Protein Detection Methods

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Detection of Genetically Modified (GM) Plants and Food

Plants
Seeds
Grain
Food

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Detection of Genetically Modified (GM) Plants and Food

Targets for Detection

DNA:
- Gene-specific PCR
- Event-specific PCR
- Southern blot
- Next Generation Sequencing

Protein(s):
- Plate format assay
- Lateral flow strip assay
- Western blot
- LC/MS

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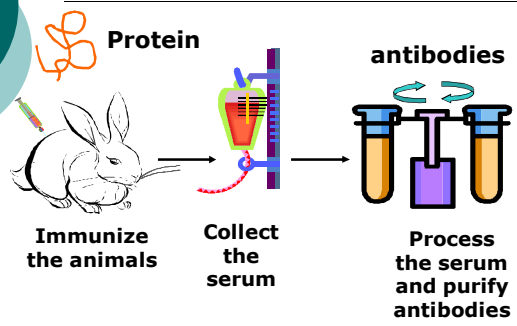
Production of Antibodies



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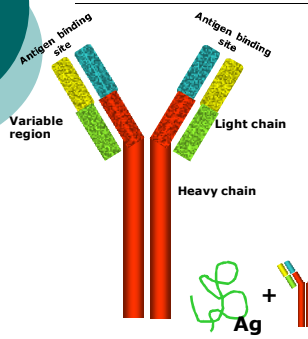
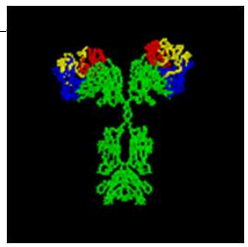
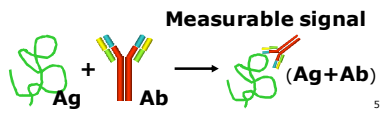
Structure of Antibody







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Types of Antibodies in Immunoassays

Polyclonal antibodies [Ab]

Monoclonal antibodies [MAb]

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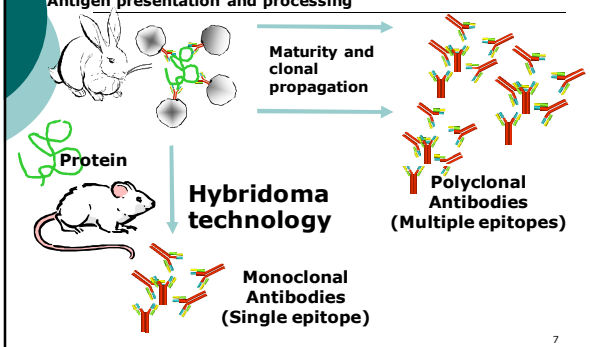
Polyclonal and Monoclonal Antibodies



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Monoclonal vs Polyclonal

Monoclonal	Polyclonal
▪ *Lot-to-lot consistency*	▪ *Lot-to-lot variability*
▪ *Indefinite supply*	▪ *More broadly reactive*
▪ *Highly specific*	▪ *Often more sensitive*
▪ *Higher initial costs*	▪ *Lower initial costs*

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Sensitivity of Immunoassay

Is a function of

- **Affinity of antibody used in the assay (strength of Ag and Ab binding)**
- **Extraction efficiency**
- **Range from 1 ng/mL to 5 µg/mL**
- **How assay is developed & optimized.**

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Sensitivity of Immunoassay

Limit of Detection (LOD)

The minimum concentration of protein that can be detected by the assay

Limit of Quantification (LOQ)

The smallest unknown concentration of protein that can be quantified reliably with specified accuracy and precision

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Advantages of Immunoassay

- **Easy sample preparation**
- **Economical and cost effective**
- **Robust, can be used in field or the lab**
- **Qualitative and quantitative applications**
- **Flexible formats available**
- **Multi-analyte capability**

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Disadvantages of Immunoassay

- **Limited to samples for which we can produce a useful antibody**
- **Limited use in GM detection in processed food – denaturing protein**
- **Validation for each matrix required**
- **Cross reactivity**
- **Some Products may not express protein in seed/grain**
  - **Low experssion**
  - **Targeted expression**

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Commonly used Immunoassays

- ✓ Western blot
- ✓ Immunostaining
- ✓ ELISA
- ✓ Lateral Flow Devices

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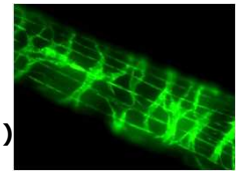
Detection of Genetically Modified (GM) Plants and Food

Western Blot and **Immunohistochemistry**

are specialized techniques, usually used in the initial stages of gene discovery (Research)



Not common for routine diagnostics

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Commonly used Immunoassays

- ✓ Western blot
- ✓ Immunostaining
- ✓ ELISA
- ✓ Lateral Flow Devices

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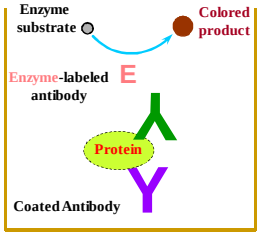
Immunoassay Methods

ELISA

The detection antibody is attached (conjugated) to an enzyme



Well of polystyrene 96-well plate

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Detection of Genetically Modified (GM) Plants and Food

ELISA

- Quantitative estimation of GM protein

- Grain, animal feed, unprocessed food and in certain cases semi-processed food

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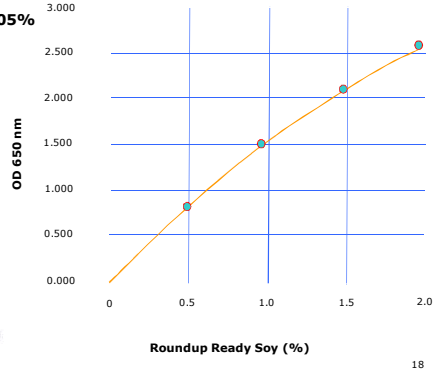
Range of quantification 0.1 to 2.0%
LOQ = 0.1%
LOD = 0.05%



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Detection of Genetically Modified (GM) Plants and Food

ELISA

- ✓ Quantitative-measure amount (%) of GM protein
- ✓ Qualitative - Yes or No answer
- Seed Quality testing
- Screening to preserve Identity

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Detection of Genetically Modified (GM) Plants and Food

ELISA

Advantages

- Economical and robust
- Easy to perform and transferred to laboratories
- Qualitative and Quantitative
- Sensitive, accurate, and reproducible
- High throughput, can be automated
- Minimum lab equipment needed
- Multi-analyte capability
- Widely accepted method

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Detection of Genetically Modified (GM) Plants and Food

ELISA

Disadvantages

- Requires several timed steps
- Not very portable
- Requires refrigerated storage

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Detection of Genetically Modified (GM) Plants and Food

ELISA - Commercial kits

Antibody coated plate

Antibody conjugate

Substrate

Buffers

Usually takes from 1 hours to 5 hours depending on the manufacturer

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Commonly used Immunoassays

- ✓ Western blot
- ✓ Immunostaining
- ✓ ELISA
- ✓ **Lateral Flow Devices**

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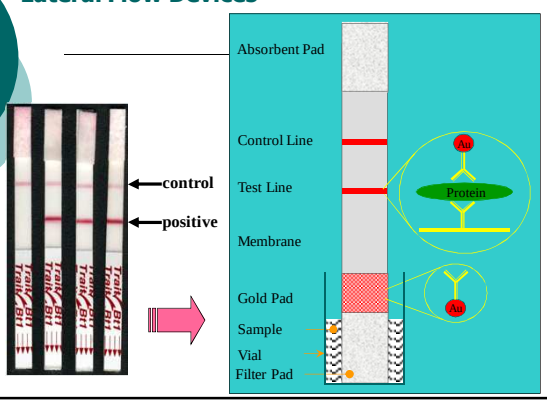
Lateral Flow Devices



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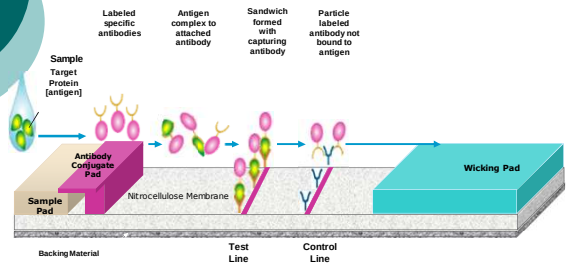
Immunoassay Methods

Lateral Flow Devices



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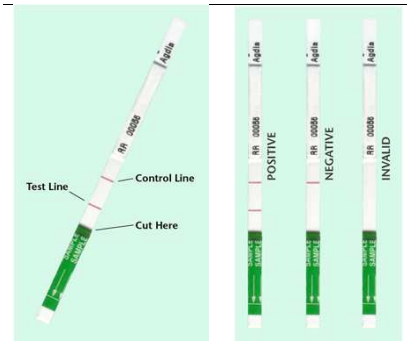
Immunoassay Methods

Lateral Flow Devices



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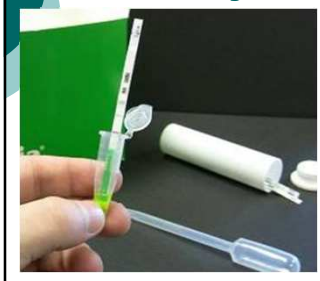
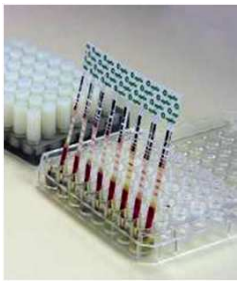
Immunoassay Methods

Lateral Flow Devices

Flexible designs



Single strips



Strip combs
For 48 wells

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Immunoassay Methods

Lateral Flow Devices

Advantages

- Portable and can be used in the field
- "Point of Care", fast, results in 5 minutes
- Robust and longer shelf life
- No refrigeration required
- Minimal training and equipment
- Can be used for semi-quantification
- Multi-analyte capability

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Immunoassay Methods

Lateral Flow Devices

Disadvantages

- More expensive than ELISA
- Less sensitive than ELISA
- Requires dry storage

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Immunoassay Methods

Lateral Flow Devices

Leaf - Breeders and seed multiplication

Snap a leaf disc into the microfuge tube

Add buffer and grind

Add ImmunoStrip

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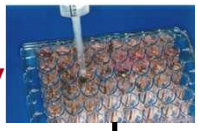
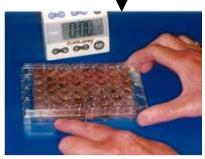
Immunoassay Methods

Lateral Flow Devices – Seed QC labs

Seed quality testing Identity Preservation





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Immunoassay Methods

Lateral Flow Devices

Adventitious Presence

Identity Preservation

Grain elevators where speed is critical

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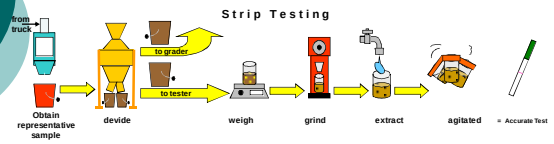
Immunoassay Methods

Lateral Flow Devices - Grain testing



Bt Cry 1Ab
Bt Cry 9C
Roundup Ready®
Bt Cry 3Bb
Bt Cry 1F
Liberty link®

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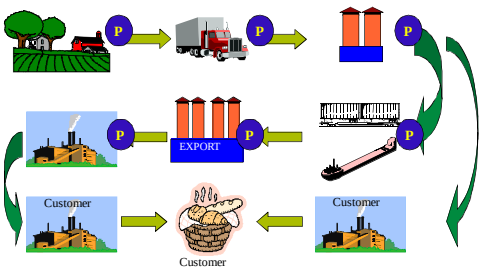
GM Protein Test in Food & Feed Supply Chain



*Source: Grothaus et al. AOAC International. **2006**, 89: 913-928.*

Detection of Genetically Modified (GM) Plants and Food

Emerging Technologies

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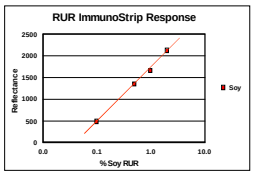
Measuring Response of Lateral Flow Devices

Readers



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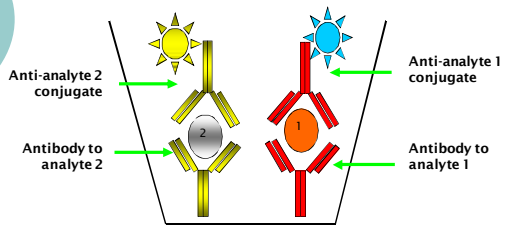
Emerging Technologies

Multi–analyte capabilities



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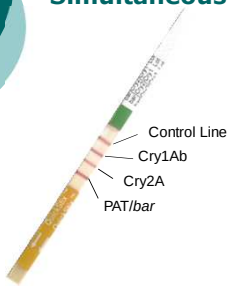
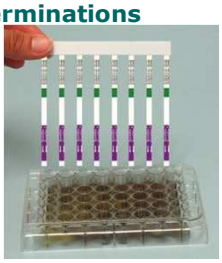
Emerging Technologies

Multiple trait strips
Simultaneous determinations





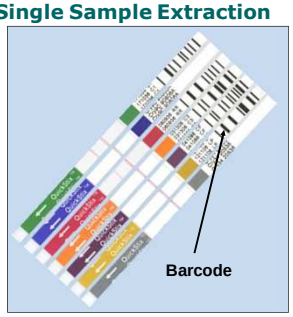
8-strip combs

Source: EnviroLogix Inc., Portland, ME

Emerging Technologies

Multiple trait strips
Combined for Single Sample Extraction

Common extraction of one large grain sample for maximum efficiency in Identity Preservation testing programs in the grain distribution system.

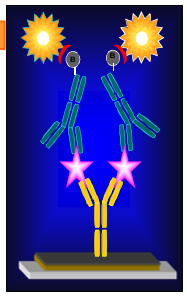


Source: EnviroLogix Inc., Portland, ME

Emerging Technologies

Multiplexed Platforms

- **Protein microarrays**
  - Glass slide format
  - Fluorescence based detection
- **Searchlight (Pierce)**
  - High protein binding plates
  - Chemiluminescence
- **MSD (Meso Scale Discovery)**
  - Special plates
  - Electro-chemiluminescence (ECL)
- **Luminex platform**
  - Colored beads
  - Flow cytometer



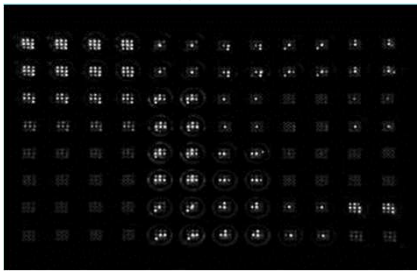
ELISA

Emerging Technologies

Antibody-based Protein Array

(SearchLight Technology)

Detect up to 16 proteins in one well

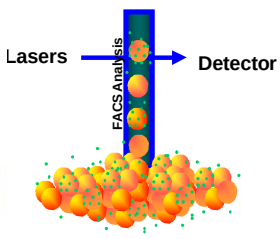


Source: www.piercenet.com

Emerging Technologies

Luminex Platform

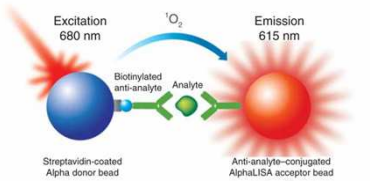
Antibody coupled to individual bead
100 colored beads available



Emerging Technologies

Homogenous Assays

AlphaLISA bead-based technology relies on PerkinElmer's exclusive amplified luminescent proximity homogeneous assay. In the presence of the analyte, the two beads come into close proximity. The excitation of the donor beads at 680 nm generates singlet oxygen molecules that trigger a series of chemical reactions in the acceptor beads resulting in a sharp peak of light emission at 615 nm.



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Emerging Technologies

Homogenous Assays

Advantages over ELISA-like technologies

- Reduced hands-on and total assay times. No washing steps required
- Large dynamic range (approximately 3 – 5 logs)
- Easy to miniaturize (volumes as low as 5ul total assay)
- Easy to automate enabling High Throughput Screening
- Quantitative detection in 96-, 384- and 1536-well microplates possible

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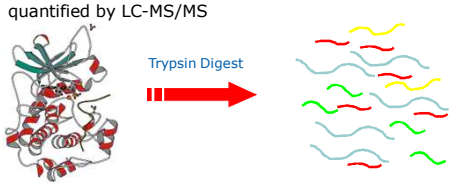
Emerging Technologies

Protein Quant by Mass Spec

» Target proteins (and tens of thousands of other proteins) are digested with trypsin (or other proteases)

» One/more signature peptides for target proteins are quantified by LC-MS/MS



Signature peptides are quantified as protein surrogates

Emerging Technologies

Protein Quant by Mass Spec

» Immunoassays widely used for protein detection/quantification, but …

- ❖ High quality antibodies are NOT always available
- ❖ It takes months to generate good antibodies
- ❖ Typically it is challenging to differentiate mutants from wild type

Technology that overcomes these challenges

» Short turnaround time for method development

» High sensitivity and high selectivity

» Multiplexing capabilities

Emerging Technologies

Comparison of Multiplexed Platforms

Technology	Microarray Printer	Sensitivity	Dynamic Range	Multiplexing
ELISA	No	~10 pg/ml	2-3 logs	none
AlphaLISA	No	Low pg/ml	3-5 logs	none
Protein Chip	Yes	Low pg/ml	3-4 logs	15-30
Luminex	No	20-50 pg/ml	3 logs	~15-20
MSD	Yes	Low pg/ml	5 log (claimed)	up to 10
Searchlight	Yes	Low pg/ml	3-4 logs	16
MS	No	Low pg/ml	3-4 logs	> 50

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Questions?

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