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Unit Automation in High Throughput Screening

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1. Introduction

This chapter will explain the unit automation approach to high throughput screening. The unit automation approach is predicated on the belief that automation is best implemented only to the extent that operations are standardized, i.e., if you standardize individual assay steps (or unit operations) they can be readily (and effectively) automated ***(1)***. If entire assay formats could be standardized, then it would make sense to automate the entire assay. However, unless assay development is severely constrained this is not possible. We have found that the majority of scientists will not agree on a standardized protocol for a particular assay format, therefore we have chosen to automate only to the level of unit operations. It should be noted that the concept of identifying, standardizing, and optimizing unit operations is applicable to fully automated screening systems as well.

2. Unit Automation Defined

2.1. Automating the Process Rather than the Assay

Unit automation can perhaps best be described by contrasting it with the alternative methodology of integrated automation. In integrated automation, samples, reagents, plates, and other consumables are supplied to an integrated system of liquid-handling instruments, detectors, and robotic plate manipulators ***(2)***. Scheduling software then controls the flow of plates through the system and conducts the entire assay, totally unattended in the best of cases. Several different assay protocols may be conducted in parallel. Human intervention is limited to feeding test samples, plates, and reagents and disposing of waste.

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In contrast, the unit automation approach is limited to automating individual workstations that perform a single unit operation. In unit automation, each workstation is an island of automation that is designed to optimally perform a single function for maximal throughput *(3)*.

2.2. Plate Handling is the Biggest Difference

The single biggest difference between unit automation and fully integrated automation is in the approach to plate handling. In unit automation, the individual workstations have dedicated plate-handling capability to move a number of plates through that particular unit operation. The plate-handling may be performed by stackers, conveyers, robotic manipulators, and carousels, or other means. The difference is that human beings are used to move the batches of plates from one unit operation to the next.

2.3. Standardization of Equipment and Methods is Key

As mentioned earlier, the key to effective implementation of automation is standardization. Standardization of both equipment and methods used is a great enabler of further improvements in effectiveness.

Standardizing on the equipment to be used for a given unit operation has many advantages and benefits.

- Identify standards for future purchases
- Identify equipment types that need investigation
- Prioritize integration and development of tools
- Reduce complexity and proliferation of required support skills
- Consolidate sourcing and service
- Toolbox for screen development
- Process for evaluating new equipment

Method standardization is as simple as picking one methodology and sticking to it wherever possible. Once a standard method has been identified, it can be optimized, tweaked, and refined. By reusing a standard methodology, progression up the learning curve is cumulative, rather than starting anew with each new assay. Departure from the standard method should only be permitted when there is a valid reason for the exception. For example, there is minimal benefit and great complexity if custom plate maps are developed for each assay to be run in dose-response mode.

3. What are Unit Operations?

Unit operations are those individual process steps that make up an assay protocol. Unit operations can be standardized in terms of both equipment uti-

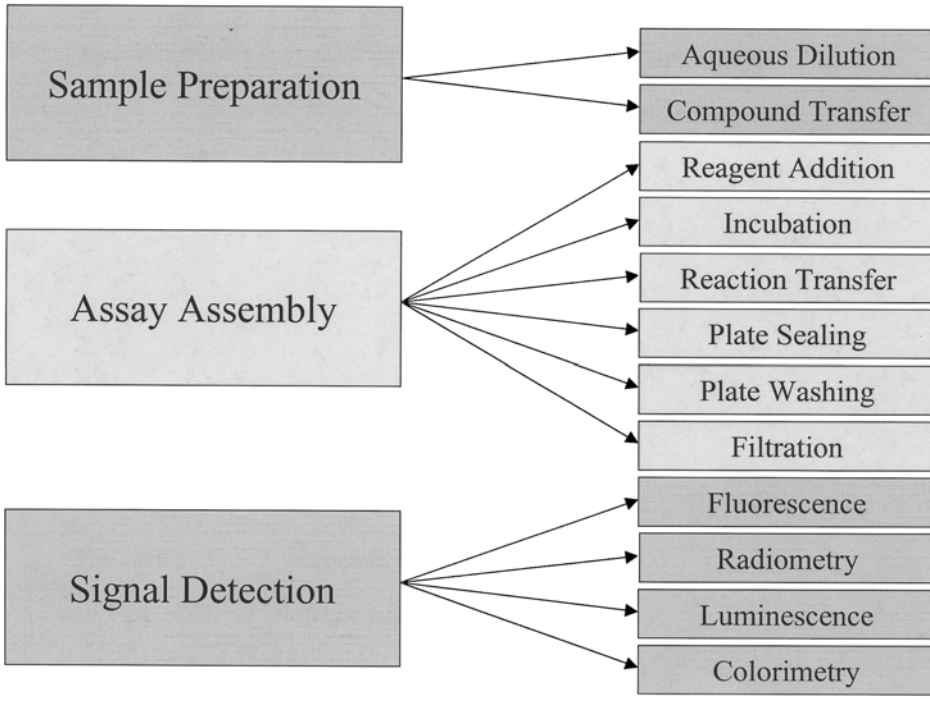


Fig. 1. Typical unit operations.

lized and methodology. Typically, unit operations in HTS can be divided into three categories: 1) sample preparation, 2) assay assembly, and 3) signal detection (*see* **Fig. 1**) ***(4)*** Very simple homogenous assay formats (sometimes referred to as mix-and-measure) may just involve additions, incubation, and reading. More complex heterogeneous formats may include many more steps involving filtration, plate-washing, plate-to-plate transfers, etc. The point is that unit operations are the building blocks that can be combined in whatever order is needed for a particular assay protocol. Once unit operations are defined and standardized, they can be effectively automated with little or no dependence on the particulars of a given assay.

Process flowcharting can be used to graphically represent an overview of an assay protocol. The format shown in **Fig. 2** uses the convention of materials flowing in from the left, waste streams exiting to the right. Additionally, information on critical parameters, equipment used, and throughput for each unit operation is represented.

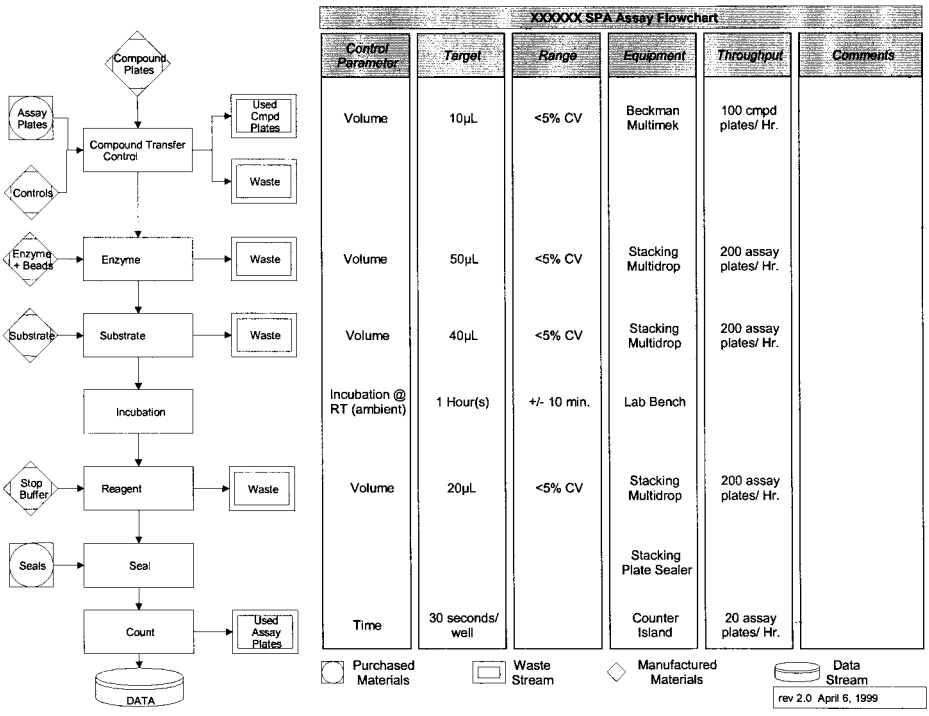


Fig. 2. Assay process flowchart.

3.1. Sample Preparation

Sample preparation unit operations include those steps necessary to provide the test samples in the proper volume, concentration, and diluent for screening. Examples include:

- Transferring aliquots of sample (generally in 100% dimethyl sulfoxide [DMSO]) from compound library stock plates to a sample plate.
- Diluting the sample plates with aqueous buffer.
- Serial dilution of test samples (for multipoint dose-response curves).
- Transferring aliquots of the diluted sample and assay controls to the assay plate.

3.2. Assay Assembly

Assay assembly unit operations include all the steps necessary to combine the test samples with all the other ingredients of the assay. Examples of assay assembly unit operations include the following.

3.2.1. Reagent Addition

A reagent addition step is described as the addition of a reagent (or mixture of reagents) in a constant volume to all wells on the plate. The control parameter is volume of the addition. In many cases, adequate mixing is achieved by the combination of the dispensing velocity and diffusion. In other case, agitation or mixing via up-and-down pipetting may be required. If reagent additions are performed by pipetting as opposed to noncontact dispensing, washing, or changing tips between plates may be required to avoid carryover.

3.2.2. Incubation

Incubation steps may be required for several purposes, including biochemical-reaction equilibrium, cell growth, scintillation proximity assay (SPA) bead settling. Control parameters are length of time and environmental conditions, commonly including temperature, humidity, and CO_2. From an automation perspective, there are many important details concerning incubation periods. What is the allowable range around the nominal incubation period? An assay with rapid-reaction kinetics might require tight control of the time period between starting the reaction and adding a stop reagent. Other reactions that go to completion may be primarily concerned with establishing a minimum incubation time. Whether or not plates can be stacked or lidded during incubation must be determined. In a fully automated system, a robotic incubator can be a complex and expensive part of the system. The interface between the incubator and the plate-handling robot is critical to ensure that the loading of one plate at a time, every few minutes, does not excessively perturb the environmental conditions that are being maintained. In contrast, with unit automation plates are loaded into the incubator in batches of a size appropriate for the particular assay. The principal concern in this case, is ensuring that if the incubator will be not just maintaining, but significantly changing the temperature of the plates, that the thermal mass of the plates loaded and the time to reach equilibrium is taken into account ***(5)***.

3.2.3. Cell Plating

A specialized form of reagent dispensing, cell plating may require special provisions to avoid contamination, maintain uniform cell suspension and minimize temperature effects while out of the incubator. For example, developing a robust process for dispensing cells into 384-well microtiter plates was problematic. An 8-channel peristaltic-type dispenser had been commonly used for dispensing cell suspensions into 96-well plates. When the same equipment was used with 384-well plates, a small but unacceptable percentage of wells had trapped air bubbles at the bottom of the well. It was determined that if a

384-well pipettor was used to "pre-wet" the plates with media, the bubble problem was completely eliminated. As long as the bottom of the well was covered with media, the cell suspension would be drawn down to the bottom without trapping air.

This is a particularly good example of the power of decoupling steps using unit automation. Even though two steps (pre-wet and cell dispensing) were required, the overall operation was much more effective. One hundred 384-well plates could be loaded onto the 384-well pipetting workstation and the media pre-wetting operation could run unattended (in about 30 min) while the cells were being harvested. The cells could then be dispensed into the pre-wetted plates in another 30 min using a reagent dispenser.

3.2.4. Reaction Transfer

In some heterogeneous assay formats it is necessary to transfer the contents from a first plate to a different plate for further processing or detection. Enzyme-linked immunosorbent assay (ELISA), filter binding, and cell based assays that require removal of supernatant from the cell layer are just a few examples. The preferred methodology is to use a multichannel pipettor (96 or 384) to effect a simultaneous transfer from all wells of a plate. In addition, it is frequently important to minimize the time (or the variability of timing) across a given batch of plates.

3.2.5. Filtration

In HTS, filtration refers to a specialized unit operation used to separate bound and unbound assay components ***(6)***. Filter plates and extraction equipment are available from numerous manufacturers (*see* Appendix A). Filter plates have a permeable membrane at the bottom of each well. When the plate is placed on a vacuum manifold, the well contents are pulled through the filter membrane and the permeate is separated from the filtrate. Generally, the permeate is waste and the filtrate is washed with buffer to remove remaining unbound material.

3.2.6. Plate Washing

Plate washing is required for many assay formats for a variety of reasons. Plate washing involves removing liquid from the wells and refilling with another liquid. All plate-washing operations are not alike. Some applications, such as coating plates with proteins, are simply concerned with effective removal of all excess residual coating. The addition and removal of the wash buffer can (and should be) quite vigorous, with relatively high flowrates to maximize throughput and leave as little residual liquid as possible.

In other cases, spent cell-culture media must be removed and replaced with fresh (or serum-containing media replaced with serum-free media). Some assay formats involve binding reactions where the unbound portion must be washed off. In other cases, cells may be loaded with dye ***(7)***. At the end of the dye-loading period, the dye which has not been incorporated into the cells must be washed off. In any of these cell-washing applications, both the addition and aspiration must be carefully controlled to avoid damaging the cell layer. Liquid-flow rates, vacuum pressure, tip speed, tip height, and aspiration time are all variables that must be optimized for best results.

3.2.7. Plate Sealing

Frequently plates are sealed, either to provide containment of radiation or other potential hazards, or prevention of evaporation or contamination. Variables that determine the choice of seal stock include heat sealing vs adhesive seals, whether the plates will need to be unsealed, compatibility with solvents, suitability for low-temperature storage. There are also seals that can be pierced and resealed. Another important consideration is making sure that adhesive residue or loose seal edges will not cause problems for plate-handling equipment.

3.3. Signal Detection

The signal-detection unit operation is the key step where the biochemical activity is quantitatively measured. From an automation standpoint, whether the method employed is colorimetric absorbance, luminescence, SPA ***(8)***, or any of the fluorescent formats is not as important as understanding the implications of throughput, cycle time, and signal stability. Reading a 384-well SPA plate on a 12 detector scintillation counter can take over 20 min per plate. But since this assay format is typically stable for 24–48 h, large batches of plates can be prepared and read on multiple counters over an extended period of time. Conversely, a cell-based chemiluminescent assay may need to be read within 30 min of lysing the cells ***(9)***. In this case, it is necessary to restrict batch size to that which can be read within 30 min, or to utilize a reader with on-board reagent addition.

In the past, most detection systems have been based on Photo-Multiplier Tubes (PMTs). For those assay formats where signal collection time is significant (> 1–2 s) higher throughput is achieved by arranging multiple PMTs (up to 12) in parallel. The next advance involves switching from measuring individual wells to imaging the entire plate using a charge-coupled device (CCD) camera. This approach enables the reading of higher-density formats such as 1536-well plates, where reading one well at a time is not feasible.

The latest generation of CCD-based plate imaging systems have added to the advantage of unit automation. While these imagers are extremely sensitive and can have very high daily throughputs (100,000s of wells per day) they are also extremely expensive ($300k–500k). If one of these imagers is incorporated in a fully automated system, it cannot easily be shared by multiple assays that are in different phases of development, HTS, or follow-up. The workstation approach is much more amenable to shared utilization by a number of users.

4. Unit Automation in HTS

4.1. HTS is Different from Clinical Automation or SAR Support

One of the significant contrasts between the application of laboratory automation to HTS compared to clinical assays is the duration of the assay. In a clinical diagnostic laboratory, new test samples arrive on some periodic basis. The assay will be run perhaps for years, until a better assay replaces it. Similarly, screening in support of a medicinal chemistry Structure-Activity Relationship (SAR) effort deals with relatively few samples at a time, but may go on for months, even years in an iterative fashion.

In contrast, the objective of HTS is to screen hundreds of thousands of compounds for activity in an assay as quickly as possible, for only once the initial run is complete can the subsequent phases of confirmation and secondary assays take place. Because of this, a typical assay runs for only days, or at most weeks in an HTS lab. Consequently, there is a limited opportunity for assay level automation to "pay" for itself. In fact, if time is required to reconfigure, reprogram, and revalidate a fully automated system for each assay, considerable cost can be incurred. On the other hand, once the methodology for a unit operation is standardized and automated, it can be re-used over and over for a multitude of different assays.

5. Advantages of Unit Automation

5.1. Flexibility

As implied in the previous section, perhaps the greatest advantage of the unit-automation approach is flexibility. The building blocks that are the automated-unit operations can be combined in whatever order and quantity are required for a specific assay. **Figure 3** shows how assays can range from the simple, homogeneous, Add/Incubate/Read type to extremely complex, heterogeneous formats involving many steps, multiple incubations, and plate-to-plate transfers. A fully integrated system that has the capability to execute very complicated assay formats will require a great deal of space, sophisticated control algorithms, and equipment that can perform all the different unit operations.

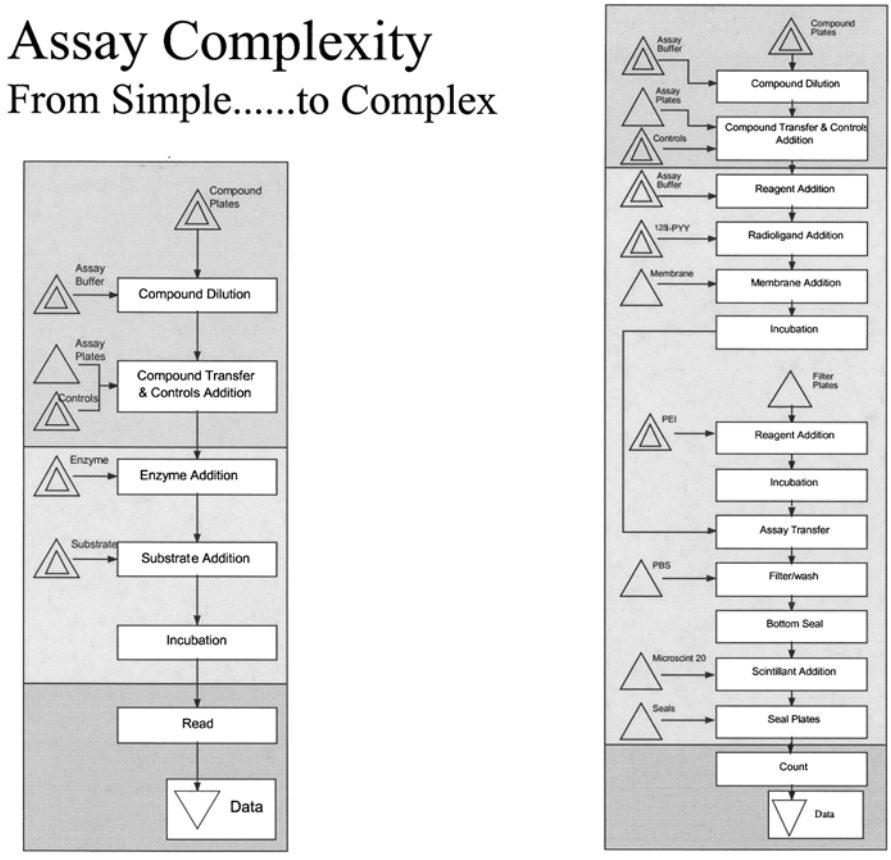


Fig. 3. Assays vary greatly in complexity.

5.2. System Reliability: Complex vs Simple and Serial vs Parallel

Robotic manipulators have become increasingly reliable and the software that controls them increasingly easy to use. However, the fact remains that in a fully automated assay system, the hardware and software that make up the robotic manipulator is, in many cases, the most complex portion of the entire system. Positional requirements involve moving plates several meters with an accuracy of tenths of millimeters, from hundreds of storage locations to dozens of instruments. On top of this is the complexity required of dynamic scheduling software to optimize utilization of both the robot and the instruments. Without a good dynamic scheduler, accrued lag time waiting on plates can add

significant time to a method. The de facto result of this additional complexity is increased downtime, whether due to planned preventive maintenance or to unanticipated hardware or software failures.

Another approach uses many, simple, dedicated plate handlers to move plates from one instrument to the next. In this approach, each plate handler serves only one "source" and one "destination." While this greatly simplifies both the hardware and software demands, it also greatly increases the potential for downtime. It must be remembered that the reliability of a system is equal to the product of the reliability of individual components ***(10,11)***. Thus, if there are 10 plate handlers in a system that each have an uptime of 99%, the expected uptime of the system is 0.99^{10} or only about 90%.

The unit automation approach avoids these additional opportunities for decreased reliability by omitting these elements entirely. Of course, this requires that people be available to move stacks of plates from one process step to the next, but especially with plates of increasing density (384, 1536, etc.) the number of operator actions required is minimized. In addition, when multiple workstations exist in a unit-automation environment, they can be used in parallel and provide redundancy in case of equipment failure.

5.3. Decoupling Facilitates Maximal Thruput

A significant advantage of unit automation is the ability to utilize available equipment to optimize thruput for a given assay ***(12)***. For example, a colorimetric absorbance plate reader can read more than two hundred 96-well plates in 1 h. For a simple enzymatic assay with short incubation times, it might be desirable to team four 96 well pipettors (at a rate of 50 plates/h each) performing the compound transfer operation and be able to easily run 1200 96 well plates in an 8-h shift. Conversely, a cell-based ELISA might use the same plate reader, but be limited to 200 plates a day due to cell culture or plate washing capacity and thus only need one 96-well pipettor. In the fully automated approach, some fixed amount of capacity for each unit operation is dedicated to an assay system. This has the inevitable result of less than optimal thruput for straightforward assays or substantial underutilization of capacity at some steps for more complicated assays. The Gantt charts shown in **Tables 1–3** portray the flow of batches in a typical biochemical SPA. These charts illustrate not only the power of parallel processing, but also how miniaturization of assays into higher-density formats (96 to 384) dramatically reduces the number of operator interventions required.

In the assay illustrated in the Gantt charts, counting is performed in a single large batch. This is accomplished by a "workstation" (shown in **Fig. 4**) that is made up of other workstations. Three Wallac Microbeta Trilux scintillation

Fig. 4. Scintillation counting workstation.

counters are fed by an ORCA plate-handling robot from carousels that can hold up to 400 plates. This approach eliminates the need to load a batch of 16 plates every 2 h and maximally utilizes both the plate handling robot and the counters. By decoupling preparation of plates from counting, plates can be prepared in batch fashion in a very short period of time and counting can continue unattended through the night.

5.4. Independent Workstations Minimize Impact of New Instruments, Technologies

HTS is a rapidly evolving discipline ***(13)***. Much of the equipment currently offered by leading instrument manufacturers was not even available three years ago (*see* Appendix B). New advances in miniaturized liquid handling, higher density plate formats, new detection technologies, proprietary reagent systems optimized for specialized detection platforms and other changes are continually emerging ***(14)***.

Table 1
96-Well Plates, 400 Compound Plates/d

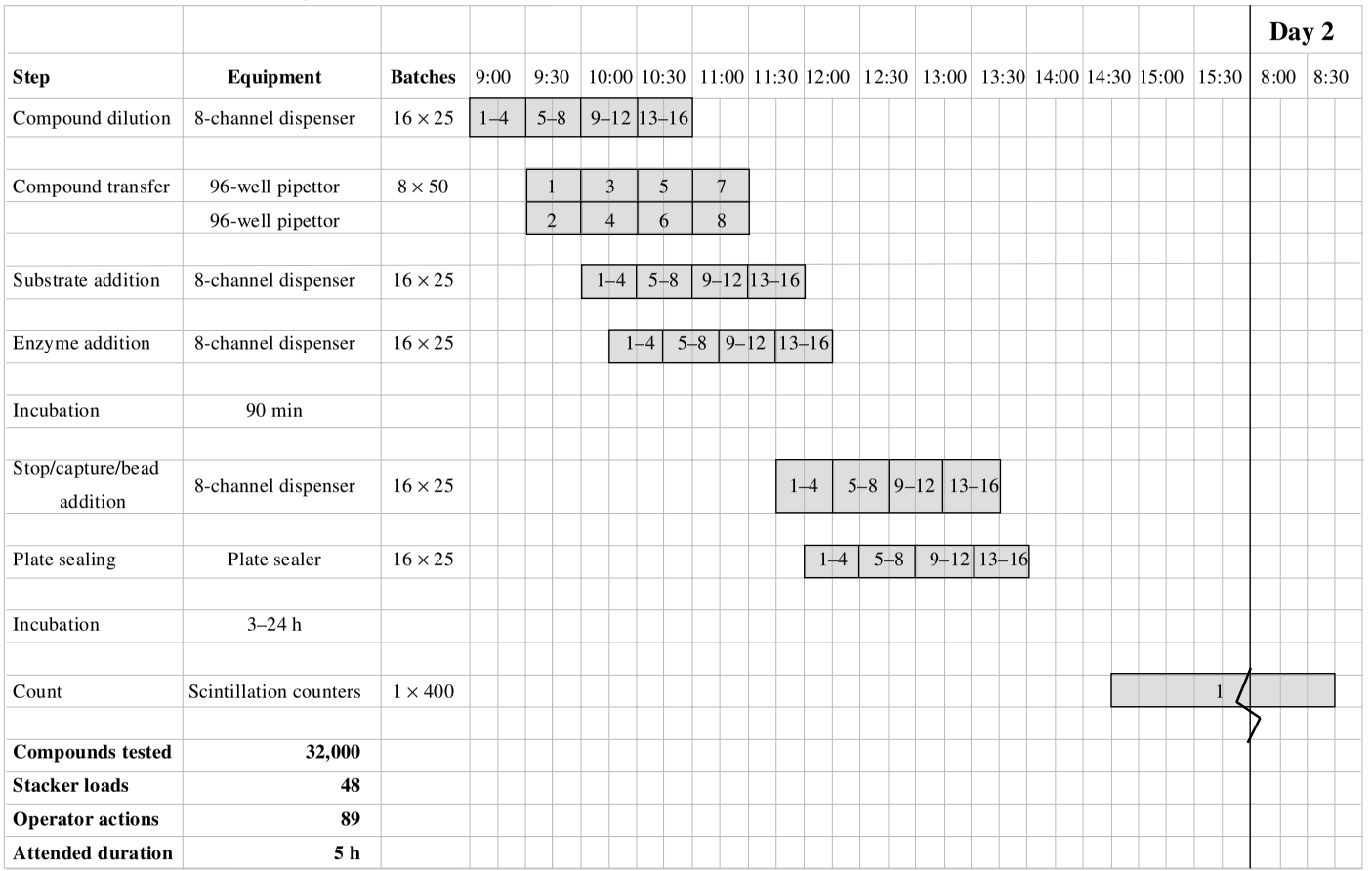


Table 2
384-Well Plates, 400 Compound Plates/d

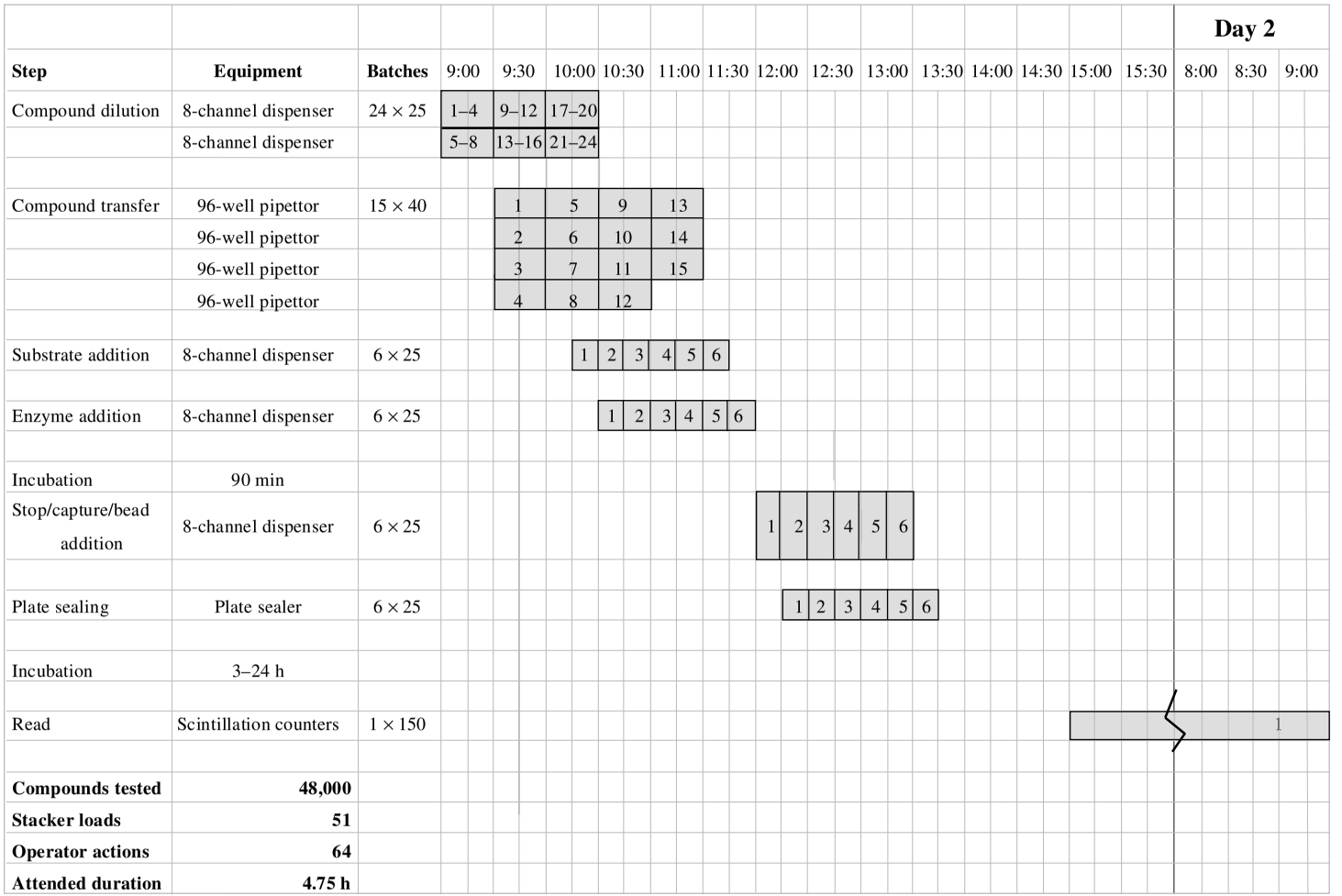
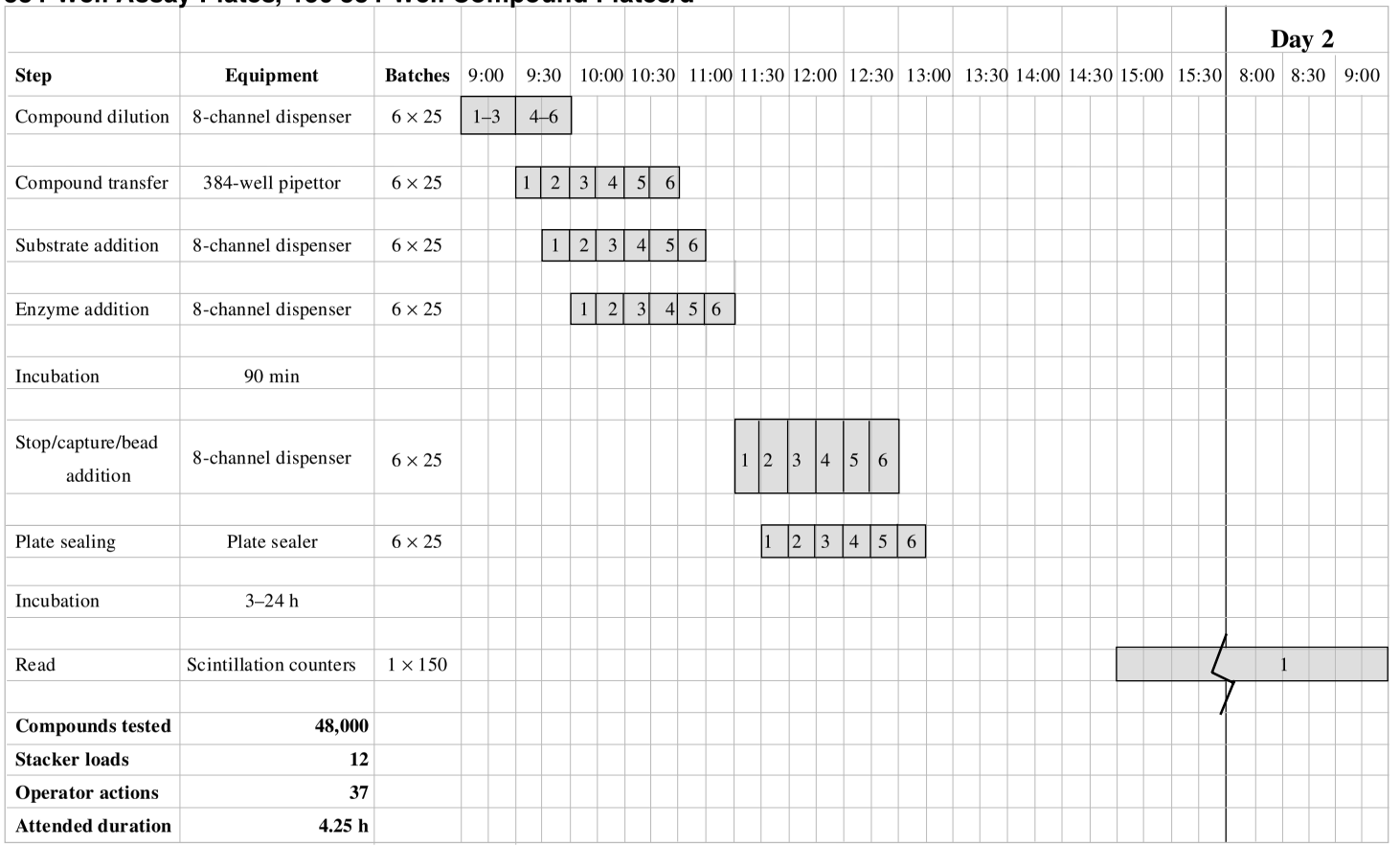


Table 3
384-Well Assay Plates, 150 384-Well Compound Plates/d



The impact of incorporating any of these advancements in a fully integrated automated screening system can be significant. Conversely, by its very nature, the unit automation approach confines the impact of changes to the specific unit operation involved. Proof-of-principle experiments, pilot runs or side-by-side comparisons vs existing methodologies can be readily performed with minimal impact to ongoing operations.

5.5. Incremental Capacity Additions Possible

Another significant advantage to the unit automation approach is the ability to add capacity in an incremental fashion. Capacity can be managed independently for each unit operation. Cycle time for a given unit operation can be measured. Cycle time multiplied by the number of workstations that can perform that operation, along with an estimate of utilization, results in the capacity (total throughput) for that unit operation. If a shortfall in capacity exists, it can be increased either by improvements that reduce cyle time, increasing utilization or, if neither of these are feasible, by adding another workstation. Conversely, in a fully integrated approach, adding capacity may very well require the addition of another complete system.

5.6. Access to Automation for Assay Development and Follow-Up

While the HTS timeline may only be days or weeks, both assay development and follow-up phases typically last for months ***(15)***. The unit automation approach allows equal access to the same capabilities throughout all phases of a project. In the fully automated approach, unless a system is dedicated to a specific assay format, it will very likely be reconfigured after each HTS target.

6. Conclusions

Whether an HTS laboratory decides to utilizes workstation automation, fully integrated systems, or a combination of both approaches can be a matter of philosophy or culture, as much as technology or economics. In any case, the principals described in this chapter can be applied. The key points to remember are:

- Automate the process, not the assay. If an automated system is designed around the needs of a particular assay, the probability that it will have to be modified for subsequent assays is substantially increased. Instead, focus on the processes used in the operations to design systems that are assay independent.
- Identifying unit operations is key. The most important component of process-based automation design is breaking the process down into generic unit operations that can be utilized for a wide variety of assay formats. Once unit operations become the focus, improvements in methodologies, capacity, cycle time, and cost will benefit any assay that utilizes those particular unit operations.
- Standardize until it hurts...but be flexible. This seemingly contradictory statement is a powerful tool for prioritization. By standardizing wherever possible, on

equipment or methodologies, you can reserve flexibility for those occasions where it really matters. Standardization promotes incremental improvements, greater familiarity, fewer errors, and reduced learning curve.

- Look for the bottlenecks. The unit operation approach makes it easier to examine processes to determine where the true bottlenecks reside. Focusing resources on the bottleneck, whether to investigate improved methodologies or to add capacity, is the only way to improve the overall output of the system. Improvements in other areas will not result in benefit if a bottleneck in another area prevents their utilization.
- Remember the bottom line. We're not in the automation business; we're not even in the HTS business. We're in the business of drug discovery. A rapidly changing, continuously evolving enterprise, where tremendous sums are spent and failure and success cannot be evaluated until years down the road. Because of this, return on investment (ROI) or other financial payback calculations can't be utilized as easily as in other ventures where automation is frequently applied. This reality increases the importance of delivering the right automation solutions for the right problems.

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Appendix A

Manufacturers of Filter Plates Used in High Throughput Screening

Manufacturer/Product	Website
Corning/Filter Plates	http://www.scienceproducts.corning.com/
Millipore/Multiscreen™	http://www.millipore.com/
Packard Biosciences/OmniFilter™	http://www.packardbioscience.com
PerkinElmer Wallac/Acro Well, Harvest Plate	http://www.wallac.com/
Whatman Polyfiltronics®/UniFilters™	http://www.whatman.com/

Appendix B

HTS Workstations Introduced Since 1998

Manufacturer	Model	Type	Date
Tecan	Polarion	Fluorescence	1998
Labsystems™	Fluoroskan Ascent FL™	Multilabel	1998
Packard	MultiPROBE® II	Pipettor	1998
Packard	PlateTrak®	Pipettor	1998
Tomtec	Quadra96/384®	Pipettor	1998
Hudson Control Group	PlateCrane™	Plate Handler	1998
Hudson Control Group	PlateSilo™	Plate Handler	1998
Packard	PlateStak	Plate Handler	1998
Amersham Pharmacia	LEADseeker Generation 1	Plate Imager	1998
Labsystems™	Nepheloskan™ Ascent™	Turbidity	1998
Titertek	MAP Series	Washer	1998
Tri Continent	Encore 2000™	Washer	1998
Labsystems™	Multiskan™ Ascent™	Absorbance	1999
Titertek®	Multidrop 384™ with S60 Stacker	Dispenser	1999
Molecular Dynamics®	Typhoon 8600	Gel Scanner	1999
Tecan	Ultra	Multilabel	1999
Wallac™	1420 VICTOR ^{2}™	Multilabel	1999
Cartesian Technologies	PegaSys™ 320	Pipettor	1999
Cartesian Technologies	PixSys™ 3200	Pipettor	1999
Cartesian Technologies	PixSys™ 5500	Pipettor	1999
Tecan	GenMate	Pipettor	1999
Amersham Pharmacia	LEADseeker Generation 2	Plate Imager	1999
Tecan	PowerWash 384	Washer	1999
Tri Continent	Multiwash II™	Washer	1999

Tri Continent	Multiwash Advantage	Washer	1999
Packard	Alphaquest®	Fluorescence	2000
Wallac™	Flite	Fluorescence	2000
Packard	Fusion®	Multilabel	2000
Tecan	Genios	Multilabel	2000
Tecan	Safire	Multilabel	2000
Wallac™	1420 VICTOR V™	Multilabel	2000
Beckman Coulter	Biomek® FX	Pipettor	2000
Labsystems™	Wellpro 384	Pipettor	2000
Packard	MultiPROBE® HTS	Pipettor	2000
Tecan	TeMo-96	Pipettor	2000
Tecan	Genesis NPS	Pipettor	2000
Tomtec	Quadra3	Pipettor	2000
Tomtec	QuadraPlus®	Pipettor	2000
Amersham Pharmacia	LEADseeker Generation 3	Plate Imager	2000
Applied Biosystems	Northstar™	Plate Imager	2000
Wallac™	ViewLux™	Plate Imager	2000
Labsystems™	Wellwash 384	Washer	2000
Tomtec	Quadra-Wash® 96/384	Washer	2000