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Commentary

Theme: Ligand Binding Assays in the 21st Century Laboratory
Guest Editors: William Nowatzke, Ago Ahene, and Chad Ray

Ligand Binding Assays in the 21st Century Laboratory: Automation

Ago B. Ahene,[1,10] Chris Morrow,[2] David Rusnak,[3] Susan Spitz,[4] Joel Usansky,[5] Holger Pils,[6] Francesca Civoli,[7] Kinnari Pandya,[7] Brian Sue,[2] Daniel Leach,[8] and John Derent[9]

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INTRODUCTION

A constant demand in the pharmaceutical industry is to accomplish more with the same or fewer resources. It is generally accepted that a major expense of an established laboratory is the personnel, which constitutes a fixed cost. Improving the efficiency of laboratory personnel through automation reduces the overall cost.

In addition to this demand, there is an expectation for the production of high-quality data with an ever-shortening turn-around time. To meet this challenge, a significant amount of pressure is brought on the analyst who must find ways to increase the efficiency and throughput with limited resources.

Large molecule bioanalysis presents unique challenges, compared to small molecule analysis, that add to the complexity of quality data generation. Firstly, the number of samples to be analyzed may be doubled or tripled when compared to bioanalysis for small molecules. For example, unlike small molecules, monoclonal antibodies typically have long half-lives necessitating the collection of many sampling time points. In addition to the bioanalysis of the therapeutic compound for pharmacokinetic purposes, these macromolecules may elicit anti-drug antibodies that have to be quantified in addition to the drug concentrations. Samples that screen positive for anti-drug antibodies using an antibody assay undergo secondary testing for confirmation and further characterization.

The second challenge in large molecule bioanalysis is due to the limited dynamic range of the calibration curves in ligand binding assays used to estimate therapeutic drug concentrations. Calibration curves for large molecules typically range from picograms per milliliter to nanograms per milliliter whereas the serum or plasma drug concentrations are often in the micrograms per milliliter to milligrams per milliliter range. This might influence the choice of assay platform. Thus, in order for samples to be quantified by the calibration curve, they have to be precisely diluted sometimes by a millionfold in a sequence of serial dilution steps.

The third challenge is the format of these assays. The 96-well plate format is commonly used, although 384 and higher well plates have been developed for a few bioanalytical assays. Because samples are run in replicate, typically fewer than 30 samples/plate (60 wells) can fit into a batch of a 96-well plate run; the remainder of the wells are taken up by the replicate calibration curve and quality control samples which are incorporated onto each plate. Execution of a manual assay may take 4 to 6 hours. Data reduction, data checking (QC), and reporting may take an additional 1 or 2 days. About 120 samples (four plates) can be processed per day by a well-trained analyst. Thus it will take 15 working days (3 weeks) to complete analysis of an 1,800-sample study. This timeframe is only applicable if samples are all available on site at the commencement of analysis. For most studies, the samples do not arrive all at once thereby reducing the assay

[1] XOMA Corporation, 2710 Seventh Street, Berkeley, California 94710, USA.
[2] Genentech, South San Francisco, California, USA.
[3] BioAgilytix Labs, Durham, North Carolina, USA.
[4] MedImmune, LLC, Gaithersburg, Maryland, USA.
[5] Thermo Fisher, Philadelphia, Pennsylvania, USA.
[6] Hamilton Company, Reno, Nevada, USA.
[7] Amgen, Thousand Oaks, California, USA.
[8] Tecan, Durham, North Carolina, USA.
[9] Janssen Research Development, LLC, Radnor, Pennsylvania, USA.
[10] To whom correspondence should be addressed. (e-mail: ahene@xoma.com)

ABBREVIATION LIST: AP, Assay plate; CFR, Code of Federal Regulations; CO_2, Carbon dioxide; cGMP, Current good manufacturing practice; DOE, Design of experiment; ELISA, Enzyme-linked immunosorbent assay; IQ, Installation qualification; IT, Information technology; LBA, Ligand binding assay; LIMS, Laboratory information management systems; OQ, Operational qualification; PK, Pharmacokinetics; PQ, Performance qualification; QC, Quality control; RFID, Radio frequency identification; SBS, Society for Biological Science; USB, Universal Serial Bus.

throughput. The daily manual pipetting has been linked to repetitive stress syndrome culminating in ergonomic injuries and loss of work days. In addition, the manual mundane tasks cause worker fatigue that leads to operator errors. Thus, there is a critical need to adopt processes that will increase efficiency, quality, and reproducibility through laboratory automation.

Individual biopharmaceutical companies have implemented automation in their bioanalytical laboratories (1). Others eschew automation due to cost, complexity, and compliance risk. However, to a large extent, the pharmaceutical industry and its supporting contract research organization companies have not yet successfully implemented highly automated systems for bioanalysis in a standardized way. One reason for this lack of standardization is the speed at which technology evolves, compared to the speed of technology integration in regulated bioanalytical laboratories. Other reasons range from the failure to explicitly delineate the best approaches for laboratory automation to the equipment vendors and the end users, and the lack of cooperation between vendors to manufacture laboratory equipment that can be interchanged in a "plug-and-play" manner.

There are many examples of competitive companies collaborating in other highly complex and technical industries. One example is the Universal Serial Bus (USB) specification (2,3) which allows external devices such as computer mouse devices, printers, external hard drives, and digital cameras to be easily and readily plugged into computers with "plug-and-play" simplicity. A second example is the standardized 96-well plate that was initiated by the Society for Biomolecular Sciences (SBS) and published by the American National Standards Institute on behalf of the SBS (4). The definition of a microtiter plate governs various characteristics of microplates including well dimensions and plate properties. This article describes the processes most generally used in the automation of ligand binding assays (LBAs), highlights the shortcomings of current laboratory automation technologies and advocates for a common set of hardware and software standards and inter-device communications that can be used to automate sample processing.

APPROACHES TO AUTOMATION

At the present time, to undertake an automation project one has to do one of the following: purchase an off-the-shelf automated system, have a system custom-made or embark on a do-it-yourself endeavor.

A discerning analyst may elect to contract out the construction of such a system to a vendor but may want to have a say in formatting the integration of components into an automation system. In some cases, the expertise for automation may reside in-house, thus the analyst may select choice components to build the desired highly tailored automated system to meet the foreseen need. Major problems involving communication between components tend to arise when components from different vendors are selected as part of the automated system. Such incompatibilities should be eliminated or at least minimized in the 21st Century laboratory.

Ideally, one would like to assemble preferred components from different vendors, plug them into a central computer (Fig. 1) and with communication software, be able to execute an assay with minimal or no tweaking. This vision can be likened to "plug-and-play" systems that exist in the computer or the audio industries where irrespective of the source of the components, the assemblage works.

In the regulated laboratory environment (e.g., CLIA, GLP, cGMP) (5–7), it is not sufficient to claim plug-and-play capabilities. Each aspect of a system including the hardware, software, users, procedures, and the environment must be carefully validated to ensure that the instrument performs the tasks for which it is designed (functional integrity) and reliability of system data (data integrity). Validation requirements for an instrument typically include installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). One must also address compliance with electronic signatures (Title 21 Code of Federal Regulations (CFR) Part 11) (8).

In many bioanalytical laboratories, the skills required to conduct system validation are often either not present or the personnel may have other competing projects that preclude timely support. As a result, the platforms can remain idle for months waiting for resource allocation to verify their validity.

From a 21st Century laboratory perspective, the goal is to define a set of minimum requirements that require verification, a list of test scripts that cover 80% of the core functionality of an automated platform, and a recommended end-user test that combines the three phases of IQ, OQ, and PQ into a simple process. By combining these processes into one that is easy to use, the laboratory need only focus on validating their procedures and environment. This will result in cycle time reduction from receipt of components to implementation.

In-House *vs* Outsourced System Development

There are three broad strategies for LBA automation, namely, do-it-yourself in-house, an off-the-shelf vendor-built system, or a collaboration between the end user and one or more vendors to build a custom system. All approaches have their pros and cons, but none can be hands-off. Even for an off-the-shelf vendor-built system, the end user must evaluate if it meets the requirements, compare vendors, and validate the system for a specific use.

Before describing some of the pros and cons of the three options, we will first describe a typical project of each type. An in-house LBA automation project would typically involve a small team with the end user, and personnel with laboratory automation, project management, software development, mechanical, and electrical engineering, compliance, and validation skills (Table I). The project team would define their requirements, identify individual hardware and software components from various vendors, identify what hardware or software would need to be customized or created from scratch, set budget and timelines, and identify additional internal resources. The team would perform the full suite of hardware and software tests to ensure that each component functions as expected. The team would then go about integrating the various components into a single

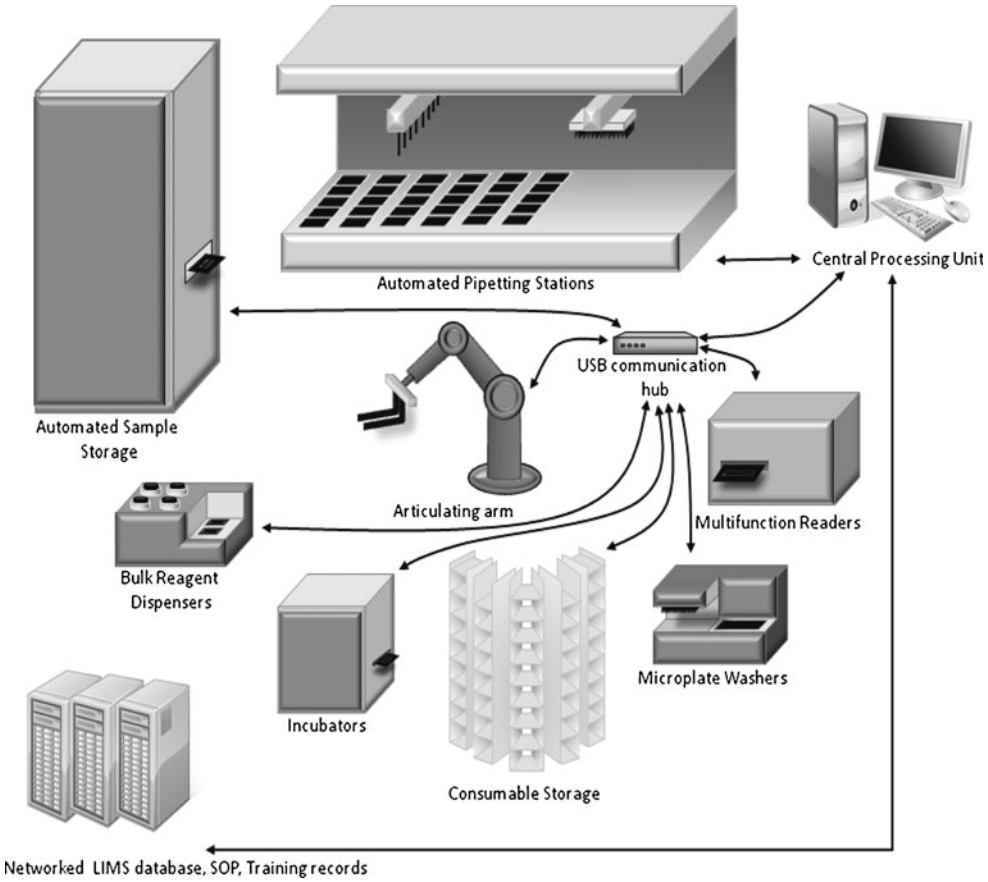

Fig. 1. Schematic of ligand binding assay automation system. All the peripheral components are connected to a central computer through an USB communication hub. Note that this depiction does not represent systems that are self-contained

system to ensure that the components interact with each other correctly, and the system as a whole meets its requirements.

Choosing a typical off-the-shelf, vendor-built LBA system would involve the same processes with the exception of software development and mechanical/electrical engineering. The project team would still need to spend considerable amount of time defining their requirements, and even more time assessing what vendors have to offer (since the vendor has to meet all requirements). Once a system has been chosen, the team would have to carefully test the system to ensure that it meets the critical requirements. This process will often identify deficiencies with vendor software or hardware that must be corrected or modified by the vendor. Once the system passes acceptance, testing, implementation, and validation begin.

The third approach entails collaboration between the end-user laboratory and a preferred automation company to build a customized system. As described above, a single automation company can rarely provide the best tools for every aspect of LBA automation (for example, liquid handling *vs.* plate washer, shaker, or an incubator). The end user and the automation expert would collaborate in choosing all automation components from solutions available on the market. This would be followed by integration of the hardware and the software by the automation company. Table I lists pros and cons of each approach.

The preferred approach depends on the skills and needs of an individual laboratory. For many smaller laboratories that may not have deep software development and engineering skills, purchasing an off-the-shelf system from a vendor probably makes most sense. For larger laboratories that do have the skills and resources a cost/benefit analysis should be performed to select the best approach. With available resources in-house, the third option which would require a more extensive collaboration between vendors is recommended. However, it is important to emphasize that any approach requires the end user to have a basic understanding of automation and the commitment to manage a complex technical project and overcome the inevitable obstacles that arise.

THE FOUR BLOCKS OF LBA AUTOMATION

Although there are some variations in how LBAs are run, the basic process can be divided into four parts: liquid handling, assay dynamics, data handling, and logistics. Liquid handling is the addition or transfer of liquids. It is comprised of reagent preparation, coating, and blocking of plates and pipetting of samples, standard curve, and quality control samples. Assay dynamics is the movement of plates from one station to another. Data handling includes the electronic (meta) data generated from each component and

Table I. Pros and Cons of In-House Developed and Vendor Developed Ligand Binding Automation System

In-house developed	Off-the-shelf vendor provided	Collaboration
Pros	Pros	Pros
Total flexibility in creating a solution	Comparatively inexpensive	Flexibility to choose from different vendors
Able to do things that vendors cannot (or does not yet support)	Vendor takes risk (if purchase order is worded correctly)	Vendor takes risk
More control over development process	Does not require depth of technical resource in-house	Most optimal end product using components already on the market
	Able to hire people who have experience with system	
	Large installed base makes bugs less likely	
	Large vendors have compliance controls in place	
Cons	Cons	Cons
Can be time-consuming and costly if staff is not qualified to take on such a project	Offered standard solutions are not flexible or hard to customize	Automation companies do not always work together
All of the project risk is taken by the end user	Limited in what vendors have to offer	
Can be difficult to validate if validation was not considered during design		
May need to develop (or hire) software development and engineering skills		
Difficult to replace trained users for fully customized systems		
Potential difficulty in managing multiple vendors		

communication commands sent between them. The logistics section describes system security and safety.

Block 1: Liquid Handling

The ultimate ideal automated system is "a walk-away" robot that runs the assay. Therefore the reagents must be prepared before use. The timing of reagent preparation and the stability must be evaluated. Ample reagent dead volume is required, particularly if a 96-channel pipettor is used. The blocking incubation step should allow for variable incubation duration so that there is some flexibility in when the robot starts processing the plates.

A typical preparation for an assay may involve a coating step, additions of samples and reagents, incubations of varying lengths of time and plate washing. Most LBA laboratories will not have the throughput to justify total automation including the reagent preparation step, but there are several key considerations to ensure an automation-friendly process later in the assay.

For example, the labware (assay plates) must be automation-compatible. This generally means that they are stiff enough to be handled by a robotic gripper. If different types of assay plates are used, the automation equipment must be programmed with the correct grip dimensions, well bottom, and pipetting volumes. Standardizing on a small number of plate types makes this process much easier.

The most tedious part of running an immunoassay is the preparation of the sample dilutions for measurement of drug concentration in samples from a toxicokinetic (TK) or a pharmacokinetic (PK) study. The liquid handler makes the biggest impact at this step in both terms of efficiency, precision, and accuracy. Even in cases where samples are run undiluted using automation to prepare sample aliquots into a 96-well format still provides significant throughput gains.

Bioanalysis laboratories must develop and validate PK assays that meet the requirements set forth in the bioanalysis guidance document including requirements for accuracy, precision and total error (9). The use of automation for the dilution of samples, standards and QCs can provide a significant benefit to meet these requirements by eliminating human error (10). By improving assay performance, more assay plates will meet plate acceptance criteria and fewer assay runs will need to be repeated, leading to additional gains in efficiency.

Sample Preparation and Loading

Depending on the downstream workflow the two common approaches used for sample preparation and assay plate loading are: (1) batch dilution and assay plate loading and (2) plate-by-plate dilution and assay plate loading

1. Batch Dilution and Assay Plate Loading: Depending on the stability of sample (neat or dilution) a certain number of samples, standards and/or controls are diluted as the first step. When the dilutions are complete, the system starts filling assay plates sequentially. This provides an efficient schedule for the immunoassay steps that follow. These samples may proceed immediately to analysis or could be stored for later assay. Plates of samples can only be diluted well in advance of the assays if sample stability supports it. In addition, this approach allows for overall efficiency gains by diluting and preparing

samples for other shared tests, such as immunogenicity, pharmacodynamic, and biomarker assays from the same aliquot during the same run of the automated instrument. The sample needs to be thawed only one time for multiple uses and can be tested in the same laboratory or distributed to other laboratories within the company or outside for analysis. Other advantages of pre-diluting samples are that the sample dilutions can be stored and repeat assays may be performed on the dilutions. The dilution plates can be used for repeats due to plate failures or individual sample failures. Automation can be used to "cherry pick" the individual samples for repeat analysis.

2. Plate-by-Plate Dilution and Assay Plate Loading: This approach is recommended if sample stability is an issue or if throughput is not critical. In this approach only the samples that will be analyzed on one assay plate right before they are loaded onto the assay plate are processed. This approach is particularly useful for samples that need to be run in real-time during a study. For biomarkers that do not have long-term stability or for studies that require a quick turn-around of small batches as the study proceeds, one may choose to dilute the samples and proceed directly to the assay. In this case, small batches of samples are loaded onto the instrument as diluted or undiluted samples and transferred onto the assay plate. The dilutions may require an intermediate plate for sample dilution before the sample is transferred to the assay plate. Whether the batch or plate-by plate dilution method is utilized, there are certain requirements for the automation. The requirements are described in Table II.

Due to the wide range of sample concentrations and the generally limited dynamic range of LBAs, samples and QCs may require dilutions anywhere from 1/1 to up to one millionfold in PK/TK studies. Each sample should be diluted into the assay range while maintaining a consistent serum concentration. This is best accomplished by using two different diluents. The serum test sample is first diluted to the assays' "minimum dilution" in a diluent that contains no serum. Subsequent dilution steps (if required) are performed in a diluent that contains the same serum concentration as the minimum dilution step. Samples are typically analyzed in duplicate at one dilution, although it is also common to analyze samples in singlicate at several serial dilutions (e.g., 1/10, 1/20, 1/40, 1/80).

Table II. Requirements for a Liquid Handling System for Sample Preparation

Eight or more independent probes
Can handle volumes of 10–5,000 μL for sample and diluent (5 μL may be required for 384-well format)
Probes can access 8 or more sample tubes with different fill levels simultaneously
Supports a variety of tubes and labware
Can hold large volumes of diluent in reservoirs with small dead volume
Can hold sufficient number of dilution blocks/tubes, assay plates, and disposable tips
Can read tube and plate barcodes
Can track volumes of sample and diluent used for preparation of dilutions
Can track positions to which samples were pipetted
Safety features (discussed in section THE FOUR BLOCKS OF LBA AUTOMATION)

With these potentially large dilutions, the question becomes how to structure the dilutions to accommodate the volume limitations of the containers and the liquid handler. Dilutions are usually done in several steps. The maximum dilution factor for one dilution step depends on the containers used for dilutions and the accuracy and precision required. Most commonly, polypropylene deep-well plates are used for sample dilutions. The maximum dilution per step is then usually limited to 1:100 (as a 10 μL+990 μL) for 96-well plates.

The challenge is to perform all the pre-dilutions in an efficient manner. The more pipetting probes that can be used simultaneously, the more samples can be diluted in a given timeframe. However, diluting in the most efficient manner does not always position the samples in a way that allows efficient assay plate loading. A solution to this problem for assays with multiple dilutions is to divide the dilution process into pre-dilutions and final dilutions. The pre-dilution steps are performed in the most efficient way in pre-dilution blocks. The final dilution step for every sample is performed into the "transfer plate" in a way so the samples can be transferred into the assay plate without any positional changes.

This same approach is used for QC dilutions, although they are generally done at the assay minimum dilution. The same principle applies to standard curve dilutions, but the standard stock is generally not prepared in serum, so there is no need for the non-serum diluent. Standards are generally diluted serially to a minimum of 8 points. The test samples, QC samples, and standard calibration samples must all be run at the same minimum dilution of serum.

Different laboratories will have varying dilution practices, but the dilution process needs to be automation-friendly. The following are some general considerations to facilitate automation:

- Minimum pipetted volume of 10 μL: it can be difficult to get good accuracy and precision with serum and plasma below 10 μL given the variations in individual serum and the potential presence of clots.
- Perform dilution in deep-well plates: It is much easier to manage several deep-well plates compared to hundreds of individual tubes.
- Limit total dilution volume to 2 mL: A liquid handling robot can easily mix 2 mL sample volumes with 1-mL pipet tips. If larger volumes are used, care must be taken to ensure adequate mixing.
- Error detection, notification, and recovery: Liquid level detection, clot detection, bubble detection, inadequate volume detection should be used to differentiate between normal and problematic aspiration/dispense steps, particularly for any liquid transfers involving 100% serum/plasma. The system should notify users of the errors or be programmed to automatically recover from the errors. At the end of the run, the user needs some way of knowing which samples were correctly diluted and which were not. This can be accomplished through a paper log that the user

fills out during the run, or ideally through an automated report that the system generates at the end of the run.
- Utilize disposable tips: Although they are more expensive, there are two key benefits of disposable tips: (1) There is no possibility of carryover, which greatly reduces assay-specific carryover testing, and (2) the system is faster since no wash steps are required.
- Managing bubbles: Many liquid handlers have trouble dealing with bubbles. They can often detect them, but they generally have no idea of the distance between the bubble surface and the liquid surface (systems that can sense liquid level via pressure can get around this particular issue). Therefore, the best practice is to avoid generating bubbles in the first place. This means avoiding multiple "blow-out" steps that are sometimes used to ensure all liquid is dispensed from the pipet tip.

In addition, any laboratory with an automated liquid handler should have a procedure to check the liquid handler's precision and accuracy with minimal time investment. This may be done gravimetrically or colorimetrically using dyes, or using commercially available calibration systems. While these systems are not the perfect system, in that they may not provide measurements of the exact liquid used in assays, they do provide feedback that the liquid handler is working within specification. They also provide valuable feedback of instrument consistency over time against itself and compared to other instruments.

Block Two: Assay Dynamics

There are two general approaches to using automation in the bioanalytical laboratory. These are fully integrated automation systems, which perform the whole assay from start to finish or the modular approach, where two or more disconnected systems are used by the analyst. The fully integrated approach is often preferred because it frees up the analyst completely, while the modular approach can provide advantages in terms of efficiency and throughput, as well as ease of use. Here we describe primarily the fully integrated system approach. Assay dynamics is the movement of plates between components such as plate washers, incubators, shakers, and plate readers and the challenge of these movements being performed in a specified sequence, at a specific temperature, and with a specified timing. This may be accomplished by an articulated arm if stand-alone components are used (Fig. 1). For a single self-contained unit, an articulated arm is not necessary.

Automation of Immunoassay Steps

There are multiple platforms for performing LBAs. These immunoassays generally have similar formats, in that a reagent or a series of reagents capture the analyte, and another reagent or reagents detect the analyte. These reagents may be added in series as in a step-wise ELISA or there may be a solution phase incubation on one plate that is added to another plate containing the capture reagent. All of these steps are amenable to automation and usually require multiple steps of multi-well pipetting.

Table III depicts an example of a fully automated workflow for a LBA that utilizes an ELISA format. Note that similar steps are performed for other types of immunoassay formats utilizing electrochemiluminescence, fluorescence, or isotopic detection. In addition, there are many variations of incubation steps.

Some considerations to facilitate automation of these steps:

- If possible, validate assays without plate sealers or lids. Although more complex robots can handle lids and even plate sealers, this greatly reduces the timing efficiency of the system and adds complexity. In this case, the user must evaluate possible evaporation effect on plate uniformity.
- Set plate washers to aspirate each well completely dry since there is no human intervention to remove any excess liquid in the plate.
- Create a software utility to track actual incubation times for each plate and each incubation since predicting timing on these systems is not usually possible.

Automation of Other Bioanalytical Procedures

Automation is generally thought to be most useful for high throughput, repetitive type applications, such as sample analysis as discussed earlier in this section. However, automation can also be used to perform

Table III. Example of Fully Automated Workflow for LBA

User loads samples from −80°C freezer (automated or standard) and updates inventory database (laboratory information management system).
User and system prepare assay plates (AP) for assay.
User selects assay and enters run parameters (coating, blocking, etc.).
User loads reagents, samples, controls, standards and labware, and starts run.
System moves AP to dispenser and runs dispense protocol to add capture reagent (target, anti-id etc.).
System moves AP to incubator for *x*min.
System moves AP to washer and runs wash protocol.
System moves AP to dispenser and runs dispense protocol to add blocking reagent.
Plates are stored until needed for assay.
User selects samples to be analyzed from the database.
User selects assay and enters run parameters (dilutions, etc.).
User loads reagents, samples, controls, standards and labware and starts run.
System performs dilutions of sample, control, and standards.
System performs AP loading.
System performs assay steps.
System moves AP to incubator for *x*min for sample incubation.
System moves AP to washer and runs wash protocol.
System moves AP to dispenser and runs dispense protocol for detection reagent (conjugated antibody, e.g., biotinylated antibody).
System repeats the incubation, wash, and reagent steps for additional reagents (e.g., HRP-streptavidin, substrate).
System moves AP to reader and runs data acquisition protocol.
System creates output files (data file, results exported from data acquisition software, plate map file, run report).

variable and complex experiments that would otherwise be too difficult to perform consistently manually or have pipetting procedures that require consistency that may not be achieved with manual pipettes. Examples include the use of automation for design of experiments (DOE), pipetting samples for incurred sample repeat analysis, and preparation of batches of standard curves and QCs.

DOE is a powerful tool that leverages statistical design to test many different factors and conditions in a systematic and efficient way and is frequently used in bioanalysis laboratories to develop LBAs (11–13). To perform a DOE manually is extremely difficult because mistakes in sample transfer are easy to make, and it is difficult to know if and where the mistakes were made. Fortunately, automated liquid handlers can be programmed to pipet the complex plate set up of the DOE. If programmed correctly, the liquid handler will properly dilute each reagent and deliver it to the correct location. This can be performed through writing instrument scripts or using worklists. The best approach is to use software which automatically translates any experimental design into liquid handling and plate movements, without requiring programming; although one example exists of such a software, it would be useful if more automation companies would provide such a solution, to allow a wider use of DOE in the bioanalytical laboratories. Table IV describes the workflow of an automated DOE.

Another example of a variable process that may benefit from automation is repeat sample analysis. Oftentimes, LBA sample bioanalysis must be repeated. If an entire plate fails, it may be easy to find the source plate of dilutions and prepare a new assay plate manually or by using automation. However, the ability to "cherry pick" specific samples within a dilution plate or a collection of vials can be tedious if attempted manually and errors can occur.

Repeat sample analysis may need to be performed on samples for incurred sample reanalysis, due to percentage CV failure or a dilution not falling into the quantifiable range of the assay. The use of automation to select those samples from vials or dilution plates makes the activity less tedious and reduces the risk of human error. Utmost benefit is derived from liquid handlers in automation as long as flexibility is built into the system. Liquid handlers could be used in applications where parameters vary, where random or cherry picking is needed, or reproducibility is required.

Table IV. A Basic Workflow of an Automated DOE for Optimizing a LBA

DOE design (factors, levels, number of conditions, etc.) is used to generate a plate format
The user loads the automated liquid handler with the necessary labware, reagents, and buffers.
The automated liquid handler performs the complex dilutions and additions of the reagents in intermediate plates according to the plate format.
A multichannel pipettor (integrated or stand-alone) transfers the diluted reagents to the assay plate.

Block Three: Data Handling

A benefit of using automation is that assay steps can be tracked by instrument logs and output files. While manual methods may provide tracking in the form of check-lists or electronic notebooks, only robotics can provide real-time tracking of events. Instruments provide a log file which may include user log-in, administrative events, and actions taken by the instrument. Generally, these files are not user friendly, but are appropriate for general troubleshooting by a trained user. These files may be generated and formatted by the instrument in such a way as to facilitate regulatory requirements. Users in regulated environments should understand the generation of their instrument log files and be prepared to tailor appropriate security and record retention policies regarding these files. Vendors must design log file recording with regulatory (particularly the FDA's Title 21 CFR Part 11) guidelines on electronic records and electronic signatures compliance in mind. It would be useful for vendors to consider the design of log and trace files as they are quickly becoming a primary audit trail for many users and not simply a troubleshooting guide for instrument service technicians and engineers.

In addition to log and trace files, instruments also may provide output files that are useful for tracking bar-coded labware including plates and tubes. Specific vendors have sometimes made these files more user friendly by providing the option to export these files in various formats including text and Microsoft Excel® that provide many options for generating user-friendly output. The benefit of barcode labels on samples, QCs, reagents, and microtiter plates is that every step of the assay can be tracked with valuable information including but not limited to volumes of additions, source destination, a time stamp, liquid handling errors, barcode scanning errors. It should be noted that while barcodes are beneficial for tracking, the quality of the barcode is a parameter that cannot be overlooked. Because barcodes are the primary link between much of the labware, reagents and sample results, the barcode must be read efficiently and accurately. One-dimensional barcode labels must be applied appropriately, the instrument must be set to read the type of barcode (e.g., Code128, Code39) the whitespace must be sufficient and the barcode lines must be clear. Constant interaction between the user and the system to enter or correct unreadable or errant barcodes rapidly provides diminishing returns to the adopter of an automated system. 2D barcodes are becoming popular, because of their smaller size and usually lower error rate. Additionally, multiple seemingly disconnected log, trace, and output files can make assay tracking and troubleshooting a time-consuming process. For regulated studies the ability to accurately recreate steps of an entire assay is required. Having these output files and associated instrument log and trace files easily integrated and readily interpretable can be critical for facile evaluation by laboratory staff, service technicians, and quality assurance.

Block Four: Logistics

The logistics of working with automation include the consideration of security and safety. The security of a bioanalysis laboratory's instruments is critical for providing

high quality, reproducible tamper-proof data. The safety of the scientists utilizing the automation must also be considered, since there are mechanical hazards, electrical hazards, and biohazardous materials present when automation is utilized for bioanalysis.

Instrument Security

Many manufacturers offer well-designed instrument-specific authentication built into the automated system software. Instrument-specific security often contains thorough system tracking and audit trail generation for ensuring the integrity of the system and the data generated from it. Looking forward, newer models of liquid handlers are taking advantage of touchscreen technology, so it is conceivable that more cutting-edge approaches such as finger scans or retinal scan identification could be used for user verification. The security could be further enhanced by merging databases that include employee training to verify that the individual has been properly trained on all aspects the study. This approach to security offers two 21st Century laboratory advantages: better quality compliance and more efficiency because the system would require far fewer manipulations by the user to achieve verification.

Automation Laboratory Safety

Implementation of an automated system requires certain considerations regarding laboratory safety. While some features of automated systems may provide potential laboratory hazards that must be mitigated, a well-designed system can result in much safer experimental manipulations. There are key elements in ensuring safety when utilizing automation that includes evaluation of the hazard and implementing safety controls. Considerations should be given to the following: (1) mechanical and electrical hazards associated with instrument design and operation, (2) manipulation of hazardous solutions including the generation of aerosols and decontamination of laboratory work surfaces. If these considerations are well-managed, the automated system should result in lower exposures to potential hazards such as biological, chemical, or radioactive materials; contaminated sharps; and repetitive tasks that may cause chronic injuries. Thus ergonomic design is another safety concern, but also a potential advantage of automated systems. Most often, automation prevents ergonomic injuries by eliminating repetitive manual pipetting and labware handling. There may, however, be other ergonomic risks still prevailing. For example, when performing bioanalysis on the samples from large studies, capping and un-capping of tubes, opening, labeling or otherwise manipulating large amounts of other labware may present risks. These risks can often be mitigated with automated tube cappers and de-cappers, automated labeling systems, and labware packaging designs that facilitate easy loading into the system. Finally, automated systems usually demand significantly more time at the computer therefore applying good "office" ergonomic design in the laboratory setting is desirable.

Users should always consider the impact of system design on both upstream and downstream processes as they pertain to laboratory safety and should regularly ensure that they are keeping up to date with safety enhancements. Safety must be of paramount concern; however, it is recognized that any laboratory operation requires a balance of efficiency and safety that is best addressed by a well documented risk management process and understanding of current safety regulations. Sources for information regarding safety practices include individual institutions' safety department and OSHA 29 CFR 1910 guidelines (14) for general industry machine guarding. Individual institutions may require review of instruments for safety issues prior to purchase or inspections prior to use.

To achieve effective, safe, and secure automated workstation, the four blocks of LBA automation, namely, liquid handling, assay dynamics, data handling, and logistics require consultation with vendors, the scientists requiring automation of assays, the IT specialists, and the institution's environmental, health, and safety engineers.

COMPONENTS OF AN AUTOMATED SYSTEM FOR LIGAND BINDING ASSAYS

In this section, the various components that constitute an automated system are discussed. Consideration is given to the hardware comprising of automated refrigerators, liquid handlers, robotic plate handlers, incubators, plate washers, dispensers, plate readers, barcode readers, sealers, and decappers. The system control software and the LBA application software are also included (Table V).

Automated Storage and Retrieval of Samples

Automated storage at 4°C and −20°C and retrieval has been successfully used in the pharmaceutical industry for many years. New in the market are the −80°C systems. These systems can automate laborious sample location manipulation and chain-of-custody tracking, but they come at considerable cost. To that end, not all companies see a positive cost/benefit ratio for automating freezer storage. Additionally, any downtime on a fully automated system requires a manual backup process.

The first major consideration for this system is whether or not the materials can be stored at −20°C instead of −80°C. If storage must be at −80°C, the system will likely retrieve racks of tubes at −80°C and transport them to a −20°C chamber in order to pick the individual tubes required for the run since −80°C is less optimal for automation. A database for such a system will probably work best if it tracks only essential information. This includes container ID and location within the store or tube location within racks. This database should be on a backed-up server. All other information relating to the container should come from the laboratory information management systems (LIMS) or other central database. These systems typically run best with a limited number of labware types, for instance, one to three tube types or one to two plate types. The automated freezer should be able to scan samples (in the −20°C chamber) with linear or 2D barcodes. 2D barcodes should be preferred as they allow

Table V. Components of an Automated System for Ligand Binding Assays—Hardware

Component	Description
Liquid handler	Holds sample tubes/plates/tube racks, calibrators/controls, assay plates, diluents, dilution blocks
	Holds backend reagents (conjugate, substrate, stop solution, or read buffer)
	Can hold assay plates for incubation
	Can read tube and tube barcodes
	Performs sample preparation (pre-dilutions)
	Loads samples/controls/calibrators onto assay plate; dispenses backend reagents into assay plate
Robotic plate handler	Can move plates between all integrated devices
Incubator	Can hold required number of assay plates
	Can heat? Chill? Shake?
	Is it dark? Humidified?
	Can gas CO_2 (for cell-based assays)
	Can read plate barcodes (optional)
Plate washer	Can wash 96-well plates? 384-well plates?
	Can handle various wash buffers
	Can perform preventative maintenance protocols to avoid malfunctions due to clogged nozzles
Dispenser	Can handle various reagents
	Has separate, reservoirs, lines, and manifolds for each reagent
	Can perform preventative maintenance protocols to avoid malfunctions due to clogged nozzles
Plate reader/data acquisition device	Can read Absorbance? Fluorescence? Luminescence?
Plate sealer	Can seal assay plates
Seal peeler	Can peel sealing tape off assay plates
Plate stackers	Can store large numbers of assay plates and dilution blocks
	Can serve as a room temperature incubation solution (used in some ultra high throughput applications)
Tube capper/decapper	Can uncap/recap sample tubes; Available for 96-tube racks with rubber caps or screw caps (0.5–1.4 mL)
	Available for 48-tube racks with screw caps (0.5–2 mL)
Control software	Controls movements, operation, and communication of system components
	Allows user to create workflows and liquid handling protocols
	Can read data from external sources (structured text files or data base files), write data to external files
LBA application software	Enables trained user to schedule and run single or multiple assays on the same or multiple sets of samples.
	Enable trained user to create new assays by entering assay parameters
	Tracks and records all activities of the system (volume transfers, plate movements, incubation times, wash protocols, data acquisition protocols etc.)
	Supports 21 CFR part 11 features

for rapid scanning and faster confirmation of sample location when in process (at a liquid handler for example).

The system should provide a virtually frost-free environment. It should have redundant power supplies and cooling systems. The store should provide some way to manually access the samples. Strong consideration should be given to two modular units in the event contents of one unit need to be transferred to another. Traditional freezers may also be used as a backup.

The system should allow a central database or LIMS to issue a pull list of samples and should be able to arrange the samples physically in the tube rack according to instructions. The system should also have a user interface that allows an operator to directly issue a command for retrieval of a list of samples. Preferably this would be an intuitive touchscreen interface. It should be possible to dynamically schedule and prioritize such pull lists to allow a large pull sequence to be interrupted so small list of urgent samples may be pulled immediately. The system should track the tube rack barcode and the sample location in that rack while the rack is in process.

Loading of the system should require minimal information as the local store database should only record container ID and location. The load procedure should communicate with the primary LIMS and should be able to reconcile receipt of expected samples and work with the LIMS to call out samples in a logical group (study, etc.) that have not been received.

Liquid Handlers

Liquid handlers typically come as either multichannel head (96 and 384) models or as independent channel models (1–16) or a combination of both. They can handle volumes from 0.1 μL to 5 mL. The decks for these liquid handlers differ in size and format which affects the capacity for plates, tubes, and reservoirs that can be handled without user interaction.

Liquid handlers often offer a way to move plates or tube racks between locations on the liquid handler and to locations outside of the liquid handler (off-deck); they come with a variety of locators that support different types of labware (plates, tubes, reservoirs).

Liquid handlers can come with disposable tips, fixed tips, or exchangeable tips. Disposable tips are the most expensive and require dedicated deck space, but are faster and have no possibility of carryover. Washing fixed tips means that the system cannot pipet while the tips are being washed. Exchangeable tips can be dropped off into a wash station while another set of tips is used. Wash stations for exchangeable tips also allow the use of different wash solutions (detergent, disinfectant, water). Wash stations for fixed as well as exchangeable tips take up space on the liquid handler deck. Wash protocols need to be optimized for elimination of carryover.

Most liquid handlers use capacitance-based liquid level detection to sense the liquid surface. It is critical that this feature work correctly since an overly sensitive setting can result in the system mistaking the wall of the sample tube for liquid, resulting in an aspiration of air instead of liquid. Another desirable feature is the ability to detect clots. Some liquid handlers are generally equipped with capacitance-based clot detection in which the system retracts the pipette tip to a specified height above the liquid and take a capacitance reading to determine if it is still in electrical contact with the liquid. If it is, then the system assumes there is a clot hanging off the end of the tip and reports an error. There are also liquid handlers which have pressure-based detection where the system reports an error if the internal pressure in the pipette tip falls below a specified threshold.

The use of liquid handlers within an automated system varies with the system requirements. For full automation and full walk-away systems, the liquid handler usually handles the sample dilutions as well as the assay plate loading and reagent addition. Depending on the throughput requirements the system may include more than one liquid handler.

Dispensers

Most reagent dispensers on the market have only one eight-channel manifold through which all of the reagents are dispensed. To avoid cross contamination rinsing and priming are required which waste precious reagents. A few dispensers can handle four reagents in dedicated lines and with dedicated manifolds, however clogging of the probes with dried up reagent may require daily maintenance and cleaning. The decision whether to use a dispenser or a liquid handler to dispense reagents becomes a question of philosophy and cost. Note that liquid handlers have the advantage of having the lower dead volume.

Robotic Plate Handlers

Plate handlers can range from small workstations with integrated robotic arms, to simple rail systems on which plates move from one location to another, to complex gantry robot arms surrounded by various instruments. They can consist of articulated arms which sometimes are mounted on rail systems to bridge longer distances. They differ in size, reach, speed, safety features, and ease of use. They come with different gripper designs that are either optimized for accessing random access shelves or stacks.

Incubators

Incubators or incubation positions are chosen based on the assay requirements and expected throughput. For incubation at room temperature without the requirement for shaking or dark incubation, plates could be incubated on locations on the deck of the liquid handler (sometimes stacked). If there is no space on the liquid handler, plates can be incubated in plate stacks or plate hotels. Plate hotels are available with shaking options and in various sizes. If the temperature has to be regulated, automated incubators are the best option. They come as heated only or as heated and chilled. Heated incubators are available as single-plate, six-plate, ten-plate, or 40–1,000-plate units from different vendors. Chilled incubators are available as 40–1,000-plate units. Some incubators have the capability of CO_2 gassing for cell-based assays

When shaking many plates at room temperature in the dark is required, the incubator should also be able to compensate for the heat generated by the shaker.

Plate Washers

While assays that do not require washing are becoming more popular, the majority of assays still require one or more wash steps. Most common plate washers are available with 8- or 96-probe manifolds for aspirating and dispensing. The 8-probe models are of advantage when strip well plates are used. Strip well plates are common for commercial kits. Washers also differ in the number of buffers they can handle and in the parameters available to customize the wash process (soak, shaking, cross-aspiration, aspirate and dispense height, aspirate and dispense speed).

Plate Readers

With new labeling and detection chemistries available every year, the variety of detection equipment has grown tremendously in the last two decades. The common detection methods, absorbance, fluorescence and luminescence, and electrochemiluminescence are applied in various ways. Absorbance readers come as filter-based or monochromator-based models. Fluorescence and luminescence are used in plate- and bead-based detectors. Electrochemiluminescence is used in plate-based readers as well as in detection chambers.

Barcode Readers for Sample and Assay Plate Tracking

Barcoding is used to track samples and plates on their path through the system. Sample tubes are barcoded with either 1D barcode labels on the side of the tube or with 2D barcodes on the bottom. Plates are tracked with 1D barcodes on the side of the plate. More and more devices feature built-in barcode readers (plate hotels and incubators, readers, liquid handlers, sample storage systems) but there are a variety of barcode readers available for integration as well.

Miscellaneous Equipment

Based on the special need of a customer, other devices can become part of the automated system. End users sometimes

prefer sealing and peeling of plates to lidding. When samples come in tube racks decapping/recapping can be automated by integrating a capper/decapper instrument. Recently, 1.6-mL vials (10×47 mm) with 2D barcodes on the bottom are available in racks that allow automated decapping and recapping. If fast mixing is required, single-plate shakers can be integrated on the liquid handler.

With the availability of automated −80°C freezers with sample management software, the user does not even have to search for samples in the freezer; sample tubes can be picked from source racks into export racks and handed off to the liquid handler for processing.

Control Software

These days, almost every device comes with some sort of control software that allows the user to define protocols or set parameters remotely rather than via the controls on the instrument. Depending on the features of the device, this control software can vary in complexity.

Control software for a washer or dispenser is usually fairly simple. Control software for plate readers that also includes data reduction features can be more complex. Liquid handlers offer so many features that their control software often has its own scripting language. Sophisticated liquid handler software includes the ability to control third-party devices via drivers and scheduling capabilities. Control software should not only be able to address third-party devices and schedule workflows but it should also be able to communicate with other software and databases. Reading and writing information from and to a variety of file formats is also expected.

LBA Application Software

The LBA application software must be simple and easy to use. It should allow users to create assays by simply entering assay parameters and saving them as an assay template.

By combining the capabilities of sophistication in liquid handlers and the flexibility and versatility of control software with the functionality of the application software of simple ELISA processors, systems can process large numbers of samples in multiple assays while enabling complex dilutions and a variety of detection mechanisms. LBA application software should support import of sample worklists with sample barcodes, dilution factors, and auxiliary information. It should support the export of data contained in the sample barcodes and auxiliary information in a way that it can be imported into a LIMS.

FUTURE DIRECTIONS

In any field, technological development is typically an interplay between end users and vendors. Vendors create new instruments and software, and end users choose the systems that best meet their needs. While professional organizations such as the American Association of Pharmaceutical Scientists cannot and should not dictate the future of technological development, they can gather and collate feedback from a large body of end users, and provide coherent direction for vendors to satisfy end-user needs.

Vendors currently provide a wide variety of systems that can accommodate all of the steps of LBAs. Nowadays, systems are available for liquid transfer from nanoliters to milliliters in plate formats as small as 3,456 wells (15). Multifunction readers are available for detection of virtually the entire electromagnetic spectrum using real-time and time-resolved methods. Computerized LIMS software can track samples from automated freezers onto an instrument system and through the data analysis process. Seemingly, the process is complete, but brief conversations with assay developers will quickly reveal their need. The greatest gap currently faced by end users is the ability to rapidly integrate the myriad of instrumentation and software choices into an efficient system capable of meeting regulatory standards. Thus, the most idyllic situation for automation is to pull together a conglomeration of disparate instruments from different companies into an assay system and have it become operational with minimal manipulation and minimal skill requirements.

The proposition of this vision has been likened to what has been referred to in the computer industry as “plug-and-play”. This state of automation will become feasible through more standardized physical and electronic interfaces. The use of touchscreen programming, which has become common in our daily lives through cell phone technology, will bring familiarity to the new entrants from the next generation to laboratory automation. The basic steps for LBA are common for most assays; therefore, the availability and invocation of universal scripts for quick editing would be invaluable. Vendors across the spectrum of instrument and software supply should be thinking in the same context as smart phone providers. Applications or scripts should be available to be easily downloaded. Such availability goes well beyond clever marketing. It impacts the likelihood that a user will purchase and develop a system. Project life cycles often dictate that an assay be operational in days or weeks. If a system requires weeks or months to integrate, customers simply cannot justify the purchase.

As any bioanalyst will attest to, the mundane but required system validation takes significant amount of resources (16). Standardization of firmware and software will offer off-the-shelf solutions to validation. In the regulated environment, access to universal scripts would streamline the validation of the entire workstation. Standardized scripts that provide not only instrument integration but integration of user identification and data transfer would be priceless. By coupling graphical touchscreen interfaces with biometric identification such as finger or retinal scans, the move from paper-based records to electronic records can be easily implemented. Further utility of the electronic identification can be harnessed when the biometric data is merged with employee training records for automatic authentication. Additionally, these biometric data can also protect the restricted areas of the laboratory. 21CFR Part 11 compliance would quickly move from a cumbersome blend of physical security, data transfer, authentication, and signing procedures to robust electronic tracking which is difficult to corrupt.

An often overlooked gain from automation occurs in user safety. Certainly, any mechanically moving part of the automated system represent a physical hazard. The safety of

laboratory personnel should be safe-guarded. This group recommends the wider use of light shields that pause when a barrier is traversed. Electronic locks that engage when the system is in operation should also be incorporated. In addition, one has to take into consideration the incidence of repetitive stress syndrome involving sample preparation and transfers. Automated liquid transfers reduce repetitive stress disorders; the use of automated tube cappers and decappers provide increases in efficiency and decreases in repetitive stress injuries.

The use of automated labeling system should be a commonplace occurrence. Reagent suppliers must consider packing designs that make easy loading onto the assay system. As most of the activities from samples receipt to the report generation need to be automated, there is a need for improved labeling to identify samples through the use of barcodes. To this end, more efficiency can be harnessed by the use of the more superior RFID tags.

In the foregoing, attributes of ideal ligand binding assay, automated system have been discussed. The technology to bring this to fruition is currently available in the hardware, electronic, and the software industries. It is a yeoman's effort for any one company or a laboratory scientist to bring it to the fore. With a concerted effort of assay scientists and vendors, future automation systems could be created which would satisfy requirements of the drug development process and also acceptable to the regulatory agencies.

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