Review Article

Theme: Best Practices for Bioanalytical Methods: Recommendations from the Global Bioanalysis Consortium

Guest Editors: Binodh DeSilva and Philip Timmerman

Assay Formats: Recommendation for Best Practices and Harmonization from the Global Bioanalysis Consortium Harmonization Team

Sherri Dudal,^{1,2,11} Daniel Baltrukonis,³ Rebecca Crisino,⁴ M. Jaya Goyal,⁵ Alison Joyce,⁶ Karolina Österlund,⁷ John Smeraglia,⁸ Yoshitaka Taniguchi,⁹ and Jihong Yang¹⁰

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Abstract. As part of the GBC (Global Bioanalysis Consortium), the L3 assay format team has focused on reviewing common platforms used to support ligand binding assays in the detection of biotherapeutics. The following review is an overview of discussions and presentations from around the globe with a group of experts from different companies to allow an international harmonization of common practices and suggestions for different platforms. Some of the major platforms include Gyrolab, Erenna, RIA, AlphaLISA, Delfia, Immuno-PCR, Luminex, BIAcore, and ELISAs. The review is meant to support bioanalysts in taking decisions between different platforms depending on the needs of the analyte with a number of recommendations to help integration of platforms into a GLP environment.

KEY WORDS: assay format; biotherapeutic; large molecule; platform; technology.

INTRODUCTION

As one of the Large Molecule harmonization teams in the Global Bioanalysis Consortium (GBC), the goal of the L3 Assay Formats team was to describe and compare different ligand binding assay (LBA) platforms that are currently being used for drug analyte sample measurement and the applicability of regulatory guidelines and white papers. The first section of this paper evaluated the current international standards published or presented by the U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA), Brazilian Health Surveillance Agency (ANVISA), Japan Bioanalysis Forum (JBF), as well as bioanalyst's input from India and China.

In the second section, a brief description of each assay platform and its pros and cons is summarized where a platform is defined as a technology-based method used to measure a protein analyte. Liquid Chromatography-Mass Spectrometry (LC-MS) methods were considered out of scope and covered by other workgroups. These platforms are compared in tabular format with a typical plate-based LBA, such as an ELISA (enzyme-linked immunosorbent assay). A generic sandwich ELISA that measures total monoclonal antibody (mAb) was chosen as the benchmark for comparison because it is a universally accepted platform in the ligand binding assay community. This typical LBA format is compared across the various platforms with minor variations due to platform differences and is based on common practices in the bioanalytical community. To get an overall understanding of the use of different platforms, Myler *et al.*⁽¹⁾ review a number of platforms in function of the needs of a given project.

Bioanalysts have specific scientific and practical criteria that guide selection of a platform. The specific biology of the project, required assay end-points, the financial cost, regulatory risk and business sustainability when investing in a new platform, are all considered in order to determine what will meet study and business needs over time. The comparison of platform characteristics provides some insight into the advantages and disadvantages for each platform. The platform characteristics reflect the current opinion of bioanalysts from the community with an overall purpose to guide and provide insight to bioanalysts when evaluating different platforms.

The third section focuses on key topics where assay validation and sample analysis on selected platforms are not

¹ Novartis Pharma AG, Novartis Institute for Biomedical Research, 144 Klybeckstrasse, 4052 Basel, Switzerland.

² F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124 Basel, 4070, Switzerland.

³ Pfizer Inc., Eastern Point Road, Groton, Connecticut 06340, USA.

⁴ Janssen, Research & Development, LLC, 145 King of Prussia Road, Radnor, Pennsylvania 19087, USA.

⁵ Biogen Idec, Cambridge, Massachusetts 02142, USA.

⁶ Pfizer Inc., 1 Burt Road, Andover, Massachusetts 01834, USA.

⁷ Gyros AB, Uppsala Science Park, 751 83, Uppsala, Sweden.

⁸ UCB Parma S.A, Avenue de l'Industrie, 1420 Braine-l'Alleud, Belgium.

⁹ Toray Research Center, Inc., 10-1, Tebiri 6-chome, Kamakura-shi, Kanagawa 248-0036, Japan.

¹⁰ Genentech, 1 DNA Way, South San Francisco, California 94080, USA.

¹¹ To whom correspondence should be addressed. (e-mail: sherri.dudal@roche.com)

adequately covered in current guidances or white papers. Platform characteristics and assay acceptance criteria that pose unique challenges compared to typical plate-based LBAs are discussed and best practices regarding these issues are suggested. The final section of the paper summarizes the results of a global industry bioanalysis survey conducted throughout multiple companies, focus groups, and GBC-sponsored social media sites. This survey compiled information related to current practices of the international community around the identified gaps in guidance for assay validation criteria and sample analysis using the highlighted assay platforms.

COMPARISON OF LIGAND BINDING ASSAY GUIDANCE FOR LARGE MOLECULES

In 2001, the FDA issued a guidance document for bioanalytical method validation (2). However, it described the acceptance criteria and assay operations for small molecules run on a LC-MS platform and did not address appropriate acceptance criteria for large molecule immunoassay measurements. Therefore, bioanalysts relied on a mixed interpretation of workshop reports that originated within the American Association of Pharmaceutical Scientists (AAPS) community as well as the original guidance document for both pre-study and within-study validation (2–6). In 2011, the EMA guideline was published where a section was dedicated to large molecule measurement using ligand binding assays (7). This is currently the most widely applied guidance for immunoassay measurement of large molecules. Also, Brazil has published a draft guidance that resembles the EMA guidance and Japan is planning to publish guidelines based on the current thinking in the international community. Recently, the FDA has published a new draft guidance, which is currently being addressed within the GBC community as well as at the Crystal City V workshop. A follow-up on the major recommendations from the L3 team will be published combined with other large and small molecule teams.

Out of the currently published guidances, the EMA guideline and the FDA guidance/AAPS workshop reports currently provide the most important information for the large molecule and ligand binding assay community. However, there are some differences between the documents from EMA and FDA/AAPS. For example, preparation of quality controls (QCs) and criteria in cross validation are described in the EMA guidance, but not in FDA/AAPS guidance/reports. On the other hand, calibration curve fitting, which is in FDA/AAPS guidance/reports, is not included in EMA guidance. Here, we summarize the major differences between the guidelines or workshop reports issued by FDA/AAPS and EMA (2–7) (Table I).

COMPARISON OF ASSAY PLATFORMS FOR THE MEASUREMENT OF LARGE MOLECULE BIOTHERAPEUTICS

A wide range of assay platforms were discussed within the L3 Assay Platform team with intensified discussions of specific platforms in smaller workgroups. Each workgroup had a designated expert lead, and at least one or two additional members, with the goal of identifying the major

pros and cons of the platform and conveying the information back to the team. Frequently, workgroups consulted colleagues in the LBA community to ensure ample opinion and expertise was acquired. The entire team would then discuss the collated information and a consensus was made. Some of the platform comparison information was available on the GBC website to allow further feedback from the LBA community. Although the list is not exhaustive of all the platforms currently being used, the following platforms were included in these discussions: ELISA, MSD (MesoScale Discovery sector imager), BIAcore (T200), RIA (radioimmunoassay), AlphaLISA®, DELFIA®, Gyrolab, Erenna®, Immuno-PCR, Luminex, and cell-based assays. The platforms were chosen to cover a range of types of assays such as solution-phase *versus* solid-phase platforms and in function of bioanalytical experience as implementation in a GLP environment requires a sound understanding of the platform and the expected performance. A recent example of platform comparisons can be found in a publication by Leary *et al.* where ELISA, MSD, AlphaLISA®, Gyrolab, and LC-MS/MS were compared with one assay format (8). Although this example is limited, the conditions such as capture and detection antibody were kept the same to have a relevant comparison between each platform. A previous paper by Soderstrom *et al.* compared four different platforms: Delfia, MSD and ELISA with different detection systems (9). The authors concluded that no one platform out-performed the others and the requirements based on the study should be the deciding factor. This is often the final defining point for the bioanalytical strategy, but it is of course advantageous to be aware of the different platforms and their ability to become GLP.

In order to implement a platform for GLP use, there should be a benefit to use the platform *versus* the local platforms in use. Since the time investment is heavy in the GLP environment, the need should be justified by first testing the platform for the intended application and performing a comparison between the two. Some of the main benefits of integrating a new platform in the GLP environment are increased sensitivity, decreased matrix effects, higher throughput, better performance in terms of precision and accuracy, lower sample volume requirements, possibility to multiplex, or better adapted for the proof of concept in the case a functional readout is required. The new method needs to be validated according to the regulatory guidelines and the instrument validated through evaluation of IQ/OQ/PQ (installation qualification/operational qualification/performance qualification) with the required documentation. Transfer of data from the new instrument to the LIMS or other in-house system needs to be traceable and validated. If an interface is not available, manual traceability checks will be required. This is of course, reviewed by the local quality assurance officer and the quality manager that evaluates instrument validation.

Although the main focus was on drug concentration assays, some platforms are currently used primarily for pharmacodynamic (PD) assays and the same criteria were applied to PD assays that measure soluble target. Briefly, each platform was described and evaluated in terms of acceptance criteria and assay set-up and the major differences highlighted in the following section. A summary of pros and

Table I. Major Differences Between FDA Guidance/AAPS Workshop Reports and EMA Guideline

Parameter	FDA/AAPS(1–5)	EMA (6)
Calibration standards	Proper weighting of the points is important to minimize bias and imprecision of interpolated values near the LLOQ and ULOQ values. Evaluate positional effects.	No comments about weighting, positional effects, and cumulative RE and CV in the guideline.
Curve fitting parameters	The cumulative RE and CV should be within 15% for each standard (within 20% at LLOQ). 4/5-parameter logistic (PL) function. Other calibration algorithms may be used (logit-log, cubic spline, etc.) if they demonstrate goodness of fit.	No comments.
Quality controls	QCs are termed validation samples (VSs). At least 2 independent determinations per run.	The term VS is not used. No comments about determination number in the guideline.
Preparation of QCs	No comments.	QCs should not be freshly prepared, but should be frozen and treated the same way as for the analysis of study samples.
Dilutional linearity	Spiked QC samples are at 100- to 1,000-fold higher concentrations than ULOQ. The precision of the cumulative back-calculated concentration should be within 20%.	No comments of detailed concentration of QCs higher than ULOQ. The precision of the final concentrations across all the dilutions should not exceed 20%.
Parallelism	Parallelism is assessed with multiple dilutions of actual study samples or with a sample representing the same matrix and analyte combination.	A high concentration study sample (preferably close to $C_{\max}$) should be diluted to at least 3 concentrations with blank matrix.
Selectivity	No comments about lipemic and haemolysed samples.	The sources should include lipemic and haemolysed samples.
Specificity (target interference)	No comments about concentration and criterion.	QCs (LLQC, ULQC) by adding increased concentrations of “related molecules” or drugs. Accuracy should be within 25% of the nominal values.
Stability	Whole-blood stability is recommended. No comments about stock solution and working solutions of the analyte standard and bracketing approach.	Stability of the stock solution and working solutions of the analyte standard should be evaluated. A bracketing approach may be considered. No comments about whole blood stability. Stability should be evaluated at each temperature study samples are stored.
Short term stability	Bench top stability should be done at room temperature (minimum 2 h) and at refrigerator temperature (2 to 8°C) (minimum 24 h).	The investigation of stability should cover short-term stability at room temperature or samples processing temperature and freeze–thaw stability.
Incurred sample reproducibility	The number of samples repeated should equal 5–10% of the total sample size, with 5% as the minimum for larger studies.	10% of the samples should be reanalyzed if number of samples is less than 1,000, and 5% reanalyzed for number of samples exceeding 1,000.
Evaluation of a critical reagent	Extension of the expiration date of critical reagents may be justified by the acceptance performance of the QC samples.	No comments about extension of the expiration date of critical reagents.
Commercial kit	No comments.	Commercial kits need to be revalidated.
Sample analysis	No comments about placement of Cs and QCs.	It may be acceptable that a set of Cs be placed in the first and the last position of one run and QCs on every single plate.
Analytical run		
In-study acceptance (QCs)	If additional sets of QCs are used in a run, then 50% of them need to be “in-range” at each concentration. The LQC should be placed above the second non-anchor standard, ~3 times the LLOQ. The MQC is placed near the mid- point (geometric not arithmetic mean) of the standard curve. The HQC should be placed below the second non-anchor point high standard and/or ~75% of the ULOQ.	No comments about additional sets of QCs and detailed positioning of each QC.
Analyst changes (ruggedness)	See robustness and ruggedness testing.	No comments.

Table I. (continued)

Parameter	FDA/AAPS(1–5)	EMA (6)
Robustness and ruggedness testing	An attempt should be made to evaluate the variety of conditions that may reflect the execution and performance of the method during in-study phase. Assessment includes incubation time tolerances, changes in analysts and batch size.	No comments.
Assay format	The assay format validated should be used during in-study validation. If a major change was done, a partial validation may be needed.	No comments.
Carry-over effect	No comments.	If robotic liquid handling systems are used, carry-over should be investigated by placing blank samples after samples with a high analyte concentration or calibration standard at the ULOQ.

cons of all platforms is shown in Table II. A brief overview of the several assay parameters and platform operations compared to a typical sandwich ELISA for measuring total mAb with colorimetric detection, which consists of the total bound and unbound mAb, was compiled in Table III.

ELISA

ELISA is the most widely used ligand binding assay platform within and outside the pharmaceutical industry. Formats include direct, indirect and sandwich assays which are often used as a basis for comparison with novel platforms in development and are run manually or semi-automated on 96 well plates where samples are measured in duplicate. Typically, a colorimetric detection agent is used but other detection agents such as luminescence and fluorescence are sometimes used. Generally, ELISA requires monitoring of lot changes for reagents such as antibodies, ligands and buffers.

MSD

The MSD platform is a micro-titer plate-based format utilizing an electrochemiluminescence (ECL) signal for detection (10). With this platform, immunoassay formats are similar to typical ELISA formats that are used for drug and target sample measurements, but it is expected that sensitivity would be increased due to the use of chemiluminescence (11) although users have not found this to be the case on a regular basis. This platform can be susceptible to hook effect when run in a homogeneous assay format. In addition, the reagents and plates are prone to lot-to-lot variations and cross-comparison between lots is strongly recommended.

RIA

Radioimmunoassay (RIA) is often a competitive binding assay where the analyte is measured through competition with radiolabeled analyte for a capture protein. This method is still actively used and in some cases modified for use in clinical trials (12). Other alternatives that are non-competitive

also exist and similar comparisons can be made to these formats. With this method, large sample runs (i.e., 200 samples) can be performed depending on reagent stability with a calibration curve and intermittent QCs (quality controls) (e.g., every 50 samples). One advantage of this platform is its large dynamic range and a disadvantage is the handling of radioactive waste.

AlphaLISA

The Amplified Luminescent Proximity Homogeneous Assay (AlphaLISA) platform utilizes a luminescent bead-based proximity format whereby singlet oxygen is transmitted from an excited streptavidin-coated donor bead to an acceptor bead if in close enough proximity. The beads are brought into proximity through the use of a biotinylated capture antibody and a secondary detection antibody conjugated to the acceptor bead. There are several considerations to make when evaluating AlphaLISA®, which includes antibody conjugated AlphaLISA® acceptor bead lot-to-lot variability, the light sensitivity of the donor beads, the minimal required dilutions (8) to reduce matrix effects due to quenching by high volumes of complex serum, and the presence of hook effect (13). One major advantage for users is the short time required for running the assay and the possibility to run a long series of samples due to the bead-based format rather than the plate-based format. Some laboratories implement 384-well plates for sample analysis with the aid of an automate.

DELFA

The Dissociation Enhanced Luminescent Immunoassay (DELFA) platform uses the same sandwich assay fundamentals as a typical ELISA, but with a time-resolved fluorescence readout from a lanthanide chelate label (Europium) that has both a large Stokes shift and a high fluorescence intensity. Reagent stability must be re-evaluated every 6 months to 1 year for expiry date extension or for comparison of a new lot.

Table II. Comparison of Ligand Binding Assay Parameters for a mAb Relative to ELISA with Colorimetric Detection

Platform	Format	Dynamic range	Matrix effects	Hook effect (unavoidable)	Sensitivity (compared to average ELISA)	Neat Sample volume
ELISA (reference platform)	Plate	2 logs	Medium	No	20 ng/mL (133 pM)	Medium
MSD	Plate	3–4 logs	Low	No	+	Medium
Biacore	Series	2 logs	Low	No	–	Medium
RIA	Series	2–3 logs	Low	No	+	High
AlphaLISA	Plate /series	2–3 logs	High	Yes	+	Low
DELFA	Plate	2 logs	Low	No	+	Medium
Gyrolab	CD/series	3–4 logs	Low	No	+	Low
Singulex Erenna	Plate/ series	3–4 logs	Low	No	++	High
Immuno-PCR	Series	3–4 logs	High	No	++	Low
Luminex	Plate	2–3 logs	Medium	No	+	Medium
Cell-based Assays	Plate/series	2 logs	High	No	–	High

Gyrolab

Gyrolab is a semi-automated non-plate based immunoassay platform with liquid handling that performs an immunoassay on a compact disc (CD) using small sample and reagent volumes. The assay is carried out on a nanoliter volume affinity capture column using a biotinylated capture reagent and a fluorophore labeled detection reagent. Sample and reagent volumes are defined within the microstructure on the CD and applied to the column by spinning the CD. On each CD, there are 96–112 microstructures, resulting in 96–112 data points, depending on the CD type. Sample processing is fully automated inside the Gyrolab workstation, where a liquid-handling arm transfers samples and reagents from micro-titer plates to the CDs and the fluorescent signal from each column is measured by a laser/detector. Up to five CDs can be processed without manual intervention.

This assay format allows for small sample and reagent requirements, rapid assay kinetics, short assay run time and extended dynamic range compared to ELISA (8,14–17). Both carry-over and reagent stability require evaluation during assay development due to the liquid handling and ‘hands-off’ aspects of the platform.

The Gyrolab workstation has been validated through the process of IQ/OQ/PQ to meet the requirement for compliance with 21 CFR Part 11 (14,16,18). The vendor offers IQ/OQ support and PQ guidance as a service.

Erenna

The Erenna® immunoassay system utilizes a two-step process to achieve ultrasensitive assays. Initially, a bead-based sandwich assay format is executed in 96-well plates, using a biotinylated capture reagent and fluorescent labeled detector. The detector is eluted off the beads and the samples are transferred to a 384-well reading plate which is loaded into the instrument. The samples are analyzed in series using capillary flow into a flow cell, where laser-induced single molecule detection occurs. The platform advantages are broad dynamic range, made possible through a unique software curve fitting algorithm that takes advantage of multiple readouts and enhanced sensitivity (19,20).

The assay time is long compared to other platforms, though some off-line automation may be utilized for washing and transfer steps. Typically used for PD but may also be utilized for drug analyte assays, the vendor supplies manufactured kits and stability must be evaluated by the end user as reagent stability is generally unknown (21). Generic kits can be bought to develop an assay with in-house reagents and plate-based formats are also compatible with the platform using 96 or 384-well plates (22). The supplier recommends calibration standards (Cs) be run in triplicate to allow for accurate analysis at low concentrations.

The Erenna can be validated through IQ/OQ procedures in combination with internal PQ procedures. If integrating the

Table III. Comparison of Platform Characteristics

Platform	Labeled reagents	Time required	Carry over , hotspots or cross-talk	Automated sample handling	Multiplexing possible	Vendor-specific reagents
ELISA (reference platform)	Yes	6 h	No	No (possibilities exist)	No	No
MSD	Yes	4 h	Yes (cross-talk)	No	Yes	Yes
Biacore	No	6 h	Yes (carry over)	Partial	No	Yes
RIA	Yes	6 h	No	No	No	No
AlphaLISA	Yes	2 h	No	No	No	Yes
DELFA	Yes	6 h	Yes (hotspots)	No	No	Yes
Gyrolab	Yes	2 h	Yes (carry over)	Partial	No	Yes
Singulex	Yes	6 h	No	No	No	Yes
Immuno-PCR	Yes	8 h	No	No	No	Yes
Luminex	Yes	6–8 h	Yes (cross-talk)	No	Yes	Yes
Cell-based Assays	Yes	Days	Yes	No	No	No

use of Erenna with LIMS for regulated bioanalysis, one option is to use one of the three signal read-outs for concentration analysis. However, dynamic range and sensitivity could be affected depending on which read-out is chosen.

Immuno-PCR (Polymerase Chain Reaction)

Immuno-PCR (Chimera) is an amplification immunoassay-based technology (23). It involves the amplification of the DNA-labeled detection antibody that leads to enhanced sensitivity. This platform can be prone to strong matrix effects if the antibodies used cross-react with components in the serum or plasma. The signal-to-noise ratio must be evaluated during assay validation and used to determine assay performance during sample analysis. Advantages of this platform include a large dynamic range and enhanced sensitivity, which is of particular interest for PD analysis. As with Erenna®, the reagents are highly dependent on the supplier and can become costly for large batches of sample analysis. Particular attention should be made for lot-to-lot variability of labeled reagents and plates as for MSD, Erenna®, and other bead-based platforms. Since the platform relies on PCR technology, caution must be taken in handling of samples, reagents, and pipette tips to avoid contamination. Once again, data analysis can be cumbersome.

Luminex

Luminex is a bead-based immunoassay that utilizes dye-defined beads that are coated with a particular anti-analyte antibody. Once the analyte is added, a fluorescently labeled antibody is added to detect positive signals for analyte measurement. This technology is used primarily for PD assays with pre-defined kits by vendors and is often used for multiplexing. Acceptance criteria are set-up for each analyte and multiple analytes are measured with one sample. There is a tendency for cross-talk between analyte beads and the assay is light sensitive so special precaution must be taken.

BIAcore

Biacore-based drug analyte assays rely on the interaction of an analyte of interest to the analyte-specific reagent that is immobilized on a sensor chip surface. The binding interaction is monitored in real-time with a read-out in relative response units (RU) that is related to the change of Surface Plasmon Resonance (SPR) angle and is determined by the change of refractive index of the thin liquid film near the sensor chip surface. The change in refractive index is proportional to the amount of mass bound to the chip surface. This real-time, label-free technology is quite different from a typical immunoassay that requires multiple incubation and wash steps as well as a labeled detection antibody for signal readout.

For preclinical drug analyte measurements, an anti-drug-specific antibody can be immobilized on the chip surface for a generic assay that will bind all mAb drug contained in the sample as it flows through the cell. Other variations can also be used for specific assays such as target immobilized on the chip.

For this platform, carry-over must be tested over ten cycles during validation and signal-to-noise ratio of maximum

signal to background should also be determined to evaluate run performance during sample analysis. Furthermore, chip stability over longer periods when performing large batches of sample analysis must also be evaluated and the method is specific to the type of chip used. As the platform runs generally overnight or longer, the stability of samples and reagents must be known for longer short-term stability periods than a typical immunoassay (over 24 h) and overall sample throughput is slow on a T200. Also, a calibration curve followed by intermittent QC samples within the run, are defined as a single run. Generally, this corresponds to one plate of Cs and samples.

Biacore can be validated through the process of IQ/OQ/PQ to meet the requirement for compliance with 21 CFR Part 11 (14,16,18). Making the Biacore instrument GLP compliant is not a straightforward process and significant time investment is involved to deal with issues such as data transfer and a proper calibration over time.

Cell-based Assays

Bioassays or cell-based assays for drug analyte measurements are rarely used due to the difficulty in obtaining accurate, precise and reproducible assays and thus, are utilized only in special cases where active biotherapeutic levels are needed. Examples of such cases include instances where the therapeutic becomes inactivated over time in blood, gains activity once released from formulation, is dosed by activity rather than mass units, or a historical or “Gold Standard” activity/bioassay exists. Deployment of cell-based assays requires specialized skill sets, additional time and resource commitment for cells and reagent management. Additionally, bioassays are inherently complex assays and performance of such assays varies merely due to the metabolic state of cells used in the assay on a given day. They are also not easy to transfer between the labs during the life cycle of therapeutic development, especially during assay validation and sample testing.

As compared to a typical LBA, performance of cell-based assays falls short in many essential parameters including dynamic range (i.e., 1 log), sensitivity, accuracy, precision, and matrix effect. This is not a general rule as a comparison study of LBA *versus* cell-based assays by Spriggs *et al.* showed that the two methods were comparable (24). Additional parameters for validation include demonstration of cell bank and cell line stability, optimal plating density, time to confluence and effect of individual serum/plasma. In addition, it is often challenging to automate or multiplex cell-based assays that can be easily achieved for LBA. The time and skills required to develop, maintain, and run such assays and associated costs also discourage the use of such assays for routine drug analyte assessment. One example is receptor occupancy assays for determining the percent of receptor occupancy that corresponds to downstream intracellular effects or secretion of certain target proteins.

For running such assays, triplicates are usually required to allow for accurate measurement in a 96-well plate format. The acceptance criteria are larger with a bias and precision of up to 30% and a total error of 40%. Many parameters for cell-based assays need to be controlled such as serum lot changes, number of cell passages, length of time in culture

Table IV. Biacore Assay Set-Up

Two calibration standard sets run in duplicate at start	QC1	QC2	QC3	QC4	QC5	QC6	Run status
	Pass	Pass	Pass	Fail	Pass	Pass	Pass, bracket approach
	Pass	Pass	Pass	Fail	Fail	Pass	Pass until QC3
	Fail	Pass	Fail	Pass	Fail	Pass	Fails
	Pass	Pass	Fail	Pass	Fail	Pass	Fails

conditions, border effects of the plate, freezing stability, and cell batch comparisons. Cells can be frozen after the first passage in a large number of aliquots to help keep the consistency of the cell line over time. A range of performance parameters for running cell-based assays should also be evaluated during validation and applied during sample analysis such as assay signal/ response expected *versus* observed, cell growth rate to evaluate cell metabolism and viability of the culture coated on the plate. This can help to control the growth phase or metabolic state of the cell and allow the cell response to stimulus / inhibition to be monitored. Relative measurements *versus* an internal control are strongly suggested. The parameters used to define assay performance will depend on the assay and the cell line used as well as the technology used for measuring cell signal / response.

KEY TOPICS AND SUGGESTED BEST PRACTICES FROM THE PLATFORM COMPARISON

Through comparison of the different platforms, unique attributes and issues were uncovered that are not highlighted in current guidances, and allowed certain platforms to be grouped by these common characteristics. For example, some platforms run sample analysis in parallel, using plates or CD's, whereas others handle readout in series. This leads to some ambiguity around placement of Cs and QC's during large run sample analysis, and what acceptance criteria should be used when a run partially fails. For platforms using liquid handling with non-disposable tips, carry-over may occur. Singlet analysis is also discussed in light of improved duplicate precision over the years. Several of the highlighted platforms have multiplexing capabilities, which poses unique challenges for assay validation and sample analysis. Finally, method transfer across multiple platforms also requires a number of considerations and is highlighted in this section.

Large Run Sample Analysis and Instrument Failure

MSD

For MSD-based sample analysis, placement of Cs and QCs is generally the same as that used for ELISA. On the other hand, it is worth noting that plates are read in sections of four wells at a time and can be read only once after the voltage is applied. Thus, if instrument failure should occur, measurements may not be acquired for an entire calibration curve or a part of the QC sets may be missing depending on the plate set-up. This will typically happen for a horizontal set-up. For a vertical set-up, one is more likely to obtain the Cs and the first QC set but not the second QC set. In both

cases, the entire plate will need to be re-done. If the instrument fails, samples may have to be re-assayed as the signal significantly decreases with time if the reading buffer has already been added to the plate.

Gyrolab

Typically a run is defined as one CD and Cs and QCs are placed on each CD. If precision and bias for QCs placed on different CDs meet acceptance criteria, a series of up to five CDs processed in an unattended run could potentially be defined as a single run (25). This requires testing and evaluation for any given format as this relies on the rapid capture of the analyte and the stability of the reagents. Acceptance criteria is applied to each CD, which means that the CD with the failed QCs and/or failed standard curve is rejected, while other CDs from the same multiple CD run can pass. If only one standard curve is included per multiple CD run and this curve fails to meet acceptance criteria, all data from this multiple CD run would be rejected. Due to the risks with a multiple CD run, this should be well-evaluated during method validation with the chosen calibration curve and QC placement for use in sample analysis. In any case, QCs must be included on each CD.

On suspicion of needle failure during a run, as can be observed by a high percentage of inter-structure CVs for example, the performance of the liquid handling device can be tested with an instrument routine maintenance test provided by the vendor. Samples, Cs or QCs transferred with a needle that did not meet acceptance criteria in the test can thereby be identified and re-analyzed in a future run. The liquid handling device has ten needles and the needle used to transfer a certain sample is listed in the analyzed data.

Some of the newer LBA platforms, including Gyrolab, offer a broader dynamic range than ELISA. In spite of this, it is still suggested to use the same number of Cs and QCs (repeat each QC level twice per CD/plate) for validation (LLOQ, LQC, MQC, HQC, ULOQ) and sample analysis (LQC, MQC, HQC) as suggested for ELISA assays.

BIAcore

The platform runs samples in series as with RIA and a plate is used for sample loading. Therefore, there are two ways to run the BIAcore for sample analysis. A 96-well set-up of Cs and QCs can be used as in ELISA or the two plates (or more) can be run as one whole run with Cs at the beginning and the sets of QCs interspersed throughout the sampling run. A partial run reading can be due to instrument failure or can be due to QC failure from an unknown cause. Instrument failure should be addressed according to what and when this occurred. In the case of QC failure for an unknown reason, a

bracket approach can be applied where all samples between two sets of accepted QCs require reanalysis as depicted in Table IV.

Overall, if 40% or more of the QC sets fail, the samples after the last set that passes until the end must be re-run. The first set of samples can only be accepted if the QC sets at the start of the run have all passed. If the QC set acceptance and failure is sporadic, the sample analysis fails and an investigation must be performed. Similar methodology would be applied to RIA as it runs with a series of tubes.

Erenna

On this platform, Cs, QC's and samples are assayed on multiple 96-well assay plates, then later transferred to one 384-well reading plate before loading into the instrument. It is recommended that a standard curve and QCs be run on each 96-well assay plate unless automation is used and %CV on duplicate or triplicate wells is consistently <10%. In this case, a similar bracket approach to Biacore can be applied to Erenna® where a maximum run is 384 wells with a standard curve in the beginning and QCs interspersed on each of the 96-well assay plates.

If the instrument fails during a run, result files would not be generated for the wells not read. If the error is discovered soon after the instrument stops reading, the instrument can be restarted and the remaining wells run. If instrument failure is not detected quickly, data from 96-well runs processed before the failure may be used if standard curve and QC acceptance criteria are met.

Luminex

Common practice is to have an in-plate standard and to run the Cs and samples in duplicate. The range of Cs is typically wider than that of ELISA to accommodate wide ranges of analyte concentrations and standard curves and QCs are determined for each analyte. It is worthy of pointing out that the anchor point on a standard curve for one particular analyte may not be the same for another analyte standard.

If the instrument fails in the middle of a run (i.e., partial run), the completed data may still be used, as long as relevant Cs and controls are successfully completed and acceptable. This is less of a concern as compared to some sequential platforms where limited QC's are placed between relatively large runs of sample analysis.

Carry-Over and Cross-Talk

Carry-over may occur with fluidic systems such as Biacore, Erenna, Luminex and Gyros and this can be tested by passing a high QC followed by a blank sample multiple times on a plate or CD. Such controls can be integrated into sample analysis runs as well with specific acceptance criteria for the blank sample. Ideally, no quantifiable levels should be detected in the blank sample and carry-over should be eliminated during assay development. If mitigation strategies fail, a small amount of carry-over may be tolerated by increasing the LLOQ and adjusting the dynamic range. Signs

of carry over include high %CV between replicates and elevated assay background levels.

For Gyrolab and other liquid-handlers more stringent wash solutions, assay buffer choice, extra wash steps and needle wash steps can be implemented to minimize carry-over during assay development. A suitable buffer for sample dilution will minimize any potential loss of analyte in the needle while a well selected wash buffer will ensure thorough cleaning of needles in between transfers.

For Erenna®, carry-over is diagnosed as a high %CV in a sample following a very high concentration sample or as an upward shift in the instrument background, which would be detected in the plate calibration run each day. Generally, the calibration plate fails if carry-over is an issue, and a cleaning procedure using bleach and urea is utilized, which strips the capillary tubing containing the high level of fluorescence. If carry-over continues to be a problem, the volume of buffer aspirated between samples can be increased. In any case, carry-over cannot be tolerated with the Erenna® system due to the low sensitivity measurements.

For MSD, cross-talk can be an issue especially for multiplex formats and the assay should be re-developed if this occurs. Inter-well cross-talk as can be observed with Luminex and should be investigated during assay development.

Singlet Analysis

Due to the improvement of the precision of duplicate analysis for LBAs and the fact that LC-MS for small molecules does not run in duplicates, it could be possible to run the samples in singlets, instead of the classical duplicate analysis for LBAs. The suggested best practice would be to run the sample analysis in singlet format if the validation proved that duplicate analysis led to a % CV for all duplicate analysis below an acceptable threshold such as 5–15%, for example, with a goal of taking the most stringent criteria. If the run is over more than one solid support, CD, or plate, it is suggested that the %CV be maintained over the entire run with a suitable intra-run precision such as the aforementioned 5–15%. The Cs and QCs should be run in duplicate during sample analysis to demonstrate the precision of the assay and maintain the accepted threshold.

Multiplexing

Multiplexing is where multiple analyte measurements can be made with the same sample at the same time in the same well, and is possible with the MSD and the Luminex platforms. Due to the nature of multiplexing, it is usually performed in the context of PD or biomarker analysis. For both of these platforms, the same rules apply and thus, Luminex will be used as an example to cover the most difficult case.

In order for a method to be validated for a multiplexing assay, quantification of each analyte needs to be evaluated in the presence of all other analytes to be included in the multiplexed assay. While most of the practices are similar to that of ELISA for assay validation, the multiplexing nature of Luminex platform requires some special considerations during method development and validation.

Standard curves and anchoring points may vary from analyte to analyte, and therefore need to be individually assessed during development to determine calibration ranges and QC levels. The curve fitting may differ for each analyte, but if possible, the curve that fits all analytes adequately should be used and the QC levels adjusted accordingly. This may change drastically for clinical samples where the various analyte concentrations are altered due to the disease progression or regression compared to the healthy donor used for assay validation. Analytes present at low levels may be diluted differently compared to those present at high levels, therefore there could be a division in how samples are treated. It may be possible for some multiplexing platforms to have a curve fit per analyte and this can be acceptable as well.

It is anticipated that sometimes not all analytes in a multiplexed assay pass acceptance criteria, and it is unclear whether all analytes need to be re-assayed. Currently, there is no guidance or industry consensus on how to approach this issue. One approach commonly used by industry is to report analyte values if their respective Cs and controls pass acceptance criteria. In order to do so, a cocktail of all the analyte Cs need to be tested together and compared to the single analyte during development, and comparability of the cocktail approach and the single analyte approach need to be established. Validation can be performed with the cocktail mixture while reporting the individual analyte results and acceptance criteria. Cross reactivity is another major concern, and therefore, it is recommended that efforts should be made to ensure that each analyte can be quantified accurately in the presence of the excess of others, and can be tested individually. Finally, looser acceptance criteria than for drug analyte assays may be required in the case of PD measurements (i.e., 30%) and this can be defined during validation.

When partial run failure occurs, analytes that pass according to the acceptance criteria should be accepted. For the analytes that do not pass, a reanalysis can be performed with all analytes, masking the accepted analytes from the previous run. This eliminates the potential subjectivity in dealing with the analyte data when multiple result sets are generated.

Method Transfer from an Elisa to Another Platform

MSD

Transfer from an ELISA to the MSD platform is relatively straightforward and is frequently performed to reduce matrix effects, enhance sensitivity or increase the dynamic range of the assay when an ELISA with a specific antibody pair cannot reach the desired sensitivity. However, just like many other assay transfers, the difference in reagents and different modifications such as ruthenium or biotin labeling can potentially impact the assay. Therefore, although the antibody pair and the format can be re-implemented with MSD, the Cs and QCs will need to be adjusted and the method fully validated.

Gyrolab

Ideally, an assay requiring small volume measurements or semi-automation should be directly developed on the Gyrolab platform. In the case where the antibody pair can be used in the same capture-detection combination, there is a greater chance

of success of the direct transfer of an ELISA assay to Gyrolab although re-optimization may be of interest to extend the dynamic range or test the sensitivity of the assay. Re-optimization includes testing antibody pair combinations, adjusting the antibody concentrations to maximize sensitivity and dynamic range, testing selectivity to minimize the MRD, and re-establishing the dynamic range and QC levels. Reagent modifications using biotin and fluorescent tag can also impact assay performance when transferring to the Gyrolab platform. In any case, a full validation is required for this platform.

BIAcore

The differences between BIAcore and ELISA are too great to allow a direct transfer and thus, development followed by a full validation is required. As the BIAcore is a fluid-based system, this does not directly compare to immunoassays which are based on a solid phase (e.g., the plate). Many aspects require development for BIAcore such as the conditions for immobilizing and regenerating the chip, reagents used for immobilization and buffers for regeneration, reagent stability, standard and QC determination as well as other aspects of ligand binding assays.

Erenna

The same antibody pair can be tested on the Erenna® platform, but assay development and re-optimization would need to go through the supplier due to their proprietary reagent conjugation methods and buffers suitable for instrument use in the case a Singulex kit is used. More recently, Singulex now produces kits for labeling reagents and coupling beads to allow the user to develop their own assays. As the platform involves bead-based technology and an elution step, the method may not be comparable to a plate-based ligand-binding assay, and would need development specifically on this platform. For less sensitive assays, it is possible to perform the method using a plate-based format. In any case, a full validation with the conjugated reagents and the adjusted calibration curve and QCs would be necessary.

Luminex

If a single analyte will be measured in the Luminex format, transfer from an ELISA format is straightforward in terms of assay layout, although new reagents (e.g., dye-coated beads, fluorescent-tagged detection antibody) need to be made, evaluated and validated. Sometimes, an additional plate (vacuum or magnetic bead plate) is needed during the washing steps. If multiple ELISA's are to be combined and transferred to a single Luminex format due to its multiplexing capability, additional studies need to be carried and efforts in optimizing assay parameters will likely require additional assay development time. Overall, Luminex in the singulate or multiplex format must be fully validated and the conditions used in ELISA provide an indication of the format and antibody pair to begin development.

Standardization of the Comparison Between Platforms

If a new method is used, the comparison of spiked sample measurements on each platform would be most

suitable to see if the same concentration can be measured (i.e., within 30% of the mean of the two or more measurements for 75% of the samples). The spiked samples must cover the whole dilution range expected from the study and QCs at the low, mid, and high end, but within the working range of both platforms.

If readily available, use of real samples for comparisons can be done with a selection of 30 samples and results from two assays must fall between 30% of each other. Samples may also be pooled before being run. However, there are several disadvantages to performing real sample comparisons between platforms. If two different measurements are observed for one sample, this is difficult to explain and will require investigation, and sample stability must be assured and enough sample volume must be available to support the investigation.

It has been common practice to perform platform or method comparisons when new methods are used during the lifetime of a project throughout the development process. Of course, it is important to assure that the analysis of the analyte represents what was previously measured, but heed must be taken in the comparison since the form of the analyte may not be exactly the same, i.e., free or total forms. An example is where an assay that measures the free analyte will not always provide the same absolute value as an assay that measures the total analyte especially when soluble target is present (26).

Different binding affinities of capture or detection reagents can lead to different measurements as can different assay stringencies. Assays may differ in the time of capture as can be seen by comparing ELISA *versus* Gyrolab where a platform with rapid capture is used *versus* a platform that allows the samples to reach equilibrium (ie ELISA). In this example, ELISA has a longer incubation with stringent wash steps whereas Gyrolab involve a rapid affinity interaction with a coated antibody or target protein. These two assay platforms may not provide the same results especially in a target capture assay where an ELISA can favor dissociation of a binding partner. Therefore, such platforms may not be comparable as they simply do not measure the same analyte form. Another comparison can be made with Biacore where, due to the stringency of the ELISA, it will capture high-affinity proteins whereas Biacore will capture both low and high-affinity proteins (27). Additionally, Biacore involves real-time analysis where the analyte is directly measured without being subjected to a series of washing steps or incubation times. Differences in assay stringency can also be observed when comparing bead-based platforms to plate-

based platforms. Therefore, relative within-study comparisons may be more informative than comparing absolute measurements. In any case, comparability may be requested between platforms and explanations on the potential differences between the methods/platforms should be highlighted to explain potential differences during the cross-validation.

SURVEY RESULTS

An international survey was sent to focus groups (AAPS, European Bioanalysis Forum, and JBF), multiple companies and posted on the GBC Linked-In site to evaluate the current practices of the international bioanalysis community related to the addressed gaps in the previous section. A total of 26 responses were collected from North America, Europe, and Asia, which represented major global pharmaceutical companies, smaller biotech companies and contracted research organizations. The use of the different platforms for the three major assay types used in bioanalysis of biotherapeutics is shown in the table below (Table V).

Although the survey results can be consulted in more detail on the GBC website, a brief summary of the results on current practices of the global bioanalysis community on key topics not addressed in guidance documents is presented here.

For defining a run, the bioanalysis community was divided on this as 48% defined a run as a plate or CD and 44% allowed for a run to be multiple plates or CDs. In platforms that run sample analysis in series such as Gyrolab, BIAcore, Erenna® and RIA, 44% would consider placing the calibration standard set at the beginning of the run and interspersing the QCs throughout the run and 32% kept a more classical approach of adding a calibration standard set at the beginning of each plate or CD with 2 sets of QCs per plate or CD. A special comment was made to include about 5% QC samples within the total number of samples run. In a series set-up, most (60%) would consider the whole run failed if one or more QC sets fail while 24% accept the bracketing approach and 8% accept the run up to the last accepted QC set.

For carry-over, 70% of the community would eliminate carry-over before validating an assay. If carry-over could not be eliminated, 15% of survey respondents would consider implementing a calculation to estimate the amount of carry-over and subtract this out from the sample concentrations.

For singlet analysis, 62.5% of the community would not perform singlet analysis if the %CV was less than 5%, although specific case studies were presented for BIAcore and Gyrolab uses for singlet sample analysis. Only 9.1% would perform singlet analysis even if the %CV was 10% or lower. According to the comments provided in the survey, most of the community appears reluctant to do singlet analysis unless the regulatory agencies accept this practice for sample analysis of biotherapeutics.

Most multiplexing is used for PD analysis (55%) as can be expected with combinatorial uses for PK/PD (4%), PK/IG (8%), IG/PD (7%) and PK/PK (7%) where PK is meant to be any drug analyte measurement. About 15% of the community does not perform multiplexing but this could be due to different operational set-ups within the company as many biomarker groups perform the majority of multiplexing. For those multiplexing, 43% would validate a cocktail of analytes

Table V. Survey Responses on Type of Platforms Currently in Use Per Assay Type

	PK assay	Immunogenicity assay	PD assay
ELISA	23	19	18
MSD	17	20	16
Gyros	10	3	6
Erenna	2	–	5
Alphalisa	4	2	2
Delfia	4	–	2
Biacore	2	11	3
Luminex	2	1	10
Cellular Assay	6	16	9

as opposed to validating each analyte separately (12%). Most respondents acknowledged that analytes may be validated in a cocktail depending on the cross-interference of the different analytes. When running multiplexing, 69% of bioanalysts would mask the analytes that had previously passed when performing reanalysis for a failed analyte run. Also, 8% would re-run only the single analyte and 4% would recalculate all results together.

In order of preference, 92% of the community prefer using spiked samples for method transfer comparison, 60% prefer real samples either alone or in combination with spiked samples and only 8% would ever use pooled samples. To validate a new platform or assay format, 44% suggest using 20–50 samples and 24% suggest using 50–100 samples and in some cases it was noted the number depended on the stage of drug development. When transferring to a new platform, 69% would completely re-validate the assay (not including stability testing) and 30% would perform a partial validation. Others would compare spiked samples (8%), real samples (15%) or both (23%) as part of the validation. The approach would depend on the type of platform transfer. Many respondents suggested a statistical method be used to determine platform comparability.

For differences in dynamic range that can be observed with the various platforms, a dynamic range that extends over 2 logs was considered by 30% to require more Cs, or both Cs and QCs (30%), more Cs at the ends of the curve (4%), or nothing different to a standard curve that expands over 2 logs (42%). Most adhered to no change in the number of calibration Cs.

CONCLUSIONS

With our team, we were able to compare and evaluate a number of bioanalytical platforms that are commonly used for drug analyte and PD analyses. From here, we described unique elements in acceptance criteria and sample run analysis not covered by current guidances on ligand-binding assays. These elements include large sample run analysis, including run definition and placement of Cs and QCs, partial run acceptance criteria if instrument failure occurs during sample analysis, carry-over, singlet and multiplex analysis as well as method transfer and comparison of results generated between platforms of the key platforms in use. The major thoughts and key points to address in the more exploratory field of cellular drug analyte assays have been discussed to allow new users to be aware of the different issues. Finally, through the use of a survey, we were able to compile feedback from pharmaceutical industry and CROs worldwide to inform the bioanalysis community on the current thinking and practices around these unique platform characteristics, and offer best practice suggestions around these issues.

From our discussions, GBC presentations, surveys, and evaluation of the different platforms, we have suggested the following best practice for running sample analysis:

1. Carry-over should preferably be eliminated during development and not calculated out in sample analysis.
2. For each platform, short-term stability data should be used to determine the duration of each run to assure sample stability.

3. A run is not limited to 96 datapoints of Cs and samples for platforms that are defined by a different support such as a CD and / or run in series such as RIA, BIAcore, Erenna®, AlphaLISA, and Gyrolab.
4. A standard curve is implemented at the start of the run with intermittent QCs integrated in the run at a reasonable frequency to validate the results on the platform. Depending on how a run is defined, Cs can be placed either on each solid support or on one plate/CD followed by multiple sample plates as a serial stream of samples with intermittent QCs. QCs should be placed regularly during a run regardless of how a run is defined, to allow for a verification of intra-run precision.
5. Singlet sample analysis can be performed as long as the %CV determined during validation is within an acceptable range (i.e. less than or equal to 15% as is currently accepted for small molecules), taking the most stringent acceptance criteria. These should be applied to QCs run in duplicate and to the intra-run precision including multiple solid supports, if the run is over multiple solid supports.
6. For method transfer, most platforms require re-validation when changing platforms or formats and even partial validations may miss some key parameters of evaluation. Therefore, it is suggested to re-validate the method to be used for sample analysis fully.
7. Multiplexing best practice? It is recommended that analytes be validated in a cocktail whenever possible. During sample analysis, if one analyte fails then all samples should be re-run and the previous passing analyte results masked.

These suggested best practices are meant to guide the community for integrating new technology into bioanalysis from an international perspective.

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Conflict of Interest A possible conflict of interest would be the role of Karolina Österland in terms of Gyrolab input, but as this was a group-driven workstream and feedback from the international community, her opinion was mixed amongst the opinion of the LBA community.

Disclaimer This article is representative of the expert opinions of the co-authors and the international community in the field of large molecule bioanalysis. It does not represent a single company viewpoint.

REFERENCES

1. Myler HA, Given A, Kolz K, Mora JR, Hristopoulos G. Biotherapeutic bioanalysis: a multi-indication case study review. *Bioanalysis*. 2011;3(6):623–43 [Review].

2. US Department of Health and Human Services, Food and Drug administration, CDER, CVM. Guidance for Industry, Bioanalytical Method Validation, 2001.
3. DeSilva B, Smith W, Weiner R, Kelley M, Smolec J, Lee B, *et al.* Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. *Pharm Res.* 2003;20(11):1885–900 [Guideline].
4. Fast DM, Kelley M, Viswanathan CT, O'Shaughnessy J, King SP, Chaudhary A, *et al.* Workshop report and follow-up—AAPS Workshop on current topics in GLP bioanalysis: assay reproducibility for incurred samples—implications of Crystal City recommendations. *AAPS J.* 2009;11(2):238–41 [Review].
5. Smolec J, DeSilva B, Smith W, Weiner R, Kelly M, Lee B, *et al.* Bioanalytical method validation for macromolecules in support of pharmacokinetic studies. *Pharm Res.* 2005;22(9):1425–31 [Review Validation Studies].
6. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, *et al.* Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. *Pharm Res.* 2007;24(10):1962–73 [Consensus Development Conference].
7. European Medicines Agency. Guideline on Bioanalytical Method Validation, 2009.
8. Leary BA, Lawrence-Henderson R, Mallozzi C, Fernandez Ocana M, Duriga N, O'Hara DM, *et al.* Bioanalytical platform comparison using a generic human IgG PK assay format. *J Immunol Methods.* 2013;397(1–2):28–36.
9. Soderstrom CI, Spriggs FP, Song W, Burrell S. Comparison of four distinct detection platforms using multiple ligand binding assay formats. *J Immunol Methods.* 2011;371(1–2):106–13 [Comparative Study Evaluation Studies].
10. Forster RJ, Bertonecello P, Keyes TE. Electrogenerated chemiluminescence. *Annu Rev Anal Chem (Palo Alto Calif).* 2009;2:359–85 [Review].
11. Lewkowich IP, Campbell JD, HayGlass KT. Comparison of chemiluminescent assays and colorimetric ELISAs for quantification of murine IL-12, human IL-4 and murine IL-4: chemiluminescent substrates provide markedly enhanced sensitivity. *J Immunol Methods.* 2001;247(1–2):111–8 [Comparative Study Research Support, Non-U.S. Gov't].
12. Sloan JH, Ackermann BJ, O'Dell M, Bowsher RR, Dean RA, Konrad RJ. Development of a novel radioimmunoassay to detect autoantibodies to amyloid beta peptides in the presence of a cross-reactive therapeutic antibody. *J Pharm Biomed Anal.* 2011;56(5):1029–34 [Research Support, Non-U.S. Gov't Validation Studies].
13. Sickert D, editor. AlphaLISA - a “no wash” high-throughput alternative to ELISA for PK, PD and immunogenicity measurement during drug development? European Bioanalytical Forum, 3rd Focus Meeting; 2012; Brussels, Belgium.
14. Fraley KJ, Abberley L, Hottenstein CS, Ulicne JJ, Citerone DR, Szapacs ME. The Gyrolab immunoassay system: a platform for automated bioanalysis and rapid sample turnaround. *Bioanalysis.* 2013;5(14):1765–74 [Research Support, Non-U.S. Gov't Validation Studies].
15. Liu R, Pillutla R, DeSilva B, Zhang YJ. Rapid development of multiple ‘fit-for-purpose’ assays on an automatic microfluidic system using a streamlined process in support of early biotherapeutics discovery programs. *Bioanalysis.* 2013;5(14):1751–63 [Research Support, Non-U.S. Gov't].
16. Liu XF, Wang X, Weaver RJ, Calliste L, Xia C, He YJ, *et al.* Validation of a Gyrolab assay for quantification of rituximab in human serum. *J Pharmacol Toxicol Methods.* 2012;65(3):107–14.
17. Mora JR, Obenauer-Kutner L, Vimal PV. Application of the Gyrolab platform to ligand-binding assays: a user's perspective. *Bioanalysis.* 2010;2(10):1711–5.
18. Patel V, Leach D, Hornberger M, Williams K, Shih J, Ma M, *et al.* Automating bioanalytical sample analysis through enhanced system integration. *Bioanalysis.* 2013;5(13):1649–59 [Research Support, Non-U.S. Gov't].
19. St Ledger K, Agee SJ, Kasaian MT, Forlow SB, Durn BL, Minyard J, *et al.* Analytical validation of a highly sensitive microparticle-based immunoassay for the quantitation of IL-13 in human serum using the Erenna immunoassay system. *J Immunol Methods.* 2009;350(1–2):161–70 [Comparative Study].
20. Todd J, Freese B, Lu A, Held D, Morey J, Livingston R, *et al.* Ultrasensitive flow-based immunoassays using single-molecule counting. *Clin Chem.* 2007;53(11):1990–5.
21. Dyleski L J, A., *et al.*, editor. Method development and validation of an alternative immunoassay platform for PK studies in a regulated environment. AAPS NBC; 2011; San Francisco, CA, USA.
22. Tarasow TM, Penny L, Patwardhan A, Hamren S, McKenna MP, Urdea MS. Microfluidic strategies applied to biomarker discovery and validation for multivariate diagnostics. *Bioanalysis.* 2011;3(19):2233–51 [Research Support, Non-U.S. Gov't Review].
23. Huber R, Eisenbraun J, Miletzki B, Adler M, Scheer R, Klein R, *et al.* Pharmacokinetics of natural mistletoe lectins after subcutaneous injection. *Eur J Clin Pharmacol.* 2010;66(9):889–97 [Clinical Trial, Phase I Research Support, Non-U.S. Gov't].
24. Spriggs F, Aggarwal P, Deng S, Soderstrom C, Donovan C. Comparison of an antibody capture and a cell capture ligand-binding assay to quantify a monoclonal therapeutic in serum. *Bioanalysis.* 2011;3(6):605–11 [Comparative Study Evaluation Studies].
25. Dula M. *et al.*, editor. Development and validation of a pharmacokinetic assay on the Gyrolab platform for use in phase II/III clinical studies. AAPS NBC; 2012; San Diego, CA, USA.
26. Lee JW, Kelley M, King LE, Yang J, Salimi-Moosavi H, Tang MT, *et al.* Bioanalytical approaches to quantify “total” and “free” therapeutic antibodies and their targets: technical challenges and PK/PD applications over the course of drug development. *Aaps J.* 2011;13(1):99–110 [Review].
27. Lofgren JA, Dhandapani S, Pennucci JJ, Abbott CM, Mytych DT, Kaliyaperumal A, *et al.* Comparing ELISA and surface plasmon resonance for assessing clinical immunogenicity of panitumumab. *J Immunol.* 2007;178(11):7467–72 [Comparative Study Controlled Clinical Trial Validation Studies].