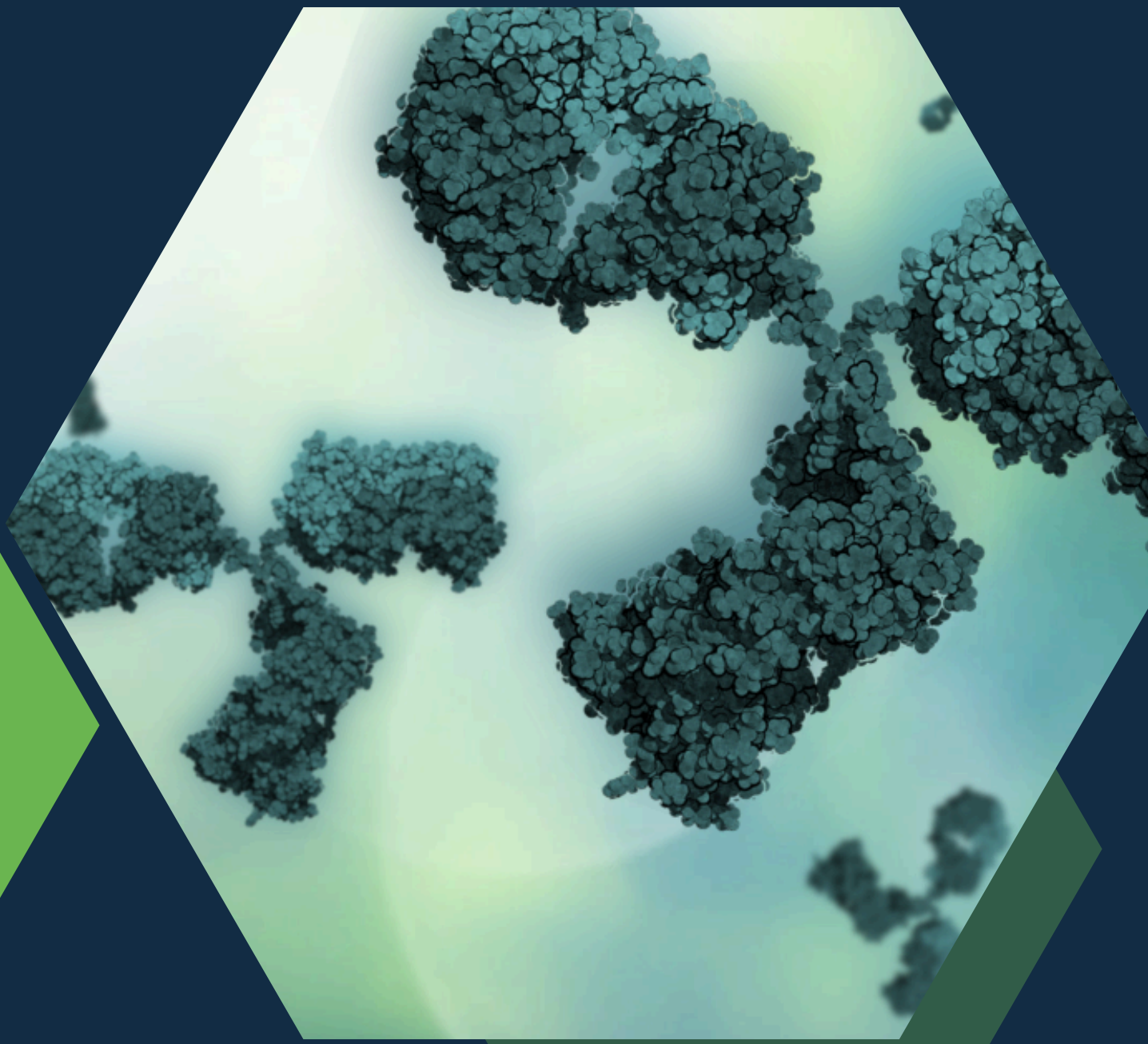
Bioanalysis ZONE

SARTORIUS



Transforming drug development with label-free, kinetic-based assays

Taylor & Francis

Contents

Technology Digest: Transforming drug development with label-free, kinetic-based assays

by Ellen Williams
Digital Editor, Bioanalysis Zone

Keywords: Assay validation; Kinetics-based assays; Ligand-binding assay (LBA); Biolayer interferometry (BLI); Surface plasmon resonance (SPR); Enzyme-linked immunosorbent spot assays (ELISA); Antibodies; Sensitivity; Selectivity; Precision; Robustness; Octet® BLI platform.

An overview of assay development and validation for kinetic-based assays

Ligand-binding assays (LBAs) are kinetic-based assays widely used to measure interactions between two molecules, quantifying proteins, antibodies and many other molecules. They are considered the industry 'gold standard' for protein-drug quantification due to their high sensitivity, selectivity and throughput capabilities [1]. Method development and validation of LBAs are critical to drug production as they preserve safety and efficacy standards. This can be achieved using either a minimal or enhanced approach, as described in the ICH Q14 guidelines [2]. In both instances, the goal is to create a control strategy that ensures the LBA functions as expected during routine usage throughout its full lifecycle [3]. Although the minimal approach is accepted by regulatory authorities and is the focus of this article, an enhanced approach may also be taken to support LBA development and lifecycle management.

A minimal approach to assay development begins with defining the assay's intended use. Initial research into the binding molecules will inform the assignment of the ligand and analyte; the ligand acts as the immobilized capture molecule, while the analyte is free in solution. Size, sensitivity and molecular properties will play a part in determining these roles. For instance, more sensitive molecules may not tolerate immobilization and should be assigned as the analyte [3]. The ligand and analyte used during assay development must be similar or ideally identical to the samples expected to be used in the validation phase. Early considerations for method development include reagent quality, purity, activity, and availability, as these factors directly influence the selection of an appropriate LBA platform. System suitability should be assessed during assay development to ensure the reliability of the assay, followed by clearly defined assay descriptions and development studies [3].

Once an assay has been sufficiently optimized to ensure the bioanalytical parameters are met, validation of the assay can commence. LBA validation ensures the assay is fit for purpose and can consistently meet defined parameters, which is crucial for maintaining data accuracy and reliability during pharmaceutical production [4]. Importantly, validation should not be considered a 'one-off' event but rather a cyclical process where each stage is revisited to enhance robustness. This is particularly true when changes in reagents, instrumentation and consumables occur, as these elements impact a method's ability to achieve its original acceptance criteria [3].

Validation parameters for kinetics-based assays

The validation of kinetics-based assays typically focuses on four parameters: specificity and selectivity, precision, sensitivity and robustness.

Specificity and selectivity

These parameters indicate whether the method measures only the intended analyte without interference from other substances. This is best evaluated using positive and negative controls. Positive controls should closely resemble or be identical to the analyte in question. For example, in an Fcγ receptor kinetics-based assay, a suitable positive control would be another antibody with the same isoform. The negative control could include process contaminants or, where the antibody being assessed is a highly purified form, an antibody with a different isoform that does not bind with the Fcγ receptor [3,4].

Precision

This parameter assesses the variability of measurements for the same sample and is typically presented as the variance, standard deviation or coefficient of variation. Precision can be tested in two ways: intra-assay and inter-assay. Intra-assay precision is assessed by performing replicates of the assay using a QC or reference standard, preferably within a single assay. Inter-assay precision is assessed in the same way but requires the use of different analysts over multiple assays [3,4,5].

Sensitivity

An assay's sensitivity refers to the lowest concentration of an analyte that can be measured while still maintaining the required accuracy and precision. The lower and upper limits of quantitation are set, and the range across which measurements provide reliable data is described [3,4].

Robustness

This refers to the assay's ability to maintain performance under varying conditions. Robustness is assessed by deliberately altering key LBA parameters, including assay temperature, the concentration and/or pH of the assay buffer and shake speed, to assess the assay's stability and reliability under different conditions [3,4,5].

Choosing the right platform for LBA validation

LBA validation can be performed using a variety of platforms, including label-free options like biosensors. Biosensors offer sensitive and selective detection of analytes including viruses, toxins, drugs and antibodies [6,7]. As their name suggests, optical biosensors utilize an optical

measurement approach, using an optical transducer system combined with a biorecognition sensing element to produce a signal equivalent to analyte concentration [6]. Optical biosensors have provided an edge over other biosensor types and conventional analytical techniques, offering direct, real-time and label-free detection [6–8]. Label-free biosensing can be achieved with a range of different platforms, including biolayer interferometry (BLI), surface plasmon resonance (SPR), isothermal titration calorimetry (ITC) and microscale thermophoresis (MST), among others.

Biolayer interferometry (BLI)

BLI measures the interference pattern of white light reflected from two surfaces: an internal reference layer and a layer of immobilized biomolecules (biocompatible surface) on the biosensor tip [9,10]. When an analyte binds to an immobilized biomolecule, the thickness of the biocompatible layer increases according to the analyte's size and affinity to the target. A change in the number of analytes bound to the biocompatible layer causes a shift in the interference pattern, producing a measurable wavelength or 'spectral' shift. This provides real-time kinetics data on binding specificity, analyte concentrations and the rate of dissociation [9,10].

Surface plasmon resonance (SPR)

An alternative optical technique to BLI for measuring molecular interactions in real-time is SPR, which detects changes in refractive index at a biosensor's surface due to mass changes on the sensor surface. It utilizes a glass substrate with a thin gold coating, where light energy couples through the gold surface at a specific angle of incidence, creating a surface plasmon wave at the sample-gold surface interface. Any changes in the refractive index, caused by molecular binding and dissociation, are directly proportional to the mass changes on the sensor surface [8].

Why use biosensor assays over ELISA?

ELISAs are label-dependent alternatives to BLI and SPR, using enzyme-linked antigens or antibodies for biomolecular detection, where enzyme activity is measured using a color-changing substrate. While ELISAs are well-established and have low upfront costs and readily available reagents, they only provide endpoint affinities [10,11]. In contrast, BLI or SPR provide real-time binding kinetics (ka and kd) and affinity (KD) data, allowing optimized association, dissociation and ligand immobilization steps [9,12]. This avoids long incubation times and allows the analysis of less stable biomolecules. Furthermore, BLI and SPR assays can characterize low-affinity analytes that would otherwise be washed away in an ELISA workflow [10,11].

Assay development and validation on the Octet® BLI platform

Despite the broad range of assay platforms available, the Octet platform, which utilizes BLI in conjunction with Dip and Read™ biosensors, offers significant advantages for developing and validating LBA assays [5,9,10–13]. By using biosensors that dip directly into samples, the design eliminates the need for fluidics, reducing sample preparation complexity and avoiding issues with clogged fluidics experienced by other platforms [11,12,14]. BLI systems can characterize a wide range of biomolecular interactions >150 Da and are suitable for larger molecular weight analytes such as viruses, nanoparticles, liposomes and cells [11]. They can characterize Fcγ receptors due to their ability to rapidly measure binding interactions from 1mM to 10pM. These assays also enable high-throughput analysis due to full automation, and have lower consumable costs, making it a cost-effective replacement for ELISA [10,12]. Multiple assay formats can be run depending on the user's required sensitivity, and the provision of real-time data encourages experimental progression [14]. Compared to ELISA, these assays provide faster time to results, which is particularly key when working with less stable analytes and have a lower assay cost as samples and reagents can be fully recovered and reused [11,12].

Kinetic characterization using BLI platforms like Octet can readily replace label-dependent methods like ELISA, but despite their advantages, there remains some hesitation within the field to move away from traditional techniques:

> *"Octet BLI systems, despite their widespread use in research and drug discovery, face challenges similar to other technologies when considered for validated assays. These challenges often include a reluctance to move away from traditional, end-point assays, which are less precise and have lower throughput compared to label-free, real-time assays. This reluctance is partly due to a lack of awareness that current regulatory guidelines for LBAs apply to Octet BLI assays and that regulators support the transition to more precise technologies," commented Stuart Knowling, PhD, Senior Scientist, Sartorius (Goettingen, Germany).*

The need for regulatory harmonization

Octet systems are not yet universally recognized for validated assays; however, current regulatory guidelines for LBAs can be applied to BLI, and regulators are predominantly supportive of this shift towards more precise technologies [3,13]. For example, the FDA Bioanalytical Method Validation: Guidance for Industry, which provides recommendations for the development, validation and in-study use of bioanalytical methods, highlights that these recommendations can be modified with appropriate justification depending on the

bioanalytical method. Consequently, this allows their application to technologies such as Octet BLI [3,4]. The inclusion of Octet BLI in regulatory guidelines such as ICH Q2(R2) also demonstrates this regulatory support [15]. To ensure consistent and efficient LBA development and validation, there is a critical need for global harmonization of regulatory guidelines. Increased awareness of the opportunities offered by new platforms will help to reinforce their applicability and usefulness in assay validation. Further support from regulatory bodies will help to accelerate this shift from traditional technologies to the exploration of newer, more efficient techniques.

Summary

The Octet BLI platform provides an excellent alternative to label-dependent technologies such as ELISA for LBA validation. By utilizing BLI, it enables real-time kinetic analysis, improving assay sensitivity, precision and throughput. Despite its advantages, further acknowledgment of its compliance with regulatory standards is necessary to encourage its broader adoption within the bioanalytical community for validated assays. However, current guidelines from the FDA and EMA can be applied, signaling regulatory support for its integration into validated assays. Global harmonization of these guidelines is essential to fully leverage the capabilities of Octet BLI in drug development.

Disclaimer

References

1. Papadimitriou A , Bansal S, Heinrich J, Staack RF. Can LC-MS/MS and ligand-binding assays live in harmony for large-molecule bioanalysis. *Bioanalysis* 6(13), 1735–1737 (2014).
2. EMA guidance. ICH Q14 Analytical procedure development - Scientific guideline. (2024). [Accessed 2 September 2024].
3. Satorius. Harmonizing Regulatory Guidelines for Assay Validation: A Focus on Kinetics-Based Assays Using Octet® BLI Systems. (2023). [Accessed 27 August 2024].
4. FDA Guidance. Bioanalytical Method Validation Guidance for Industry. (2018). [Accessed 29 August 2024].
5. Cameron C, Peacor B, Oien N, Cheeseman A, Smedley J, Laughlin J, Apiyo DO. Sartorius. Octet® Potency Assay: Development, Qualification and Validation Strategies. (2021). [Accessed 28 August 2024].
6. Damborsky P, Švitel J, Katrlík J.Optical biosensors. *Essays Biochem.* 30(60), 91–100 (2016).
7. Singh AK, Mittal S, Das M, Saharia A, Tiwari M. Optical biosensors: a decade in review - ScienceDirect. *Alex. Eng.* J. 67, 673–691 (2023).
8. Singh P. SPR Biosensors: Historical Perspectives and Current Challenges. *Sensors and Actuators B: Chemical.* 229, 110–130 (2016).
9. Jug A, Bratkovič T, Ilaš J. Biolayer interferometry and its applications in drug discovery and development. *TrAC*. 176 (2024).
10. Sartorius. Enhanced Productivity and Labor Efficiency in Lot Release and In-Process Testing of Biologics in GxP Laboratories. (2021). [Accessed 28 August 2024].
11. Sartorius. Label-Free Detection Technologies, Key Considerations, and Applications. (2023). [Accessed 27 August 2024].
12. Sartorius. Octet® BLI Systems – Unmatched Versatility for Discovery, Development and Quality Control. (2021). [Accessed 27 August 2024].
13. Sartorius GxP compliance tools. GxP Compliance Tools for Octet® Systems. (2024). [Accessed 28/08/2024].
14. Sartorius BLI products. https://www.sartorius.com/en/products/biolayer-interferometry. (2024). [Accessed 28/08/2024].
15. EMA Guidance. ICH Q2(R2) Validation of analytical procedures - Scientific guideline. (2024). [Accessed 29 August 2024].

SARTORIUS

Application Guide

June 1, 2023

Keywords or phrases:
Method Development, Validation, Quality Control, QC, GxP, GMP, Ligand-Binding, USP Chapter <1108>

Ligand Binding Assays That Meet Compliance with Octet® BLI Systems

David Apiyo, PhD, Fremont, CA

Correspondence
Email: octet@sartorius.com

Abstract

Octet® Bio-Layer Interferometry (BLI) systems enable analytical assessment of biologics in various stages of the development workflow beginning with discovery and early selection to validation, manufacturing and quality control. The instrument's configuration and sample plate format coupled with real-time analysis allows for rapid assay method development. In the last couple of years, Octet® BLI systems have been used by multiple organizations to generate supporting data submitted to various regulatory bodies for the approval of different biologics drug candidates. In addition, Octet® BLI systems have been cited in the United States Pharmacopeia General Chapters as an appropriate technique for ligand-based Fc-receptor binding studies.[1]

Analytical instruments that offer simplicity and speed can greatly enhance operations under compliance requirements as they reduce the risk of errors while enhancing productivity.

Sartorius offers Octet® GxP packages to support complete GMP compliant implementation. The packages include a GMP qualified Octet® BLI system, 21 CFR Part 11 software with audit trails, software validation package, installation, and operational qualification (IQOQ) protocols and kits, user guides, performance qualification (PQ) protocols and kits, and biosensor validation support. The Octet® platform's design and configuration coupled with its ease of use allows for enhanced productivity. The capacity to test for instance QC potency samples increases to 40X more samples over manual ELISA and up to 16X more samples per day compared to the majority of SPR platforms.[2]

Benefits of the Octet® BLI Platform in GMP

- **Parallel processing of samples — reduced assay development time**
  Faster analysis across many samples allows for faster decision making and is especially useful in assay method development when multiple conditions must be investigated for method qualification.

- **Direct binding assay — minimal sample prep, automated assay without manual steps**
  Assay setup is easy and fast; complete walkaway while experiment is running and minimal manual intervention reduces the risk of human errors.

- **Few moving parts that require maintenance — QC environment ready**
  Octet® BLI systems instruments require very low maintenance. There are no microfluidics that need to be guarded against clogs, contamination, or leaking. Preventive maintenance is recommended annually for the Octet® R8 instrument and bi-annually for the Octet® RH16 and Octet® RH96 instruments.

- **Off the shelf biosensors such as Protein A (Pro A), Protein G (ProG), Anti-human Capture (AHC) and Anti-mouse Capture (AMC) are available for direct capture of ligands. High precision streptavidin (SAX and SAX2) biosensors deliver enhanced precision for GMP applications**
  Octet® Bio-Layer Interferometry (BLI) systems offer an advanced, fast, robust and fluidics-free approach for screening and characterizing molecular interactions such as protein-protein and protein-small molecule analysis. The Octet® BLI platform enables a huge variety of applications performed at various stages of biologics development - from early selection to validation to manufacturing and quality control, e.g. concentration determination of the active analyte, ligand binding, or contaminant testing for lot release and in-process testing. To read more, please refer to the Application Note: **Octet® Potency Assay: Development and Validation Strategies.**

It is estimated that for a typical ligand binding method, an Octet® R8 system will be 2X faster for method development than traditional ELISAs or 4-channel SPR instruments. The sample plate format coupled with real-time analysis and high-throughput readout allows for a more rapid assessment of different assay conditions, that in-turn help speed up assay optimization. Qualification parameters such as ligand immobilization concentration, assay shake speed (similar to flow-rate evaluation in SPR based methods) and effects of temperature are first assessed before the method is evaluated for robustness around more typical parameters such as biosensor and reagent lots, different analysts and day-to-day variability amongst other parameters. Sartorius provides a biosensor lot reservation service for select biosensors which is reserved only for GMP assays method validation.

Examples of Approved Drugs Using Octet® BLI Technology

Octet® BLI is continuously being used to generate supporting data for various drug regulatory submissions including new drug applications (NDA) and biologics license applications (BLA). Applications range from specificity assessment to affinity characterization and binding epitope confirmation in blocking assays.

Octet® BLI data was used as a part of supporting data for the assessment of functional activity of pembrolizumab (Keytruda; EMEA approved in 2015). Keytruda as a monotherapy is indicated for the treatment of advanced (unresectable or metastatic) melanoma in adults. The active substance of Keytruda is pembrolizumab, which is a humanized monoclonal antibody that binds to human PD-1 and blocks the interaction between PD-1 receptor and its ligands. It is an IgG4 monoclonal antibody with Class-II mechanism of action (binding to cell-bound antigen not involving Fc effector function). The Octet® together with ELISA were used for in vitro binding studies of pembrolizumab.[3]

Tecentriq, on the other hand, was approved by the EMA in 2017 and later by the FDA in 2018. Tecentriq's active substance is atezolizumab which is an Anti-PD-L1 monoclonal antibody. It is used in the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy and other cancers. The Octet® system was used for supporting data in the binding characterization of the drug to its receptor PD-L1 amongst other studies.

In addition to these approved drugs, there are multiple instances where the Octet® system has been used along

with other techniques to provide supporting orthogonal data for various drug applications. For example, Theramex Ireland Limited applied for marketing authorization with the EMA for Livogiva in 2019 with a positive opinion of granting the same provided in 2020. Livogiva is a biosimilar to Forsteo and is used for the treatment of Osteoporosis. The active substance is Teriparatide, PTH (1-34) the biologically active 34-amino acid N-terminal fragment of the 84-amino acid native parathyroid hormone, PTH (1-84). The Octet® BLI platform was used to verify biosimilarity between the two drugs in functional binding assays.[4]

STADA Arzneimittel AG filed an application for marketing authorisation with the EMA for Oyavas in early 2020 with a positive response obtained in January 2021. Oyavas has been developed as a similar biological medicinal product (biosimilar) to the reference medicinal product Avastin, which contains bevacizumab as the active substance. Bevacizumab is a recombinant humanized monoclonal antibody, which specifically binds to human vascular endothelial growth factor (VEGF), preventing its interaction with VEGF receptors (VEGFRs) on the surface of endothelial cells. In this case, the Octet® system was used for confirming specificity of the biosimilar through functional binding assessment with irrelevant antigens.[5] Many other examples of drug development with Octet® BLI data used for supporting characterization data exist in literature (Table 1).

Table 1
Examples of Approved Drugs Utilizing Octet® BLI Data in Their Applications

Drug Name	Target	Drug Modality	Sponsor	Indications	Application	Regulatory Agency	Year of Approval
Keytruda | Pembrolizumab	PD-1	mAb	Merck	Non-small cell lung cancer	Affinity Characterization	EMA	2015
Tecentriq | Atezolizumab	PD-L1	mRNA Vaccine	Roche	Non-small cell lung cancer	Specificity	EMA	2017
Comirnaty | BNT162b2	COVID-19	mAb	Pfizer	COVID-19	Affinity Characterization	EMA	2020
Ultomiris | Ravulizumab	Human C5	Peptide Biosimilar	Alexion	Paroxysmal nocturnal hemoglobinuria (PNH)	Affinity Characterization	EMA	2019
Livogiva | Teriparatide	PTH	mAb Cocktail	Teva	Osteoporosis	Affinity Characterization	EMA	2020
Atoltivimab, maftivimab, and odesivimab-ebgn	EBOV glycoprotein	mAb Biosimilar	Regeneron	Ebola virus	Competititon studies	FDA	2020
Oyavas | Bevacizumab	VEGF-A	mAb	Mabxience	Non-small cell lung cancer	Specificity | Affinity Characterization	EMA	2021
Jemperli | Dostarlimab	PD-1	mAb	GSK	Endometrial cancer	Affinity Characterization	EMA	2021
Regdanvimab	S protein	mAb	Celltrion	COVID-19	Affinity Characterization | Blocking Assay	EMA	2021
Xevudy | Sotrovimab	S protein	mAb	GSK | Vir Biotechnology	COVID-19	Affinity Characterization	EMA	2021
Nuvaxovid	COVID-19	Vaccine	Novavax	COVID-19	Affinity Characterization	EMA	2022
Kimmtrak | Tebentafusp	CD3 | gp100	TCR	Immunocore	Uveal melanoma (UM)	Specificity	EMA	2022
Tixagevimab | Cilgavimab	COVID-19	mAb Combination	AstraZeneca	COVID-19	Affinity Characterization | Blocking Assay	FDA	2022
Opdualag (nivolumab | relatlimab)	LAG-3 | PD1	mAb Combination	BMS	Melanoma	Blocking Assay	EMA	2022

Bio-Layer Interferometry (BLI) in USP General Chapter <1108>[1]

Regulatory bodies such as the FDA, EMA, NMPA and others do not typically recommend or refer to specific product, brand, or vendors in their guidance on analytical technologies. References are however typically made of certain technologies in some USP chapters as a general guidance. These references are based on general concepts around the application in question. The United States Pharmacopeia (USP) has recently released a new general chapter <1108> which for the first time cites the use of biolayer interferometry in key ligand binding applications, specifically in *in-vitro* assays that assess complex Fc domain interactions with molecules such as C1q (a subcomponent of the C1 complex of classical pathway of complement activation that is involved in antibody dependent immune function) and Fc-receptor molecules that act as regulators of immune response amongst other functions. BLI is further cited as a label-free technique that provides more precise kinetic data and that can measure a relatively wide range of binding affinities. It can incorporate the use of a reference material to express relative equilibrium dissociation constant (K_D); a useful parameter in comparing a reference material and a new lot during manufacturing.

References

1. USP-NF <1108>; **Assays to evaluate fragment crystallizable (FC) – mediated effector function**
2. **Enhanced productivity and labor efficiency in lot release and in-process testing of biologics in GxP laboratories;** Sartorius White paper
3. **https://www.ema.europa.eu/en/documents/assessment-report/keytruda-epar-public-assessment-report_en.pdf**
4. **LIVOGIVA, INN-teriparatide** (europa.eu)
5. **Oyavas - INN-bevacizumab** (europa.eu)

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 650 322 1360
Toll-free +1 888 OCTET 75

Find out more: www.sartorius.com/octet-support

For questions, email: AskAScientist@sartorius.com

SARTORIUS

White Paper

May 20, 2021

Keywords or phrases:
Octet® Instrument, 21 CFR Part 11, BLI, PQ, IQOQ, GMP, GxP, Software Validation, Method validation, Instrument Qualification, QC, Potency Assays, Regulatory Compliance

Enhanced Productivity and Labor Efficiency in Lot Release and In-Process Testing of Biologics in GxP Laboratories

David Apiyo, Fremont, CA

Correspondence
Email: octet@sartorius.com

Abstract

Octet® systems come with distinct advantages including ease of use, throughput, low maintenance, and microfluids-free configurations, enabling them to provide GxP users with enhanced productivity and labor efficiency for lot release and in-process testing of Biologics. Octet® instruments can be equipped with Octet® 21 CFR Part 11 Software for compliance with regulatory requirements. Compliance is further enhanced with the availability of instrument qualification kits including IQOQ and PQ kits that ensure the platform performs as stipulated. This whitepaper includes a few examples in which Octet® systems have been used under GxP compliance for different applications and shows the relative benefits over alternate technologies.

Key Considerations

- **Enhanced productivity:** Enhances productivity by increasing the capacity to run 20X–40X and 8X–16X more QC potency samples testing/day than ELISA and SPR* respectively.
- **Cost savings:** Reduced hands-on operation allows for 10X less analyst hands-on time than ELISA, resulting in FTE cost savings of > $40,000*.
- **Time savings:** Robust instrument resulting in significant maintenance cost savings and low downtime compared to SPR; no fluidics means no clogging of samples and less instrument downtime. You will not need a second backup instrument to support uninterrupted operation.
- **Ease of use:** The Octet® platform is approximately 2X faster than ELISA and SPR* in method validation for ligand binding and potency assays.
- **Complete Octet® GxP package:** Comes with all requirements for GMP compliance – IQOQ protocols and kits, user guides, performance qualification (PQ) protocols and kits, Octet® 21 CFR Part 11 Software with audit trails, software validation package and biosensor validation support. Octet® R8 and Octet® RH16 systems come with specifically optimized performance qualification kits.
- **Successful use:** Currently validated by and used in multiple CROs and CDMOs world-wide.

* Please contact Sartorius for details.

Introduction

Bio-Layer Interferometry (BLI) is an optical technique that analyzes the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on the biosensor tip and an internal reference layer (Figure 1A). Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real time (Figure 1A and 1B). The binding between a ligand immobilized on the biosensor surface and an analyte in solution produces an increase in optical thickness measured as a wavelength shift, Δ λ (Figure 1C).

Sartorius' Octet® instruments utilize BLI technology to monitor biomolecular interactions in real time. They are an ideal replacement for ELISA and HPLC techniques for the quantification of antibodies and recombinant proteins and are especially suitable for product potency lot release assays. The platebased and non-fluidic format also offers GxP users distinct advantages over comparative SPR based techniques. Octet® systems provide higher throughput, with the flexibility to run 2 to 96 samples simultaneously, and better sample versatility, including the ability to analyze crude samples and more tolerance to diverse sample matrices. In addition, ease of use, low maintenance and high data precision speeds time to results throughout the drug development process. The Octet® platform is particularly well-suited for GxP and QC laboratories.

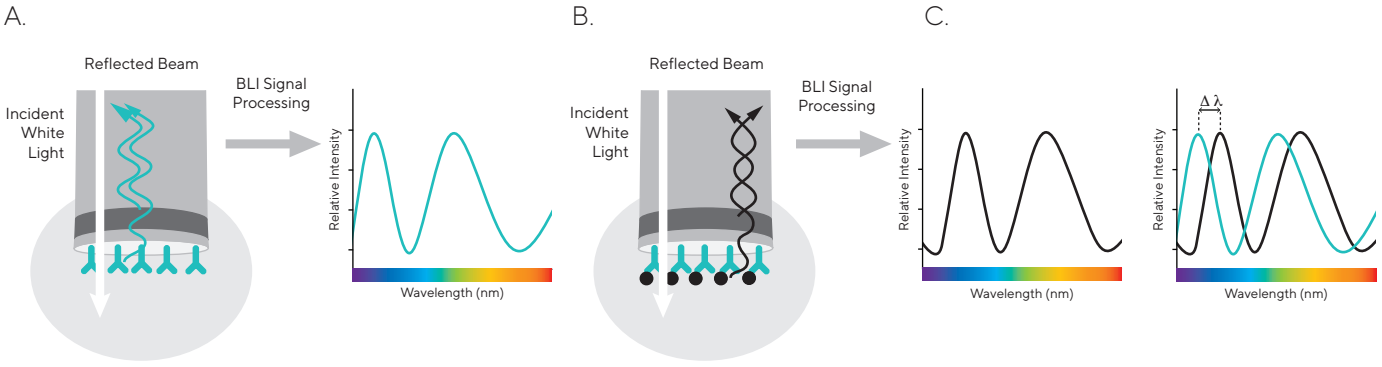


Figure 1: Relative intensity of the light reflection pattern from the two surfaces on the biosensor. Octet® sytems with BLI technology measure the difference in reflected light's wavelength (Δλ) between the two surfaces.

Figure 2: Sartorius' Octet® systems meet a wide range of throughput needs. The higher throughput Octet® RH16 and RH96 systems analyze either 16 or 96 samples at a time, respectively (top). The lower throughput Octet® R Series systems analyze either 2, 4 or 8 samples at the same time respectively (bottom).

Analytical Instruments in the GxP Environment

Analytical methods that provide reliable and accurate data are necessary to ensure quality standards are met during the production and release of drug products. In order to ensure data accuracy, it is critical that the performance of the applied analytical instruments, the computer systems used, and the developed methods for data acquisition meet required specifications.

Several official regulatory guidelines including Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP) and ISO Standards exist and provide guidance on meeting compliance.

Sartorius, an ISO 9001 certified company, offers Octet® 21 CFR Part 11 Software and full GxP products and services for Octet® instruments (Figure 2). These include:

- Octet® CFR Software and Sartorius FB server features for secure, traceable electronic record keeping that enable compliant data acquisition and data analysis in laboratories working under GxP and 21 CFR Part 11 regulations.
- IQOQ/PQ packages that ensure that Octet® systems are qualified and operate as intended and that the performance meets specifications.
- Customer-run software validation support
- Biosensor validation support services
- Technical support assistance

Octet® Instrument Qualification

Instrument qualification is critical to meeting compliance guidelines in any analytical laboratory developing, qualifying and validating assays in the GxP environment. Sartorius offers a rigorous installation qualification and operational qualificaton (IQOQ) kit that includes a user guide, reagents, and documentation required for instrument installation and qualification. In addition, a comprehensive performance qualification (PQ) kit is also available that can be used for performance qualification and periodic system checks. The IQOQ and PQ kits can be purchased separately at the time of instrument installation or any other time for regular system performance checks to ensure calibration and maintenance of the instrument.

Installation Qualification and Operational Qualification (IQOQ)

The IQOQ Kit provides a checklist of instrument components and documented verification that the Octet® instrument, accessories and computer system as supplied and installed, comply with Sartorius' specifications. It also provides a comprehensive and documented verification that the Octet® instrument operates as intended. It verifies that various critical operational parameters such as the optical system, alignment of instrument, plate, temperature and sample plate shake speed meet the stipulated specifications. The IQOQ Kit can be added at time of instrument purchase or at any other time during the lifespan of the Octet® instrument. Sartorius recommends the IQOQ procedure to be repeated after an instrument move or repair and following a software upgrade.

Performance Qualification (PQ)

The PQ Kit is used to verify that the Octet® instrument is fully functional after installation. System functionality is tested for both quantitation and kinetics analysis. The kit contains biological samples, reagents and biosensors for each analyses and is used to demonstrate that consistent and reproducible results are obtained within product specifications.

The qualification process for kinetics applications is two-fold:

1. Assess instrument performance for ligand loading onto the biosensor surface with stipulated loading acceptance criteria
2. Assess instrument performance for analyte binding to the immobilized ligand with stipulated acceptance criteria.

The qualification process for quantitation applications asseses performance of known concentrations of Prostate Specific Antigen (PSA) used in the generation of a standard reference curve. Assay performance is evaluated against stipulated percent acceptance variation statistics.

Octet® CFR Software and Software Validation Services

Software validation and the use of 21 CFR Part 11-compliant software are regulatory requirements prior to using any analytical instruments in a GxP environment. Octet® CFR Software includes features such as controlled user access and electronic signatures. In addition, the Sartorius FB Server module manages the information recorded during user sessions. Software validation provides users with the confidence that data integrity and accuracy are always maintained. The Software Validation services provide a single source for meeting all software compliance needs, including demonstration that the Octet® Software is compliant with the 21 CFR part 11 final rule. This validation package includes an instruction manual that provides guidelines on how to verify functions in the FB Server monitor and Octet® Analysis Studio Software. In addition, it provides example data sets for the comparison of Octet® software-generated data to other software such as Microsoft® Excel® and GRAPHPAD PRISM™.

High-Precision Biomolecular Analysis

BLI is a widely adopted technique in biopharmaceutical development and manufacturing. The throughput and flexibility of the Octet® platform has helped accelerate almost every stage of the drug development workflow (Figure 3) in leading and start-up biotherapeutic companies, enabling informed decisions to be made earlier.

The comprehensive tool set for compliance make Octet® systems ideal in regulated quality control (QC) labs for concentration and impurity analysis in both upstream and downstream processes. In addition, Octet® systems have become a solution platform for kinetic and potency analysis of drug-target and drug-Fc receptor(s) (FcγRI, FcγRIIa, FcRIII and FcRn) interactions, and for stability analysis by assessing changes in activity in stressed and forced degradation samples. The high-throughput capability and flexibility of the platform enables rapid completion of assay method development through design of experiments (DOE) essential in identifying and controlling critical quality attributes (CQAs) that ensure consistent and reliable quality of biopharmaceutical products.

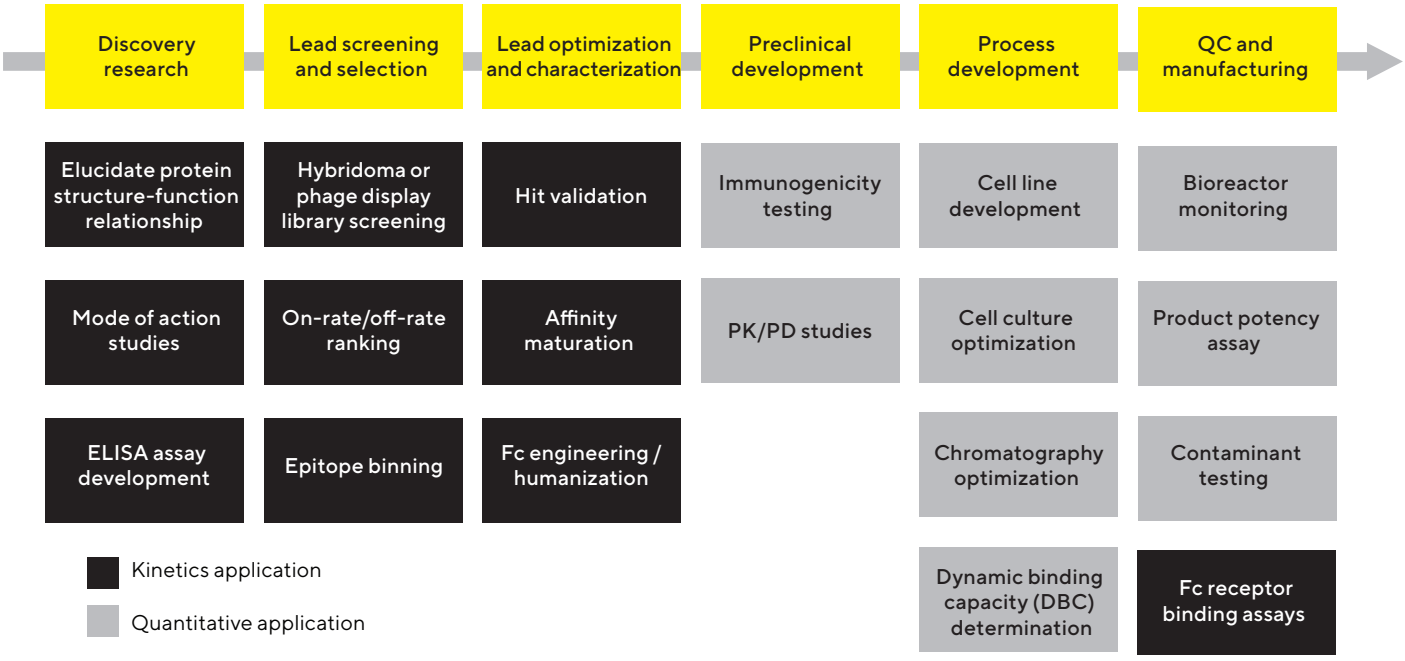


Figure 3: Select applications areas of Octet® systems in biotherapeutics development.

Biosensor Validation Services

Sartorius provides GxP users with biosensor support during the method validation process. Customers are able to order multiple lots of a biosensor for assay method qualification and validation, and can reserve one of the lots for future purchase.

The service is available for the following biosensor types: Protein A (ProA), Streptavidin (SA), High Precision Streptavidin (SAX), Ni-NTA (NTA) and Anti-Mouse Capture (AMC). Customers can order up to five different biosensor lots, up to 20 trays from each lot for evaluation, and reserve up to 40 additional trays for future purchase.

High Precision Streptavidin 2.0 (SAX2) Biosensors

Sartorius provides GxP users with High Precision Streptavidin 2.0 (SAX2) Biosensors developed and qualified for applications in downstream drug discovery and regulated environments that have stringent assay precision requirements. SAX2 is QC-tested for the biotinylated molecule to meet precisioncontrolled coefficient of variation (CV) specification of ≤ 4% within lot and CV range of 20% across lots. SAX2 is suitable for both kinetics and quantitation assays when used with biotinylated ligands.

Concentration Determination

The Octet® platform provides a convenient and reliable analytical method for measuring antibody and protein concentrations. The simple Dip and Read approach enables streamlined workflows and rapid quantitation of 96 samples in as little as 5 minutes on the Octet® RH96 system or 80 samples in < 30 minutes for the Octet® R8 system. Samples can be analyzed in cell culture supernatant or in complex media, thus removing the need for purification.

In a typical quantitation assay, biosensors coated with capture molecules are dipped into samples in the sample plate and the on-rate is measured in real time. The measured on-rate is then used to determine the concentration of the target protein. This is done by comparing the binding signals obtained from a set of known analyte concentrations which are used to generate a standard curve with the signals from the unknown samples. Commonly used biosensors for antibody and protein concentration determination include Protein A- and Protein G-coated Biosensors and Anti-Human IgG (AHQ) and Anti-Mouse IgG (AMQ) Biosensors for antibody or Fc based protein molecules. Anti-HIS (HIS2), Anti-GST (GST) and High Precision Streptavidin Biosensors (SAX) are used for quantitation of recombinant proteins. Octet® instruments measure the unique binding properties of interacting pairs of molecules; hence the calibration with external standards used with techniques like OD 280 measurements are not needed. The instrument's performance is verified through well-characterized materials that can be used to confirm the system is running within set specifications. Since the binding properties of each specific interaction pair is unique, a stan dard or reference curve using the purified version of the analyte in matching matrix is required. As the association curve of a binding event is used to quantify the analyte in the sample, only the amount of biomolecule that really binds to the immobilized ligand on the biosensor is taken into consideration.

In quantitation assays, precision, linearity and accuracy are key parameters that must be demonstrated in order for the assay to be validated for use in manufacturing. In addition, the limit of quantitation (LOQ) should be determined to increase confidence in the assay's performance. As an example, the Octet® system was used to determine method precision and accuracy in the quantitation of recombinant insulin.[1] High Precision Streptavidin Biosensors (SAX) were coated with anti-insulin antibody and used to bind purified insulin samples. Known insulin sample concentrations were prepared in Sartorius' 1X Kinetic Buffer at concentrations ranging from 0–50 µg/mL and used in binding assays (Figure 4A) to generate a standard/reference curve (Figure 4B). Three samples (25, 6.25 and 1.56 µg/mL) of insulin were also spiked in 1X Kinetics Buffer and treated as unknown samples. All samples were run in triplicate. Data was analyzed using the initial slope binding rate analysis mode in Octet® Analysis Studio Software. The standard samples exhibited concentration % CVs of < 3% (Table 1), while the unknown samples exhibited concentration % CVs well below 5% indicating excellent precision. Dose recovery for the unknown samples was found to be within 90–110%, indicating excellent method accuracy (Table 2). A similar approach can be used for other recombinant proteins.

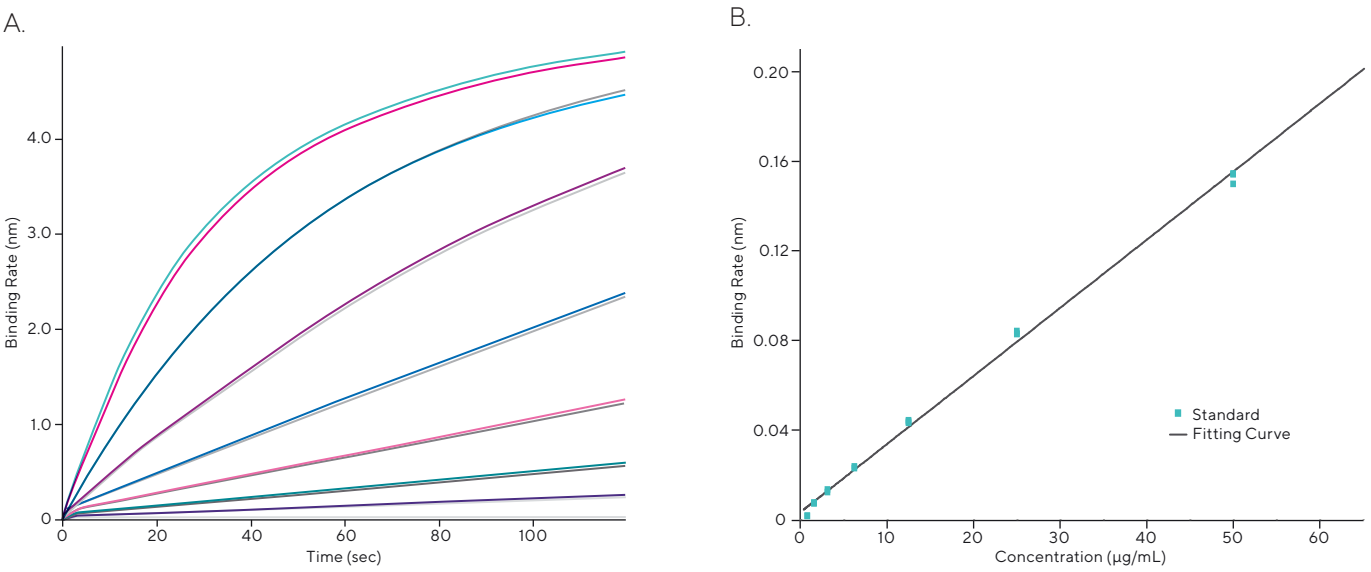


Figure 4: Binding of known concentrations of recombinant insulin (A) used to generate a standard/reference curve (B) for the quantitation of recombinant insulin. SAX Biosensors loaded with anti-insulin were used for the studies.

Table 1: Insulin quantitation. Standard samples exhibit excellent precision at < 3% CV.

Conc. µg/mL	Binding rate, nm/s	Conc. avg, µg/mL	Conc. % CV
50	1.02	50	2.39
25	0.5	25	1.88
12.5	0.1872	12.5	1.24
6.25	0.0611	6.2	1.48
3.13	0.0189	3.1	0.3476
1.56	0.0058	1.6	2.83
0.78	0.0012	0.8	0.2831
0	0.0001	0	0

Table 2: Insulin quantitation. Unknown samples, n=3, exhibit excellent precision and accuracy.

Conc. µg/mL	Binding rate, nm/s	Conc. avg, µg/mL	Conc. % CV	% Recovery
25	0.5182	25.9	1.41	104
6.25	0.064	6.4	0.1638	102
1.56	0.0066	1.7	1.4	106

High-precision Ligand Binding and Potency Assays

Ligand binding kinetics assays are increasingly finding use as batch lot release methods; especially in potency assays where an assessment of product stability and function can be made. In these binding kinetics studies, the interaction is often assessed through the measurement of either the affinity of the analyte/drug product to a receptor or ligand immobilized on the bisensor surface or by monitoring the drug product's binding response signals (Req) as a function of concentration and comparing it to a control reference product for relative potency assesments[2]. Sartorius offers ready-to-use biosensors such as Ni-NTA and FAB2G Biosensors for the immobilization of different panels of Fc gamma receptors that can in turn be used to bind drug products for potency assesment.[3]

In all cases, reproducibility measured through precision and accuracy are key metrics. Octet® systems are highly suitable for these assays as they decrease method development time significantly compared to SPR and ELISA techniques. An overlay of replicate data (Figure 5) for the binding of an FcRn molecule to an IgG, with the IgG immobilized onto Anti-Human Fab-CH1 (FAB2G) Biosensors shows excellent reproducibility.

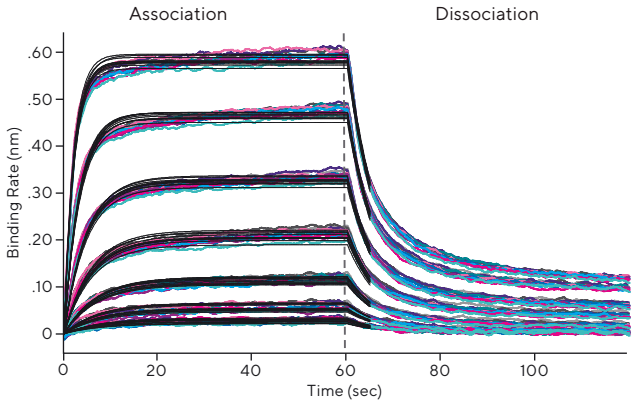


Figure 5: Overlay of several replicates of FcRn-IgG interactions on the Octet® platform using FAB2G Biosensors. Fitting of data traces was done using a 1:1 model with global fitting and a 5-second dissociation step (fit lines in magenta).

Stability and Forced Degradation Studies

Octet® platforms can also be used for developing stability-indicating methods, and are suitable for measuring and distinguishing between fully-functional drug products and those whose binding activities have been affected by degradation. In one study, the Octet® platform was used to distinguish between native and deactivated antigen and showed reduced binding activity of the deactivated antigen (ref), hence proving the assay was stability indicating.
In another study[4] (Figure 6), the affinity of an IgG1 to an Fc gamma receptor IIIa molecule was shown to decrease with increasing percent deglycosylation, further indicating that Octet® systems are suitable for use in developing stability-indicating methods.

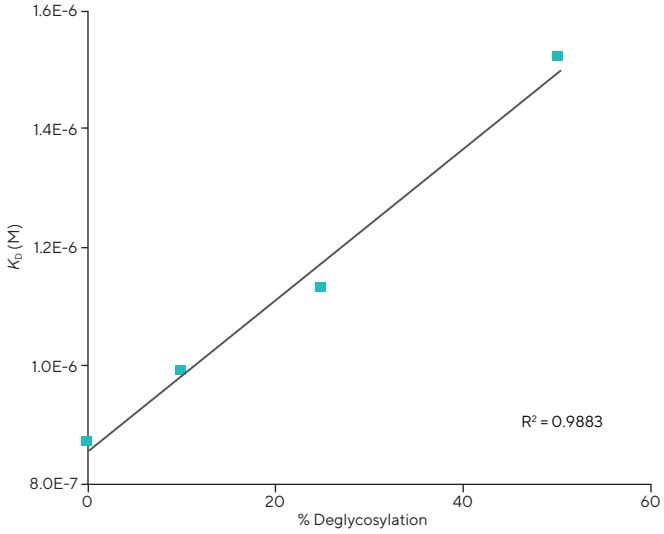


Figure 6: Affinity analysis of the binding of an IgG1 to an Fc gamma receptor as a function of deglycosylation.

Technical Support

Sartorius offers comprehensive technical and service support for Octet® system users in the GxP environment. Field Applications Scientists and Engineers are trained to provide guidance in various aspects of compliance, including assay development and qualification. Performance certification (PC) that includes instrument preventive maintenance and the necessary documentation is also available to GxP users, and is recommended every 6 months.

Summary

Sartorius offers Octet® system users complete qualfication, validation and support solutions that ensure compliance in the regulatory space, and allow rapid development, optimization and validation of assay methods for various applications in GLP and QC laboratories. A summary of product offerings and assay recommendations are listed in Table 3.

References

1. Steve Turner et al., Sartorius Application Note: Customized Quantitation of Recombinant Therapeutic Proteins Using High Precison Streptavidin (SAX) Biosensors.
2. Nathan Oien et al., KBI Potency webinar: From Screening to QC: Development considerations for Octet® methods.
3. Michael Sadik et al., Bioprocessing Online, Accelerate Biopharmaceutical Development with Novel Analytical Techniques, April 28th, 2017.
4. Renee Tobias et al; Sartorius Application Note: Analysis of FcRn-Antibody Interaction on the Octet® Platform.

Table 3: Sartorius Octet® platform GxP product offerings and typical GxP/QC applications.

Item	Description
IQOQ Kit and service	Includes items required for the verification and documentation of instrument components and for the qualifcation of instrument operational features
PQ Kit	Includes biosensors and reagents required to verify that the instrument performance meets specifications in binding and quantitation applications
Octet® CFR Software	21 CFR Part 11 data acquisition and analysis software that ensures compliance with 21 CFR Part 11 regulations
Software validation support	Sartorius offers user support during validation of Octet® Software
Biosensors for validation support	Sartorius will work with customers to sequester multiple lots of biosensors (for a select group of biosensors) during assay method qualification and method validation processes
Key recommended Octet® platform GxP applications	Lot release, in-process testing and investigational new drug (IND) testing assays, potency assays, ligand binding, stability, detection limit and titer determination methods

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 888 OCTET 75
Or +1 650 322 1360

For further contacts, visit
www.sartorius.com/octet-support

WP-41-0293 Rev F

SARTORIUS

Application Note

March 15, 2021

Keywords or phrases:
Octet®, Bio-Layer Interferometry, BLI, Quality control, Octet® QC, GxP, Method development, Method qualification, Method validation, Ligand binding, Design of experiments, DOE, FcγRIIIa binding

Octet® Potency Assay: Development, Qualification and Validation Strategies

Carson Cameron[1], Brendan Peacor[1], Nathan Oien[1], Andrew Cheeseman[1], Jimmy Smedley[1]
John Laughlin[2], David O. Apiyo[2]

1. KBI Biopharma, Durham, NC
2. Sartorius, Fremont, CA

Correspondence
Email: octet@sartorius.com

Abstract

The determination of drug analyte characteristics can be affected by the test method used. In antibody drug candidate ligand binding potency assays for example, FcγRIIIa binding is a common characteristic tested to assess the potency of the drug. Factors such as the amount of FcγRIIIa ligand immobilized on the biosensor for assessing its affinity to the drug molecule, assay temperature and assay flow rate or shaking speed (for plate based platforms) among others can affect the binding behavior and in turn the accuracy of the method. These factors typically need to be evaluated in the pre-qualification stage of an analytical method development. While they can be evaluated as single variables, an ideal analytical platform is one that allows the user to evaluate multiple factors in-tandem in a design of experiment (DOE) to enable an understanding of the effect of their interactions to the output parameters. The Octet® platform is highly suited for a fast evaluation of the interactions between these potential key assay inputs and allows for relatively fast time to results due to its high-throughput and ease of use.

KBI BIOPHARMA

Introduction

Kinetic analysis of biomolecular interactions is critical during drug discovery and development. The affinity of an interaction directly affects the dose required for a biopharmaceutical to be effective. Real-time data on the kinetics and affinity of binding can provide useful information at every stage of biopharmaceutical reagent development. Moreover, understanding the mechanism of binding can provide insights into the desirability of a drug candidate during development, including implications for the drug's stability upon complex formation with its binding target. Binding kinetics assays and specifically affinity constant (K_D) analysis are increasingly being used for biological product lot release. Regulatory requirements necessitate that such products be QC tested using methods that have been appropriately developed, qualified and validated under GMP conditions.

In this application note, we discuss the strategies for the development and validation of a potency assay using Octet® systems. We have highlighted the Octet® system's ease-of-use and fast time to results by showcasing strategies for the development and validation of a method for evaluating the binding of an Fc gamma receptor III molecule to the widely characterized NISTmAb.

Fc receptors are widely distributed cell-surface proteins that act as communication points between effector antibodies and their biological implements. There are three classes of Fc receptors, which bind to antibodies through their Fc region and impart different activities including Fc-gamma receptor I (CD64) that is responsible for phagocytosis and the activation of monocytic cell lines, Fc-gamma receptor II (CD32) that is mainly responsible for antibody-dependent cellular phagocytosis and Fc-gamma receptor III (CD16), which is responsible for antibody-dependent cellular cytoxicity (ADCC). Glycosylation and other modifications to the Fc region of an antibody can affect Fc gamma receptor binding hence these receptor molecules are a good tool for evaluating antibody drug efficacy and for antibody product lot release assessment. In this application note, we use affinity constant (K_D) as the reportable parameter to determine Percent Relative Potency to a reference lot.

Bio-Layer Interferometry

The Octet® platform utilizes a Dip and Read format in combination with Bio-Layer Interferometry (BLI) to monitor the interactions between biological molecules. BLI is an optical technique where white light incident to a reflective biosensor surface immobilized with a ligand results in changing interference patterns of the reflected white light detected upon the interaction between the ligand and the analyte which is kept in solution. Binding events between the ligand and the analyte result in an increase in optical thickness on the tip of the biosensor that can be measured as a wavelength shift from the reference surface, and is a proportional measure of the change in thickness of the biological layer (Figure 1).

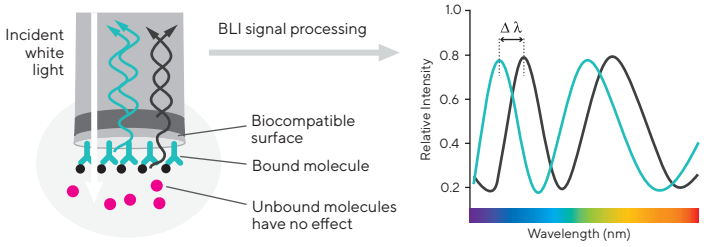


Figure 1: Relative intensity of the light reflection pattern from the two surfaces on the biosensor. Octet® BLI systems measure the difference in reflected light's wavelength (Δλ) between the two surfaces.

Materials and Reagents

Materials and reagents for the studies:

Material/reagent	Vendor	Catalog #
Ni-NTA Biosensors	Sartorius	18-5102
FcγRIIIa	R&D Systems	4325-FC-050
NISTmAb	National Institute of Standards and Technology (NIST)	RM8671
96-well plates	Griener Bio-One	655209
384-well plates	Griener Bio-One	781209
Octet® RH96 system	Sartorius	NA
Diluent (1X PBS, 0.1%Tween 20, 0.2% BSA)	KBI (prepared day of use)	NA

Method Development

Determination of analyte characteristics, including potency, can be affected by the test method used. Factors such as the amount of FcγRIIIa captured on the biosensor for the detection of the analyte, the temperature and shaking speed of the reaction, the sample matrix, and the equilibration time allowed for binding can affect the binding behavior. Each of these factors can also impact critical assay attributes such as accuracy. The Octet® platform is highly suited for a fast evaluation of the interactions between these potential key assay inputs and allows for relatively high-throughput method development. Three key input variables: temperature, shake speed and ligand loading density, were identified to be critical to the performance of the potency assay and were evaluated in a mini-design of experiment (DOE) prior to establishing the method. Each variable was examined at three or four levels. To establish assay performance at these conditions, the analyte concentration, sample matrix and assay step run times were maintained at constant values. A control condition was set at the Sartorius default conditions for ligand binding assay (kinetics) with the shaking speed at 1,000 RPM and temperature at 30°C for these studies.

Optimal assay behavior can be split into four distinct parts for kinetic determination assays:

1. The baseline(s) must be flat and absent of upward or downward drift (Figure 2, 0–60 seconds).
2. The loading density should be adequate to ensure sufficient protein is loaded on the biosensor, but not too high as to cause steric hindrance of the subsequent binding event. Multiple concentrations of the loading protein (Figure 2) are typically evaluated, often with a following association step although this is not mandatory. In general, low loading density is recommended when using Ni-NTA Biosensors.
3. The association step should show a concentration-dependent signal over at least 1.5 orders of magnitude (in concentration) as seen in Figure 2 (60 to 360 seconds). This step should also not show binding heterogeneity unless it is known that there is a 2:1 binding event occurring as indicated by a sigmoidal curve (similar to Figure 2) followed by a linear increase in signal rather than a plateau.
4. The dissociation step should show at least a 5% drop in binding signal (Figure 3, 300 to 900 seconds).

Data Acquisition Setup

For most of the kinetics experiments, a 384-well plate was used to enable high-throughput development (example shown in Figure 4). Additional buffer and loading wells were added as needed. Control points were performed at the beginning of a run prior to setting the shake speed or temperature to ensure no mechanical effects (*i.e.,* degradation due to increased temperature) on the control sample.

Biosensor Selection

Ni-NTA Biosensors were selected for this assay for robustness, ease of use, and to take advantage of the commercial availability of poly-histidine tagged (HIS-tag) receptors. FcγRIIIa is available commercially with many different purification conjugates. Using FcγRIIIa with a histidine tag at the C-terminus ensured the optimal orientation of the protein binding to the biosensor and provided the most distance between the biolayer of the biosensor and the binding site to prevent hindrance of FcγRIIIa binding to NISTmAb.

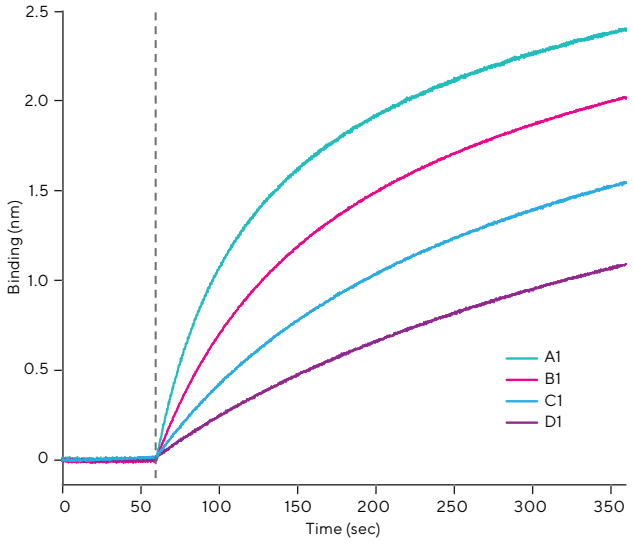


Figure 2: Four concentrations (10, 5, 2.5 and 1.25 µg/mL) were chosen for evaluation while targeting 1 nm response over 300 seconds. The 1.25 µg/mL concentration was chosen for further development. The baseline (first 60 seconds) and the loading step (60 to 360 seconds) for all four concentrations are shown.

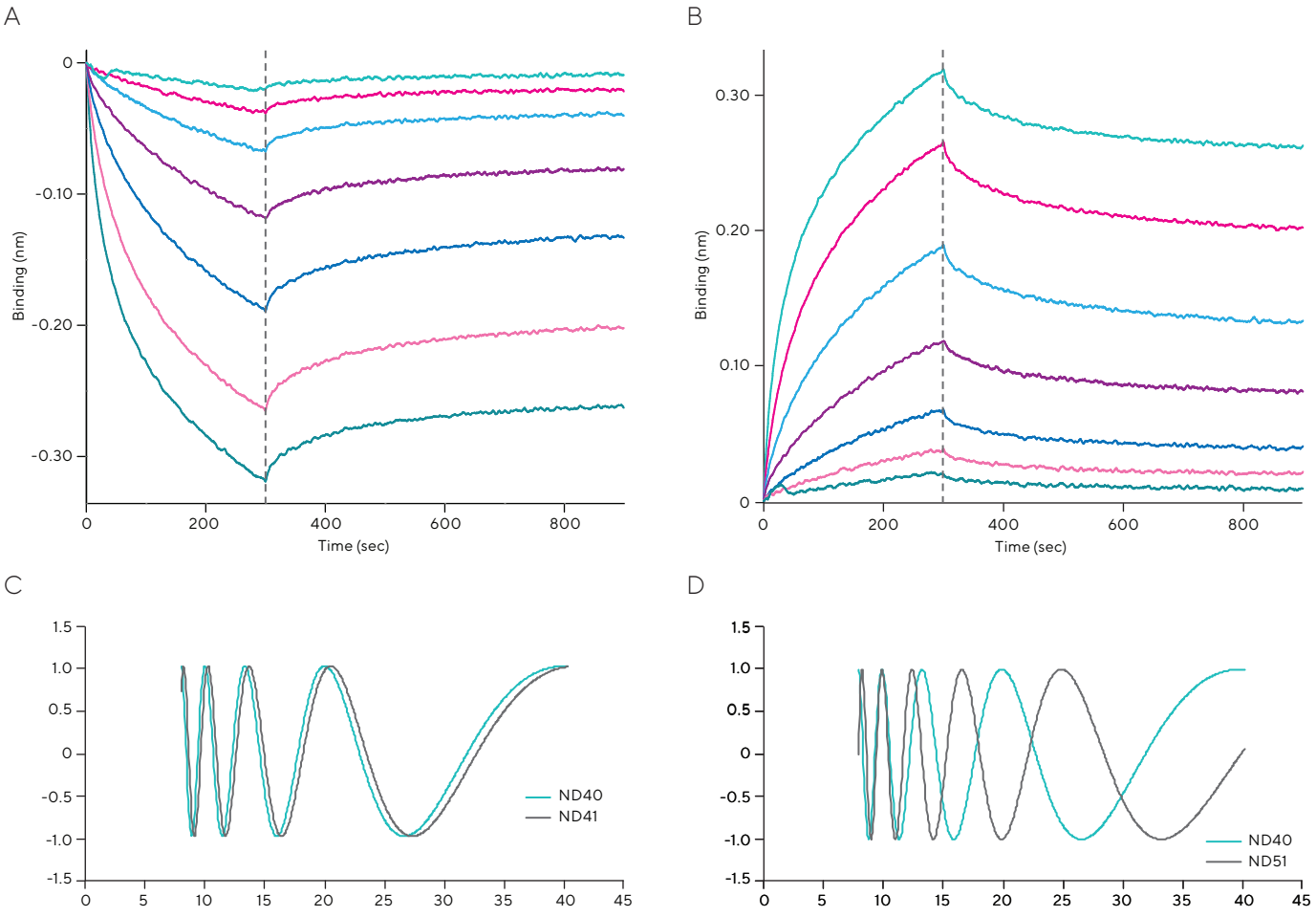


Figure 3: A) Inverted signal of NISTmAb associating to a FcγRIIIa bound to the bisosensor tip (loading of FcγRIIIa not shown). B) Flipped data from A using the "Flip Data" feature on Octet® Analysis Studio Software. C) Typical wavelength shift (left to right) from a small change in optical thickness (ND = Optical Thickness). D) Wavelength shift from a large change in optical thickness (right to left) resulting in an inverted signal.

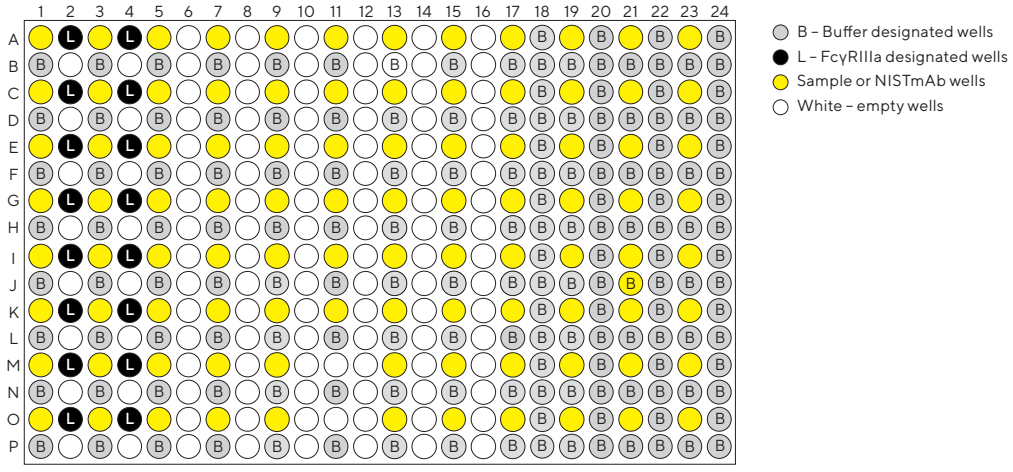


Figure 4: Sample Diluent was used in buffer wells and the zero point of all curves. 8-point curves (including zero point) were plated in a single column. Each baseline step had designated wells, and each curve used previously unused buffer wells for the dissociation step.

Ligand Density Assessment

Proteins such as Fc receptors typically provide optimal loading at concentrations <10 µg/mL. For this assessment, four concentrations were analyzed in duplicate for optimal signal and lack of saturation of the sensor (Figure 4). A FcγRIIIa concentration of 1.5 µg/mL was initially chosen as the ideal loading concentration based on a signal of 1 nm after 360 seconds, a typical initial benchmark.

Antibody Binding

NISTmAb concentration scouting was performed beginning with a range of 500 to 1.56 µg/mL. Curve shape, R_{Max}, Chi^2, R^2, Global Fit vs Local Fit, and Steady State were all considered when determining the optimal antibody binding. These attributes were also evaluated when establishing data processing parameters. The working range was determined to be 200 to 3.125 µg/mL based on acceptable assay performance. During the association step, signal inversion occurred. Signal inversion is a phenomena that arises when the optical thickness at the tip of the biosensor experiences a large change (Figure 4).[1] This is usually attributed to large molecules or complexes binding to a biosensor and is indicated as a decrease in signal. To verify that binding is occurring and not dissociation, the decrease in signal should be concentration-dependent and often a following step should be included, such as a dissociation step. The final assay steps are shown in Table 1 and were used for the theoretical pre-qualification/validation assessment.

Table 1: Octet® assay steps.

Step	Step type	Time (s)	Shaker speed (RPM)
1	Baseline	60	1,000
2	Loading	300	1,000
3	Baseline 2	120	1,000
4	Association	300	1,000
5	Dissociation	600	1,000

Pre-Qualification/Validation Assessment

For NISTmAb binding to FcγRIIIa, a short screening assay was performed as described above to determine the optimal FcγRIIIa loading concentration and NISTmAb concentration range. A DOE was then planned (Table 2) to determine the optimal loading concentration and if the platform conditions (30°C and 1,000 RPM) were suitable. A control preparation was performed at these platform conditions (including 1.5 µg/mL loading) to observe day-to-day repeatability and to calculate Percentage Relative Potency. The DOE approach, coupled with the fast assay time of the Octet® platform allows for the method parameters to be scouted in minimal time.

Table 2: Pre-qualification/validation development DOE.

Parameter	Range	Number of points
Temperature	28–35°C	4
Loading concentration	0.75–3.0 µg/mL	4
Shake speed	800–1,200 RPM	3

The results were analyzed using statistical analysis software which showed the optimal conditions for this assay were a 1.1 (±0.1) µg/mL loading concentration for FcγRIIIa, a 1,000 (±100) RPM plate shake speed, and a 30°C (29.5-31.5°C) assay temperature.

Critical Process Parameter Assessment

The pre-qualification DOE also provided the data required to assess specificity, precision/repeatability, and the working range of the assay. A diluent blank was performed as part of each NISTmAb curve. These blanks all demonstrated no matrix interference, indicating specificity of the assay. Due to the nature of the DOE, evaluating precision required assessing the data points from center points of the DOE. The average Percentage Relative Potency was 91% with a %RSD of 7%, suggesting good precision of the assay. Further, all points in the DOE showed R^2 ≥0.97, suggesting the working range of the assay (200 µg/mL to 3.125 µg/mL) is suitable for qualification.

Hydration of the biosensors was also evaluated. The baseline signal immediately after biosensor hydration of 10, 15, and 20 minutes was comparable, demonstrating that a 10 minute hydration time was suitable for the final method.

Method Qualification

Method Qualification, while not always required, can be a useful tool in early phases of drug development and provide critical data leading up to a validation. In general, the qualification of a potency method involves evaluating linearity, specificity, accuracy, precision, and range. Method Qualification also serves to set system suitability criteria for the assay as well as sample acceptance criteria for release testing and/or stability samples. For instance, the results from the accuracy calculations may allow for a criterion of 70% to 130% relative potency for test articles. When test samples meet this criterion, they are considered equivalent to reference. The results of an Octet® Percentage Relative Potency method qualification generally allow criteria to be set for: R^2, X^2, maximum response signal, minimum response signal, and a range of Percentage Relative Potency (potency comparison to reference). Typically, the results from running a qualified method (during development or stability experiments, etc.) in conjunction with the process (purification, culture, etc.) can provide the data to set criteria for a validation protocol. Validation of a method is performed after qualification and includes similar parameters to the qualification but with well-defined acceptance criteria in addition to validation specific parameters such as robustness.

Method Validation

Method Validations are completed to ensure an analytical method is suitable for its intended purpose. This provides an assurance of reliability for routine testing in GMP environment. Validation involves comprehensive protocol-driven experiments that evaluate and document the performance of an assay: As this method was being established as a potency assay, linearity, specificity, accuracy, precision, range, robustness, and ruggedness were evaluated as recommended by ICH Guideline Q2 (R1)[3] "Validation of Analytical Procedures: Text and Methodology."

Linearity is the expected relationship between known potencies of samples and their measured values using a range of 50% to 150% of the nominal relative potency, but treating their nominal concentration as 100%. Five levels were tested over the 50% to 150% range including 100%. The R^2 values of the resulting curves were all ≥0.95, indicating good linearity.

Accuracy is the degree of closeness to the expected value and was determined using results obtained from the linearity studies by calculating the percent recovery for each linearity level. For example; a Percentage Relative Potency of 46% at the 50% linearity level returns a 92% recovery. The average %recovery was calculated to be 97% with a range of 85% to 118% recovery. These results showed the method was accurate.

Precision is the variability in the data from replicate determinations under normal assay conditions. Repeatability of the method was assessed by testing multiple preparations at the nominal concentration. The average relative potency was 101% with a %RSD of 6%. Intermediate precision of the method was assessed using a second analyst to test multiple preparations at the nominal concentration. The average relative potency between two analysts was 101% with a %RSD of 8%. These results were within the expected limit.

The range of the method is demonstrated when precision, accuracy and linearity of the method show suitable performance. Suitable performance was demonstrated spanning the working range of 50% to 150% of the nominal potency. This corresponded to 100 µg/mL to 300 µg/mL for the highest concentration of the dose-response curve.

Specificity of the method was verified by testing a buffer blank and a generic non-human antibody, both diluted in the same scheme as NISTmAb. The Percentage Relative Potency of the blank and generic antibody were determined to be not-comparable to NISTmAb and specificity of the method was confirmed.

Robustness of this assay was evaluated by testing the working range of the parameters generated by the results of the development DOE. This involved making small but deliberate changes to the assay loading concentration, shake speed, and temperature. These changes in methodology returned results within 70% to 130% proving the method is robust.

Ruggedness of this assay was tested by evaluating normal test conditions that may vary over time. To test ruggedness of the assay, a DOE was performed on the parameters with the most risk for variance. This included biosensor lot, FcγRIIIa lot, and analyst to analyst variability (Tables 3 and 4).

The results of the DOE were analyzed by performing a Fit Least Squares analysis. The results of this analysis are shown in Figure 5. The Effect Summary Table showed no statistically significant interactions (*i.e.,* all ρ values were greater than 0.05). The Prediction Profile and Interaction Profiler showed no clear substantial trends between different variables. The effects of this DOE prove the method is rugged.

Table 3: Ruggedness DOE.

Parameter	# of Points
Biosensor lots:	5
FcγR(III)A lots:	2
Analysts:	3

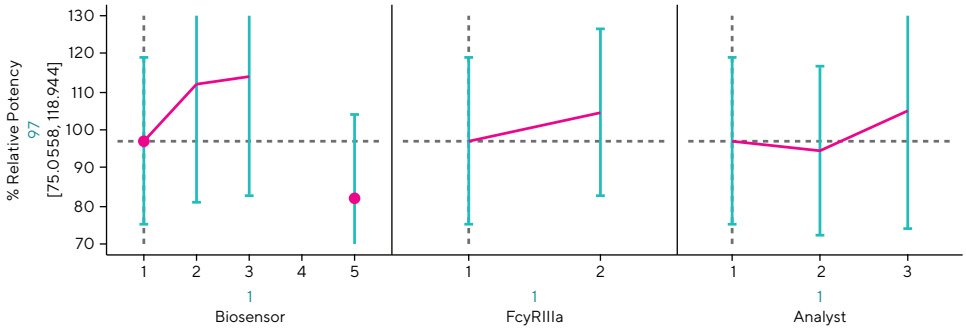
Table 4: A 30 run DOE showing the various combination of parameters tested.

Run	Biosensor	FcyR(III)A	Analyst	K_D (nM)	%Relative potency
1	1	2	1	25	100%
2	1	2	3	21	93%
3	5	1	1	31	82%
4	1	2	2	30	109%
5	1	1	2	28	103%
6	2	1	1	23	112%
7	3	2	2	25	91%
8	4	2	3	24	80%
9	5	2	1	21	119%
10	3	1	1	22	114%
11	5	1	2	30	110%
12	3	2	3	22	88%
13	5	2	2	26	96%
14	3	2	1	20	127%
15	5	1	2	28	113%
16	1	1	2	22	86%
17	4	2	1	23	108%
18	3	1	2	27	105%
19	5	2	1	24	104%
20	1	1	1	19	109%
21	1	2	2	25	98%
22	1	1	3	18	105%
23	4	2	3	18	109%
24	3	1	2	30	115%
25	3	2	2	22	86%
26	1	2	1	23	109%
27	5	1	1	31	82%
28	5	2	2	25	97%
29	1	1	1	25	85%
30	3	2	1	33	76%

A Effect summary table

Source	LogWorth	PValue
Biosensor*FcyRIIIa	0.677	0.21029
Biosensor*Analyst	0.629	0.23475
Biosensor	0.611	0.24497
Biosensor*FcyRIIIa*Analyst	0.551	0.28098
FcyRIIIa	0.216	0.60810
FcyRIIIa*Analyst	0.171	0.67448
Analyst	0.079	0.83416

B Prediction profiler



C Interaction profiler

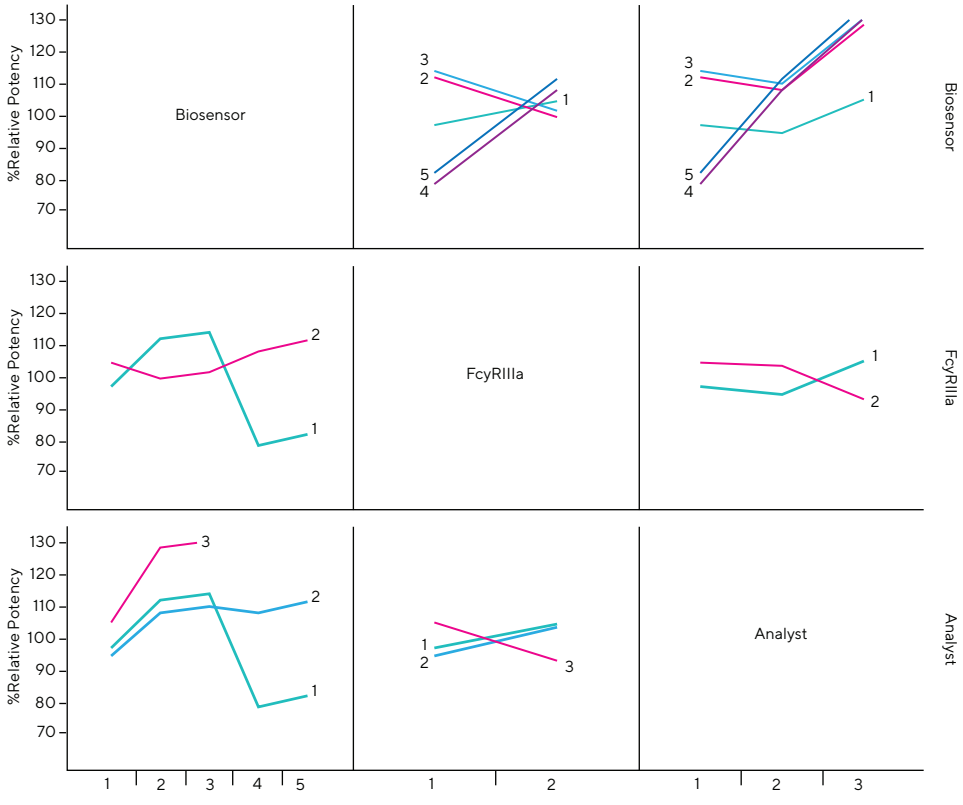


Figure 5: A) Effect screen of multiple parameters showing no significant interactions. LogWorth = -Log (p-Value). B) Prediction Profiler showing results are not able to predict future trends in data. C) Interaction Profiler showing the interactions between two variables have no predictable effect on %Relative Potency.

Results

A Percentage Relative Potency method for FcyRIIIa has been developed and analyzed in a representative method validation. For this validation exercise, the representative raw data can be seen in Figure 6 and analyzed results in Table 5. The results show that this method is linear, specific, accurate, precise and robust over a specific range in accordance with ICH Guidelines Q2 (R1).[3]

Table 5: Results of the validation exercise.

Parameter	Reportable result	Result
Linearity	R^2 of triplicate preps	$R^2 \geq 0.95$
Specificity	Diluent and non-specific mAb comparable to reference	Not comparable
Accuracy	%Recovery of linearity preparations	85% to 118% recovery
Repeatability	Average %relative potency and %RSD	Average = 101%, %RSD = 6%
Intermediate precision	Average %relative potency and %RSD of Analyst I and Analyst II	Average = 101%, %RSD = 8%
Range	Method range	50% to 150% for highest concentration
Robustness	%Relative potency at modified conditions	70% to 130% Relative Potency

Octet® Systems in GxP Laboratories

The use of Octet® systems in GxP laboratories is constantly expanding. KBI Biopharma has successfully developed 30+ methods on the Octet® platform used for titer, potency, kinetics, and identity testing. Many of these methods are being used to support Manufacturing, Drug Substance or Drug Product Release testing, and Long-term Stability testing in a GxP environment. While the assay and sample acceptance criteria are dependent on the method variability as well as the process variability, these methods generally exhibit ≤10 %RSD between replicates over long term testing.

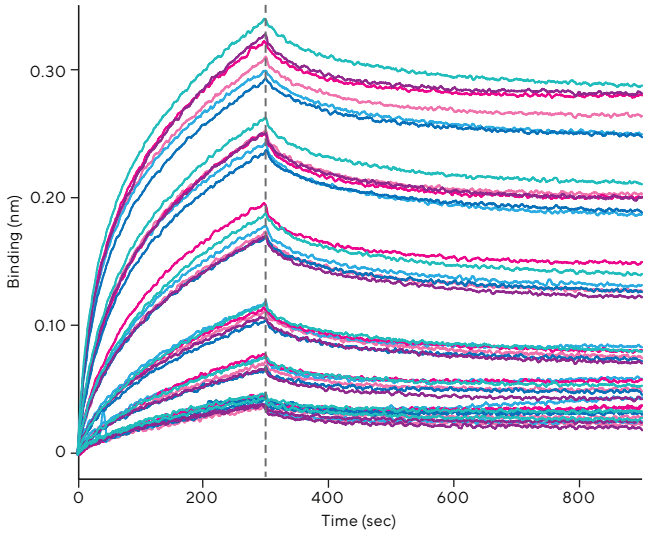


Figure 6: Replicate binding curves (n=6) of NISTmAb binding to FcγRIIIa.

Conclusion

Functional biological activity is a critical quality attribute (CQA) essential to verifying the potency of a drug molecule.[2] Potency assays can be used throughout the development process in comparability and formulation studies, and are required for release of every lot of therapeutic protein. The Octet® platform offers a fast, accurate, and robust solution for measuring potency of a drug molecule. Here we have described considerations for the development of a Percentage Relative Potency method capable of early-phase comparability studies and subsequent method validation for lot release. With the speed of the Octet® RH96 system, we could rapidly achieve Design of Experiment results which led to development, optimization, and potential validation practices.

References

1. Application and Comparison of Algorithms for the Evaluation of Interferograms, G. Kraus and G. Gauglitz Fresenious' J. Anal. Chem., 349: 399–402, 1994.
2. ICH Guideline Q6B, "Specifications: Test Procedures and Acceptance Criteria for Biotechnological/ Biological Products."
3. ICH Guideline Q2R1, "Validation of Analytical Procedures: Text and Methodology."

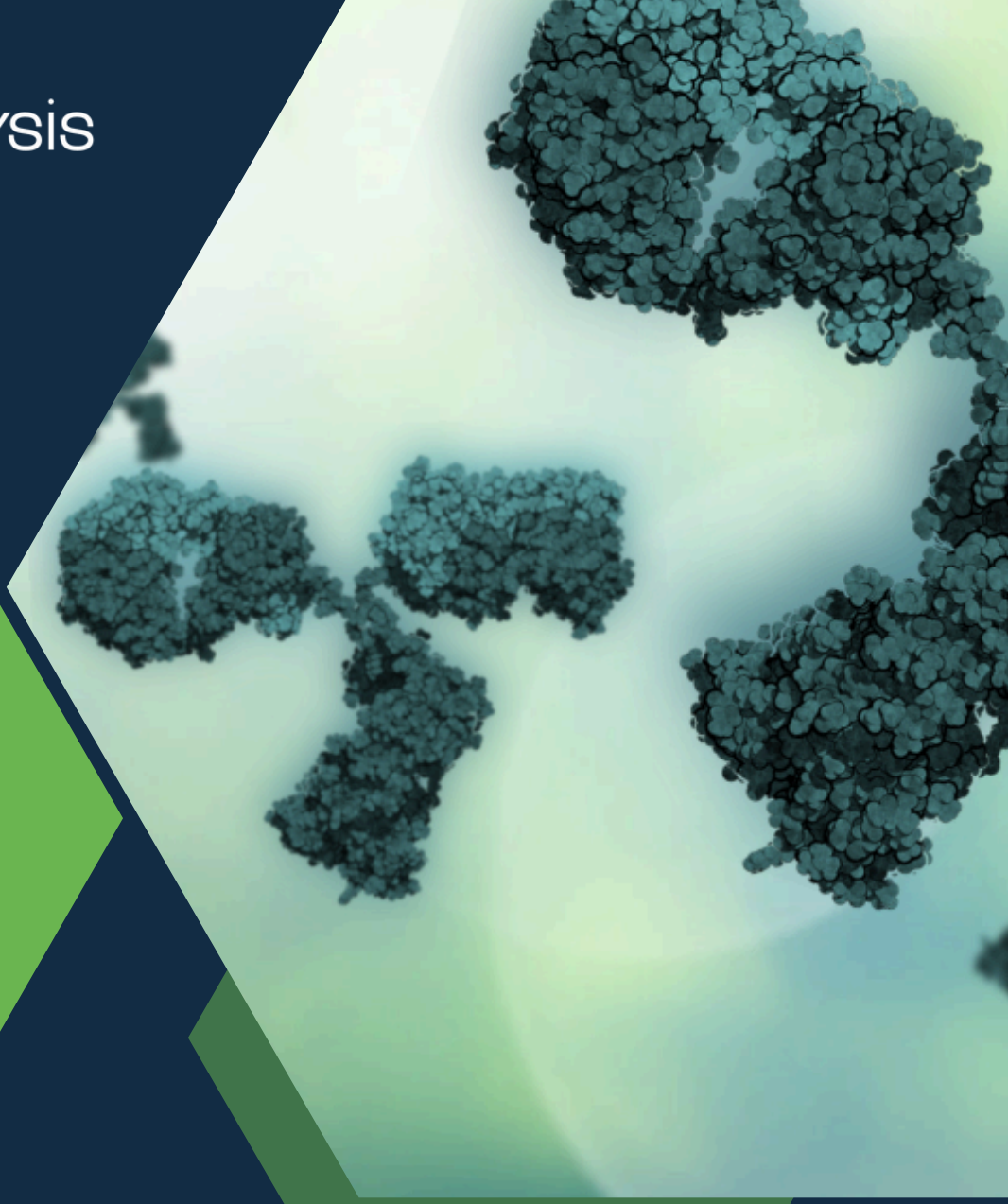
Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 888 OCTET 75
Or +1 650 322 1360

For further contacts, visit
www.sartorius.com/octet-support

41-0298-AN Rev C

Bioanalysis ZONE



Contact us

Editorial department
editor@bioanalysis-zone.com

Business Development and Support
advertising@future-science-group.com

This supplement is brought to you by Bioanalysis Zone in association with:

SARTORIUS