



Enzyme Linked Immunosorbent Assay (ELISA)

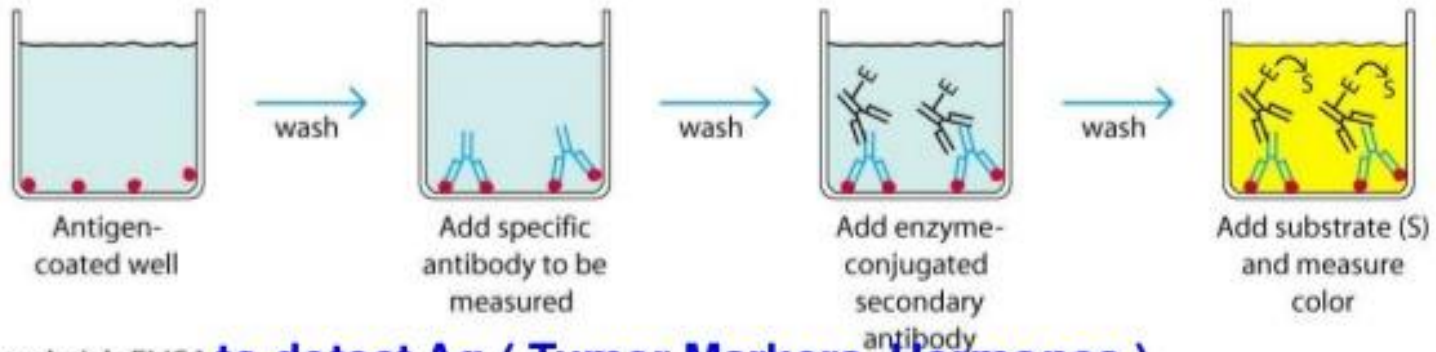
Assistant prof. Dr. heba fadhil hassan

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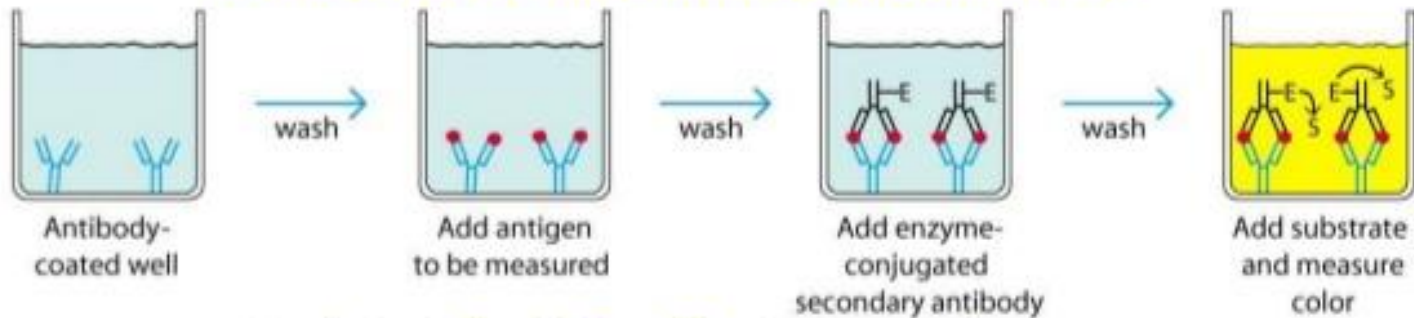
- Enzyme Linked Immunosorbent Assay (ELISA)
- Term Was Coined By Engvall and Pearlmann in 1971
- Different Types
 - direct
 - Indirect (**Detect Ab to Ag coating plastic**)
 - Sandwich (**Detect Ag to Ab coating plastic**)
 - Competitive

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- **Enzymes Commonly Used: HRP (Horse Radish Peroxidase) And AKP (Alkaline Phosphatase)**
 - **Substrate is TMB Tetramethylbenzidine (Chromogen)**

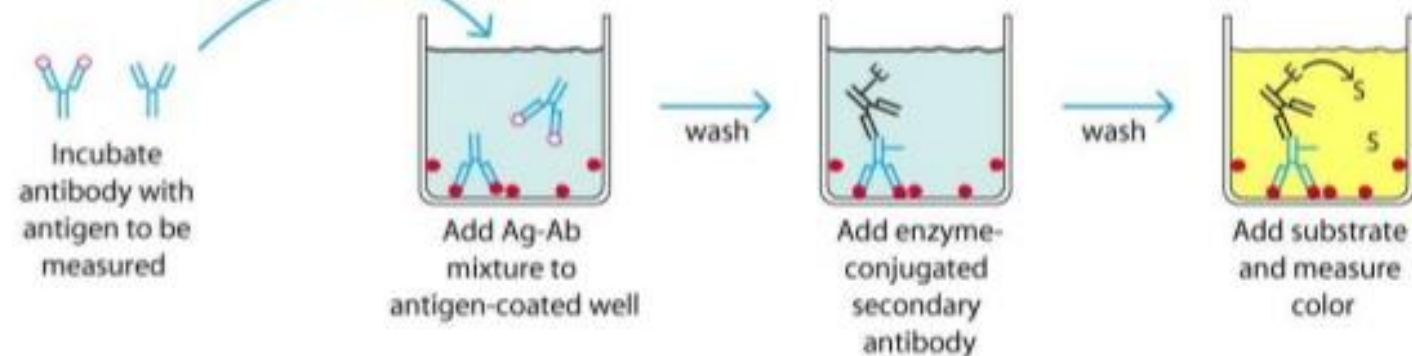
(a) Indirect ELISA to detect Ab (HIV, HCV)



(b) Sandwich ELISA to detect Ag (Tumor Markers, Hormones)



(c) Competitive ELISA to detect Ag (Free Testosterone)

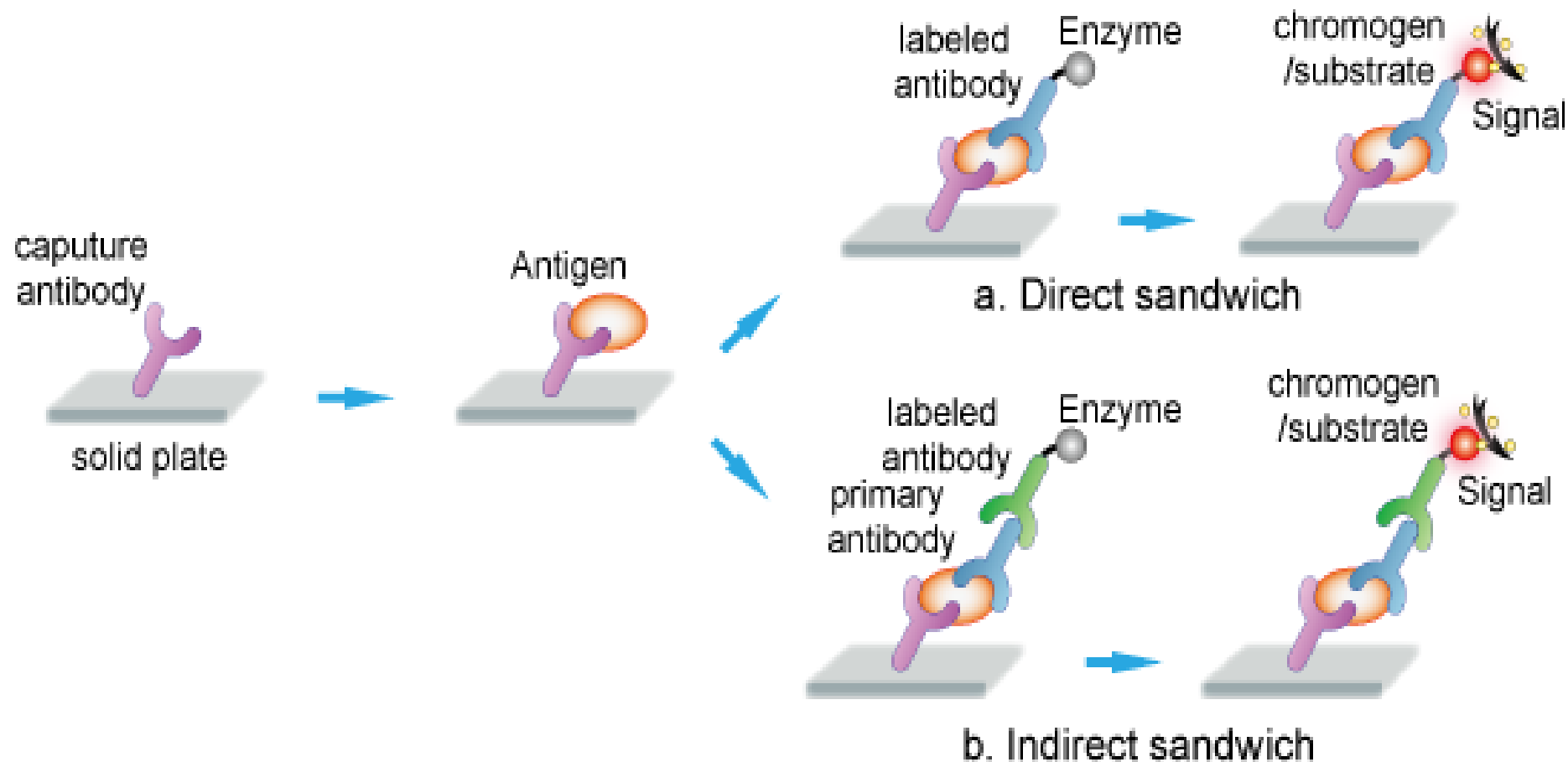


Comparison between Indirect Sandwich & Competitive ELISA

Sandwich ELISA

- 2 Antibodies Required
- Must Recognize Different Epitopes
- 1st Antibody Is Referred To As Capture Ab
- 2nd Antibody Detection Ab
- 2nd Antibody Is Biotinylated
- **Enzymes Commonly Used: HRP (Horse Radish Peroxidase) And AKP (Alkaline Phosphatase)**
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Sandwich ELISA

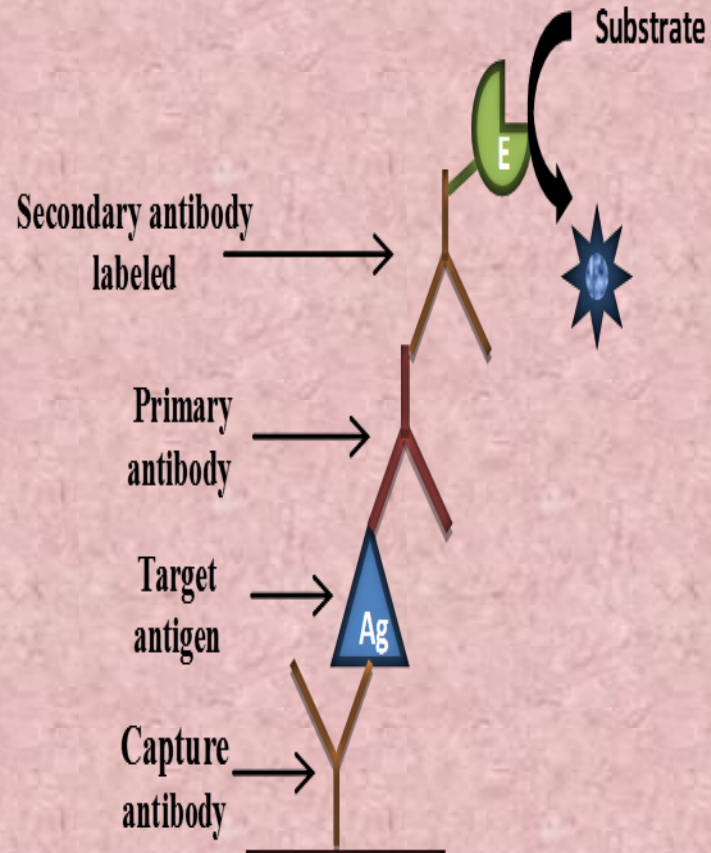


1. Capture antibody is coated onto wells by passive adsorption and incubation.

2. Antigen sample is added and incubated with capture antibody

3. labeled antibody (a)/primary antibody and labeled antibody (b) is added and incubated with antigen

4. Substrate / chromophore is added and colour develops.



Sandwich ELISA

Sandwich ELISA

The viral antigen is sandwiched between two antibodies namely capture and detection antibodies. For the assay both the antibodies should target different epitopes of the antigen. This is followed by addition of enzyme conjugated anti-species immunoglobulin. On adding suitable chromogen-substrate colour reaction develops.

Advantages

Assay is quantitative, amount of viral antigen can be detected

Assay has high sensitivity and specificity

More samples can be tested at the same time

Disadvantages

Need ELISA reader for result interpretation; not possible under field conditions

The method is time consuming and labourious.

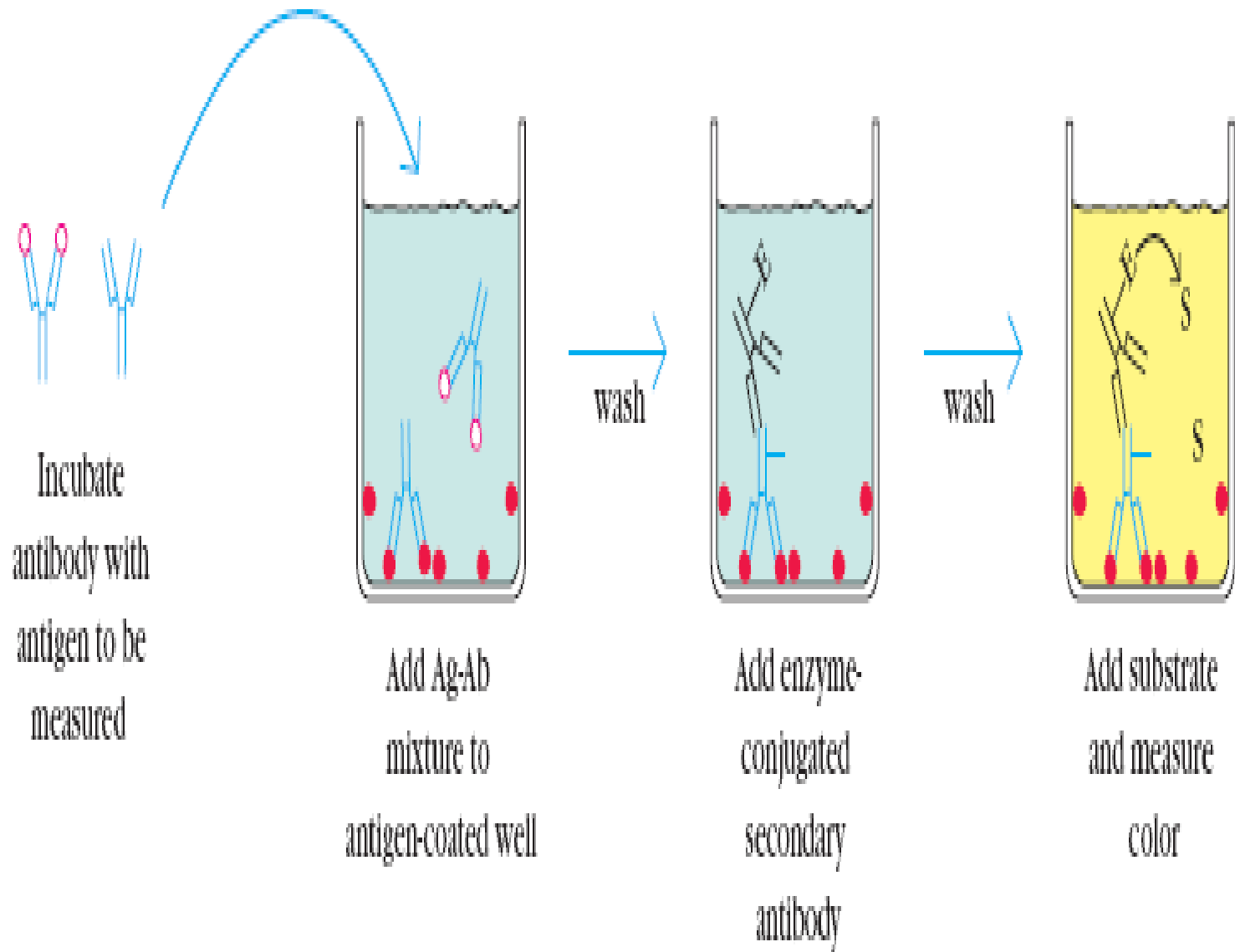
Applications:

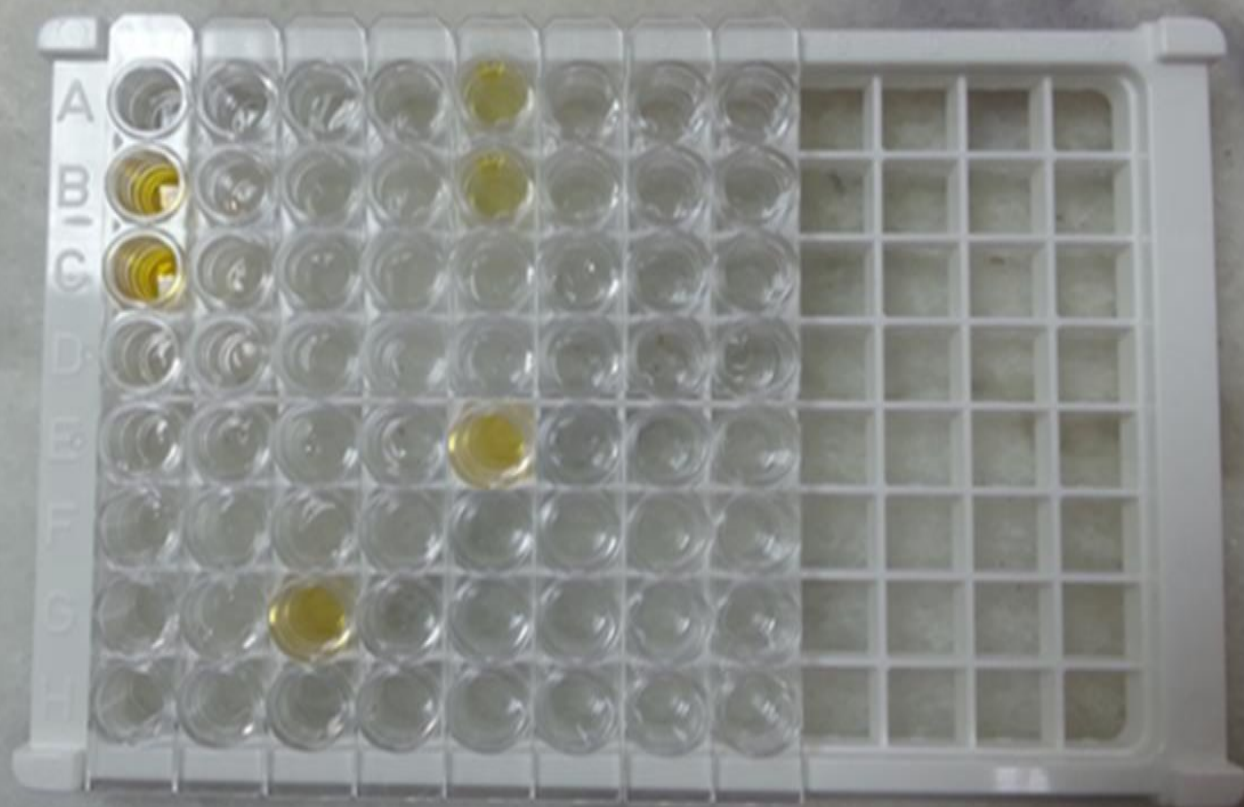
Used in diagnosis of PPR, Bluetongue, FMD etc

Competitive ELISA (cont.)

- The more antigen in the sample, the more primary unlabeled antibody will be bound and thus less available to bind to the antigen in the well plate.
- Secondary enzyme-linked antibody is added followed by substrate.
- Absence of color indicates a positive sample.
- **Advantage:** High sensitivity to compositional differences in complex antigen mixtures, even when the specific detecting antibody is present in relatively small amounts.

(c) Competitive ELISA





Results

- ▶ After reading the results the standard curve is drawn where the concentration is plotted on the X-axis and the absorbance on the Y-axis

