Food Allergen Analysis for a Risk-Based Future: Where do we need to go and how do we get there?

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A Risk-Based Future for Food Allergens: Are Analytical Methods Ready?

- On the verge of a major transition for food allergen management

- Zero tolerance → decisions from quantitative data

Are quantitative methods available?
Are methods sensitive enough?

Transitioning Analytical Functions for a Risk-Based Future

Zero-Tolerance Mindset

Is there detectable allergen in this product?

Is there a quantifiable amount of allergen in this product?

Qualitative use of Quantitative Methods

Risk-Based Mindset

Is the amount of allergen in this product above or below a given action level?

How much allergen is in this product?

Quantitative use of Quantitative Methods

Is the amount of allergen in a product above or below an action limit?	How much of an allergen is present in a product?
↓	↓
Is the method sensitive enough to detect the allergen source in the product?	Can the method accurately quantify the amount of allergen source present in the product?
↓	↓
What is the likelihood of making the wrong decision?	What is the likelihood of providing an incorrect result?

Core Method Performance Characteristics

Underrated?

Overrated?

Method Limits

Sampling

Confidence

Recovery

Precision

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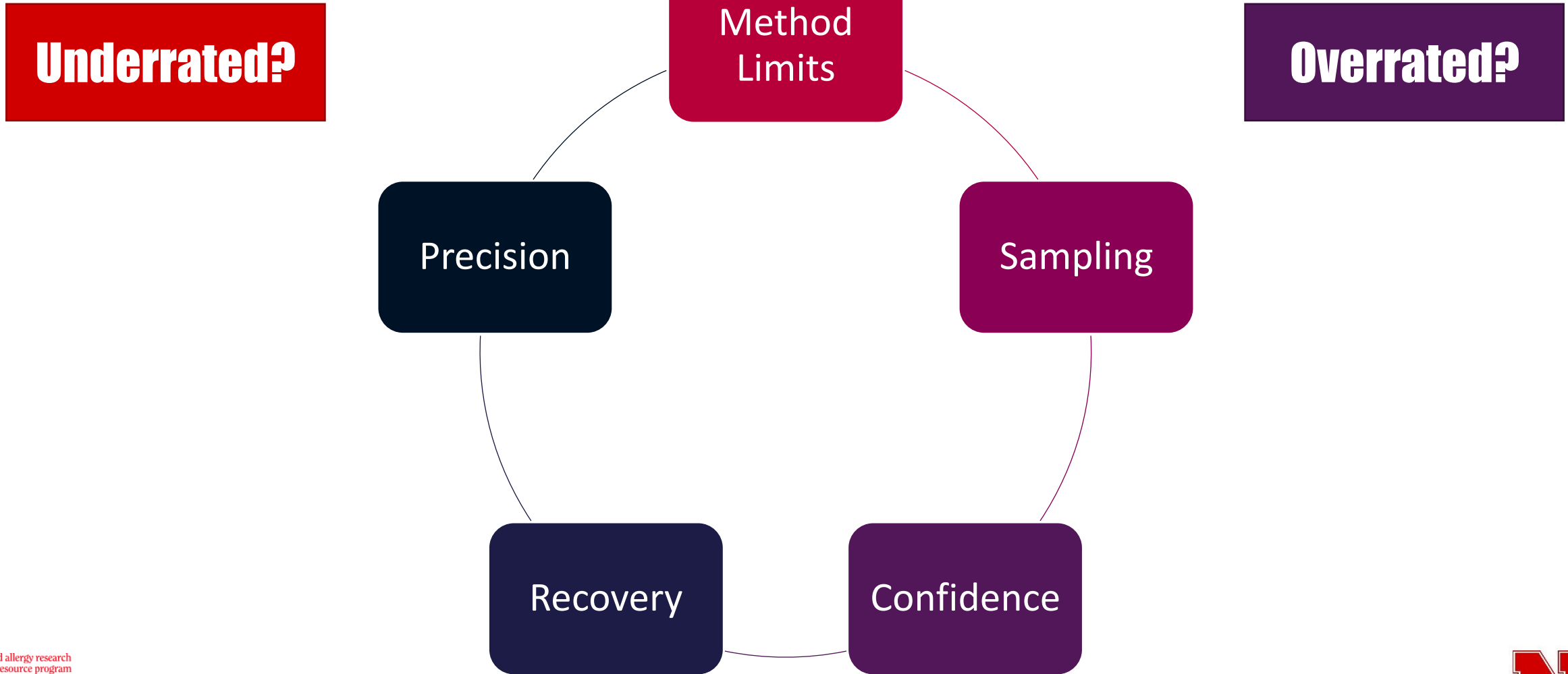
Method Limits: Are methods sensitive enough?
Need to align statistical concepts with end user expectations

Statistical Concepts	Current: **Overrated**	User Expectations

What is the concentration below which a method cannot distinguish a true positive from a true blank?

LOD ≠

What is the lowest concentration at which the allergen will be detected?

Below what concentration does precision of quantification become unacceptable?

LOQ ≠

What is the lowest point on the calibration curve?

Recovery

Underrated

Diversity circle with surrounding items:
- Baked Goods
- Meat
- Chocolate
- Candy? Supplements?
- Retorted
- Extruded
- Fermented
- Spices, Colors…

- Affects:
  - Accuracy
  - Method Limits

- Not included in traditional limit estimation methods
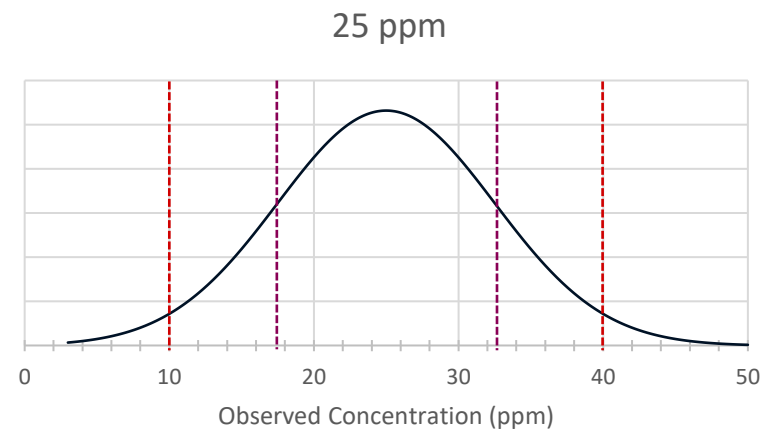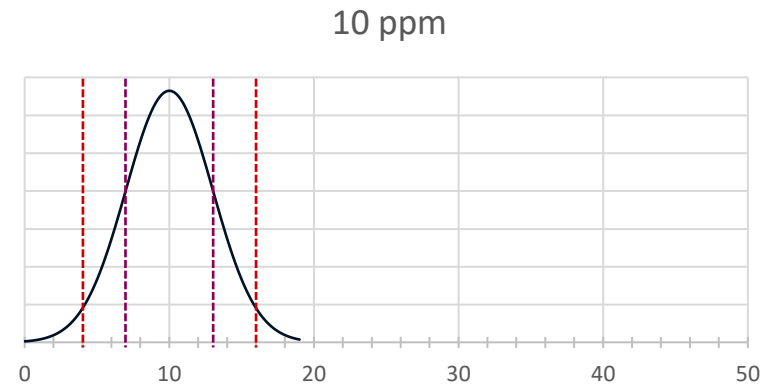
Precision

Underrated

- Repeatability
- Reproducibility
- Intermediate Precision
- Relative Standard Deviation:
  - Expectation
 - Relationship with concentration
- Affects:
  - Method Limits
  - Confidence
  - Sampling

30% RSD

5 ppm

10 ppm

25 ppm

Observed Concentration (ppm)
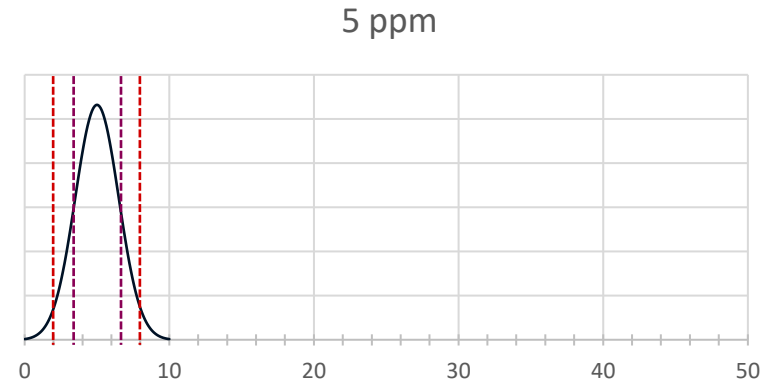
Learn Lessons from Others:
Mind the ~~Gap~~

- Precision observed with test kit calibrants vs. food materials
- Laboratory sample homogeneity
- Test portion size

- Sampling   **Underrated**

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Gluten in Oats
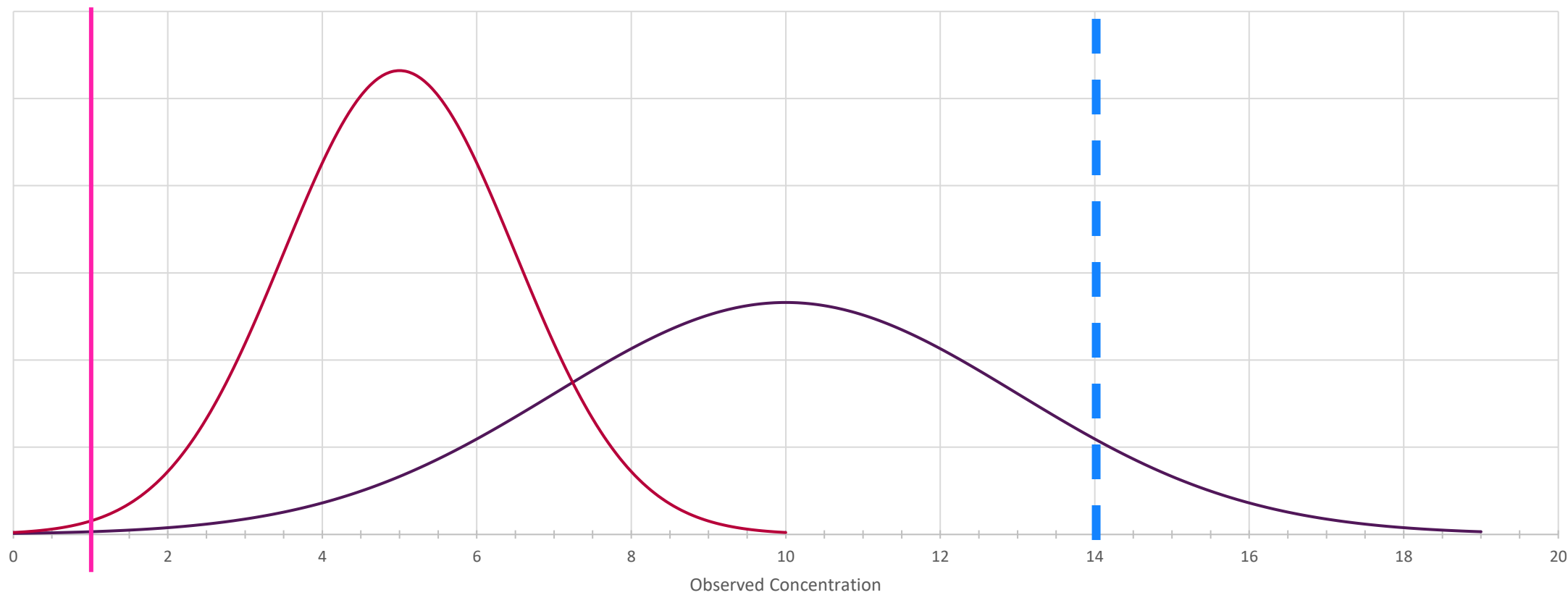
- Fritz et al. 2017 data
- Repeated test portions from market samples with initial results between 5-20 ppm
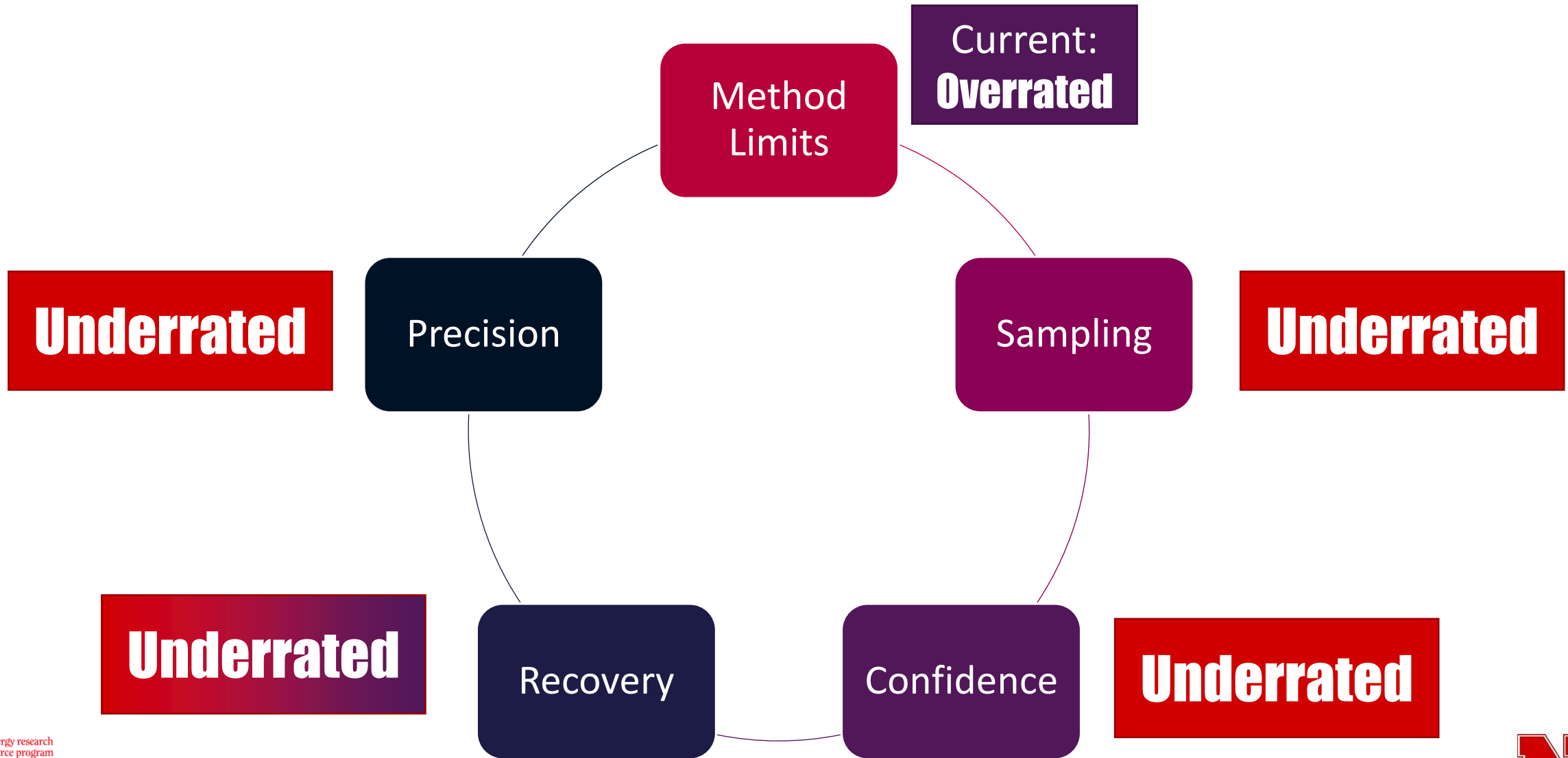- %CV: 33-256%

Precision and Decisions

Observed Concentration (ppm)
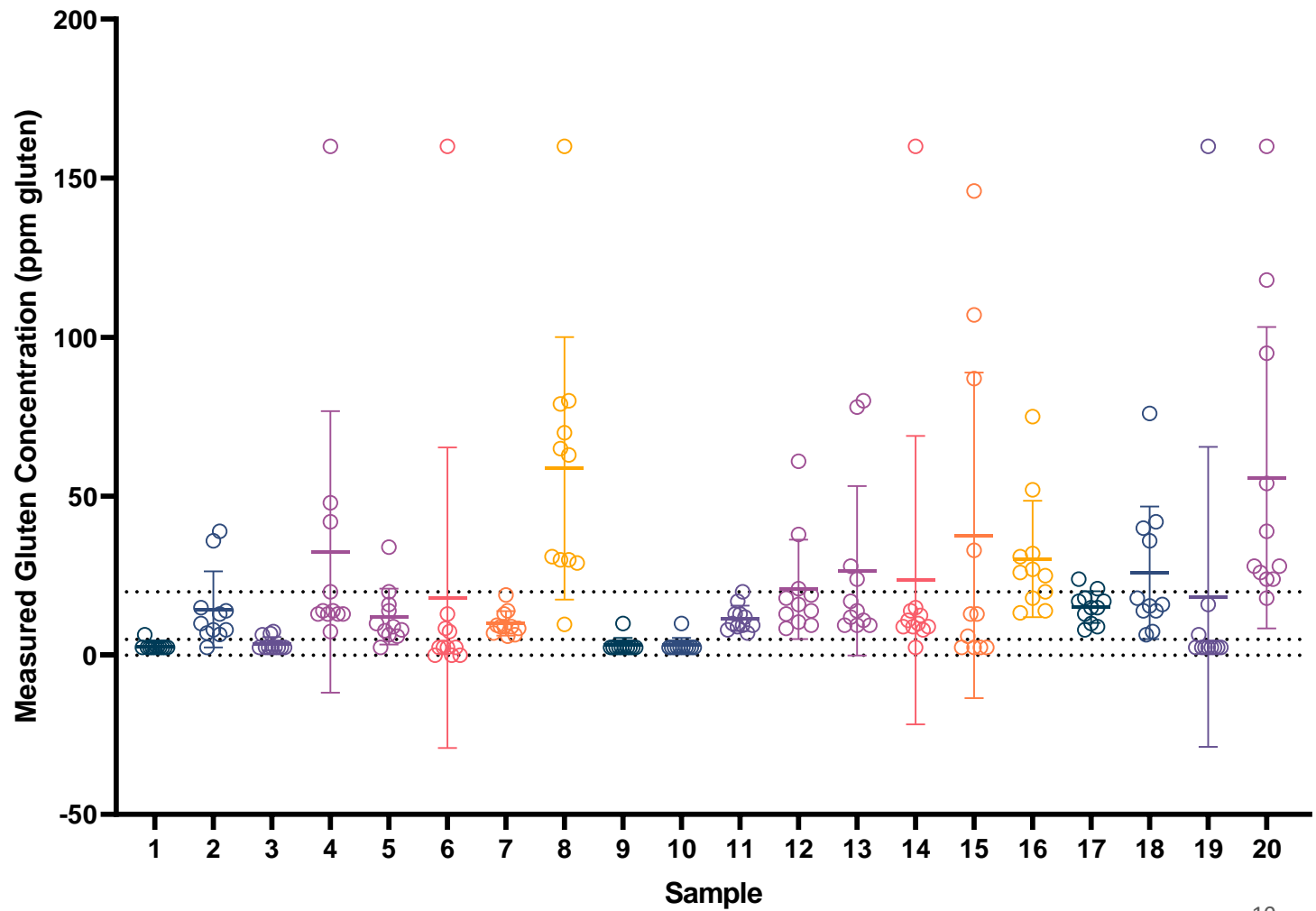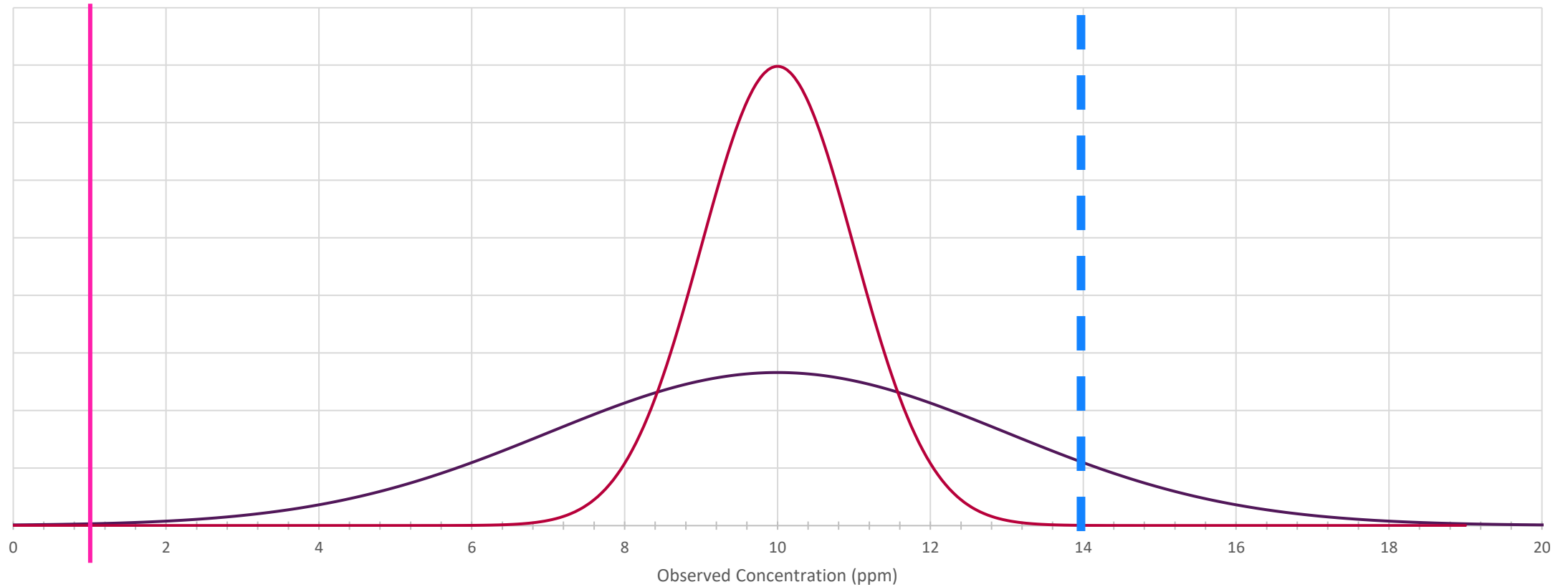
Precision and Decisions... and Recovery?

Observed Concentration

Confidence

How certain do we want to be that a product contains allergen at a concentration above/below an action level?

Core Method Performance Characteristics

14

Will Results be Comparable?

Progress In Progress

- Consistent requirements for reporting units:
  - mg total protein from the allergenic source/ kg food matrix (ppm total protein)
- Path towards collaborative studies
 - Different laboratories, same method

Comparison Challenges

- Same laboratory, different method
- Different laboratories, different methods
- Matrix diversity

Mass Spectrometry to the Rescue?

MS Quantification of Milk Protein in Cookie Matrices

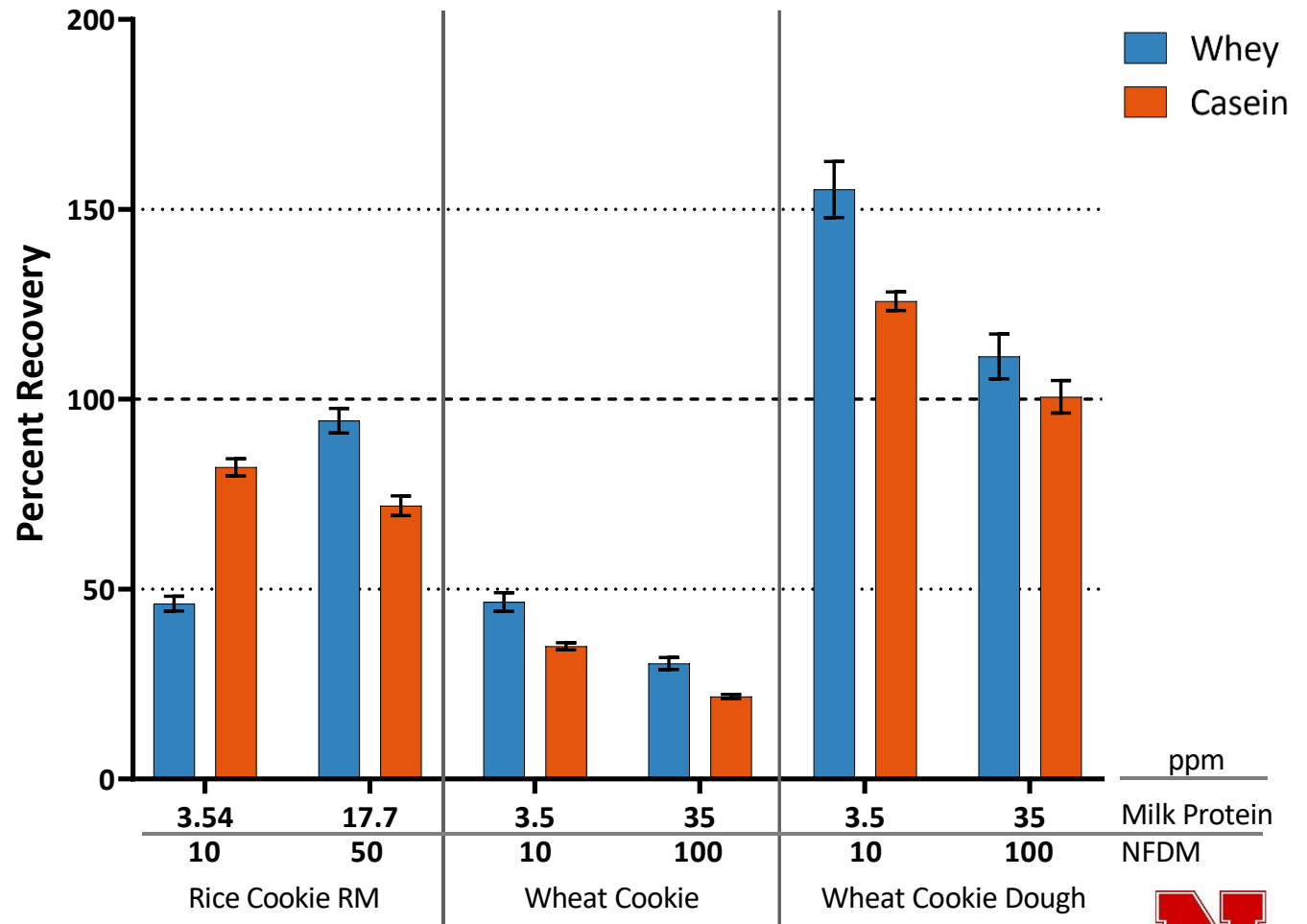NFDM External, Matrix-Independent Standard Curve with Internal Standards

Legend: LIV, VLV, LSF, VLP, AVP, GPF

Axes: Log$_{10}$ response (L/H) vs Log10 conc (ppm)
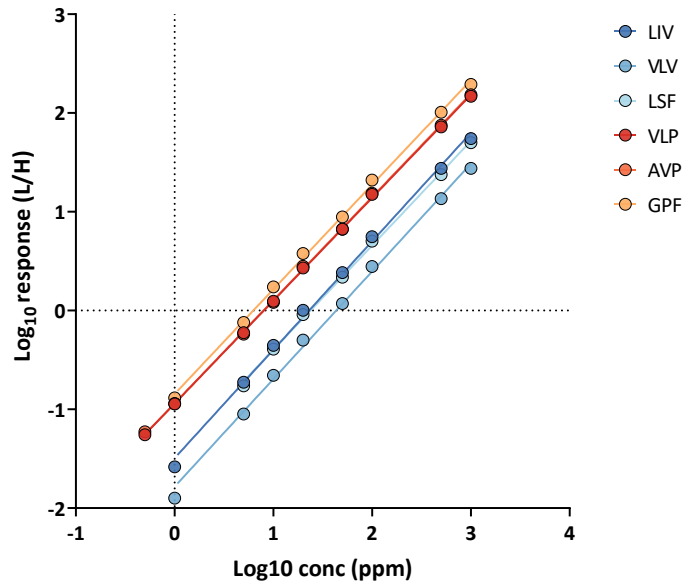
Incurred Cookie Matrix

Dough incurred with 1, 10, 100 ppm NFDM → 10 min, 400 °F → Cookies incurred with 1, 10, 100 ppm NFDM

Cookies

Legend: Whey (blue), Casein (orange)

Y-axis: Percent Recovery

	Rice Cookie RM		Wheat Cookie		Wheat Cookie Dough	
Milk Protein	3.54	17.7	3.5	35	3.5	35
NFDM	10	50	10	100	10	100

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Quantification of Incurred Milk Protein

| NFDM Concentration	Sample Type	ELISA 1: Total Milk	ELISA 3: BLG	ELISA 4: Casein	MS-PRM*
10 ppm	Cookie	BLQ	BLQ	15.0%	35.0% (Casein) **46.3% (Whey)**
	Dough	101.5%	BLQ	83.0%	125.8% (Casein) **155.2% (Whey)**
100 ppm	Cookie	7.4%	BLQ	-	21.7% (Casein) **30.4% (Whey)**
	Dough	85.5%	42.7%	-	95.8% (Casein) **111.3% (Whey)**

Milk Quantification with Matrix-Independent Calibration

Method capable of detecting and quantifying whey and casein peptides in a variety of processed matrices
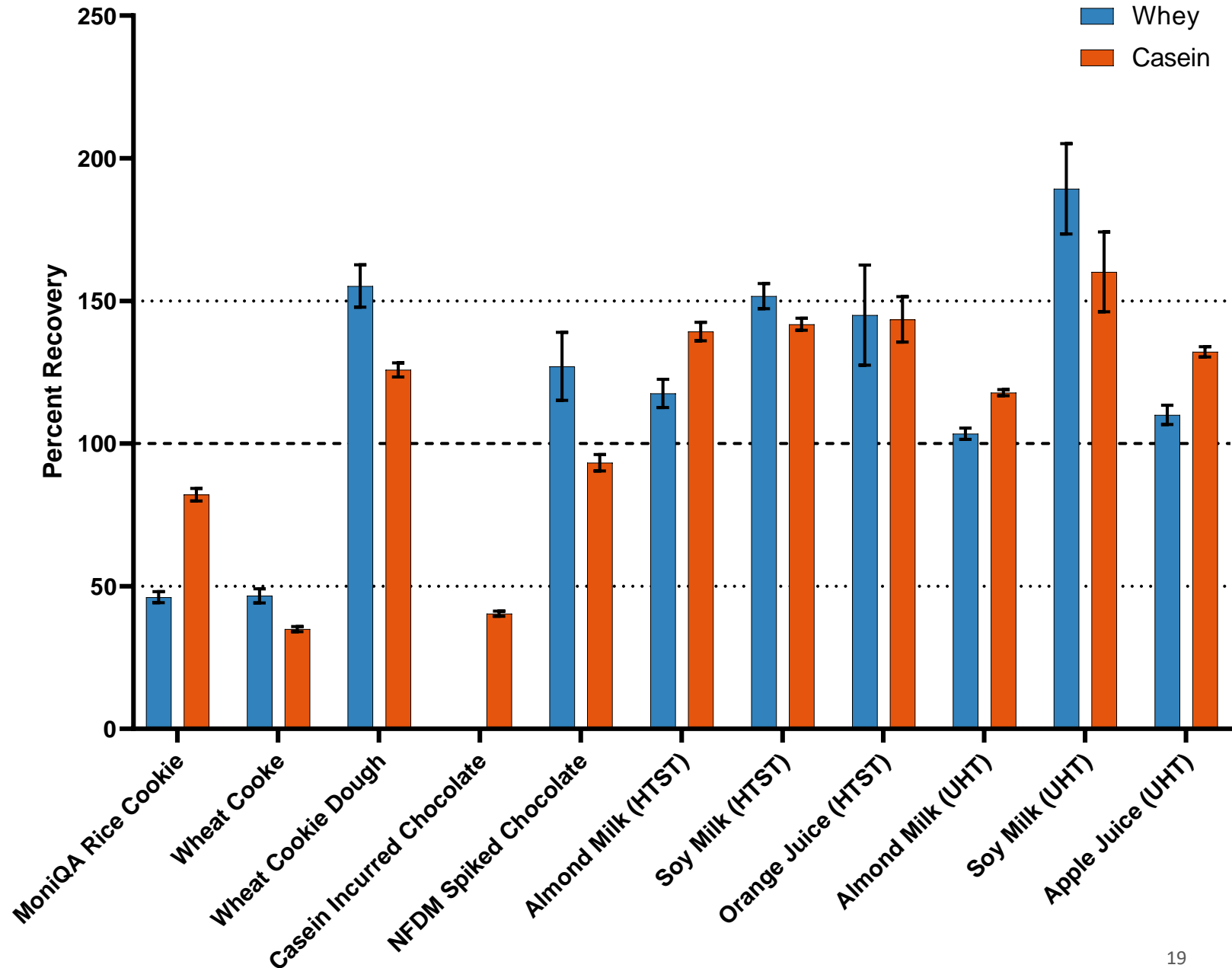
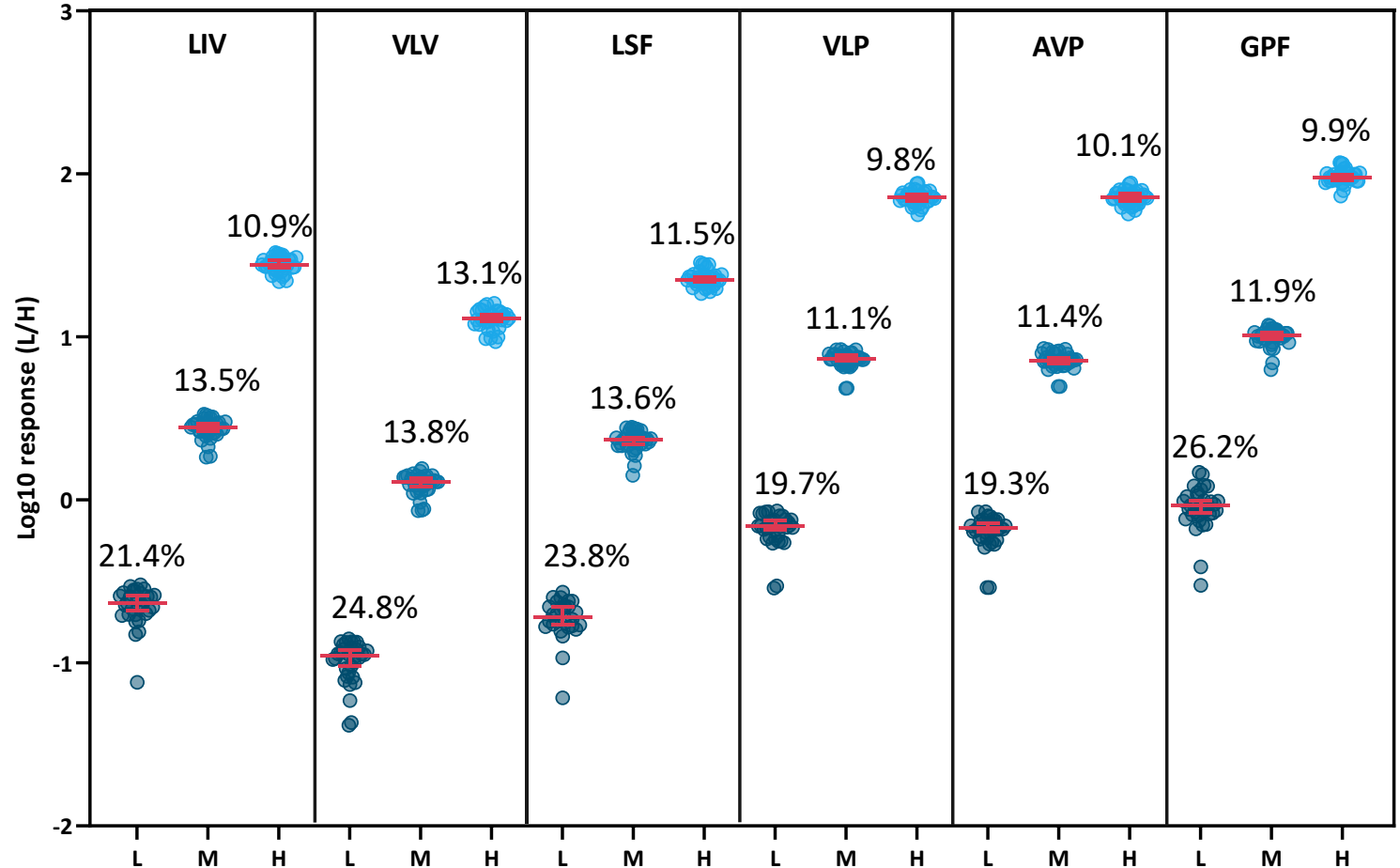# Can methods be run by multiple users?

- Calibration curves
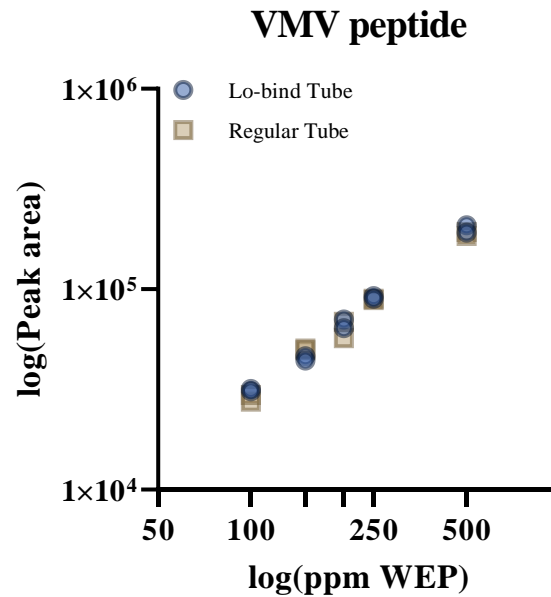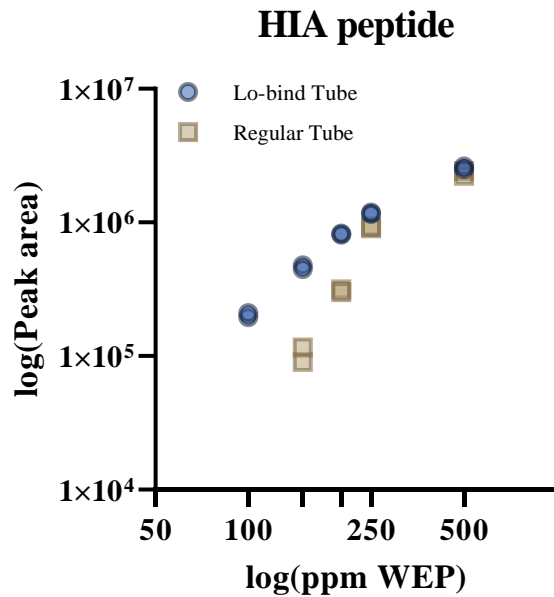- Reference materials
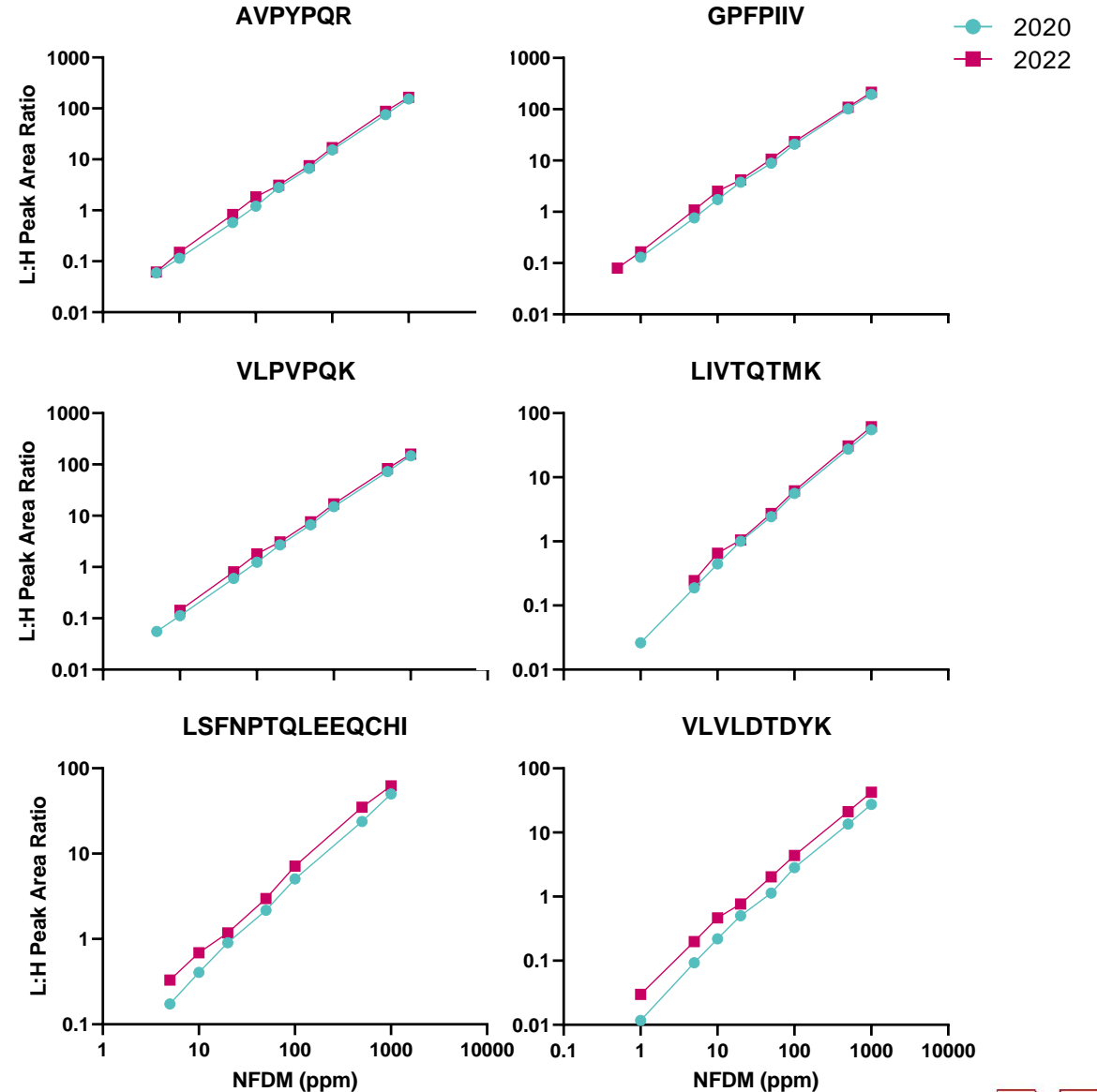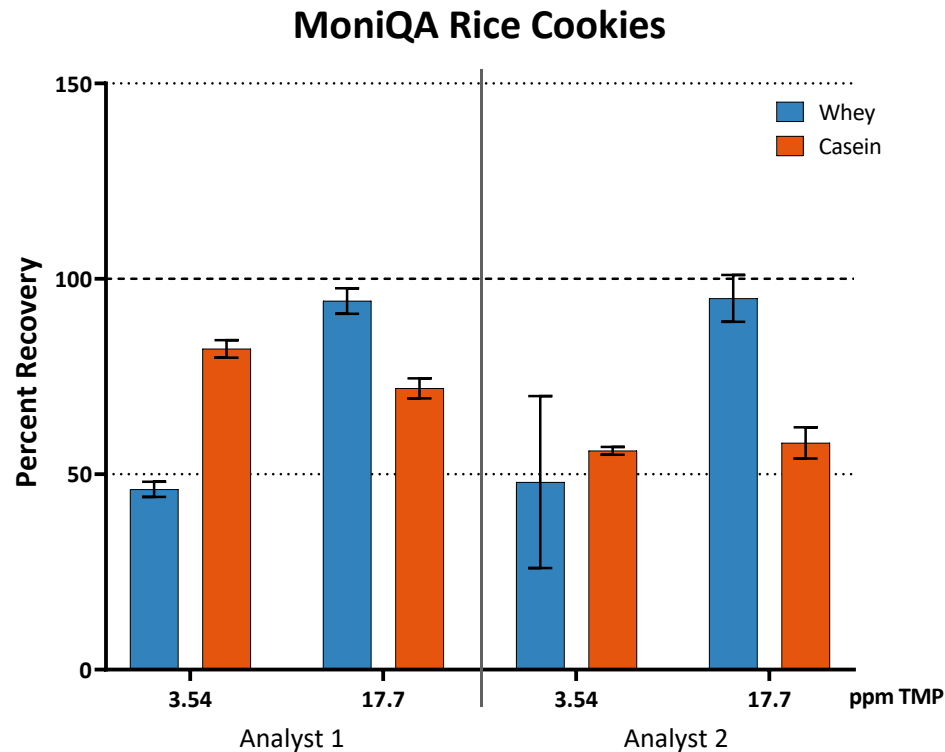
Are methods stable over time?

QC calibration runs:

- Low: 1.75 ppm TMP
- Mid: 17.5 ppm TMP
- High: 175 ppm TMP
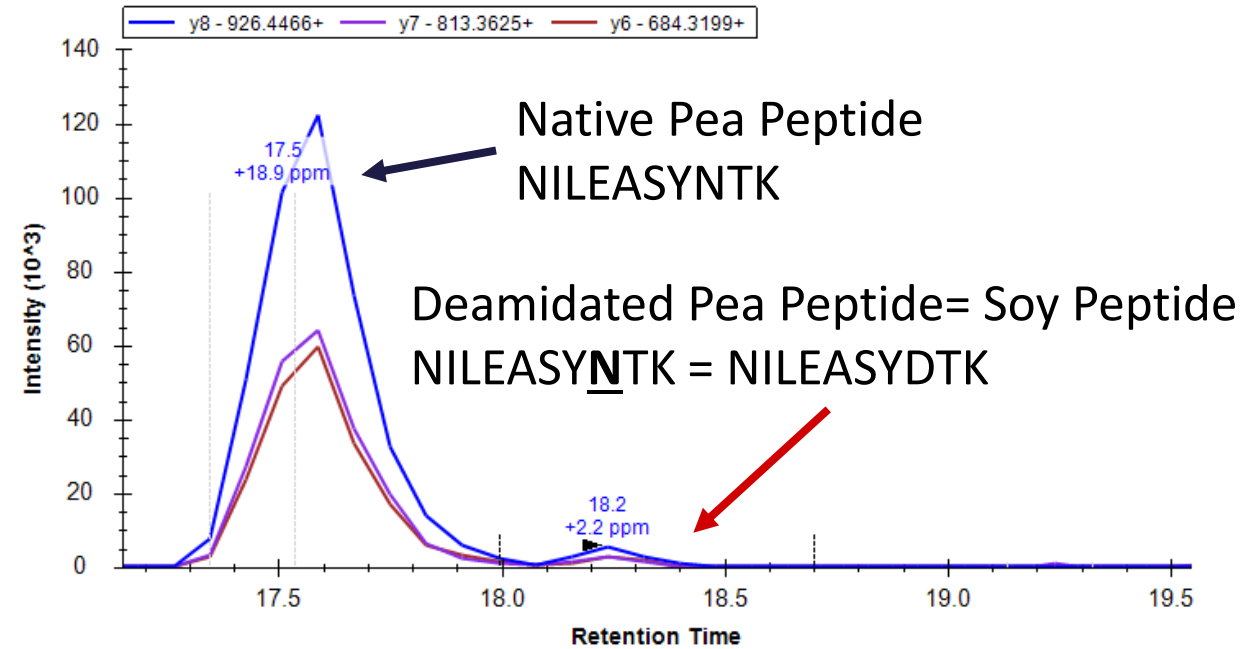- 1-year time period
- n = 34

But...

Effects of Plasticware:
Egg Method Development

Specificity Challenges:
Soy Detection in Pea Milk

Native Pea Peptide
NILEASYNTK

Deamidated Pea Peptide= Soy Peptide
NILEASY**N**TK = NILEASYDTK

Mass Spectrometry Needs

- Understanding Matrix and Processing Effects
- Specificity and Sensitivity Measures
- Comparability of Methods and Results
- Multi-laboratory Studies

Food Allergen Analysis Method Needs

- Clear consensus on method performance characteristics
  - Parameter definitions
  - Estimation procedures
  - Needed performance

- More published data on method performance in foods

- Interlaboratory Method Evaluations & Best Practices

Thank you!
Questions?

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