

## Auckland Allergen Bureau Conference October 2008

Allergen Analysis and its role in Food

Allergen Control in Production Facilities

( working title – what is allergen analysis and does it work anyway ? )



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## Allergen Analysis

- Routine analysis – appropriate use of allergen analysis
- Application for production facilities
- Current research and developments in allergen testing



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## Allergen Analysis -A Useful Tool with a Bad Reputation



- Most current technologies that are routinely used are more like blunt instruments than fine surgical instruments
- Despite the kits producers best efforts one size does not fit all .

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## Allergen Analysis

- most chemical and immunological methods that detect proteins can be used for allergen detection but not all are appropriate for routine application in the food industry
  - Kjeldahl, HPLC, Mass spectrophotometry , Capillary electrophoresis
  - Immunodiffusion, Counterelectrophoresis,
  - Radioimmunoassay Immunoblotting

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## Techniques Applicable for Routine Analysis



- Non specific
  - ATP – bioluminescence or Protein swabs
- Specific
  - Target either the allergen itself or a marker that indicates the presence of the allergenic food .
  - Markers include
    - Specific proteins
    - DNA fragments
- **Enzyme-Linked Immunosorbent Assay (ELISA)**
- **Polymerase Chain Reaction (PCR)** in the form of a real-time PCR or in combination with an ELISA

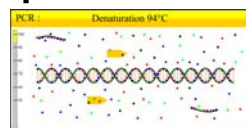


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## Basis of PCR techniques

- Sub-sample test material
- Extract and purify DNA
- Polymerase Chain Reaction (PCR) amplification
- Detection of amplified DNA
- Interpretation of results
- detects DNA sequences that are indicative of the presence of the allergenic species.
  - PCR allergen tests can verify or clarify an ELISA result
  - detect allergens such as fish and shellfish for which no ELISA test is currently available
    - useful for testing highly processed food products containing hydrolyzed proteins..



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## PCR Systems Available

- Kits
  - Almond  
PCR-ELISA And Real Time PCR Kit
  - Peanut
  - Soya
  - Milk
  - Wheat and Gluten
- Available but not as commercial kits
  - Hazelnut
  - Tree nuts
  - Brazil
  - Macadamia
  - Pecan
  - Walnut
- buckwheat, sesame, peanut, pecan, walnut, celery, freshwater and salt water fish and shellfish.
  - detects the presence of classes of shellfish such as bivalves (oyster, mussel, clam) or crustaceans (crab, shrimp, lobster, prawn)
- fish as a class
  - including halibut, salmon, perch, shark, swordfish, catfish, tuna and tilapia.

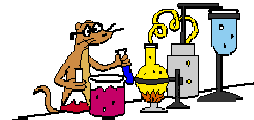


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## Immunoassay

- Allergen is the antigen
- Create an antibody to the allergen
- Coat a stable surface with the antibody
- React antigen with antibody
- Use marker to detect the reaction



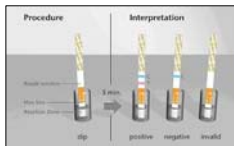
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## ELISA or GLISA Kits

- Enzyme Linked Immuno-Sorbent assays
- Kits detect a range of proteins
- Ideally designed to detect the allergenic proteins of the target allergen.
- They are designed to be
  - Specific ( minimal false positives )
  - Sensitive ( able to detect very low levels of allergen – ppm level)



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## ELISA Kits Available Internationally

- ELISA SYSTEMS
- Neogen
- Pro Lab Diagnostics
- R Biopharm
- Tepnel
- Hallmark/HAVEN
- This list is not exhaustive. Others are available

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## Testing Available Via ELISA Technique

**Milk - Total Milk , Casein and Beta -Lactoglobulin**  
**Egg**  
**Soy**  
**Wheat ( barley and rye ) - gluten**  
**Peanuts**  
**Shellfish**  
**Tree Nuts – hazelnut, almond available**  
**Buckwheat**  
**Lupin Pea**  
**Fish - via frog protein ( GAD3 Still coming )**  
**Mustard**

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## Published Data and Approvals

Few kits have either US , UK or Australian approval status due in part to

- the lack of reliable standards for spiking studies and assessments
- Lack of reference standard methodologies for assessment of performance
- **New Standard** –British Standards Institution has just published a new standard  
08/30182372 DC - BS EN 15842. Foodstuffs. Detection of food allergens. General considerations and validation of methods
- **Health Canada** – The Compendium of Food Allergen Methodologies
  - [http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/allergen/index\\_e.html](http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/allergen/index_e.html)

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## Published Data and Approvals

- **Gluten- Tepnel BioSystems, Ltd. BioKits Gluten Assay Kit**  
– **AOAC Official Method** for food, meat **N.B 160 ppm or above** .
- Codex Alimentarius Commission have accepted the RidaScreen Gliadin test method (using the R5 monoclonal antibody) as a Codex Defining Method of Analysis (Type I).
- **Gluten- R Biopharm Rida screen Gliadin – AOAC approval –**
  - RIDASCREEN® Gliadin test at 5 ppm
- **Neogen, R Biopharm, Tepnel**
  - AOAC-RI Approval for peanut detection.
- Food Safety and Quality  
European Commission  
Institute for Reference Materials and Measurements  
(EC – JRC – IRMM)

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## Papers and Accreditation

- International proficiency programs are now available for peanut, gluten ,milk , hazelnut and egg.
- NATA has accepted peanut allergen testing and Gluten analysis as valid analytical areas and accreditation is available with compliance with normal NATA criteria, verification data and on site assessment
  - Currently only for peanut and gluten and only in a limited number of matrices .
- **Use of Detergents and Sanitizers to remove Proteins ( allergens) from Food Contact Surfaces** by Christian A. Remus Johnson Diversey
- **Food Allergen detection methods and the challenge to protect food allergic consumers**
  - Arjon J.van Hengel Anal Bioanal.Chem (2007) 389:111-118 E.C., Directorate General Joint Research Centre , IRMM Belgium
- **Cleaning and Other Control and Validation Strategies To Prevent Allergen Cross-Contact in Food-Processing Operations**
- **Authors:** Jackson, Lauren S.<sup>1</sup>; Journal of Food Protection®, Volume 71, Number 2, February 2008 , pp. 445-458(14)
- **Detection of Allergens in Food** – Stef Koppelman and Sue L .Hefle

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## Allergen Analysis



- Approach is currently loosely based on microbial principles
  - Utilise the same criteria in terms of representative sampling
  - Risk based sample numbers
    - Visually clean is insufficient to ensure allergen carry over will not take place
    - Microbiologically clean – not allergenically clean



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## Appropriate Application of Analysis

- Investigative or Preventative Measures
  - Identify a suspect process
  - Isolate a cross contact point
  - Identify a contaminating ingredient
  - Raw Material Control - Confirm a client COA
  - Identify the volume of push thru product required
- Reactive Measures
  - Clarify a consumer complaint
  - Identify an unintentional inclusion of allergenic material

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## Analysis



- Validation Measures
  - Validate cleaning
  - Examine finished product
  - Verify control procedures are effective
  - Verify an allergen free claim
    - ( other processes and procedures required )
  
- To differentiate between real and potential risk

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## Appropriate Analysis

- Raw material
- Production surfaces
  - Swabs - presence / absence – Don't push it !
- Cleaning solutions
- Finished products
  - Protective clothing
  - Cleaning cloths

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## Targeted Analysis



- In order to obtain value from testing you need to know what you are looking for
  - Consider the allergen of concern
    - Blanket testing is expensive and in many cases unnecessary
  - It is important to target the correct allergen
  - It is important to consider the nature of the matrix



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## Appropriate Analysis

- Issues to consider
  - Allergen must be in detectable form
    - Most kits are not suitable for detection of highly hydrolysed protein
  - Heat Treatment
    - in some cases may render the allergen undetectable but still present in the food and **STILL** allergenic.



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## Appropriate Analysis

- If protein fraction is removed during processing  
Protein fraction may be the target of the detection system therefore kit no longer detect the allergen but food remains allergenic
  - E.g. soy sauce
    - Fermented – reduces allergenicity but some retained
    - Refined oils
  - ELISA Systems kit for Soy residue – kit inserts state that hydrolysed soy may not be detected .(Herrian et al 1993)

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## Kits

- The kit must be designed to detect the allergen of interest
  - E.g. a Casein kit will detect casein but not whey .
  - Milk kits can look for Casein, Beta Lactoglobulin or both
- Different kits for the same allergen “look” for different allergenic proteins
  - Tepnel – Conarchin
  - ELISA Systems – Ara h1 and Ara h2
- ELISA Systems Soy kit detects soy trypsin inhibitor and soy flour proteins
- Both ELISA SYSTEMS and Tepnel soy kits can cross react with chickpeas
- Tepnel Sesame kit – detects seed storage protein
  - sesame seeds need to be crushed to make detectable protein available
- Specific challenge for each allergen

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## Matrix Issues

- The nature of the matrix will impact on the suitability of the assay
  - Tannins
  - Polyphenols
  - Salts , sugars, phosphates,
  - Oils
- Production facility provides co packing for a range of products including their own
  - Production of a sauce base with almond meal
  - Scheduled to produce and pack a minced herb product
  - Determine whether ELISA techniques appropriate to analyze finished product



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## Informed testing where kits are not available- Modeling



- Model cleaning validations around currently used allergens
- Characteristics differ so need to consider the nature of the allergen
- Use a component of similar consistency
- Use a component of significant proportion in the product
- Use a component for which there is a validated test kit with a credible pedigree

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## Post Production Cleanup Failure- Potential versus Real Risk



- Production Facility – produced peanut containing product – adherence to cleaning protocol questioned . Equipment visually clean
- Suspect Sauce produced post peanut product , post clean sample submitted – sample positive ( greater than 25 ppm peanut )
- Retained alternative product used as neg control – negative
- Confirmed suspicion that inappropriate cleaning had occurred .

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## Scheduling Failure

- Schedule indicates that egg containing product produced after egg allergen free product
- Testing confirmed scheduling failure – egg present at high levels indicating egg product produced first with insufficient cleaning to remove although equipment visually clean.
- Egg containing product - > 25 ppm .
- Post egg product 2.5 – 5 ppm .

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## Sampling Issues

- Distribution is not homogenous
  - May concentrate in the first part of the run
  - OR
  - May be impacted by hang ups in the system which result in "random" dumping of allergen
    - Associated with worn gaskets, valves , washers
- May be particulate and therefore distributed irregularly
- May be due to inadequate cleaning
  - Use known microbial hot spots
  - testing for presence of allergens cannot be regarded as a substitute for the precautions and preventive measures



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## Suggested Testing Regime

Comprehensive Food Allergen Verification ( fifth edition )

- Mixed facility with peanuts as an ingredient
  - Supplier required to verify non peanut ingredients as peanut free
- AND
- **Testing of raw material**
  - Every 5 th load of incoming raw material **screened** tested for peanut allergen – not accepted until negative result received .
- Testing of Food contact surfaces**
  - Post peanut biscuit , processing equipment cleaned
  - Post clean and prior to next run each piece of equipment swabbed for peanut residue and **screened**. . If positive recleaned and swabbed and tested again .
- **Quantitative testing** –
  - Final product testing

»Neogen corporation and FARRP

»( Food Allergy Research and Resources Program )



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## Application



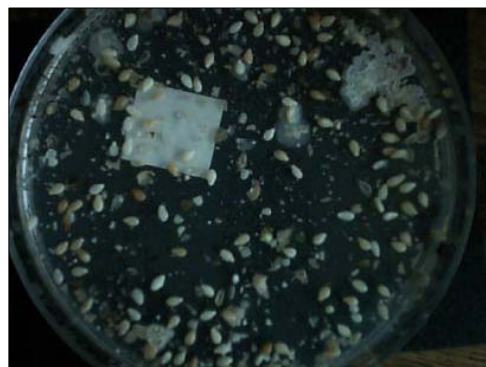
- Air monitoring – IAQ modifications
- Settle plates for aerial contamination and investigation of dissemination
- Swabbing of surfaces



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## Settle Plates



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## The Package Approach

- from atp to pcr and all the acronyms in between !!
  - Use atp or alternatives to monitor cleaning effectiveness in real time
  - Use pre and post clean swabs or test strips to examine allergen related cleaning effectiveness
  - Examine finished product with ELISA to confirm allergen status
  - Confirm

“ unexpected results “with pcr



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## The Future and Emerging Technologies

- Biosensors
  - Immobilisation of a target molecule to a chip or sensor
  - Binding interaction can be measured quantitatively
  - Detection and quantification measured by changes in refractive index .
  - short analysis time and highly automated
- Improved and expanded ELISA based technologies –
  - rapid screening of surfaces
- Improved and more cost effective PCR and Real Time PCR
- Nanotechnology
- Technology
  - Reliable techniques for use on line or at real time
  - Cost effective confirmation techniques
  - Increased sensitivity

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## An Australian Standard

- Widespread use of the kits requires standardisation for obvious reasons
- Two types of kits with AOAC approval
  - Peanut
  - Gluten
- Australian standard under development
  - FT-024-00-03 - Allergens Working Group
  - Allow adaptation and application to emerging technologies
- Standard is only the beginning



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## Considerations

- Requirement for agreement and recommendation for sampling plans
- Guidance on sampling procedures
- Information and guidance on application to industry
- Uniformity in reporting of results
- VITAL -under the Allergen Bureau
  - new working group to investigate the application of analysis to Action levels – include industry analysts , private analysts , kit producers – both national and international
- New technologies change the proteins and therefore make it difficult to detect i.e. gamma radiation
- New R and D – derivatives of celery add flavour but highly modified may not be detectable



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## Food Allergen Control Training Analysis

formed to provide

- expert advice and consultative services on food borne allergens for the food manufacturing, food service and associated supply industries.
- specialist training in food allergen awareness
- specialist analysis services for food borne allergens
- consultation in strategies for allergen control within factory and food service environments.

experience in this area over the past five years under the umbrella of EML Consulting Services Qld and hold NATA accreditation under EMLQ.

We are passionate about the opportunity to expand and develop our services to industry.



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