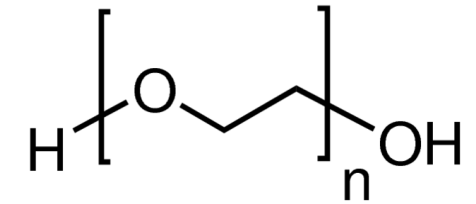# Development of an Anti-PEG Antibody Assay for Assessing Immunogenicity of PEGylated Proteins and Lipid Nanoparticles

Sam Willcox, Senior Scientist, Labcorp Drug Development
EBF Open Symposium, Barcelona 2021

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Polyethylene Glycol(PEG) –
A Synthetic, Hydrophilic Biocompatible Polymer

What is it used for in drug development?

Stability	Half-Life	Solubility	Linking/Cross Linking (ADCs)

Surface Coating (LNPs)

Currently 26 FDA-approved PEGylated biologic drugs; 30% of these have been approved in the last 3 years

Anti-Polyethylene Glycol(PEG) Antibodies

Why are these important?

Anti-PEG IgG and IgM are shown to account for efficacy loss due to accelerated blood clearance

Hypersensitivity reactions documented entailed in severe allergic reactions

A level of pre-existing anti-PEG antibody expected in the healthy population

First reported existence of anti-PEG antibodies in 1985, 10 years before first the PEGylated drug was FDA approved

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Lipid Nano-Particles (LNPs) and Liposomes

What are these?

- Specialized delivery vehicles which enhance the capability of active pharmaceutical ingredients

- Often used to delivery different genetic payload used in gene therapies such as siRNA, mRNA and saRNA

- Commonly composed of PEGylated phospholipid to improve circulation time and shield from blood plasma proteins

- Gained wide-spread prominence within the public domain due to the BioNtech/Pfizer and Moderna Vaccines, which both use the technology

The global liposome drug delivery market was valued at **US$ 3.6 Bn** in **2018** and is estimated to grow to over **~ $ 8 Bn** by **2027**

A

- PEG-lipid
- cationic lipid
- helper lipid
- cholesterol
- nucleic acid
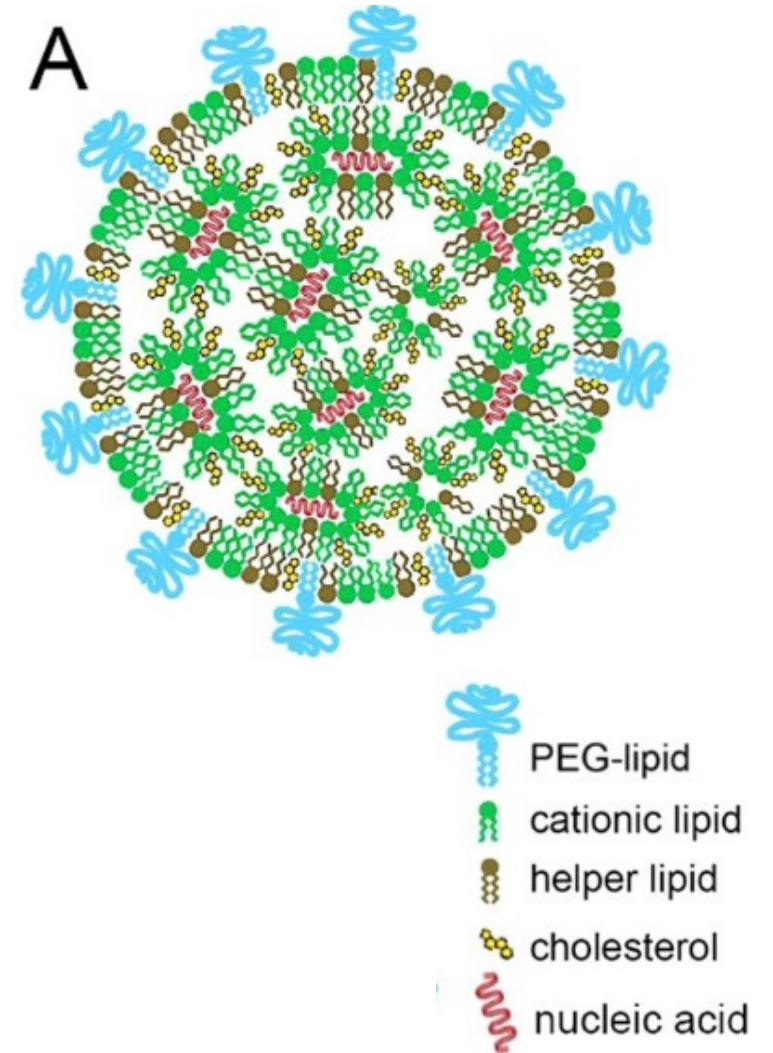
Suggested structures of lipid nanoparticle nucleic acid carriers: nucleic acids organized in inverse lipid micelles inside the nanoparticle
Image Source: https://pubs.acs.org/doi/10.1021/acsnano.1c04996

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Assay Development - Aims

Fit For Purpose

- Meets scientific requirements and current regulatory expectations

Adaptability

- Multiple modalities/variations – Plug and Play

Cheap, Quick and Easily Transferable

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Assay Development – Expected Challenges

Sourcing Appropriate Reagents

- Positive Control Selection, Generic reagents required

Pre-existing Antibodies

- Cut-point strategy?

Potential Contamination

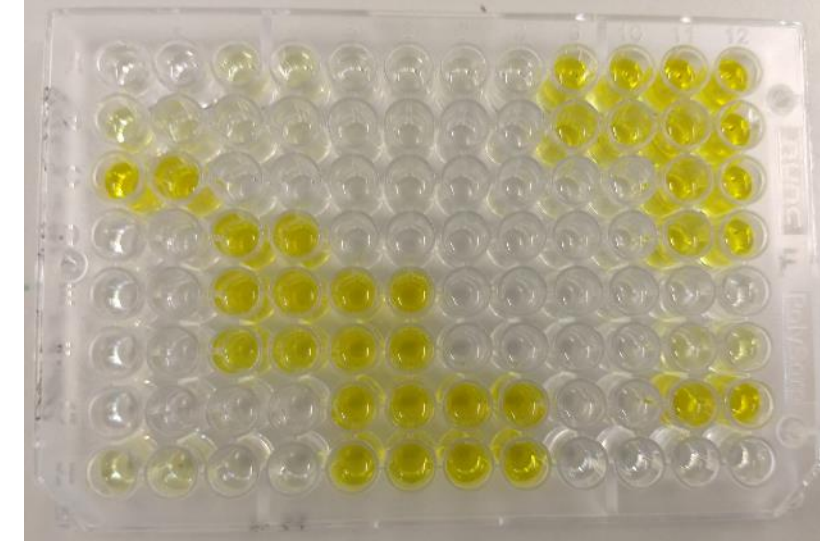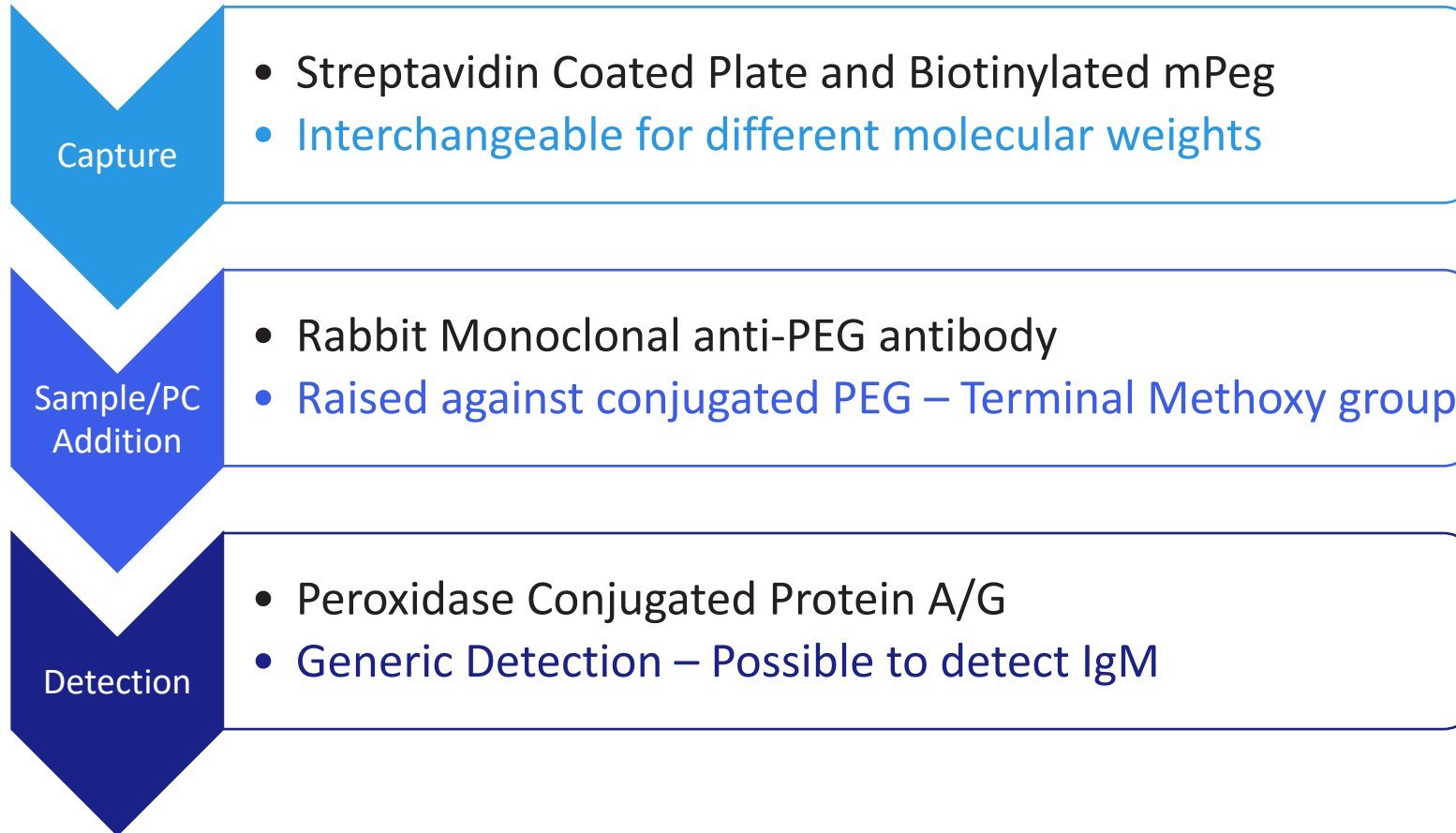- PEG component included in many standard buffers

Sensitivity/Drug Tolerance

- Can't have everything!

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Assay Outline – ELISA

Simple, cheap, wide range of generic reagents available

Capture
- Streptavidin Coated Plate and Biotinylated mPeg
- Interchangeable for different molecular weights

Sample/PC Addition
- Rabbit Monoclonal anti-PEG antibody
- Raised against conjugated PEG – Terminal Methoxy group

Detection
- Peroxidase Conjugated Protein A/G
- Generic Detection – Possible to detect IgM

Tested in-house:
- Neulasta (Pegfilgrastim)
- Generic Lipid Nanoparticles (Poly A)
- Multiple Sponsor projects

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Assay Issues and Solutions: 1 – High Matrix Background

Streptavidin, Biotin and Protein A/G all love to bind!

PC/Sample	Concentration (ng/mL)	Observed Response (AU)					
		5%BSA PBS (Assay Buffer)	5%BSA PBS + HAMA Blocker	Casein	Seablock	3% Non-Fat Milk, 1x PBS, 1M NaCl	Super Block
		1 in 50	1 in 50	1 in 50	1 in 50	1 in 50	1 in 50
Buffer Blank	0	0.001	0.821	0.000	0.002	0.003	0.000
Buffer LPC	100	0.493	0.997	0.506	0.420	0.431	0.298
Buffer HPC	250	3.473	3.185	3.672	3.048	3.227	3.102
Matrix Blank	0	0.354	0.898	0.126	0.265	0.081	0.232
Matrix LPC	100	0.746	1.100	0.617	0.575	0.501	0.540
Matrix HPC	2500	3.533	3.250	3.564	3.206	3.364	3.147
PC Level				Matrix Signal:Noise			
LPC		2.107	1.225	4.897	2.170	6.185	2.328
HPC		9.980	3.619	28.286	12.098	41.531	13.565

Assay Issues and Solutions: 1 – High Matrix Background

Streptavidin, Biotin and Protein A/G all love to bind!

Matrix NC/PCs
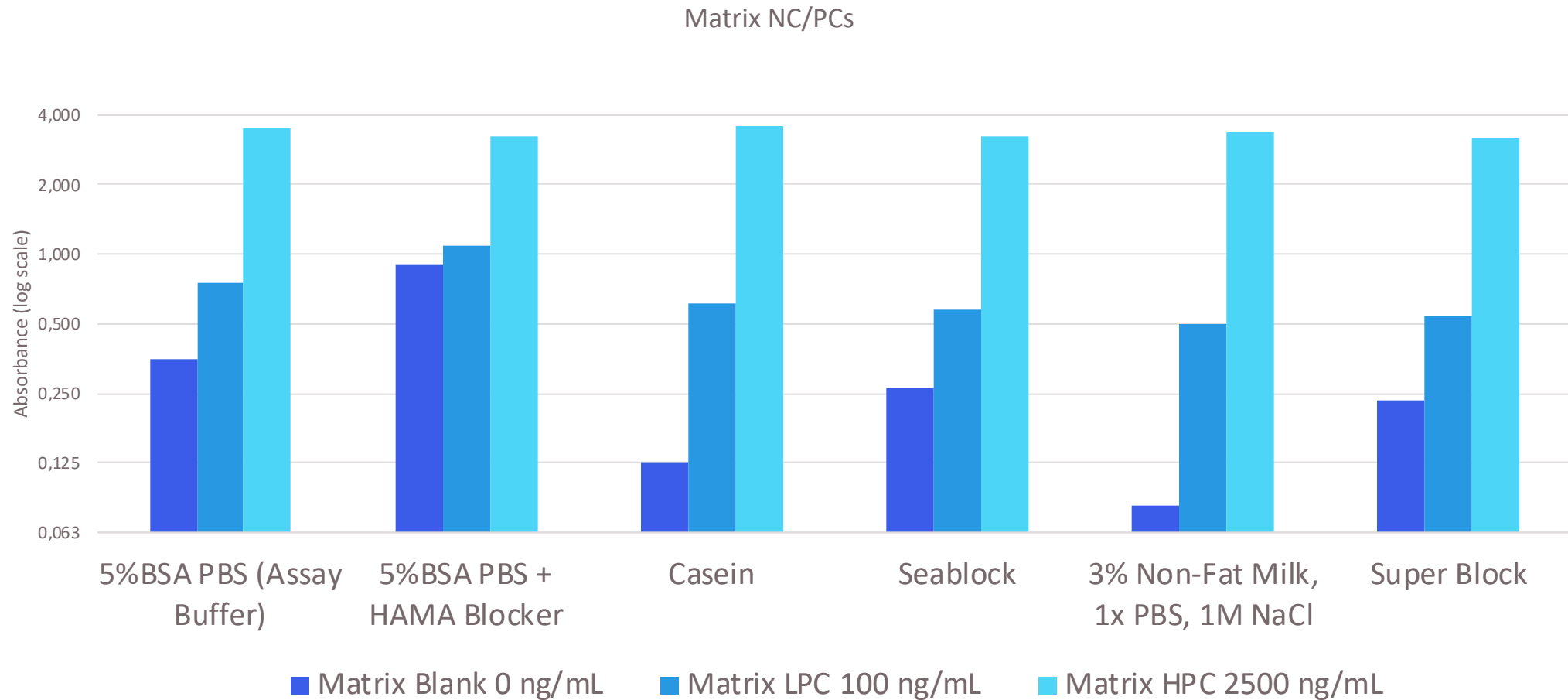
Assay Successes: 1 – Plug and Play

## Assay can be adapted for different PEG molecular weights 2kDA – 20kDA

PC Level (ng/mL)	Coating (mPeg Biotin)			Over-All Statistics		
	2kDA	5kDA	20kDA	Mean (AU)	Standard deviation (n-1)	Precision (%)
	Observed Response (AU)					
LPC: 100	0.269	0.309	0.270	0.277	0.030	10.7
	0.256	0.309	0.265			
	0.260	0.325	0.266			
	0.219	0.317	0.272			
	0.246	0.306	0.266			
HPC: 2000	2.525	2.806	2.672	2.667	0.203	7.6
	2.658	2.772	2.689			
	2.517	2.885	2.743			
	2.249	2.865	2.733			
	2.257	2.910	2.721			

Generic Screening/Titre Tiers particularly beneficial for quick sample TAT & preclinical studies.

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Assay Issues and Solutions: 2 – Pre-Existing Antibodies

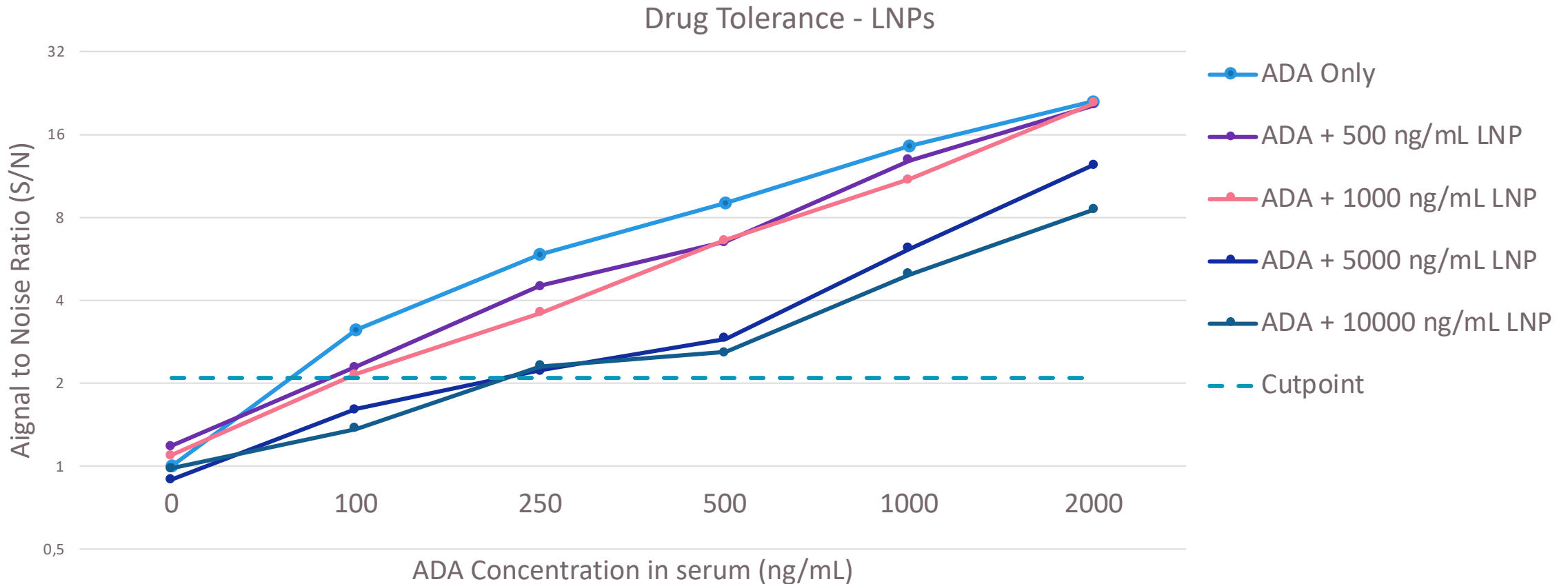## Level in healthy population – 0.2 – 75%?!

Matrix Screening for Outliers/Cut-Point Generation
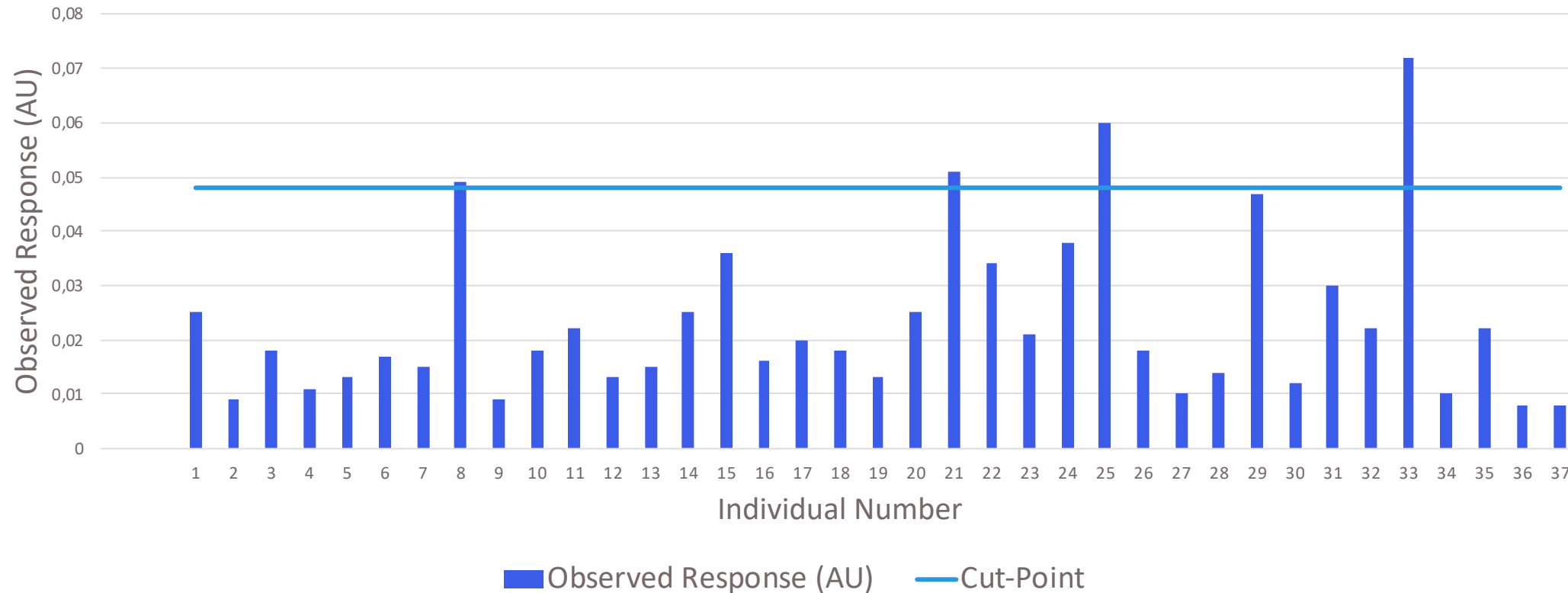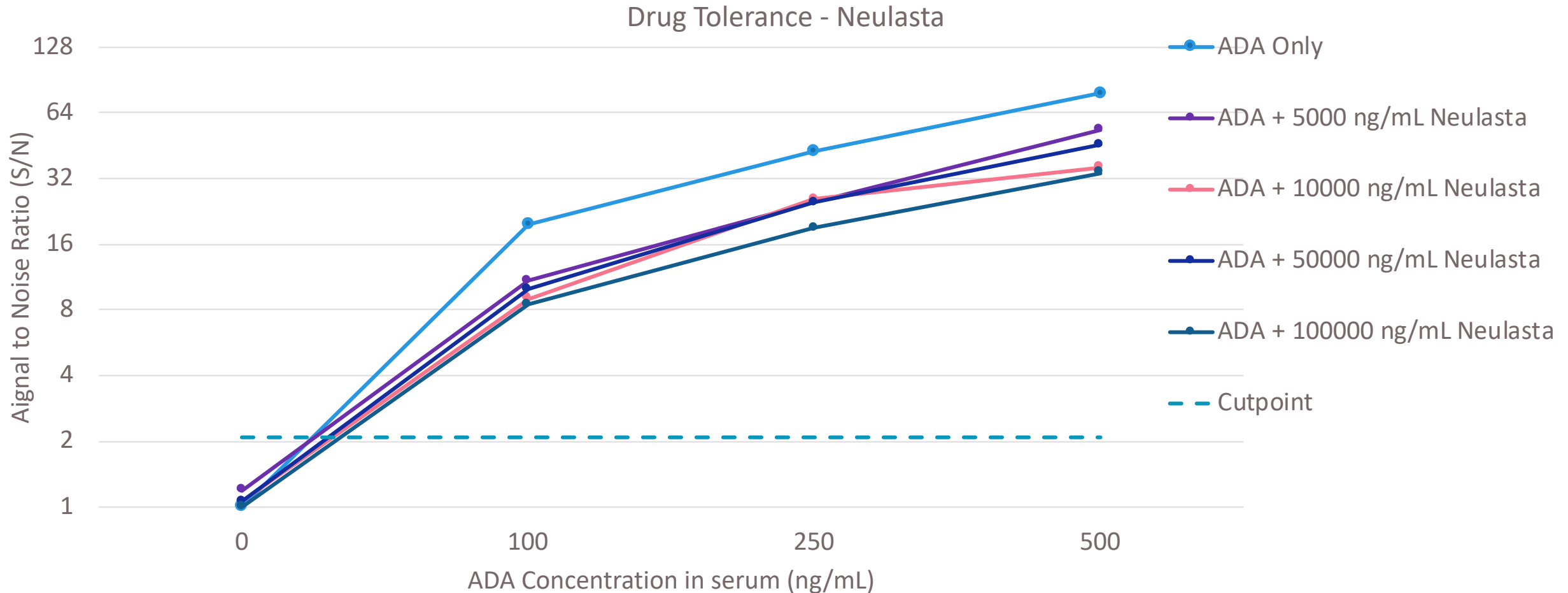
Screening False - Positive rate of ~11%

Assay Successes: 2 – Drug Tolerance, LNPs

Advantage of using with LNPs – quick clearance compared to mAbs

Drug Tolerance - LNPs

Neulasta elimination Half-life 15-80hrs. LNPs 12-72hrs. Typical ADA sampling timelines 2-3 Weeks

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Assay Successes: 2 – Drug Tolerance, Neulasta

Drug Tolerance - Neulasta

Neulasta elimination Half-life 15-80hrs. LNPs 12-72hrs. Typical ADA sampling timelines 2-3 Weeks

Conclusions – Assay Successful

Scientific and Regulatory Needs Met

Plug-and-Play Assay for Multiple Modalities Achieved

Drug Tolerance Acceptable

ACP/CCPs Workable Despite Pre-Existing Antibodies

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Acknowledgements

Anas Tomeh
Daniel Dyer
Wei Huang
Allan Watkinson
Robert Nelson

Thank you for your time

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Questions?