9th EBF Young Scientist Symposium

How to adapt an ADA method to a vaccine efficacy study

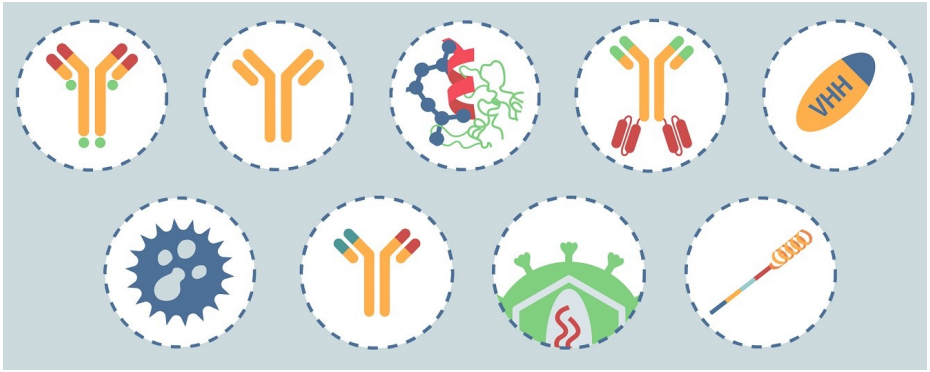
Swiss BioQuant

Sabrina Zadic

What are biotherapeutics?

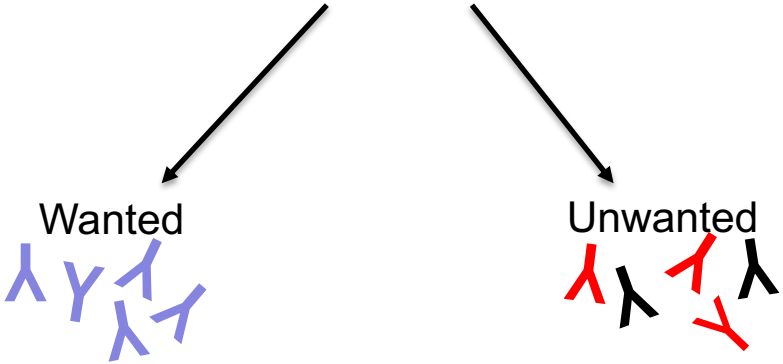
According to the **NLM-National Institutes of Health**, biotherapeutics are “antibody-drug cell therapy products where the active substance is extracted or produced from a biological source by a biotherapeutic technology.”

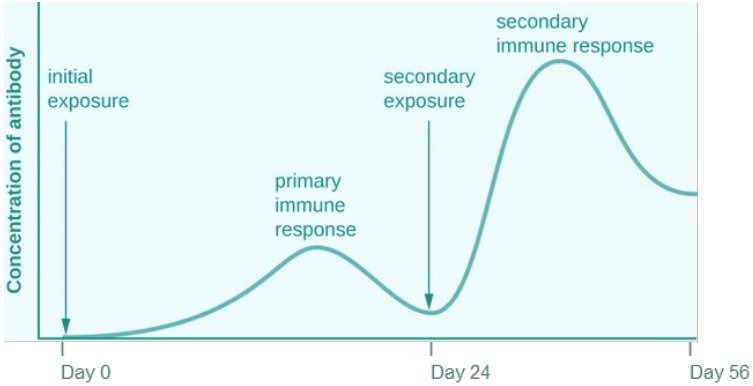
Biotherapeutic products include proteins and hormones, monoclonal antibodies, cytokines, antibodies, gene cell therapy products, vaccines, stem cell therapies, and more.



Immunogenicity and immune response

Immunogenicity is the ability of a “non-self” substance to stimulate the immune system by provoking an **immune response**





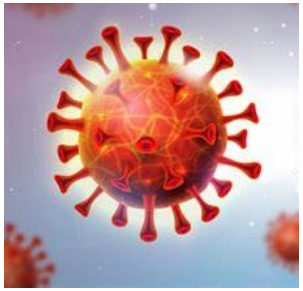
Immunogenicity Assays

Nowadays immunogenicity assays are mostly used to monitor and detect the unwanted response of the immune system, but the same type of assay can be used for a different purpose or rather to measure the wanted reaction of the immune system after a vaccination

ADA Investigation criteria

1. Screening cut point (SCP) and Confirmatory cut point (CCP)
2. Sensitivity and Precision
3. Selectivity
4. Drug tolerance
5. High dose hook effect
6. Stability

Corona Virus

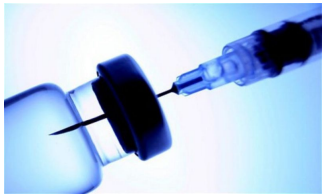


COVID-19 pandemic:

The disease is mainly transmitted via respiratory route

and the infection can occur over longer distances

→ Many novel candidate vaccines have been proposed

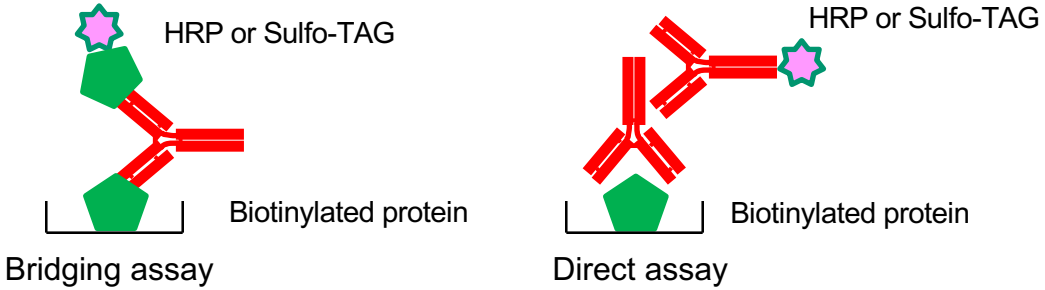


High request for new methods able to detect the immune response

Case Study

Purpose of the study

- The purpose of this study was a validation of an analytical method for the determination of antibodies against SARS-COV-2 S protein (omicron variant) in rat serum by an electrochemi-luminescence immunoassay (ECLIA).



Direct MSD immunoassay was used

Case Study

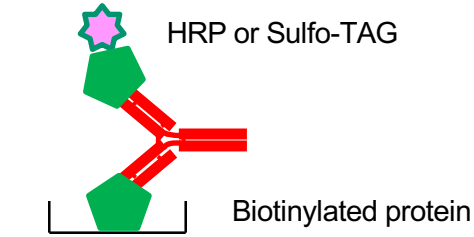
Method development on MSD platform

Fit for purpose method development was needed

1. Set-up

- Coating: SARS-CoV-2 trimer protein
- Detection: SulfoTAG SARS-CoV-2 trimer protein

No Signal



Bridging assay

Coating and detection have been tested at different concentrations

Case Study

Direct assay on MSD platform

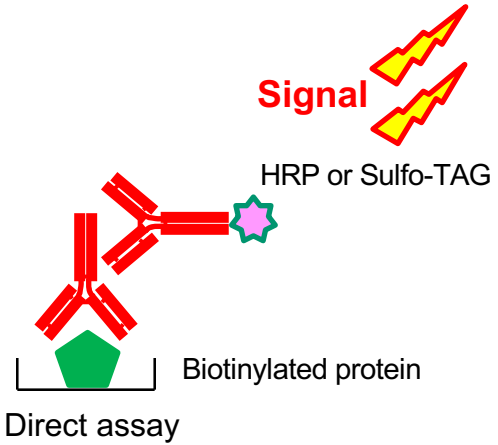
2. Set-up

Direct immunoassay on the MSD platform:

- Coating→ SARS-CoV-2 (Omicron) S trimer Protein
- Detection→ Goat anti Rat IgG HRP

Different conditions have been tested:

- Coating concentrations
- Different blockers
- Detection antibodies concentrations
- Increasing of washing steps
- Different MRDs



High background and low S/N ratio

Case Study

Direct assay on MSD platform

- MSD standard plate
- Coating: SARS-CoV-2 (Omicron) S1+S2 trimer Protein (ECD, His Tag) in 1xPBS (from 2 different lot numbers)
- Blocking: 5% MSD Blocker A or 5% MSD Blocker A + 1% human serum
- MRD: 100 and 500
- Detection: SulfoTAG Goat anti-rat IgG (H+L)

Plate layout

ng/mL	1	2	3	4	5	6	7	8
A	0	5% MSD	0	5% MSD	0	5% MSD	0	5% MSD
B	100	Blocker A	100	Blocker A	100	Blocker A	100	Blocker A
C	500	+ 1%	500	+ 1%	500	+ 1%	500	+ 1%
D	25000	human	25000	human	25000	human	25000	human
E	0		0		0		0	
F	100	5% MSD	100	5% MSD	100	5% MSD	100	5% MSD
G	500	Blocker A	500	Blocker A	500	Blocker A	500	Blocker A
H	25000		25000		25000		25000	
MRD	100				500			

S/N ratio

	1	2	3	4	5	6	7	8
A	1.00	5% MSD	1.00	5% MSD	1.00	5% MSD	1.00	5% MSD
B	0.78	Blocker A	0.75	Blocker A	0.95	Blocker A	0.94	Blocker A
C	1.34	+ 1%	1.37	+ 1%	**1.01**	+ 1%	1.03	+ 1%
D	1.71	human	1.84	human	4.84	human	5.41	human
E	1.00		1.00		1.00		1.00	
F	0.66	5% MSD	0.58	5% MSD	0.96	5% MSD	0.95	5% MSD
G	1.51	Blocker A	1.45	Blocker A	1.11	Blocker A	**1.10**	Blocker A
H	2.31		2.49		5.04		5.57	
MRD	100				500			

Case Study

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5% MSD Blocker A
MRD 500

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G	500	Blocker A	500	Blocker A	500	Blocker A	500	Blocker A
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H	2.31		2.49		5.04		5.57	
MRD	100				500			

Still high background due to the presence of cross-reacting antibodies in the individual serum, but after the vaccination the amount of antibodies will be much higher: not a typical ADA study !

Case Study

Sample testing from the pre-tox study

- With the same method we analyzed 6 samples provided by the sponsor

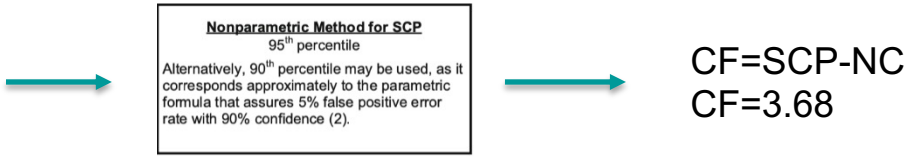
	Mean ECLU signal
Pre-dose	3037
Pre-dose	2809
Pre-dose	2830
22 days post-treatment	330701
22 days post-treatment	415478
22 days post-treatment	353094

Average fold increase: 127

Case Study

1-The Screening (SCP) and Confirmatory (CCP) assay cut point were determinied in six batches

1. Analyze NC data, identify and exclude outliers
2. Analyse 25 individuals by 2 different analysts in 6 different runs
3. Normalize data as ration of response signal to noise. All further analysis is on Log (S/n) scale, if no transformation is needed, then S-N may be used
4. Use the box plot analysis to identify and exclude outliers and re-evaluate the distributions
5. Neither the signal nor the log-transformed data were normally distributed after Shapiro-Wilk analysis



Case Study

1-The Screening (SCP) and Confirmatory (CCP) assay cut point were determinied in six batches

- Proposed PCs (dilution curve prepared with surrogate positive control)

Analyte	PC1 [ng/mL]	PC2 [ng/mL]	PC3 [ng/mL]	PC4 [ng/mL]	PC5 [ng/mL]	PC6 [ng/mL]	PC7 [ng/mL]
surrogate standard	1000	2000	4000	8000	16000	32000	64000

- Proposed preliminary QCs

Analyte	pLQC [ng/mL]	pHQC [ng/mL]
surrogate standard	5000	50000

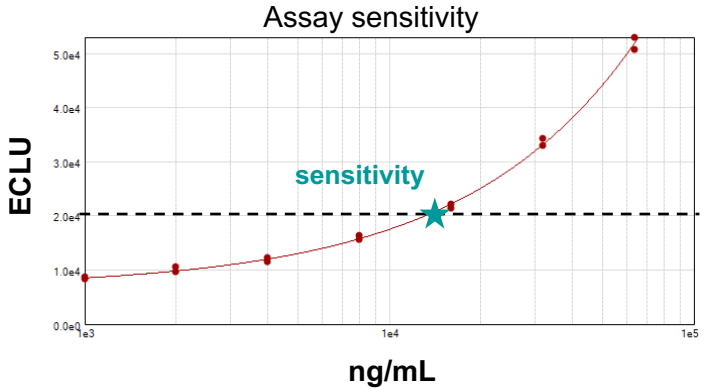
The determination of the CCP was performed together with the determination of the SCP

The same individual blank matrix samples used for the determination of the SCP, were incubated with excess of drug

Case Study

2-Sensitivity and precision of the screening assay

Sensitivity is determined interpolating the plate-specific SCP to the dilution curve



Assay sensitivity=19700 ng/mL

One QC level has been selected: **50000 ng/mL**

This concentration has been used to determine additional parameters: drug tolerance, stability, sensitivity, HDH

Case Study

Sample testing in the tox study - Confirmatory assay results

SBQ-no	Group	Gender	Animal	Day	Batch	CSS	CID	% ID	Result
S0061	3	male	31	44	4	274000	76800	72.0	POSITIVE
S0062	3	male	32	44	4	259000	48400	81.3	POSITIVE
S0063	3	male	33	44	4	268000	59100	77.9	POSITIVE
S0064	3	male	34	44	4	335000	112000	66.6	POSITIVE
S0065	3	male	35	44	4	149000	41600	72.1	POSITIVE
S0066	3	male	36	44	4	334000	89800	73.1	POSITIVE
S0067	3	male	37	44	4	255000	76100	70.2	POSITIVE
S0068	3	male	38	44	4	269000	55400	79.4	POSITIVE
S0069	3	male	39	44	4	286000	80300	71.9	POSITIVE
S0070	3	male	40	44	4	390000	147000	62.3	POSITIVE
S0071	3	female	76	44	5	175000	39900	77.2	POSITIVE
S0072	3	female	77	44	4	222000	51400	76.8	POSITIVE
S0073	3	female	78	44	4	276000	72900	73.6	POSITIVE
S0074	3	female	79	44	5	179000	25300	85.9	POSITIVE
S0075	3	female	80	44	4	325000	105000	67.7	POSITIVE
S0076	3	female	81	44	4	244000	84500	65.4	POSITIVE
S0077	3	female	82	44	4	166000	47400	71.4	POSITIVE
S0078	3	female	83	44	4	325000	96200	70.4	POSITIVE
S0079	3	female	84	44	4	286000	97300	66.0	POSITIVE
S0080	3	female	85	44	5	127000	22800	82.0	POSITIVE
S0081	3R	male	41	57	5	61800	7440	88.0	POSITIVE
S0082	3R	male	42	57	5	80600	7760	90.4	POSITIVE
S0083	3R	male	43	57	5	163000	18100	88.9	POSITIVE
S0084	3R	male	44	57	5	167000	19000	88.6	POSITIVE
S0085	3R	male	45	57	5	183000	17600	90.4	POSITIVE
S0086	3R	female	86	57	5	230000	36800	84.0	POSITIVE
S0087	3R	female	87	57	5	180000	27100	84.9	POSITIVE
S0088	3R	female	88	57	5	176000	30800	82.5	POSITIVE
S0089	3R	female	89	57	5	219000	29200	86.7	POSITIVE
S0090	3R	female	90	57	5	195000	30000	84.6	POSITIVE

All treated animals have been confirmed positive

Screening assay	ECLU signal (mean)
Pre-dose	608
44 days post-treatment	893600

Average fold increase 1470

CSS: confirmatory screening signal
CID: conf ID signal
%ID: % Immunodepletion

Conclusions

- Covid-19 pandemic: high request for new methods able to detect the immune response
- Direct immunoassay was used
- Coating and detection have been tested at different concentrations
- Still high background due to the presence of cross-reactive antibodies in the individual serum however this has not impacted the suitability of the method since vaccinated animals showed very high signals
- After the analysis of the 6 cut point runs, the sensitivity of the assay (and as well the calculated LQC) was much higher then the initial planned, for this reason only a single QC level was used in 4 sets.

It`s possible to use an ADA method for a vaccine efficacy study!

Acknowledgment

LBA analysis group and all SBQ members

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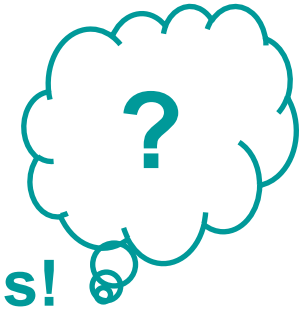
Mark Enzler (CSO)



Time for questions!