Detection of Food Allergens using ELISA

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**Food Allergens**

- Food allergies affect 6% of children, and less than 4% of adults
- Prevalence of allergies has been rising over recent years – *Why??*
- Health Canada has identified the following 9 allergens as priority food allergens that account for about 90% of severe reactions to food;

<table>
<thead>
<tr>
<th>Peanut</th>
<th>Treenut</th>
<th>Sulphites</th>
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<tbody>
<tr>
<td>Milk</td>
<td>Egg</td>
<td></td>
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<tr>
<td>Soy</td>
<td>Fish (Crustacean, Shellfish)</td>
<td></td>
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<tr>
<td>Wheat</td>
<td>Sesame seeds</td>
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- Within these groups of ingredients there are a number of allergenic components such as casein and beta-lactoglobulin, both components of milk
- Beyond this, different individuals are allergic to different regions, or epitopes, of those protein components.
What is an allergic response?

• An allergic response is an immune response to components of foods (as it can be to other antigens such as pollen and wood dust) that elicits an adverse effect. These immune response can include the following;
  • IgE-mediated hypersensitivity
  • Cell-mediated hypersensitivity
  • Combined IgE- and cell-mediated hypersensitivity

• Symptoms can range from itchy hands or mouth, to swelling of larynx, respiratory distress, and/or abnormal heart rhythm.

• A food allergies resulting in an IgE-mediated immune response are the most severe and life-threatening.
**Schematic of Allergic Reaction**

**Sensitization**

- Allergen + B-Cells and T-Cells
  - IgE Production

**Elicitation**

- IgE + Mast Cells
  - Activated Mast Cells + Food Allergen
    - Release of Mediators
      - SYMPTOMS
The Antibody: How is it specific?

Antigen binding site

Antigen binding site
How a sandwich Enzyme-linked Immunosorbent assay works?

1. Prepare microtitre plate
2. Add dilute/extracted sample or standard
3. Wash unbound sample material
How a sandwich Enzyme-linked Immunosorbent assay works?

- Add Enzyme Conjugate
- Antibody-Antigen-antibody Complex formed
- Substrate/chromogen added
How a sandwich Enzyme-linked Immunosorbent assay works?

chromogen converted by enzyme to produce blue colour

Acid stop solution

Measure absorbance at 450 nm
A Commercial Method for a Quantitative Gliadin ELISA

1. 100 µL of sample or standard add to well.
   - Incubated 30 min at room temperature
   - Liquid poured out, and wells are washed to removed all that is unbound to the specific antibodies.

2. 100 µL of diluted enzyme conjugate added to each well.
   - Incubate 30 min at room temperature
   - Liquid poured out, and the wells are washed to removed all that is not part of the antibody-antigen-antibody (sandwich) complex.

3. 50 µL of substrate and 50 µL of chromogen is added to each well.
   - Gently mix and incubate for 30 minutes at room temperature in the dark.

4. 100 µL of stop reagent (phosphoric acid) added to each well

5. After colour change, the absorbance is measured at 450 nm.
Limits of ELISA

- In-house Brazilnut ELISA - 0.06 to 2.0 ppm
- In-house Hazelnut ELISA - 0.5 to 100 ppm
- Commercial gluten(gliadin) ELISA - 1.5 to 80 ppm

- Use of monoclonal antibodies (MAb) is very specific, but requires another plate to be run for other allergens

- An assay using polyclonal antibodies can target a number of antigens in a sample, but you won’t know which allergenic components it is detecting.
Limits of ELISA

- Sample preparation for a 96-well microplate (80 samples + 16 standards) requires about 2 hours. The ELISA then takes about 1 to 2 hr to complete, depending on the protocol.

- About one to two full 96-well plates can be run in one day (varies with protocol)

- Variation due to temperature, fast kinetics, and analyst and/or instrumentation differences.
Detection of Undeclared Milk Ingredients in Infant Cereals

- Milk is one of the most common food allergies in children.
- 2-4% of infants are milk allergic.
- Food allergies and labelling issues continue to be a concern for government, consumers, and the food industry.
- Two of the main allergenic proteins in milk are casein and beta-lactoglobulin (BLG), found in whey.
- A small market survey of infant cereals using two commercial ELISA kits specific for the following:
  - Casein
  - Casein and beta-lactoglobulin
Detection of Undeclared Milk Ingredients in Infant Cereals

Results for Burnaby Infant Cereal Samples

- Casein
- Casein and beta-lactoglobulin
Detection of Undeclared Milk Ingredients in Infant Cereals

Results for Ottawa Infant Cereal Samples

- Allergen Concentration (ppm)

- Casein
- Casein + Beta-lactoglobulin

Ottawa Samples

AQ01 AQ02 AQ03 AQ04 AQ05 AQ06 AQ07 AQ08 AQ09 AQ10 AQ11 AQ12 AQ13 AQ14 AQ15 AQ16 AQ17 AQ18 AQ19

0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0
A Robust Method

- The specificity of ELISA makes it very robust, but because different antibody-based assays recognize different epitopes, variable results may be obtained using different systems.
- A wide variety of foods make it difficult to quantify proteins present as the varying food matrices can prevent good extraction of allergens, reducing detection, but not significantly affecting allergenicity.
- The Allergen Methods Committee is therefore involved in developing formalized guidelines and performance criteria for allergen methods.
- And, to develop and evaluate alternatives to ELISA-based methodologies for confirmation of results, using protein separation techniques coupled with Mass Spectrometry, or using DNA-based techniques.
**Trace levels?**

- ELISA level of detection is generally at the low ppm level, with some assays sensitive in the ppb level.

- Advancements in technology are taking immuno-based techniques to unprecedented depths, as with a new immunoassay based on multiphoton detection of radiolabelled proteins - 40 zeptomoles!!

- Ultra-sensitive quantitative measurement of biomarkers as low as a few femtogram/mL
  - useful for detection of prions, HIV, cytokines related to diseases