# BASIC TECHNIQUES IN MICROBIOLOGY

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# INTRODUCTION

**Good quality diagnostic test**

1. Clinically relevant

2. Reliable

3. Reproducible

4. Turn around time

5. Cost benefit ratio

# INTRODUCTION

## Factors affecting quality of test:

1. Personnel factors

2. Environmental factors

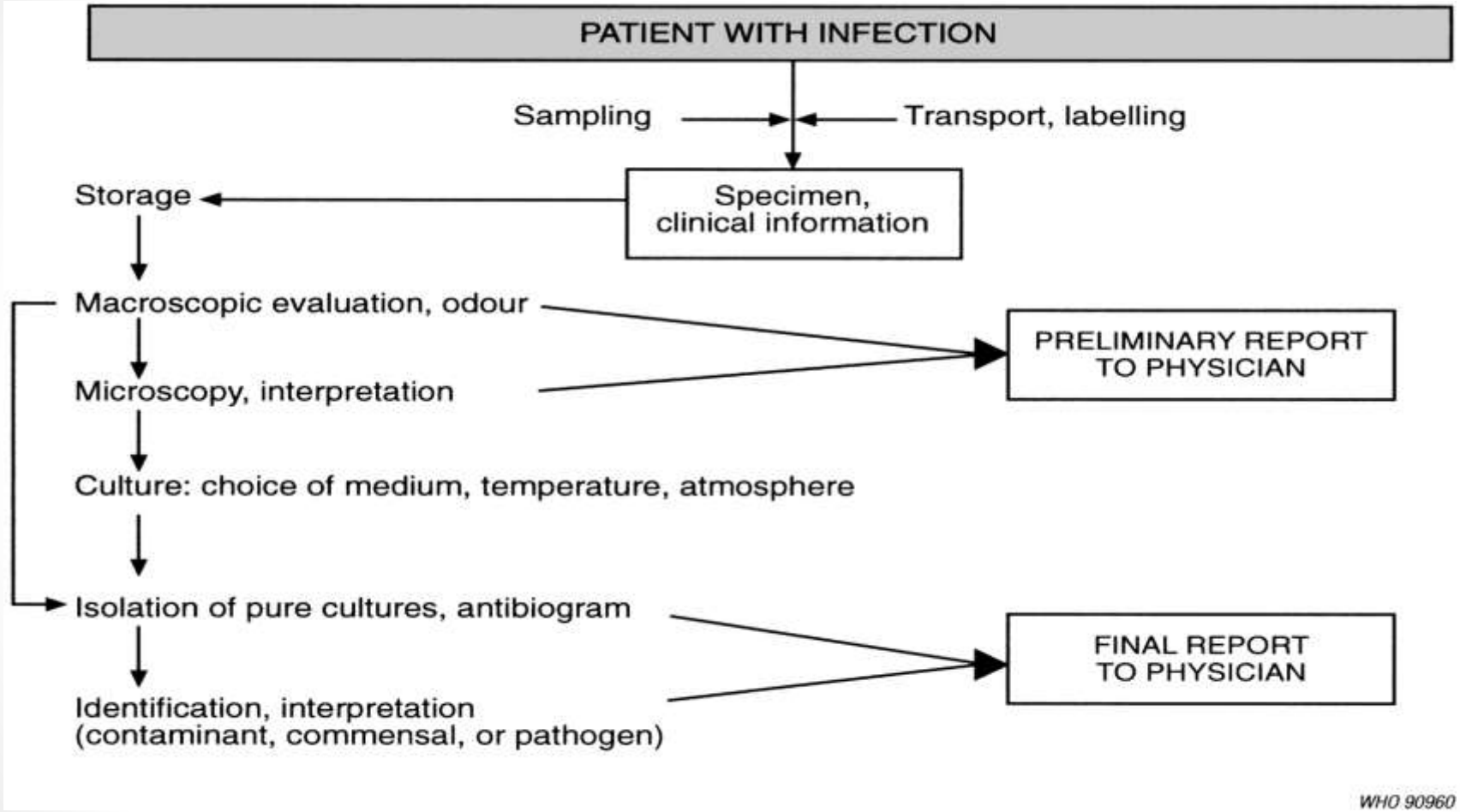
3. Specimen quality

4. Laboratory materials and testing methods

5. Examination and reading

6. Reporting

# PROCEDURAL FLOW



# BACTERIOLOGY

# GRAM'S STAINING

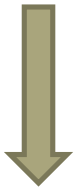
- Differential method of staining
- Differentiates between Gram positive and Gram negative bacteria.
- **PRINCIPLE** -Gram-positive bacteria have cell walls that contain thick layers of peptidoglycan . These stain purple.

- Gram-negative bacteria have walls with thin layers of peptidoglycan, and high lipid content. These stain pink.

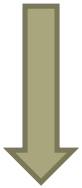
# Cont….

- **PROCEDURE**

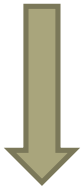
Crystal violet (Primary stain)



Gram’s Iodine(Mordant/Fixer)



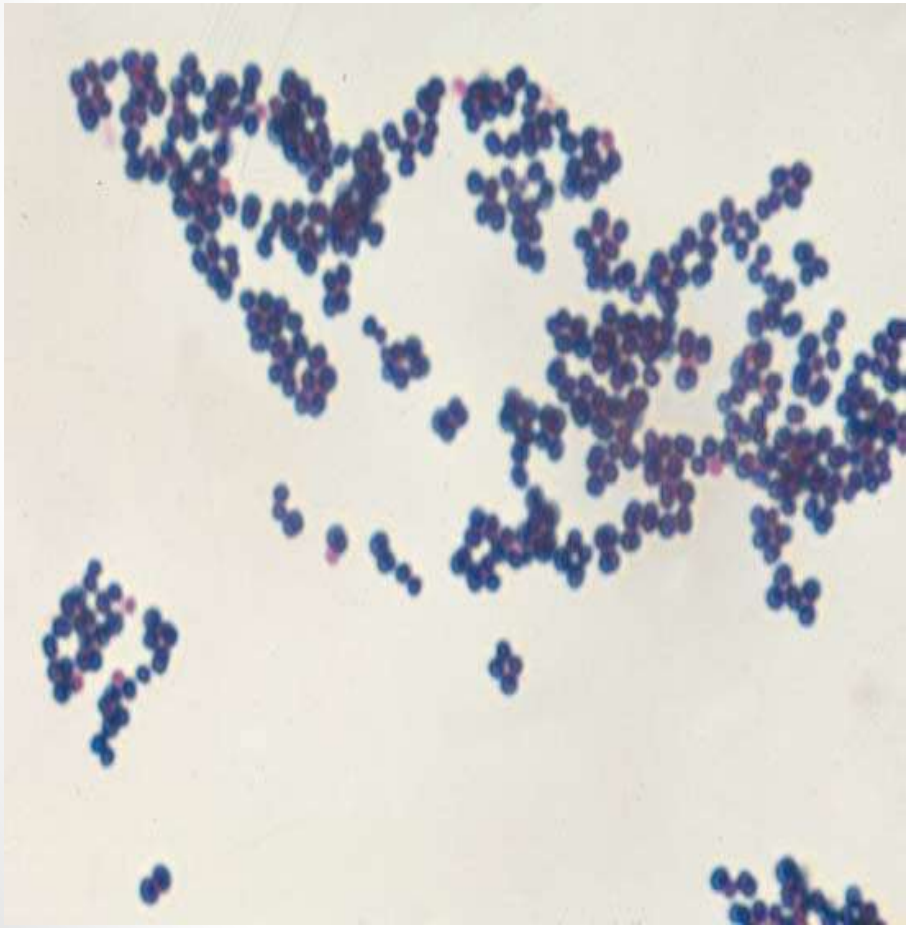
Acetone alcohol (Decolorizer)



Safranin (Counter stain)

# Cont….

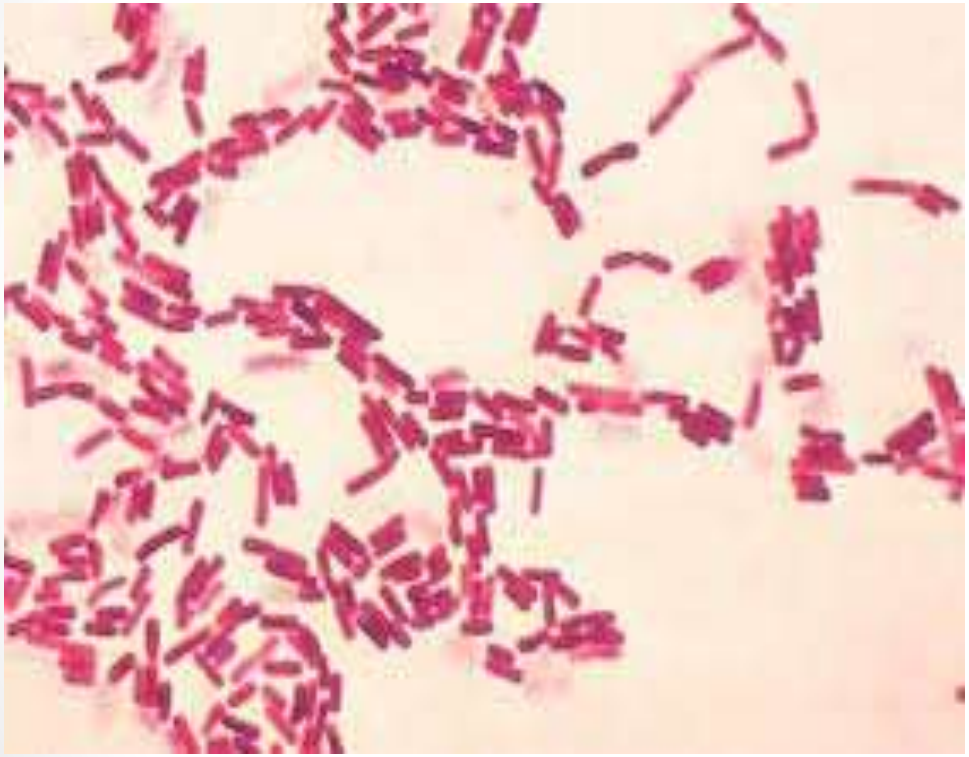
## GRAM POSITIVE BACTERIA



Example: Staphylococci
Streptococci
Micrococci

# Cont….

## GRAM NEGATIVE BACTERIA



Example: Escherichia coli
Proteus
Pseudomonas
Klebsiella

# Media: Providing Nutrients in the Laboratory

- **Culture media**: artificial substances used for growth, isolation and identification of micro-organisms.

- **Classification of media**

1. Physical state
2. Chemical composition
3. Functional type

# Cont…

Media :

1. Enriched media
2. Enrichment media
3. Selective media
4. Differential media
5. Anaerobic media
6. Transport media


# TECHNIQUES OF CULTURE

- Five basic techniques to manipulate, grow, examine, and characterize microorganisms in the laboratory.

1. Inoculation
2. Incubation
3. Isolation
4. Inspection
5. Identification

# Conti…

- **Inoculation**: procedure of introducing an inoculum of specimen on culture media.


- **Methods of inoculation:**

A. Liquid specimen- 1 or 2 drops

B. Tissue – direct implantation

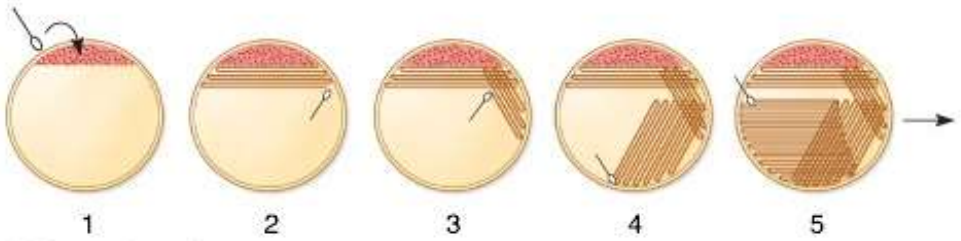
C. Swabs- rolling the swab

# Cont…

- **Streak plate method**: Is designed to provide a continuous dilution of the specimen in order to give isolated colonies



**Note:** This method only works if the spreading tool (usually an inoculating loop) is resterilized after each of steps 1–4.




(a) **Steps in a Streak Plate**

(b)

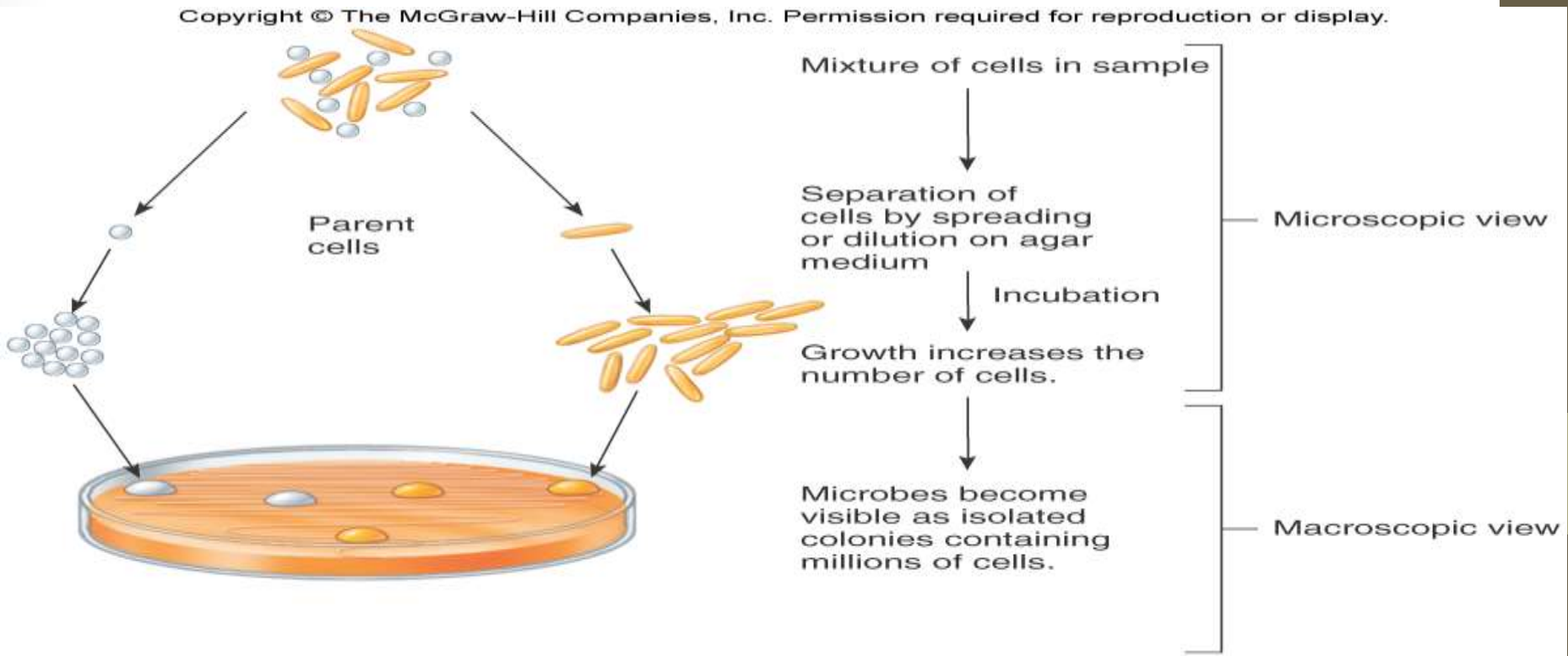
# Cont...

- **Incubation**:

1. Incubated in inverted position.
2. Optimum growth temperature is 35℃.
3. Visible colonies observed in about 18 hours.
4. Relative humidity around 70-80%.

# Cont...

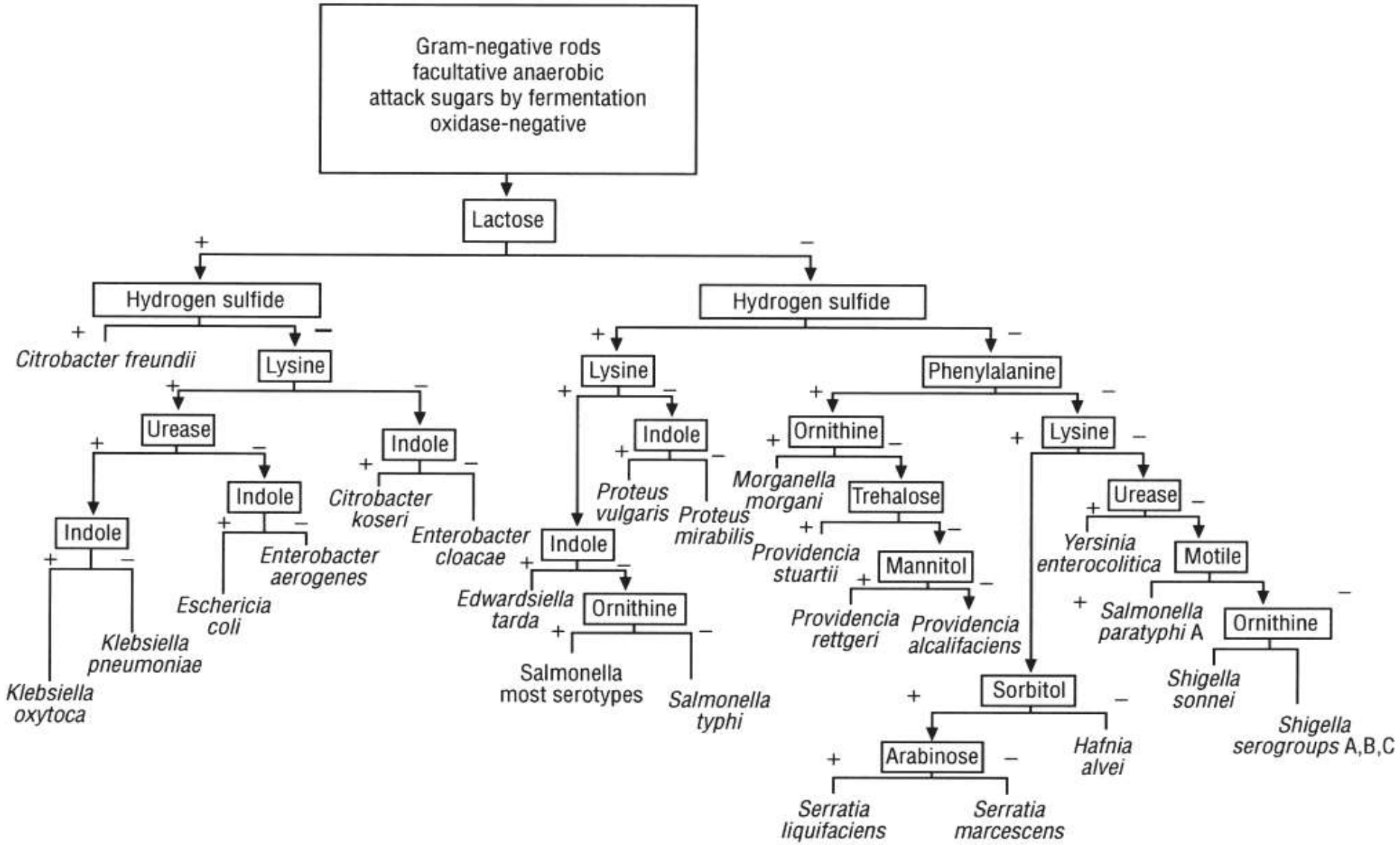
- **Isolation**: separating one species from another. If colony is formed from a single cell, the colony contains cells from just that single species.



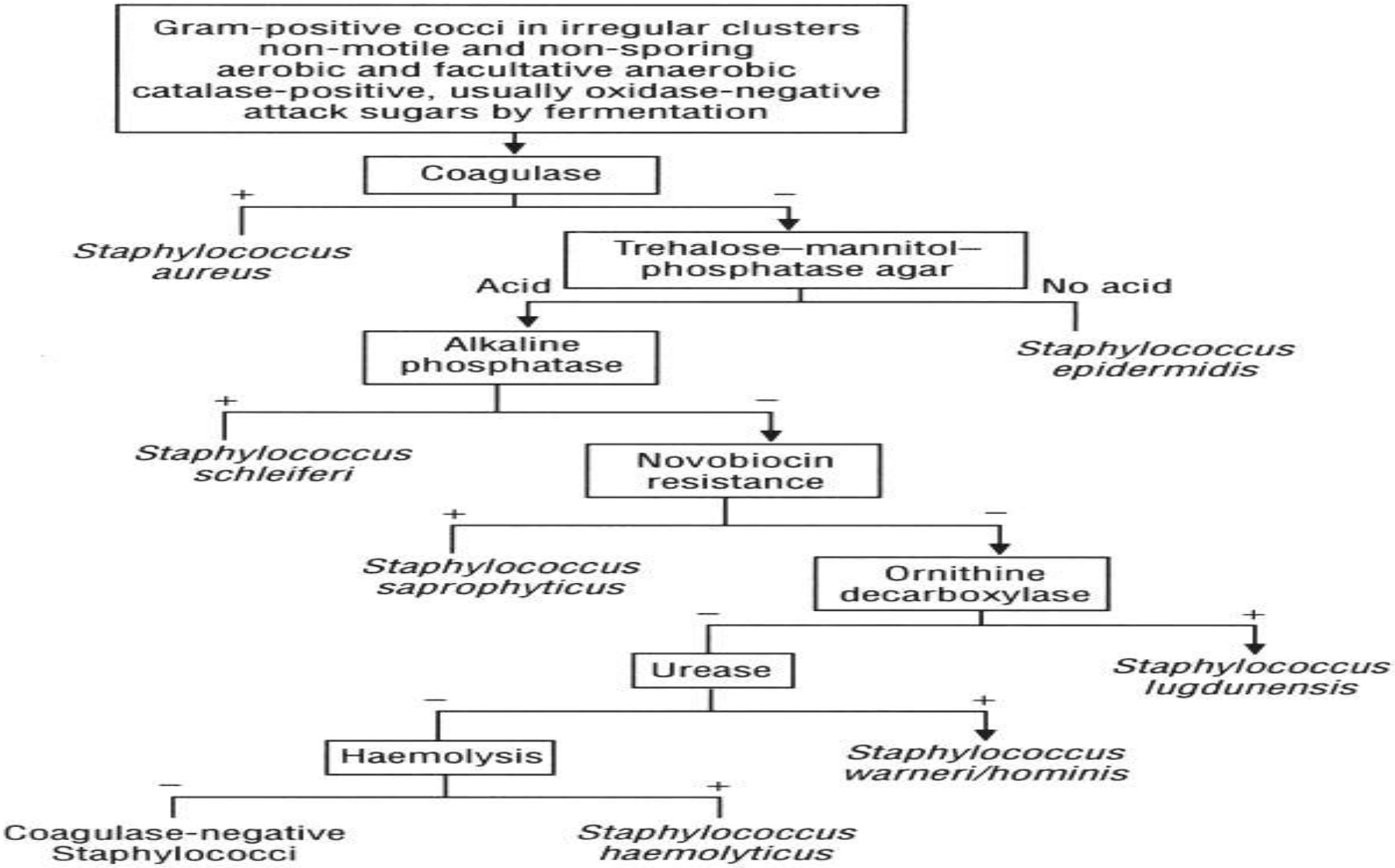
# Cont…

- **Inspection and Identification**: Observing appearance as well as biochemical reactions and sometimes genetic analysis or immunologic testing to identify the organisms in a culture.


# Flow chart for *Enterobacteriaceae*



# Flow chart for *Staphylococcus*



# Flow chart for *Streptococcus*

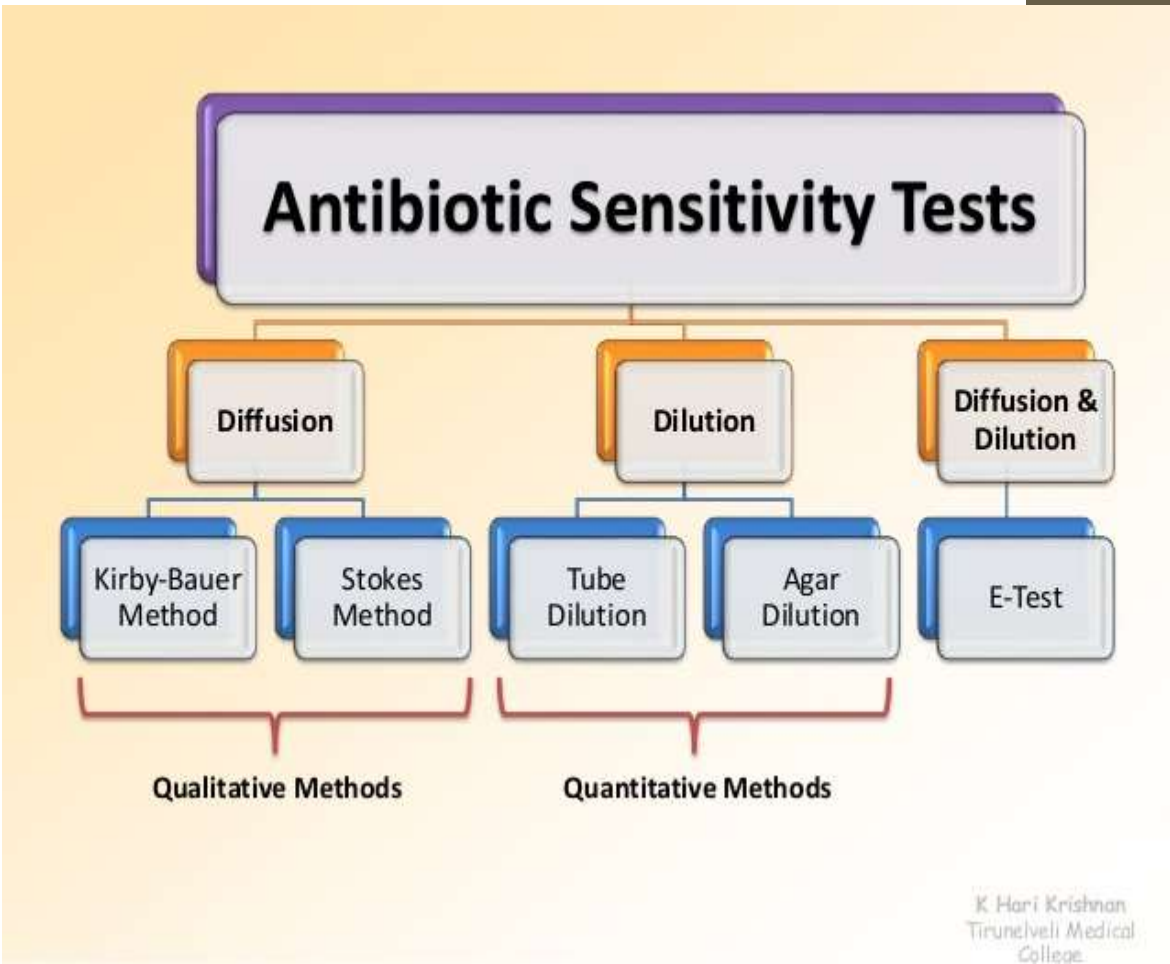
| Species | *S. pyogenes* | *S. agalactiae* | *E. faecalis* var. *zymogenes*[a] | Others |
|---|---|---|---|---|
| Lancefield group | A | B | D | C, G, F |
| Haemolysis | β | β[b] | β | β |
| Zone around the differential bacitracin disc | + | 0[c] | 0[c] | 0[d] |
| Bile–aesculin agar (growth & blackening) | 0 | 0 | + | 0 |
| Reverse CAMP test | 0 | + | 0 | 0 |
| Co-trimoxazole[e] susceptibility | 0 | 0 | 0 | + |
| PYR test[f] | + | 0 | + | 0 |

# ANTIMICROBIAL SUSCEPTIBILITY TESTING

- Measures the ability of an antimicrobial agent to inhibit bacterial growth in vitro.

- **Two methods:**

1. Dilution method
2. Diffusion method

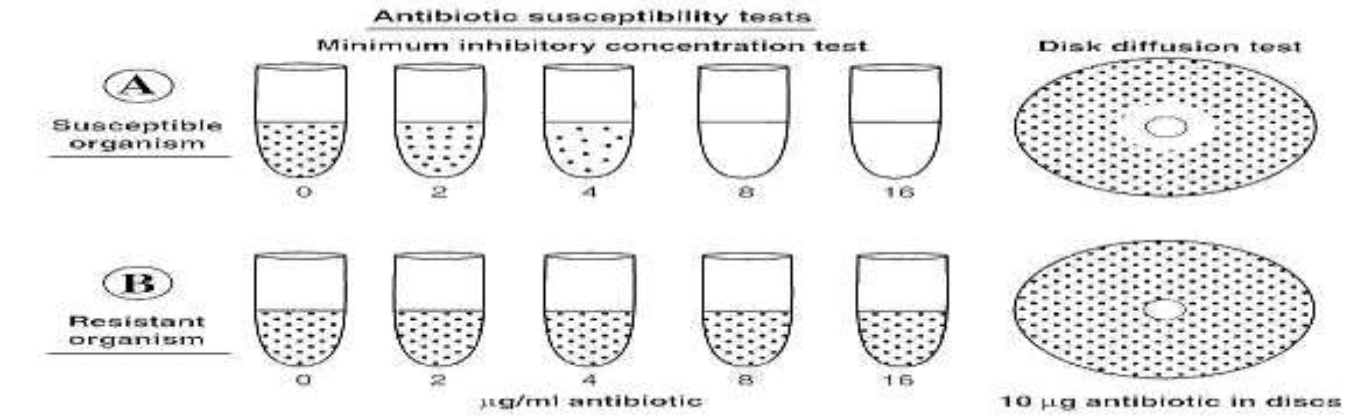


# Cont...

## Dilution method

- Dilutions of antimicrobial agent is added either into broth or agar medium
- Inoculate test organism
- MIC (Minimum inhibitory concentration):

  Check for lowest concentration of antimicrobial agent dilution which prevents visible growth of organism after over night incubation.



# Cont...

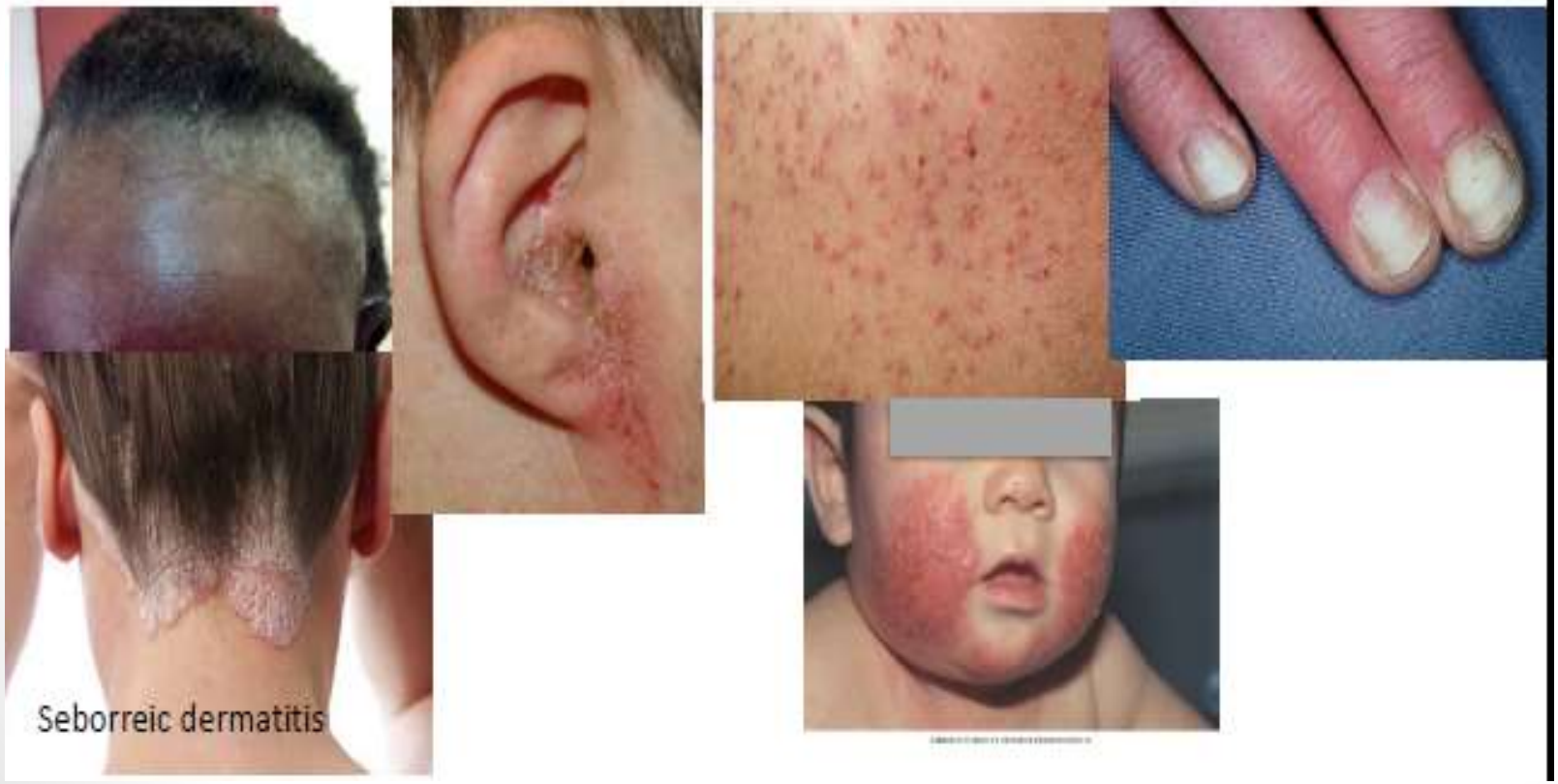
## Diffusion method

- Lawn culture of test organism on culture medium
- Placed antimicrobial impregnated paper disc on agar medium
- Observe zone of inhibition and compare with CLSI standards.

# MYCOLOGY

# Laboratory procedures for the diagnosis of fungal infection

As with other microbial infections, the diagnosis of fungal infections relies upon a combination of **clinical observation** and **lab investigation**.



# Laboratory procedures for the diagnosis of fungal infection

Lab methods for the diagnosis of fungal infections remain based on the three broad approaches:

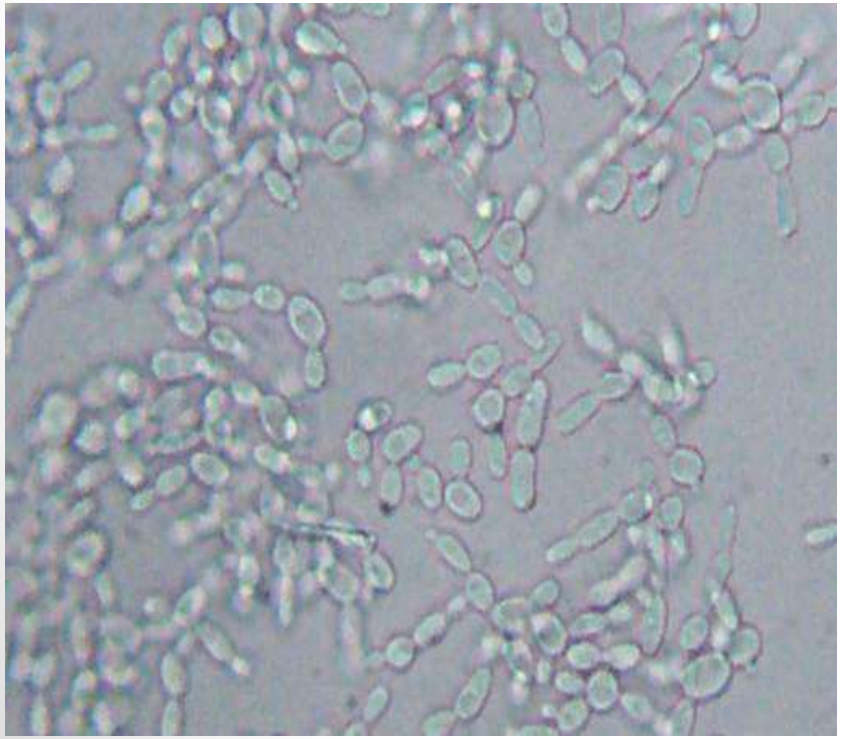
- (i) Microscopic detection of the etiological agent in clinical material
- (ii) Isolation and identification of the etiological agent in culture
- (iii) The detection of a serological response

# Cont...

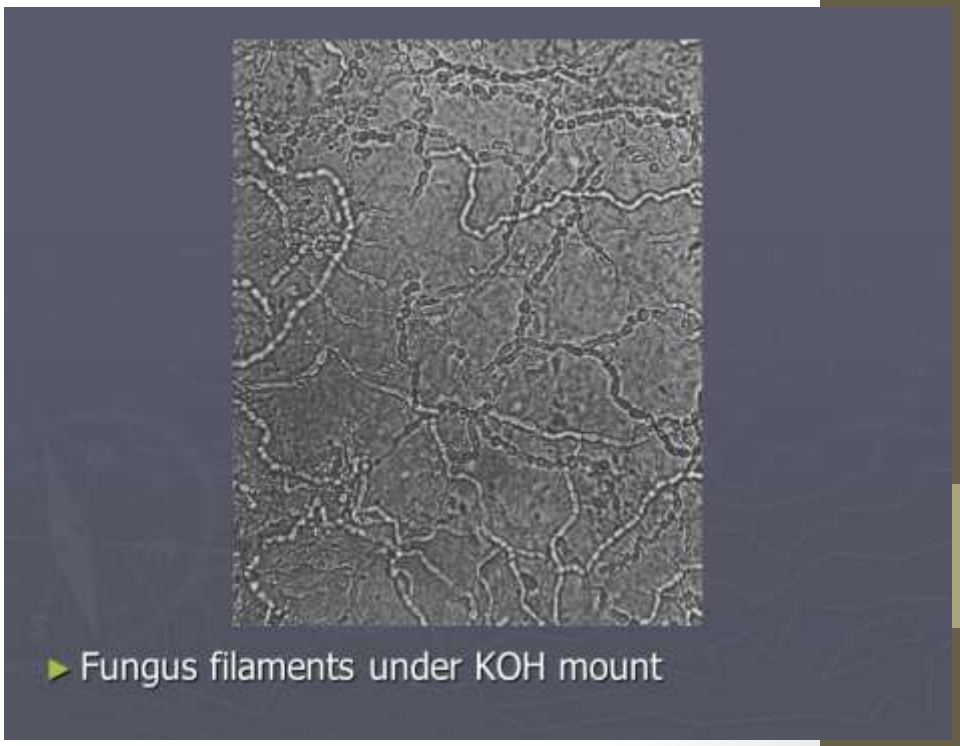
- **Microscopic examination**

## Direct examination

### Wet Mount



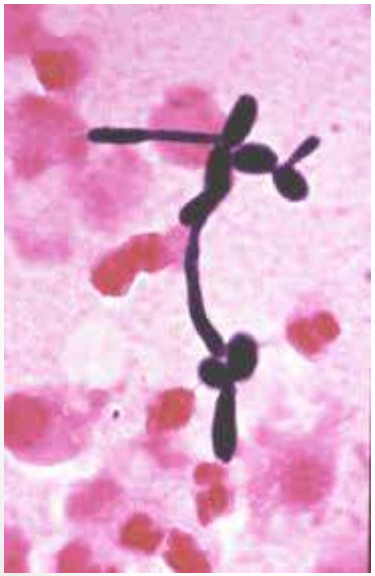
### KOH mount



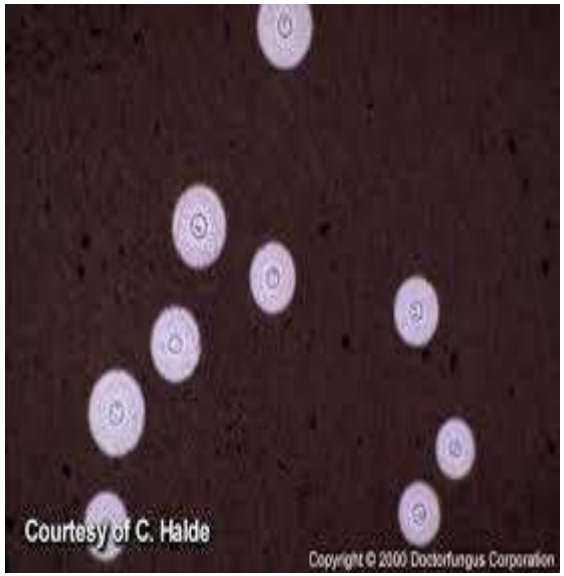
# Cont…

- Microscopic examination
- Staining

Gram



India ink




CFW

# Cont...

| Medium | Fungus |
|---|---|
| **Sabouraud's dextrose agar (SDA)** | Universal medium for fungus |
| SDA with **Chloramphenicol and Gentamicin(inhibits bacterial growth).** | |
| **Brain heart infusion agar (BHI)** | Dimorphic fungi |
| **Potato-dextrose agar (PDA), Corn-meal agar** | **Induce sporulation** |
| Dermatophyte test medium | Dermatophytes |
| **Caffeic Acid Agar , Birdseed Agar** | ***Cryptococcus spp.*** |

# Fungal Culture

- **Temperature** – Room temp.(25-30℃),
  37 ℃ for dimorphic fungi

- Aerobic environment

- **Time**- Yeast grows in overnight incubation
  Saprophytes in some days.
  Pathogenic fungi may require 2-3 weeks

- Growth is generally kept for 4 weeks.

# Fungal Identification

1.Macroscopic examination

2.Microscopic examination

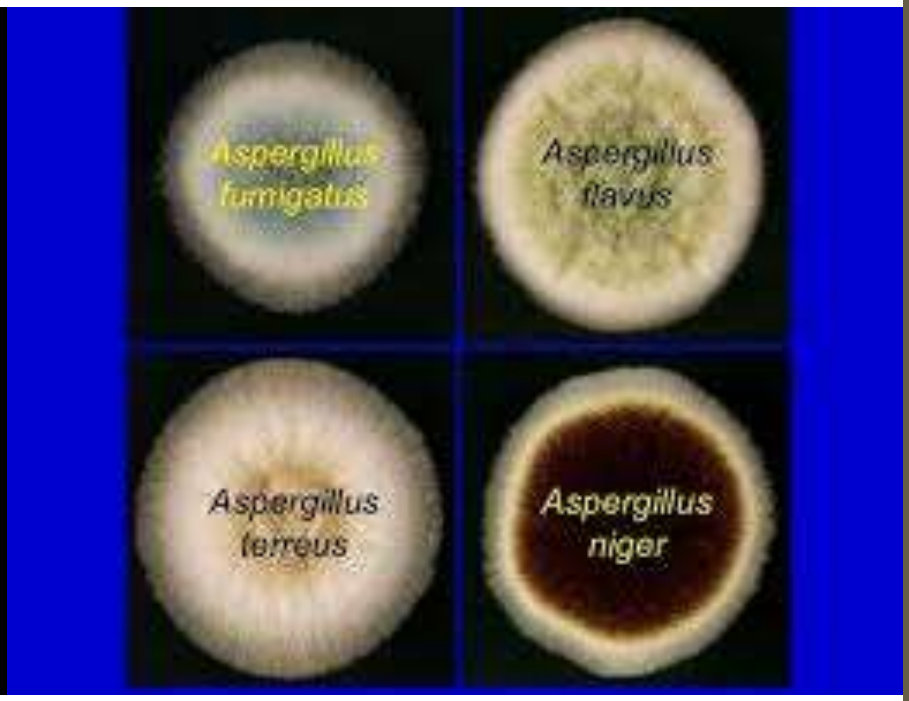
Identification tests-

**A. Yeast-** Germ tube, Sugar assimilation and sugar fermentation

**B. Mycelial fungi-** LPCB mount, Slide culture

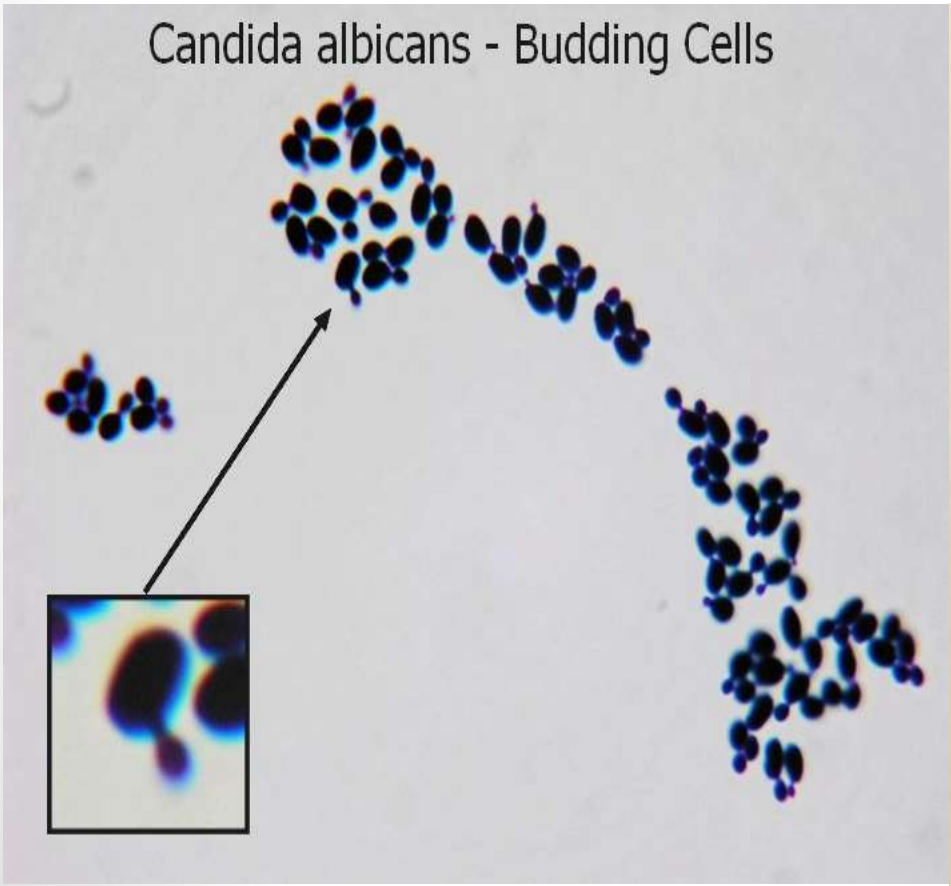
# Cont...

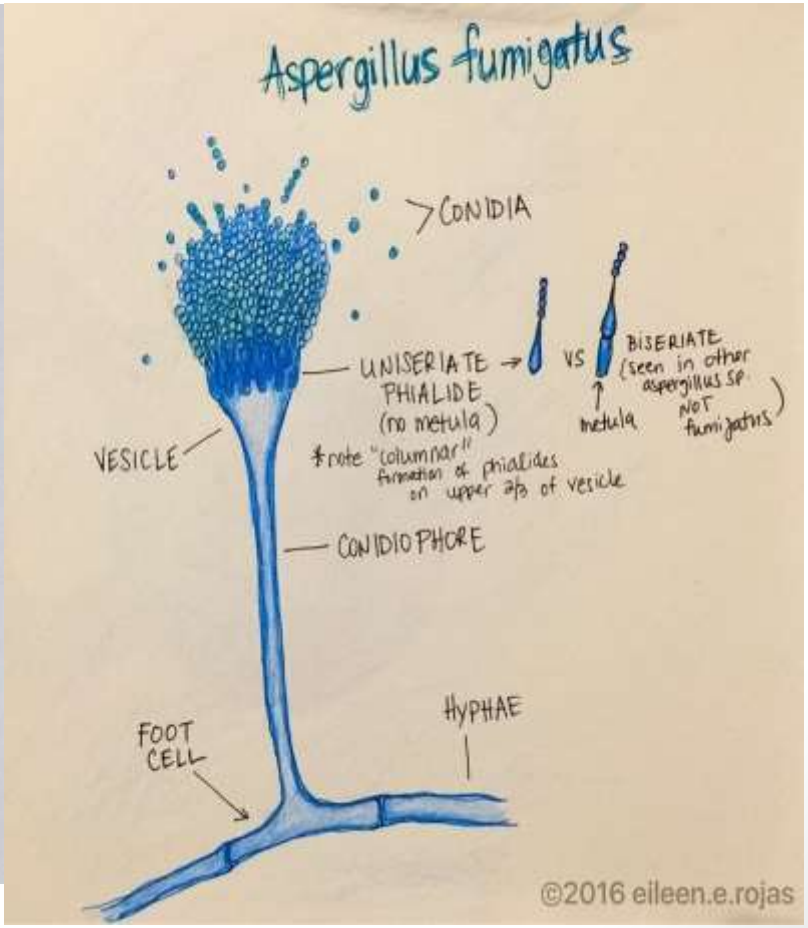
- **Macroscopic examination :**



# Cont…

- Microscopic examination:

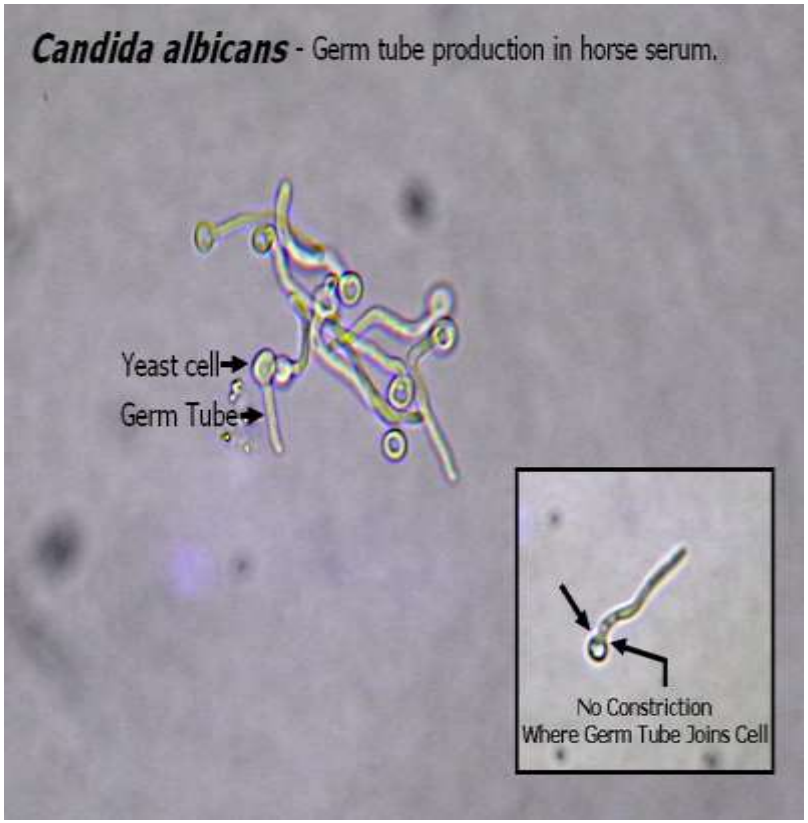


# Cont...

- Microscopic examination:

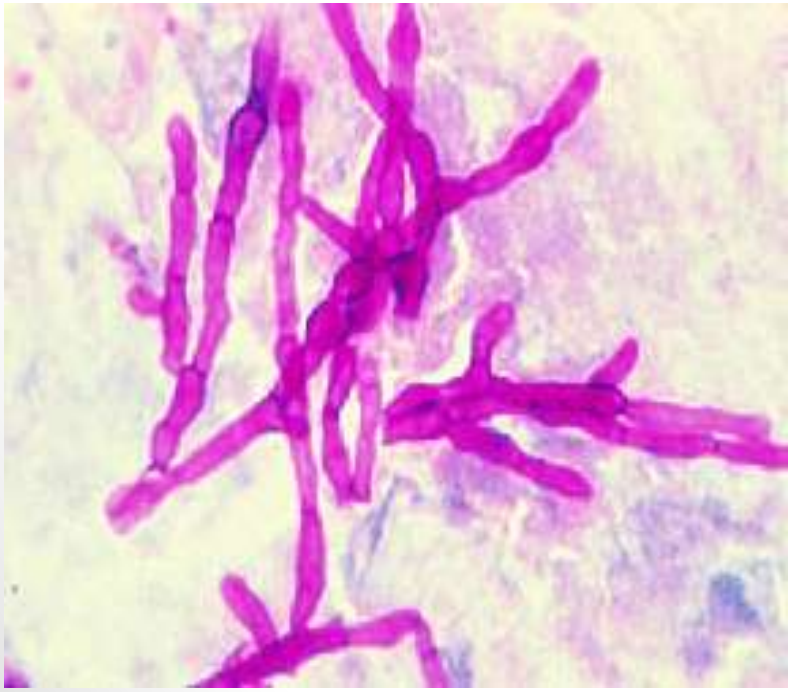
**Germ tube test**



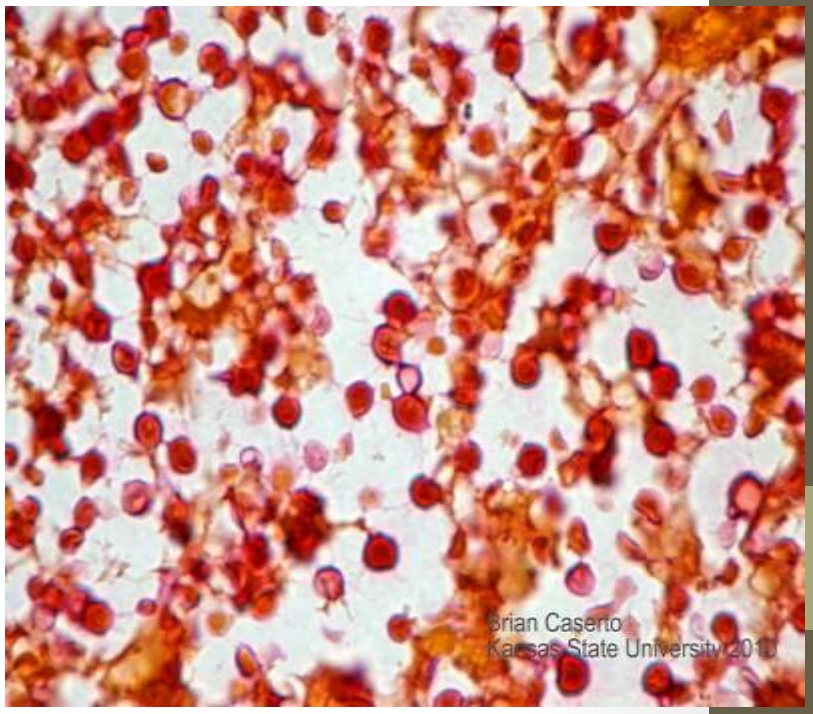
# Cont...

- Microscopic examination:

**PAS**



**Mayer Mucicarmine**



# MYCOBACTERIOLOGY

# ZIEHL-NEELSEN STAIN(ACID FAST STAIN)

- **PRINCIPLE-**

Acid – fast bacilli are difficult to stain because of the lipid content of the cell wall.

The exact nature of this unique staining reaction is not completely understood, but it is believed that the phenol dissolves the lipid sufficiently to allow penetration of the primary stain.

The cell wall retains the primary stain even after exposure to the decolorizing agent, acid-alcohol.

This resistance to decolorizing by acid-alcohol is required for an organism to be termed acid fast.

A counterstain is employed to highlight the stained organisms for the microscopist's recognition

# Cont…

- **PROCEDURE**

Sputum smear

↓

Carbol fuschin (Primary stain)

↓

Intermittent heating( Fixing)

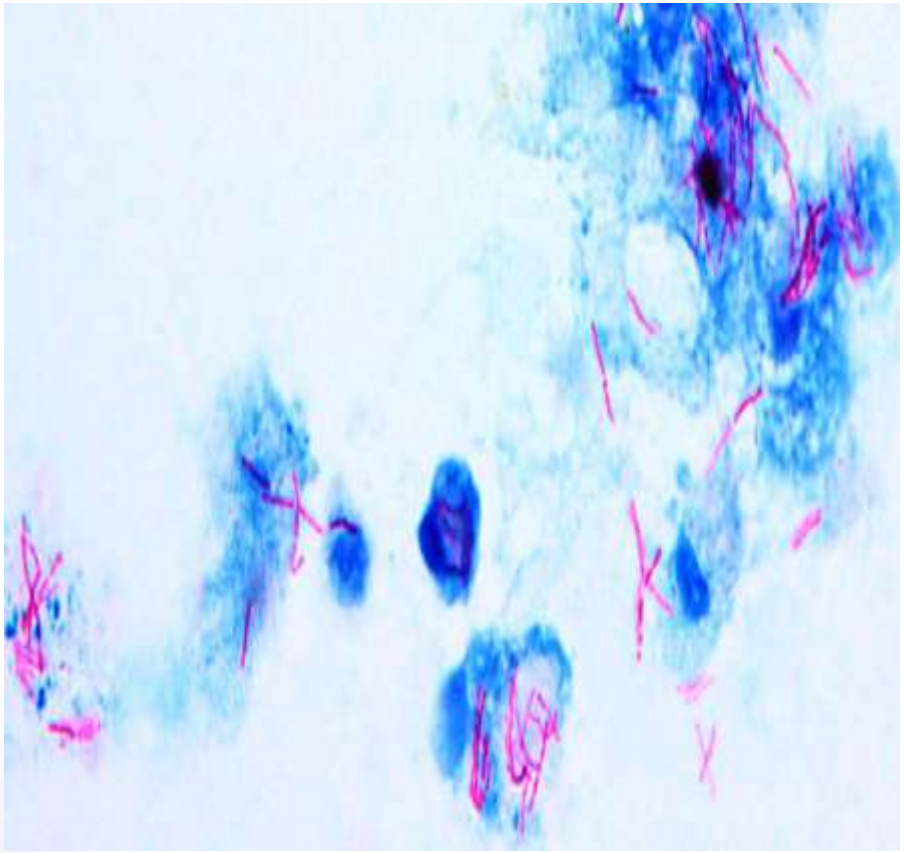
↓

20% $H_2SO4$/ 3% Acid(HCL) alcohol (Decolorizer)

↓

Methylene blue (Counter stain)

# Cont…



Acid fast bacilli

# PARASITOLOGICAL EXAMINATION

- **Diagnostic methods-**

**1.Clinical**

**2.Laboratory-** For confirmation of clinical suspicion.

- For detection of suspected Infection.

# Cont...

- Stool Examination-

1. Macroscopic examination

2. Microscopic examination

A. Wet mount

B. Iodine mount

C. Concentration techniques-
   Floatation method
   Sedimentation method


Thank
You