Needs and challenges in HIGH THROUGHPUT SCREENING

High throughput screening (HTS) groups face similar challenges as the pharmaceutical and biotechnology industry as a whole faces. HTS groups are looking for ways to improve effectiveness in lead discovery, increase their productivity and operate under tight spending budgets. To help meet these challenges, HTS directors seek to develop more relevant screens and assays, automate more processes, apply their skills and expertise to other functional areas, and efficiently manage the data they collect in their operations. These are some of the findings documented in HighTech Business Decisions recently published study, 'High Throughput Screening 2007: New Strategies, Success Rates, and Use of Enabling Technologies'.

By William Downey, Dr Helen Nicely and Dr Lynne Sopchak

In its study, HighTech Business Decisions interviewed 55 directors of HTS laboratories from pharmaceutical and biotechnology companies and academic centres or research institutes worldwide. In addition, it interviewed representatives from 32 major HTS supplier companies to gain their perspective of the industry and to understand new products and services available to meet the ever-expanding challenges of the industry.

This article highlights some of the thoughts by the HTS directors concerning areas for improvement, unmet needs in primary and secondary screening, suppliers' new products that address some of those needs, and finally, the HTS directors' opinions on new technologies.

More successful screening operations

The HTS directors discussed what would make their screening operations more successful or productive. As can be expected, the HTS directors cite various actions and technologies that would improve their screening operations. A summary of the top four items mentioned by the HTS directors is shown in **Figure 1**. The most often mentioned needs are robust assays and larger staffs, followed by improvement in liquid handling equipment and processes and better compound libraries. The HTS directors also noted other areas that would help make their screening operations more successful, including the need for improved software, more user-friendly and rugged equipment along with better integration of robotics.

A few selected comments regarding opinions by the HTS directors about areas for improving the success of their screening operations are as follows:

"Increased assay sensitivity is something that would help our screening. Something better would be fantastic. Making screens more sensitive to inhibitors would be useful for us."

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"We are always looking for more biologically relevant assays – primary cells or phenotypic assays, for example."

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"We always think we want newer technologies. On the other hand, people have questions about spending a lot on technology. The upfront investment for new technology can be quite high. The instruments often cost $0.25 to $0.50 million. The specialised plates are also an issue. We found the new technology comes with a much higher sensitivity. Our consensus is the new technology can't just be a convenience; it has to have better sensitivity. Label-free binding allows things that weren't possible before, the sensitivity wasn't there. The latest technology gives 1 to 2 orders of magnitude in improved sensitivity. If it only saves time, we will be cautious about using such a technology. But if there is no other alternative then we consider it."

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"The key to success is a meaningful assay. That means the biology needs to be clearer and we need to develop smarter assays."

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"Someone who can tell me the most relevant form of a protein to use in a biological assay: It is the biggest problem in screening. We can never be sure if the artificial set-up in a well or a test tube reflects the real *in vivo* situation."

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"Additional personnel in the data analysis group for primary screening would make our operation more successful and productive. In 2004, we got funding for assay development and that helped drive assay development forward."

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"We need more people. Over the years we have achieved a level of efficiency with new equipment and tools but now we need more hands."

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"More compounds and a better quality compound library. Compound library enhancement that would cover more chemical space would be helpful."

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"I think that we would focus on the quality of the compound collection. Our screening capability is pretty good, but what you screen determines the quality of the output."

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"In one word, infrastructure would make our screening operation more successful and productive. In general, things would run smoother if we could improve data management, improve communication within the disease areas, and increase our understanding of diseases and biology."

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Unmet needs

Primary screening

Following-up on the areas that would make their screening operations more successful, the HTS directors described areas where they see major unmet needs in primary and secondary screening operations. As might be expected, the needs of the HTS directors are as varied as the operations, tasks, goals and strategies pursued by the various HTS groups. While individual organisational needs vary, there are common segments where unmet needs exist. As noted by one HTS director, overall, the industry suppliers are doing a good job in meeting their customers' needs; however, the challenges lie in improving the accuracy and knowledge, while offering relevant cost-effective solutions.

In primary screening, one-third of the HTS directors are looking for improved assays, specifically the HTS directors require robust assays with improves predictability. Other unmet needs in primary screening noted by the HTS directors include the need for better data management tools and improvements in productivity and automation, improved compound libraries and improved detection methods. An overview of the unmet needs in primary screening by major category is shown in **Figure 2**.

Figure 1: Top items to make screening more successful

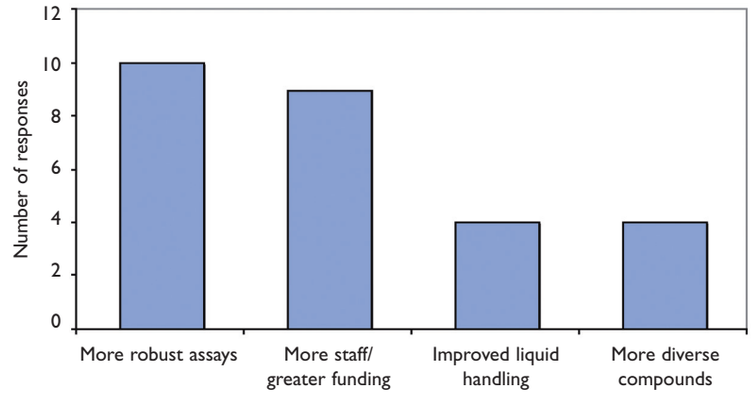
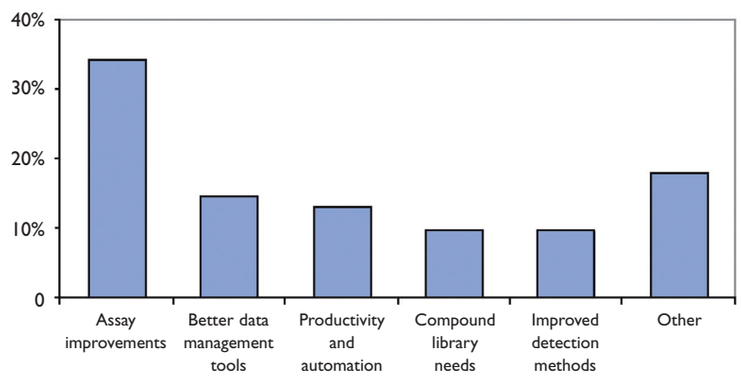


Figure 2: Unmet needs in primary screening



A few selected comments regarding unmet needs in primary screening by HTS directors surveyed at leading HTS laboratories, follow:

"There is no biggest unmet need, there are pockets of need. The quality of plates is one area. Every company claims to have an SBS-compatible plate, but we can do simple tests to show that this is not true. Another pocket of need is the lack of innovation in new detection methods. Label-free detection is still full of artifacts. The unmet need is for companies, such as Promega and Invitrogen, to think of cool, smart ways of doing things within the screening well. The availability of smart decision-making software is also a need. We look for trends; we do not have software to do that. We have to do it manually, which is time consuming. We are doing okay otherwise."

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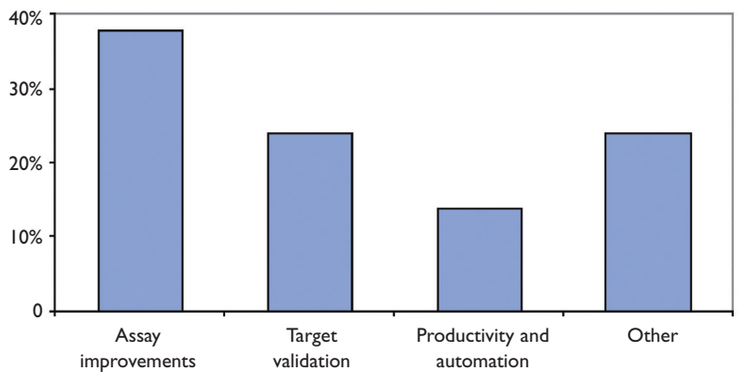
Figure 3: Unmet needs in secondary screening



"For primary screening, we need to eliminate non-specific responses or false positives. We end up weeding through a lot of junk."

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"The primary screening as it is done is pretty mature. The question is the relevance of it. Why are people going to HCS? They think it is a more adequate model of disease rather than just an enzyme in a well. They are moving to more phenotypic readouts, not biochemical read outs. In HCS it seems there are severe throughput limitations."

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"The biggest unmet need for primary screening is knowing if we have the right compounds, robots that will do it all, anything that will make their assays more robust, and increase the quality of the data so we have more confidence in our results."

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"We haven't been pushing screening to its limits because we are building our capacity. For us data analysis is an issue and the connectivity of instrumentation and software analysis tools. We want to increase the amount of screening we do, but data analysis is a bottleneck for us."

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"The biggest unmet need is non-contact dispensing. We, the HTS community, need to collect more than one data point per well. We also need to improve the richness of data by running more panels of assays against more possible targets. We shouldn't limit the panel of assays to the secondary screen. Of course, more data will result in a need for more data handling power."

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Secondary screening

In addition to highlighting unmet needs in primary screening, the HTS directors also note several unmet needs in secondary screening. For the most part, the most important unmet needs in secondary screening mirror those of primary screening, with 38% of the HTS directors mentioning the need for improved assays. Improvements being sought in secondary screening cover a wide range of areas including better understanding of biological functionality, more

physiologically relevant assays, and more rapid assay development are some of the areas where the HTS directors are looking for improvements. Similarly, just as with primary screening, the HTS directors see a need for improvement in automation tools and software that will increase productivity. An overview of the unmet needs in primary screening by major category is shown in **Figure 3**.

Below are selected comments from HTS directors regarding their unmet needs in secondary screening:

"In secondary screening, the biggest unmet need is the availability of cell lines that are physiologically relevant. Both human and animal cell lines that are physiologically relevant are important so we can follow them through cell-based systems, animal models and toxicology activity."

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"For secondary screening, capacity is an issue. People are continuing to make triage decisions on the number of compounds we can carry forward into secondary screening. If we could screen a broader collection of hits through various secondary assays we may have more success."

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"For secondary screening the biggest unmet need for cell-based assays is testing in the appropriate contexts, and for biochemical assays, it is kinetic assay analysis."

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"For secondary screening, throughput of *in vivo* studies is a limitation. If I could change one thing in drug discovery it would be the ability to put a higher number of compounds into *in vivo* models."

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Suppliers

As mentioned previously, the industry's suppliers are working to meet the needs of the HTS groups by introducing new products and software. To meet the needs for improved assays, many companies such as Promega Corporation, Invitrogen Corporation, Cisbio International and MDS Analytical Technologies have all recently introduced new assay-related products. Promega Corporation (www.promega.com) has a major new product that is a cell-based 384 assay for CYP 3A4 induction in cells. John Watson, PhD, Director, Cellular Analysis, notes: "The key technological leap is the ability to perform cell-based induction and inhibition assays with using CYP substrates and human hepatocytes. All previous 3A4 substrates have shown cross reactivity with other CYPs, thus preventing their use when more than one CYP is present." Promega Corporation has also developed a luciferace-based biosensor technology that works through a circularly permutated complementation method which allows the monitoring of intracellular changes such as cyclic AMP levels.

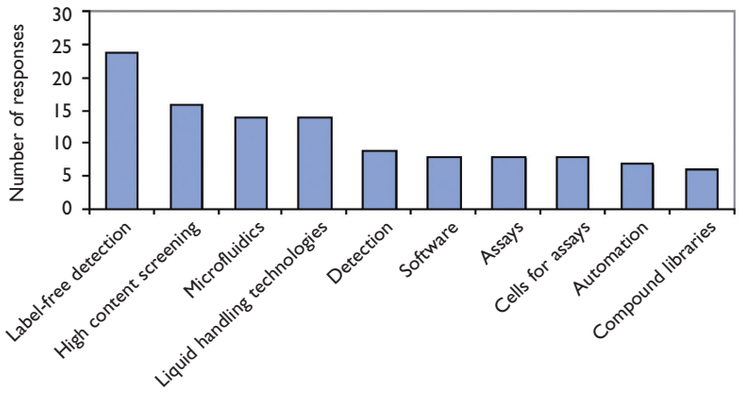
Invitrogen Corporation (www.invitrogen.com) has several new products for improved assays including mTOR kinase and LanthaScreen™ TR-FRET assay – the first commercially available active mTOR kinase protein and assay for a key disease relevant kinase. LanthScreen™ GFP Cell Lines – cellular kinase assays that enable the HTS Labs to monitor protein kinase activity within the complex intracellular milieu. Invitrogen also offers Tango™ GPCR assays which allows isolation of target GPCR signal to be separated from the background of endogenous GPCRs, thus reducing the number of false positives during HTS.

For cell-based assays, Cisbio International (www.cisbiointernational.com) launched in 2006 a new monoclonal antibody-based concept specifically for cell-based assays screening serine/threonine and tyrosine. The platform is based on a combination of four biotinylated substrates, a proprietary monoclonal antibody and the company's patented HTRF technology. This platform can be used for kinase selectivity assays and high-throughput screening.

Recently, MDS Analytical Technologies (www.mdsintl.com) released the FLIPRTETRA® plus which will enable fluorescence and luminescence. In the reagents area it has introduced an assay for potassium ion channel specifically for its FLIPR® instruments. It has also introduced a neurotransmitter transporter uptake assay kit, which is a fluorescence-based cell assay for serotonin, norepinephrine and dopamine neurotransmitters.

In the areas of improved automation, Beckman Coulter, Inc (www.beckman.com) offers Industrial Robotics Solutions for HTS based upon a robotic arm from Motoman USA. These systems are robust, customised systems to provide optimal process solutions. Other equipment from Beckman Coulter includes PARADIGM™ Detection Platform, which addresses the delicate balance between sensitivity and flexibility by offering both filter and monochromator (currently only used for absorbance) in a single instrument. In meeting the industry's needs for higher throughputs and improved productivity, Beckman Coulter, through its recent acquisition of Aurora Discovery, offers the BioRAPTR FRD and PicoRAPTR microfluidic workstations that provide small-volume bulk

Figure 4: Technologies with the largest impact on HTS



dispense and compound transfer/direct serial dilution for plate densities up to 3456.

In 2007, Labcyte, Inc (www.labcyte.com) introduced the Echo® 555 liquid handler, which allows for the routine use of higher density, lower well-volume micro well plates and provides better, more precise and more accurate results. The Echo® 555 is also capable of transferring fluids to as many as 660,000 wells per day with its unique 'touchless' technology. Another product from Labcyte is The POD™ 810 plate assembler. The POD™ 810 is a plate-on-demand integrated system that includes plate storage, robotics, controller software and optional bulk filler, plate centrifuge and plate sealer, which automatically assembles plates as desired for dose-response experiments, reformatting, cherry-picking or simple plate copying.

New technologies

On a final note, the HTS directors provided comments about new technologies and the technologies they expect will have the biggest impact on HTS in the future. The HTS directors mentioned a wide variety of technologies depending upon their own organisation's needs and strategies. The most often mentioned technologies that will have the largest impact on HTS are the adoption of label-free detection, high content screening and microfluidics. Other areas where technology will have a major impact on HTS operations include improvements in software and assays, including cell-based assays. A summary of these responses area shown in **Figure 4**.

Below are selected comments from HTS directors regarding their opinions about the technologies that will have the most impact on HTS:

"Label-free technologies will have the biggest impact on HTS. We will be going to an image-based system for primary screening. We will be evaluating label-free technologies, looking for success in primary screening. Right now it costs too much and it is not robust."

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"HCS may allow us to get a better picture of how a target is biologically relevant. The tools are being developed to make it better but the cost of instrumentation is a problem. Right now HCS is being used selectively but we need to use it more widely. We are looking for sensitive readouts of biological samples. For our lab, we are looking for technology that will allow us to substitute a biochemical screen instead of a cell-based assay."

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"The technologies that will have the largest impact on HTS in the future are miniaturisation of liquid handling, such as microfluidics. We expect to adopt microfluidics technologies and imaging-based plate readers that can capture a plate image, rather than well-by-well – higher throughput being the driver."

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Concluding remarks

The directors at high throughput screening laboratories see the needs for improved assays, better automation tools and improved software as some of the areas that can make their screening operations more successful. The need for new tools and software is being met by many of the suppliers in the industry as they introduce a host of new products and services into the market. Over the next several years, The HTS directors see the adoption of label-free detection systems, high content screening, and new liquid handling technologies as having the largest impact on their operations. DDW

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