Guidelines for Validation and Use of Immunoassays for Determination of Introduced Proteins in Biotechnology Enhanced Crops and Derived Food Ingredients

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AEIC Analytical Environmental Immunochemical Consortium

About 27 member organizations, primarily:

- **agricultural biotechnology companies**
- **crop protection companies**
- **immunoassay kit manufacturers**

www.immunochem.org

AEIC Collaboration

- **Abraxis, LLC**
- **Dow AgroSciences, LLC**
- **Monsanto Company**
- **Pioneer Hi-Bred International**
- **Strategic Diagnostics Inc.**
- **Zeneca Ag Products**

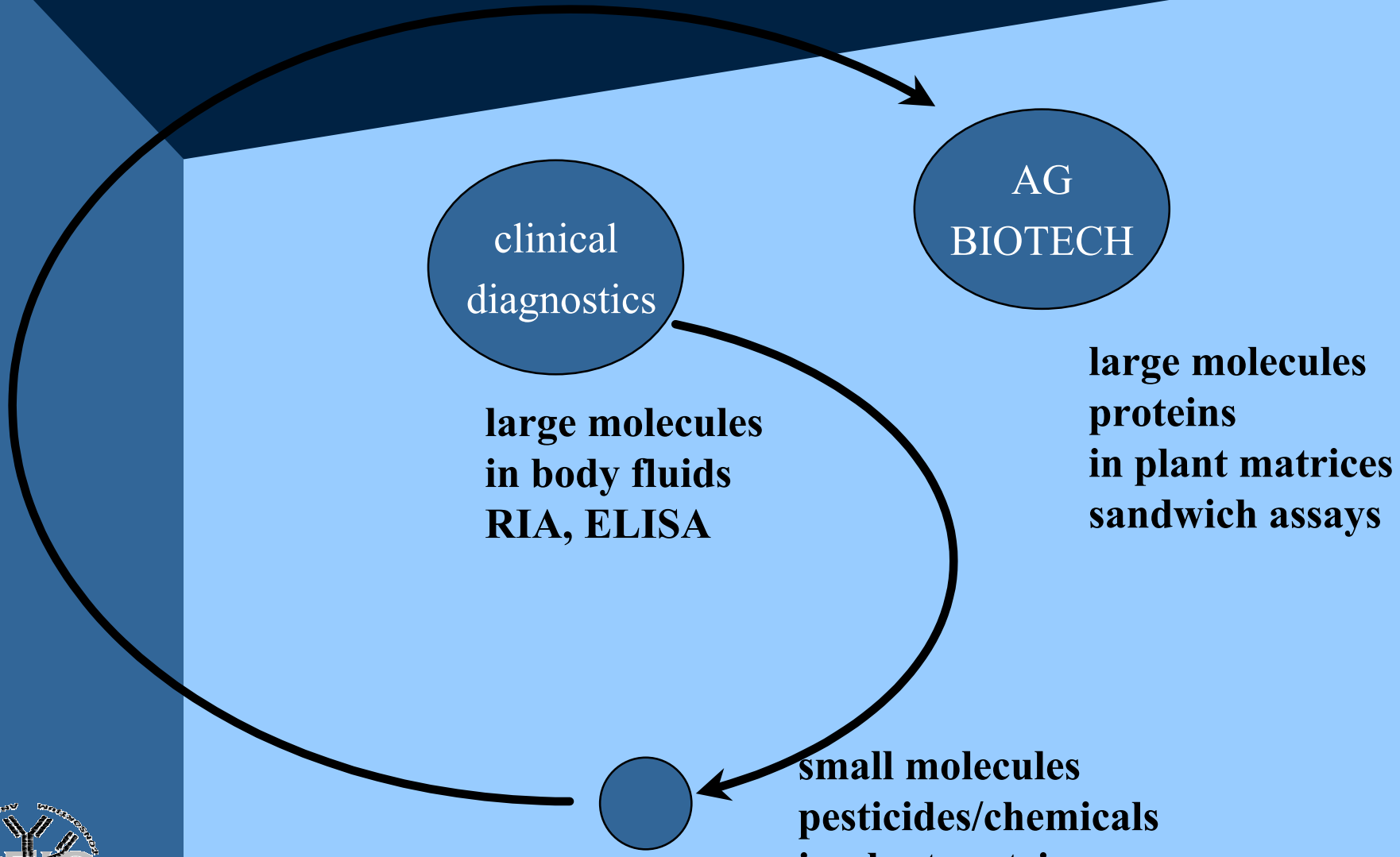
Analytical Environmental Immunochemical Consortium

Mission:

To advance and support immunoanalytical technologies, the AEIC will:

- Provide a consensus voice for applications to agricultural, biotechnology and environmental industries
- Develop educational programs
- Furnish scientific expertise
- Establish performance standards for immunochemical methods

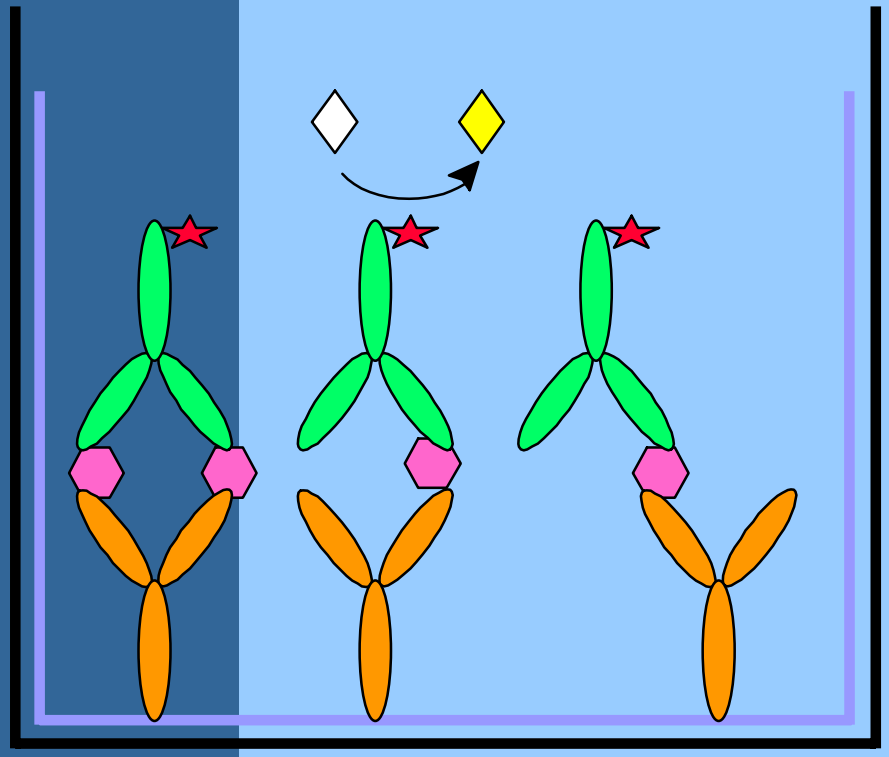
Immunoassay Evolution

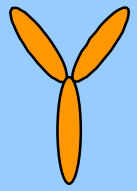


Immunoassay

- **the specific binding of an antibody with its target analyte**
- **detection is achieved by the addition of a signal-generating component (reporter label)**
  - radioactivity (RIA)
  - enzymes (EIA, ELISA)
  - colloidal gold/latex
  - fluorescence
  - phosphorescence
  - chemiluminescence
  - bioluminescence

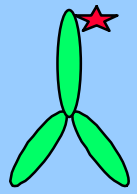
Sandwich ELISA





Capture Antibody

Analyte (protein of interest)



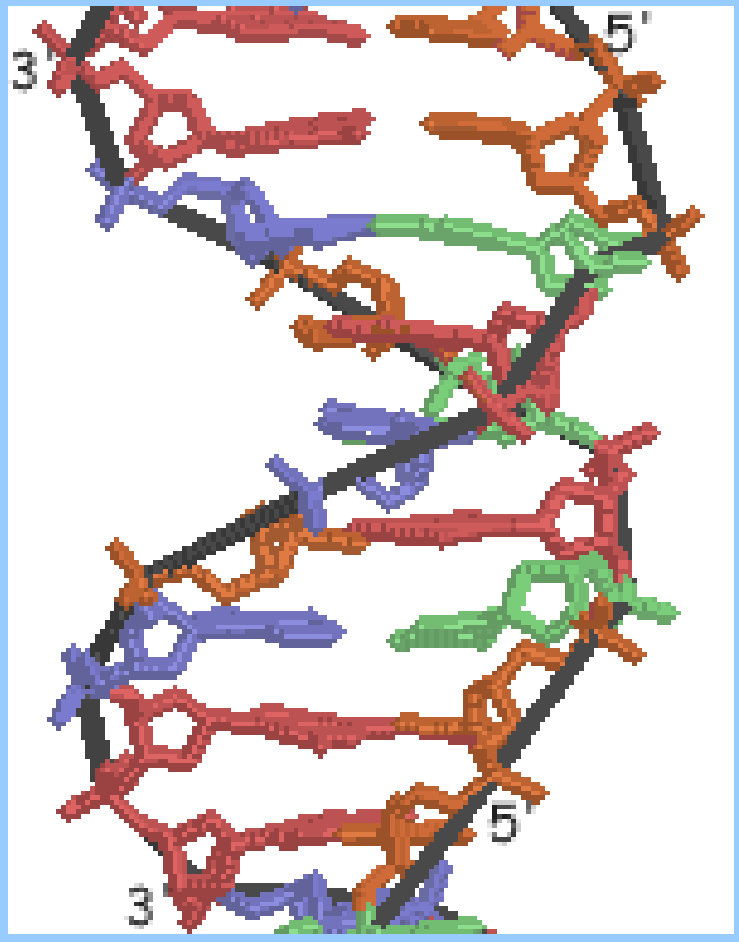
Labeled Antibody

Substrate

Product

Biotechnology Enhanced Crops

- **One or more genes are inserted into the plant genome by recombinant DNA techniques**
- **The new genes often code for the production of new proteins**
- **These introduced proteins (gene products) can be detected by immunoassay**



Ag Biotech Applications for Immunoassay

- **Ag Biotech Product Development**
  - Screening/Transformant identification
  - Line selection/Plant breeding
  - Seed quality control (QC)

- **Regulatory Approval**
  - Protein expression/Product characterization studies
  - Assessments of food, feed and environmental characteristics
  - Determining concentrations for toxicology studies

Ag Biotech Applications for Immunoassay

- **Approved Product**
  - Product support/stewardship
  - Intellectual property protection
  - Seed QC
- **Grain Handling/Distribution System**
  - Identification of transgenic products at grain elevator
  - Testing at other transfer points in supply chain
  - Identity preservation

Ag Biotech Applications for Immunoassay

- **Food Ingredient Testing**
  - Raw agricultural commodities
  - Processed food fractions

Method Validation and Use

- **Sampling**
  - Appropriate
  - Representative
- **Standardized Reference Materials**
  - Purified protein standards
  - Commodity to be tested containing known proportion of transgenic material

Method Performance Characteristics

- **Accuracy**
  - Recovery experiments
- **Extraction efficiency**
  - "Incurred" analyte vs. spiked
  - Exhaustive extraction
- **Precision**
  - Verify linearity of response
  - Measure variation among replicates

Method Performance Characteristics

- **Method Reproducibility**
  - Variation among replicates
    - within the test and among separate tests
    - among analysts
    - day to day
- **Sensitivity**
  - Limit of Detection (LOD)
    - response vs. background noise
  - Adequate sample size

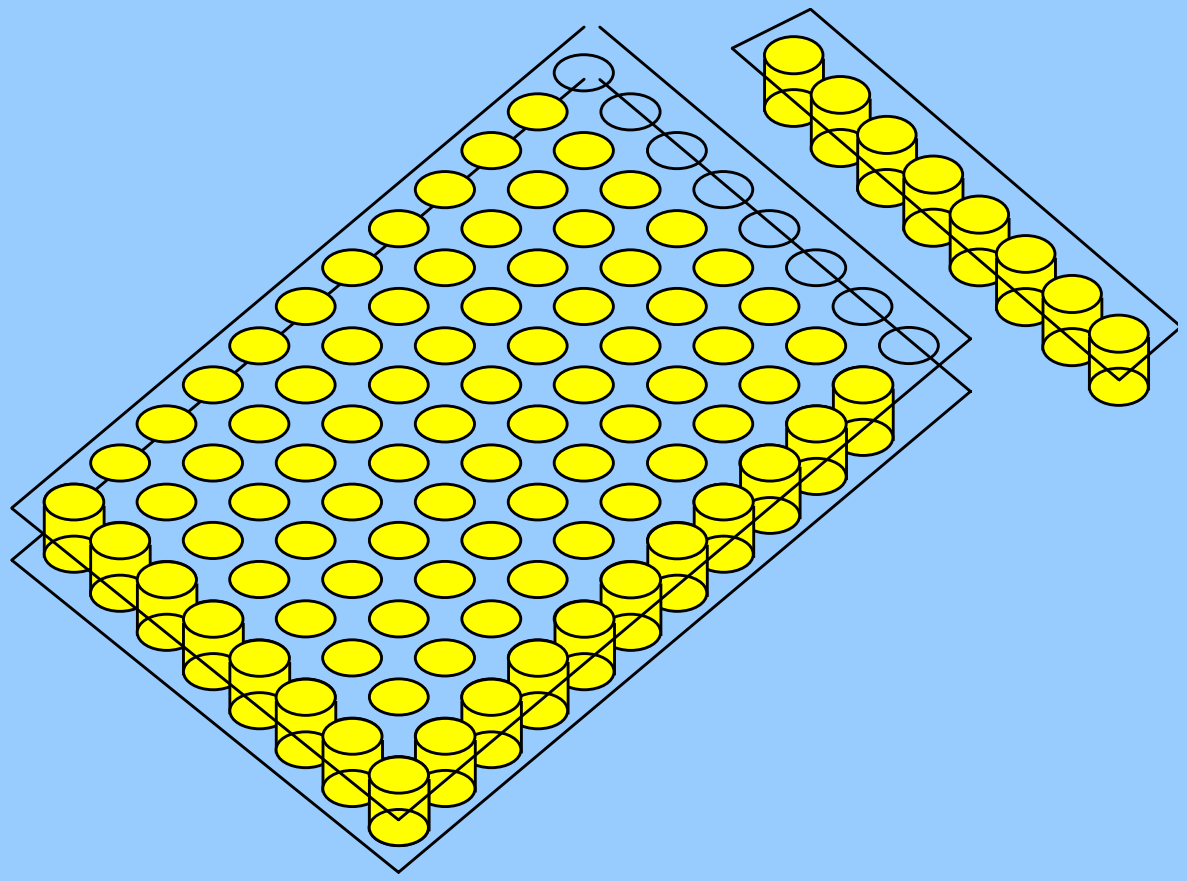
Method Performance Characteristics

- **Specificity**
  - Interferences from reagents or labware
  - Matrix effects
  - Cross-reactivity
- **Ruggedness**
  - Independent validation
  - Round-robin or ring testing
  - Compare material from various sources

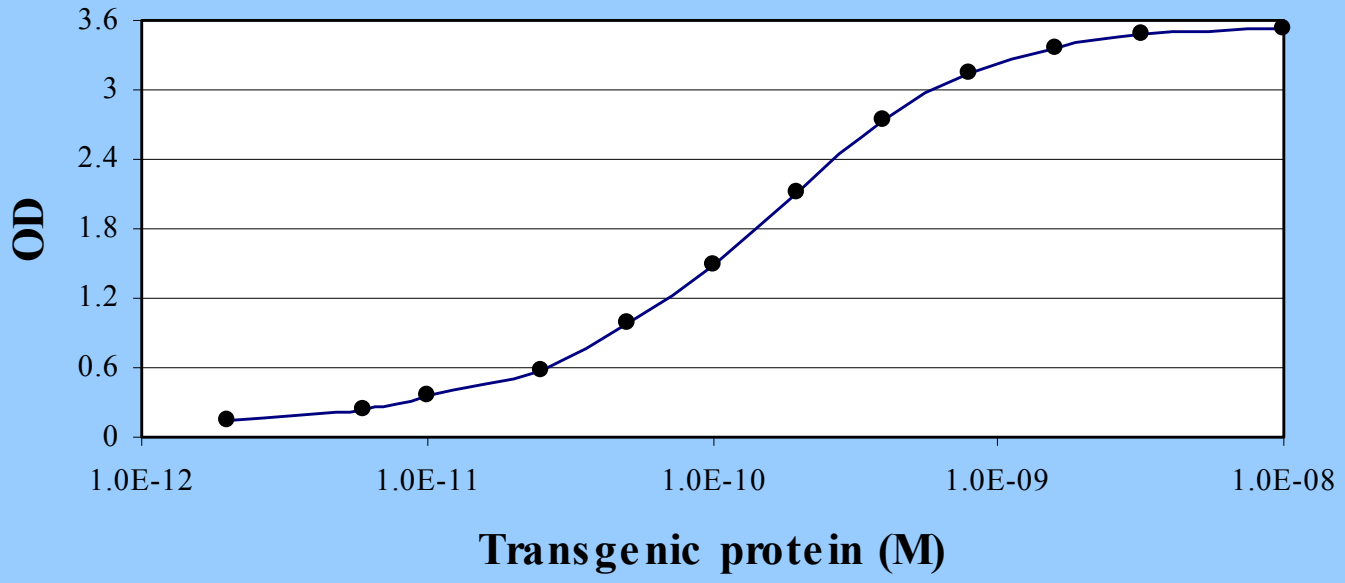
Considerations

- **Analyte stability**
- **Method sensitivity**
- **Difficult matrices**
- **Defined testing objective**
  - Qualitative or yes/no
  - Semi-quantitative or threshold
  - Quantitative

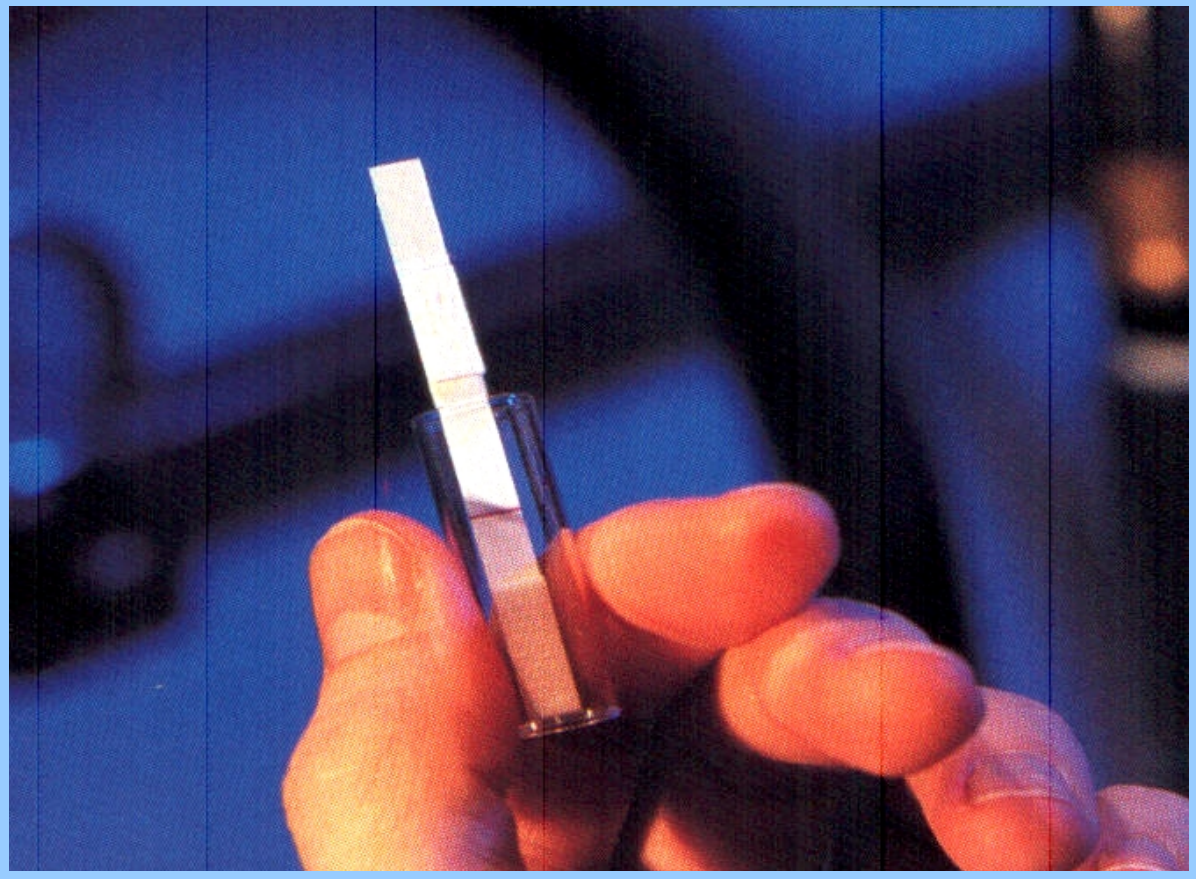
Microplate ELISA



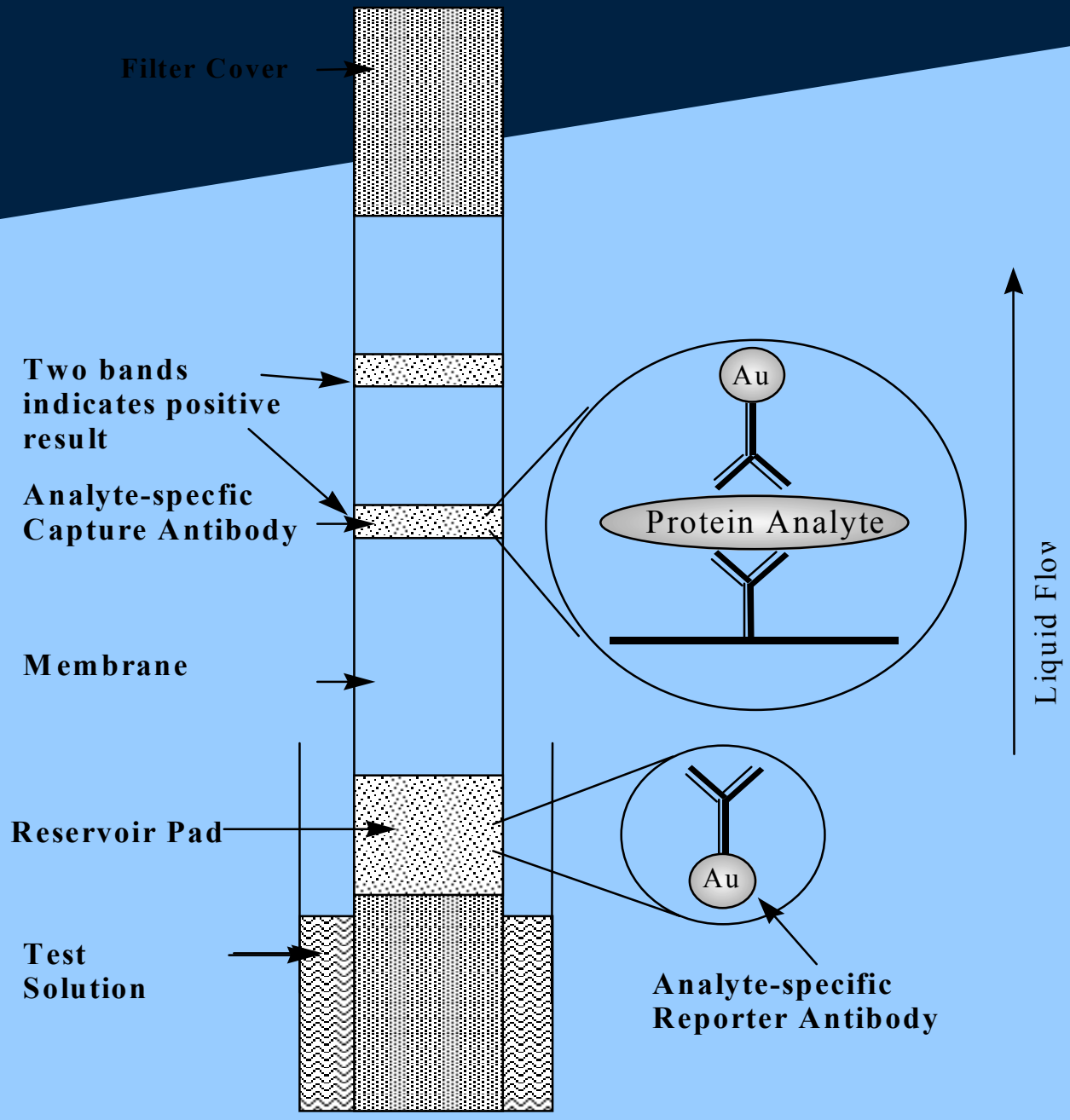
STANDARD CURVE FOR SANDWICH ELISA



Immunoassay Strip Test
Lateral Flow Device



Lateral Flow Strip Immunoassay



Conclusion

- **Immunoassays are extremely useful for Ag Biotech**
  - both microplate and lateral flow devices
  - for quantitative and threshold applications
  - for product development and support
- **Like any analytical method**
  - Immunoassays must be validated
  - Performance characteristics demonstrated and described

Acknowledgements

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