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Compound Library Management

An Overview of an Automated System

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1. Introduction

The Automated Liquid Sample Bank (ALSB) is a key component of our strategy to increase drug-discovery productivity and to reduce manning requirements wherever possible.

1.1. Need for Automation in Sample Supply

Discovery management had set a challenging target for growth in candidate generation, and in order to achieve this, a coordinated strategy to increase our lead-seeking capabilities was developed. This included significant investments in combinatorial chemistry, both internally, and externally with Arqule, in order to enrich our files with thousands of drug-like molecules amenable to combinatorial follow-up. As a result of this, the then-current compound file was expected to increase by some 50% by end 2000 and it was essential that these compounds be exposed to as many of our lead seeking targets as possible. A parallel initiative, involving collaborations with Aurora and Evotec, will increase the annual number of high-throughput screens (HTS) run.

This plan implies an enormous increase in sample handling and throughput to meet the large increase in the number of HTS to be run against a growing number of biological targets, and the increase in the size of the file against which they would be run. Our ability to dispense samples for test on the scale required to support this effort was far beyond our existing manual capabilities. Our system for sample dispensation was a mixture of manual and semi-automated effort: labor intensive and prone to human error. Since our existing liquid store was contained in deep well microtiter plates, follow up of actives

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required manual handling of large numbers of compounds to access the few required and meant that recourse to dry samples was the only solution for the compilation of a targeted subset, which was impractical for sets larger than several thousand compounds. If manning resource for sample preparation was not to become excessive (and we estimated that an increase of 10 FTEs would be required to meet the predicted requirements), or sample provision to prove a bottleneck in the process, the requirement for automation of the storage and dispensing process was clear.

1.2. A Secure Store

The composition of our drug file was also changing. Where once dry sample had been the major storage form for the file, increasingly high-speed chemistry was producing many compounds but in smaller quantities, such that weighing and dispensing from a dry store was not viable. The only source of the majority of our compound file soon would be from a liquid stock and it was essential that this could be securely stored and efficiently used.

1.3. Requirements of the System

Projections of the likely storage requirements, needs of HTS, and follow-up, led to a specification of an ALSB) that would have an initial capacity to store up to approx 2.8 million samples, would deliver up to 44,000 compounds per day for screening, and could support our HTS initiatives for at least seven years (specification fixed in 1997). By streamlining sample provision, ALSB was expected to make a significant impact on the number of HTS-derived CANs (compounds submitted for development) in our portfolio, and hence increase our overall productivity. ALSB would also keep us at the forefront in exploiting high-speed technologies and would provide the first step in developing a worldwide Pfizer strategy for dispensing screening samples.

The ALSB was designed to store all compounds as liquid samples at –20°C, with robotic retrieval and distribution to a range of screens via computer control. Compounds are stored individually in tubes, held in rack arrays, such that single tubes or an entire rack can be selected. Therefore HTS sets can be compiled quickly by extracting and sampling from entire racks, while thousands of samples can be assembled for screening in any order, individually, or as structurally related families, by tube-wise selection, almost 100 times faster than the existing system. Moreover, since compounds are stored in individual tubes, retrieval of active samples for follow-up of HTS is much more efficient than manual operation, that relies on retrieving and thawing entire deep-well plates. Importantly, the database of compound inventory usage and biological activity for all samples would be integrated into central information technology (IT) systems such as DISCUS (our global screening database), for rapid and reliable

dissemination of information. ALSB would also be integrated with in-house compound ordering and experiment creation and analysis software to provide a smooth workflow for the scientist with a strong audit trail for compound and test result. As part of a world-wide strategy for global compound ordering and management ALSB can also be used to furnish those samples not available locally to Pfizer Global R & D centers in the USA, Europe, and Japan.

2. The Manufacture and the Design

2.1. The Idea

Once the case for an ALSB had been made (albeit not at this stage approved), the team produced a summary specification of how we saw the final product. The key goals of the system were that it should:

- Maintain a bank of samples.
- Maintain an inventory of stored samples.
- Accept orders for samples and schedule preparation.
- Prepare ordered samples by transferring the required volume from the stocks held in the bank to mother plates. If required, dilute the mother plates and produce (multiple) daughter plates.
- Interface with existing equipment and software.
- Achieve critical success factors that would assure controlled and reliable operation.
- Be a commercially realistic package both for Pfizer and for the vendor.

As we were planning to use this specification as the basis for the document that would be sent out to potential suppliers of the system, we were very careful to avoid indicating possible solutions to the goals listed previously. In this way we hoped to encourage companies to identify creative solutions rather than to redevelop our preconceived ideas.

2.2. The Tendering Process

The user requirements document was distributed to 20 companies, in both Europe and North America, together with a letter inviting the submission of tenders to design, build, and install an ALSB at the Sandwich, UK, Pfizer site. Of these only six companies were prepared to undertake the complete project, several others indicated a desire to involve themselves as subcontractors to the main contractor.

A working party involving key members of Pfizer's chemistry, biology, IT, and engineering departments was commissioned to review in detail the design-specification documents of those companies offering complete solutions. The outcome of this working party was to identify a short list of three companies. Upon notification, the three companies were invited to Sandwich to present

their solution to Pfizer's ALSB requirement, and demonstrate that they were competent to undertake a project of such magnitude.

In the interim period between notification and presentation, representatives from Pfizer accepted invitations to the production facilities of each company to inspect projects in development and to the sites of previous customers who where willing to demonstrate already completed projects. In addition, Pfizer legal and financial teams investigated the viability and credibility of each of the three candidate companies.

After on-site presentations from each of the three competing companies, there followed an intensive series of internal meetings at which the case of each was minutely examined. Not surprisingly, the solution offered to the problem by each of the tendering companies was quite different in concept, though certain basic threads ran through each solution: frozen storage, single rather than multiple sample copies, tube based system, etc. For a variety of reasons, including the quality of their engineering and their reputation built on the large-scale products they had supplied to other customers, Pfizer selected the tender of RTS Thurnall (then Thurnall plc) and requested that they begin preparing a detailed proposal of how they would achieve the project objectives.

2.3. From Idea to Design

One of the more difficult aspects of the ALSB project was communication of concepts and ideas. A team of biologists and chemists had conceived how a system might automatically provide samples for biological testing. This scientific concept now had to be translated into terms that would allow a team of hardware and software engineers to identify the technical solution. The Functional Design Specification, a close look at each functional element in each area of the system, was based on a mutually agreed System Design Concept, which gave a clear description of the system input and outputs. The preparation of these documents required several hundred man-hours of discussion and debate, much of which concerned the appreciation of the relevant 'business' of each other's industries. We learned from this exercise that it is virtually impossible to explain points in too much detail. We realized, too late in some cases, that as HTS specialists, we had thought that there was only one sensible way of doing some things; however, it was wrong to assume that an engineer would have the same understanding as a biologist. From an engineer's viewpoint, alternative methods were perfectly logical, but could have caused great practical problems for the biologists.

3. Development and Building – What, Why, and How?

For reasons of compound stability, we elected that samples should be stored in 100% dimethyl sulphoxide (DMSO) at –20°C. In addition the system should

prepare daughter plates for screening (a 'daughter' plate being defined as the screen ready plate derived by dilution from an intermediate 'mother' plate, which is derived by dilution from the 4 m*M* source stored in the ALSB), and that these daughter plates should be prepared in as timely a manner as possible. Therefore, at the highest level, the ALSB was to consist of a frozen store, a station in which to thaw the samples, and liquid handling robotics, all connected by a transport system. **Figure 1** shows the final layout of the system.

An attractive feature of the RTS Thurnall solution was the use of tried and tested components throughout the system. For example the Flexlink conveyor system (Flexlink Systems Ltd., Milton Keynes, UK) connecting the various parts of the ALSB is identical to that found in hundreds of factories throughout the world. Again, samples were to be stored in vertical paternoster carousels, which are widely used in warehouses and stores in many industries. The principle behind this approach was to build reliability into the system at the planning stage.

3.1. Cold Store

The cold store is a 500 m^3 room maintained at –20°C. This room houses the samples, held in racks of 90 tubes, together with about 18 tons of plant and robotics. Forty-three racks are held on each storage tray, which are held in banks of 3 at 60 levels on each of 4 Paternoster storage carousels. Multiplying up, $90 \times 43 \times 3 \times 60 \times 4$ gives the capacity of the system: in excess of 2.7 million samples. Following an order, the carousel level containing the sample will rotate to a preset position where one of 12 tray-pulling robots will pull the entire tray of 43 racks out of the carousel. The picking mechanism of one of the twin overhead gantry robots (one robot serving 2 carousels) will then select either an individual tube from a rack, or the entire rack of samples, depending upon the order makeup. These gantry robots weigh approximately one ton, are around 3 meters long, and, over an operating envelope of 9 sq. m., can position with an accuracy of 1 tenth of a millimeter. Individual tubes are placed into a transport rack, or entire racks are selected by the robots and the racks of tubes, identified by bar code, are placed on the cold-store outfeed conveyor and transported to the to the defrost oven.

3.2. Defrost Station

Since samples in 100% DMSO at –20°C will certainly be solid, prior to aspiration the samples are gently thawed into the liquid state in the defrost station. Racks of samples are transferred from the Input/Output port connecting the defrost station with the cold store, to the next available shelf of a Carbolite Paternoster oven (Carbolite Furnaces Ltd., Sheffield, UK) contained in the defrost station. After a pre-set time, the rack of samples, now thawed, is

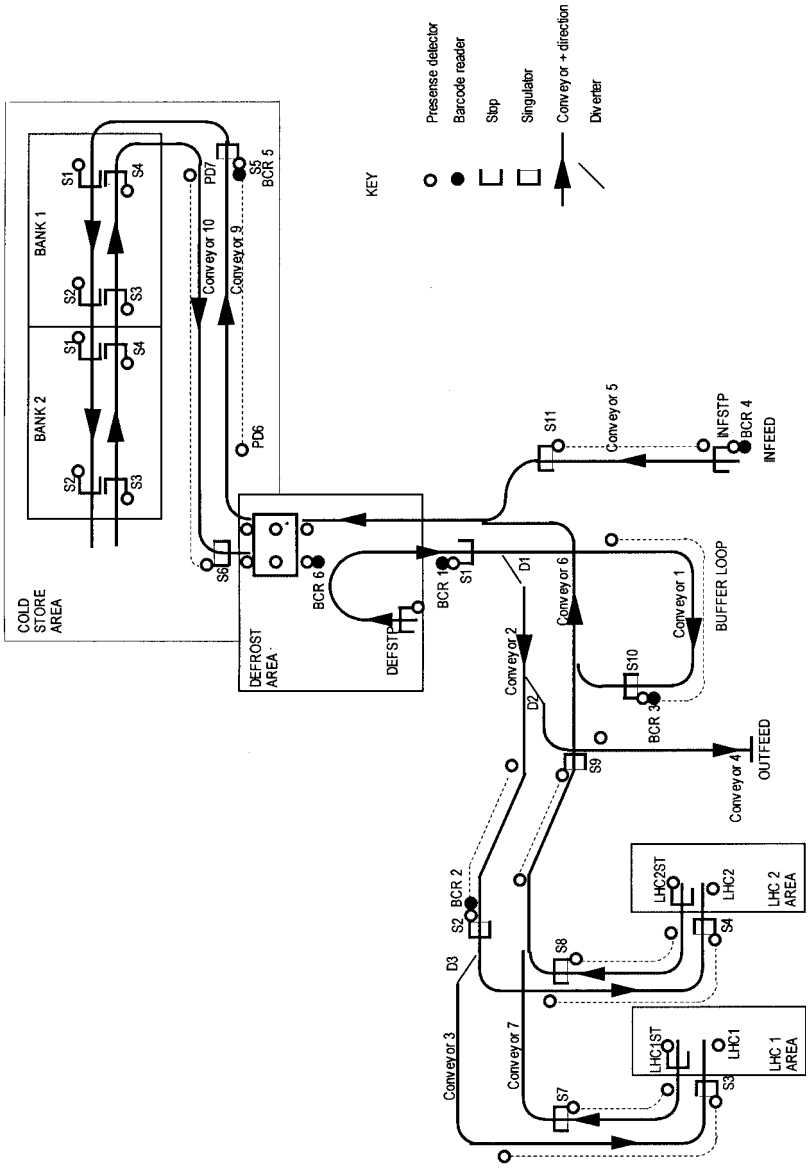

Fig. 1. Compound library management – an overview of an automated system.

picked and placed on the conveyor leading into the laboratory environment of the ALSB. The operating temperature of the oven, and the time samples are contained within it have been selected to minimize thermal exposure, while ensuring the entire rack of frozen samples are completely thawed. In addition, the defrost station atmosphere is maintained at 15% relative humidity to reduce ingress of water into the cold-store environment.

Samples being returned to storage bypass the oven and are carried straight back into the cold store, passing under a high velocity air stream to evaporate any post liquid-handling micro-droplets that might possibly adhere to the top of the septum seal of the tubes.

3.3. Liquid-Handling Cells

The ALSB system has twin liquid handling cells in which the samples are aspirated, diluted, and dispensed into the format specified in the originating order. The hardware configuration of each cell is identical, and is shown in **Fig. 2**. Selection of which liquid handling cell a sample rack is sent to is made by the system software with priority to keep samples from the same order together. If required, it is possible to manually override the system selection.

Incoming racks of tubes are picked up by a 6-axis Stäubli robotic arm (Stäubli Unimation Ltd., Telford, UK) that traverses the length of the cell. As part of the failsafe design, status checks occur at all stages, and the initial operation of the Stäubli robot is to run the rack of tubes it has picked past the on-board barcode reader to ensure the samples are those specified at this stage of the order process. The rack is placed on one of nine positions on twin platens designed to locate in the operating envelope of a Tecan Genesis robotic sample processor (Tecan UK, Reading, UK). This twin platen arrangement allows the Genesis to work on the contents of one platen while the Stäubli robot unloads or loads the second platen. Dilution algorithm software calculates the most efficient and compound sparing aspirate-dispense protocol to achieve the delivered volume, compound concentration and DMSO concentration, in the output plates specified in the original order.

The Stäubli robot collects the appropriate labware from infeed stack locations, checking each selection by scanning its type identifying bar code. Prior to liquid handling, the system sprays the order information on a blank label on the final destination (daughter) plate. Once all labware is assembled the platen retracts and the Genesis probes pierce the tube septa to aspirate the sample. All destination racks are open but since the source tubes are sealed the probes are designed with an outer jacket open to the atmosphere to equalize pressure each side of the sample tube.

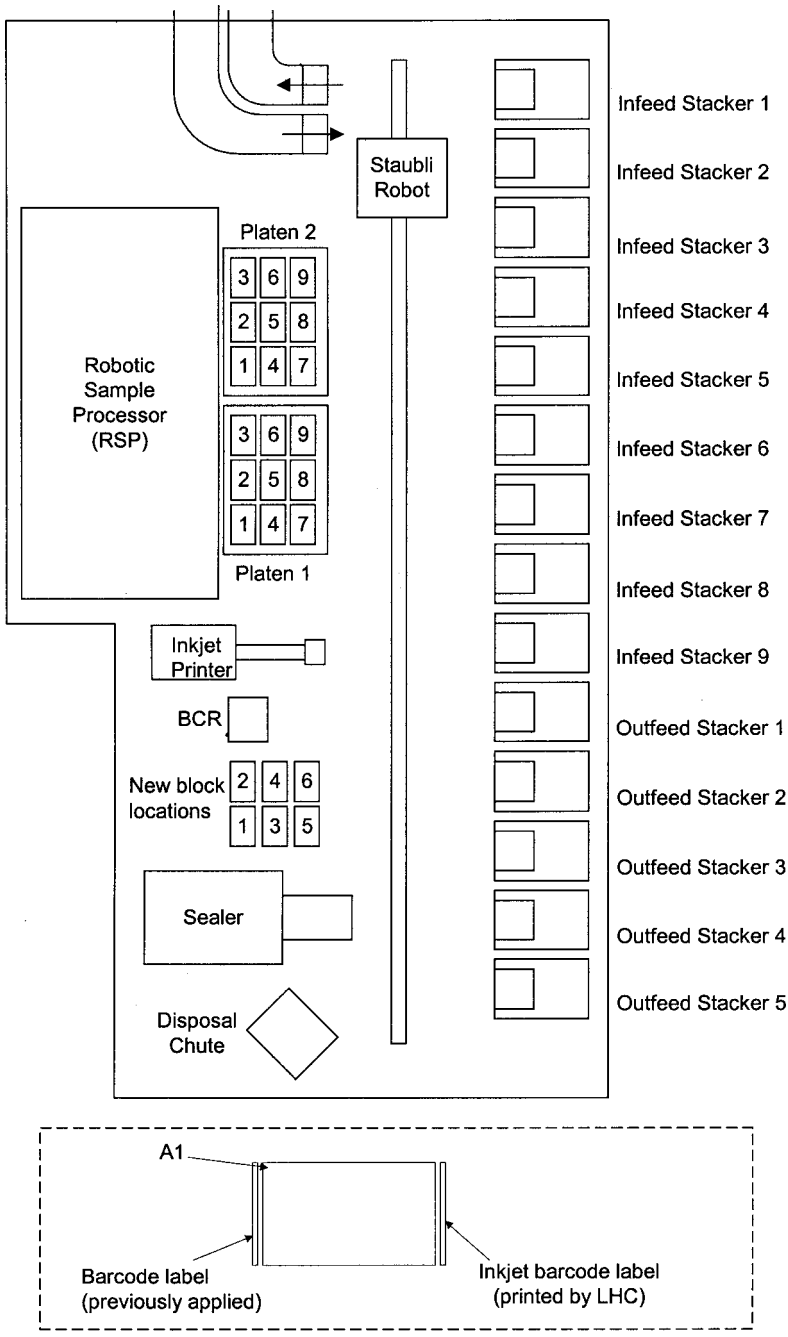

Fig. 2. Hardware configuration of an ALSB liquid handling cell.

A further modification of the Genesis is a grid held above the tube racks through which the probes can pass. This grid prevents tubes being carried with the probes once they retract through the septa. Once all liquid handling is complete, the transport rack and tubes is placed on the outfeed conveyor loop for return to the cold store. The volume field for each tube in the database is decremented by the amount aspirated. Labware used in the order is sealed, daughter plates being delivered to the outfeed stacks, and any labware used for intermediate dilutions dropped into the disposal bin. The capacity of each liquid-handling cell allows up to 1200 microplates to be available at one time, together with an outfeed capacity of 700 plates.

3.4. Transport System

The three major components of the ALSB—cold store, defrost station, and liquid-handling cells—are connected by a series of 10 Flexlink conveyor systems. In order to optimize the use of space, the conveyors are on two levels, the upper being outfeed and the lower level for infeed, relative to the cold store. Components situated along the transport system include:

- Barcode readers to confirm the correct identity of each rack at critical points in the system.
- Diverters, which direct sample racks along the selected path when there is a junction point, e.g., to divert to either of the liquid-handling cells.
- Singulators, which allow only one rack at a time to feed into certain areas, such as the pickup point between the cold store and defrost station.
- Presence Detectors, which pick up a signal from a downstream unit that a rack is in transit, and will alert the operator should the correct labware not be detected within a certain time period.
- Software-controlled retractable stops prevent buffer loops and the defrost station from overfilling.

With safety a key consideration in the ALSB, all drive wheels and conveyor stub ends are guarded, and the track itself is contained within a series of hinged Perspex covers, which, once the relevant conveyor section is halted, can be lifted for cleaning and maintenance. Emergency stop buttons are widely distributed across the system.

3.5. Controlling Software

The software hierarchy of the ALSB is shown in **Fig. 3**.

The Sun Ultra server (Sun Microsystems Inc., Palo Alto, CA) operates using the Solaris UNIX platform, with external disc storage comprising twinned disc sets operating in RAID 0+1 mode and an Oracle RDBS (Oracle Corp., Redwood Shores, CA) providing data storage.

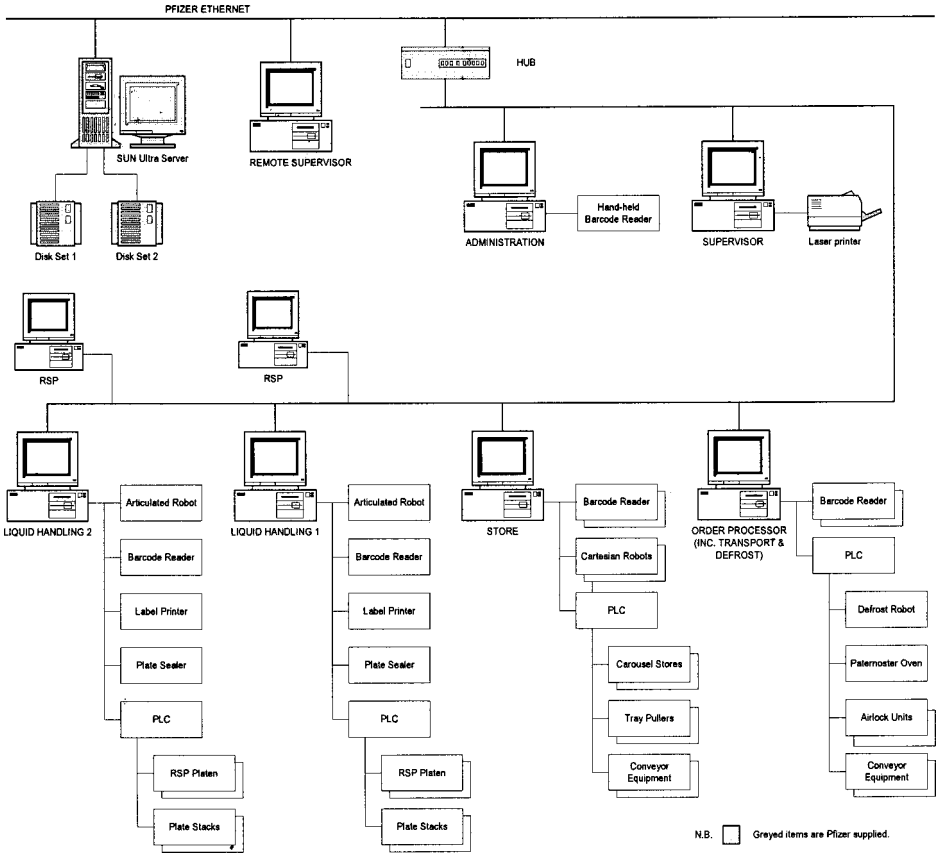

Fig. 3. Software hierarchy of the ALSB.

The controlling and processing computers, and the main ALSB database server, are networked using TCP/IP communications over an Ethernet based Local Area Network (LAN). A bridge connects this LAN to the Pfizer network.

Each 'Application Server' is dedicated to a particular major component of the ALSB, and the status of each these component can be monitored by accessing the relevant server PC. In normal use, these PCs remain locked in the service panels, but should an alarm or a need for maintenance arise, each component can be switched to manual control in order to diagnose and usually rectify the fault.

The Administrator Terminal is the hub of the ALSB from which an order's status can be checked, database queries made, and new samples entered. The administration system is provided with a Graphical User Interface (GUI), which

allows the operator to menu select the various programs that manage the ALSB inventory. Data processing and verification are performed by this system against the ALSB database, and the external Pfizer corporate database, for every administration order. A hand-held barcode scanner aids the identification of specified blocks for entry or replacement. Within the program the operator is provided with selection lists as a guide to performing other transactions based upon predefined criteria for the order type.

A further PC is dedicated to System Control and Data Acquisition (SCADA) software. SCADA supplies a schematic overview of the ALSB in its entirety, and allows the operator a real time snapshot of all the system's components, and the position of any sample racks currently being used in an order. SCADA also interfaces with the Pfizer-specific Building and Environmental Management System (BEMS) and alerts an operator should an alarm situation occur at any time during 24/7 operation.

3.6. Compound Ordering Software

The Order Processor validates orders submitted to the ALSB database and performs short-term scheduling of these orders. This PC processes and schedules orders issued from the Administration System and constantly monitors the order queues in the ALSB triggering the initiation of the next order. The Order Processor monitors and updates the status of all orders issued to the ALSB. Only the administrator is able to generate orders directly via this interface, customers of the system have two purpose built user interfaces which give access to the system; these are described in **Subheading 7.**

4. Labware

4.1. Design

At an early stage in the functional design program, Pfizer agreed with RTS Thurnall that Pfizer would take responsibility for the supply of the storage media that would contain the liquid samples in ALSB. RTS Thurnall did, however, supply specifications as to required size and tolerance of each component. As the labware would be used across the total operating temperature range of the system, these tolerances turned out to be more difficult to achieve than originally imagined. At its most basic, the storage medium specified required a microtiter formatted rack bar-coded at one end, with various slots and holes in the body of the rack to allow robotic grippers to operate, air to circulate, and for the rack to locate, without slippage, on a storage tray. The rack would contain 1 mL polypropylene tubes, each carrying a unique (human readable) identifier, and sealed with a silicon-rubber septum, and conform to a maximum weight specification.

4.2. The Tubes

A tube already available from Marsh Biomedical conformed to the tube specification, and we decided to design the rest of the storage system around this tube. However, it did not carry an identifier, and although in theory tube marking was not necessary, as the software would track all tubes, we wanted the 'feel good' factor that the tubes could, if necessary, be physically identified. In practice, tube identification has proved to be an extremely useful feature. At the time we were designing the storage medium, the uniquely identifiable tubes currently on the market were not available (uniquely identified tubes now available from Integra Biosciences, Traxis® and Matrix Technologies, Trakmate®). Although several manufacturers made claim, none proved able to legibly and durably print or adhere either an electronic or human readable label on the tubes. One remaining possibility was to laser-etch a number on the side of each tube. We had practical experience that this was possible, but concerns that this might not be practical for the large numbers of tubes that we had to prepare.

The challenge was to design and build a machine to laser-etch a unique identifier on each of 1 million tubes, and to electronically associate that number with what would be the rack number and rack position of that tube. We decided that the tube number should be driven by the rack in which the tube was located, thus the tube in position A1 of Rack 1 should be numbered 1, while H12 in rack 1 would be number 96. Tubes numbers in each following rack would increment by 96, therefore it would be possible to predict a tube number by knowing its rack number and location and likewise given the tube number, its rack number and location would be a given. Our solution was to purchase the Marsh tubes already racked and to build a machine to pick up tubes, eight at a time from the source rack, pass them in front of a laser-gun etcher, and transfer the tubes to a destination rack. The laser gun was programmed to etch an incrementing number calculated from the rack number, for each tube. As the software disallowed the repetition of any number, we now had generated a set of uniquely identified tubes.

4.3. The Racks

The destination or storage racks mentioned earlier presented us with another set of challenges The ALSB design allows not only whole racks of tubes to be selected, but also individual tubes. Therefore a key factor in the rack design was that tubes could be freely pulled from, and inserted into, their storage racks over the 40°C range of operating temperature. If the surface of the rack face was not perfectly flat, the tubes would 'stick.' In addition, the holes in the rack could not be oversize otherwise the tubes would have too much lateral move-

ment and may not lie perfectly vertical. Additionally, as we were limited on the mass of the rack, we could not use excessive material in the moulding. After much consultation with a local toolmaker, many prototypes, and reworks of the moulding tool, we finally produced a satisfactory rack. The finished product was a two-piece design with the base locating into the body of the rack. The weight was within limit and tubes could be removed and replaced freely across the specified range of temperatures. However, when the rack was used at –20°C we discovered that for approx 1 in 1000 of the units the rack base became detached from the rack body under operational conditions. There was no option but to recall the entire production run of 10,000 units and have the two components spot-welded together. After several design iterations, we now had our racks.

4.4. The Tube Septa

Each sample tube in the ALSB was to be sealed with a silicon-rubber septum, the performance specification of these septa necessarily being very stringent. Operationally, samples are aspirated from the tubes through a Tecan Genesis probe designed for septum piercing. The septa were required to remain airtight after 160 penetrations by the probe, and during that period show no sign of coring or of being pushed into the tube. We found that once the septum had been pierced for a first time, subsequent piercing tended to follow the original track through the septum. We were able capitalize on this feature by designing a funnel-shaped guide above the tube, so that probes tended to pierce in the same area of the septum each time. We also required that no biologically or chemically active substances would be leached from the septa during contact with DMSO. Again, we were fortunate to have a specialist local company who were contracted to work with us through many prototypes, until we identified the optimum material and manufacturing process to produce the septa we required. We now had all the components of the storage media. All that remained was to put them together and transfer our samples into this ALSB compatible labware.

5. Preparation of Samples

5.1. Transfer of the Legacy Set of Compounds

Samples at Pfizer are stored as both dry compounds and as presolubilized solutions, and it is from the latter bank that HTS and follow-up screening are sourced. It was this legacy set of some half-million samples that was to be transferred to the new labware. The liquid-handling challenge was daunting, and each sample had to be positively and accurately identified in its new location, and this information loaded into the ALSB database. In addition we were required to populate a sample-volume field in this database, a parameter

that, for the vast majority of our legacy set, was unrecorded. All samples were stored in deep-well microtiter plates, and without a computer-legible label of content. The first task was to transfer the entire contents of each plate into the ALSB tube sets using a Quadra 96 dispenser. At each transfer, the identity of the source plate was associated with the destination ALSB tube set id using a PC networked with our corporate compound database. To ensure accuracy, we ran a two-person buddy system, with each association being checked prior to it being committed. The task was simple, but the magnitude daunting, as we had around 6000 deep-well plates to transfer.

We had made the decision to insert septa into the tubes after liquid transfer, and our research workshop designed and built a machine with a rotating cutter that removed a septum from a moulding of 96 septa and spun it into a destination tube. We later commissioned an engineering company to build two further machines based on the initial design of this ingenious prototype.

Perhaps the most difficult task was the final one of accurately estimating the volume of liquid sample in each tube, and again, reporting this to the corporate database. After much deliberation over technique, a Swedish company devised a system that picked up 8 tubes at a time, and took a real-time image of the tubes. Software measured the height between the base of the tube and the meniscus of the liquid, and knowing the tube's geometry, calculated the contained volume. In double-blind trials the device proved to be highly accurate for both clear and colored solutions. Moreover, the robotics of the system allowed volume estimation at a rate of over 1500 tubes per hour, much greater than that which could be achieved by weighing, the main alternative we had considered.

The project of designing the ALSB compatible labware, transferring our legacy sample set, assimilating the new data into corporate and ALSB databases involved a Pfizer team of over 20 engineers, scientists, and information technologists and took almost two years to complete. Despite the demands made on sample supply logistics by the transfer process, during that time it was necessary and indeed we were still able to resource our entire ongoing battery of high throughput and follow up screening programs.

5.2. Methods for New Samples

During the period of legacy sample transfer, our synthetic chemistry and sample-acquisition programs were still supplying samples for file enrichment. Together with an external software house we developed a liquid-handling program that manipulated mass and molecular-weight parameters from flat files to solubilize new samples to an exact molarity. The program then transfers a recorded volume to a rack of ALSB tubes that are sealed and entered into the

system. Solid-sample solubilization, distribution, and database update is now a fully automated, continuous process requiring very little operator intervention. Acquired liquid samples simply require transfer to ALSB labware and an electronic association of the incoming sample details with the destination ALSB rack identifier.

6. Milestones in the Project

6.1. Acceptance Testing: The Test Document

Jointly with RTS Thurnall, a battery of tests designed to comprehensively examine all aspects of the ALSB's function, operation, and performance were devised. This Acceptance Test Script (ATS) was used as a common testing scenario to ensure that the machine performed to specification, to the satisfaction of both RTS Thurnall and Pfizer, after factory build and again following subsequent site installation.

The ATS comprised 7 main sections and included a total of 97 discrete tests. These sections and what they contained are summarized in **Table 1**.

In order to operate the tests, a purpose-specific database of sample information and order types was designed, created, and loaded into the ALSB: together with hundreds of racks containing dummy samples.

6.2. IFAT (Internal Factory Acceptance Test)

The final stage of the build program at RTS Thurnall's Manchester factory was to ensure the machine performed in accordance with the functional design specification to which it was built, and that the system design would in fact deliver when translated from concept to actual process. This initial stage of acceptance testing, performed by RTS Thurnall staff, inevitably identified a number of hardware and software problems requiring system modifications. These problems, together with a diagnosis of the root cause, and the eventual solutions, were recorded and satisfactory resolution of all System Trouble Notes (STNs) became a condition of system acceptance.

After several weeks testing, RTS Thurnall indicated that IFAT had been satisfactorily completed, and invited a team from Pfizer to rerun the battery of test defined in the ATS.

6.3. CFAT (Customer Factory Acceptance Test)

A team of four Pfizer scientists, with an expertise covering liquid handling, biological screening, and information technology, worked together with a team of mechanical and software engineers from RTS Thurnall to conduct CFAT. The combined test team spent 2 wk during April 1999, working 15 h per day,

Table 1
Acceptance Test Script Scope

Administrator form operation	Functionality of all GUI forms that allow the administrator to set orders.
Administrator transactions	Testing of all sample withdrawal, replacement, and replenishment capabilities described in the specification.
Daughter plate orders	Ensure the system delivers all permissible variations of daughter-plate format.
Access procedures	The various elements within the ALSB must be accessible, but only when the system is in a safe condition.
Replenishment of consumable items	Demonstration that all consumable storage modules are accessible, operable, and give suitable alerts when running low.
Alarm conditions and recovery from failure	Simulation of a wide range of hard-ware, software, and service failures, to demonstrate that the equipment fails safe and the system is recoverable.
Performance testing	Does the system deliver its required output within the time frames specified.

conducting an identical set of tests to those performed at IFAT. This testing culminated in acceptance of the system performance by Pfizer. The work was very demanding, but the camaraderie between the two teams was tremendous, which further cemented the strong relationship that had been built up between the two companies during design and development, and which proved to be a great asset in the final stages of the project.

With the customer factory acceptance testing complete, the onus was now on RTS Thurnall to dismantle the equipment, ship it to Pfizer's Sandwich site, and then begin a program of installation and commissioning. RTS Thurnall indicated that they were ready for the final test phase to commence in December 1999.

6.4. SAT (Site Acceptance Test)

The Pfizer team now prepared to run the ATS for the third time. The major difference between CFAT and SAT was that the cold store was now at its operating temperature of –20°C, whereas during factory testing the store had been at room temperature. This made a big difference to the timing of various performance tests, as each test involving liquid handling had to be preceded by a 3-h thawing cycle. Not only did Pfizer require all 97 tests be successfully completed, they also insisted that all STNs raised during the test and commissioning periods—a total of 437—be satisfactorily resolved.

On January 20, 2000, almost five years to the day the original concept document had been presented to senior management, Pfizer formally accepted delivery of the ALSB.

As a stand-alone system, ALSB worked, but it was still necessary to complete the integration of ALSB with Pfizer software packages DISCUS, ECADA, and IMSO, without which ALSB could not be used.

6.5. PINT (Pfizer Integration)

Integration testing was conducted using test instances of the Pfizer databases. DISCUS (Discovery Universal Server), which holds the compound inventory; ECADA (Experiment Creation and Data Analysis), which manages HTS data; and the compound ordering application IMSO (Inventory Management and Sample Ordering), which provides both a direct user interface for ALSB plus an interface to connect HTS experiment creation with ordering from ALSB. Details of the connectivity of these applications are shown in **Fig. 4**. For the purposes of testing, these databases were populated with dummy data to ensure that incompatibilities could be identified and resolved before interfacing ALSB with the live applications and databases. This stage in the implementation of ALSB was not made easier by being conducted across the Y2K changeover period, which meant double testing in many cases and scarcity of IT resource to resolve problems. Good planning did, however, pay dividends and this part of the testing procedure was completed successfully on schedule.

7. User Interfaces

Introduction of the ALSB at Sandwich not only meant having a new repository for liquid samples, and major changes in the working practises of the liquid-handling team, it also meant substantial changes for the screening scientists who would be the customers of ALSB. Prior to automation of sample selection and preparation, scientists had ordered their samples by a variety of means including telephone, e-mail, and electronic ordering. Automation of the

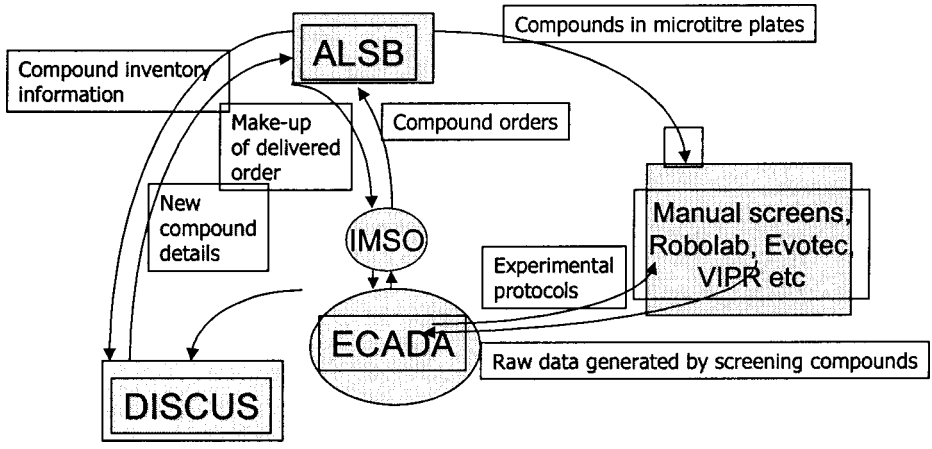

Fig. 4. Compound library management – an overview of an automated system.

task meant that all ordering had to become electronic, a substantial change for users. Luckily, there were also substantial benefits to the users of this new system with which to sweeten the change.

Previously, if users required samples for HTS, they could expect to have to give several weeks notice to the liquid-handling team in order that their samples would be ready when required; ALSB means that samples are available much faster. Users requiring bespoke selection of compounds had no alternative but to have these weighed as dry samples to order by our Compound Control Centre and expect to wait several months should the selected set be more than a few thousand compounds; again ALSB is much faster. To allow users to gain full benefit of the capabilities of ALSB, we realized it was essential to give them efficient and user-friendly ordering tools as their interface with the system. Two applications, which have been designed and built in-house, are available to users to select and order their samples, each with a specific range of functionality.

7.1. IMSO (Inventory Management and Sample Ordering)

IMSO is our basic ordering application that handles both solid and liquid compound requests as well as stores orders for chemicals. Some of the functionality of IMSO is not directly relevant to the ALSB, but it does provide a common front end for selection of both dry and liquid samples for test. Within IMSO, users can:

- Select individual compounds or racks of compounds based on the compound number or the rack name (all racks of compounds have logical prefixes denoting specific ‘sources’ of compound).

- Conduct substructure searches (IMSO uses the structural searching powers of MDL's ISIS (MDL Information Systems Inc., San Leandru, CA) and build a selection on the basis of the search.
- IMSO can import a text-file of compound numbers so that a selection compiled by any other means can be submitted as an order to ALSB.

When selections of individual compounds are submitted, IMSO checks the ALSB location of each compound in the selection. If the majority of compounds on a rack are selected by an order, IMSO will suggest to the user that the whole rack is supplied (which is a more efficient operation for the robotics) but the user can decline if they so choose. Within IMSO, the user selects the plate type in which they want to receive their samples (from a selection available within ALSB), the concentration of compound and the % DMSO which they require, and the volume of solution required. It is the users' responsibility to take account of any further dilutions, etc., to which their samples will be subject and they must select the volume and concentration required accordingly.

7.2. ECADA (Experiment Creation and Data Analysis)

ECADA is our main data tracking and analysis tool for in vitro screening. Primarily designed to handle HTS, ECADA also serves lower throughput projects if the in vitro assay records similar parameters to a HTS (e.g., % compound effect at a range of, or a single, concentration). Its primary purpose is to support the screening process and, as such, it handles setting up of screens and experiments within those screens, ordering the compounds that will be used in these experiments and checking on the status of compound orders. ECADA captures the raw data generated by the experiments, assists the user to check the quality of the data, and analyse and validate the data for transfer to the corporate database.

Via ECADA scientists can:

- Select compounds for screening by compound number, by plate identifier or by compound set (for example compounds targeted to specific target types, or sets coming from specific locations).
- In cases where the number of compounds selected cannot conveniently be screened in one day, ECADA will schedule experiments at rates selected by the experimenter and place orders against ALSB for just in time delivery.
- ECADA can import text files to create an order list.
- Can automatically create a new ALSB order based on the active compounds from a previous experiment.

Because ALSB selects compounds within an order in the most efficient picking order, ECADA has to construct the experiment to match the order sequence of compounds supplied by ALSB. All this is transparent to the user.

7.3. Provision for Automated Systems as Customer

The customer of the ALSB output is not always a screening scientist. Many of our HTS screens are now run on fully automated systems such as Robolab and Beckman-Sagian linear tracks and Evotec uHTS devices. In some cases, the output from ALSB requires reformatting before delivery to the scientist and, here too, the recipient of the ALSB output plate is a robotic system. All output plates from ALSB bear a human readable label printed directly onto the plates at time of liquid handling in ALSB. This label gives details of the number of plates in the order and sequence of the individual plate, plus recipient's name, etc. The same information is also held in a barcode printed on the label again at liquid handling which the other automated devices can read. The barcode gives information about what should happen to this plate and which compounds it contains, so, for example, should plates get out of sequence during running of the assay, the data will still be associated with the correct compound via the barcode check. Reformatting systems could also apply various dilution or reformatting regimes to intermediate plates arriving from ALSB according to details on each barcode (*see* **Subheading 8.2.**). However, selection of a bar-coding symbology is not a trivial detail in integrating systems one with another. Code EAN 128 was selected as the barcode system which ALSB would utilize and this has meant that we have preferred to have the other elements of our automation (screening robotic, etc.) making use of the same system. On occasion, this has proved to be less simple than one might expect and, with hindsight, selection of a symbology system in more common use in the pharmaceutical industry would have been preferred.

8. Capacity

8.1. Input and Output Metrics

As described in **Subheading 3.** this first stage of ALSB has a capacity of greater than 2.7 million samples. We estimated this capacity to be sufficient until at least 2004 at current rate of sample acquisition (although since this estimate the merger of Pfizer and Warner Lambert has taken place and is likely to demand a re-estimate). The design of the system allows for incorporation of a second cold store at a later date, with space for up to 4 more paternosters that would increase capacity to 5.6 million individual samples.

Our output requirements for ALSB were based on estimated screening requirements over the next five years and, necessarily, involved a lot of best guesses. Does anything change so fast as HTS technology? Central to our philosophy was the idea that ALSB should be kept simple, should not be constantly altered to support each new requirement, but rather that we would

obtain speed from a simple ALSB output and flexibility from ancillary systems that could modify that initial output. Thus, ALSB stores compounds in a 96-place array, and produces output in this same array, though the majority of our screening currently demands a 384 array. As stated previously, ALSB operates in two modes: rack-based (where the customer requires all, or nearly all, of the compounds in a rack) or tube-based (where a customized selection of compounds for screening from a wide range of racks is made). A conservative estimate of ALSB output rate for rack-based orders is 600 racks per day (limited generally by liquid-handling rate) and for tube-based orders is 6000 tubes per day (limited by tube-picking robotics). Most days a mix of tube- and rack-based orders results thus maximizing the usage of both liquid handling and robotic tube picking. We judged these to be adequate throughput rates to satisfy screening at least until 2005. This is based on likely number and rate of screens to be run, likely number of compounds to be screened, which leads to an estimate of rack-based orders. In addition, the likely follow-up rate per HTS and potential use of targeted subsets, etc., leads to estimates of tube-based requirements. Our usage strategy is also designed to be compound sparing. Although there are liquid-handling devices on the market that can aspirate and dispense much smaller volumes than 5 µL, most would be unable to pierce the re-sealable caps that close our tubes.

Additionally we chose to keep this as our lowest aspirate volume via the Tecan Genesis robots that form the liquid-handling system for ALSB because, in our experience, Genesis liquid handlers offer reliability, accuracy, and precision at the 5 µL level and this is all important in a 24×7 walkaway system. In the case of most HTS, 5 µL of 4 m*M* compound, however diluted, will be in vast excess to the requirements of the screen and hence wasteful of compound. Currently, the vast majority of our HTS utilise the same screening set of compounds, therefore it is possible to use the same intermediate plates (at, say, 200 µ*M*) to supply multiple HTS at primary screen level with ALSB being used to restock these intermediate plates as required.

8.2. Supporting Systems

Plate replication, (to produce many HTS sets from one ALSB output plate) is handled by 96/384 and 384/384 liquid-handling systems and allows us to make full use of the ALSB output while retaining flexibility over dilution buffer, % DMSO, and final assay concentration to suit the individual screen. Our main workhorse for this type of work is the Matrix Platemate (in Europe now marketed as CyBi-well from CyBio) although we also use Tomtec Quadra and Packard Platetrak systems for similar tasks. At present, operators take the output from ALSB and, from knowledge of the contents of the output and

knowledge of the required HTS input plate, program the Platemate to transfer the appropriate volume of compound, plus correct diluent to the target plate(s). To remove the potential for human error in this process, we are developing a system whereby the Platemate, following barcode reading, will automatically select the appropriate liquid-handling program. The selection is being based on the output and target information which has been written to database tables by ALSB (output information) and by ECADA (target information).

An additional customer for ALSB output is our Evotec uHTS system. This screening system consists of two parts, Mitona, which reformats 96- or 384-well plates into Evotec format nanocarriers (2280 well, 1536 of which are compound containing), and Scarina, which carries out the HTS. The output of ALSB can go directly into Mitona where each nanocarrier is compiled from the contents of sixteen, 96-well microtiter plates. Additionally, it is possible to make replicate copies of each nanocarrier (this is the most efficient way in terms of both speed and compound usage of preparing a large number of plates with Mitona), which act as a source of screening plates for future assays.

In both examples, one production of a full HTS file by ALSB can supply many complete primary HTS.

9. Changing Processes: Need for Confidence

Before our ALSB was completed and open for use, we realized that we needed to educate our scientists to these new ways of working, which would be necessary in order to take full advantage of what ALSB has to offer. Scientists who had always used dry compound and had solubilized and diluted their own samples would now be relying on the fidelity of a sample produced by an automated system. Further, scientists had to be convinced that a compound stored long-term, in 100% DMSO, at –20°C, which had possibly been through several freeze-thaw cycles, was as good as the freshly weighed sample they might previously have received. We tried to address this challenge in a number of ways.

9.1. Publicity

Prior to the launch of ALSB, we attended and presented at communications meetings for most of those departments that we knew would be customers of ALSB. The presentation that we developed was altered at each meeting, tailored to suit the needs of the audience. Thus, for chemists we focused on abilities to select a subset of compounds based on structural searching and on quality-control aspects, which would ensure the integrity of the chemical file. Biologists were mainly interested in how to order compounds by screening set and what assay-plate formats could be supplied. We also spent considerable

effort explaining the business need for the system to our IT helpdesk, database managers, facilities managers, and mechanical engineering support—all of those people who would have an impact on the support of the system—to gain their commitment to the critical importance of this new technology.

9.2. Liquid/Solid IC_{50}s

Those of us working in HTS at Pfizer have been used to receiving all of our screening samples in liquid form for many years; this was the only way the rate of sample supply could be raised to meet the demands of HTS. However many of our therapeutic zone colleagues were accustomed to receiving dry sample, which they could weigh, dissolve, and dilute themselves to prepare fresh solutions as required. Comparative data was gathered using samples from liquid store and the corresponding freshly made solution from dry sample. Most of this data was produced by colleagues in HTS who were persuaded to include liquid and dry sourced samples in their IC_{50} tests for follow-up to primary HTS.

In general, the data show that data from liquid and solid IC_{50} are comparable to that from repeat testing, on different days, of dry samples. Also, there is no consistent trend in potency data from solid and liquid samples since approx 50% of cases showed a higher IC_{50} value from dry compared with liquid samples while 50% showed the reverse. This is not to say that all samples store well at –20°C in DMSO (or under any other conditions), but that, in general, this is a suitable set of storage conditions. Confirming compound fidelity using a biological endpoint limits the data to that which can be gathered for compounds with activity against a particular biological target and is an indirect measure of compound integrity. Inevitably, this set of compounds will already have some similar structural features and so may not be representative of the file as a whole. The variations of a biological assay also impinge on the data gathered, lead us to make assumptions about the reason for the biological activity, and render it less precise than we would wish. Our desire to have a direct quantitative measure of compound integrity is fulfilled using on-line LC-MS quantification of compounds from ALSB.

9.3. LC-MS Purity/Identity Checking

Pfizer's compound file, like that of most other Pharmas, is a heterogeneous collection. Historically, all compounds were stored dry, in some quantity, with a replicate liquid inventory being constructed during the last ten years, which was available alongside the dry sample. For many samples in our file, this is still the case and so comparisons by LC-MS of the two samples are possible and the integrity of the liquid sample after variable storage periods can be assessed (always assuming that the integrity of the dry sample is not in doubt).

For newer samples entering the compound file, in particular those generated by high speed chemistry, mass of sample dictates that only a liquid sample can be held, there being insufficient material generated to permit weighing and dispensing of dry sample. For these samples, LC-MS trace of the original sample is available to compare with that generated after a period of storage. Until recently, speed of analysis of samples meant that only those interesting actives from a primary screen would be subject to structural confirmation and purity check. However, newer technology, comprising reduction in size and increase in capacity, has allowed a LC-MS with sufficient throughput to permit purity checking in quantity to be installed alongside ALSB. We plan to sample a proportion of all samples ordered from ALSB on an ongoing basis allowing us to build up a purity profile of our liquid file. We shall also be sampling a representative set of compounds on a periodic basis to gauge the changes in compound integrity over time, which may allow us to build up knowledge as to structural classes that are unstable even under such storage. This structure and purity checking will also help gain the confidence of biologists and chemists alike in the integrity of liquid samples.

10. Conclusion

This brief article has tried to encapsulate over five years of work not only from a dedicated core team, but also from over a hundred other scientists, technologists, and engineers who contributed along the route from concept to reality. The project was often difficult, frequently frustrating, but never anything less than a fascinating challenge. At time of writing we have been operating ALSB for a number of months and appreciate we are still on the steep part of the learning curve of how to get the best out of the system. A meeting called shortly after the system went live, to discuss possible enhancements, produced a list of over 40 items! A valuable lesson was learned that with a project of this magnitude, you cannot expect to get it right from day one. However, the entire team is convinced that from an ALSB derived liquid sample, we can produce solid data.

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