A fast and high precision influenza vaccine potency assay

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Overview

Vaccines are biological preparations that contain agents resembling disease causing microorganisms, and can improve immunity against a specific disease. They are typically prepared from inactivated or weakened forms of the microbe or its toxins, or surface proteins. Classical vaccines against the influenza virus are developed in embryonated hen eggs and may include whole virus, split virus or a purified subunit with every component other than hemagglutinin (HA) or neuraminidase (NA) removed.[1] The target molecule for the protective immune response triggered by vaccination is generally accepted to be the HA molecule; a glycoprotein found on the surface of the influenza virus. Measuring the vaccine potency or the biologically active components is critical to the determination of the vaccine's effective dose. In addition, the stability of the vaccine has major impact on its usage for immunization programs worldwide. Although real-time stability studies under different storage conditions is preferable, thermal stability testing using potency assays with samples subjected to heat or environmental stress conditions can be used as predicators of vaccine stability over time.[2]

A fast and accurate determination of vaccine titer during manufacturing is important in understanding vaccine development process performance, and for correctly scaling each process step. The Single Radial Immunodiffusion (SRID) technique has been the most commonly used technique for vaccine titer determination. However, SRID is time consuming and generally exhibits poor precision. An alternative assay that can speed up the analysis process and provide accurate and precise potency data on different vaccine strains is therefore desirable. The Octet® platform's Bio-Layer Interferometry (BLI) technology combines the high-throughput characteristics of a 96-well or 384-well plate format with improvements in precision and reproducibility and is derived from a simpler and more direct vaccine/antigen–antibody binding measurement method. They provide process development groups with a robust and easy to use alternative to the SRID method. BLI reduces the assay time from days to just a few hours for a 96-well plate of samples.

Figure 1: Octet system with sample plate loaded.

The relative standard deviation and dynamic range of a vaccine titre assay was tested for the influenza virus using the Octet platform and was found to be better than that encountered with SRID. Unlike in the SRID technique where detergents are used to expose the target HA molecule, with Octet systems, samples are analyzed in their natural state without the use of detergents. As a result, Octet systems are capable of analyzing whole virus, split virions and recombinant HA vaccine samples.

Materials and reagents

- Samples of inactivated virus and antibody standards were purchased from NIBSC, South Mimms, UK. Split virion vaccine samples were provided by Sanofi Pasteur, France, and recombinant hemagglutinin vaccine samples and antibodies were provided by Protein Sciences, USA.
- Sample Diluent (Part No 18-1048), Protein A and G biosensors (Part No 18-5010 and 18-5082 respectively), were provided by ForteBio.
- Black polypropylene 96-well sample plates from Greiner, Part No 655209 (Sigma Aldrich Part No M9685) were used.

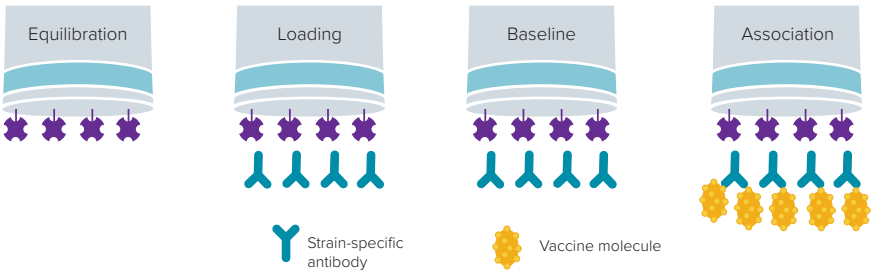

Figure 2: Assay workflow for the influenza vaccine titer assay. The assay is run using the Advanced quantitation setup in the Octet Data Acquisition software.

Method

The assay is based on the binding of the vaccine sample to polyclonal antibodies that recognize the influenza epitopes presented by the vaccine. Protein A or Protein G derivatized biosensors are first used to load the specific polyclonal antibody from the serum antibodies (Figure 2). The antibody-immobilized biosensors are then dipped into the vaccine samples and a response signal that depends on binding epitope recognition and vaccine concentration is registered.

To determine the optimal antibody concentration to capture onto the biosensor, a serial dilution of the serum antibody sample was performed using ForteBio Sample Diluent.

Figure 3 shows the biosensor binding profiles of a range of antibody dilutions starting from 1/10 to 1 /2000 of the neat antibody samples obtained from NIBSC as California/7/2009 (H1N1); an A strain inactivated virus standard and antibody pair. Typical Octet assay antibody loading time is 300 seconds with a shake speed ranging from 400–1000 RPM. The antibody loading time can vary significantly from virus strain to strain and should be evaluated.

While high precision Streptavidin (SAX) biosensors are recommended for multi-step quantitation assays, Protein A or G biosensors can be used especially when dealing with non-purified IgG samples. The biosensors were first tested in five replicates for each lot in a biosensor lot to lot robustness study for antibody loading (Figure 4, Table 1). The biosensors were found to be highly robust to loading variations. However, it is critical to include a referencing biosensor (zero analyte) in each assay to subtract off potential post antibody baseline drift. The second stage of the assay optimization involves dipping the antibody-loaded biosensors into a fixed concentration of the vaccine sample to monitor response (Figure 5). Both antibody loading and antigen binding during assay optimization are

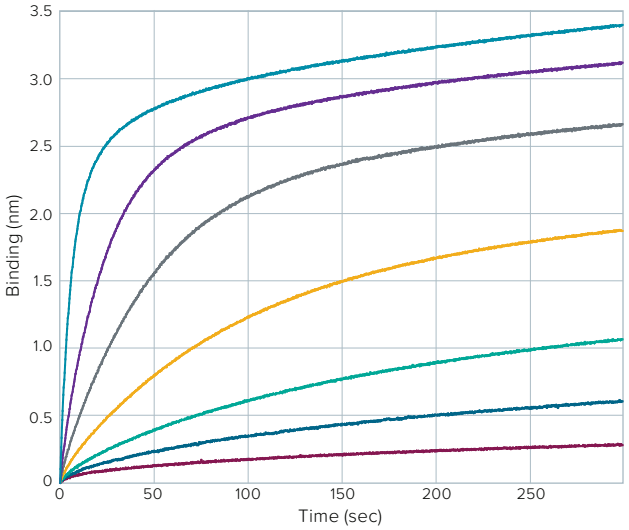

Figure 3: Antibody Load Scouting using Protein A biosensors – A/California/7/2009(H1N1).

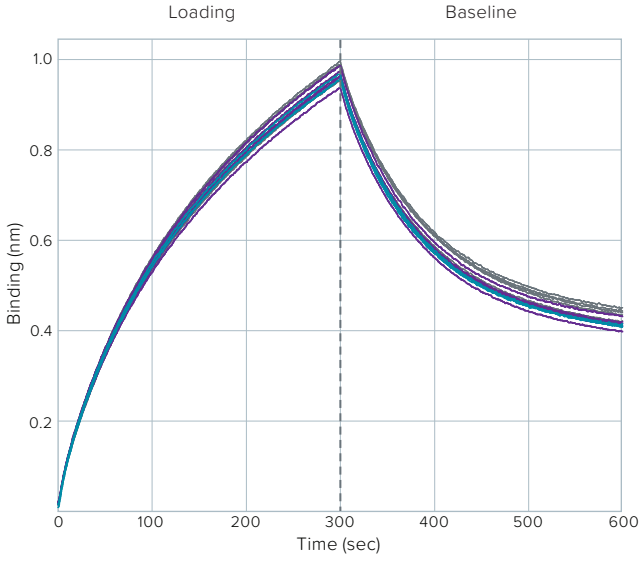

Figure 4: Antibody loading at 1/250 dilution using different lots of Protein A biosensors.

Biosensors	Average response	% CV
Lot 1	0.489	2.41
Lot 2	0.463	2.63
Lot 3	0.455	1.53
Lot to Lot % CV		3.78

Table 1: Protein A biosensors lot to lot loading response monitored at T = 500 seconds

performed in sequence on the Octet instrument using the Advanced Quantitation experimental setup in the Octet Data Acquisition software.

The lowest coating antibody concentration (1/250 dilution in this example) that gives maximal vaccine binding should be selected for the studies. For influenza virus, the sample binding step is typically 300 seconds except for split virion analysis, where the step should take less than 30 seconds as virus heterogeneity can occur with time. To determine titer, a standard curve is required and is generated using a titration of standard samples whose concentration is known. The response signals obtained from the standard samples are analyzed by calculating the binding rate for the initial slope of the binding curve. The measured binding rate is then plotted as a function of the standard samples concentration and the data fit to a dose response equation resulting in a standard curve from which unknown sample titre can be determined.

The antigen binding response for the three most concentrated antibody solutions overlap (Figure 5). The lack of distinction in response from these three antibody coating concentrations suggest biosensor binding saturation, which may imply that steric hindrance could play a role in antigen binding. As a result, the next lower antibody concentration should be selected for the studies.

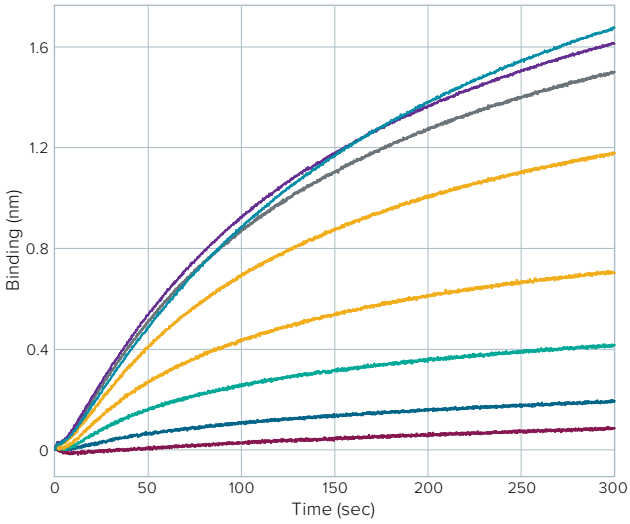

Figure 5: Fixed concentration of antigen bound to the different antibody concentrations from Figure 3 – A/California/7/2009 (H1N1).

Tips for optimizing vaccine titer assays

- Select the appropriate biosensors for the study. For immobilization of non-purified samples such as serum antibodies, Protein A or Protein G biosensors (ProA or ProG) are recommended. When purified samples are available for capture, it is preferable to use High-Precision Streptavidin biosensors (SAX). In this case, the purified capture molecule should be biotinylated prior to use.
- Use the same batch/lot number of biosensors for both the standard curve and samples.
- Ensure that biosensors are hydrated in assay buffer for at least 10 minutes.
- Determine the shake speed for both the antibody loading and the antigen binding steps to obtain optimal conditions. Two shake speeds, 400 RPM and 1000 RPM, are recommended for evaluation prior to the start of the assay. The same shaking speed should be used for both the standard curve and unknown concentration samples.
- Standard curves should have a minimum of eight data points, including a reference or zero concentration point for data subtraction, and should be run in replicates. The reference data should be acquired using media similar to the vaccine analyte.
- Check raw data for overlapping binding curves in both the antibody loading and antigen binding scouting steps. This will highlight data that cannot be differentiated at these levels. For antibody loading, choose the highest concentration of antibody that does not overlap (typically around 2 nm binding). For antigen binding, discount all standard levels that overlap. Figure 5 shows examples of these overlaps in binding curves.
- In some cases, depending on size, particles such as viruses will generate a negative signal, so the acquired data will need to be 'flipped' in the data analysis window prior to fitting.
- Read time should be set at 300 seconds for inactivated and recombinant hemagglutinin vaccine samples. Split Virion sample read times should be ascertained from the raw data (typically < 30 seconds), and the appropriate window used to capture the linear region in the positive binding response.
- Antibody and vaccine samples give different results for each strain, and so conditions such as shake speed, analysis time, antibody loading and linear range should be evaluated for each strain. Typical conditions for the assay are shown in Table 2.

	Antibody loading			Vaccine binding		
	Whole virus	Recombinant hemagglutinin	Split virion	Whole virus	Recombinant hemagglutinin	Split virion
Assay time (s)	300	300	300	300	300	30
Hold time (s)	600	600	600	N/A	N/A	N/A
Shake speed (rpm)	400					

Table 2: Typical conditions for vaccine titer assays. Hold time refers to temperature equilibration time with samples in the instrument prior to the start of the assay.

Results – inactivated virus

The virus standard was tested from a range of 1 to 140 µg/mL based on the HA concentration provided by NIBSC. The linear range was established to be between 5 and 75 µg/mL with a linear regression value of 0.9951, as shown in Figure 6. Once the linear range and antibody loading concentration are determined, the assay is ready to be used for sample titre determination.

Stability indicating assay – heat denatured samples

A robust assay capable of distinguishing between native and deactivated antigen would also be suitable as a stability assessment assay. Such an assay can then be used to determine the stability of the sample under accelerated degradation conditions such as temperature, pH and oxidative conditions.

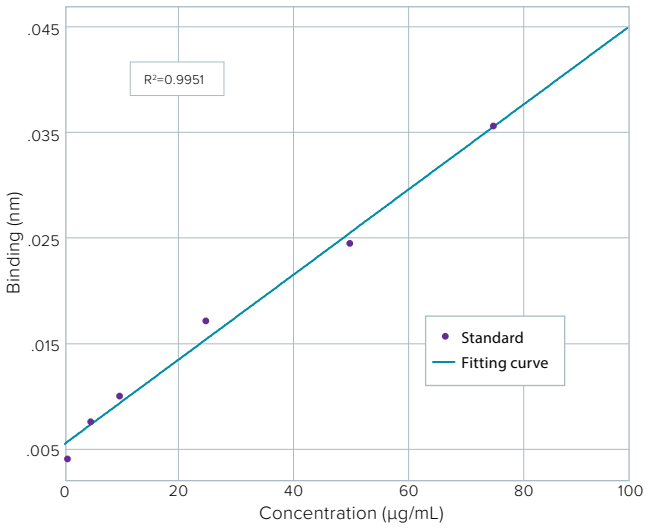

Figure 6: Linear range of A/H1N1 California virus standard layout.

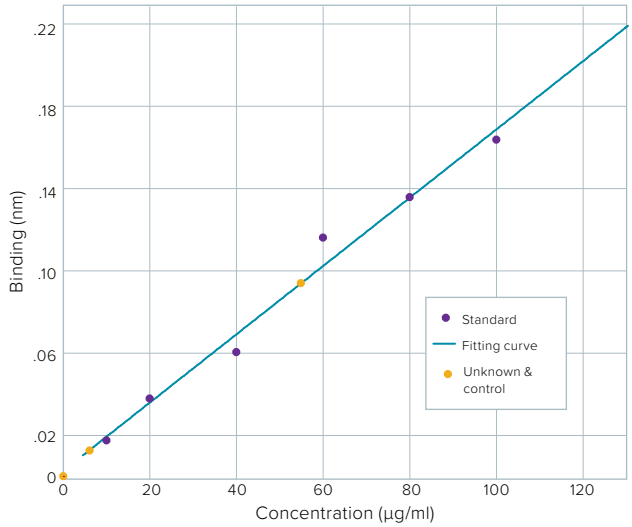

Figure 7: Heat denatured H3/TX recombinant hemaglutinin sample vs. control. Both samples are shown in red against the standard curve points shown in blue.

The recombinant hemagglutinin H3/TX sample was diluted to 50 µg/mL using ForteBio Sample Diluent and was divided into two aliquots. One aliquot was treated at 95 °C for five minutes while the other was left at room temperature as a control. The samples were then analyzed using the using the method described above with the H3/TX antibody bound to the biosensors. Figure 7 shows that the native sample gave an average recovery concentration of 53.6 µg/mL, while the heat-treated sample showed a loss of response to an average recovery concentration of 7.2 µg/mL. This proves the assay can successfully test heat-treated, stability-indicating samples and can distinguish between native and deactivated antigen.

A comparison between srid and Octet analysis for split virion samples

SRID, the most often used technique for influenza virus titre, is a gel-based assay that is easy to use and relatively inexpensive to setup. It can, however, take as long as three days to run, and can produce subjective data.[3] Antigen diffusion into the agarose gel alone can take as long as 16 hours.[4]

Due to differences in sample processing, the two techniques measure different molecular aspects of the sample. SRID measures the HA content after lysing with zwittergent 314, while the Octet system measures the diluted sample as is. The Octet platform is a much quicker technique with easier sample processing, involving only a simple dilution in Sample Diluent. It also analyzes the vaccine sample itself and not a secondary sample that has been changed by the addition of a reagent. Precision and dynamic range produced by the Octet assay is also significantly better than what can be obtained from SRID.

Platform	Assay time for 96-samples
SRID	Several days
Octet RED96	< 180 min
Octet RED384	< 90 min
Octet HTX	< 20 min

Table 3: SRID technique and Octet platforms assay time comparison

B/Massachusetts								
	Octet assay (µg/mL)				SRID (µg/mL)			
Replicate group	Conc. avg	High	Low	Conc. %CV	Conc. avg	High	Low	Conc. %CV
A	723.65	821	658	8.84	711	792	620	8.04
B	664.18	753	589	7.97	697	850	554	13.20
C	737.58	796	686	5.21	697	802	538	10.04

Table 4: Comparison of SRID vs Octet assay data for split virion vaccine samples.

Conclusion

Octet platform assays offer a number of advantages over the SRID assay for vaccine titre analysis:

- **Higher quality data:** The Octet assay has a wider dynamic range (5–75 µg/mL), with greater precision and accuracy.
- **Faster results with plate-based format:** It uses a 96 or 384-well plate format and can analyze a full plate in under three hours, including sample preparation time.
- **Simple sample prep:** Samples only need to be diluted with no complex sample preparation. In-process and purified samples can be analyzed without encountering matrix effects.
- **Direct vaccine measurement:** the data collected is for the vaccine itself and not a derived analyte produced from denaturing the sample with reagents such as Zwittergent 314.
- **Use the same assay for all strains:** A change in vaccine strains does not require a change in equipment or method. A limited requalification with the appropriate biosensor and strain specific antibody is sufficient.
- **Determine vaccine stability:** The assay can analyze heat inactivated samples, hence can also be used as a vaccine stability indicating technique.

References

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