Influenza Vaccine Titre Determination Using Bio-Layer Interferometry (BLI)

D.W. Wheatley[1], D. Saunders[1], J.H. Welsh[1], E. Matthews[2], I. K. Srivastava[2], M. M. J. Cox[2], D. Apiyo[3]

[1] Pall Life Sciences, Portsmouth/UK; [2] Protein Sciences Corp, Meriden/USA; [3] Sartorius, Fremont USA

* Corresponding author: David.Apiyo@sartorius.com

Introduction

Fast, accurate determination of vaccine titre during influenza vaccine manufacture is important in understanding process performance and correctly scaling each process step. Traditionally Single Radial Immunodiffusion (SRID) assays have been used as the 'gold standard' but require very skilled operators to obtain reproducible results and is relatively low throughput. ELISAs have also been used to determine titre but have lower precision and dynamic range. Bio-Layer Interferometry (BLI) technology, used on BLitz® and Octet® systems, combines the high-throughput characteristics of a 96-well plate based ELISA assay in conjunction with improvements in accuracy and repeatability derived from a simpler direct measurement of mass transfer on binding.

The assay is based on the binding of the vaccine to polyclonal antibodies that recognise the influenza epitopes presented by the vaccine. The polyclonal antibody is bound to a protein G or protein A derivatized biosensor depending on the animal source of the antibody. This configuration gives increased flexibility by allowing swift changes between vaccines derived from different viral strains by simply binding the paired antibody for the new strain to a biosensor without the need for derivatization. Hence, the assay is suitable for the rapid changes in the viral strains represented in a vaccine. A robust assay, capable of determining vaccine titre from various process stages has been developed and has been shown to be applicable to both live attenuated and synthetic vaccines.

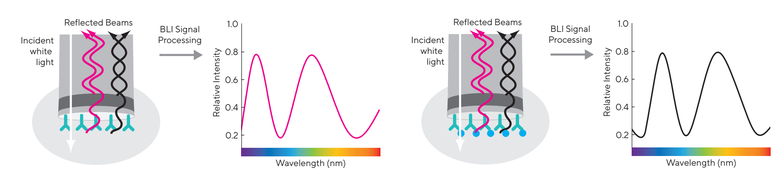

Figure 1: Principles of Bio-Layer Interferometry.

Figure 2: The Octet® family of instruments and BLItz® system.

Bio-Layer Interferometry

- Analyzes the interference pattern of white light reflected from two surfaces, the ligand at the surface of the tip and an internal reference layer.
- The binding of an analyte in solution to the ligand produces an increase in optical thickness at the biosensor tip, which results in a wavelength shift and changes in the interference pattern.
- Interactions are measured in real-time, providing the ability to monitor binding specificity, rates of association and dissociation or concentration.
- Only molecules binding to or dissociating from the biosensor can generate a response. Unbound molecules, refractive index or flow rate changes do not affect the interference pattern. Therefore Octet® and BLitz® systems have the capability to perform in crude samples.

Virus Vaccine Manufacturing

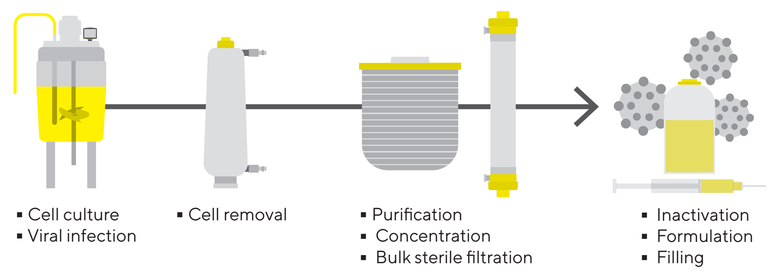

Figure 3: Virus vaccine manufacturing process.

At each stage of vaccine manufacturing, analysis of the in-process samples is required. Current methods are off-line and take hours or days to get the information required. At-line or quicker analysis techniques would allow for a faster response and faster decision making.

Single Radial Immunodiffusion (SRID)

Pros

- Industry wide 'Gold Standard' assay.
- Relatively easy to use.
- Inexpensive to set up.

Cons

- Assay can take up to 3 days
- Limited sample throughput.
- Narrow dynamic range.
- Data can be subjective and open to interpretation.
- Poor precision and accuracy due to imperfections in laying the gel.

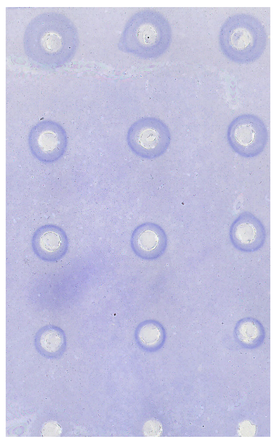

Figure 4: SRID data.

BLI Sample Preparation and Analysis Time < 3 Hours!

Samples taken directly from purification steps have been assayed successfully with zero sample clean up, having been only diluted with a proprietary Sartorius sample buffer. Protein A and Protein G biosensors have been successfully used in the assay. The system uses well plate technology allowing for high sample throughput.

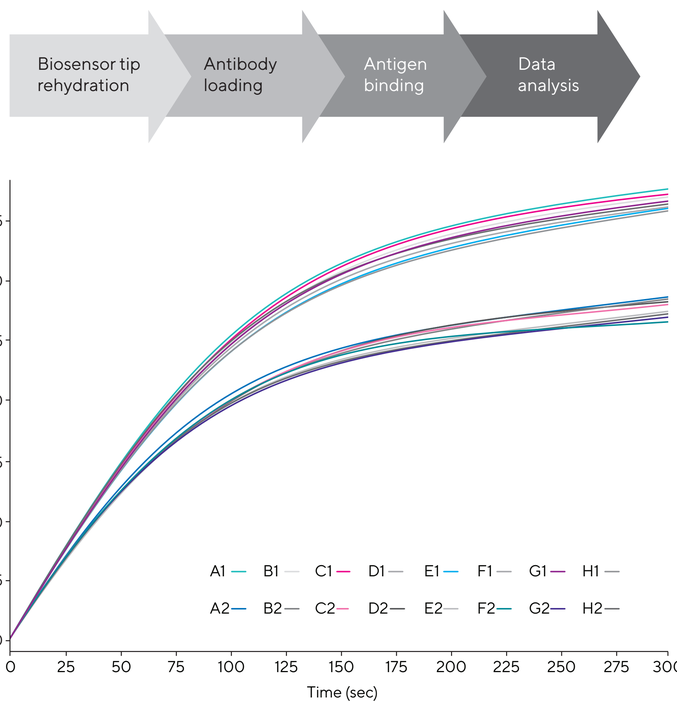

Figure 5: Data traces from two concentrations of antibody, demonstrating loading precision.

Figure 5 shows 2 different concentrations of antibody, demonstrating the level of precision that can be expected when loading antibody to the biosensors.

Typical Antigen Calibration Curves for rHA 'A' Strain Influenza Antigens

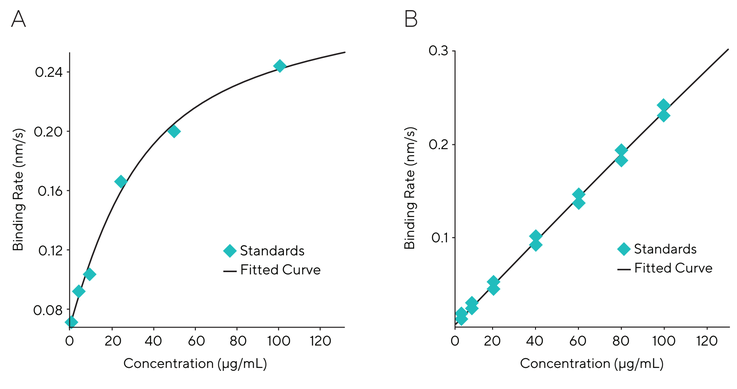

Figure 6: A) H1 recombinant haemagglutinin, standard curve, $R^2 = 0.9942$.
B) H3 recombinant haemagglutinin, standard curve, $R^2 = 0.9981$.

Typical Calibration Curves for rHA 'B' Strain and Attenuated Intact H1N1 Influenza Antigens

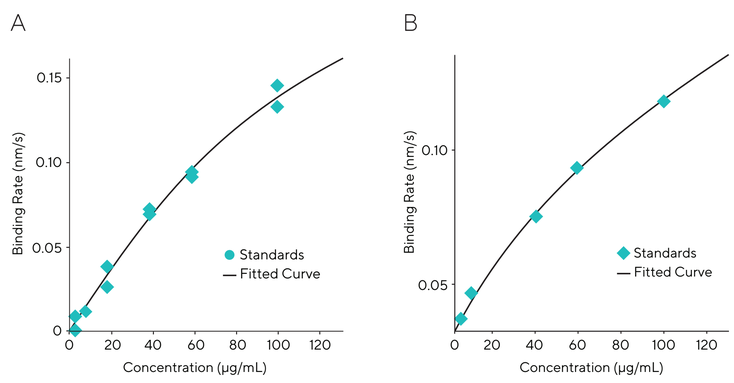

Figure 7: A) BBris recombinant haemagglutinin, standard curve, $R^2 = 0.9981$.
B) H1N1 attenuated virus, standard curve, $R^2 = 0.9979$.

Precision Data

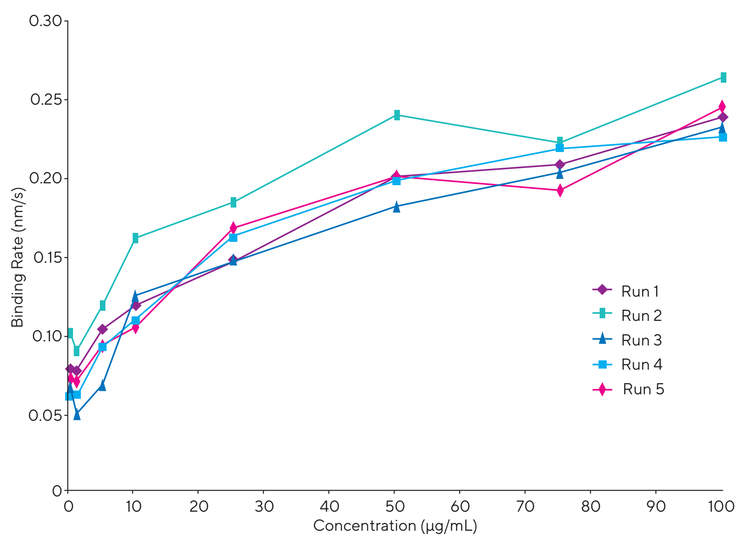

Figure 8: Precision data. CVs for each standard are < 7% in the range 10–100 µg/mL. R^2 values for each curve were >0.97.

Potency Testing: Heat Denaturation

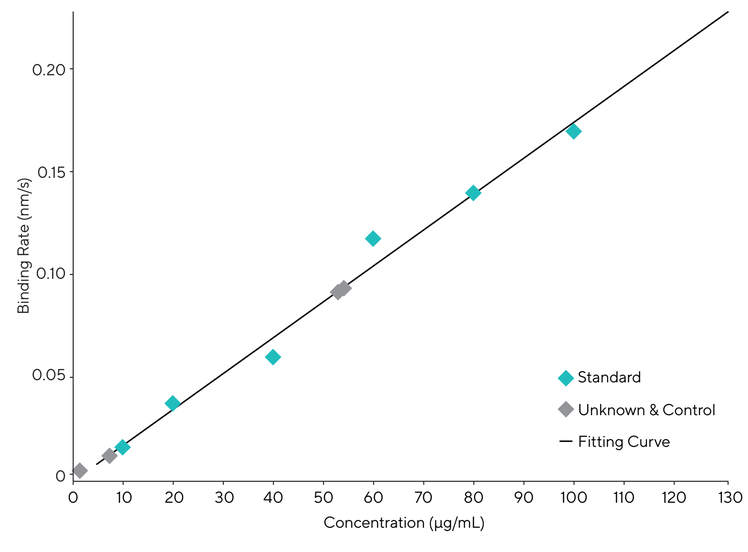

Figure 9: Native samples gave an average result of 53.6 µg/mL . The heat treated samples showed a loss of response to an average value of 7.2 µg/mL, proving they had been inactivated and that the assay can also test for vaccine potency.

Conclusion: Rapid Analysis for Virus Titre Results

- Total analysis time of less than 3 hours on the Octet® systems.
- The Blitz® platform enabled at-line sampling in process development laboratories.
- A change in the vaccine strains or formulation only required a limited requalification of the assay and did not require a change the equipment or the method.
- In-process samples and purified samples were both analyzed without matrix effect issues.
- The assay analyzed heat inactivated samples and can therefore test for vaccine potency.