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Allergen Analysis, Why and what to test for ?

*Technical and information workshop for application of
analysis to allergen management*

Sydney NSW,

22 February, 2011



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Why are food allergies important? : Prevalence

- ❑ Food allergies and celiac disease affect over 1.6 Million Canadians
 - 5 – 6% of children and 3-4% of adults have food allergies
 - Asthma is linked to sulphite sensitivity : about 200,000 asthmatics have a sulphite sensitivity
 - Celiac disease affects about 340,000 Canadians (1% of the population) and is underdiagnosed

Food allergies, sulphite sensitivity and celiac disease are considered significant public health issues



Why are food allergies important? : Impacts

- ❑ The health impacts are serious : hospitalisation, anaphylaxis and **death** (100-200 deaths per year in the US).
- ❑ About 12% allergic reactions to foods need treatment in emergency rooms
- ❑ Celiac disease is associated with multiple complications including osteoporosis and cancer
- ❑ These conditions impact individuals, families and social circles

Food allergies and celiac disease are life long and incurable conditions – Avoidance is the only line of defense



Costs and Burden of food allergies

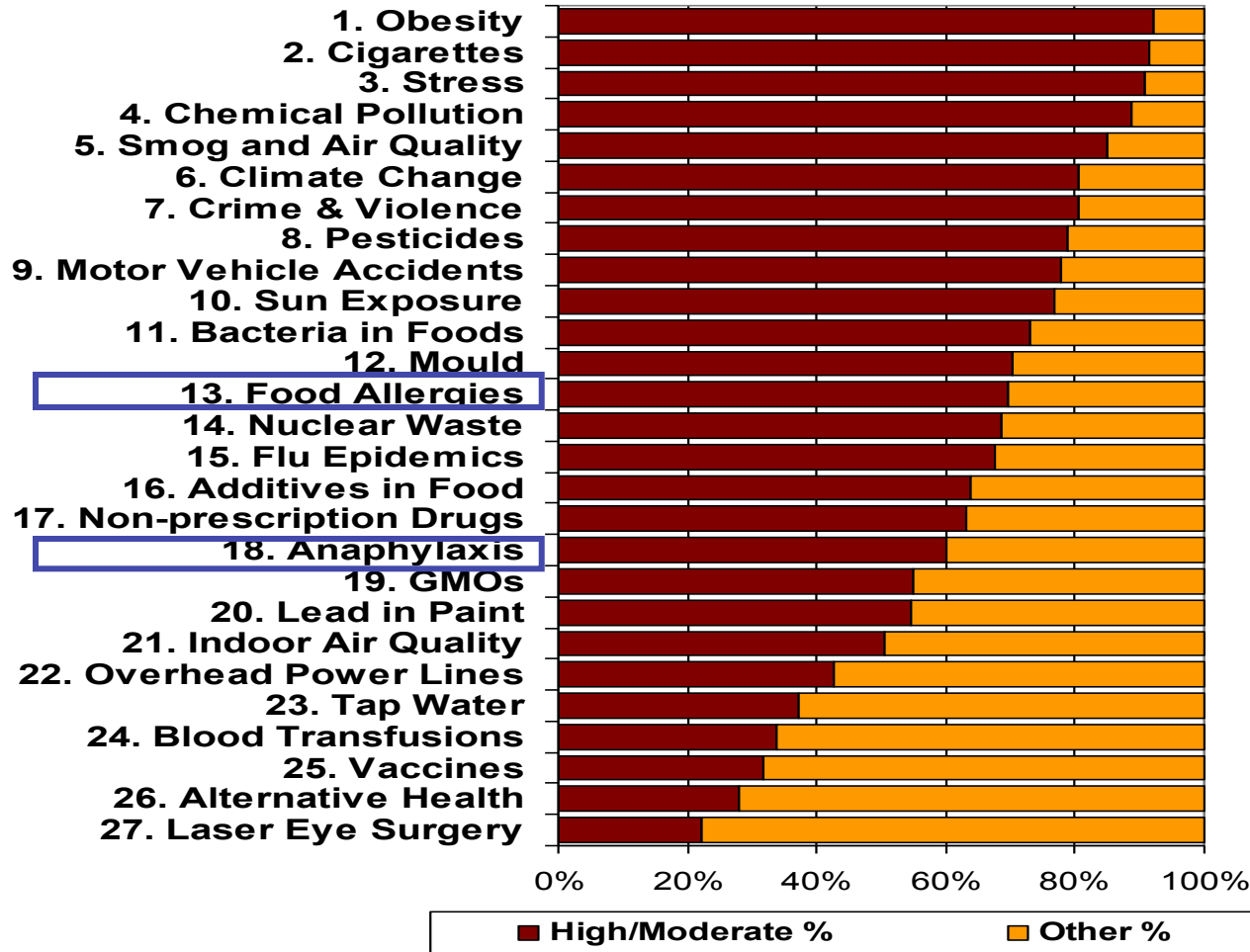
- ❑ Costs for emergency room visits / hospital stays due to allergic reactions* estimated to be about \$5.4M/ year
 - Estimate over 14,500 Emergency Room visits and nearly 400 requiring hospital stay
- ❑ Recent studies suggest \$5000 (CAD) of additional direct and indirect costs to households with food sensitivities, **for a total of \$5.4B ****

Prevention of food allergy incidents may help reduce the cost of this burden on individuals, family and society



* (not including costs for celiac disease and sulphite induced asthma)

Canadians consider food allergy a high risk



In Canadians' understanding : food allergies are considered **a higher risk** than nuclear waste or flu pandemics

(From Elliott and al., 2009)



Why are changes to labelling regulations needed ?

- ❑ Labels are the only link for consumers to know the composition of prepackaged foods
- ❑ **1/3 of those who experienced a food allergy incident attributed it to a problem with the label**
- ❑ Allergic consumers and their caregivers need:
 - Reliable/accurate labelling (no doubt should be left for allergens)
 - Clear and consistent labelling (no shopping dictionary should be needed)

While ingredient labelling is mandatory for most prepackaged foods, there are instances where allergen ingredients are not readily disclosed and remain « hidden » for consumers



Regulations to enhance labelling requirements

- ❑ A number of countries have enacted regulations and legislation to ensure that major allergens are not omitted from the list of ingredients:
 - Labelling of ingredients
 - Possible indication of unavoidable cross-contamination incidents
 - Indication of products destined to food allergic consumers (e.g. « Gluten Free », « peanut free » foods
- ❑ Compliance verification against these rules makes require the use of analytical methods
- ❑ Allergen testing is becoming more important for
 - Food industry : to ascertain and document compliance
 - Food Safety regulators : to support food safety investigations



Example of omission from labels

Verification of composition for complex mixtures : Seasonings, spices ..

Ingredients : Potatoes, sunflower oil, salt, **seasonings**

Allergen labelling requirements

Ingredients : Potatoes, sunflower oil, salt, **seasonings (milk)**

OR

Ingredients : Potatoes, sunflower oil, salt, seasonings

Contains : milk



Seasonings are a multi-component ingredient which can contain allergen sources



AVOIDANCE

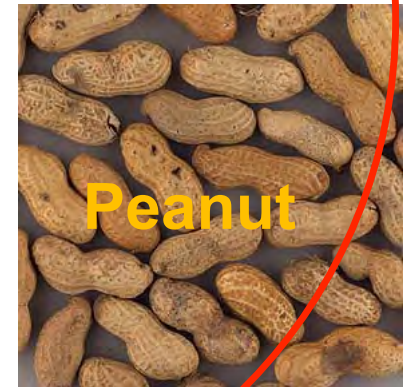
The Key to Preventing Potentially Serious Health Consequences

- ❑ Consumers depend on the information provided on the label to avoid the food where allergen, gluten sources are used as ingredients, or to identify those foods that are known to be suitable for their condition: “



Analytical methods are key for ...

- ✓ Validation of sanitation practices by food processors
- ✓ Compliance verification against established ingredient declaration requirements, application of GMP practices
- ✓ Enforcement by food safety regulators



Analytes : Markers of allergens / Gluten

Proteins involved in Adverse reactions

Proteins not involved in adverse reactions, but characteristic of the commodity

DNA markers

- ✓ What markers should methods target ?
- ✓ Will one assay protect all consumers?



Examples of Food Allergens and Specific allergenic proteins

Allergen	Specific proteins
Peanut	Ara h 1, Ara h 2 (7S storage proteins) Ara h 3, Ara h 4 (11S storage proteins)
Hazelnut	Cor a 9
Cow's Milk	Bos d 8 (α and β -casein) Bos d 5 (beta-lactoglobulin)
Hen's Egg	Gal d 1 (hen's egg ovomucoid) Gal d 2 (hen's egg ovalbumin) Gal d 4 (hen's egg lysozyme)
Brazilnut	Ber e 1 (2S albumin from Brazilnut)



Antibody-based methods

- ❑ Exploits the specificity of antibody-antigen interaction
- ❑ Enables specificity and selectivity
- ❑ Various signal amplification techniques enable low level detection
- ❑ Adapted for the determination of analytes in complex mixtures



Immunochemical-Based Methods

- ELISA
- Immunoblotting
- Biosensors / SPR
- Lateral flow device / Dipstick



Analytical Challenges – Food matrices

- ✓ Processing Effects
 - Modification of proteins
 - Denaturing of proteins
 - Hydrolysis of proteins
- ✓ Matrix Effects
 - Chemical interferences
 - Extraction of proteins
 - Denaturing and Hydrolysis of proteins



Analytical Challenges - Example

Detection of Mustard, Egg, Milk, and Gluten in Salad Dressing Using Enzyme-Linked Immunosorbent Assays (ELISAs)

POI-WAH LEE, LYNN M. NIEMANN, DEBRA M. LAMBRECHT, JULIE A. NORDLEE, AND STEVE L. TAYLOR

ABSTRACT: Enzyme-linked immunosorbent assay (ELISA) is a commonly used method for the detection of trace amounts of potentially allergenic protein residues in foods. However, food matrices and processing conditions can affect the detection of protein residues. The effects of acidity on the detectability of several allergenic proteins commonly found in salad dressing using ELISAs was investigated. First, recovery experiments were performed on salad dressing formulated with 0 to 1000 ppm mustard flour (mustard). The mean percent recovery for mustard from the salad dressing was only $7.7\% \pm 1.6\%$. When the pH of the salad dressing was adjusted to pH 7 prior to spiking with mustard, recovery improved to $94.1\% \pm 7.6\%$. However, if the pH was adjusted to pH 7 after spiking and extraction, the recovery was only $11.1\% \pm 1.7\%$. When vinegar was spiked with mustard flour at pH 3, 3.5, and 4, detectability of mustard was lowest at pH 3. Basic extraction of mustard proteins from salad dressing did not improve the mustard detection. Acidic salad dressing matrices reduced the detectability of mustard by the mustard ELISA probably because of acid precipitation of mustard proteins that renders them insoluble and nonextractable. Commercial salad dressings containing 100 ppm (mg/kg) of egg, milk, or gluten were analyzed every 2 to 4 d for 90 d using 3 commercially available ELISAs. A decrease in the detection of the egg, milk, and gluten in the salad dressing upon storage was observed. Our study highlighted the importance of evaluating the utility of various ELISAs for specific food matrices and the recovery as a function of product storage.

Keywords: acidity, allergenic food proteins, detection, ELISA, salad dressing

JOURNAL OF FOOD SCIENCE—Vol. 74, Nr. 5, 2009



Food Allergen testing can also be used for

□ Development of data to support exposure assessment:

- Example : Detection of low level occurrence of gluten sources in foods sought by celiac individuals (part of a Gluten free diet)
- Gathering data to support analysis of low level chronic exposure to gluten, as part of a Gluten –free diet
- Support to development of possible MLs for Gluten in « Gluten-free » foods

□ Development of Compliance thresholds for other allergens:

- In the absence of thresholds based on completed safety assessments, practical risk management thresholds are set on the basis of analytical methods capabilities



Food Allergen methods are key for

- 1. Rapid Response:** supporting quantitative (deterministic) assessments in response to food safety investigations (system available 24hr/day, 7day/week to support food recalls related to undeclared allergens).
- 2. Prevention of adverse reactions:** development of compliance policies and quality assurance measures
 - ❖ supports development of and updates to food labelling standards
 - ❖ Supports education and outreach to industry (implementation of food safety control practices / verification of effectiveness of sanitation practices)



Thank-you!

For more information, please visit us @

www.healthcanada.gc.ca/foodallergies

www.Meniga.org





고맙습니다 谢谢 תודה!
mahalo *děkuji*

Thank You

شكرا köszönöm gracias
Ευχαριστώ merci
どうもありがとう danke

