

Antigenic distance and breadth of vaccine response

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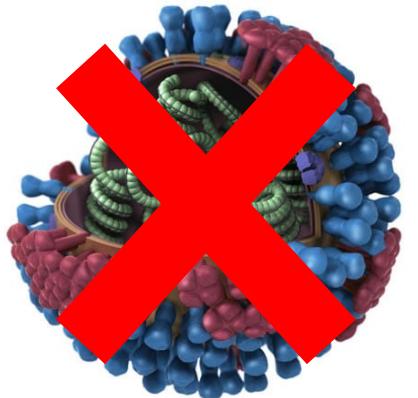
Overview

- Introduction and motivation
- Proposed method
- Simulation study
- Real data analysis with UGAFluVac data
- Some fun little case studies (time permitting)

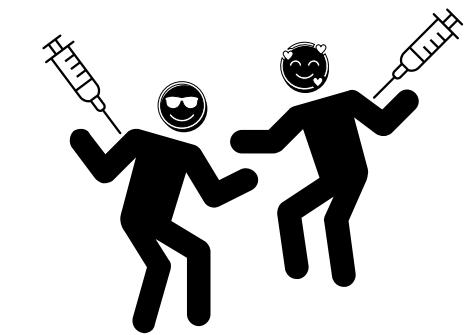
Overarching questions

- How can we convince you that our method is *useful*?
- What do you want to see to show that our method is *valid*?

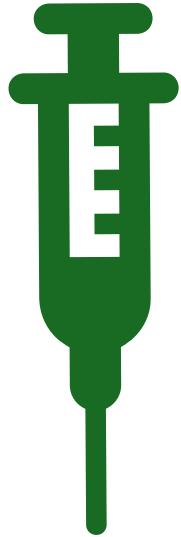
Part 1: Background



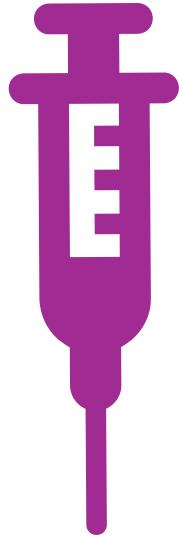
The universal flu vaccine



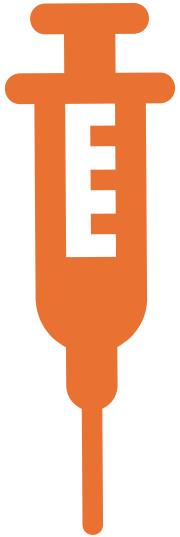
Recombinant



Stem-based



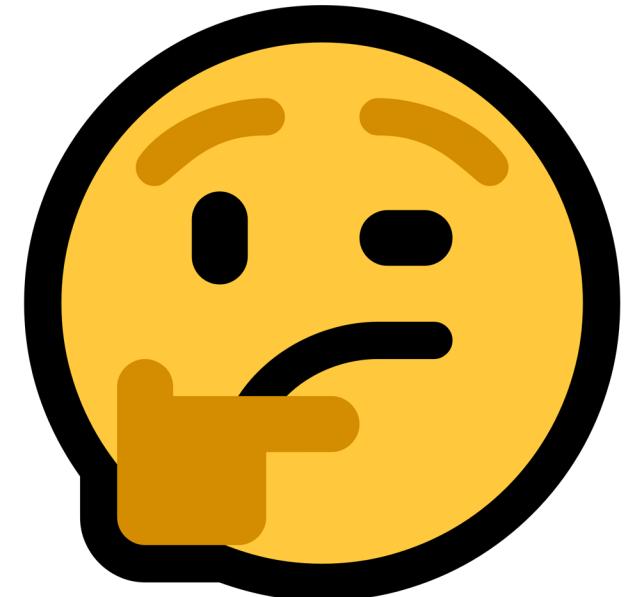
Adjuvanted



High dose



...and others!



**Which next-gen broadly reactive
influenza vaccine do I get?**

How do we measure breadth of response?

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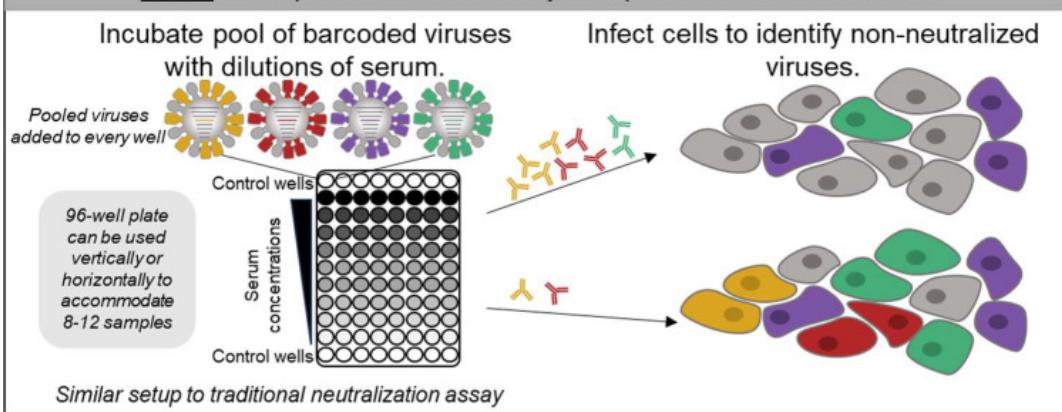
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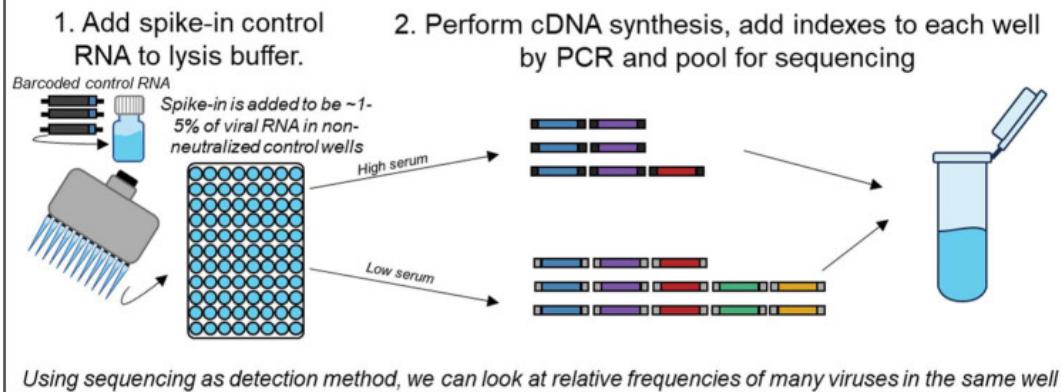
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(Everything in this presentation is HAI since that's the data we have right now.)

Day 1: Set up neutralization assay with pool of barcoded viruses.

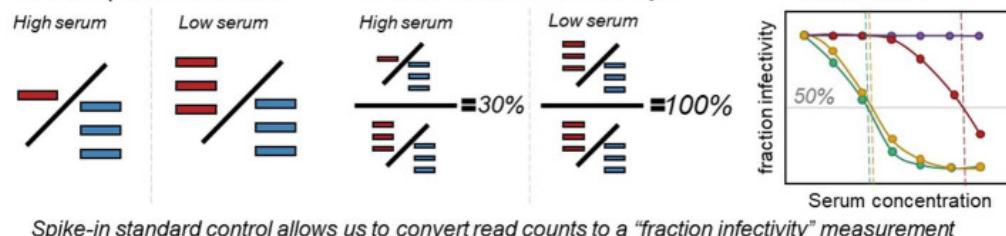


Day 2: Extract viral RNA and prepare samples for next-generation sequencing.



Analysis: Determine fraction infectivity for each variant at each concentration and fit a curve to calculate an NT50 for each virus

1. Normalize read counts for each variant in each well to the spike-in control.
2. Normalize corrected counts to a no-serum control to find "fraction infectivity".
3. Fit neutralization curves to calculate NT50s for each virus

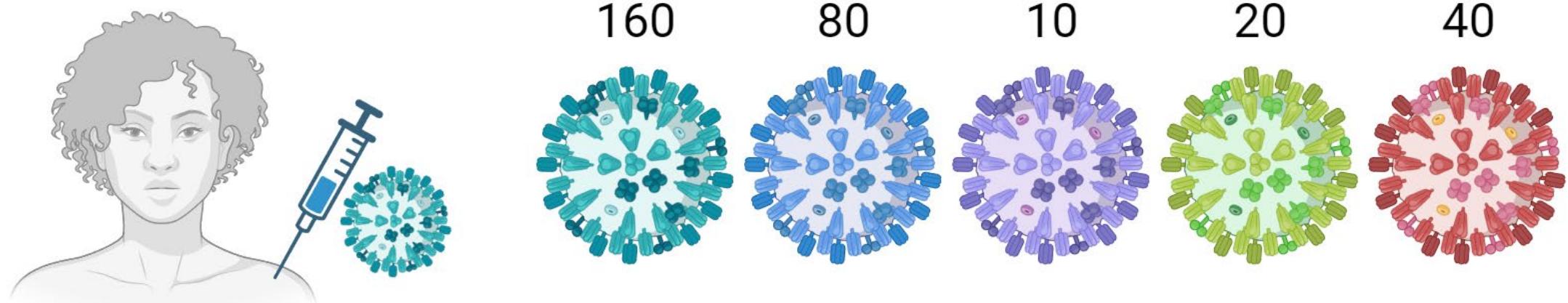


New future assay?

- The multiplex assay seems like a promising way to analyze many strains at once.
- Everything we discuss will be about HAI, but the new assay should be a drop-in replacement to our method.
- True for (theoretically) any assay e.g. ELISA or NAI.

Loes AN, et al. High-throughput sequencing-based neutralization assay reveals how repeated vaccinations impact titers to recent human H1N1 influenza strains. J Virol. 2024 Oct 22;98(10):e0068924. doi: 10.1128/jvi.00689-24. Epub 2024 Sep 24. PMID: 39315814; PMCID: PMC11494878.

OK, we ran the cohort study...
What do we do with the titers we collect?



1. Magnitude: response to the homologous strain.

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2. **Breadth:** responses to heterologous strains.

- 1. Magnitude:** response to the homologous strain.
- 2. Breadth:** responses to heterologous strains.
- 3. Overall strength:** can we combine magnitude and breadth into one measurement of vaccine “strength” or “goodness”?

Current methods

How do we measure the response?

- **Magnitude**: geometric mean titer of homologous responses.

$$\exp\left(\frac{1}{n} \sum_{i=1}^n \ln \text{titer}_{i,j=0}\right)$$

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- **Breadth:** seroconversion rate across all strains

$$\frac{1}{n} \sum_{i=1}^n \sum_{j=0}^k I(\text{seroconverted}_{i,j})$$

How do we measure the response?

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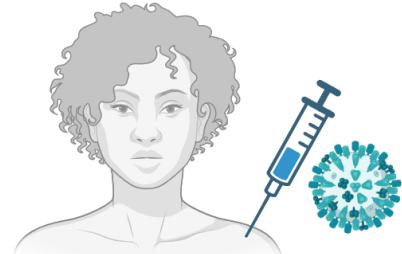
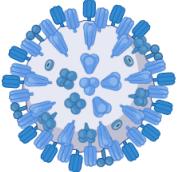
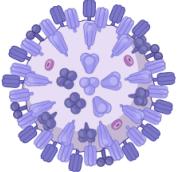
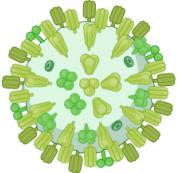
$$\exp\left(\frac{1}{n} \sum_{i=1}^n \ln \text{titer}_{i,j=0}\right)$$

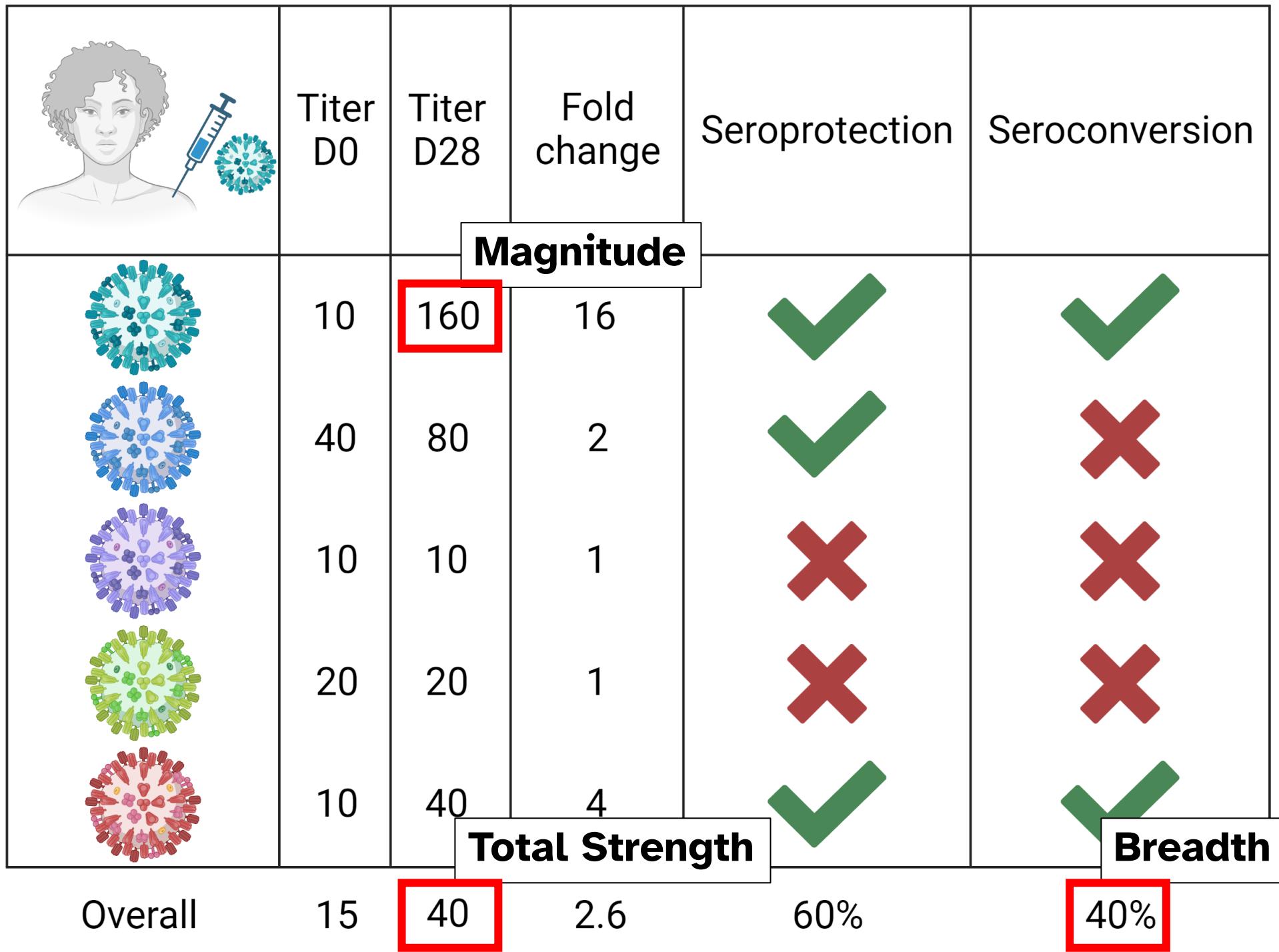
- Breadth: seroconversion rate across all strains

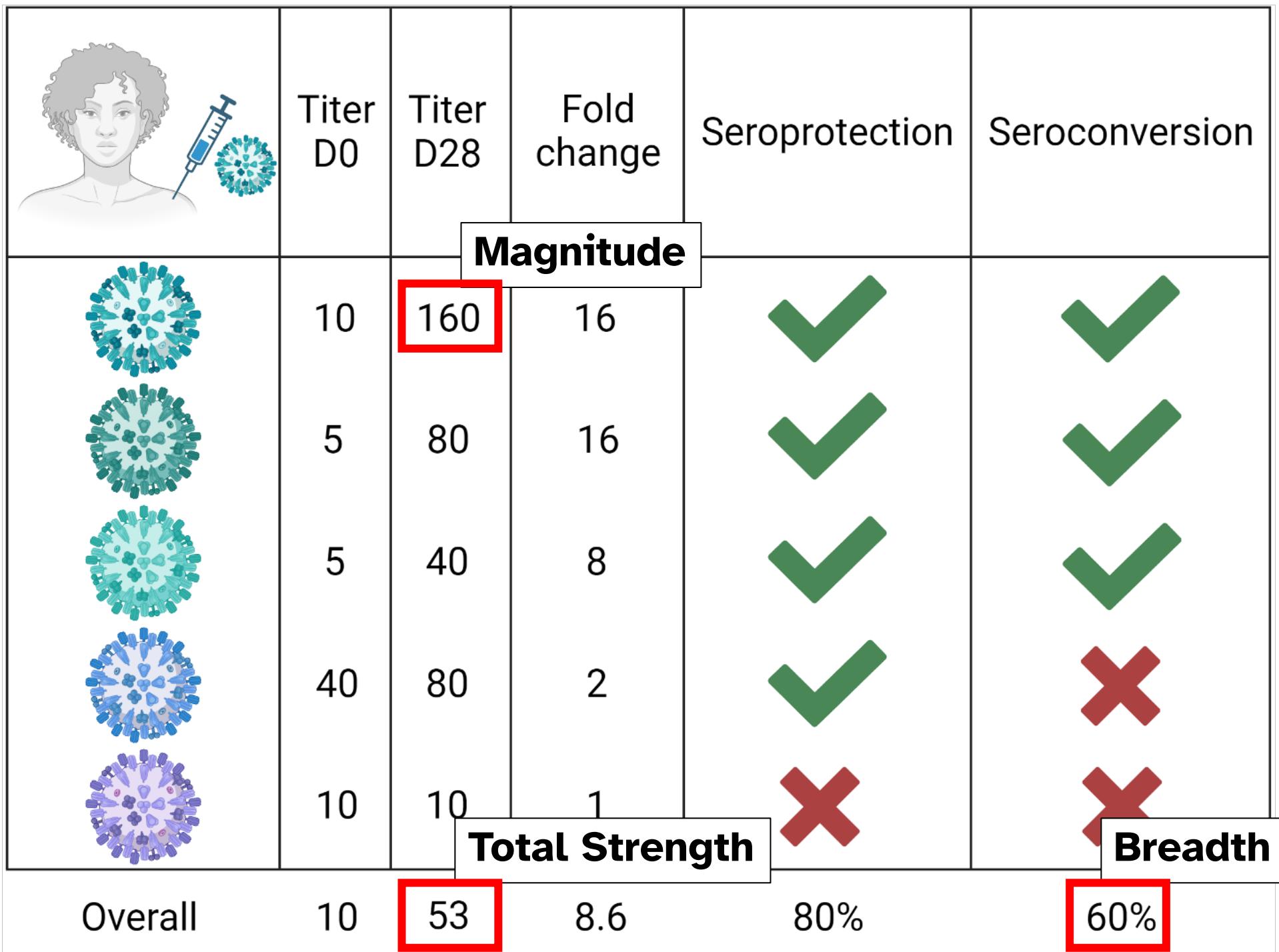
$$\frac{1}{n} \sum_{i=1}^n \sum_{j=0}^k I(\text{seroconverted}_{i,j})$$

- Overall **strength**: GMT across all strains.

$$\exp\left(\frac{1}{n} \sum_{i=1}^n \sum_{j=0}^k \ln \text{titer}_{i,j}\right)$$

	Titer D0	Titer D28	Fold change	Seroprotection	Seroconversion
	Overall	15	40	2.6	60%
	10	160	16		
	40	80	2		
	10	10	1		
	20	20	1		
	10	40	4		

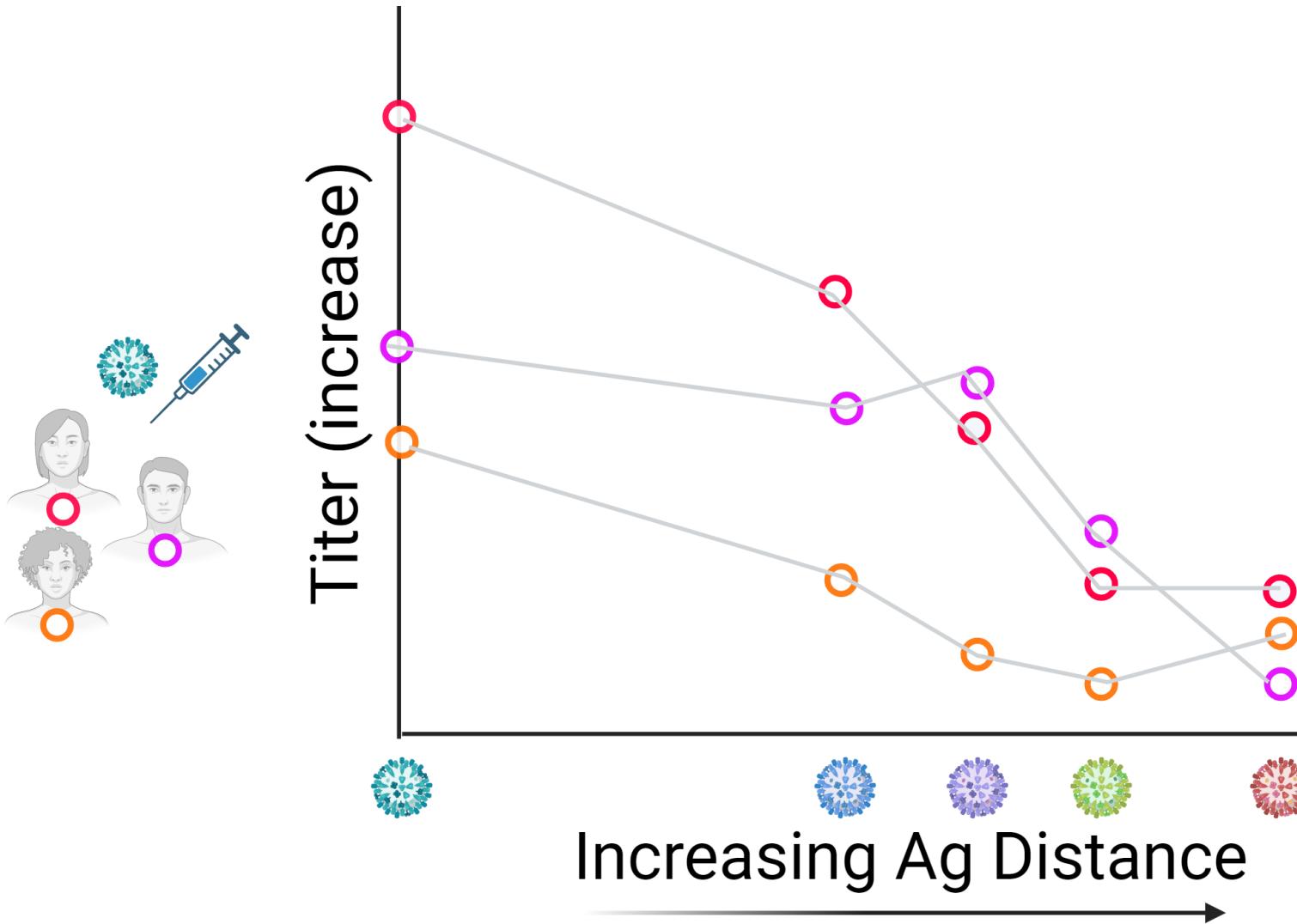




Selection of panel strains can
completely change results
even if nothing else changes!

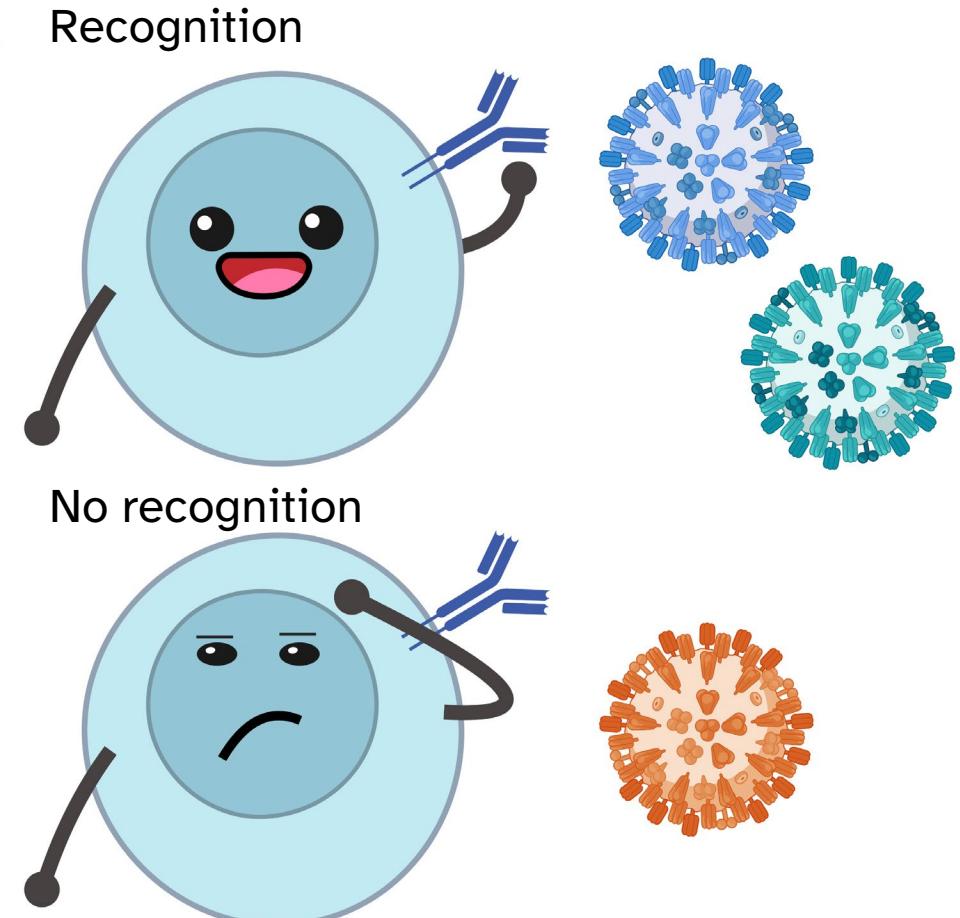
Proposed method

Antibody landscape: titer vs. antigenic distance for all participants.



Antigenic distance:

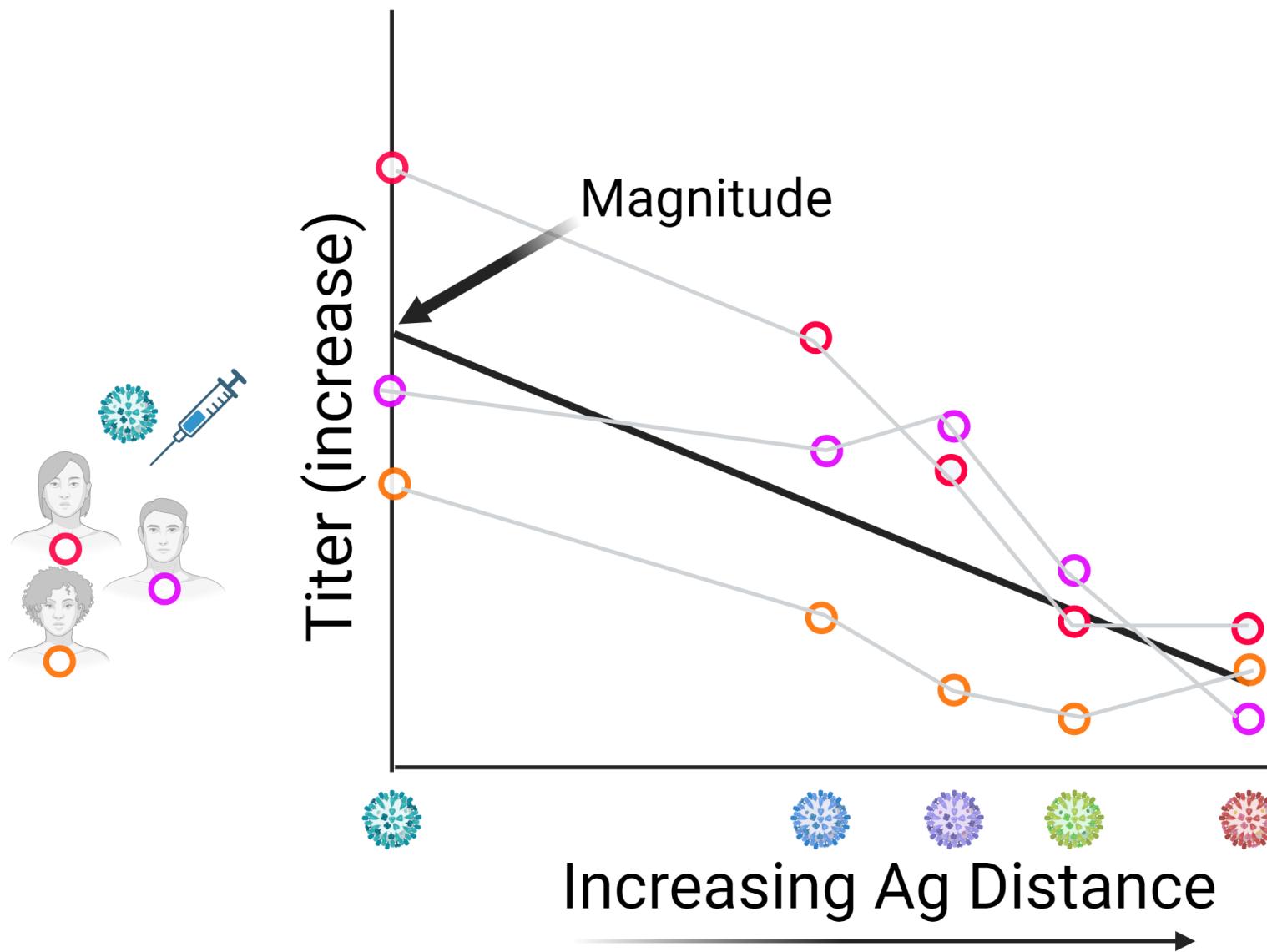
how different are two strains?



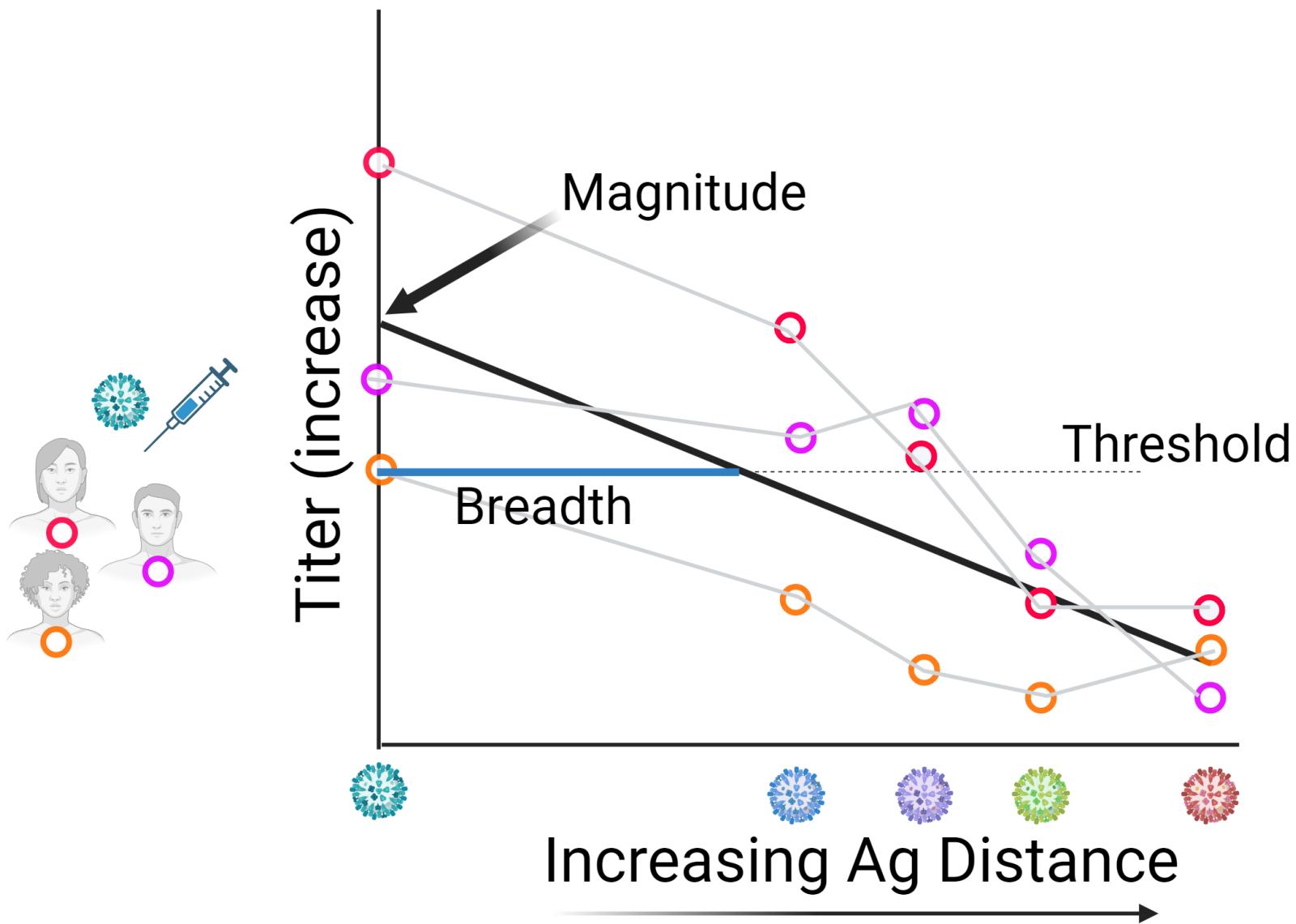
- **Temporal** method: absolute difference in years of strain isolation.
- **Sequence** method: based on genetic or protein sequence comparison.
 - E.g. Hamming distance or *p*-Epitope
 - Biochemical or evolutionary weighted methods like Grantham distance.
- **Antigenic cartography**: use previously computed ferret maps or build our own.

Sidenote: we are developing a pipeline to calculate all these distances and share our results.

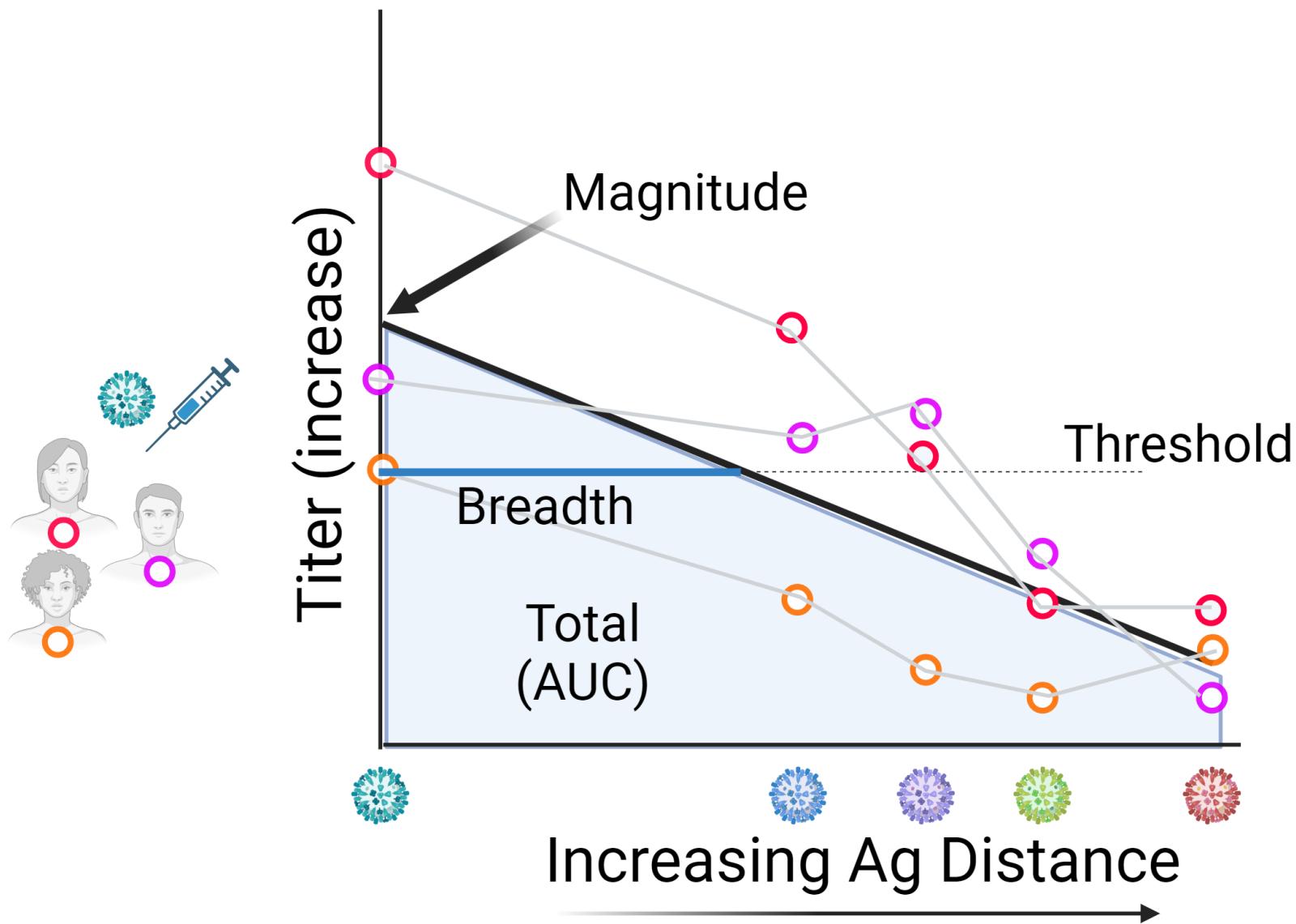
Magnitude: regression line intercept



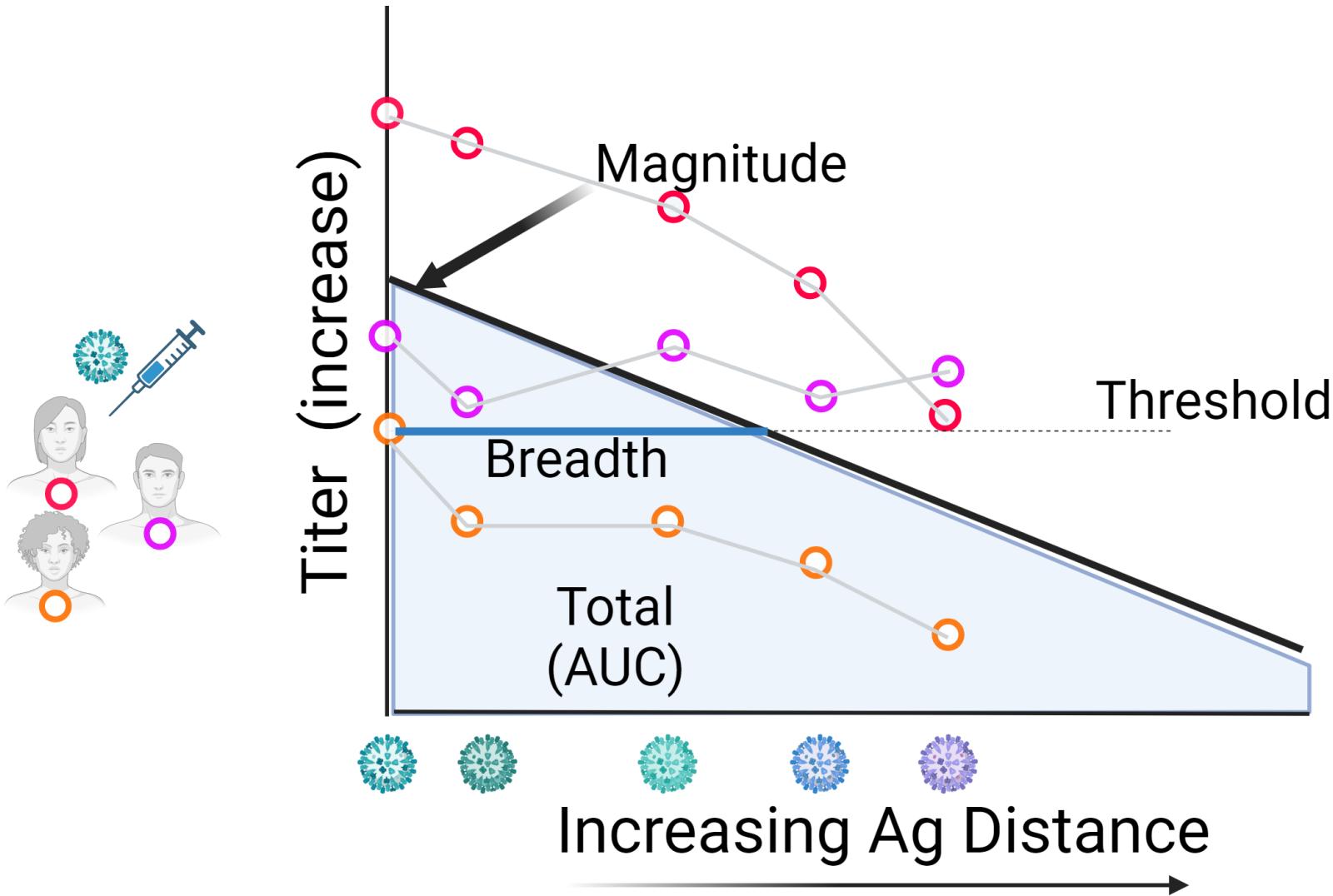
Breadth: prop. of line above threshold



Total strength: area under the curve



We predict this will be robust across multiple panels!



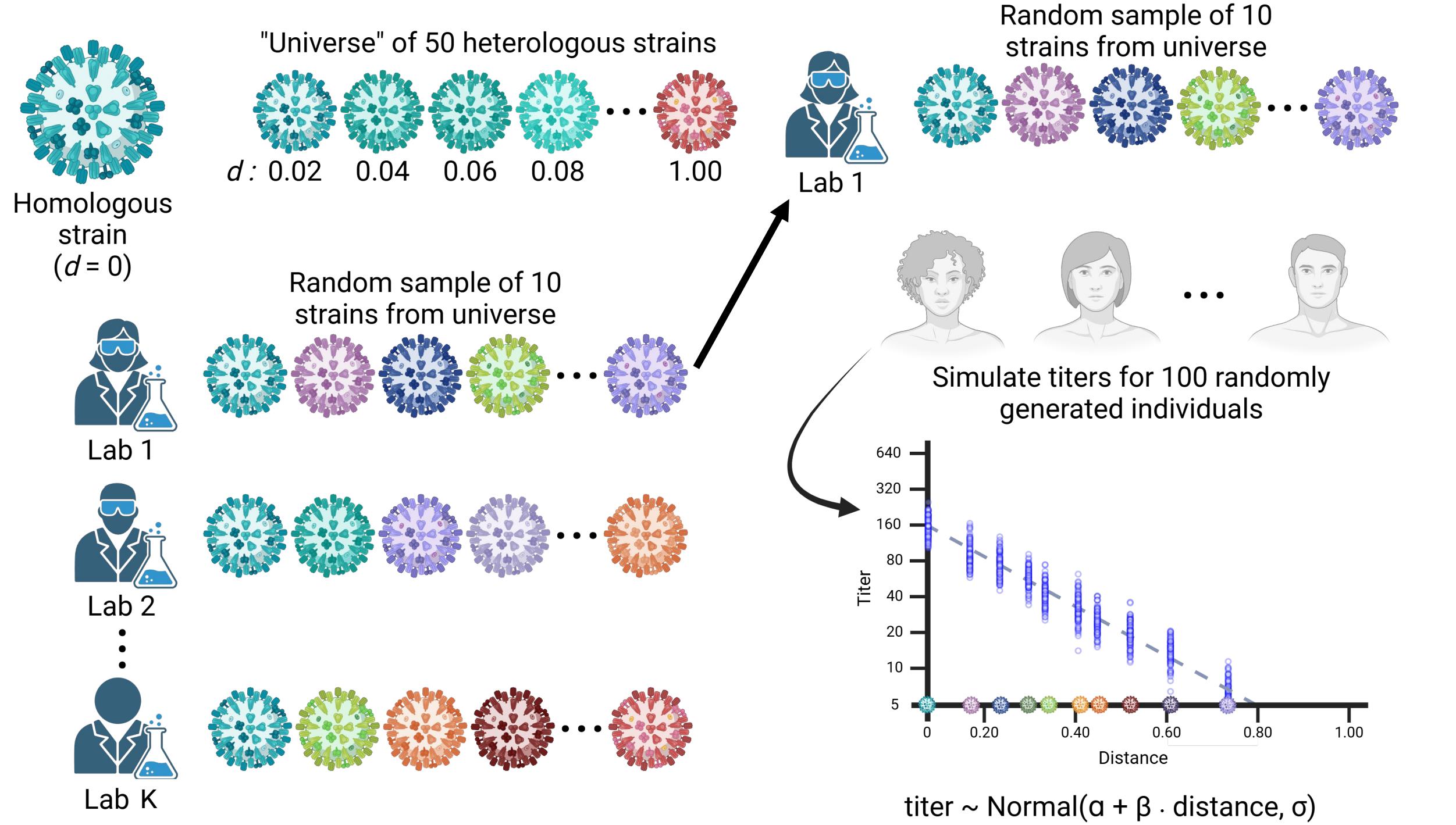
We expect our methods to be more robust across multiple labs.

	Current method	Proposed method
Magnitude	Homologous GMT	Intercept
Breadth	Overall SCR	Fraction above threshold
Overall strength	Overall GMT	AUC

How do we show that AUC is more robust than GMT across strains?

1. **Simulation study:** generate simulated “lab studies” based on our model assumptions and see if AUC is more robust (and when our assumptions fail).
2. **Subsampling analysis:** using UGAFluVac data, subsample multiple smaller studies with different panels and calculate metrics on each.

Part 2: Simulation Study



Next we join the MLM downline

- **Multilevel modeling:** we fit a model where slope and intercept vary by individuals and used pooled estimates to adjust for between-subjects variance (aka hierarchical, mixed effects).

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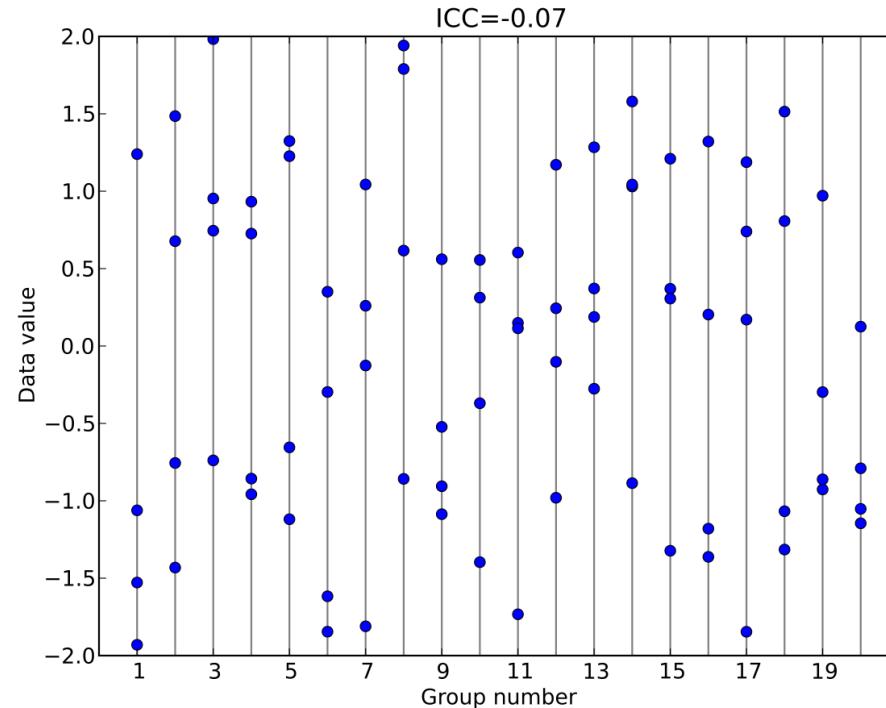
- **Multilevel modeling:** we fit a model where slope and intercept vary by individuals and used pooled estimates to adjust for between-subjects variance (aka hierarchical, mixed effects).
 - We fit independent models for each subtype and season
 - $y_{i,s} = \beta_0 + b_{i,0} + (\beta_1 + b_{i,1}) \cdot \text{distance}_s + \varepsilon_{i,s}$
 - $i = 1, \dots, n$ indexes subjects; $s = 1, \dots, S$ indexes strains

Next we fit the model to the data

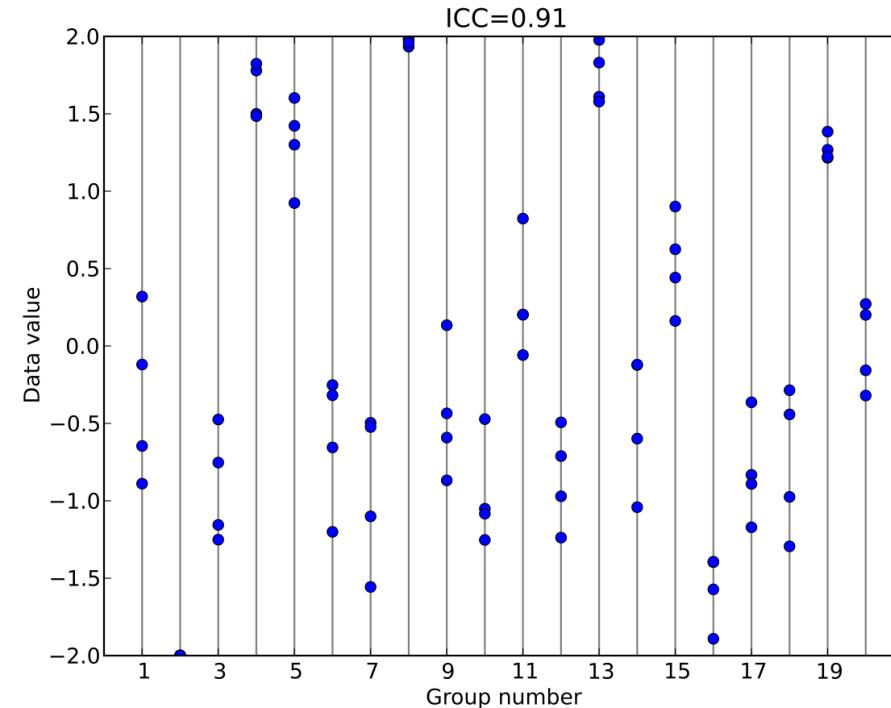
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 - $i = 1, \dots, n$ indexes subjects; $s = 1, \dots, S$ indexes strains
- **Censoring correction:** the limit of detection and binning inherent to HAI and other lab assays can create false uncertainty, so we correct the estimated likelihood for censoring.

Intraclass correlation measures between vs. within group variance

**Low ICC: within > between
(groups don't explain variation)**



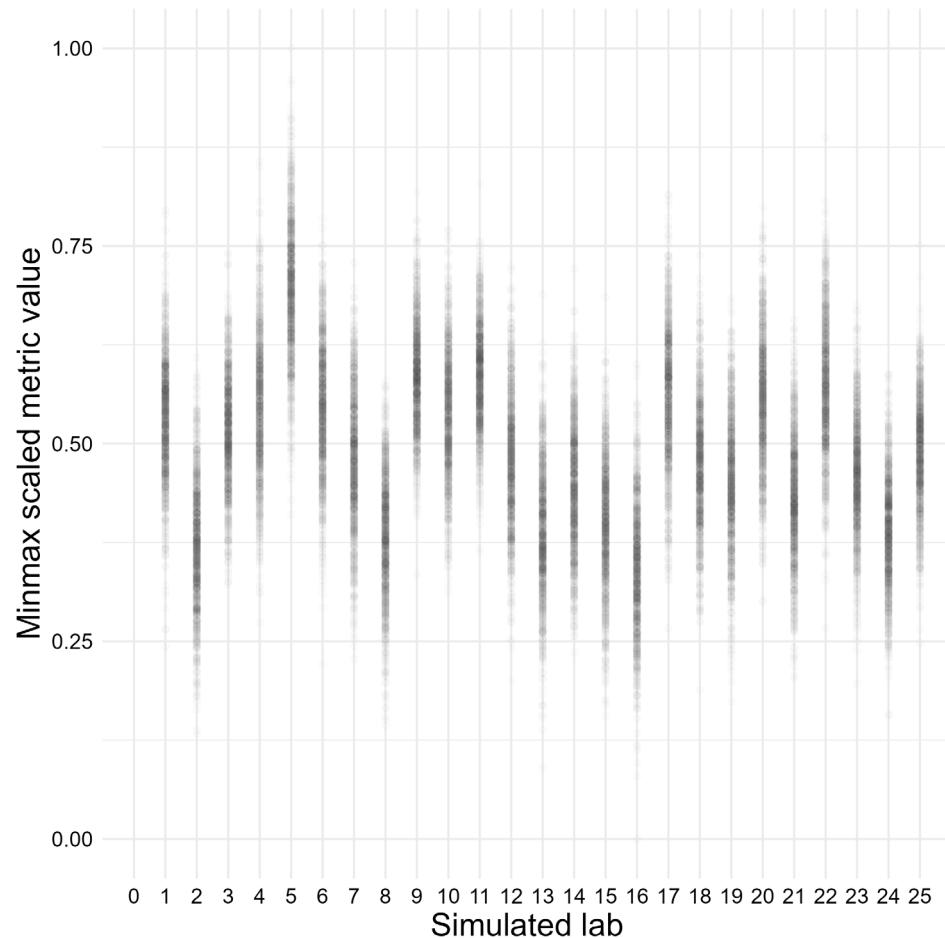
**High ICC: between > within
(groups vary together)**



Simulation study ICCs

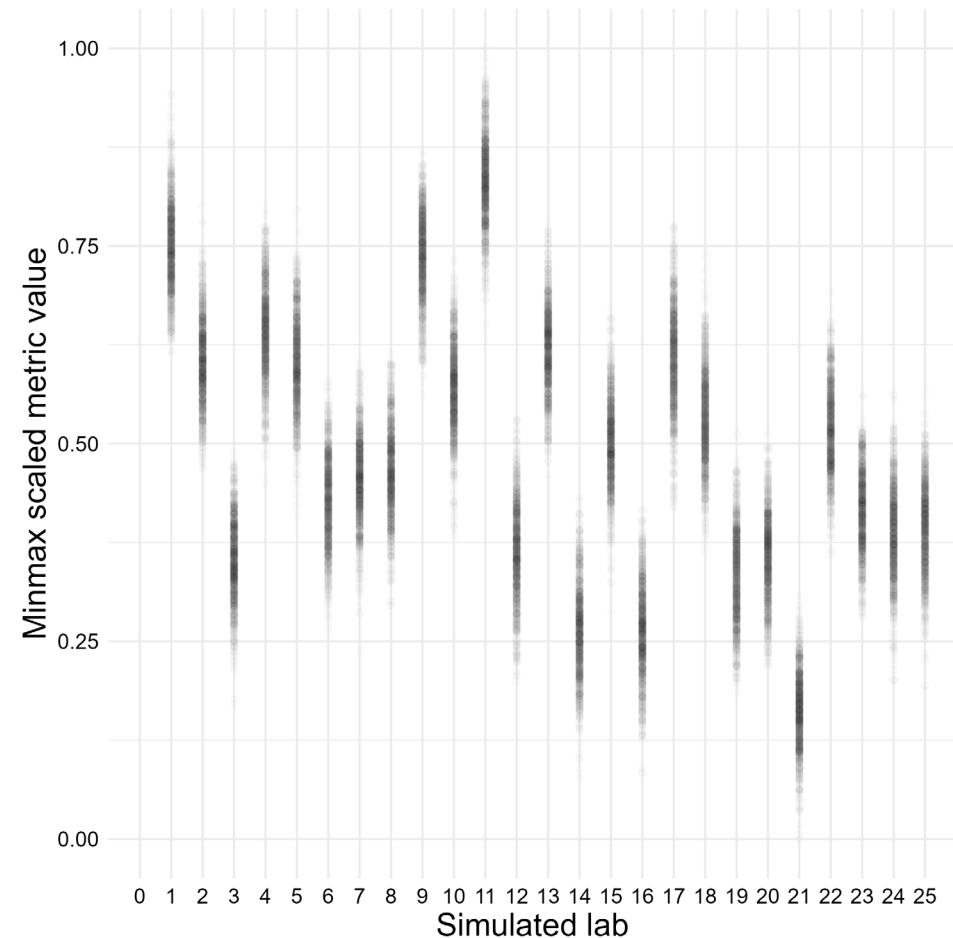
AUC (our metric):

ICC: 0.52 (0.42, 0.60)



GMT (standard metric):

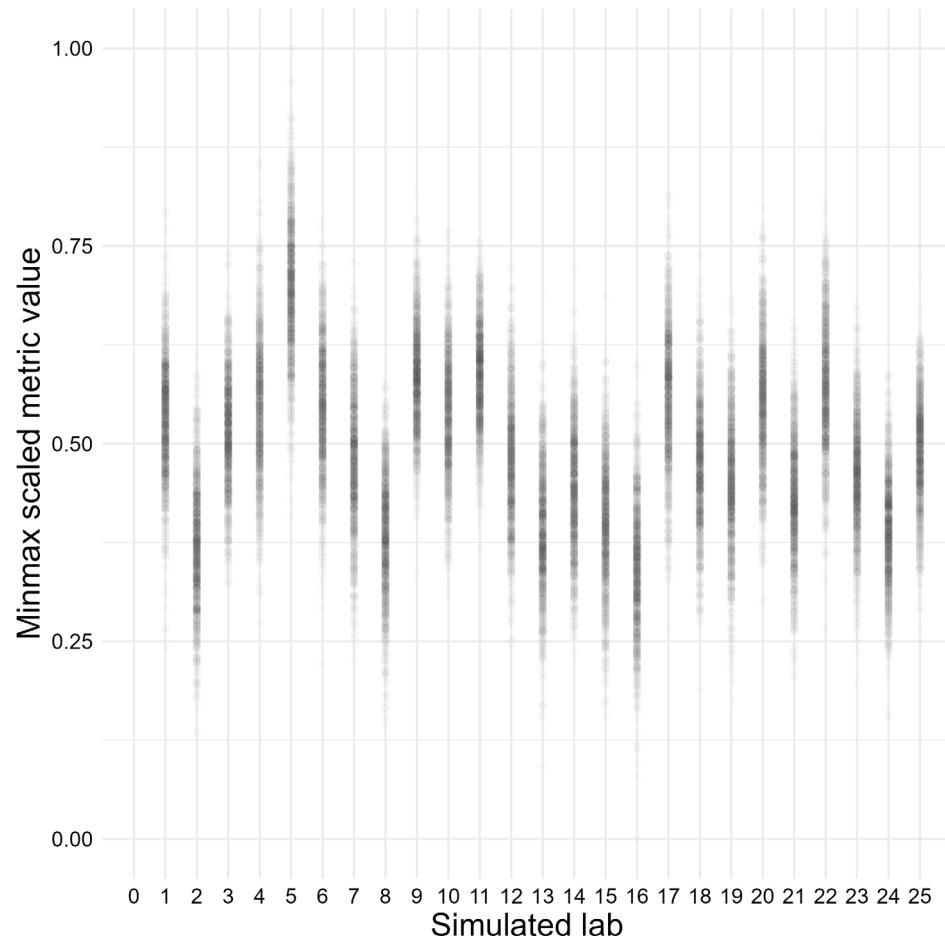
ICC: 0.92 (0.90, 0.94)



Simulation study ICCs

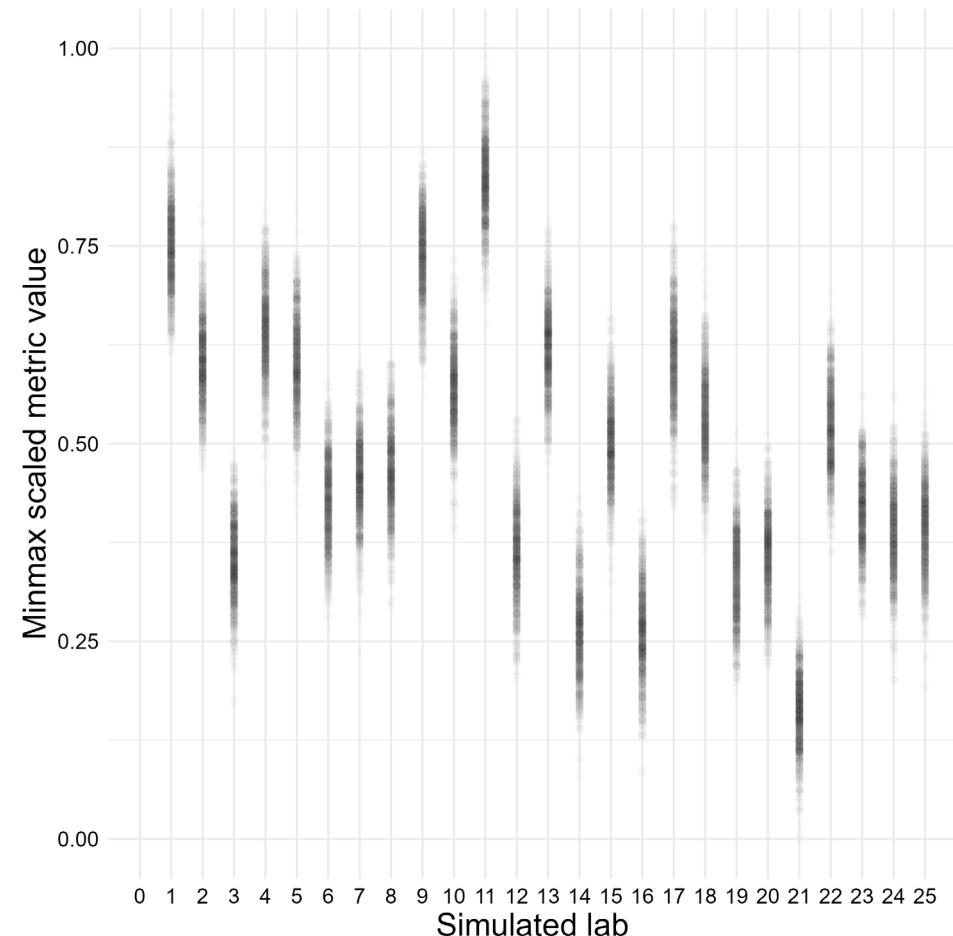
AUC (our metric):

Labs explain ~50% of variation



GMT (standard metric):

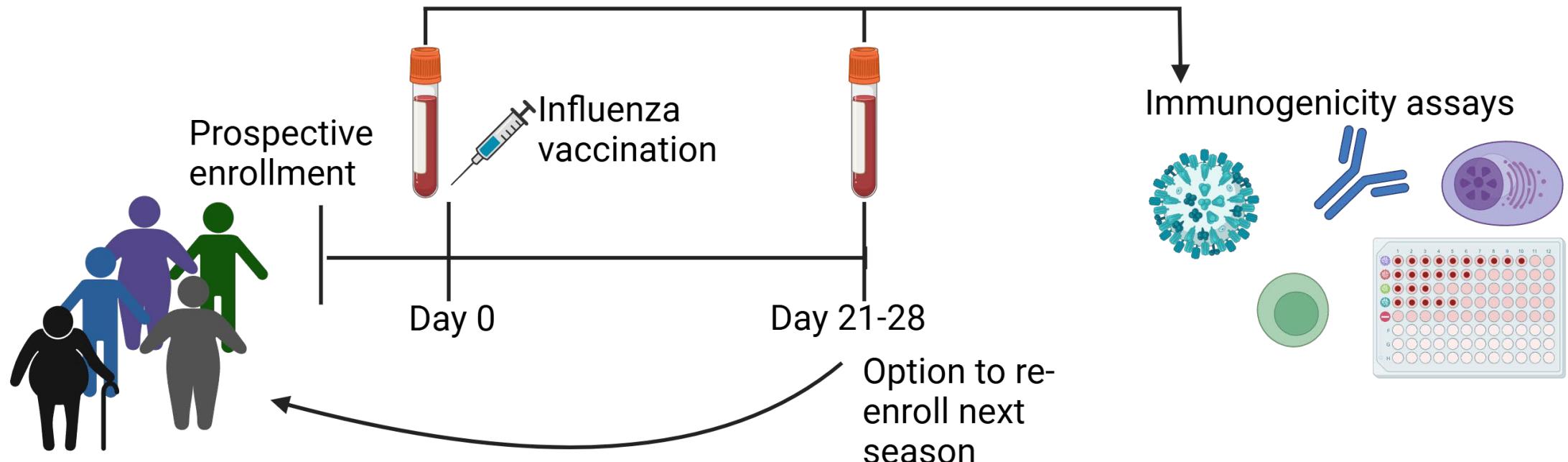
Labs explain ~90% of variation



In the simulation, AUC looks
more robust to subsample
than across-strain GMT!

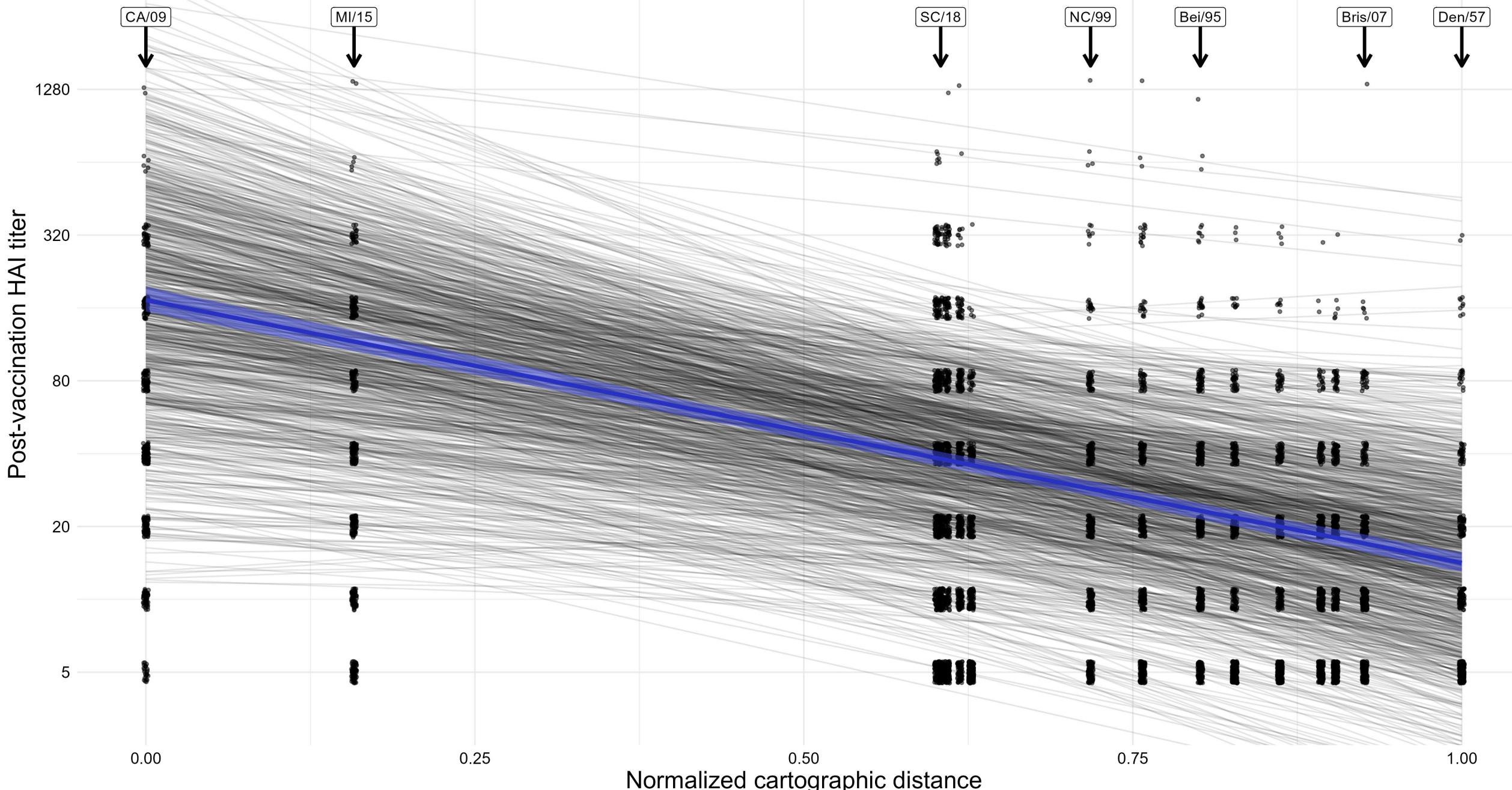
Part 3: Real Data Analysis

UGAFluVac dataset

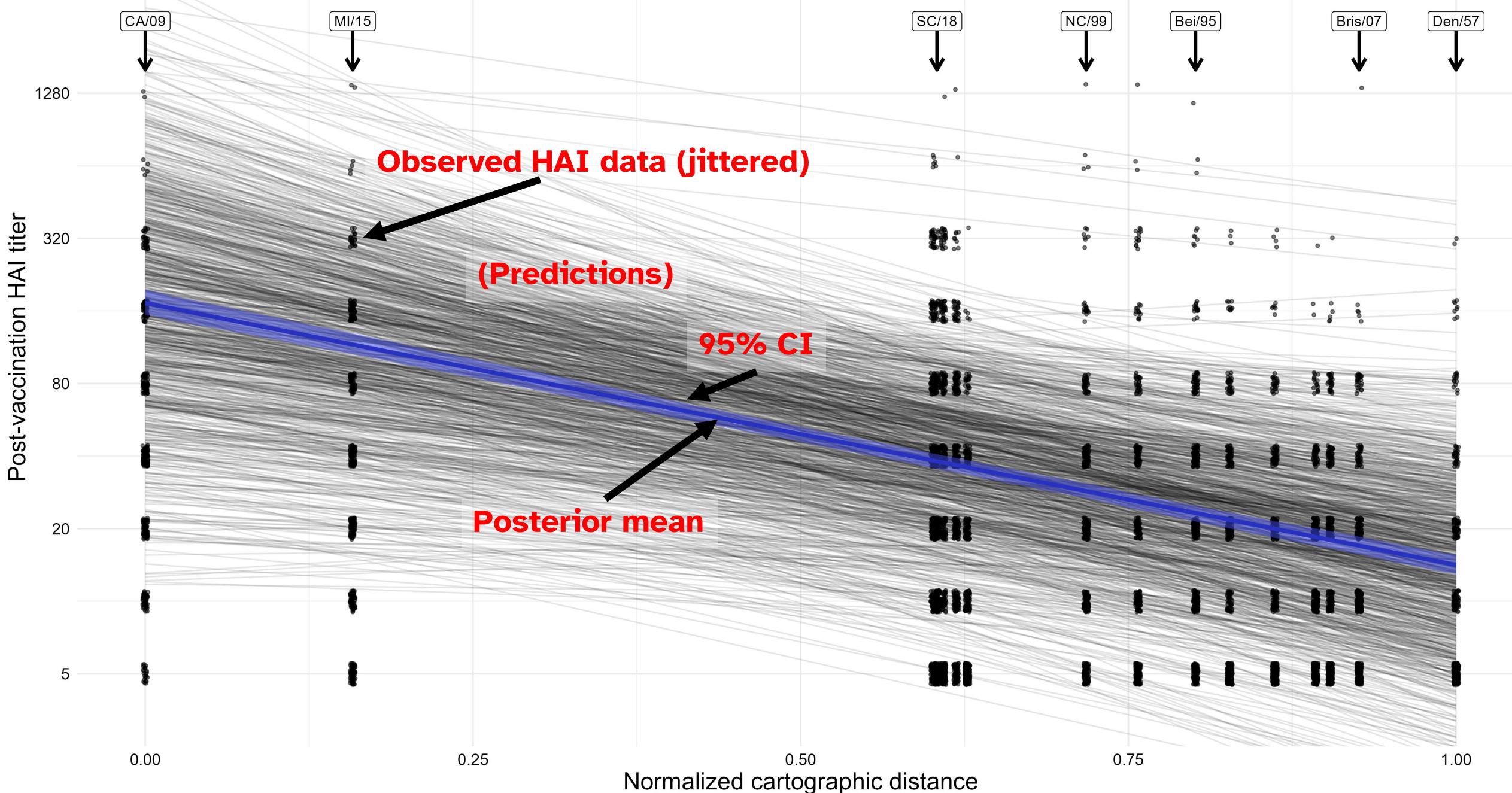


- Led by Ted Ross, part of DIVERsity
- Collected a panel of historical HAI assays from 2013/14 – 2017/18.

2016/17 H1N1 antibody landscape



2016/17 H1N1 antibody landscape



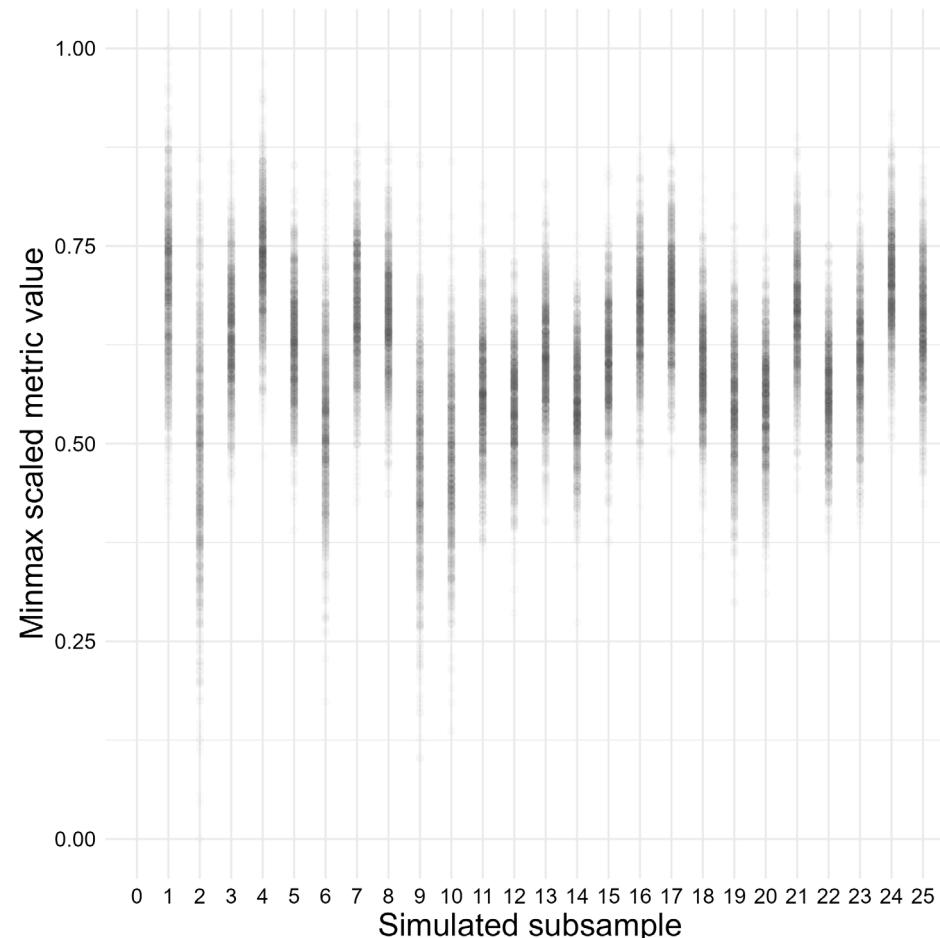
Subsampling methods

- Pick a subcohort from one season (so there's no vaccine changes), treat all four subtypes in the vaccine separately.
- From full cohort, choose 9 heterologous strains and 100 individuals to create one subsample, add the homologous strain data as well.
- Repeat this 25 times to get 25 “studies”.
- Follow analysis strategy for simulations – fit a multilevel model, get AUC and GMT samples, calculate ICC.

Real data subsampling ICCs

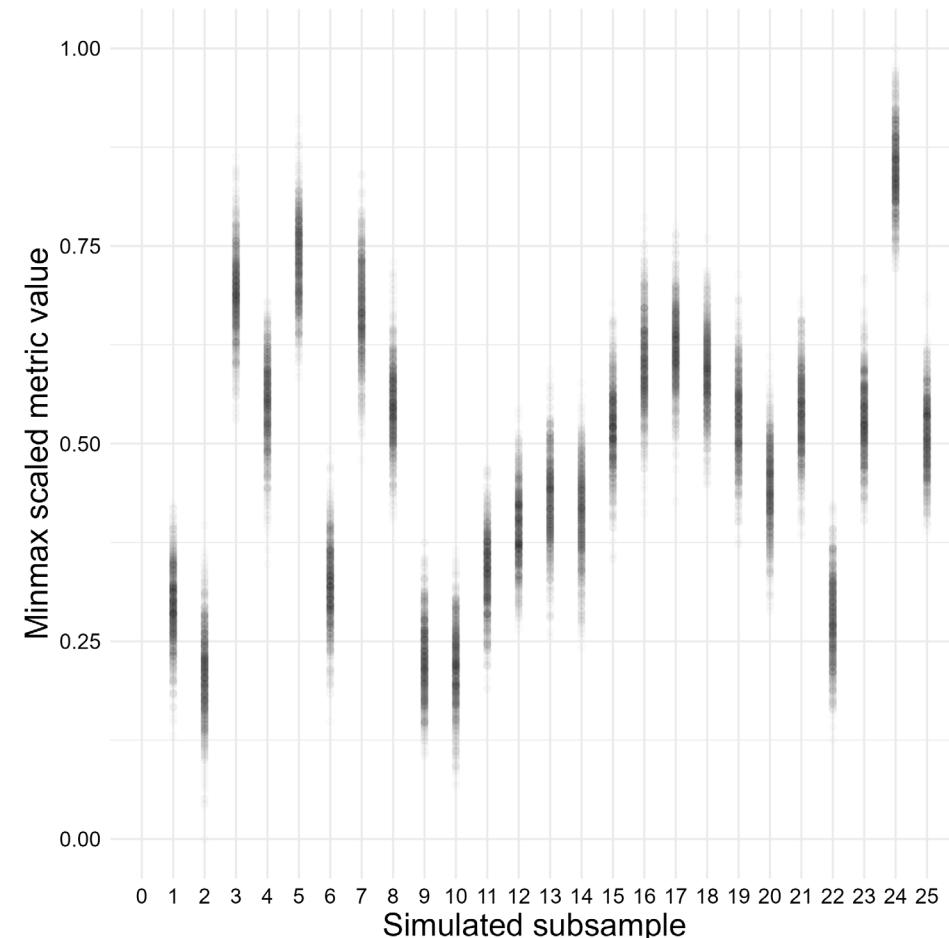
AUC (our metric):

ICC: 0.44 (0.22, 0.64)



GMT (standard metric):

ICC: 0.91 (0.82, 0.95)



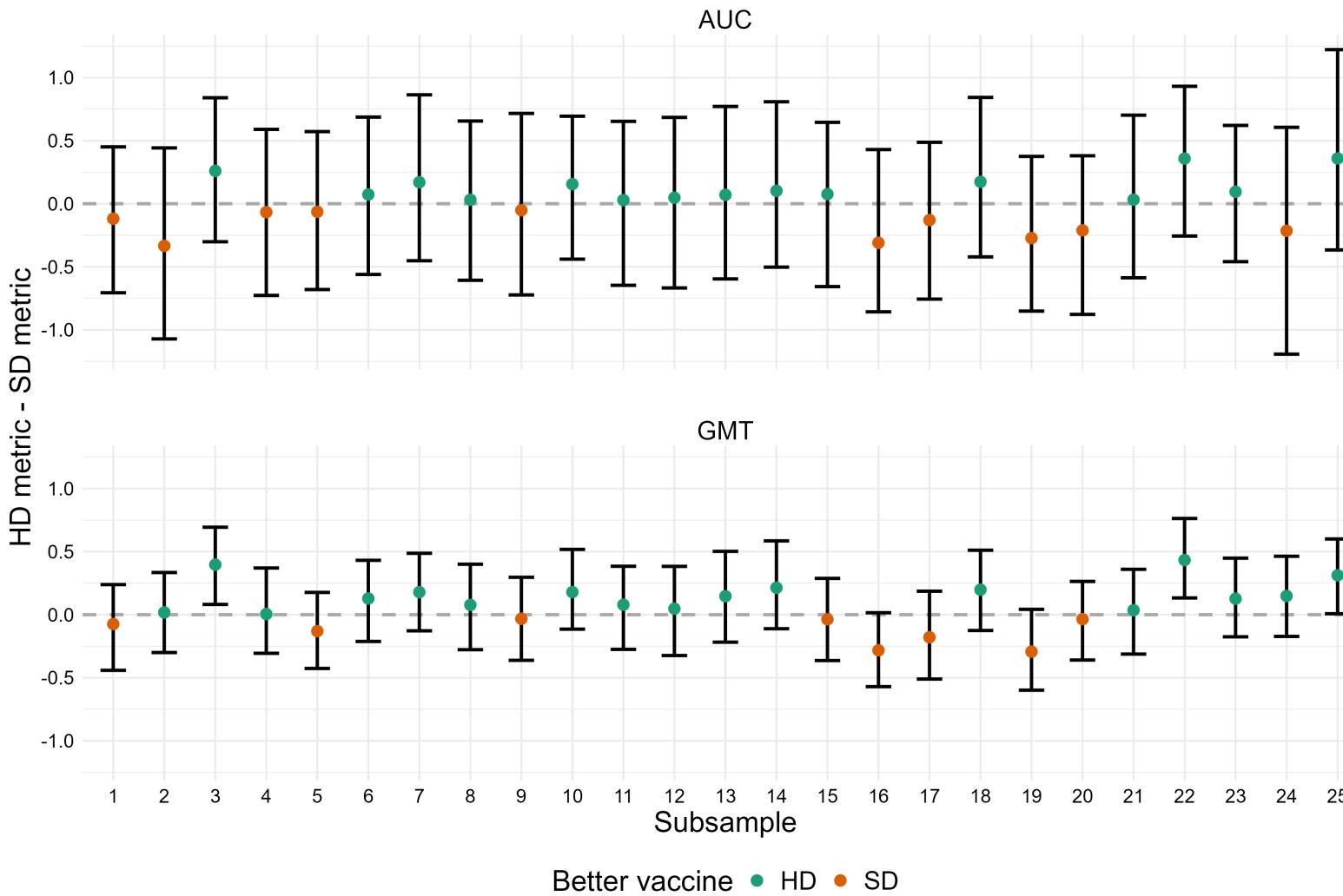
If we account for censoring,
the real data match the
simulation!

Data not shown: not accounting for censoring falsely reduces the variance of both measures, but seems to reduce GMT variance more.

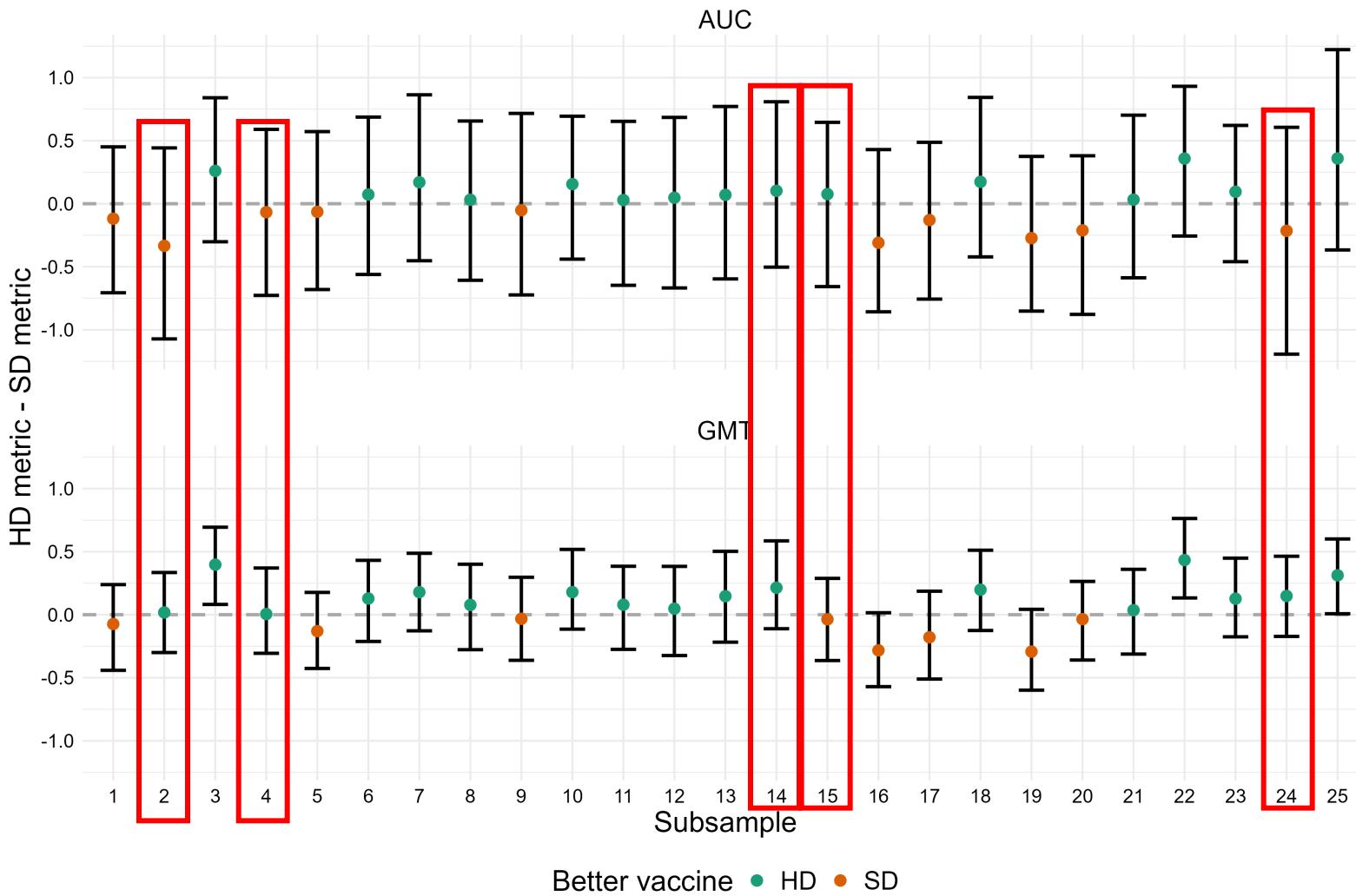
Example: HD vs SD

- We show data for 2016/17 flu season only
- Only included individuals 65 and older who were allowed to choose a high dose vaccine
- We create 10 “studies”, i.e. subsampled panels of strains.
- There were only 34 people ≥ 65 , so we subsampled 30 SD and 30 HD subjects per subsampled study. (This will make our subsamples more correlated, unfortunately.)
- Which vaccine would each conclude is more broadly reactive if they used GMT, and if they used AUC?

Some subsamples would change conclusions, but all have high uncertainty



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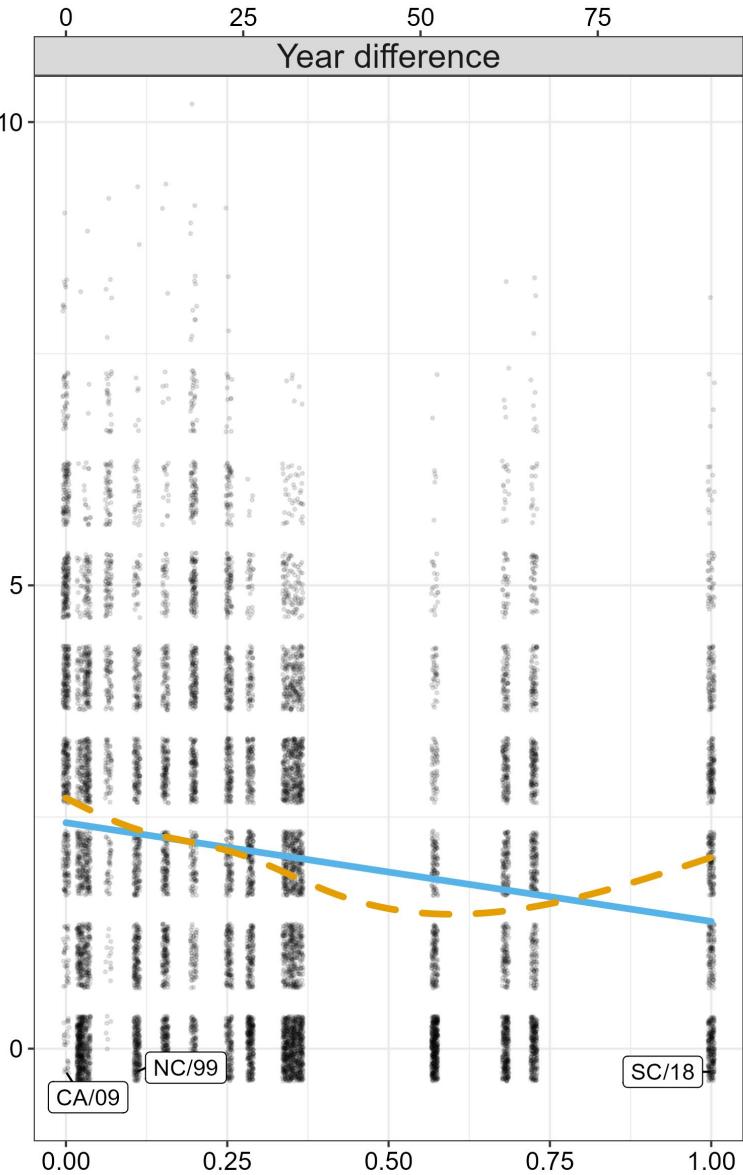
- Higher sample size would help out here.
- Other seasons or pooling seasons together could be useful.
- Some point estimates do change, so it seems reasonable to say that the metric matters!

Future directions with AUC

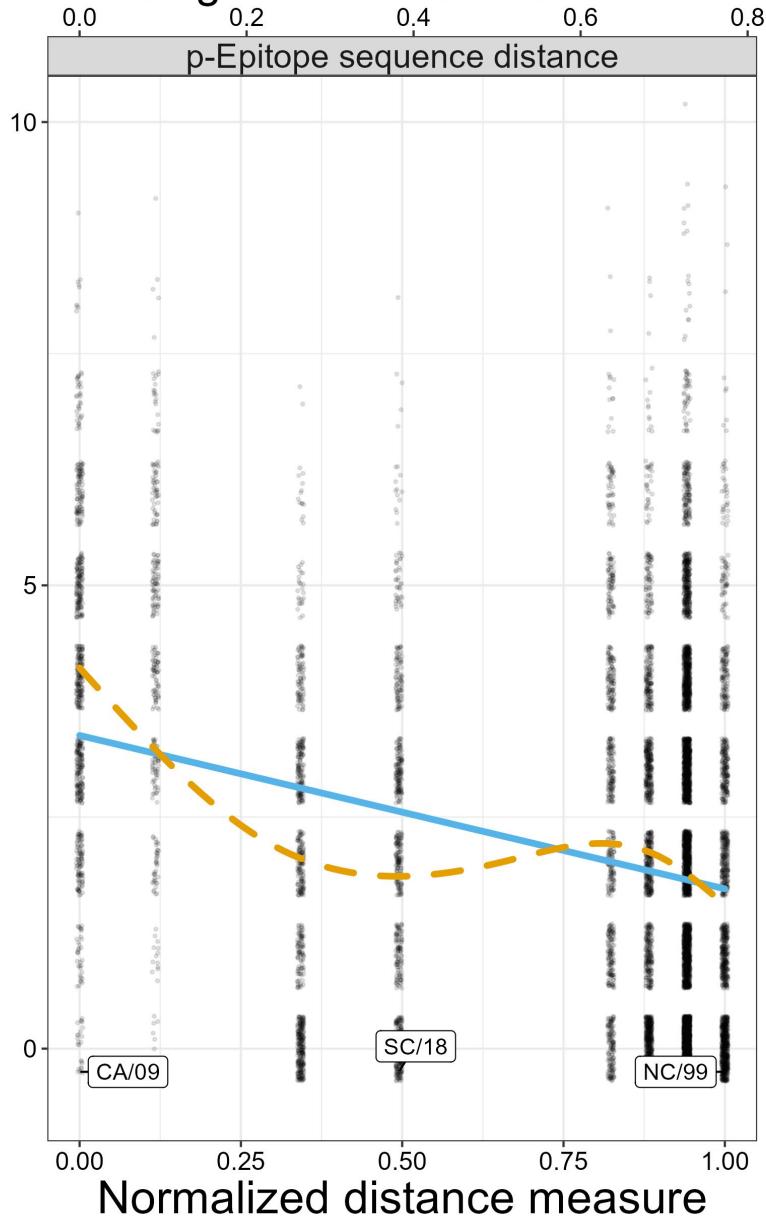
- Compare multiple distance measures to see which seem best / most predictive of a good vaccine candidate.
- Explore summary landscape – is a linear model good enough?

H1N1-California-2009 (n = 773)

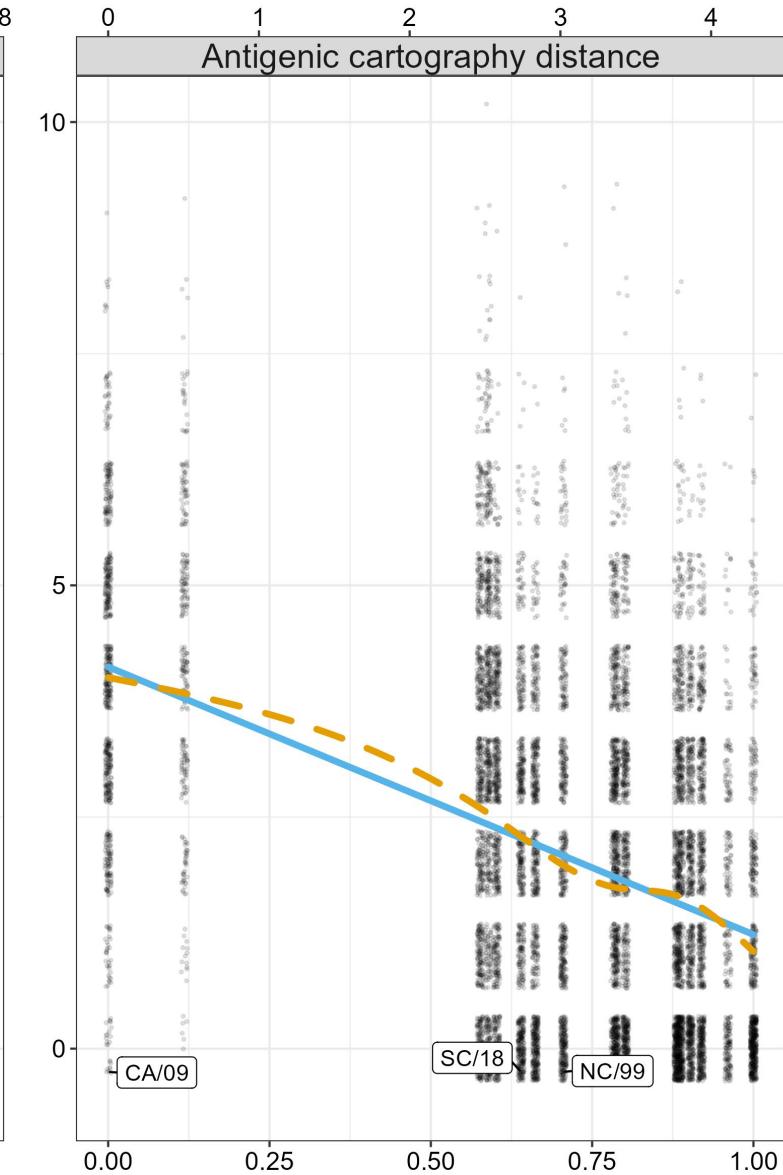
titer (log₂ HAI)



Original distance measure

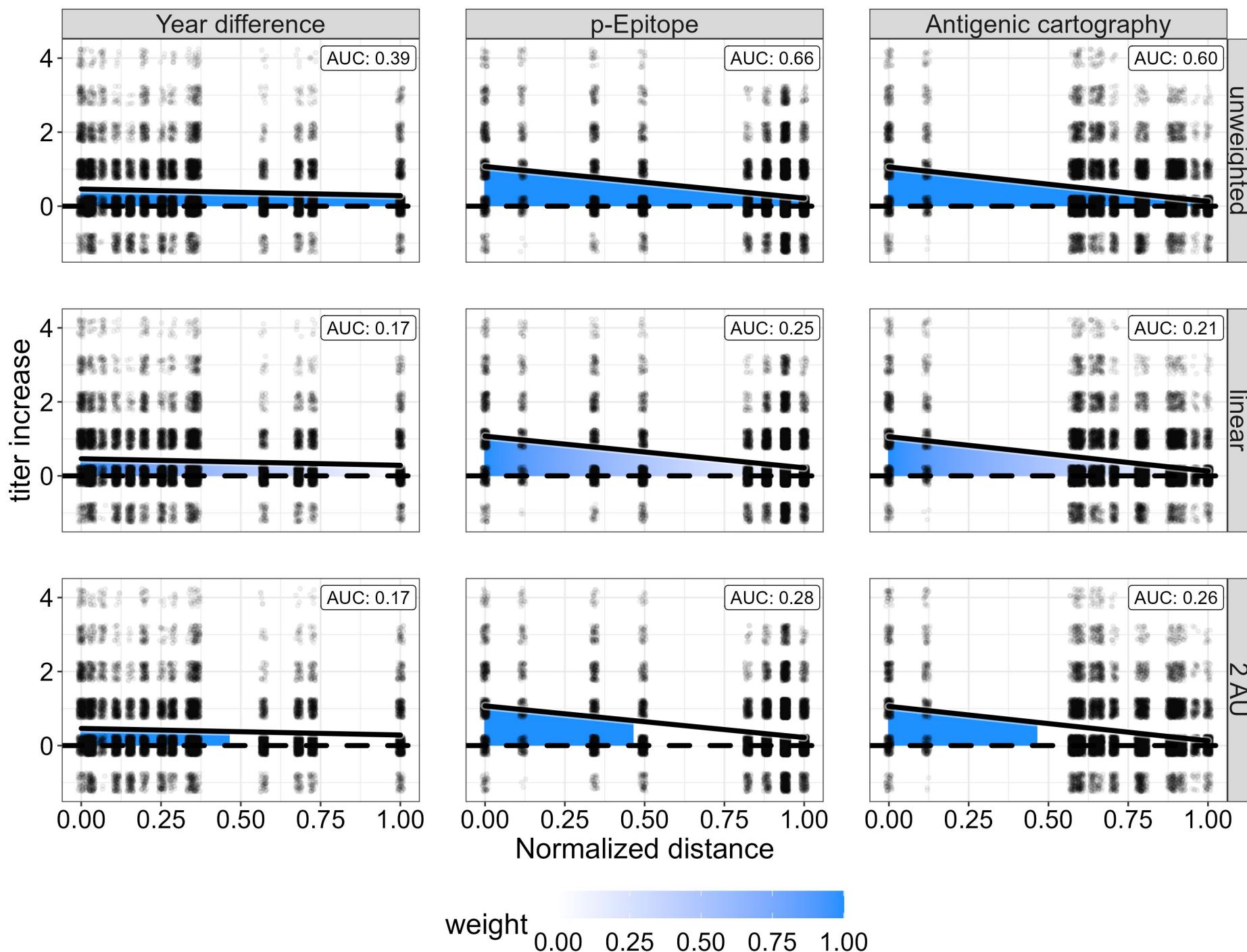


Antigenic cartography distance



Future directions with AUC

- Compare multiple distance measures to see which seem best / most predictive of a good vaccine candidate.
- Explore summary landscape – is a linear model good enough?
- Experiment with weighting schemes.



Future directions with AUC

- Compare multiple distance measures to see which seem best / most predictive of a good vaccine candidate.
- Explore summary landscape – is a linear model good enough?
- Experiment with weighting schemes.
- We need heterologous data that tests old serum samples against “future” strains to really test our hypotheses.
- E.g. take serum samples from 2016/17, test HAI against MI/15, Brisbane/18, GM/19, and so on.

Final thoughts

- Correct for censoring and don't use year-based difference
- AUC seems to be more robust to the panel sampling problem than current methods
- Variance components (from multilevel models) seem to be a useful approach to understand sampling variation
 - E.g. analyzing how important between-individual variation is
- Understanding the causal effect of distance could help us use distance for vaccine selection.

**WIP: variance partitioning
(time permitting)**

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- Approach 1: model variance components

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 - Within vs. between lab variation as we showed
 - Multiple sources of sampling variation: individuals, labs, vaccines

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- Approach 1: model variance components
 - Within vs. between lab variation as we showed
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- Approach 2: variance in posterior predictions
 - Compare variation explained by observed effects vs. random variation
 - Emily's question about interindividual variance

Approach 1: variance components

$$y_{ijk\dots} = \mathbf{X}\beta + b_{\text{subject}[i]} + b_{\text{lab}[j]} + b_{\text{vaccine}[k]} + \dots + \varepsilon_{ijk\dots}$$

$$b_{\text{whatever}} \sim \text{Normal}(0, \sigma^2_{\text{whatever}})$$

$$\varepsilon_{ijk\dots} \sim \text{Normal}(0, \sigma^2)$$

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- I promise it isn't as scary as it looks!

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$$\varepsilon_{ijk\dots} \sim \text{Normal}(0, \sigma^2)$$

- I promise it isn't as scary as it looks!
- Right now, we only have data from one lab.
- But we can add between-lab variance in simulations.

Approach 2: Effect of individual variation

$$y_{i,s} = \beta_0 + b_{i,0} + (\beta_1 + b_{i,1}) \cdot \text{distance}_s + \varepsilon_{i,s}$$

- Betas: population effects for intercept and distance
- Little b's: each individual gets their own effect representing departure from the population intercept and effect of distance.
- Fit model, get (counterfactual) predictions on dataset.

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$$\Delta_\sigma = \frac{\text{Var}(\hat{\beta}_0 + 0 + (\hat{\beta}_1 + 0) \cdot \text{distance})}{\text{Var}(\hat{\beta}_0 + \hat{b}_{i,0} + (\hat{\beta}_1 + \hat{b}_{i,1}) \cdot \text{distance})}$$

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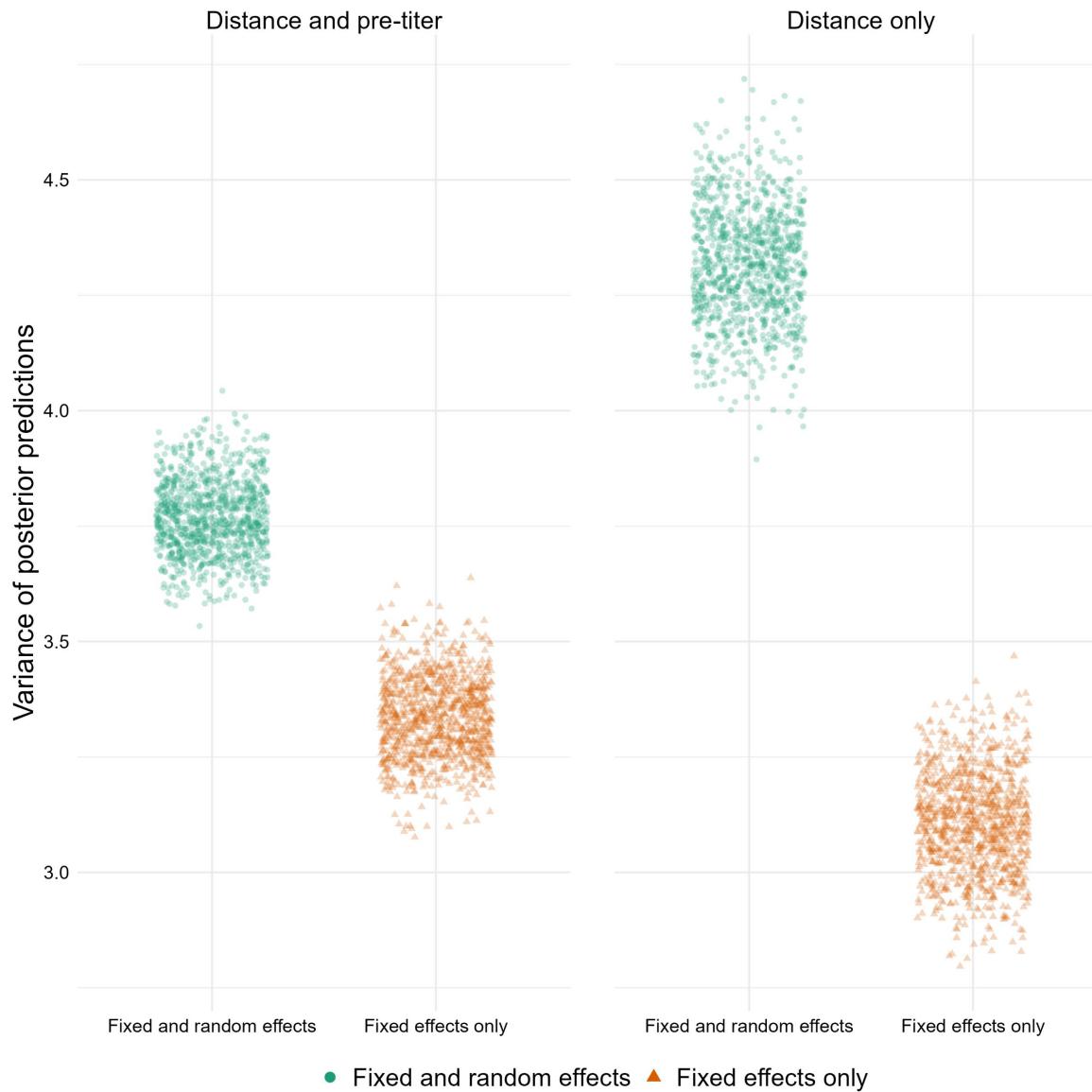
$$\Delta_\sigma = \frac{\text{Var}(\text{all predictions ignoring random effects})}{\text{Var}(\text{all predictions with random effects})}$$

Approach 2: Effect of individual variation

$$\Delta_{\sigma} = \frac{\text{Var(all predictions ignoring random effects)}}{\text{Var(all predictions with random effects)}}$$

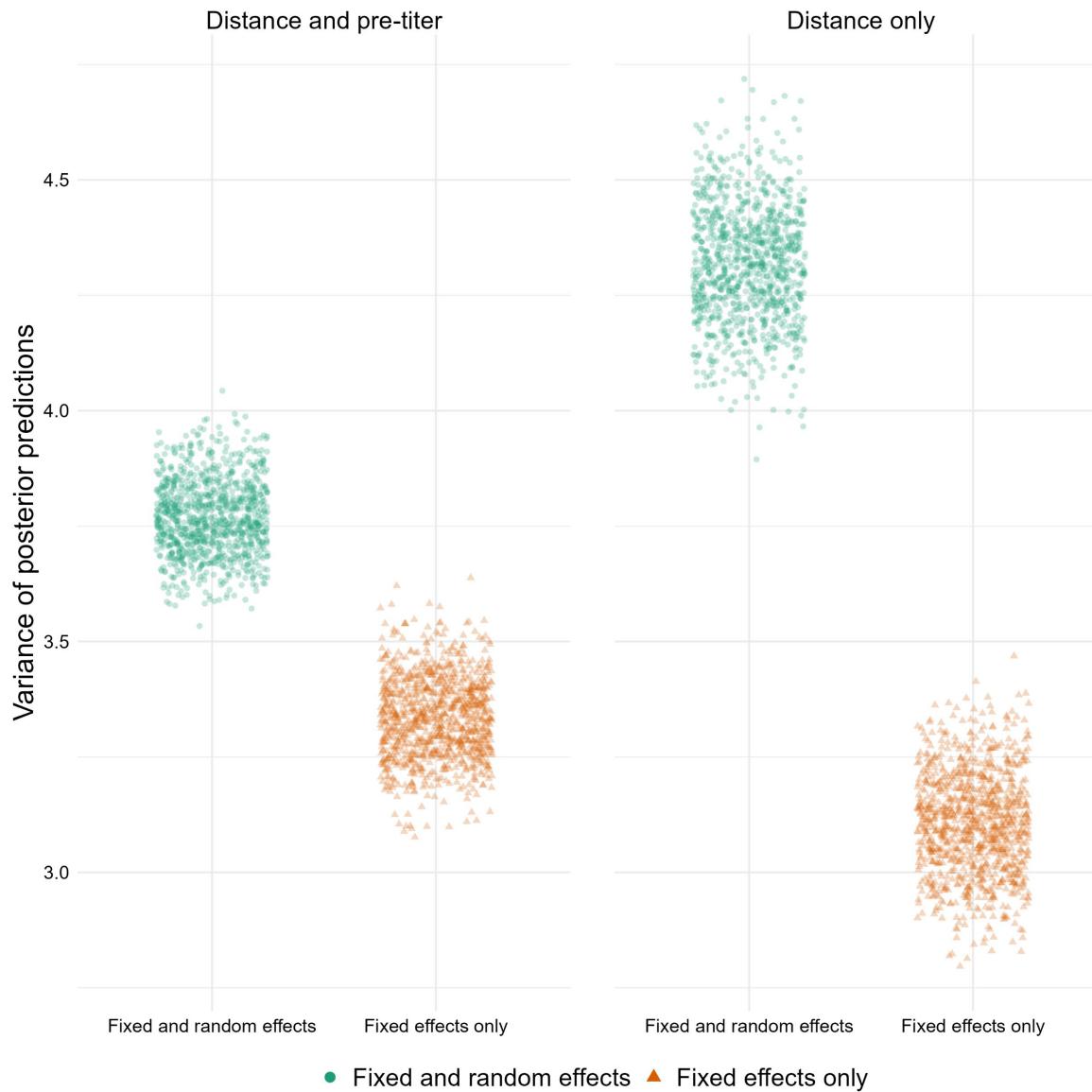
- **“Fraction of variance explained by fixed effects only”**
- Can provide insight about how important between-individual variance is, compared to observed effects like distance.
- $\Delta_{\sigma} \approx 1 \rightarrow$ Including interindividual variance doesn't do much.
- $\Delta_{\sigma} \gg 1 \rightarrow$ Individual variance becomes more important than fixed effects.
- If you do it Bayesian, you can get uncertainty estimates for free.

Interindividual variance in UGAFluVac



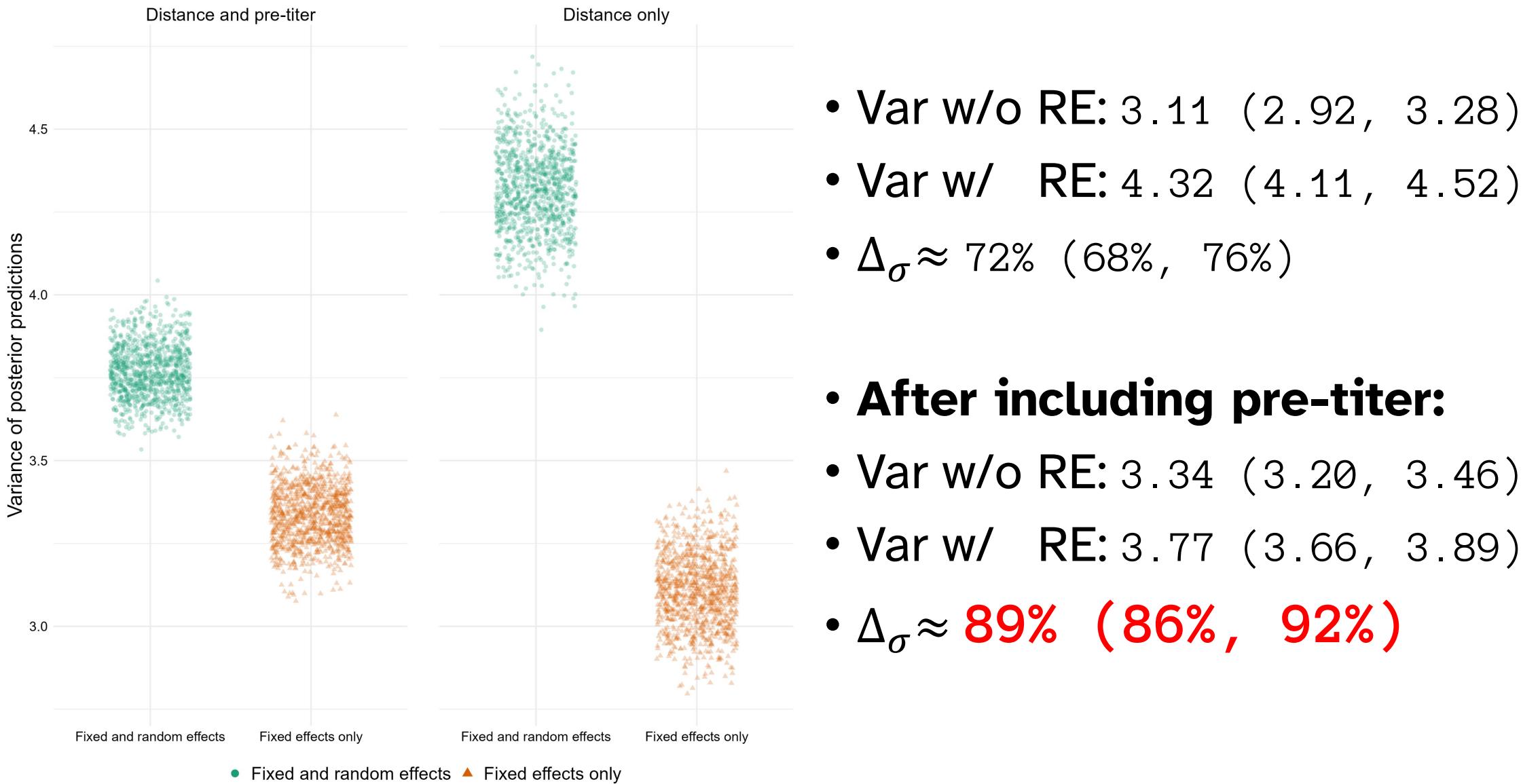
- Var w/o RE: 3.11 (2.92, 3.28)
- Var w/ RE: 4.32 (4.11, 4.52)
- $\Delta_\sigma \approx 72\%$ (68%, 76%)
- **After including pre-titer:**
- Var w/o RE: 3.34 (3.20, 3.46)
- Var w/ RE: 3.77 (3.66, 3.89)
- $\Delta_\sigma \approx 89\%$ (86%, 92%)

Interindividual variance in UGAFluVac

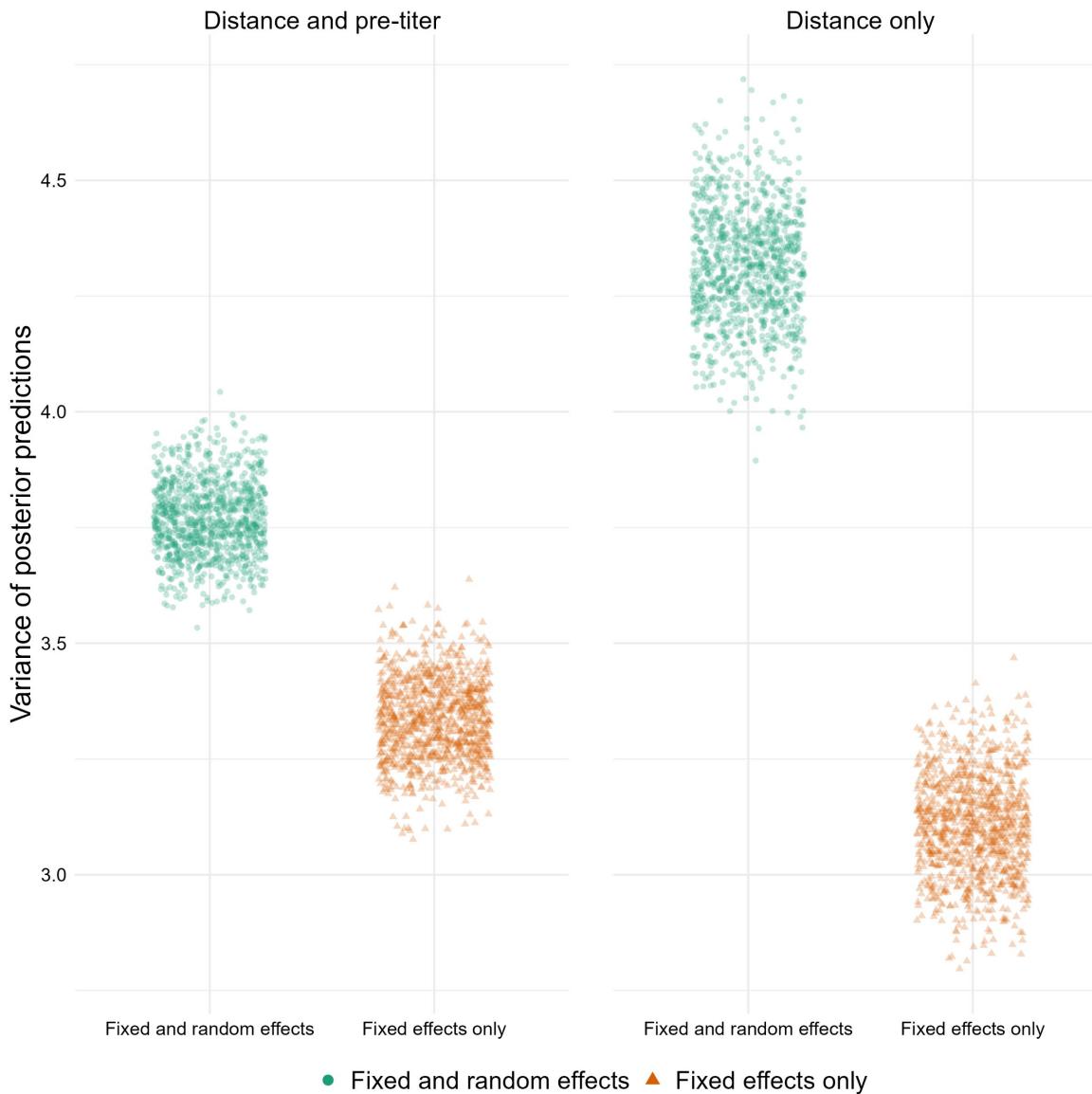


- Var w/o RE: 3.11 (2.92, 3.28)
 - Var w/ RE: 4.32 (4.11, 4.52)
 - $\Delta_\sigma \approx 72\% \text{ (68%, 76%)}$
-
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Interindividual variance in UGAFluVac



Interindividual variance in UGAFluVac



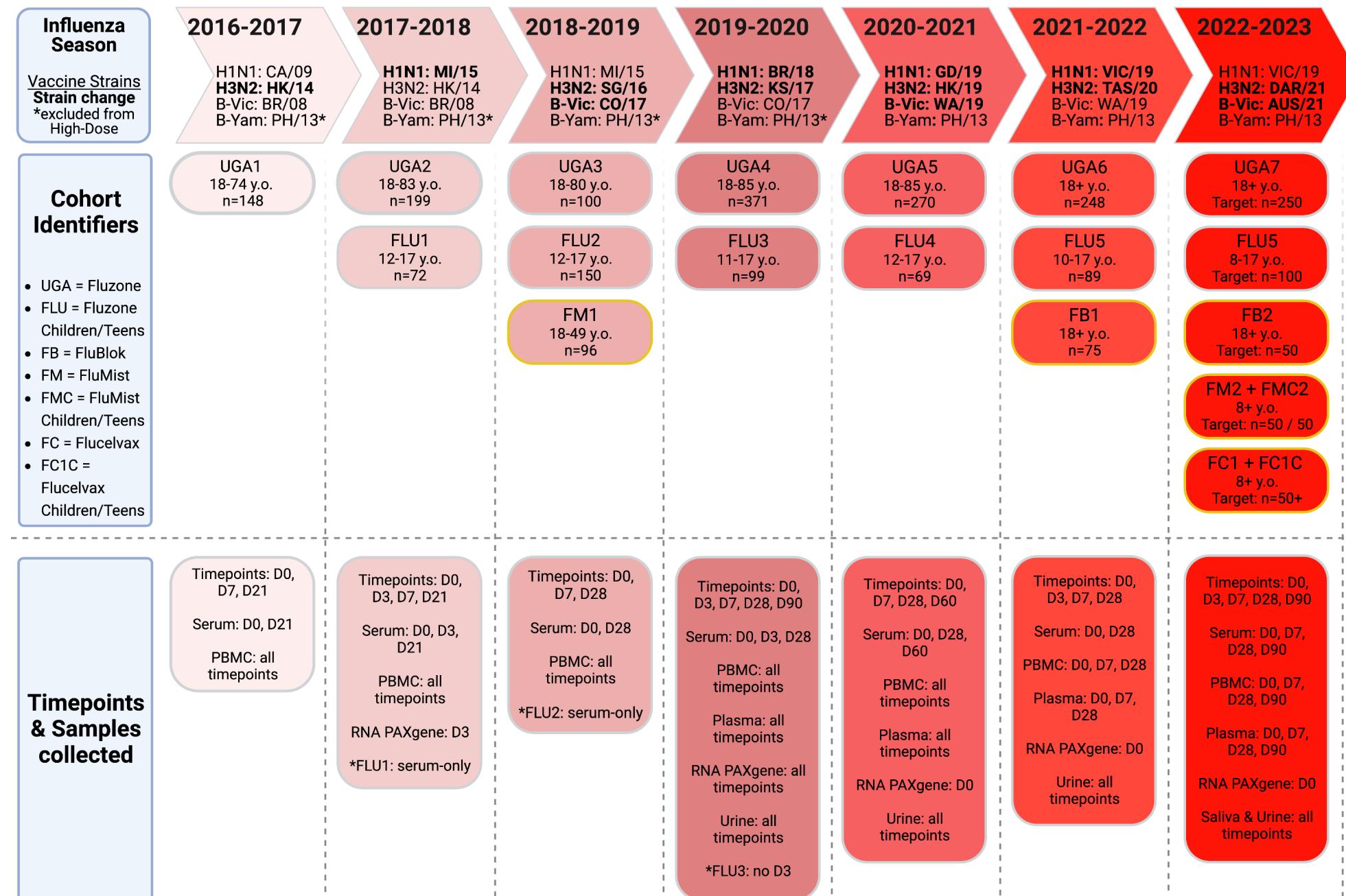
- Var w/o RE: 3.11 (2.92, 3.28)
- Var w/ RE: 4.32 (4.11, 4.52)
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- **After including pre-titer:**

- Var w/o RE: 3.34 (3.20, 3.46)
- Var w/ RE: 3.77 (3.66, 3.89)
- $\Delta_\sigma \approx 89\%$ (86%, 92%)

Some seasons/subtypes showed larger differences!

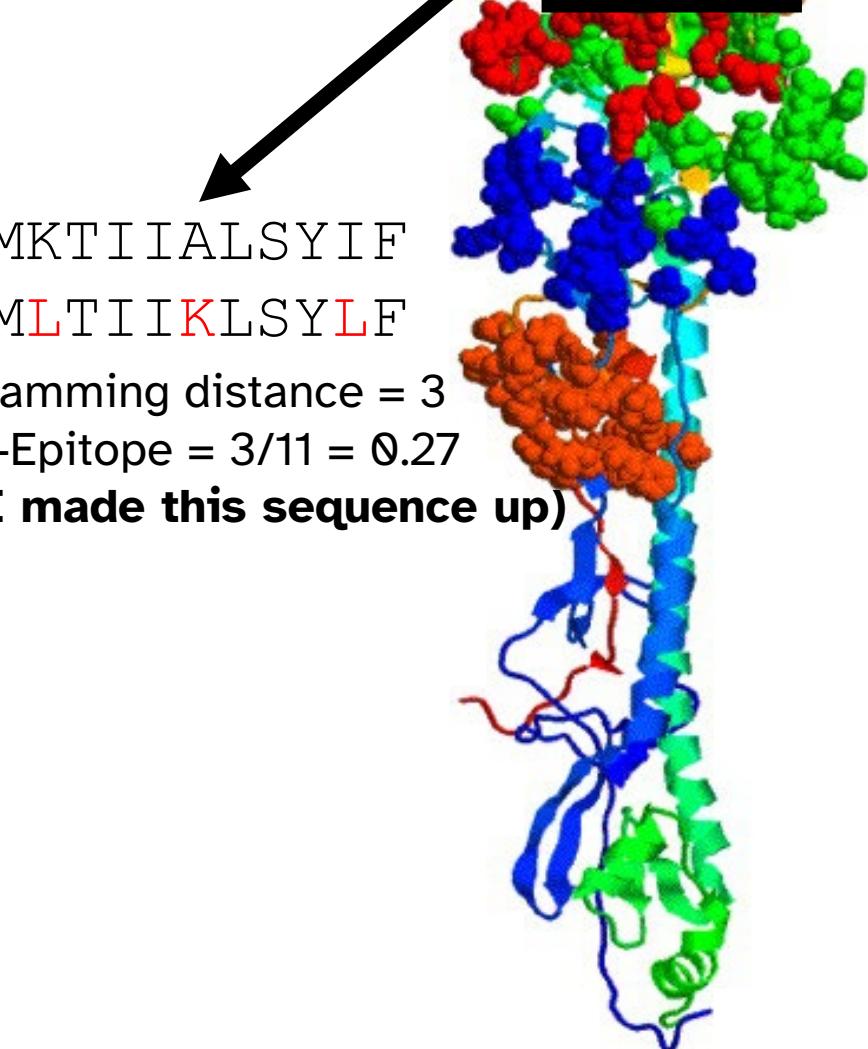
Extra info



“UGAFluVac”: this, plus a similar study from 2013 – 2016 also by Ted Ross ⁸¹

Antigenic distance

(dominant) *p*-Epitope method



Temporal method



A/H3N2/Aichi/2/1968

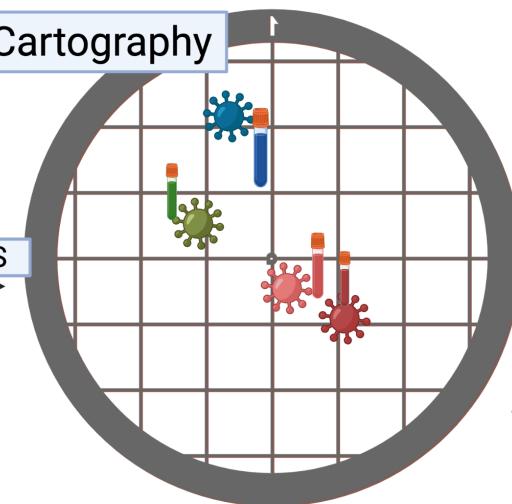


A/H3N2/Kansas/14/2017

$$|2017 - 1968| = 49$$

Antigenic cartography method

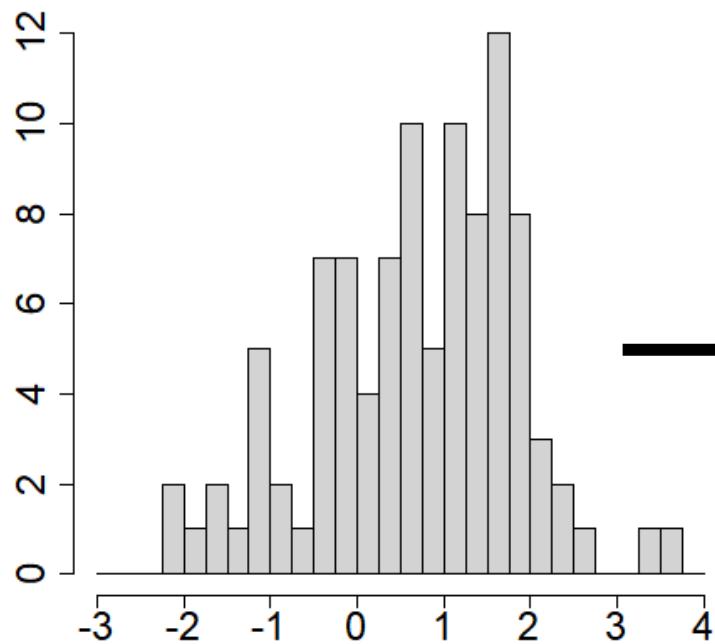
HAI Matrix	Viruses			
	640	320	40	10
Viruses	640	320	20	10
Sera	10	80	640	160
	40	40	320	320



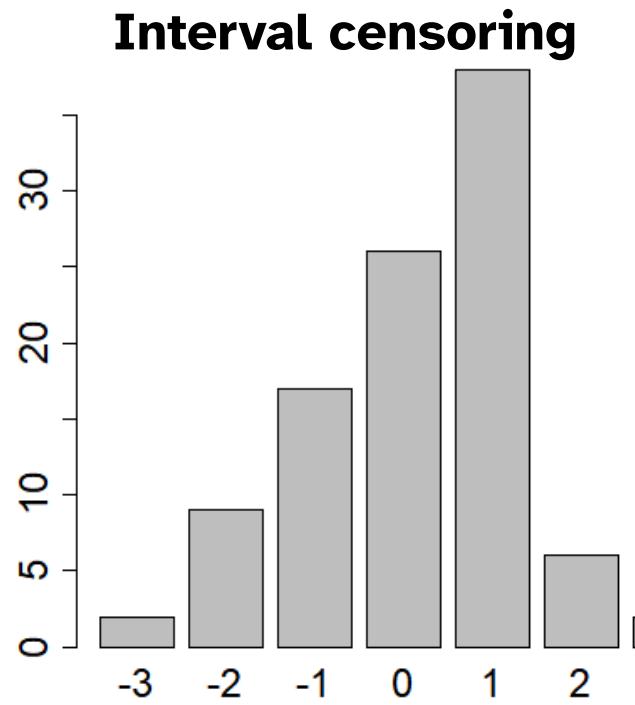
■ Similar HAI Profile
■ Close on map

■ Similar but not as close HAI Profile
■ Distant from viruses and sera that didn't have similar profiles

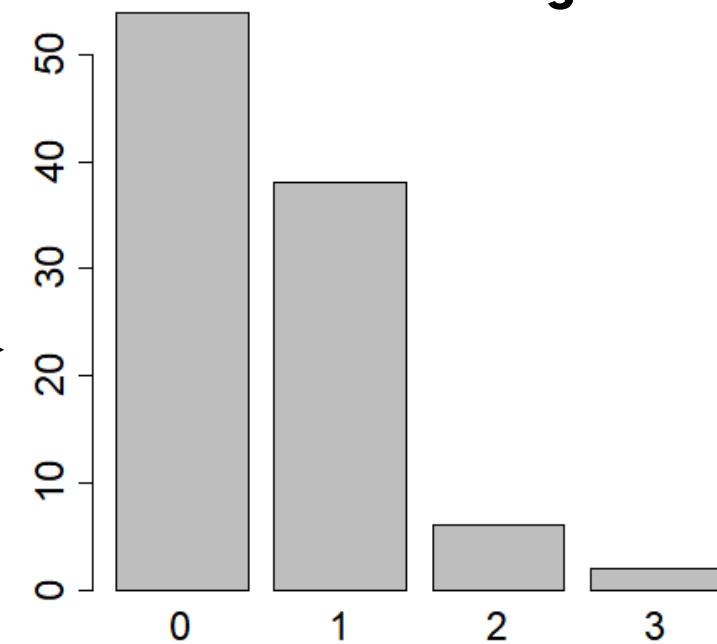
Protein structure from Gupta, Earl, and Deem. Vaccine 2006.
Cartography figure made by Amanda Skarlupka.



Continuous “underlying”
titer



Floored titer
(round down)



Floored titer
with LoD

Interval censoring

Left censoring



ICC Calculation

1. Calculate AUC or GMT for each lab/subsample
2. Fit another mixed-effects model (this is a computational trick to easily get the variances we need)

$$\text{AUC or GMT} = \beta_0 + b_{\text{lab}} + \varepsilon; b_{\text{lab}} \sim \text{Normal}(0, \sigma_{\text{lab}}^2); \varepsilon \sim \text{Normal}(0, \sigma^2)$$

3. Calculate ICC from variances:

$$\widehat{\text{ICC}} = \frac{\widehat{\sigma}_{\text{lab}}^2}{\widehat{\sigma}_{\text{lab}}^2 + \widehat{\sigma}^2}$$

4. Get a bootstrap CI for frequentist model*, or if you do it Bayesian you get posterior distributions of variances.

*You probably need more labs or you'll get a boundary convergence error and I would cry

HD/SD comparison ICCs

<u>ICC</u>	GMT	AUC
HD	0.77 (0.65, 0.87)	0.62 (0.44, 0.74)
SD	0.71 (0.30, 0.82)	0.36 (0.20, 0.51)

- Despite the high uncertainty in contrasts, AUC was still more robust for AUC than SD.
- Metrics were more robust on SD data than HD? This could be sampling variation or an interesting lead.

Multilevel modeling

- There is some “universe” of possible influenza strains that exist in different freezers around the world.
- For a given subtype $T = t$ and year $Y = y$, we can compute the antigenic distance between all of these strains and the vaccine component strain using one or more methods. Let $D(s | t, y)$ map strain s to antigenic distance.
- Every lab ($l = 1, \dots, L$) group that runs a heterologous data cohort study uses some subsample of these strains ($s = 1, \dots, S_l$) and measures titers for subjects $i = 1, \dots, N_l$.
- Then, the post-vaccination titers for that lab group are $y_{i,s} = f(D(s | t, y)) + g(\text{covariates}) + b_i + \text{error}$.

Multilevel modeling

- For now, we assume the effects of covariates are negligible and we assume the effect of distance is linear. Then our model is
- $y_{i,s} = b_0 + b_{0,i} + (b_1 + b_{1,i}) * D(s | t,y) + \text{error}_{i,s}$
- This is a **linear mixed effects model** and is estimable whenever we have at least two measurements per subject (per subtype per year).
- The outcome y has left-censored (below LoD) measurements and interval censored measurements, so we apply a likelihood correction to adjust for censoring.
- The effect of pre-vaccination titer is NOT negligible but might be ignorable for our purposes.

Approach 1: variance components

$$y_{ijk\dots} = \mathbf{X}\beta + b_{\text{subject}[i]} + b_{\text{lab}[j]} + b_{\text{vaccine}[k]} + \dots + \varepsilon_{ijk\dots}$$

$$b_{\text{whatever}} \sim \text{Normal}(0, \sigma^2_{\text{whatever}})$$

$$\varepsilon_{ijk\dots} \sim \text{Normal}(0, \sigma^2)$$

- Looks scary!

Approach 1: variance components

$$y_{ijk\dots} = \mathbf{X}\beta + b_{\text{subject}[i]} + b_{\text{lab}[j]} + b_{\text{vaccine}[k]} + \dots + \varepsilon_{ijk\dots}$$

$$b_{\text{whatever}} \sim \text{Normal}(0, \sigma^2_{\text{whatever}})$$

$$\varepsilon_{ijk\dots} \sim \text{Normal}(0, \sigma^2)$$

- Looks scary!
- But it's not too bad once you get past all the b's.

Approach 1: variance components

Fixed effects

(regular regression part)

$$y_{ijk\dots} = \boxed{X\beta} + b_{\text{subject}[i]} + b_{\text{lab}[j]} + b_{\text{vaccine}[k]} + \dots + \varepsilon_{ijk\dots}$$

$$b_{\text{whatever}} \sim \text{Normal}(0, \sigma^2_{\text{whatever}})$$

$$\varepsilon_{ijk\dots} \sim \text{Normal}(0, \sigma^2)$$

- Looks scary!
- But it's not too bad once you get past all the b's.

Approach 1: variance components

Random (intercept) effects
(cool and fun part)

$$y_{ijk\dots} = \mathbf{X}\beta + [b_{\text{subject}[i]} + b_{\text{lab}[j]} + b_{\text{vaccine}[k]}] + \dots + \varepsilon_{ijk\dots}$$

$$b_{\text{whatever}} \sim \text{Normal}(0, \sigma^2_{\text{whatever}})$$

$$\varepsilon_{ijk\dots} \sim \text{Normal}(0, \sigma^2)$$

- Looks scary!
- But it's not too bad once you get past all the b's.

Approach 1: variance components

$$y_{ijk\dots} = \mathbf{X}\beta + b_{\text{subject}[i]} + b_{\text{lab}[j]} + b_{\text{vaccine}[k]} + \dots + \boxed{\varepsilon_{ijk\dots}}$$

$$b_{\text{whatever}} \sim \text{Normal}(0, \sigma^2_{\text{whatever}})$$

$$\varepsilon_{ijk\dots} \sim \text{Normal}(0, \sigma^2)$$

**Residual unexplained variance
(the part that makes it statistics)**

Approach 1: variance components

$$y_{ijk\dots} = \mathbf{X}\beta + b_{\text{subject}[i]} + b_{\text{lab}[j]} + b_{\text{vaccine}[k]} + \dots + \varepsilon_{ijk\dots}$$

$$b_{\text{whatever}} \sim \text{Normal}(0, \sigma^2_{\text{whatever}})$$

$$\varepsilon_{ijk\dots} \sim \text{Normal}(0, \sigma^2)$$

All those (subjects/labs/whatevers) have some effect...

- And it changes for each one
- And we only have a subset of the possible subjects/labs/whatevers

Approach 1: variance components

$$y_{ijk\dots} = \mathbf{X}\beta + b_{\text{subject}[i]} + b_{\text{lab}[j]} + b_{\text{vaccine}[k]} + \dots + \varepsilon_{ijk\dots}$$

$$b_{\text{whatever}} \sim \text{Normal}(0, \sigma^2_{\text{whatever}})$$

$$\varepsilon_{ijk\dots} \sim \text{Normal}(0, \sigma^2)$$

Sources of variation add together!

Approach 1: variance components

$$y_{ijk\dots} = \mathbf{X}\beta + b_{\text{subject}[i]} + b_{\text{lab}[j]} + b_{\text{vaccine}[k]} + \dots + \varepsilon_{ijk\dots}$$

$$b_{\text{whatever}} \sim \text{Normal}(0, \sigma^2_{\text{whatever}})$$

$$\varepsilon_{ijk\dots} \sim \text{Normal}(0, \sigma^2)$$

Sources of variation add together!

$$\text{Total variation in } y|x = \sigma^2 + \sum_{\text{whatever}} \sigma^2_{\text{whatever}}$$