Compound storage evolution: unlocking the right combination

Delivering the correct compound, at the right concentration, to the correct place, at the expected time across different facilities, should be the aim of all liquid sample management teams. This article discusses how the team at Wyeth, went about streamlining and automating their compound management process.

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The paradigm for drug discovery and High Throughput Screening (HTS) has changed significantly in recent years. The number of compounds routinely screened has grown exponentially as increased synthetic chemistry efforts and new therapeutic foci have contributed to the expansion of compound libraries. In the past decade there has been major investment in systems for automated storage and retrieval of compounds. Although now the initial rush has rather subsided, the focus has shifted to the expansion, upgrade or replacement of existing systems to allow pharmaceutical companies to adapt and grow their facilities as their screening demands evolve. In earlier years, our Liquid Sample Management group at Wyeth worked with vendors to source innovative automation tools to transform our compound management facilities, and as our HTS strategies increased in complexity and number, our team had to re-evaluate these to gear up for expansion and relocation.

Chapter 1 – The Wyeth, Pearl River story

Very early on in the evolutionary process at Wyeth we set ourselves the goal of 'delivering the right compound at the right concentration to the right place at the right time'. It was recognised there would be some significant hurdles to overcome to get there but the benefits to the internal screening community would be invaluable. Essentially, our Liquid Sample Management group initiated a process of investigation and evaluation to source a selection of automation providers and technologies for an integrated compound storage and handling system that would increase throughput, maintain long-term compound integrity, improve reliability with complete inventory control and offer unattended operation. Moreover, the contemporary R&D climate and rapidly growing screening demands dictated that the future proofing of products was essential.

Key drivers for automation

In 1999, following the acquisition of American Cyanamid/Lederle and the subsequent inheritance of a varied and extensive compound library located at numerous sites, our Liquid Sample Management group began a process of consolidation. Obviously, the key objectives of such a project were influenced not only by corporate strategy and increasing demand for ROI but also the combined research needs. With a growing compound library, chemists at Wyeth were placing increasing priority on the development or purchase of a system that could efficiently maintain compound integrity. This, together with the fact that a strategic focus had been placed on 'ramping up' HTS and increasing

throughput, with a commitment to invest in automation our team was ready to explore the marketplace for new and innovative technologies for compound storage, retrieval and preparation. This would add value to the substantial efforts of the upstream medicinal chemistry teams and the downstream drug discovery scientists.

The key driver was to streamline the processes involved by using automation and we knew we needed to:

- Consolidate the diverse range of storage formats and compound libraries – both established and acquired.
- Standardise compound storage format.
- Bring all compound storage to one site.
- Replace ageing fleet of more than 70 obsolete freezers where compounds were manually retrieved, with a state-of-the-art system.
- Improve and maintain integrity of expanding compound library through effective environmental control.
- Reduce time spent on manual/semi-automated sample preparation. (In 1999, 50% of screeners time was spent on this task.)
- Reduce the number of plates. (The facility had its compound library in a combination of 96-well storage plates and screening plates in 384-well format. Additionally, with 15-20 copies of each, there were thousands of working plates in the system.)
- Improve the cherry picking of hits as this was the weakest link in the HTS set-up.

Finally, as the facility in Pearl River, NY, was serving six therapeutic areas at four different sites, of which two HTS laboratories were 75 miles apart, there was a high probability that the facility would, in time, either need to be expanded or moved. Thus the project was initiated under the guiding principle that the facility would be modified and systems incorporated with a view to expansion and possible relocation.

Out with the old... replacing rapidly ageing technologies

It was clear to the team that we needed to standardise and automate our compound storage facilitates and replace our room of cavernous, rather archaic freezers with a state-of-the-art alternative that could be fully integrated with the screening workflow. However, we were reluctant to embark on a journey, common to many at the time, of incorporating monolithic and inflexible systems that used complex robotics.

Internal evaluations enabled the team to put together a list of requirements to present to potential vendors. To begin the process, an evaluation to determine the preferred storage temperature was initiated. A preliminary stability study on approximately 70 compounds over a period of seven

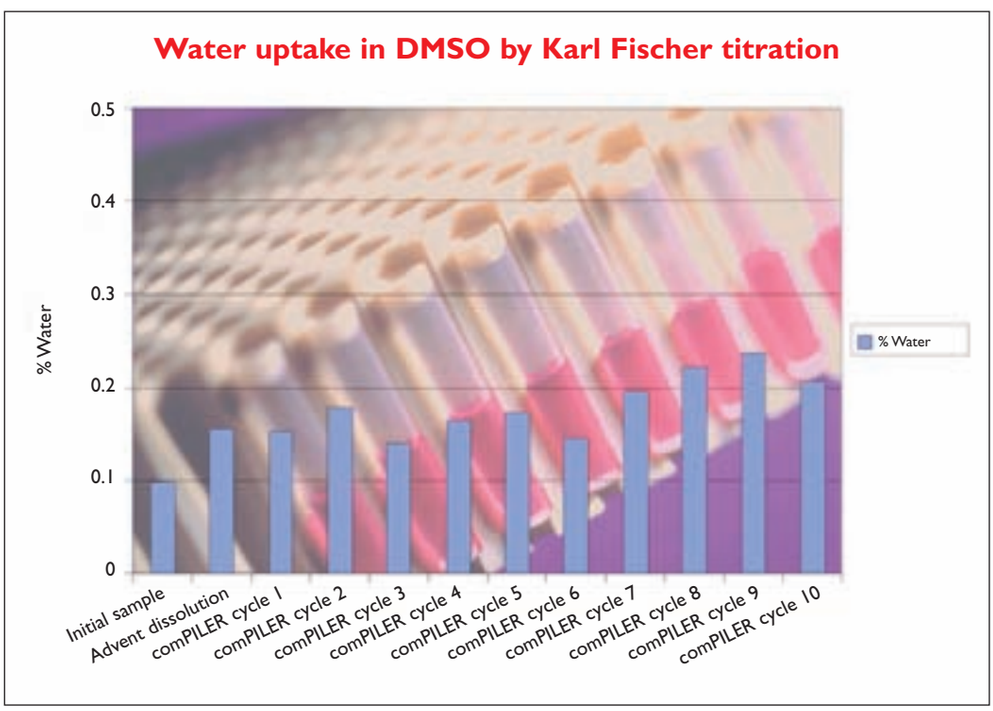

Figure 1

months, and three different storage temperatures (-80˚C, -20˚C and 4˚C), demonstrated that -20˚C was the optimal storage temperature. Further review and analysis revealed our core objectives to be as follows:

- Automated storage and random access for one million samples in plates/tubes at -20˚C.
- Automated retrieval and replication of plates.
- Automated sealing/unsealing and bar-coding.
- Disposable pipette tips to minimise contamination of samples.
- Mechanical plate/tube seals.
- Integrated hit-picking from microtubes.
- Stable environment – dry, inert gas to prevent the uptake of water and oxygen.
- Modular, flexible system to serve remote sites.

Optimising sample integrity

To address the issue of sample integrity the team first looked at how dimethyl sulfoxide (DMSO) solutions were prepared and stored. Our researchers at Wyeth, along with many others in the industry at the time, were having growing concerns about the exposure of solutions during the preparation and storage of stock compound solutions. Thus, the priority was to find a system that could facilitate storage in an inert atmosphere, obviating the rapid uptake of atmospheric water or oxygen that is normally attributed to the breathable nature of the polypropylene vials and the hygroscopic nature of DMSO. As a consequence we partnered with Advent Design Corp to develop a system that allowed the dry compounds to be flushed with nitrogen before dissolving them in DMSO, which itself had been distilled in nitrogen prior to pipetting into the polypropylene vials. Following installation of the Advent system in late 2001, we began the long process of compound dissolution and reformatting. Consequently all compound powders could be provided in 1.4ml tubes and following preparation, presented as 30mM DMSO solutions in microtubes.

To achieve an inert storage environment the team toyed with the extravagant idea of a nitrogen-filled storage room and colossal (both in size and price) stand alone nitrogen-storage systems before turning to a respected British company, TTP LabTech, with its unique storage solution, comPOUND. The incorporation of two comPOUND units provided solutions to many of the key objectives, including improved sample stability through the -20˚C storage of up to 100,000 1.4ml tubes or 200,000 0.5ml microtubes per unit in an inert, dry, dark environment. In each comPOUND unit samples are stored in a series of 26 carousels enveloped in nitrogen and held in a hermetically sealed unit, approximately 8ft in height. These systems are compact, which simplified the installation procedure, and their modularity not only reduced the scale and initial cost of the system but provided a viable upgrade path as the library grew. Both comPOUND units were linked in parallel, with the option of adding more systems to this set-up with time and increasing the system throughput with the number of stores linked. As such, the initial installation of two comPOUND modules was rapidly followed by a third in late 2002.

Facilitating rapid sample retrieval

Using this system and its random access format meant that requested sample tubes could be rapidly retrieved and cherry picked from either comPOUND store for reformatting into microplates, via a pneumatic delivery system (not a robotic arm as with many of the existing systems) and delivered into racks. Individual sample selection also mitigates any unnecessary freeze-thaw cycles, inherent with plate-based systems. Furthermore, to facilitate efficient sample tracking every sample tube carries an individual DataMatrix 2D barcode which is read upon exit and re-entry to the store, thus making it nigh on impossible to select and plate out the wrong sample. To further streamline the process and relieve our researchers of the manual pick up of assembled racks, TTP LabTech then developed comPILER, a rack assembly and screening preparation device. This automated store-to-plate processor, assembles racks of tubes in an arraying station where they are then passed through a thawing tunnel, and centrifuged prior to automated decapping and extraction. All decapped tubes are immediately purged with argon gas to provide an inert plug against the atmosphere prior to pipetting and reformatting using a liquid handler. For this, TTP LabTech collaborated with TekCel (now Hamilton Storage Technologies, Inc) to incorporate its Assay TekBench (ATB) liquid handler and develop an integrated hybrid system for tube-to-plate transfer, allowing our team at Wyeth to make a new HTS library in 384-well format. One of the main advantages here was that the whole comPILER/ATB system did not need to be kept inert and dry, only the tubes being processed were exposed to the microenvironment. This not only prevented the uptake of atmospheric water (**Figure 1**), but it was of course better

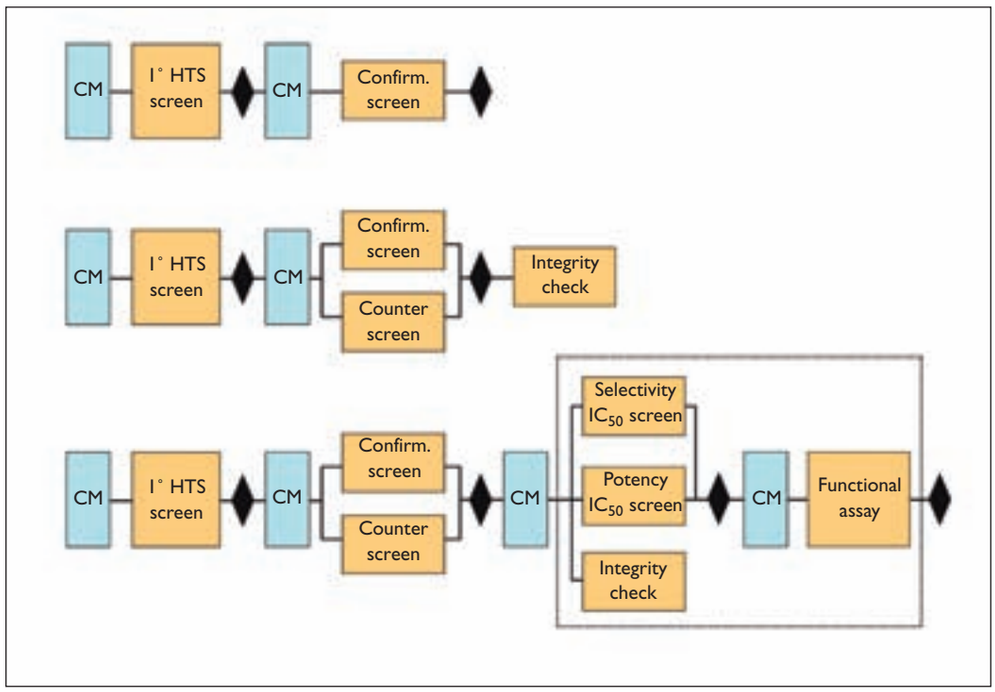

Figure 2
Evolution of HTS at Wyeth

from a system reliability, maintenance and cost points of view.

As it stood, with the purchase of the Advent Design system, three comPOUND stores and one comPILER from TTP LabTech and the TekCel Assay TekBench, our team had acquired a robust and self-contained platform for unattended operation. Existing compound libraries could be amalgamated, and sample reformatting and preparation fully automated for subsequent microplate storage and transfer to various screening sites. These new installations became key to the successful management and supply of compounds across the Wyeth screening facilities.

Effective HTS campaign management

As the Wyeth screening strategy moved to increase the overall number of HTS campaigns and screens per annum, so the number and complexity of screening steps increased (**Figure 2**). Through the implementation of strategic HTS campaigns for specific therapeutic or target groups, many steps of the screening process at Wyeth were, and still are, revisited. Simply, following the identification of hit compounds from primary screens, compounds that undergo additional hit assessment through secondary confirmation and counter screens must again be retrieved from storage. The same applies for successful compounds passed to potency and selectivity IC50 screens and compound integrity testing using analytical chemistry. Effective hits are then tested and further validated using cell-based assays. In essence, in one Wyeth HTS campaign a compound maybe accessed and re-analysed up to five times, and the primary concern was to maintain sample integrity over this timeframe. comPOUND allowed the effective retrieval of microtube subsets for retest and subsequent secondary screens but a real focus for the team was to prepare ultra low-volume, single-use, disposable compound tubes, that would negate the need for the multiple access of any one sample and would optimise sample integrity over the entire screening campaign. However, as the screening activity continued to ramp up into 2004, so began internal discussions to look at the relocation of the facilities and the subsequent evaluations of systems for a new site in Collegeville, PA. This provided the team with the opportunity to address these new requirements by looking at new technologies for integration.

Chapter 2 – Relocation

Further consolidation

In preparation for relocation and upgrade of our facilities in 2006, our Liquid Sample Management group at Wyeth initiated a program in 2004, working with selected vendors, to further improve and expand our set-up. The successful and ongoing expansion of our existing primary TTP LabTech

System history and expansion

● 2002-2003	comPILER plus comPOUNDS 1-5 in PRI
● 2004	comPOUND 6 added in PRL
● 2006	comPLETE system relocated
	comPOUNDS 7 & 8 added major pms
● Late 2006	comPOUNDS 9 & 10 added
● 2007	V11 plate processor added
	2-D plate barcode scanner added

Figure 3
Timeline for comPOUND modules added

comPOUND/comPILER platform (**Figure 3**), together with its modular nature meant it was integral to the relocation process. With scope to add another seven comPOUND modules to the existing comPOUND/comPILER platform, there was still much room for growth. Locating a picotube store to facilitate ultra-low volume single-use sample storage was of paramount importance and integrating liquid handlers into the compound management stages adjacent to each of the five steps of the HTS campaign became a priority.

In preparation for the move, our team sourced the Solar PicoTube storage and microplate generation system from The Automation Partnership (TAP). Using Matrix picotubes alongside multiple types and formats of microplates, the system enabled the single shot sample handling and microplate generation that was so much needed.

Figure 4
Compound management platform Collegeville, PA

Furthermore, when combined with the TAP BasePlateXR for assay ready plate production, sealing and temporary storage (using PlateSafes), we had identified a system which operated at an even higher throughput and had the potential to supply follow-up screening plates to the HTS group at a projected rate of 50,000 samples accessed per day. As each campaign comprised of roughly 5,000 microplates, which had previously been manually collected and transported across the lab from one step of a campaign to another, the incorporation of this system would, and did revolutionise the screening workflow. Compounds were now retrieved from storage in comPOUND using the comPOUND/comPILER platform, and transferred from a microtube format to 384-well master plates. These were then passed to the TAP platform (HomeBase and BasePlateXR) for picotube preparation and storage (in single access format), and for the production of replicate daughter plates for storage prior to screening.

Current operation

Following the relocation and upgrade programme of 2006, we have expanded our storage capacity significantly to approximately 1 million compound samples (**Figure 4**). Today, with 10 comPOUND systems and one comPILER, we have vastly increased our throughput to the point that we can retrieve, process and re-store approximately 10,000 compounds a day. TTP LabTech has also integrated the storage and retrieval platform with a Velocity 11 plate processor for the preparation of mother plates to be used for Picotube and daughter plate generation. New software developed for this integration has also allowed the collation of individual plate data and the recent addition of a 2D rack barcode reader into comPILER has vastly improved compound tracking. Each compound is checked on removal from comPOUND, on receipt at comPILER and on re-entry to storage. Furthermore, all tracking data is linked to the databases of both the TTP LabTech and TAP systems and fed into the Wyeth corporate Oracle database. It is thus possible to track every sample, and identify its storage/screening format, volume and location. The TTP LabTech database can provide 'transaction' information on each plate passed to the TAP system which is logged for each chemical compound solution, ie number of freeze-thaw cycles, etc. Additionally, full tracking can be accessed and downloaded in the form of reports by simply requesting it from the system. An invaluable tool to aid the accommodation of the rapid increase in compound requests.

Meeting previously unmet internal and external demands

The entire internal screening community has realised the benefit of this streamlined system. Today, valuable compounds are in frozen storage in dry DMSO, processes are better defined, cherry-picking is 10-fold faster and minimal sample volumes are available for all customers. Furthermore, with automated downstream storage and integrated liquid handling, screeners not only have easy access to mother/working plates in a screen ready format, but also have the ability to rapidly process these for replicate daughter plate formation. As such, multiple groups have gained access to the screening facility at the Collegeville site, and compound requests have increased exponentially across numerous groups, ie for primary screening plate production, HTS confirmation, hit characterisation in research areas (RAs) and supporting groups, and analytical chemistry. The deposition of whole screening libraries into one central repository has allowed the biologists to access any compound or group of compounds they require in a screen ready format. As the number of HTS campaigns has continued to rise (**Figure 5**) (40 campaigns a year is the present total; equating to 700,000 compounds per campaign in primary screen [2000 plates], and 350,000 samples in follow-up assays [2000 plates]) and the downstream steps have also increased in complexity, so our automated systems for compound storage, retrieval and preparation have come into their own. DDW

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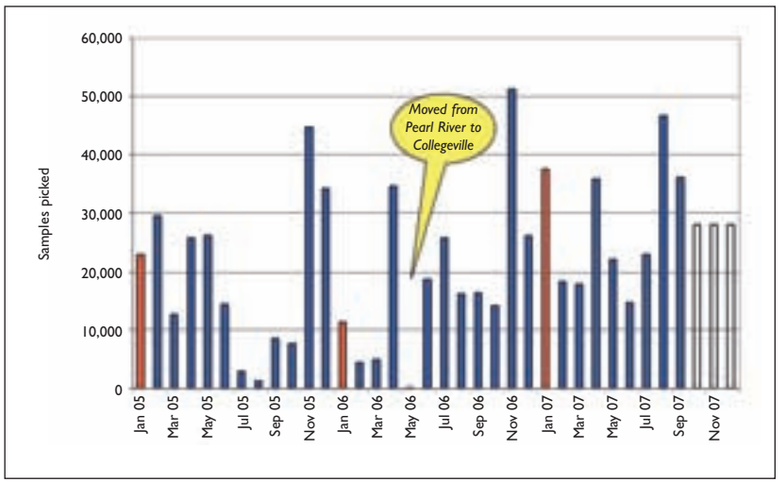
Dr John Morin *directs the HTS, compound management and cell culture functions for Screening Sciences at Wyeth. After receiving a PhD in Biochemistry at Purdue, he trained in Molecular biology at the University of Vermont and UCSF before joining Wyeth's biotechnology group in 1984. His career spans the development of viral vectors in live vaccines, hit to lead activities in an antiviral chemotherapy screening programme, and positions of increasing responsibility in Wyeth's core screening groups.*

Dr Jeff Paslay *leads the Screening Sciences department at Wyeth spanning compound management, HTS, assay development, hit-to-lead characterisation and profiling. After receiving a PhD in Molecular and Cellular Biology from the University of Alabama in Birmingham and completing a Postdoctoral fellowship in Immunology at Washington University, he joined The Upjohn Company in 1981. His career has spanned the pharmaceutical, biotechnology and contact research industries with positions at The Upjohn Company, MDS Panlabs, Cellomics, Abgenix and Wyeth.*



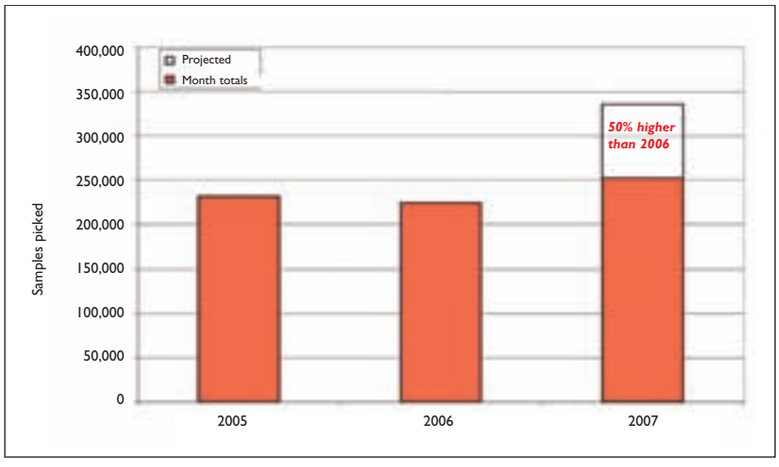


Figure 5: Registration monthly totals (above) and registration yearly totals (below)