

Immunogenicity Titer Analysis – What are the options?

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2019, 20, 21, 22, ...

Since SARS-CoV-2 covid "everyone" has become an expert in:

- Vaccination
- Boosting
- Antibodies
- Neutralizing Antibodies
- Titer
- PPE (surgical- and FFP2 masks)
- • •



Titer

- Strength of a solution or the concentration of a substance in solution (as determined by titration).
- Measure of the concentration of a substance in a sample. Titer testing employs serial dilution to obtain approximate quantitative information from an analytical procedure that inherently only evaluates as positive or negative. The titer corresponds to the highest dilution factor that still yields a positive reading and is expressed as a ratio (e.g. 1:200).
- 3. In textile engineering, titer is also a synonym for linear density (weight per unit of length).



Questions

- Why is it important to know antibody titers?
- What can we learn from this?



Figure P. Chamberlain (2022)



Titer: Availability of ADA Magnitude Data Enables

- Subset analysis of ADA impact on PK, safety, and efficacy
- Monitoring of immune response progression, especially in subjects with pre-existing antibodies
- Comparison of ADA response characteristics between biosimilar and innovator

Titer is currently the most used variable for ADA magnitude determination.



Subset Analysis by Quantiles

- E.g. analysis by ADA titer tertile* category
 - Low Titer (Maximum ADA Titer < T1), Mid Titer (T1≤Maximum ADA Titer ≤T2), High Titer (Maximum ADA Titer>T2)
 - T1 = lower tertile of maximum post-baseline ADA
 - T2 = upper tertile of maximum post-baseline ADA

*Either of two points that divide an ordered distribution into three parts, each containing a third of the population.





Monitor Immune Response Progression

- Determine a treatment-induced/boosted ADA response
 - In a subject with an ADA negative pre-dose sample, a treatment-induced ADA response is defined as any post-dose sample being positive in the ADA confirmatory assay
 - In a subject with an ADA positive pre-dose sample, a treatment-induced (boosted)
 ADA response is defined as an x-fold increase (the minimum significant ratio) in
 titers from the pre-dose assessment compared to a post-dose assessment



Comparison of ADA Response Characteristics

An example where the ADA response characteristics is compared between a biosimilar and innovator drug.

Anti-Drug Antibody Titers by ADA Status, Treatment and Visit





More Questions

- Who is interested in this data and why?
- What is the challenge measuring ADA titers?
- Are there alternatives to determine ADA magnitude?
- When we use an alternative, will the "end users" accept it?

Microtiter Plate Capacity is Different for Each Analysis Tiercaring for life



Confirmatory, and especially titer measurements, can significantly impact sample throughput and costs / time of a study and reporting of the results.

1st Publication of Assay Signal as an Alternative to Titer



The authors around Marta Starcevic (Amgen) published their work in 2017

Conditions for using S/N instead of titer

- Linearity or appropriate dose-response within range of study responses
- Acceptable inter-assay precision
- Presence of expected level of drug does not change assay signal or S/N > 30%

For reprint orders, please contact reprints	@future-science.com	Biodnalys
Assay signal as an alter assessment of magniter antibody response	rnative to titer for ude of an antidrug	
Background: Titer methods are common of an antidrug antibody response. Assay circumstances under which use of signal-t- well defined. Results: We validated both therapeutics. SNK orrelated strongly with on a per subject basis. Analysis of impact of yielded the same result using either me precision, good linearity, and adequate circumstances, assay S/N is a valid alternatio of an antidrug antibody response. First draft submitted: 24 August 2017, Accept Published online: 12 October 2017	hly used to characterize the magnitude S/N is an appealing alternative, but the >-noise (S/N) is appropriate have not been titer and S/N-based methods for several iter both in aggregate and when examined antibody magnitude on pharmacokinetics thod. Each assay demonstrated excellent drug tolerance. Condusion: Under these to titer for assessment of the magnitude ed for publication: 22 September 2017;	Marta Starcevic Manning «1.3 Mark A Kroenke ² , Stephanie A Lee ³ , Simon E Harrison ³ , Sarah A Hoofring Daniel T Mytch ² & Vibba Jawa ^{2,4} (¹)Hamacakinetics & Drug Metabolism, Angen, Thouand Dale, CA 9120, UI Cincal Immunology, Angen, Thouas Calit, CA 9120, UIA 2001-34, Photased Oals, CA 9120, UI 2001-34, Photased Oals, CA 9120, UIA Currenty, Biologics & Nacche Bionalytics, Merck, Kenherch, N 107203, UIA
Keywords: antidrug antibody • immunogenicity • S/N • titer		*Author for correspondence: Tel.: +1 805 447 4987
Key terms Immunogenicity: the ability of an antigen to administration to humans or animals. A ntidrug antibody: an antibody elicited by a specific for the therapeutic. A ntidrug antibody magnitude: a surrogate n antibodies detected in patient samples. Signal-to-noise (SNN): assay response generated by the response generated by the negative of the reciprocal of the highest sample di assay, or the reciprocal of the highest sample di assay.	induce an adaptive immune response after dministration of a protein therapeutic which is neasure of the concentration of antidrug ted by the sample or positive control divided ontrol analyzed on the same plast. Ution that still gives a positive result in the derived from interpolation from the sample	mdarox@anges.com
The assessment of antidrug antibody (ADA) responses in clinical samples is a regulatory expectation, and an important component of the adrty evaluation of a new therapeutic. ADAs can have wide variety of consequences ranging from complete neuralization of drug to enhanced exposure, and for this reason it is important to carefully assess how ADA may impact plarmacokinetics (PK), safety, and efficacy (i). A variety of ADA detection	(ECL)-based approach is the most com- monly used, especially for monoclonal anti- body therapeutica [23]. Historically, ADA assays have been purely qualitative, with a positive or negative result reported based on statistically derived assay cut points. How- ever, there are cases where it is important to provide some measure of the magnitude of the immune response. The availability of quasi-quantitative ADA data enables strati- fication by available lowed ning to available.	



Measuring the ADA Magnitude by Titer vs Signal

Titer

- Serial dilution of the sample may reduce drug impact on final result
- Well accepted within the medical community and with regulatory agencies

Signal

Significantly fewer resources, time and cost



Optimization

ADA assay response values to gauge the titer can help to reduce titer dilution curves to fewer points. It is important to balance the risk of inconclusive results with the increase of sample throughput.



Heena Rijhwani https://medium.com/analytics-vidhya/optimization-acb996a4623c



A First Assessment Using a Preceding Study



Both analyses did show good concordance.







Frequency Plot of % CV



Furthermore, the frequency plot of % CV supports concordance of both wells.



Singlicate Analysis

According to the European Bioanalysis Forum (Barfield et al. Bioanalysis 2020), singlicate analysis can be employed for sample analysis after a validation is in place (singlicate analysis should be assessed as part of method development and only progressed to validation if successful).

Exploratory analysis to assess whether the level of precision of a single well analysis will be sufficient for the assessment of titers in future immunogenicity analyses.

To evaluate the potential impact of samples with high % CV or inconclusive results.



Objective of the Evaluation

To compare titer results obtained using a single well approach (singlicate) with titer results obtained using a duplicated well approach. For the purpose of this exploratory analysis, the following single well analyses will be considered:

- 1. Use of titer results obtained from single well (well-1) for all samples
- 2. Use of titer results obtained from single well (well-2) for all samples
- For each sample, derivation of "worst" titer defined as the titer obtained from the well (well-1 or well-2) associated with the largest difference as compared to titer obtained based on the duplicated analysis.

The results obtained from the 3 different approaches will be compared to the titer results obtained using a duplicated approach by means of summary statistics. No formal statistical comparison will be performed.



Comparison Approach

Titer values, based on duplicated well analysis will be compared descriptively to the following titer values based on single well analysis.

- Titer based on well-1 results
- Titer based on well-2 results
- Titer based on worst case scenario, derived as:
- Select only samples with results associated with macro code="TiterAssay" (removal of inconclusive assay results)
- 2. For each sample, compute the difference between titer based on average of wells:
 - a) ("FinalCalc" variable) and titer based on well-1 ("1st Well Titer" variable)
 - b) ("FinalCalc" variable) and titer based on well-2 ("2nd Well Titer" variable)
- 3. For each sample, identify the well associated with the largest difference
- 4. For each sample, derive the worst-case titer value by assigning titer result of well identified in step 3



Comparison Outcome Figures



Histogram of Titer Results According to Type of Analysis Duplicate Wells, Singlicate Well-1, Singlicate Well-2, Singlicate Worst Case





Outcome

- A total of 45 (21%) samples, of the 214 analyzed samples, would be associated with a different titer value when using the worst case singlicate analysis
- A difference of maximum one "level of dilution" has been observed between the duplicate and the "worst case" singlicate approach*
- Similar titer distribution is observed between the 4 approaches

Suggesting for this assay is that the use of a single well analysis approach is acceptable for titer determination purposes.

*The MSR (minimum significant ratio, i.e. the smallest fold change between the titers of any two anti-drug antibody positive samples that is considered significant) is around 2 for this assay.

Conclusion and Advantages of Singlicate Titer Analysis

In a real-world example (~4000 immunogenicity samples, high positive ADA incidence), using singlicate titer analysis saved ~6 weeks and ~USD300K without compromising the data quality <u>and</u> maintaining the familiar variable of "ADA Titer".



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