accommodating cells in HTS

High Throughput Screening has become an important and integrated part of drug discovery at most pharmaceutical and many biotechnology companies worldwide, and is now entrenched in the drug discovery process. To produce high quality leads, centralised HTS laboratories are expanding their overall role in drug discovery, and have become more closely aligned with the project teams, therapeutic areas and medicinal chemistry departments. Most notable in this push for higher quality leads is an increasing use of cell-based assays in high throughput mode.

By Sandra Fox

The results of a recent survey of 51 HTS directors at pharmaceutical and biotechnology companies indicate, for the first time, that cell-based assays will represent more than half of the assays screened in HTS laboratories by 2005. Currently, almost all HTS laboratories conduct at least some cell-based assays. Cell-based assays can provide functional read-outs, permeability and cytotoxicity information, and provide a better prediction of how a compound will behave in the cellular environment. The average current and future use of cell-based assays, as a percentage of all screening, is shown in **Figure 1** by level of throughput. For purposes of the study, HTS laboratories screening 100,000 or more wells per week are in the high throughput group, while those screening fewer than 100,000 wells per week are in the medium throughput group.

Cell-based assay challenges

Screening cell-based assays in an HTS laboratory can be challenging. HTS directors were asked to describe the most prevalent problems encountered with cell-based assays, and most say that producing, growing and maintaining stable cell lines in culture causes the most difficulty. Additional challenges noted include ensuring that a hit is relevant for the target, maintaining stable expression levels and miniaturising cell-based assays, along with a host of others.

To alleviate some of these problems, the directors made several suggestions regarding how suppliers can help. The suggestion mentioned most often is the development of an inexpensive automated system for growing and maintaining stable reproducible cell cultures. Suppliers in the industry are well aware of these problems and many others, and have developed numerous new products to address them.

Flow cytometry for high throughput applications

Vera Imper, PhD, VP of Business Development at BD Biosciences, says: "Our customers have expressed needs for increased automation, higher sample throughput, the ability to work with

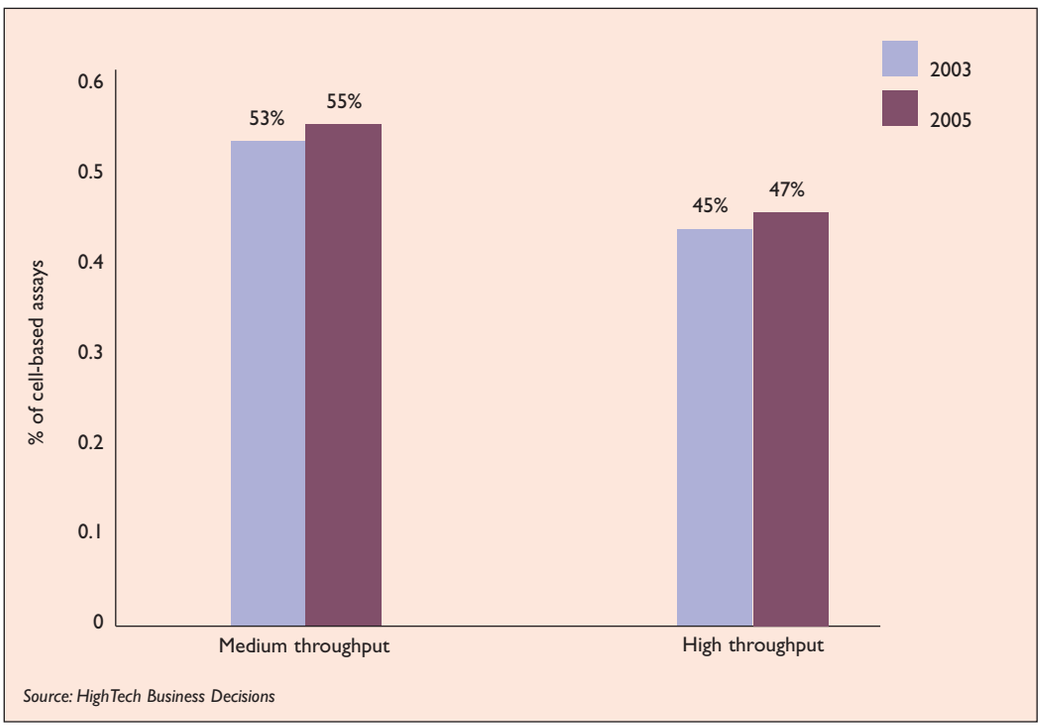


Figure 1
Average percentage use of cell-based assays at HTS laboratories

smaller sample volumes, improved sensitivity, ease-of-use, robustness, multiplex capabilities, the ability to evaluate compound solubility, assay platforms that allow understanding of interactions of many cell types and the ability to monitor events in living cells. BD Biosciences has recently launched several products that meet these, as well as other, needs."

The BD FACSAria™ cell sorter is an instrument platform that brings several technologies together, including cuvette-based cell sorting, efficient optics allowing the use of air-cooled lasers and self-contained fluidics and digital electronics to improve multicolour analysis and sorting. Sample acquisition rates are up to 70,000 events per second. The BD FACSArray™ bioanalyser is composed of an analyser with a 96-well plate sampler, an optical detection unit, a computer workstation and acquisition and analysis software. Acquisition rates for this instrument are up to 15,000 events per second. A dual-laser highly sensitive optical detection system allows the combination of several very bright fluorophores in one sample. Imper says: "Because cell sorting – when used as a preparative device – can improve sensitivity, we believe flow cytometry is poised to make substantial contributions to cell-based assays and high content research." The BD™ HTS loader allows scientists to work with multiwell plates, offering the first walk away sample introduction device for 96- and 384-well plates. The BD Gentest™ solubility scanner is a modified flow cytometer that directly measures the formation of precipitates.

Imper says: "We have a catalogue of thousands of fluorochrome-conjugated monoclonal antibodies, neoprotein reporter systems and bead array assays for multiplex analysis. Through our Clontech unit we offer two product lines ideally suited for use in HTS cell screening applications." BD Living Colors™ novel fluorescent proteins (NFPs) offer direct visualisation of subcellular events without the need for added cofactors or chemical staining that older methods require.

Increasing automation for cell-based assays

Beckman Coulter offers automated cell-based assay solutions using the Biomek® FX Assay Workstation. New designs ensure that all of the automated steps to manipulate cells – seeding, feeding and the performance of the cell-based assay – can be done in a sterile environment. Cell plates are transported to an integrated CO_2 incubator or an integrated multi-mode detector using a new conveyor ALP (Automated Labware Positioner). Software with scheduling capability ensures that plates are processed at appropriate times.

David Daniels, PhD, Applications Marketing Manager at Beckman Coulter, says: "It is key to

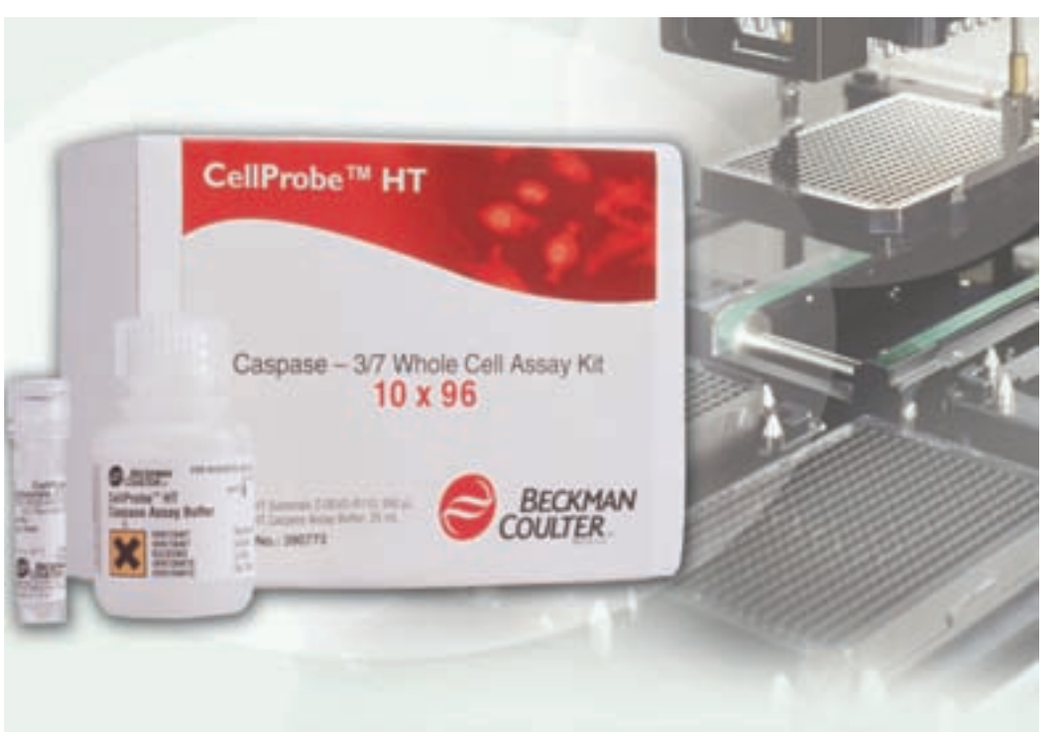
help researchers automate, standardise and simplify cell-based assays. Beckman Coulter's recent acquisition of the high throughput microscopy system for cellular imaging from Q3DM, the EIDAQ™ 100, complements our automated flow cytometer product the FC500 and extends our solutions for cellular analysis." The FC500 flow cytometer has been fitted with Beckman's Multi-Platform Loader (MPL) adapter to make it amendable to automation.

For automated cell viability analysis, Beckman Coulter's Vi-CELL™ provides video imaging of the trypan blue dye exclusion method in a flow-through cell for ADME/Tox studies. A new automated tissue culture flask adapter has been made for the Allegra® X-12 Series Benchtop Centrifuges to enable trypsinised cells to be pelleted directly in the flask without the need to transfer them to a centrifuge tube. Daniels says: "We focus on providing automated solutions and have automated several cell-based assays such as our CellProbe™ HT Caspase- 3/7 Whole Cell Assay Kit. This high-throughput assay streamlines liquid handling by eliminating cell lysis and washing steps and it is easily automated." Beckman Coulter has also automated the CaCo-2 cell permeability assays, which are difficult to automate because the cells must be propagated for 21 days with multiple rounds of feeding in a sterile environment.

Cell-based assays to assess function in primary human cells

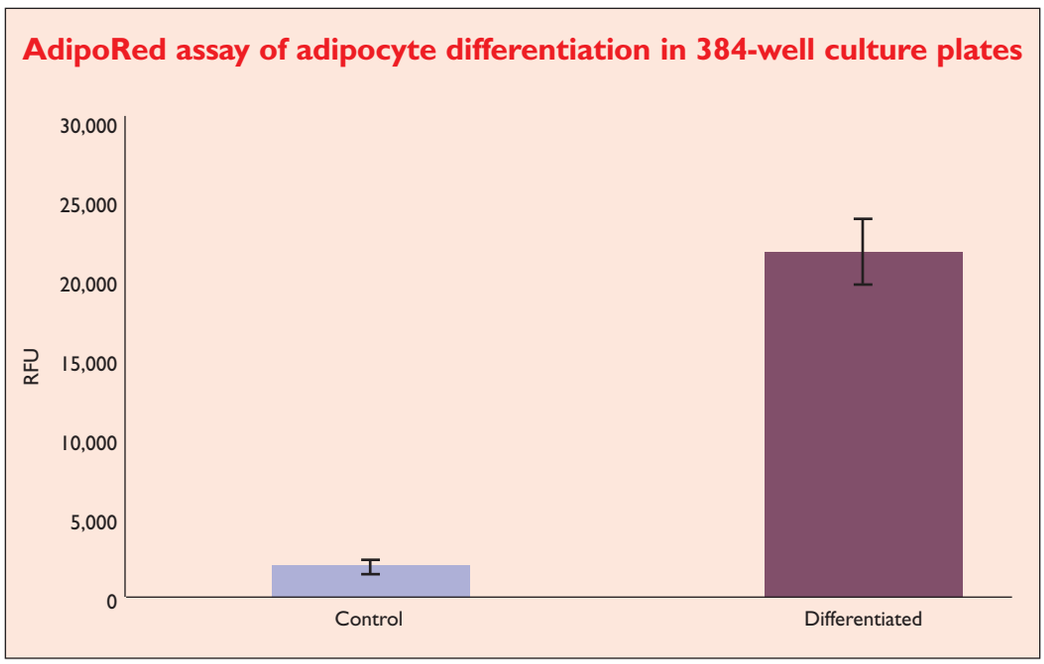
A key goal for many of the new cell-based assay programmes in HTS laboratories is to more clearly understand the effect of compounds and potential drugs on targets under physiologically relevant conditions. Markus Hunkeler, Director of Marketing, Cell Biology, at Cambrex, says: "To address this need, we are focused on developing unique assays to assess function in primary human cells and therefore generate the most relevant answers to questions of drug performance and toxicity."

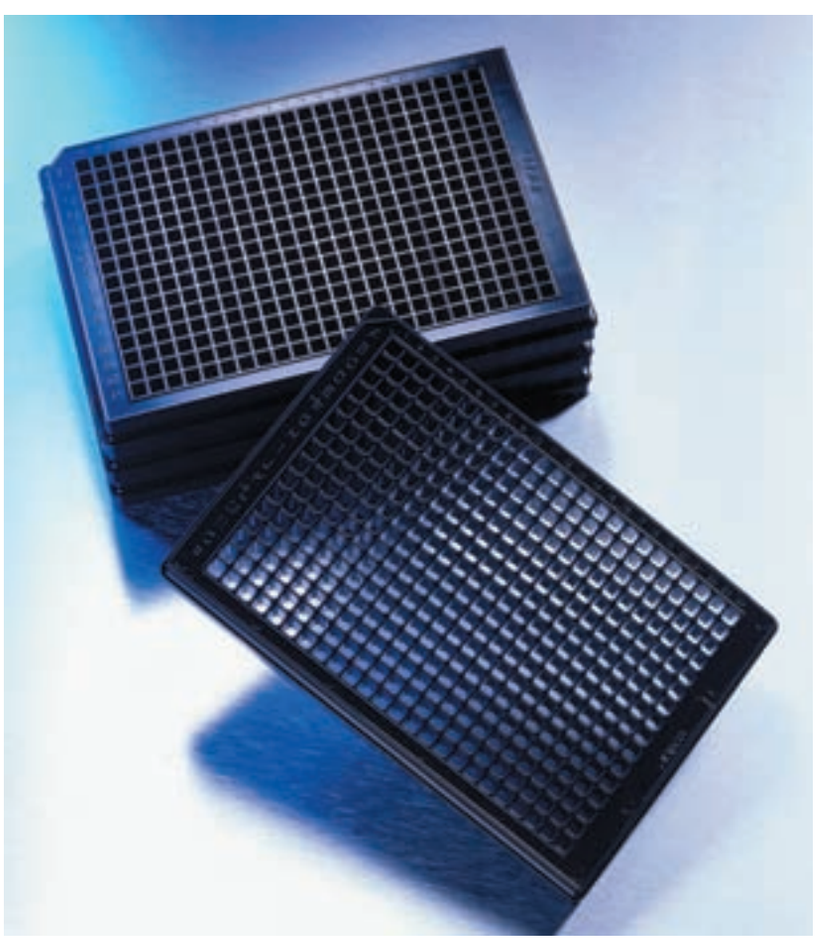
Cambrex has introduced several new cell-based assays, including the AdipoRed™ Assay, which is a homogeneous assay of preadipocyte differentiation for use in drug discovery in the fields of diabetes and obesity. The AdipoRed Assay Reagent provides a sensitive fluorescent signal that quantitatively measures the accumulation of intracellular lipid droplets, a morphological and biochemical criterion characteristic of the terminally differentiated adipocyte. The assay can be done in both 96- and 384-well plates and is read in a robotics-compatible plate reader. When used with Cambrex's primary human preadipocytes in 384-well plates, the cost of cells and reagent is less than $0.20 per well. **Figure 2** is a graph of primary human preadipocyte differentiation over time as assayed by the AdipoRed Reagent with cells cultured in a 384-well plate.



Beckman Coulter's CellProbe™ HT Caspase- 3/7 Whole Cell Assay Kit

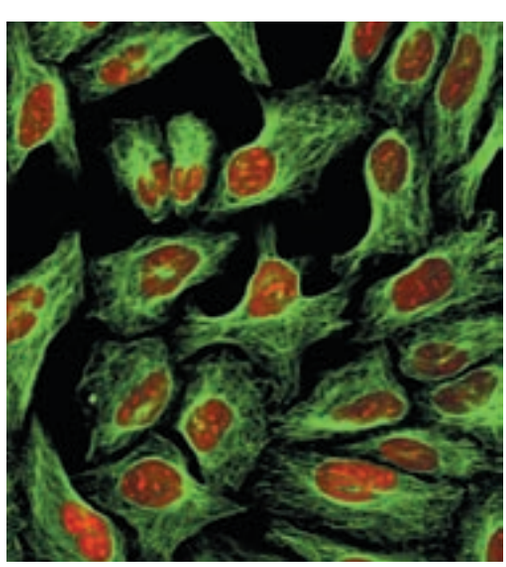
Figure 2
Measurement of triglyceride accumulation in differentiating primary human adipocytes. Preadipocytes were seeded at 3,000 cells/well in a 384-well cell culture plate in 40µl/well of Preadipocyte Growth Medium. After 24 hours, differentiation was induced by the addition of an additional 40µl of 2X Adipocyte Differentiation Medium. Control (undifferentiated) cells received an additional 40µl of growth medium. After an additional 10 days of culture, culture supernatants were aspirated and each well was rinsed with 80µl of PBS. Wells were then filled with 80µl of PBS and 1.5µl of AdipoRed-384 reagent was added. After 10 minutes, the fluorescence was read at 485nm (excitation) and 572nm (emission). Data are presented as mean +/- standard deviation





Left: Corning Life Sciences' Corning® 384-well Optical Imaging Microplate

Below: Immunofluorescent Staining of Microtubules and Nuclei. Cells plated on Corning Optical Imaging microplates. Images taken at 40x on the Amersham IN Cell Analyzer 3000



Cambrex has also introduced a series of CELISA™ assays of hematopoietic precursor differentiation for use in both high throughput toxicity screening and drug discovery. The CELISA assays, which are available for the measurement of the differentiation of the myeloid, erythroid and megakaryocytic lineages, are done in 96-well plates and are read in a (time-resolved) fluorescent plate reader. Hunkeler says: "The new assays replace the more subjective and very labour-intensive methylcellulose-based colony assays. Throughput of the CELISA assays is greatly increased by use of cell-culture-compatible filter plates that permit plating, growth and differentiation, assay processing and removal of the europium-labelled probe in a rapid and efficient manner."

High throughput sub-cellular imaging microplates

Corning Life Sciences recently introduced the Corning® 384-well Optical Imaging Microplate for high throughput and high content subcellular imaging. Debra Hoover, PhD, Applications Manager at Corning Life Sciences, says: "In response to customer needs, Corning developed a unique polymer microplate as an alternative for the unsatisfactory growth and attachment that can be observed on glass bottom microplates. Specifically, the well bottom thickness of the Optical Imaging microplate is optimised for laser-based confocal optical imaging using the Amersham IN Cell Analyzer 3000."

By using an innovative molding process, Corning designed this microplate to have dimensional stability with flat well bottoms and well-to-well uniformity for reproducible ultra sharp, high resolution imaging. This plate can be used for any high-content screening application including Transfluor ™ assays, and can be used on a variety of imaging systems.

Hoover says: "The Corning Optical Imaging microplate will be fully launched to the screening market in 2004." Corning is currently working with customers to evaluate application of these microplates with various imaging systems.

Ultra-high throughput cell-based screening and hit profiling

With a throughput of more than one million wells per day, the Kalypsys uHTS System typically runs an entire cell-based screen in a single day with one operator, eliminating much of the cellular and reagent variability of traditional cell-based screening campaigns. Kevin Lustig, PhD, Director of Lead Discovery at Kalypsys, says: "Initially designed in 1999 and now in its third generation, our uHTS System has run over 100 cell-based screens to date

Molecular Devices' AquaMax™ DW4 microplate washer and dispenser

in 1536-well plate format. These screens have included cellular assays for targets such as GPCRs, ion channels, kinases and nuclear receptors, in addition to cellular assays that examine complex pathways within cultured or primary human cells." Also unique to the Kalypsys uHTS System is a proprietary technology that enables 1536-well cell-based assays (5µL volume) to be carried up to five days without significant edge effects.

Kalypsys has recently expanded the repertoire of assay and detection formats for the uHTS System. In addition to standard luminescence, fluorescence, FP, TR-FRET and absorbance homogeneous assay formats, Kalypsys has developed proprietary technologies to perform heterogeneous, radiometric, and ion channel assays in a 1536-well format. The system has an open architecture and it can accommodate almost any third-party reader or device for cell-based or biochemical assays.

Lustig says: "Our scale and ability to accommodate almost any assay or detection format has enabled our internal drug discovery programmes to run multiple screens per target and to profile screening hits in multiple follow-up assays that assess safety, selectivity and potency. Extensive profiling prior to lead optimisation has had a dramatic impact on lead series selection at Kalypsys, and should ultimately reduce the failure rate of our pre-clinical and clinical development candidates."

Easier analysis, washing and dispensing

Molecular Devices, well known for cell-based calcium mobilisation and membrane potential assays for FLIPR® and FlexStation™ systems, continues to broaden its cellular assay offerings. It recently launched new application modules for Neurite Outgrowth, Angiogenesis and Proliferation for the Discovery-1™ system. Director of Marketing at Molecular Devices Michael Biros says: "These new software modules for our automated microscopy system significantly reduce the complexity of analysing images from screening campaigns. The modules each consist of a single dialog box for setting analysis parameters, replacing fairly sophisticated macros that our customers previously used. The Discovery-1 software, which is a super-set of the popular MetaMorph® image analysis package, now lets users analyse with either the turn-key modules or design very flexible custom macros." Molecular Devices is planning on introducing additional application modules in 2004, starting with Granularity.

Molecular Devices is also expected to launch in early 2004 the compact AquaMax™ DW4

microplate washer and dispenser to expand the company's line of liquid handling products. The AquaMax DW4 provides flexibility for ELISA and cell applications by allowing the user to dispense up to four different reagents from separate fluid channels and select various rows for dispensing. Marketing Manager at Molecular Devices Christopher Silva says: "With the AquaMax DW4, low-volume, precise dispensing is accurate down to 0.5ml in 384-well and 1536-well microplates. In addition, washing has been optimised to give a low residual volume of less than 1ml and is available to handle 1536-well plates."

Cellular assays for high throughput screening

Target specific cellular assays for Nuclear Hormone Receptors and G-protein coupled receptors (GPCRs) are currently offered as 'off-the-shelf' solutions for drug screening under the PanVera brand of drug discovery products. John Printen, Chief Scientist for Drug Discovery at PanVera, says: "In 2003 we introduced three new assays for the Mineralacorticoid receptor (MR), Glucocorticoid Receptor (GR) and Androgen Receptor (AR), and in 2004 we will release nuclear receptor assays including one for the Progesterone receptor which completes the steroid hormone receptor set, PPARgamma, FXR and LXR alpha/beta."

Printen says: "We offer a number of assays for therapeutically relevant GPCRs, and this will be a major area of focus for product development in 2004." The PanVera cellular assays are reporter gene assays constructed with the Beta-lactamase technology (BLA), which enables development of highly robust assays that can be miniaturised for high throughput compound screening. The BLA assay read-out can be performed in live cells and it is ratiometric, due to the FRET read-out of PanVera's proprietary fluorescent BLA substrates. Ratiometric read-outs improve assay performance by normalising for loading and cell density.

Fully automated cell culture maintenance

Achieving a reliable and consistent supply of cells for use in cell-based assays represents a significant bottleneck in the HTS environment. Scaling up manual culture methods to better meet this demand is possible, however this often requires development of new processes and protocols, adding time and expense to the project. Rick Luedke, Proteomics Marketing Manager at Tecan, says: "Our new Cellerity, designed for cell and protein production applications, provides a high throughput solution for fully automated cell culture maintenance, cell-line expansion and harvesting with minimal process development."

Based on Tecan's next-generation Freedom EVO robotic liquid handling platform, the Cellerity features simultaneous processing of up to eight flasks with the flexible eight-tip liquid handling arm

Tecan's next-generation Freedom EVO robotic liquid handling platform

(LiHa), rapid plating of cells into 96-, 384- and 1536-well plates with the 96/384 channel multi-pipetting option (TeMo), refrigerated storage with an in-line warmer for dispensing up to eight different medias and Tecan's AutoLoader™ stacker for easy loading of new flasks. With a large capacity for consumables including media and flasks, the system can provide days of unattended operation. Luedke says: "A fully integrated cell counting module, with an optional imaging package, precisely monitors the growth rate of cells, taking the guesswork out of replating and seeding activities."

Developed in collaboration with leading labware manufacturers, the Cellerity uses a novel T-Flask. An on-deck device enables fully automated rotation, shaking and knocking of these septa-pierceable flasks. Fully enclosed in a CleanBench-level laminar flow hood, the Cellerity uses an air-lock for interfacing to a high capacity CO_2-incubator for storage of the plates and flasks.

Luedke says: "Offering complete control over the culture environment helps to ensure optimal metabolic conditions for superior growth performance and consistency from passage to passage. For maximum efficiency and workflow, the Cellerity includes a software package combining a user-friendly web-based user interface with sophisticated scheduling and tracking functionality, providing the freedom to effectively manage the complex logistics of maintaining multiple cell lines simultaneously."

Generating potency and selectivity profiles

Vitra Bioscience recently introduced the CellCard™ System to enable researchers to examine multiple variables such as potency and selectivity from standard cellular assays. The CellCard System can generate potency and specificity profiles using up to 10 targets or cell lines in the same assay well. Heidi Bullock, Senior Product Marketing Manager at Vitra Bioscience, says: "With this information, selective compounds are identified early in the process and compounds can be prioritised. This increases the opportunity to examine compounds that otherwise would have been viewed as false negatives and discarded in a standard sequential screen that only focused on potency. It also enables researchers to identify non-selective compounds earlier by running multiple targets or cell types per well."

Because more data can be gathered from a single lot of cells, it is feasible to use human primary cells to evaluate multiple leads prior to final drug candidate selection. Additionally, the CellCard approach provides a unique ability to assay multiple sources of primary cells. Leads are validated as active on the native target and cells from several different patients can be used for potential efficacy testing. Also, several cell types could be used for potential *in vitro* toxicity testing.

Bullock says: "The CellCard System provides a parallel approach for cell-based assays that enables a more comprehensive data set to be generated. Selectivity and compound potency profiles can be acquired simultaneously using up to 10 different targets or cell types in a standard microtiter well." With the CellCard System, physiologically relevant data can be produced through the screening of primary human cells and an informative panel of data is generated, increasing the chances of identifying potent and selective lead compounds with a greater chance of clinical success.

Industrialisation of the HTS laboratory has been an important trend that must now include cell-based assays as scientists seek both higher quality leads and higher throughput. New products that automate cell-based assays, from seeding, feeding, sorting and maintaining cells to the performance of newly developed assays for higher throughput, subcellular imaging, uHTS systems for cells, and more comprehensive data collection and analysis tools, indicate that suppliers are stepping up to the plate to support the increasing use of cell-based assays in HTS. DDW

***Sandra Fox** is President and founder of HighTech Business Decisions, a consulting firm specialising in customised market analysis, industry reports and customer loyalty studies for suppliers serving the pharmaceutical and biotechnology industries. The company recently published the report High Throughput Screening 2003: Improving Strategies, Technologies, and Productivity. Tel: +1 925-631-0920. Web: www.hightechdecisions.com*