Core system model: understanding the impact of reliability on high-throughput screening systems

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Laboratory automation is an evolving technology that has grown in complexity and sophistication over the past decade. High-throughput screening (HTS) is the discipline that has driven the technology to the point at which principles can be stated about how much and what is appropriate to automate in an HTS laboratory. The following analysis discusses the principles that are central to a successful and productive HTS system. Furthermore, an approach to designing and programming HTS systems to achieve a higher degree of reliability than has been experienced in the past is described.

Laboratory automation has matured over the past 10 years to the point where a wide variety of possibilities exists for building a 'fully automated research laboratory'. Individuals in the area of high-throughput screening (HTS) pioneered the development of robotics technology in the drive to reduce the time-to-market for drug development. The technology originated with simple automated workstations, and matured through the late 1980s and early 1990s into large, sophisticated, robotic systems that attempted to remove as much human involvement as possible in HTS assays. These complex systems were 'one-offs', with little uniformity in hardware or software programming. They were typically given names like 'the beast' and required in-house experts to run and maintain them.

Nowadays, a large amount of experience suggests that these large systems, although designed to do everything, are not the optimum approach for maximizing productivity in HTS or most other types of laboratory automation. Rather, taking a step back in overall complexity dramatically improves overall productivity and achieves the major objective: processing more samples. This article describes the evidence based on the fundamentals of device and system reliability[1] that supports the concept of smaller, focused, systems. This concept has been promoted by Zymark (Hopkinton, MA, USA), utilized in Tecan Trak systems, and describes the efforts of Beckman Instruments and Sagian (a division of Beckman) to improve the reliability of HTS systems by developing standardized robotic systems, termed 'core systems'.

Device reliability

HTS systems are composed of a series of devices physically linked by a transport mechanism, usually a robotic arm, that performs a series of tasks within an HTS assay. It is impossible to provide a specific value for individual device reliability within these systems because every system is different and the interaction between system devices can vary. However, some generalizations can be made in order to understand individual device reliability.

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Device reliability can be broken down into three primary components: the functional quality of the device (i.e. how well it functions independently), the ease of physical interaction between the device and the transport mechanism, and the software routines used to control the device within the system. The functional quality of a device is typically not a major issue in HTS systems as long as quality manufacturers are used. Ideally, the system will utilize the best available components to accomplish a given task. These components are typically supplied from a variety of vendors and evolve over time. Some devices evolve to take advantage of, and offer, improving technology, and some technologies evolve to replace those that were once dominant. A system must be able to accommodate multiple vendors' devices to allow for adaptation to different needs and changing technology.

Problems occur when the individual task that a device must perform includes steps that cannot be monitored or when the success of the overall task cannot be detected and reported. An example of such a task/device situation is cell harvesting (or other types of filtration), which is usually conducted under the watchful eye of an operator. Filtration is frequently requested in HTS systems, which poses numerous problems as the rate of filtration in all 96 wells is difficult to control, clogging of individual wells cannot be directly monitored, and overfilling of a clogged well can contaminate adjacent wells on subsequent washes. Devices that perform functions requiring close human supervision to ensure proper operation should be avoided if possible or taken 'off line'. Alternatively, techniques such as solid-phase, homogeneous assay systems solve a complex task by eliminating the need to perform a separation or filtration step. Therefore, when considering a device for integration into an HTS system, it is critical to consider the complexity of the task and how well the task is monitored and controlled by the device. Bearing in mind the old saying 'keep it simple' is a very intelligent approach to selecting devices and assay formats for HTS systems.

Physical interactions between the transport mechanism and the device are typically made reliable by use of an articulated arm robot as the transport mechanism to simulate the type of motions a human would perform to load/unload the device. Also, the device manufacturer will usually supply a robot-compatible version of the instrument if access is too restrictive even for an articulated arm. The software routines used to integrate a device into a system can be a source of problems, reducing the reliability of a given station. A reliable communication routine is usually the result of a suitably designed operating program for the device where the appropriate lower language links (serial, DDE, or OLE) have been made available. The number, complexity and uniqueness of the software procedures that interact with the device are also critical to the overall performance of the device. In custom systems, there are typically many procedures that must be individually 'fine tuned'. A more reliable approach is to modularize and standardize the design of the software routines for each device. With this approach, the same well-tested routines for a specific device can be used regardless of the configuration of the rest of the system. Once this approach is adopted, an individual component can be fully tested, independently of its integration into a given system. In effect, systems become a mixed and matched grouping of standardized devices and supporting software routines. This approach not only provides higher reliability through more-focused testing, but also allows for easy integration of additional devices at a future time.

A secondary factor in device reliability revolves around the required maintenance for a device. This includes everything from changing bulbs in a detector to avoiding crystallization within a plate washer. If not properly addressed, these issues can cause a system to halt operation as effectively as a defective code or a catastrophic equipment failure. Most systems will semi-automate avoidance of some of these problems, such as a device not being on, by the operator simply 'touching' each device before start up so that the operator can be notified of such an error before leaving the system. Other maintenance or set-up issues may not be so easily checked by the system on start up. HTS laboratory personnel will formulate checklists to be addressed before start up in order to avoid these other errors. However, with increasing numbers of devices on a system, these checklists can become counter-productive or simply not comprehensive enough to avoid making mistakes. Therefore, as systems get larger, the probability that some maintenance issues will be overlooked increases, which consequently decreases the effective reliability of the devices on a system.

System reliability

The reliability of the entire system is dependent upon the reliability of the individual devices and their configuration. 'Reliability' is usually defined as the ability of a device or system to perform a required function under stated conditions for a specified time[2]. Because of the short period of time (<5 years) that most HTS systems have been in operation, it

is not possible to predict the lifetime or rate of degradation of a specific device or system. However, we can apply the principles of probability to produce a model of how to configure an HTS system properly, irrespective of specific vendor's devices. To eliminate the highly variable degrees of reliability found in devices used in HTS systems and to make the analysis unbiased, we shall use standard values for the reliability of each device found in an HTS system: R_n = 99.9, 99.8, 99.5 or 99.0%, where R is the hypothetical percentage up-time of the device (n).

System reliability is a statistical measure of the probability that a single device within a collection of devices will fail[3]. This value is an 'inherent reliability', which means it is the maximum reliability for that system and not necessarily what is observed. Furthermore, how a system is configured, in a series, parallel and/or redundant fashion, can have a dramatic effect on the measured reliability for the system. Unfortunately, as we shall demonstrate, HTS systems by their nature are configured in the least reliable way.

HTS assays define HTS systems

When we examine a bioassay, we recognize that it is composed of a series of sequential steps that are independent: pipette buffer, add compound, shake, add substrate, incubate, read. However, if one step fails, the assay fails. HTS systems behave in the same fashion – they are serial systems. As each device in an HTS system is independent, the probability of the system functioning is the product of the reliability of the devices, i.e. $R_s = R_1R_2...R_n$, where n is the number of devices.

When we look at HTS systems hypothetically and use the device reliability factors described previously, we generate reliability curves based strictly on the number of system devices, as shown in Figure 1. There are three important features that we can conclude from this. First, the more complex the HTS system is, the less reliable it is. This concept is the foundation of the core system concept. Second, if the number of assay steps dictates the number of devices used in an HTS system, then the more complex the assay the less reliably the HTS system will perform. This effect is the primary reason why simple, nonseparation-based assays, such as scintillation proximity, fluorescence polarization and fluorescence energy transfer/quenching techniques, are so commonly employed in HTS systems. Third, concurrent assays (performing two assays simultaneously) will increase the number of devices used and the number of steps in the combined assays, and this will reduce system

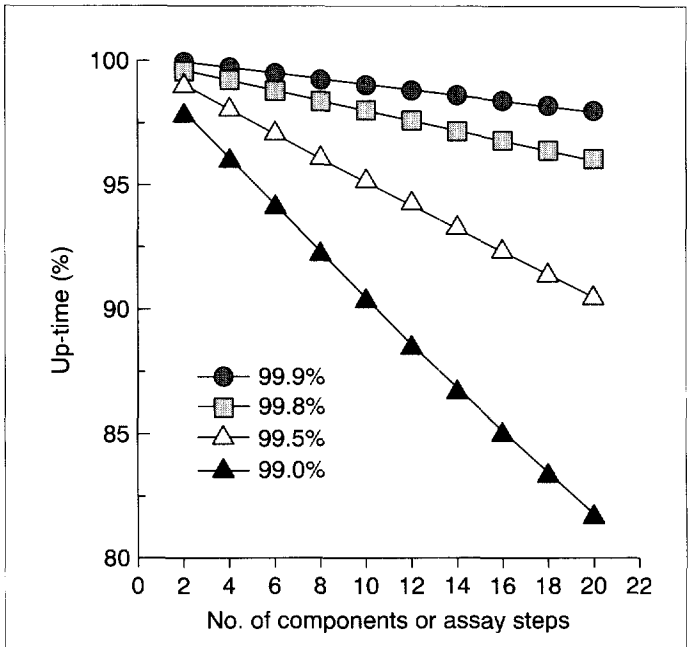

Figure 1. *Effect of device reliability and system complexity on overall reliability for serial systems. Reliability curves based on the number of components for each serial system.*

reliability. Unfortunately, serially designed systems are the least reliable approach to configuring devices in a system.

The core system concept

A core system is defined as a robotic system assembled from a variety of robotic-compatible hardware components with standardized software modules not normally found in individual custom systems. The components and their programming modules have been used and tested extensively in previous custom and early core systems. Core systems are designed in both software architecture and in the constraints placed on hardware positioning such that the mode of interaction between the robot and the various devices, or the interactions between the various devices, is independent of the overall system configuration. In this sense, each component is a modular addition to the system and, if proven to work in one system configuration, will work in any other core system configuration. Unlike a typical custom system, which is uniquely programmed for a specific set of components, all core systems utilize the same programming and devices. This creates a 'pool' of users employing the same components and associated software modules. This differentiates core systems from a custom system because the end-user of a custom system is the only 'test subject' for

that system and must live through the unproductive alpha, beta and debugging phases that occur with any new instrument or system. The reliability of a core system is further enhanced by utilizing a selected set of peripherals to accomplish various functions. This approach limits the choice of components but fosters familiarity with the selected peripherals, enhancing support and ultimate device reliability to the end-user. Although not essential, reducing the number of components in a core system improves the ultimate reliability of the system.

Table 1. Definition of devices that comprised the smaller core systems and the large custom system[a]

Core systems			Custom
Enzyme	SPA	Cell-based	
Biomek 2000	Biomek 2000	Biomek 2000	Biomek 2000
ORCA	ORCA	ORCA	ORCA
Plate/tip carousel	Plate/tip carousel	Plate/tip carousel	Multipette
Plate shaker	Plate shaker	Plate shaker	Plate shaker
Lid station	Lid station	Lid station	Lid station
Plate washer	Plate washer	Plate washer	Plate washer
Plate reader	Scintillation reader	Fluorescence reader	Plate reader
Incubator	Incubator	CO_2 incubator	Scintillation/ luminescence reader
	Plate sealer	Luminescence reader	Fluorescence reader
			Incubator
			CO_2 incubator
			Plate sealer
			Tip carousels (2)
			Plate carousel

[a]Biomek 2000 and ORCA, products of Beckman and Sagian, a Division of Beckman, respectively; SPA, scintillation proximity assay.

Theoretical comparative analysis

To support the philosophy that smaller, multiple systems are more productive than a single large complex system, the following comparative analysis was performed by configuring one large, multifunctional system and three smaller application-specific systems comprising the components described in Table 1. These systems were identical in components and type of programming software, thus eliminating variable performance from different components and focusing on the primary differences: size and complexity. Although the analysis was performed using Beckman products, the relative differences in productivity between approaches would be similar if performed with different equipment, as has been reported by Zymark[4]. Furthermore, our definition of system reliability is in fact the start-up reliability of the systems over the first 2 years. Large complex systems can be made reliable; however, if an HTS group decides to acquire such a system, they will be the system designer and only testing site. This can dramatically reduce the productivity and overall value of such systems. It should be noted, however, that large complex systems can perform assays using only a subset of the available devices, similar to a small system, but the inherent reliability of the devices will still be reduced for a variety of reasons, ranging from how the software handles the interactions between devices to how well maintained a less frequently used device is. These types of factors are peculiar to specific systems and the operating procedures of end-user sites and cannot be addressed within the scope of this study.

For the analysis, three different assay types commonly used in high-throughput screens were used: an enzymatic end-point assay, a scintillation proximity assay (SPA) and a cell-based gene reporter assay. Box 1 describes the steps and timing for each assay. Comparison of implementation time, theoretical throughput and reliability allowed calculation of the maximum theoretical productivity that two HTS laboratories could achieve using either one large or three three smaller systems. Finally, a cost/benefit ratio was calculated to demonstrate which approach was optimal.

Throughput

To determine maximum throughput, the assays described in Box 1 were programmed using SAMI™ system methods development software. SAMI employs a simple-to-use graphical interface to build methods, and powerful scheduling algorithms to maximize the work flow in HTS assays (Figure 2). To mimic real laboratory conditions, the assays were designed for overnight operation on the large complex system and two 12 h runs per smaller system, even though these smaller systems can be run overnight with expanded capacity. Each day, 2 h was allowed for setting up the large complex system and 1 h per run was allowed for the smaller systems. Each assay plate contained 16 controls (two columns) and 80 assays. Compounds were assayed in duplicate on the same plate, resulting in two assay plates per compound plate. Analysis of the assays in Table 2

Box 1. High-throughput screening assays used for throughput and productivity analysis

Enzymatic assay

- Add buffer/enzyme solution to assay plates
- Bar-code read compound plate and assay plates
- Add compounds to assay plates
- Mix
- Add substrate
- Mix
- Incubate for 30 min
- Add stop solution
- Read plate
- Trash plate

SPA binding assay

- Add buffer/SPA solution to assay plates
- Bar-code read compound plate and assay plates
- Add compounds to assay plates
- Mix
- Add radioactive ligand
- Seal plate
- Mix
- Incubate for 30 min
- Add stop solution
- Read plate
- Trash plate

Cell-based luciferase assay

- Wash assay plates with fresh buffer
- Bar-code read compound plate and assay plates
- Add compounds to assay plates
- Incubate for 1 h
- Add ligand
- Incubate for 5 h
- Wash cells
- Lyse cells
- Mix
- Add luciferase substrate
- Read plate
- Trash plate

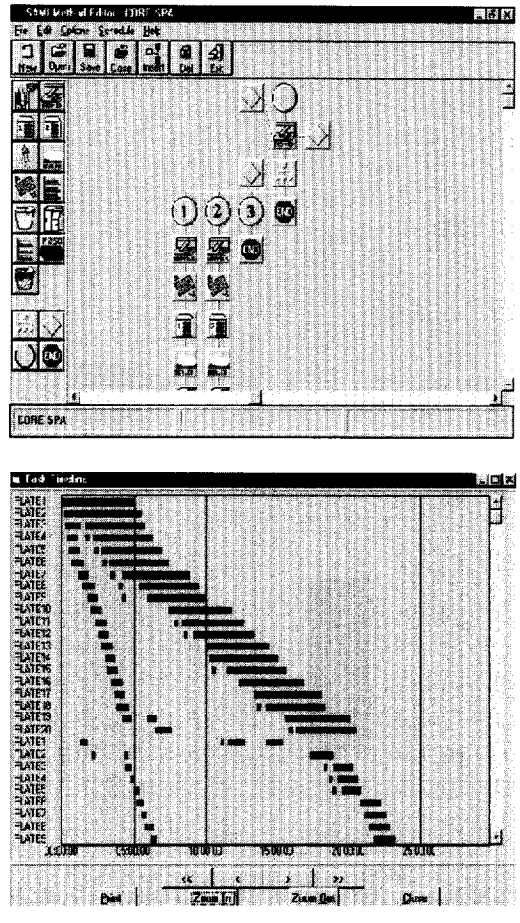

Figure 2. *SAMI system methods development software for (a) building high throughput screening (HTS) programs and (b) scheduling the multiple tasks in an HTS assay.*

Table 2. Comparison of throughput between the core systems and the large custom system[a]

Assay	Core	Custom
Enzyme	9,984	10,016
SPA[b]	7,680	7,488
Cell-based	3,840	3,264

[a]Core systems were run twice per 24 h period and the large custom system was run continuously for 24 h.
[b]SPA, scintillation proximity assay.

demonstrates that the throughput was similar for each system and was independent of the assay tested. It should be noted that these assays were run batchwise, using only a subset of the large complex system to perform each assay type.

Batch versus concurrent assays

A second perceived advantage of a large complex system is that multiple different assays can be run simultaneously on the one system. To examine this scenario, the throughput of performing two assays simultaneously was compared with performing them in a batchwise approach. Table 3 shows the throughput using each approach. Substantial improvement in throughput was observed only when one assay had a very long incubation time (>5 h). Under these conditions there was enough time to perform a second assay while the first assay was incubating. However, concurrent assays are difficult to perform for practical reasons such as correctly setting up a system with a variety of different reagents.

Table 3. Comparison of batchwise versus concurrent assay screen approaches for a large custom system[a]

Assay	Batchwise	Concurrent
Cell/enzyme	13,280	14,592
Cell/SPA[b]	10,752	12, 672

[a]Throughput values are for a 48 h period performing the assays concurrently or sequentially, one assay per day.
[b]SPA, scintillation proximity assay.

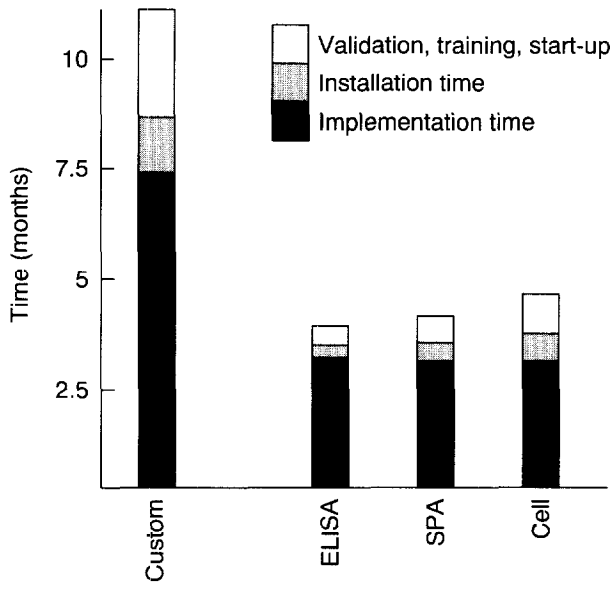

Figure 3. *Comparison of implementation time for a large complex high-throughput screening system versus application-specific smaller systems (as defined in Table 1). ELISA, enzyme-linked immunosorbent assay; SPA, scintillation proximity assay.*

Implementation time

When the decision has been made to automate, the first problem is the extensive time required to define, develop, program, deliver and validate a large complex system. Even under the best of circumstances it can take 8–24 months from the time of order for a large complex system to become fully operational. However, as all the components in a core system are already defined and their programming is modular in character, the start-up time is only 3–4 months (Figure 3). Consequently, an HTS laboratory using core systems can perform 8–9 months of screening that could not have been done using a large complex system. The impact of this lost productivity is discussed below.

Reliability

Reliability is difficult to compare between systems because components and software can vary dramatically. In addition, the level of the operator's expertise significantly affects overall reliability. Nonetheless, by applying the reliability principles described previously, it is possible to generate expected reliability values for each type of system. Figure 4 shows the percentage up-times of the various components of the HTS system that were defined for the analysis. If a 99.5% up-time per component is assumed, then the reliability value for the large complex system is 92.8% up-time. It is important to understand that the inverse of these data expresses the probability of a system breaking down. Therefore, a 15-component system will have a 7.2% probability, or a 1 in 14 chance, of failure. A system with this type of reliability will fail at least once in a month of continuous operation. Furthermore, down-time is typically composed of a diagnostic phase and a repair phase. With complex

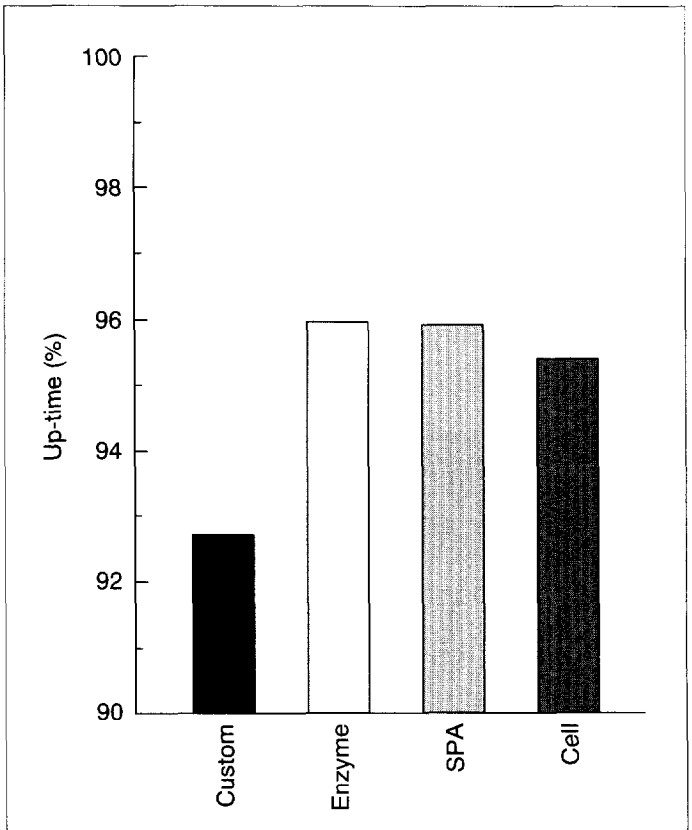

Figure 4. *Comparison of the theoretical reliability values for a large complex system versus application-specific smaller systems. The values are based on a defined device reliability of 99.5%. SPA, scintillation proximity assay.*

systems (e.g. large systems), more time is spent troubleshooting before making repairs. Thus, not only are large systems more likely to break down, but once down they require longer to repair.

Productivity: effect of start-up and down-time

The productivity of an HTS laboratory will be directly related to the flow of different assays and how well developed the assays are for the robotic systems. In the analysis described above, productivity was expressed as the total cumulative assay points over a period of 2 years. To mimic the dynamic environment of an HTS laboratory, a cycle of the three assays described above was used. The large complex system employed concurrent assays when possible, and the three core systems ran each assay simultaneously. The effects of implementation time and reliability were factored into the analysis. The 99.8% theoretical reliability curve (Figure 1) was used to estimate the frequency of breakdowns per month for each system. The down-time per breakdown was defined as 5 days for the large complex system and 3 days for the core systems, and the total down-time per year was then averaged per month and subtracted from the maximum monthly productivity. To make the values closer to reality, assays were performed on a 5 day per week schedule, and systems were serviced 2 days per month.

The data were calculated for two, three or four individual core systems versus one large complex system that contained all the functionality of the different core systems. Figure 5 expresses the data as the cumulative number of assay points generated from the time the systems were ordered from the vendor. As the core systems were delivered and running long before the large complex system, more than a million assay points were generated using the core systems over this time period. However, more importantly, the slope of the curves for the multiple core systems was greater than that for the large complex system, indicating that the large complex system could never catch up with or exceed the productivity of the core systems.

Cost/benefit

If the cumulative assay point values at 1 and 2 years are paired with the overall cost of each system configuration, the cost/benefit ratios can be compared (see Table 4). The data, expressed as the number of assay points per dollar of system, show that although multiple core systems cost 30–50% more than one large complex system, their value in productivity exceeds large complex systems by 200–500%.

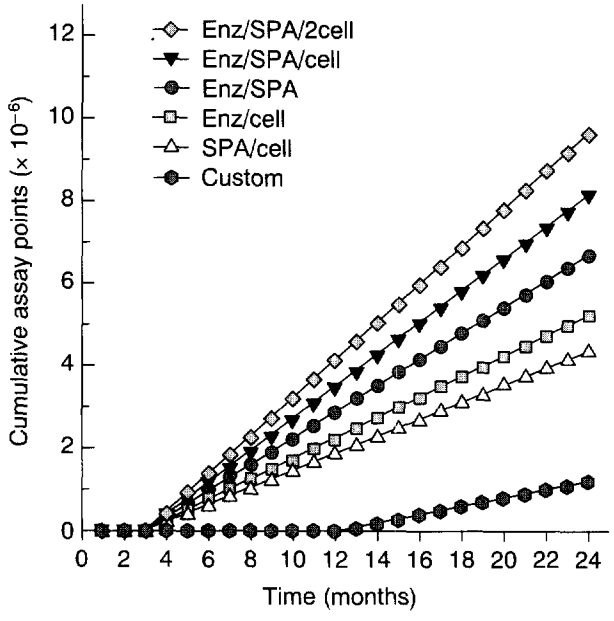

Figure 5. *Theoretical productivity curves for core and large complex high-throughput screening systems. The cumulative number of assays performed per month was compared between a large complex system and different configurations of multiple core systems. The values are based on a defined device reliability of 99.8%. Enz, enzyme; SPA, scintillation proximity assay.*

Intangible benefits associated with reliability

Additional benefits occur with increased reliability. In particular, reliable systems generate a greater degree of confidence, and scientists will desire, not avoid, working with the systems. There have been many cases in which a large complex system has become obsolete because the research scientists refused to risk wasting an assay that was difficult to develop. Additionally, the cost of a 'crash' in lost precious reagents (which can be quite substantial) was not factored into our analysis. This cost can be much greater for large, high-capacity systems than for smaller, focused systems[5].

As reliable systems can be equated with predictable systems, the application of program management techniques is possible. Previously, planning high-throughput screens was at best an estimate but, with the development of reliable systems, interim goals and long-range planning become more realistic. HTS can now be viewed almost as a manufacturing process, generating data based on the number of orders (assays) submitted.

Table 4. A comparison of the cost/benefit ratios for multiple core systems and a large custom system

System configuration	Estimated cost ($)	No. assays/cost of system[a] 12 months	24 months
Custom	500,000	0.21	2.5
Enzyme	200,000	9.0 (42.9)	18.9 (7.6)
SPA[b]	300,000	4.6 (21.9)	9.7 (3.9)
Cell	400,000	1.7 (8.1)	3.6 (1.4)
Enzyme/SPA	500,000	6.4 (30.5)	13.4 (5.4)
Cell/enzyme	600,000	4.2 (20.0)	8.7 (3.5)
Cell/SPA	700,000	3.0 (14.3)	6.2 (2.5)
(1) Cell/enzyme/SPA	800,000	4.3 (20.5)	9.0 (3.6)
(2) Cell/enzyme/SPA	1,100,000	3.5 (16.7)	7.4 (3.0)

[a]In parentheses, the ratio of the core system(s) cost/benefit value to the custom system at 12 and 24 months after decision on a system. These values represent the theoretical fold increase in productivity achieved with core systems compared with a large custom system. Note that the values for the individual core enzyme system are skewed because the enzyme assays are typically the highest throughput and the least expensive systems to purchase.
[b]SPA, scintillation proximity assay.

Future role for large complex systems

The HTS laboratory should not be the environment for product development and invention. However, standardized systems such as core systems will not perform all assays or incorporate new liquid handling or detection technologies before market acceptance with established reliability. This resistance to change is both a strength and a weakness. Standardizing robotic systems forces new technology to be 'proven' in custom systems before incorporation into a standardized system. The approach of using multiple small systems has the drawback of requiring additional laboratory space, and lack of sufficient capacity may cause problems with work shifts when performing 2–12 h runs. However, recent expansion of core system devices has made it possible to configure a system for continuous operation. Custom systems will always play a role in advancing the technology to improve the process. As new technology demonstrates its usefulness in HTS facilities, incorporation of the technology into the core systems and upgrading existing systems becomes a productive option.

Conclusions

HTS is the initial phase in the drug discovery process, for which the objective is to discover chemical compounds that have activity in relevant bioassays. Until recently, the technology for achieving the type of throughput necessary to make this approach feasible was novel and custom in origin. Standardized systems attempt to eliminate the problems with custom systems by standardizing the components and modularizing the programming to produce sophisticated HTS systems quickly and reliably. With the advent of the core system approach, the need to struggle with the many problems associated with a sophisticated large complex system is dramatically reduced, and HTS laboratories can focus more of their efforts on the discovery of new drugs.

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In short...

Genzyme Corp. and **Bayer AG**, Leverkusen, Germany have announced a collaboration whereby the identification of new drug development candidates could be accelerated. Genzyme's extensive small molecule compound library will be analyzed by Bayer's high-speed robotic screening methods. The agreement is potentially worth $35 million for Genzyme – Bayer will pay Genzyme an up-front fee for access to its library of >1 million small molecules and, once biologically active compounds are identified and development candidates have been selected, milestone payments will be made as the new drugs proceed through clinical trials. Dr Wolfgang Hartwig, head of worldwide pharmaceutical research at Bayer AG (Wuppertal, Germany) said, 'This partnership is a further important contribution to our efforts to develop new drugs faster and more cost effectively'.