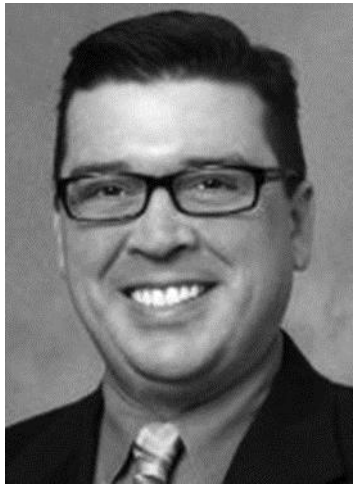

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THE 2016 GLUTEN-FREE STAKEHOLDER UPDATE & PLANNING SESSION PRESENTED IN COOPERATION WITH:

Agriculture and Agri-Food Canada

Canadian Grain Commission

Growing Forward 2

Prairie Oat Growers Association

Pulse Canada

CAPABILITIES AND CHALLENGES FOR DETECTION OF GLUTEN BY IMMUNOASSAY

Scott Radcliffe, Technical Support Scientist, Romer Labs

Sept. 27, 2016

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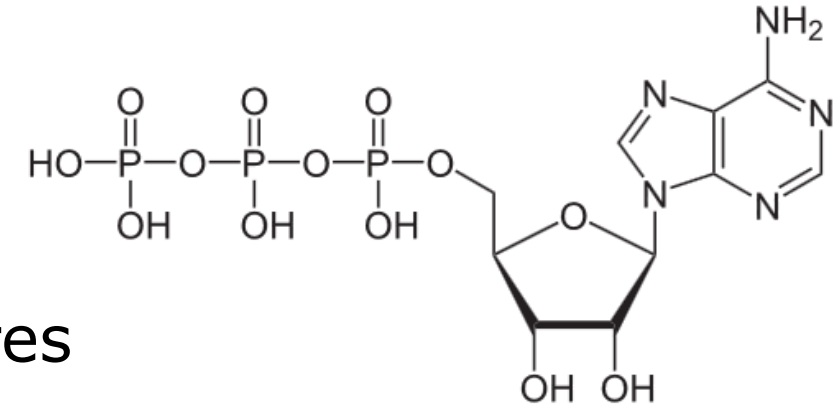
ALLERGEN DETECTION METHODS

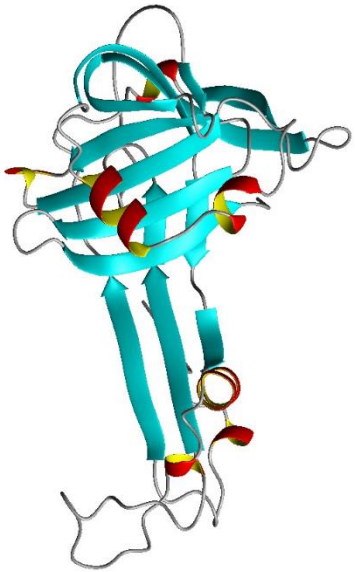
ATP SWAB

- Energy transport molecule found in all living cells
- Rapid & inexpensive, requires luminometer
- Result correlates surface cleanliness
- ATP is not a protein or allergen
- *Negative ATP test ≠ Negative for allergen protein*



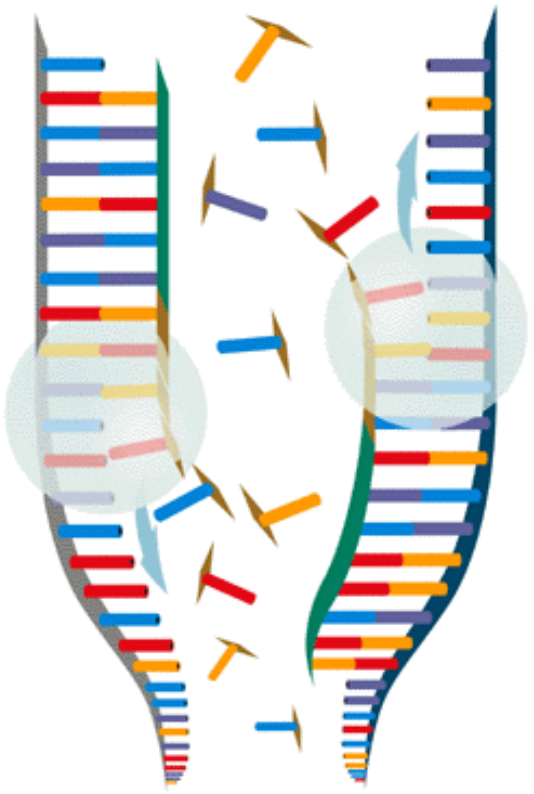
PROTEIN SWAB

- Rapid, inexpensive
- Chemical rxn with protein residue causes color change from green to purple
- Since allergens are protein, better indicator allergens removed than ATP
- Non-specific, less sensitive than allergen immunoassays



POLYMERASE CHAIN REACTION (PCR)

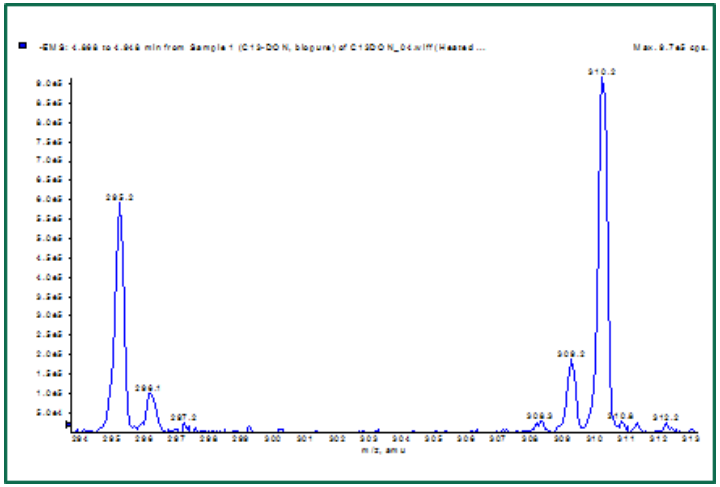
- Highly allergen-specific, highly sensitive
- Detects presence of DNA coding for allergen proteins
- Costly equipment, specialized, longer protocol
- *Does not directly detect allergenic proteins*
  - *some processing results in DNA↓, protein↑*



LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

- Highly specific, extremely sensitive, quantitative
- Detects peptide fragments of allergenic proteins

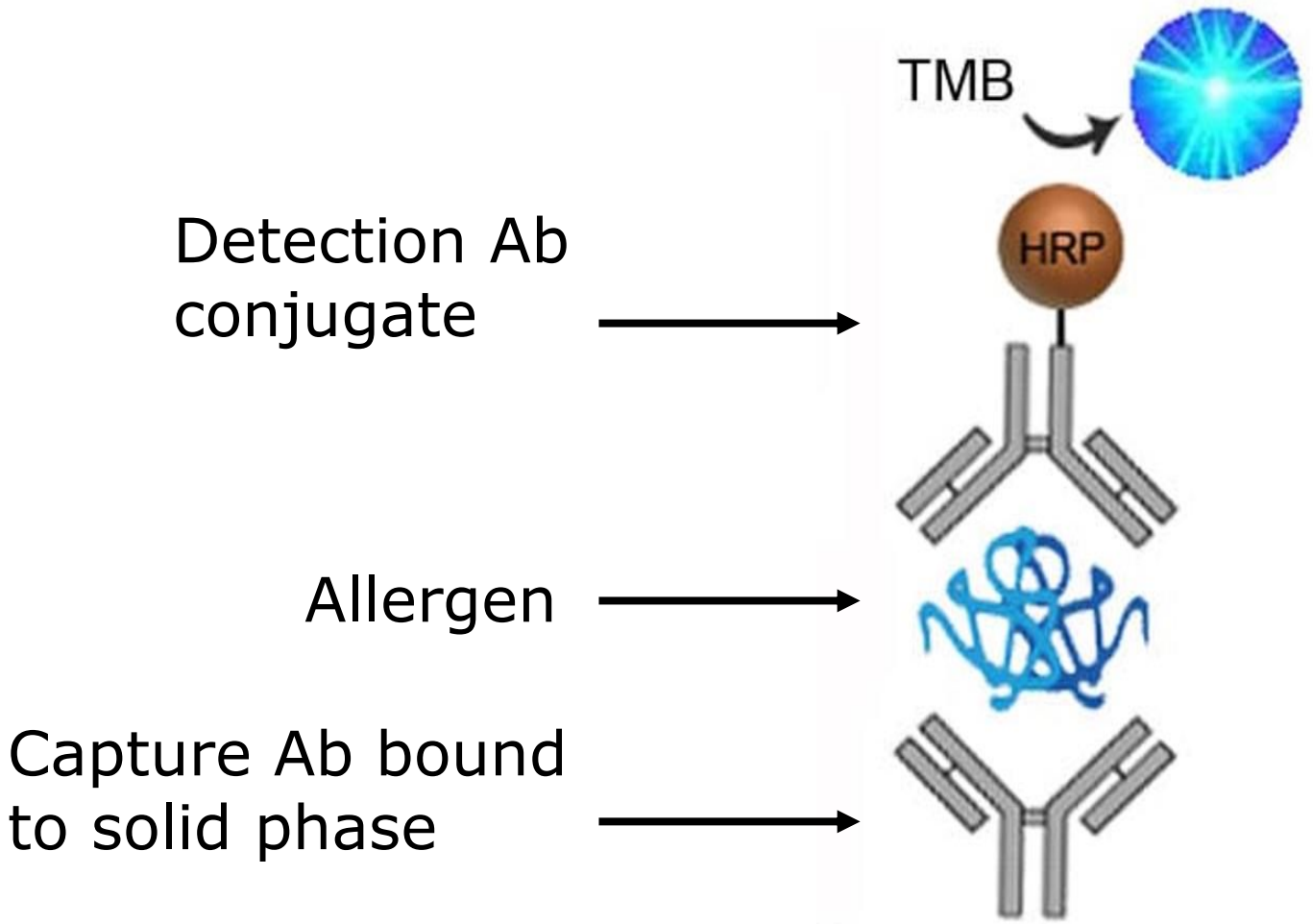
- Equipment costly, large and highly specialized
- Limited number of labs performing LC-MS/MS testing for food allergens
- Ability to detect heavily processed allergen protein fragments



IMMUNOASSAYS

- Allergen-specific, very sensitive
- Detects using antibodies against specific allergen protein
- Quantitative (ELISA) or qualitative (LFD)
- Can be used for both cleaning verification and validation (ingredients, finished product also)

SANDWICH IMMUNOASSAY

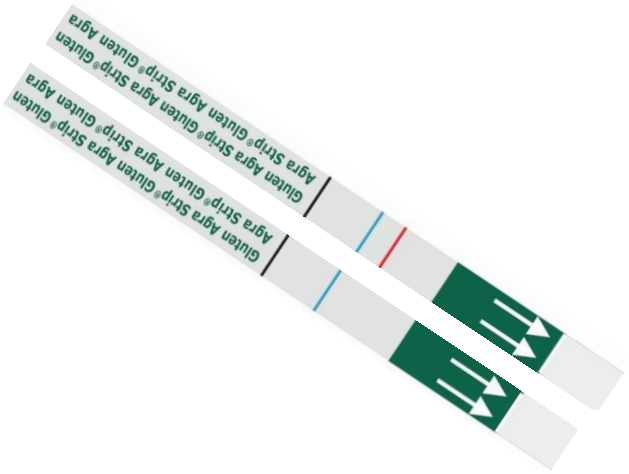


ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

- Quantitative
- Some additional lab equipment, experience/training necessary
- 45-120 min test time
- Sample result extrapolated from standard curve
- Can test raw ingredient, finished product, swabs, rinses

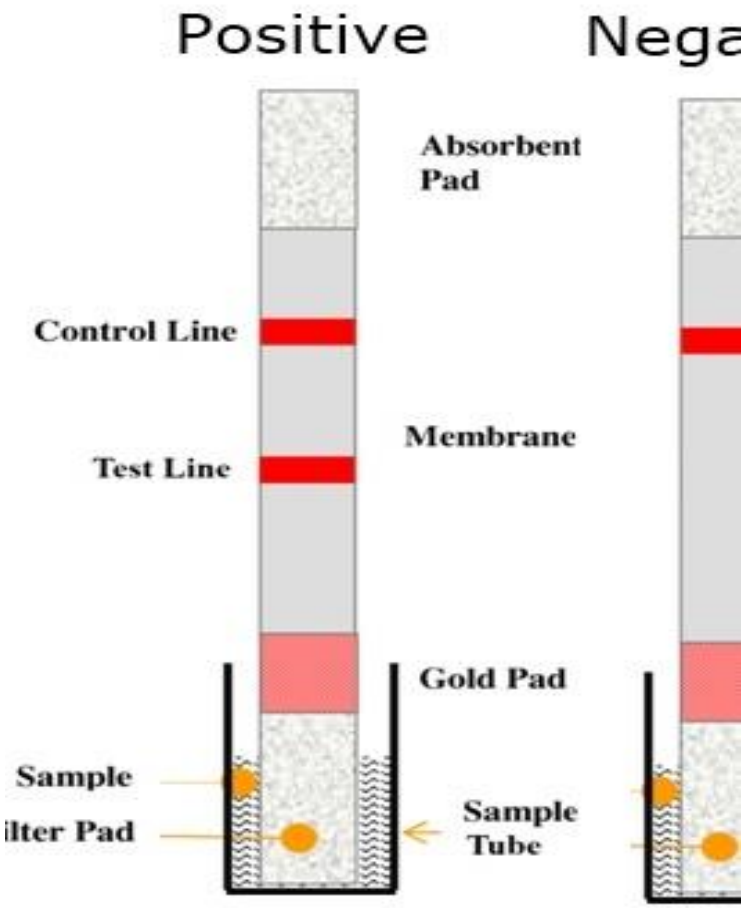
LATERAL FLOW DEVICE (LFD OR STRIP TEST)

- Qualitative: ≥ or < LOD
- Rapid time to result
- No or little additional equipment
- Does not require lab



LATERAL FLOW DEVICE (LFD OR STRIP TEST)

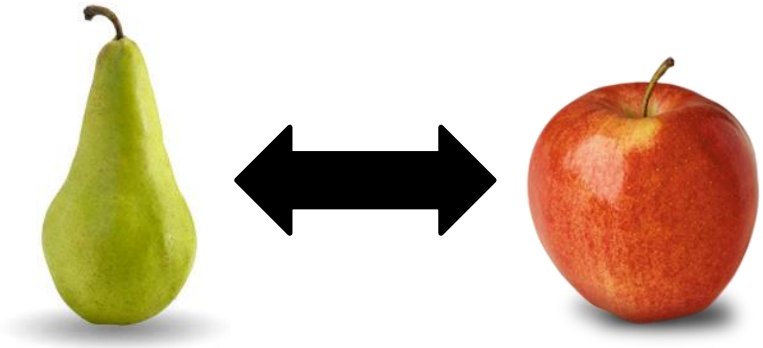
- LFD Result Interpretation
  - Limit of detection (LOD) varies between allergens, manufacturers
  - Test line presence indicates allergen conc. ≥ LOD
  - Test line absence indicates allergen conc. < LOD
  - Weak test line is still a positive



THE PROBLEM WITH ALLERGEN METHODS…

Comparability!

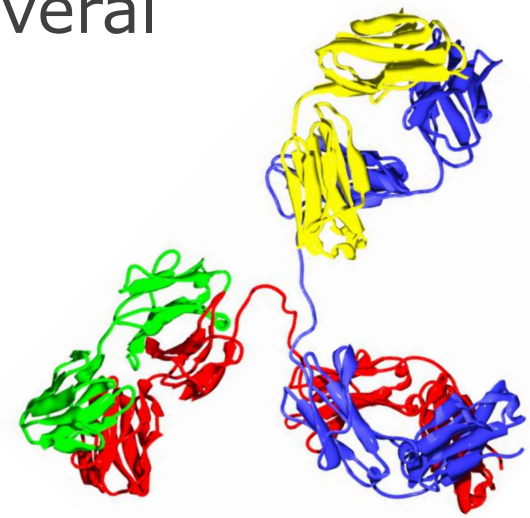
- A variety of factors contributes to the different performance of test methods and test kits within the same method for the same allergen.



Which kit's result is correct?

FACTORS INFLUENCING IMMUNOASSAY MEASUREMENTS

- Antibodies
  - Monoclonal or polyclonal
  - Target of the antibody: single/several proteins, fractionated, modified, synthesized
  - Material used for immunization

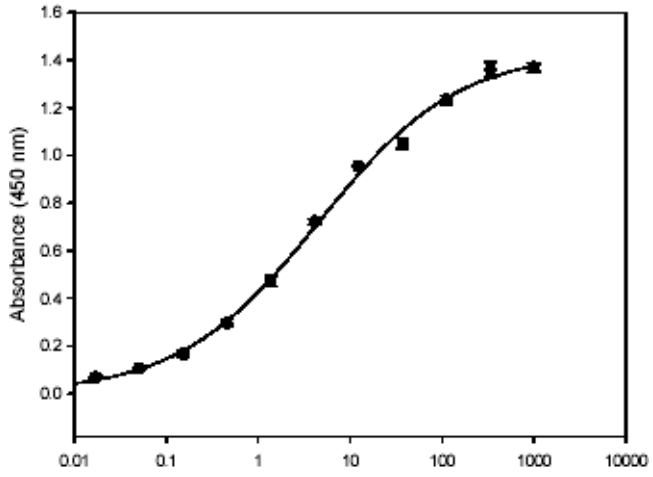


TESTING FOR GLUTEN - ANTIBODIES

- mAb 401.21 (Skerritt)
  - 1st generation, raised against wheat
  - Majority of reactivity to glutelins, ω-gliadin
  - Difficulty detecting barley hordein
- R5 (Mendez)
  - Raised against rye secalin, cross-reacts with wheat gliadin
  - Cross-reactivity to soy and lupin proteins reported
- G12 (Morón)
  - Raised against immunotoxic 33-mer of wheat gliadin
  - Cross-reacts strongly to homologous immunotoxic peptides in rye and barley
  - Cross-reactivity to oat avenin*

FACTORS INFLUENCING IMMUNOASSAY MEASUREMENTS

- Calibrators
  - Match the antibodies of the kit
  - Not the same as sample material
- Extraction
  - What is extracted (efficiency)?
  - Antibody can only detect what has previously been detected

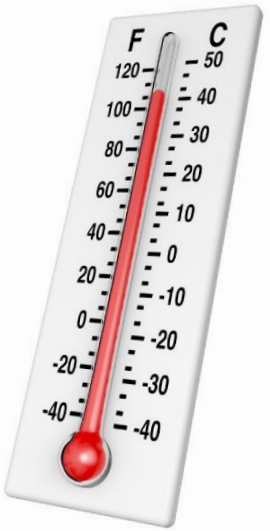


FACTORS INFLUENCING ELISA COMPARABILITY

- Limit of Detection (LOD)
  - Lowest amount of allergen to be distinguished from a true blank
  - LOD mostly determined in buffer
  - May be completely different in food samples
- Recovery
  - Ideally 80 – 120%
  - Not possible for all matrices (pH, salts, polyphenols,…)
  - Spiked vs. incurred samples

FACTORS INFLUENCING IMMUNOASSAY COMPARABILITY

- Immunological test system
  - Environmental influences
  - Experimental conditions
  - Stability affected by transport & storage conditions
  - Robustness, ruggedness



SAMPLING FOOD COMMODITY

- Sample from beginning, middle and end of lot
- Take a fairly large sample – at least 100 g, but 500 g to 1 kg is preferable
- Thoroughly mix sample to homogenize
- Subsample a smaller portion, and grind if necessary to as fine as possible
- Mix to homogenize subsample
- Take portions for testing according to manufacturer's assay instructions

IMMUNOASSAY MATRIX VALIDATION PARAMETERS

- Important to validate that matrix does not cross-react or interfere with assay (selectivity)
- Accuracy, precision and recovery across range of assay
- LOD/LOQ will vary, and should be established for each new matrix
- Incurred allergen reference material would be ideal, but very little available
- Often necessary to spike

SOURCES OF IMMUNOASSAY INTERFERENCE

- High protein concentration
- High lipid concentration
- High polysaccharide concentration
- Extremes of pH
  - May push past pH-buffering capacity of kit reagents
- Reactive biomolecules
  - Polyphenols
  - Tannins
  - Anthocyanins

MATRIX CHOICES: ALLERGEN MATERIAL

- Incurred
  - Better option – allergen processed along with food matrix
  - True evaluation of extraction efficiency
  - Difficult to design/predict allergen levels precisely
  - Challenge for method developer
  - Some incurred RM available for purchase
  - No CRM

MATRIX CHOICES: ALLERGEN MATERIAL

- Spike
  - Necessary for matrices which cannot be produced as incurred
  - Over-recovery of spike
  - Useful for measuring precision of detection portion, interference
  - Allergen extract vs whole allergen
  - Homogeneity

SUMMARY

- **Understand that every gluten detection method has strengths and weaknesses…no perfect method**
- **Know capabilities and limitations of any detection method prior to implementation**
- **Validate that method will detect gluten in your matrices**
- **When in doubt, contact tech services dept. of the kit manufacturer**

THANK YOU!

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