

Technology – different allergen detection methods

- **ELISA** – main test used to detect allergens via detecting the allergenic protein, or specific biomarkers targeted that coexist with the allergen in the product
 - Gluten
 - Soy
 - Peanut
 - Milk
 - Egg
 - Tree Nuts*
- **Lateral Flow** – mainly used for checking machinery is allergenic free, and as a back up in case there are no ELISA kits available to test for a allergen
- **PCR** – used as a alternative to ELISA and when no ELISA kits available or reliable

*Tree Nuts have to be separated into the individual nut test



Tree Nut – what we can and can not detect

We can currently detect

Almond
Brazil Nut
Cashew
Hazelnut
Sesame
Pistachio
Walnut

We cannot detect

Beechnut
Bush nut
Butternut
Chestnut
Coconut
Fibert
Ginko Nut
Hickory Nut
Lichee nut
Macadamia Nut
Nangai nut
Pecan
Pine
Shea nut



Allergen kits – what to look for

- **Detection limits**
 - ppm on total allergen or allergen protein
- **Matrix interference – ability to detect on the food matrixes tested**
 - Cross contamination
 - Milk on egg white matrix
 - False negatives
 - Crustacean test of molluscs
- **Safety**
 - MSDS on the chemicals used in the allergen kit



Allergen kits – what to look for

- **Validation information**

- Tested on a wide range on matrixes

Matrix	OD	(mg/kg) Milk Protein
α-lactalbumin	0.140	<LOQ
lactoferrin	0.125	<LOQ
wheat flour	0.090	<LOQ
oat flour	0.093	<LOQ
rice flour	0.092	<LOQ
maize flour	0.080	<LOQ

Limit of detection (LOQ)

- Spike recoveries

Sample Matrix	Spike material	Calculated as milk protein (mg/kg)	Milk Protein concentration measured (mg/kg)	Recovery (%)
Spikes with milk powder				
Heated samples				
bread	NIST SRM 15:49 SMP	381.0	429.2	112.7
mixed ground meat	NIST SRM 15:49 SMP	463.0	482.3	104.2
white bread	NIST SRM 15:49 SMP	734.0	588.5	80.2

These are small selections of R-Biopharm's Quick Milk ELISA validation report



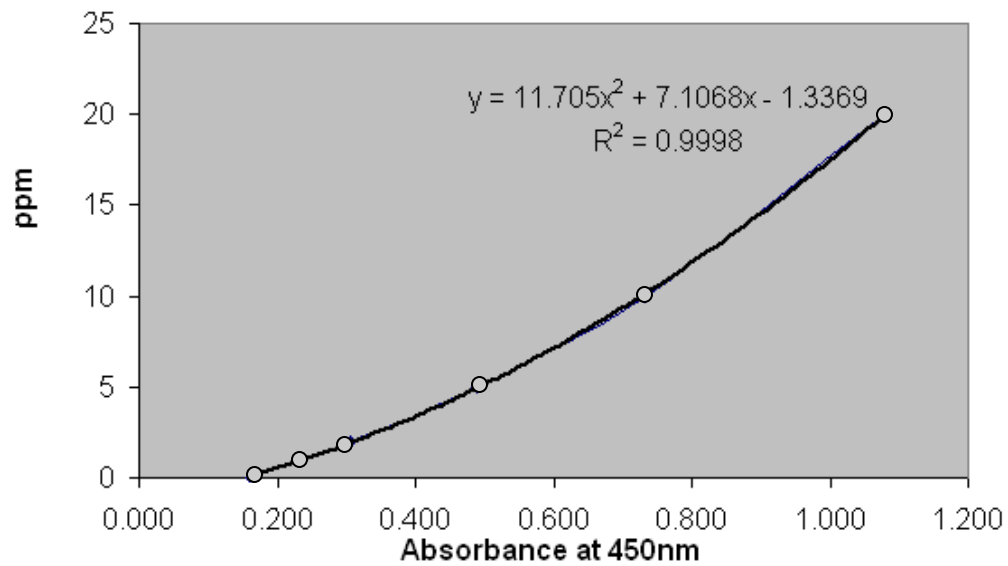
Results - Interpretation and Validation

- **Validate all the standards and 3 samples internally before we can start running the kit**
- **Internal and external standards as well as a spike solution**
- **Calculate the internal measurement uncertainty (MU) on all the absorbance readings for the standards and the end allergen result**
- **Validate any new sample/matrix which isn't of the manufacturers validation report**
- **Allergen results worked out on a calibration curve must be run for every batch of allergens**



Results - Interpretation and Validation

- Run a calibration curve each batch



Problems with methods and processes

- **Matrix interference**

- Gums and gels
- High fat samples

- **Processed foods**

- To break down lattice structures and extract the allergen
- Ability to detect the broken down allergenic marker

- **Cross contamination between kits and samples**

- Soy with gluten and milk
- Cross contamination during preparation
- Extraction solution testing positive for other allergens

- **Presented with a matrix which hasn't been validated from the manufacturer**



Misconceptions

- **A Not Detected (N/D) result doesn't mean that there is no allergen present in the sample**
- **Even though we test a product for allergen, manufacturers still need to have systems in place to stop allergen cross contamination**
- **International and local allergens laws**



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