Methods for Detecting and Measuring Ag Biotech Products

AEIC
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AEIC is pleased to provide the following slide presentation for use in educational or training applications associated with detection methods for biotech products. Due to the size of the file, this presentation is provided as a PDF, which does not allow for any changes in content. For a copy of the presentation on a CD please contact AEIC.

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- EnviroLogix
- GeneScan
- Medallion Labs
- Monsanto
- SDI
- USFDA
Ag Biotech Crops

- **Transgenic plants have:**
  - Novel trait (e.g., herbicide resistance)
  - May express novel protein
  - Novel DNA

- **Novel DNA and protein may be found in:**
  - Plant tissues
  - Seed/Grain
  - Food ingredients and food products
Biotech Crops 2004

- **2 major traits**
  - Insect protection - *Bacillus thuringiensis* (Bt)
  - Herbicide tolerance
    - Roundup Ready (RUR)
    - Liberty Link (LL)
    - Bromoxynil tolerance (BXN)

- **4 major crops**
  - Corn - Bt, RUR, LL
  - Soy - RUR
  - Canola - RUR, LL
  - Cotton - Bt, RUR, Bxn
Testing in Support of Labeling Biotech Foods

- **Consumer Choice**

- **Approved Events – Quantitative and threshold testing**
  - European Food Labeling Law
    - Labeling began April 10, 2000 and updated April 2004
    - Threshold adventitious < 0.9% - “genetically modified”
  - Japanese Food Labeling Law
    - Labeling began April, 2001
    - Threshold guidelines set at 5%

- **Unapproved Events - Detection**
  - Zero tolerance e.g. StarLink
  - European Food Labeling Law
    - Threshold adventitious < 0.5% - "genetically modified"
Determining Concentration of Biotech Ingredients in Foods

- Results are reported in terms of % Ag Biotech
  e.g. 1 Biotech corn kernel in 99 negative = 1%
- Decisions are based on regulated thresholds
  (given in weight %)
- Testing is based on detection/quantitation of novel DNA or protein
- Ag Biotech concentrations are estimated from protein concentration
- DNA can be measured in relative terms, i.e. % Roundup® Ready soybeans with respect to total soybean
Commonly Used Detection Methods

DNA-based methods
PCR

Protein-based methods
Immunoassay (ELISA)
Applications of PCR

Qualitative

- A “YES” or “NO” answer
- Can look for specific DNA event such as RUR, MON810, etc. or
- Can look for generic elements such as NOS, 35S
- Applicable to a zero tolerance situation

Quantitative

- A determination of the percent of GM DNA present
- Relates amount of GM DNA to species DNA
- Real time quantitation by laser during PCR process
- Can be specific or a general screen
PCR – Uses

- Genetic purity testing
- Disease Diagnosis
- Forensic Medicine
- Molecular Evolution
- Gene Cloning
- DNA sequencing
Applications of PCR

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Qualitative Detection

Specific Reaction for Roundup Ready® (RUR) Soy

Detection of Roundup Ready® soy-DNA (128 bp amplicon). The samples RUR soy, whole meal, lecithin, raw oil, and cakes contain genetically modified DNA.
Quantitative Detection

- Real-time PCR measures the amount of PCR product at each and every amplification cycle
- Amplification plot is a curve that represents the accumulation of product over the duration of the entire PCR reaction
- A standard curve is generated that plots the cycle threshold values against starting amounts of DNA
- Measurement of DNA is done by fluorescing DNA molecules (TaqMan™, Molecular Beacon, SYBR Green)
PCR - Advantages

- High sensitivity
- Can detect and quantify specific traits
- Capable of detecting groups of traits through the use of common genetic elements (e.g. promotor or terminator)
- Higher stability of DNA (than of proteins) permits analysis of most mixed and processed foods
PCR - Disadvantages

- High cost per determination
- Requires sophisticated equipment and procedures
- Requires highly skilled and well trained personnel
- PCR reactions can be extremely sensitive to low levels of contaminating DNA template that will result in false positive reactions.
  - “Accidental PCR Template or amplicon carry-over”
    (e.g. Reagents, pipettes, tips, fingers)
  - 10 pg of contaminating target DNA can produce 1 μg of product (25 cycles)
- Standardization across labs and protocols is still under discussion
- Labor intensive steps, needs time to complete (3 days or more)
Immunoassays

- $6 Billion Industry Worldwide
- 2.5 Billion Tests Sold Annually
- Highly Quantitative
- Recognized by Regulatory Agencies
- Flexible Test Formats
- Diverse Markets and Applications
Other Immunoassay Markets

- Agricultural
- Environmental
- Veterinary
- **Food**
- Industrial
- Pharmaceutical
- Water Quality

- Microorganisms
- Allergens
- Hormones
- Toxins
- Pesticides
- Antibiotics
- Species identification
- Food adulteration
- Quality
Clinical Diagnostic Immunoassays

- In Use >30 Years
- Basis for Critical Human Health Decisions
  - Disease diagnosis (AIDS, Hepatitis, PSA)
  - Therapeutic drug monitoring
  - Drug abuse screening
  - Over 70 clinical analytes tested by immunoassay
  - Home pregnancy tests
- Highly Reliable
Reactivity of 2 Different ELISA to RUR Toasted Soy Meal

Soymeal was toasted for 60 min. at 100 °C

\[ y = -0.1567x^2 + 1.3795x + 0.0656 \]

\[ R = 0.9999 \]
Reactivity of 2 Different ELISA to RUR Toasted Soy Meal

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Antibody detects toasted soymeal
Antibody not selective for toasted soymeal

0.1% LOD
Advantages of Immunoassay Methods

- Directly measure biologically active protein of interest
- Reliable
- Quantitative analysis
- Qualitative analysis
- High sample throughput
- Easy to perform and transfer to other laboratories
- Widely accepted method by regulatory agencies
- Established use in food industry
- Cost effective
- Timely Analysis
Disadvantages of Immunoassay Methods

- No single method will detect all biotechnology-derived products (DNA or protein-based)
- Methods are trait specific and must be validated for each matrix (DNA and Protein-based)
- Limited to use of protein containing processed ingredients and final food products
- Some products may not express a detectable protein in grain
- Antibodies may cross react
Summary of Protein Testing by Immunoassay

- Immunoassays are quantitative analytical methods
- Flexible format provides for diverse applications
- Commercial methods are available for detection of biotech products
  Raw agricultural commodities
  Processed food fractions
- Methods have been validated internationally
- Accurate, reliable and timely analysis
Biotech Immunoassay Method Validations

- **Collaborative studies**
  - AACC
    - MON810 Cry1Ab ELISA – ground corn
    - StarLink Cry9c ELISA – corn flour and meal
  - Joint Research Centre, European Union
    - Roundup Ready® ELISA
      - IRMM ground soybean certified reference materials
      - Soy toasted meal, protein isolate, defatted flakes
  - FDA
    - StarLink Cry9c ELISA – processed food fractions

- **USDA Certification**
  - Cry9c strip tests – corn kernels
  - Cry9c ELISA - ground corn, meal, flour
  - CP4EPSPS Strip tests - ground corn, soybeans
Factors Effecting Use of Protein Immunoassays

- No protein – no Immunoassay
- Very low level expression (e.g. Bt 176)
- Crossreactivity (e.g. GA21 Roundup® Ready corn)
  - Modified corn EPSPS – 2 amino acids of 445 different from native corn EPSPS
Factors Effecting Quantitation Using Immunoassays

- Method performance characteristics (e.g. precision, accuracy)
- Variability of protein expression levels
  - Within an event
  - Between events expressing same protein (e.g. Cry1Ab)
- Varied effects of sample processing on protein conformation and antibody binding
## Commercially Available Protein-based Tests For Commercial Ag Biotech Traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>Agdia</th>
<th>EnviroLogix</th>
<th>Neogen</th>
<th>SDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP4 EPSPS</td>
<td>E,S</td>
<td>E,S</td>
<td>S</td>
<td>E¹,S</td>
</tr>
<tr>
<td>Cry1Ab</td>
<td>E,S</td>
<td>E,S</td>
<td>E,S</td>
<td>E²,S</td>
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<tr>
<td>Cry9C</td>
<td>E³,S</td>
<td>E,S</td>
<td>E,S</td>
<td>E³,S</td>
</tr>
<tr>
<td>Cry2A</td>
<td>E,S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cry3A</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK603</td>
<td>S</td>
<td></td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>


E  ELISA
S  strip

¹validated by JRC ring test for Roundup Ready  Soy bean
²validated by AACC ring test for YieldGard  (Mon810) Corn
³official method AACC international collaborative trial
Advantages of Immunoassay Methods

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- Quantitative analysis
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Clinical vs. Environmental Immunoassay

The Sample

- Clinical
  - Urine, blood, saliva

- Environmental, Agriculture (more diverse matrices)
  - Water
  - Soil extracts
  - Plant extracts
  - Animal products/tissues - blood, urine, milk, meat
  - Food
  - Industrial processes and effluents
Monoclonal vs. Polyclonal Antibodies

<table>
<thead>
<tr>
<th>Monoclonal</th>
<th>Polyclonal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot-to-lot consistency</td>
<td>Lot-to-lot variability</td>
</tr>
<tr>
<td>Indefinite supply</td>
<td>More broadly reactive</td>
</tr>
<tr>
<td>Highly specific</td>
<td>Often more sensitive</td>
</tr>
<tr>
<td>Longer lead time</td>
<td>Shorter lead times</td>
</tr>
<tr>
<td>Higher initial costs</td>
<td>Lower initial costs</td>
</tr>
</tbody>
</table>

Selection is based on application, time and money
Immunoassay Formats

- **ELISA, Striptests, Western Blot**

- **Each format has advantages and disadvantages**
  - Fully automated – clinical analyzers
  - Laboratory kits - ELISA
  - Field tests – “Strip tests”
  - ‘Research’ methods – Western blot

- **Choice of method is determined by specific application**
  - Performance specifications
  - Ease-of-use (user training)
  - Testing location
  - Cost per test
  - Batch size, testing frequency
  - Turnaround time
  - Equipment costs
## Comparison of PCR, ELISA and Strip Methods

<table>
<thead>
<tr>
<th></th>
<th>PCR</th>
<th>ELISA</th>
<th>Strips</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Design</strong></td>
<td>DNA/Line specific</td>
<td>Protein specific</td>
<td>Protein specific</td>
</tr>
<tr>
<td><strong>Requirements</strong></td>
<td>Sophisticated</td>
<td>Moderate</td>
<td>Easy</td>
</tr>
<tr>
<td><strong>Assay time</strong></td>
<td>3-14 days</td>
<td>2-7 days</td>
<td>5-10 min</td>
</tr>
<tr>
<td><strong>Sample cost</strong></td>
<td>$400-600</td>
<td>$100+</td>
<td>$7-10</td>
</tr>
<tr>
<td><strong>Availability</strong></td>
<td>Published/Testing Co</td>
<td>Diagnostic Co.</td>
<td>Diagnostic Co.</td>
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<tr>
<td><strong>Validation</strong></td>
<td>JRC (qualitative)</td>
<td>JRC/USDA</td>
<td>USDA</td>
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<tr>
<td><strong>Application</strong></td>
<td>Qualitative/semi-quant</td>
<td>Qual/semi/quant</td>
<td>Qual/ test/</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>compliance with</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>threshold</td>
</tr>
<tr>
<td><strong>Limitations</strong></td>
<td>Basic pH, heat, false pos,</td>
<td>heat, extraction,</td>
<td>Same as ELISA</td>
</tr>
<tr>
<td></td>
<td>false neg, dynamic sys</td>
<td>reference materials</td>
<td>Pro-zone,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Protein dependent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 sample/test</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>~&gt;0.1% GMO</td>
<td>~&gt;0.3% GMO</td>
<td>~1% GMO</td>
</tr>
</tbody>
</table>