Common Serology Tests and their problems

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Serology

- What is the definition?
- A. Amplification and detection of genetic material
- B. Cell culture of viruses
- C. Measuring antibody or antigen in body fluids
- D. Microscopic examination of body fluids

Detection

- Detection of antigen-antibody complex
- Antigen-antibody complex requires specific conditions
  - temperature
  - pH
- Complex may be directly visible or invisible

Detection

Directly visible – agglutination, precipitation

Invisible

- requires specific probes (enzyme-labelled anti-immunoglobulin, isotope-labelled anti-immunoglobulin, etc.)
- binds Ag-Ab complex and amplifys signals
- signals can be measured by naked eyes or specific equipment e.g. in ELISA, RIA, IFA

Methods for Ag-Ab detection

- Precipitation
- Agglutination
- Hemagglutination and hemagglutination inhibition
- Viral neutralization test
- Radio-immunoassays
- ELISA
- Immunoflourescence
- Immunoblotting
- Immunochromatography

Precipitation

Principle

- soluble antigen combines with its specific antibody
- antigen-antibody complex is too large to stay in solution and precipitates

Examples

- flocculation test
- immuno-diffusion test
- counter-immuno-electrophoresis (CIEP)

Flocculation test (precipitation reaction)

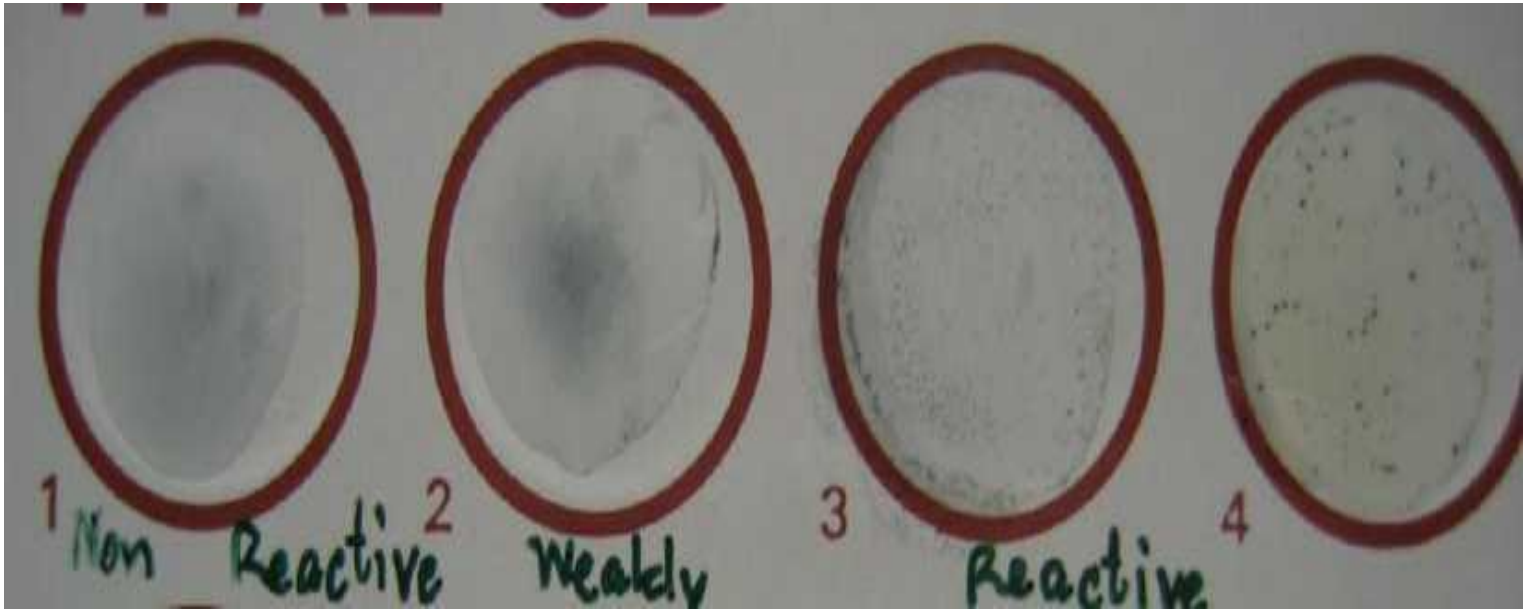
Principle

- precipitate, a concentrate of fine particles, is usually visible (macroscopically or microscopically) because the precipitated product is forced to remain suspended

Examples

- VDRL slide flocculation test
- RPR card test
- Kahn's test for syphilis

Flocculation test
(A precipitation reaction)



(1) Non Reactive **(2) Weakly Reactive** **(3,4) Reactive**

RPR card test

Precipitation: Performance, applications

- Advantages
  - sensitive for antigen detection
- Limited applications
- Time taken - 10 minutes

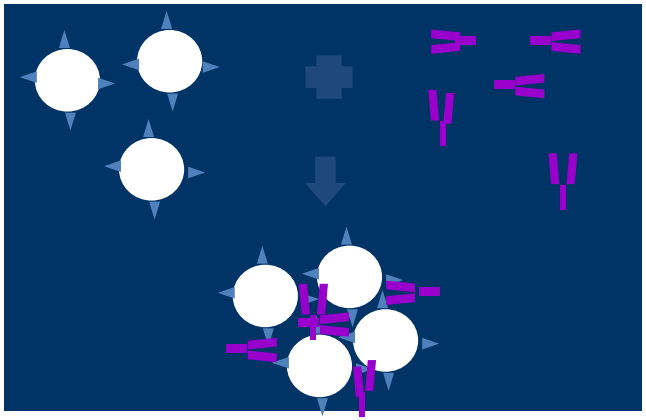
Direct agglutination

Principle

- combination of an insoluble particulate antigen with its soluble antibody
  - forms antigen-antibody complex
  - particles clump/agglutinate
- used for antigen detection

Examples

  - bacterial agglutination tests for sero-typing and sero-grouping e.g., *Vibrio cholerae, Salmonella spp*



Ag-Ab complex

Positive Negative

Passive (indirect) agglutination

Principle

- precipitation reaction converted into agglutination - coating antigen onto the surface of carrier particles like red blood cells, latex, gelatin, bentonite
  - background clears

Examples of types

- latex agglutination
- co-agglutination
- passive hemagglutination (treated red blood cells made resistant)

Examples of tests - agglutination for leptospirosis
Widal test (typhoid fever)

Reverse passive agglutination

Principle

- antigen binds to soluble antibody coated on carrier particles and results in agglutination
- detects antigens

Example

- detecting cholera toxin

Slide Agglutination

- Uniform suspension of particulate antigen and appropriate antiserum
- Positive result - Clumping of particles and clearing of the drop
- Typing of bacterial isolates
- Blood groups and typing

TUBE AGGLUTINATION

- Standard quantitative method
- Particulate antigen and equal volume of serial dilution of antiserum
- Agglutination titre
- Diagnosis - Typhoid, brucellosis, typhus fever

HETEROPHILE AGGLUTINATION

Weil–Felix - Typhus

Typhus, rickettsiae and Proteus

Streptococcus MG agglutination - Primary atypical pneumonia

Paul-Bunnel test – Infectious mononucleosis

Agglutination: Performance, applications

Advantages

- sensitive for antibody detection

Limitations

- Prozone phenomenon:
  - requires the right combination of quantities of antigen and antibody
  - handled through dilution to improve the match

Time taken

- 10-30 minutes

ELISA

- Which of the following is true?
- A. Fluorescent dyes illuminated by UV lights are used to show the specific combination of an antigen with its antibody
- B. Enzyme system is used to show binding of antigen with its antibody
- C. Radioactively labeled antigens are used as competitors to measure very low concentrations of antigens or antibody
- D. Visible clumping together by binding of antigen-antibody

Enzyme-linked immunosorbant assay (ELISA)

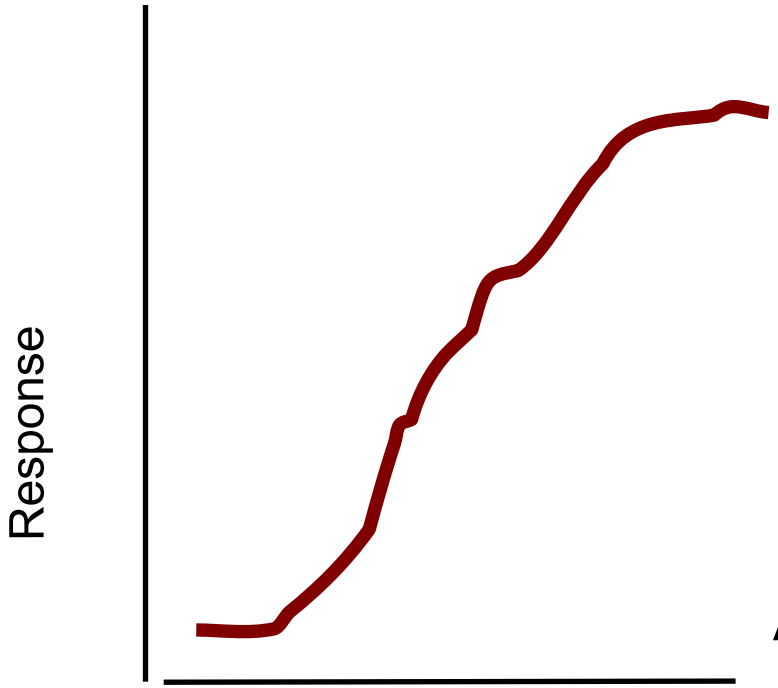
Principle

- use of enzyme-labelled immunoglobulin to detect antigens or antibodies
- signals are developed by the action of hydrolyzing enzyme on chromogenic substrate
- optical density measured by micro-plate reader

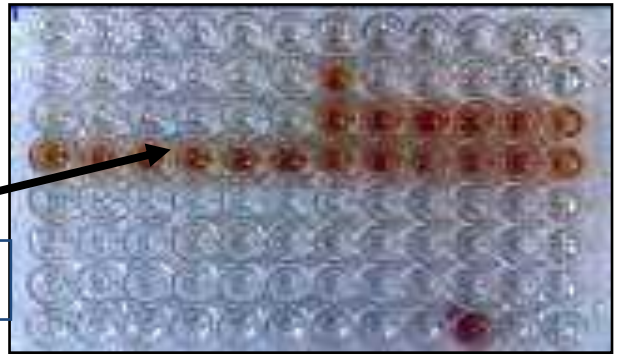
Examples

- Hepatitis A (Anti-HAV-IgM, anti-HAV IgG)

ELISA



Micro-plate reader



96-well micro-plate

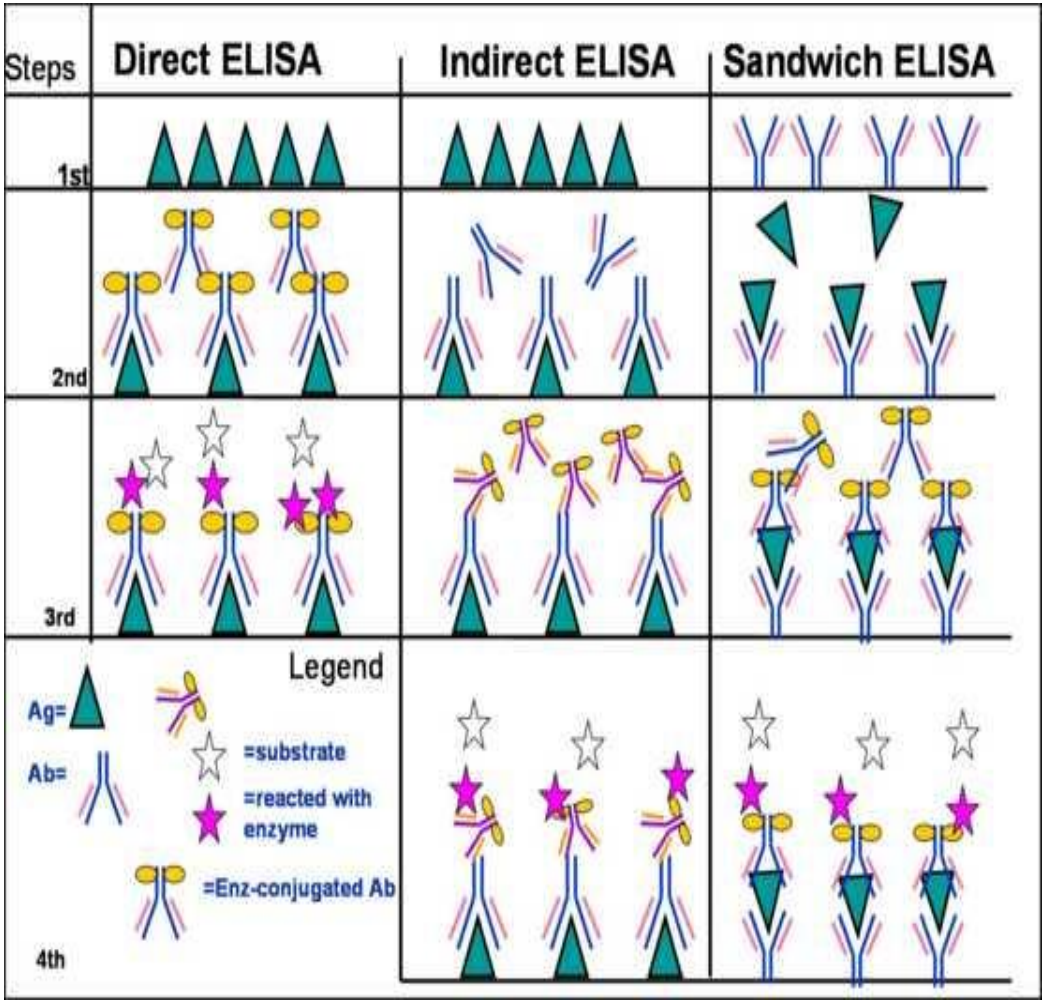
Types of ELISA

Competitive

- Antigen or antibody are labelled with enzyme and allowed to compete with unlabeled ones (in patient serum) for binding to the same target
- No need to remove the excess/unbound Ag or Ab from the reaction plate or tubes)

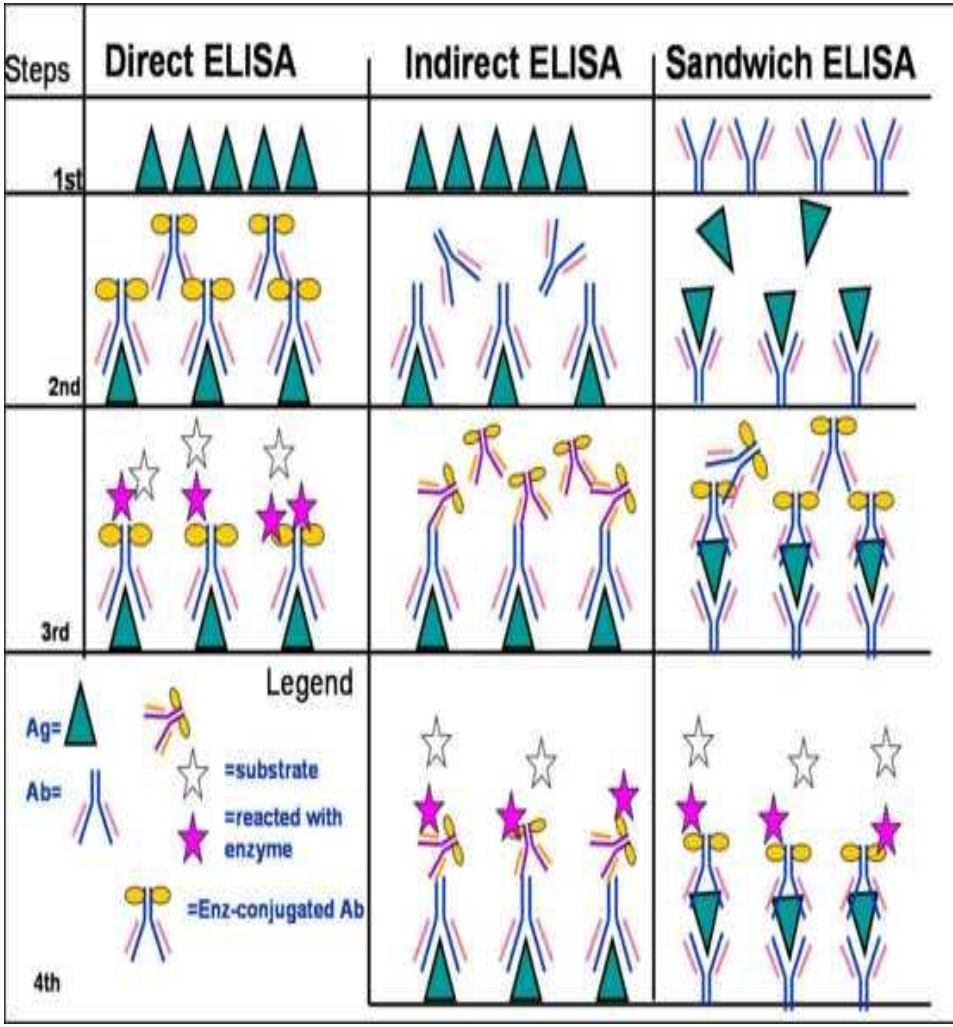
Non-competitive

- must remove excess/unbound Ag or Ab before every step of reactions



Types of ELISA used in the detection of antigens and antibodies

- Direct ELISA
- Indirect ELISA
- Sandwich ELISA
- Ab Capture ELISA (similar to sandwich ELISA but in 1st step, anti-Ig (M or G) is coated on the plate
- Then antibodies in patient serum are allowed to capture in next step



ELISA: Performance, applications

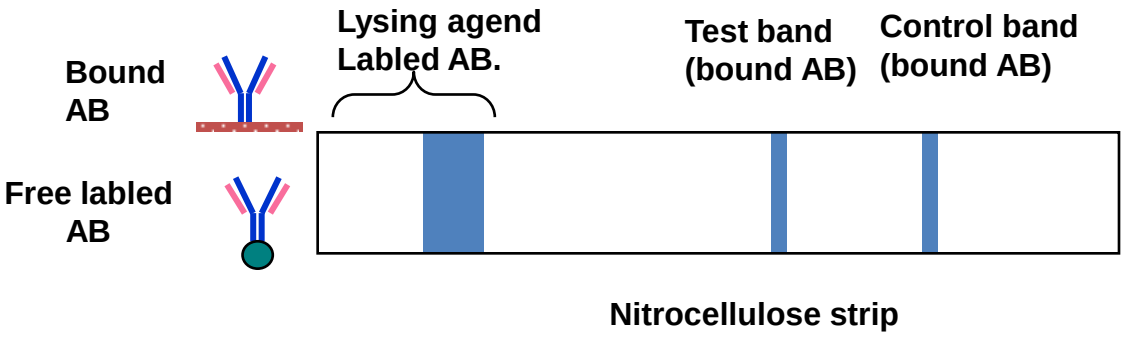
- Advantages
  - Automated, inexpensive
  - Objective
  - Small quantities required
  - Class specific antibodies measurable
- Limitations
  - Expensive initial investment
  - Variable sensitivity / specificity of variable tests
  - Cross contamination
- Time taken - 1 day

Performance comparison of various ELISAs for antibody detection

Performance characteristic	Non-competitive ELISA	Competitive ELISA	Capture ELISA
Purpose	**Antibody**	**Antibody**	**Best for class specific antibody**
Sensitivity	**++**	**++**	**++**
Specificity	**++**	**++**	**+++**
Cost	**+**	**++**	**+++**
Ease of performance	**++**	**+++**	**++**
Time taken	**++**	**+**	**+++**

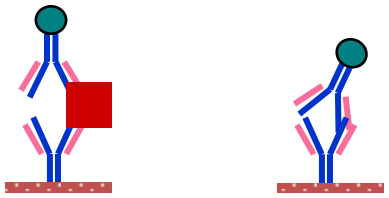
Immuno-chromatography: Principle (1)

- Dye-labelled antibody, specific for target antigen, is present on the lower end of nitrocellulose strip or in a plastic well provided with the strip.
- Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line
- Either antibody specific for the labelled antibody, or antigen, is bound at the control line



Immuno-chromatography: Principle (2)

- If antigen is present, some labelled antibody will be trapped on the test line
- Excess-labelled antibody is trapped on the control line



Labelled AB-AG-complex Captured by bound AB of test band

Labelled AB-AG-complex Captured by bound AB of control band

Captured Ag-labelled Ab-complex

Captured labelled Ab

Immuno-chromatography: Performance, applications

- Advantages
  - Commercially available
  - Single use, rapid test
  - Easy to perform
  - Can detect antigen or antibody
  - Can be used in the field
- Limitations
  - Cost
  - Concern validated data
- Time taken - 1 hour

Interpretation of antigen detection tests

- In general, detection of the antigen denotes a presence of the pathogen
- More important in some of parasitic and fungal diseases

Antigen test	Interpretation
Positive	**•Current or recent infection**
Negative	**•No infection** **•Insufficient number of organisms** **•Sensitivity of testing is low (Consider test by test)**

Interpretation of a single, acute IgM test

IgM test	Interpretation
Negative	•**No current infection**
Positive (Newborn)	•**Congenital infection**
Positive (Adult)	•**Primary or current infection**

Interpretation of two, acute and convalescent IgG tests *

Test	Interpretation
Negative	• **No current infection** • **Past infection** • **Immuno-suppression**
Positive (4-fold rise or fall in titer)	• **Recent infection**

*** Convalescent serum collected 2-4 weeks after onset**

Interpretation of a single IgG test

Test	Interpretation
Negative	•**No exposure or immuno-suppression**
Positive (Newborn)	•**Maternal antibodies crossed the placenta**
Positive (Adult)	•**Evidence of infection at some un-determined time** •**Infection in some cases (e.g., rabies, legionella, Ehrlichia)** •**May be significant if immuno-suppression (e.g., AIDS)**

*** Collected between onset and convalescence**

Elements influencing the sensitivity and specificity of a given test kit

- Test format
  - Precipitation versus IFA, Rapid test versus ELISA
- Purity of the antigen used
  - Crude versus purified antigen versus synthetic peptides
- Type of the antibody used
  - Polyclonal versus monoclonal antibodies
- Interfering substances in the sample
  - Presence of rheumatoid factor in the serum of the patient
- Similarity in antigenic composition of pathogens
  - Cross reactivity

Sources of Error

Test Phase	Source of Error	Result
Extraction Phase Applies to throat and nasopharyngeal swabs.	Failing to mix the swab thoroughly with the extraction reagents. Not leaving the swab in the extraction reagent for the required amount of time. Inadequate expression of the liquid from the swab after extraction.	Insufficient specimen is obtained. May lead to a false-negative test result.
Wash Phase Applies to some procedures.	Insufficient washing of the test surface.	Debris left behind may be misinterpreted as a positive result.
Test Phase Single-use devices.	Not adding the required amount of specimen to the test device.	Insufficient specimen could lead to a false-negative test result.
	Using a test device that has discoloration in the test area prior to applying sample.	False-positive test if the color is interpreted as a positive result. False-negative if the color masks a weak positive reaction.
Test Phase Agglutination-based procedures.	Failing to spread the specimen across the entire test area or not rotating the test for the required amount of time.	Specimen cannot mix properly with the reagent. May be read as a false-negative or a false-positive.
Test Phase Single use devices and agglutination-based procedures.	Holding a dispensing device incorrectly so that an insufficient volume is dispensed.	Incorrect proportion of specimen to reagent may cause an erroneous test result.
	Using specimens or reagents that are not at room temperature at the time of testing.	As test conditions differ from the manufacturer's tested and approved procedure, the results are not reliable.
	Using expired test reagents.	Reagent reactivity has lessened and may result in a false-negative result.

HIV

- Screening test
- Confirmatory test

Window period for HIV

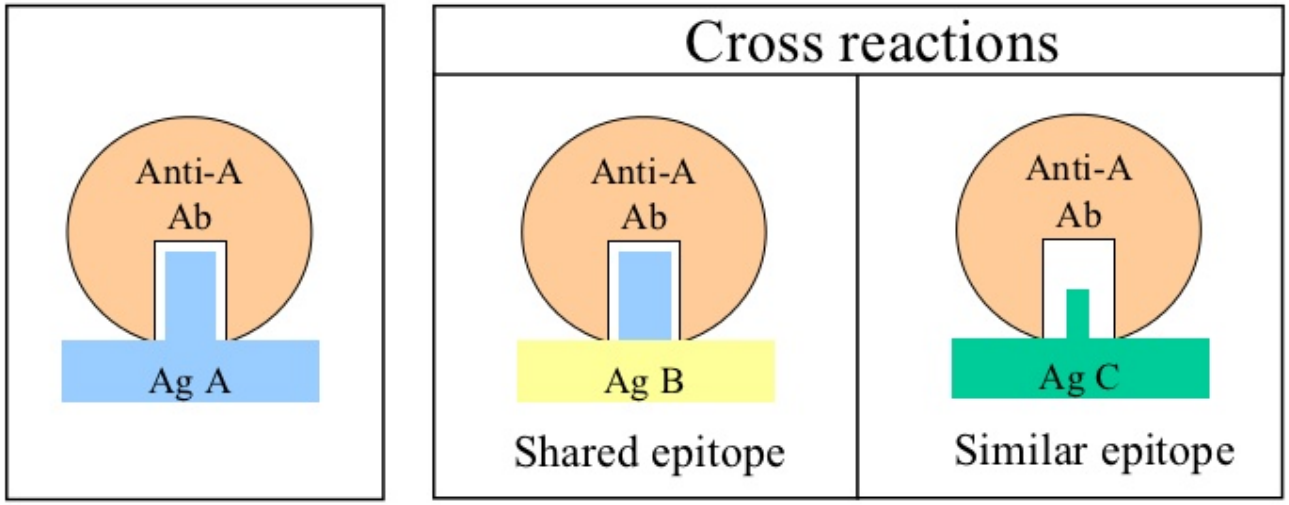
- 6 to 12 weeks for the antibody (1^{st}, 2^{nd}, 3^{rd} generation assays)to be positive

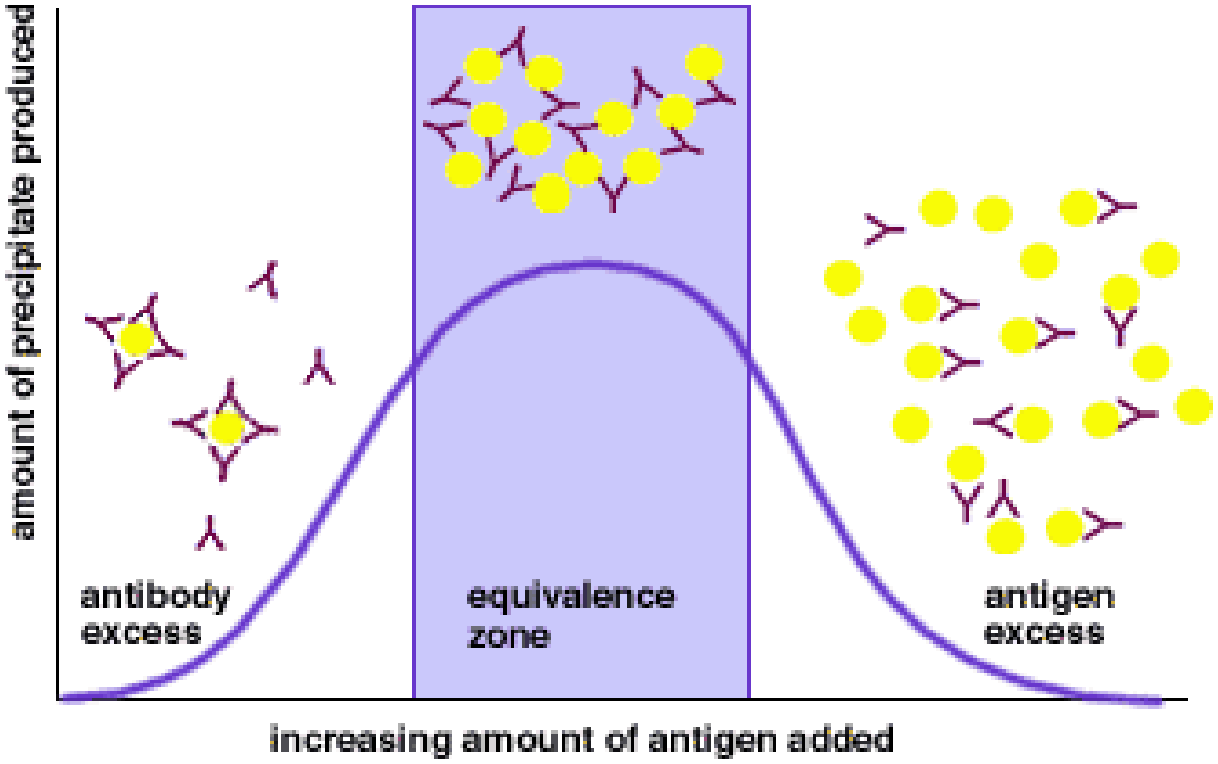
- Using antibody plus p24 antigen (4^{th} generation test) the test could be positive in 16 days

- False negative IgM
- Persistent IgM
- Cross reactive antibodies

Cross Reactivity

- The ability of an individual Ab combining site to react with more than one antigenic determinant.
- The ability of a population of Ab molecules to react with more than one Ag




amount of precipitate produced
antibody excess
equivalence zone
antigen excess
increasing amount of antigen added

- Prozone - Zone of antibody excess
- Zone of equivalence
- Postzone - Zone of antigen excess

THANK YOU