Development of an automated high-throughput screening system: a case history

David Harding, Martyn Banks, Simon Fogarty and Alastair Binnie

The automation of high-throughput screening (HTS) is progressing rapidly in the drug discovery world. This report describes how one such automated HTS facility was developed, with emphasis on the flexibility of the final system and the design process required to develop the system to an 'industrial' level of reliability and robustness. The facility was developed to meet specific customer requirements within a short timescale, and the project life cycle is described to show how the initial requirements were converted into a working system.

The two robot systems described here have been supplied to Glaxo Wellcome Research and Development (GWRD; Stevenage, UK) by Thurnall (Manchester, UK). In summary, their purpose is to increase the screening capacity of the Lead Discovery Unit. The background and planned application of the two robot systems is described in more detail by others[1].

High-throughput screening (HTS) involves the testing of a wide range of diverse chemical entities for activity against specific disease targets. A biological assay must possess certain key characteristics in order to be run successfully as a high-throughput screen. It must be robust, cost-effective, user-friendly, safe and amenable to acceptably high throughput. Because automation and robotics play an increasing role in high-throughput screening, assays increasingly have to meet a further criterion – they must be amenable to automation. Within the Lead Discovery Unit at Glaxo Wellcome, assay design groups are responsible for developing new and modifying existing assays to render them suitable for automated HTS applications.

The commissioning of the new systems has increased the importance of developing robot-friendly, generic, homogeneous assays involving as few steps as possible – for example, Scintillation Proximity Assay[2] (Amersham International, Amersham, UK). The two systems screen samples in microtitre plates (or 'plates'), the first system handling isotopic assays ('hot' system), the second non-isotopic assays ('cold' system). Traditionally, plates used in HTS incorporate 96 assay wells, but the drive to increase throughput yet reduce the considerable costs associated with the HTS process has led to the miniaturization of assay formats. One such advance is development of plates with a 384-well format with the same 'footprint' as conventional plates, which enables an equivalent screen to be completed using one quarter of the number of plates. However, because not every screen will be run in 384-well format, it was imperative that the new systems were configured to cope with both 96- and 384-well plates. In addition, the system had to be flexible enough to accommodate future developments, such as 864-well plates.

The two systems are identical except for their plate readers. The hot system incorporates two TopCount Scintillation Counters (Canberra Packard, Reading, UK), and the cold system an SLT Spectra Image colorimeter (Tecan UK, Reading) and an SLT Fluostar fluorimeter (Tecan UK, Reading).

David Harding*, Thurnall, Northbank Industrial Park, Irlam, Manchester, UK M44 5BL. **Martyn Banks**, **Simon Fogarty** and **Alastair Binnie**, Glaxo Wellcome Medicines Research Centre, Gunnels Wood Road, Stevenage, UK SG1 2NY. *tel: +44 161 777 2000, fax: +44 161 775 0539, e-mail: d.harding@thurnall.co.uk

  1359-6446/97/$17.00. PII:S1359-6446(97)01082-9

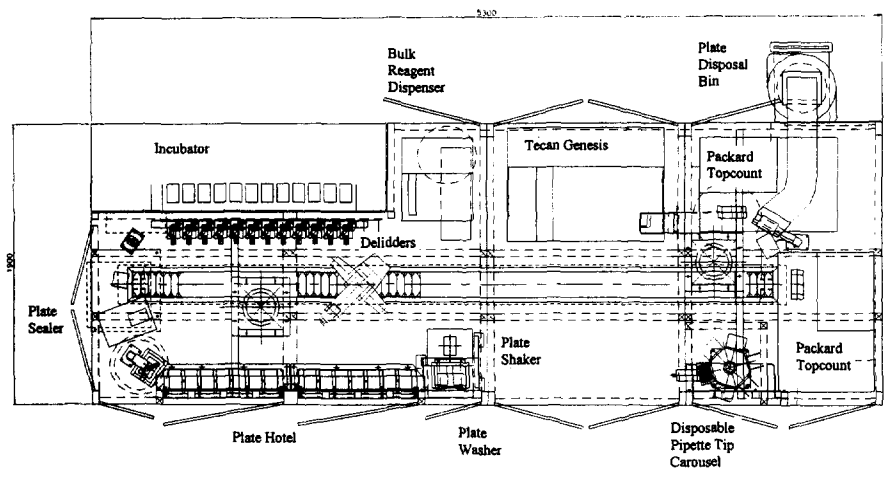

Figure 1. *Hot system – plan view. Where possible, standard equipment has been used; however, the incubator and bulk reagent dispenser are a special design.*

System overview

The hot system is shown in Figures 1 and 2, and the main elements of the system are listed in Box 1. Where possible, standard equipment has been used, but in some cases equipment was designed specifically for the system: notably, the bulk reagent dispenser and the 37°C incubator, which are described below. Other special designs were produced for the plate hotel, plate delidders/lidders, disposal chutes and the pipette tip supply carousel. There are, however, clear advantages in using standard equipment, such as reduced risk, known performance, proven field operation, good availability of spare parts and well defined maintenance requirements.

Figure 2. *Hot system, showing the incubator and plate hotel in the foreground.*

The system is designed to be 'CE' marked; it meets the relevant European Standards on Safety of Machinery, Robotics and Electromagnetic Compatibility. The most obvious signs of this are the system guarding and interlocked doors that prevent user access while the system is running.

Special purpose incubator

The 37°C incubator was specially designed for this project. It was adapted from a previous, well proven design for heating and cooling samples (Figure 3). It is not an easy task to store a large number of samples in an incubator so they can be retrieved on a random-access basis, especially because the retrieval mechanism must cause minimal disturbance to the incubator atmosphere. The solution produced for this project involved a number of shelves mounted on a nylon chain, driven round a vertical loop. The shelves pivot on the chain so that they remain horizontal as the paternoster mechanism moves the next shelf into position for robot access. Rear access is available for human checking and maintenance, robot access is via an automatic front shutter, allowing access to one shelf at a time. The shelves are moved into position by a stepper motor. The shutter remains open for the minimum time to allow robot access.

Bulk reagent dispenser

In the initial stages of the project, modelling showed that dispensing reagents on a single robotic sample processor (RSP) would become a bottleneck for system throughput. This is partly because of the mode of operation of such a system – the RSP has eight pipette tips that aspirate reagent from a reagent reservoir and then dispense this into the microtitre plate. This requires several transfers between the reservoir and plate, which is time consuming. In addition, if the RSP is dispensing more than one reagent, then significant time must be spent working or replacing the pipette tips. Therefore, a device dedicated to dispensing reagents and standards was designed. The main features of this device are its three sets of eight channels, each set dedicated to dispensing a single reagent for the duration of a run. Three eight-channel syringes dispense the reagents via an

Box 1. Key elements of the high-throughput systems.

- Plate hotel. Samples (microtitre plates) are loaded here by the user and may be returned here at the end of the assay. In addition, the user may allocate space for incubation at room temperature during the assay, and secondary plate input for use in plate-to-plate transfers on the robotic sample processor.
- Tecan Genesis (Tecan UK, Reading) robotic sample processor (RSP). This has eight probes with individual syringes for reagent addition and plate-to-plate transfers.
- Bulk reagent dispenser unit with three sets of eight syringes. Each set of syringes has a common drive, allowing the same amount of reagent to be dispensed to eight wells simultaneously. The reagents pass through the syringe itself with a separate set of syringes being used for each reagent. This unit is used for rapid dispensing of reagent across a microtitre plate.
- 37°C incubator.
- SLT 96PW (Tecan UK, Reading) plate washer.
- Plate sealer (hot system only). This unit applies an adhesive transparent label over the plate to minimize the risk of splashing potentially dangerous/radioactive reagents when microtitre plates are discarded. Assays may be run with or without lids. The use of lids minimizes evaporation in the incubator.
- Packard TopCount (Canberra Packard, Reading, UK) scintillation counters (two units, hot system only).
- SLT Spectra Image (Tecan UK, Reading) colour reader (cold system only).
- SLT Fluostar (Tecan UK, Reading) fluorescence reader (cold system only).
- Plate shaker.
- Disposable pipette tip supply carousel. This provides a supply of pipette tips for the Tecan Genesis.
- Plate delidder/lidder.
- Plate, plate lid, pipette tip and pipette tip rack disposal chutes. The plate waste chute and bin are designed to minimize the risk to users from radioactive reagents.

autosampler, and during assay setup the user may define the volumes dispensed and the columns to be dispensed to.

The bulk reagent dispenser is typically twice as fast as the robotic sample processor for dispensing reagents, and is suitable for many applications. However, the robotic sample processor is still required for more sophisticated operations such as dispensing using disposable pipette tips, or plate-to-plate transfer.

Scheduling software

Scheduling software[1] is required to command each device or instrument to take the required action at the appropriate time. In essence, each sample is processed through a defined sequence of steps, and the scheduling software must track the progress of each sample through each step. When a device or instrument has completed its task, the next step must be selected and executed.

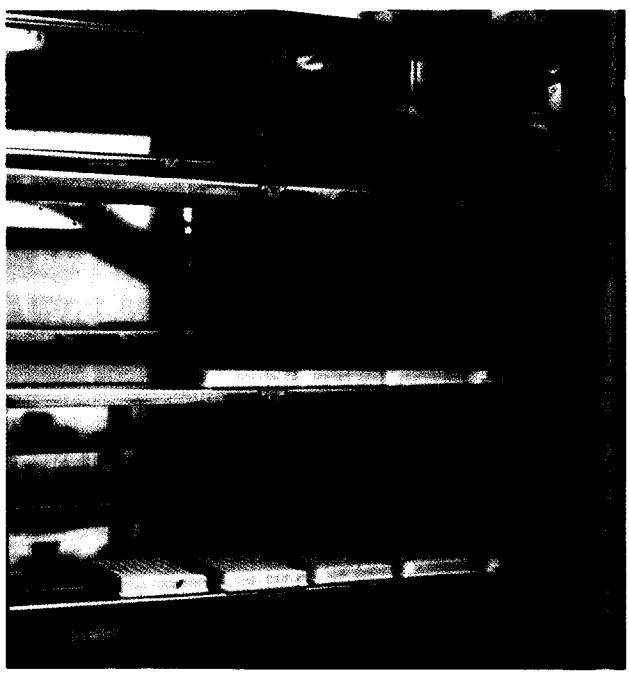

Figure 3. *Paternoster incubator. Three of the 18 shelves are shown, each holds 11 plates. Shelves are rotated by a nylon chain for presentation at a robot access shutter.*

Requirements for scheduling software. While the concept of such scheduling software is straightforward, there are some key requirements if such software is to be easy to use and productive:

- ease of creation and editing of schedules;
- ability to test schedules 'off line', i.e. without using up time on the robot system;
- reliability of sample tracking, so that sample and results can be easily linked;
- error recovery to minimize the impact of human error or instrument failure;
- ability to 'pause' a run (to allow safe user access to the system) and then continue the run; and
- robustness in the event of power failure.

The need for robustness and error recovery cannot be overstated. Because typical assays include incubation periods of

1–8 h (and sometimes longer), a HTS system will only be productive if runs can last several days if necessary.

The SPRINT scheduling system. Sample Preparation Robot using Industrial Technology (SPRINT)[3] has been developed over a number of years by Thurnall PLC; SPRINT 2, which has been introduced recently, has been used on the Glaxo Wellcome system (Figure 4). The main elements of SPRINT are:

- standard software and hardware core, proven in use on many projects;
- user-friendly Windows™ user interface;
- user-configurable assays or schedules;
- easy data integration via standard relational database;
- throughput analysis reports using Microsoft Project;
- simulation modes; and
- user-friendly device setup and test facilities.

The core of the SPRINT software is based around three schedulers that control how samples are processed. These schedulers are:

- a batch scheduler (controls the input of samples into the robot system),
- a sample scheduler (controls the processing of an individual sample), and
- a cell scheduler (controls the use of the robot system or 'cell' resources).

Typically the user will define a sample schedule consisting of a number of operations, such as:

1 Transfer plate from hotel to robotic sample processor.
2 Dispense reagent.
3 Transfer plate from robotic sample processor to 37°C incubator.
4 Incubate.
5 Transfer plate from 37°C incubator to bulk reagent dispenser.
6 Dispense reagent.
7 Transfer plate to shaker.

Each operation has certain associated parameters, such as reagent volumes or incubation period. The user can then initiate a run of a number of plates using this sample schedule. However, it is often useful to be able to have samples in the same run processed by different sample schedules. For example, in a screening application where only a small number of plates are to be processed, but incubation times are long, the system would not be working at full capacity if only one sample schedule could run at once. To allow more than one sample schedule to run simultaneously, the user may define batch schedules, which define how sample schedules are combined to make up the complete batch for that run.

While SPRINT is running, the cell scheduler controls the allocation of resources so as to optimize the processing of the sample schedules. For example, in many assays it is important to minimize the delay between a sample being due for retrieval from an incubator and the next operation. This can be controlled by increasing the priority of this operation, to ensure that the robot services this sample next, rather than another sample waiting for a lower priority operation (for example, transfer from the plate hotel to the first reagent addition).

SPRINT software can run under any of the current Microsoft operating systems (Windows™ 95, 3.1 or NT). In addition, data storage using the Microsoft Access database allows the users to set up their own queries for results, throughput reports, schedules etc. The users may define such queries in their own Access database or in any tool supporting ODBC (Open DataBase Connectivity). Examples of such queries are:

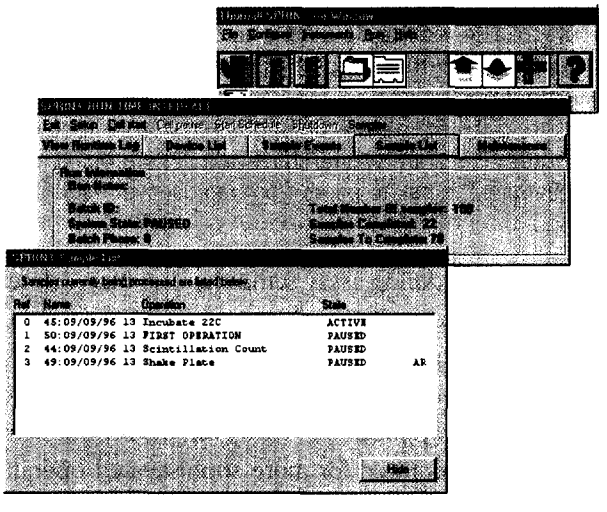

Figure 4. *Screen dump of SPRINT at runtime. SPRINT has a number of system status displays – this particular window shows the program of the samples currently being processed.*

- all the plate reader results for a certain run,
- all the plate reader results for certain wells (the wells with standards) outside a certain tolerance band, or
- results of all standards for a run exported to a spreadsheet for further analysis.

In addition, various standard database reports are provided (for example, a sample schedule listing giving operations and their parameters), but users can easily develop their own using the standard Access database reporting tool. The advantages of using a standard database (as opposed to a bespoke file format) are clear. However, the potential disadvantage may be speed – if data access is too slow, sample throughput might be reduced. Particular care was taken when developing SPRINT to optimize database-intensive tasks so that such problems do not occur.

In justifying the purchase of automation data management systems such as SPRINT, sample throughput is usually critical. SPRINT has been developed to aid throughput optimization:

- Off-line simulations can be set up well before the system is built. These use the same SPRINT scheduling software that the final system will use, thus minimizing any errors in throughput estimates.
- Simulations can again be run during system construction to optimize detailed design for system throughput.
- When the user is developing sample schedules, these can be simulated off line (either on the control system PC or another desktop PC), thus allowing throughput assessment and optimization as well as proving the schedule before using time on the robot system.
- Throughput reports can be exported to Microsoft Project (Figure 5) to allow graphical analysis of a real or a simulated run. This is particularly useful when optimizing throughputs for an assay that has no obvious bottleneck.

Project life cycle

The HTS facility described in this paper was developed to meet specific user requirements. The project life cycle[4] and the major aspects of the client–contractor relationship underlying this project are described below.

The project started with the development of a User Requirements Specification (URS) within GWRD. This was then issued to potential contractors. After these companies had returned quotations (or declined to quote), a short list of suppliers was drawn up and each company invited to present their offer. This was followed by a further cycle of updated URS and quotations before the order was placed. Important issues in the vendor selection were the ability to provide a working system, minimizing the time from installation to productive use; the proposed technical solution; and the ability to follow a well defined project life cycle and produce a system that meets the user requirements.

Although some months elapsed between issuing the first revision of this specification and placing an order, this period was very valuable; for example, the client's URS and Thurnall's quoted specification were each refined. It is most cost-effective to make design changes as far 'upstream' as possible, when neither party has committed significant resources.

Once the order was placed, Thurnall developed the Functional Design, which consisted of mechanical drawings, and a Functional Design Specification. These documents were developed in conjunction with GWRD, so that formal approval was rapid. Once the Functional Design was approved, the project progressed to detailed design. Although formal approval was not requested during detailed design, regular client–contractor design reviews ensured that requirements were correctly interpreted.

Change control

In the course of the project a range of questions arose that required formal client input – these arose both before approval of the Functional Design and afterwards, as the detailed design, manufacture and test phases progressed. To ensure that these points were fully considered by both

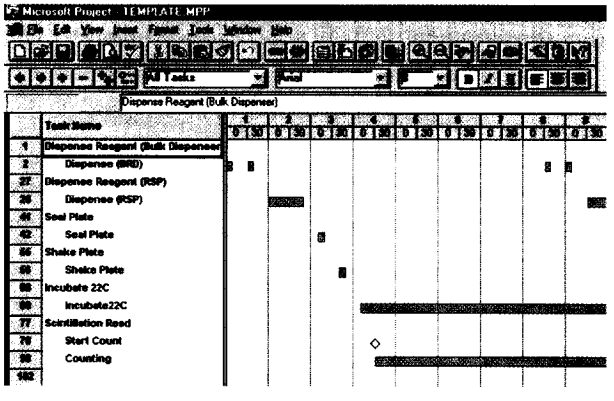

Figure 5. *Typical Microsoft Project throughput report. The chart shows time (minutes/seconds) along the horizontal axis and operations along the vertical axis. The usual MS Project functions (such as print, save, modify, and timescales) are available as required.*

parties, Technical Query notes were issued and a formal response made. All queries were logged and tracked to ensure a satisfactory resolution for all parties. Where queries resulted in changes to the Functional Design, these documents were amended accordingly. This exchange is essential for both parties. It ensures that everyone involved knows how the design is developing so that potential problems can be resolved early.

System testing

The project completion and handover involves rigorous testing to ensure that the system meets the agreed specification. Testing is performed sequentially as outlined below. First, when the Functional Design Specification is approved, a Functional Acceptance Test document is written and submitted for client approval. All formal testing is performed according to this approved document. Once the engineer responsible for system development has tested all aspects of the system fully, the Internal Factory Acceptance Tests are formally witnessed by a different engineer. The Customer Factory Acceptance Tests are then performed, witnessed by a representative of GWRD – the intention being to bring in a 'fresh pair of eyes' to look at the system at each stage. Only when both parties are happy that the system is completed and signed off is the system shipped to site. At site, once the system is installed, Site Acceptance Tests are again witnessed by GWRD – this ensures that the system is correctly installed, and marks handover to the client.

Any such project is accompanied by risks. In particular, technical risks can lead to late delivery, escalating costs and poor performance, if not addressed early in the project. Various development activities were undertaken to ensure that the final acceptance tests proceeded smoothly. For example, as described above, the bulk reagent dispenser was developed specially for this project. During each stage of its development dispense-accuracy trials were undertaken, and minor design modifications were made to ensure accurate operation.

Safety issues

Robot systems, and particularly those handling hazardous reagents, must be safe to operate. While this is really a matter of good engineering practice, the advent of common European standards and 'CE marking' has heightened awareness of the issue in Europe. The approach taken in this project was to design safety in from the start. While it is reasonable to expect that the contractor producing a system is able to design and build an electrically and mechanically safe system, it is likely that the client will have superior knowledge of the particular compounds and reagents likely to be processed. Thus, a joint approach was taken to reviewing the hazards associated with the materials being processed, and as with mechanical and electrical safety, this was designed in at the start. Examples of features designed in to reduce the hazards from the compounds and reagents under test include a 'bellows' arrangement covering the robot track to minimize any decontamination required in the event of spillage, and a simple mechanism to prevent plates sliding down the waste chute when the plate waste bin is being changed.

Summary

The design and customer–client interaction procedures described above have enabled GWRD to introduce a state-of-the-art, flexible and safe high-throughput screening system into the Lead Discovery Unit. The system offers the potential for high assay throughput, for a wide range of assays, at a low cost per assay using 'production style' screening. Continuous operation – 24 h per day, 7 days a week – was a major factor in the cost-benefit analysis. This, combined with the ability to use 96- and 384-well plates (together with an upgrade path to 864-well plates) will ensure the life span of the system and justify the investment costs.

ACKNOWLEDGEMENTS

We thank our colleagues in the project teams at Glaxo Wellcome and Thurnall for their commitment to making this project a success. Glaxo Wellcome: Tim Linsdell, Martin Valler, Kirsten Mills, Shane O'Brien and John Houston. Thurnall: Andrew Ormrod, Ian Gravener, Graham Clough, Tony Smith, Nick Harrison, Dave Gorton, Paul Hughes, George Barlow and Dawn English.

Trademarks

Windows and Windows NT are trademarks of the Microsoft Corporation.

REFERENCES

1 Banks M.N. *et al. Automated High Throughput Screening to Aid the Drug Hunter at Glaxo Wellcome* International Symposium on Laboratory Automation and Robotics Proceedings (in press)
2 Cook, N.D. (1996) *Drug Discovery Today* 1, 287–294
3 Wyatt, A. (1996) *MipTec '96*, June, Basel, Switzerland
4 Waine, D. *A Structured Approach To The Development of Software* Internal paper available from Thurnall PLC