Modular automation for screening a cost/benefit analysis

Cost is now a key driver for pharmaceutical companies and in many respects shapes the capital, revenue and resource decisions that have to be made during the drug discovery process. Where companies are resource rich, the need for fully automated screening platforms is reduced and workstation-based systems tend to be more abundant. For companies which are resource limited, fully integrated screening systems tend to provide a good solution but require a high capital investment. As new flexible automation systems are being developed it is now possible to balance the high capital cost of fully automated systems with both revenue and resource savings across wider business needs. This makes fully automated solutions more attractive to big pharma as benefits can be delivered back to the business more quickly.

By Dr Jonathan Wingfield

At AstraZeneca, biochemical secondary screening for the Cancer UK department was centralised in 2006[1]. Initially the aim was to reduce the resource required to deliver the biochemical data across the range of projects within the cancer portfolio. The capability as tasked with generating all the routine biochemical screening data for all the cancer projects post high throughput screening up to the nomination of a candidate drug. This covers the lead identification, lead generation and lead optimisation phases of the drug discovery process. Each phase requires very different levels of biochemistry support. During lead identification, large numbers of compounds are screened in dose response format relatively infrequently – typically up to 1,000 compounds might be tested over a short period against a single target. Once focused chemistry is applied during lead generation the level of screening often decreases, 50-100 compounds may require testing each month, but these may require selectivity testing across multiple target assays. As chemistry homes in on defined chemical series, the number of compounds that need testing reduces again. It is probable that only a handful of compounds are made each week, however, data needs to be generated on these compounds very quickly. A significant challenge for the centralised biochemical screening capability was to be able to support projects across all these phases with differing demands for data delivery schedules. The capability must have the ability to deliver large packages but also react quickly to late stage projects that require small amounts of data with a fast turnaround time. Within two years the centralised capability was delivering 30% more data to the business with half the resource head count. The equivalent of six full time members of staff were liberated from routine biochemical screening activities and returned to bioscience activities.

Compound preparation

The initial aim of the centralised capability was to provide a rapid turnaround on data. The major bottleneck in data delivery was compound preparation, on average the team was expected to generate 2-3,000 IC50 curves per week. To enable this level of data delivery most of the automation used by the team was dedicated to compound plate production. Typically compound preparation would be carried out overnight so that plates would be ready for the bioscientist first thing in the morning, so the automation had to be robust enough to run reliably unattended. A simple automation module, consisting of a Labcyte Echo 550 acoustic dispenser fed by a CRS F3 articulated arm, linked to Liconic carousels as a storage location, was built. Overlord from PAA was used as control software to manage plate movements and cherry picking of compounds from the source plates into destination assay plates. The major cost of this system was the Labcyte acoustic dispenser, however, there was a significant increase in the quality of data delivered to projects. The first unit installed replaced four Tecan Genesis liquid handling systems and reduced the demand for Dimethyl Sulphoxide (solvent) from 42l per day to almost zero. It is estimated that the annual cost saving in moving to acoustic dispensing was in the region of £160,000 – within two years these savings offset the capital cost of the automation unit.

Bulk reagent addition

The screening team were expected to run an average of nine assays per day. The dispenser of choice was the Molecular Devices AquaMax DW4. These were relatively cheap units with the ability to dispense up to four reagents accurately and quickly. By adding both enzyme and substrate through different liquid channels to each plate in one pass, a 384 well plate could be processed in less than 30 seconds. The typical number of plates within each assay batch was only five (~120 compounds) so dispensing reagents only required 2.5 minutes. At first the DW4 units were fed by CRS F3 arms, however, it became clear that it was much faster to process plates by hand. The transfer time of plates in to and out of a carousel via the automated arm would take up to 1.5 minutes per plate, which was much slower than moving plates by hand. As a result members of the team declined the option of using automation.

The AquaMax enabled the assay volume to be reduced to 12ul, which significantly reduced the reagent costs (revenue spend) compared with the traditional 50ul 384 well assay. Historically, compounds had been tested in duplicate with the duplicates being present on the same plate within the same run. Having moved to acoustic droplet ejection for compound dosing and more accurate bulk reagent dispensing, it became clear that duplicates

within runs were not necessary. Changing to single point testing at each dose of the concentration response effectively doubles the number of compounds that can be tested on each plate. All actives were retested so that an N of 2 was generated against these compounds. These changes resulted in an increase in capacity of 30% while remaining revenue neutral.

Throughout 2007 the revenue cost for delivering secondary screening data across the cancer department was monitored. The typical batch size for each assay was 5 x 384 well plates, each plate required 2.3ml for each reagent. Therefore, on average each batch required only 11.5ml of each reagent. The dead volume of the AquaMax DW4 is ~8ml and in many cases a sacrificial plate was run through each assay to ensure the dispensers were completely primed. Typically 10.5ml of reagent was being wasted just in priming the dispensing device, it was estimated that almost half of the revenue budget for biochemical reagents was being lost annually. While the benefits of the AquaMax DW4 were speed and flexibility, the downside was dead volume and reagent costs. For high throughput screening where large numbers of plates are processed in each run, the dead volume is not a significant issue, however, in a secondary screening environment where small number of plates are processed through large numbers of assays, each day this became a significant revenue burden.

Move to low dead volume dispensing

Reducing the revenue burden became a significant driver for change. Replacing the AquaMax DW4 dispensers with a low volume dispensing system became a priority. The biochemical screening capability considered several different types of dispensers. In addition to the reduced dead volume the dispenser had to be able to process plates quickly with the ability to add enzyme and substrate to the assay plates in one pass of the plate. 1536 capability would be advantageous as this would allow the assays to be reduced in volume from 12ul to 3ul. The Idex Nanodrop Express was purchased to meet these new challenges. This device has aspirate and dispense capability with a

wash cycle applied at the end of each dispense. While the Nanodrop is slower than the Aquamax DW4 at dispensing, it has a 2 x 8 configuration that enables two different reagents to be aspirated and dispensed during one pass over the assay plate. In addition, the wash function after dispensing ensures that reagents are not held in the head, this reduces the risk of tips blocking. Reagents can be supplied in a wide range of containers so that the dead volume can be minimised as each tip only aspirates the volume required to be dispensed plus a 7% excess. This also allows a very large number of reagents to be available to the dispense head, a 384 well reagent plate could allow for 384 different reagents to be available to the head. Each tip is controlled independently so a different reagent can be dispensed by each tip if necessary making the Nanodrop useful for factoral experimental design as well as routine bulk reagent addition. The unit is capable of accurate 96, 384 and 1536 well dispensing in the nl through ul range. By comparison with the Aquamax DW4, the Nanodrop Express is considerably more expensive, but this additional cost is offset by the reagent saving – typically the dead volume is only 1ml, effectively reducing the reagent loss by 85%. Again within two years the capital cost of purchasing the dispenser would be recovered.

Higher density formats

Moving biochemical assays into 1536 well formats for secondary screening initially seemed like an unnecessary activity. Considering that the typical test batch size was about 5 x 384 this would equate to only 2 x 1536 well plates. The plasticware costs are similar to run the assays in either format, the reagent volumes are typically ¼ in 1536 well format. Only 2.5ml of reagent are required per 1536 well plate, including a 1ml dead volume, resulting in a minimum reagent volume of only 3.5ml. Since it requires 10.5ml to prime the AquaMax DW4, it clearly makes economic sense to test even very small numbers of compounds in 1536 well format rather than in 384 well format. Processing 1-2 plates per assay also means that reagent stability becomes less of an issue, moving ¼ of the plates through a process will require less time. With reasonable incubation periods it becomes possible to interleaf several different 1536 well assays. Clearly this has advantages for a secondary screening capability, where many assays are run daily, the issue becomes how to track and manage these assays. Automation offers the solution to tracking plates through multiple assays, reducing the number of plates means fewer plate movements and therefore higher probability of success.

Large scale automation

During 2008 it became clear that the focus of the local biochemical secondary screening automation would move away from compound preparation. Acoustic droplet ejection was being rolled out within AstraZeneca's central compound management group. This allowed the secondary screening capability to consider how to effectively deploy automation to address the issues with 1536 well plates. The automation package would have to be flexible enough to enable up to nine assays to be run each day, would need to be able to handle current 384 well assays and 1536 well assays, adaptable enough to work for different screening technologies and target classes and, finally, be affordable. The demand for secondary screening within

the cancer department had not been considered high enough to warrant the investment in a large scale automation system in the past. However, the secondary screening team supporting the cardiovascular research area was co-localised in the same building as the cancer department's secondary screening capability. Both departments have made significant investments in automation and technology independently in the past. Combining the expanding needs of these two capabilities moving forward would justify the purchase of a new automation platform. In addition, aligning the automation needs of the two teams would be more cost-effective, since it would reduce the overall amount of capital equipment that would be required if the teams continued to function independently. A good example of this was that between the two teams there were five PerkinElmer Envision readers, both teams carrying one additional reader as a spare in case of emergency. A similar situation occurred with dispensers, plate washers and incubators. Spreading the cost of the automation system between two departments would reduce the cost of the system to a more manageable level for both departments. However, this would add an additional level of complexity in that the system would have to service two departments with very different automation needs.

At the Society for Biomolecular Screening meeting in St Louis in 2008, HighRes Biosolutions was presenting its highly flexible MicroStar automation system. This looked like a platform that could address most of our issues. Central to the system are two Staubli TX60 arms, each mounted on a six-sided MicroStar pod. Each pod has five sides available for peripheral devices while the last position is used as a hand over station between the two arms. The peripheral devices (readers, plate washers, dispensers, incubators, etc) can be mounted on movable carts. The carts are docked to the MicroStar pods via floor plates which supply power, data communications and gases. By moving carts between docking plates the user is able to change the configuration of the automation system quickly. It is possible to run the two arms independently if necessary so that each pod can run a different assay. It is also possible to use the arms for different functions, one pod can prepare compound plates while the other pod adds the biologicals. Cart-based devices are not limited to being used only on the automation system, carts can be taken off line and external power and gases can be supplied to allow the device to be used anywhere in the laboratory.

The peripheral devices stand on turntables which enable an operator to use the devices even when the robotic arms are building assays. The Cellario control software knows which way the device is facing and, therefore, if the arms can safely access the device. This effectively means that integrated devices are not locked down during a run and can still be used off line. This reduces the need for additional stand alone devices in the lab and allows assay development teams to utilise the same dispensers or readers that will be part of the final automation solution. Most of the devices that were required to populate carts were already available within the laboratory and could be retrospectively mounted on to the carts once the automaton system had been installed. Since the peripheral devices were already being used by the screening teams it was thought that there would be less resistance to using the automation platform once installed. In addition there would be less need to re-optimise assays when transferring them to the automation system as the only difference between the two processes was that an articulated arm was moving the plates between devices.

AstraZeneca agreed to purchase a dual pod MicroStar system in September 2008. To reduce the impact of the capital purchase it was decided that the peripheral devices to be docked around the system would be those already in use in the laboratory. However, this presented a problem because it would not be possible to supply these to HighRes Biosolutions in its Boston, USA facility for a typical factory acceptance test. Once the arms had been mounted on to the pods, equipment was utilised locally from vendors and devices owned by HighRes to enable testing before the unit was shipped late in November 2008.

After testing in the US, the automation system was broken down into modules ready to be shipped to the UK. By shipping the system in modules rather than as components, the time required to reassemble the system on site would be reduced. The modules were delivered to the laboratory on 5th January 2009 and the system was assembled and supplied with services by 9th January. During the following week, devices were taken out of service in the lab and mounted on to carts to reduce operational down time. Once the devices were placed on carts, robot access movements were taught and the system was ready for site acceptance testing during the week commencing January 19th. Careful co-ordination was required during this final phase so that devices could still be accessed for generating data off line while leaving time to carry out the required site acceptance testing and training. The system was handed over to AstraZeneca on 9th February 2009.

References

1 Wingfield, J, Jones, D, Clark, R and Simpson, P. American Drug Discovery 3(3), 24-30.

By the end of February the system was routinely running 1536 well assays and several of the 384 well assays had been transferred on to the system. The 'user friendly' software interface coupled with familiarity with the peripheral hardware enabled a rapid user uptake. Initial teething problems interfacing the control PC with the local IT security were quickly overcome and users began to gain confidence with the system. Several of the more labour intensive ELISA assays were run using the system and operators were confident enough in the system to allow these to run unattended over night. Currently, the presence of only one Nanodrop Express unit on the device is limiting the number of assays being run on the system, though this is being addressed.

Within secondary biochemical screening supporting the cancer department there has been a 46% decrease in revenue spend during the first half of 2009 compared to the same period in 2008. This saving is directly attributed to the deployment of the HighRes Biosolutions system, converting several expensive assays to 1536 well format and moving to the low dead volume Nanodrop Express dispenser. In addition, the utilisation of capital equipment more widely across different departments will significantly reduce the capital expenditure over future years.

Conclusions

The centralisation of secondary screening within one capability led to a significant decrease in resource required to generate the required biochemical screening data. In addition, once centralised it was possible to analyse the various stages of the screening process to identify areas where cost savings and quality improvements could be made. Initially there was a drive to acoustic droplet ejection technology to increase the quality and throughput of compounds preparation. While this had a significant capital expense, the savings made by reducing solvent use and plasticware were shown to offset the cost of purchase within two years.

Switching to low dead volume reagent dispensing for bulk reagent additions again had a significant impact on revenue and has enabled moving to higher density assay formats. Extending the principle of centralisation to encompass cross department working offers an additional avenue for capital cost reduction. Purchasing large scale automation that can support the differing needs of two diverse research areas can be challenging, but ultimately if the automation is both flexible and adaptable can provide long term cost savings.

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Dr Jonathan Wingfield *received his PhD from the University of Wales in 1990. He carried out post doctoral research at Childrens Hospital in Cincinnati before joining the Yamanouchi Research Institute in Oxford, UK. While working in Oxford he established an automation system to build and test assays for high throughput screening. In 2000 Jonathan joined the Cancer Department at AstraZeneca as part of a team dedicated to support secondary screening automation. By 2003 the value of automation in a post high throughput screening setting had been recognised, Jonathan was tasked with leading a team dedicated to providing automation solutions to projects in the lead generation phase. Under Jonathan's leadership the team evolved into a centralised biochemical screening capability in 2005. This team is responsible for generating all the biochemical data for cancer projects within AstraZeneca.*