

HIGH THROUGHPUT SCREENING, SCHEDULING AND SUCCESS

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ABSTRACT

There are two stages to a successful high throughput screening run; setting up, running and collating the data at the end of the run. However at first you need to determine if you require a real-time or pre-emptive scheduler. Both types of scheduler will be described with a selection scheme for the most appropriate.

Setting up the schedule should be easy. However as protocols become more complex, methodology becomes very important for the reliability of any method to ensure the reliability of the application.

Running the schedule requires protocol selection, worklist incorporation, protocol execution and end-of-run data collation. Within this section lies the key to seamless integration with LIMS and data management systems.

Possible integration techniques required between automation hardware and data systems will be discussed, as well as automated system control from external computer systems. Bridging software that undertakes this link between automation hardware and LIMS will be described. OVERLORD™ is a laboratory automated workcell control package that currently has over 130 instrument drivers. As the product is supplied from an independent software supplier, all the major pipetting workstations and laboratory robot systems are supported. As a bridge between the data source and the data store and manipulation, OVERLORD™ is a very powerful tool.

AUTOMATED GC-MS ANALYSIS FOR DRUG DETECTION IN SPORT

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ABSTRACT

Maintaining the integrity of the horseracing industry in relation to drug misuse and medication control is of prime importance to racing jurisdictions world wide and is governed by international rules. These rules do not name individual substances but define prohibited substances as substances capable, at any time, of acting on any one or more of the mammalian body systems e.g. the nervous system, the respiratory system, the endocrine system etc. Thus laboratories providing a service to the animal sports industry are required to provide coverage for a very wide range of drugs, in theory, any drug that could act upon the major mammalian body systems.

The body fluid of choice for providing this service is urine. HFL at the current time is analysing about 20000 samples per annum of which 95% are urine samples. Thus the laboratory faces the challenge of a high sample throughput, albeit with a single major biological matrix, and the need to cover a very broad spectrum of drugs in its efforts to provide an effective, efficient and cost effective service to the animal sports industry. This challenge can be addressed by automation of laboratory procedures.

Drug screening services generally involve sample pre-treatment (hydrolysis of conjugates), isolation of the drug from the biological fluid and possible sample purification prior to analysis. The sample extraction procedures have been simplified and rendered amenable to automation by the development of solid phase extraction (SPE). SPE, with mixed mode cartridges, allows for group separation of drugs thus introducing an element of purification into the extraction procedure. Modern automated SPE equipment also provides facilities for solvent removal and reagent dispensing e.g. derivatising reagents.

The application of bench top gas chromatographic-mass spectrometric (GC-MS) systems to drug screening is well established for SPE extracts obtained off-line. HFL has utilised the advantages of the programmable temperature vapouriser (PTV) injector to directly couple automated SPE extraction systems to the GC-MS. The system thus provides group separation of drugs (basic/acidic) using mixed mode cartridges, removal of the elution solvents, derivatisation and GC-MS analysis without any manual intervention. Automated processing of the mass spectral data through the use of in-house generated libraries then provides a sample-in/results-out process for drug detection with no manual input.

HFL is now also a major provider of bioanalytical services to the pharmaceutical industry. High sample throughput and the ability to provide high quality quantitative data are essential to providing an effective and efficient service. To meet this demand HFL uses 8 tip MultiPROBE robotic processing, often with 96 well plate technology, for all stages of the quantitative analytical process; preparation of calibration standards and QC samples, addition of internal marker followed by protein precipitation, solid phase extraction or liquid/liquid extraction. Extracts are then available for LC-MS or LC/MSMS analysis.

Providing biomarker services is also a growth area for HFL and again high throughput robotic sampling handling processes, including 384 well plate technology, have been adopted to provide a timely and cost-effective service to the pharmaceutical industry.

AUTOMATION OF SCRAPIE ANALYSIS BY MASS SPECTROMETRY

Jim Thomson, LGC

ABSTRACT

Scrapie is a disease of sheep in the class of transmissible spongiform encephalopathies (TSE), which includes BSE in cattle and Creutzfeldt-Jakob disease (CJD) in humans. EU legislation now requires member states to implement a scrapie control and eradication program based on selective breeding, which will be compulsory from April 2005. The National Scrapie Plan for Great Britain (NSP-GB) is a government scheme developed by the DEFRA, with the aim of increasing the level of scrapie resistance in the national sheep flock to the extent necessary to eventually eradicate the disease.

The cornerstone of the scheme is a genotyping program to identify whether individual sheep are genetically resistant or susceptible to scrapie and to use this information as the basis of a controlled breeding program. Previous studies have shown that 4 key single nucleotide polymorphisms (SNPs) within the prion protein (PrP) gene can greatly influence scrapie susceptibility. These SNPs modify three codons for amino acids at positions 136 (Alanine [A] or Valine [V]), 154 (Arginine [R] or Histidine [H]) and 171 (R, H or Glutamine [Q]).

To deliver this, a fully automated dedicated genotyping facility has been built at LGC, centred on a MALDI-TOF mass spectrometric detection platform from Sequenom. Three separate robotic modules process the samples in the pre-amplification laboratory area: (1) accessioning and primary tube sampling, (2) isolation of DNA in 96 well format, (3) DNA quantification and PCR setup in 384 well format. Following PCR and a multiplex primer extension, a further robot module prepares target chips for analysis in the MALDI-TOF mass spectrometer. Up to ten 384 position chips (3840 samples) can be analysed in a single run in around 3.5 hours. Automated analysis software identifies expected mass peaks and assigns genotypes. The laboratory process is controlled by a Beckman LIMS system which manages the bar-code sample tracking, provides and checks batch QC information and dispatches electronic reports to the customer for all completed approved samples.

EVOLUTION OF AUTOMATION IN A BIOANALYTICAL DEPARTMENT

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ABSTRACT

Bioanalysis has evolved rapidly over the past 15 years with the advent of mass spectrometry combined with automation of the sample preparation and analysis.

Automation has been limited to two major areas, sample introduction and sample preparation. Automated sample introduction, in the guise of autosamplers, has been in use for many years and is now routinely used, whereas automated sample preparation has seen rapid advances over the past ten years.

In this presentation the evolution of sample preparation and the consequent automation for bioanalysis will be discussed using examples from three different laboratories. In particular the use of liquid handling robots will be examined.

AUTOMATION IN HIGH THROUGHPUT GENOTYPING

Jon Curtis
Systems Group Applied Technology GSK

ABSTRACT

SNP based genotyping is still primarily cost and, to a lesser extent a throughput constrained technology. A number of elegant highly multiplexed approaches to solving the current bottlenecks exist. Our approach was to miniaturise single-plex reactions conducted to discriminate between the alleles, thereby reducing the reagent consumption and thus cost with no reduction in quality, producing a highly flexible platform. At GSK we embarked on and completed a round of technology development that has enabled us to robustly miniaturize a number of fluorescent-based SNP Genotyping chemistries to sub microlitre levels at high-throughput. In order to achieve this we have designed, developed and produced a 1536 well polypropylene microtitre plate. This plate has a 3 μ l well volume and has been shown to be suitable for robust sub μ l PCR reactions.

This high throughput platform is now used at Kbiosciences and we will present the technology and data obtained from this platform.

AUTOMATION IN HIGH THROUGHPUT CELL BIOLOGY

Sid Shaikh
GlaxoSmithKline

ABSTRACT

This case study will describe the automation used to perform high throughput biological assays.

The automated platform designed to run these assays uses an industrial robot to move plates between peripheral equipment. These peripherals perform the following basic process steps Incubation, Liquid aspiration and dispensing, drug addition, antibody addition, fixation, plate sealing, plate washing, plate tracking, plate lid and de-lid. The assays are carried out in a sterile safety interlocked enclosure.

The assays to be performed contain time critical steps. For example within 'Assay A' a drug is in contact with the cells for 33 minutes. Irrespective of what day 'Assay A' is run, what other assays are scheduled around it, or the equipment availability, whenever assay A is run, **all time critical steps must be repeatable**. In this case this means that the drug must be in contact with the cells for 33 minutes. Resource critical schedulers cannot meet this requirement, if equipment is free then drug contact time may be shorter, if equipment is busy then drug contact time may be longer. This level of uncertainty will produce poor data.

The automation platform is controlled using 'Overlord Scheduler' a time critical software package produced through a partnership between GlaxoSmithKline and Process Analysis and Automation. 'Overlord Scheduler' ensures that assays are scheduled so that all time criticalities are met, certain assays may even contain overlapping time criticalities. The automation platform is a central walk up system for HTB. 'Overlord Scheduler' allows multiple users to run different assays on the same automation platform with built in security to prevent one user's assay interfering with another user's assay.

AUTOMATION FOR SAMPLE MANAGEMENT, SCREENING AND CELL CULTURE IN THE DRUG DISCOVERY PROCESS

Ralph Strandmann
The Automation Partnership

ABSTRACT

Automation systems are important for a number of aspects of lead identification.

- 1) Automated detection systems allow more and more information to be generated, especially from cell based assays, which increases the likelihood of identifying high quality leads.
- 2) Miniaturisation: to reduce operational spend within campaigns, ever smaller assays volumes require very small volumes of reagents. TAP's automation equipment now enables this accurate dispensing of nanoliter volumes.
- 3) Increasing numbers of assays requires automation for plate handling and pipetting, enabling a high degree of reproducibility and rapid turnaround times without additional FTE.

Whilst as a company we are interested and working on all aspects of automation, I will concentrate in this talk on the high throughput solutions.

In order to achieve high throughput with good Z' the following are important:

- 1) Compound collection quality and storage conditions
- 2) Assay systems, and the processes in automated assay systems that optimise uniformity
- 3) Homogenous cell populations in the case of cell based assays

Please enjoy a presentation of solutions that have been implemented at GSK and other major pharma companies for this.