Department of Microbiology

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Microbiology

- Why carry out Microbial Testing
- For use by regulatory agencies and food companies
- -to determine the quality and safety of food products

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Receipt of samples for microbiological analysis

Physical condition of the sample container or package

Adequacy of the sample

Mode of transport to the laboratory

Absence of any added preservative for analysis

Note sample details especially ingredients.

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Microbiological testing can be grouped into the following categories:

- 1- Testing for detection of specific organisms (*Listeria monocytogenes, Salmonella, etc)*
- 2- Enumerating specific organisms (Staphylococcus aureus, etc.)
- 3- Enumerating groups of organisms (coliforms, fecal coliforms)
- 4- Enumerating all organisms which will grow under certain defined conditions (aerobic plate count)
- 5- Testing for the presence of microbial toxins (*Clostridium botulinum)*
- 6- Testing for the presence of microbial metabolites (histamine)

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Detection of Food-Borne Pathogens

Must be rapid and sensitive

Methods include:

- culture techniques – may be too slow
- immunological techniques - very sensitive

Molecular techniques

- probes used to detect specific DNA or RNA
- sensitive and specific

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Conventional Culture techniques

Plate count method

- **Pour plate**
- **Spread plate**

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

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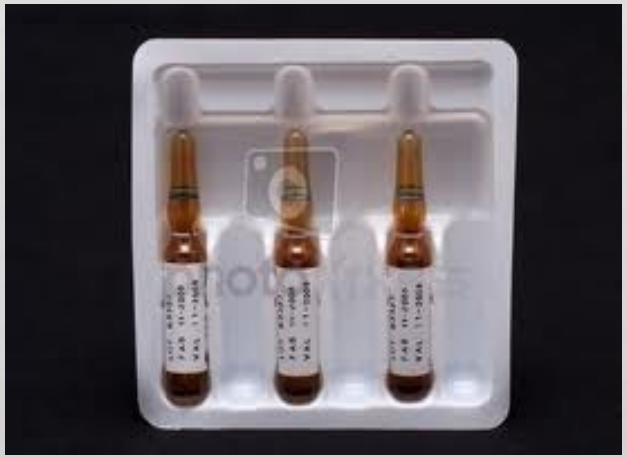
Stock Culture Maintenance and Storage

- Effective maintenance of stock cultures is essential for QC, method validation and research purposes
- Repeated sub-culturing may eventually lead to contamination, loss of viability and genotypic/phenotypic changes
- Freeze-drying and cryogenic storage are preferred, but may not be practical for smaller laboratories



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PROCEDURE FOR REVIVAL

Freeze dried cultures are obtained in ampoules.

↓

Mark the ampoule near the middle of the cotton wool with a sharp file.

↓

Disinfect the surface around the mark with alcohol

↓

Wrap a thick cotton wool / gauge around the ampoule and break at the marked area

↓

Gently remove the top of the ampoule and remove the cotton

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PROCEDURE FOR REVIVAL

add about 0.3 to 0.4 ml of Nutrient broth medium to make a suspension of the culture

↓

Streak a few drops of the suspension to Nutrient agar medium in a petri plate

↓

remaining suspension may be transferred to 5 ml of Nutrient broth medium in a test tube

↓

Incubate at 37°C for 12h under aerobic conditions

↓

The growth obtained thus is referred to as reference stock.

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SUBCULTURING AND STORAGE

Reference stocks obtained by reviving the reference cultures should be preserved as glycerol stocks.

Sub-culturing is recommended every 30 days.

Glycerol stocks may be stored up to a period of three years.

To prepare working stocks, aseptically inoculate the pure culture from glycerol stocks to agar slants

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Testing – Sample Preparation

Preparation of sample homogenate

To Make 1:10 dilution

↓

Weigh 50±g of sample and add 450ml diluent into stomacher bag

↓

Mix in the stomacher for 30-60 sec

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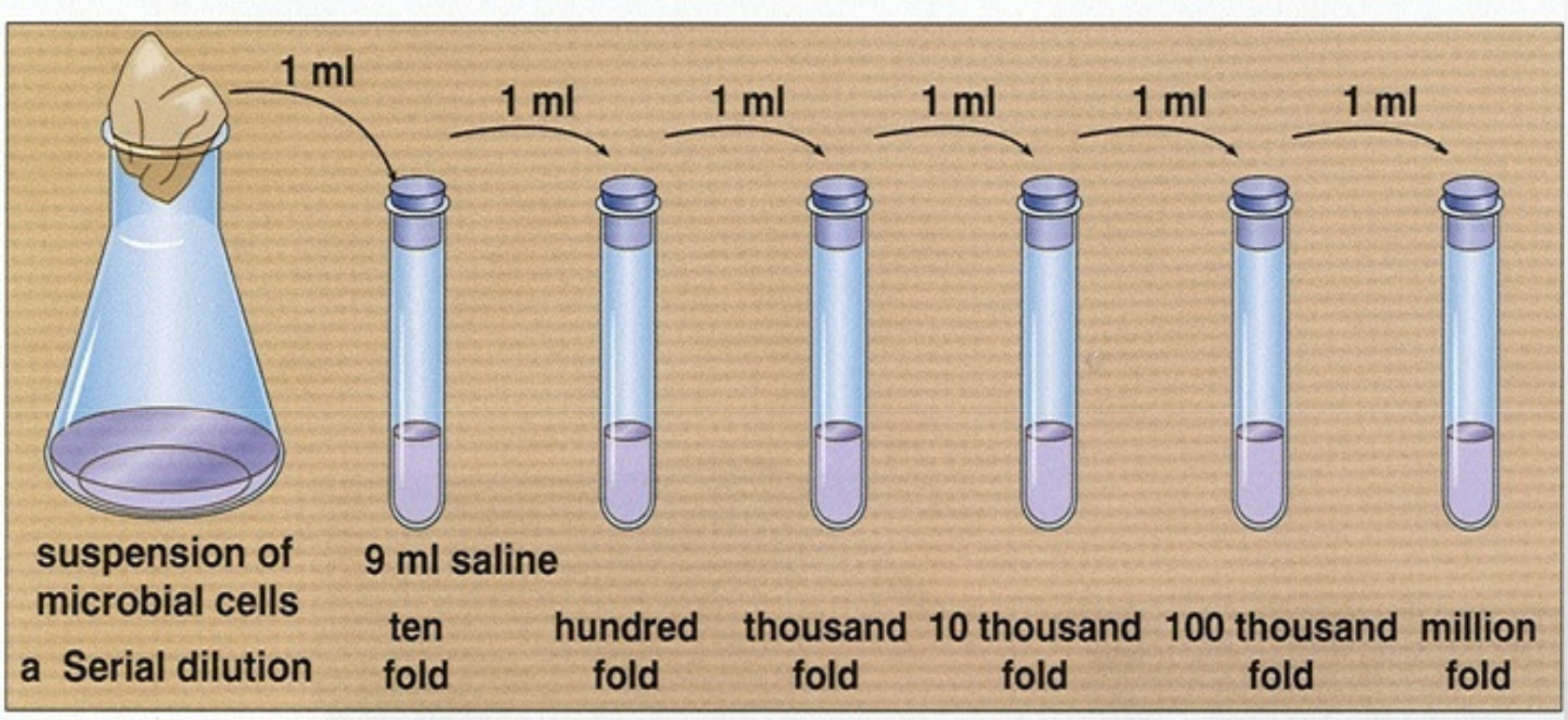
Testing – Preparation of Serial Dilutions



a Serial dilution

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Testing – Food Analysis

Microbiological Methods

Broad Microbial Groups

- Aerobic Mesophilic plate count
- Coli form count
- Yeast & Mould count

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Testing – Aerobic Mesophilic plate count

Sample homogenate

↓

Sample serial dilution

↓

Plating (Pour plate technique)

↓

Incubation

↓

Observation

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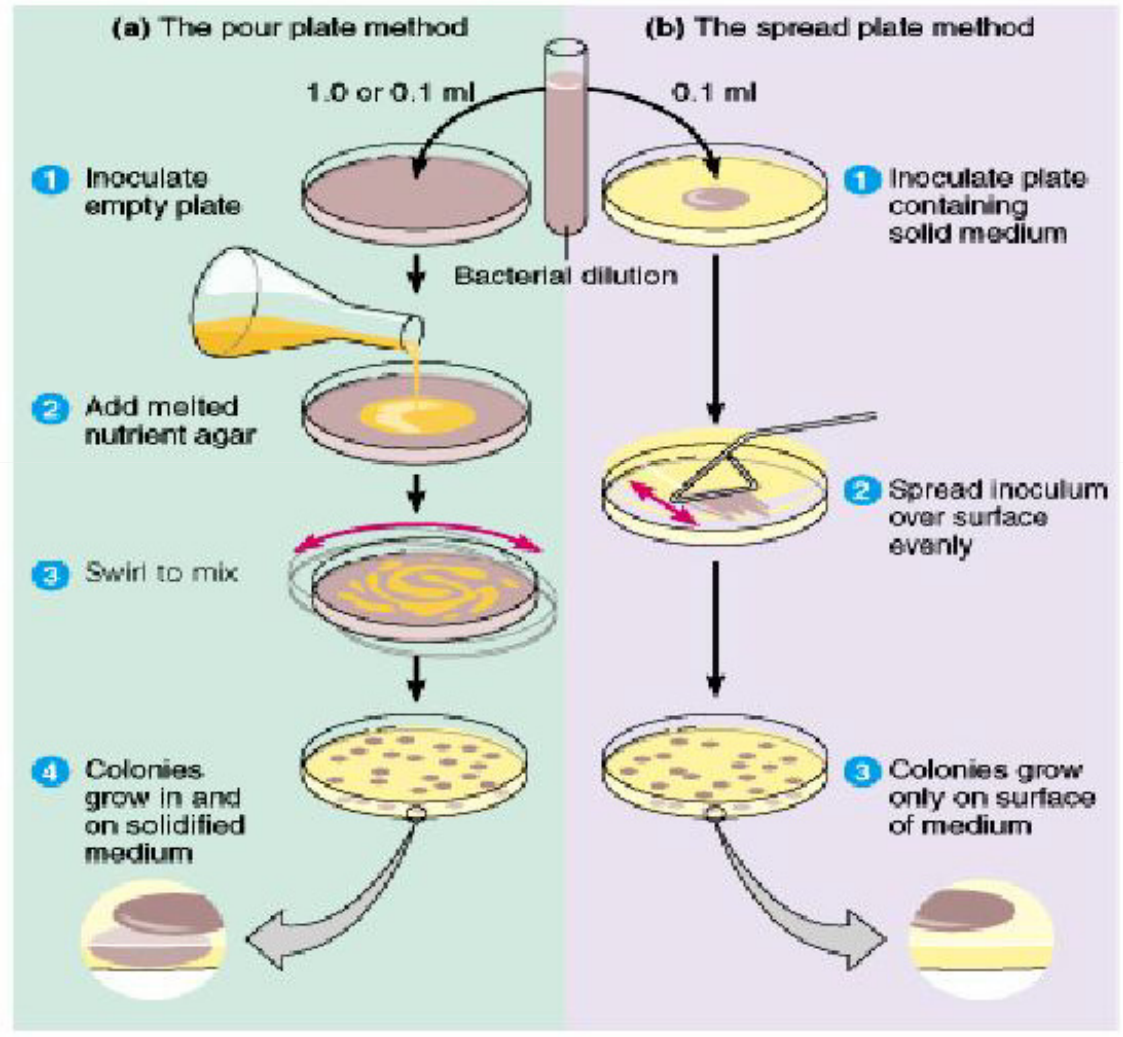
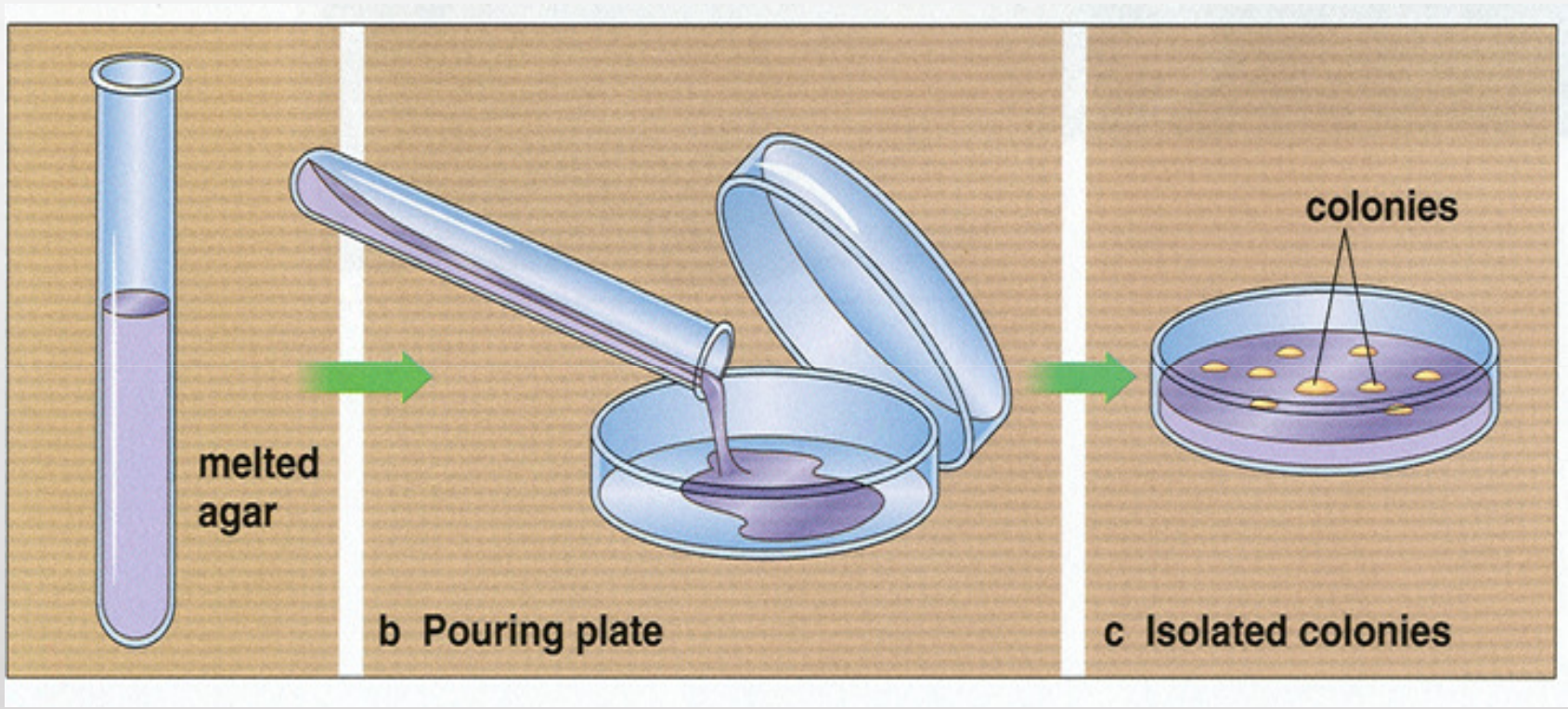
Plate count method



Testing – Pour plate technique



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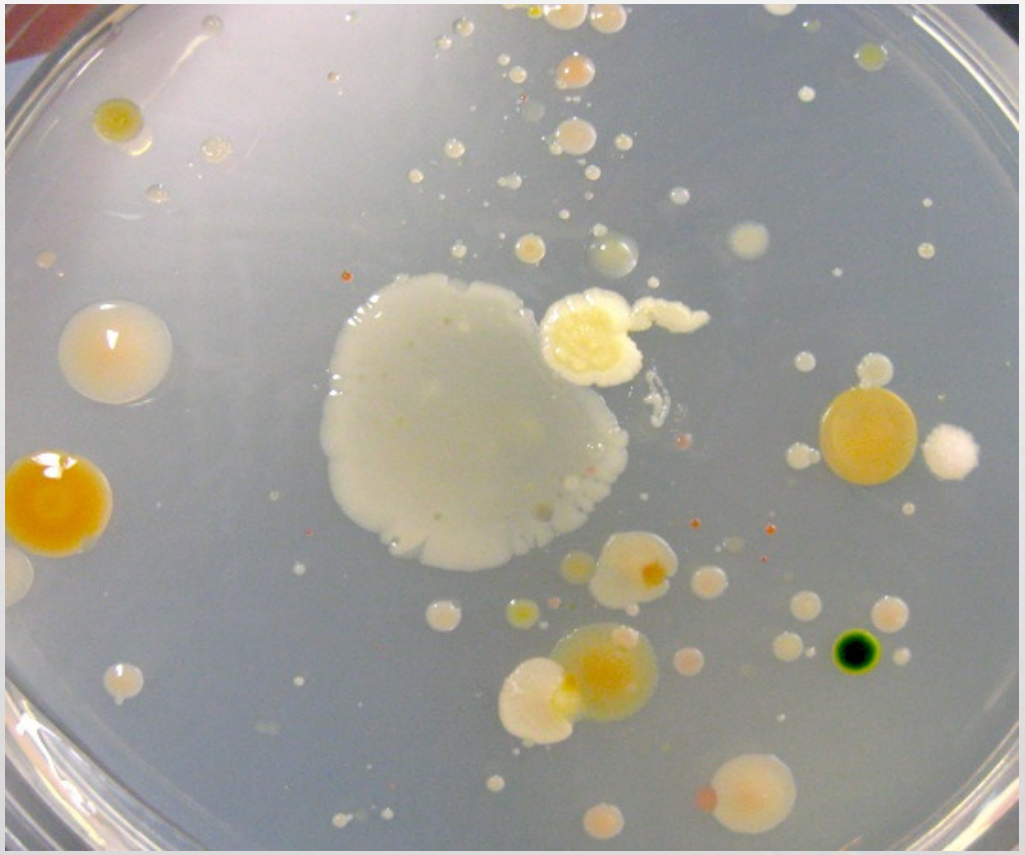
Testing – Observation



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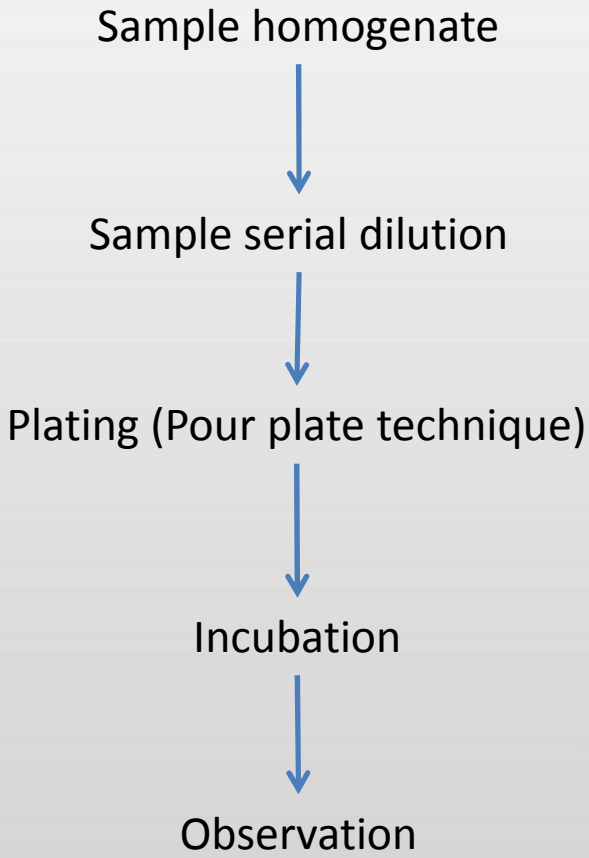
Testing – Detect Coliforms

Detection of Coliforms by Plating Technique



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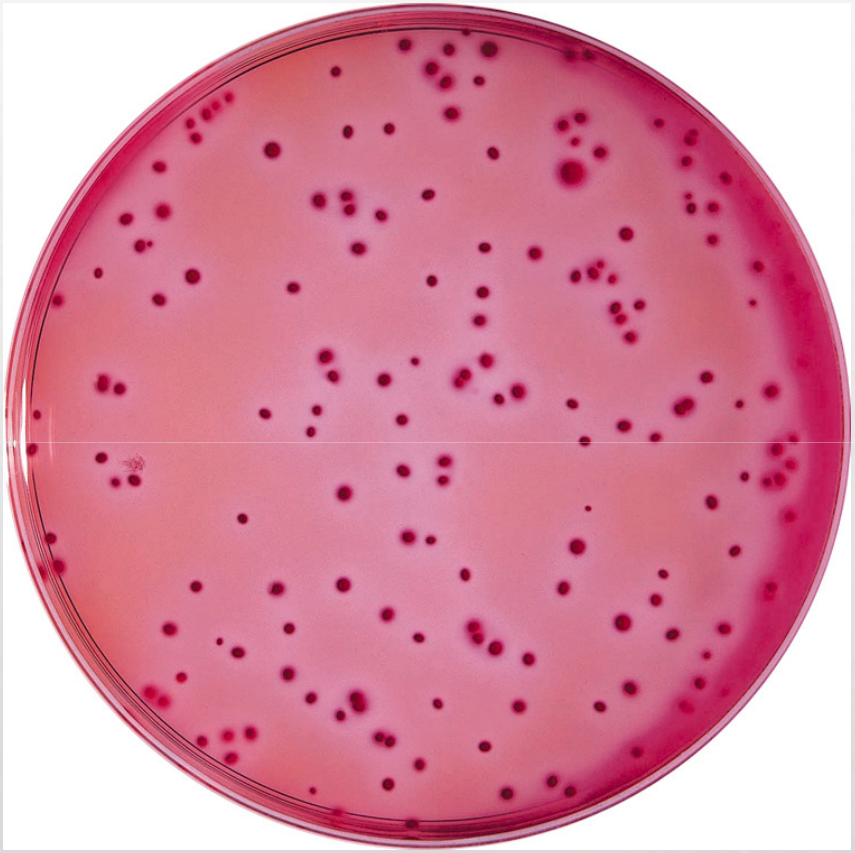
Testing – Coliforms Observation



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Testing – Yeast and Mould

Determination of Yeast and Mold

Sample homogenate

↓

Sample serial dilution

↓

Plating (Pour plate technique)

↓

Incubation

↓

Observation

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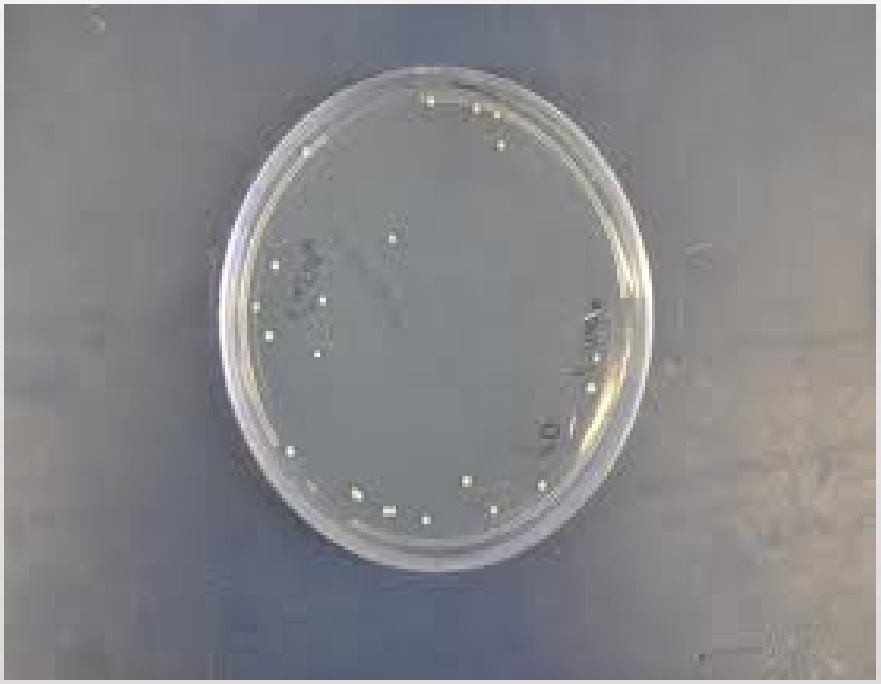
Testing – Yeast and Mould Observation



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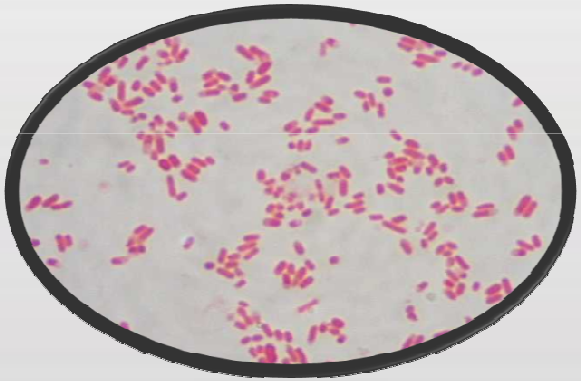
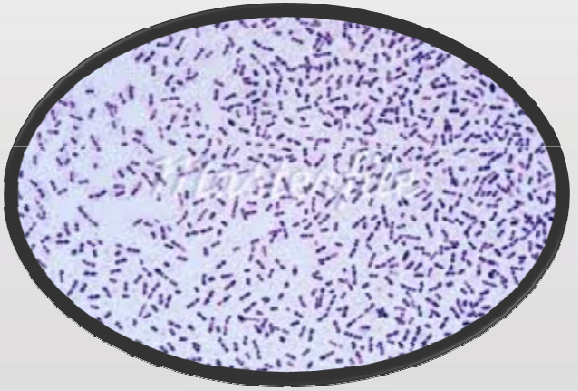
Testing – Pathogens

E.Coli



Staphylococcus aures



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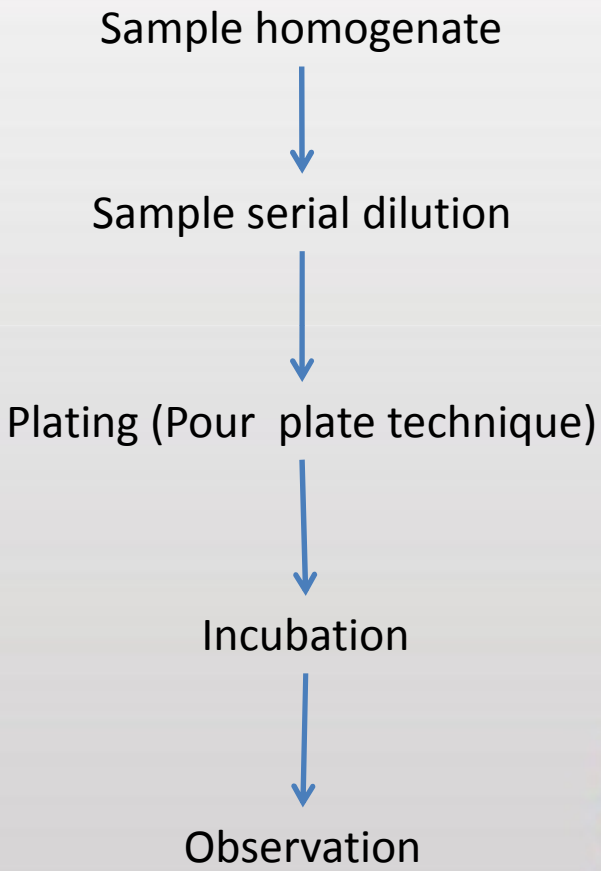
Testing – Escherichia

Enumeration of Escherichia Coli in Foods



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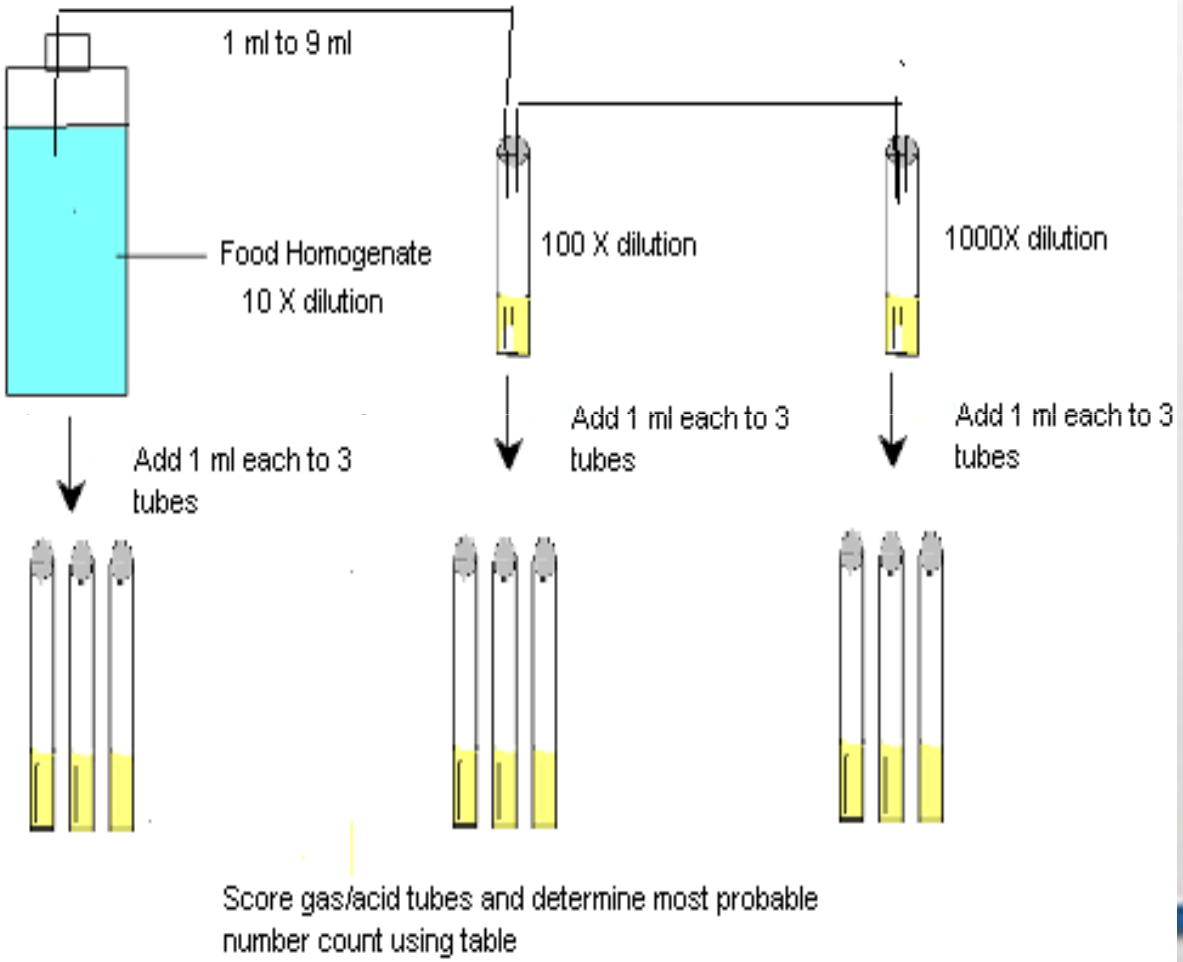
Most Probable Number Technique

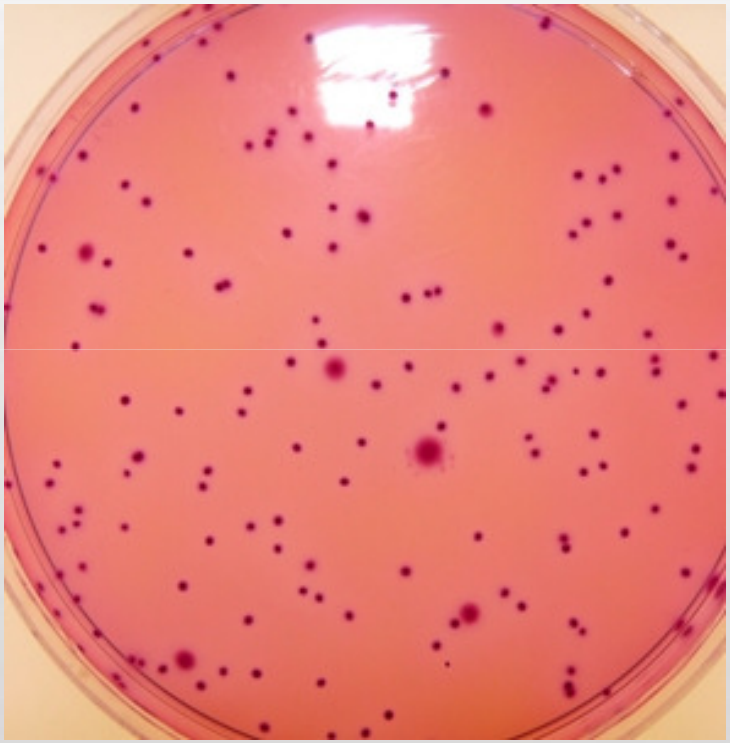
Low count

- **Coliform**
- ***E.coli***
- ***S. aureus***
- **Faecal streptococci**



Testing – Spread Plate Technique

Observation



E.Coli on VRBA MUG

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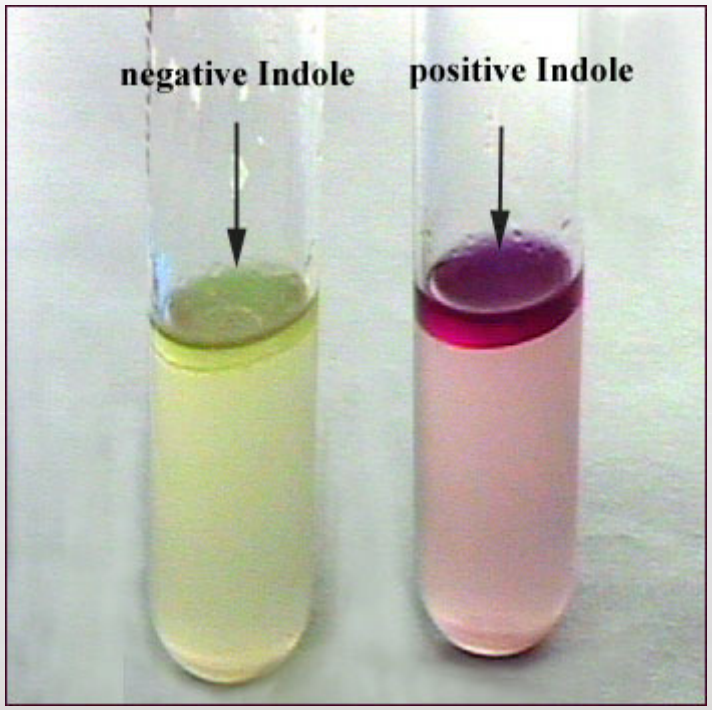
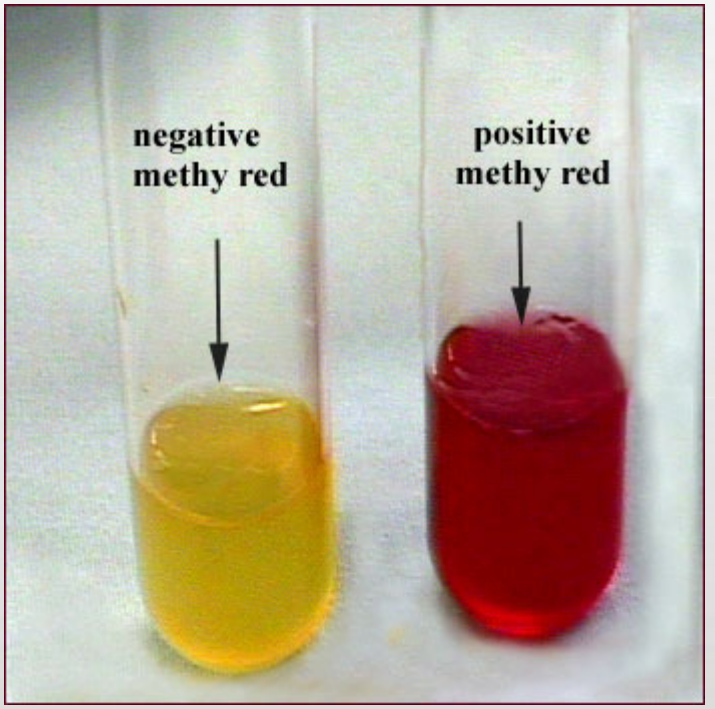
Testing – E. Coli - Biochemical Confirmation



Indole test



Methyl red test

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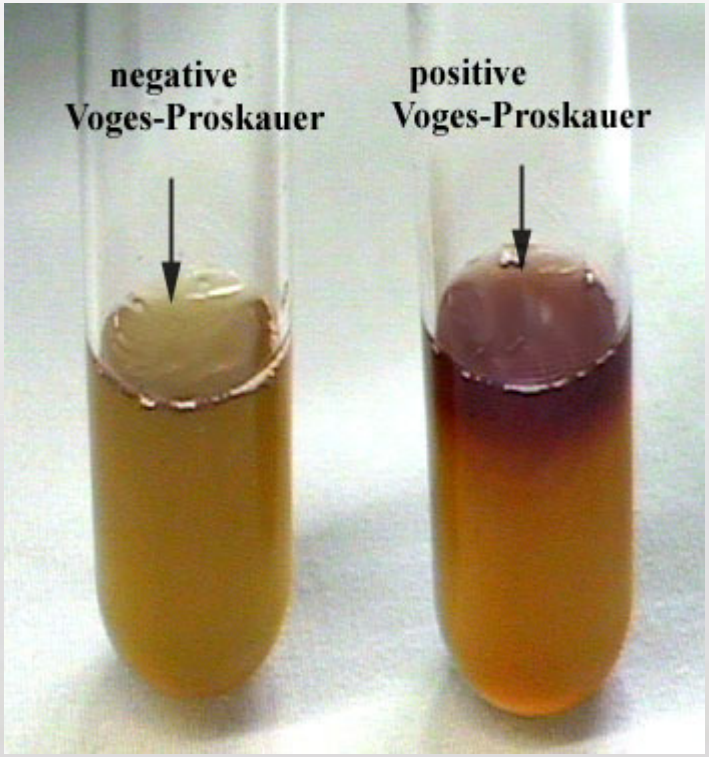
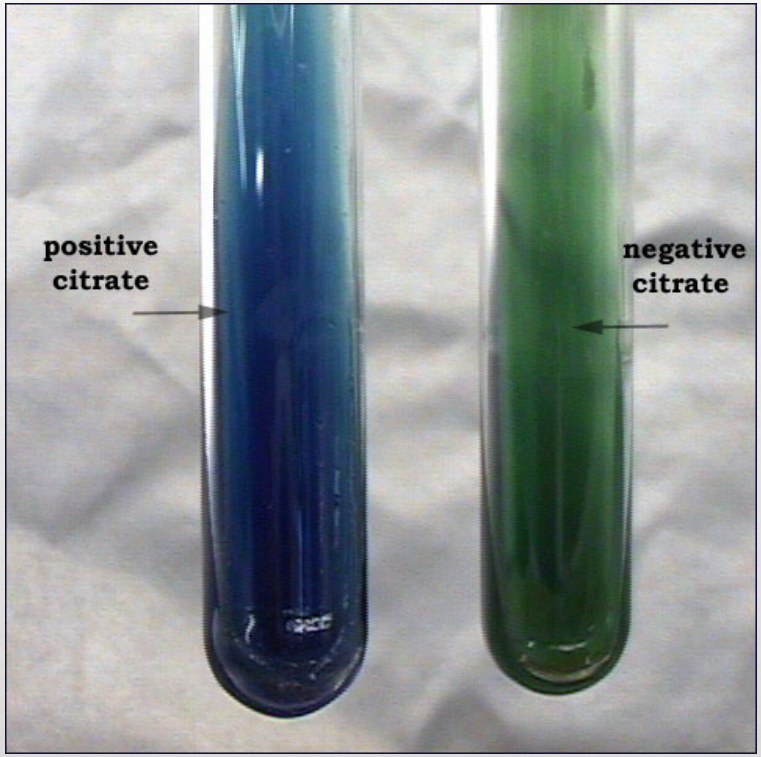
Testing – E. Coli - Biochemical Confirmation

Continue …



Voges-proskauer



Citrate test

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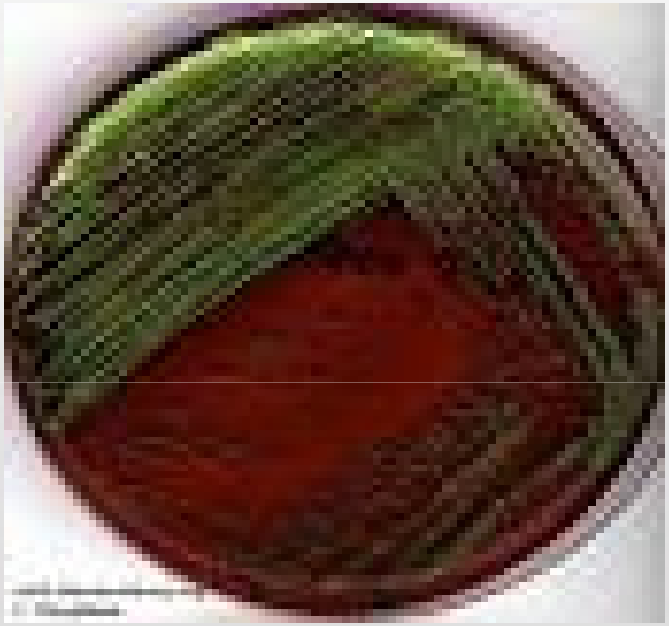
Testing – E. Coli - Biochemical Confirmation

Continue …

E.Coli on Mac conkey



E.Coli on EMB

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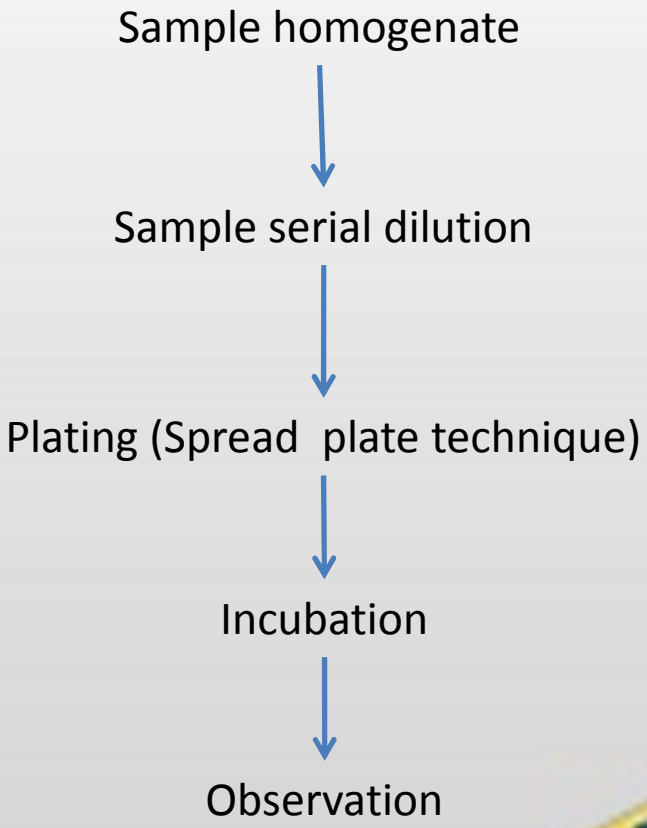
Testing – Staphylococcus Aureus

Detection of Staphylococcus Aureus



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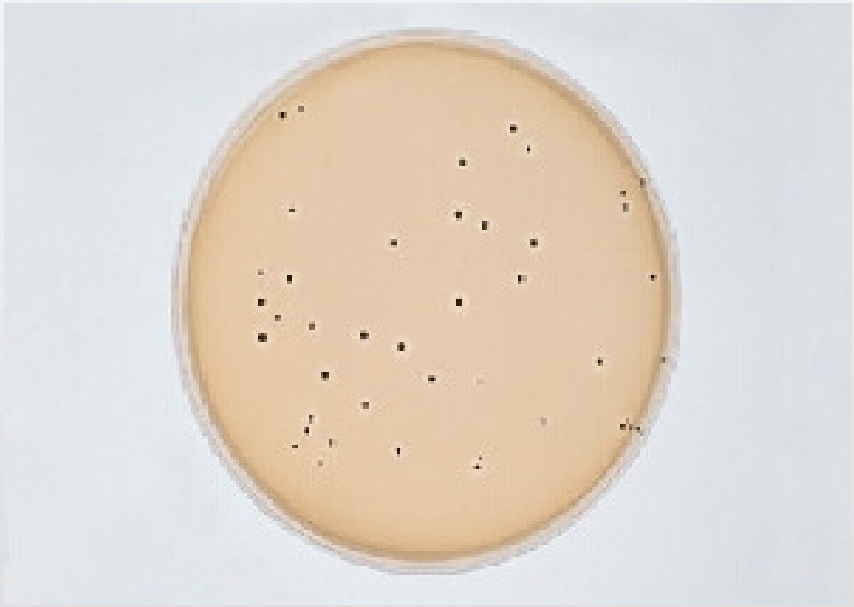
Staphylococcus Aureus



BPA Plate

MSA Plate

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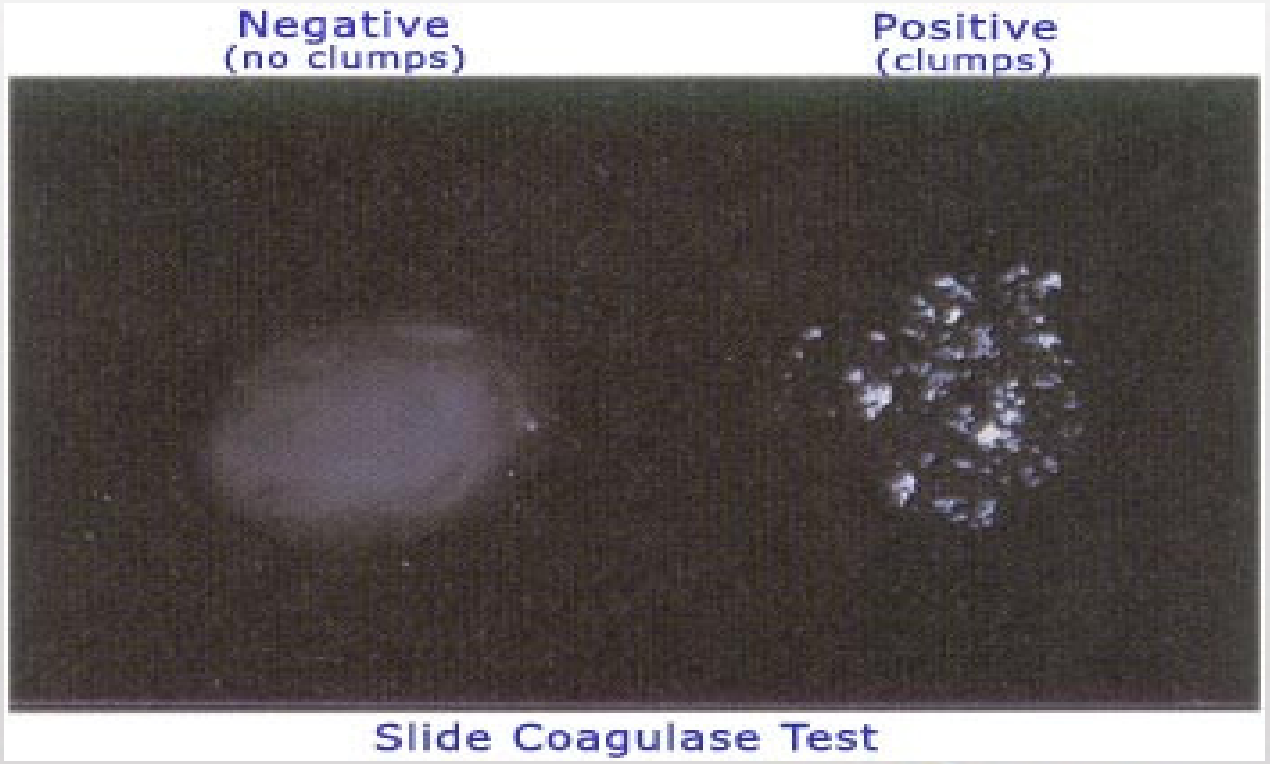
Testing – Serological Confirmation

Serological Confirmation by Coagulase Test



Slide Coagulase Test

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