RESOLIAN

Proven expertise.
Worldwide access.

RESOLIAN

A singlicate immunogenicity method to detect anti-PEG antibodies: Pre and post dose of pegylated therapies

Issa Jyamubandi, PhD
Principal Scientist

Acknowledgements

RESOLIAN
BIOANALYTICS

A singlicate immunogenicity method to detect anti-PEG antibodies: Pre and post dose of pegylated therapies

Authors: Issa Jyamubandi, Aanya Aamir, Jyotsna Kaur, Alen Sylvester, Richard Hughes, Sophia Li

Abstract

Aims: Pre-existing anti-PEG (Polyethylene glycols) antibodies (APA) may affect the efficacy and safety of pegylated compounds. Omontys® and Krystexxa® withdrawal, and SARS-CoV-2 RNA vaccines anaphylaxis were all linked to APA. This project aimed to develop and validate a method to detect total antibodies against PEG, pre and post dose.

Materials and Methods: The repetitive linear PEG structure prevented the use of a bridging homogenous format; hence the requirement to use a Solid Phase Extraction and Acid Dissociation (SPEAD) assay coupled with the MSD (Meso Scale Discovery®) platform.

Results and conclusion: Using singlicate analysis, the method was validated to successfully detect APA pre- and post-dose, with a crucial aspect of the method being the preparation of an appropriate negative control (NC).

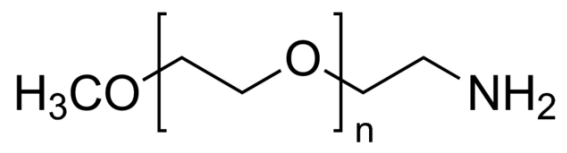
Overview

- Background
- Method development
- Validation
- Future consideration
- Conclusion

Background

RESOLIAN
BIOANALYTICS

- Polyethylene glycols (PEG)



- Classified as GRAS (Generally Recognized as Safe) by the FDA in 1973
- 36 FDA-approved PEGylated therapeutic drugs
- Increase drug solubility, half-life and decrease self-aggregation
- Used in many household products due to different properties

Commercial use

- **PEG-12**: Provides consistency to the product
- **PEG-40 Castor Oil**: Prevents the liquids in our products from separating

- **PEG-6 to PEG-9**

PEG-100

- **PEG-20 to PEG-40**

Clinical impact

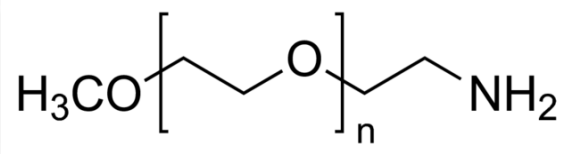
RESOLIAN BIOANALYTICS

- Anti-PEG prevalence varies (0.2% - 72%) depending on method used
- Impact on the therapeutic drug efficacy and safety
- Krystexxa® and Omontys® were both withdrawn because of strong immunogenicity against protein and PEG moieties
- 41% of patient treated with Krystexxa® developed APA
- Individual vaccinated with SARS-CoV-2 RNA that experienced anaphylaxis also had APA, potentially due to the PEG moiety in the lipid nanoparticle
    - What impact is that going to have on Pegylated therapeutic?

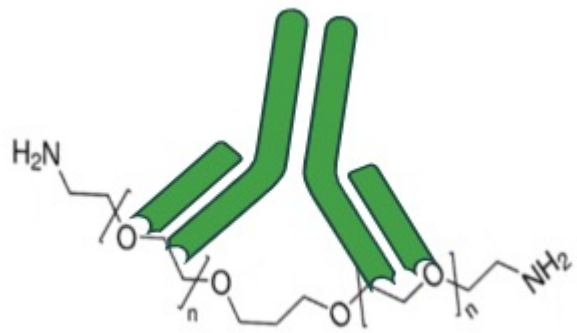
Assays to detect APA

RESOLIAN BIOANALYTICS

- Initially tried APA commercial kits
- They are direct assays and are isotype specific (IgG or IgM)
- The standard homogenous ADA assay approach was not able to detect APA consistently
- ~700 Da of PEG (16 PEG monomers) is sufficient to interact with the APA fab paratope (Justin et al. 2020)
- Suggesting that larger MW PEG may occupy both APA paratopes due to the repetitive sequence



Method development

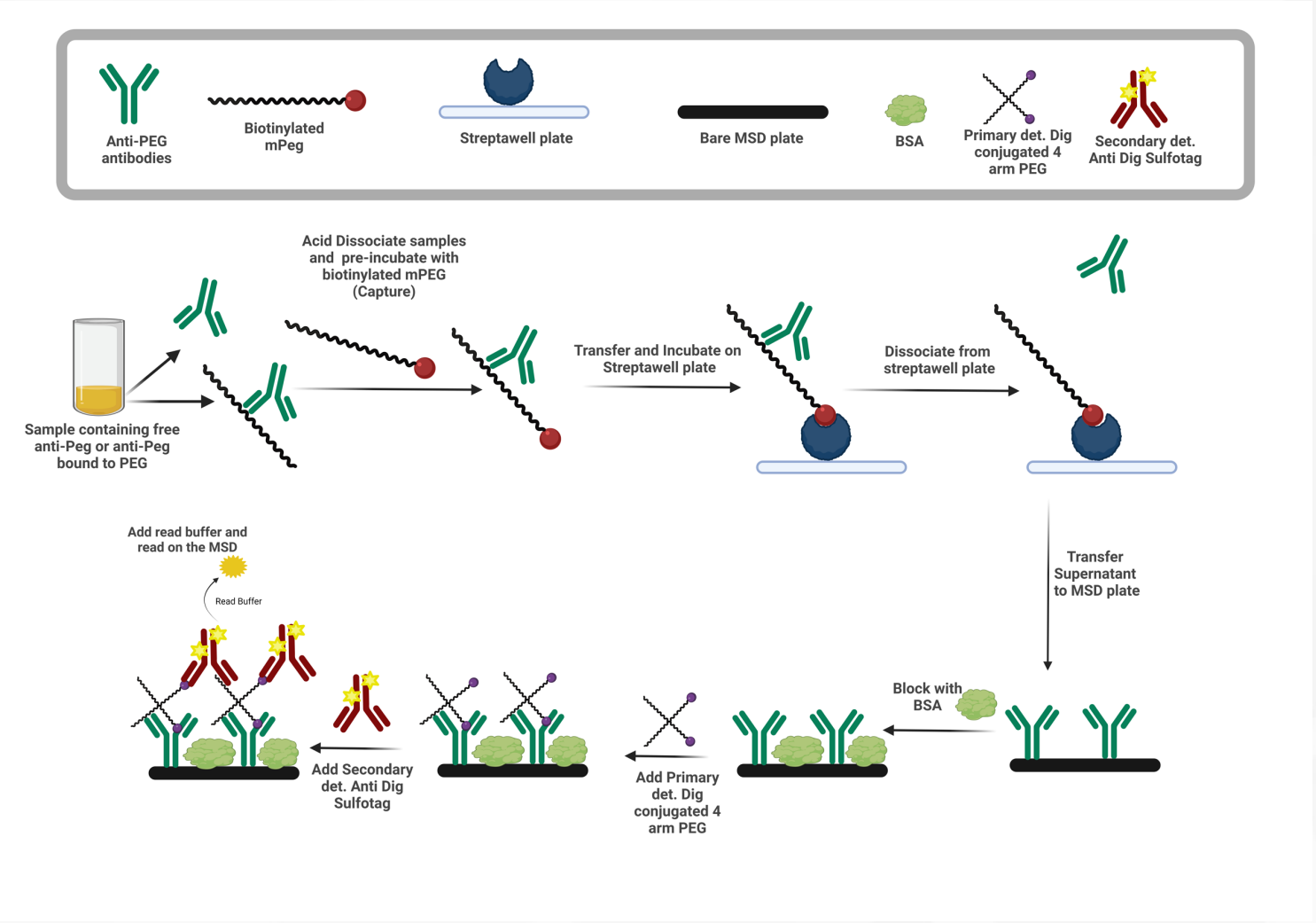


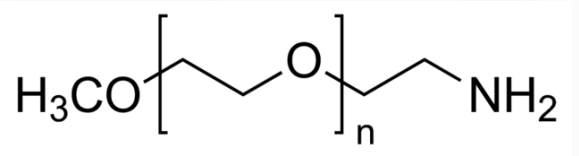
Method development

- We tried to develop a direct assay inhouse using protein A/G
- High baseline was observed
- Aimed to develop an alternative assay that:
  - Can detect all anti-PEG isotype
  - Can detect anti-methoxy and PEG backbone antibodies
  - Use Generic reagents and that can be adapted using any PEGylated compound

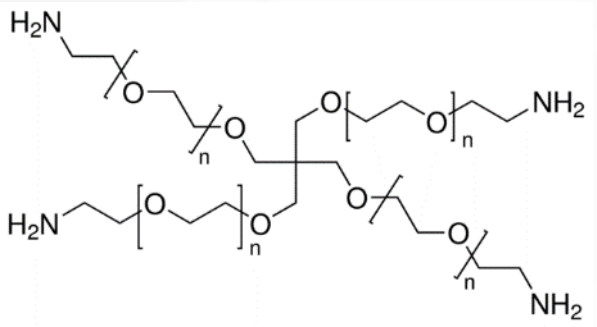
SPEAD method to detect APA

RESOLIAN BIOANALYTICS





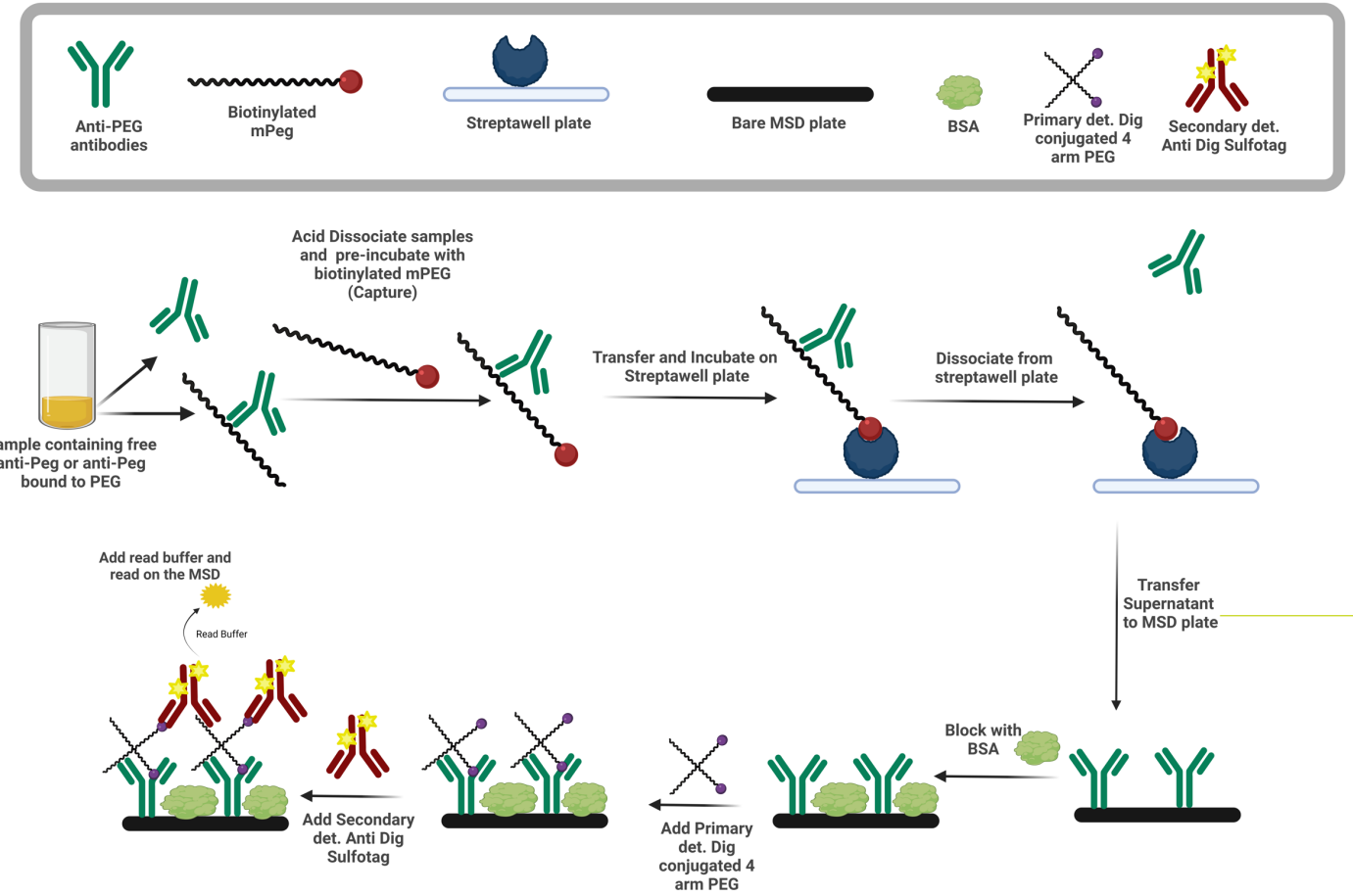
Capture

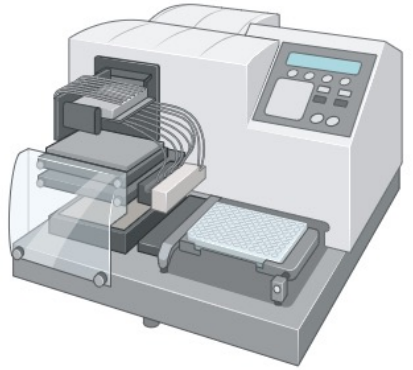


Detection

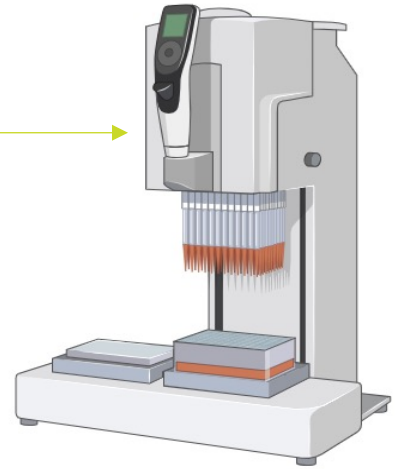
SPEAD method to detect APA

RESOLIAN BIOANALYTICS



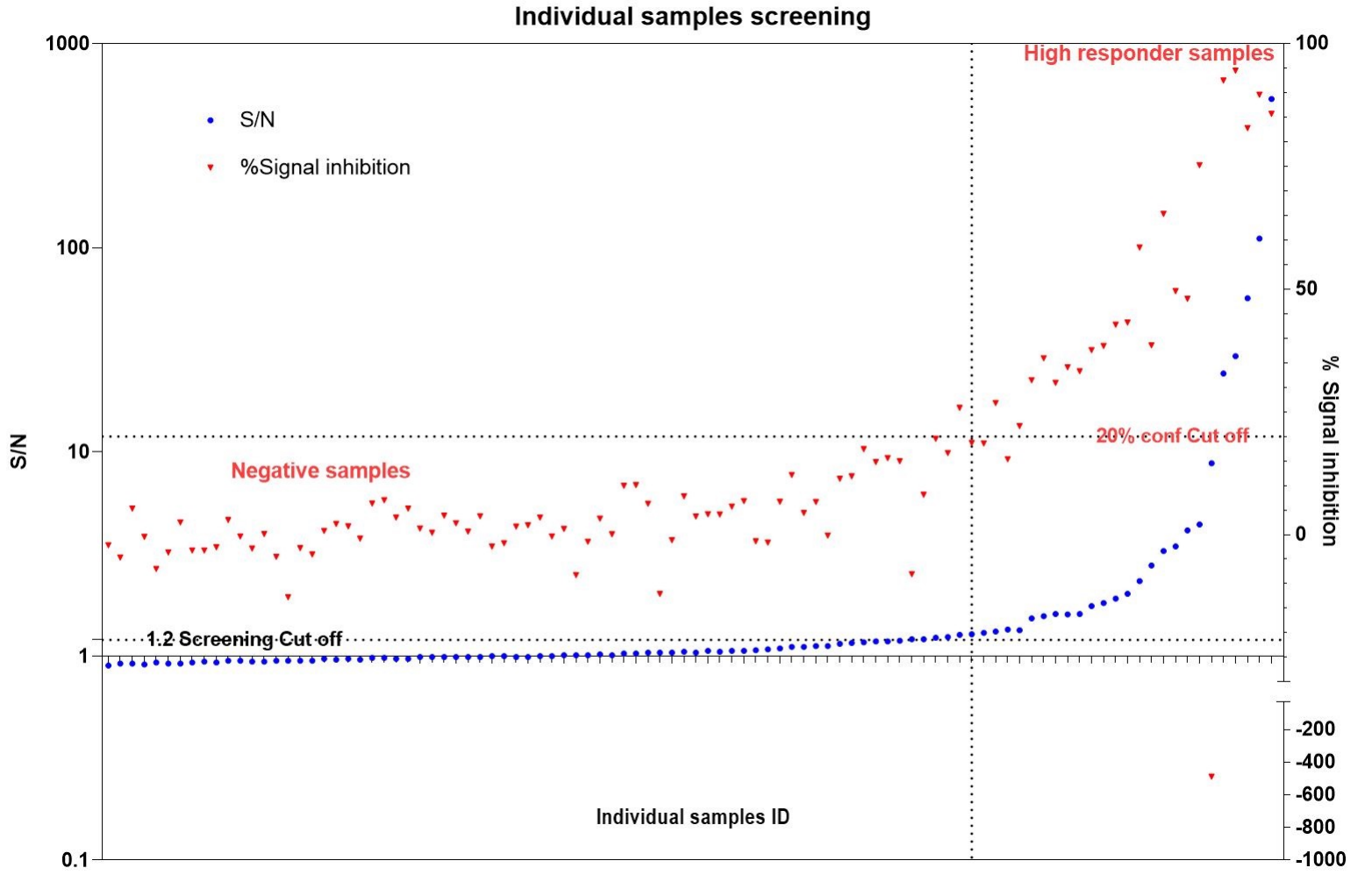


Dedicated Plate washer



Used the integra to improve precision and consistency

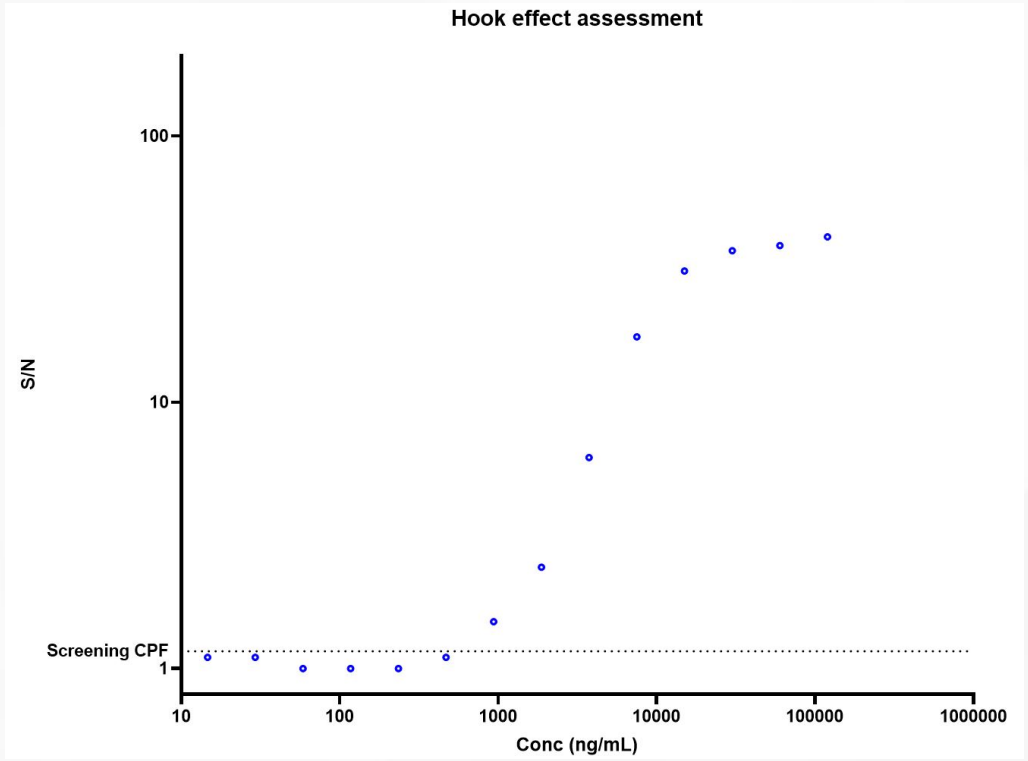
APA Screening

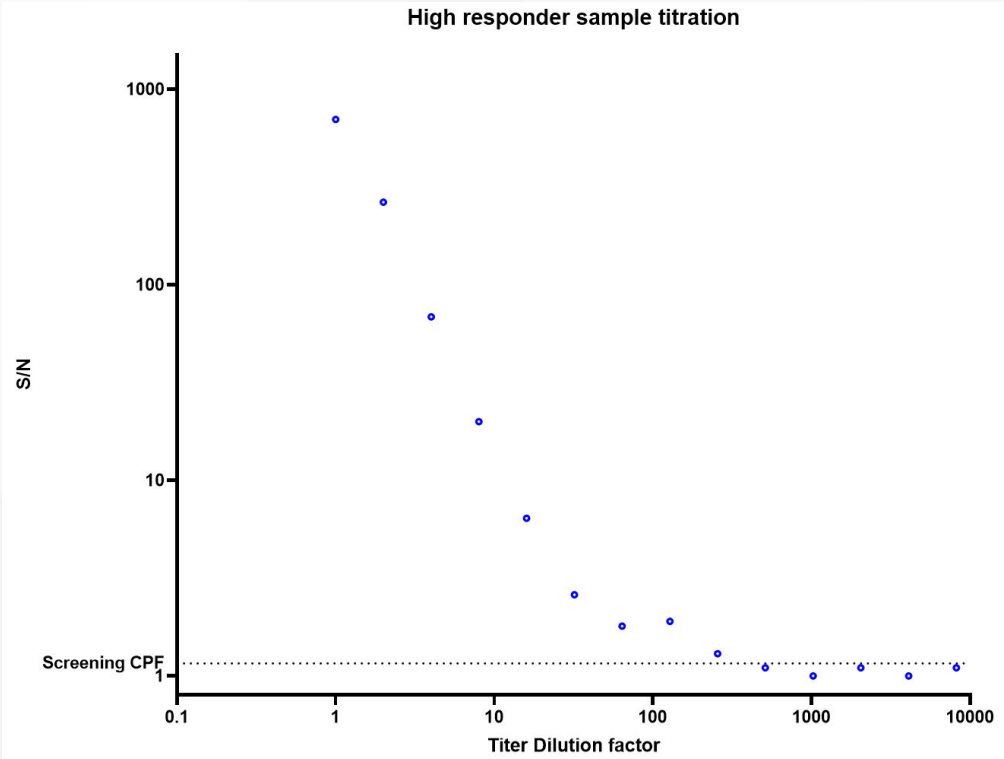


- Assay optimized in the screening and confirmatory
- Individuals screening using a generic 1.2 Screening CP and 20% Confirmatory CP
- 32% identified as positive
- Negative samples used for making the NC and CP assessment
- High responder samples used for human anti-PEG purification

Commercial APA vs HR sample

RESOLIAN BIOANALYTICS



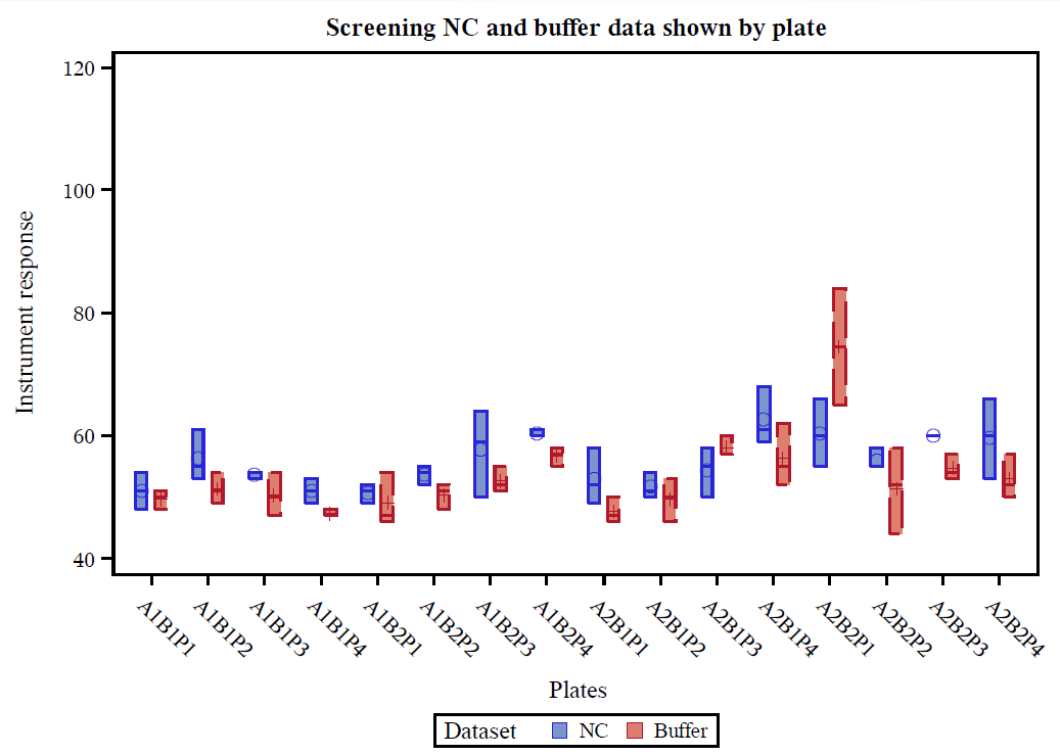


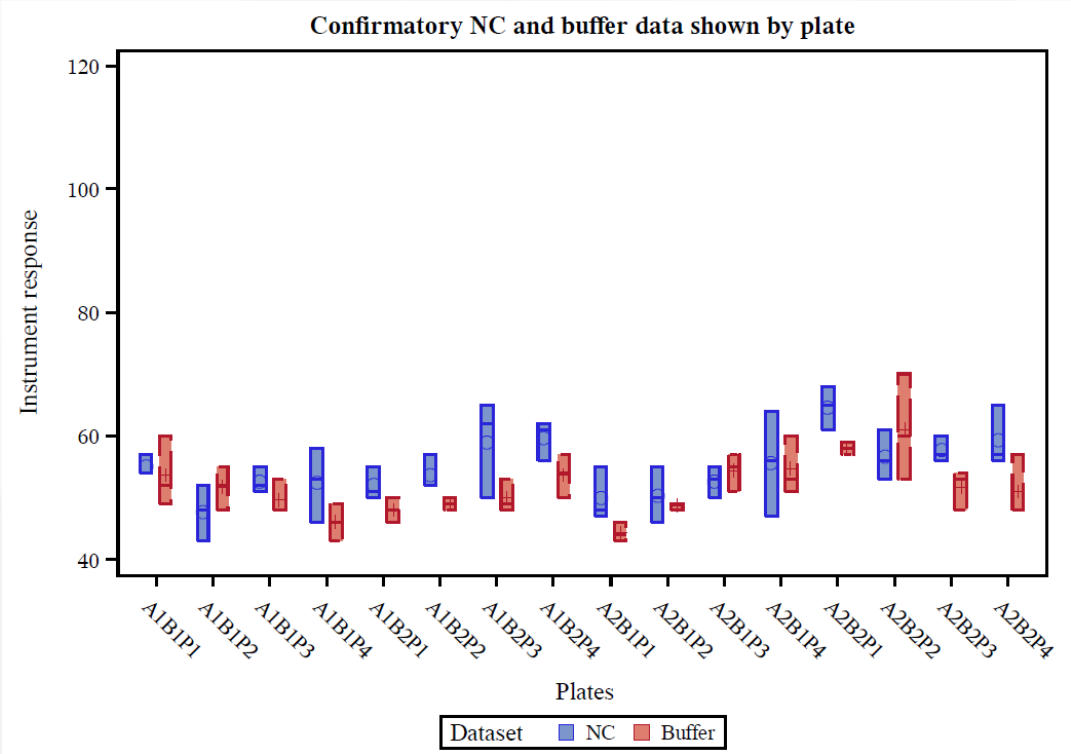
- High responder sample demonstrated higher signal compared to the commercial pool
- Demonstrating that surrogate antibodies do not exhibit the same properties as endogenous antibodies

Validation

NC vs Buffer comparison

RESOLIAN BIOANALYTICS





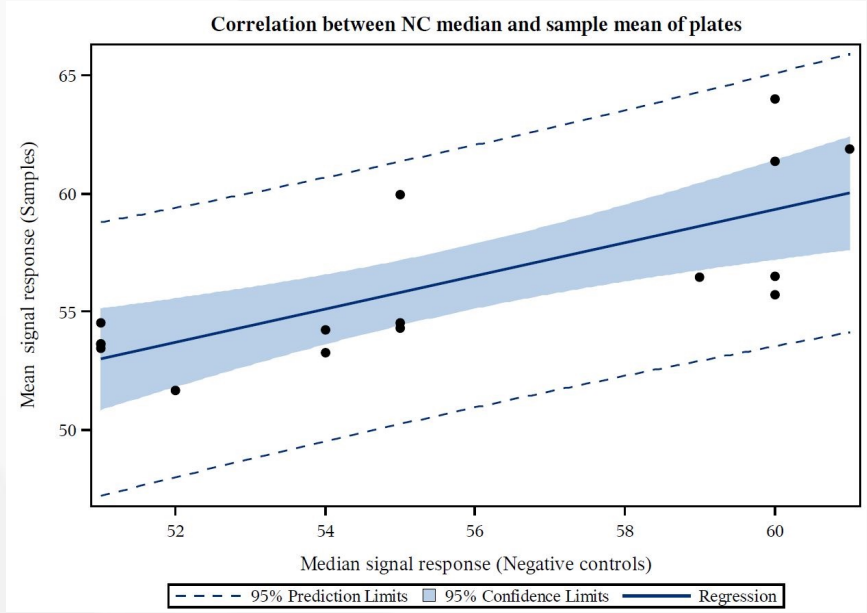
- NC and the buffer were demonstrated to have a strong correlation in the screening and the confirmatory assay
- Confirming that the NC is truly negative

Cut Point Plates and NC correlation

RESOLIAN BIOANALYTICS

	Sample order				
		S1-S14	S15-S28	S29-S42	S43-56
Day 1	Plate 1	√	√		
	Plate 2			√	√
	Plate 3		√	√	
	Plate 4	√			√
Day 2	Plate 1			√	√
	Plate 2	√	√		
	Plate 3	√			√
	Plate 4		√	√	

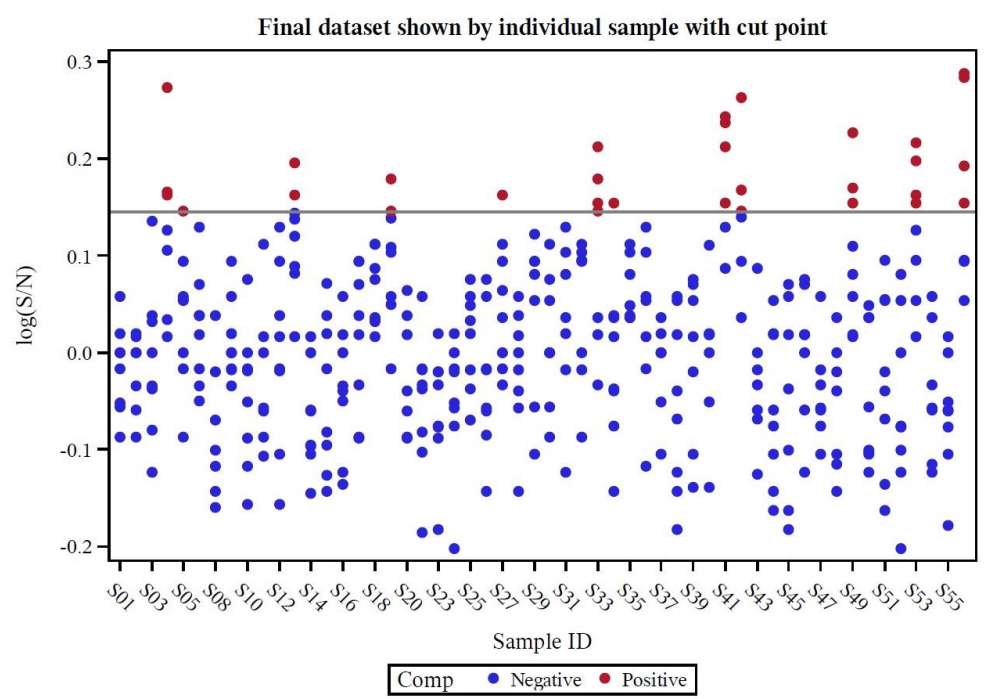
Plate order	Analyst 1 Run Order	Analyst 2 Run Order
Day 1	P1, P2, P3, P4	P4, P3, P2, P1
Day 2	P4, P3, P2, P1	P1, P2, P3, P4

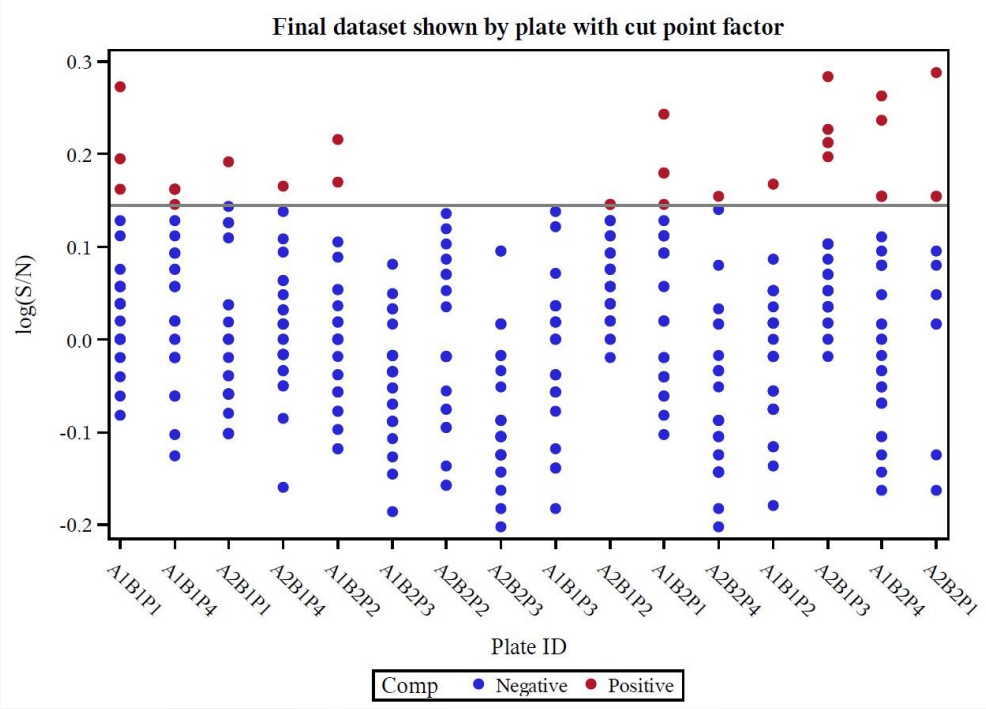


- Singlicate assessment using this design generated 448 data points in two days instead of 306 data points using duplicate in three days
- The NC was demonstrated to correlate with individual samples variation

Screening Cut Point

RESOLIAN BIOANALYTICS

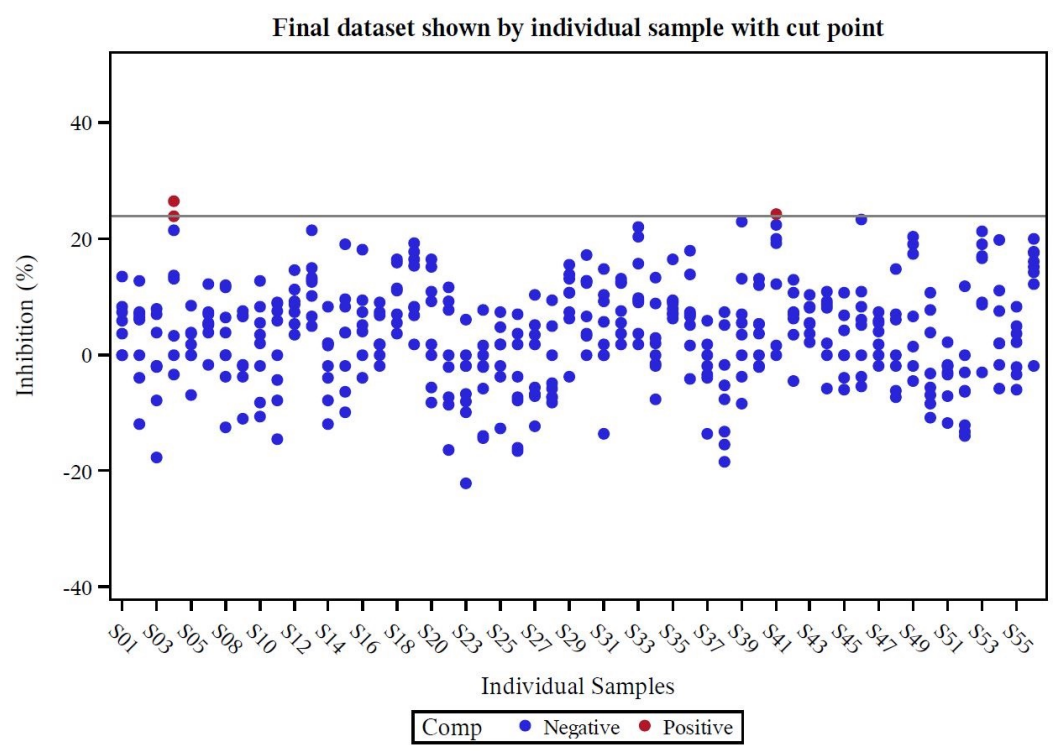


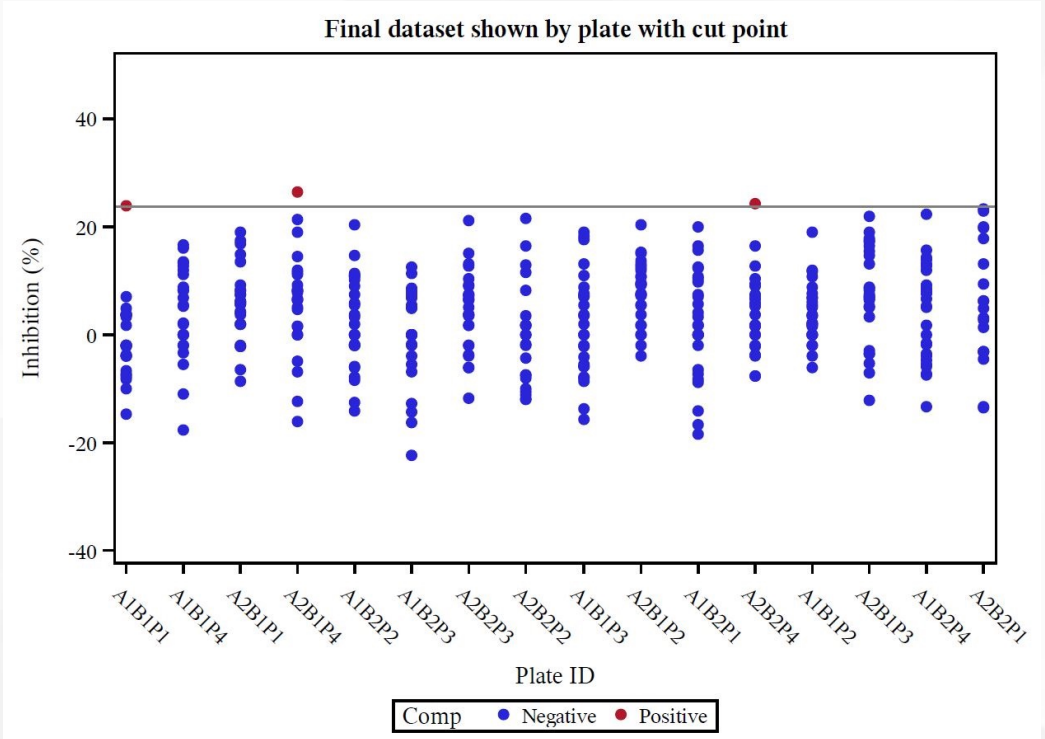


- The robust estimate was used to determine the cut point factor (CPF) of 1.16.
- The false positive samples were spread across all plates, analysts and days.

Confirmatory Cut Point

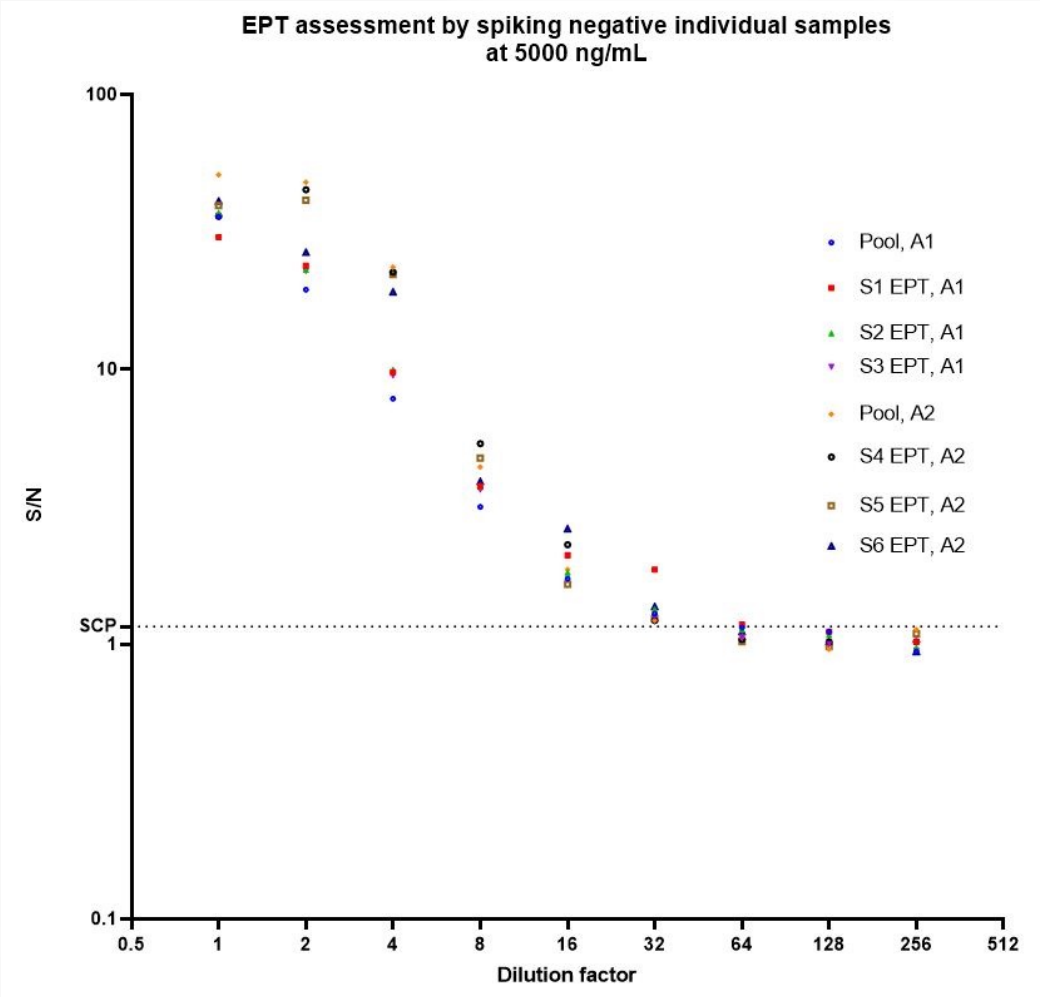
RESOLIAN BIOANALYTICS





- The confirmatory cut point was determined to be 23.8%
- The false positive samples were spread across different plates, analysts and days.

EPT assessment



- Six individual samples identified as negatives and the NC
- Spiked at 5000 ng/mL of commercial anti-PEG
- Serially diluted two-fold
- Titre cut point the same as the sCPF of 1.16
- All the six individual samples and pool serum control were within the acceptance criteria of Median EPT ±1.

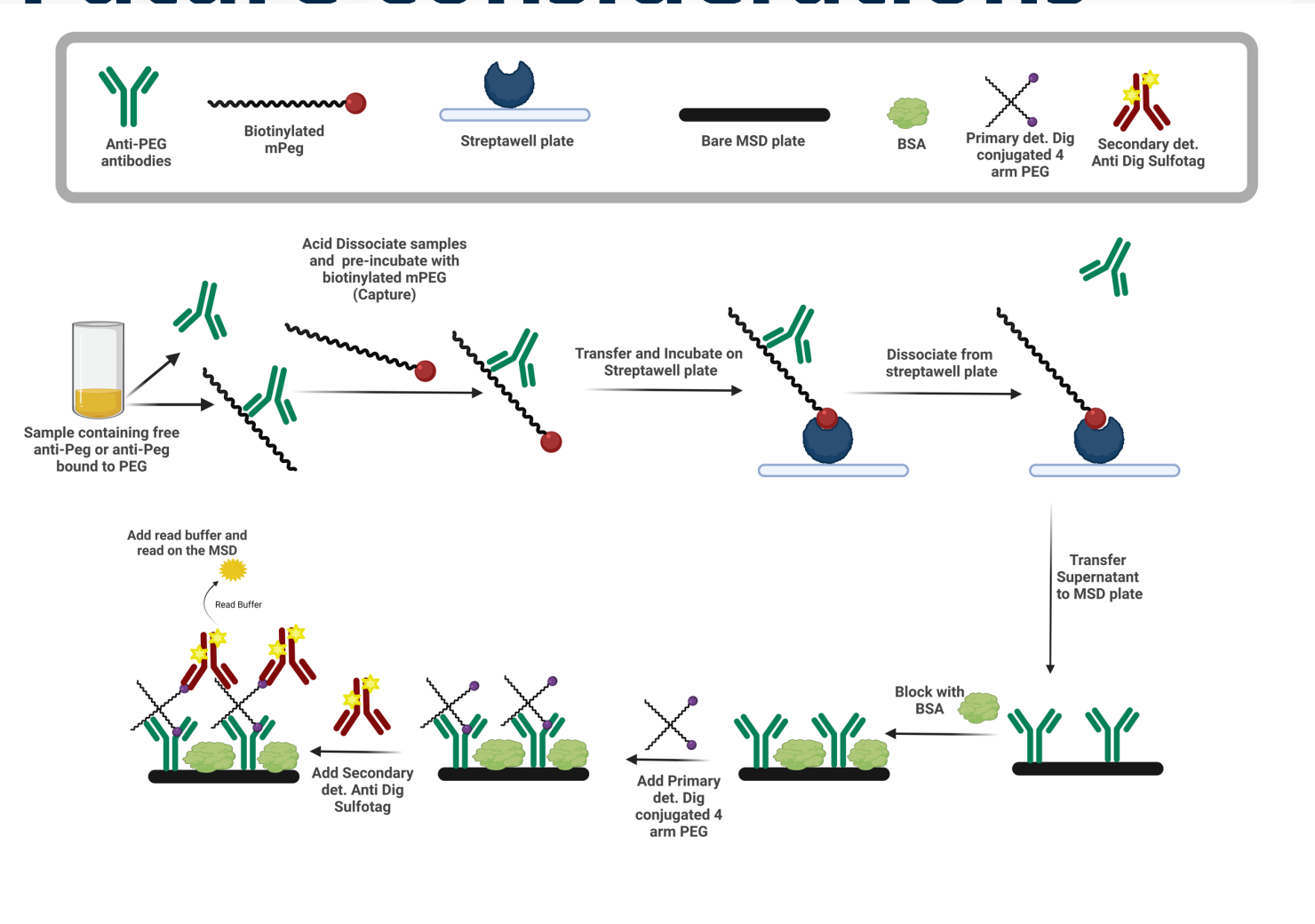
Precision and selectivity

Intra Assay Precision	Level	Conc. (ng/mL)	Screen % CV signal response (PC & NC)		Confirm % CV signal response (PC & NC)
	HPC	5000	16.3		21.0
	MPC	500	12.4		7.2
	LPC	300	14.7		3.2
	NC	N/A	6.3		4.8
Inter Assay Precision	Level	Conc. (ng/mL)	Screen % CV S/NC ratio (PC), signal (NC)		Confirm % CV % Inhibition (PC), signal (NC)
	HPC	5000	15.3		3.0
	MPC	500	18.3		7.3
	LPC	300	17.9		17.3
	NC	N/A	7.2		N/A
Selectivity	Tier	Pop.	CP (pop)	PC level	Met criteria
	Screen	Healthy matrix	1.16	Blank	10/10
				LPC (300 ng/mL)	10/10
	Conf.		23.8%	Blank	10/10
				LPC (300 ng/mL)	10/10
Haemolysis Selectivity	No effect up to 300 ng/mL (LPC)				
Lipemic Selectivity	No effect up to 300 ng/mL (LPC)				

- Intra assay precision acceptable across all the PCs and the NC
- Confirming that the use of Singlicate has no impact
- Inter assay precision acceptable across all the PCs and the NC
- Selectivity acceptable in the screening and confirmatory

Future considerations

Future considerations



Biotinylated Pegylated compound

Capture

Sulfo-tag Pegylated compound

Detection

Conclusion

RESOLIAN
BIOANALYTICS

- Homogenous assay are not suitable to assess anti-peg antibodies
- This generic SPEAD method can be used to assess APA in samples pre and post dose of pegylated therapeutic
- NC screening is crucial for assays with pre-existing antibodies
- The use of singlicate generates 448 data points instead of the 306 and can be performed over 2 days instead of 3 days
- The use of SPEAD method can avoid development of multiple assays to characterise ADA for pegylated compounds
- The use of purified human ADA should be encouraged where possible

Acknowledgements

RESOLIAN BIOANALYTICS

A singlicate immunogenicity method to detect anti-PEG antibodies: Pre and post dose of pegylated therapies

Authors: Issa Jyamubandi, Aanya Aamir, Jyotsna Kaur, Alen Sylvester, Richard Hughes, Sophia Li

Abstract

Aims: Pre-existing anti-PEG (Polyethylene glycols) antibodies (APA) may affect the efficacy and safety of pegylated compounds. Omontys® and Krystexxa® withdrawal, and SARS-CoV-2 RNA vaccines anaphylaxis were all linked to APA. This project aimed to develop and validate a method to detect total antibodies against PEG, pre and post dose.

Materials and Methods: The repetitive linear PEG structure prevented the use of a bridging homogenous format; hence the requirement to use a Solid Phase Extraction and Acid Dissociation (SPEAD) assay coupled with the MSD (Meso Scale Discovery®) platform.

Results and conclusion: Using singlicate analysis, the method was validated to successfully detect APA pre- and post-dose, with a crucial aspect of the method being the preparation of an appropriate negative control (NC).

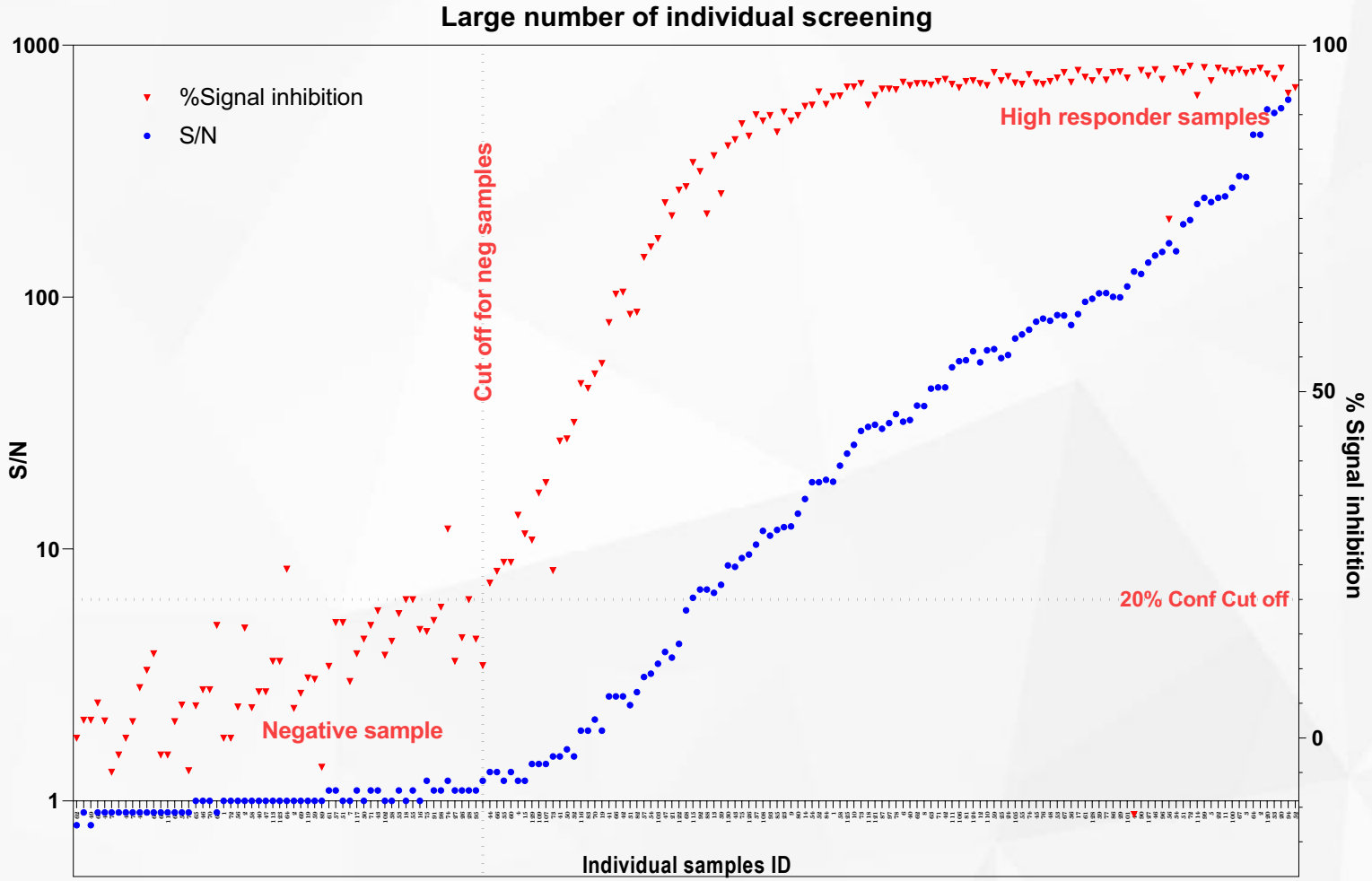
LIAN means integrity

RESOLIAN

Resolvers
Resolute

Anti-AAV antibodies detection, Screening

RESOLIAN BIOANALYTICS



- The high prevalence of anti-AAV in the general population (>70%) meant production of a true negative control (NC) serum pool was challenging.

  The identification of a true NC pool was an essential reagent for the successful validation of the immunogenicity assay to detect anti-AAV antibodies in human matrix, pre- and post-gene therapy.