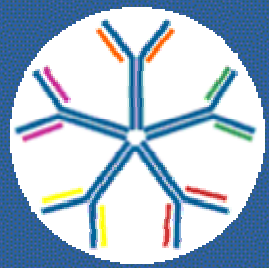
Methods for Detecting and Measuring Ag Biotech Products



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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- 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

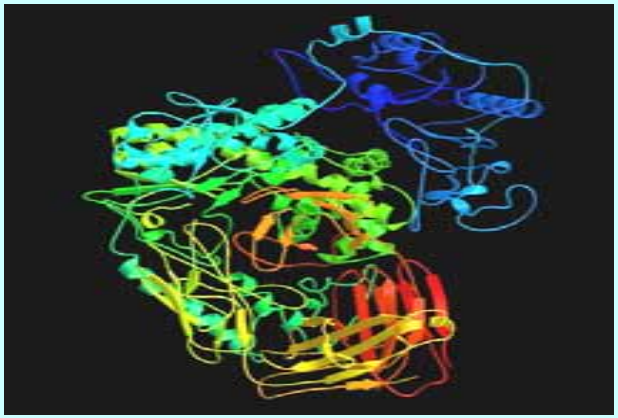
- **Consumer Choice**
- **Approved Events – Quantitative and threshold testing**
  - European Food Labeling Law
    - Labeling began April 10, 2000 and updated April 2004
    - Threshold adventitious < 0.9% - "genetically modified"
  - Japanese Food Labeling Law
    - Labeling began April, 2001
    - 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

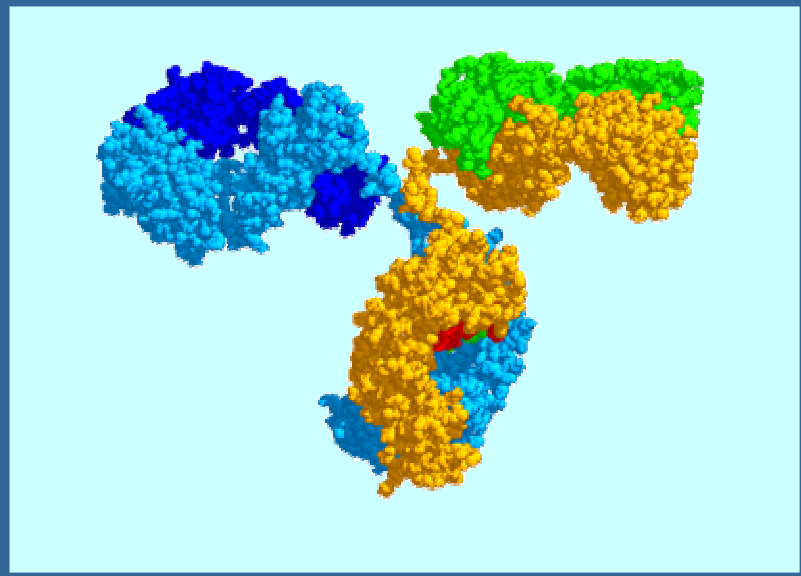
- **Results are reported in terms of % Ag Biotech**
  e.g. 1 Biotech corn kernel in 99 negative = 1%
- **Decisions are based on regulated thresholds (given in weight %)**
- **Testing is based on detection/quantitation of novel DNA or protein**
- **Ag Biotech concentrations are estimated from protein 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

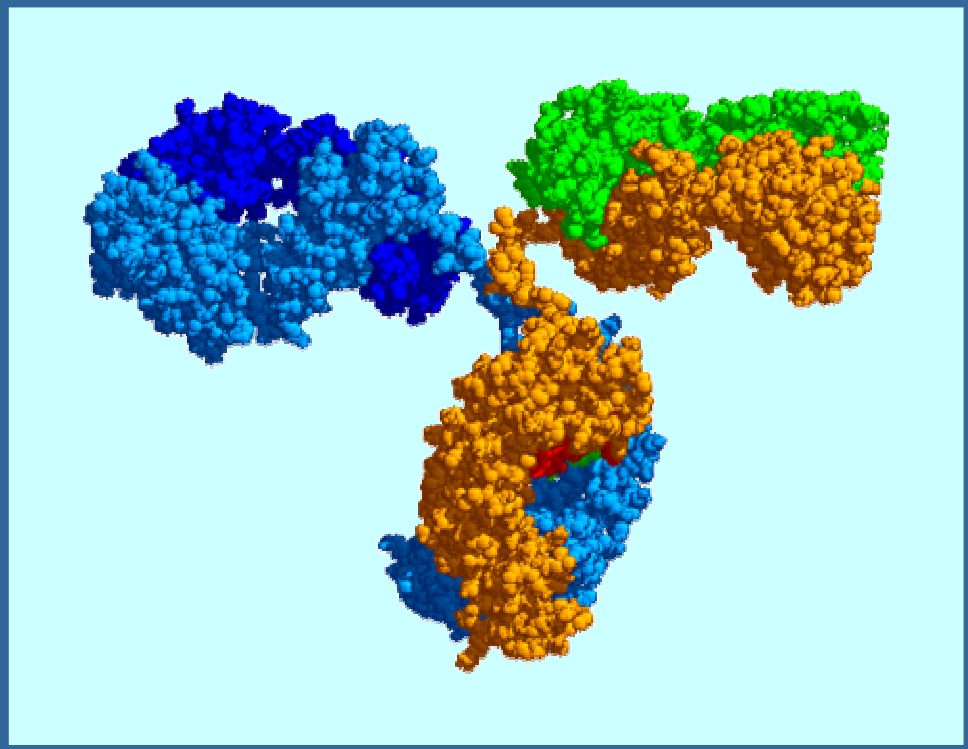


Protein-based methods
Immunoassay (ELISA)



Immunoassay

An immunoassay is an analytical method which uses antibodies as reagents to quantitate specific analytes (proteins)



Tertiary Structure of an Antibody Molecule

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

Lateral Flow Format

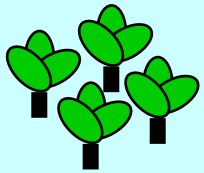
- 5 minute yes/no results
- Simple procedure for testing anywhere

Microwell Plate Format

- Quantitative
- 1-2 hours
- Plate reader required

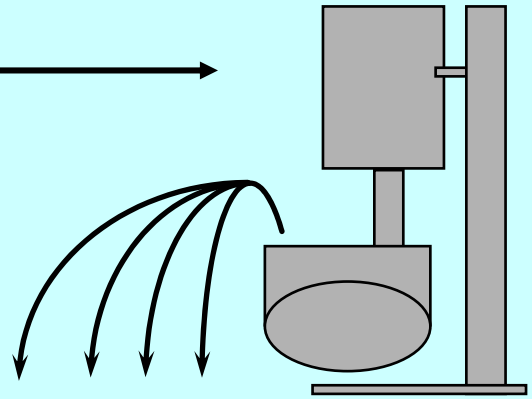
Steps in Determining Protein Expression by ELISA

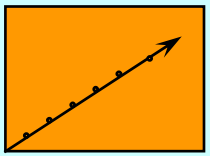
Step 1
Sample plant tissue



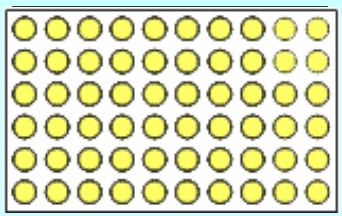
Step 2
Weigh Sample Aliquots

Step 3
Extract Samples

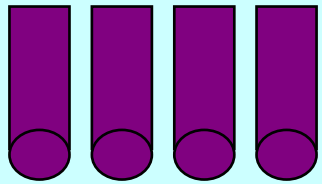




Step 6
Data analysis



Step 5
ELISA Analysis



Step 4
Aliquot extracts for analysis
and sample back-ups

Advantages of Immunoassay Methods

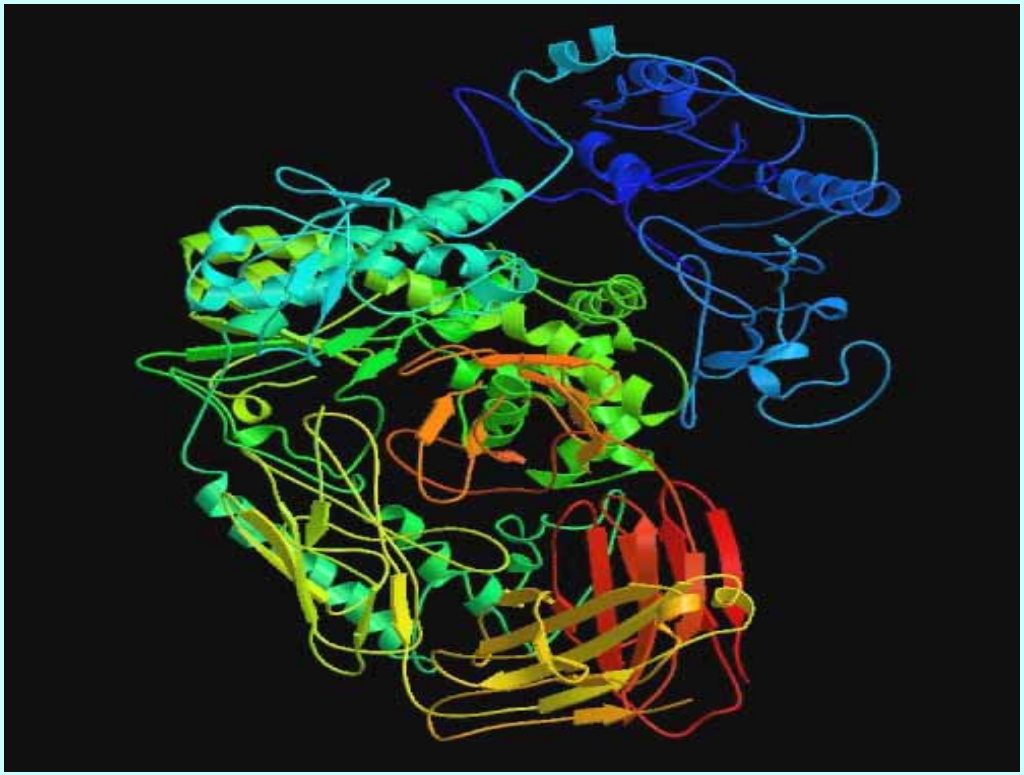
- Directly measure biologically active protein of interest
- Reliable
- Quantitative analysis
- Qualitative analysis
- High sample throughput
- Easy to perform and transfer to other laboratories
- Widely accepted method by regulatory agencies
- Established use in food industry
- Cost effective
- Timely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

PCR - Polymerase Chain Reaction

Technique that allows amplification of specific DNA sequences millions of times in a few hours. DNA sequences unique to GM traits can be detected and measured.



Applications of PCR

Qualitative

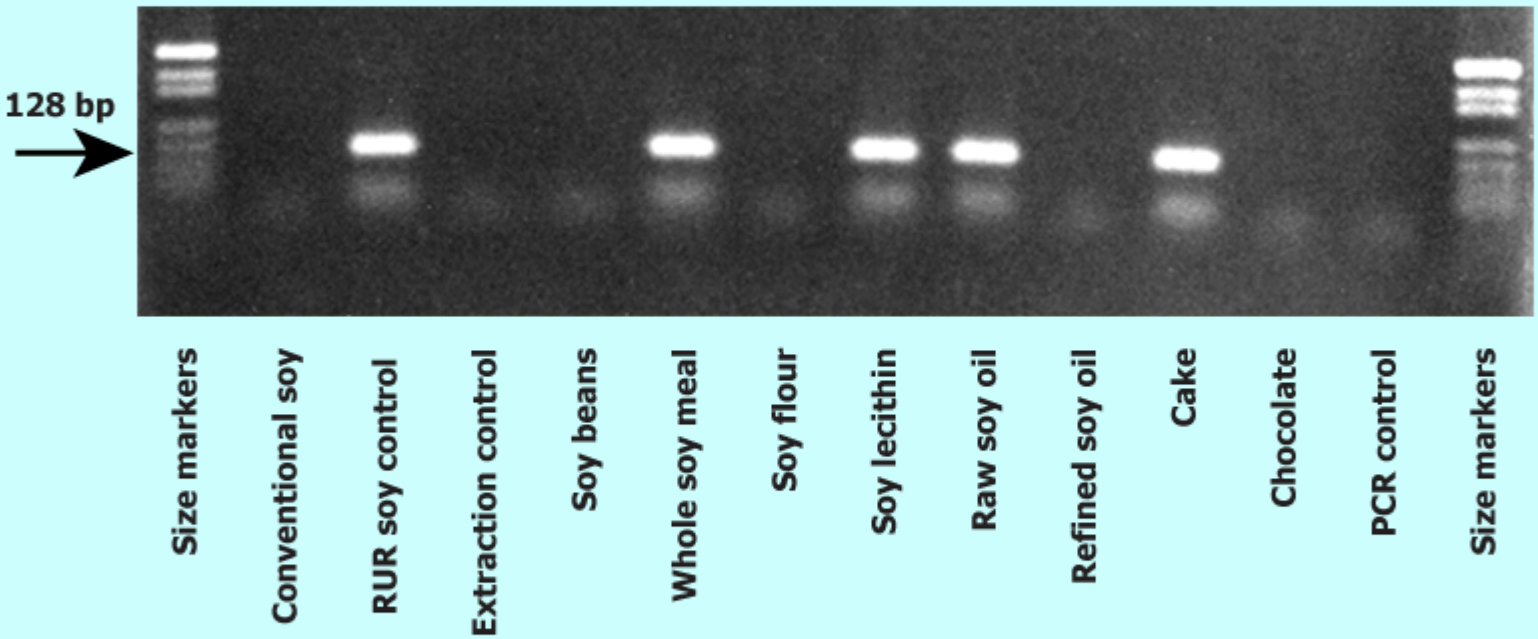
- A "YES" or "NO" answer
- Can look for specific DNA event such as RUR, MON810, etc. or
- Can look for generic elements such as NOS, 35S
- Applicable to a zero tolerance situation

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

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)**

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