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 an allergen

- **PCR** – used as an 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

<table>
<thead>
<tr>
<th>Matrix</th>
<th>OD</th>
<th>(mg/kg) Milk Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-lactalbumin</td>
<td>0.140</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>lactoferrin</td>
<td>0.125</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>wheat flour</td>
<td>0.090</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>oat flour</td>
<td>0.093</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>rice flour</td>
<td>0.092</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>maize flour</td>
<td>0.080</td>
<td>&lt;LOQ</td>
</tr>
</tbody>
</table>

Limit of detection (LOQ)

- **Spike recoveries**

<table>
<thead>
<tr>
<th>Sample Matrix</th>
<th>Spike material</th>
<th>Calculated as milk protein (mg/kg)</th>
<th>Milk Protein concentration measured (mg/kg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spikes with milk powder</td>
<td>NIST SRM 15:49 SMP</td>
<td>381.0</td>
<td>429.2</td>
<td>112.7</td>
</tr>
<tr>
<td>Heated samples</td>
<td>NIST SRM 15:49 SMP</td>
<td>463.0</td>
<td>482.3</td>
<td>104.2</td>
</tr>
<tr>
<td>white bread</td>
<td>NIST SRM 15:49 SMP</td>
<td>734.0</td>
<td>588.5</td>
<td>80.2</td>
</tr>
</tbody>
</table>

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

\[ y = 11.705x^2 + 7.1068x - 1.3369 \]

\[ R^2 = 0.9998 \]
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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