



# ADA Testing in Singlicate?

NCS 2024 Conference

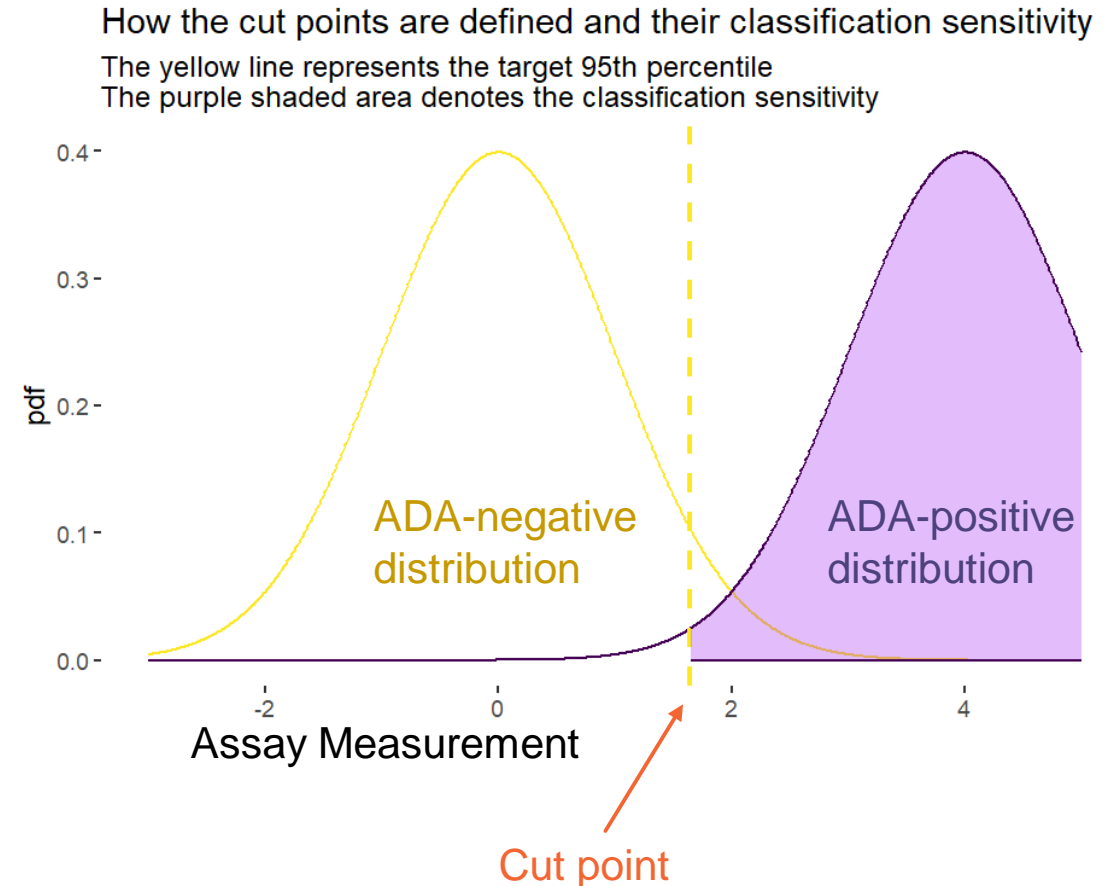
Joe Watson, GSK

# Outline

1. What is ADA testing?
2. Singlicate ADA testing: what do we know?
3. What don't we know?
4. Simulation study
5. Conclusions

# What is ADA testing?

- ADA = Anti-Drug Antibody
- ADA immunogenicity testing is a regulatory requirement
- Helps establish the clinical efficacy and safety of therapeutic biologics.
- ADA may accelerate or prolong clearance (PK), reduce efficacy, or pose safety risks
- Elevated ADA levels in **clinical** samples are established using two Type I error-controlled 'cut points' calculated using drug-naïve (**validation**) samples.



# ADA screening and confirmation

Two datasets and two stages of testing

Validation data: cut points defined	Clinical data: cut points applied
[Grey shaded area]	[Grey shaded area]
[Orange shaded area]	

## Example plate map for ADA screening assay

Duplicate analysis limits to ~ 40 samples per plate for screening

Commonly **test** all samples **in duplicate** and compute the **sample mean**

	Col 1-2	Col 3-4	Col 5-6	Col 7-8	Col 9-10	Col 11-12
A	ASSAY CONTROLS	Sa-03	Sa-11	Sa-19	Sa-27	Sa-35
B		Sa-04	Sa-12	Sa-20	Sa-28	Sa-36
C		Sa-05	Sa-13	Sa-21	Sa-29	Sa-37
D		Sa-06	Sa-14	Sa-22	Sa-30	Sa-38
E		Sa-07	Sa-15	Sa-23	Sa-31	Sa-39
F		Sa-08	Sa-16	Sa-24	Sa-32	Sa-40
G	Sa-01	Sa-09	Sa-17	Sa-25	Sa-33	Sa-41
H	Sa-02	Sa-10	Sa-18	Sa-26	Sa-34	Sa-42



'Sa' =  
Sample

# Regulatory Expectations

Number of replicates not specified



18 May 2017  
EMA/CHMP/BMWP/14327/2006 Rev 1  
Committee for Medicinal Products for Human Use (CHMP)

Guideline on Immunogenicity assessment of therapeutic proteins

Immunogenicity Testing  
of Therapeutic Protein  
Products — Developing  
and Validating Assays for  
Anti-Drug Antibody  
Detection

Guidance for Industry

U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)  
Center for Biologics Evaluation and Research (CBER)

January 2019  
Pharmaceutical Quality/CMC

# Regulatory Expectations

## USP

GENERAL CHAPTERS ▶ [GENERAL INFORMATION](#) ▶ *<1106> IMMUNOGENICITY ASSAYS—DESIGN AND VALIDATION OF IMMUNOASSAYS TO DETECT ANTI-DRUG ANTIBODIES*

### **<1106> IMMUNOGENICITY ASSAYS—DESIGN AND VALIDATION OF IMMUNOASSAYS TO DETECT ANTI-DRUG ANTIBODIES**

“One should use the same number of test and control sample replicates during validation as are used in the assay during routine use.”

## The obvious question

Can ADA testing be done in singlicate?

- Clinical ADA testing expensive (~ 100s of plates, 10,000s samples) and time-consuming (~ years),
- Switching to singlicate could ~ halve the costs
- ADA measurements have become much more precise over time

	Col 1-2	Col 3-4	Col 5-6	Col 7-8	Col 9-10	Col 11-12
A	ASSAY CONTROLS	Sa-03	Sa-11	Sa-19	Sa-27	Sa-35
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H	Sa-02	Sa-10	Sa-18	Sa-26	Sa-34	Sa-42



A large, flowing orange graphic that starts as a thick, rounded shape on the left and tapers into a thin, curved line extending towards the right. It has a slight gradient and a soft shadow effect.

**What has been done before?**

**GSK**

## Jiang et. al 2021

The first statistical treatment for tackling the question

Research Article

For reprint orders, please contact: [reprints@future-science.com](mailto:reprints@future-science.com)

Bioanalysis

### Feasibility of singlicate-based analysis in bridging ADA assay on Meso-Scale Discovery platform: comparison with duplicate analysis

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## Jiang et.al 2021 approach

- Tackle the question: *How similar would cut points estimated using singlicate validation data be to those that would have been estimated using duplicate-averaged data?*
- They conclude: If the data are normal, the well-to-well coefficient of variation  $< 10\%$ , and well-to-well variability represents  $< 25\%$  of the total variability, then the impact to the cut point is marginal
- In a case study with the well-to-well variance comprising  $\sim 3\%$  of the total, they find:
  - Screening cut point = 1.19 vs 1.18
  - Confirmatory cut point 19.3% vs 19.4%
- Open question: *How well do these cut points perform **when applied to singlicate clinical data?***



# Our contribution

How we chose to frame the question

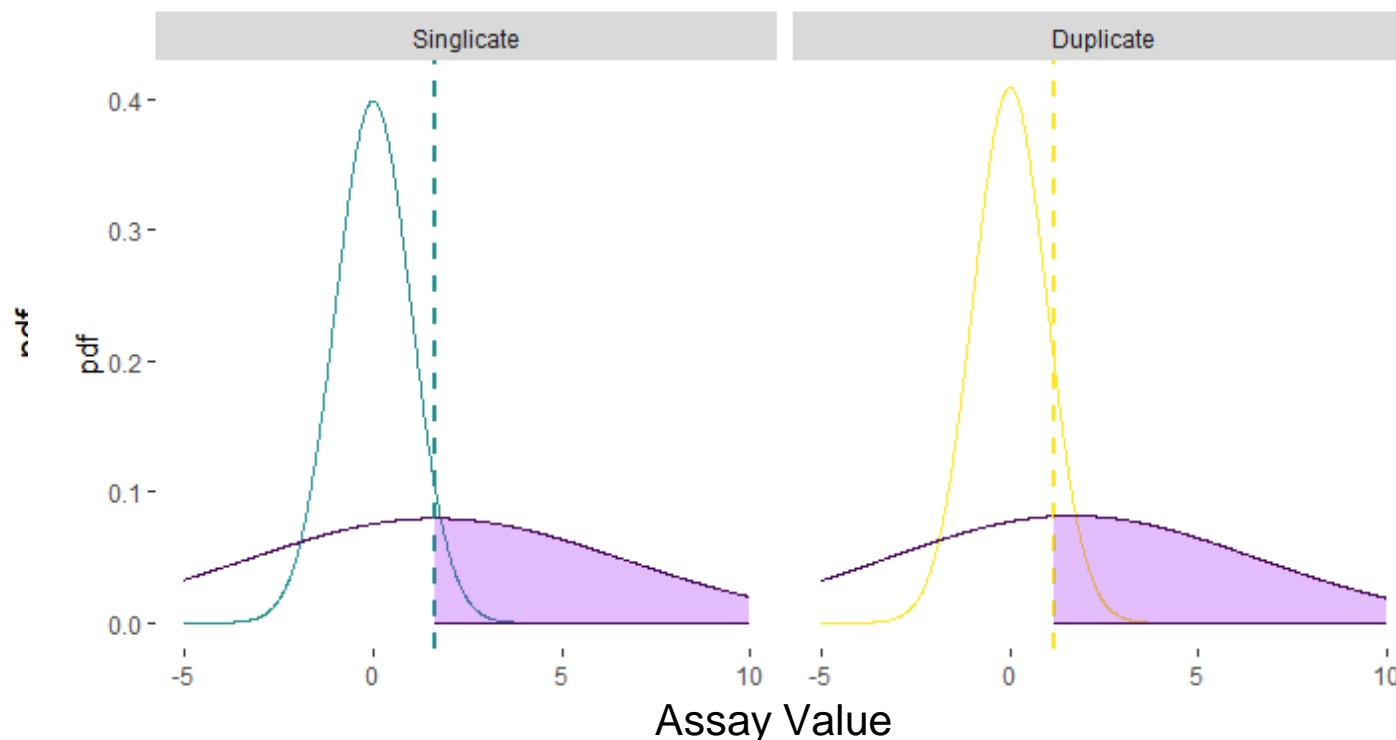
**GSK**

# How many *more* ADA positive cases may be missed in singlicate?

- Challenge: the drop in sensitivity depends strongly on the (**unknown**) mean and SD of the ADA-positive distribution and the (**estimable**) proportion of total variance that is well-well, denoted  $\omega$ .
- Solution: Compute an **upper bound** across a 'large' family of ADA-positive distributions

Drop in sensitivity depends on the relative size of well-well variation

Loss in sensitivity equals the difference in the areas beneath the curves.  
This equals 0.0034, implying an additional 0.34 out of every 100 ADA-positive samples will be missed here. Well-well variability explains 10% of total





# Simulation Study

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# How big could the drop in sensitivity be?

## ADA stage 1 (screening)

- The maximum sensitivity decline observed across the family of distributions is computed for each pixel
- Account for sampling error (10,000s simulations per pixel)

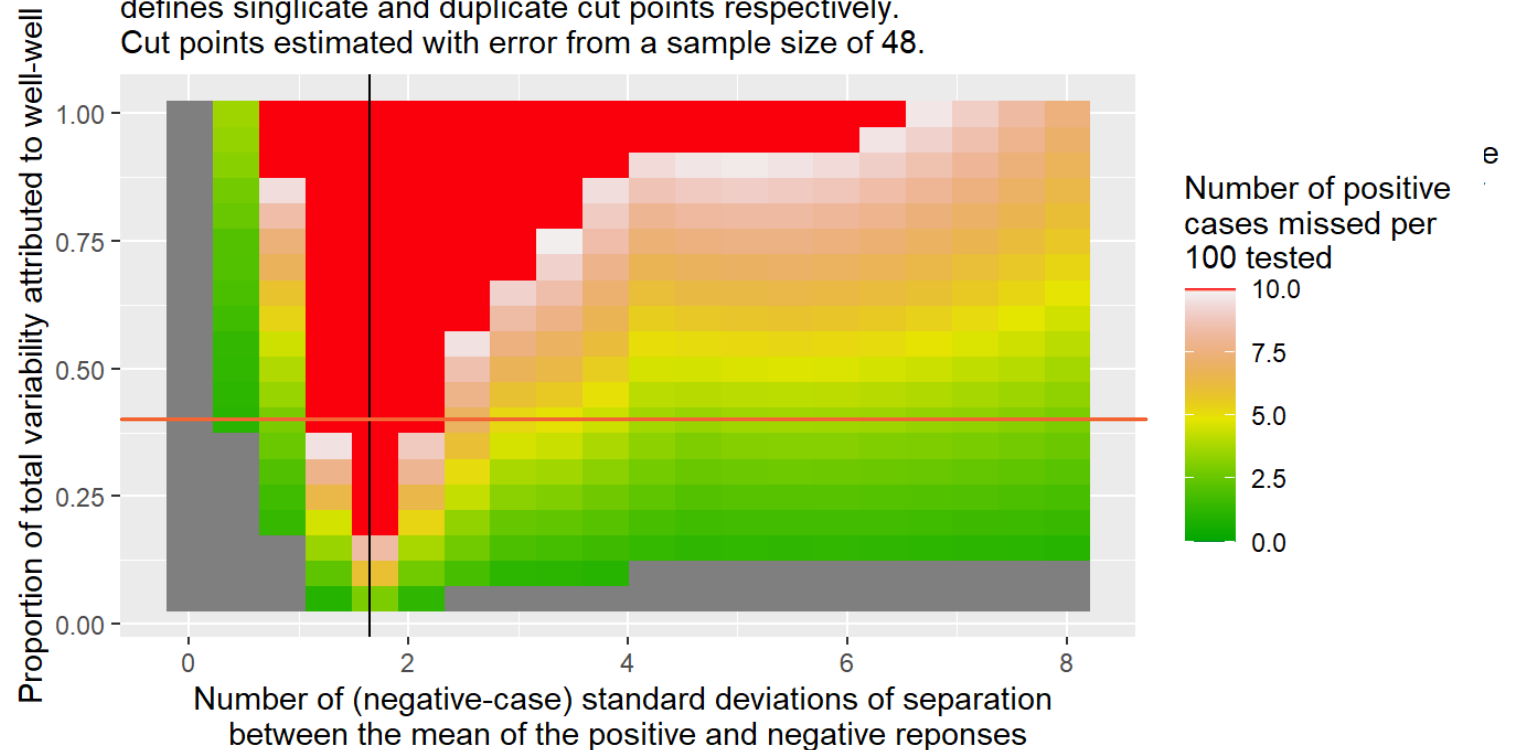
How many more positive cases may be missed if singlicate data are used both for screening and defining the cutpoint vs. duplicate?

All pixels where fewer than 1 cases is missed/gained are coloured grey. Standard deviation of positive cases varied between 0.2 and 5 times the negative response and the highest sensitivity loss chosen.

95th percentile of negative cases defines cut point (shown as vertical black line)

Point estimated 95th percentile of negative cases assuming normality defines singlicate and duplicate cut points respectively.

Cut points estimated with error from a sample size of 48.



# How big could the drop in sensitivity be?

## ADA stage 2 (confirmation)

- The target percentile changes from 95 to 99 for confirmation.
- At the black line (the cut point), ~50% of the ADA positives are already being missed in duplicate

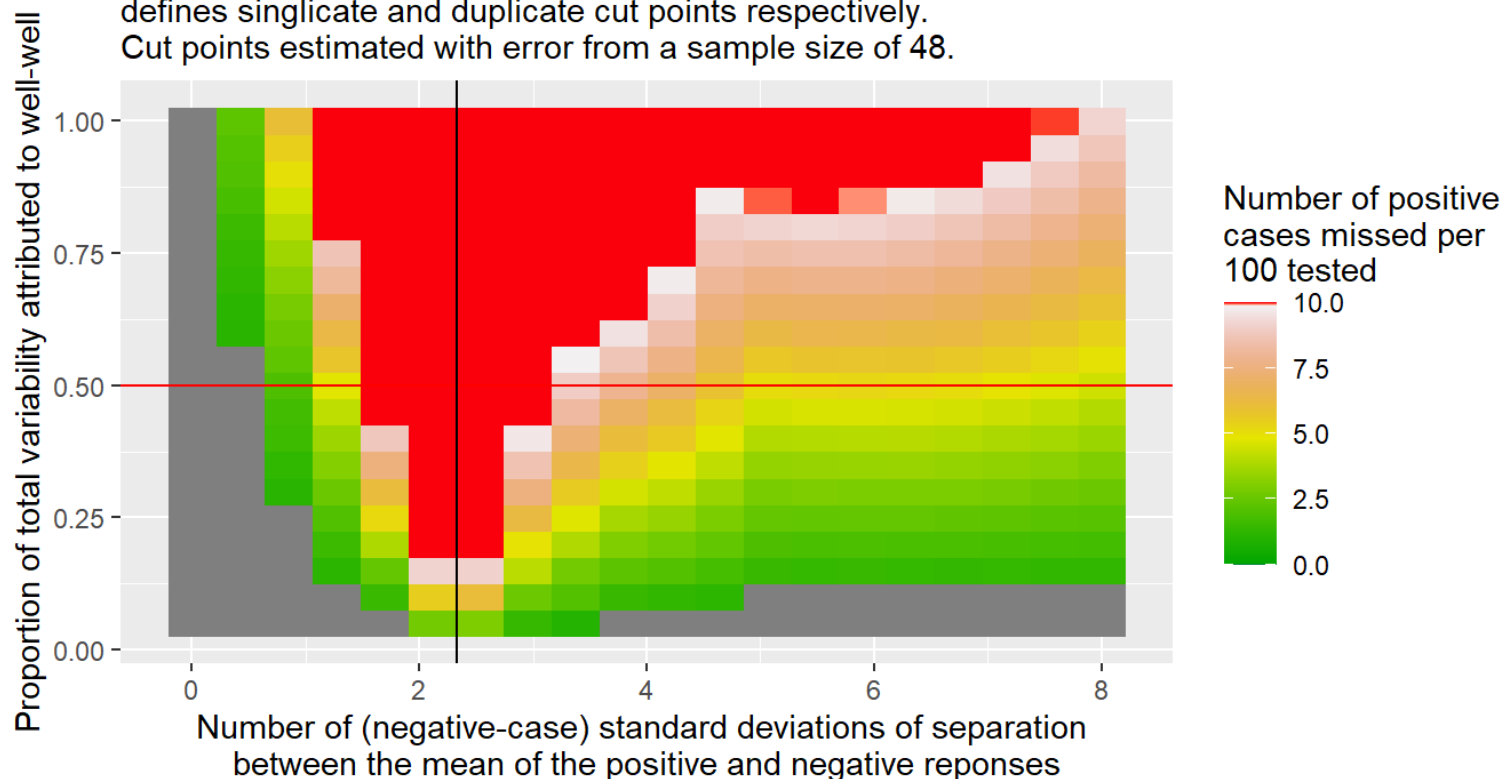
How many more positive cases may be missed if singlicate data are used both for confirmation and defining the cutpoint vs. duplicate?

All pixels where fewer than 1 cases is missed/gained are coloured grey. Standard deviation of positive cases varied between 0.2 and 5 times the negative response and the highest sensitivity loss chosen.

99th percentile of negative cases defines cut point (shown as vertical black line)

Point estimated 99th percentile of negative cases assuming normality defines singlicate and duplicate cut points respectively.

Cut points estimated with error from a sample size of 48.

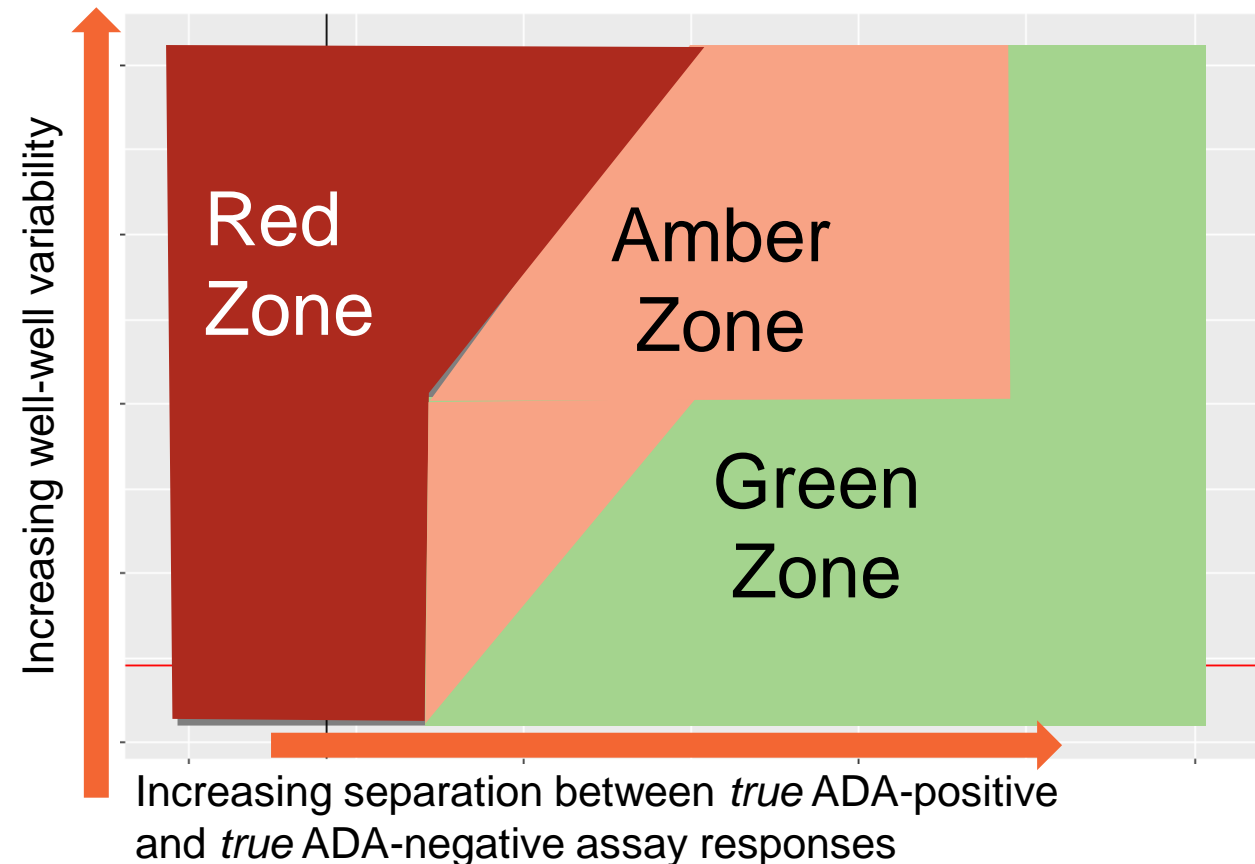




# Can we define a region where the loss in sensitivity is acceptable?

- Do we expect ADA positive assay responses close to the cut points?
- Do values of  $\omega$  exist for which the max sensitivity declines are tolerable (e.g.  $\omega < 20\%$ )?
- If the answer is no, can we modify the cut points to err on the side of caution?

How likely are each of these zones to occur in practice?



# Lowering the target percentile by 1% eliminates sensitivity loss

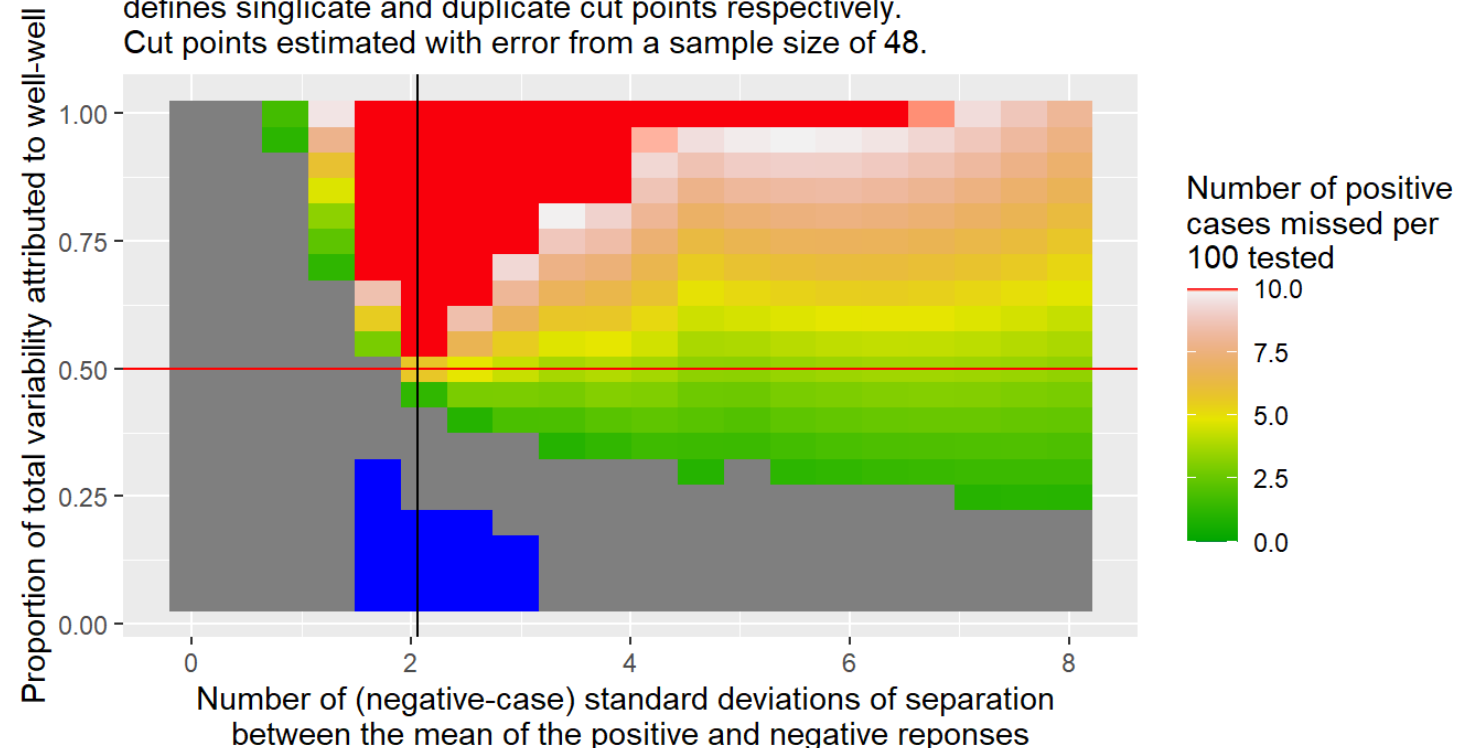
ADA stages 1 and 2 (screening + confirmation)

- Lower target percentiles from 95% to 94% for screening, and from 99% to 98% for confirmation
- We see an increased size in the acceptable  $\omega$  regions

How many more positive cases may be missed if singlicate data are used both for confirmation and defining the cutpoint vs. duplicate?

All pixels where fewer than 1 cases is missed/gained are coloured grey. Standard deviation of positive cases varied between 0.2 and 5 times the negative response and the highest sensitivity loss chosen.

95th percentile of negative cases defines cut point (shown as vertical black line) Point estimated 98th and 99th percentile of negative cases assuming normality defines singlicate and duplicate cut points respectively. Cut points estimated with error from a sample size of 48.



Note: Blue pixels indicate gains in sensitivity of > 1%

# The cost/benefit of lowering the target percentiles

- Benefits:
  - Sensitivity losses caused by singlicate testing are reduced for all  $\omega$
  - Increased range of 'acceptable'  $\omega$  values (e.g. from <20% to <40%)
  - Increased number of studies 'suitable' for singlicate
  - Reduced average cost of ADA testing
- Costs:
  - Additional false positives at both stages
  - Additional 1% of ADA negatives are tested twice
  - Additional number of false 'confirmed positives' (ADA negative samples which both screen and confirm positive)
- Important:
  - In practice, subjects are screened at multiple timepoints! Sensitivity declines may be lower



# Conclusions and next steps

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## Main conclusions

When might we be able to run things in singlicate

Good	Bad
proportion of well-well variability is low (~10%)	proportion of well-well variability is high (>50%)
<i>true</i> ADA-positive assay values <i>rarely</i> lie close to the cutpoint	<i>true</i> ADA-positive assay values <i>commonly</i> lie close to the cutpoint

- **The proportion of well-well variability can be estimated for a given dataset!\***
- **We believe the risk of switching to singlicate can be low, especially if the percentile is lowered**

# Acknowledgements

## GSK

George Gunn (Senior Scientific Director, Immunogenicity)

Kim Reese (Associate Director, Immunogenicity)

Devin Root (Senior Scientist, Immunogenicity)

Ann Schwartz (Investigator, Validation Coordinator, Immunogenicity)

Eric Yang (VP, Biomarker and Bioanalytical Platforms)

Buffy Hudson-Curtis (Statistics Director, Dev Biostats NCTS Research Stats)

Andrew Gehman (Associate Director, Dev Biostats NCTS Research Stats)

Owen Leete (Principal Statistician, Dev Biostats NCTS Research Stats)

Brady Nifong (Principal Statistician, Dev Biostats NCTS Research Stats)

Austin Cole (Principal Statistician, Dev Biostats NCTS Research Stats)

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**Bonus Slides**

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# How can we estimate the means and SDs of the distributions?

## Mixture distributions

- Fit mixture distribution **jointly to validation and clinical data** to estimate means and SDs of ADA-positive and ADA-negative distributions:
  - Validation data contain only ADA-negative:
    - $Y^- \sim N(\mu^-, \sigma^-)$
  - Clinical data contain a mixture of ADA-positive and ADA-negative data
    - $Y \sim p^+ N(\mu^+, \sigma^+) + (1 - p^+) N(\mu^-, \sigma^-)$
  - Estimate  $\mu^+, \sigma^+$  and hence the expected sensitivity loss in real data (account for analyst, plate, etc.,)
  - Make a go/no-go decision on switching to singlicate
- Do the financial savings of singlicate ADA outweigh the additional practical challenges?
- Either requires ongoing back-and-forth between ADA scientist(s) and statistician(s)
- **Or**, requires software development for scientist(s) to use (e.g. Shiny App)