Detection of Microbe in Food : Quanlitative methods-I

Cultural and Rapid Immunoassay based methods

Qualitative methods

- Qualitative methods comprises of methods for detection of specific microbe species detection among total population.
- Eg. Food borne *Salmonella*, *E. coli* O157:H7. Two types

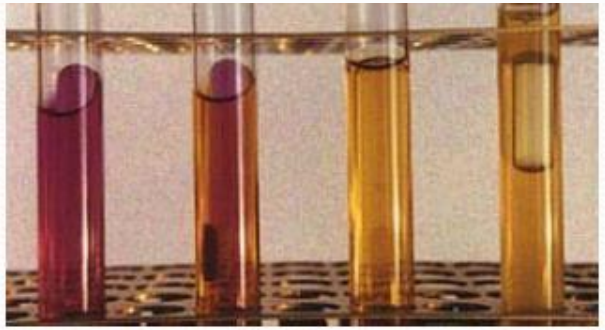
1. **Cultural methods**: Involves Selective enrichment and isolation on selective media, followed by confirming the suspicious bacterial colonies by biochemical tests.
2. **Rapid methods:** different rapid methods with high sensitivity and specificity have been developed to overcome the limitations of conventional methods for the detection and identification of foodborne pathogens.

Cultural methods

- Generally regarded as the 'gold standard' for microbiological analysis of food.
- Involves detecting the foodborne bacteria pathogens present in food are based on culturing the microorganisms on agar plates followed by standard biochemical identifications
- Simple, inexpensive and but can be time consuming as they depend on the ability of the microorganisms to grow in different culture media such as pre-enrichment media, selective enrichment media and selective plating media.
- Usually conventional methods require 2 to 3 days for preliminary identification and more than a week for confirmation of the species of the pathogens

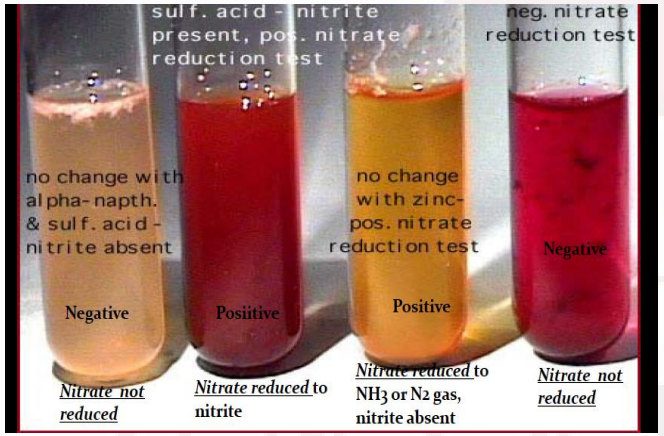
Biochemical identification systems

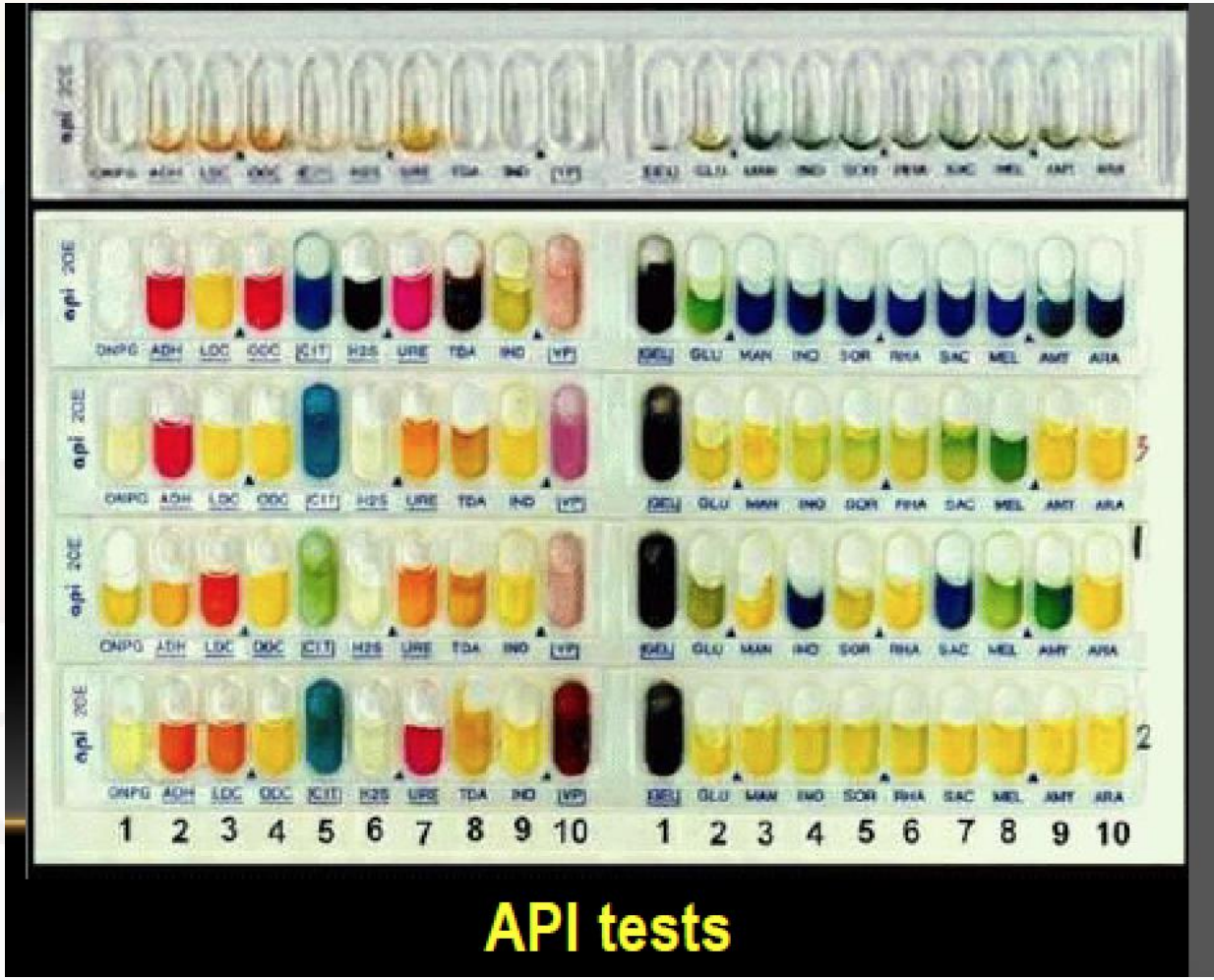
Carbohydrate Utilization test



TSI test

Nitrate reduction test





Rapid Biochemical identification kits

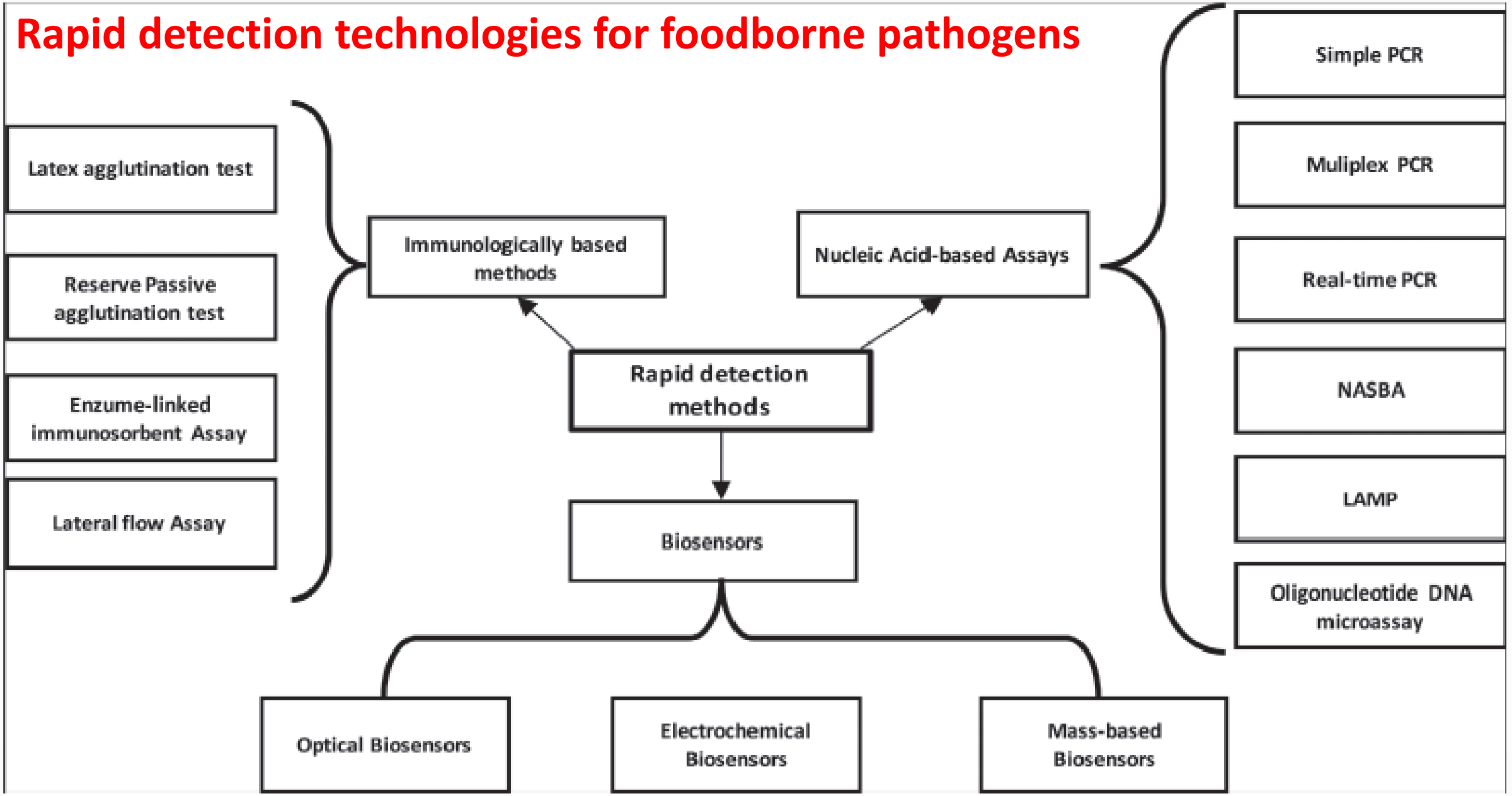
- **culture-based methods** still represent the first choice for many food testing laboratories.
- they are sensitive, inexpensive, easy to use.
- give both qualitative or quantitative information on the number and type of viable microorganisms present in the food samples
- However, culture-based analysis of food is generally not a rapid process.
- They are time consuming, laborious and have limited detection capability if microorganisms is in an injured state or a VBNC (Viable But Non Culturable) state

Rapid Detection methods

Need for rapid Tests

- To provide immediate information on the possible presence of pathogen in raw material and finished products.
- Low numbers of pathogenic bacteria are often present in complex biological environment along with many other non-pathogenic organisms. Even a single pathogen can be infectious.
- For monitoring of process control, cleaning and hygienic practices during manufacture
- To reduce human errors and to save time and labor cost

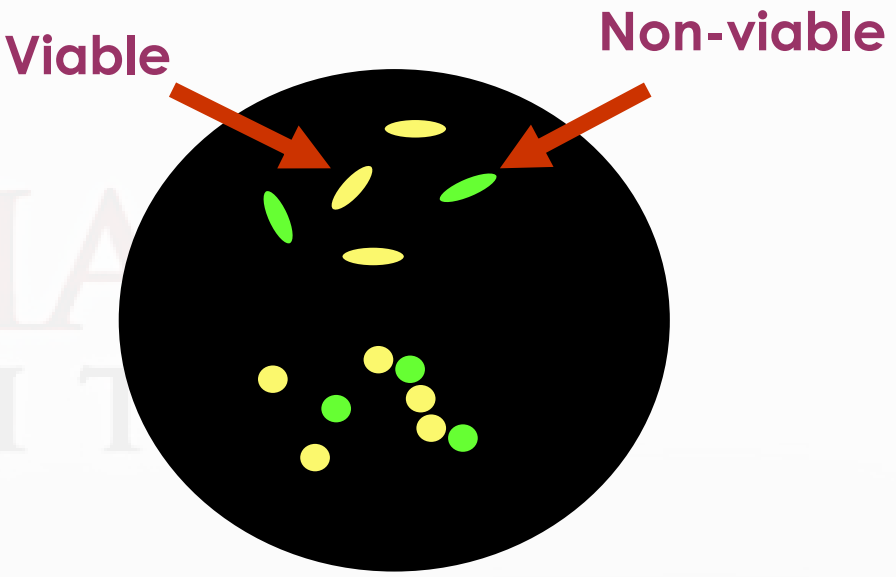
Rapid detection technologies for foodborne pathogens



http://dx.doi.org/10.5772/intechopen.74421

Direct epifluorescent filter technique (DEFT)

- Liquid food is filtered through membrane filter and a fluorescent dye (Acridine orange) is poured through filter
- Epifluorescent microscopy counting (manual or automatic)
- Acridine orange is a nucleic acid binding dye, gives orange fluorescence when bound to RNA and green fluorescence when bound to DNA
- Distinguish Viable cell from Non-viable cell
- Viable cells: RNA > DNA --- orange
- Non viable cells: DNA > RNA ---green



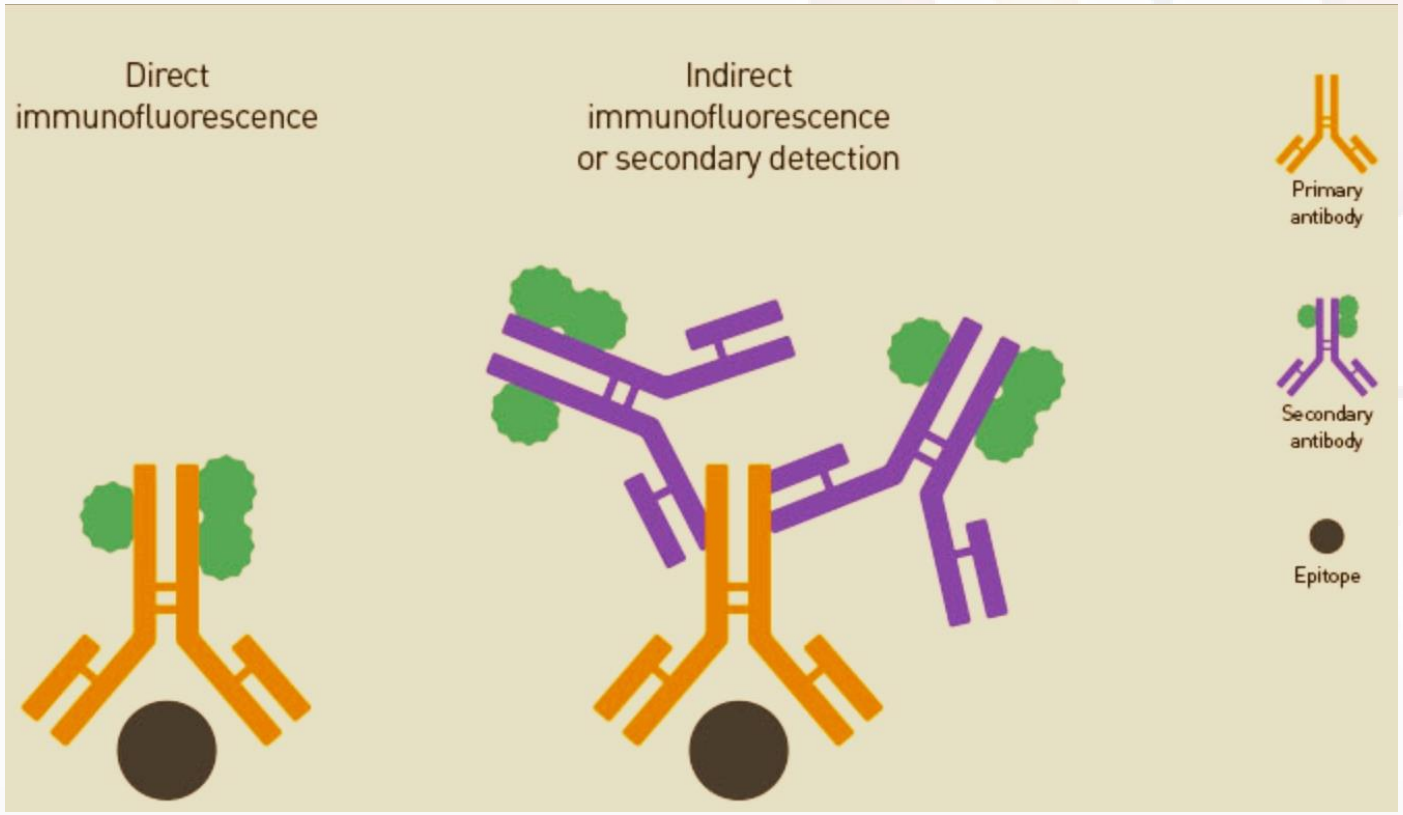
Immunoassays

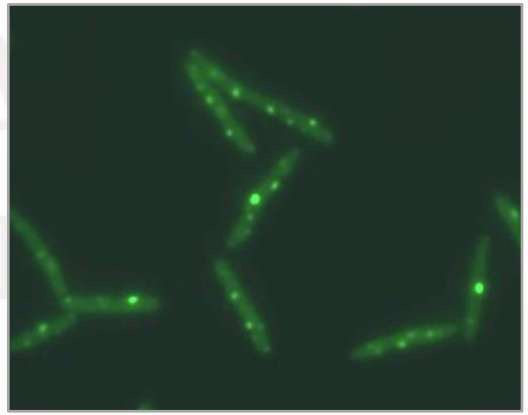
- The binding strength of a particular antibody to its antigen determines the sensitivity and specificity of immunological-based methods.
- Immunological-based methods involve the use of polyclonal and monoclona antibodies
- Ag-Ab interaction that can be detected by clumping, agglutination, color formation from chromogenic substrate, formation of immunoband or fluorescence

➢ **Immunofluorescence**
➢ **RPLA**
➢ **Enzyme immunoassay (EIA or ELISA)**
➢ **Lateral flow assay**
➢ **Magnetic Immunobeads**

Immunofluorescence

- Fluorescence conjugated antibody (against somatic/flagellar Ag of pathogen) mixed with sample on a glass slide and observed under fluorescent microscope

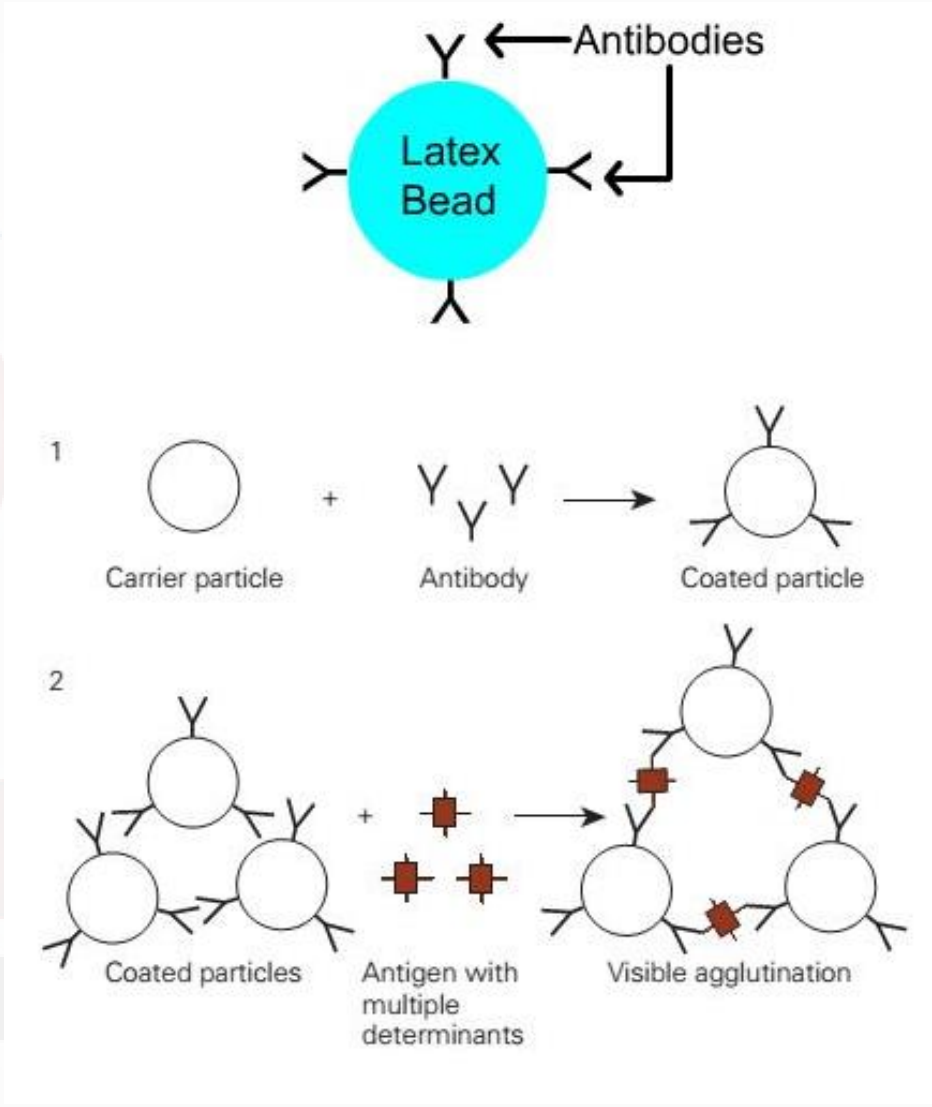




Reverse passive latex agglutination (RPLA) test

To detect toxins of several food born pathogens eg. *Closteridium perfringes*, *Bacillus cereus*

- Antibody to specific toxin is immobilized on latex particles and mixed with sample in a microtitre plate.
- If antigen is present then visible agglutination is observed. Diffused pattern: toxin +ve; Ring/button: -ve
- Advantages: Simple, rapid and Cost effective

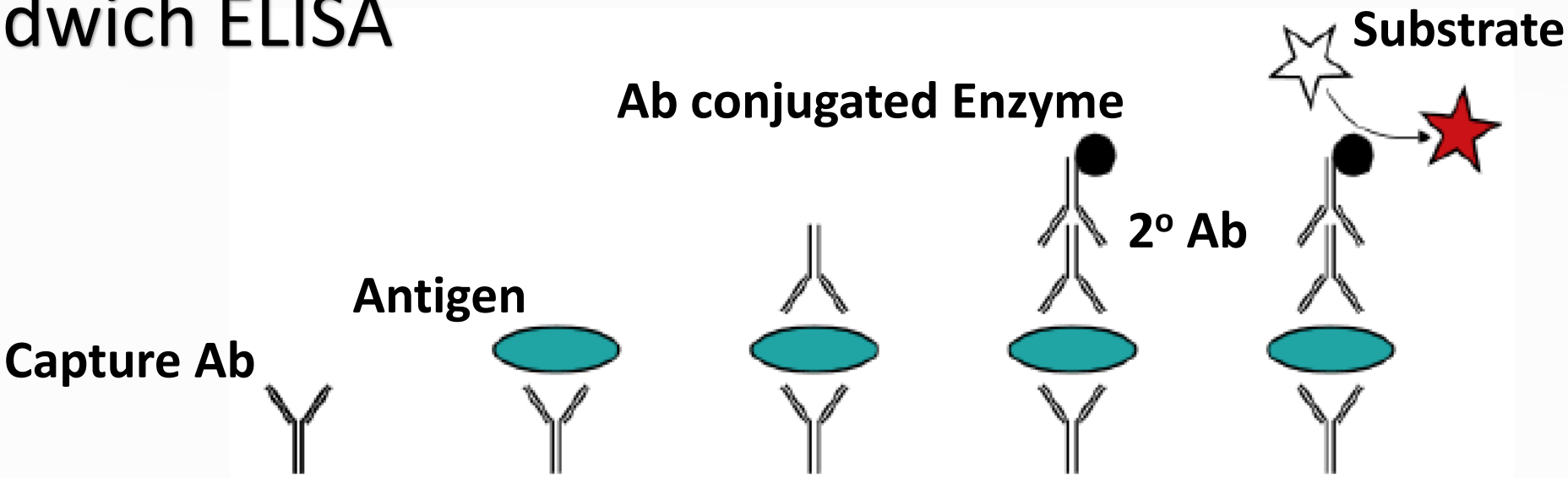


https://slideplayer.com/slide

Enzyme Linked immunosorbant assay (ELISA)

- Most commonly used immunological methods for the detection of food borne pathogens.. *Listeria*, *Salmonella*, *Camphylobacter jejuni or* specific toxin
  - Types: Indirect ELISA, Sandwich ELISA, Competitive ELISA
  - Also commonly used for the detection of toxins present in foods such as *Clostridium perfringens* α, β, and ε toxin, staphylococcal enteroxins A, B, C, and E,botulinum toxins and *Escherichia coli* enterotoxins
  - high-throughput and automated ELISA systems such as VIDAS (BioMerieux) and Assurance EIA (BioControl) are available for the detection of foodborne pathogens

Sandwich ELISA



Enzyme

Alkaline phosphatase (ALP), Horse Radish Peroxidase (HRP)

Substrate

Tetramethylbenzidine (TMB) + 30% H2O2

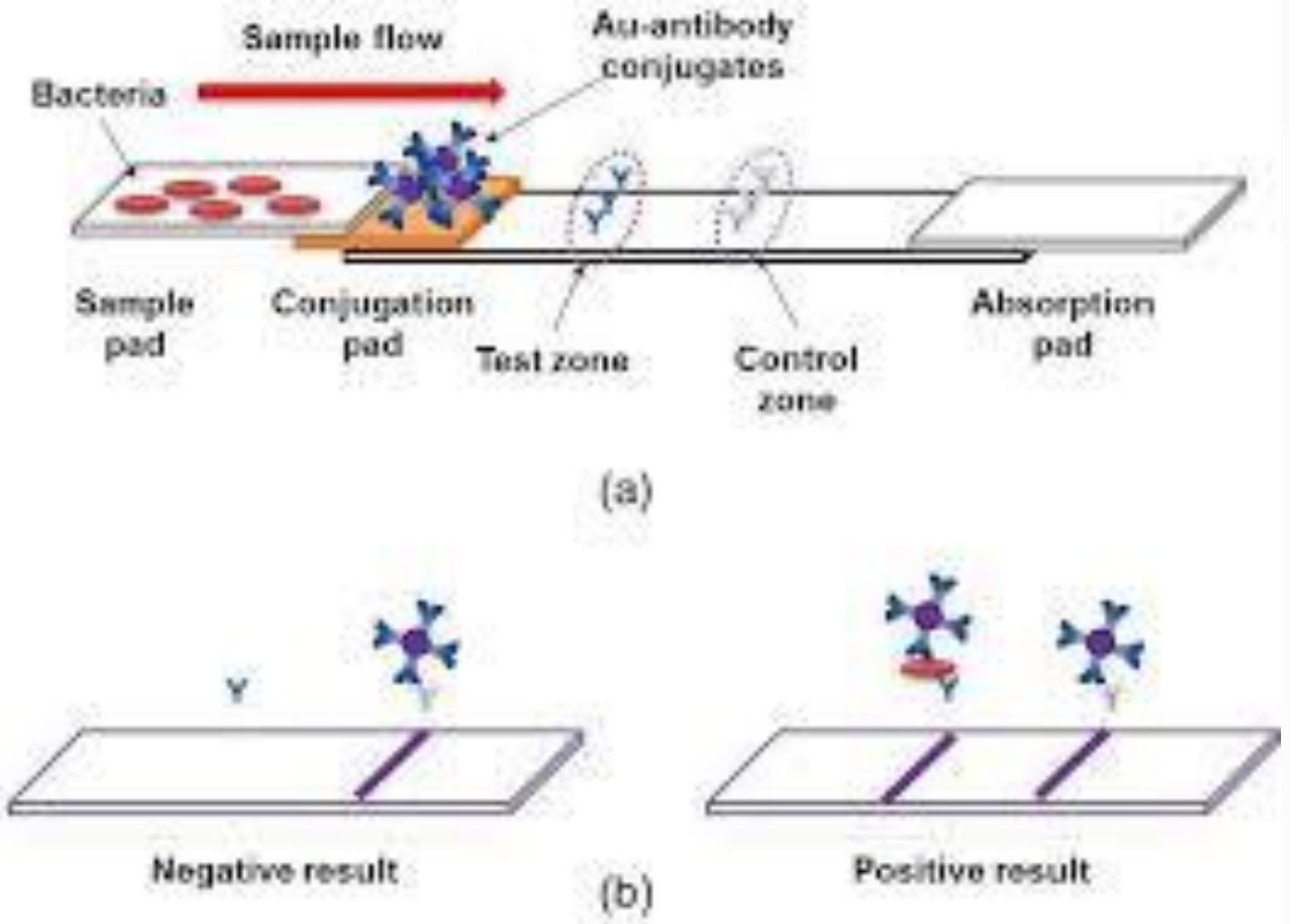
Azinobis sulphonic acid (ABTS)

o-phenylinediamine (OPD), p-nitrophenyl phosphate

Lateral flow assay

- LFA is low cost, reliable, easy-to-operate, sensitive, specific, and allows the detection of bacteria (*Escherichiacoli* O157 ,*Staphylococcus aureus, Campylobacter jejuni , Listeria* spp.and *Salmonella*
- It can also be used to detect toxins which may cause food- borne diseases such as brevetoxins and staphylococcal enterotoxin B
- Method using dipstick and immunochromatographic strips have been developed for rapid onsite detection of foodborne pathogens.
- The detection of foodborne pathogens by lateral flow immunoassay employs labels such as monodisperse latex, col- loidal gold, carbon and fluorescent tags

Lateral flow assay procedure



- Commercial LFA strips are also available eg: Reveal® test kits (Neogen) for *Listeria, Salmonella* and *Escherichiacoli* O157

https://www.e-sciencecentral.org/

Immunomagnetic Separation (IMS)

It is an established technique used for recovery of microorganisms, cells, proteins etc. using specific antibodies immobilized on paramagnetic beads.
Developed for detection of *Salmonella* spp. *Listeria* spp. *Listeria monocytogenes E. coli, Legionella* spp. and *Cryptosporidia*.

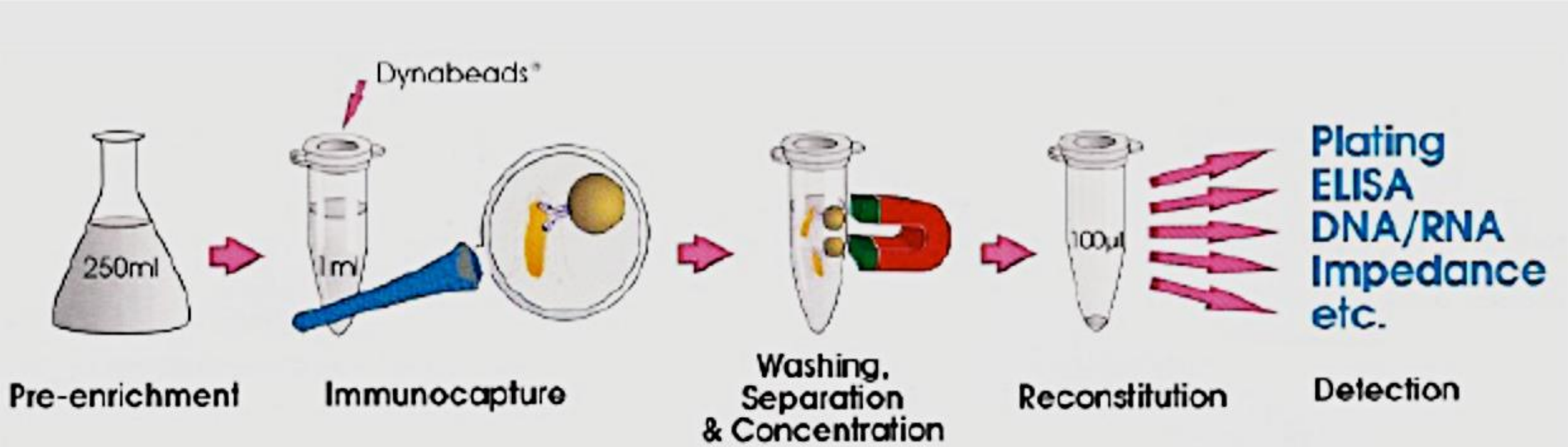
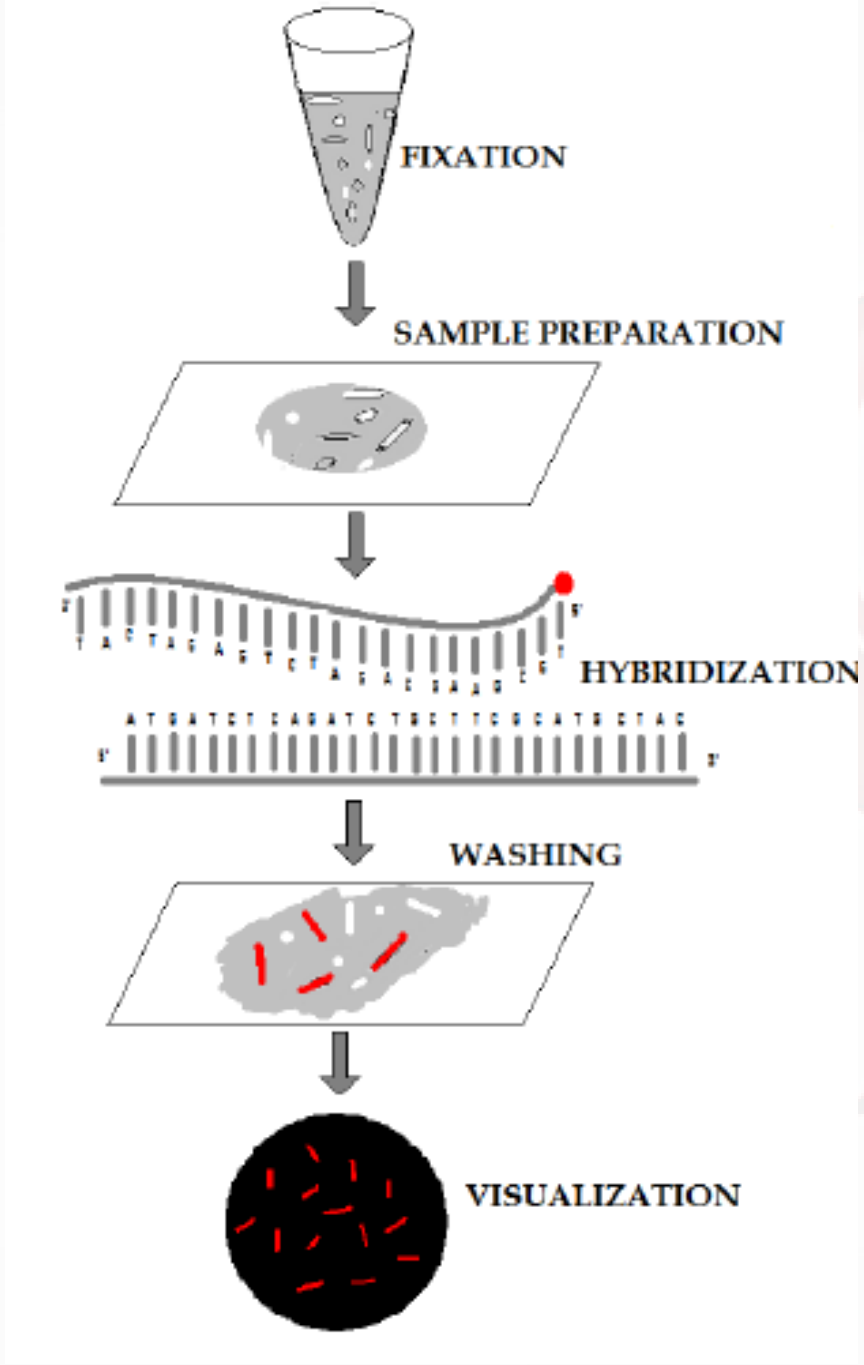


Figure 1. Protocol for detection of bacterial pathogens from food matrices (Dynal).

Source: Wirtanen et al 2007

Fluorescent in situ hybridisation (FISH)

- Rapid and sensitive technique
- detection of both cultivable and non cultivable micro-organisms
- Determine complex microbial communities.
- *Staphyl.* spp., *Escherichia coli*, *Salmonella* spp., *Campylobacter*,



Steps involved

- Fixation and permeabilisation of the bacterial cells,
- Hybridisation of the probe and the target sequence,
- Stringency washes to remove excess probes;
- Visual detection & documentation by fluorescent microscopy

https://www.researchgate.net/figure/Flow-chart-of-a-typical-FISH-procedure-The-FISH-technique-consists-in-hybridization-of_fig1_235752267

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