Agilent Instrumentation in Biological Food Testing

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Food Safety and Food Quality

FOOD SAFETY AND QUALITY ARE A GLOBAL CONCERN.

Major Areas of Biological Testing:

- Authenticity
- Foodborne Pathogens
- Allergen Detection
- GMO Testing
Foodborne Pathogens & Spoilage

Contamination of food with pathogenic or toxin producing bacteria or funghi are a major concern in food safety.

- Major pathogenic bacteria: E. coli O157, toxin producing E. coli (VTEC, EHEC), Salmonella, Listeria, Campylobacter, Yersinia enterocolitica, Enterobacteriaceae,...

  Recent examples:
  - Egg scandal in the US - contamination with Salmonella
  - Infection through cantaloupe in the US – contamination with Listeria
  - Bean sprouts - E. coli O104 outbreak Germany (EHEC)

Many commercial assays available for pathogen detection validated on the Mx platform (eg. foodproof® kits/Biotecon, Adiafood® kits/AES Chemunex and more)

Spoilage causing organisms are a major concern in food quality.

- Spoilage organisms include funghi (eg. aspergillus, yeast), bacteria (eg. Clostridia, Pseudomonas)
- Testing is important for the beverages and canned/preserved foods industry

Consumer health concerns, outbreak control and quality control drive the need for testing
mPCR - Pathogens in Food
- Salmonella, Shigella and Yersinia pseudotuberculosis

Lane 2: Salmonella: invA gene, 275bp
Lane 3: Shigella: ipah gene, 610bp
Lane 4: Yersinia pseudotuberculosis: inv gene, 440bp

Data by JING Jian-zhou, College of Food and Biological Engineering, Zhengzhou
10.1109/ICBBE.2010.5516375
GMO Detection

- In 2008, 144 GM events in 24 crops had regulatory approval, 33 GM crops are commercialized worldwide.
- Numbers are growing fast: 90+ events are awaiting final development and approval.
- Many countries impose strict rules on planting GM crops or selling GMO containing food and feed products.

**US:** Voluntary labeling

**EU:** Strict approval and labeling requirements:
If more than 0.9% of an approved GMO is present, labeling is required (Novel Food Directive).

**Japan:** Approved GM only, unapproved GM up to 1% in feed, zero tolerance for non-approved GM in food.

- Unauthorized GM events are a problem.
- Currently, QPCR based detection and quantification is the method of choice.

Agilent Technologies
Endpoint detection uncommon due to requirement for quantification if positive

Larger set of markers can be assessed per sample (multiplexing) and well compared to QPCR

Protein based finger-printing method available for transgenic soybeans on the Bioanalyzer
GMO Detection – Endpoint Detection Multiplex Assay for Soya

80bp: soya lectin gene
Monsanto Roundup-Ready Marker:
EPS-Phosphat Synthase (Salmonella)

Reference Material

Agilent Application Note 5988-4070EN
Allergen Detection

Growing concern over health risks by consumption of allergenic compounds in food.

-US: 8 allergens require labeling - Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282, Title II):

<table>
<thead>
<tr>
<th>Milk</th>
<th>Egg</th>
<th>Fish</th>
<th>Crustaceans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree nuts</td>
<td>Wheat</td>
<td>Peanuts</td>
<td>Soybeans</td>
</tr>
</tbody>
</table>

-EU: 14 allergens require labeling - Allergenic foods listed in Annex IIIa of the labeling directive:

<table>
<thead>
<tr>
<th>Gluten</th>
<th>Milk/Lactose</th>
<th>Egg</th>
<th>Fish</th>
<th>Crustaceans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanuts</td>
<td>Soybeans</td>
<td>Nuts</td>
<td>Celery</td>
<td>Mustard</td>
</tr>
<tr>
<td>Sesame Seeds</td>
<td>SO₂ &amp; sulphites</td>
<td>Lupin</td>
<td>Molluscs</td>
<td></td>
</tr>
</tbody>
</table>

Detection by direct (allergenic protein – ELISA or MS) or indirect (DNA - PCR) means...
Soy is used in many food items eg. as replacement for milk

Although indirect evidence soy DNA detection performs equally well as protein based methods

PCR is more quantitative than immunochemical methods even though DNA concentration might not accurately reflect the quantity of allergenic protein
Food Authenticity

**Which species is part of my product?**
- Multiple methods available today
- Protein or DNA based
- Application in analysis of fraud, mislabeling or substitution

**Where does my product or its ingredients come from?**
- Scientifically more challenging – Not readily available
- Trace minerals, fatty acid analysis (MS) or DNA based using polymorphisms
- No general approach available – solutions on a species by species basis
Genomic DNA Isolation: < 1 h (depends on # samples)

Amplification of species ID target (CytB): ~ 1.5 h

Generation of species specific patterns (RFLP): ~ 2.5 h

Pattern analysis using RFLP Decoder Software: ~ 6 h from sample to result
Authenticity – Meat Speciation

- **Multiplexed PCR and 2100 Bioanalyzer detection**

- **It is known that primers used in the Agilent Fish ID solution may also produce results from meat**

- **Pork, wild boar, beef, lamb, turkey and chicken meat was tested**

- **Species specific and meat specific primer pairs have been tested**


Authenticity – Basmati Rice

Analysis of non-basmati/basmati rice admixtures using two microsatellite primer sets.
Over 40 varieties of UK-grown varieties were analyzed. Bioanalyzer files were imported into the Phoretix 1D Advanced and 1D Database (Nonlinear Dynamics Ltd) Software for pattern-matching.

Phoretix 1D Database dendrogram generated from Bioanalyzer electropherogram .csv-files.
Food Authenticity: Milk

Analysis of Admixtures possible by Isoelectric Focussing (IEF):

- α-lactalbumin
- β-lactoglobulin
- β-casein
- α-casein
- κ-casein

[Graph showing peaks and molecular weights]
Future Technologies: Principle of MassCode Detection

1. RT, PCR amplification of target sequences with mass-tagged primers
   - MassCode tagged primers
   - Random nonamers
   - Reverse Transcription
   - PCR

2. Purification of amplicons to remove excess tagged primers & primer dimers
   - DNA purification plate

3. Automated sample injection w/ in-line photo-release of MassCode tags and MS detection
   - Control RNA
   - Target RNA
   - UV cleavage of MassCode tags from amplicons
   - Ultraviolet Light
   - Ionization (CI) & detection

4. Identification of MassCode tags – detection of target
   - Control vs. Target
   - Relative Abundance
   - Mass (amu)
   - Pos. TH
   - Neg. TH

[Diagram showing the process flow with labeled steps and visual representations of each step]
MassCode Applications

- Proof of concept work published in Public Library of Science (PLoS):
  14plex assay designed to subtype a select panel of *Salmonella enterica* serogroups and serovars

- True liquid array no solid support required

- QPCR like setup and analysis overlay facilitates technology access for non-chemists and improves ease-of-use dramatically

- Multiple projects to develop panels for a variety of application fields including food and feed analysis:
  - Pathogen screening and typing
  - GMO screening
Summary of Food Testing Applications

2100 Bioanalyzer – Sensitive Endpoint Analysis
- Small footprint, broad range of robust and high performing assays
- Ability to resolve and analyze complex pattern profiles
- Analysis of multiplex reactions with a large number of markers
- Protein as well as DNA or RNA analysis

MX3005 real-time QPCR and Brilliant Reagents
- Up to 5 targets, quantitative real-time detection
- Examples in GMO, pathogen and allergen detection
- Validated commercial assays available as well as innovative solutions by academic labs

Versatile, Accurate and High Performing Tools
- Agilent delivers tools that can successfully be applied to any food testing application
- Numerous publications in peer-reviewed journals and Agilent application notes available
- Fish Species Identification kit available as workflow solution combining instrumentation and consumables
- MassCode are high potential PCR-MS technologies
Thank you!

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