



Use of Serology in Plant Disease Diagnostics

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Workshop



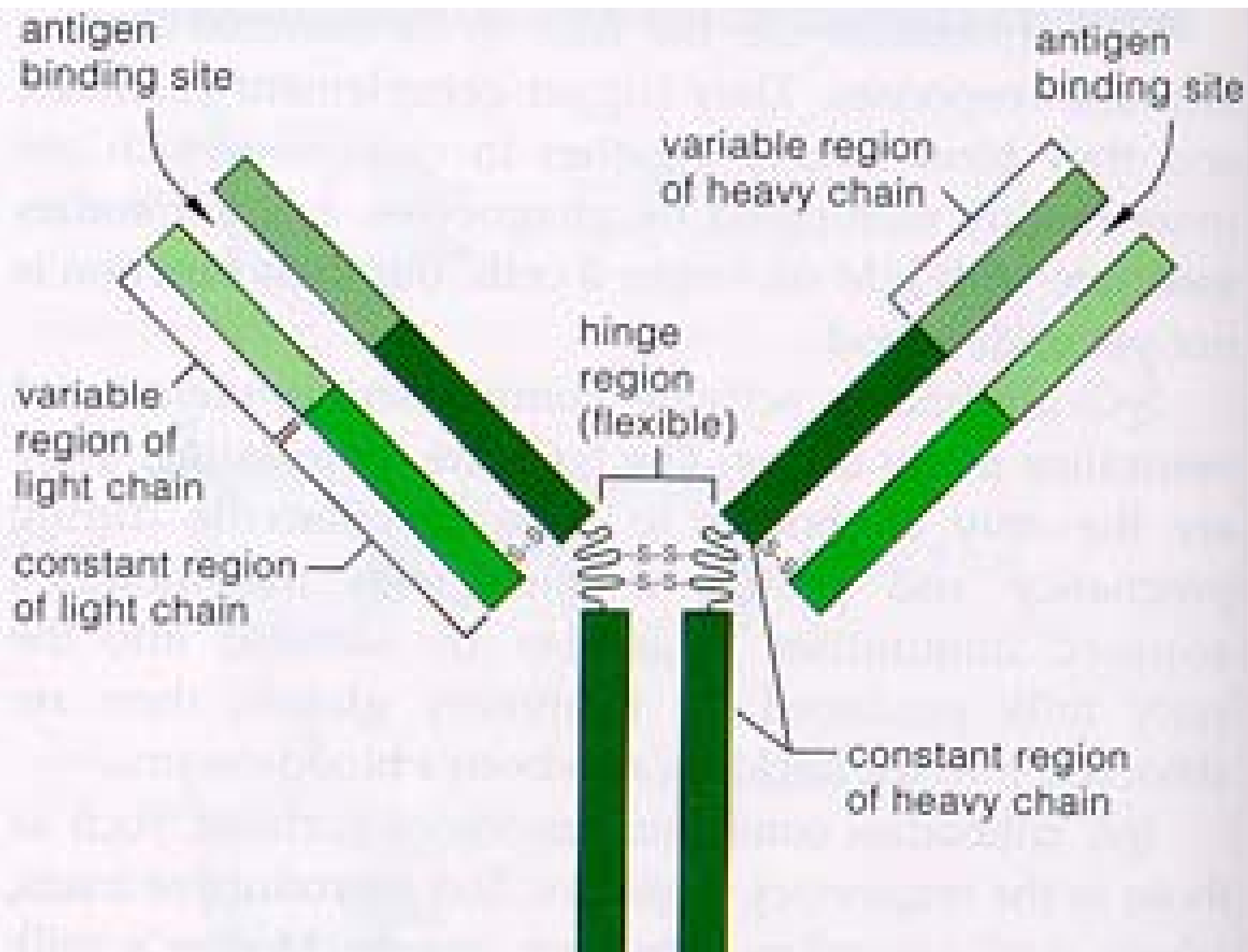
Immunological Assays

- Rapid
- Sensitive
- Specific
- Diagnose diseases
- Detect pathogens

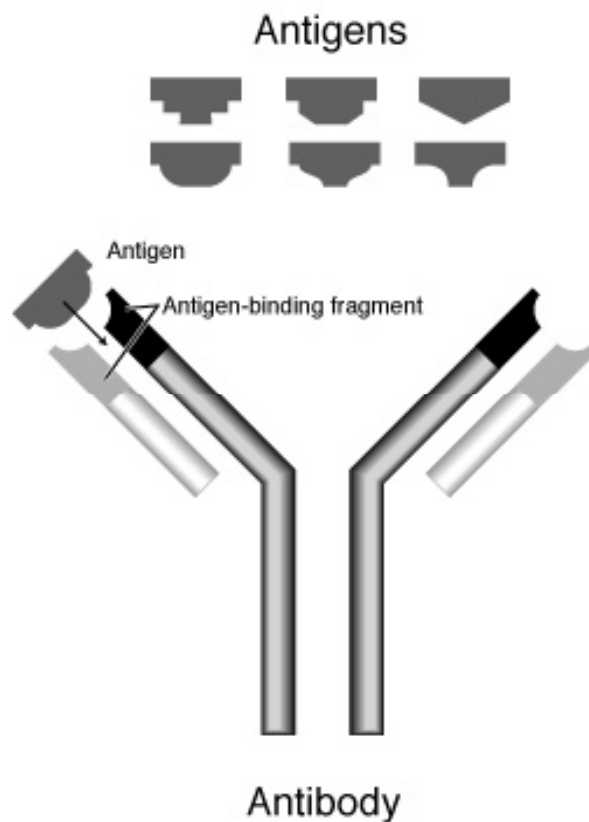


Immunoassays are based on antibodies....

- Mammals produce antibodies that specifically recognize binding sites (epitopes) on proteins, glycoproteins, lipopolysaccharides, carbohydrates (antigens)
 - Polyclonal antibodies
 - Monoclonal antibodies
- Antibodies specifically bind antigens
- Bound antibodies are detected with various markers

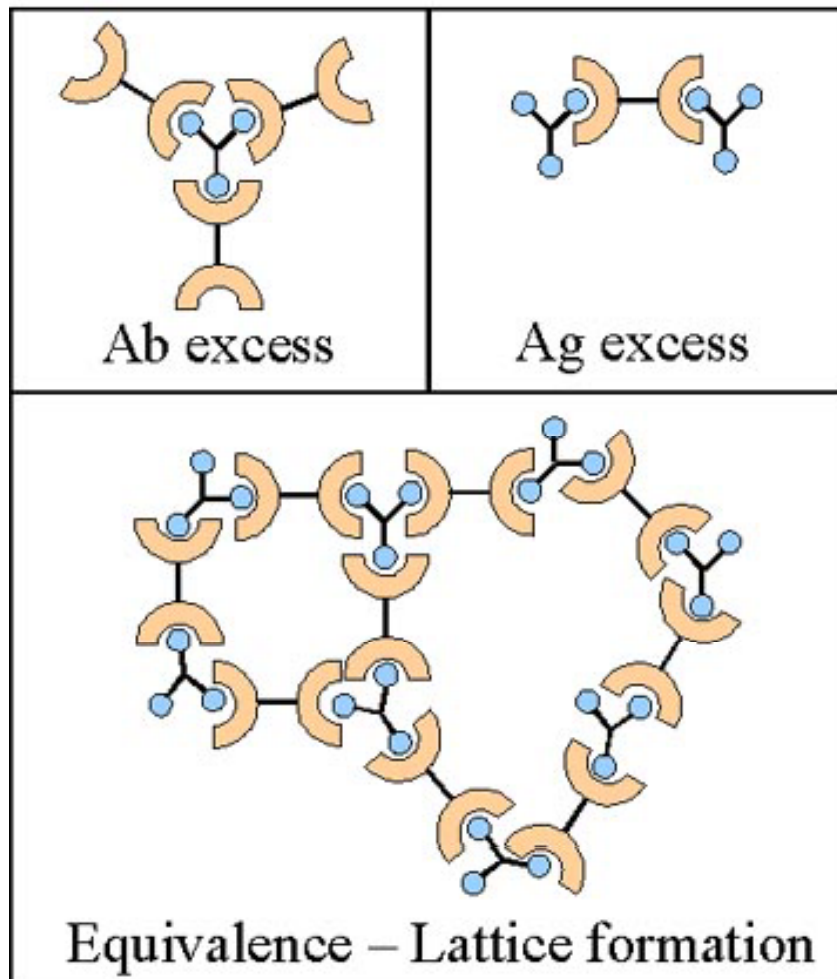


Antibody-Antigen Binding



- n **Lock and Key Concept**
 - Key (*i.e.* the Ag) fits into
 - Lock (*i.e.* the Ab)
- n Ab binding site in the Fab variable regions of the heavy and light chains
- n Ab has two identical binding sites
- n Ab: heterogeneous population with different specificities
- n Reaction **ONLY** if Ab & Ag has same determinants

Antibody-Antigen Binding



- Antibodies bind antigens
- Bonds are non-covalent, reversible
 - Hydrophobic
 - Hydrogen
 - Electrostatic
 - Van der Waal's

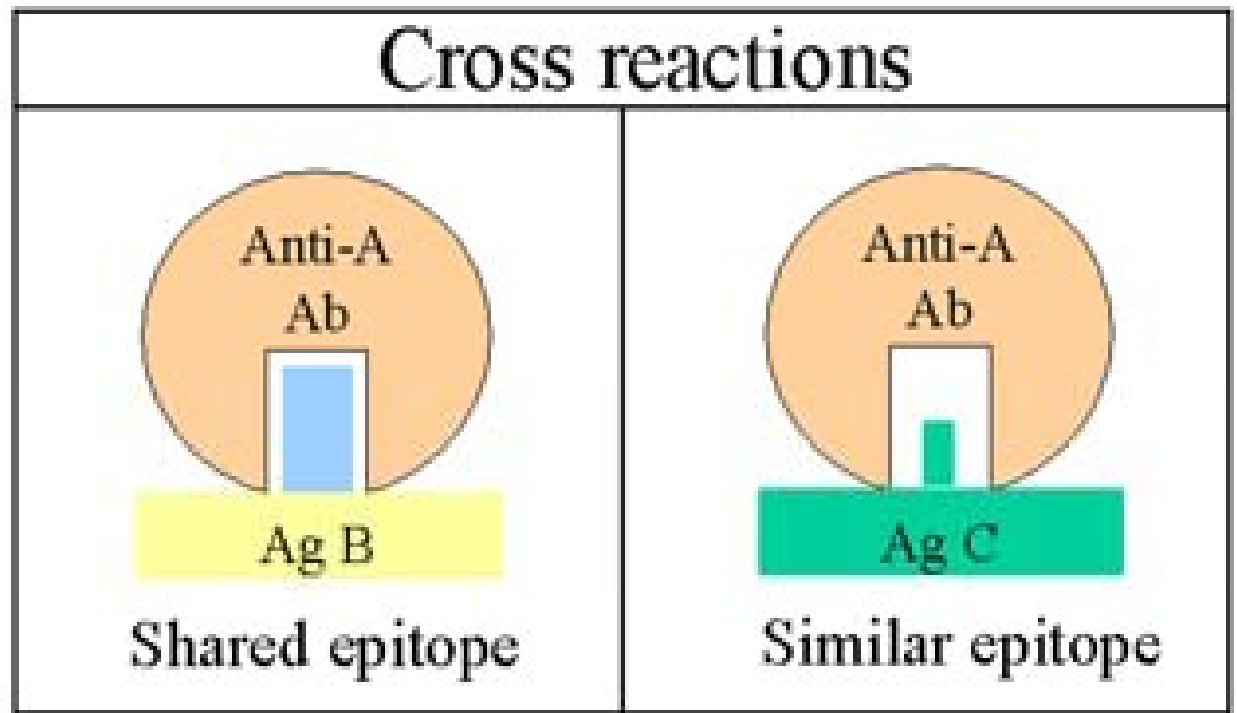
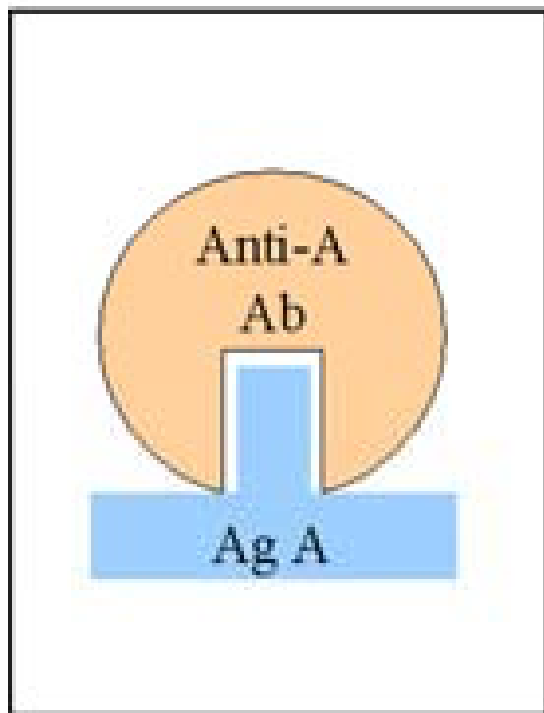
Reaction Specificity

- n Antibody reacts with only one antigenic determinant
- n Population of antibodies react with only one antigen
- n Antibodies can distinguish differences in the
 - primary structure of an antigen
 - isomeric forms of an antigen
 - secondary and tertiary structure of an antigen

Cross-Reactivity

- n Antibody reacts with more than one antigenic determinant
- n Population of antibodies react with more than one antigen
- n Cross reacting antigen shares an epitope in common with the immunizing antigen
- n Epitope is structurally similar to one on the immunizing antigen

Cross-Reactivity



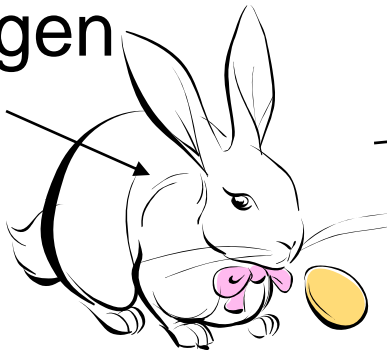
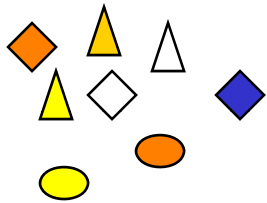


Polyclonal Antibodies

- Immunogens (preparations containing antigens that are used to immunize an animal)
 - Various degrees of purification of immunogens
 - Whole cells
 - Cell (surface) washings
 - Virus particles
 - Broken cells
 - Purified cell components
- Immunogens injected into animals for antibody production

Polyclonal antibody production

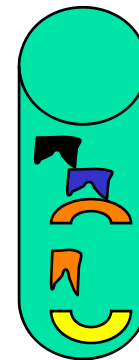
Immunogen



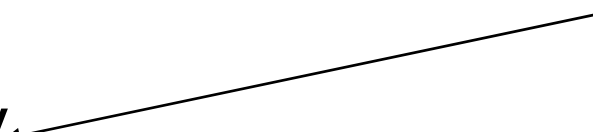
Collect blood; separate serum



Purify antiserum



Immunoassay





Characteristics of polyclonal antisera

- High sensitivity
- Varying specificity - depending on purity of immunogen/number of epitopes
- May vary from batch to batch

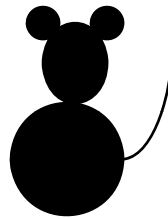
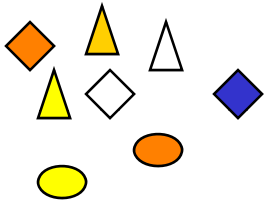


Monoclonal antibodies

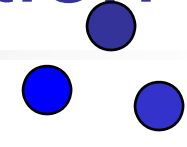
- Single type of antibody
- Highly specific
 - Recognize single epitope
- Sensitivity varies
- Produced by hybridoma cell lines that are theoretically immortal

Monoclonal antibody production

Immunogen

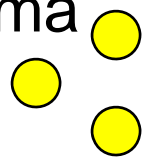


Remove spleen;
Isolate B-lymphocytes



Fusion

Myeloma
cells



Immunoassay

Select hybridomas; screen
for specificity



Immunoassay formats

- Enzyme-linked immunosorbent assay (ELISA)
 - Enzyme conjugated to antibody = marker
 - Alkaline phosphatase
 - Peroxidase
- Lateral flow immunoassay
 - Ab-Ag binding occurs as mixture flows through solid phase in liquid
- Immunofluorescence
 - Fluorescent molecule marks Ab-Ag reaction



ELISA

- Positive reaction indicated by enzymatic reaction with chromogenic substrate = color change
 - Antigen capture/plate-trapped antigen
 - Antigen bound to solid phase
 - Indirect vs. direct
 - Direct = detecting antibody conjugated with enzyme
 - Indirect = enzyme conjugated to secondary antibody
 - Sandwich ELISA (double antibody, triple antibody)

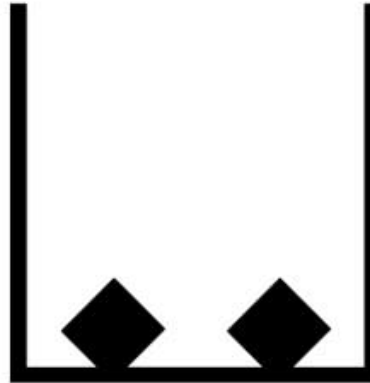
Indirect, Plate-trapped Antigen

ELISA

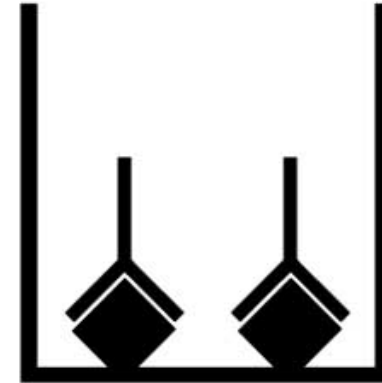
Primary Ab specific
to Antigen

Secondary Ab produced
in a different species,
e.g. goat - specific to
primary Ab

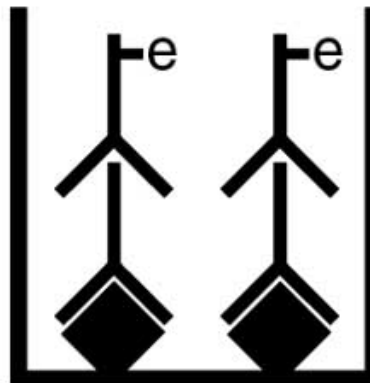
Step 1
Specific antigen is attached to
a solid-phase surface



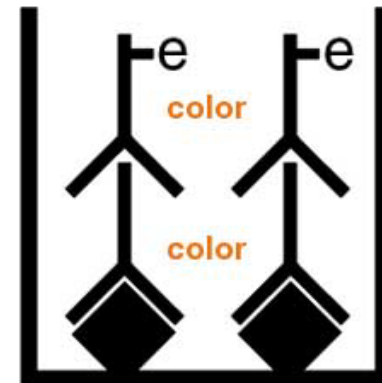
Step 2
Test specimen is added, which may
or may not contain the antibody



Step 3
An enzyme-labeled antibody
specific to the test antibody is
added (conjugate)



Step 4
Chromogenic substrate is added,
which in the presence of the
enzyme, changes color.

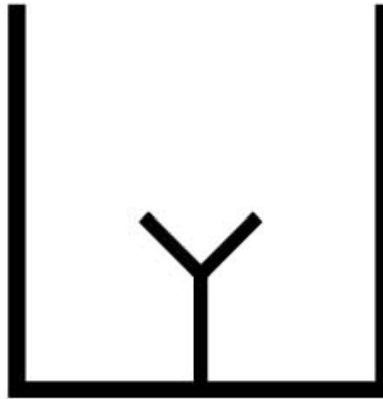


Direct, Double Ab Sandwich ELISA

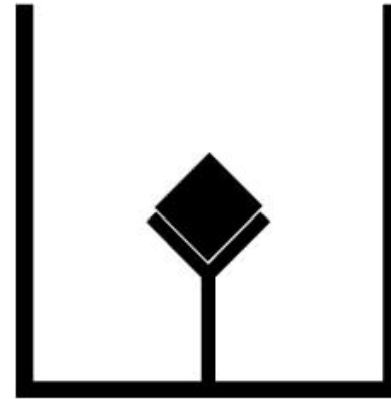


DAS-ELISA

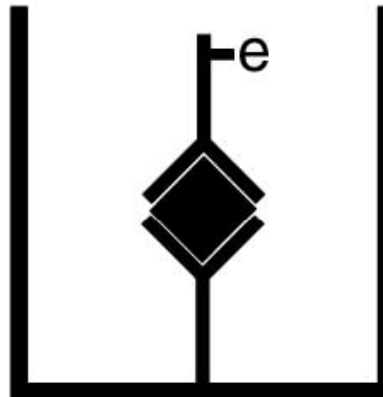
Step 1
Antigen-specific antibody is attached to a solid-phase surface



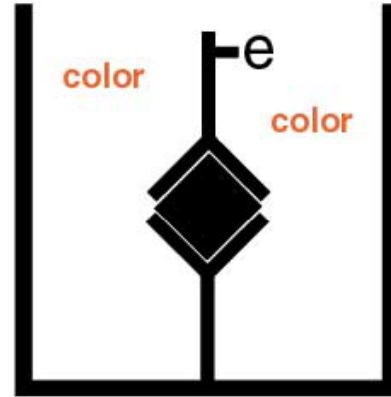
Step 2
Test specimen is added, which may or may not contain the antigen



Step 3
An enzyme-labeled antibody specific to the antigen is added (conjugate)



Step 4
Chromogenic substrate is added, which in the presence of the enzyme, changes color.

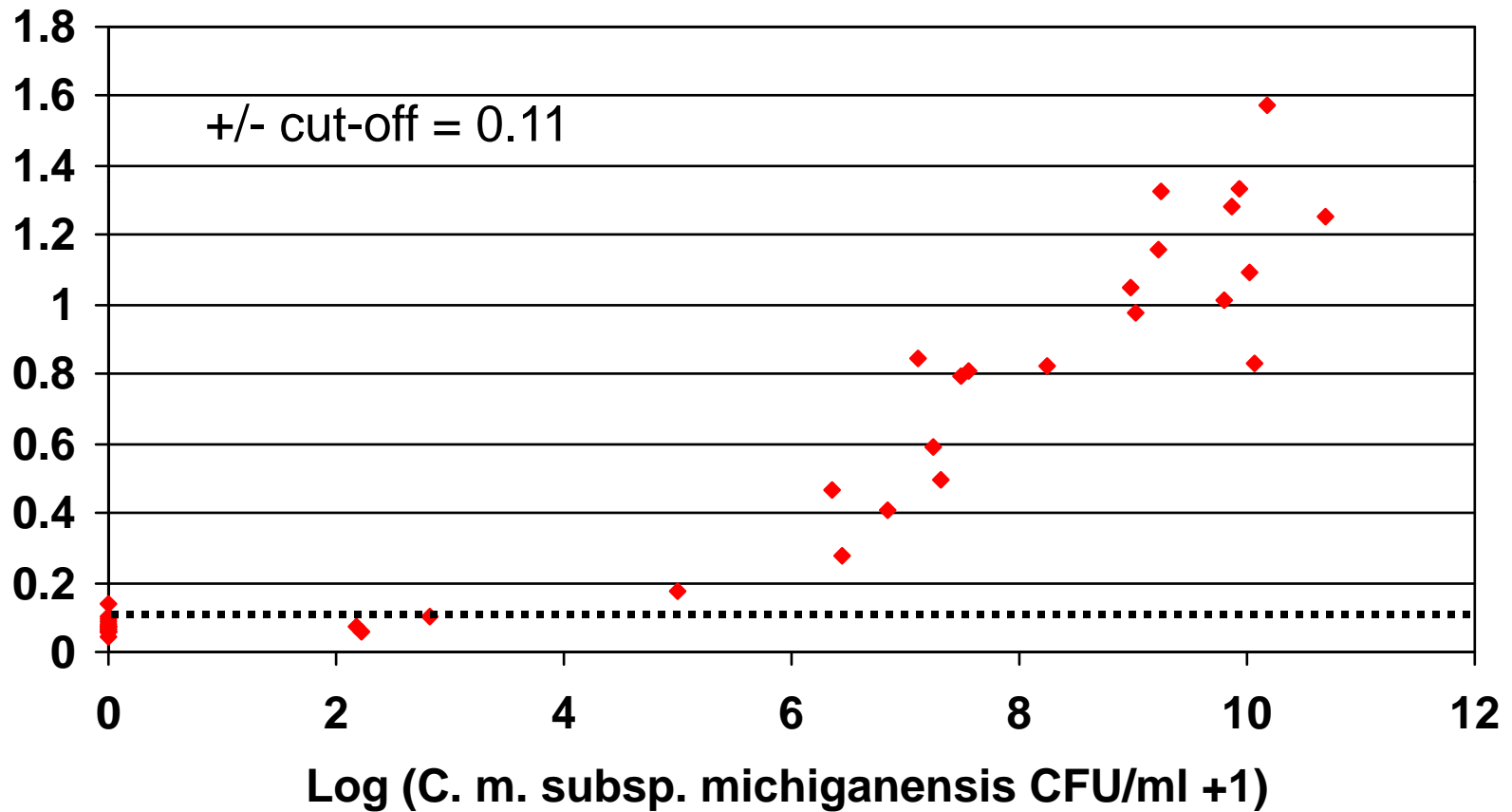


Multiwell Immunoassay



- Many commercially available
- Most detect various viruses
- Also for bacteria, fungi

Sensitivity of ELISA-bacteria





Interpretation of ELISA

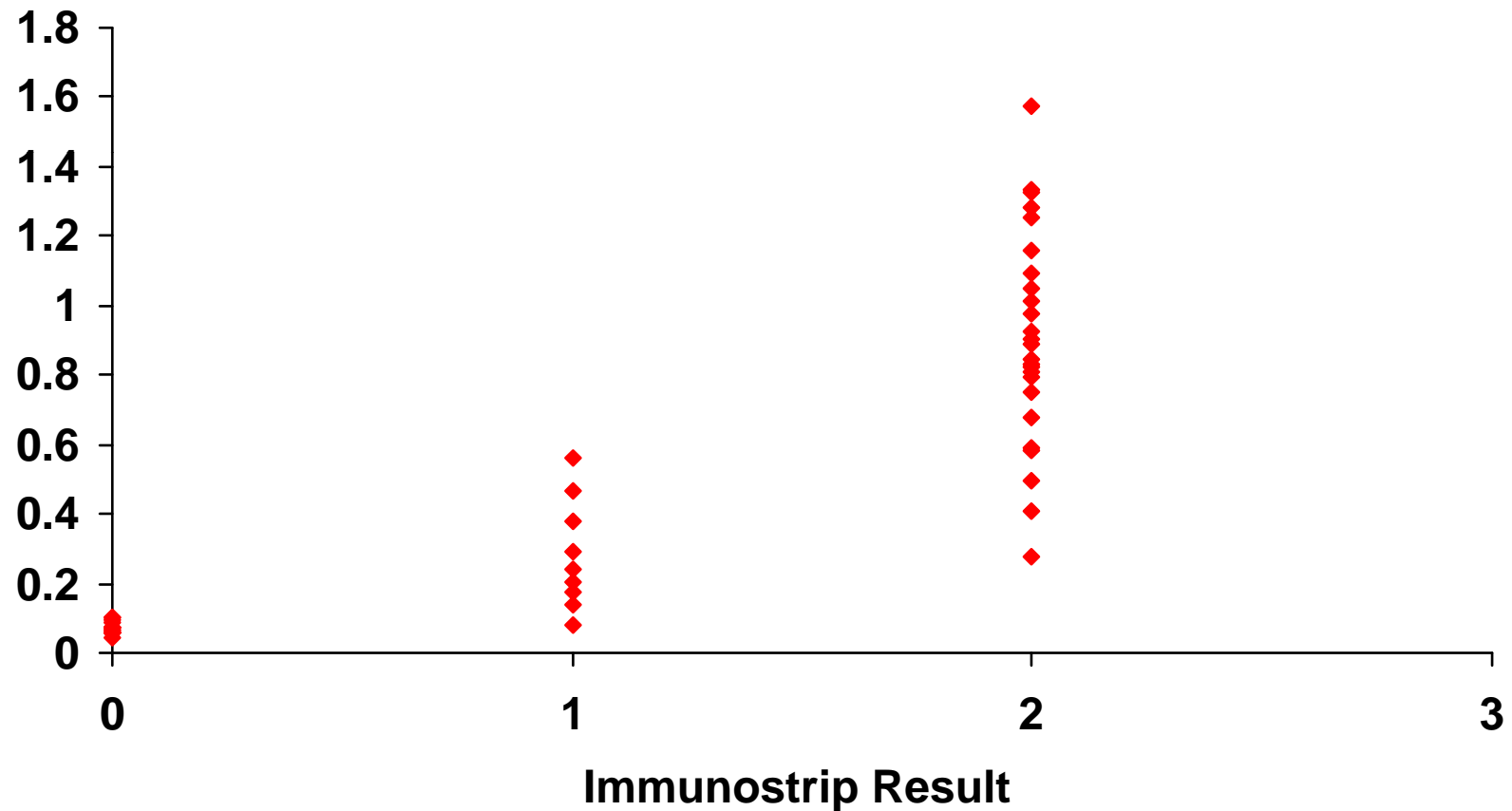
- What is a positive result?
 - 2-3X negative control
 - Negative control + 2-3x standard deviation of mean for negative control
- What is a false positive?
- What is a false negative?
- What is “background”?
- How does sampling affect the outcome?

Immunostrip (Lateral Flow) Assays



- Very fast - 3-5 minutes
- Sensitive
- Some are available commercially
- Extracts diffuse through paper strips
- Marker may be gold microparticles

ELISA vs. Lateral Flow - Cmm



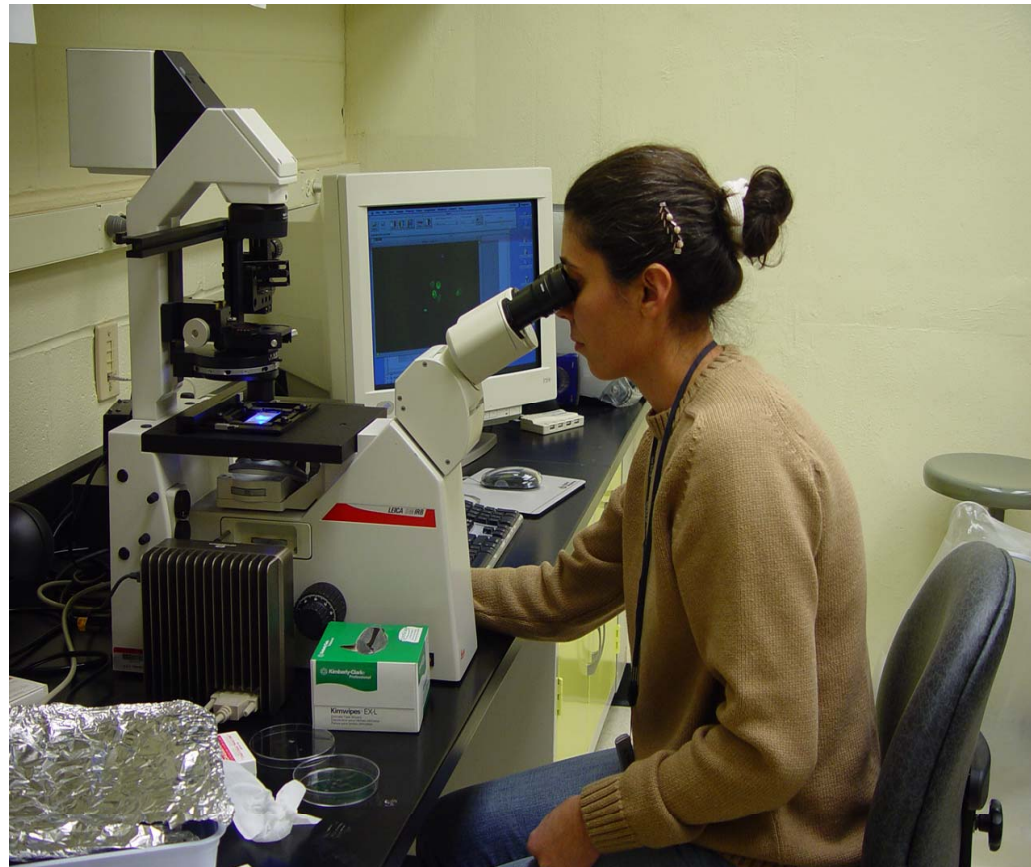
0 = negative; 1 = weak positive; 2 = positive

Immunofluorescence (IF)

Assays

- Generally used for bacterial, fungal spore identification
- Fluorescent dye is the marker
- Similar formats, binding washing step as ELISA
- Generally combined with epifluorescent microscopy

Epifluorescent Microscopy





Soybean Rust IF Assay

Apply 1° Antibody in PBS-Tween



Incubate 2 hr, 22C



Wash 6X, PBS-Tween



Apply 2° Antibody (Alexa Fluor 488, 1:500) in PBS-Tween

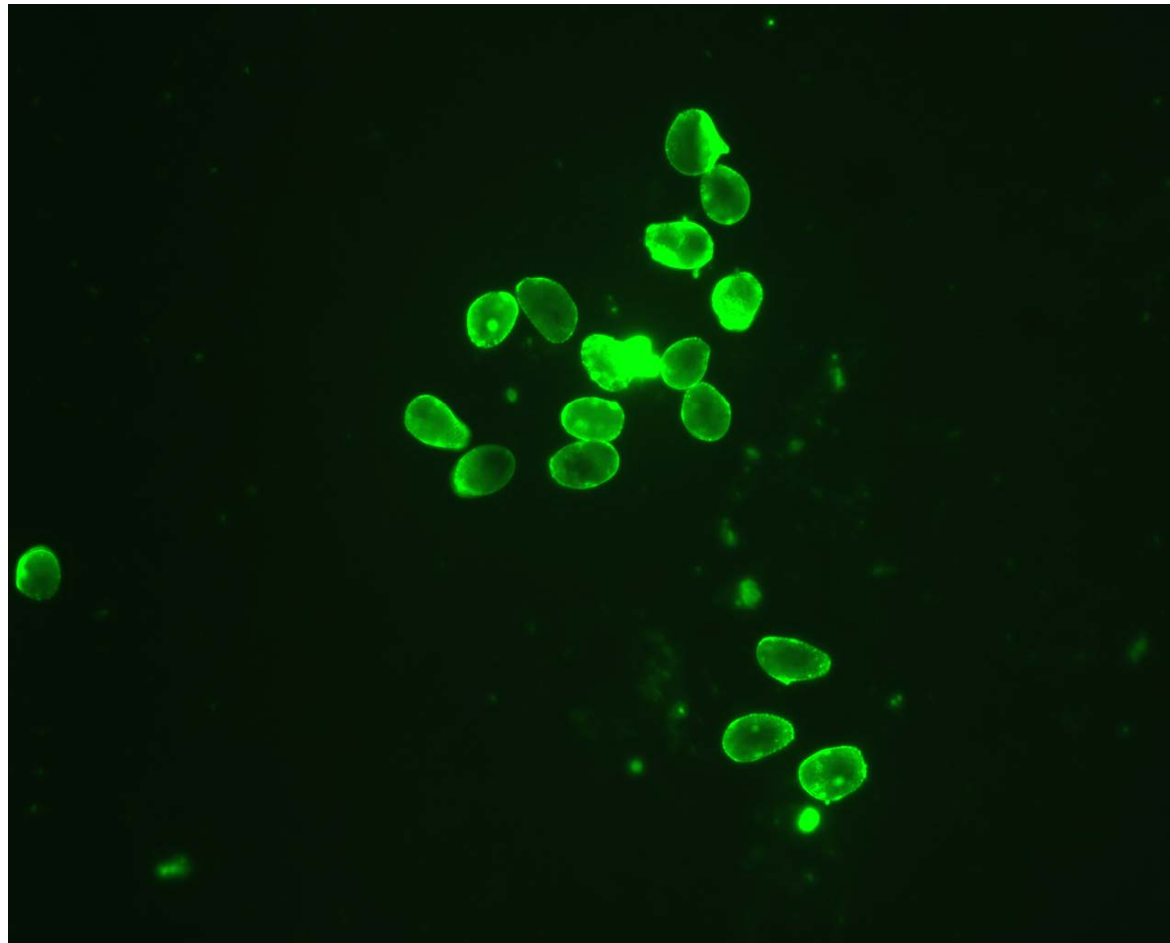


Incubate 1 hr, 22 C in dark

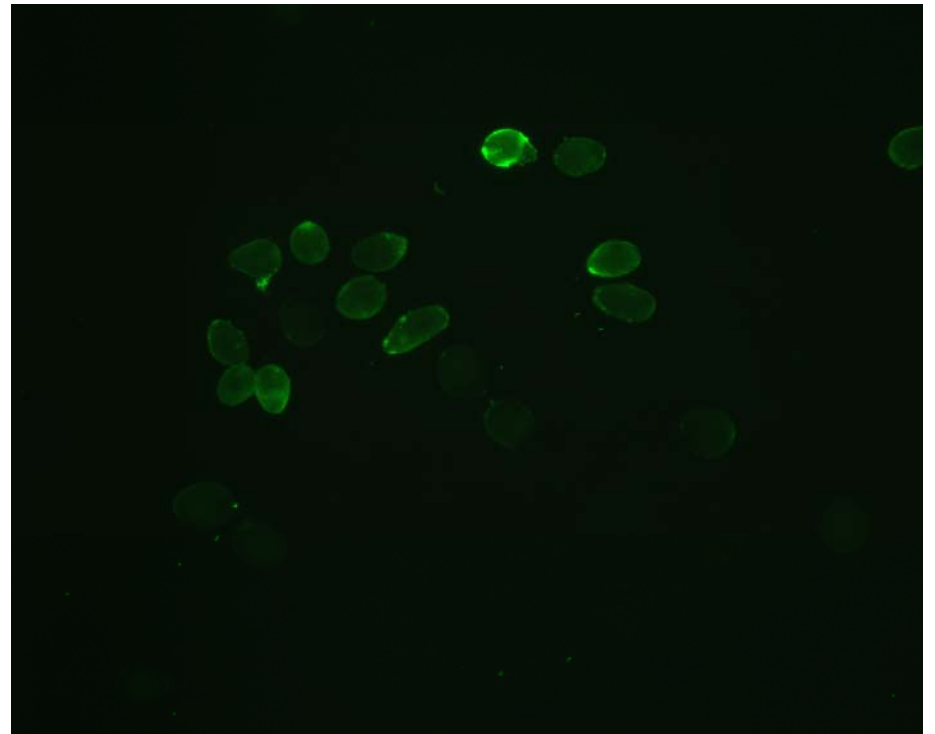
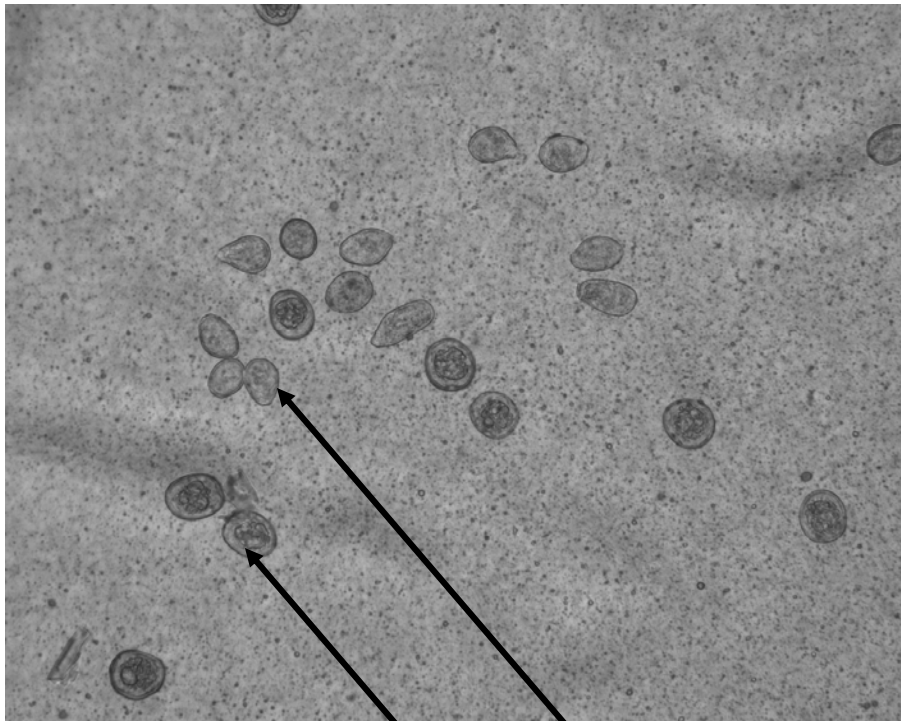


Wash 6X, PBS-Tween

Phakopsora pachyrhizi



IF Specificity



P. pachyrhizi

Puccinia graminis

Spore Capture/IF



- Spores captured in air sampler
- Spores attach to double-stick tape on slides
- IF carried out on slides



Advantages of Immunoassay

- Speed
- Ease of use
- Portability
- Sensitivity
- Specificity
- Quantitation possible



Disadvantages of Immunoassay

- Relative sensitivity - may be less than other methods
- Specificity varies among products - risk of false + or false -
- Cost