Food Microbes & Detection Methods

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Food Microbiology

- food is an ecosystem and microorganisms play a key role in the stability of that ecosystem
- microorganisms are introduced to the food ecosystem from the soil, harvesting, handling, storage, and packaging

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Food Microbiology

- fermentation: good food microbiology

  food that have been intentionally altered such as sour cream, cheese, beer

  any desirable change a microorganism makes to food

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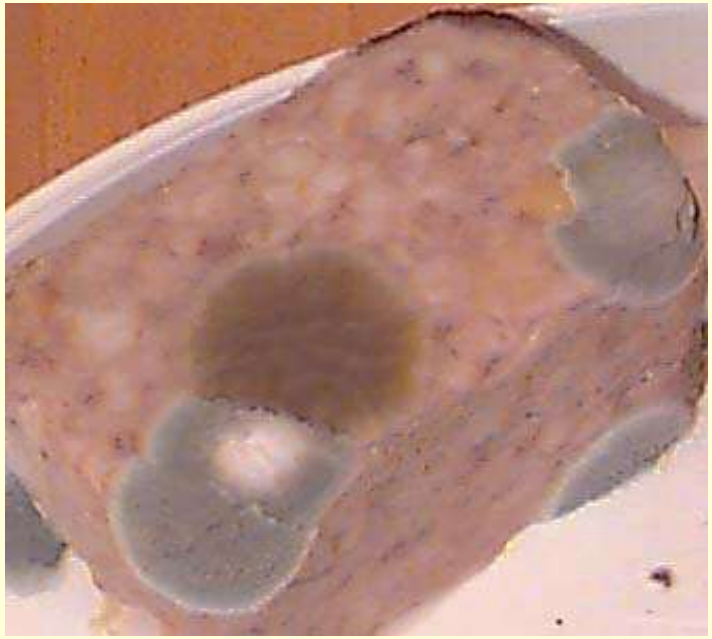
Food Microbiology

- spoilage: bad food microbiology
  - undesirable changes to food; sour milk, moldy bread
  - preservatives and refrigeration inhibit the growth of microorganisms



Moldy Spam

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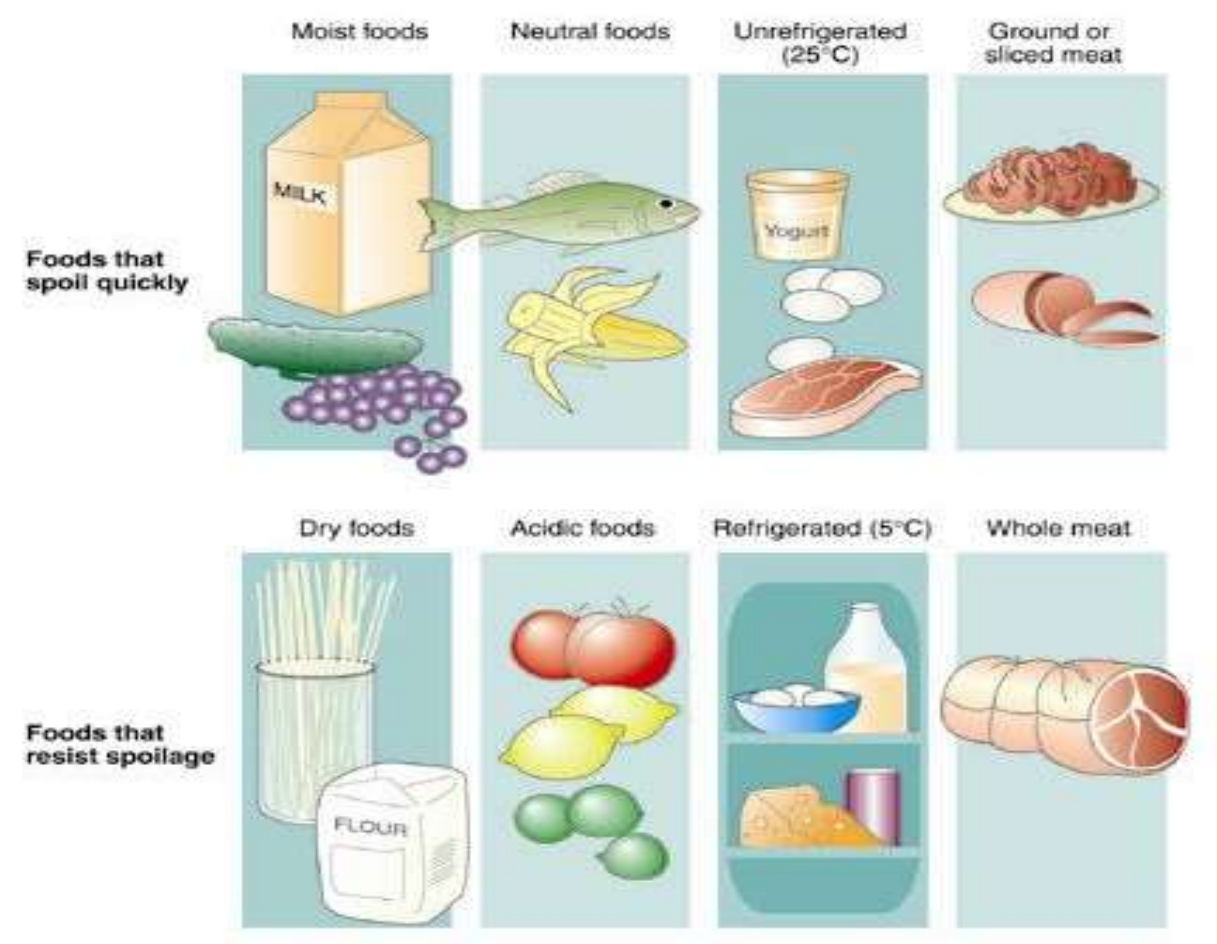
Conditions for Spoilage

- Water
- pH
- Physical structure
- Oxygen
- temperature



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Microorganisms in Food

- factors that affect the presence of microorganisms in food include

  intrinsic

  extrinsic

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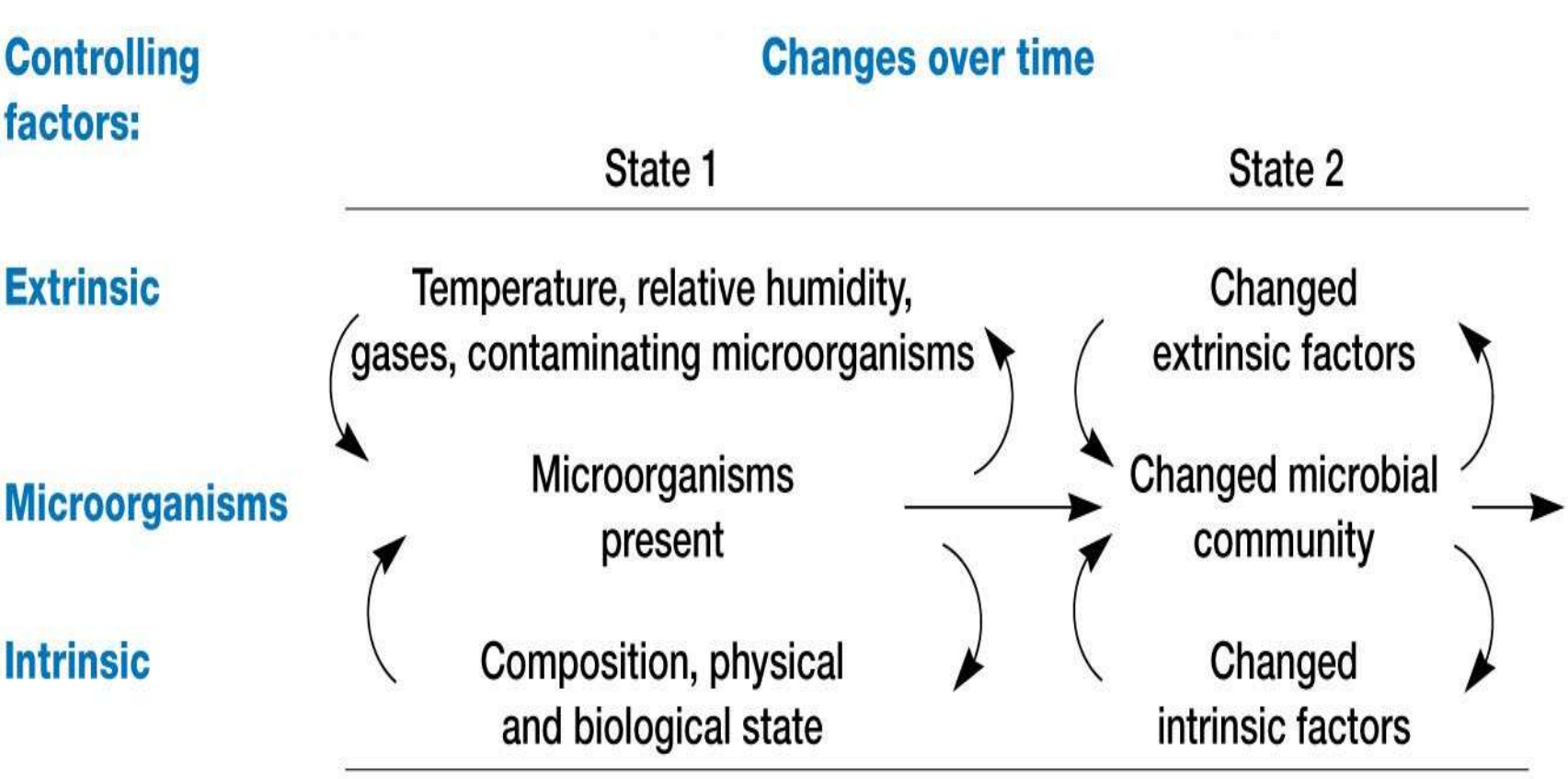
Microorganism Growth in Foods



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Intrinsic Factors

- composition
- pH
- presence and availability of water
- oxidation-reduction potential
- altered by cooking
- physical structure
- presence of antimicrobial substances

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Composition and pH

- proteolysis and anaerobic breakdown of proteins, yielding foul-smelling amine compounds
- pH impacts make up of microbial community and therefore types of chemical reactions that occur when microbes grow in food

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Putrefaction

Table 41.1 Differences in Spoilage Processes in Relation to Food Characteristics

Substrate	Food Example	Chemical Reactions or Processes[a]	Typical Products and Effects
Pectin	Fruits	Pectinolysis	Methanol, uronic acids (loss of fruit structure, soft rots)
Proteins	Meat	Proteolysis, deamination	Amino acids, peptides, amines, H_2S, ammonia, indole (bitterness, souring, bad odor, sliminess)
Carbohydrates	Starchy foods	Hydrolysis, fermentations	Organic acids, CO_2, mixed alcohols (souring, acidification)
Lipids	Butter	Hydrolysis, fatty acid degradation	Glycerol and mixed fatty acids (rancidity, bitterness)

[a]Other reactions also occur during the spoilage of these substrates.

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Intrinsic Growth Factors

- **pH**

  many species of bacteria are inhibited by low pH, including most pathogens

Testing soil pH

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Intrinsic growth factors: naturally present in food

- **water availability** is measured as water activity (a_w), the amount available in the food

  - most microorganisms require an a_w of 0.90 or above for growth
  - fungi can grow with a a_w of 0.80
  - fresh food have an a_w 0.98

Water availability

- in general, lower water activity inhibits microbial growth
- water activity lowered by:

  drying

  addition of salt or sugar
- osmophilic microorganisms

  prefer high osmotic pressure
- xerophilic microorganisms

  prefer low water activity

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Intrinsic Growth Factors

- **biological barriers:**
- shells, rinds protect foods from invading microorganisms

antimicrobial chemicals: naturally occurring in some foods egg whites have lysozyme which will destroy lysozyme susceptible bacteria

Physical structure

- grinding and mixing increase surface area and distribute microbes

  promotes microbial growth
- outer skin of vegetables and fruits slows microbial growth

Antimicrobial substances

- coumarins – fruits and vegetables
- lysozyme – cow's milk and eggs
- aldehydic and phenolic compounds – herbs and spices
- allicin – garlic
- polyphenols – green and black teas

Extrinsic Factors: environmental conditions

- temperature of storage

  below freezing water is unavailable for microorganisms

  low temperatures (above freezing) enzyme reactions are non-existent or slow

  refrigerated food microbial growth is likely psychrophiles

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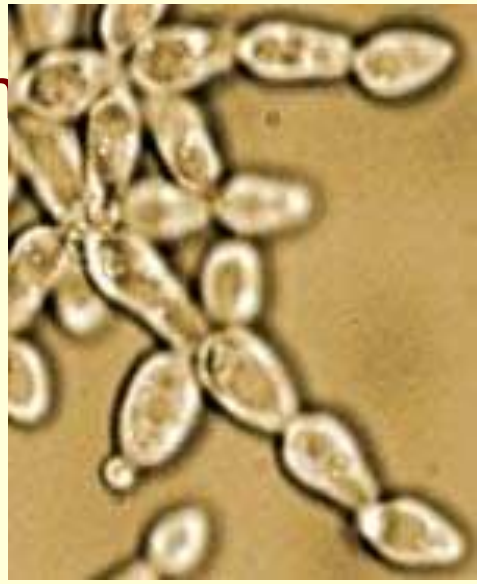
Microorganisms in Food Production



- using microorganisms for food production has been done for thousands of years
  cheese, yeast, beer
- microorganisms used in food often produce an acidic by-product as a result of metabolism
- can inhibit growth of many spoilage microorganisms
- can inhibit growth of many foodborne pathogens

Yeast cells

Food Spoilage: undesirable changes in food

- smell bad, taste bad, look bad
- probably are not harmful
- microorganisms that cause food spoilage compete with pathogens

  in the case of food spoilage vs. pathogens, the spoilers are winning

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Foodborne Intoxication

- illness from microbial exotoxin

  microorganism does not cause the illness, the toxin released by the microorganism does

- common exotoxin producing microorganisms

  Staphylococcus aureus

  Clostridium botulinum

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Foodborne Infection

- requires consumption of microorganism
- symptomatic about 1 day following ingestion of contaminated food
- common foodborne infecting
- microorganisms

Salmonella and *Campylobacter*

- poultry product infections

Escherichia coli 0157:H7

- undercooked hamburger

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Campylobacter

Salmonella

Food Preservation: preventing growth and metabolic activities of microorganisms

- spices, salting, drying are methods that have been around for years
- most common methods of current food preservation are

  high temperature treatment

  low-temperature storage

  antimicrobial chemicals

  irradiation

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Extrinsic Factors

- atmosphere: presence or absence of O_2

  obligate aerobes (need O_2) won't grow in sealed containers

  - may allow growth of anaerobic microbes

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Extrinsic Factors

- temperature

  lower temperatures retard microbial growth

- relative humidity

  higher levels promote microbial growth

- Atmosphere oxygen promotes growth

  modified atmosphere packaging (MAP)

  - use of shrink wrap and vacuum technologies to package food in controlled atmospheres

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Microbial Growth and Food Spoilage

- food spoilage

  results from growth of microbes in food

  - alters food visibly and in other ways, rendering it unsuitable for consumption

  involves predictable succession of microbes

  different foods undergo different types of spoilage processes

  toxins are sometimes produced

  - algal toxins may contaminate shellfish and finfish

Microbiological Examination of Foods

Traditional method

- Plate counts
- Membrane filtration
- Most probable number
- Direct microscopic count
- Dye reduction tests
- Indicator

Rapid Method

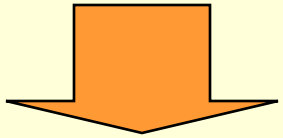
Direct epifluorescent filter technique (DEFT)

Electrical impedance

Enzyme-linked immunosorbent assay(ELISA)

Plate count method

Standard plate count (SPC)
Aerobic plate count (APC)



Total bacteria count (TBC)
Total viable count (TVC)

"Live"

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Plate count method

- Diluent
  - 0.85%NaCl
  - 0.1% peptone
  - Phosphate buffer
- Medium
  - Elective medium
  - Selective medium
  - General
- Petri dish plate Replication

- Pour plate
- Spread plate
- Drop plate

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Plate count depends on

- Diluent
- Food homogenate
- Dilution series
- Medium
- Plating method
- Incubate conditions

Baird-Parker Agar

Selective agent
- Sodium tellulite
- Lithium chloride

Elective agent
- Sodium pyruvate
- Glycine

Diagnostic agent
- Egg yolk

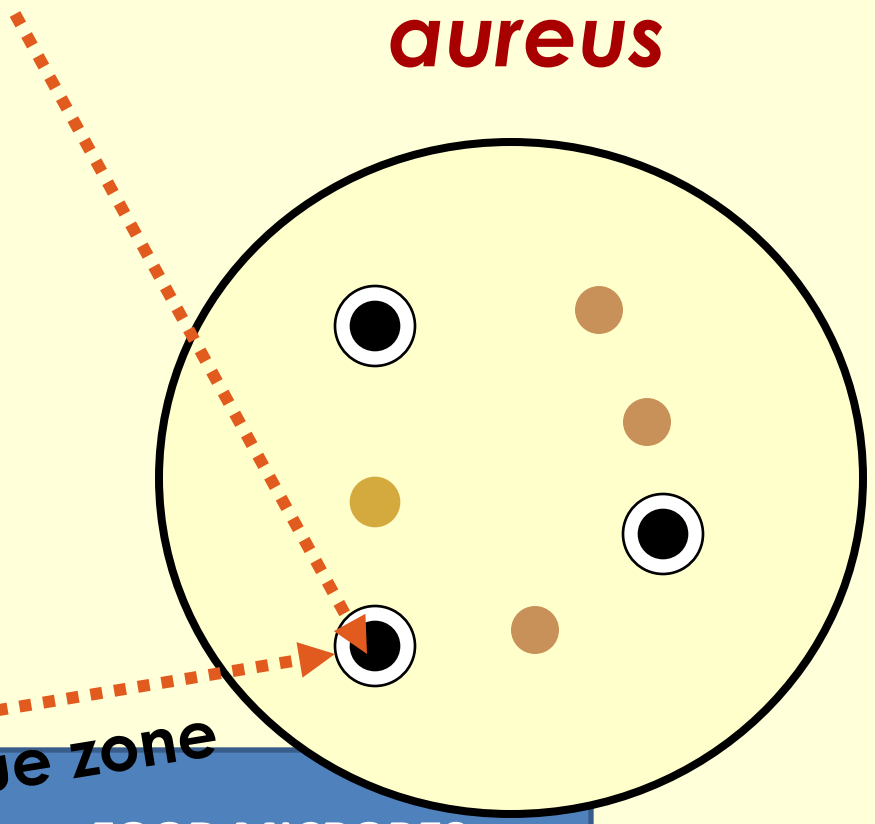
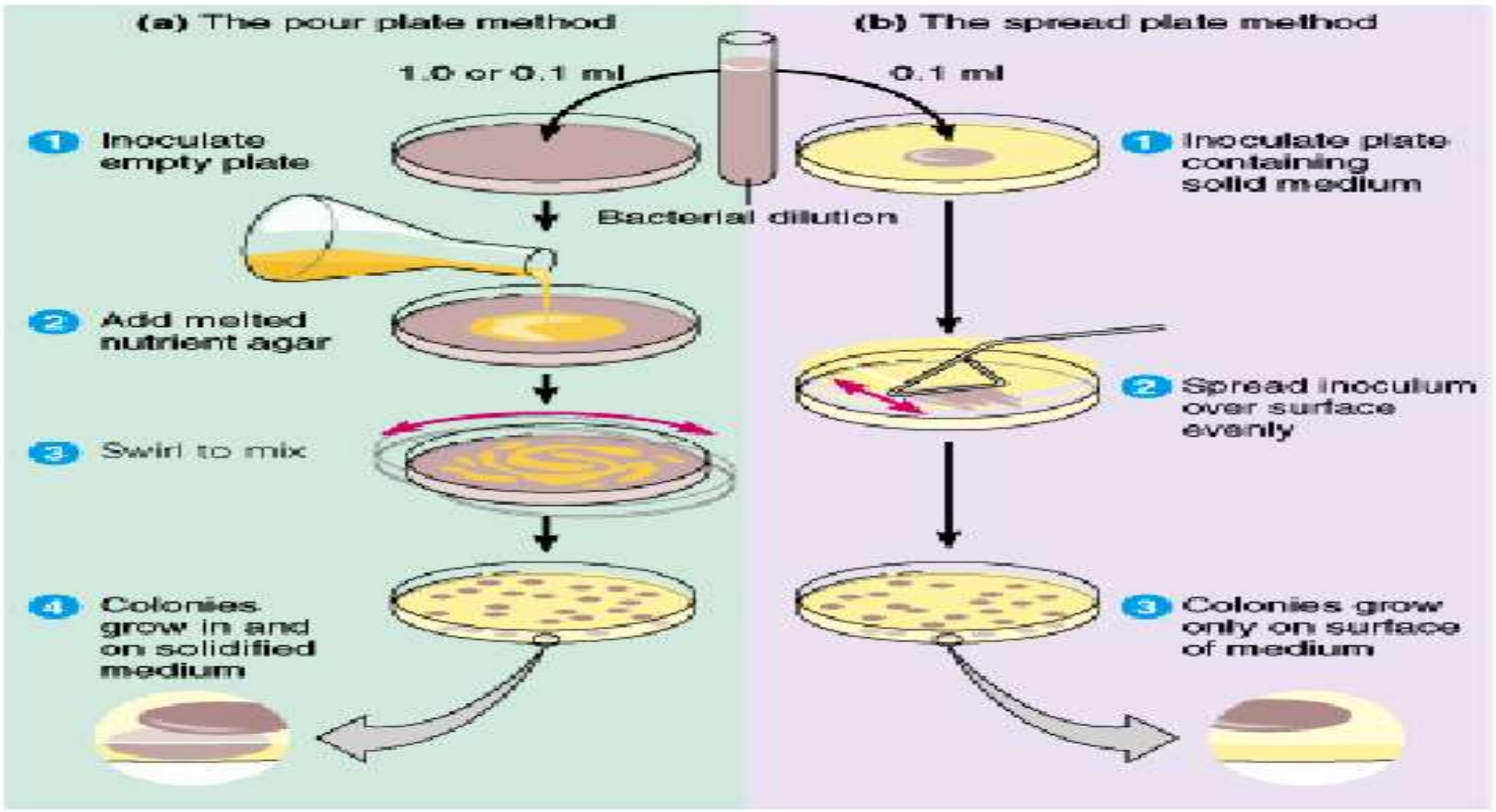


Plate count method



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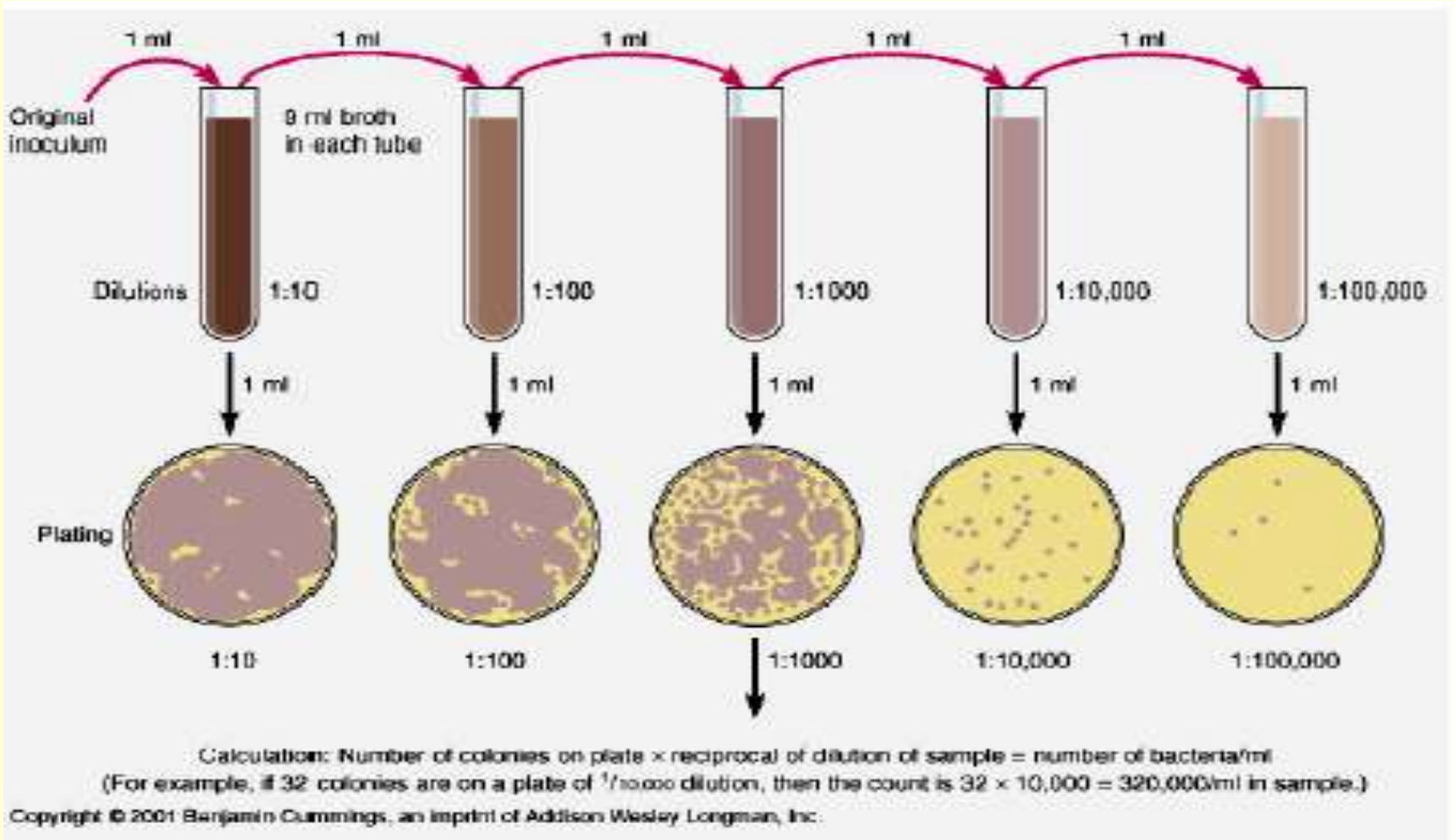
Pour plate



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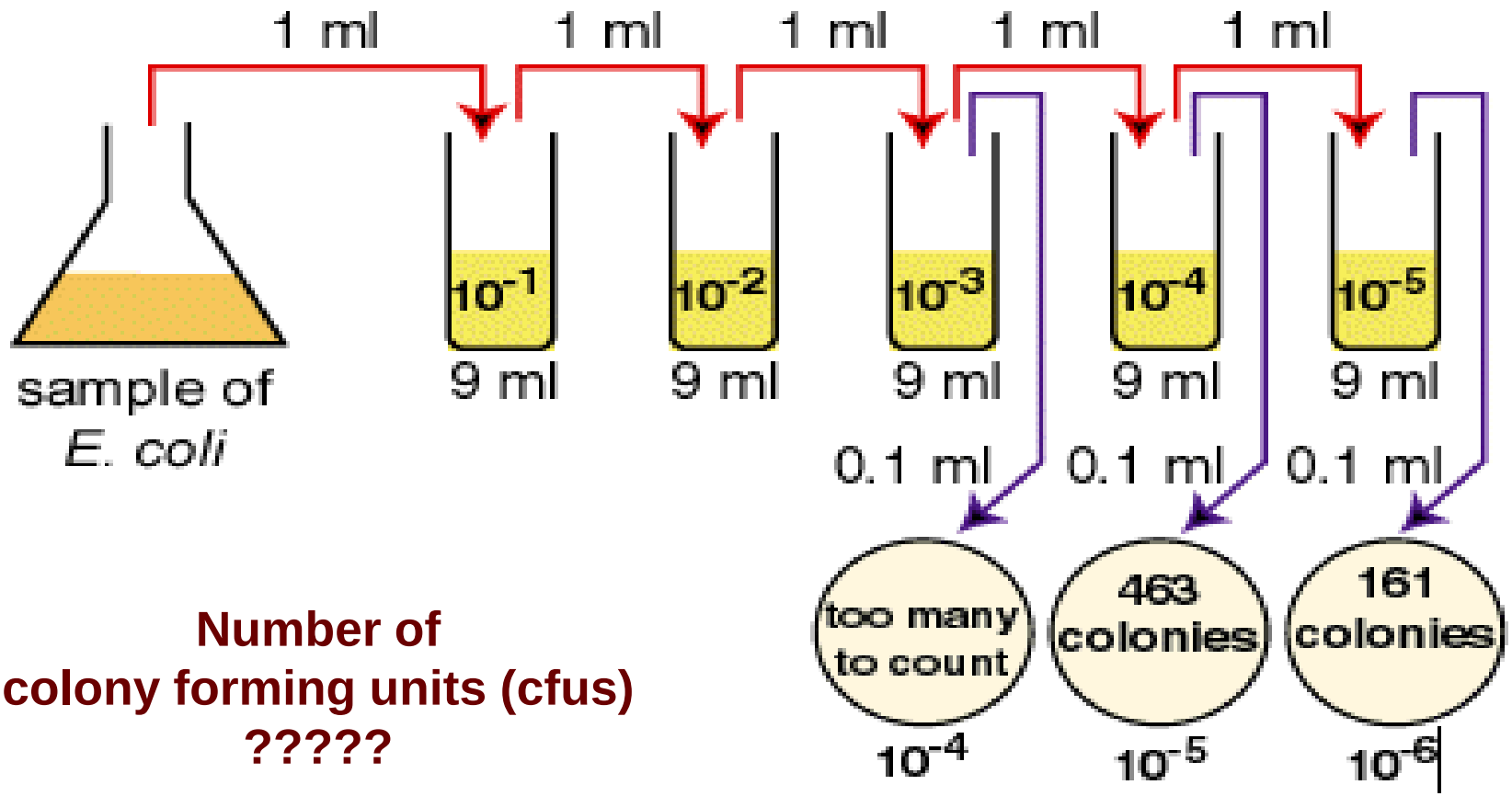
Spread plate



Number of colony forming units (cfus) ?????

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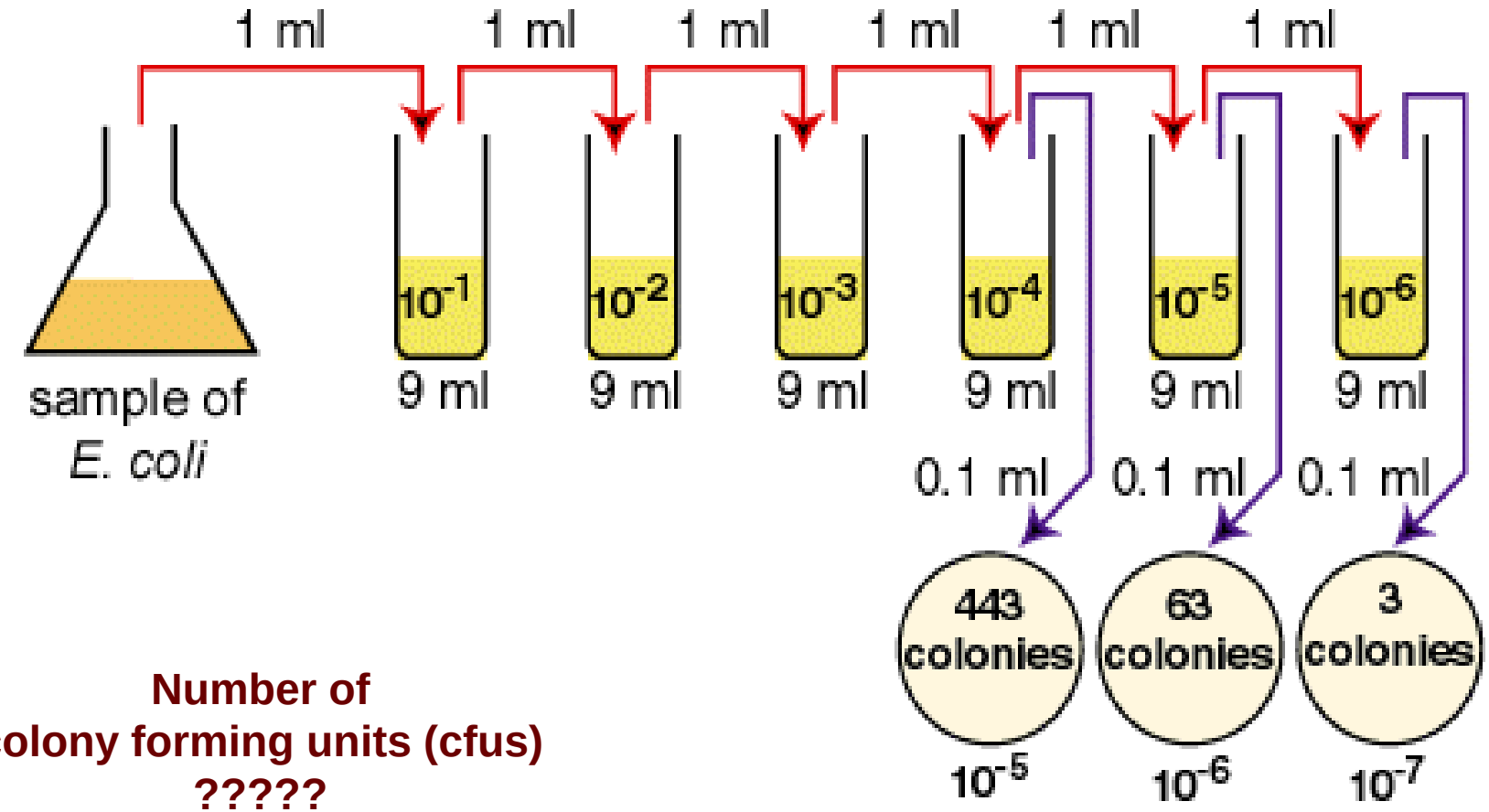
Spread plate



Number of colony forming units (cfus) ?????

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Drop plate

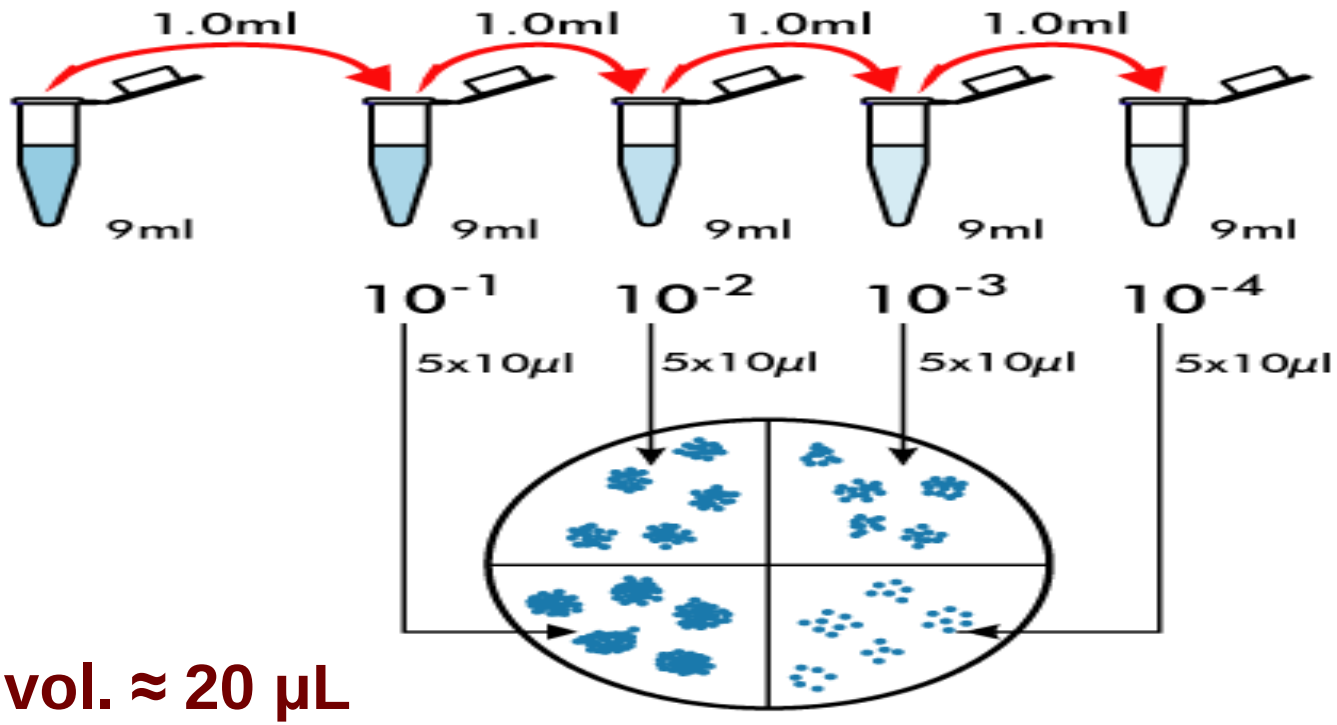

Figure 1. Dilution series followed by drop-plating techniques

- **Sample:**
  - **Small vol. ≈ 20 µL**
- **Cost**

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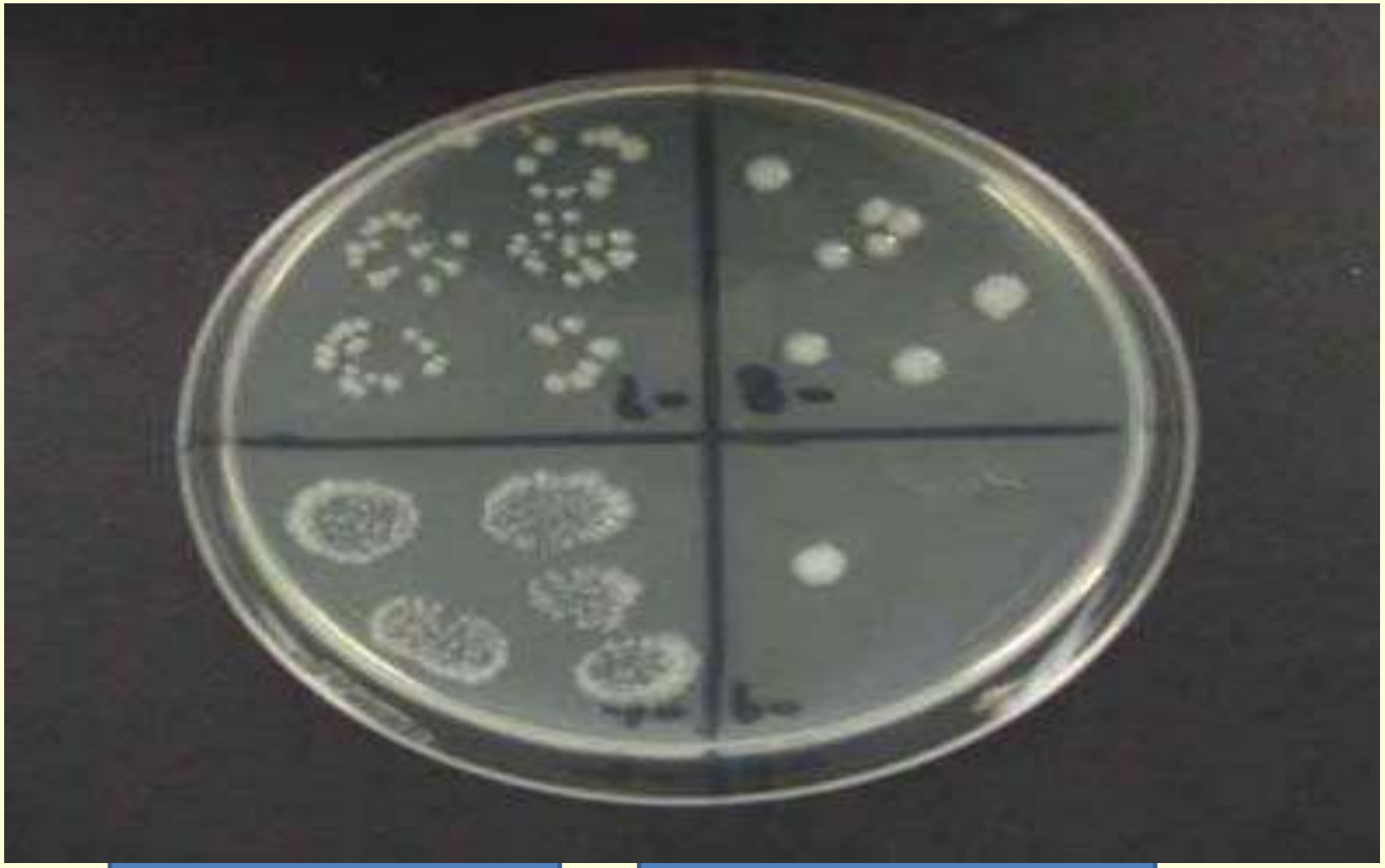
Drop plate



Application of plate count

- Check quality of RM & final products
- Check condition hygiene
- Estimate storage life of products
- Determine

  Production

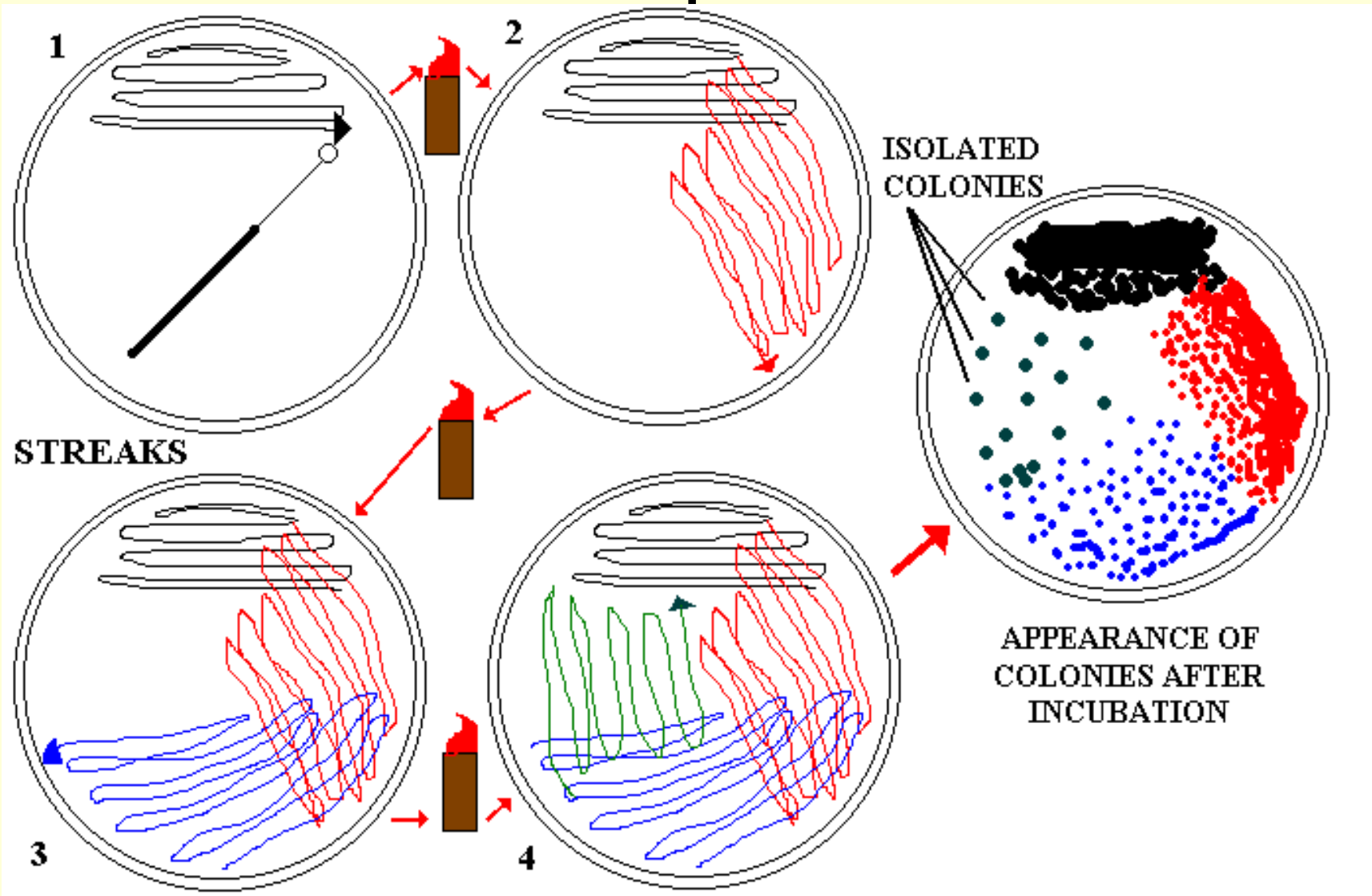
  Transport

  Storage
- Determine pathogens

Selection of media in food microbiology

Medium	Use
Plate count agar	Aerobic mesophilic count
MacConkey broth	MPN of coliforms in water
Brilliant green/Lactose/Bile broth	MPN of coliforms in food
Baird Parker agar	*Staphylococcus aureus*
Thiosulfate/Bile/Citrate/agar	*Vibrio* sp.

Streak plate

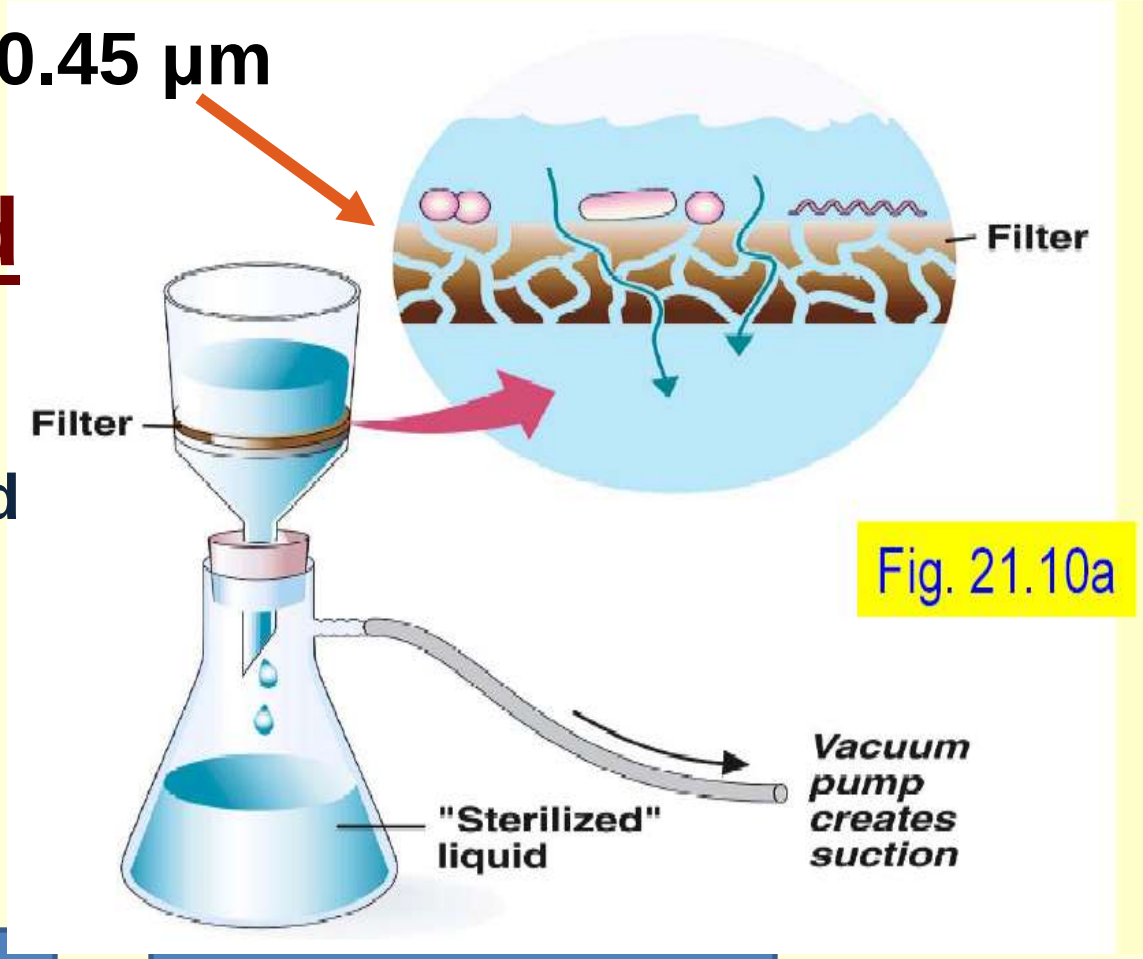


Filtration

Liquid food

- Low number of MO. Large volume of food

- Count
- Sterilize



Most probable number

Most probable number (MPN)

Multiple tube techniques

- **Pathogen**
  - **Number too low**

- **Coliform**
- ***Escherichia coli***
- ***Staphylococcus aureus***
- **Feacal streptococci**

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Most probable number

Medium	Organisms assessed
Lauryl sulfate tryptose broth	Coliforms
MacConkey purple broth	Coliforms
EC broth	Faecal coliform
Glucose azide	Faecal streptococci
Minerals modified glutamate medium	Coliforms
Baird-Parker broth	*Staphylococcus aureus*

Microscopic count

Direct microscopic count (DMCs)

Small sample (0.01 ml) & rapid

Optical light microscope

Total cell

- living & dead cells

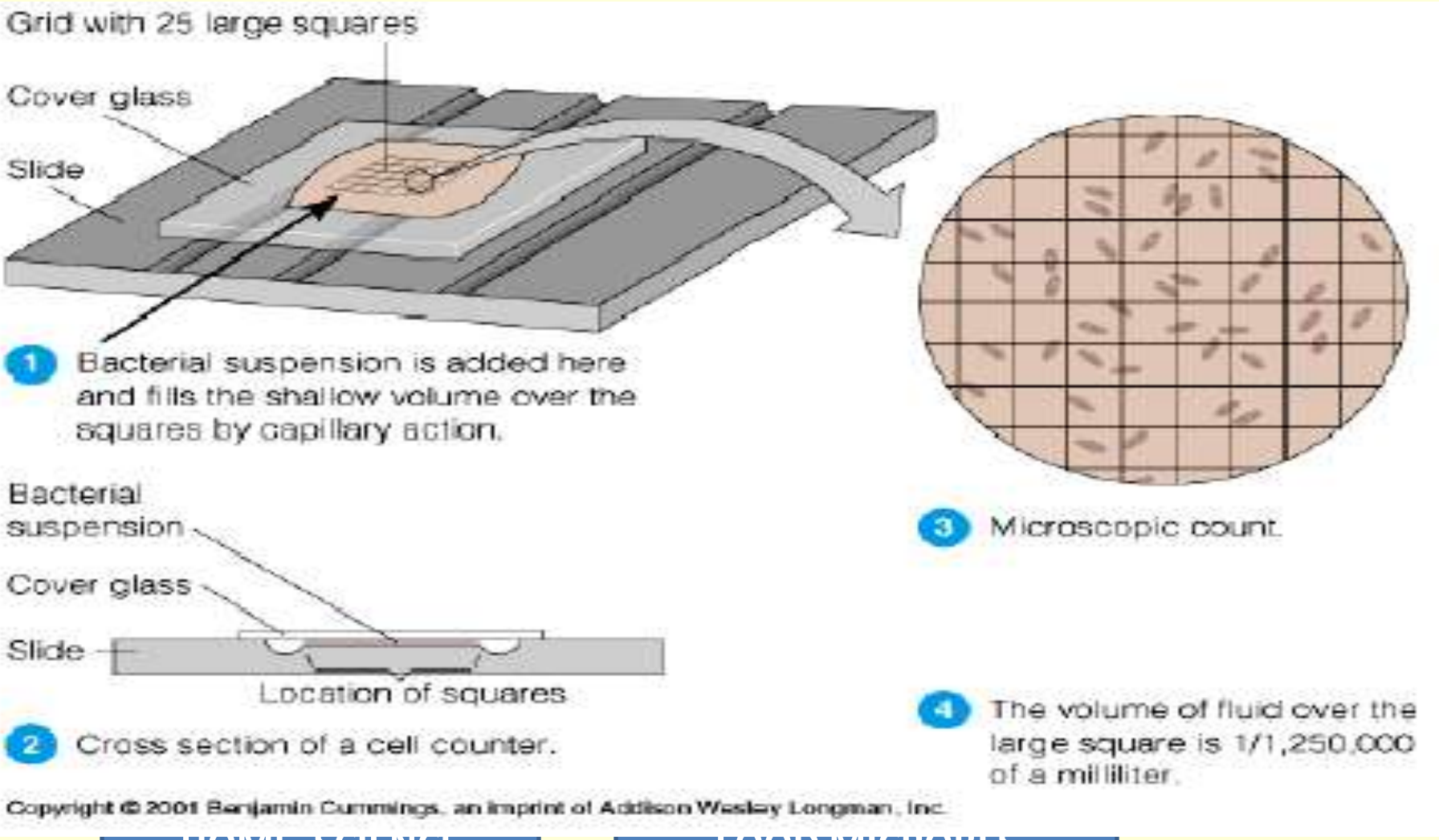
Foods

- Liquid
- Semi-solid

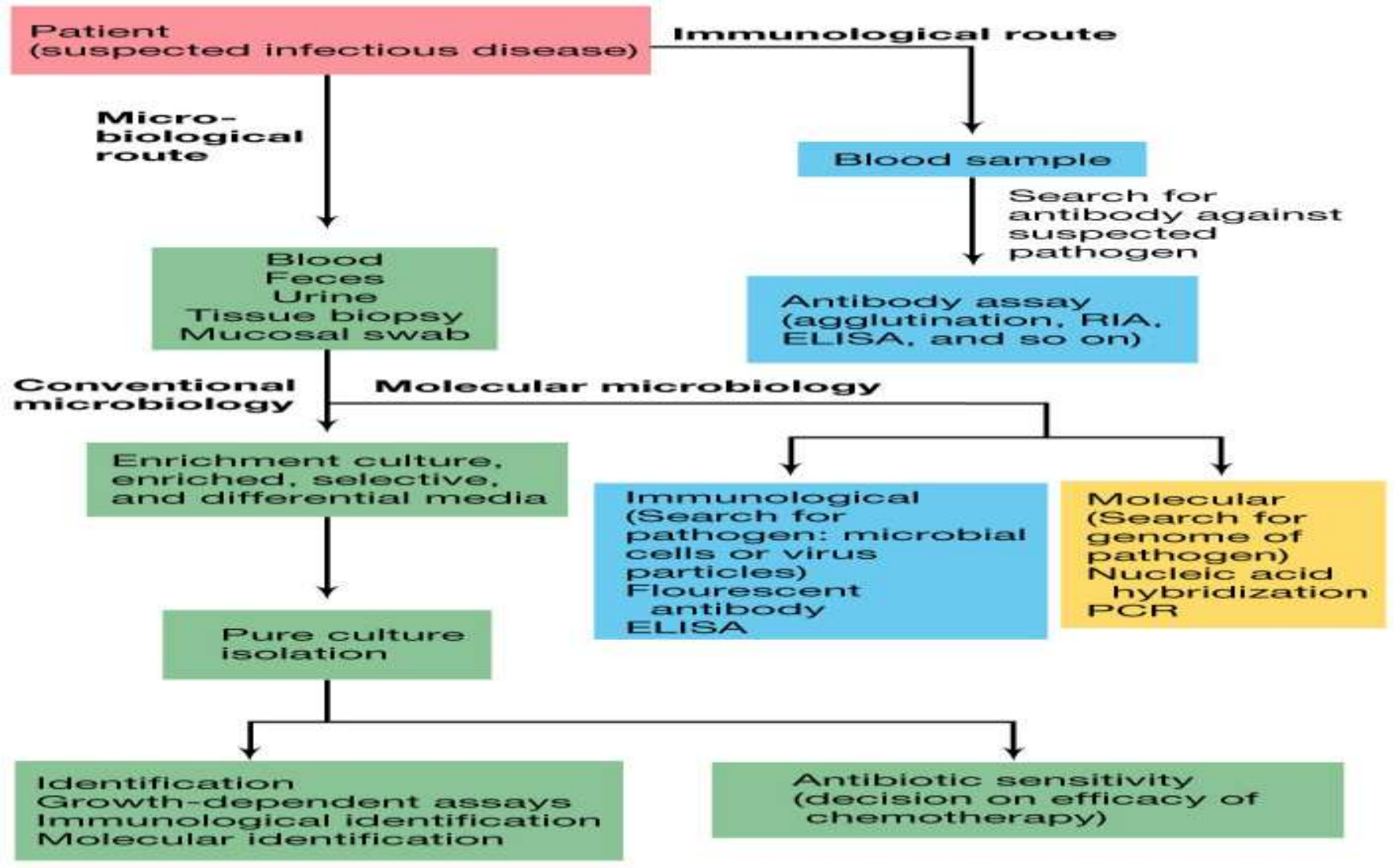
Ex.

- Milk
- Wine
- Yogurt starter
- Tomato sauce
  - Howard mold

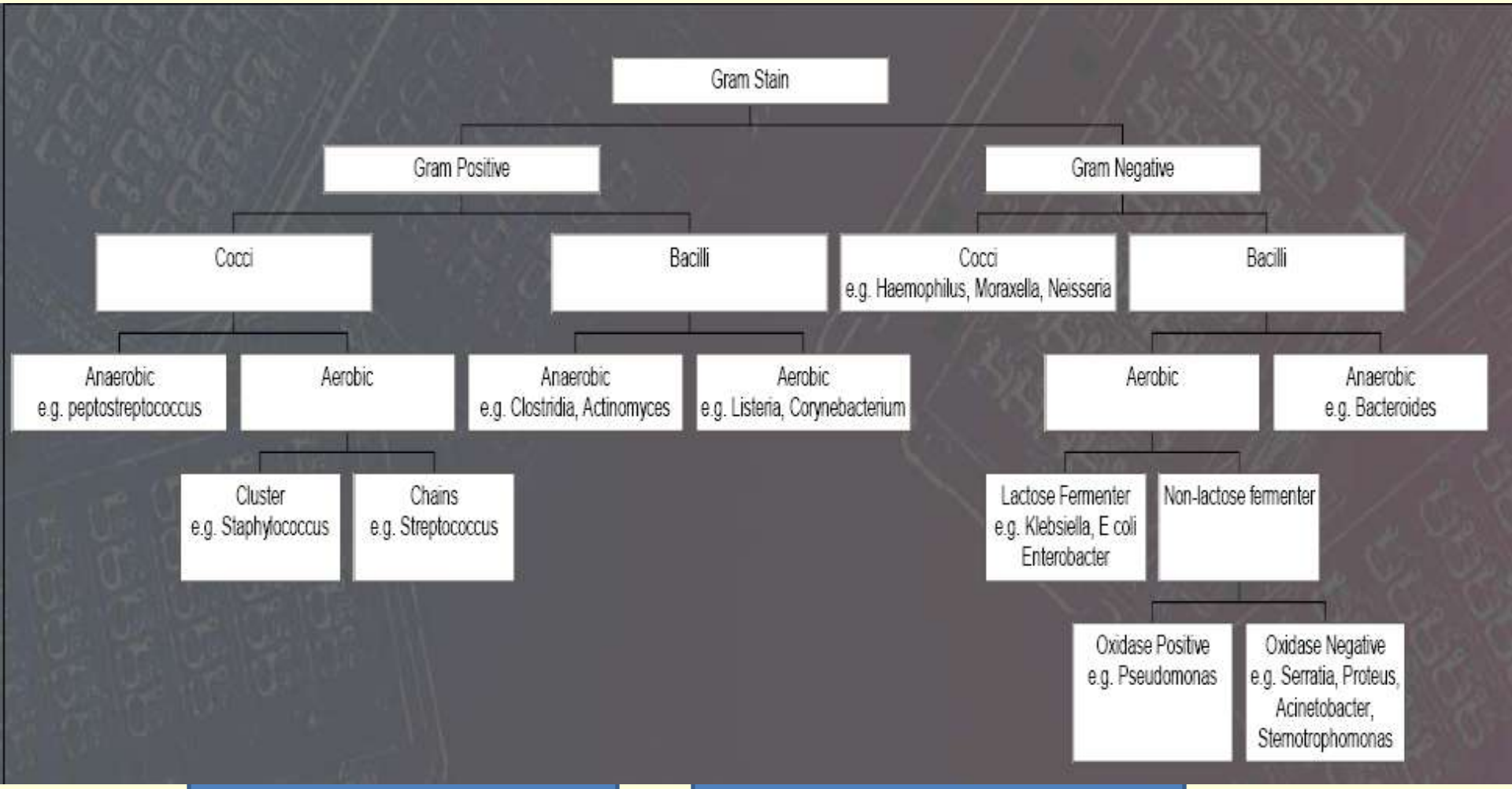
Microscopic count



Microbe Identification Scheme



Bacterial Classification



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Process

- Specimen collection
- Specimen receipt
- Specimen processing
- Testing
- Interpretation
- Reporting

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Microbe Identification

- The successful identification of microbe depends on:
  - Using the proper aseptic techniques.
  - Correctly obtaining the specimen.
  - Correctly handling the specimen
  - Quickly transporting the specimen to the lab.
  - Once the specimen reaches the lab it is cultured and identified
  - Use care and tact to avoid patient harm

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Microbe Identification

- Identification measures include:
  - Microscopy (staining)
  - growth on enrichment, selective, differential or characteristic media
  - specimen biochemical test (rapid test methods)
  - immunological techniques
  - molecular (genotypic) methods.
- After the microbe is identified for clinical samples it is used in susceptibility tests to find which method of control is most effective.

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Different Identification methods

1) Staining Reactions
2) Cultural Characteristics
3) Resistance
4) Metabolism
5) Biochemical properties

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Staining Reactions

The presence of certain structures and staining reactions aids in their identification and classification

1) To render microscopic and semitransparent objects visible
2) To reveal their shape and size
3) To produce specific physical or chemical reactions
4) To produce specific physical or chemical reactions.

Staining Reactions

- Simple staining bring out the morphology the best
- Differential and special stains are necessary to bring out characteristics like flagella, capsules, spores and metachromatic granules.
- Gram stain divides bacteria into Gram positive and Gram negative
- Ziehl-Neelsen stain divides them into acid fast and non acid fast
- Fluorescent dyes bring out special characteristics and fluorescent antibody technique enables to identify them.

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Cultural Characteristics

- Provides additional information for the identification of the bacterium. The characters revealed in different types of media are noted.
- While studying colonies on solid media following characteristics are observed :

  Size, Shape, Margins, Surface, Their elevations, Edge, colour, structure, consistency.

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Cultural Characteristics

- In fluid medium following characteristics are observed :

  Degree of growth – Absence, scanty, moderate, abundant etc.

  presence of turbidity and its nature

  presence of deposit and its character

  Nature of surface growth

  Ease of disintegration and odour

MacConkey's Agar

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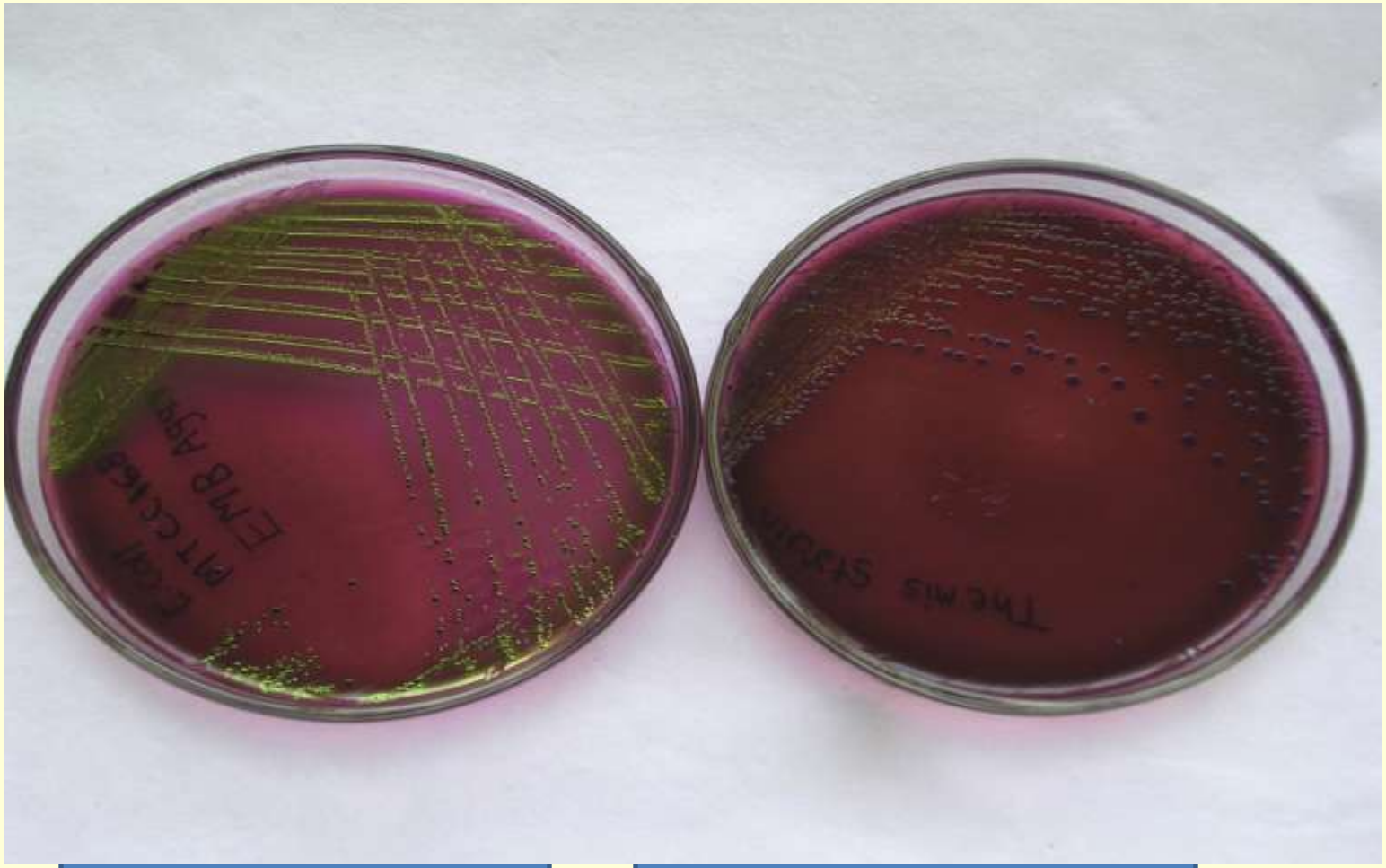
Plates showing differentiating characteristics



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Resistance

The resistance of the organism is tested against number of parameters which helps differentiation and identification of the organisms

- Heat
- Low concentration of disinfectants
- Antibiotics
- Chemotherapeutic agents
- Bacteriocins etc.

Metabolism

- To classify and differentiate species following aspects are studied

  - Requirements of oxygen
  - The need for CO_2
  - Capacity to form pigments
  - power of haemolysis

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Biochemical properties

- Tests for Metabolism of Carbohydrates and related compounds
- Tests for Metabolism of Proteins and Amino acids
- Test for metabolism of Lipids
- Tests for Enzymes
- Combined Tests

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Biochemical properties

Tests for Metabolism of Carbohydrates and related compounds

- Tests to distinguish b/w aerobic and anaerobic breakdown of carbohydrates

  - O/F test depends upon the use of a semi-solid tubed medium containing the carbohydrate (usually glucose) along with the pH indicator

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Biochemical properties

- Tests to show the range of carbohydrates and related compounds that can be attacked

  - A large variety of carbohydrate compounds are used and they are often regarded as ‘sugars’

  Sugar fermentation – Acid production

  Litmus milk – Acid or alkali production, clot formation, peptonisation or saponification. Disruption of clot due to gas production.

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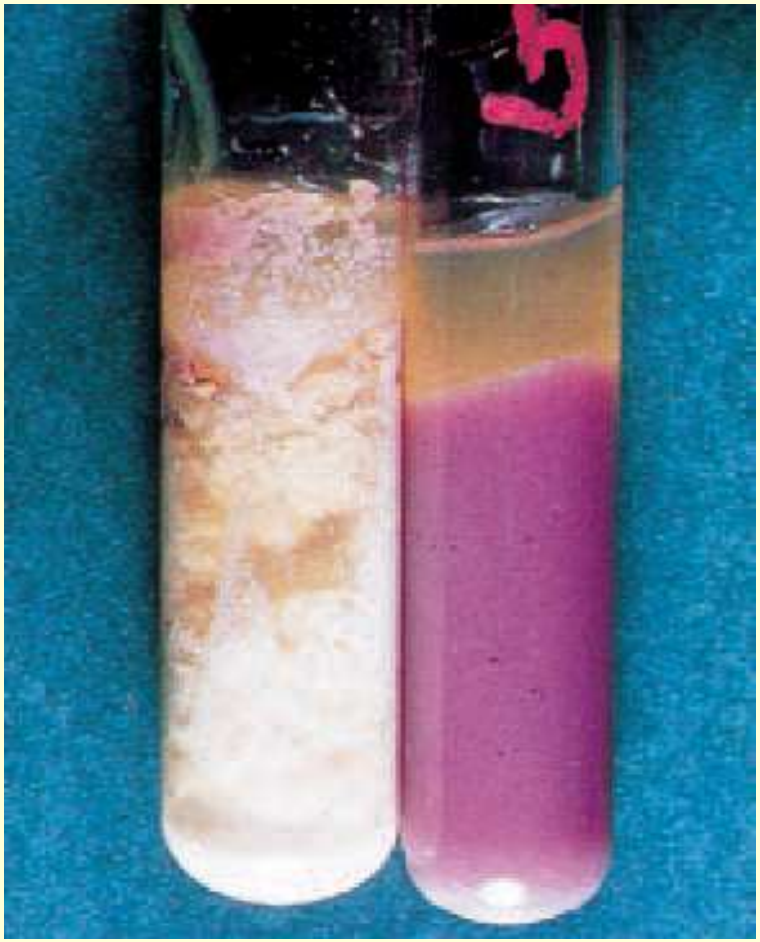
Biochemical properties



Stormy Fermentation of Litmus Milk.

The tube on the left shows fermentation; the tube on the right is negative for stormy fermentation.

Used for the identification of *Clostridium* species.

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Sugar fermentation
Acid and gas production

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Biochemical properties

Tests for Metabolism of Carbohydrates and related compounds

- Tests for specific breakdown products

  - Methyl red test – To detect Acid production during Glucose fermentation.

  -

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Biochemical properties

- Voges-Proskauer test – Depends on the production of acetyle methylcarbional from pyruvic acid, as an intermediate stage in its conversion to 2:3 butylene glycol.

- Tests to show ability to utilize particular substrate

  Citrate utilization – Ability to use citrate as a sole source of carbon and Ammonia as a sole source of Nitrogen.

Differentiation of two organisms

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Biochemical properties

- Miscellaneous tests
- Antibiotic tolerance (resistance) test, dye tolerance and other chemical inhibition tests

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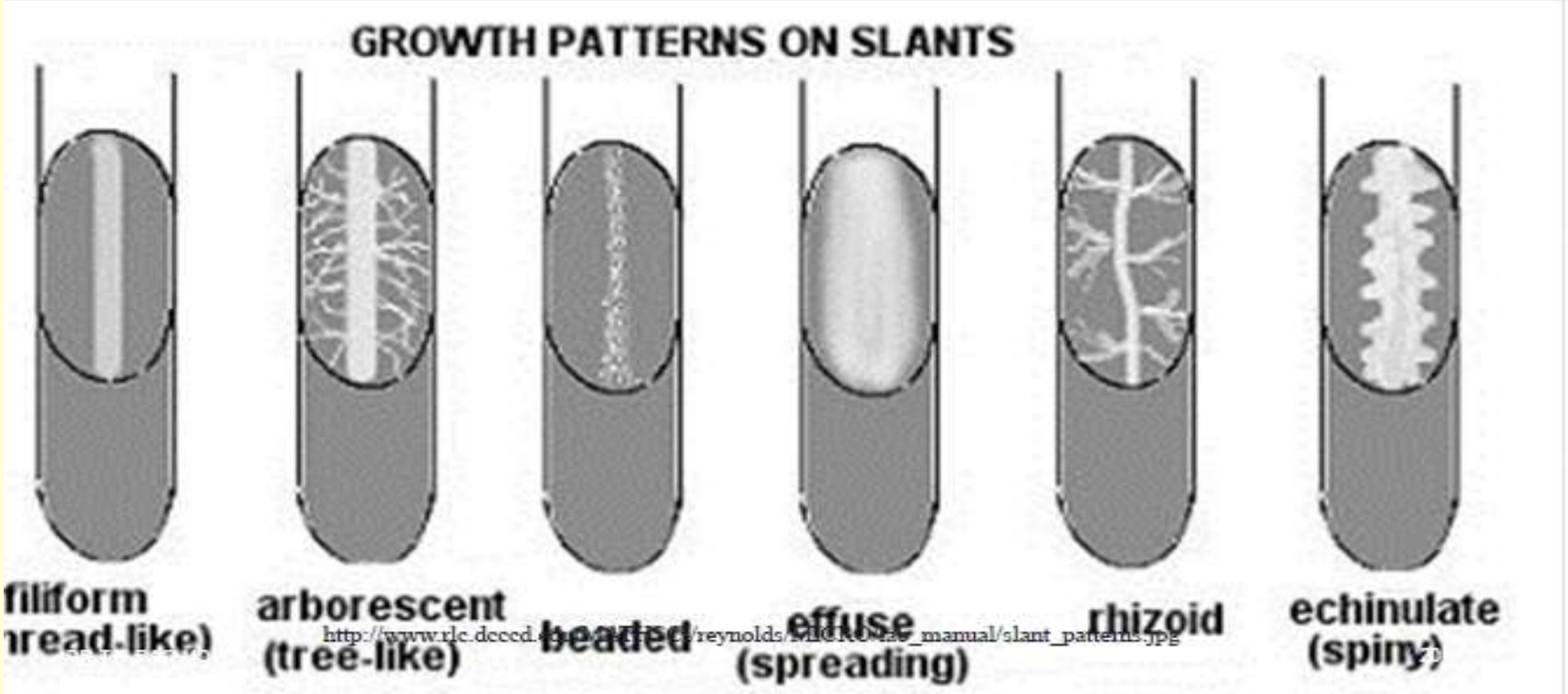
Biochemical properties

- KCN test – Ability to grow in a medium containing KCN. (Should be handled carefully)
- Detection of motility

  - Slide test (Hanging drop technique)

  -Tube test (Semisolid Agar)

SLANT OBSERVATION



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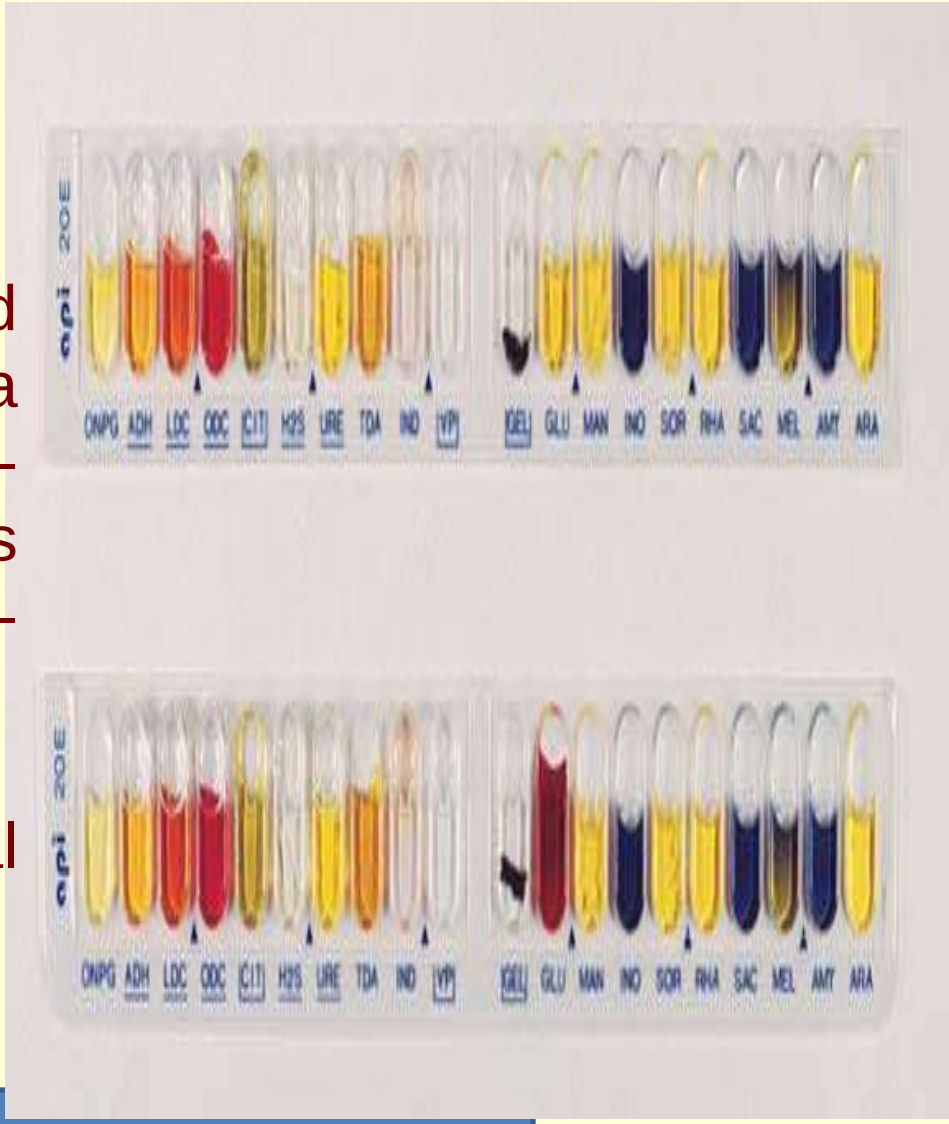
API Strips - Rapid Tests

Commercial miniaturized biochemical test panels - Cover a significant number of clinically-important groups of bacteria, as well as food- and water-associated microorganisms.

The earliest, is the Analytical Profile Index (API) panel.



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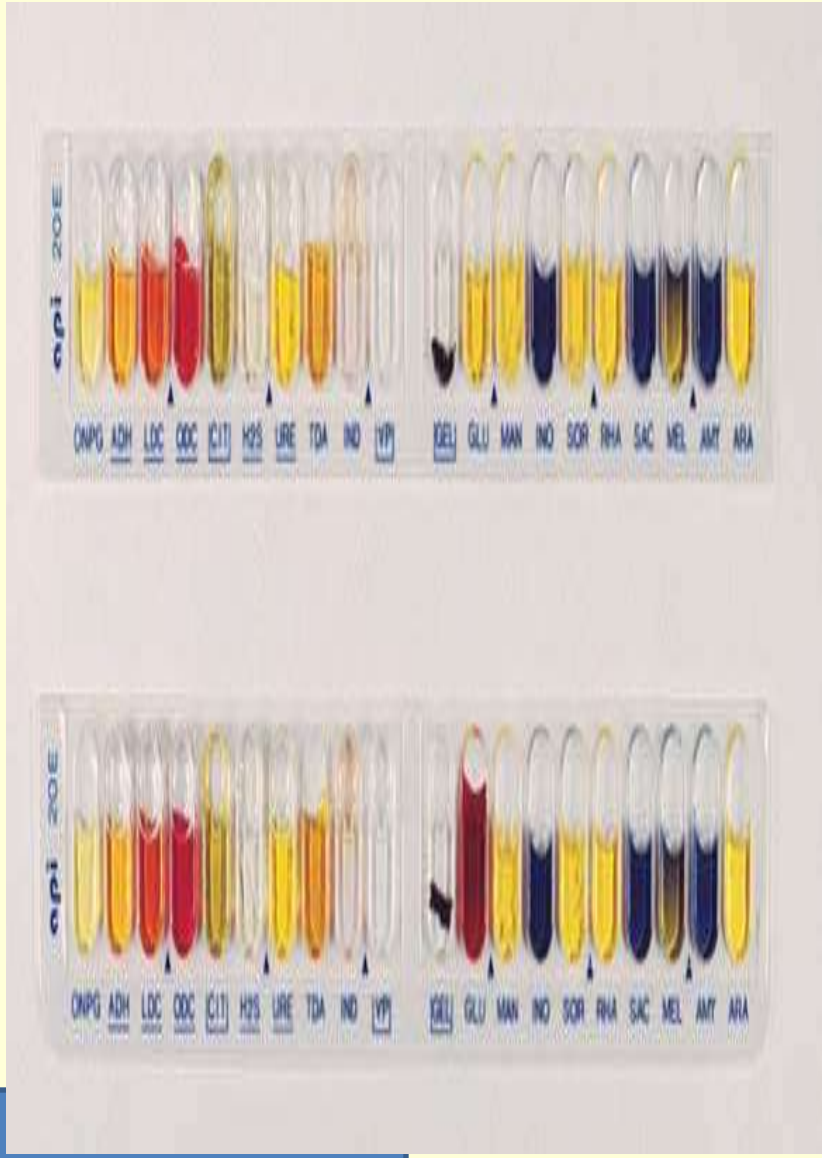
API Strips - Rapid Tests

Different test panels are prepared in **dehydrated forms** which are reconstituted upon use by addition of bacterial suspensions. After incubation, positive test results are scored as a seven-digit number (profile). Identity of the bacterium is then easily derived from the database with the relevant cumulative profile code book or software.



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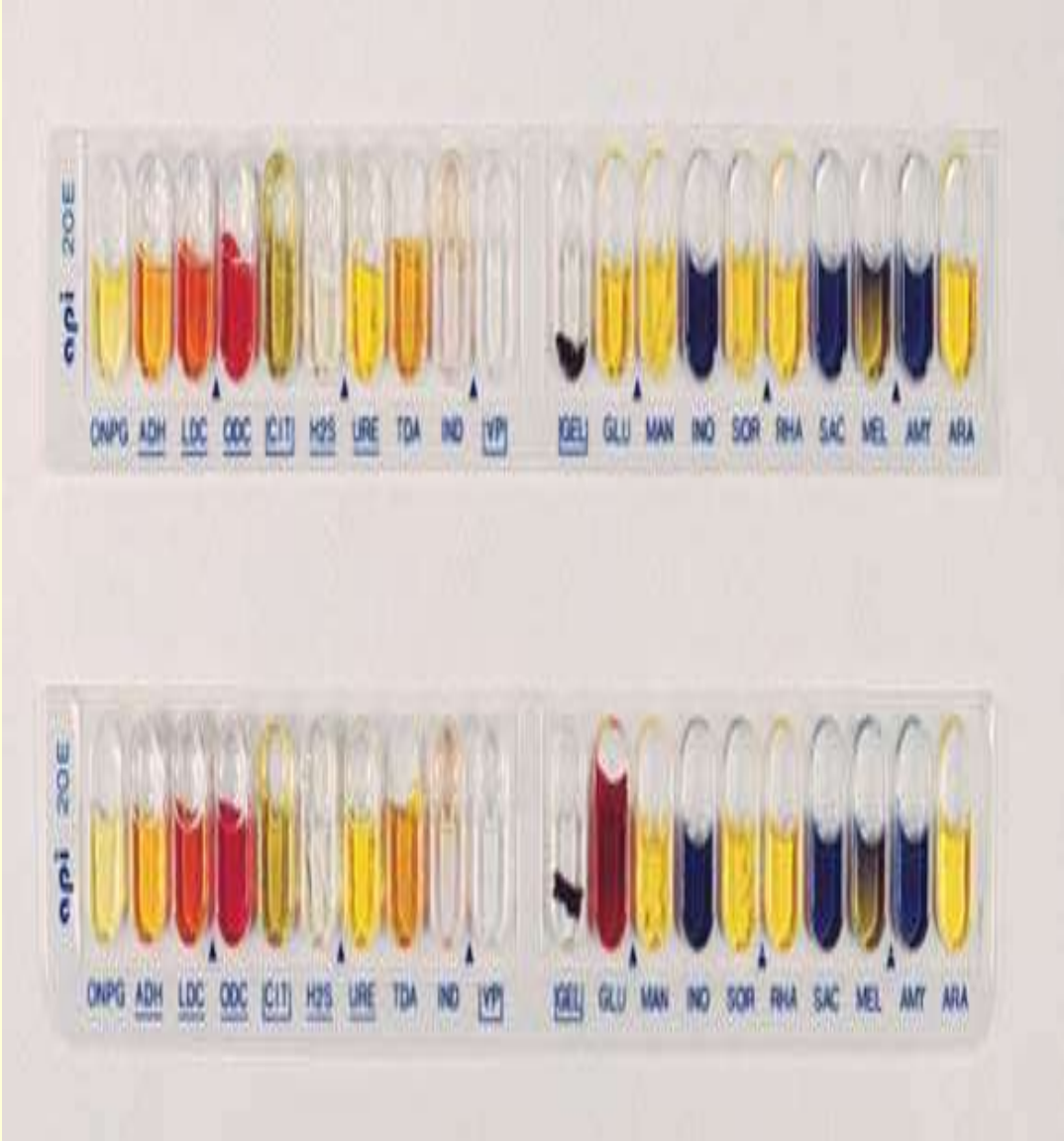
API Strips - Rapid Tests

Identification of Enterobacteriaceae using API 20E, a standardized microplate method. Positive and negative reactions are shown by color reactions.



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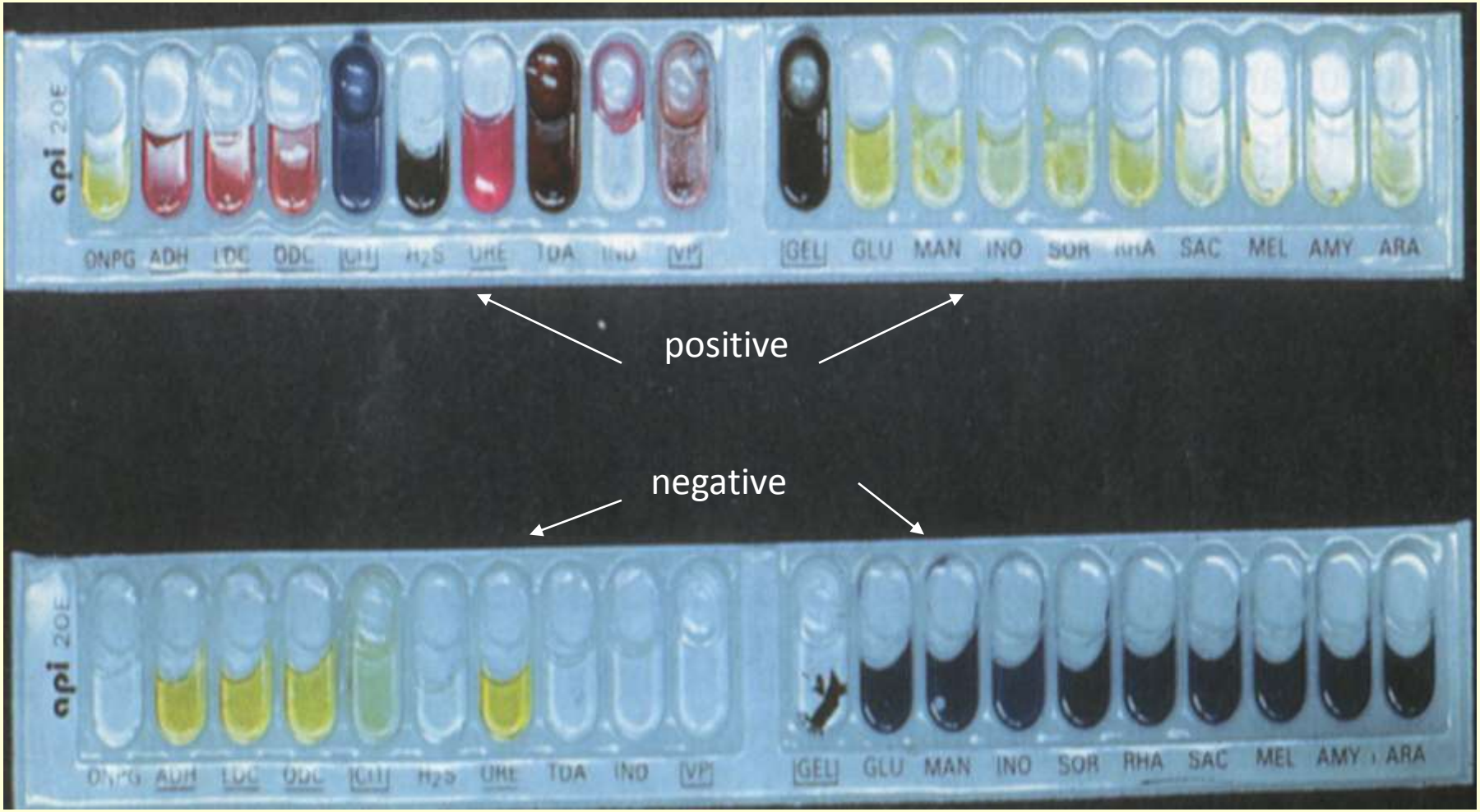
Rapid Tests



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Rapid Tests

ONPG (β galactosidase); ADH (arginine dihydrolase); LDC (lysine decarboxylase); ODC (ornithine decarboxylase); CIT (citrate utilization); H_2S (hydrogen disulphide production); URE (urease); TDA (tryptophan deaminase); IND (indole production); VP (Voges Proskauer test for acetoin); GEL (gelatin liquefaction); the fermentation of glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), sucrose (SAC); Melibiose (MEL), amygdalin (AMY), and arabinose (ARA); and OXI (oxidase).

ELISA

Antigen – conjugate enzyme

Antibody – conjugate enzyme

Pathogen

- *Salmonella*
- *Listeria*
- *S. aureus*

Toxin

- Staphylococcal
- Botulinum toxin
- Mycotoxin

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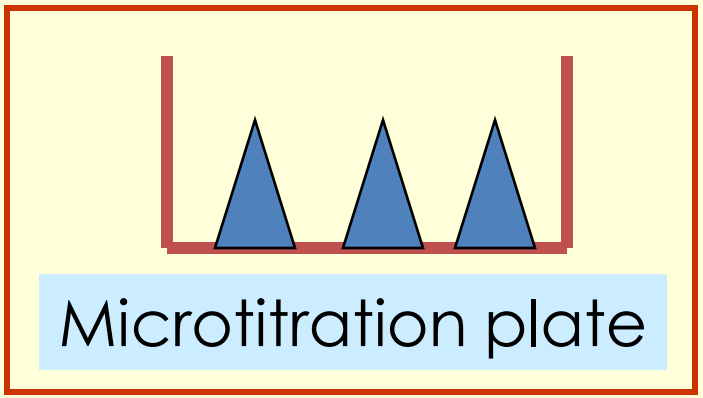
Enzyme-linked Immunosorbent Assay



Antibody

Antigen(toxin)

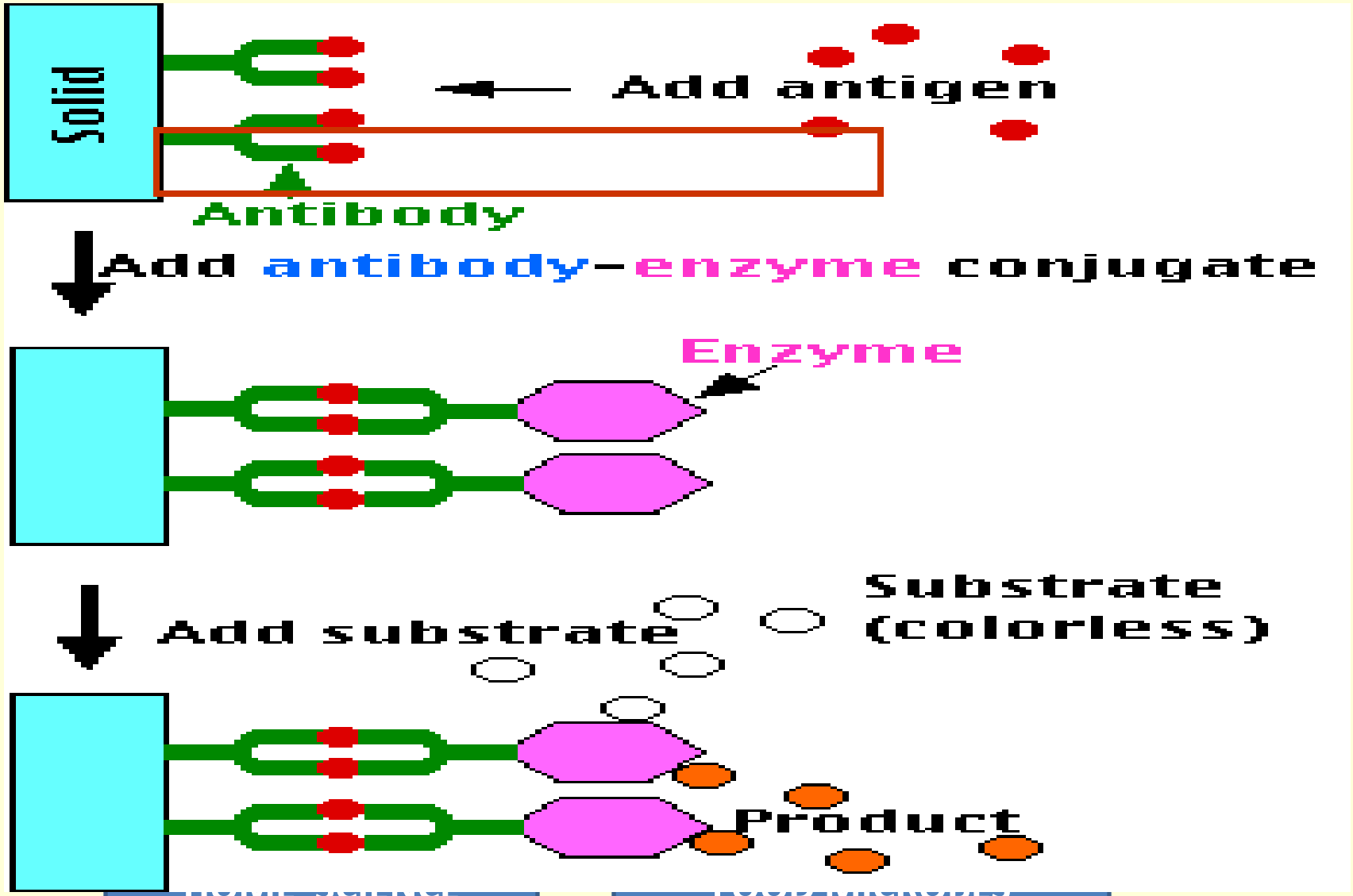
Enzyme

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

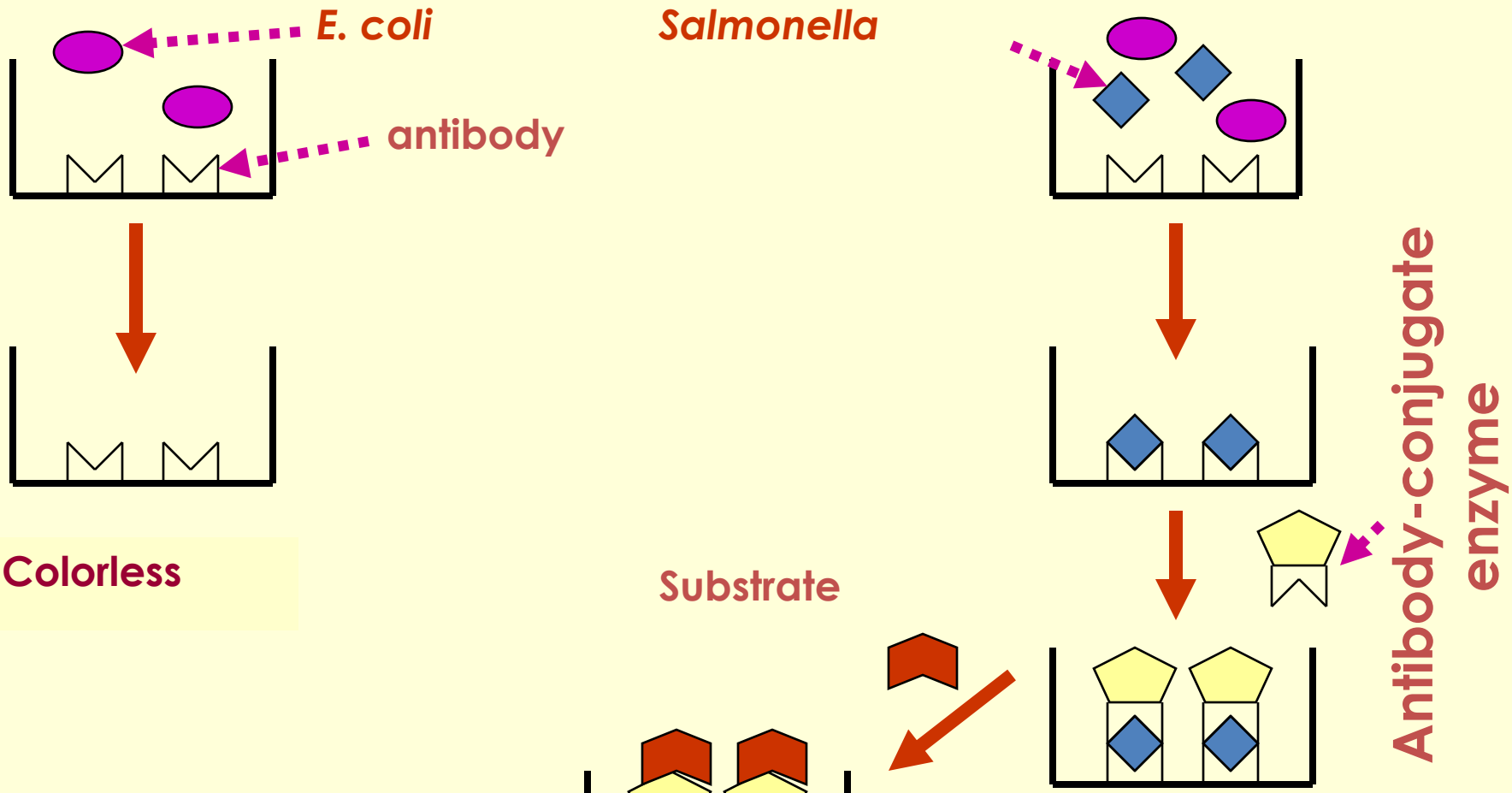
Substrate

- Tetramethylbenzidine (TMB) + 30% H2O2
- Azinobis sulphonic acid (ABTS)
- o-phenylinediamine (OPD)
- p-nitrophenyl phosphate

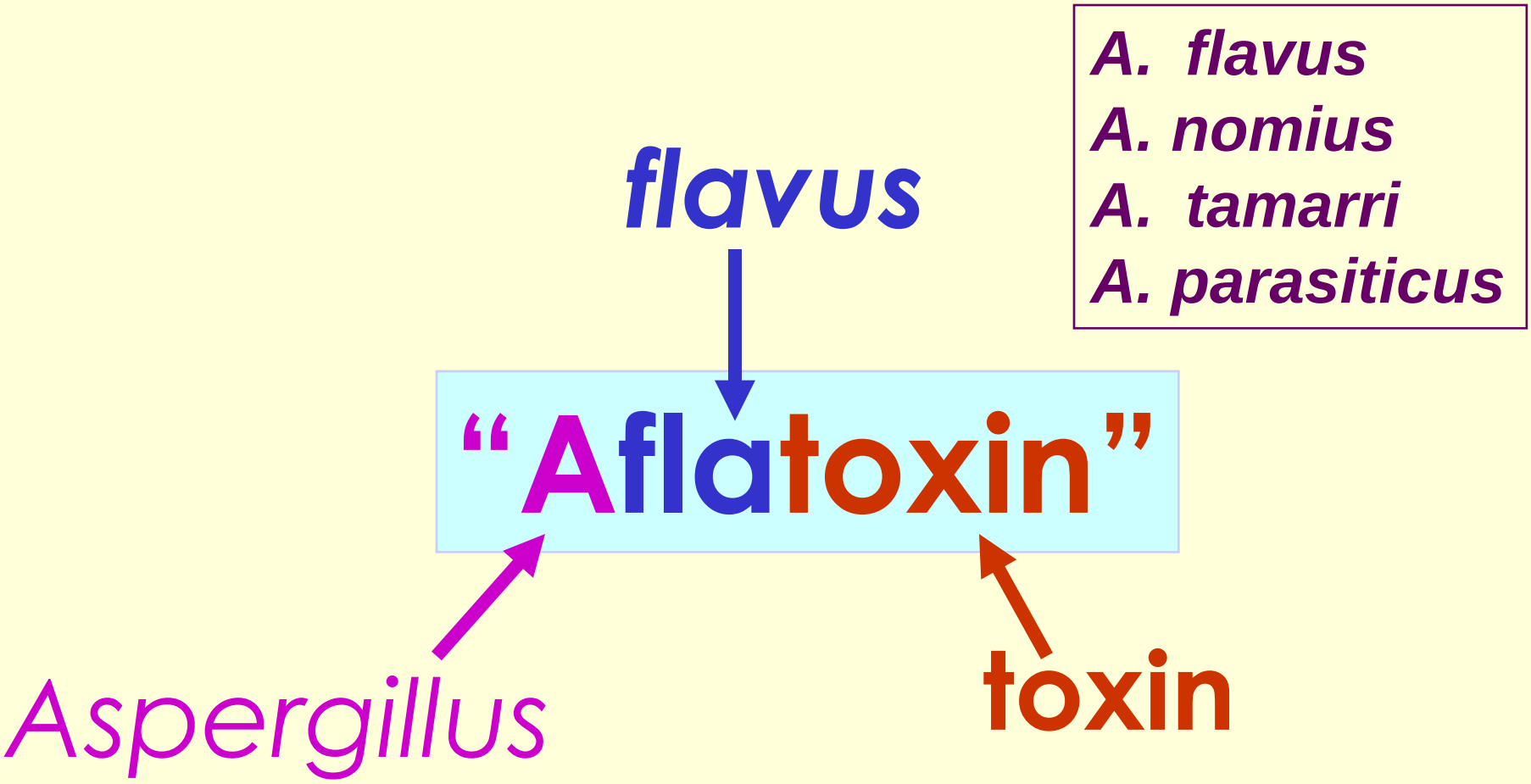
“ELISA”


Solid
Add antigen
Antibody
Add antibody–enzyme conjugate
Enzyme
Add substrate
Substrate (colorless)
Product

Sandwich-ELISA



Aflatoxin

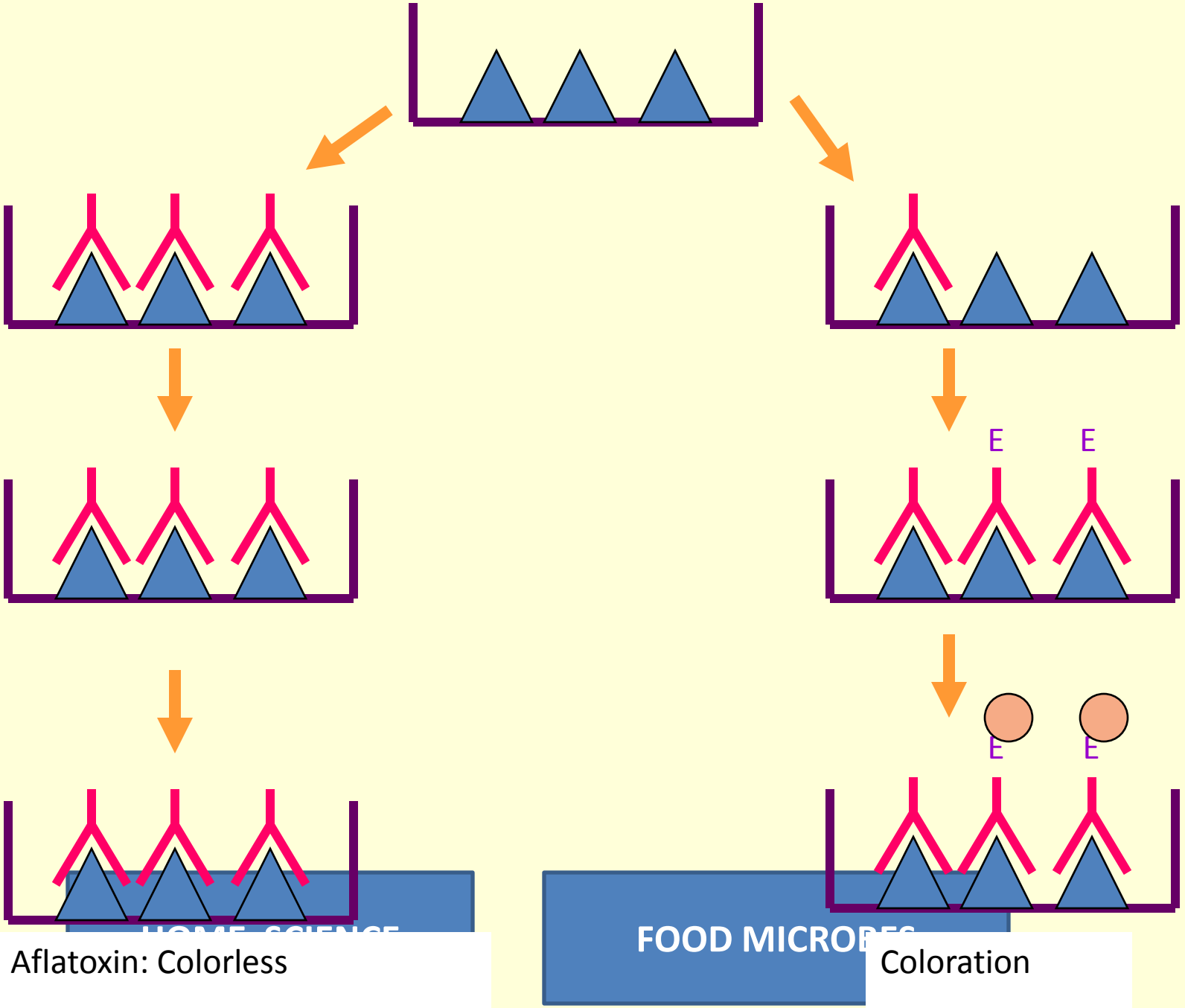

A. flavus
A. nomius
A. tamarri
A. parasiticus
flavus
"Aflatoxin"
Aspergillus
toxin

Aspergillus flavus conidiophore

Walnut infected with Aspergillus flavus

Atoxigenic A. flavus biocontrol strain growing on kernels of wheat

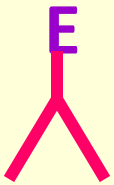
http://msa.ars.usda.gov/la/srrc/aflatoxin/afcrsp.htm


E
E
E
E
FOOD MICROBES
Aflatoxin: Colorless
Coloration

“ELISA”

Antibody

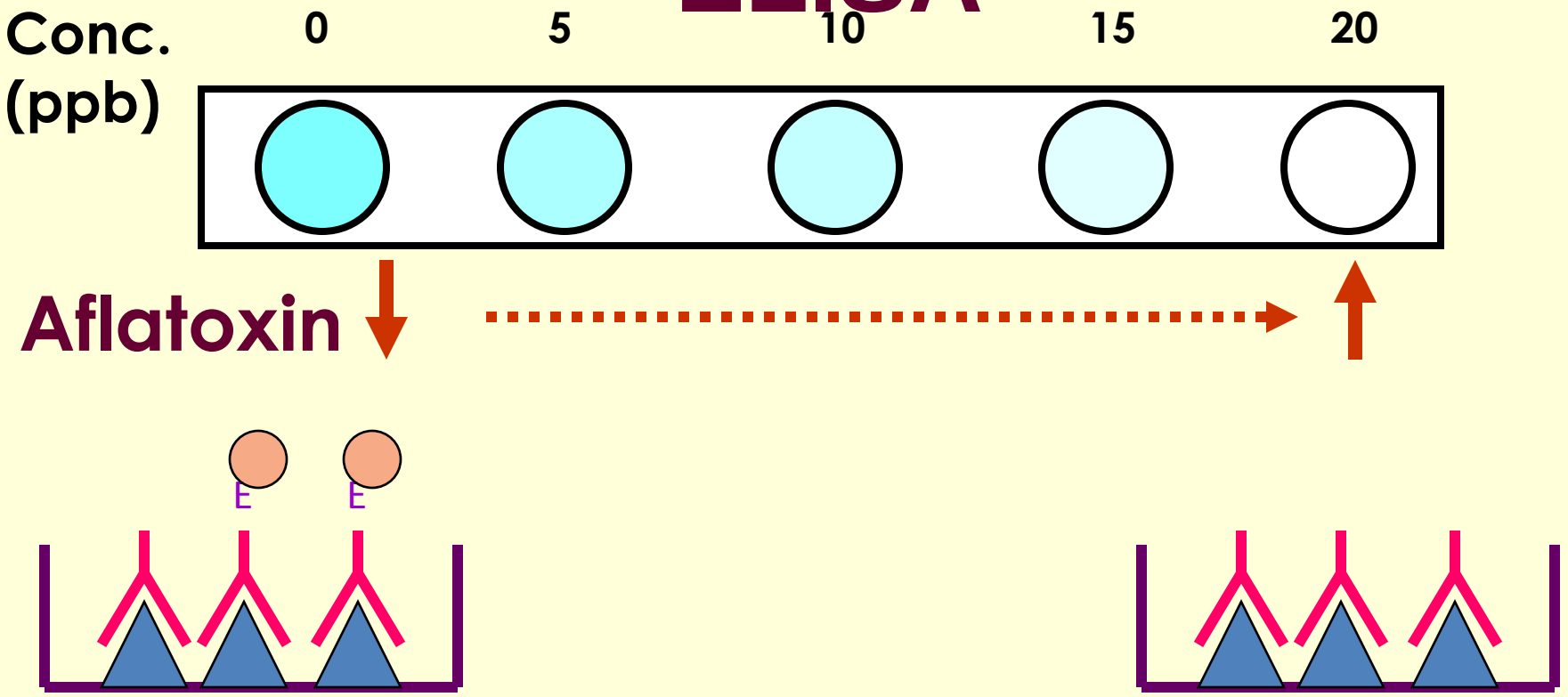
Aflatoxin (free toxin)



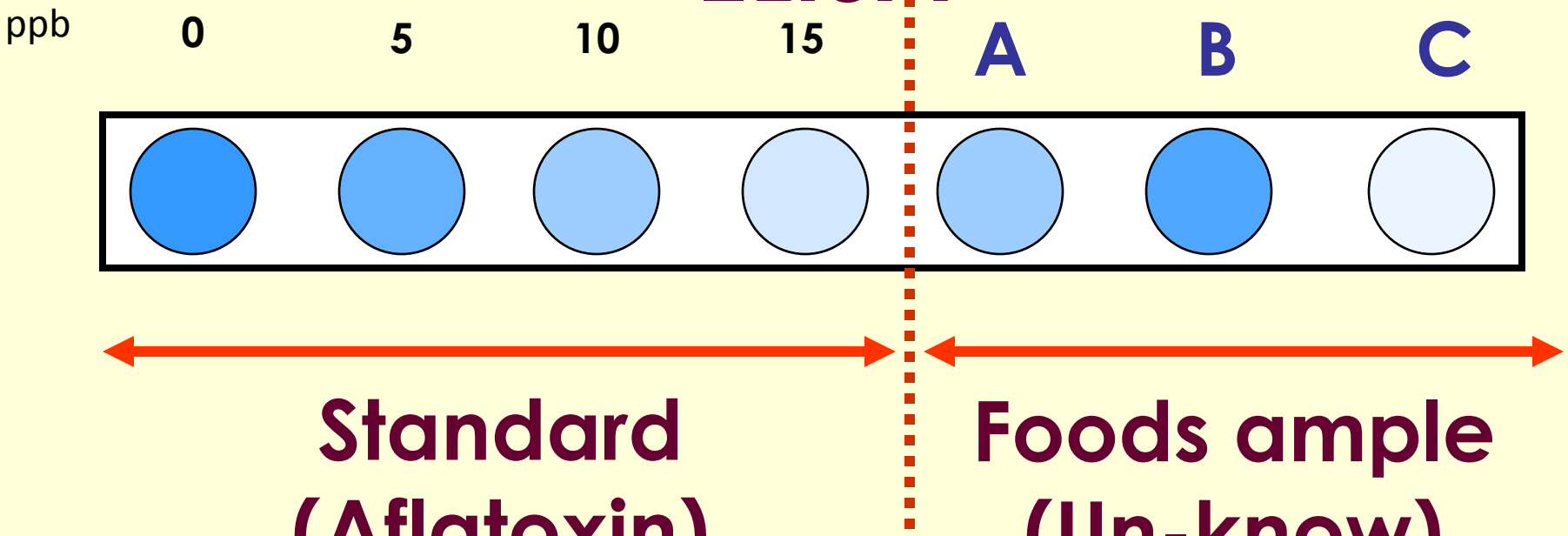
Aflatoxin-enzyme labeled (labeled toxin)

Substrate

Direct Competitive ELISA



Direct Competitive ELISA



Standard (Aflatoxin)

Foods ample (Un-know)

A= ?????? Ppb

B= ?????? Ppb

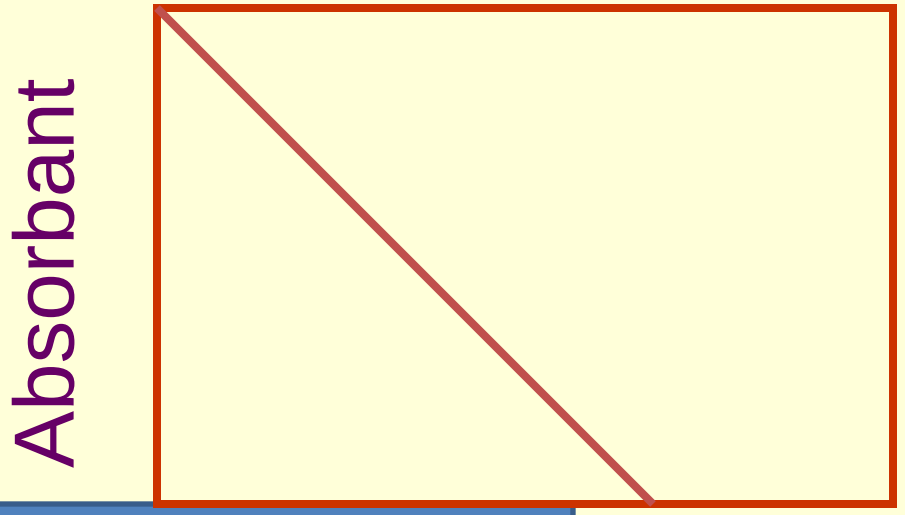
C= ?????? ppb

ELISA

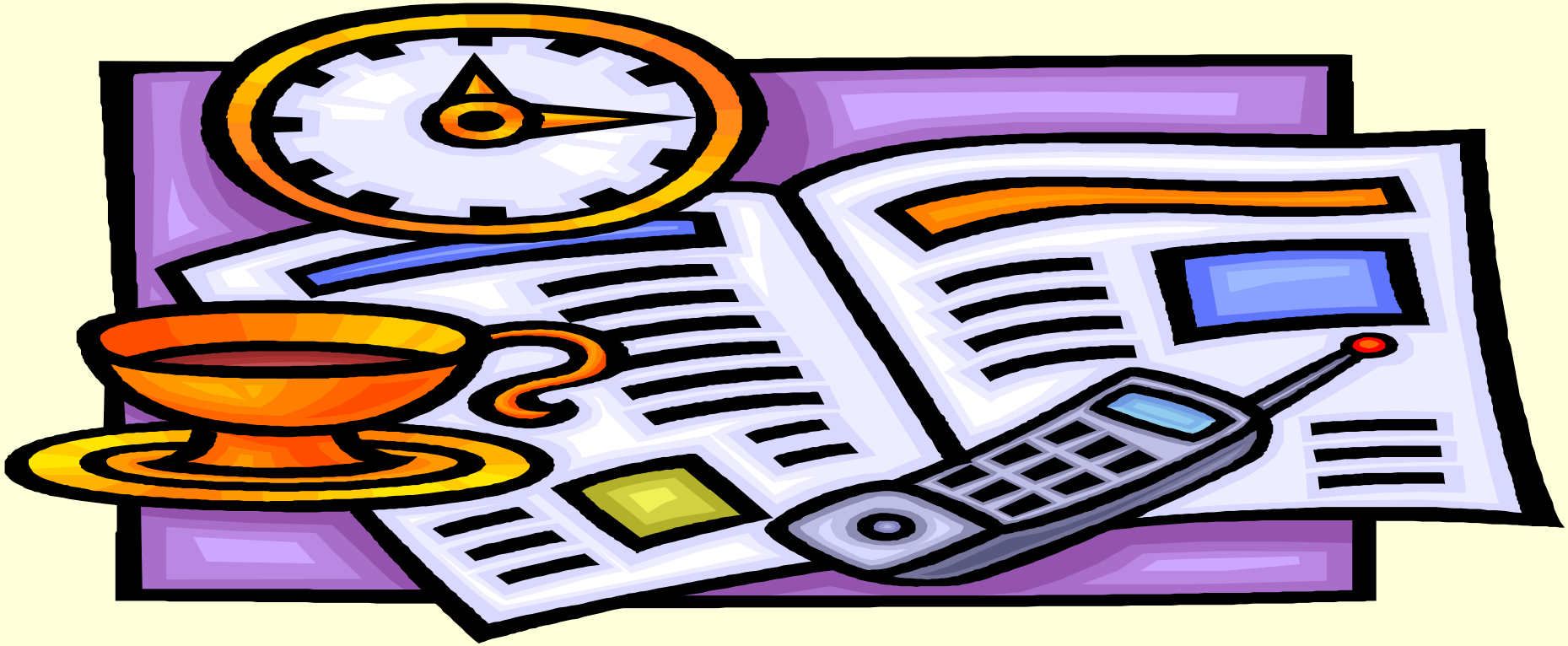
Qualitative result ⇢ color

Quantitative result

- Micro ELISA reader
- Spectrophotometer
- Standard curve



Questions?



THANK YOU

HOME SCIENCE

FOOD MICROBES