Methods for Food Allergen Detection and Quantitation

LGC

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Assertion

- Testing is now considered to be a ‘minimum standard of care’ for the management of Food Allergens.

- Could many of the problems of allergen management be avoided by **taking control ?**

What are the options for testing ?

Risk Analysis

- **Studies indicate prevalence of food allergy is rising in conjunction with consumer and media awareness**
- Growth in Allergen free market (e.g. US $ 4 Billion by 2008)
- Company's own manufacturing errors are the major reason for product recalls
- The purpose of Risk Analysis ultimately is to identify and implement ways of **"Risk Reduction"**

Allergens responsible for over 90% of all allergic reactions

- Cereals containing gluten
- Crustaceans
- Egg
- Milk/Dairy products (including lactose)
- Fish
- Peanuts
- Soybeans
- Nuts
- Celery
- Mustard
- Sesame seeds

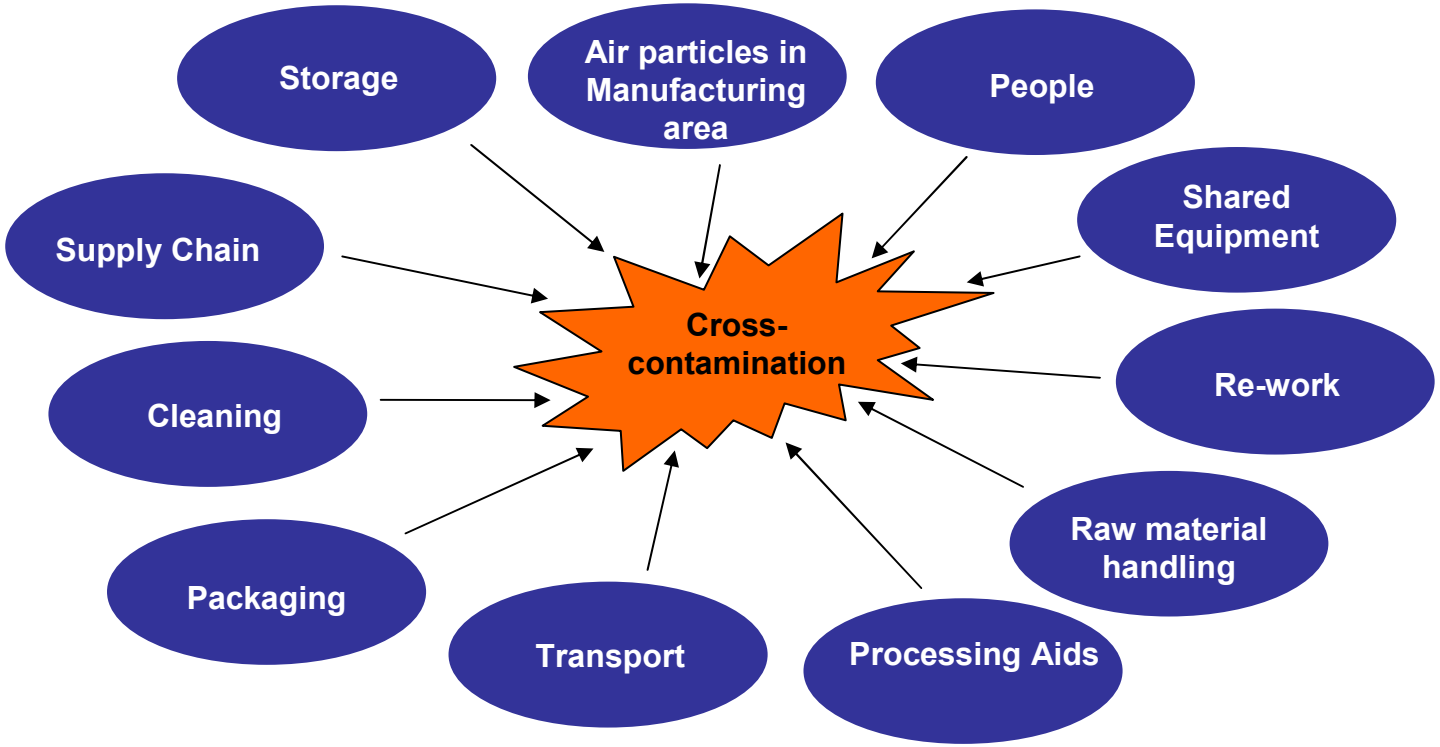
Threshold levels

Allergen*	Milk	Egg	Soya^	Fish	Peanuts	Tree nuts	Sesame Seed	Crustacea	Gluten#
FSANZ Action Level 1 (ppm)	<5	<2	<10	<20	<2	<2	<2	<2	<20
FSANZ Action Level 2 (ppm)	5 - 50	2 - 20	10 -100	20 -200	2 -20	2 -20	2 -20	2 -20	20 -100
FSANZ Action Level 3 (ppm)	>50	>20	>100	>200	>20	>20	>20	>20	>100

*mg/kg (ppm) of total protein
Gluten includes all Gluten type proteins as defined in the Food Standards Code
^ Action levels for Soy is highly conservative

The VITAL Action Levels are:
Action Level 1 – precautionary cross contact statement is not required for the relevant allergen under evaluation.
Action Level 2 – precautionary cross contact statement is required for the relevant allergen using the standard VITAL statement.
Action Level 3 – significant levels of the allergen are likely to be present. Labelling of the relevant allergen as present is appropriate.
The VITAL cross contact statement is “May be present XXX”

Sources of Cross Contamination



Source: UK Food Standards Agency, 2006

Methods

- **Physicochemical methods**
  - Kjeldahl nitrogen
  - High performance liquid chromatography (HPLC)
  - Mass spectrometry
  - Capillary electrophoresis
  - Polymerase chain reaction (PCR) for allergen-specific DNA
- **Immunological methods**
  - Immunodiffusion
  - Counterelectrophoresis
  - Radioimmunoassay
  - Enzyme-linked immunosorbent assay (ELISA)
  - Radioallergosorbent inhibition
  - Immunoblotting

Available Methods

ALLERGEN	TEST KITS			SERVICE
	Hand-held test	ELISA	DNA	External Lab
Celery			✓	✓
Crustacea / Shellfish	✓ *	✓*	✓	✓
Egg	✓ *	✓ *		✓
Fish			✓	✓
Gluten	✓ *	✓ *		✓
Lupin		✓	✓	✓
Milk	✓ *	✓ *	✓	✓
Mustard			✓	✓
Peanuts	✓ *	✓ *	✓	✓
Sesame		✓ *	✓	✓
Soya		✓	✓	✓
Sulphites				✓
	Tree Nuts			
- Almond	✓ *	✓ *	✓	✓
- Hazelnut	✓ *	✓ *	✓	✓
- Walnut		✓ *	✓	✓
- Others			✓	✓

* Environmental swabs have been validated for testing with these methods

Tepnel
Research Products & Services

Criteria for Laboratory tests

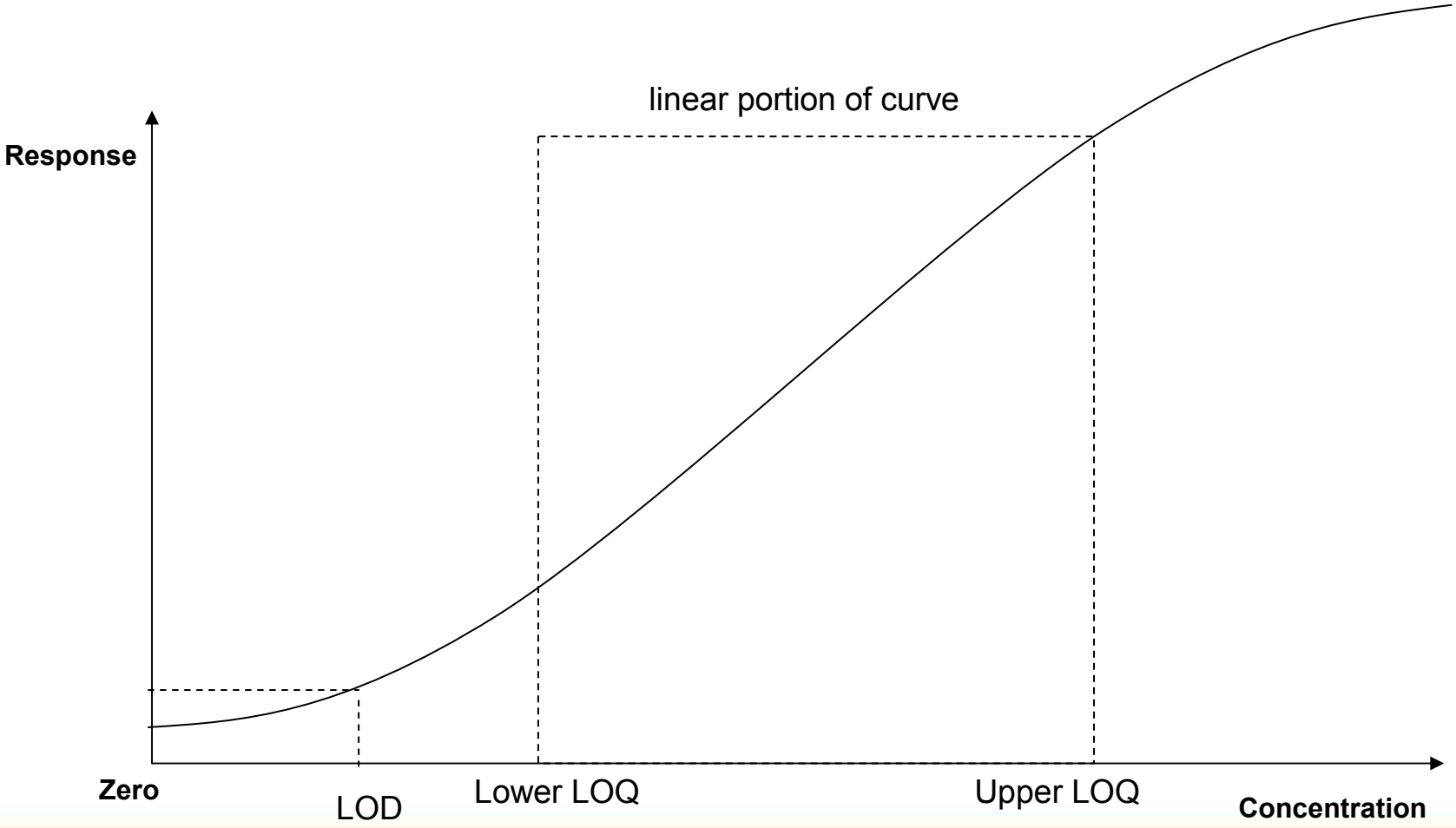
1. Specificity	Target identified & any cross reactivities
2. Units of measurement	Food protein, food residue, DNA
3. Limit of Detection (LOD)	Theoretical or diluted sample extract
4. Limit of Quantitation (LOQ)	Reliable Standard curve
5. Sensitivity	Ability to differentiate low from high levels
6. Validation	Precision, matrix effects, robustness
7. Approvals	Laboratory and / or test kit, Proficiency Trials

Calibration based on defined reference material

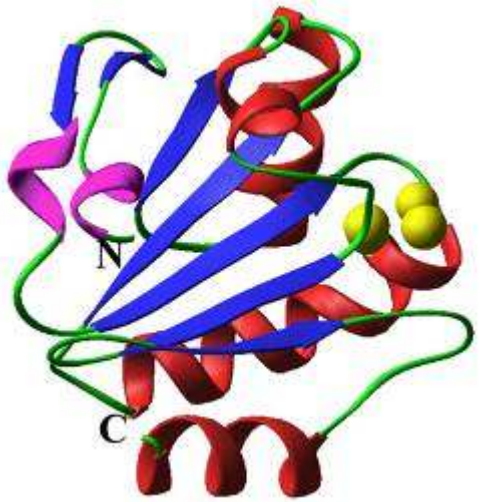
Analytical Recommendations

- Decide how much sampling & how much testing?
- Ensure test portions are as homogenous as possible
- Avoid cross contamination during sample preparation
- Ensure assay steps are followed as outlined in insert – particular attention to shaking incubations
- Always perform spike recovery experiments when testing new or unusual matrices

How Sensitive ?



Protein v Food Residue



versus

CEN/TC 275/WG 12
Food allergens

The method shall be validated for the matrix to be analysed and standards available for establishing a standard curve from which calculation of the protein content in test samples is performed.

Immuno-reactivity v. Allergenicity

Three major allergens recognised by 50% allergic individuals: Ara h 1, Ara h 2 & Ara h 3/ h 4.
Minor ones: Ara h 5, h 6 & h 7.

Peanut comprises >20% – <40% protein; 20 – 25% of this is target protein.
Assumed "target protein" composition of 10% w/w

Arachis hypogea: Runner, Valencia, Spanish, Virginia

Assay detects: Ara h 1 (Conarchin)

BIOKITS Peanut Assay Standard Curve

SPECIFICATION (SOP 1K022)	RESULT / O.D.	COMPLIANCE
MEAN NEGATIVE	**0.177**	**COMPLIES**
MEAN STANDARD 1	**0.350**	**COMPLIES**
MEAN STANDARD 5	**2.673**	**COMPLIES**
MEAN REFERENCE CONTROLS (-VE'S)	**0.132**	**COMPLIES**
MEAN BISCUIT CRUMB	**0.171**	**COMPLIES**
MEAN SPIKE CONTROL (PPM)	**4.8 PPM**	**COMPLIES**

Zero OD <0.2

Max Binding OD > 1.5

Standards %CV <20%

Spike SRM2387 ~5ppm

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 030402

BIOKITS PEANUT ASSAY AOAC-RI Validation

LOD	< 0.1 ppm Peanut Content
Reproducibility/Repeatability	Intra Assay <10% CV Inter Assay < 10% CV
Cross reactivity	None (in 40 commodities tested)
Detection of peanut content	Biscuits; breakfast cereal; ice cream; milk choc; plain choc; milled nuts (almonds) 100% correct results
Mean spike recovery	>85 %

*** Samples of this test kit model were independently evaluated by the AOAC Research Institute and were found to perform to the producer's specifications as stated in the test kit's descriptive insert. The producer certifies this kit conforms in all respects to the specifications originally evaluated by the AOAC Research Institute as detailed in Performance Tested certificate number 030402.**

Poms et al. (Aug 02)

280-400%