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High-Throughput Screening of Antibody Reagents to Support Discovery and Applied Research Into Emerging Clade H5N1.2.3.4.4b

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Abstract

During the production of antibodies for therapeutics or vaccine development, generated libraries are screened to assess and determine candidate quality attributes such as titer, binding kinetics and other biophysical properties as a critical first step to enable the selection of promising candidates early in the process for further development. In avian flu, the emergence of the antigen HPAI H5N1 clade has been cause of concern as it has shown exceptional ability to spread amongst different host species, including bovine and other mammals. To facilitate the production of suitable reagents for research and other applications against this emergent flu antigen, anti-hemagglutinin (HA) monoclonal antibodies were generated and later characterized in an automated, high-throughput setting using the 96-channel Octet® RH96, which is capable of screening 96 antibody samples simultaneously. The instrument was integrated with a Biosero® robotic arm to speed the assessment of competitive binding of the antibody clones to a biosensor immobilized with H5N1 antigen. This was achieved in an 18-hour unattended epitope binning run with a 72 × 72 binning matrix.

Introduction

The antibody discovery process often involves the screening of early-stage candidates that range from hundreds to thousands of clones and is aimed at selecting those clones suitable for cell line development and manufacturing. Key antibody attributes are screened early in development to avoid costly late-stage failures. These attributes include binding specificity to target, antigen binding epitopes, kinetics and binding affinities in addition to other critical quality attributes like glycosylation profiles, and overall developability and manufacturability amongst other critical quality attributes. Screening often starts with a simple target binding analysis followed by kinetics characterization of the primary hits. Unfavorable hits with weak or sub-optimal target binding affinities, low target selectivity, poor biochemical or biophysical attributes need to be eliminated early in the discovery process. In addition to titer and kinetics screening, a critical and useful characterization in selecting optimal candidates is epitope binning. In this application note, we describe the use of the high-throughput Octet® RH96 BLI instrument as a useful tool for an automated and high-throughput screening of antibodies generated against an influenza antigen.

Highly pathogenic avian influenza (HPAI) is commonly found in wild birds and poses risks to both animals and humans. It typically spreads zoonotically and can, at times, mutate to infect mammals, including humans. The HPAI H5N1 clade 2.3.4.4b first emerged around 2020 and, by 2023, had spread across all continents. In 2024, this clade was reported to cause widespread infections in wild birds and livestock, with severe outbreaks in poultry [Elsmo EJ, Wünschmann A, Beckmen KB, *et al*]. Additional outbreaks included extensive infections in bovine herds and detection of H5N1 in milk, as well as cases in other mammals, including limited human infections. The exceptional ability of this H5N1 clade to cross species barriers has raised significant concern and a call to develop additional strategies to mitigate future outbreaks [Burrough ER; Graziosi, Xie Z.]. In this study, we aimed to develop research tools to support the interrogation of the hemagglutinin (HA) protein from clade 2.3.4.4b HPAI H5N1. Our goal was to generate anti-HA monoclonal antibodies and identify non-competing mAbs suitable for downstream assay development. To this end, we undertook an effort to produce a large cohort of anti-HA monoclonal hybridomas antibodies using commercially available recombinant HA from clade 2.3.4.4b.

The epitope binning process enables the classification of antibody candidates into distinct bins, with each bin representing a subclass of antibodies having similar binding characteristics.

There are quite a few epitope binning technologies in the market, each offering distinct advantages to users that can be used to efficiently bin antibodies. The binning process can however be hampered when the purification of large numbers of hybridoma or phage display samples is required prior to the analysis. This is because most analytical techniques are unable to collect accurate measurements in crude matrices due to high non-specific binding or low resolution. Label-free techniques are better suited for these types of samples but for systems that use fluidics, crude samples may cause fluidic blockages thereby preventing robust data collection.

Fluidic-free biolayer interferometry (BLI) can be used to perform epitope binning analysis using cross-competition assays where competitive binding of antibody pairs is assessed. These assays can be easily extended to characterizing large antibody libraries.

High-Throughput, Label-Free Antibody Screening Utilizing BLI

The Octet® RH96 combines fast assay turnaround, flexible assay design, and parallel, independent biosensors in a fluidics-free dip-and-read format—ideal for high-throughput screening, including crude or non-purified samples. Its working principle is BLI, a label-free technology that measures molecular interactions in real time for the purpose of quantitation and kinetic analysis. Binding analyses can be performed in standard 96-well or standard 384-well as well as in tilted-bottom 384 well microplates depending on throughput requirements and available sample volume. The 384-well tilted-bottom micro plates (Sartorius, Part No. 18-5076) only require 40 µL of sample or reagent per well. The system's ability to read 8, 16, 32, 48 or 96 wells in parallel enables tailoring of assay design to maximize analytical throughput or sensitivity. The 8 and 16 biosensor modes provide high sensitivity for measuring small molecules or peptide binding interactions and protein quantitation down to ng/mL for 1-step or pg/mL for multi-step assays.

Materials and Methods

Antibody Generation

Female BALB/c mice (6–7 weeks old) were immunized through a series of four intraperitoneal injections with Avian Influenza A(H5N1) clade 2.3.4.4b hemagglutinin (HA) protein (The Native Antigen Company, part no. REC32116-500) with Complete Freund's Adjuvant. Following each boost, sera were collected and analyzed by ELISA and detected using peroxidase conjugated anti mouse IgG (Rockland®, part no 610-403-C46) and TMB substrate (Rockland, part no TMBE-1000). Three mice with strong ELISA responses to HA were selected for hybridoma generation. Spleens were harvested, processed, and fused with SP2/0-Ag14 cells via electrofusion, then plated into 96-well plates containing complete growth medium supplemented with HAT selection (hypoxanthine-aminopterin-thymidine). Cultures were maintained at 37 °C and 9% CO_2 for up to 21 days. During this period, clones were monitored by size, media color change and screened by ELISA. Positive clones, reactive to HA, were expanded and further characterized by ELISA for antigen reactivity and isotype determination. A counter screen was also performed against avian influenza A(H3N2)/Cambodia/e0826360/2020 HA (The Native Antigen Company, part no. REC31951-500). Reactive clones, primarily of the IgG isotype, were subcloned by limiting dilution. Verified monoclonal clones were expanded for cryopreservation, and terminal supernatants were collected for downstream analyses.

Antibody Titer Determination and Off-Rate Screening

Titer determination and off-rate ranking of the clones were performed using the high-throughput BLI instrument. Samples were loaded onto black Greiner flat bottom 384-well plates at 80 µL per well in their crude matrix. For titer, Protein A biosensors were first hydrated in Dulbecco's Modified Eagle Medium (DMEM) (1X) for 10 minutes before dipping into samples that include mouse IgG samples run in duplicates as standards and the different antibody clones as test samples. The method involved a simple one-step 2-minute quantitation assay. For antibody clone off-rate screening, His-tagged H5N1 2.3.4.4b HA antigen (Native Antigen, P/N REC32116-500, lot# 24082215P) was immobilized onto HIS1K biosensors at 7 µg/mL. Antigen samples were prepared in Sartorius' 1x kinetic buffer (1KB) while antibody samples were analyzed in their crude matrix using the same 384 well sample plates used for antibody titer. The immobilized antigen biosensors were dipped into the antibody clones and off-rate monitored through a dissociation step by dipping the biosensors in 1x KB.

Automation Setup

The Octet® BLI epitope binning assay was automated using Biosero's Green Button Go® Scheduler (GBG®), a dynamic scheduling software featuring an intuitive graphical user interface, a massive device driver library, and best-in-class error recovery. Sample plates and biosensor trays were handled using a collaborative robot with an integrated barcode scanner. Two static plate hotels with a capacity for 44 plates, were used to host 27 plates for the binning assays while a dedicated biosensor tray hotel allowed for 8 biosensor trays to be loaded simultaneously. A regrip nest and a Q Instruments Bioshake were present to allow for plate orientation changes and plate shaking, respectively.

The GBG Scheduler interacted directly with the BLI Discovery software, enabling a robust automation experience and maximum walkaway time. Appropriate Octet® method files were saved and were called from the Scheduler by checking the plate barcodes as they were loaded onto the Octet® instrument, ensuring that each plate underwent the correct experiment. The overall workflow was as follows:

- Sample and reagents were aliquoted onto black Greiner 384-well microtiter and together with biosensor trays were loaded onto hotels. Biosensor regeneration was enabled in the method with each biosensor tray re-used four times (based on regeneration cycles studies)
- The runtime parameters and workflow options were selected at startup
- The plate barcodes in hotel were scanned to verify loading
- Biosensor pre-wet plates were loaded onto the Octet®
- Sample and reagent plates were loaded next for each run as defined in the method and scheduler. These included plate lids (to prevent sample evaporation overtime); lidding and de-lidding are programed in the plate loading process as needed.

Figure 1: *Schematic of the Octet® RH96 integrated to a Biosero® automation system for sample plates loading.*

Binning Method Steps

1. Pre-conditioning (3 cycles of regeneration in 10mM glycine buffer pH 1.7 and 1xKB for neutralization))
2. H5N1 Antigen loading (300s)
3. Antibody clone 1 binding (300s)
4. Antibody clone 2 binding (420s)
5. Regeneration (3 cycles of regeneration in 10mM glycine buffer pH1.7 and 1xKB for neutralization)

Affinity Characterization

A set of clones was selected from the different bins generated off the epitope binning analysis and were subjected to affinity characterization. For these studies, the assay orientation was reversed to allow for the capture of the non-purified antibody clone samples with the purified antigen in solution. The antigen was diluted in 10x KB in a two- fold dose response manner. The selected antibody clone samples were captured onto Anti-mouse Capture 2 (AMC2) biosensors followed by a dip of the biosensors into 10x KB for baseline generation, a dip into the antigen for the association step (600s) and finally a dip back into 10 KB (900s) for the dissociation step.

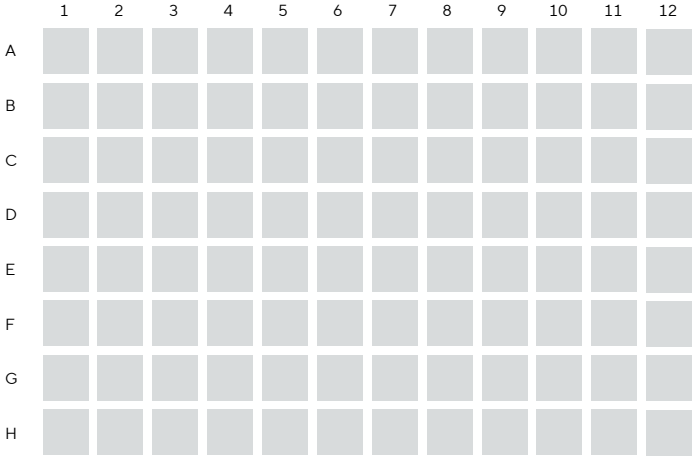

Figure 2A: *Biosensor Tray configuration with all 96 biosensors used in-tandem in each high-throughput assay.*

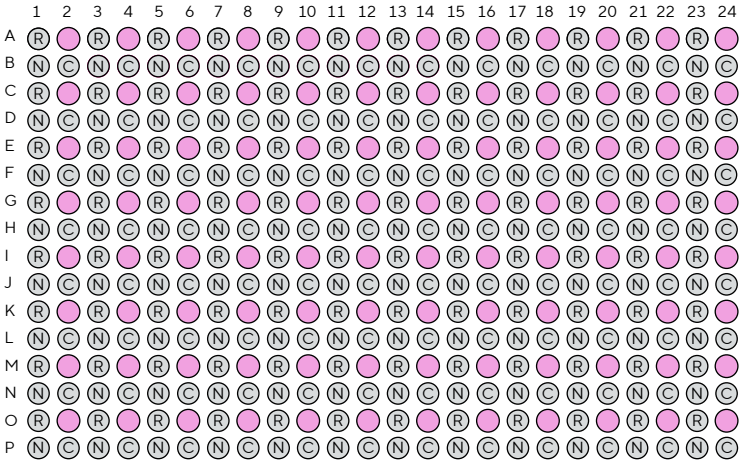

Figure 2B: *Schematic of Reagents Plate 1 loaded with the Antigen (96 wells), regeneration buffers (96 wells each) and antibodies clones 1-8 in rows B, D, F, H, J, L, N and P; 12 wells for each clone across the plate rows. Each reagent plate was used twice with two different sample plates. 9 reagents plates in total were used for the entire automated assay.*

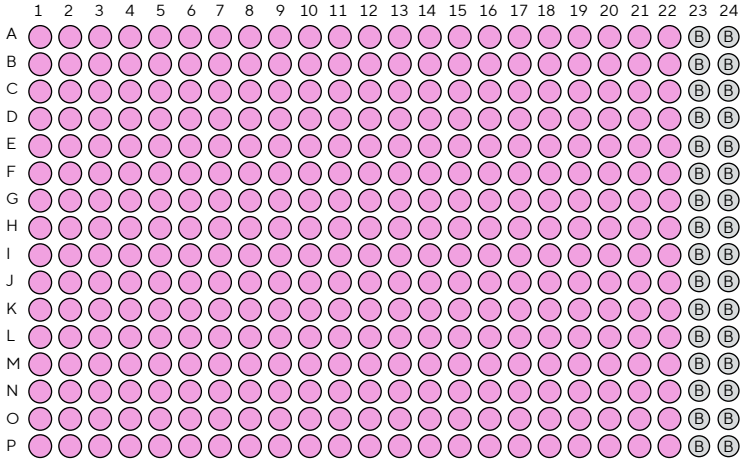

Figure 2C: *Schematic of samples Plate 1 loaded with antibody clones 1 – 44. Two clones are loaded in 8 wells per column in a well alternating design. Ie Clone 1 is loaded in wells A1, C1, E1, G1, I1, K1, M1,and O1 while clone 2 is loaded in wells B1, D1, F1, H1, J1, L1, N1 and P1. 18 Sample plates in total were used for the entire automated assay.*

Results and Discussions

The main goal of this application note was to demonstrate the utility of the Octet® high-throughput instruments in enabling an automated high-throughput antibody screening with epitope binning of antibody clones generated off an avian H5N1 antigen used as a case study. In addition to epitope binning, antibody clone titer and off-rate screening were performed as potential screening applications that often require high-throughput analytical platforms especially when a large library of clones is involved. In these studies, titer and off-rate screening experiments were deemed relatively easy and quick experiments to run on the Octet® RH96 and were as a result performed without automation.

Antibody Titer

The Octet® BLI platform provides a comprehensive characterization tool across a range of applications in various stages of drug development, including antibody and protein quantitation. The platform circumvents the limitations of ELISA and HPLC platforms, enabling informed decisions to be made earlier in bioprocess development. It is a key analytical tool for quantitation in R&D and in up-stream and down-stream process development.

The simple dip and read sample analysis approach that allows for the use of 96 or 384-well plate formats enables streamlined workflows and rapid quantitation of as many as 96 samples in as little as 2 minutes depending on the instrument. Sample purification is not a requirement. In a typical quantitation assay, biosensors coated with capture molecules are simply dipped into analyte samples. The resultant binding, a function of sample concentration, is later analyzed using either the rate of the initial slope of binding or the equilibrium of binding both of which depend on the sample concentration. To quantify samples, a standard curve is generated using the binding rates of known concentrations of the same analyte as the unknown. The unknown sample concentrations are extrapolated from the standard curve.

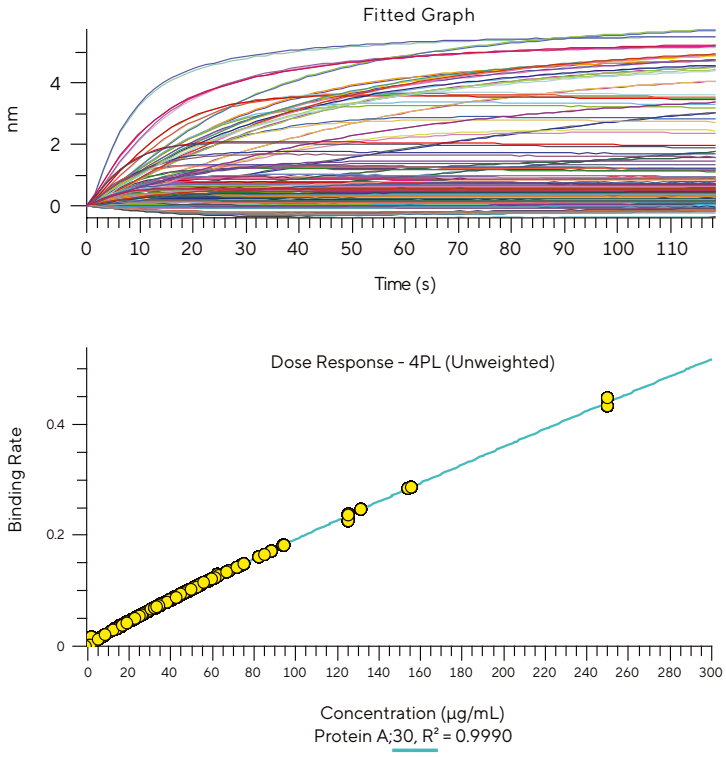

Figure 3: *Protein A biosensor binding curves (top) from both standard reference samples (purified mouse IgG at known concentrations) and hybridoma culture supernatant test samples. The binding curves underwent an initial binding rate analysis to generate a standard curve from which the test sample titers were estimated (data not shown). The bottom figure shows the standard and test samples concentrations plotted on the standard curve.*

Antibody Off-Rate Ranking

A typical bottleneck in antibody lead candidate discovery is the ranking of candidates in a large library obtained after panning based on antibody binding strength. The sheer large number of candidates that may need to be assessed often makes it prohibitively expensive and time consuming to rank these antibodies by comparison to purified samples with known sample concentrations. Off-rate ranking for identification of candidates with slow off-rates is therefore an ideal method for rapid ranking of clones as the binding off-rate is a parameter independent of analyte sample concentration. A slow off-rate is indicative of the desirable higher affinity. The dip and read configuration of the BLI platform allows for antibody off-rate ranking to be efficiently performed by immobilizing the antigen onto the selected biosensor surface followed by a quick dip into the antibody samples in their supernatant and a subsequent dip into buffer to monitor dissociation (Figure 4). The buffer dip step is later analyzed in the analysis software to extract off-rates.

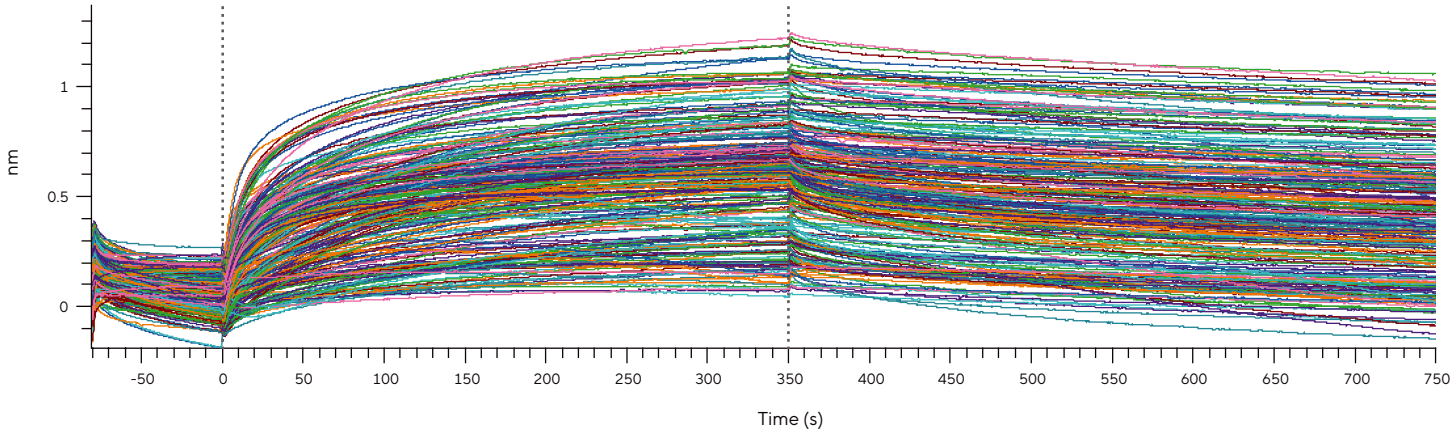

Figure 4: *Off-rate analysis ranking (performed at Rockland Immunochemicals Inc.) showing both association and dissociation steps of clones binding to immobilized H5N1 antigen. The dissociation step is next analyzed (Table 1) using the Octet® software to identify clones with the slowest off-rates (best binders).*

Clone ID	kd (S^{-1})	Clone ID	kd (S^{-1})	Clone ID	kd (S^{-1})	Clone ID	kd (S^{-1})
33F2.E4.F7	1.64E-05	1A5.D6.C9.E9	6.86E-05	20E10.B12	1.01E-02	8B5.D11	6.03E-03
1H11.H8.E2	3.33E-03	12E4.G2.D6	4.40E-03	1D4.G1	7.90E-04	28F5.F8	1.08E-01
29C7	6.85E-03	14D6	7.97E-03	8B2.F2.F3	5.44E-03	29D11.D12	2.94E-03
28D5.F1.G3	2.40E-05	3C1.G8.B8	7.47E-05	30F1.B8.F9	1.01E-02	16C2.E1.D4	6.04E-03
46C1.D10	3.40E-03	4D10.E10	4.42E-03	17C5.E7	1.02E-03	14E5.D8.C11	1.23E-01
25A5.D5.E6	6.89E-03	3C11.D1.C6	8.59E-03	5G2.F2.F6	5.50E-03	22G11.C2.B9	2.95E-03
12D5.E6.D2.F8	2.70E-05	28B3.C8	8.11E-05	14E2.E11.F3	1.07E-02	24H5.C9	6.10E-03
23G2.B4	3.63E-03	1C10	4.53E-03	18A.E4.E3	1.06E-03	20D5.A5.C11	8.76E-01
9D5	6.89E-03	12D5	8.67E-03	12B12	5.73E-03	9E7.C6.C4	3.01E-03
12B12.H10.D2	3.30E-05	10A7.F3.C2	9.93E-05	2A2.E1	1.99E-02	17A10.G5.B1	6.16E-03
28A4	3.82E-03	20G3.B11.F1	4.66E-03	14E11.A4	1.09E-03	13A6.A10.B5	2.26E+00
49F4	6.97E-03	29C12	8.73E-03	20D5	5.75E-03	1D8.C7.D3.B11	3.16E-03
43G4.E8.G8	3.82E-05	11F9.E1.A8	2.07E-04	14F8.G4.E2	2.53E-02	9A8.E6.E7.D10.B6	6.38E-03
23F2.C1	3.89E-03	5A5.F3.C7	4.80E-03	22B9.E11.D3	2.00E-03	8B5.D11.C10	2.32E+00
33F2.E4	7.04E-03	7G7.GH12.H3	9.00E-03	6G9	5.87E-03	3A5.G3.A2	3.18E-03
34A10.E5.D6	3.97E-05	47G3	2.14E-04	9C6.G3	3.00E-02	25A9.H6.D3	6.44E-03
27A8.E6	4.15E-03	44B11	5.08E-03	1E1.C3.D4	2.27E-03	14D6.C3.E3.G5	2.38E+00
6E9.E6.G3	7.23E-03	29.E.9	9.12E-03	11D6.C11.F2.C7.F11	5.94E-03	23A10.D5.C7	3.22E-03
3B10.D10.B7	6.64E-05	9G12.F11	2.23E-04	24H5.C9.H10	3.65E-02	10B4.D7.E7	6.46E-03
3G1.E9	4.16E-03	9E7.C6.F10	5.13E-03	44D6	2.38E-03	21A11	9.80E+01
30C5	7.66E-03	22A8.D2.F5	9.55E-03	3C3	5.95E-03	1C5	3.27E-03
6F1	6.72E-05	5F2.C9	4.29E-04	6E1.C4.F2	3.84E-02	50B7	6.65E-03
20E8.H4.A9	4.38E-03	11F12	5.24E-03	17G6.C5	2.83E-03	23A2.B4	3.29E-03
23D12	7.83E-03	17A10.G5.H1	9.69E-03	28D5.C2	5.96E-03	2G9	6.65E-03
22D2.C2.F8	5.25E-03	1H8.D3	5.91E-04	30E10.F9	6.04E-02	24E4.G4.F4.C2	2.89E-03

Table 1: *Off-rate analysis results for 100 antibody clones subjected to a binding analysis to enable the elimination of weak binders. Of these, 72 clones were selected from the off-rate analysis and subjected to the automated epitope binning analysis.*

Antibody High-Throughput Epitope Binning

Epitope binning assays are used to classify populations of monoclonal antibodies by how and where they interact with the antigen. Competitive binding assays are performed to identify pairs of antibodies that bind to the same or to overlapping epitopes on the antigen. The goal of a typical epitope binning or cross-blocking experiment is to identify antibodies which bind to different or identical epitopes on the antigen. By competing antibodies against one another in a pairwise and combinatorial format, antibodies with distinct blocking behaviors can be discriminated and assigned to "bins". If the binding of one mAb to the antigen prevents the binding of another mAb, then these mAbs are considered to bind to similar or overlapping epitopes. Conversely, if the binding of a mAb to the antigen does not interfere with the binding of another mAb, then they are considered to be binding to distinct, non-overlapping epitopes. Two criteria must be fulfilled in order to assign mAbs into the same cluster: first, all mAbs in the same cluster should block each other's ability to bind the antigen; second, all mAbs in the same cluster should have similar blocking profiles when paired with other mAbs in the panel (Abdiche et al, 2009). The result is matrix of pairwise binders and blockers. The Octet® RH96's high-throughput capability together with its automation ready configuration makes it a convenient analytical tool for performing epitope binning experiments.

In these studies, 72 antibody clones were subjected to a competition assay using the in-tandem assign design (Figure 5). Samples were loaded into the Octet® RH96 instrument using the Biosero® automation device as described in the method section. The automation process enabled the 72 x 72 matrix to be completed in a total of 18 hrs without human intervention.

For this study, 72 antibody clones were selected from the off-rate screening data. However, as part of the goal of the study was to investigate the utility of the Octet® RH96 for high-throughput screening, a subset of antibody clones with relatively weaker binding to the antigen was included. The weaker binders were eliminated off the binning analysis through the binding threshold setting in the analysis software. As can be seen in Figure 6A, the assay was run sequentially, first was the antigen binding step followed by the primary antibody and lastly the secondary antibody. The analysis software was later used to set a binding threshold using response data from self-binding observed across the panel of antibody clones. The secondary antibody binding response was used for binning analysis. A threshold of 0.12 nm shift was determined as the cut-off point below which any response signal was considered as non-specific or determined as no binding.

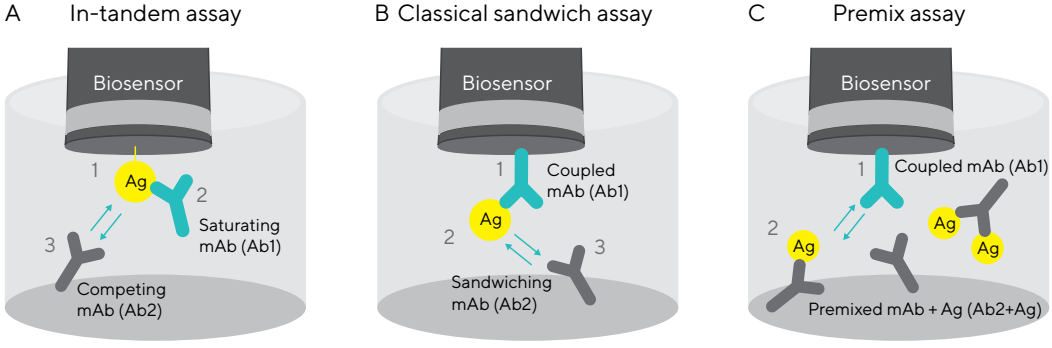

Figure 5: *The three different formats for epitope binning assay design on Octet® instruments: A: In-tandem format where the antigen is immobilized onto the biosensor tip; B: Classical sandwich format where the primary antibody is first captured onto the biosensor surface and C: the premix format where the secondary antibody is premixed with the antigen.*

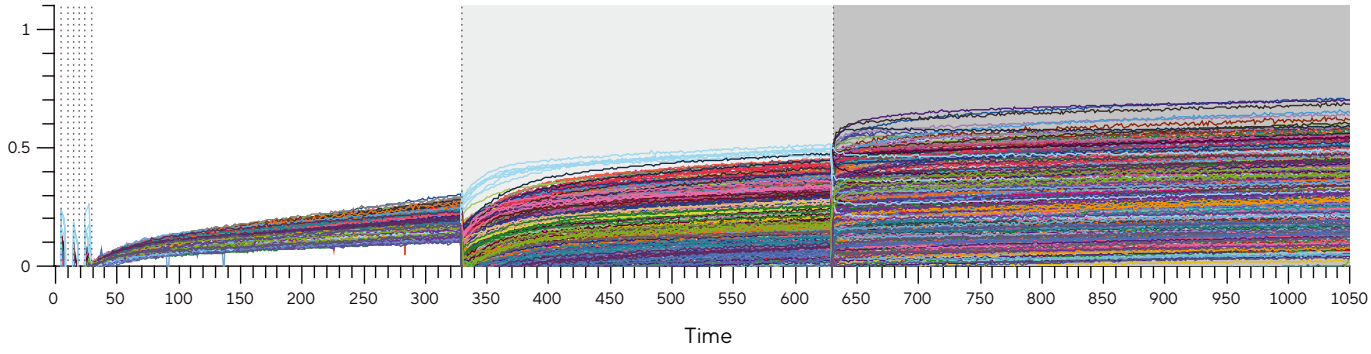

Figure 6A: *Raw data obtained from the 72 × 72 in-tandem binning assay with the His-tagged H5N1 antigen immobilized onto HIS1K biosensors at 7 µg/mL followed by a dip of the biosensors into antibody clone 1 followed sequentially by a dip into antibody clone 2.*

Figure 6B shows binding response observed from the second antibodies binding when the first antibody is already bound. Signals above the red line (threshold) are treated as positive binding and are used to determine a binding matrix (data not shown). As can be seen in Figures 6C; although this was a relatively large matrix, there was little diversity in the binding behavior of the 72 clones. The binning data revealed 4 unique bins containing 9 antibody clones with the rest of the antibody clones clustered together as potentially having similar characteristics.

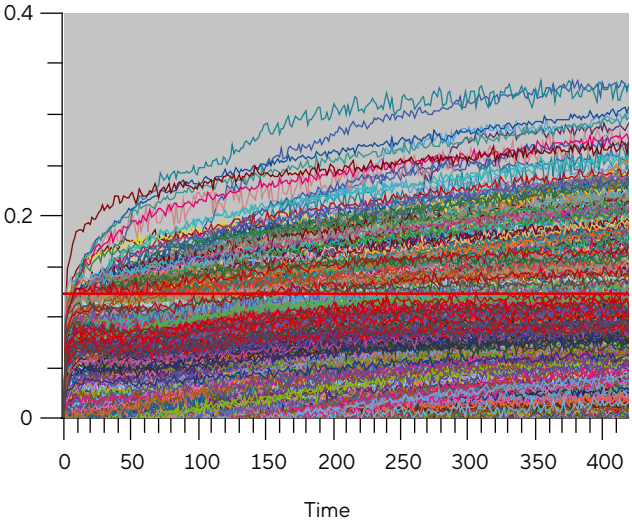

Figure 6B: *Secondary antibody clones binding step used in the binning analysis. The red line represents the binding threshold (above which positive binding is determined).*

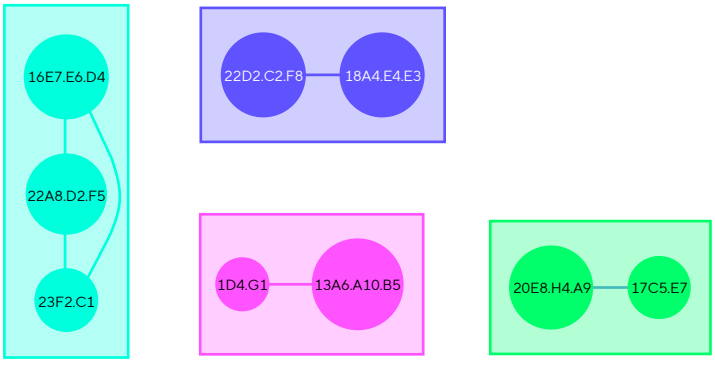

Figure 6C: *Unique bins as determined from the secondary antibody binding data in Figure 6B. Each bin represents a class of antibodies that block each other but which do not compete for binding with antibodies in the other bins as they bind to different epitopes on the antigen.*

Final Clone ID	Isotype	H5N1	H3N2
1D4.G1	IgG1/Kappa	+++	+++
13A6.A10.B5	IgG1/Kappa	+++	-
16E7.E6.D4	IgG1/Kappa	+++	+++
22A8.D2.F5	IgG2b/Kappa	+++	-
23F2.C1	IgG1/Kappa	+++	-
18A4.E4.E3	IgG1/Kappa	+++	+++
22D2.C2.F8	IgG1/Kappa	+++	+++
17C5.E7	IgG1/Kappa	+++	-
20E8.H4.A9	IgG1/Kappa	+++	-

Table 2: *ELISA specificity assay data reveals that antibody clones 13A6.A10.B5 belonging to one bin and clones 22A8. D2.F2, 23F2.C1 belonging to a separate bin and clones 17C5. E7, 20E8.H4.A9 belonging to a third bin exhibit specificity towards the H5N1 antigen. The rest of the clones exhibit significant response to the non-specific H3N2 antigen to be deemed false positives at least based on ELISA studies.*

Affinity Characterization - Selecting Antibody Clones

In successful large library binning studies where antibodies have been identified to belong to different bins, it can be concluded that these antibodies have different properties and can be used as potential reagents for diagnostic assays where a capture antibody and a detection antibody are needed. Antibodies that block one another could on the other hand be subjected to neutralization assays as they could act as effective therapeutics for a target antigen in a disease state or for vaccine development. To ensure potency and efficacy, further characterization studies that include affinity characterization are performed. To show the utility for the Octet® BLI instrument, the antibody clones identified in the various bins were subjected to a dose response antigen binding studies to determine their affinity constants. In addition, a specificity assay was run on an ELISA system to ascertain specificity of the selected antibody clones to the target H5N1 antigen using an H3N2 antigen (Table 2). In the assays, each clone was immobilized to an anti-mouse capture AMC2 biosensor at normalized concentrations (5 µg/mL) followed by a dip of the biosensors into a titration of the H5N1 (two-fold serial dilution from 300 nM – 75 nM). Any antibody that exhibited significant non-specific binding as determined from the ELISA study was eliminated from the study. Figure 7 shows an example of the data obtained from the study for a couple of the antibody clones. Those that exhibited non-specific binding as observed are also not included in these studies.

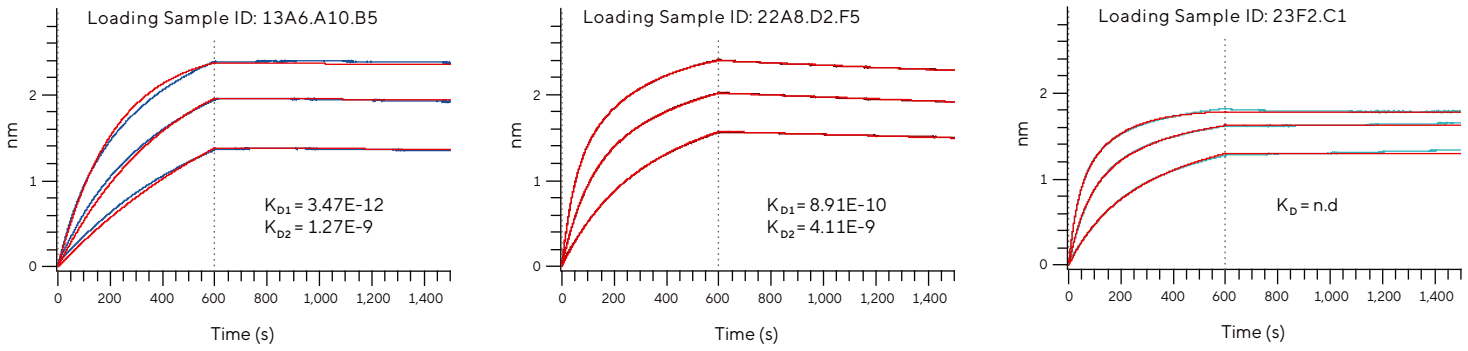

Figure 7: *An example of affinity characterization of select antibody clones from the binning studies. The approach can be used to characterize a large library of antibody clones. In these case studies, 3 top concentrations were fit using the Octet® analysis with lower concentrations excluded due to the need for further assay optimization. As can be observed, the diversity in binding characteristics can be used to select the optimal clone(s) for further development.*

Conclusion

The goal of these studies was to evaluate research tools that could support the development of an immunoassay tool for the interrogation of the hemagglutinin (HA) protein from clade 2.3.4.4b HPAI H5N1 **(https://www.rockland.com/resources/h5n1-antibodies/)**. This was achieved by evaluating selected candidates from a hybridoma monoclonal antibody campaign to identify mAbs that reacted non-competitively for later use in assay development. The anti-HA monoclonal antibodies were successfully developed at Rockland Immunochemicals and subjected to a variety of characterization studies using the high-throughput Octet® RH96. A 72 x 72 mAb epitope binning assay method developed to identify competition amongst the antibody clones was successfully automated on the Octet® RH96 using a Biosero® robotic arm with the entire assay completed in 18 hours. In addition, the Octet® BLI platform was used to rapidly screen for antibody expression and off-rate ranking to enable the determination of multiple antibody clone attributes from one instrument. These data in combination with data obtained off an ELISA platform as complimentary technique can facilitate an informed decision in the selection of optimal clones for further development. Rockland has made a subset of these H5N1 HA antibodies commercially available.

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