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IS ADA-TIERED APPROACH APPLICABLE TO THERAPEUTICS WITH PRE-EXISTING ANTIBODIES?

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OVERVIEW

01 **Background/ Guideline**

02 **Case study 01: Anti-Drug pre-existing**

03 **Case study 02: Anti-Peg pre-existing**

04 **Conclusion**

BACKGROUND

- **Pre-existing antibodies** are immunoglobulins that are either specific or cross-reactive with biotherapeutic compound.
- These antibodies can have various impact to **the safety and efficacy of therapeutic antibodies**
- Well document pre-existing antibodies includes those against AAV and those against PEG
- Pre-existing Anti-AAV can be due to naturally occurring AAV infection
- Anti-PEG maybe due to products that we are exposed to
- There is a high prevalence of anti-AAV in the general population (>70%) and anti-PEG (up to 70% depending on the method used)

GUIDELINE

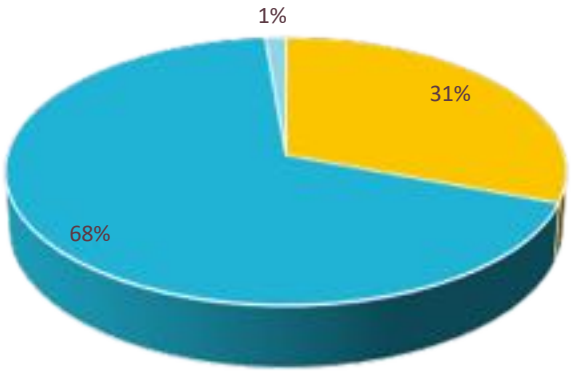
- **FDA 2019 Guidance:** In subjects that have pre-existing ADA, treatment-boosted ADA responses may be identified…. **A cut-point for defining the treatment-boosted responses should be determined.** For example, a boosted ADA response may be defined as a titer that is two dilution steps greater than the pre-treatment titer, when two-fold dilutions are used to determine the titer.
- …An alternative to the qualitative screening assay approach may be needed to **assess the quantity and quality** of ADA when pre-existing antibodies are present.
- **EMA 2017 Guideline**: No clear information on how to deal with pre-existing antibody but acknowledges the presence of pre-existing antibody.
- **WRIB**
  - 2024 White Paper on recent issues in bioanalysis
  - With an increased prevalence of pre-existing anti-PEG antibodies should these assays be developed and **validated more like vaccine assays**?

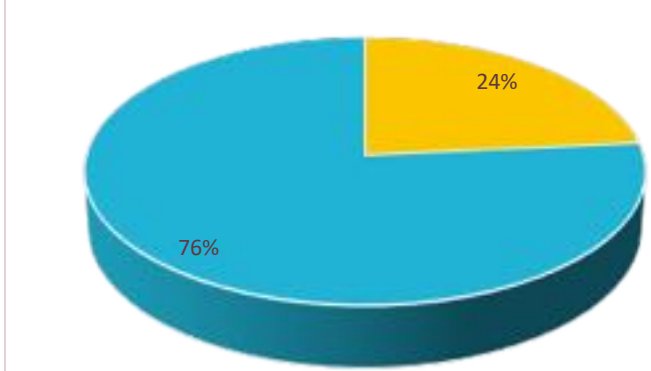
CASE STUDY 01

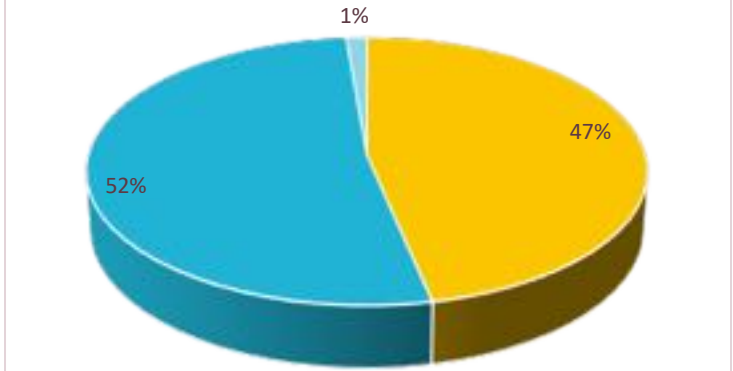
CASE STUDY 01: MULTISPECIFIC THERAPEUTIC WITH PRE-EXISTING

- A homogenous bridging assay format was developed
- A large number of High responder were observed and attributed to pre-existing
- Pre-existing antibody were not characterise as study was in phase I
- Complex molecule requiring potentially three domains characterization to identify the route cause of high response observed in drug naïve individuals
- Despite high number of pre-existing the sponsor wanted to follow the standard tiered approach (Screening, Confirmatory and titer)
- Individual samples pre-screened to create a low responder pool (negative control)

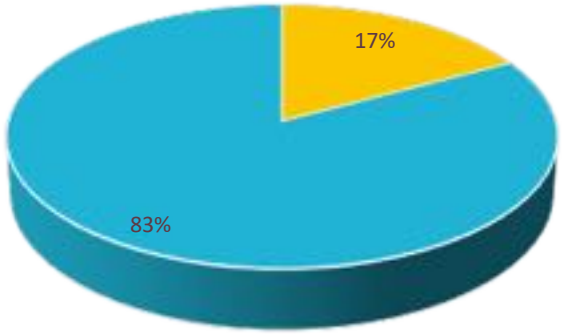
PREDOSE SAMPLES ASSESSMENT

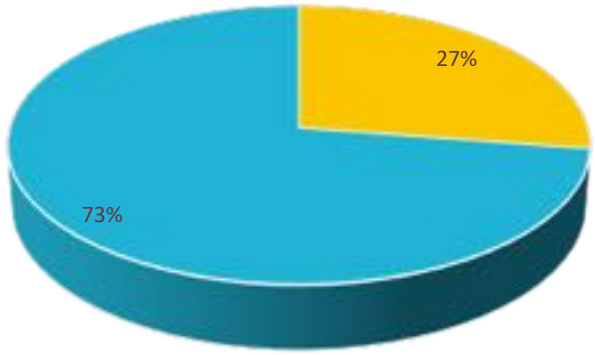


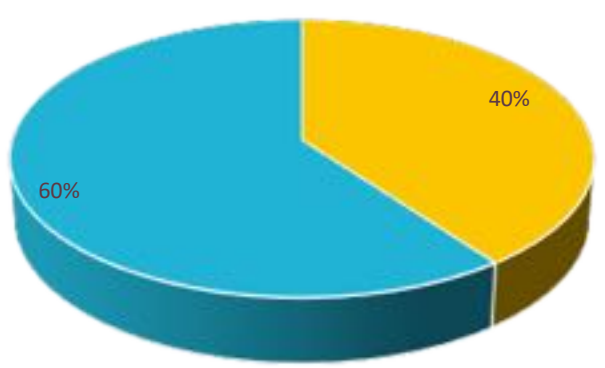




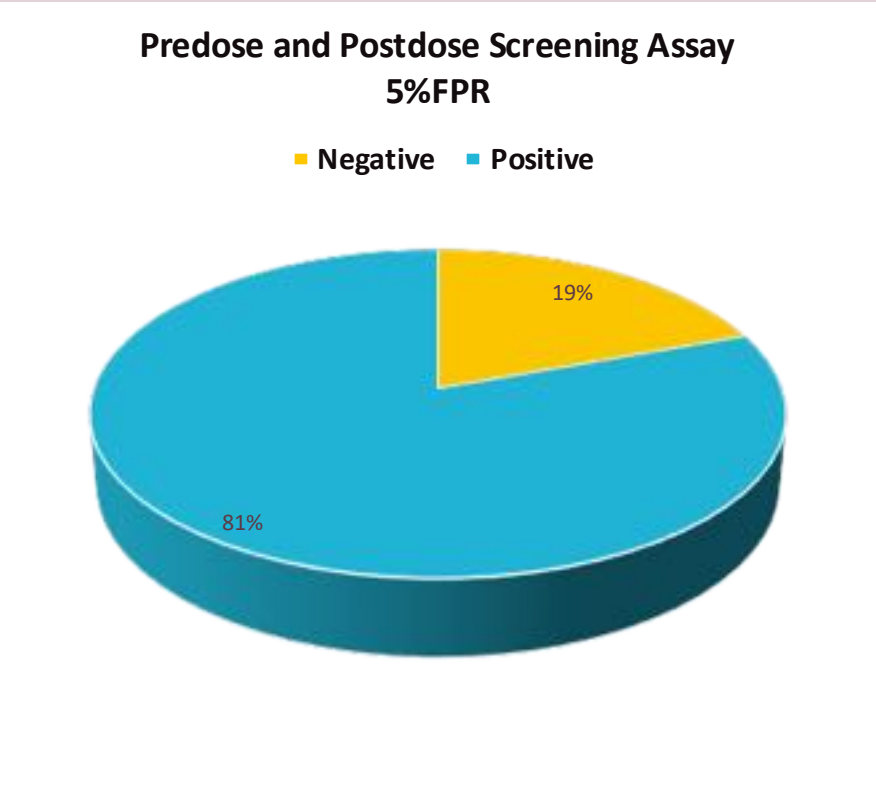
POST DOSE SAMPLES ASSESSMENT





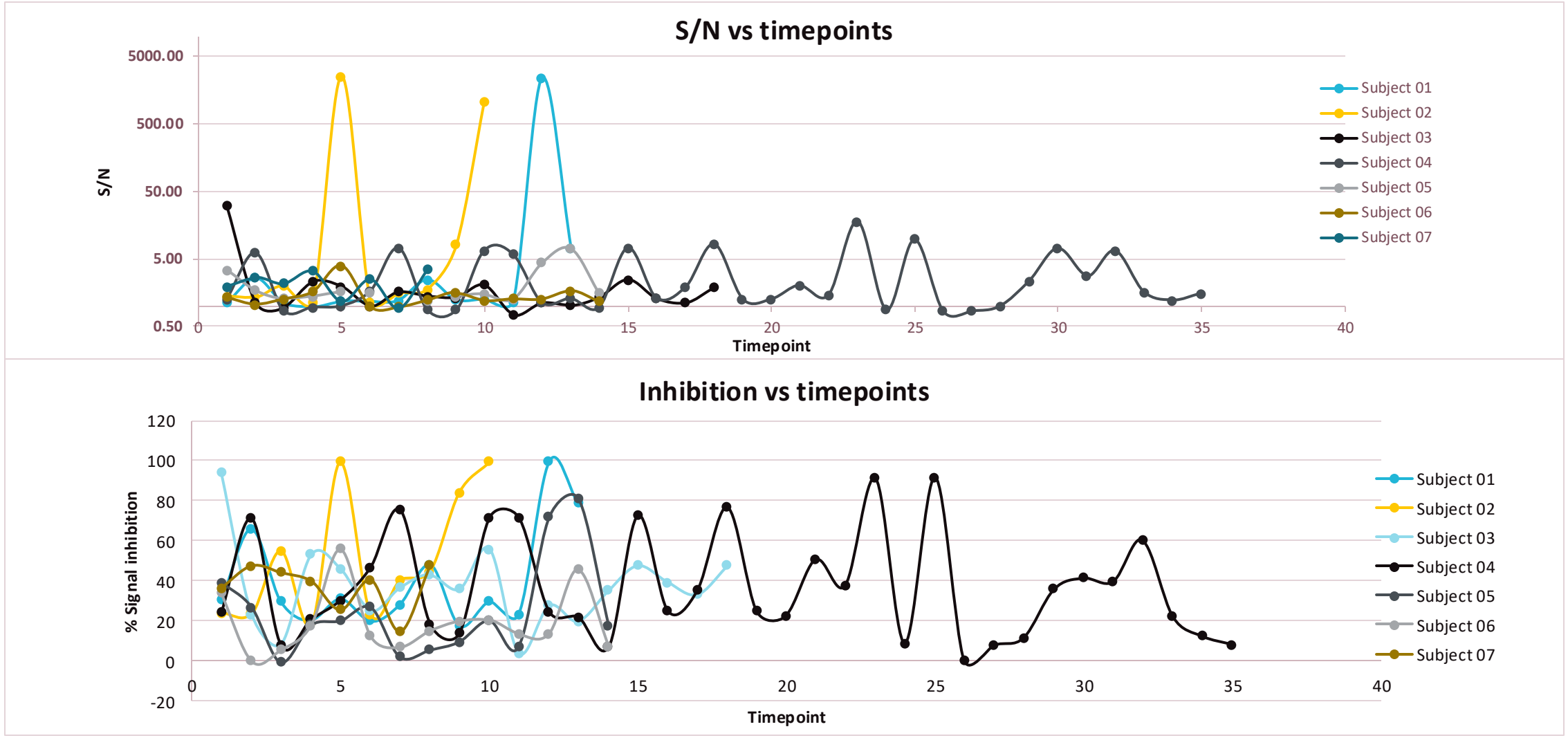


OVERALL CONFIRMATORY ASSAY ASSESSMENT



- Cost and practicality consideration
- Screening assay at 5% FPR to remove negative samples
- Confirmatory assay to remove any false positive
- Followed by the titer to determine the immunogenicity magnitude
- But when 81% of samples screening positive are these three tiers helping or increasing the cost and time?

FURTHER LOOK IN THE DATA



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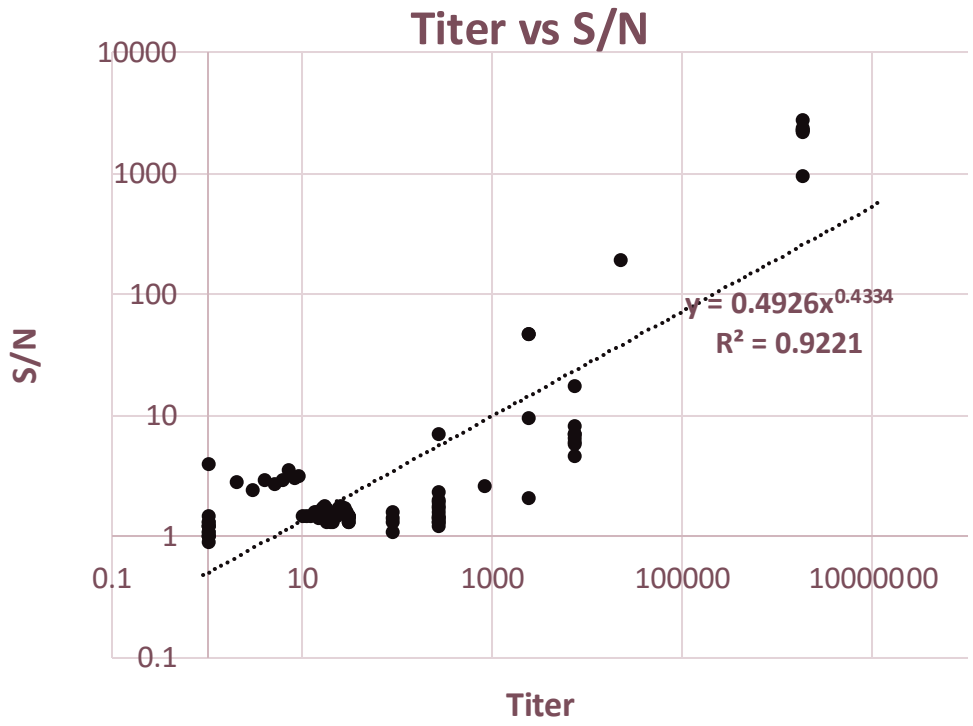
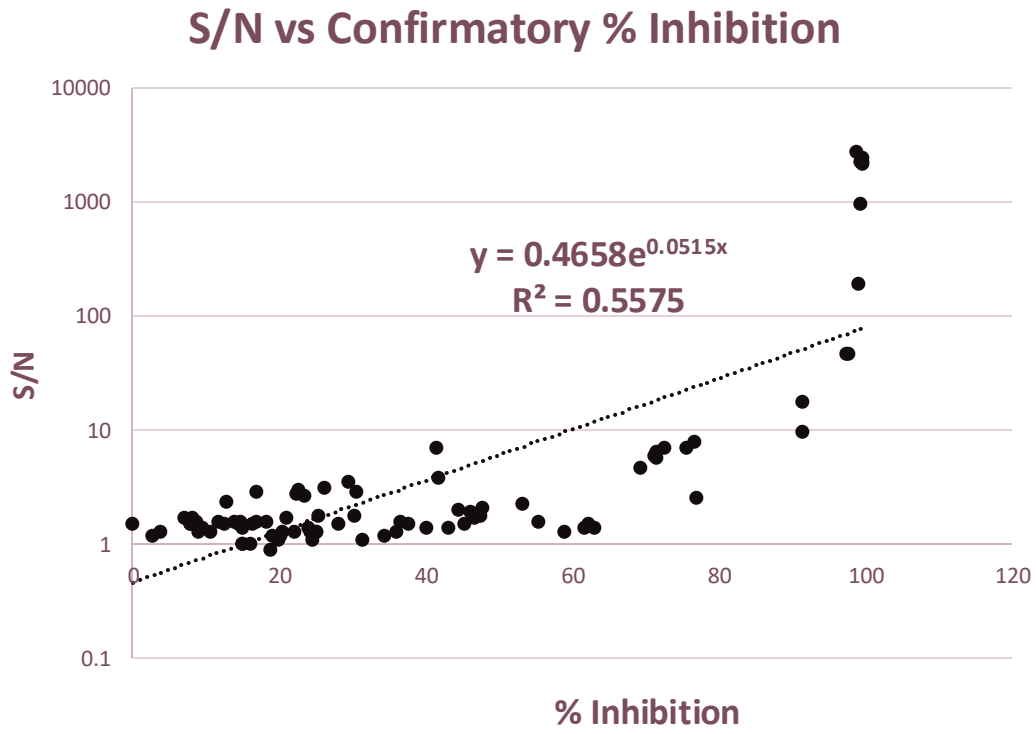
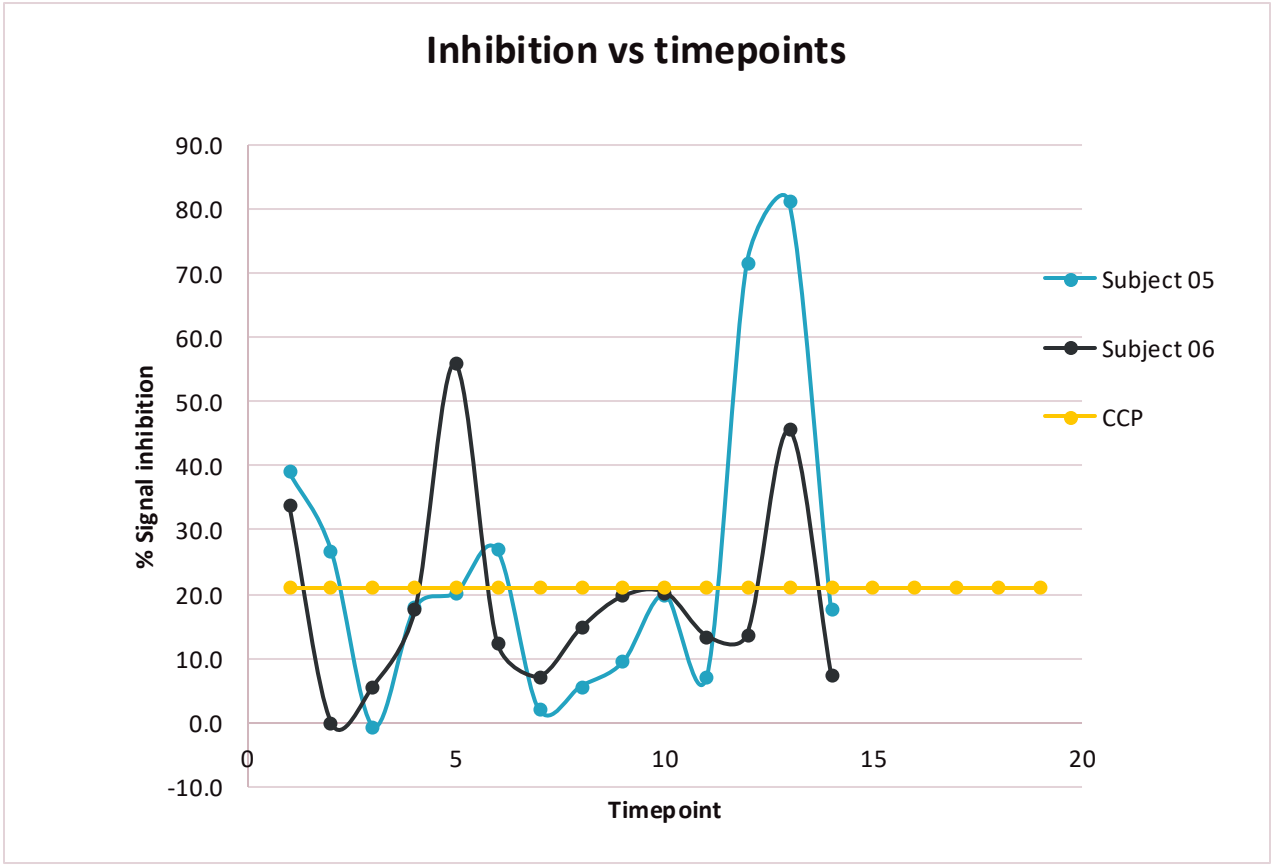




- Similar trend seen when the S/N was compared to titer (R2=0.9211)
- Similar trend seen when the S/N was compared to confirmatory inhibition, but the range was narrow (0-100%)_

ADDITIONAL CONSIDERATION AND CONCLUSION



- Titer and S/N data correlated
- Homogenous assay are very specific
- Removing the need of a confirmatory assay
- Confirmatory cut point could mask underlying biological impact

CASE STUDY 02

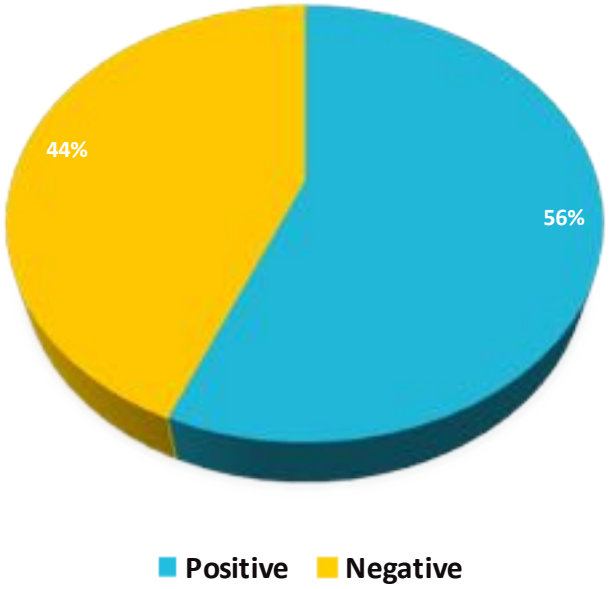
CASE STUDY 02: PRE-EXISTING ANTI-PEG TO LNP USED AS VEHICLE IN GENE DELIVERY

- High prevalence (0.2-70%) depending on the method used
- Exposure to various household and pharmaceutical products containing PEG may be the root cause
- PEG present on many therapeutic compound is very diverse
- Making it harder to develop one assay to feet all requirements
- ~700 Da of PEG (16 PEG monomers) is sufficient to interact with the APA fab paratope (Justin et al. 2020)
- Making the development of a standard homogenous ADA assay complicated for molecule containing larger MW PEG

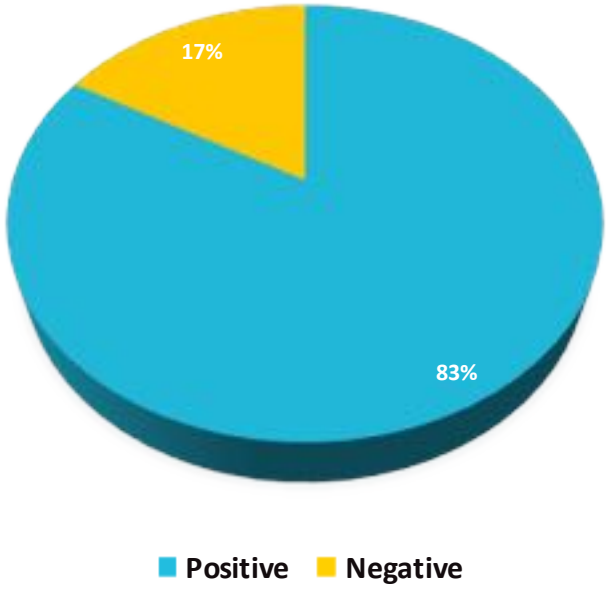
CASE STUDY 01: PRE-EXISTING TO LNP USED FOR GENE DELIVERY

- A direct ELISA assay format was developed
- Individual samples pre-screened to create a negative control

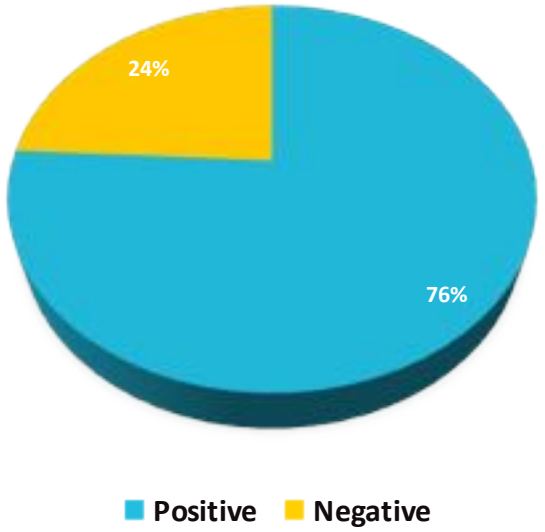
Predose screening assay



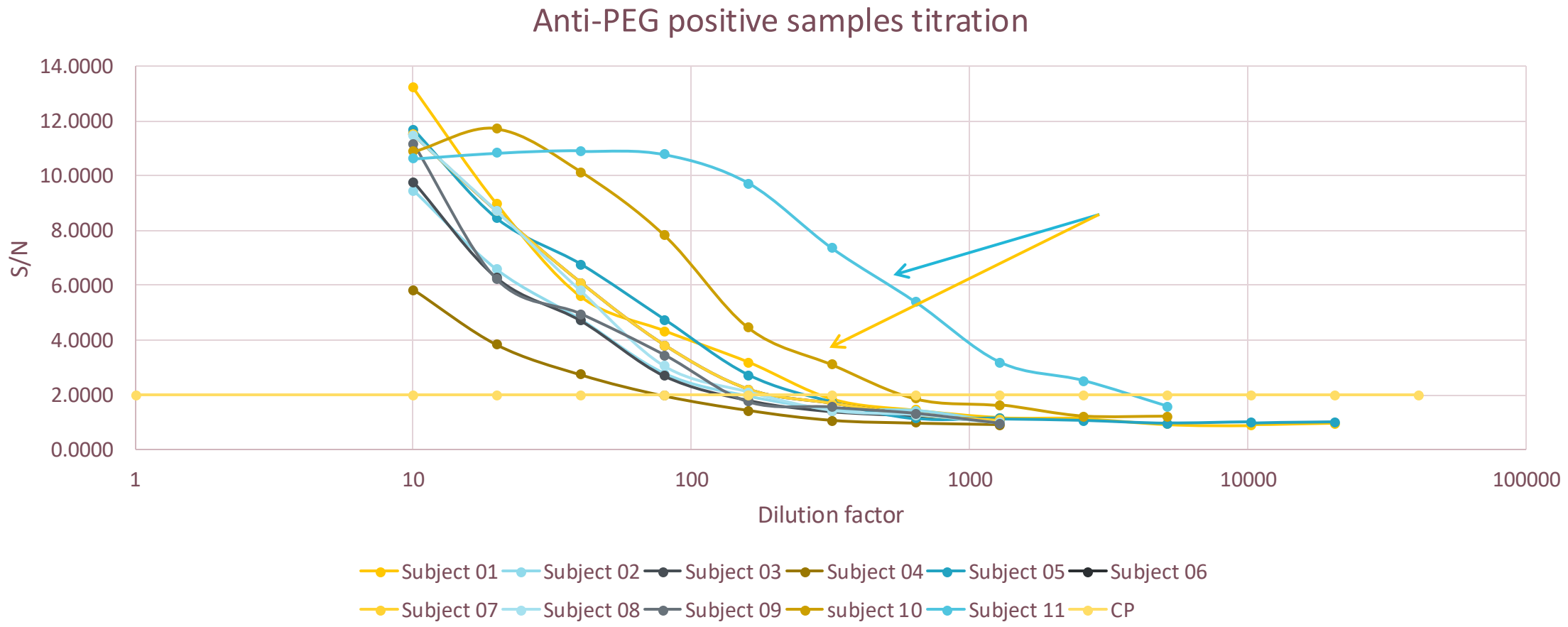
Postdose screening assay



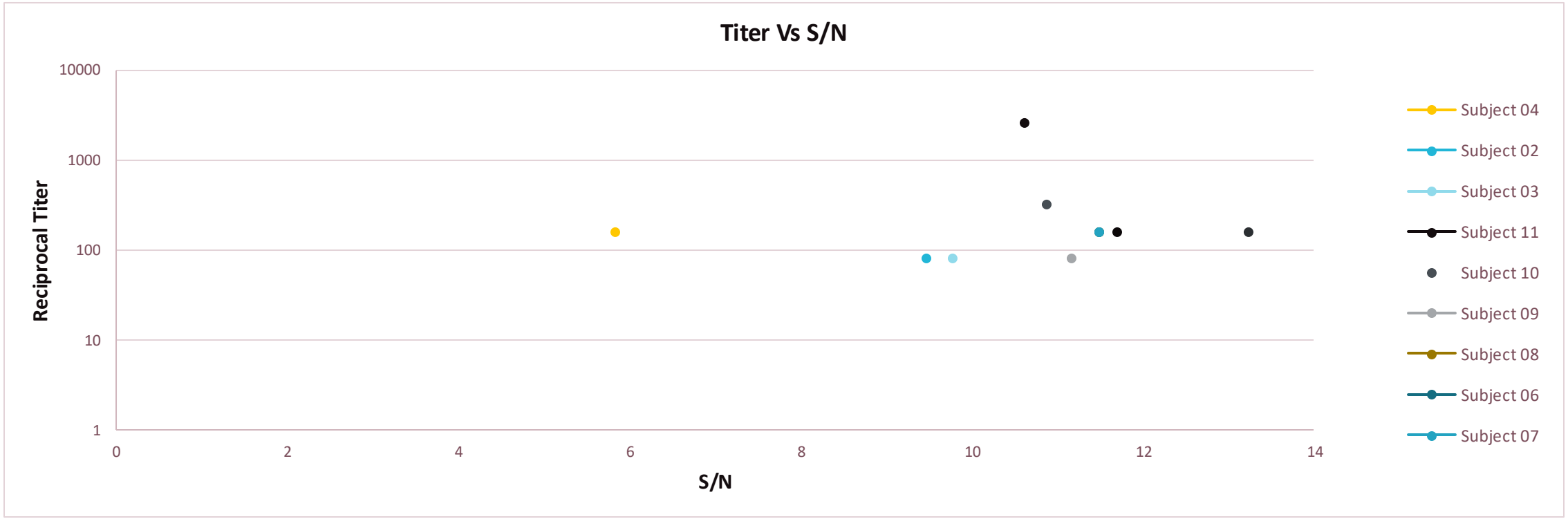
Pre and Postdose screening assay



TITER ASSESSMENT FOLLOWING THE SCREENING ASSAY



COMPARISON OF S/N AGAINST TITER



- There was no clear correlation between the S/N and the titer assessed
- Looking at the data this may be due to a narrow assay dynamic range as it was a calorimetric ELISA assay

CONCLUSION

- Are pre-existing antibodies well characterised?
- ADA assay with pre-existing antibodies may not benefit from the additional confirmatory assay but may lead to higher cost and time
- The use of cut points can mask true underlying biological effect
- The use of S/N as a single tier would be recommended
- The decision should be based on the assay performance
  - Good dynamic range and no hook effect

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Thank You!

ANY QUESTIONS?

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