



Biacore systems in vaccine development and production

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Biacore™ systems in vaccine development and production



The growing public awareness of a potential pandemic requiring rapid treatment of millions of healthy individuals has spurred renewed interest in vaccines. Vaccine development timelines vary from being only a fraction of other biotherapeutics to decades of research and efforts to find an effective vaccine. Vaccination campaigns mean that often very large quantities need to be manufactured, maintained, and distributed. To step up to the challenge, the vaccine industry is adopting new development methods as well as improved and more flexible manufacturing principles to increase production efficiency while assuring vaccine safety and efficacy. The demand for more *in vitro* characterization techniques has increased, particularly in routine testing, in line with the trend towards decreasing animal testing. Throughout the development process, biomolecular interaction data provided by Biacore systems contribute to detailed understanding of a vaccine's properties and characteristics (Fig 1).

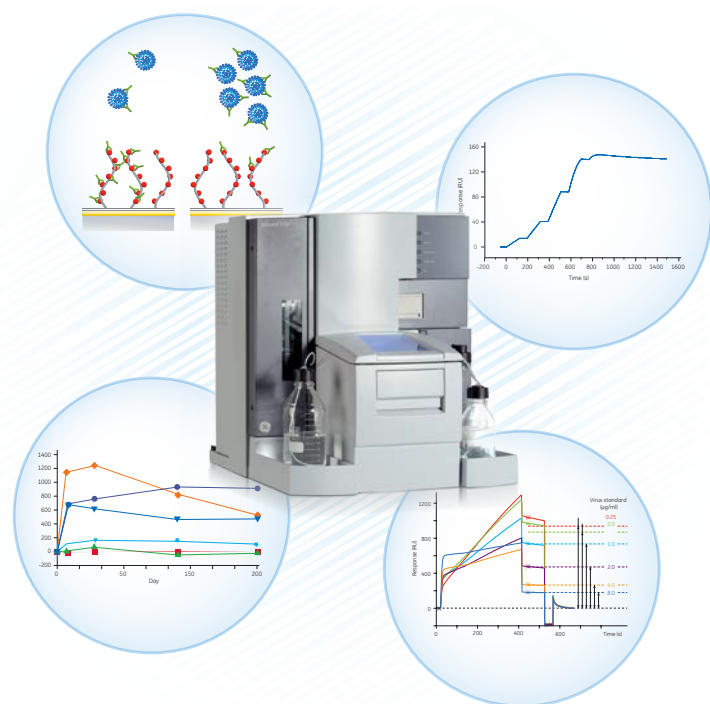


Fig 1. The vaccine development process is supported by Biacore from basic research to production and QC. Detailed information is obtained from analyses such as binding kinetics, specificity, immune responses, epitope mapping, and concentration in vaccine research and discovery.

Biacore interaction data is used in areas such as:

- Vaccine design and characterization
- Immune response studies
- Analyses during the vaccine production process and QC

This white paper describes how Biacore assays are successfully employed to design vaccines and adjuvants with desired properties, to speed up optimization of process parameters, and to perform batch release tests with great accuracy and precision.

Vaccine design and characterization

Vaccine design and identification of suitable antigens for vaccine candidates depends on research that elucidates structural and functional interactions between pathogens and the host immune system. Surface plasmon resonance (SPR) advances vaccine research and design by providing accurate, precise, and rich characterization data about binding between viral epitopes and elicited antibodies. This is exemplified below for the design of future vaccines against HIV-1, Epstein Barr virus, and influenza. In addition to these examples, Biacore systems are also used for other vaccines including MERS (1), malaria (2), meningitis (3), tetanus (4), and diphtheria toxin carrier protein (5).

Antigenic structure and epitope interrelationships of the HIV-1 Env trimer

Biacore systems are used extensively in HIV research as demonstrated recently by Yasmeen *et al* (6) where multiple Biacore assay formats are presented giving insights into the binding of neutralizing antibodies to different soluble cleaved HIV-1 trimer constructs. The trimeric envelope glycoproteins on HIV-1 virions are the target for neutralizing antibodies and further investigations by these researchers present a comprehensive antigenicity analysis performed using ELISA. This analysis was then extended to studies of cross-competition using a Biacore system (7).

In these analyses, an anti-His antibody (His Capture Kit, GE Healthcare) was first immobilized to the sensor chip. Histidine-tagged (His-tagged) trimers were then captured to the antibody, followed by sequential injections of two different antibodies using dual inject/co-inject (Fig 2). The dual inject/co-inject functionality injects two different antibody solutions immediately one after the other, without any dissociation in between. For example, in the right-hand panel in Figure 2B, the light blue curve shows injection of antibody PGT151 directly followed by antibody 35O22, both antibodies giving rise to significant binding.

In a separate analysis (same right hand panel, Fig 2B) antibody 35O22 was then injected using a single injection (dark blue, overlaid). 35O55 binding was of a similar level when injected on its own as when injected after PGT151 had already bound. This means that these two antibodies do not compete for the same binding site but bind to separate epitopes. In contrast antibody 35O22 bound significantly more when injected on its own than when it was injected directly after antibody 8ANC195 had bound (Fig 2A, right panel) indicating that these two antibodies compete for the same epitope. The antibodies all displayed strong self-competition, showing that the epitope was nearly saturated at the used concentration (Fig 2A, left panel, Fig 2B, middle panel, and Fig 2C, right panel).

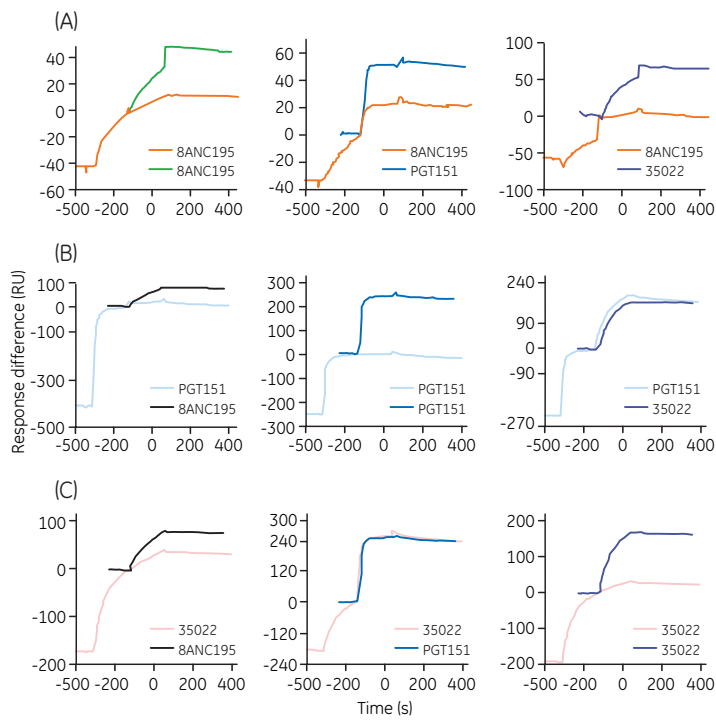


Fig 2. Antibody cross-competition analysis of binding to a HIV-1 Env trimer mimic. His-tagged trimer constructs were first captured to an anti-His antibody on the sensor chip. Two antibodies were then injected in sequence and the level of the second injection was compared to a single injection (overlayed) of the same antibody. (A) Competition between 8ANC195 (competitor) and PGT151 and 35022. (B) Competition between PGT151 (competitor) and 8ANC195 and 35022. (C) Competition between 35022 (competitor) and 8ANC195 and PGT151*.

* Reproduced from the article Comprehensive Antigenic Map of a Cleaved Soluble HIV-1 Envelope Trimer by Derking R, Ozorowski G, Sliepen K, Yasmeen A, Cupo A, Torres JL, et al. *PLoS Pathog.* **11**(3): e1004767. doi:10.1371/journal.ppat.1004767 (2015).

Results from structural studies, ELISA, and Biacore high-resolution epitope mapping generated an antigenic map of three defined epitope clusters as well as the relationship between the clusters. New epitopes were also revealed and the authors demonstrate that antibody binding can be complex involving also other areas of the trimer.

Derkins *et al* (7) list three recent developments that have facilitated studies of the antigenic structure of the HIV-1 Env trimer:

1. Many broadly neutralizing antibodies have been isolated.
2. The generation of a trimer mimics expressing most neutralizing antibody epitopes.
3. Facile binding assays allowing oriented immobilization of trimers.

Using these tools together with structural analysis the steric and allosteric relationships between the known antigenic sites were defined. This information may aid in design of new HIV-1 vaccines as well as in selecting the composition of future therapeutic antibody cocktails.

Epstein Barr virus vaccine design; importance of antigen presentation

Epstein-Barr virus (EBV) is associated with infectious mononucleosis and a variety of malignancies. It represents a major global health problem but still a vaccine is not available. EBV glycoprotein 350/220 (gp350) is the major target of immunity mediating attachment to B cells through complement receptor 2 (CR2/CD21).

In an article by Kanekiyo *et al* (8), the authors created self-assembling nanoparticles on which different domains of gp350 were displayed in a symmetric array (Fig 3). The nanoparticles were used to focus the presentation of the CR2-binding domain such that potent neutralizing antibodies could be elicited.

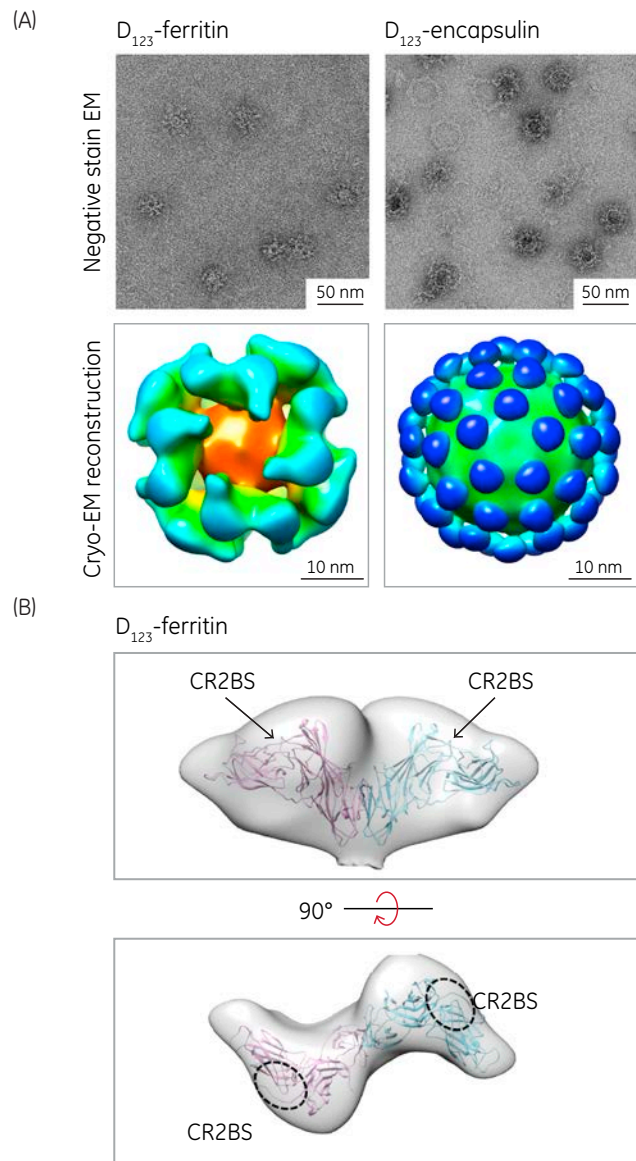


Fig 3. Self-assembling nanoparticles D₁₂₃-ferritin and D₁₂₃-encapsulin both displaying the CR2BS domain (reproduced under license agreement 3711870826672 with Elsevier).

In this paper, results using a Biacore system confirmed the presence of neutralizing antibodies with specificity for the immunogenic domain in sera from individuals immunized with the nanoparticles (Fig 4). gp350 WT protein was first immobilized to the sensor chip followed by injection of saturating concentrations of mAbs 721A (known to bind to CR2BS) or 2L10 (non-neutralizing anti-gp350 mAb). Immune sera were then injected (Fig 4A).

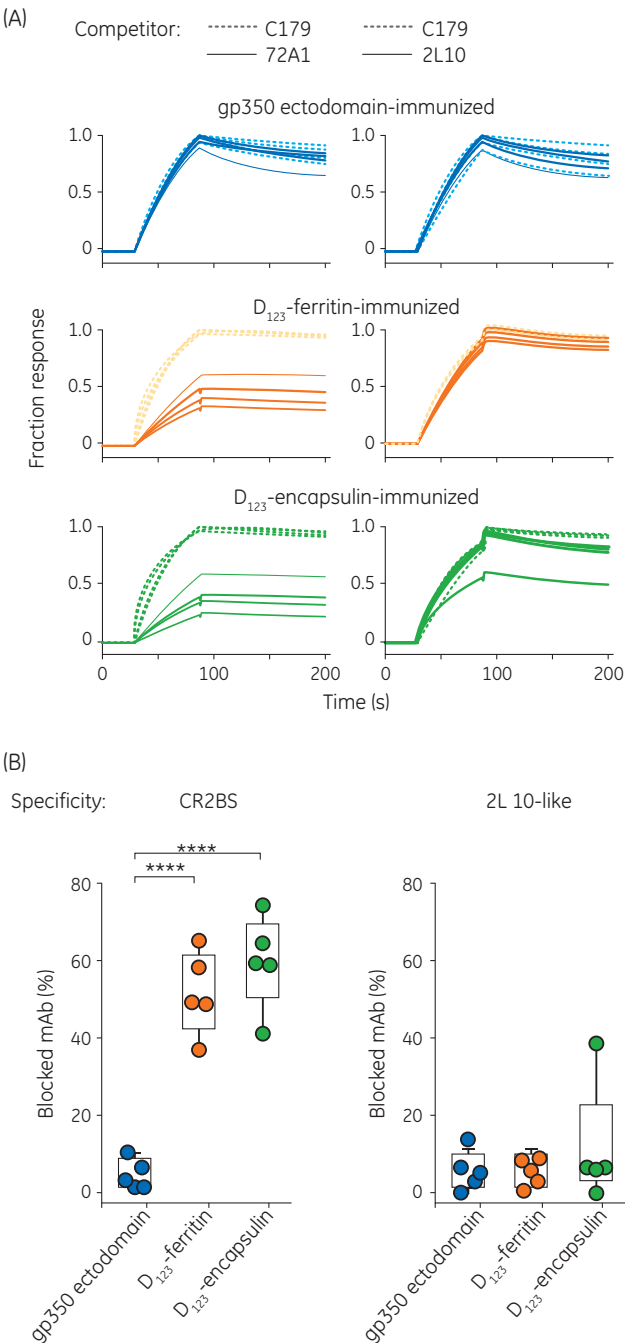


Fig 4. Detection of neutralizing antibodies with specificity for the CR2BS domain in immune sera, using a Biacore system. (A) Cross-competition assay of immune sera with saturating levels of mAbs 721A and 2L10 shown for different immunization groups. Each curve represents an individual serum. All data were normalized against C179-saturated curves (an irrelevant mAb) and are shown as fraction response. (B) Specificity of gp350-binding antibodies in immune sera displayed as percent inhibition by 721A or 2L10 (reproduced under license agreement 3711870826672 with Elsevier).

Results showed that immune sera from individuals immunized with the nanoparticles displayed decreased binding to the domain where mAb 721 was already bound and thus competed for the immunogenic area CR2BS (as opposed to sera from immunization with the gp350 ectodomain alone). The authors' approach to vaccine design, via an arrayed presentation of a conserved viral entry domain, may be applied to other viruses and emphasizes the importance of antigen presentation.

Influenza vaccine; antibody affinity maturation

Interactions between immune complexes (ICs) and Fc receptors (FcRs) mediate an array of cellular processes needed for maturation of vaccine-induced antibody responses. The type of FcRs involved and the structures of the Fc domain in the IC determine the type of cell signaling that will occur. IgG subclass and Fc glycan composition in turn determines the Fc structure. In a recent study by Wang *et al* (9) the authors characterized changes in the structures of Fc domains (IgG subclass and Fc glycan composition) on IgGs elicited by vaccination with the trivalent influenza vaccine. The results were further compared to the effect of immunizations with different types of immune complexes containing either sialylated Fc (sFc) or asialylated Fc (aFc) using a variety of *in vivo* and *in vitro* assays. In a Biacore experiment, purified serum IgG elicited from priming wild type or CD23^{-/-} mice with either sFc or aFc in hemagglutinin (HA) immune complexes, were immobilized to the sensor chip. HA was then injected and allowed to bind to the various sera IgGs on the surface, after which the IgG HA off-rate constants (k_d) were estimated. Figure 5 shows the general principle for off-rate estimations, displaying two different off-rates.

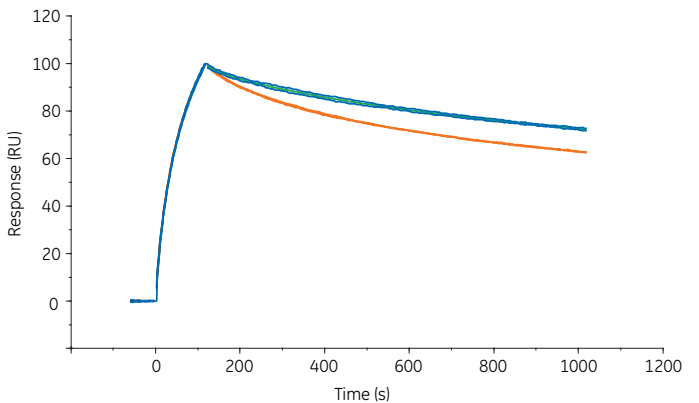


Fig 5. Overlay sensorgram showing binding (time 0 to 120 s) and dissociation (time 120 to 1000 s). Slow dissociation (blue curve) results in a lower off-rate constant (k_d) than the red curve.

A sialylated immune complex (sIC) priming protocol in wild-type mice elicited approximately 10- to 20-fold higher affinity (lower k_d) IgGs over asialylated immune complex (aIC) priming or sIC priming in CD23^{-/-} mice. Antibodies with higher affinity/ lower k_d against the highly conserved stalk domain of HA was elicited when priming was performed with sFc as opposed to aFc. This is significant because anti-stalk antibodies can mediate broad protection against influenza viruses. Further, the high-affinity IgGs were specific for CD23.

The results obtained with a Biacore system together with other *in vitro* and *in vivo* results led the authors to suggest a novel pathway for affinity maturation. Exploiting this pathway immunization with sICs could elicit high-affinity broadly neutralizing antibodies in future research.

Characterize the immune response; efficacy of adjuvant

This example describes a clinical study on screening serum samples to monitor and characterize the antibody response following vaccination against influenza virus with and without addition of the adjuvant, Matrix-M (10).

Two groups of elderly people were given the seasonal Vaxigrip™ influenza vaccine, either with or without adjuvant. Samples were taken prior to vaccination (day 0), and then 7, 28, 90, and 150 days after vaccination. Biacore 4000 was used to analyze 550 serum samples for their antibody response against hemagglutinin (HA) from the Brisbane B, California H1N1, and Perth H3N2 strains of influenza and Vaxigrip vaccine. The parallel configuration of Biacore 4000 enabled simultaneous screening of the binding between four different serum samples and four immobilized HA/vaccines in each cycle. Binding to HA and Vaxigrip vaccine was plotted against time for each individual (Fig 6). Most of the individuals peaked in antibody response at day 28. Some of them maintained fairly high antibody responses throughout the study, but, in most cases, the antibody responses declined after day 28.

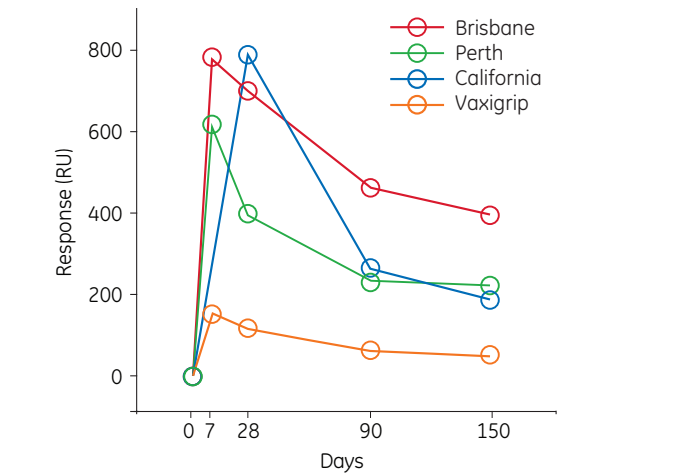


Fig 6. Response profiles from Biacore system showing responses over time for one individual. Samples were taken on days 0, 7, 28, 90, and 150.

Statistical analysis of Biacore data showed that the group who were administrated vaccine with adjuvant developed statistically more anti-Brisbane, anti-California, and anti-Vaxigrip antibodies at day 7 and 28 than the group that were provided with the regular vaccine formulation (Fig 7).

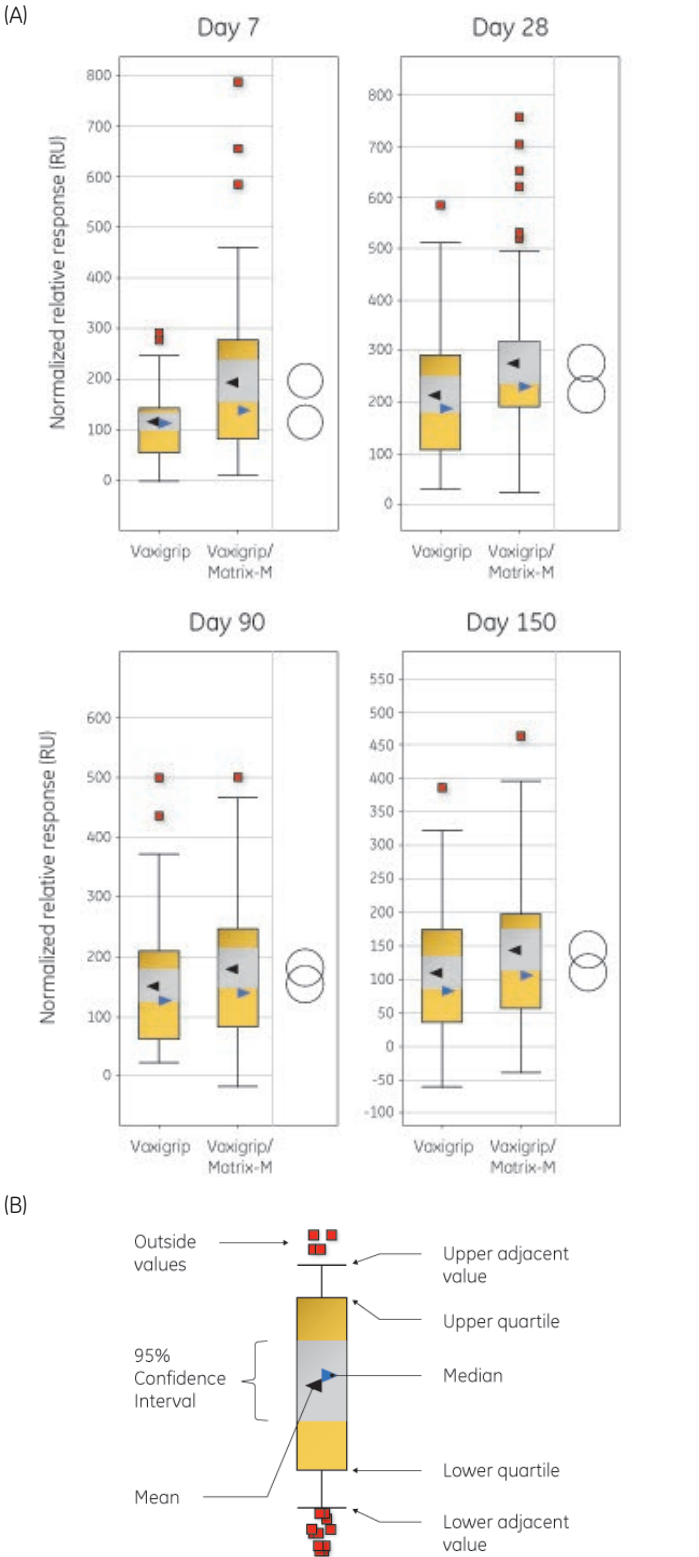


Fig 7. Statistical analysis of the anti-Brisbane responses from two groups administered with Vaxigrip vaccine, one with and one without Matrix-M adjuvant. The comparison circles display whether the group means for all pairs are significantly different from each other or not.

The immune responses were also characterized with respect to class and subclass distribution (10), a type of experiment recently performed also by Honda-Okubo *et al* (11) for influenza vaccine responses.

Characterization of immune responses using Biacore in this way offered several advantages in addition to the results verifying the effect of the adjuvant: Detailed information of the immune response in vaccinated individuals were obtained and the parallel analysis and high degree of automation made Biacore 4000 an efficient screening and characterization tool. Further, Biacore systems have the advantage of also detecting early immune responses that are characterized by antibodies with low affinity. Such immune responses are easily missed by alternative end-point assay-based techniques, due to losses during washing procedures, etc.

Analyses during the vaccine production process and QC

High quality analytical tools are required to measure the recovery, yield, and purity of the virus in vaccine process control and batch release as shown below in vaccines for polio, influenza, and virus-like particles (VLPs).

Polio vaccines

Poliovirus destroys motor neurons in the central nervous system causing irreversible paralysis. Thanks to extensive vaccination programs the risk of contracting poliomyelitis has been reduced drastically. However, this highly infectious disease has not yet been fully eradicated.

Polioviruses are small particles lacking envelope with a surface composed of 60 copies each of four capsid proteins. There are three serotypes which are all able to cause poliomyelitis, thus developed vaccines are trivalent. At Sanofi Pasteur, a Biacore assay was developed to be able to follow in real time the trivalent polio vaccine production process and to monitor the quality of the final product (12).

Monoclonal antibodies specific to each of the three viral types, along with a negative control antibody, were captured to a sensor chip via an anti-mouse IgG antibody using the Mouse Antibody Capture Kit (GE Healthcare) (Fig 8). The virus sample was then injected over all flow cells revealing sample specificity (data not shown).

The D-antigen, present on all serotypes, induces protective antibodies. Mild heating as well as other treatments may convert the D-antigen to the C or H forms which do not induce protective antibodies. Potency was determined by measuring native D-antigen content and heat treatment experiments showed the assay to be specific for this antigen form. The method was calibrated through an analysis of the European Working Standard (EWS, ref. P2160000) for trivalent poliovirus vaccines (Fig 9).

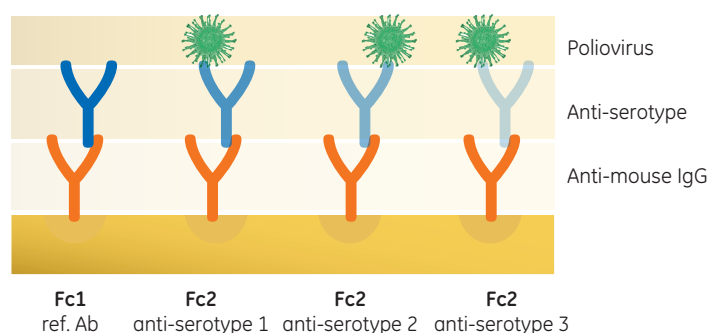


Fig 8. Assay principle. Monoclonal antibodies specific to each of the three viral types along with a negative control antibody were captured to a sensor chip via an anti-mouse IgG antibody.

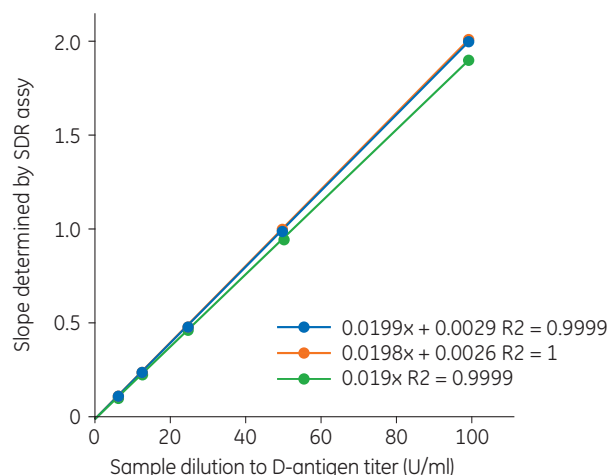


Fig 9. Linearity and reproducibility of type 1 response. EWS samples were diluted to different titers and injected on the sensor chip. Binding rate slopes were plotted against antigen titer. Triplicates on three different days for each serotype were analyzed. The response was linear to the antigen concentration (reproduced under license agreement 3711871374915 with Elsevier).

Accuracy and robustness of the assay was also investigated and the assay was found to have both high accuracy and a high level of robustness (data not shown).

Resulting Biacore data thus revealed assay specificity as well as quantitation levels and potency. The authors report several advantages that Biacore systems offer over the established ELISA assay including automation, high reproducibility, reduced work load, and reduced sample amounts. Further, the rapid protocol allowed monitoring of the industrial production process almost in real time.

Influenza vaccine

A challenge in influenza vaccine development is that the analytical tools available for quantitation of influenza virus are not satisfactory (13). The most commonly used method, single radial immuno diffusion (SRID), has major limitations due to low sensitivity, high variation, and being very labor intensive. Biacore systems can solve many of the analytical problems in vaccine process development and batch release. For example, Biacore T200 was used for the quantitation of virus hemagglutinin (HA) and host cell proteins (HCPs) in an influenza vaccine process (14, 15, 16).

The Biacore HA method showed higher sensitivity, precision, and recovery when compared to SRID (Table 1). In addition, the hands-on and analysis time was significantly reduced and for batch-release the three vaccine strains A/H1N1, A/H3N2, and B could be analyzed in one experiment.

Table 1. Comparison of Biacore and SRID assays

	Biacore	SRID
Standard curve range	0.5 to 10 µg HA/ml	5 to 30 µg HA/ml
Sensitivity		
Limit of detection	0.5 µg HA/ml	5 µg HA/ml
Limit of quantitation	1.0 µg HA/ml	10 µg HA/ml
Precision		
Number of samples CV < 5%	97	18
Recovery	95% to 105%	90% to 110%
Time for 100 samples		
Hands-on	1 to 2 h	6 to 8 h
Total	~ 17 to 18 h	~ 20 to 22 h

The results show that assays performed on Biacore systems have the potential to significantly improve vaccine development and manufacturing operations, as a complement to or replacement of existing methodologies.

VLP based vaccines

The use of VLPs with recombinant vaccines is popular. Currently, human vaccines against three different virus types use recombinant VLPs as the antigen: hepatitis B virus, human papillomavirus, and hepatitis E virus. VLPs are self-assembling bionanoparticles, typically 20 to 60 nm in diameter, that are successfully used as prophylactic vaccine antigens because they expose multiple epitopes on their surface and thereby mimic the native virions (17).

Gardasil™ (Merck Sharp and Dohme) and Cervarix™ (GlaxoSmithKline) are two prophylactic VLP based vaccines developed for the prevention of cervical cancer. Both manufacturers use Biacore systems to detect antigen availability in the VLPs (18, 19). Sanofi Pasteur have also used Biacore instrumentation to verify that adsorption of aluminium hydroxide gel does not have any negative effect on the surface of HbsAg particles, (20).

Shown here is an example from Merck Sharp and Dohme, for the VLP based vaccine against Hepatitis B, where mabs were used for probing structural changes during analysis of recombinant based VLP maturation (17, 21). A Biacore system and ELISA were used for tracking development of clinically relevant epitopes. In the Biacore assay, rabbit anti-mouse Fc antibodies were first immobilized to the sensor chip. The neutralizing mAbs RF1 or A1.2 were then captured to the surface followed by injection of rHBsAg VLPs (Fig 10). Results were compared to a reference lot.

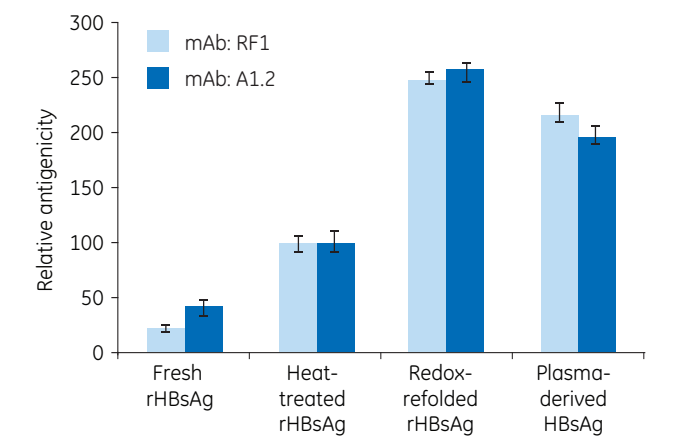


Fig 10. Measurement of rHBsAg VLP binding to the neutralizing mAbs RF1 and A1.2 using a Biacore system. Binding affinities were interpreted as relative antigenicity, with plasma-derived HBsAg particles as the reference*.

* Reproduced from the article Toolbox for Non-Intrusive Structural and Functional Analysis of Recombinant VLP Based Vaccines: A Case Study with Hepatitis B Vaccine by Mulder AM, Carragher B, Towne V, Meng Y, Wang Y, et al. PLoS ONE 7(4): e33235. doi:10.1371/journal.pone.0033235 (2012).

Results showed that the clinically relevant epitopes are sensitive to heat and/or redox treatment. Redox-treated rHBsAg VLPs showed comparable antigenicity to plasma-derived particles while fresh and heat-treated rHBsAg showed decreased antigenicity.

At Merck Sharp and Dohme, these types of experiments enabled quantitative monitoring of changes during bioprocessing and storage as well as for final product bound to adjuvant and the authors conclude that these methods are beneficial for all stages in vaccine development and manufacturing.

Conclusion

Biacore systems are widely used to design vaccines and adjuvants with the desired properties for the treatment of viral malignancies such as HIV, polio, influenza, hepatitis, and human papillomavirus. Automating processes to reduce assay time and sample volumes, these high-sensitivity instruments deliver accurate, highly reproducible, and quantitative binding data throughout the development process. Analyses such as high-resolution epitope mapping, kinetics/affinity, selectivity, and concentration enable researchers to develop a more detailed understanding of a vaccine’s properties and characteristics while ensuring that vaccine safety and efficacy is maintained.

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GE Healthcare Bio-Sciences AB, Björkgatan 30, SE-751 84 Uppsala, Sweden

GE Healthcare Bio-Sciences Corp. 100 Results Way, Marlborough, MA 01752, USA

GE Healthcare Dharmacon, Inc., 2650 Crescent Dr., Lafayette, CO 80026, USA

HyClone Laboratories, Inc., 925 W 1800 S, Logan, UT 84321, USA

GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare Japan Corporation, Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan

For local office contact information, visit www.gelifesciences.com/contact

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GE Healthcare UK Ltd,
Amersham Place,
Little Chalfont,
Buckinghamshire,
HP7 9NA, UK