An alternative approach to the classic anti-drug antibody (ADA) titer assay used in a clinical trial

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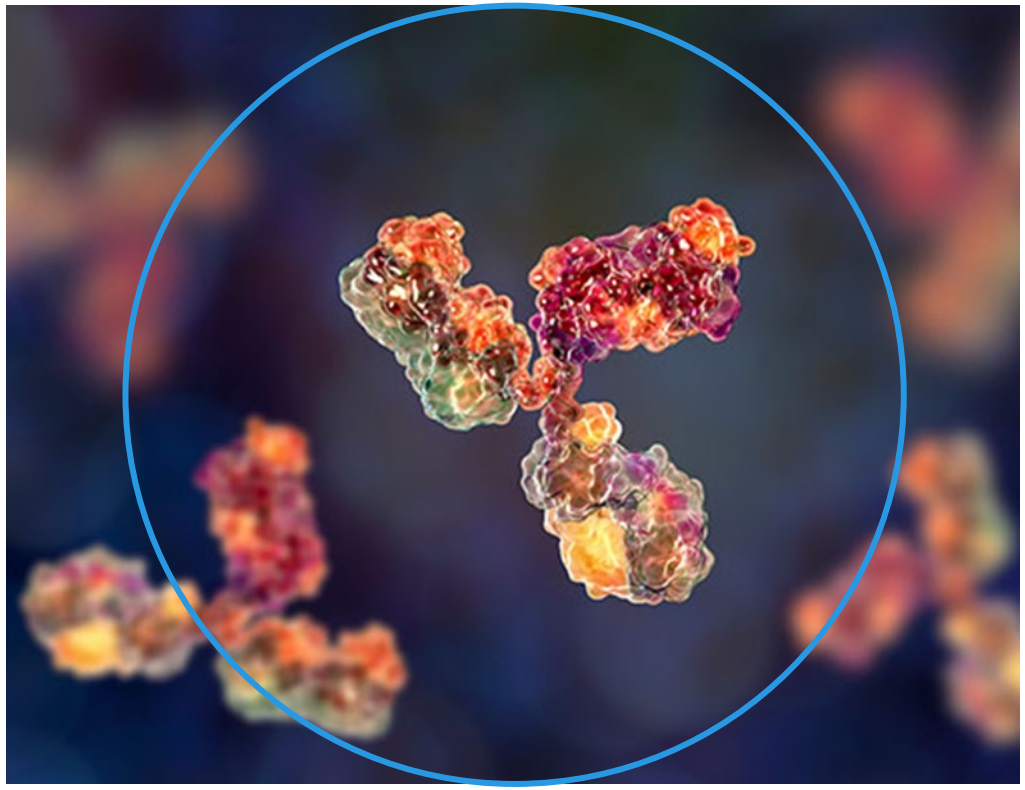
Immunogenicity

The ability of a particular substance to provoke an immune response in the body.

It can be:

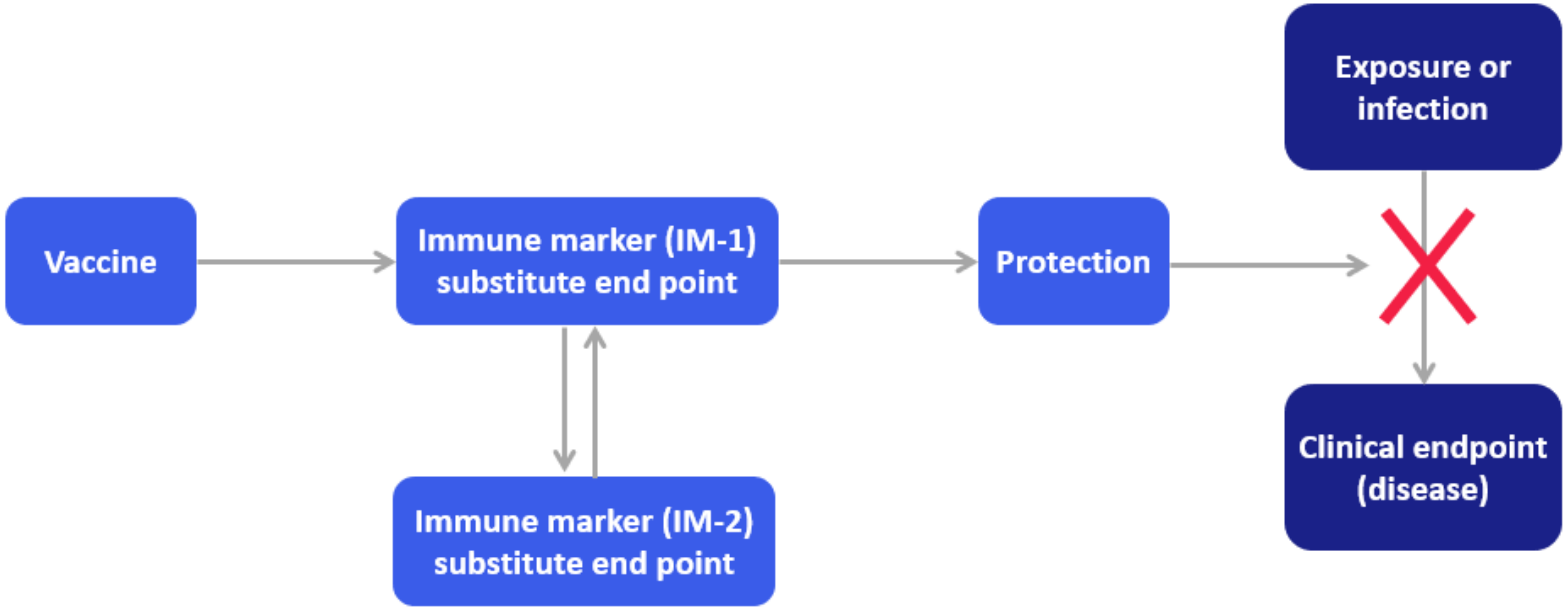
- Required: when assessing a vaccine
- Unwanted: response to a therapeutic

Monitoring the immune response against a therapeutic is critical for any clinical trial, as it can affect both efficacy and safety.



https://www.scientistlive.com/content/antibody-development-service-announced

Induction of protective immunity by a preventive vaccine



Arrows imply direct causal relationship

A preventative vaccine is a biological product that can be used to induce an immune response that provides protection against infection and/or disease on subsequent exposure to a pathogen

Therapeutic vaccines

- Used after a disease or infection has already occurred as a treatment to certain diseases or to slow their progression
- First approved therapeutic vaccine in 2010 (Sipuleucel-T, commercial name PROVENGE®)
- Immunotherapy treatment option
- To induce an immune response, improve clinical outcome
- Can be cellular, nucleic acid, virus-based, etc.

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Screening ADA vs. titer ADA assays

ADA assays

- Routinely used when assessing a therapeutic
- Positive ADA response is unwanted and could be a cause for concern
- Qualitative

Titer ADA assays

- Used when assessing a vaccine (both preventative and therapeutic)
- Positive ADA response is required
- Measure the scale of an anti-drug antibody response
- Quasi-quantitative

Key elements of ADA assays

- Cut point (screening of naïve population)
- Minimum required dilution (MRD)
- Sensitivity and drug tolerance
- Specificity and selectivity
- Confirmatory assay

Key elements of titer ADA assays

- Cut point (for example: mean buffer blank + 3x SD)
- At least 5 dilutions (ideally 2 above and 3 below the cut point)
- Minimum significant ratio (to establish validation criteria for quality controls)
- End point titer (EPT): the highest dilution that gives a reading above the cut point
- No requirement for confirmatory assay

Case Study

- Previous experience in the preclinical phase
- Titer assay to measure the immune response to a therapeutic vaccine in a clinical trial
- No surrogate positive control available
- ADAs against the target naturally present in the matrix

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In pursuit of an assay

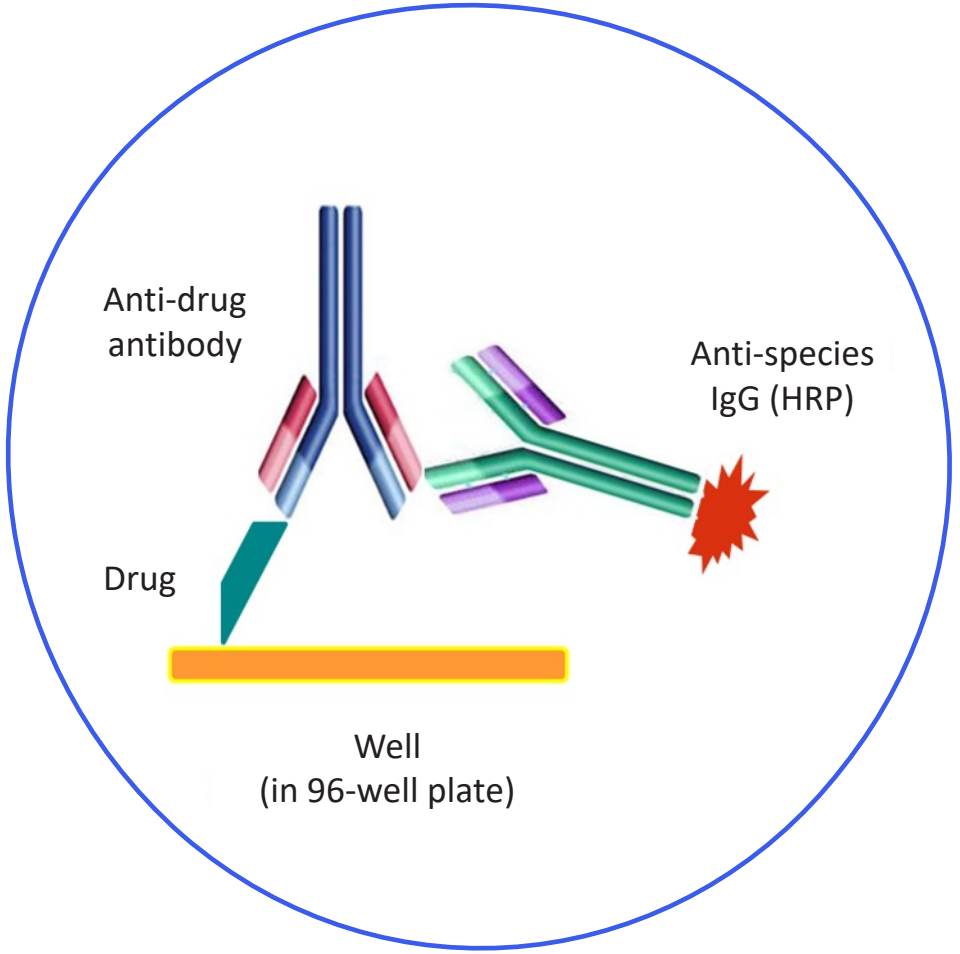
In the beginning

- Classic ADA titer approach:

  Direct ELISA: a surrogate of the test item used as a capture reagent. Anti-species IgG HRP (Horseradish peroxidase) as a detection reagent

Main issue

- High background (including high endogenous levels) masking the specific instrument response

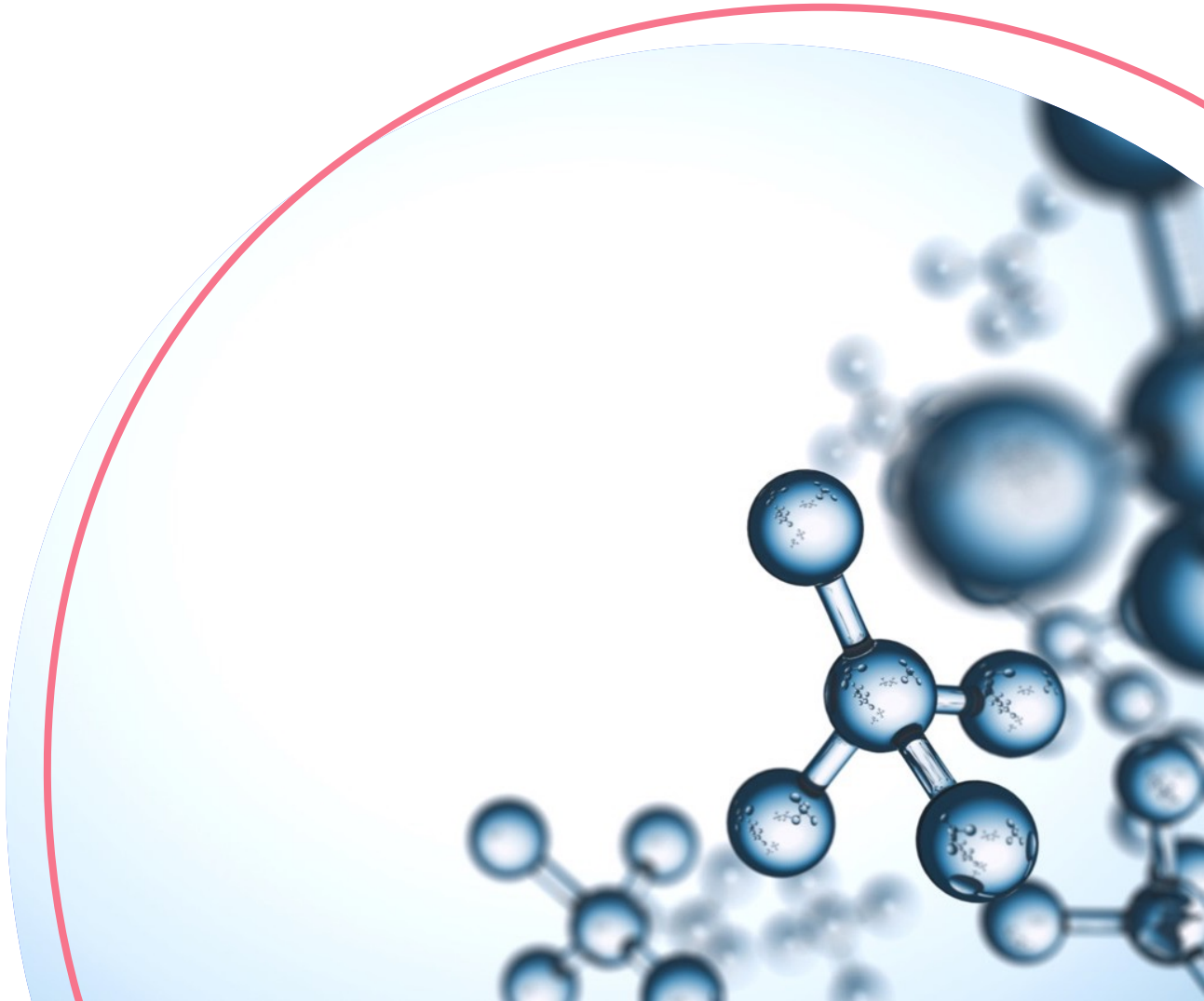


Strategies to reduce the high background

- Change of the blocking buffer and investigation different blocking proteins
- Further dilutions of samples
- Optimisation of critical regents (labelled antibodies, etc.)
- Increased number of washing cycles
- Investigation of using diluted negative matrix to calculate the cut point instead of using assay buffer
- Positive control: pooled patients population matrix
- Various formats
- Different reagents

Other strategies that were not investigated

- MRD (not applicable for titer assays)
- Confirmatory assay



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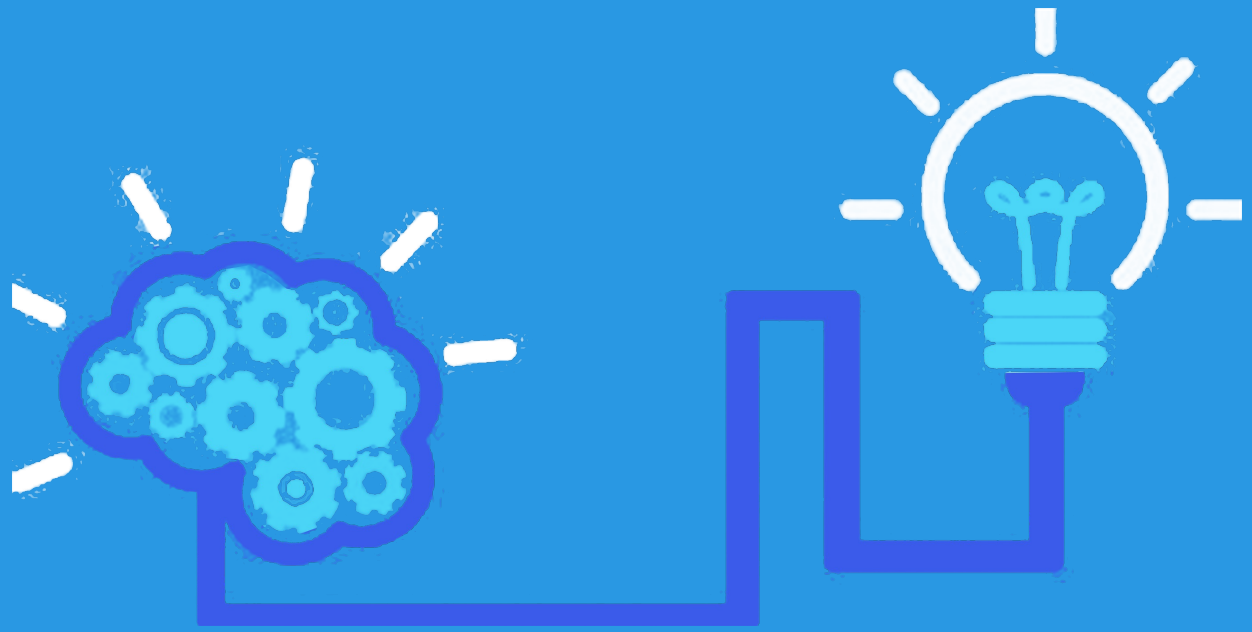
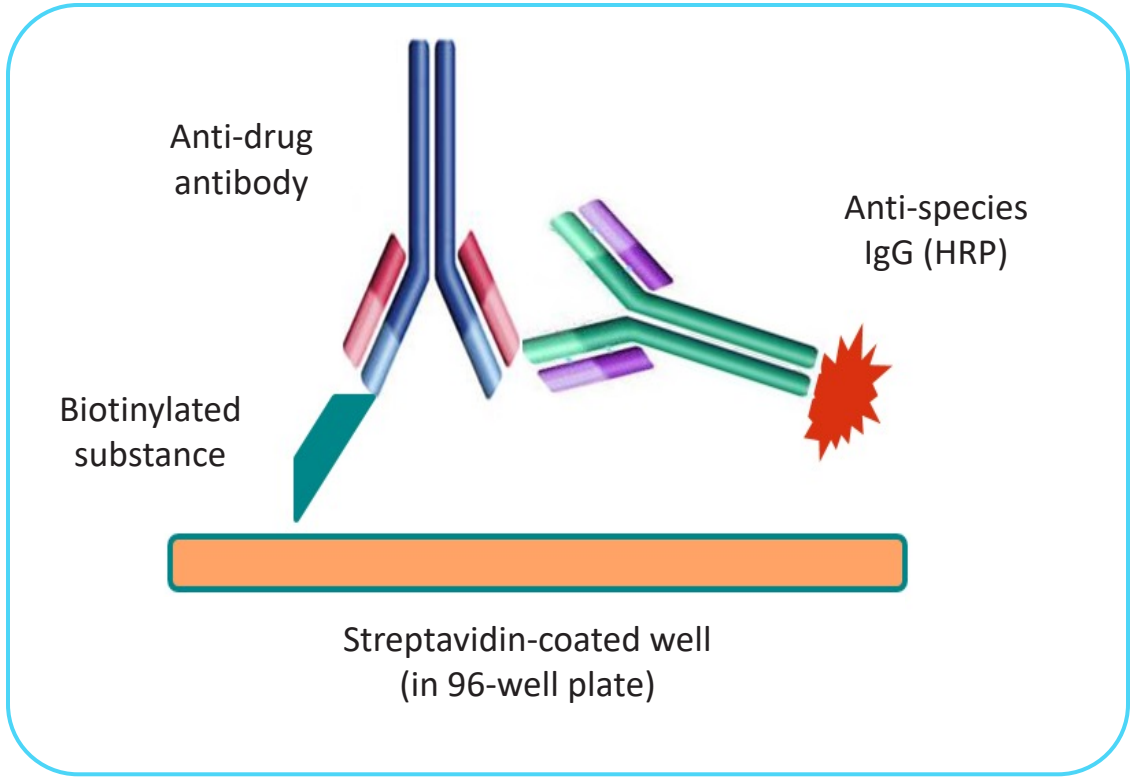


When the usual strategies to reduce the high background fail to improve the assay…

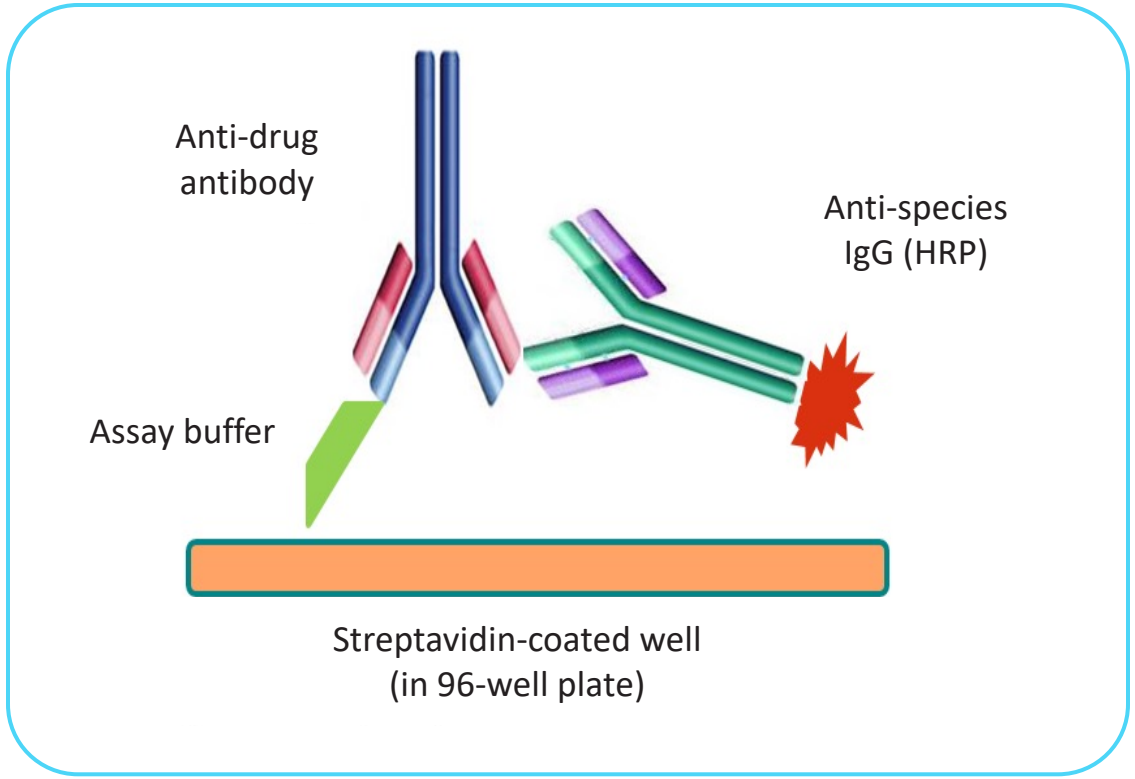
…it is time to revise what we have…
…and introduce an alternative approach…

https://asktraining.com.sg/8-ways-to-improve-your-problem-solving-skills/

Assay format for each side of the 96-well plate



Total instrument response



Non-specific instrument response

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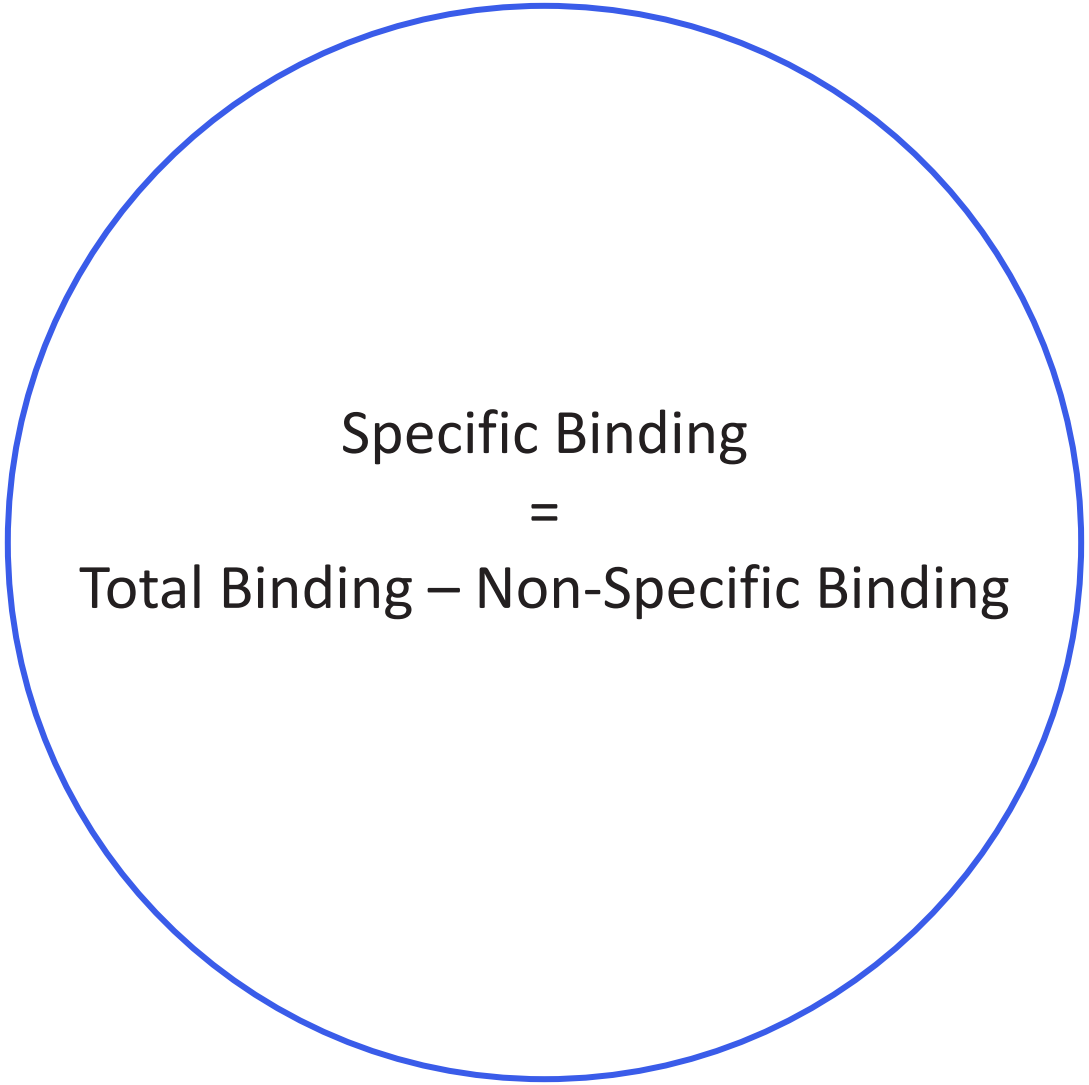
Solution



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Example of plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC 1 in 10	PC 1 in 20	PC 1 in 40	PC 1 in 80	PC 1 in 160	Assay Buffer	PC 1 in 10	PC 1 in 20	PC 1 in 40	PC 1 in 80	PC 1 in 160	Assay Buffer
B	PC 1 in 10	PC 1 in 20	PC 1 in 40	PC 1 in 80	PC 1 in 160	Assay Buffer	PC 1 in 10	PC 1 in 20	PC 1 in 40	PC 1 in 80	PC 1 in 160	Assay Buffer
C	NC 1 in 10	NC 1 in 20	NC 1 in 40	NC 1 in 80	NC 1 in 160	Assay Buffer	NC 1 in 10	NC 1 in 20	NC 1 in 40	NC 1 in 80	NC 1 in 160	Assay Buffer
D	NC 1 in 10	NC 1 in 20	NC 1 in 40	NC 1 in 80	NC 1 in 160	Assay Buffer	NC 1 in 10	NC 1 in 20	NC 1 in 40	NC 1 in 80	NC 1 in 160	Assay Buffer
E	PC 1 in 10	PC 1 in 20	PC 1 in 40	PC 1 in 80	PC 1 in 160	Assay Buffer	PC 1 in 10	PC 1 in 20	PC 1 in 40	PC 1 in 80	PC 1 in 160	Assay Buffer
F	PC 1 in 10	PC 1 in 20	PC 1 in 40	PC 1 in 80	PC 1 in 160	Assay Buffer	PC 1 in 10	PC 1 in 20	PC 1 in 40	PC 1 in 80	PC 1 in 160	Assay Buffer
G	NC 1 in 10	NC 1 in 20	NC 1 in 40	NC 1 in 80	NC 1 in 160	Assay Buffer	NC 1 in 10	NC 1 in 20	NC 1 in 40	NC 1 in 80	NC 1 in 160	Assay Buffer
H	NC 1 in 10	NC 1 in 20	NC 1 in 40	NC 1 in 80	NC 1 in 160	Assay Buffer	NC 1 in 10	NC 1 in 20	NC 1 in 40	NC 1 in 80	NC 1 in 160	Assay Buffer
	Biotinylated Substance coated (providing total instrument response)						Assay Buffer coated (providing non-specific instrument response)					

PC: Positive Control
NC: Negative Control

Data analysis?

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC 1 in 10	PC 1 in 20	PC 1 in 40	PC 1 in 80	PC 1 in 160	Assay Buffer	PC 1 in 10	PC 1 in 20	PC 1 in 40	PC 1 in 80	PC 1 in 160	Assay Buffer
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	Biotinylated Substance coated (providing total instrument response)						Assay Buffer coated (providing non-specific instrument response)					

Instrument response (A1) – Instrument response A-7 = Specific instrument response for sample PC 1 in 10

Cut point = 3 x Assay buffer mean instrument response

PC: Positive Control

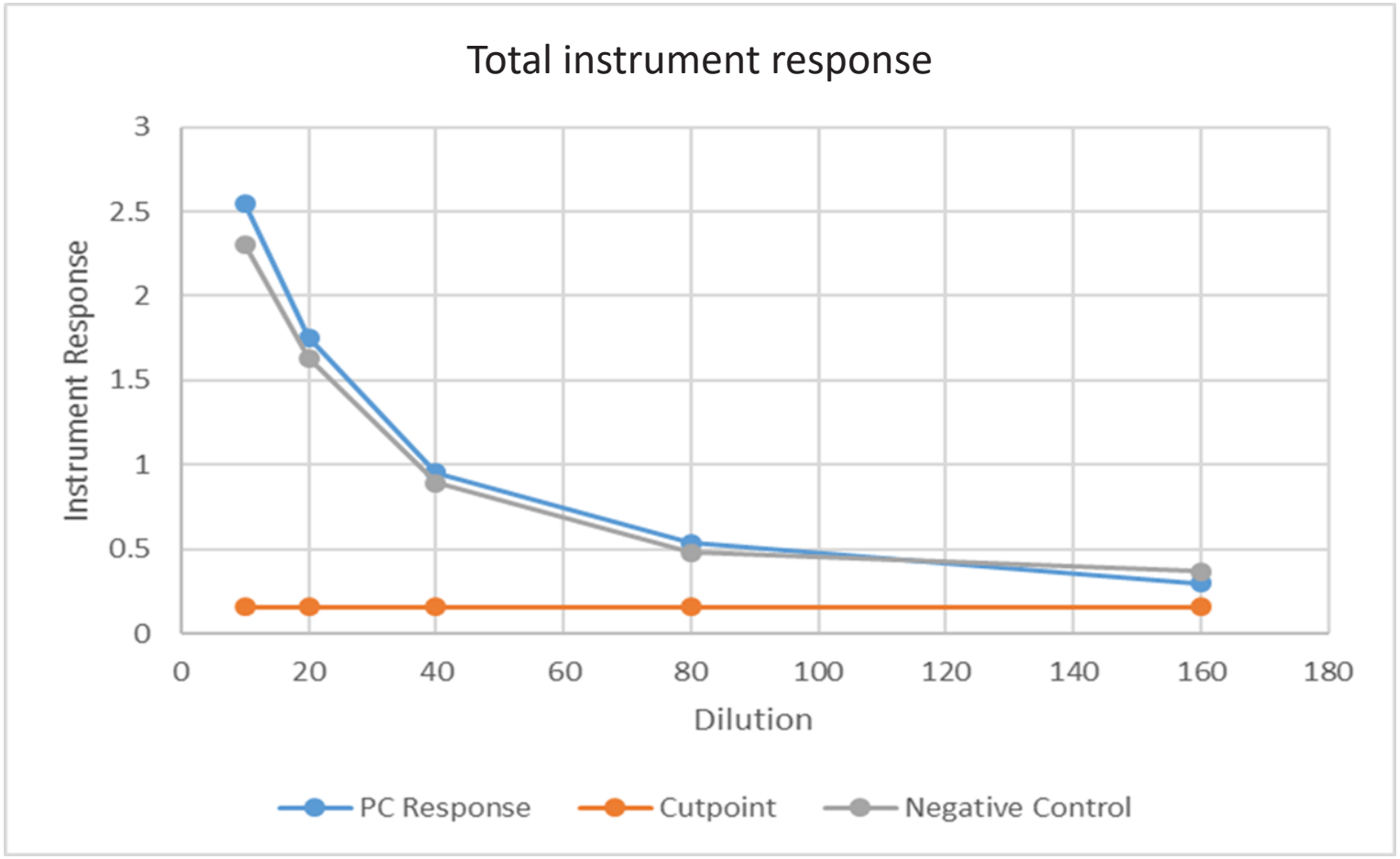
NC: Negative Control

Titer determination

- Prepare a positive control sample
- Serially dilute the sample 1 in 2 at least five times
- Analyze each dilution in singlicate
- Analyze in parallel with negative control
- Calculate the specific instrument response
- Determine the end point titer from the specific instrument response

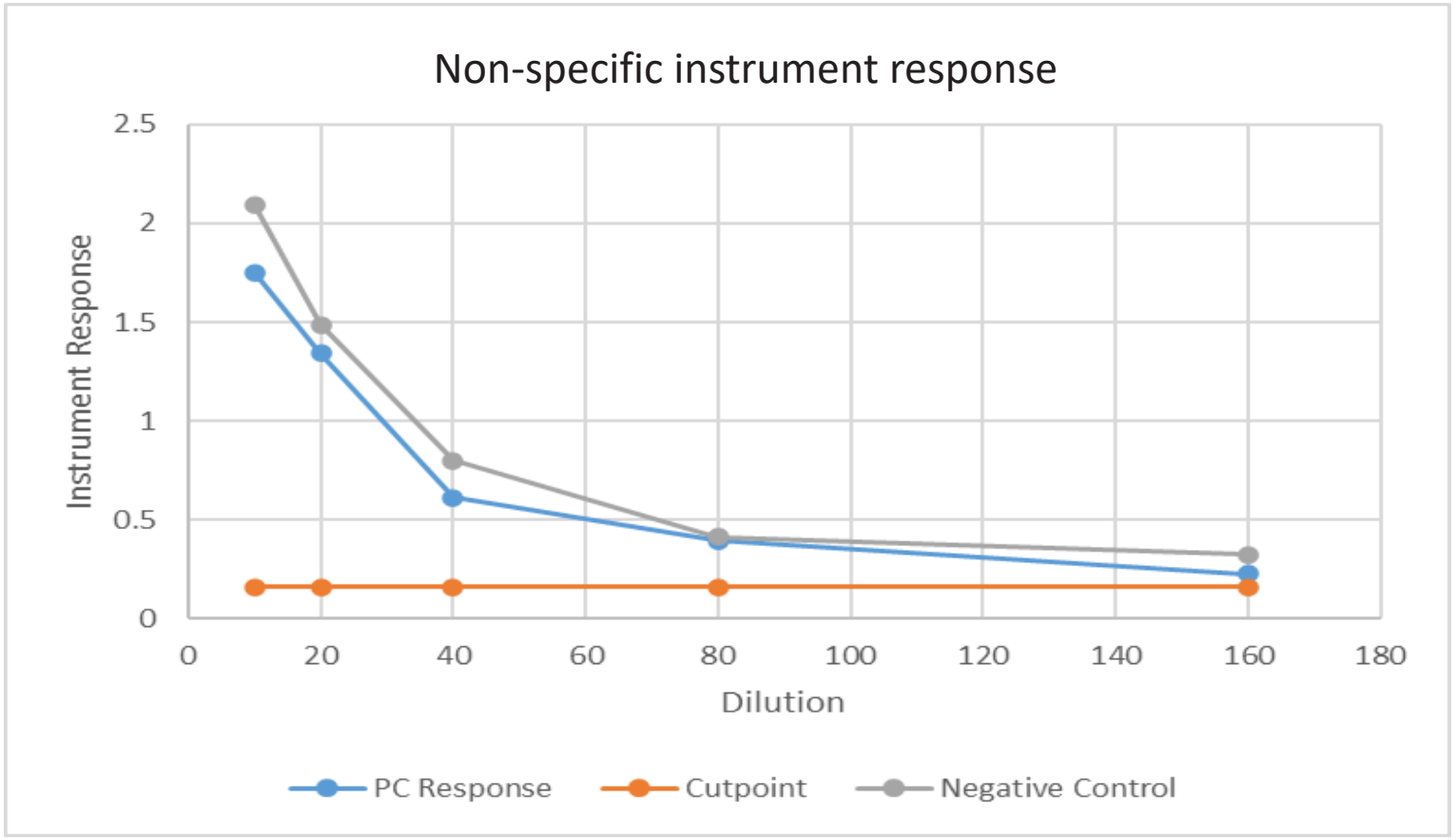
Measurement of total instrument response

	Positive Control	Negative Control	
Dilution	Total Instrument Response		Cut Point
1 in 10	2.55012	2.30517	0.1605
1 in 20	1.75589	1.6331	
1 in 40	0.95378	0.89141	
1 in 80	0.53597	0.48017	
1 in 160	0.29982	0.3691	



Measurement of non-specific instrument response

	PC	NC	
Dilution	Non-Specific Instrument Response		Cut Point
10	1.75272	2.0953	0.1605
20	1.34359	1.48449	0.1605
40	0.61278	0.80011	0.1605
80	0.3961	0.41537	0.1605
160	0.2264	0.3235	0.1605

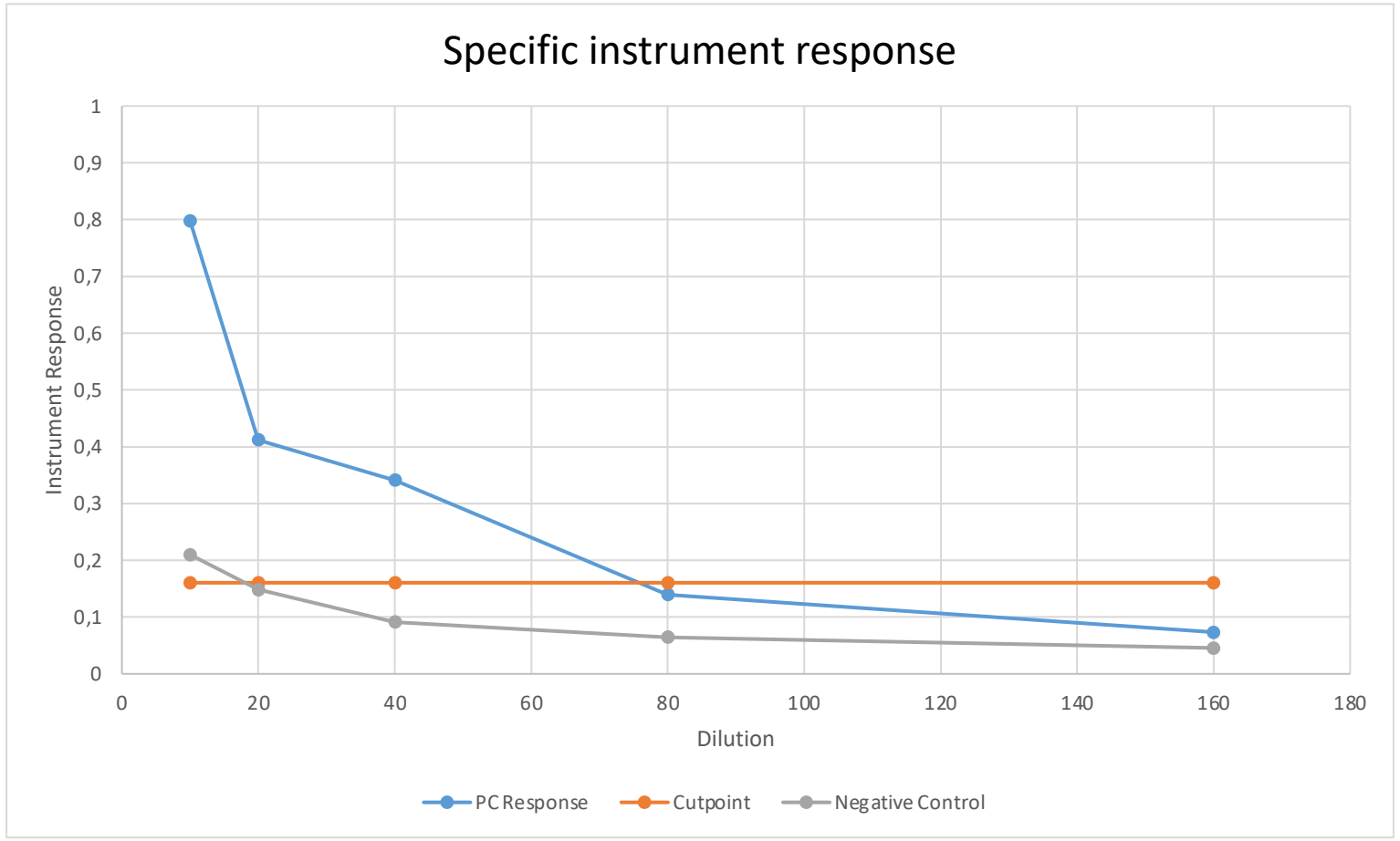


Subtraction of the specific instrument response

	Positive Control	Negative Control	
Dilution	**Specific Instrument Response**		**Cut Point**
1 in 10	0.7974	0.20987	0.1605
1 in 20	0.4123	0.14861	
1 in 40	0.341	0.0913	
1 in 80	0.13987	0.0648	
1 in 160	0.07342	0.0456	

Results

EPT Positive control	1 in 40
EPT Negative control	1 in 10



Final assay format

- Singlicate analysis (%CV was confirmed to be lower than 20%)
- Positive and negative end point titer control range established
- Cut point set (3x mean buffer blank)
- Final data presented as end point titer (for example, 1 in 20)
- Specific binding was calculated as follows:

  Specific Binding = Total Binding – Non-Specific Binding

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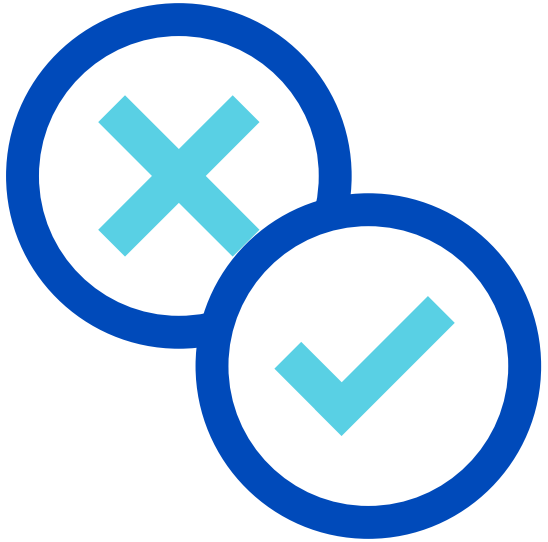
Advantages and disadvantages of the assay

Advantages

- A robust assay was developed and validated despite the high background
- Assay was fit for purpose

Disadvantages

- Reduced number of samples analyzed on a single plate
- More resources required, which results in increased cost
- Recommendation to analyze the baseline samples along with post-dose samples to remove assay variability concerns
- Increased time for data processing (use of current LIMS system not possible)



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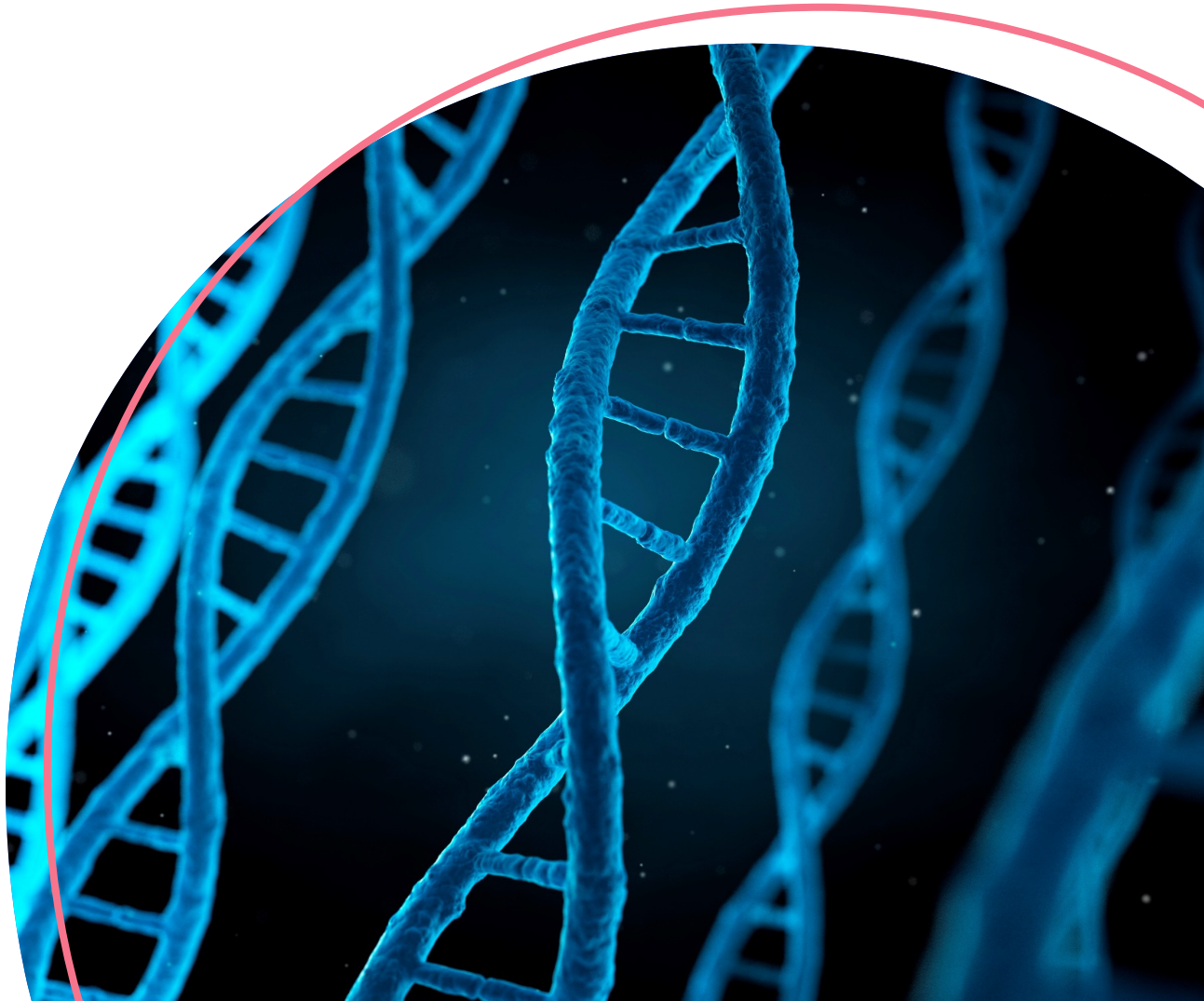
Conclusions

- Developing an ADA titer assay with high background/pre-existing antibodies is feasible even when the standard strategies to reduce the background do not work
- Context of use approach followed: the assay was fit for the purpose required in the clinical trial
- Specific signal can be calculated by subtraction from the total signal and instead of directly obtained



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Acknowledgements

- Zhe Liu
- Dimitra Kalamida
- Emma-Claire Leonard
- Karen Cadwallader
- James Munday
- I&I Huntingdon Team

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Thank you.