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

Lisa Seavers, BSc (Hons)
Desislava Galeva, MSc

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Immunology and Immunotoxicology

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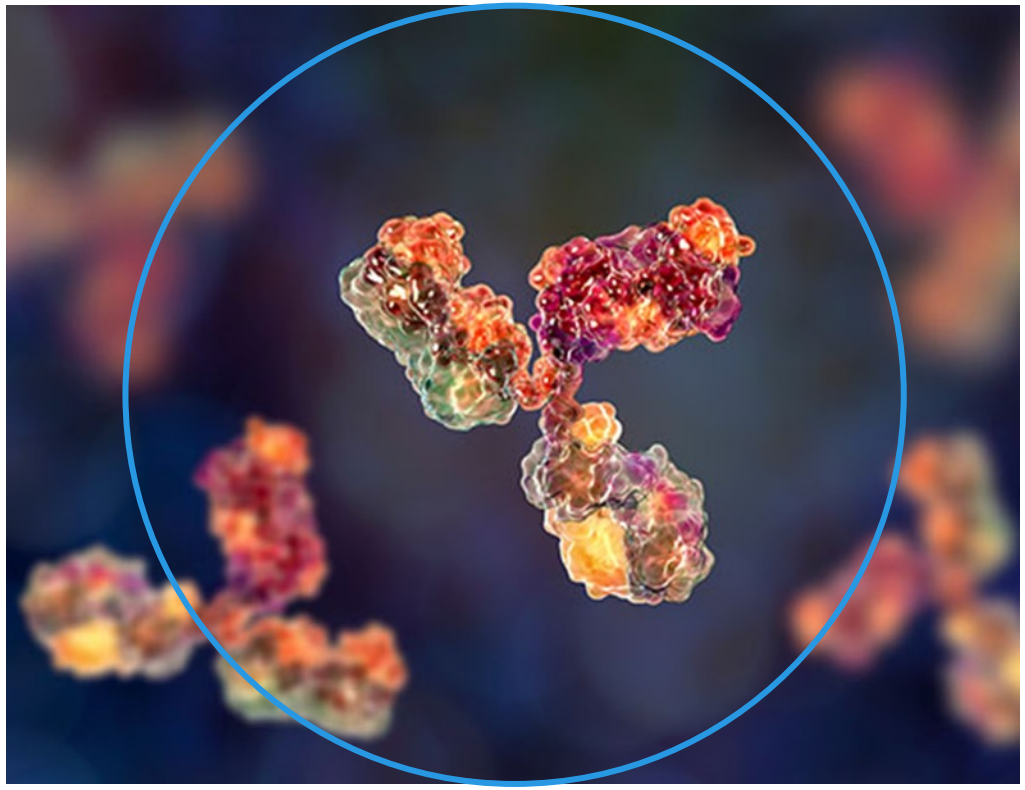
Immunogenicity

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

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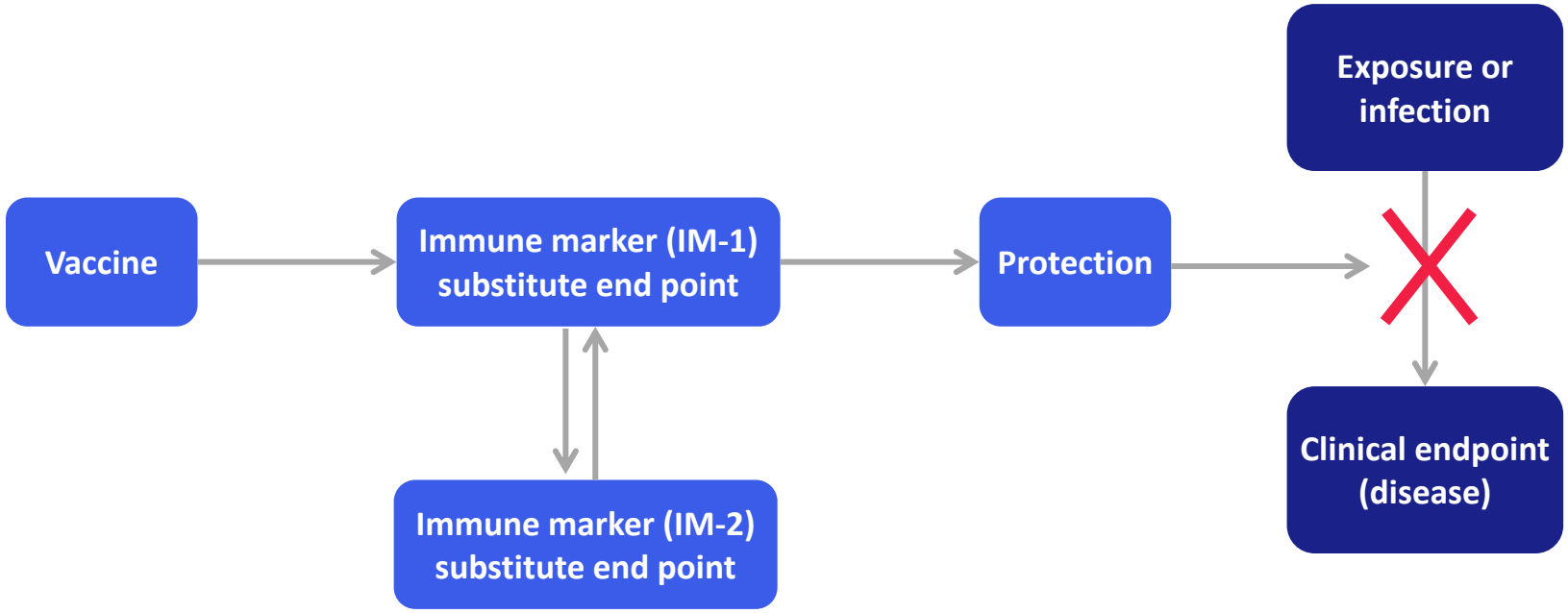
Induction of protective immunity by a preventive vaccine



Arrows imply direct causal relationships

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Therapeutic vaccines

- 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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Assay requirements

- 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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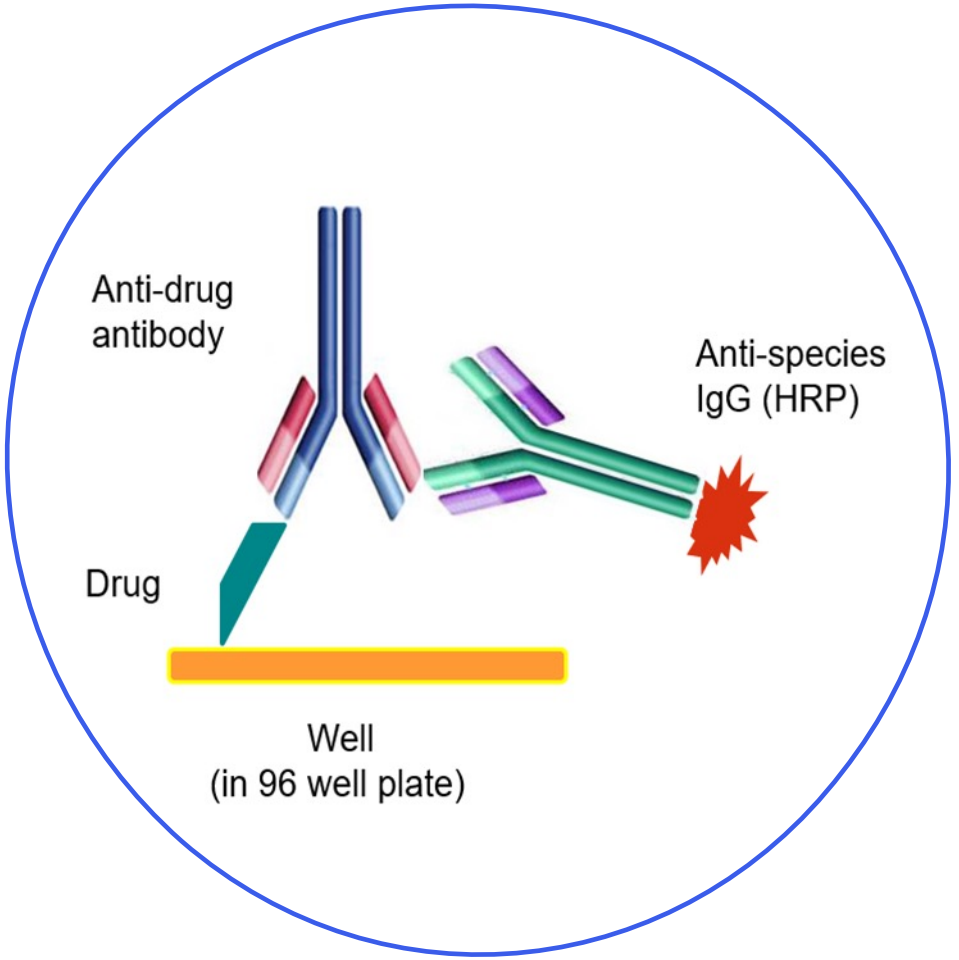
In pursuit of an assay

- Classic ADA titer approach: a surrogate of the test item used as a capture reagent. Anti-species IgG HRP (Horseradish peroxidase) as a detection reagent
- Investigation of using diluted negative matrix to calculate the cut point
- Positive control: pooled patients population matrix
- During the course of the method development various different formats and reagents were assessed; however, they were not suitable for the assay



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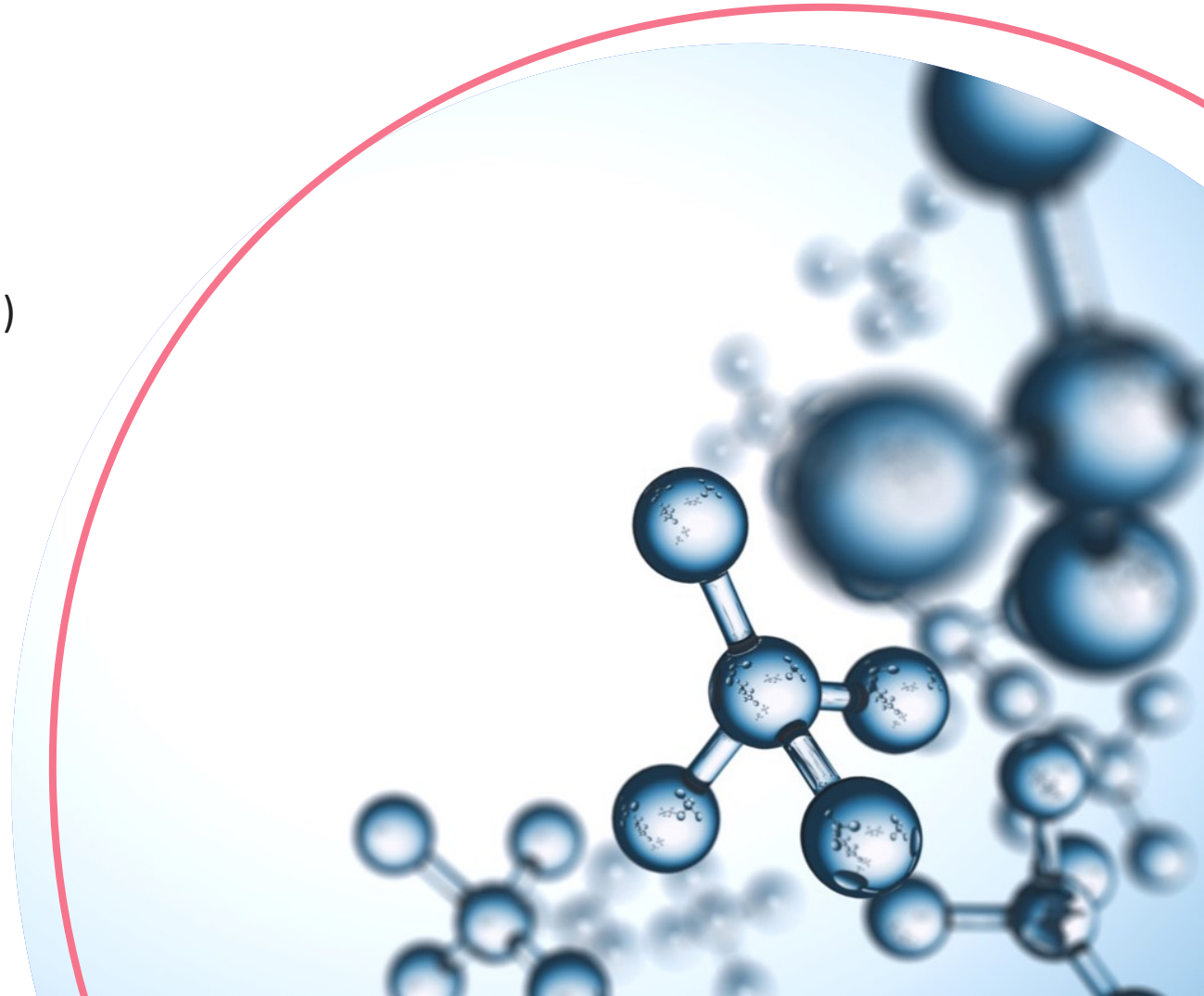
Common challenge: High background (high endogenous levels)

Potential solutions

- Change of the blocking buffer and investigation into different blocking proteins
- Further dilutions of samples
- Optimisation of critical reagents (labelled antibodies, etc.)
- Increased number of washing cycles



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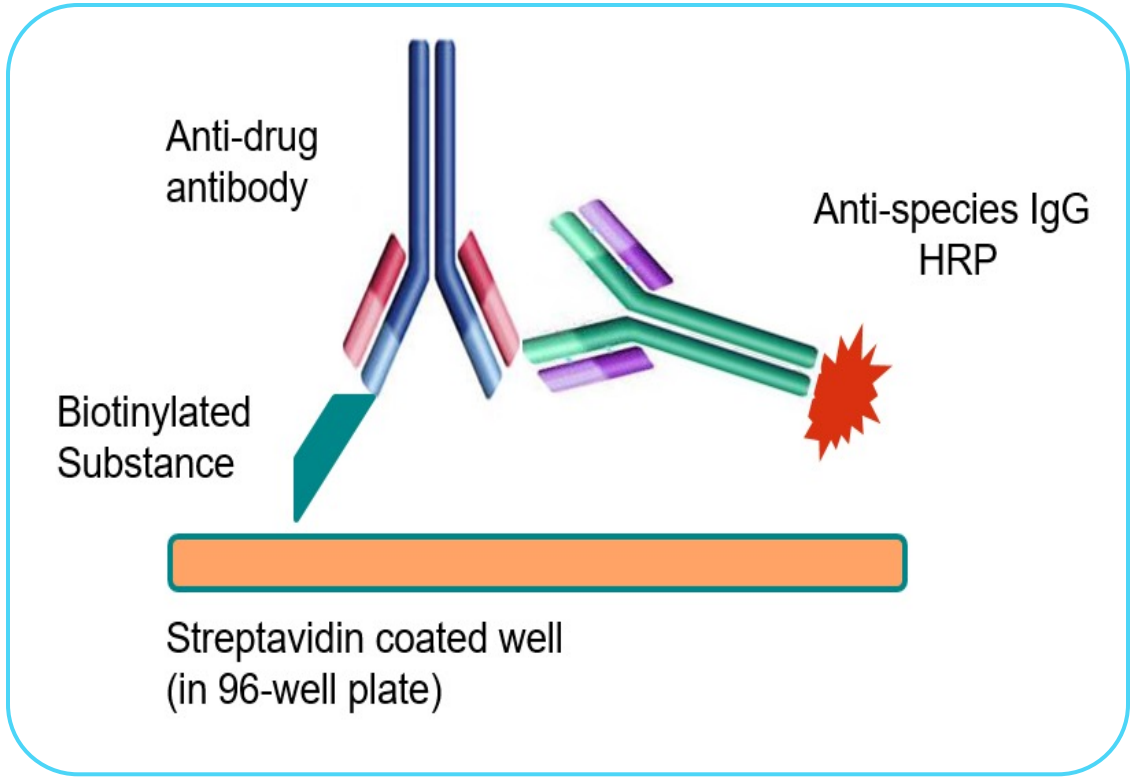
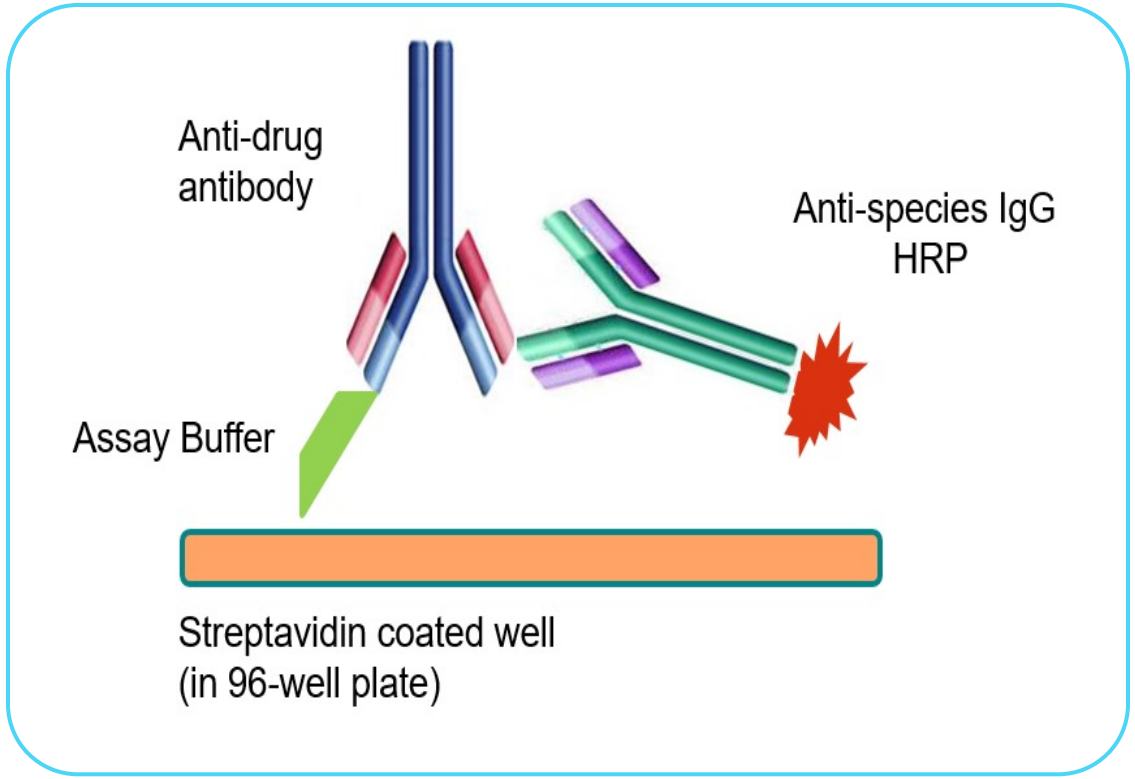
Assay format of each side of the 96-well plate



Total instrument response



Non-specific instrument response

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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)					

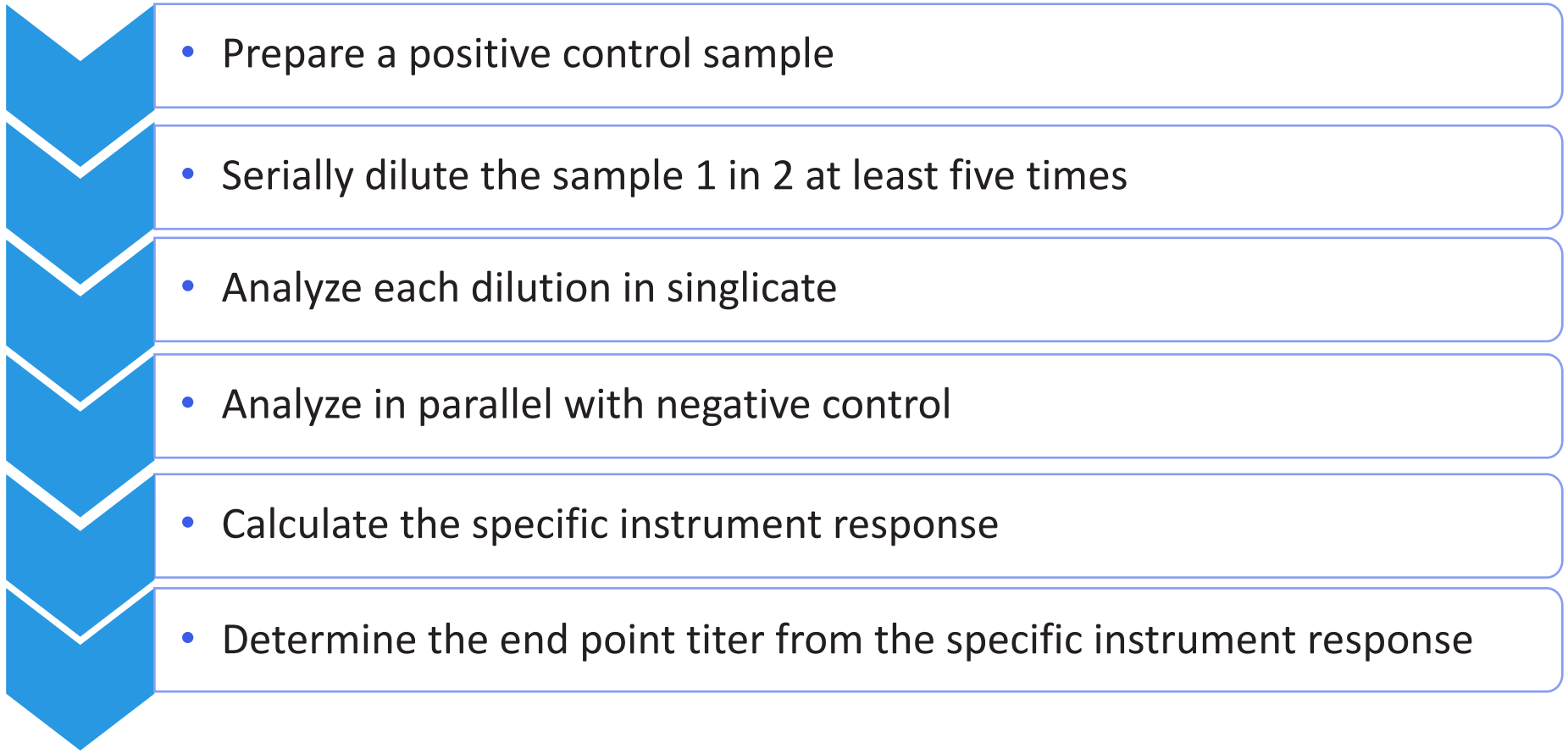
PC: Positive Control
NC: Negative Control

Assay format

- A quasi quantitative titer assay to determine scale of response in specific positive anti-drug responders
- Response is combination of concentration and affinity/avidity of antibodies in sample
- End point titer (EPT) = the reciprocal of the highest dilution that gives a reading above the cut point
- Cut point set (e.g., mean buffer blank + 3StdDev)
- Specific binding was calculated as follows:

  Specific Binding = Total Binding - Non-Specific Binding

Titer determination



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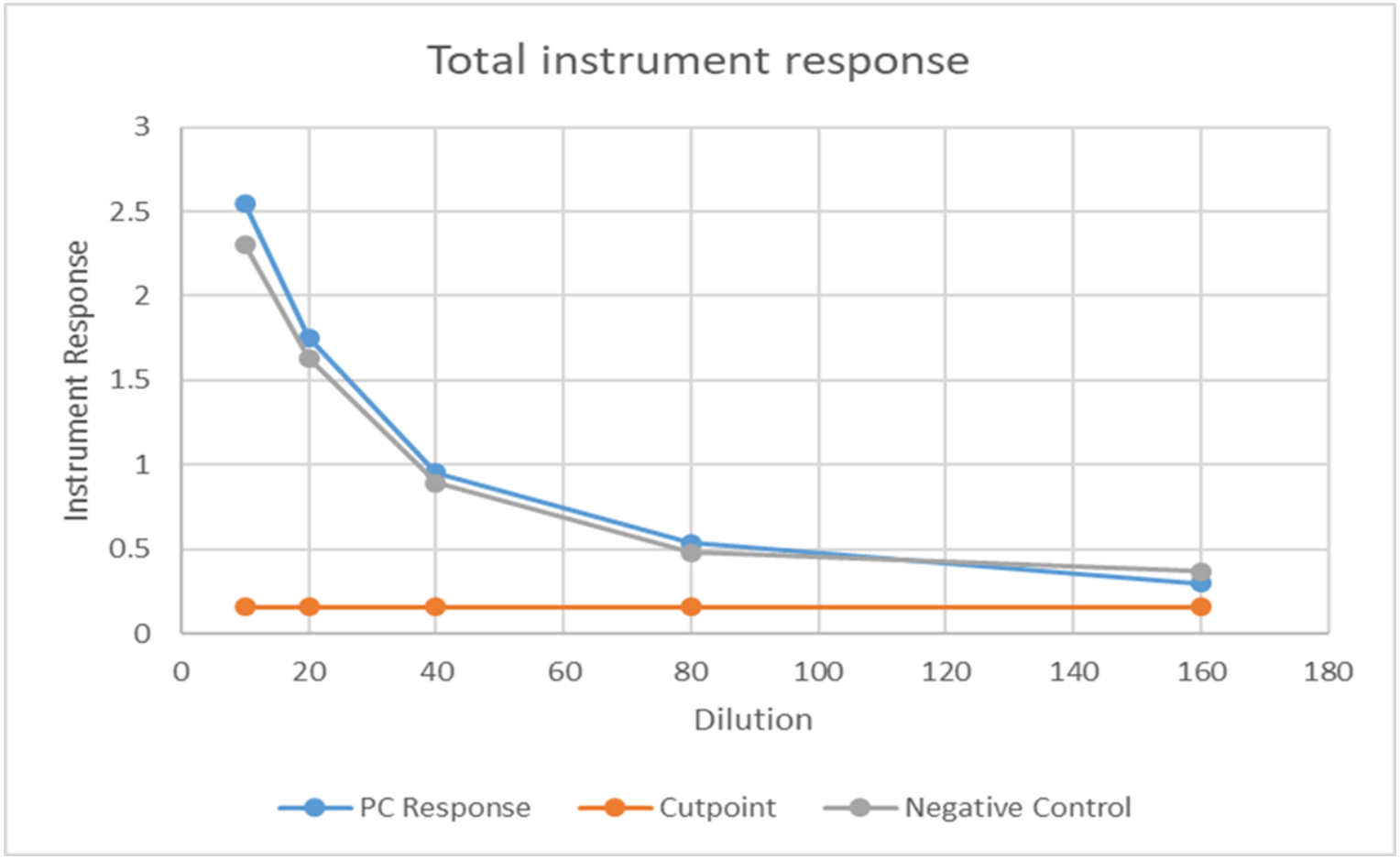
Measurement of total instrument response

	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	



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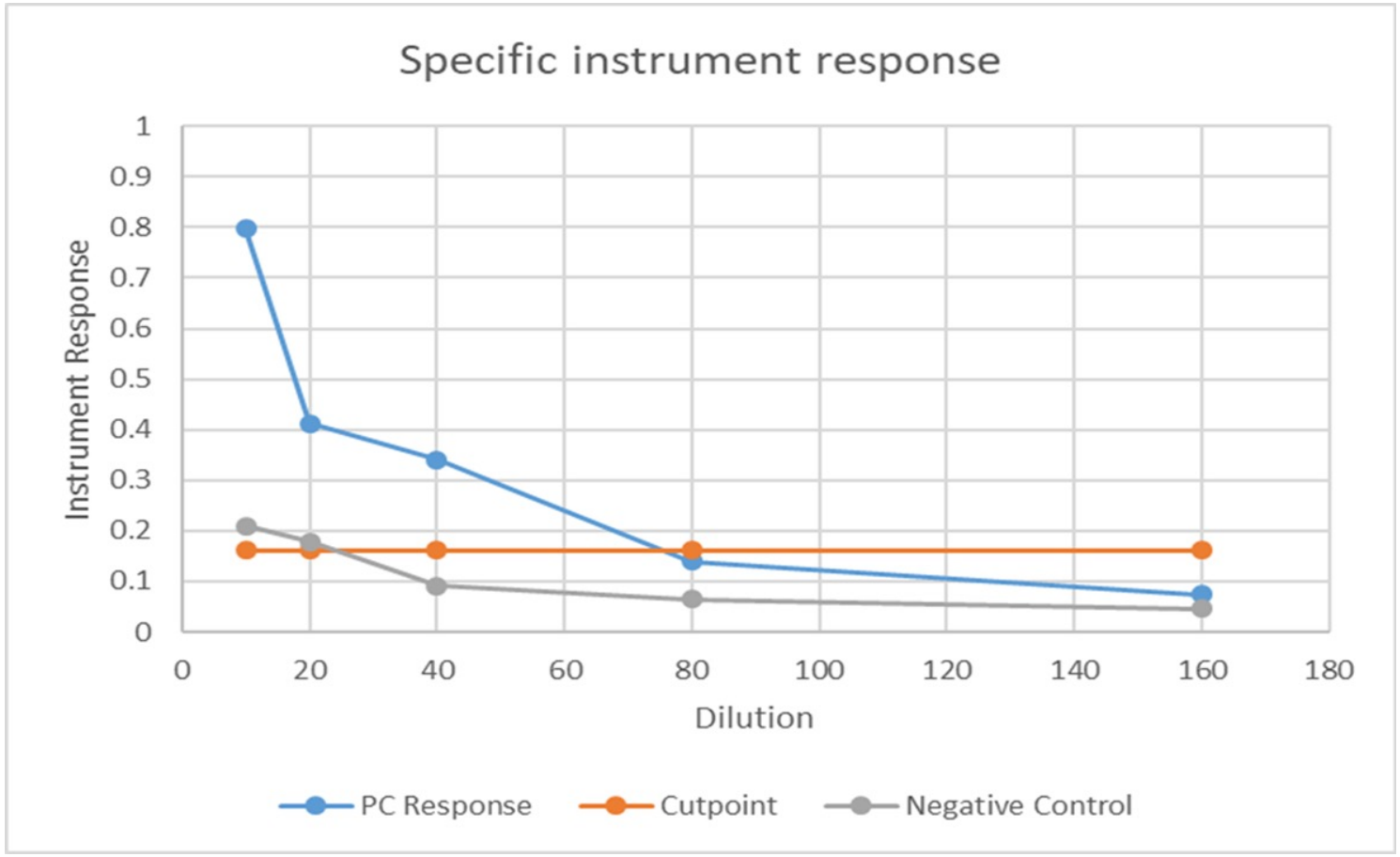
Measurement of specific instrument response

	Positive Control	Negative Control	
Dilution	Specific Instrument Response		Cut Point
1 in 10	0.7974	0.20987	0.1605
1 in 20	0.4123	0.17861	
1 in 40	0.341	0.0913	
1 in 80	0.13987	0.0648	
1 in 160	0.07342	0.0456	



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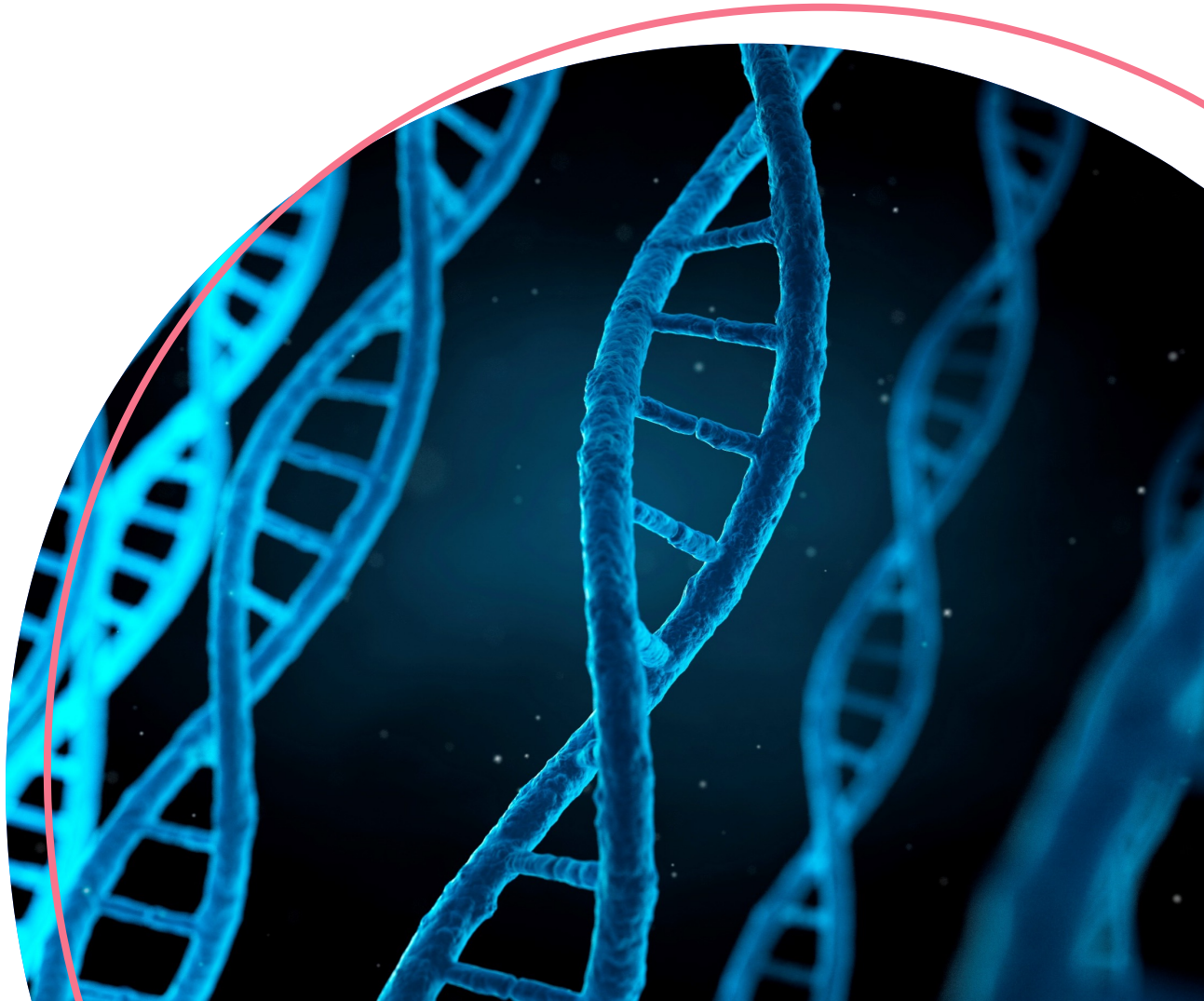
Conclusions

- Developing an ADA titer assay is feasible even with pre-existing antibodies
- Context of use approach followed: the assay was fit for the purpose required in the clinical trial
- Calculating the specific signal by subtraction from the total signal



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Thank you.

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