

The Challenges of Allergen Analysis in a Global Market

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DTS FACTA

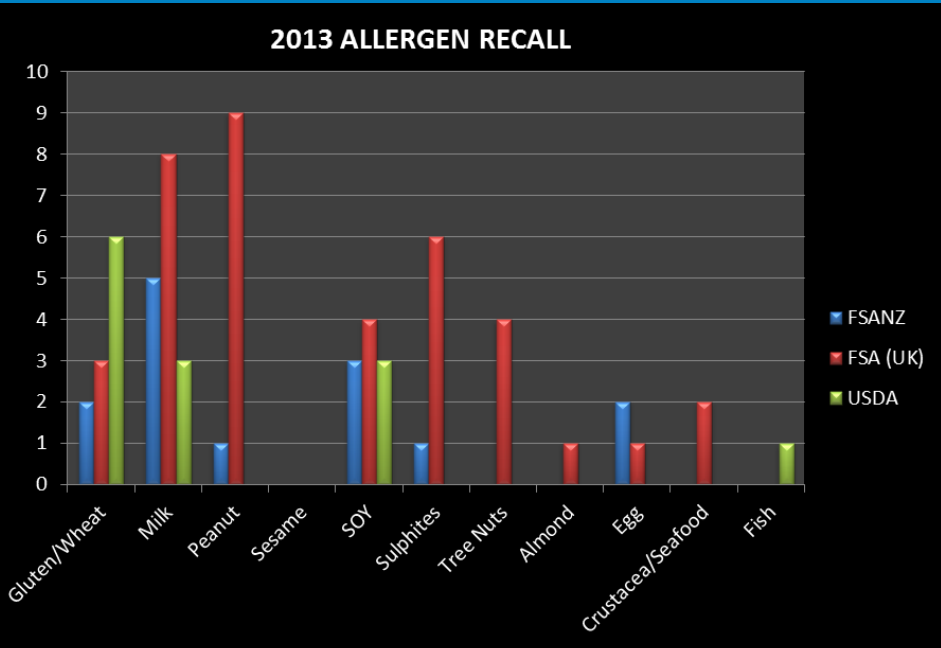
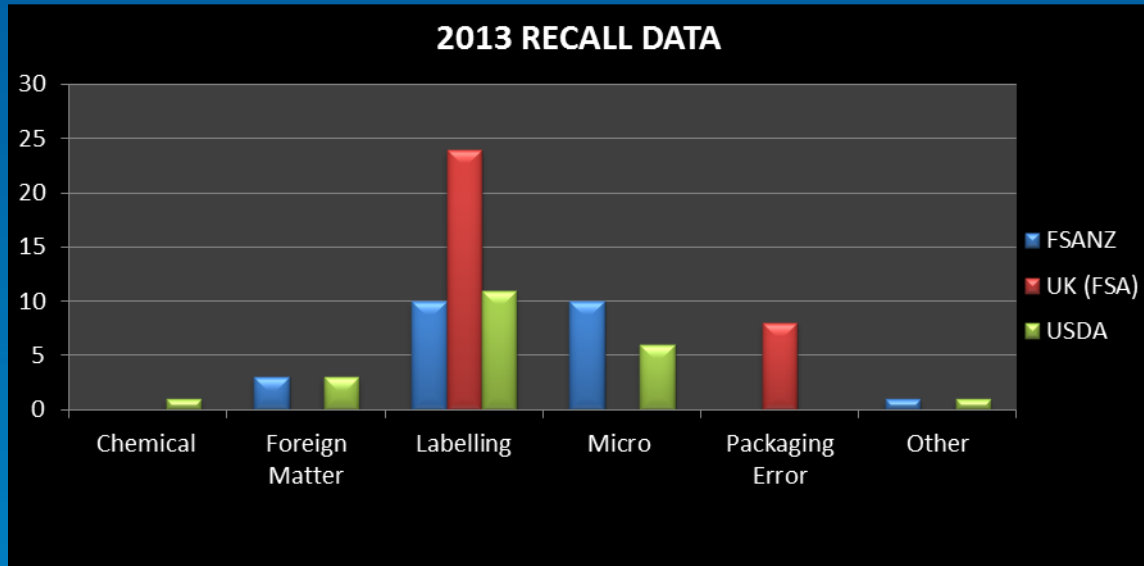
DTS FACTA is a unit within DTS Food Laboratories and was formed to provide analysis and expert advice and consultative services on food borne allergens for the food manufacturing, food service and associated supply industries. We provide training in food allergen awareness, analysis services for food borne allergens and consultation in strategies for allergen control within factory and food service environments.

We are endorsed by the Allergen Bureau to deliver VITAL® training and also provide specialised allergen risk assessment in food production facilities .

We have over 10 years experience in this area and hold the broadest range of National accreditation (National Association of Testing Authorities) - for the performance of a range of allergen analysis .

We work closely with both industry and regulatory laboratories and have been involved in Industry working groups both nationally and internationally

Recalls



Challenges of the global environment

- Geographical differences
- Population variation
- International labelling and safety expectations
- Lack of confidence around precautionary labels
- New studies
 - “In this era of globalization, it is not only populations that migrate but also foods, as people adopt foreign diets and import exotic products”

Incidence







- Variable data
- Variable mechanisms for recording
- Variable definitions
- Changing regulatory environment
- Allergy Nation

Allergic patterns

- Australia
- US
- China and Hong Kong
- Europe
- SE Asia
 - The differences are critical as the patterns determines the direction of method and kit developers

Legislation , time lines and geographical differences

- Reflects the risk of the jurisdiction's population
- International community expects an international approach
 - A chocolate is a chocolate is a chocolate

Codex	United States	Japan	Canada	The EU	Australia	China	Malaysia	Singapore	Thailand
<p>Cereals containing gluten</p> <p>Crustacea</p> <p>Egg</p> <p>Fish</p> <p>Peanuts and soybeans</p> <p>Milk</p> <p>Tree nuts (and all their products)</p> <p>Sulphites</p>	<p>As per Codex</p> <p>But states wheat</p> <p>Tree nut defn includes Coconut</p>	<p>Mandatory by ministerial ordinance—</p> <p>Eggs</p> <p>Milk</p> <p>Wheat</p> <p>Buckwheat</p> <p>Peanuts.</p>  <p>Recent changes</p>	<p>As per Codex</p> <p>And sesame seeds</p>	<p>As per Codex</p> <p>And sesame seeds</p> <p>• Added Celery</p> <p>Mustard</p> <p>Molluscs</p> <p>Lupin</p>	<p>As per Codex</p> <p>And sesame seeds</p> <p>Changes coming</p>	<p>AS per Codex</p> 	<p>Codex</p> <p>Variation cereal including wheat, rye, barley and oat</p> <p>nut and nut product including peanut and soybean;</p> <p>fish and fish product;</p> <p>milk and milk product (inc lactose)</p> <p>Egg and egg product.</p>	<p>Codex cereals containing gluten crustacea eggs fish peanuts, soybeans milk including lactose tree nuts sulphites</p>	<p>Codex Implementation Pending</p>
<p>General STD for Labelling of Prepackaged Foods</p> <p>Codex Alimentarius</p>	<p>Food Allergen and Consumer Protection Act 2004</p> 	<p>Law concerning Standardization and Proper Quality Labeling of Agricultural and Forestry Products April 2002 (Law No.175 of 1950, hereinafter referred to as "JAS Law"),</p>	<p>Amendment to Food Allergen Labelling regulations published in 2011. in force in August 2012</p> 	<p>EC Directive: 2000/13/EC</p> <p>Introduced in 2003</p> 	<p>FSANZ 2000 (enforced in 2002)</p> 	<p>General Administration of Quality Supervision, Inspection and Quarantine</p>	<p>http://fsq.moh.gov.my/v4/images/filepicker_users/5ec35272cb-78/Perundang-an/Akta%20dan%20Peraturan/Food_Regs_1985/Regulation%2011.pdf [Regulation 11 (5)]</p> <p>http://fsq.moh.gov.my/v4/images/filepicker_users/5ec35272cb-78/Perundang-an/Akta%20dan%20Peraturan/Food_Regs_1985/Regulation%2013.pdf [Regulation 4 (ea)]</p>	<p>http://www.ava.gov.sg/NR/rdonlyres/0CA18578-7610-4917-BB67-C7DF4B96504B/26460/2web_FoodRegulations_3Sep2013.pdf [Regulation 4 (ea)]</p>	<p>http://www.wtocenter.org.tw/SmartKMS/fileviewer?id=135404</p>

Analysis Techniques for Food Allergens

- **ELISA** and Immuno-Sorbent test strips
- **PCR**
- Mass Spectrophotometry
 - LC- MS MS
- Biosensors

Techniques applicable for routine analysis

- Non specific
 - ATP – bioluminescence
 - Protein
- Specific
 - Target either the allergen itself or a marker that indicates the presence of the allergenic food .
 - Markers include
 - Specific proteins
 - DNA fragments
 - ELISA variants
 - PCR



Key Analytical Considerations

- What method
- What calibrator
- What sensitivity
- What matrix
- Reference materials
- Sampling plan
- Sample handling
- Company pedigree and available information
- External publication and Approvals
- Matrix work
- Sensitivity and Related products
- Proficiencies

Sensitivity isn't everything

- Method harmonisation
- Decreased detectability has implication for application to action or threshold levels .
- Matrix interference and cooking impacts mean analysis alone is inappropriate
- Method variability or MOU
 - Impact of MOU – labelling based on interpretation of levels and requires informed response around uncertainty issues

Kit Comparisons

- In the absence of reference standards, proficiency programs essential for comparing kit performance
- Reported Results Dependent on Kit Source
- Valid Comparison Dependent on Knowing Standards Used and how Results Reported
- Requires conversion between protein and commodity
- Critical to know reporting units for application to VITAL®
- Some assays are not so technically straightforward

Enzyme Linked Immuno Sorbent Assays Available


- Almond
 - Buckwheat
 - Crustacea (Tropomyosin)
 - Gluten
 - Lupin
 - Peanut
 - Soy
 - Walnut
 - Pistachio
 - Coconut
 - Increasing number of reputable kit producers responsive to industry needs
- Beta Lactoglobulin
 - Casein
 - Egg and Lysozyme
 - Hazelnut
 - Mustard
 - Sesame
 - Total Milk
 - Cashew
 - Fish (cod species)
 - Macadamia

Poster Data


• Lupin

• Poppy

• Caramels



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Approaching Zero... Matrix Inhibition and Assessment of Low Concentration Allergens

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ABSTRACT

TITLE: Approaching Zero: Matrix Inhibition and Assessment of Low Concentration Allergens

BACKGROUND: A survey of 18 products presents the use of increasingly lower detection levels for a number of allergens with varying degrees of cross reactivity data available. While the 18 products provide validation data for a number of matrices, the survey encompasses the multitude of matrices now being presented for analysis. The following emphasis on non-ratified evaluation probes the boundaries of 18 performance particularly around the Limit of Quantification. A number of examples will be presented to illustrate the point and highlight the challenge for the analytical and food industries.

OBJECTIVE: To investigate a number of low level positive results to examine the potential for cross reactivity.

METHODS: A range of ELISA based kits were used to assess low level positive results. The laboratory protocol for investigation of suspected false positives was applied. This protocol includes repeat analysis, inhibition modification and investigation using alternative assays.

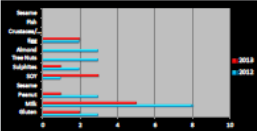
RESULTS: These three investigations yielded a range of results demonstrating that low level positive results for a variety of allergens require thorough investigation before action is taken based on the assay results.

INTRODUCTION

Allergen related results continue to be a significant contributor to the number of recalls in Australia and New Zealand (see Figs 1 and 2). In response to this, many companies have instituted rigorous monitoring of both imported goods and raw materials. While many of the samples submitted for analysis have been tested culturally and constitute part of the suite of validated matrices, not all samples prove to be suitable for particular analyses. This poster sets out three examples of positive low level results that were further investigated to assess the validity of the analytical result:

- 1) A low level positive result for egg in a commercial sample containing caramel
- 2) A low level positive result for macadamia in a biscuit containing poppy seeds
- 3) A low level positive result for egg in a commercial sample containing caramel

Fig 1. FRAC1 (Australia New Zealand) Allergen Recall Frequency (By Allergen)



Investigation One : Soy and Lupin Cross Reactivity

Sample	OD	Results (ppm)
10% Soy Flour (In the Flour)	0.064	0
10% Soy Flour (In the Flour)	1.329	2.3
10% Soy Flour (In the Flour)	1.57	2.4
1% Peanut (In the Flour)	0.07	0
1% Peanut (In the Flour)	0.06	0
1% Soy Flour (In the Flour)	0.06	0
1% Soy Flour (In the Flour)	1.1	2.1
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Table 1. Soy flour and the Lupin ELISA assay.

*Samples submitted as part of a routine surveillance of a pastry ingredient gave a low level positive result to the Lupin assay.

*Validation of sample levels did not give the expected change in signal intensity. Components of the commercial ingredients were therefore investigated for the origin of the signal and soy flour was subsequently implicated.

*Soy flour from another source was diluted in the flour to varying concentrations and analysed. The test of signal dilution over a 100 fold dilution is consistent with a matrix effect rather than specific antibody binding.

*The laboratory has completed a comprehensive Limit of Reporting (LOR) for the lupin assay in products suspected of containing soy flour, with further investigation of the kit and the nature of the cross reactivity is carried out.

Fig 2. Poppy Seeds and the Macadamia ELISA assay.

- > Most positive samples submitted as part of routine, routine allergen surveillance.
- > Matrix for macadamia
- > No obvious source of cross contamination in production facility and relatively newly received kit components ingredients of biscuit investigated.
- > Poppy seeds, a rarely encountered matrix, confirmed as source of signal.
- > Dilution had little impact on signal suggesting non-specific antibody binding.
- > Further samples containing poppy also positive for macadamia.
- > Lower level of cross reactivity, also seen with biscuits.

Fig 3. Biscuits Mix (A) and Caramel (B) analysed using various commercial Egg ELISA Assays

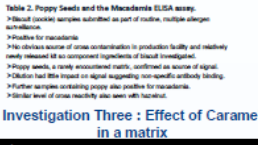
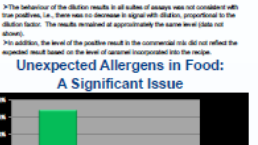


Fig 3. Biscuits Mix (A) and Caramel (B) analysed using various commercial Egg ELISA Assays

- > A number of assay kits were utilised for egg analysis using kit A and yielded positive results ranging from 1 to 2.3 ppm.
- > The component ingredients were investigated and yielded a positive result for the caramel.
- > These alternative kits were assessed and the original mix (Fig 3a) and the caramel (Fig 3b) analysed using these alternative kits.
- > The kits yielded results significantly. The divergence of result is most likely accounted for by the varying dilution ratios and/or the efficacy of extraction additives to reduce the impact of matrix effect.
- > A range of dilutions of both the commercial product and caramel samples were performed to examine linearity in dilution results.

Unexpected Allergens in Food: A Significant Issue




CONCLUSIONS


It is vital for analytical laboratories to identify additional low level positive results. Where positive results occur in related matrices, consideration should be given to the likelihood of specific cross reactivity between related matrices. While cross reactivity might be included in the user's key and logs, the appropriate cross reactivity between assay kits and matrices is more complex. This is especially true where matrix specific positive results are observed. This is especially true where matrix specific positive results are observed. This is especially true where matrix specific positive results are observed.

References

1. Common allergen strategies in hazelnut, eye grain, sesame seeds, lent and poppy seeds.
2. Common allergen strategies in hazelnut, eye grain, sesame seeds, lent and poppy seeds.
3. Common allergen strategies in hazelnut, eye grain, sesame seeds, lent and poppy seeds.
4. Common allergen strategies in hazelnut, eye grain, sesame seeds, lent and poppy seeds.
5. Common allergen strategies in hazelnut, eye grain, sesame seeds, lent and poppy seeds.
6. Common allergen strategies in hazelnut, eye grain, sesame seeds, lent and poppy seeds.
7. Common allergen strategies in hazelnut, eye grain, sesame seeds, lent and poppy seeds.
8. Common allergen strategies in hazelnut, eye grain, sesame seeds, lent and poppy seeds.
9. Common allergen strategies in hazelnut, eye grain, sesame seeds, lent and poppy seeds.
10. Common allergen strategies in hazelnut, eye grain, sesame seeds, lent and poppy seeds.



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Robust Sampling Plans



Distribution usually not homogenous

- May concentrate in first part of the run due to presence of previous product
- Hang ups in the system may result in “random” dumping of allergen
- May be particulate and therefore distributed irregularly in the sample itself

Test multiple samples at different points of production run

Avoid batching of samples for analysis as dilution will prevent identification of push through allergen from previous product run.

Sanitation swabs for aerial contamination– apply the target principle



Appropriate Applications

- use analysis to confirm assumptions
- use analysis for assessment of allergen profile of raw materials
- use analysis to confirm validation of cleaning and critical control points and assess cross contact
- Use to confirm allergen status of the final product
- Use analysis for monitoring of change impact
- Use analysis to confirm assumptions made during the implementation of a risk assessment process (eg VITAL[®])

Analytical Don'ts



- Don't take an analytical snap shot
- Don't consider analysis as the only option
- Don't simply compare a number to the grid
- Don't examine analytical results in the absence of a thorough risk assessment Always more than the grid
- Numbers alone can be misleading

International Tools

Allergen Detection Methods - The Compendium of Food Allergen Methodologies- Health Canada

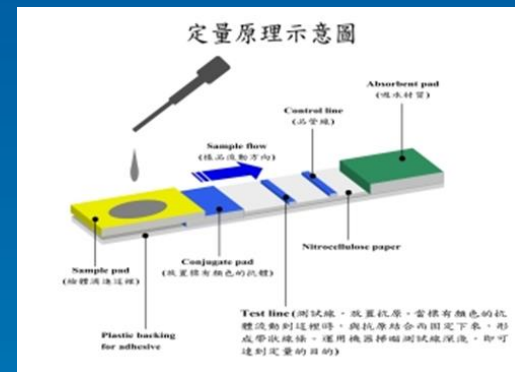
Analyte of Interest	Method Evaluated	Date Evaluated
Sesame	Elisa Systems Sesame	March 2010
Casein	Elisa Systems Casein	December 2006
Beta Lactoglobulin	Elisa Systems Beta Lacto.	March 2006
Egg	Neogen Veratox for Egg	July 2004
Almond	Neogen Veratox for Almond	December 2004 (update !)
Hazelnut	Performance Evaluation R-Biopharm RIDASCREEN® FAST	2009

<http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/allergen/index-eng.php#list>

- "Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices,"
 - Michael Abbott et al
- AOAC AFNOR CEN
- MoniQA

Current testing trends

- Overall increase in analysis
- Increase non risk based analysis
- Increased use of strip based tests
 - Surface and product
- ELISA remains most common method
- Increased interest in pcr (not US or Canada)
- International push towards Mass Spectrometry in various forms
 - May become gold standard although currently few fully validated methods (egg, milk)



What's happening.

- The AT- SIG
- Technical Workshops
- International communication thru Facebook
 - Search on Facebook for AOAC and follow links to Allergen Group AOAC – electronic newsletter
- and Linked In.
 - Allergens in a nutshell
- International Meetings
- International Ring Trails
- MoniQA Reference Materials working group

The future

- Harmonisation
- What might that look like
- Where do we as food safety experts want to go
- Regulation versus voluntary guidelines for both precautionary labels and methods
- Best if we all walk in the same step

