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

1. Strength of a solution or the concentration of a substance in solution (as determined by titration).

2. 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?
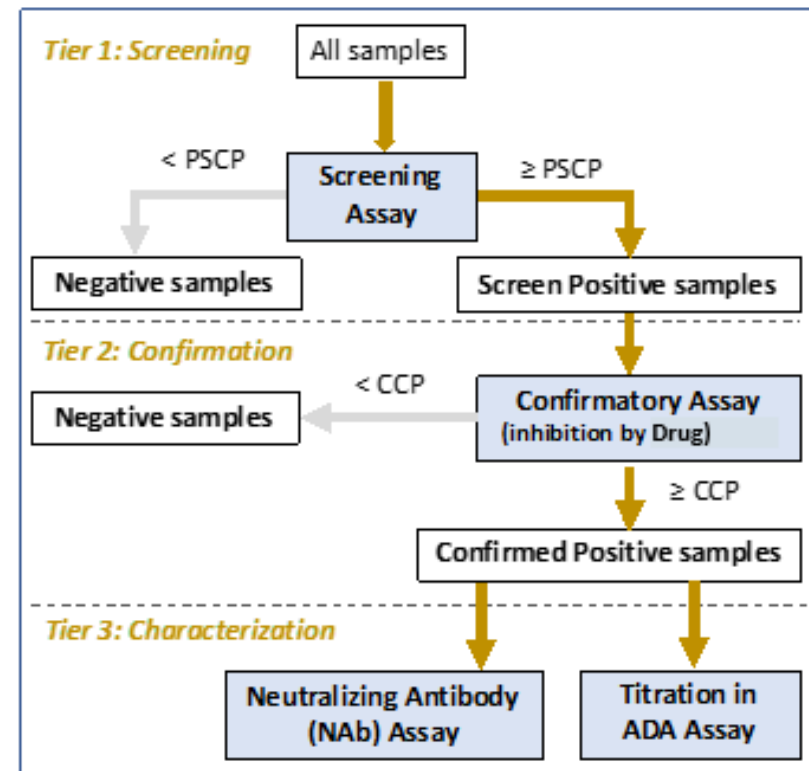
- What can we learn from this?

Figure P. Chamberlain (2022)

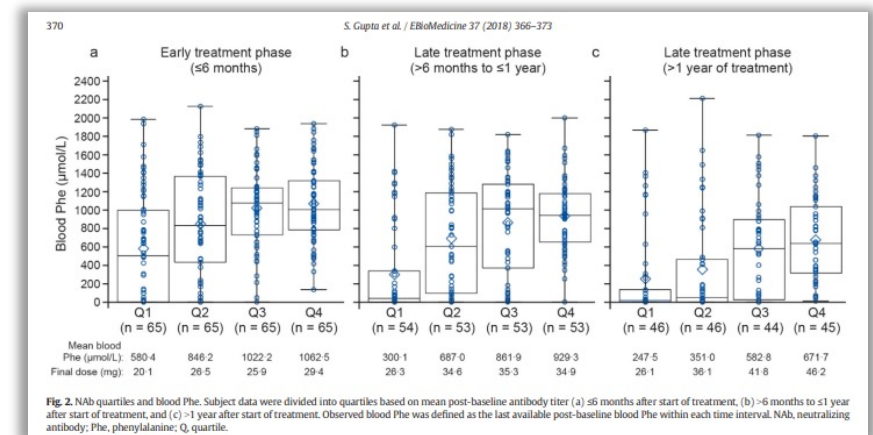# Titer: Availability of ADA Magnitude Data Enables

- 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.

Fig. 2. NAb quartiles and blood Phe. Subject data were divided into quartiles based on mean post-baseline antibody titer (a) ≤6 months after start of treatment, (b) >6 months to ≤1 year after start of treatment, and (c) >1 year after start of treatment. Observed blood Phe was defined as the last available post-baseline blood Phe within each time interval. NAb, neutralizing antibody; Phe, phenylalanine; Q, quartile.

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.
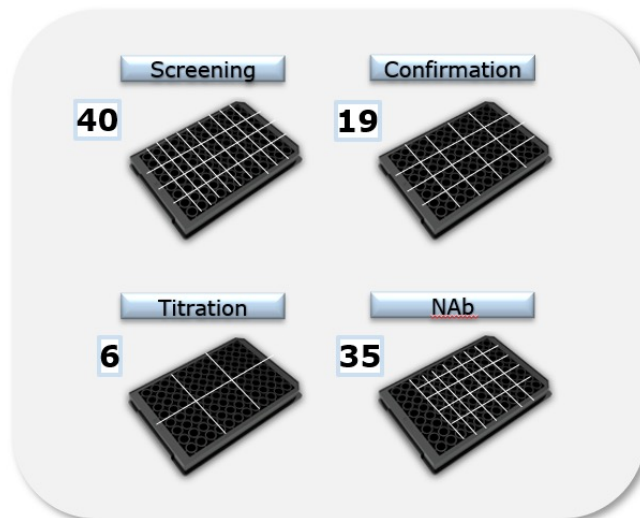

Anti-Drug Antibody Titers by ADA Status, Treatment and Visit
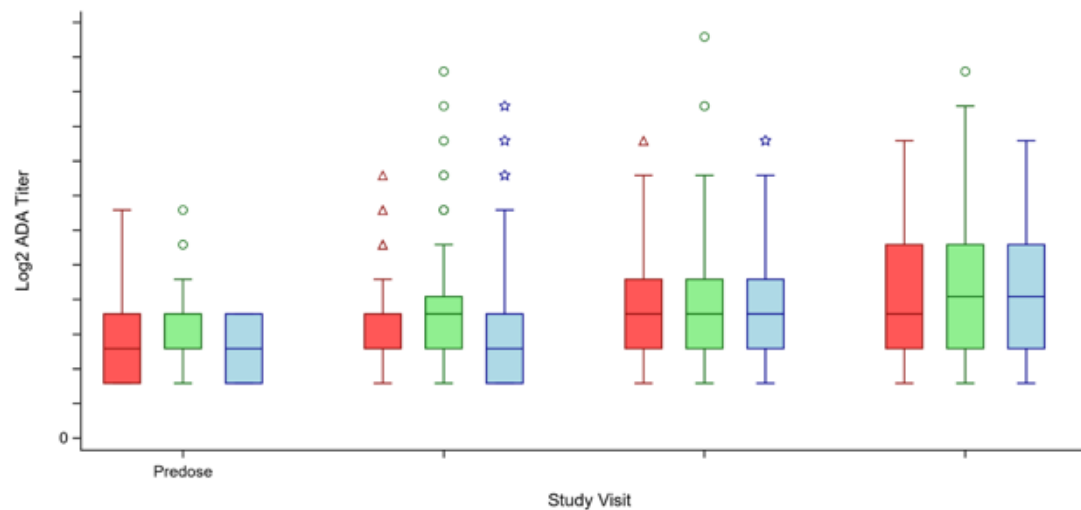
More Questions

- 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 Tier

	Samples per plate	Cost per plate	Cost per sample
Screening	40		USD 50
Confirmation	19	USD 2000	USD 105
Titration	6		USD 333
NAb	35	USD 2500	USD 70

Number of Analytical Runs
for two different scenarios of ADA positivity
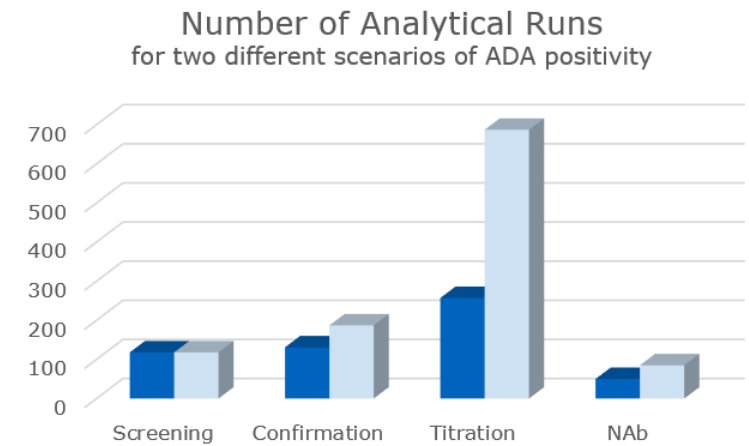
Confirmatory, and especially titer measurements, can significantly impact sample throughput and costs / time of a study and reporting of the results.

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%
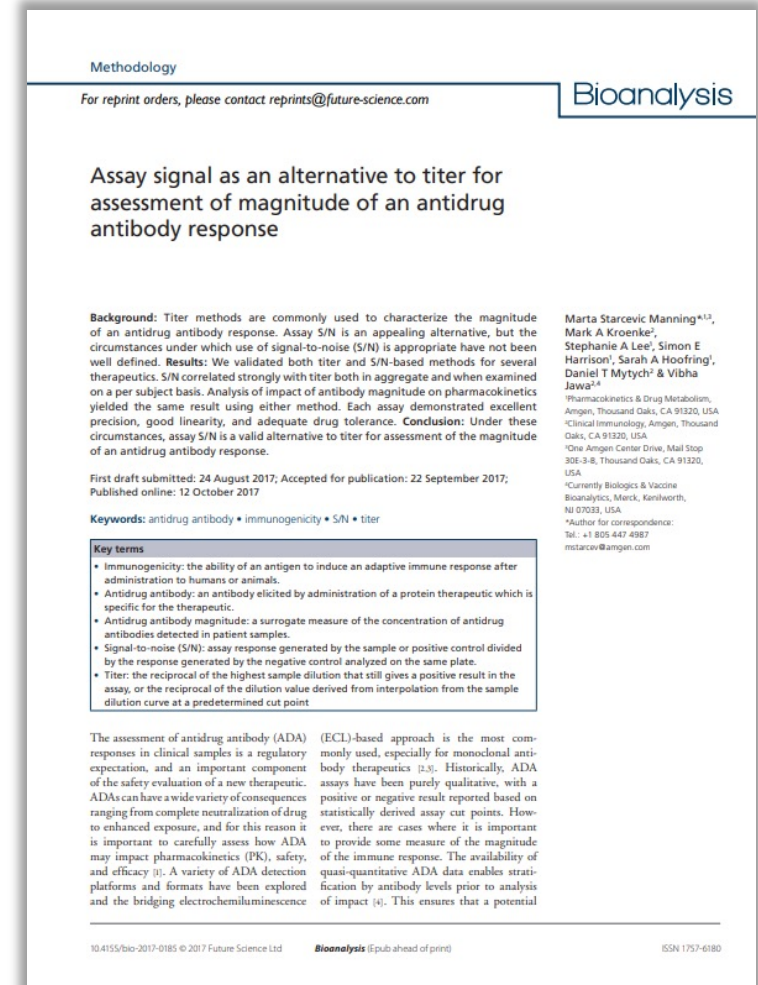
# Measuring the ADA Magnitude by Titer vs Signal

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
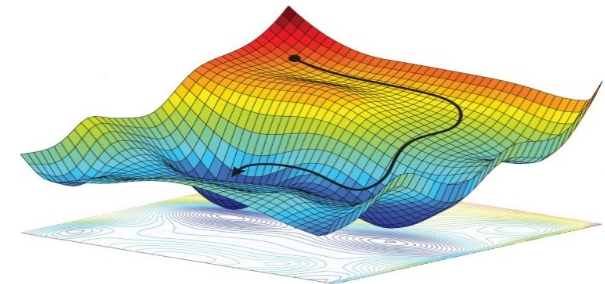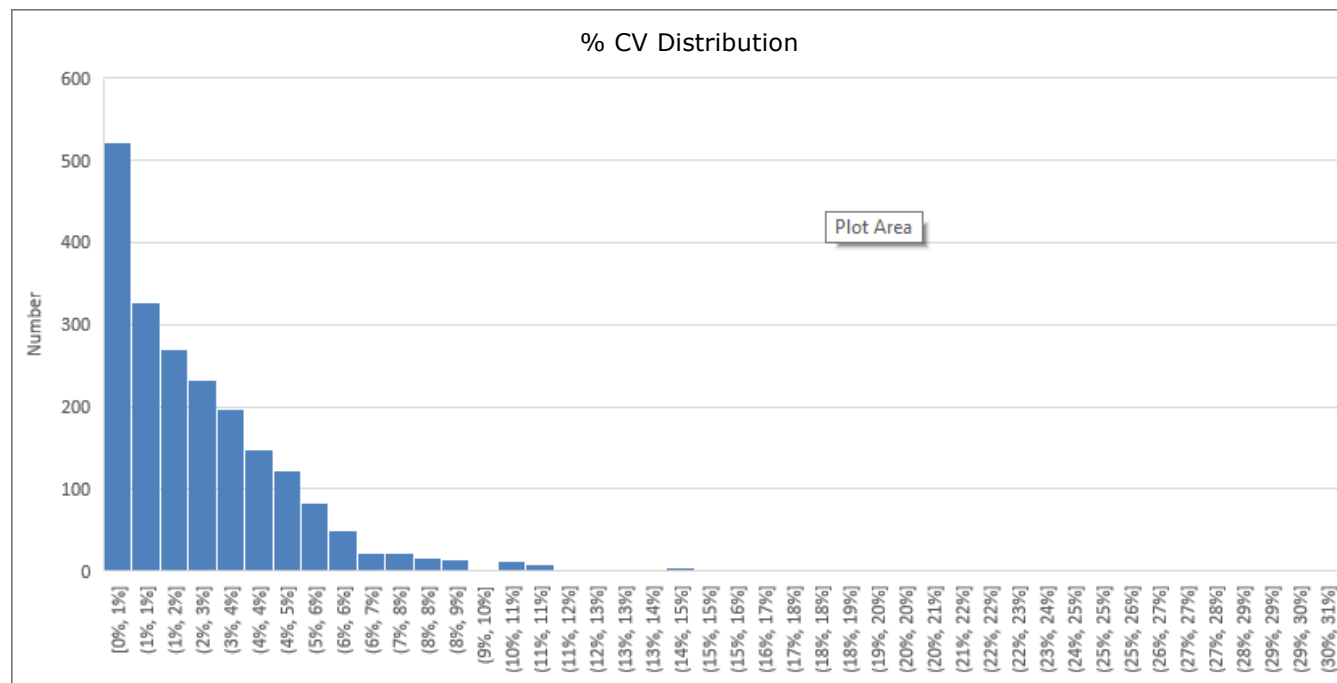
A First Assessment Using a Preceding Study

ADA titer results (duplicate) of a large

preceding study were analyzed:

a) Comparing inter-well correlation and

b) Comparing the final titer results with

each single well result

**Both analyses did show
good concordance.**

Inter-well correlation

Final Titer vs. Single-well Titers

Frequency Plot of % CV

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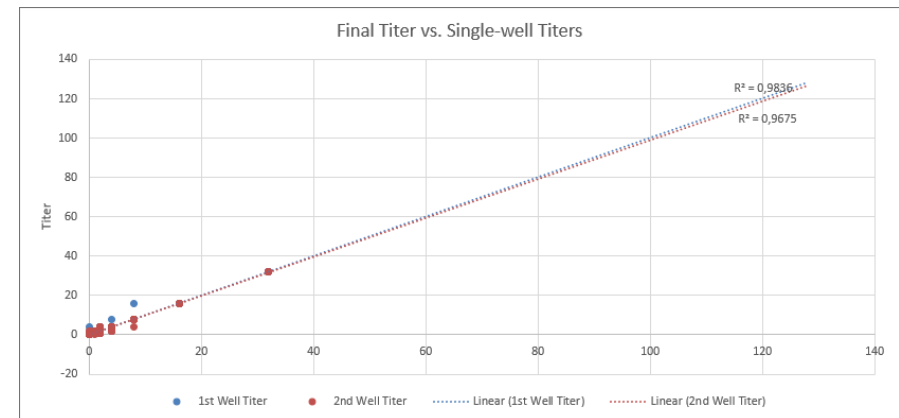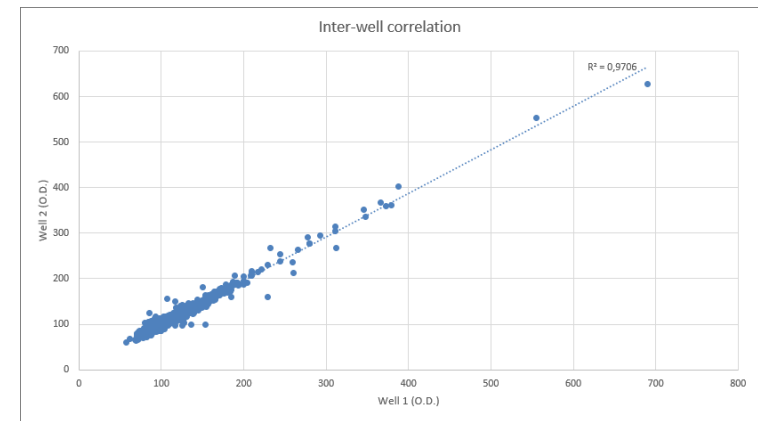
Furthermore, the frequency plot of % CV supports concordance of both wells.

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

3. 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:

1. 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

Box Plot of ADA Titer
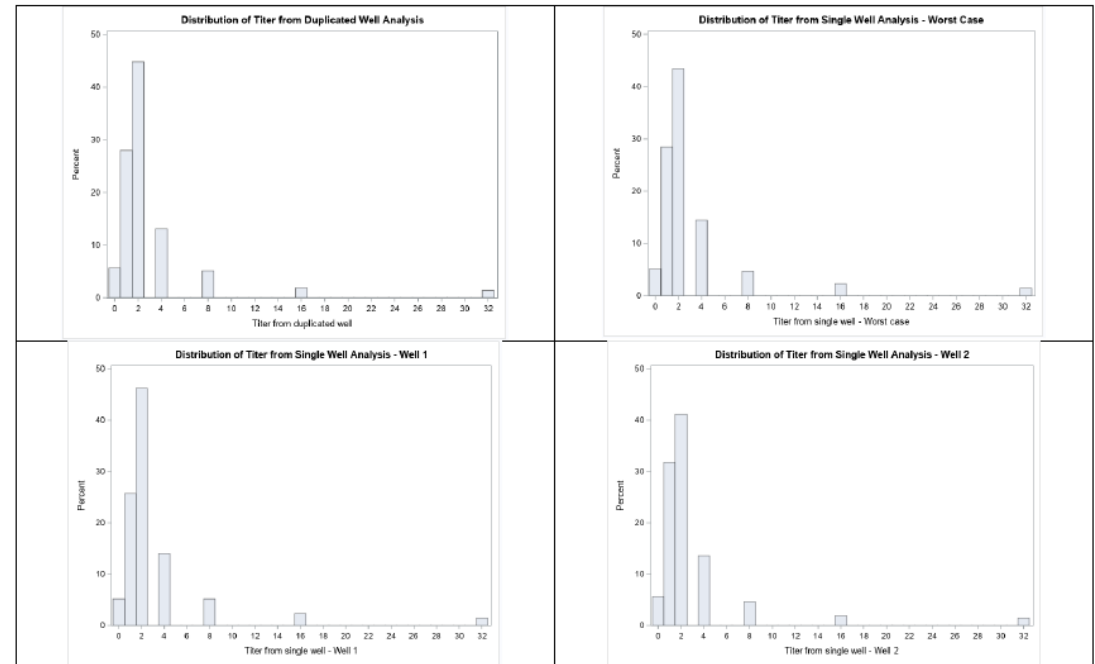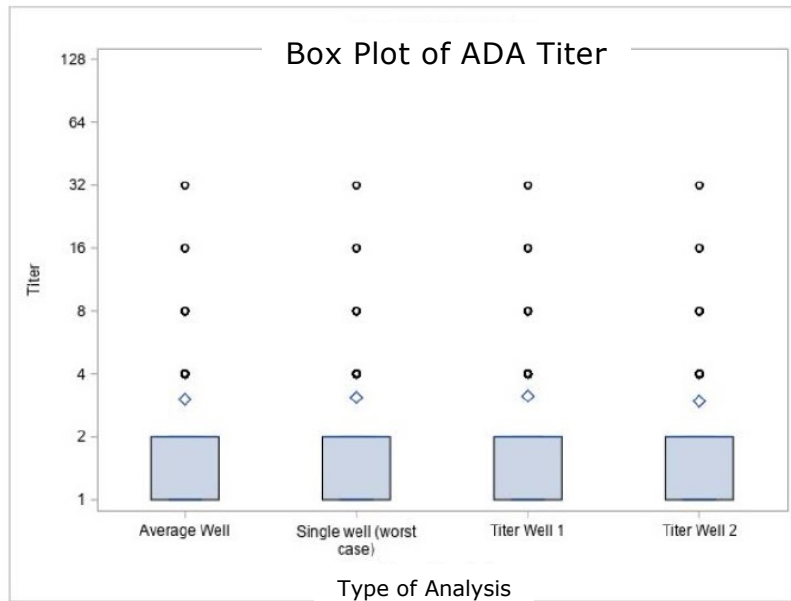
Outcome

- 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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