Development of a method for detection of anti-drug antibodies against the PEG component of a lipid nanoparticle drug product

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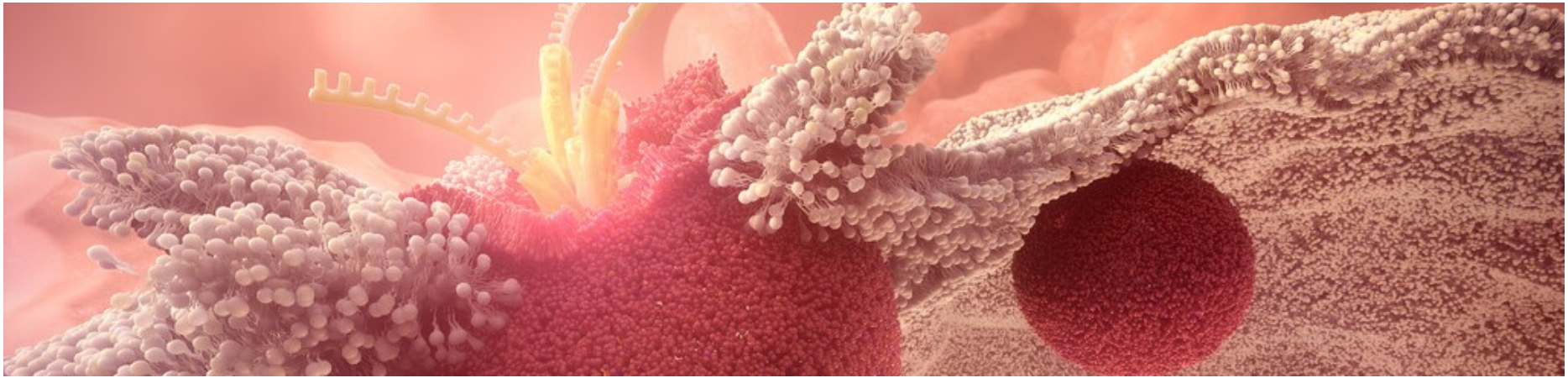
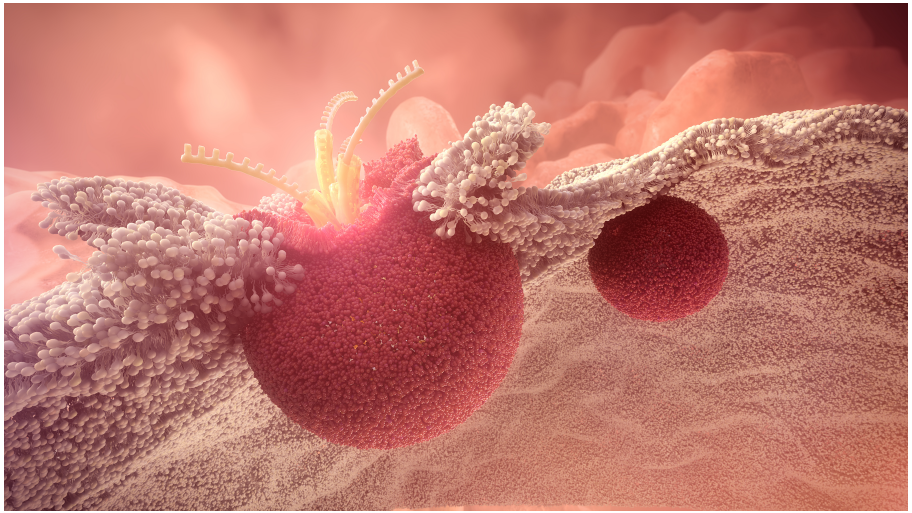
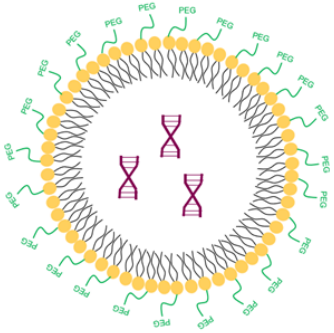


Lipid nanoparticle (LNP) drug delivery

- Lipid nanoparticles can be utilised to deliver a variety of nucleic acid based drugs, including:
  - siRNA
  - mRNA
  - saRNA

- Specialised delivery vehicle
  - Fuses with biological membranes, allowing effective delivery of RNA drug into target cells
  - All LNPs utilise PEGylated phospholipids to aid stability of the LNP
  - Protects RNA from nuclease degradation





Anti-drug antibody (ADA) assessments of LNP based drug product

- Two types of immunogenicity assessments required for LNP based drugs:
  - Derived protein ADA
  - Anti-PEG ADA to the LNP construct
  - Anti-RNA not required – RNA if released from LNP would be rapidly degraded by RNAase

Anti-PEG ADA assay challenges

- Anti-PEG assessments are a known challenge within the bioanalysis community
  - Unable to add detergent to assay buffers
  - Sourcing a suitable positive control
  - Pre-existing anti-PEG antibodies & high variability between individuals

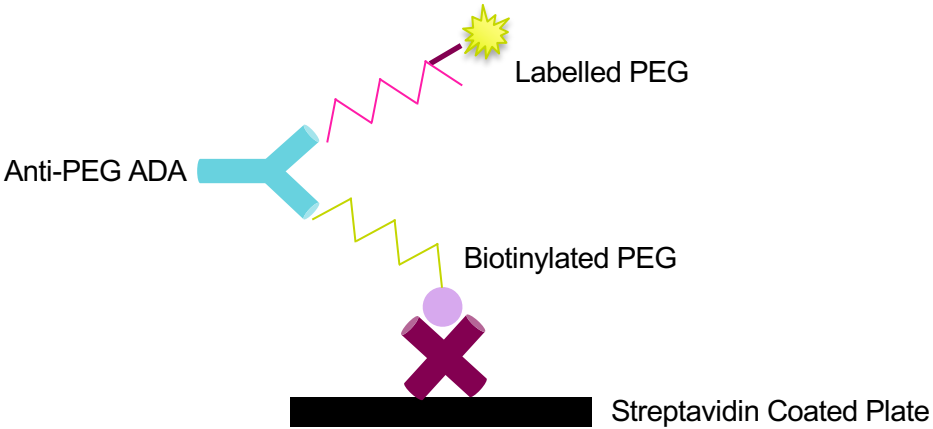
- Initial assay formats assessed were ECL and ELISA

  - Unable to biotinylate or ruthenium label drug product LNP

  - Initially attempted to utilise commercial biotinylated-PEG and labelled PEG detection to create generic anti-PEG ADA assay

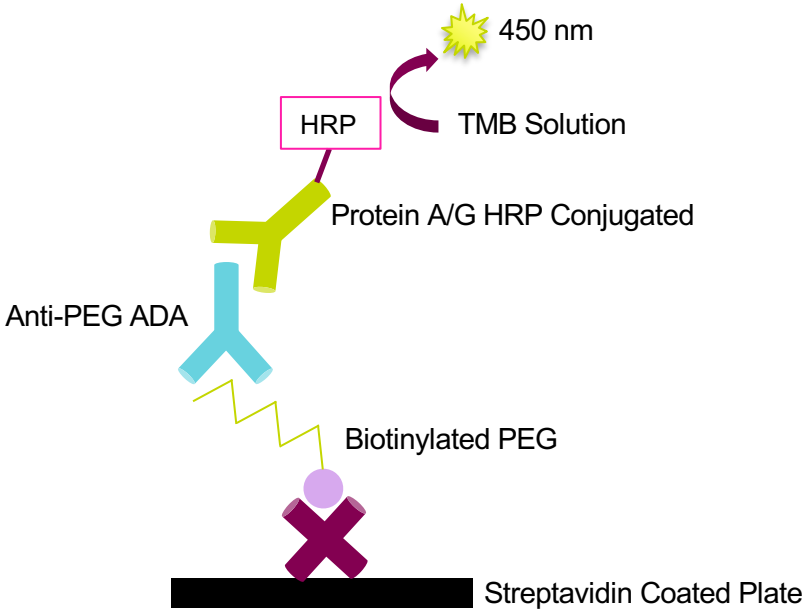
Initial formats tested unsuccessfully

1) ECL Homogenous



No Signal Observed

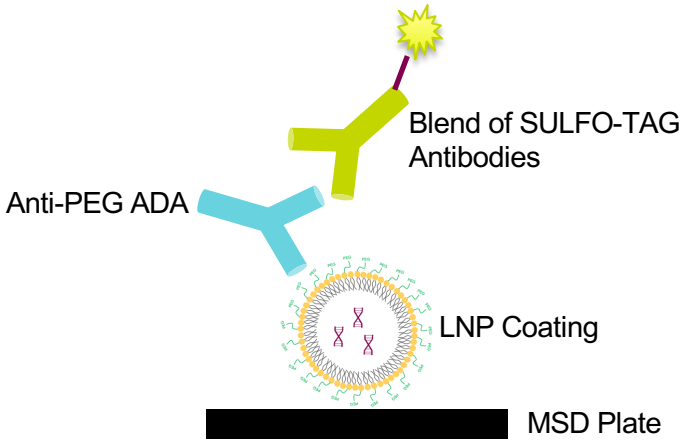
2) ELISA Absorbance



Only Non-specific Signal

Direct format for anti-PEG assay successful

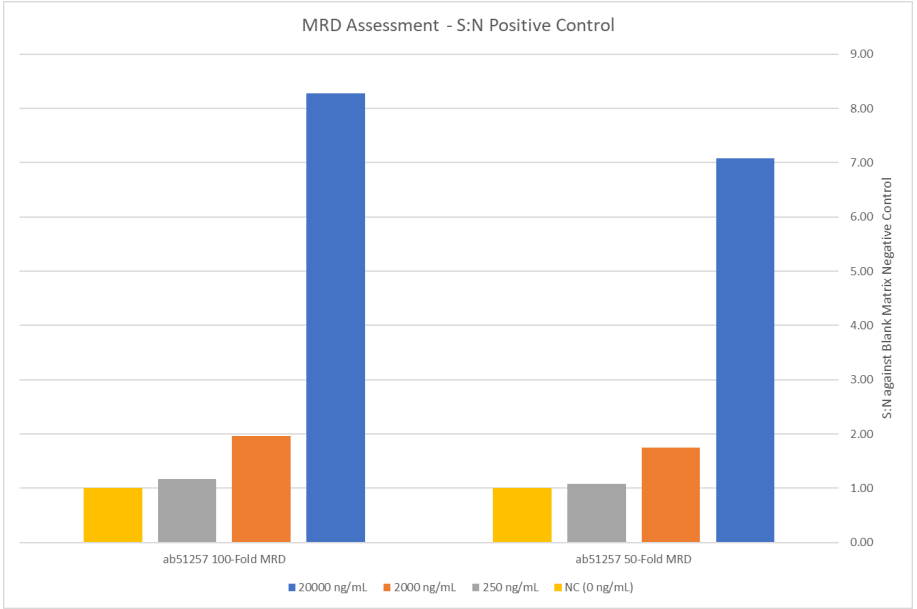
- Approach utilises drug product as capture component coated onto MSD plates
  - First assessment of format demonstrated potential
  - Clear that extensive further optimisation was required
- Unable to source human anti-PEG antibody to act as positive control (PC), therefore various non-human commercially available anti-PEG antibodies evaluated
- Requires blend of ruthenium labelled detection reagents:
  - Assay needs to detect human anti-PEG ADA
  - But PC will be non-human (rabbit)
- Initial assessments indicated to use 1 in 100 fold MRD



MRD at 100-Fold optimal

- MRD was compared at 50-Fold and 100-Fold during first test of the planned direct format
  - S/N at LPC indicated that method should be optimised utilising 100-Fold
  - 100-Fold also beneficial as reduced “high background” signal

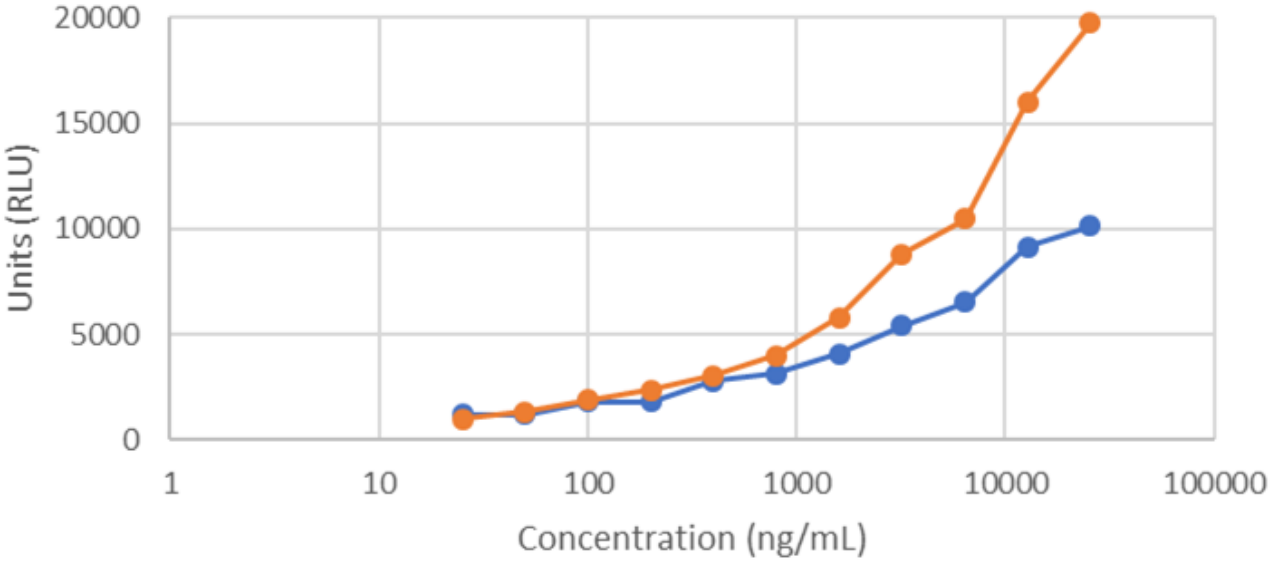
PC Concentration	50-Fold MRD	100-Fold MRD
20000 ng/mL	7.08	8.28
2000 ng/mL	1.74	1.96
250 ng/mL	1.08	1.17
NC (0 ng/mL)	1.00	1.00



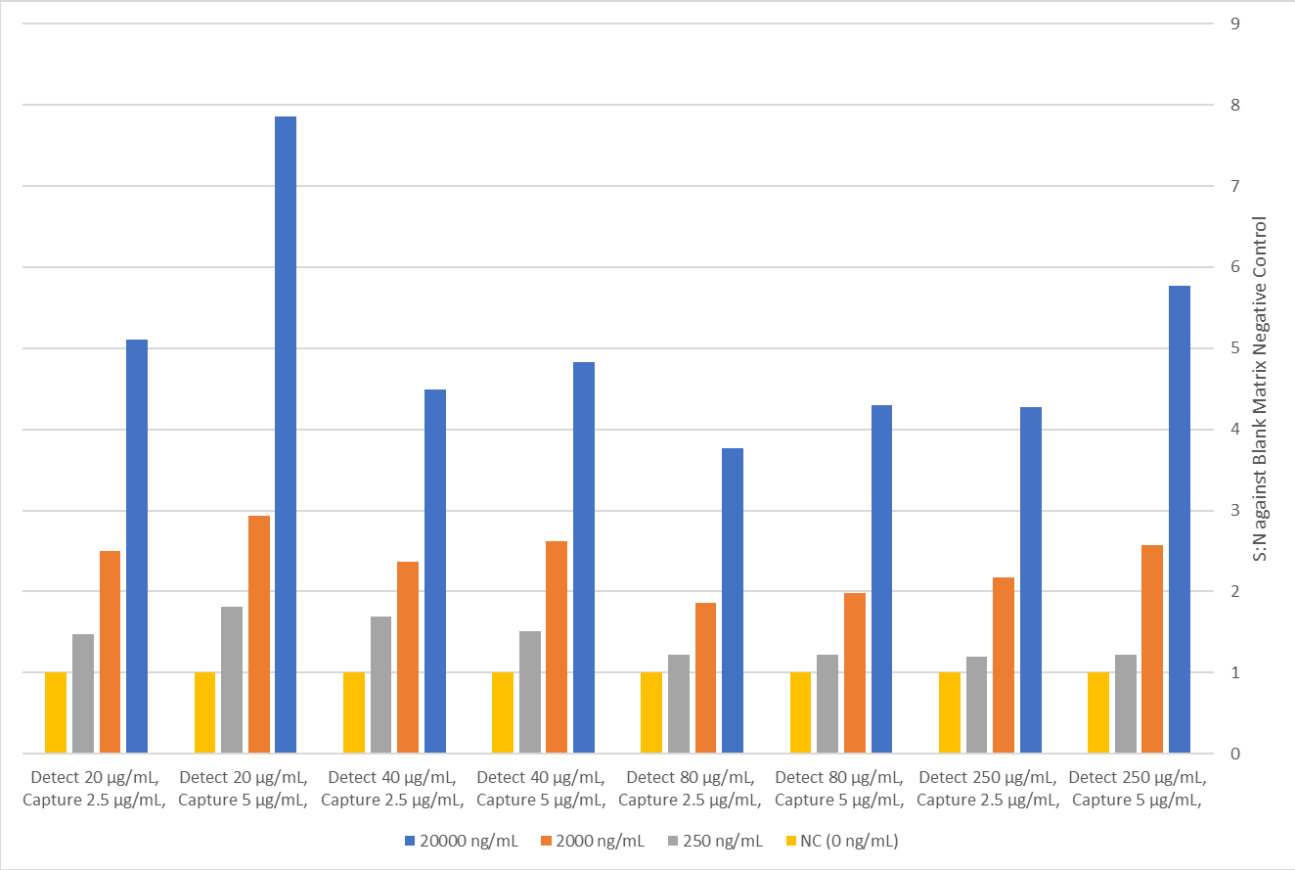
- MRD was again compared after stages of optimisation
  - 25-Fold and 100-Fold
  - Precision was poor with 25-Fold therefore 100-Fold confirmed

Positive control with most favourable performance chosen

- Sensitivity of two commercial rabbit monoclonal anti-PEG antibodies were compared

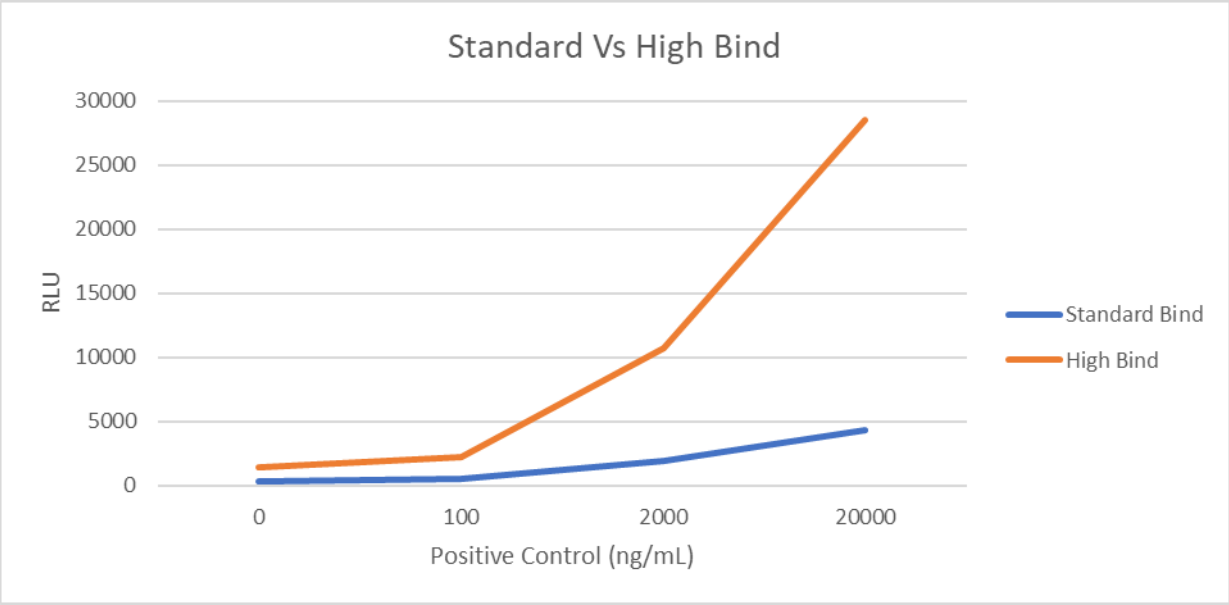


Optimised capture & detection concentration selected



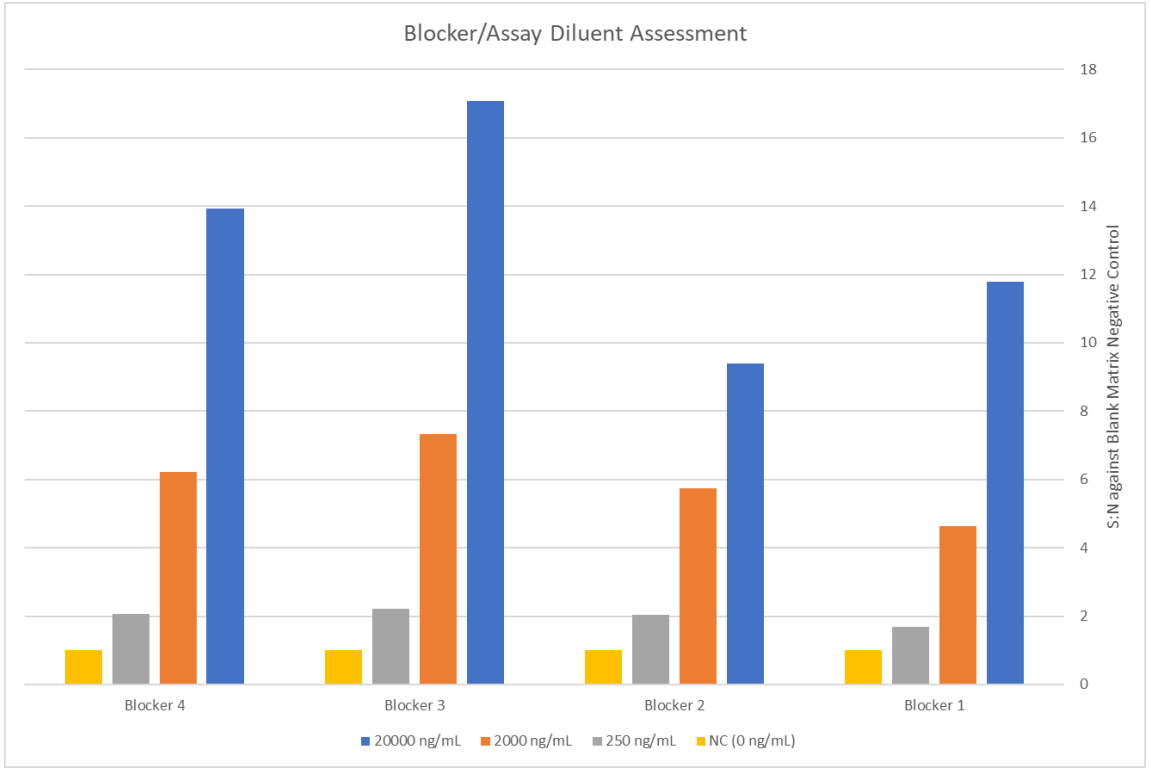
High bind MSD plates performed better than standard bind

- Method development completed on high bind MSD plates, except when compared with standard bind plates



Blocking/assay reagents selected based on best S:N and reduced non-specific background

- Four different blockers assessed as blocking buffer & assay diluent



Confirmatory diluent with 2 µg/mL drug product optimum

- Confirmatory diluent at 8, 4, 2, 1 µg/mL assessed
  - Complete inhibition is not achievable
    - Results across several runs demonstrate that 2 µg/mL is sufficient to provide the maximum achievable % inhibition ~ 90%
    - Data from 8 & 4 µg/mL do not present further % inhibition, therefore 2 µg/mL chosen

Confirmatory diluent at 1 µg/mL

	HPC	MPC	LPC	NC
	20000 ng/mL	2000 ng/mL	250 ng/mL	N/A
Duplicate 1 % Inhibition	74.3	79.0	26.4	-2.9
Duplicate 2 % Inhibition	71.9	78.4	34.6	23.2

Confirmatory diluent at 2 µg/mL

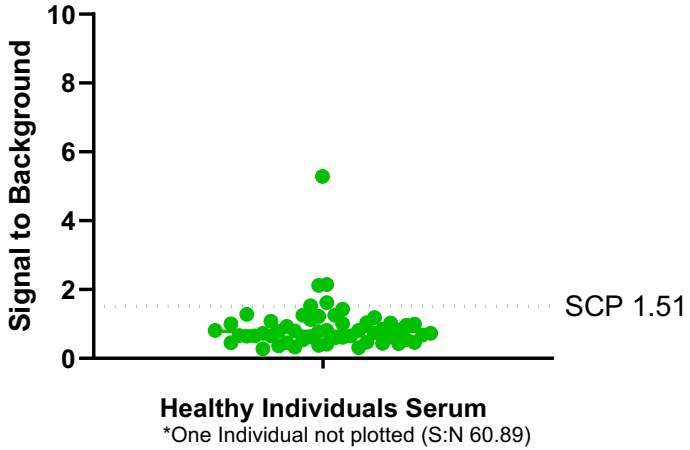
	HPC	MPC	LPC	NC
	20000 ng/mL	2000 ng/mL	250 ng/mL	N/A
Duplicate 1 % Inhibition	90.3	86.8	56.6	10.1
Duplicate 2 % Inhibition	89.5	86.6	57.1	25.0

Estimated screening and confirmatory cut points

- Estimated screening cut point and confirmatory cut point generated by assessment of 56 individual human serum
  - Two individuals excluded from calculations as response units outside 3rd interquartile range
    - Biological outliers with probable pre-existing anti-PEG antibodies
    - These will be excluded from pool when preparing NC for validation

- Screening Cut Point (5% FPR): 1.51

- Confirmatory Cut Point (1% FPR): 55.4%



- Based on these estimated cut-points:
  - Six individuals would screen positive (including the two pre-existing)
  - Two individuals would confirm positive (the two individuals with pre-existing)

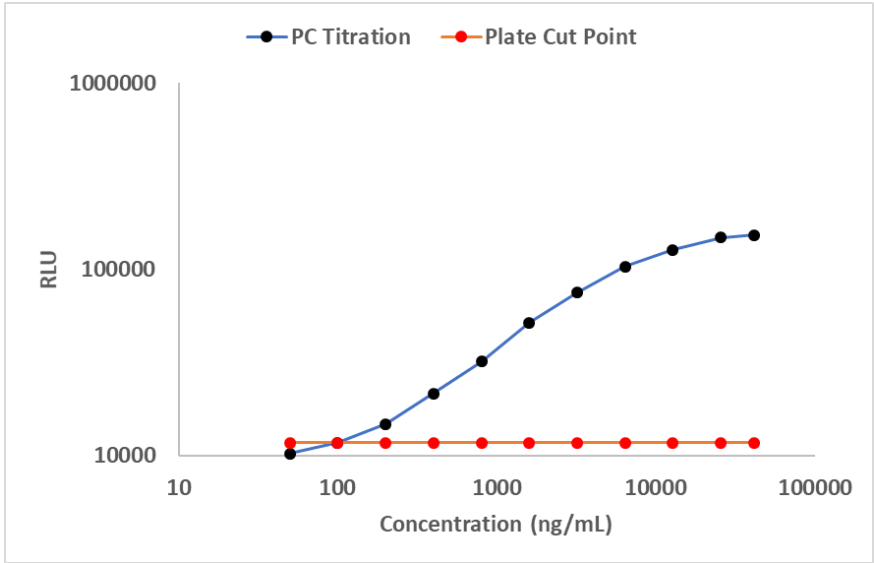
Predicted sensitivity, hook effect and titration assessments demonstrate acceptable method performance

- Sensitivity, hook effect and titration of the two pre-existing individuals assessed using predicted SCP of 1.51

- No hook effect evident up to 41300 ng/mL
  - As high as can be assessed maintaining 95% matrix

- Sensitivity demonstrated at 100 ng/mL (just!)
  - This is much lower than anticipated before optimisation

- Two healthy individuals with pre-existing both titrate down in an acceptable manner



Conclusions:

- A drug specific LNP anti-PEG ADA assay has been developed balancing the need for optimal achievable sensitivity, a suitable dynamic range and performance in terms of reliable precision

- Individual serum assessments for screening & confirmatory cut point estimated, and data demonstrate high variability of individual responses within healthy population
  - Use of additional individuals for cut point setting
  - Excluding individuals with pre-existing anti-PEG from the negative pool

Acknowledgements:

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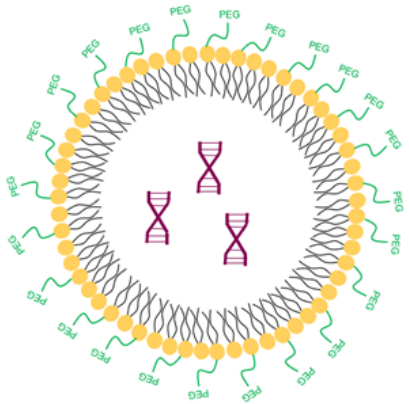

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