model can process a range of different assay endpoints such as ELISA and imaging based assays. After plates have been processed they are removed from the workcells to be read using a range of different plate readers, either standalone or part of a HighRes high content imaging workcell.

As we have broken the screening into three separate parts - dosing, assay, and read - no single workcell is a major process bottleneck. This provides flexibility in being able to run multiple assays, bringing new screens online, or for running out of hours. To ensure that, as well as high efficiency, data quality is maintained and improved through adopting this approach, custom tools have introduced for data analysis and assay monitoring.

This poster will give an overview of what has been implemented highlighting the most recent introduction and their impact.

1. Nautilus

- ASG currently supports 38 different cell assays covering a diverse Oncology pipeline.
- Up to 5K sample requests are received per month and via the improvements outlined in this poster there has been >100% increase in number of IC50s generated despite a reduced number of FTEs (Figure 1).
- ASG has many different global customers, each submitting sample requests to single or multiple assays.
- Nautilus is our customised LIMS solution, handling all aspects of these requests through sample batching, ordering process and testing maintaining a track of the sample at each stage.

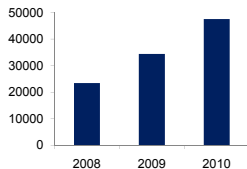


Figure 1. Number of cell assay IC50 values generated as a consequence of process improvement

2. Cells

- Increased flexibility from ~50 : 50 split between use of SelectT delivered and assay ready cryopreserved cells. There is still a need for manual culture of a small number of difficult cell lines.
- Assay ready cells are large scale cultured or bulk transfected cryopreserved cells delivered from AZ's central DECS group, in sufficient numbers per vial to run an assay minimising the need for routine cell culture.
- Assay ready cells are revived directly into assay plates following a short culture period are ready to use in the screen.
- Screening has moved from 96 well based manually cultured cells to 384 well using assay ready or SelectT delivered cells
- We have seen good correlation between manual and assay ready cells but have seen benefit in cost reductions and increased flexibility (Figure 2).

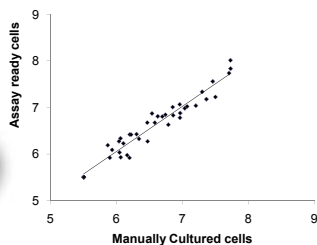


Figure 2. Correlation of pIC50s generated using either manually cultured or assay ready cells

3. Integrated Echo PAA Workcell

- This Workcell was a redesign on a pre-existing system to meet the changing requirements of our secondary cell screening group.
- The improved system was delivered in 2009 incorporating acoustic compound dosing, reagent addition, plate washing, incubation, and storage.
- All devices on the Workcell are controlled by Overlord 2 software (PAA) and Batch Manager software.
- Batch Manager is a custom designed interface that allows management of runs and keeps the user updated on the stage and location of each plate.
- Software has a runtime information tab and labware status button and a improved error recovery allowing a batch to be recovered mid-run and recommenced from desired stage.



Photograph of Integrated Echo Workcell

Cell dosing features include;

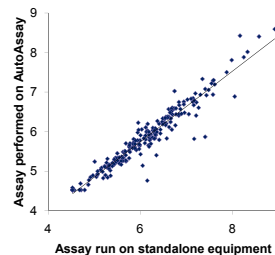
- Double Echo acoustic dispensing allowing an overlay of two separate dispensing patterns on a single destination plate with the option of simultaneous additions or consecutive additions.
- Echo software can handle multiple liquid classes both DMSO based and aqueous compounds or reagents.
- Assays that require fixation accommodated by inclusion of Multidrop combi and a Biotek ELX-405 plate washer connected to a Brandel waste unit. On completion of the process final plate storage is at 4°C.

4. AutoAssay PAA WorkCell

- Workcells designed to automate virtually any 96/384 well format assay involving a liquid dispenser and/or plate washer with multiple steps and incubation times.
- Two workcells were delivered in 2008 and were successfully used in routine screening delivering greatest benefit to long duration assays and allowing assays to be run out of hours.
- Each workcell has two Hamilton 8-way valve system attached to a Multidrop combi allowing assay of standard format to be batched together saving on reagent.



Photograph of AutoAssay Workcell



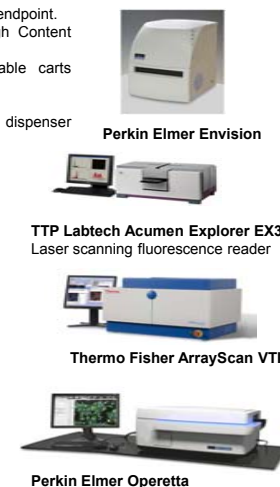
Correlation of pIC50s generated using either AutoAssay or standalone equipment

5. Plate Reading

- Flexibility - a variety of different walk up and read readers to suit endpoint.
- 2010 investment in a HighRes NanoCell 4-sided system- High Content Screening hub
- ThermoFisher ArrayScan & PerkinElmer Operetta on dockable carts surround a robotic arm
- Carts can be undocked to enable walk up and read functionality
- Flexibility of the dock able carts means plate washer or liquid dispenser could be put onto the cell as required.



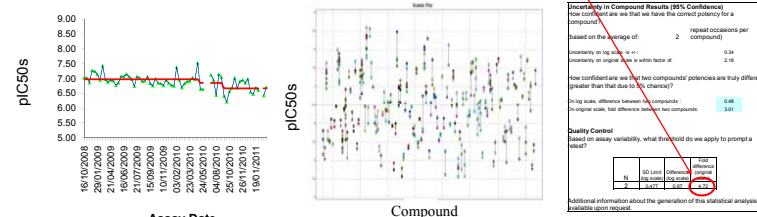
Photograph of High Content Workcell



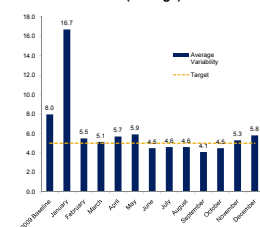
6. Data Analysis

- Introduction of this multi-workcell approach to cell screening delivers great flexibility - but we need to ensure that data quality is maintained or improved.
- Other factors in data analysis that needed to be considered were to remove subjectivity out of assay data analysis, to reduce time taken for routine data analysis and a way to flag issues with kit affecting assays quickly to minimise impact across multiple assays.
- A number of tools are now in place to enable scientists to analyse data quickly and efficiently.

- Manhattan Tool** monitors long-term assay performance and trends this links to IBIS (global database store for all screening data) and can monitor multiple compounds and export to Excel and Spotfire.
- Assay Variability Tool** monitors variability of IC50 values for compounds on separate occasions and users this to set repeat thresholds for each assay.



2010 Cell Assay Variability (average)



Summary

Moving to a workcell environment has delivered increased flexibility in ASG screening & a year-on-year increase in productivity.

There has been Improved performance and increase consistency of assays since introduction of new data analysis & QC tools, these have helped also to identify areas for assay improvement.

Acknowledgments

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