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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.

AEIC wishes to acknowledge the following companies and organizations for their contributions to the slides included in this presentation:

- 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"



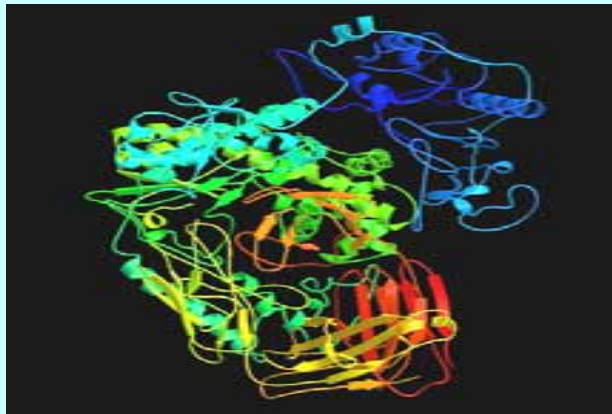
Determining Concentration of Biotech Ingredients in Foods

- **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**

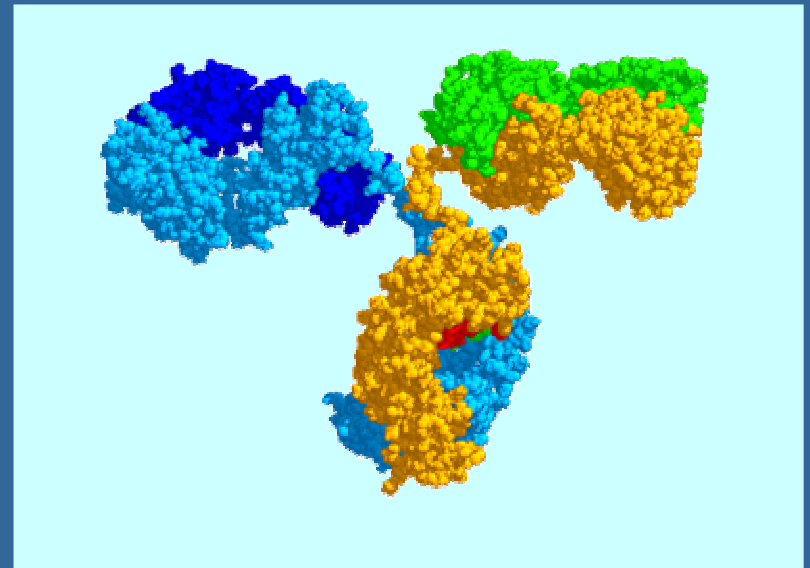


Commonly Used Detection Methods

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**



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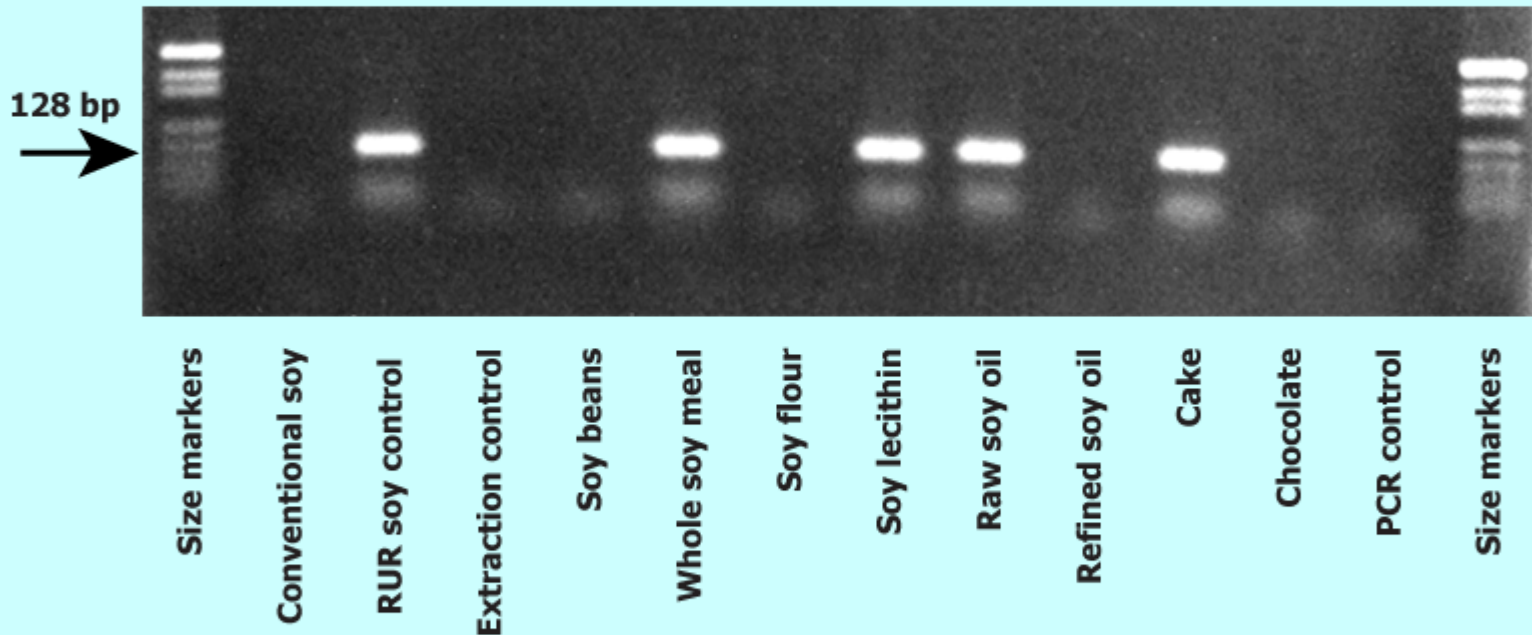
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Qualitative Detection

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 μ g 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

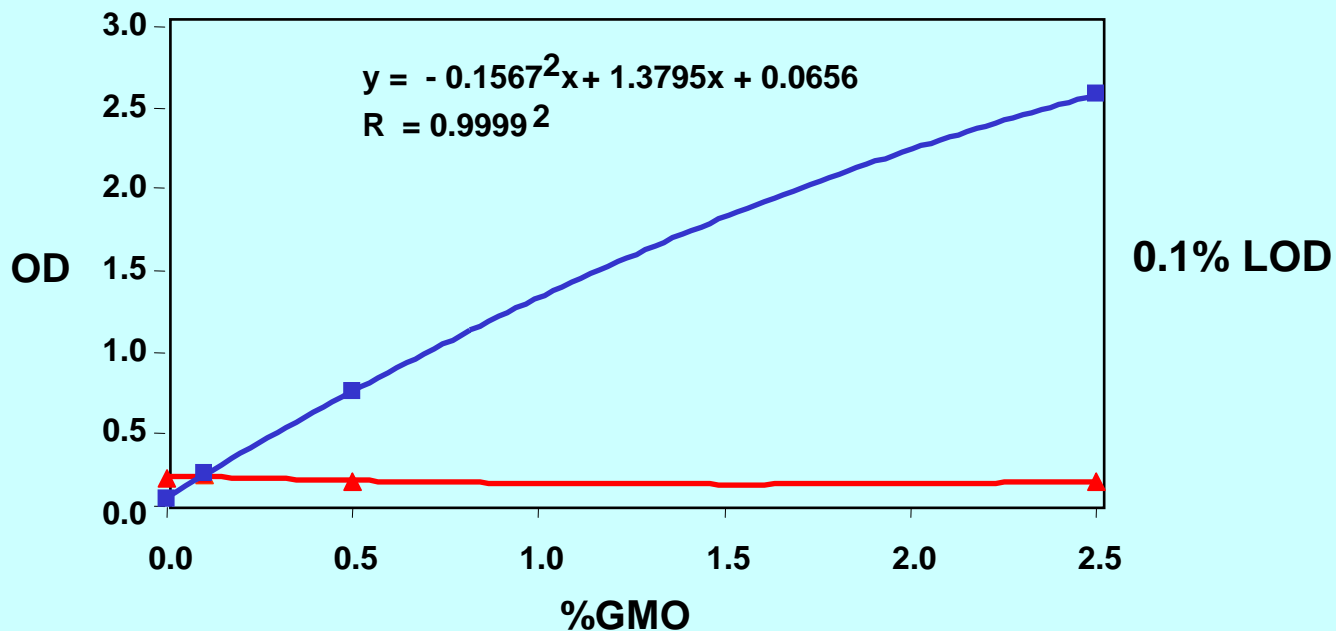


Clinical Diagnostic Immunoassays

- **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

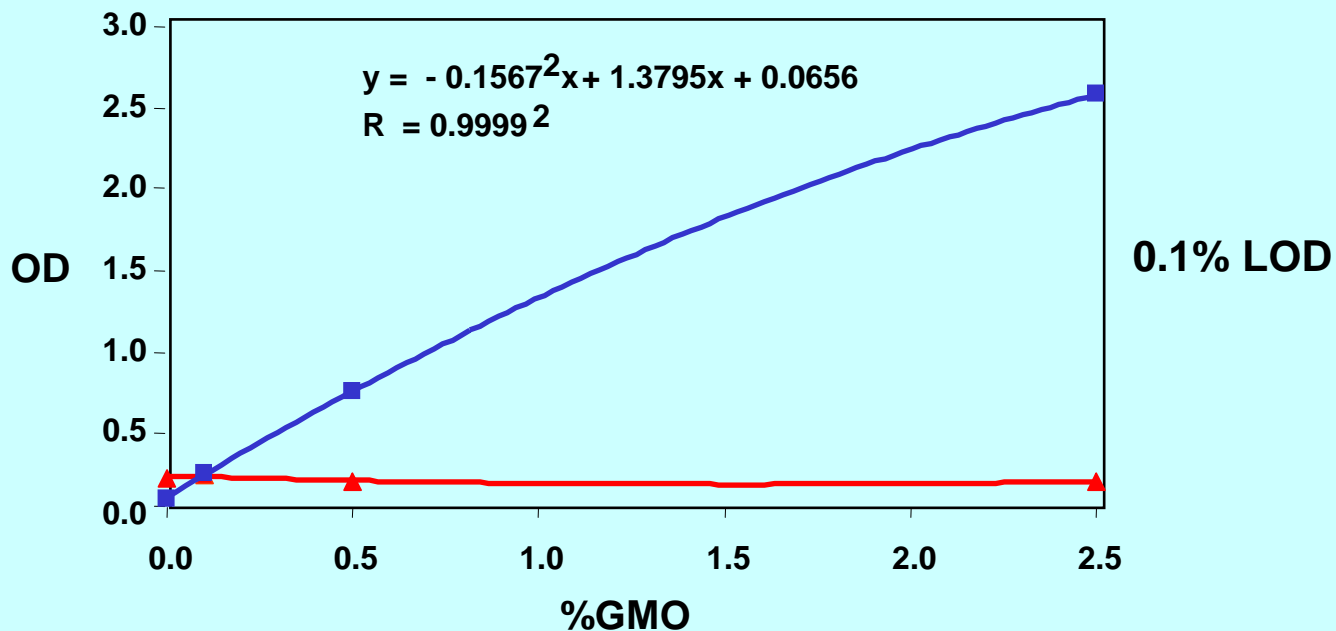


- Antibody detects toasted soymeal
- Antibody not selective for toasted soymeal

Soymeal was toasted for 60 min. at 100 °C



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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

Trait	Agdia	EnviroLogix	Neogen	SDI
CP4 EPSPS		E,S	S	E ¹ ,S
Cry1Ab	E,S	E,S	E,S	E ² ,S
Cry9C		E ³ ,S	E,S	E ³ ,S
Cry2A		E,S		
Cry3A	E			
NK603		S	S	S

www.agdia.com

www.envirollogix.com

www.neogen.com

www.sdix.com

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



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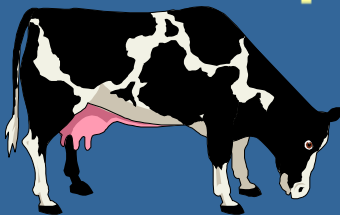
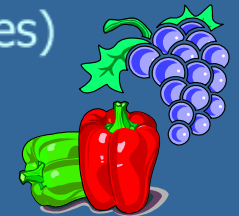
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Clinical vs. Environmental Immunoassay

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

Monoclonal

- Lot-to-lot consistency
- Indefinite supply
- Highly specific
- Longer lead time
- Higher initial costs

Polyclonal

- Lot-to-lot variability
- More broadly reactive
- Often more sensitive
- Shorter lead times
- Lower initial costs

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

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

