# ADA Testing in Singlicate?

NCS 2024 Conference

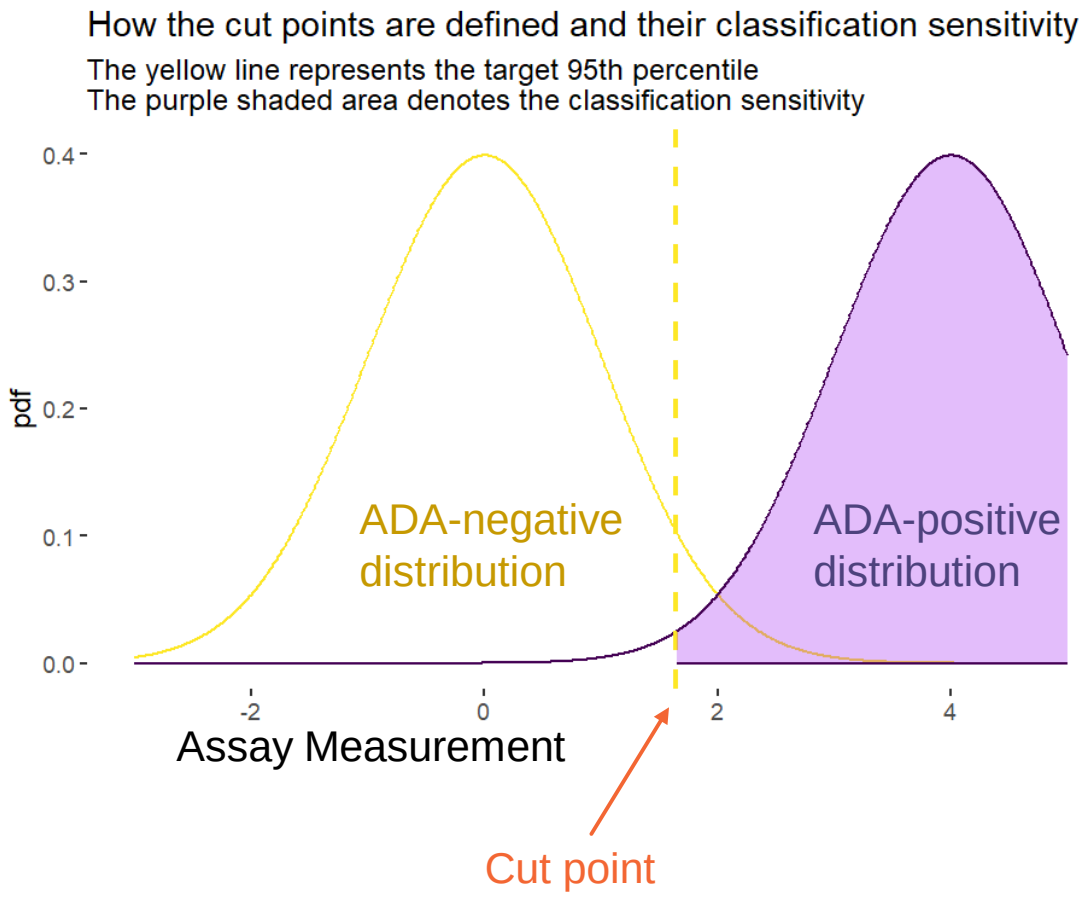
Joe Watson, GSK

# 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 |
| --- | --- |
| | |
| | |

# 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

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

18 May 2017
EMEA/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."

<1106> IMMUNOGENICITY ASSAYS - DESIGN AND VALIDATION OF IMMUNOASSAYS TO DETECT ANTI-DRUG ANTIBODIES. Aug 2013

# 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 |
|---|---|---|---|---|---|---|
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# What has been done before?

# Jiang et. al 2021

The first statistical treatment for tackling the question

Research Article

Bioanalysis

For reprint orders, please contact: reprints@future-science.com

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

Zhihua Jiang*[,1], John Kamerud[1], Zhiping You[2], Soma Basak[1], Elena Seletskaia[1], Gregory S Steeno[2] & Boris Gorovits[1]

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[2]Early Clinical Development, Pfizer Inc., Groton, CT 06340, USA

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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-well coefficient of variation < 10%, and well-well variability represents < 25% of the total variability, then the impact to the cut point is marginal

- In a case study with the well-well variance comprising ~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****?*

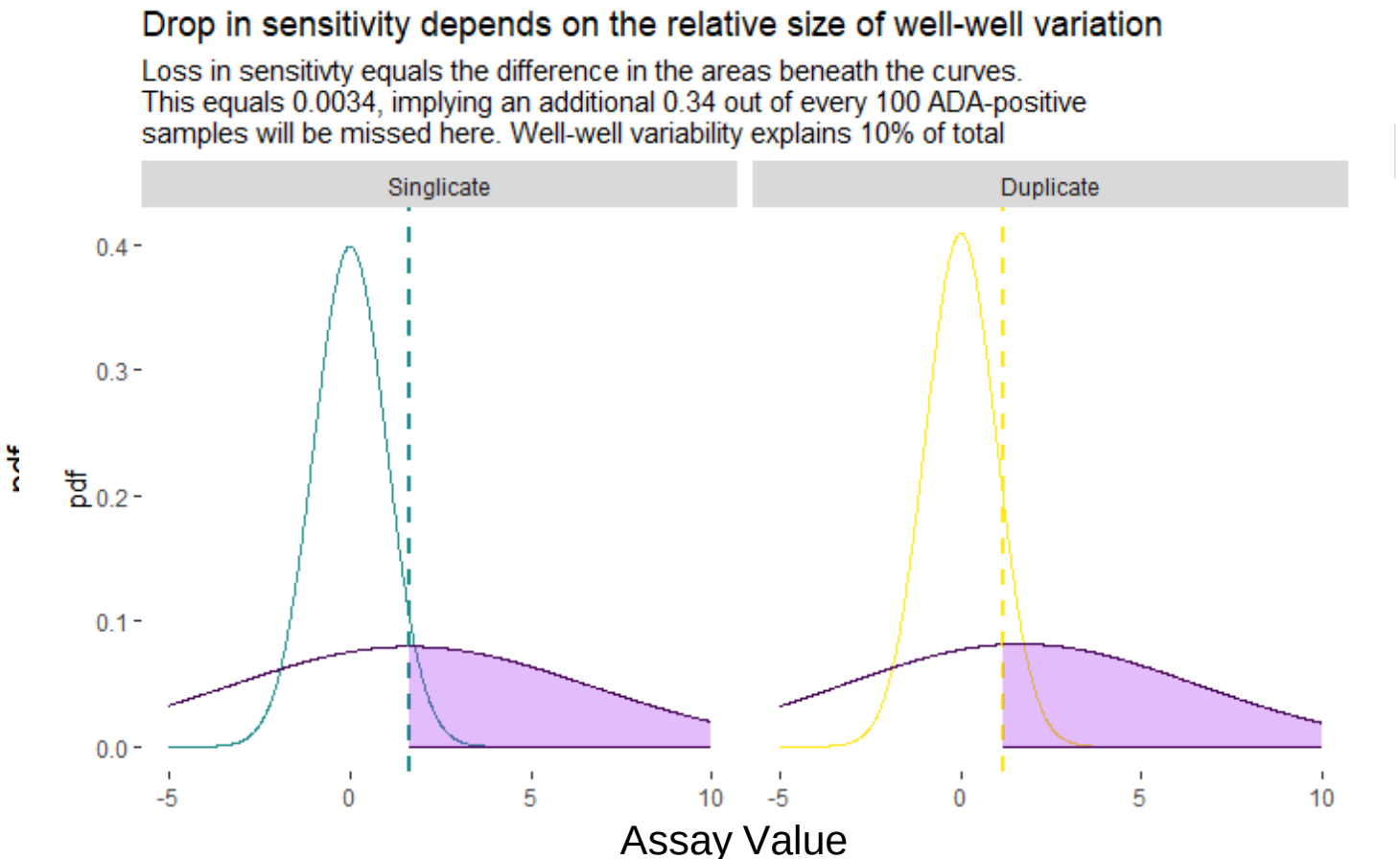
# Our contribution

## How we chose to frame the question

# 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



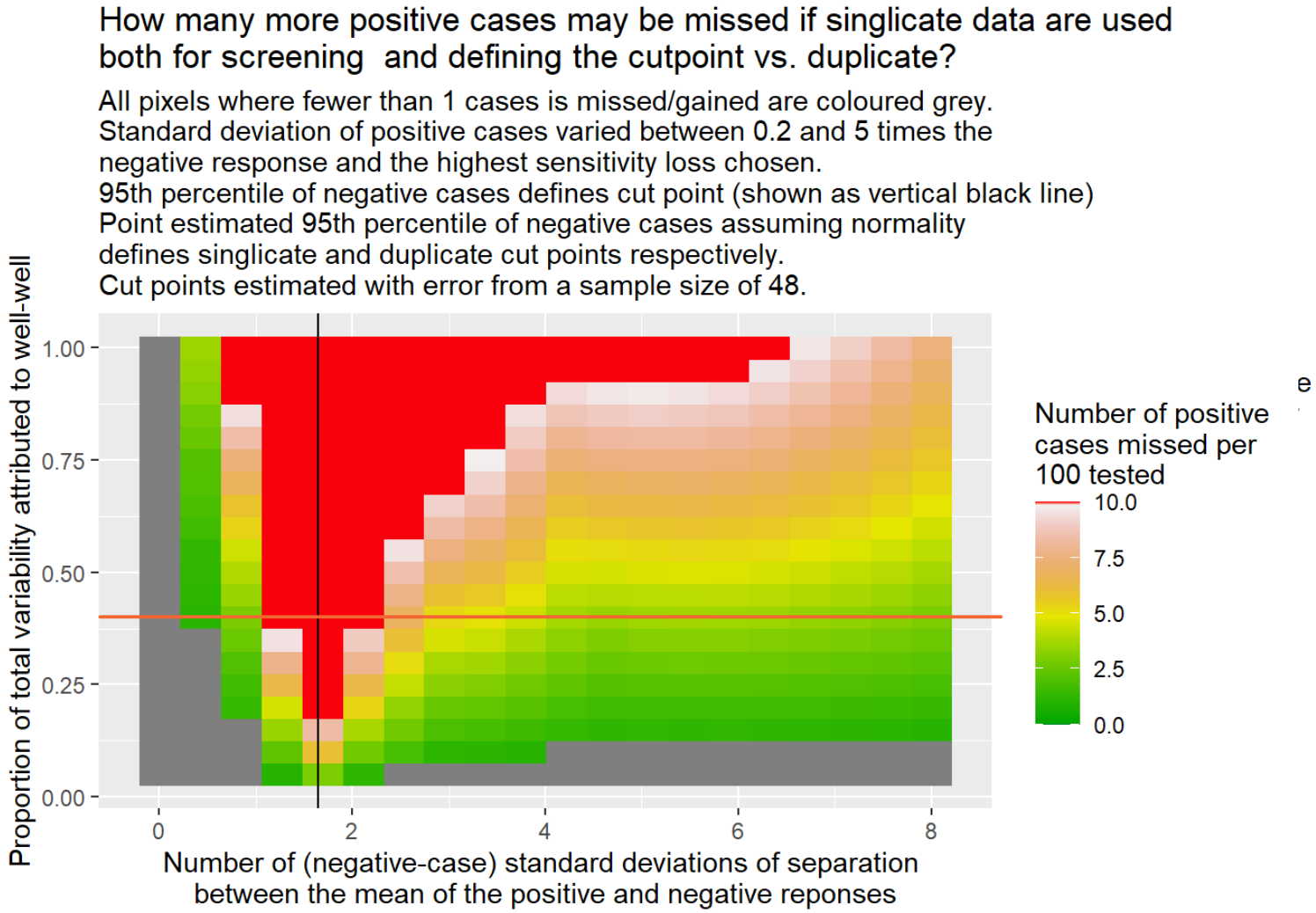
# Simulation Study

GSK

# How big could the drop in sensitivity be?

## ADA stage 1 (screening)

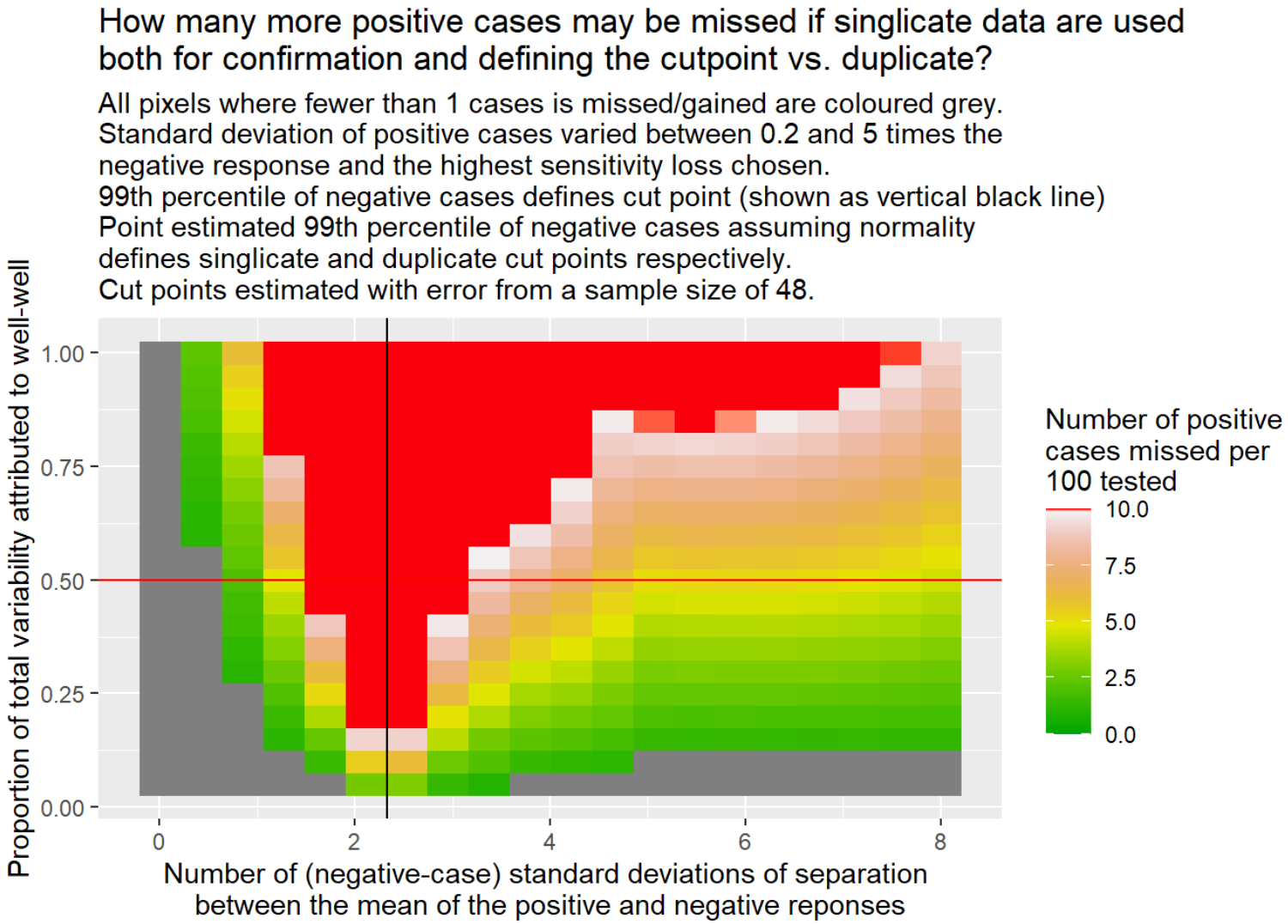
- The maximum sensitivity decline observed across the family of distributions is computed for each pixel
- Account for sampling error (10,000s simulations per pixel)



# How big could the drop in sensitivity be?

ADA stage 2 (confirmation)

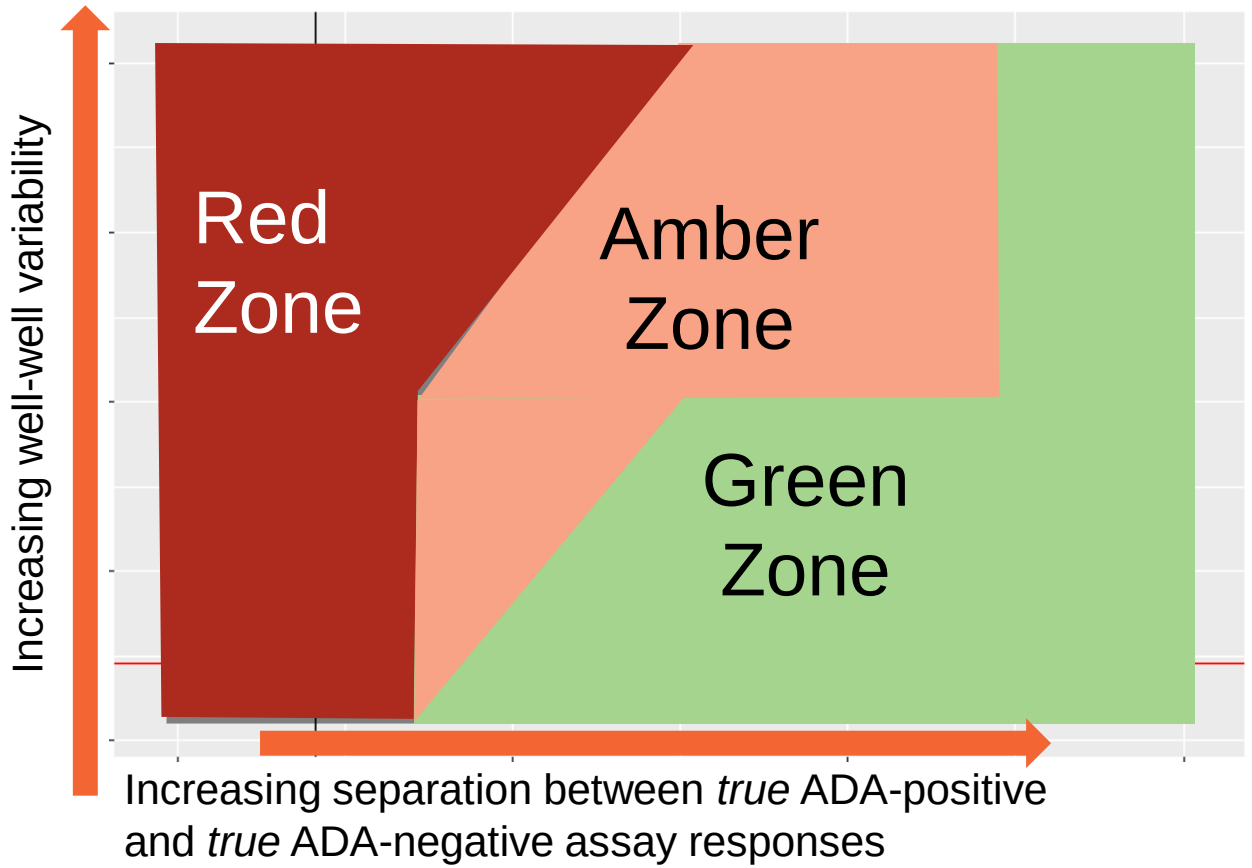
- The target percentile changes from 95 to 99 for confirmation.
- At the black line (the cut point), ~50% of the ADA positives are already being missed in duplicate



# 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?

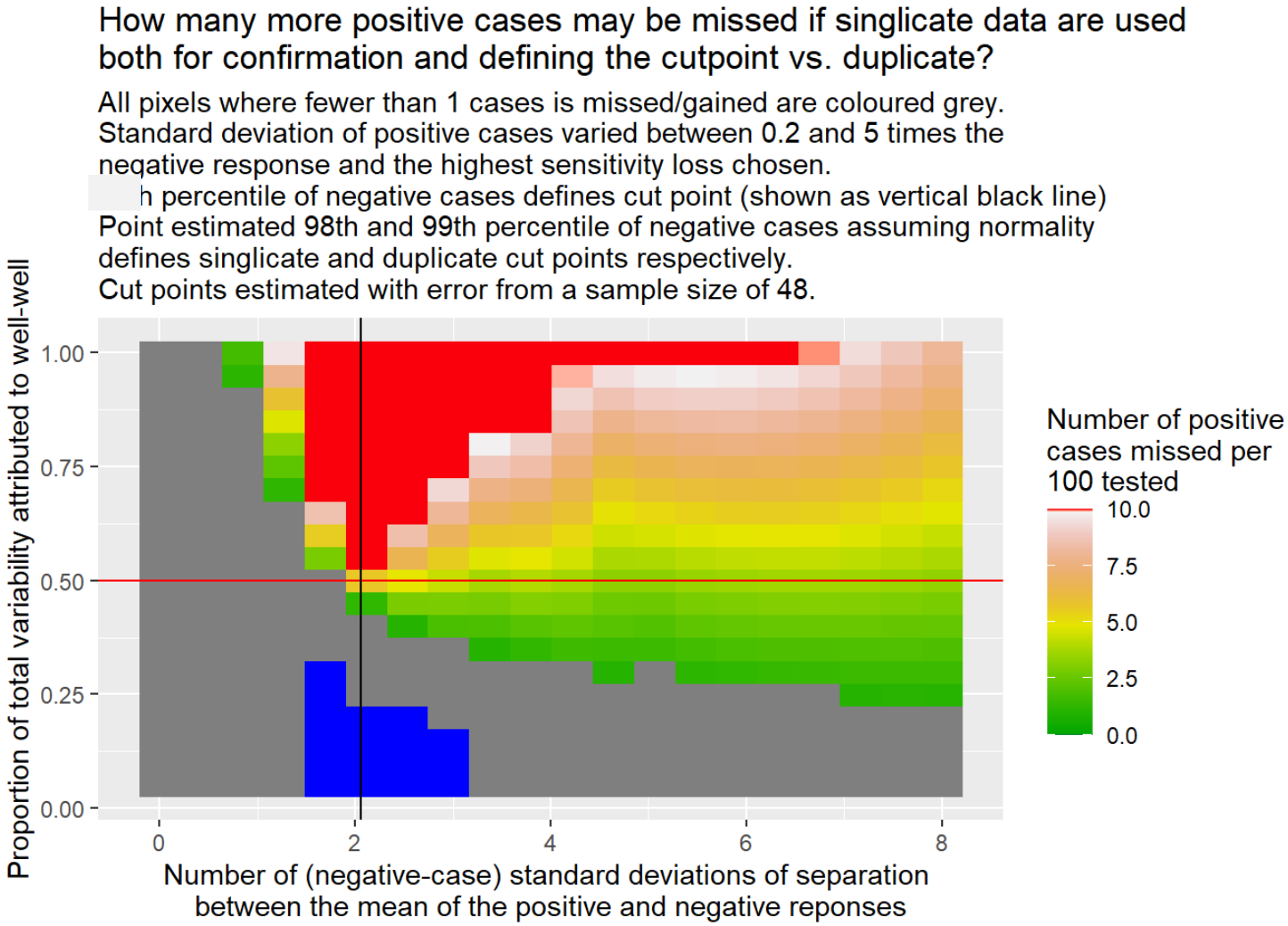


# Lowering the target percentile by 1% eliminates sensitivity loss

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




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

# 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%) |
| *true* ADA-positive assay values *rarely* lie close to the cutpoint | *true* ADA-positive assay values *commonly* 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

# 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)