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Laboratory Manager
Bia Diagnostics

THE 2016 GLUTEN-FREE STAKEHOLDER UPDATE & PLANNING SESSION PRESENTED IN COOPERATION WITH:

Agriculture and Agri-Food Canada

Canadian Grain Commission

Growing Forward 2

Prairie Oat Growers Association

Pulse Canada

COMPLEXITY OF GLUTEN ANALYSIS IN DIVERSE MATRIXES

Luke Emerson-Mason, Laboratory Manager, Bia Diagnostics
9/27/2016

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Bia Diagnostics

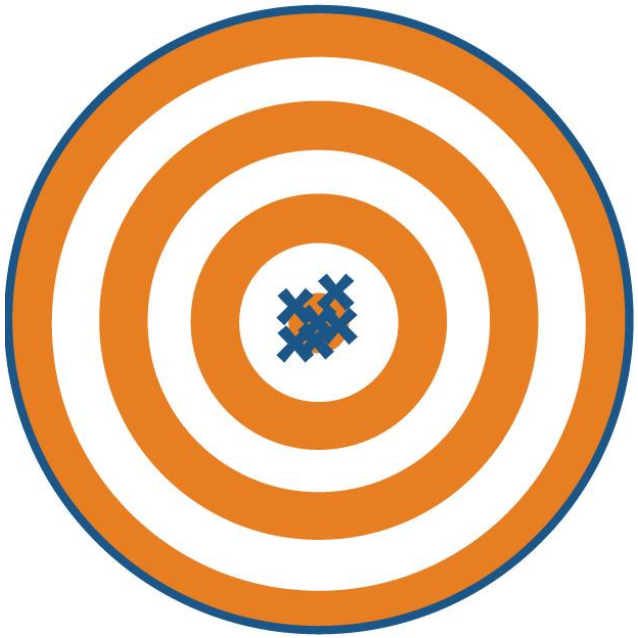
Leaders in Food Allergen Analysis

ISO 17025 Accredited allergen testing lab
Analyzing 50+ samples/day for Gluten
Same day analysis standard

PRIMARY GOAL - PRODUCE RESULTS THAT ARE BOTH PRECISE AND ACCURATE

- Critical points to achieve this are :

- Sampling
- Extraction
- Method of Analysis



SAMPLING- BEST WAY TO OBTAIN, HOMOGENIZE, AND PREPARE THE SAMPLE

- Approximately 1 wheat seed in 500-9,000 seeds of otherwise gluten free grain can produce ~30ppm of gluten contamination!
- Needle in the Haystack problem; How do we find 1 gram of wheat in ~10,000g's of gluten free grain?

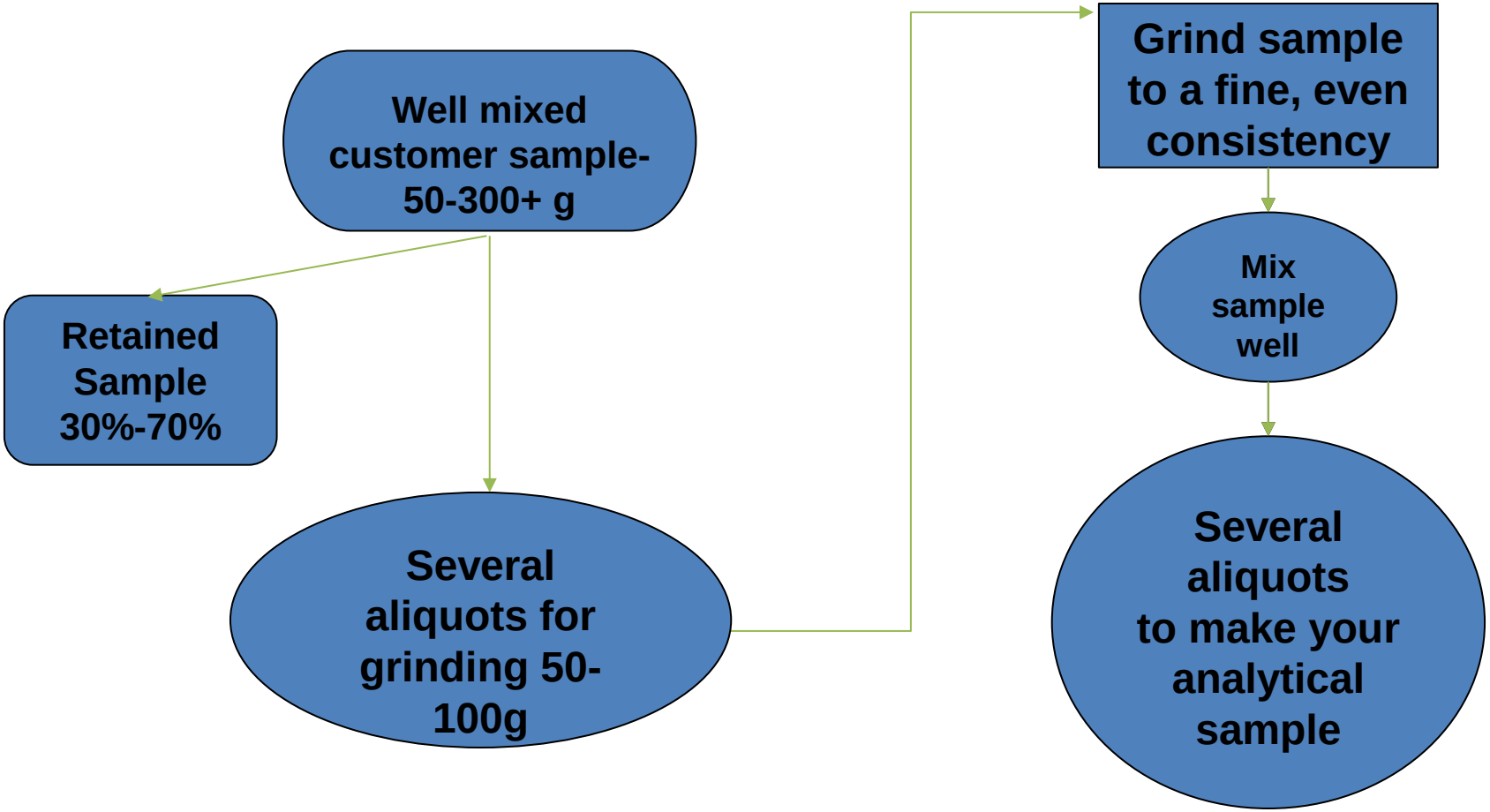
SAMPLING- BEST WAY TO OBTAIN, HOMOGENIZE, AND PREPARE THE SAMPLE

- Decide on the best way to homogenize the sample
  - Liquid – Mix well
  - Semi-solid – Stomacher or Food Processor
  - Solid – Grind to a fine powder – Mill, Coffee Grinder or Food Processor

FACTORS EFFECTING THE EFFICIENCY OF HOMOGENIZING YOUR SAMPLE

- Wheat, Rye and Barley might have higher or lower density than the sample matrix, causing sample stratification
- Particle size – how uniform are the particle sizes after homogenization
- Processing of foods
  - Moisture, gums, starches, polyphenols, tannins, flavonoids, etc.
  - Heating, extruding, boiling, frying and baking

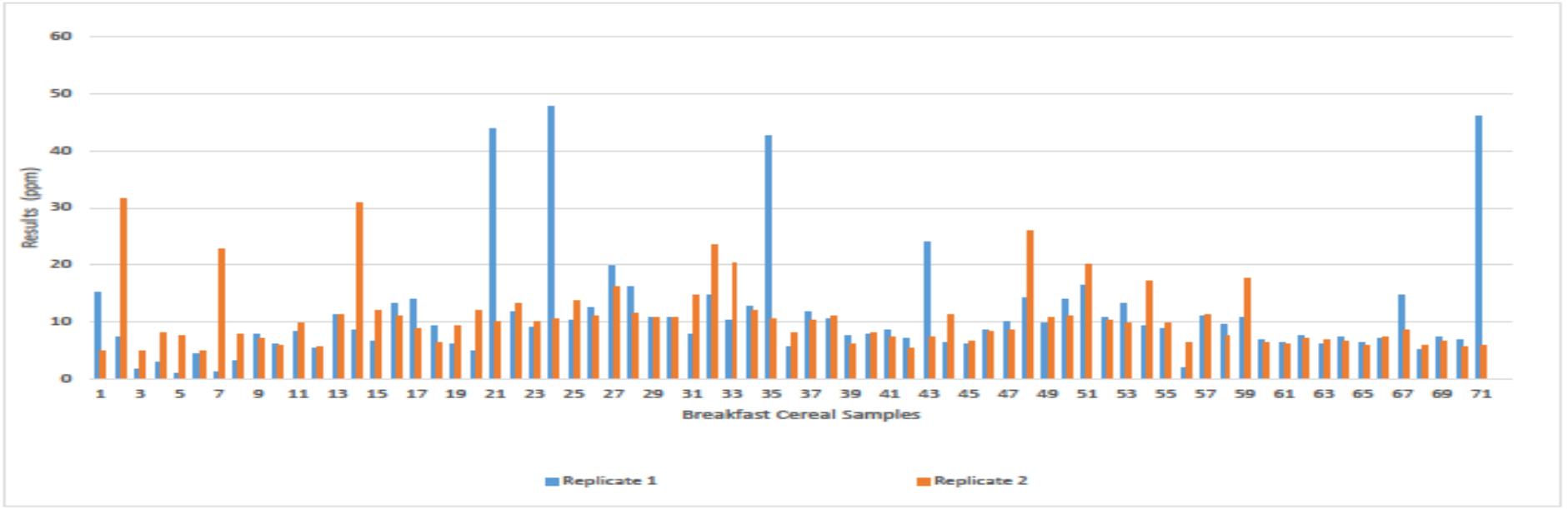
SAMPLE PREP FLOWCHART



VARIANCE IN SAMPLING– CASE STUDY #1

- Cereal from customer analyzed by Bia Diagnostics
- 71 separate samples arrived pre-ground
- Each sample was reground and homogenized and extracted in duplicate with Cocktail solution (0.25g sample sizes)
- Each extract was tested by the Gluten R7001 AOAC-OMA 2012.01 ELISA method manufactured by R-Biopharm
- Some “homogenized” samples had as much as 40ppm variance between extracts

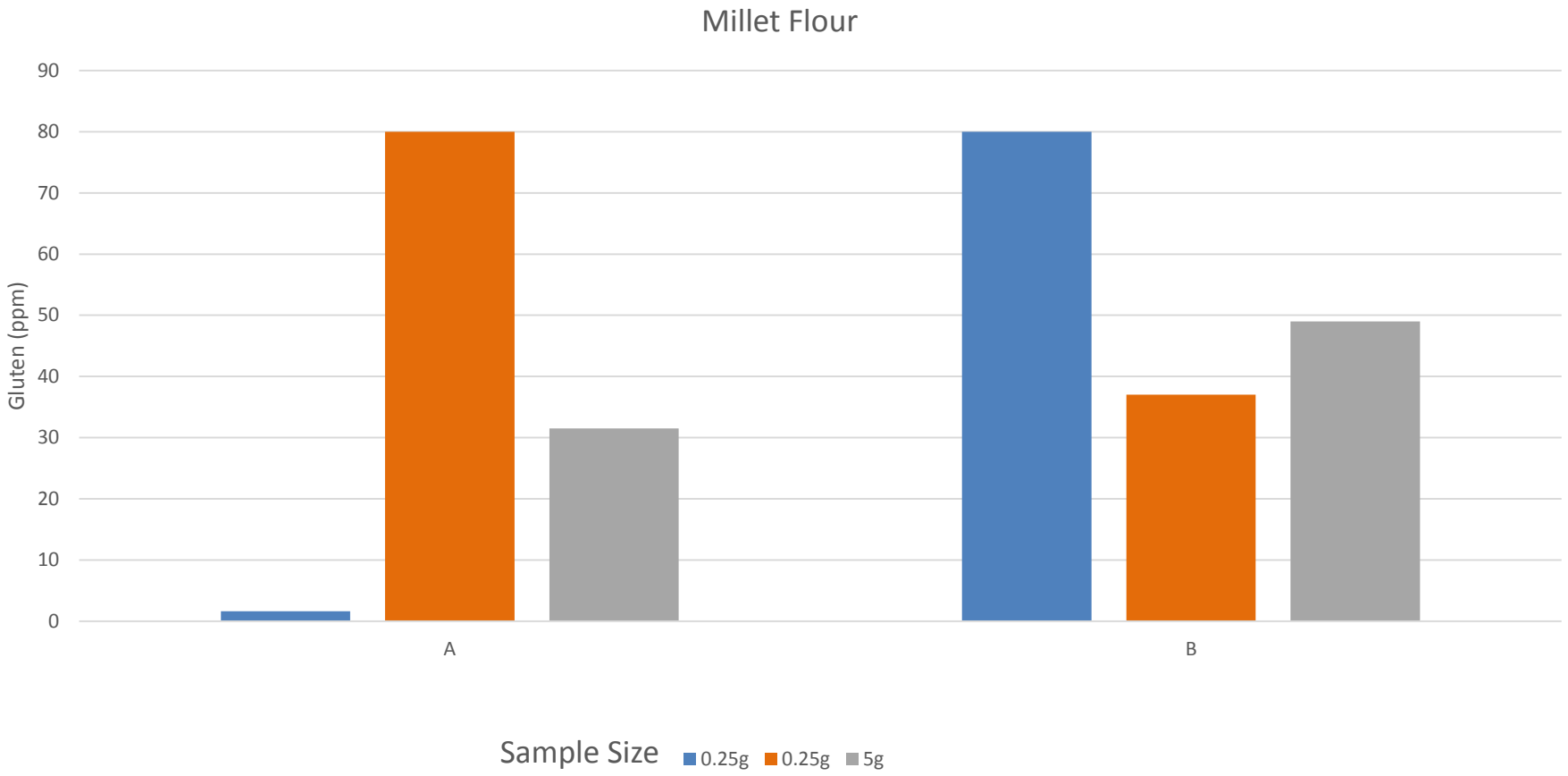
VARIANCE IN SAMPLING CASE STUDY #1- 71 HETEROGENEOUS CEREAL SAMPLES



VARIANCE IN SAMPLING– CASE STUDY #2

- Millet Flour Sample from Customer Analyzed by Bia Diagnostics
- Two sample portions were homogenized, A and B (100g each)
  - Each sample portion was extracted in duplicate with Cocktail solution (0.25g sample sizes)
  - Each extract was tested by the Gluten R7001 AOAC-OMA 2012.01
- Portion A extracts (0.25g) had results of <5ppm, >80ppm
- Portion B extracts (0.25g) had results of >80ppm, 37.5ppm
- 5 gram extracts of A and B were then analyzed
- Portion A (5g) had a result of 31.5ppm
- Portion B (5g) had a result of 49ppm

VARIANCE IN SAMPLING– CASE STUDY #2



EXTRACTION/TEST METHODS

- R-biopharm Gluten R7001 OMA 2012.01
  - Sandwich ELISA Utilizing R-5 Monoclonal Antibody
  - Cocktail (patented) extraction solution, 0.25g sample size
  - R-biopharm recommends 1g sample size for Oats
  - Nonfat Dry Milk additive recommended for polyphenol and tannin containing samples (chocolate, spices) as a blocking agent
  - Calibrated to Prolamin Working Group Gliadin standard
- Alternative extraction methods
  - 60% Ethanol
  - “GEB”, 60% Ethanol + Fish Gelatin and PVP
  - Scaled-up sample sizes (0.5-5g)

OTHER GLUTEN ELISA METHODS

- R-biopharm Gluten Competitive R7021
  - Competitive Assay Utilizing R5 Monoclonal Antibody
  - 60% ethanol extraction (+ Fish Gelatin for beer/ fermented products)
  - Detects hydrolyzed gluten proteins
  - Calibrated to a “Hydrolysate mixture of wheat, rye and barley”

OTHER GLUTEN ELISA METHODS

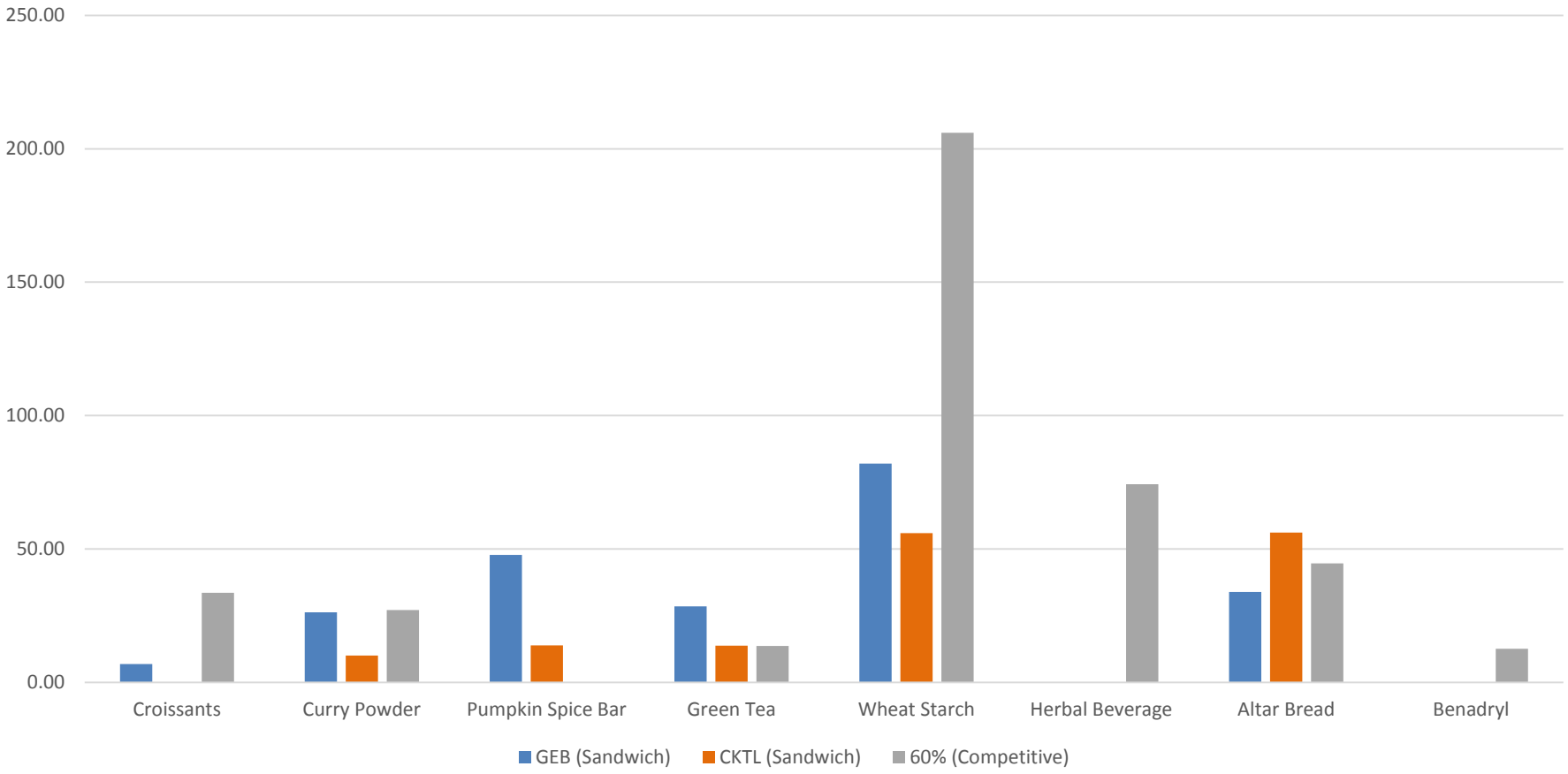
- Romer Agraquant Gluten, G12 antibody
- ELISA Systems Gliadin, Skerritt antibody
- Neogen Veratox Gluten, Proprietary antibody
- Morinaga Wheat Protein, Proprietary antibody
- Elution Technologies Gluten, Proprietary antibody

COMPARING METHODS – CASE STUDY

- Complex matrixes submitted to Bia Diagnostics for Gluten testing by a watchdog group
- Each matrix tested in duplicate with R7001 with Cocktail and GEB extractions, as well as R7021 with 60% ethanol extraction (BLD = Below Limit of Detection)

Sample Matrix	GEB (Sandwich)	CKTL (Sandwich)	60% (Competitive)
Croissants	6.85	BLD	33.60
Curry Powder	26.31	10.10	27.15
Pumpkin Spice Bar	47.82	13.86	BLD
Green Tea	28.52	13.76	13.64
Wheat Starch	82.00	55.94	206.00
Herbal Beverage	BLD	BLD	74.31
Altar Bread	33.87	56.21	44.66
Benadryl	BLD	BLD	12.60

COMPARING METHODS – CASE STUDY



INTERFERENCE

- False Negatives
- Non-Specific Binding
- How to rule these out?
  - +/- Incurred Matrixes - Impractical
  - Spike Recoveries
  - Linear Regression
  - Heat Deactivation (Enzymes)

INTERFERENCE – CASE STUDIES

- Spike Recoveries – Protein Bars

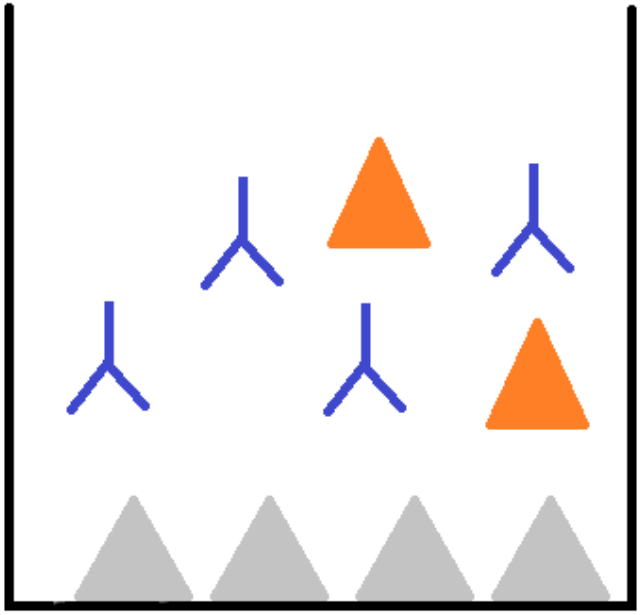
Sample	Result - Unspiked	Result - spike with 27ppm Gluten	% Recovery
Nutrition Bar 1	<5ppm	27.1ppm	100
Nutrition Bar 2	<5ppm	26.4ppm	98
Nutrition Bar 3	<5ppm	26.3ppm	97

- Non-Specific Binding - Millet

Sample	Extra Dilution	Result	Calculated Concentration
Millet	N/A	20ppm	20ppm
Millet	1/2	15ppm	30ppm
Millet	1/4	10ppm	40ppm
Millet+NFDM	N/A	<5ppm	<5ppm

ENZYME INTERFERENCE – CASE STUDY

- Probiotic Pills – Initially Positive ~80ppm on Competitive Gluten
- Suspected enzyme activity could interfere with Competitive Assay



ENZYME INTERFERENCE – CASE STUDY

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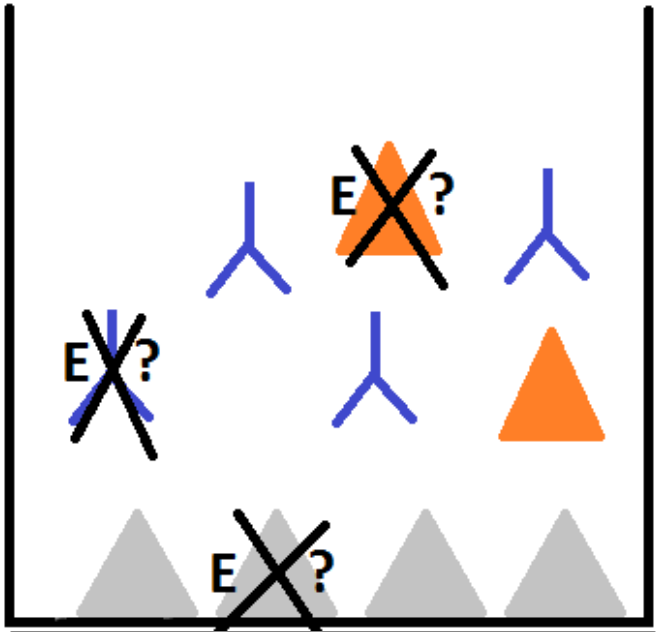


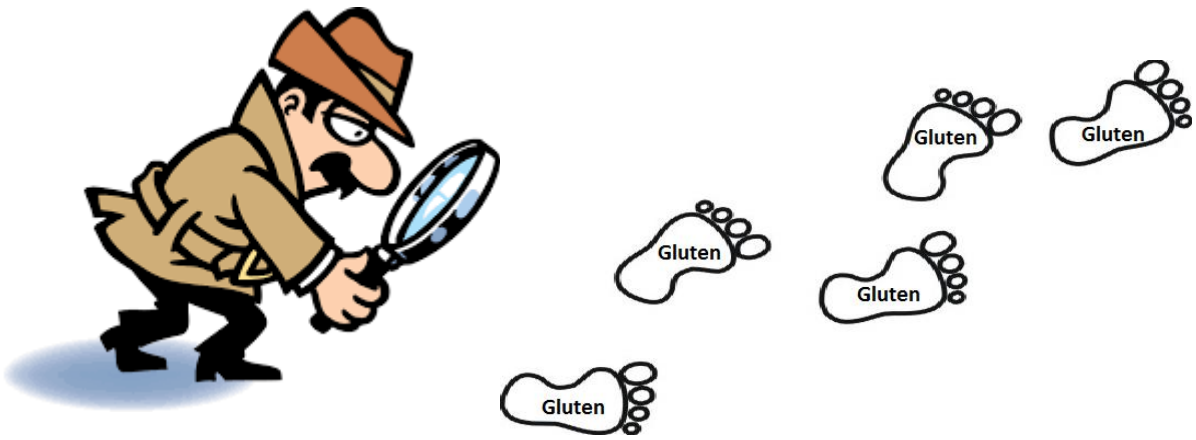
ENZYME INTERFERENCE – CASE STUDY

- Spiked the sample, then heated 25 mins @ 100C to deactivate enzymes
- After HD, sample had a result BLD with 67% spike recovery

Sample	Result (ppm)
Probiotics	80.5
Probiotics w/ HD	BLD
Probiotics w/ HD, spiked	22.2
Spike w/ HD	32.9

CHALLENGES

- No Perfect Method – ELISA, LFD, PCR, LCMS
- Need for Reference Materials and Clinically Relevant Standards
- Analysis is only as good as the sampling and extraction procedures



BEST WAY TO TEST?

- Representative sampling
  - Ingredients
  - Beginning, Middle, End of production run
- Large analytical sample size (5+ grams)
  - Expensive
- Incurred matrices as positive controls
  - Impractical

THANK YOU!

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