# IDENTIFICATION OF BACTERIA

# INTRODUCTION

- Identification includes the following

- Morphology depends on:
- Strain, culture medium, temp&time of incubation, age of culture, no of sub cultures.

# Staining Reactions

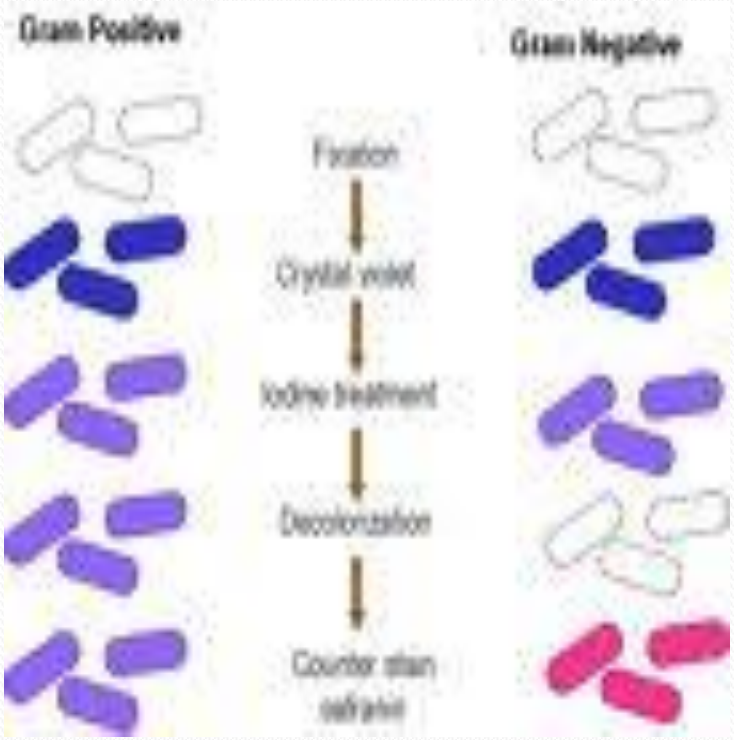
Simple stain: Morphology

Diffential stain: Gram's stain – cell wall

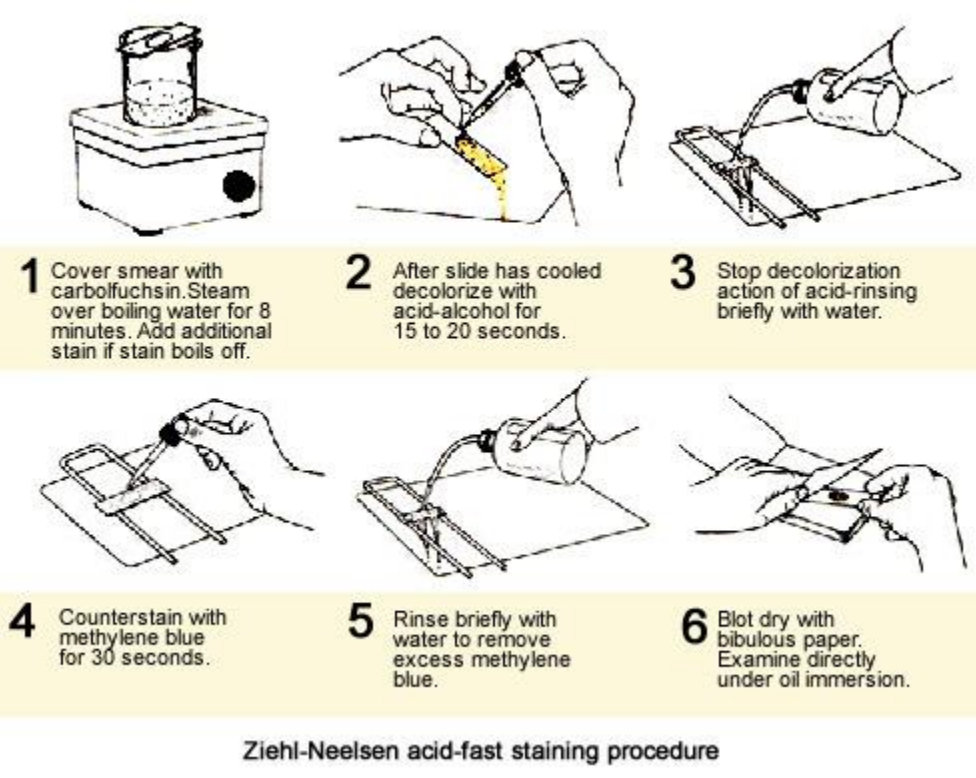
Ziehl-Neeisen- acid fastness

Fluorescent stain- surface Ag

# GRAM STAIN


Gram Positive
Gram Negative
Fixation
Crystal violet
Iodine treatment
Decolorization
Counter stain
safranin

# ZIEHL NEELSEN




Ziehl-Neelsen acid-fast staining procedure

The shape (morphology) of bacterial colonies on an agar plate is a valuable aid to identifying the bacteria.

Whole colony:

Punctiform

Circular

Rhizoid

Irregular

Filamentous

Edge:

Entire

Undulate

Lobate

Filamentous

Curled

Surface:

Smooth, glistening

Rough

Wrinkled

Dry, powdery

Elevation:

Flat

Raised

Convex

Pulvinate

Umbonate

# RESISTANCE

Resistance to:

- Heat
- disinfectants
- antibiotics
- bacteriocin
- chemo therapeutic agents

# METABOLISM

Need of oxygen ,carbondi oxide.

Pigment  production

Haemolysis

# BIO CHEM REACTIONS

Sugar media shows a change of color to yellow

Gas production detected by Durham's tube

# BIOCHEMICAL REACTIONS

SUGAR FERMENTATION TEST

# LITMUS MILK TEST

Acid/Alkali/No change.

Clotting of milk and disruption by gas-Stormy fermentation

# LITMUS MILK TEST

# INDOLE PRODUCT ION

Indole is produced from tryptophan &when 0.5% of Kovac's reagent is added a pink/red indicates a positive test.

# INDOLE PRODUCTION

I

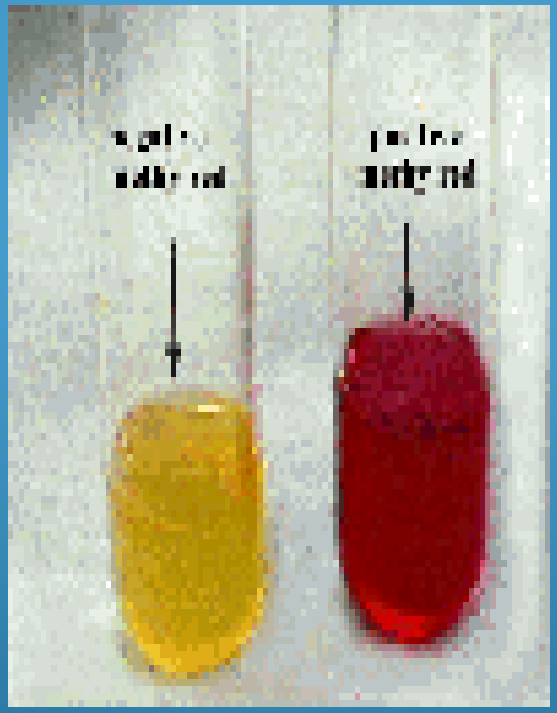
# METHYL RED TEST

Detects acid production and indicates Ph <4.5.

Red is positive.

Yellow is negative.

# METHYL RED TEST



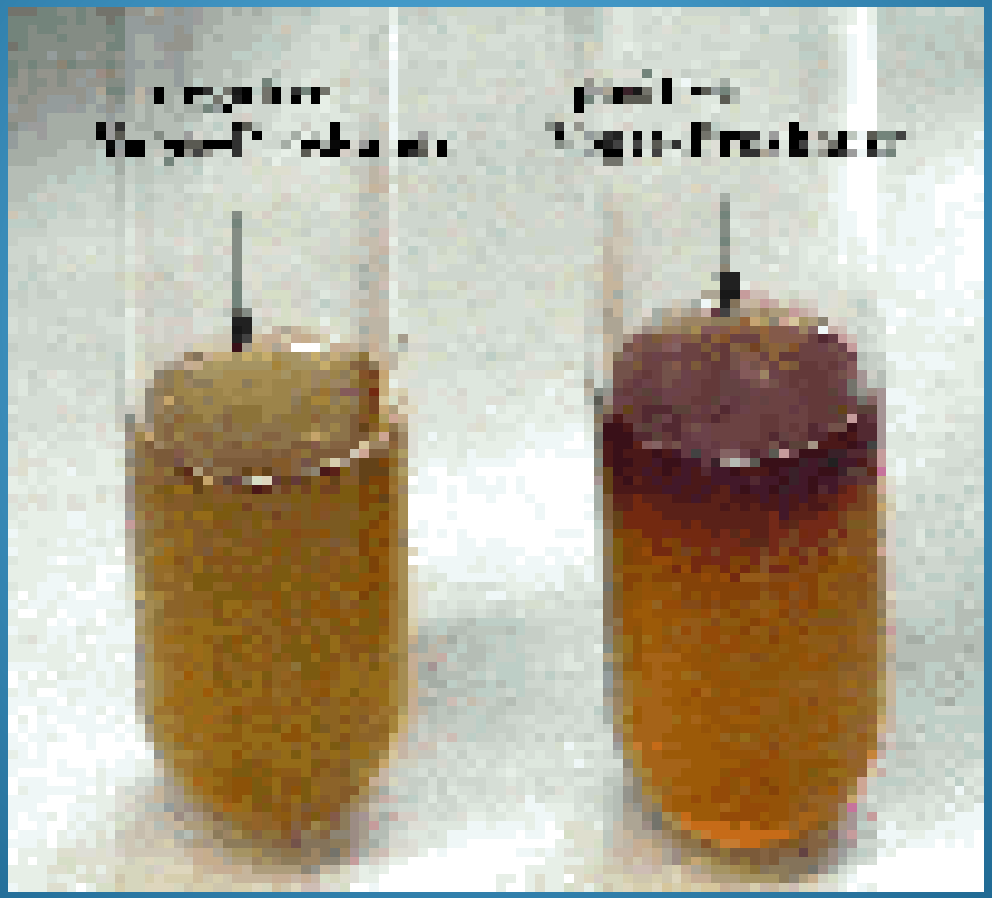
# VOGES-PROSKAUER

Detects production of acetyl methylcarbinol from pyruvate.

Pink color is positive.

Colorless is negative.

# VOGES –PROSKAUER TEST



# CITRATE UTILISATION

Citrate as the sole source of carbon.

Blue is positive..

Green is negative.

# CITRATE UTILISATION

# NITRATE REDUCTION

After 5 days of incubation in 1% pot nitrate.

Red color  positive.

No change  negative.

Detects the enzyme nitrate reductase.

# NITRATE REDUCTION

# AMMONIA PRODUCTION

Add NESSLER'S reagent to 5 days old culture .

Brown color is  positive.

Faint yellow color is  negative.

# UREASE TEST

Christensen's medium  is used.

Urease producing bacteria brings color change.

Purple pink color is positive.

No change is negative.

# UREASE TEST

# HYDROGEN SULPHIDE

$H_2S$ is produced from sulphur containing amino acids.

In the presence of lead acetate/ferric ammonium citrate/ferrous citrate black color is produced.

Black color is positive.

No change is negative.

# HYDROGEN SULPHIDE

# CATALASE PRODUCTION

Expose colonies to hydrogen peroxide.

Brisk effervescence is positive.

No change is negative.

# CATALASE PRODUCTION

# OXIDASE PRODUCTION

Detects the enzyme cytochrome oxidase.

1-1.5% tetramethyl p phylene diamine Hcl when poured on colonies.

Purple color is positive.

No change is negative.

# OXIDASE PRODUCTION

# METHYLENE BLUE TEST

One drop of 1% methylene blue is added to culture & incubate .

Complete decolorisation  is positive.

# METHYLENE BLUE TEST

# EGG YOLK REACTION

Organisms producing lecithinase  when grown in egg yolk medium form colonies surrounded by a zone of clearence.

# EGG YOLK REACTION

# GROWTH IN KCN

To detect growth of aero tolerant enteric bacilli

# COMPOSITE MEDIA

Single medium exhibits many properties.

TSI indicates utilization of sugars{glucose, sucrose, lactose},peptones, gas production.

# COMPOSITE MEDIA

TSI

# ANTIGENIC STRUCTURE

Using specific sera organisms can be identified based on serological reactions.

# BACTERIOPHAGE TYPING

Done to enable intra species typing.

# PATHOGENECITY

Inoculating test strains in lab animals to determine pathogenecity.

Currently in –vitro tests are available.

# RAPID IDENTIFICATION METHODS

Automated methods-API/BACTEC/ VITEK.

PCR/PFGE/RFLP.

Toxin production /anaerobic identification by HPLC.

Gene sequencing.