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Instrument Comparability Assessment: Kinetics Precision Assessment in Ligand Binding Assays on the GxP-Compliant Octet® RED96e and Octet® R8 Instruments

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Introduction

To ensure the quality of pharmaceutical and other biological products that support clinical trials and post market assay activities, it is important to identify the specific critical processes and quality attributes early in development and to put in place methods for their evaluation throughout the life cycle of product development and release. The International Conference on Harmonization (ICH) Q2(R1)[1] publication provides guidance on the validation of analytical technologies and helps form the basis of key quality attributes and parameters that need to be assessed for pharmaceuticals intended for use in humans. The ICH Q2(R1) publication details multiple parameters that need to be established and assessed during the validation of analytical procedures that are included as part of the registration process during applications for pharmaceutical drugs and biologics in general.

These parameters often need to be established during assay method development and qualification and prior to method validation. They include, but are not limited to, assessing specificity, accuracy and precision and are dependent on the type of assay to be validated. For example, while quantitation assays require that the method in use should exhibit linearity,

accuracy and limits of quantitation among other parameters, there is a bigger emphasis on specificity and precision when it comes to ligand binding assays where affinity constants are the main output parameter in evaluation (FDA Bioanalytical method validation).

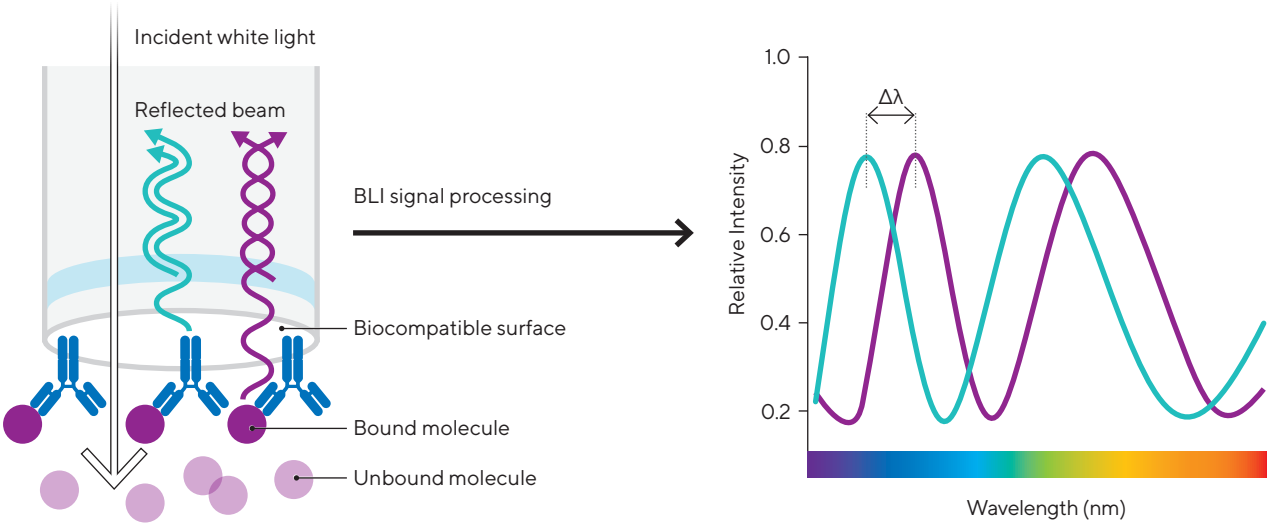
While this whitepaper does not discuss these specific requirements in detail, it nevertheless focuses on comparability assessment of Octet® RED96e and Octet® R8 instruments—the Bio-Layer Interferometry (BLI) analytical platforms commonly used in establishing supporting data and in some cases lot-release methods for pharmaceutical products and other biologics through ligand binding assays.

Bio-Layer Interferometry (BLI) Technology

Bio-Layer Interferometry (BLI) 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. 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. 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 1). In BLI, biomolecular interactions between two binding partners is converted into response signals in real-time allowing users to design methods that can help in rapidly optimizing binding assays of various biological molecules. BLI is a label-free technology that allows users to determine association and dissociation rate constants in kinetic characterization experiments; key determinants in affinity constant derivation and information not available with end-point analysis techniques such as ELISA. In addition, BLI can be used for protein quantitation determination, hence it can be applied at various stages of drug development allowing users to circumvent limitations of ELISA and HPLC platforms.

Figure 1

Relative Intensity of Light Reflection From the Two Biosensor Surfaces



Note. BLI is an optical technique that measures interference patterns of white light reflected from two surfaces on the tip of a biosensor. Interactions between biological molecules at the tip of the biosensor causes a shift in interference patterns and is measured in real-time.

Octet® RED96e Versus Octet® R8

Octet® systems utilize a standard microplate format that enables high-throughput, automated binding analysis directly from 96- and/or 384-well plates and great flexibility in assay design. The RED96e and the R8 are both 8-channel instruments capable of analyzing up to 8 samples in tandem and are compatible with 96-well micro-titer sample plates.

The RED96e system was developed to operate reliably in a regulated environment and came with features that included a sample plate cover and a wide range of sample temperature control (15–40 °C) allowing the instrument to run un-attended for up to 12 hours. The R8 on the other hand was introduced into the market as part of the modular Octet® R series that are available in configurations of 2, 4 or 8 channels (Figure 2). The R-series is designed to provide Octet® users with the flexibility of having a field upgrade performed of their lower through-put instruments (R2 and R4) to the 8-channel Octet® R8 (R2→R4, R2→R8 or R4→R8) as their through-put needs increase. The R8 maintains identical features to RED96e and comes with GxP compatible products as illustrated below.

Figure 2 and Table 1
The Modular Octet® R Series: R2, R4 and R8

	Octet® R2	Octet® R4	Octet® R8
Number of spectrometers	2	4	8
Maximum simultaneous reads	2	4	8
Temperature control	15–40 °C	15–40 °C	15–40 °C
Evaporation control	No	No	Yes
Upgradeability	Yes	Yes	No
GxP package availability	No	No	Yes

Note. Lower read heads can be upgraded to a higher configuration at user site

Octet® R8 GxP Compliance

GxP users are often looking for analytical instruments that offer simplicity, innovation and speed, and have small lab footprints with user-friendly software that allows for automation. Other key considerations include robust instruments that experience minimal downtime while at the same time allowing for ease of use; properties that are desirable in QC environments. For QC assays, the technology also needs to demonstrate methods that are stability indicating. The Octet® R8 is designed in a configuration that provides simplicity and ease of use, and yet allows for enhanced productivity by increasing the capacity to test QC and other samples.

Sartorius's Octet® R8 GxP package comes with all requirements for GMP compliance including IQOQ protocols and kits, user guides, performance qualification (PQ) protocols and kits, 21 CFR Part 11 software with audit trails, software validation package and biosensor validation support.

Octet® Comparability Studies: Octet® RED96e vs Octet® R8

It should be noted that the first step in the development of a ligand binding assay using label-free technologies such as the BLI is the selection of an appropriate assay format. This includes the selection of the solid-surface or biosensor chemistry in the case of the Octet® platform; the determination of the molecule to be immobilized onto the biosensor and the optimization and subsequent qualification of assay condition such as buffers, pH, ligand immobilization concentrations, etc. This white paper is intended to showcase a comparison between the performance of a pre-developed and qualified ligand binding assay on the Octet® RED96e and its R8 counterpart. While a typically qualified ligand binding assay would need to assess multiple qualification parameters including specificity, accuracy, robustness and precision among others, here, we focus only on precision assessment by comparing parameters that include equilibrium dissociation and other rate constants determined by multiple analysts on both instruments, to showcase comparability.

Materials and Methods

Materials

- Instruments: Octet® R8 and Octet® RED96e instrument with Octet® BLI Discovery and Analysis Studio Software.
- Samples: Biotinylated Anti HER2 Antibody (R&D systems; Cat. No. BAF1129) and HER2 receptor protein (ACRO Biosystems; Cat. No. HE2-H5212).
- Biosensors: High Precision Streptavidin 2.0 (SAX 2.0; Cat. No. 18-0047)
- Sample plates: 96-well, black, flat bottom microplate, Greiner Bio-One Part No. 655209
- Buffers: 20 mM Tris-Triton, pH 7.4 (Sartorius)

Methods

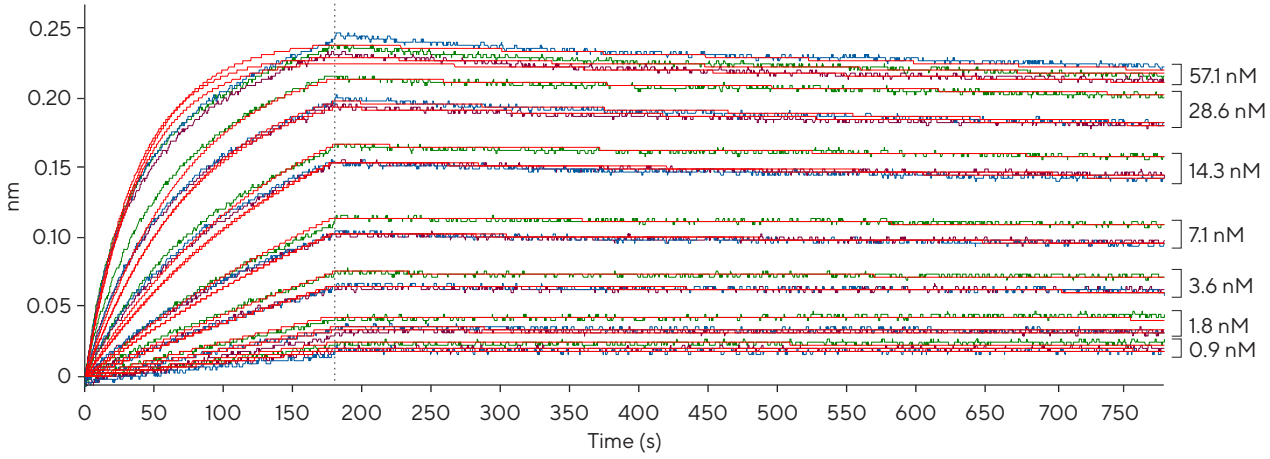
To provide information on the comparability of the binding kinetics data between Octet® R8 and Octet® RED96e a binding kinetics assay was performed by 3 analysts on both systems using the same reagents and a pre-established Octet® kinetics method. Prior to the start of the assay, the SAX2.0 biosensors were hydrated (pre-wet) for at least 10 minutes in the assay buffer (20 mM Tris-Triton buffer, pH 7.4). The assay included an initial baseline (biosensors equilibration) step where the hydrated biosensors were dipped in assay buffer for 120 seconds followed by a dip in the biotinylated anti-HER2 antibody @ 1 µg/mL for 400 seconds (ligand load step). A second baseline step where the biosensors were dipped in assay buffer for 180 seconds to remove any non-specifically bound substances in the ligand loading step was performed before the ligand-bound biosensors were dipped for 180 seconds in HER2 analyte protein samples. Samples were tested at concentrations ranging from 57.1 nM–0 nM (2-fold dilution) during the association step. A final dissociation step of 1800 seconds in assay buffer was run to establish analyte off-rates.

Figures 3–5 show overlayed replicates of binding curves obtained by 3 different analysts on the two instruments; RED96e (A) and R8 (B) and fitted to generate precision comparison data as captured in Tables 2–4 (full dissociation steps not shown).

Figure 3

HER2 to Anti-HER2 Binding Analysis Obtained by Analyst 1

A. Octet® RED96e



B. Octet® R8

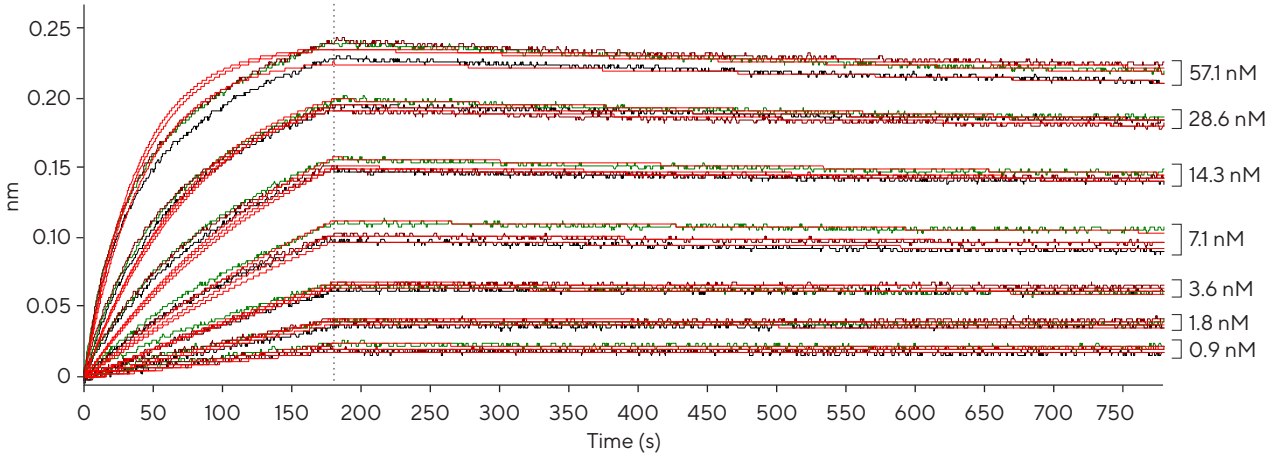
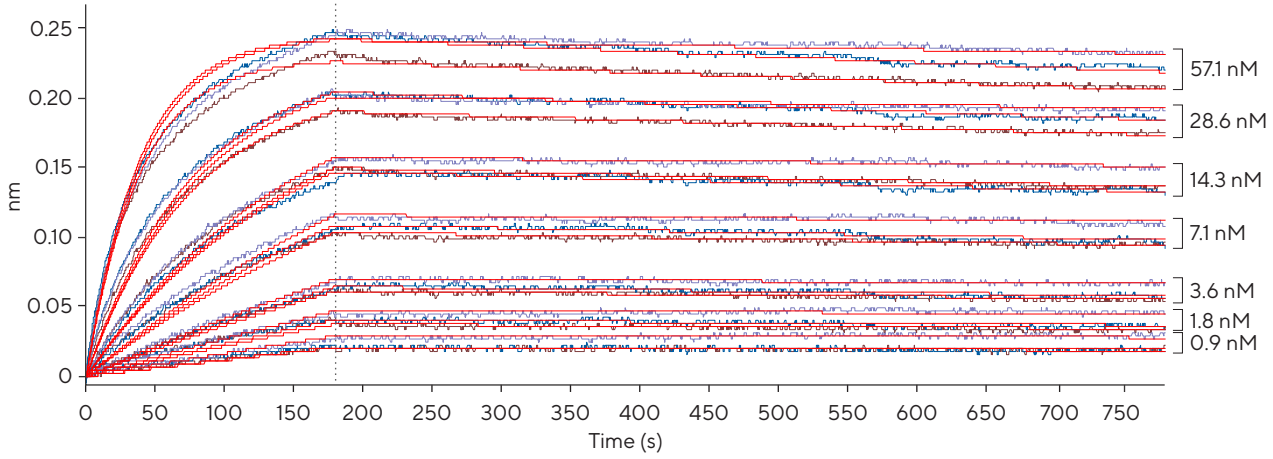


Figure 4

HER2 to Anti-HER2 Binding Analysis Obtained by Analyst 2

A. Octet® RED96e



B. Octet® R8

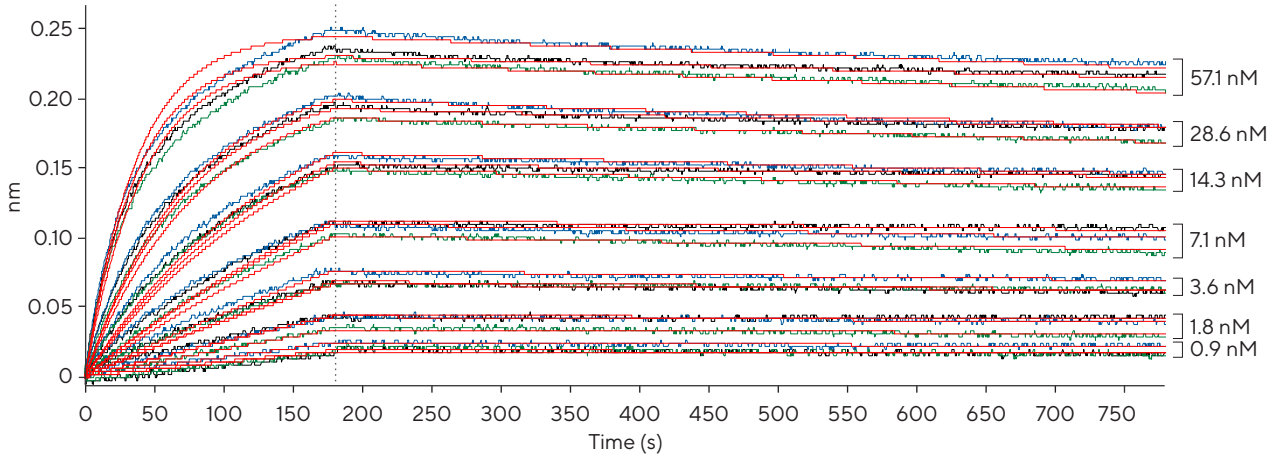
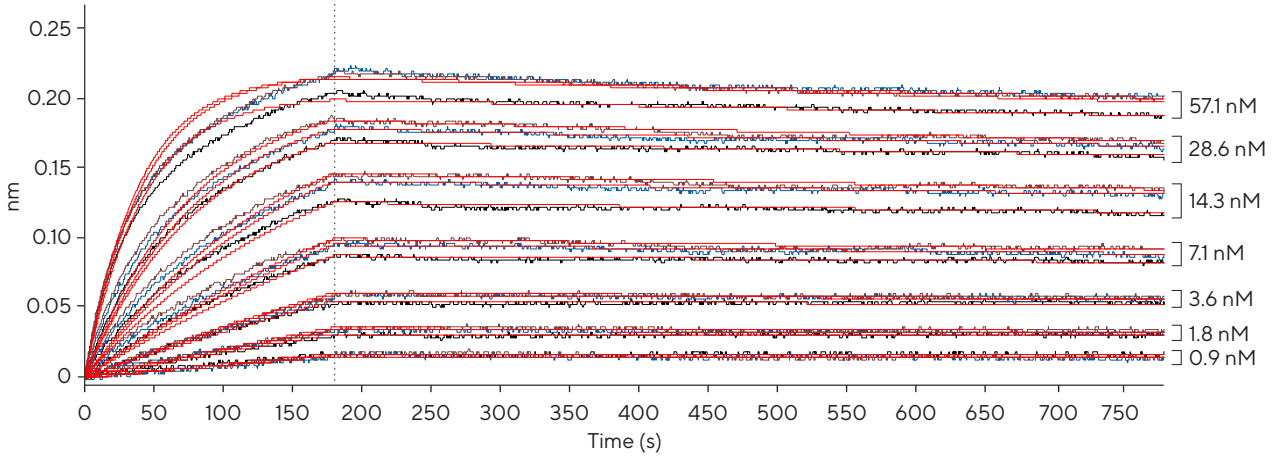


Figure 5

HER2 to Anti-HER2 Binding Analysis Obtained by Analyst 3

A. Octet® RED96e



B. Octet® R8

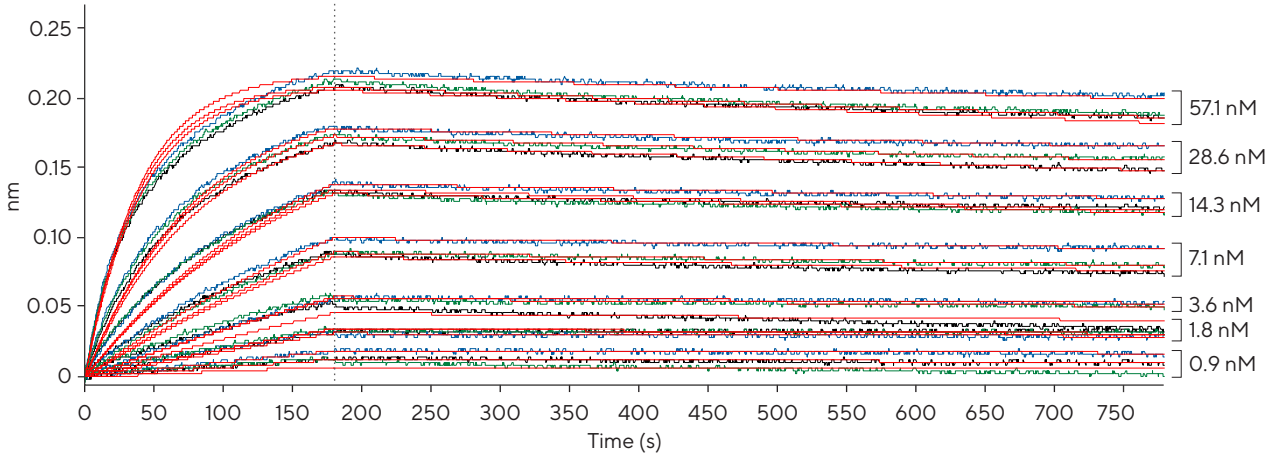


Table 2

Intra-Analyst Variation (% CV) of the Measure Binding Kinetics Parameters of Affinity (K_D), Association Rate (k_a) and Dissociation Rate (k_d) on the Octet® RED96e

	K_D (M)			k_a ($M^{-1}s^{-1}$)			k_d (s^{-1})		
	Mean	**STDEV**	**% CV**	**Mean**	**STDEV**	**% CV**	**Mean**	**STDEV**	**% CV**
Analyst 1	2.75E-10	4.68E-11	17.00	4.31E+05	1.65E+04	3.82	1.18E-04	1.56E-05	13.22
Analyst 2	3.61E-10	2.44E-11	6.75	4.55E+05	1.98E+03	0.44	1.64E-04	1.04E-05	6.32
Analyst 3	3.04E-10	5.59E-12	1.84	4.37E+05	2.01E+04	4.59	1.33E-04	8.49E-06	6.38

Table 3

Intra-Analyst Variation (% CV) of the Measure Binding Kinetics Parameters of Affinity (K_D), Association Rate (k_a) and Dissociation Rate (k_d) on the Octet® R8

	K_D (M)			k_a ($M^{-1}s^{-1}$)			k_d (s^{-1})		
	Mean	**STDEV**	**% CV**	**Mean**	**STDEV**	**% CV**	**Mean**	**STDEV**	**% CV**
Analyst 1	2.69E-10	2.64E-11	9.81	4.16E+05	1.42E+04	3.41	1.12E-04	1.485E-05	13.23
Analyst 2	3.67E-10	2.76E-11	7.51	4.46E+05	3.42E+04	7.66	1.63E-04	2.828E-07	0.17
Analyst 3	4.39E-10	4.38E-11	10.00	4.39E+05	1.34E+04	3.05	1.92E-04	1.344E-05	6.99

Table 4

Inter-Analyst Variation (% CV) of the Measure Binding Kinetics Parameters of Affinity (K_D), Association Rate (k_a) and Dissociation Rate (k_d) on the Octet® R8 and Octet® RED96e

	K_D (M)			k_a ($M^{-1}s^{-1}$)			k_d (s^{-1})		
	Mean	**STDEV**	**% CV**	**Mean**	**STDEV**	**% CV**	**Mean**	**STDEV**	**% CV**
Octet® R8	4.03E-10	5.05E-11	12.53	4.34E+05	1.53E+04	3.53	2.051E-05	1.78E-04	11.54
Octet® RED96e	3.33E-10	4.09E-11	12.29	4.46E+05	1.24E+04	2.79	2.23E-05	1.49E-04	15.01

Benefits of the Octet® Platform in 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® 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. It is estimated that for a typical ligand binding method, an Octet® system will be 2X faster for method development than traditional ELISA. The sample plate format coupled with real-time analysis and the high-throughput readout allows for a more rapid assessment of different assay conditions, that in-turn help speed up assay optimization. Benefits include:

1. Parallel processing of samples—reduced assay development time.
2. 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.
3. One-step 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.
4. Few moving parts that require maintenance—QC environment ready. Octet® instruments require very low maintenance. There are no microfluidics that need to be guarded against clogs, contamination, and leaking.
5. Off the shelf biosensors such as Protein A (Pro A), Protein G (ProG), Anti-human Capture (AHC2) and Anti-mouse Capture (AMC) are available for direct capture of ligands. High precision streptavidin (SAX and SAX2.0) biosensors deliver enhanced precision for GMP applications.
6. Comprehensive screening and characterization tool across a diverse range of applications—antibody and protein quantitation, Fc-receptor binding, contaminant testing and general product characterization.

Examples of Approved Drugs Using the Octet® Instrument

Octet® data was used as part supporting data for the assessment of functional activity of pembrolizumab (Keytruda; EMEA approved in 2015). Keytruda as 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 invitro binding studies of pembrolizumab.[2]

The Anti-PD-L1 monoclonal antibody Tecentriq (atezolizumab) was approved by EMEA in 2017 and later by the FDA in 2018. 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. Octet® instruments were used for supporting data in the binding characterization of the drug to its receptor PD-L1 among other studies.

In addition to these approved drugs, there are multiple instances where the Octet® has been used along with other techniques to provide supporting data for a various drug applications. For example, Theramex Ireland Limited applied for marketing authorization at 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 gradient 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® was used to verify biosimilarity between the two drugs in functional binding assay.[3]

STADA Arzneimittel AG filled an application for marketing authorization at 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® was used for confirming specificity of the biosimilar through functional binding assessment with irrelevant antigens.[4] Many other examples of drugs developed with the Octet® BLI used for supporting characterization data exist in literature.

Conclusion

A Kinetics Ligand binding comparability assay using HER2 receptor binding to anti-HER2 as a model system and focused on the key ICH guideline on precision analysis for such studies indicates that there are insignificant differences in performance between the two instruments. The CVs of < 20% observed for the various parameters studied are similar to what would be expected within the same instrument for the same analyst for a high affinity binding study implying that there are no detectable differences between the Octet® R8 and the RED96e. Since the data shows that the Octet® R8 and the RED96e produce similar results when using identical binding pairs and since there are no feature differences between the two instruments, Octet® users currently possessing the RED96e and who need to replace the instrument with a newer version or who need additional instruments due to emerging needs should feel confident that methods developed on the RED96e can be successfully transferred onto Octet® R8 without any impact on performance. Moreover, data derived from both instruments should be applicable for regulatory filing to support BLA, NDA or any other product related regulatory application.

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