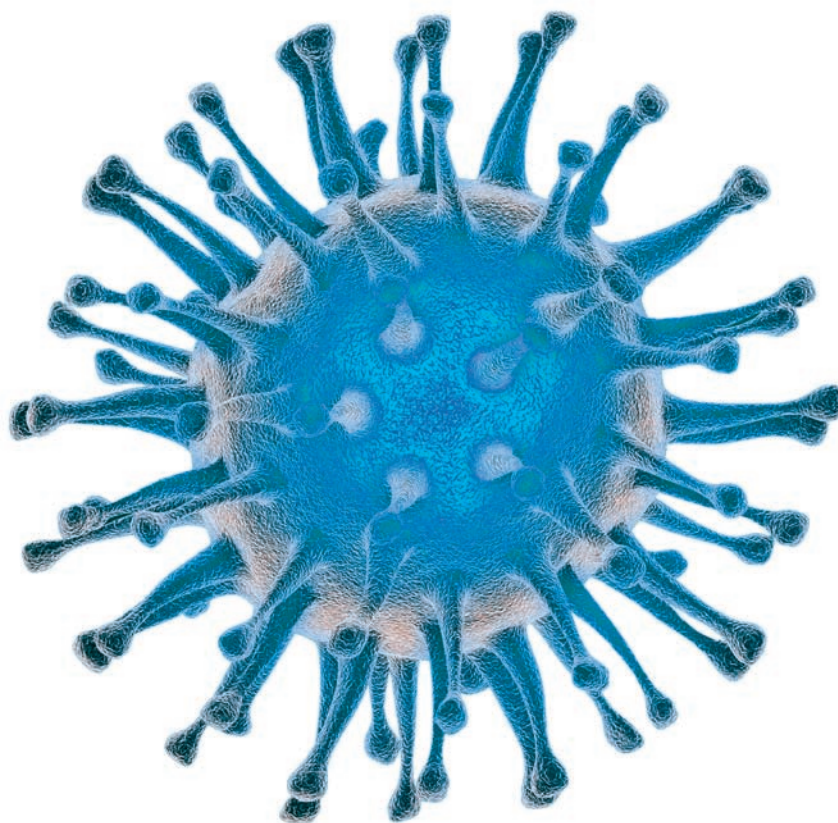


Label-free interaction and stability analysis in vaccine design and development



Analytical tools for modern vaccines

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 are a fraction of other biotherapeutics and very large quantities need to be manufactured, maintained, and distributed in a short time. To step up to the challenge, the vaccine industry is adopting new development methods as well as improved, more flexible manufacturing principles to increase production efficiency while assuring vaccine safety and efficacy.

Throughout the development process, biomolecular interaction- and stability data provided by Biacore™ and MicroCal™ systems contribute to detailed understanding of a vaccine's properties and characteristics (Fig 1).

Label-free interaction and stability data is used in areas such as:

- Vaccine research and discovery
- Formulation development
- Efficacy testing/immune response studies
- Manufacturing and QC tests

This white paper exemplifies how label-free, biophysical assays are successfully employed to design vaccines with desired properties, to speed up optimization of process parameters and formulation, and to perform batch release tests with great accuracy and precision.

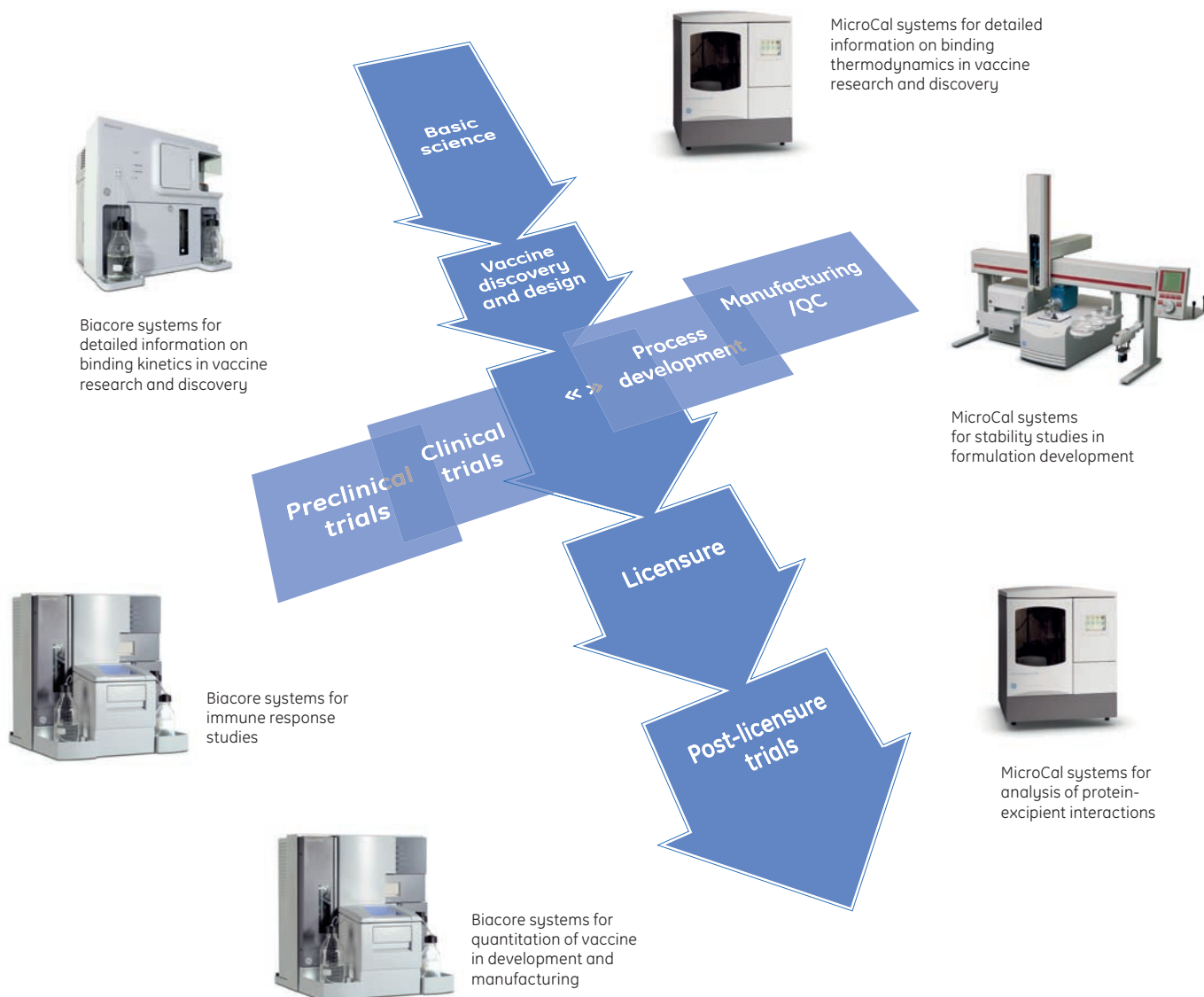


Fig 1. The vaccine development process is supported by Biacore and MicroCal from basic research to licensure.

Identify, explore, and characterize

The 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) and isothermal titration calorimetry (ITC) are orthogonal and complementary methods that advance vaccine research and design by providing accurate, precise, and rich characterization data about binding between viral epitopes and elicited antibodies (Fig 2).

Examples from scientific literature

Binding properties such as kinetics and thermodynamics are important parameters in early research and structure-guided design of protein-based vaccines. This is demonstrated below by studies of HIV, however, similar approaches have also been applied to malaria, influenza, hepatitis, and cancer (1).

The glycoprotein gp120 on the surface of the HIV envelope and the transmembrane glycoprotein gp41 are important for virus entry into the host cell and have been identified as targets for HIV vaccine development. Work on gp120 is challenging and very few broadly neutralizing antibodies (BNAb) are known due to frequent mutations in many antibody binding epitopes. In studies by Zhou *et al.* (2, 3),

a combination of ITC and SPR was successfully employed to find and characterize a conserved region of gp120 that binds to broadly neutralizing antibodies, making it a potential candidate for a gp120-derived vaccine.

Brunel *et al.* (4) investigated the interaction between the transmembrane protein gp41 and BNAb 4E10 with the purpose of identifying a peptide antigen for HIV vaccine development. By using SPR to study the variation in binding strength and kinetics for a set of peptides interacting with 4E10, the group determined the smallest number of essential amino acids required to obtain sufficient antigenicity while keeping the risk of unwanted immunogenicity to a minimum.

Sun *et al.* (5) studied structure and interaction between BNAb 4E10 and the membrane-proximal ectodomain region (MPER) of viral gp41 in a lipid environment, to gain insight into MPER antigenicity, immunogenicity, and viral function for purposes of vaccine design. Various biophysical methods were employed to reveal how an epitope buried in the membrane can be recognized by an antibody and demonstrates experimentally that membrane embedded MPER undergoes conformational change upon 4E10 binding in the lipid environment. To assess the potential importance of membrane binding on the 4E10 interaction with MPER, SPR and ITC were used in complementary experiments to unravel the kinetics and energetics of binding.

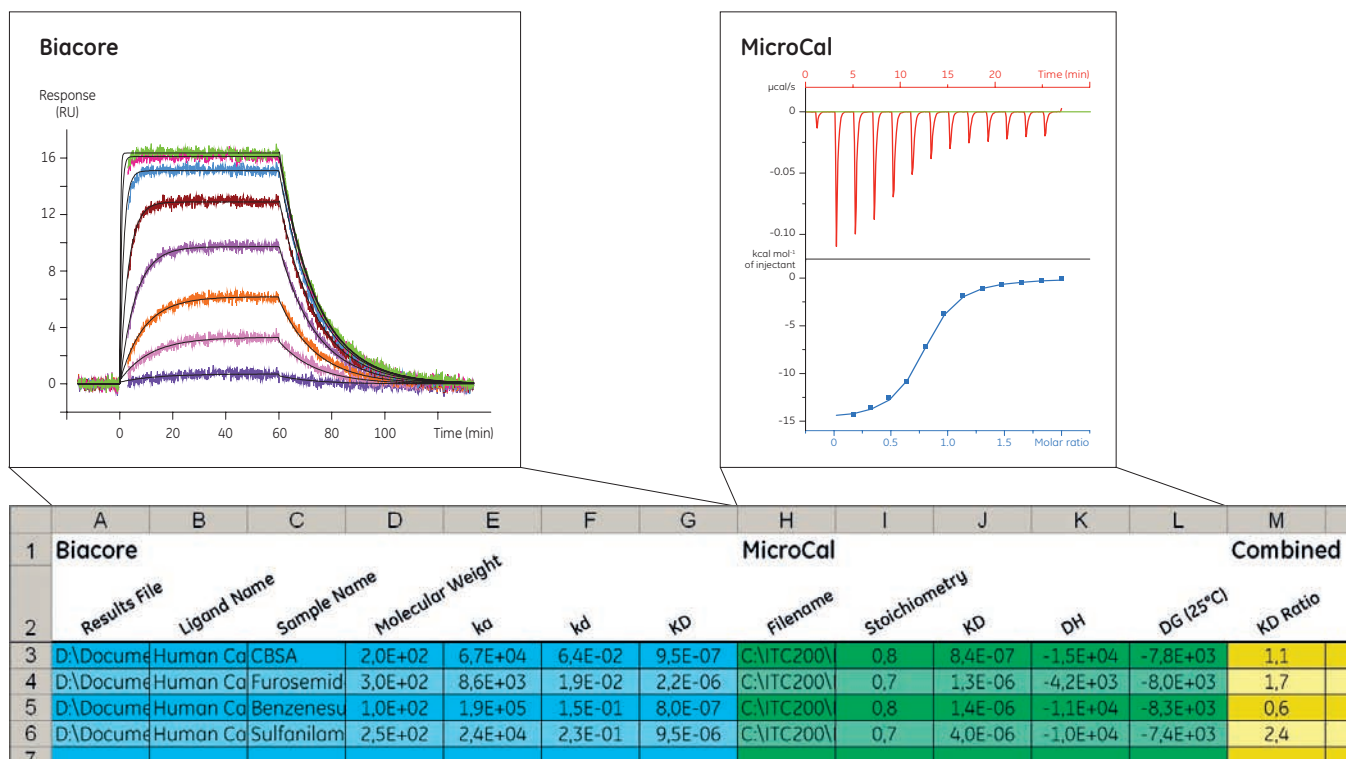


Fig 2. Bia2iTC software uses affinity data from Biacore experiments to guide efficient MicroCal iTC_{200} experimental design. This orthogonal approach builds confidence in the determined values and also helps in designing high quality ITC experiments with reduced time and material spent on optimizing conditions. Affinity, kinetic, and thermodynamic data is automatically exported for comparative data analysis in Microsoft™ Excel™.

Accelerate vaccine formulations development

Retaining biomolecular stability and activity during processing and formulation are essential for protein-based vaccines. One of the more important aspects of formulation development is the selection of excipients at optimal concentrations to provide extended shelf life while maintaining safety. Isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC) provide the ability to:

- Determine stabilizing conditions of a protein-based vaccine during formulation development
- Understand the mechanism(s) of protein-excipient interactions

Easily performed stability-indicating assays

By utilizing DSC and ITC, GlaxoSmithKline R&D (6) revealed that the structural integrity of a protein (ProX) was best maintained at a pH 5.7 in the presence of phenol, an antimicrobial agent. At this pH, the protein's tertiary structure remained intact (Fig 3).

As a protein stability-indicating assay, DSC facilitates the prediction of favorable formulations earlier in development compared to other commonly used techniques. By quickly screening various formulation buffers, DSC can provide considerable cost and time savings in preformulation development.

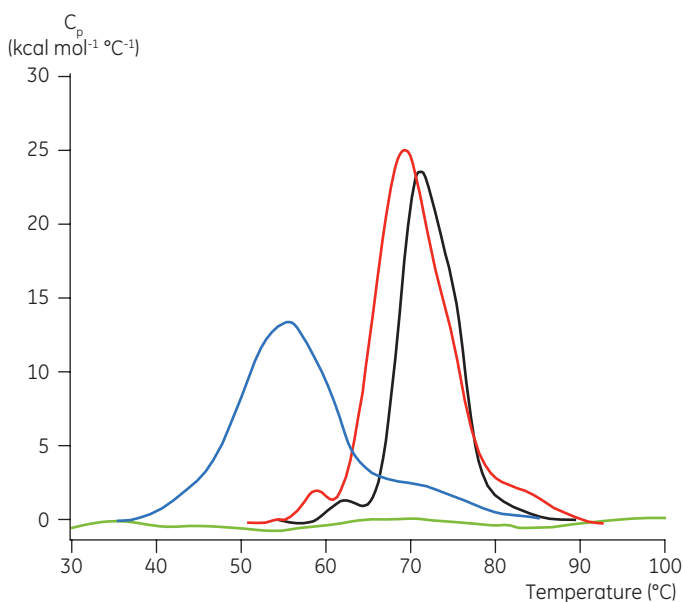


Fig 3. Heat capacity versus temperature (measured by DSC) for 1 mg/ml ProX in formulation buffer with 0.005% (v/v) phenol at pH 5.7 (black), and with 0% phenol at pH 5.7 (red), 4.5 (blue), and 3.5 (green). A higher transition midpoint (T_m) indicates a more stable protein, suggesting that the 0.005% (v/v) phenol at pH 5.7 is the preferred choice. Courtesy GlaxoSmithKline R&D (PA, USA).

Analysis of protein-excipient interactions

Microcalorimetry is increasingly used to explore protein-excipient interactions in the design and optimization of biotherapeutic and protein-based vaccine formulations. GlaxoSmithKline R&D used ITC to reveal how polysorbate-80, a surfactant commonly used to stabilize proteins, is surface adsorbed and aggregates with ProX. ITC provided information on the binding affinity constant, the enthalpy of binding and the number of binding sites per ProX molecule. Based on the isotherm, it was concluded that the polysorbate-80 binding sites were saturated at a molar ratio of surfactant per molecule of approximately 10 (Fig 4).

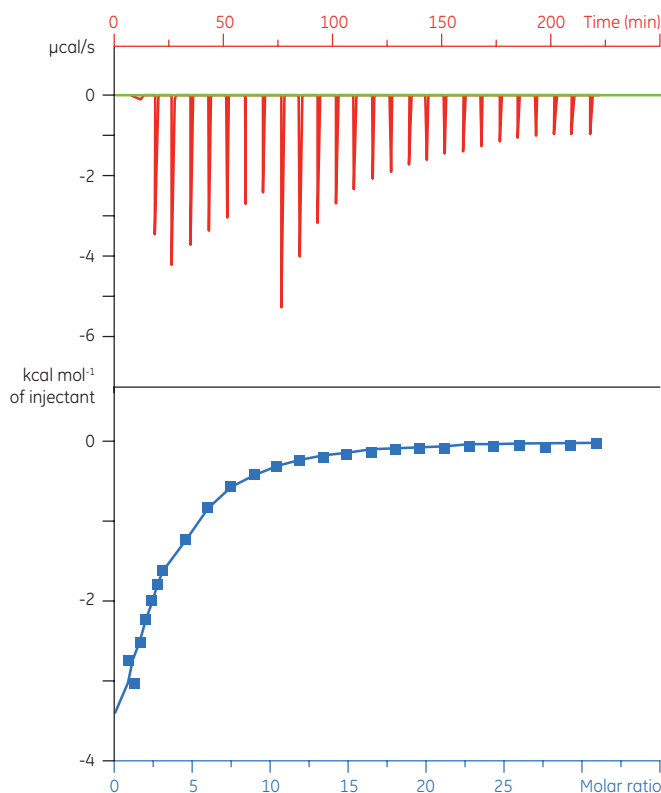


Fig 4. Differential power ($\mu\text{cal/sec}$) versus time for titration of 50 mM polysorbate-80 into 25 mg/ml ProX, as measured by ITC. Injections 9 to 12 were 3.8-fold greater in volume compared to injections 1 to 8. Courtesy GlaxoSmithKline R&D (PA, USA).

Knowledge of these binding parameters is useful for determining the lowest possible concentration of bound excipient needed to saturate the protein and attain the stabilizing effect. Keeping excipient amounts low is desirable since any additive is a potential risk for patient safety.

In another data set, ITC was used to evaluate the thermodynamics of any measurable binding between ProX and the preservative phenol. It was shown that binding of phenol to ProX reaches full saturation at molar ratios higher than 50.

The combination of ITC and DSC data helped to create a formulation having optimal antimicrobial capability and improved thermal stability.

Fully characterize the immune response

Successful development of a vaccine requires optimization of vaccine variants and immunization regimes, characterization of serum responses over extended time periods, and assessment of the biological effectiveness of the antibodies produced. Label-free interaction analysis using a Biacore system offers a number of advantages in immune response studies.

- Rapidly detect antibody response to vaccine candidates directly from serum samples
- Obtain multiple immune response characteristics (e.g., specific binding levels, binding stability, isotype, and subclass determination)
- Optimize immunization regimes (e.g., dosing schedules and adjuvant type and concentration)

Comprehensive characterization of immune response and optimization of immunization regimes

Detection of immune responses with Biacore systems has 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.

Biacore systems also enable comprehensive characterization of the immune response. Antibody maturation can be studied via assessment of antibody isotype and binding stability, and the changes in serum response patterns monitored following multiple immunizations.

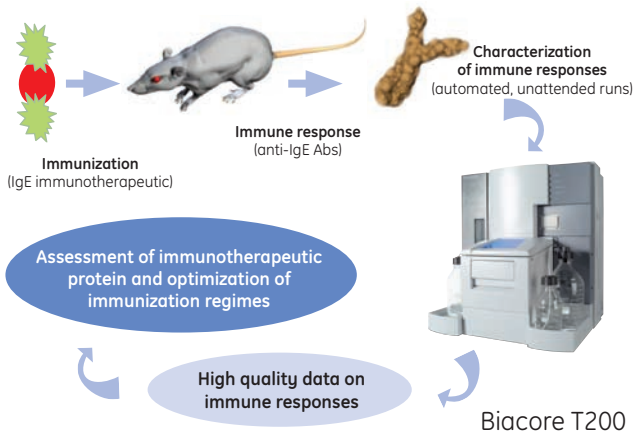


Fig 5. A Biacore system was used to characterize immune responses in a study aimed at developing an immunotherapeutic approach against atopic allergies and asthma.

The following example demonstrates how a Biacore assay was used to characterize immune responses in a study aimed at developing a general immunotherapeutic approach against atopic allergies and asthma (7).

Development involved the testing of different recombinant IgE proteins in a variety of animal species, dosing schedules, and adjuvants (Fig 5). Figures 6 and 7 show how some of these factors affected the level and character of the resulting immune responses.

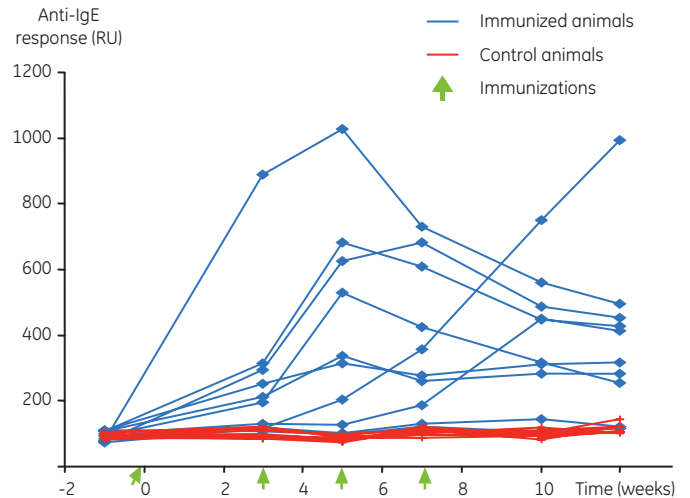


Fig 6. Rat serum responses to multiple immunizations with the immunotherapeutic protein using Adj-1. The anti-IgE response was measured in terms of binding response to full-length rat IgE immobilized on the sensor surface. Courtesy Resistentia Pharmaceuticals AB (Uppsala, Sweden).

Results from the Biacore assay were directly compared with the results from standard ELISAs run on the same samples. Comparison of the data sets showed a good correlation coefficient (data not shown).

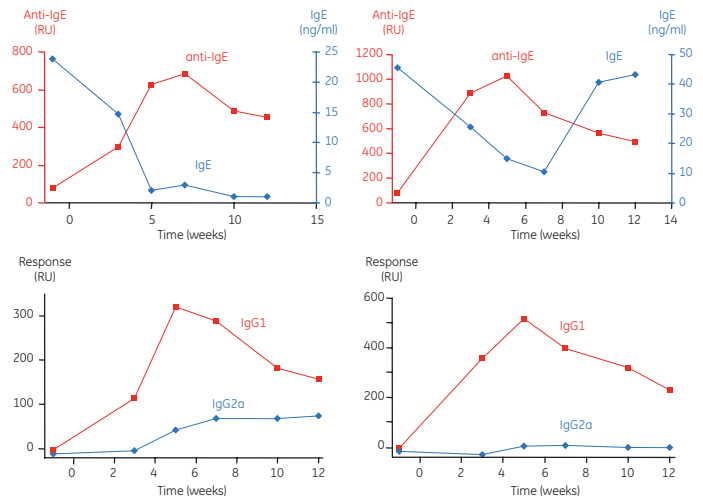


Fig 7. Comparison of total rat anti-IgE responses, circulating IgE levels, and anti-IgE subclass composition in two animals. Courtesy Resistentia Pharmaceuticals AB (Uppsala, Sweden).

Although the immune response level is one important parameter, alterations in isotype (i.e., IgG, IgA, IgM) or subclass (e.g., IgG1, IgG2a) distribution are equally important, since they indicate the maturation of the immune response. In this study, preliminary isotyping showed little or no IgA or IgM, and therefore focused on IgG subclass determinations.

Develop processes and batch release tests

High quality analytical tools are required to measure the recovery, yield, and purity of the virus in vaccine process control and batch release. A problem in influenza vaccine development is that the analytical tools available for quantitation of influenza virus are not satisfactory. 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 (8, 9). Quantitation of HA was compared to SRID (Table 1).

Accurate quantitation of influenza virus was achieved using an inhibition assay. The Biacore HA method showed higher sensitivity, precision, and recovery when compared to SRID (Table 2). In addition, the hands on and analysis time was significantly reduced, and for batch release three strains (A/H1N1, A/H3N2 and B) could be analyzed in one experiment (Fig 8).

Table 1. Quantitation of process samples; results from Biacore and SRID assays

Strain	Sample Type	Biacore HA (µg/ml)	CV (%) n=2	SRID HA (µg/ml)	CV (%) n=2
B					
Brisbane/3/2007	TBV*	31	0.4	33	7
	TBV*	43	0.6	36	5
	TBV*	43	2	30	8
	MBV*	260	0.02	244	16
A/H1N1					
Solomon Islands/3/2006	Harvest	8.3	10	8.0	7
	UFD	78	4	76	5
	TBV*	50	4	40	5
New Caledonia/20/99	Start 1	47	2	58	11
	Start 2	18	1	23	na
	Eluate 2	63	2	61	6
	Start 3	20	2	22	31
	Eluate 3	47	1	45	17
PR/8/34	Harvest	7.4	1	<LOD	na
	UFD	20	9	19	21
A/H3N2					
Wisconsin/67/2005	Harvest	1.3	4	<LOD	na
	NFF	1.0	2	<LOD	na
	DF	4.3	1	<LOD	na
	TBV	28	0.2	27	3
	TBV	38	0.3	35	8

TBV = trivalent bulk vaccine, MBV = monovalent bulk vaccine, Harvest = supernatant from infected MDCK cell culture, UFD = ultra- and diafiltration, LOD = limit of detection, Start = UFD filtrated virus diluted ten-fold in 10 mM NaP pH 7.4, Eluate 2 and Eluate 3 = fractions from chromatography of Start 2 and Start 3 in ~0.5 M NaCl, NFF = normal-flow filtration, DF = diafiltration, na = not analyzed in replicates.

* from Solvay Pharmaceuticals

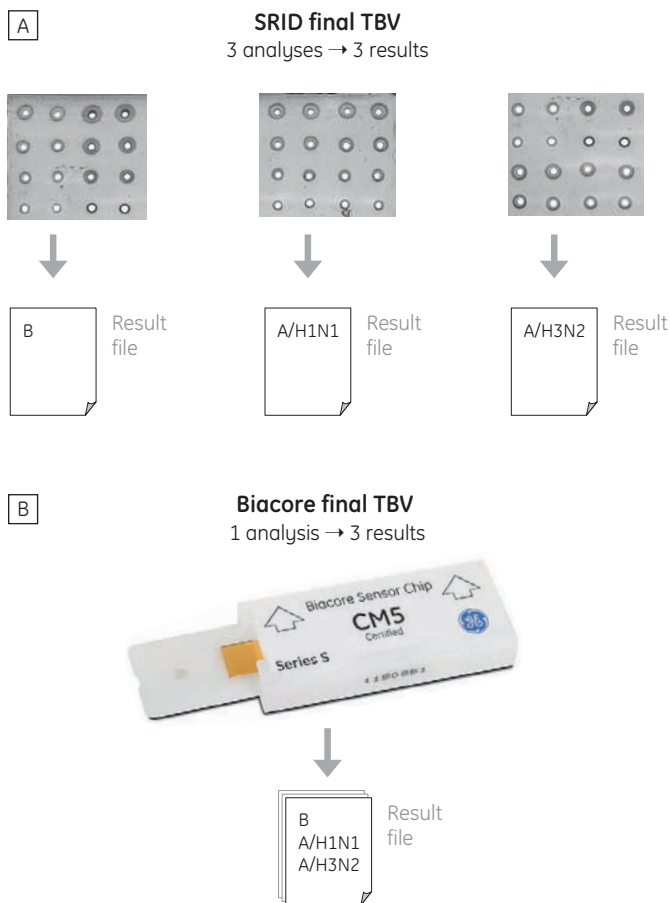


Fig 8. Using a Biacore assay, it was possible to simultaneously analyze the three virus strains in one experiment. The SRID assay required three different analyses.

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 (Table 2).

Table 2. 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:		
LOD*	0.5 µg HA/ml	5 µg HA/ml
LOQ†	1.0 µg HA/ml	10 µg HA/ml
Precision (no. 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

* LOD = Limit of detection

† LOQ = Limit of quantitation

Overview of technologies



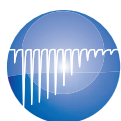
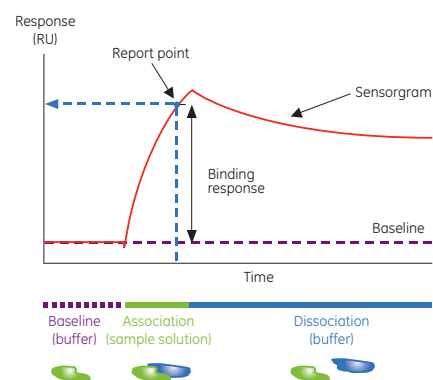
Binding strength and kinetics with Biacore systems

Biacore systems monitor molecular interactions in real-time using surface plasmon resonance (SPR). Without the need for labels, Biacore provides information on affinity, kinetics, and specificity of molecular interactions. The active concentration of biomolecules can also be determined.

One of the interacting molecules is immobilized onto a sensor surface, while the other molecule flows over the sensor surface in solution. Any interaction between the two is detected in real-time via changes in mass concentration close to the sensor surface and binding data is presented in a sensorgram where SPR responses in resonance units (RU) are plotted versus time.

The formation and dissociation of complexes are followed during the course of an interaction, with the binding kinetics (k_a , k_d) revealed by the shape of the binding curve.

More information can be found at www.gelifesciences.com/biacore



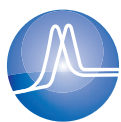
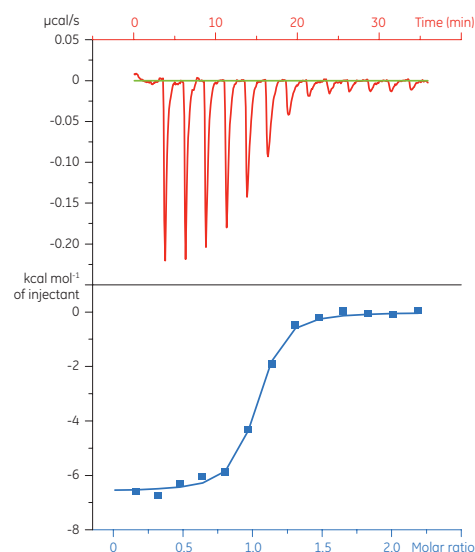
Binding strength and energetics with MicroCal ITC systems

Isothermal titration calorimetry (ITC) determines many binding parameters (n , K_D , ΔH , and ΔS) in a single experiment without the need for labels.

A solution of one biomolecule is titrated with a solution of its binding partner and the heat released upon interaction (ΔH) is monitored over time. Each peak represents a heat change associated with the injection of a small volume of sample. As successive amounts of sample are titrated into the ITC cell, the quantity of heat absorbed or released is in direct proportion to the amount of binding. At saturation, the heat signal diminishes until only heats of dilution are observed.

The binding curve, based on heat plotted against the ratio of ligand and binding partner in the cell, is analyzed to determine K_D , n , and ΔH .

More information can be found at www.gelifesciences.com/microcal

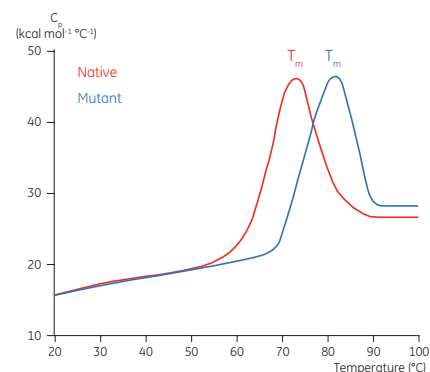


Protein stability with MicroCal DSC systems

In differential scanning calorimetry (DSC), as a biomolecule is heated at a constant rate, the heat change associated with thermal denaturation can be determined for proteins in their native state.

The measured transition midpoint (T_m) provides a quick and easy indication of stability, which can be used throughout the course of biotherapeutic development and formulation. Increases or decreases in thermal stability is observed as T_m shifts. A higher T_m reflects higher thermal stability, which correlates well with long-term stability.

More information can be found at www.gelifesciences.com/microcal



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