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Commentary

Theme: Ligand Binding Assays in the 21st Century Laboratory
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Ligand Binding Assays in the 21st Century Laboratory: Platforms

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INTRODUCTION

The 21st Century Bioanalytical Laboratory Platforms initiative in the BIOTEC section of the American Association of Pharmaceutical Scientists (AAPS) began with a pre-conference workshop held in Seattle, WA in June of 2009. This workshop brought together members of the pharmaceutical and biotechnology industries, with instrument and reagent manufacturers to discuss the current and potential future state of the bioanalytical laboratories supporting biologics development. At the conclusion of the workshop, four sub-teams were formed to further develop the ideas and concepts raised during the 2-day workshop. The sub-teams are reagents, automation, e-solutions, and platforms. This paper discusses the critical attributes of a research and development ligand binding assay (LBA) platform and the desired characteristics new platforms should strive to offer in the future. This paper is not intended to be a review and comparison of the current platforms on the market, as this has been done and published elsewhere (1–9).

The platforms team consists of a balanced cross-section of the industry with representatives from pharmaceutical, biotechnology, contract research organizations, and instrument manufacturers. The Platforms team have collaborated to discuss and arrive at a consensus regarding the most useful characteristics of a bioanalytical platform for biologics. We present here the results of these discussions.

A platform is the technology employed in an analytical method to transduce a biochemical event into a measureable output or signal. This signal allows the bioanalytical scientist to accurately and reproducibly make measurements to analyze different aspects of a specific biologic target (therapeutic, biomarker, and anti-drug antibody) such as its pharmacokinetics, immunogenicity, potency, or effect of biomarkers. An instrument is the tool utilized minimally to measure a platform's output and convert the resultant signal into interpretable information the analytical scientist can use but can incorporate other aspects such as liquid handling. Many platforms employ optical signals including the absorbance of light through a medium (10) or the emission of fluorescence (10) or luminescence (11). A variety of light detectors are used to measure these optical signals including photo diodes, charge-coupled device cameras, and photo-

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ABBREVIATIONS: ADA, Anti-drug antibody; CFR, Code of federal regulations; LBA, Ligand binding assay; PK, Pharmacokinetics.

Table I. Commonly Used Platforms

Commonly used platforms	Output	Detection mode	Example detection molecules
Chromogenic LBA	Optical density	Absorbance	TMB, AP
Luminescence	Relative light units (RLU)	Photon counting	Luminol
Time-resolved fluorescence	Relative light units	Photon counting	Europium chelate, Alexa Fluor®
Electrochemiluminesence	Relative light units	Photon counting	Ruthenium chelate
Label-free	Varies	Varies	Label-free (none)
Fluorescence polarization	Relative units	Photon counting	Fluorescein
Luminescence proximity assay	Relative light units	Photon counting	Europium chelate, Alexa Fluor®
Time-resolved fluorescence proximity Assay	Relative light units	Photon counting	Europium chelate, Alexa Fluor®
Imaging in combination with existing platforms	Image	Combination	Combination

multiplier tubes (Table I). Sections in this paper provide details on the desirable analytical characteristics, multiplexing, platform flexibility and throughput, desirable instrument characteristics, and finally, life cycle management of the ideal LBA platform.

The analytical characteristics of today's ligand binding assays are primarily influenced by three major factors—the quality of the reagents, assay format, and the choice of the analytical platform. This paper describes only those aspects derived from the analytical platform.

DESIRABLE ANALYTICAL CHARACTERISTICS

The analytical performance is critical for an LBA platform to be able to support pharmacokinetics (PK) (12), immunogenicity (13), biomarker investigation (14), and potency determination (15) throughout the life cycle of a biologic from discovery to commercialization. At a minimum, the following characteristics should be considered for new LBA platforms: sensitivity, precision, dynamic range, matrix interference, robustness and ruggedness, total assay time, and suitability for measurement of a wide variety of biotherapeutics (*e.g.*, monoclonal antibody, proteins, peptides, oligonucleotides, *etc.*). Additional characteristics to be considered may include multiplexing, throughput, adaptability to automation, and ease and cost of implementation.

Sensitivity

Clearly, an LBA platform must be sufficiently sensitive to detect the analyte measured in a specific application, regardless of whether the application is PK measurements, immunogenicity assessments, biomarker assessments, or potency determinations (16). The ideal platform would allow for some modification to sensitivity without either an assay format or platform change. The development of more potent therapeutics used at lower doses has driven a need for more sensitive platforms capable of quantifying low drug concentrations in a variety of matrices (serum, plasma, cerebral spinal fluid, *etc.*). Pharmacodynamic studies employing the measurement of biomarkers also benefit from highly sensitive platforms, particularly for biomarkers that are present at low levels. A sensitive platform can benefit immunogenicity assessment at sufficiently low ranges to monitor drug safety. Finally, sensitive platforms may better evaluate the impact of immunogenicity on pharmacokinetics, pharmacodynamics, and safety of the biotherapeutics (17).

Lowering background while increasing the signal-to-noise ratio in an LBA platform using relevant matrices will achieve greater sensitivity. An example of a strategy is utilization of a longer emission half life following excitation while maintaining high control in signal collection (18) as well as the measurement of a fluorescent signal at a shorter emission wavelength than that of the excitation (19).

Precision

A high level of precision is required to generate reproducible bioanalytical results for the measurement of drug, biomarker concentrations, and detection of immunogenic responses. Precision for LBAs analytical assays used in support regulated studies should be less than 20% coefficient of variation (12,20). A platform with high level of precision could reduce the random error of the analytical method, thus facilitating better analytical precision and more accurate monitoring of therapeutic and biomarker concentrations, immune responses, and potency measurements.

Wide Dynamic Range

A wide dynamic range is an important factor that can be achieved preferably through signal linearity or alternatively through the use of mathematical algorithms to extend usable outputs. A platform with a large signal range provides a tool for development of an analytical method potentially with a wide dynamic range. Because of the wide variety of studies during drug development, wide dosing ranges and the increased potency of today's therapeutics, a dynamic range exceeding 3 logs may be beneficial for PK assessments and monitoring biomarker modulation. For example, IL-6 levels in serum or plasma of sepsis patients can be 3 to 4 logs higher than non-sepsis patients (21), a wide dynamic range could reduce the number of dilutions required. Likewise, in order to ensure appropriate monitoring of anti-drug antibody (ADA) responses and due to variations of the magnitude of an immune response, a large dynamic range is useful.

Matrix Interference

The analytical performance of an LBA platform can be greatly impacted by matrix interference. A desirable LBA platform should have tolerance to biological matrix interference (22) and to circulating drug for biomarker and immunogenicity assessments (*i.e.*, high drug tolerance, able to withstand acid

disassociation, *etc.*) (23,24). Tolerance to biological matrices, such as serum components affecting signal output, allows for more sensitive assays to be developed, whereas greater drug tolerance could greatly enhance detection of immune response in study subjects (17). Additional tools for the management of chemical noise (correction or subtraction of background) could be incorporated into the platform's instrumentation and data analysis software. Existing technology can limit the detection of ADA to the recovery phase/nontreatment phases due to high levels of drug interference.

Ruggedness

An LBA platform should demonstrate adequate durability to perform reproducibly under slight changes in humidity, temperature, and other environmental or physical conditions including inter-instrument and inter-laboratory transfers. These parameters define the ruggedness of a platform and ensure consistent inter- and intra-laboratory performances which are becoming increasingly critical due to a number of trends including globalization of some pharmaceutical and biotechnology companies as well as an increase in outsourcing of bioanalytical studies to multiple organizations (25).

Total Assay Time

Ideally, a new platform should strive to generate results in 1 h or less in conjunction with the use of automation. Many automated clinical diagnostic platforms offer short total assay time (*e.g.*, <1 h) (26). However, these diagnostic platforms are not as flexible as open systems and the assay menus are often limited to only a few well-characterized analytes. The challenge is to form collaborations with diagnostic platform developers in order to identify and apply innovative strategies to more flexible systems.

Multi-modality

Finally, it is desirable for an LBA platform to be capable of measuring a wide variety of biologics, from peptides to macromolecules in a wide variety of biological matrices such as plasma, serum, whole blood, urine, CSF, saliva, and tissue extract. Additionally, an ideal LBA platform would be able to analyze not only individual molecules, but also molecules presented on cells, viruses, and bacteria.

MULTIPLEXING

The aforementioned desired analytical characteristics should be incorporated in any platform designed with multiplexing in mind. This section describes additional characteristics specific to multiplexing.

Multiplexing allows for the measurement of multiple analytes from a single sample and is particularly desirable for biomarker assessments. When evaluating multiplexing capabilities, there are considerations and preferable traits for the platform in respect to the dynamic range, sensitivity, matrix tolerance, on-board data reduction analysis, and the number of analytes which can be measured in one sample well. An ideal multiplex LBA platform, in combination with reagents (see "Ligand Binding Assays in the 21st Century I: Recommendations for Characterization and supply of Critical Reagents" in this issue of the AAPS Journal), would be expected to maintain its performance characteristics similar to the respective single assay should regardless of multiplex density (*i.e.*, 2-plex *vs* 10-plex).

Analyte levels can vary in different sample populations, thus the dynamic range of a platform will impact the ability to multiplex because different target analytes may be present a very different levels. For example, it may be impractical to use a platform with 2 logs of dynamic range to multiplex the measurements of two analytes whose concentrations differ by more than 2 logs, since a different level of sample dilution would be required for each analyte. Other desired attributes of a multiplexed platform include, but are not limited to: minimal cross-talk, option to limit data collection of assays in a multiplex panel that are relevant to a particular study, ability to collect data in a reasonable time frame, and an on-board data reduction software.

FLEXIBILITY AND THROUGHPUT

Increasing demands on LBA laboratories to produce high quality data with fewer resources has necessitated the need for flexible platforms that can accommodate high throughput processes and be utilized throughout the life cycle of drug development, starting from target discovery and validation through clinical and post-marketing surveillance. In evaluating the flexibility and throughput of LBA platforms, several factors are recommended to be considered, including two previously discussed topics: large dynamic range and multiplexing capability. Additional factors, discussed in this section, include automation compatibility, sample processing time, volume of data per analytical run, consumables (*i.e.*, reagents and labware), and global accessibility.

A number of bioanalytical laboratories are implementing automation into daily operations to either deal with lower available sample volumes or increase throughput by reducing human intervention. Because of the increased adoption of automation, a primary requirement for any platform is compatibility with either available automation systems or integration of independent automation components into the instrument. Further information regarding the optimal automation systems for the twenty-first century laboratory can be found in "Ligand Binding Assays in the 21st Century Laboratory: Automation" in this issue of the AAPS journal.

During various drug development phases, limitations in available sample volumes may necessitate a platform that allows for the evaluation of multiple different analytes simultaneously and/or assay miniaturization (*e.g.*, 384+ well plates or other substrates). A key consideration when evaluating miniaturization is the increased need for automation, as manual processing becomes impractical.

Reducing total assay time either by minimizing incubation times and/or the number of washes directly impacts the throughput of a platform. New platforms should have the flexibility to utilize either heterogeneous or homogeneous assay formats to reduce washing and number of incubations or implement assay miniaturization techniques to reduce time required for to reach equilibrium (27).

Another factor to consider is the availability and consistent quality of the consumables. Since the assays developed will be

used throughout the life cycle of drug development as well as post-marketing surveillance and can span many years, it is important to use platforms and consumables that are widely available from sustainable sources, can be manufactured reproducibly, and are stable for long periods of time. Non-proprietary, multi-source reagents, ease of labeling methods as well as multiple labeling chemistries that can be prepared in-house or available from multiple vendors would be preferable (28). Where proprietary technology offers enabling capabilities, business stability and customer commitment of the vendor should also be an important driver.

A final consideration for an ideal platform is its global availability. The platform should be able to accommodate and be readily accessible to different geographical regions around the world with respect to consumables, service availability, and regulatory requirements.

These attributes and considerations are not the only aspects of a flexible platform but are only a starting point. Some concepts not discussed in detail in this paper are decreasing assay development time, reducing the number of replicates, and fewer standard and control points.

DESIRABLE INSTRUMENT CHARACTERISTICS

Instrument Installation and Routine Operation

The ideal instrument housing the aforementioned platform should be user friendly, require minimal training, and have a short installation time. Electrical, computer, and network connections should be adaptable to the systems globally. In the case of automation, the instrument should have smooth interface between the hardware and software, requiring no additional programming to be written thus making it a "Plug-n-Play" style system.

Once the initial installation is complete, routine operation should be simple. The operator should be able to select a protocol and "click" to run the assay. There should be sufficient flexibility in creation of protocols to adjust critical parameters for providing optimal performance including: where appropriate, physical (temperature, humidity, *etc.*), and functional (detection mode, range of sensitivity, *etc.*) parameters.

Training required for operation, troubleshooting, safety, quality control, and maintenance should be minimal and will depend on regulatory requirements. Initial training by the manufacturer should be accompanied with training certificates at the time of instrument installation.

Technical support for the instrument platform should be of highest quality. On-line technical support should be available 24 h a day, 7 days a week in all countries where the instrument has been distributed. On-site service support should be available within a reasonable amount of time contractually defined at the time of instrument purchase. Troubleshooting documents should be available on-line and with the instrument.

Physical Characteristics of an Instrument

The instrument should be sturdy. Physical attributes such as size, weight, and footprint are important characteristics of the instrument. Bench top instruments are usually preferred over larger instruments. Stand alone instruments should comply with local environmental, health, and safety requirements and be easily accessible for both operation and servicing/repairs.

Many spare parts/components such as light sources, columns, filters, or PMTs have a finite life span and replacement parts should be readily available for purchase. The life of the major parts should be long enough to support long-term studies, or when replacement is required, there should be minimal variability in performance. When this is not possible or if a component is discontinued, information regarding a reliable, reproducible replacement should be provided by the instrument manufacturer.

Service and Maintenance

Manufacturers should provide validation services for instruments to be used in a regulatory environment. This service should include installation qualification and operational qualification tests. Additional care should be taken when selecting an instrument to ensure physical standards are available that can provide information on the accuracy of wavelength, well-to-well precision, linearity, detector sensitivity, noise, positional bias for microplate or other substrate, and other parameters affecting the assay performance.

Service contracts should be readily available at affordable rates and minimally include preventative maintenance followed with acceptable performance certification. Periodic servicing and replacement of parts should be available and appropriate re-qualification documentation provided. Daily maintenance should be minimal, detailed by the manufacturer, with well-documented procedures (*i.e.*, user and technical manuals up-to-date). Built-in reminders for service or calibration should be incorporated in the software running the instrument where applicable.

Software

Software used to run the instrument should be feasible to validate in a straight forward manner. Compliance compatibility information for the software (for example for FDA CFR 21 Part 11) should be available and appropriate technical controls should be tested and well documented. Appropriate system documentation and life-cycle management procedures should be available for audit by both the customer and the FDA if required. All upgrades, firmware, hardware, or software should be easily validated by either the vendor or end user. Any improvements/upgrades to the software and firmware should be retro-compatible so as not to render a large number of customer's equipment obsolete. Other requirements regarding software in the twenty-first century bioanalytical laboratory can be found in the paper: "Ligand Binding Assays in the 21st Century Laboratory: Recommendations for an Automated Data Interchange Process" in this issue of the AAPS Journal.

LIFE CYCLE SUPPORT

It is important to consider a life cycle plan early for a new platform and instrument used in a drug development

program. A thorough assessment of risk factors and a mitigation plan can lead to greater success of new platform implementation (29). Although it is important to have innovation within the industry, a best case scenario would be to have multiple vendors for a particular platform and associated consumables/reagents, thus lowering potential risks, making incorporation of the platform into an operation a lower risk venture (28). This is not always the case and therefore other business risk factors would also need to be taken into consideration when choosing an assay platform and instrument (30,31), for example, company size and capacity, financial stability, business model, long-term business strategy (32), and regulatory compliance (28).

The quality of both critical and platform specific reagents needed to support the platform is as important as the platform itself and also needs to be considered during the risk assessment stage. The quality of a reagent from a vendor should be assessed by evaluating the batch-to-batch reproducibility and whether the vendor has jurisdiction over the raw materials and the material that are part of their final products or kits. Proper documentation as well as regulatory and production processes should be in place to assure the quality of all reagents over long periods of time. Finally, it is important to determine if the vendor has the capacity to meet the demands of multiple clients for critical reagents. As mentioned earlier, ideally, the generation of platform specific buffers as well as any required critical reagents should be vendor independent if possible. For further information regarding reagent generation and characterization, refer to the "Ligand Binding Assays in the 21st Century: Recommendations for Characterization and Supply of Critical Reagents" in this issue of the AAPS Journal.

The instrumentation used to support the platform should be robust and have a reliable and knowledgeable service support team that can respond in a short period of time. This would require having a sufficient number of service engineers per number of instruments in each geographical area of service. It is also important that if the service support is to be subcontracted, many subcontractors have the technology and are as capable as the vendor to address the problems and the required repairs.

TOTAL PACKAGE

In summary, the users are looking for an open and rugged platform that permits flexible method development, is potentially compatible with multiplexing, and achieves a wide dynamic range with sufficient sensitivity and a short total assay time (Table II). Within ligand binding technology, instruments for PK, biomarker, or immunogenicity evaluation should be able to support a variety of platforms, *e.g.*, plate reader with the capability of colorimetric, electrochemiluminescence, chemiluminescence, and fluorescence endpoints. Ideally, one instrument should be capable of handling various platforms while meeting appropriate regulatory requirements.

CONCLUSION

This paper serves as an introduction for a broader discussion. The authors hope discussions continue over the next few years about how industry, academia, contract laboratories, and platform and instrument manufacturers can work together to better serve the end-users. The authors recognize the platform(s) described in the above sections would be a substantial undertaking to develop and commercialize for a sensible price within a realistic amount of time. This being the case, it is recommended during the development of new platforms to address the critical elements of a drug development program (fit-for-purpose). Therefore, it is recommended to establish strong relationships between end-users and manufacturers. To further mitigate risk, we encourage the formation of a consortium enabling partnerships among all stakeholders.

Table II. Summary of the ideal LBA platform

Platform attribute	Measure of success
Sensitivity	• Dependent on use of platform (*i.e.*, biomarker, pharmacokinetic, and anti-drug antibody measurements) • Capable of quantifying low analyte concentrations of drug and biomarkers in a variety of matrices
Dynamic range	• Greater than 3 log range
Precision	• Less than 2% variability for the instrument signal of internal standard
Ruggedness	• Consistent performance under varied laboratory conditions • Tolerance to biological interferences
Total assay time	• Results in 1 h or less
Multiplexing	• Should have capabilities • Minimal cross-talk due to detection mechanisms • Select assays most relevant and not collect data for assays not relevant to a particular study
Flexibility/throughput	• Automation compatible • Capable of assay miniaturization (*e.g.*, 384+ well plates or other substrate) • Utilization of various solid supports • Ability to run in low and high throughput environments • Possible to use throughout the life cycle of drug development
Multi-modality	• Measure wide variety of therapeutics including proteins, peptides, antibodies, *etc.* • Ability to measure in wide variety of matrices
Life cycle support	• Multiple sources for reagent availability • Ability to label reagents in-house

Collaborations with diagnostic companies may help in the understanding of the critical processes used to ensure assay and instrument reproducibility and ruggedness. This style of collaboration would not require opening existing systems, but rather encourage the use of knowhow for creation of a new family of platforms for drug development.

There are several aspects of platforms and instruments that should be further explored including collaborations with e-solutions, automation, and reagent sections of the 21C laboratory action programming committee, to develop fully seamless and flexible systems leading to novel and highly robust platforms.

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