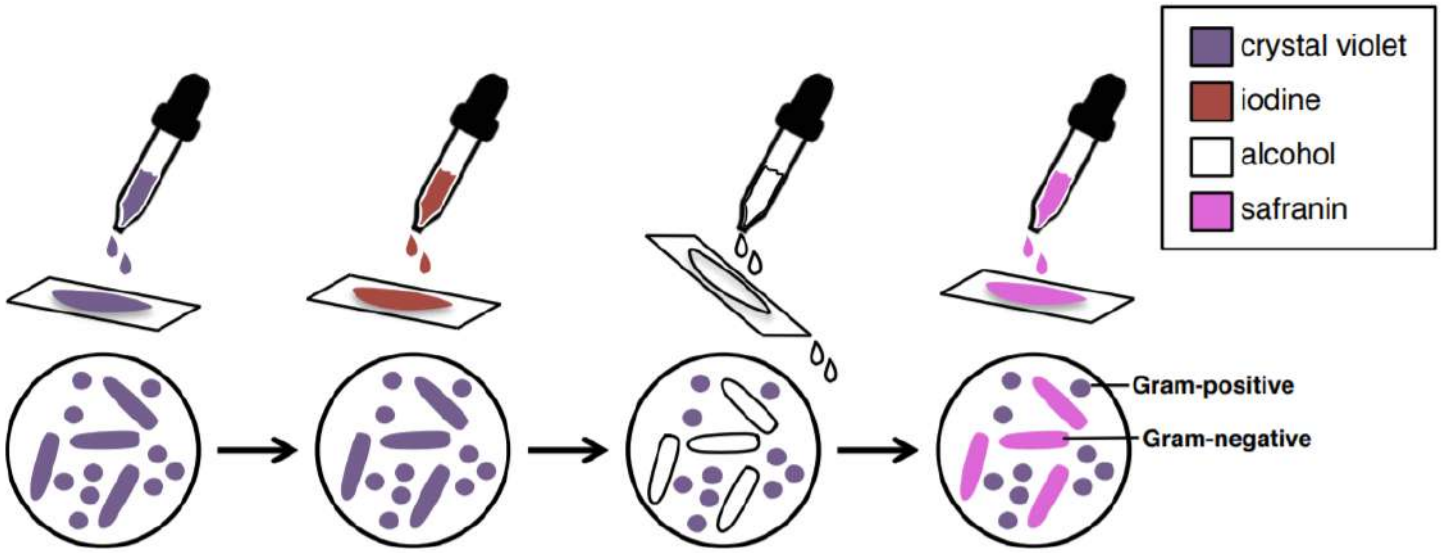
GRAM STAINING

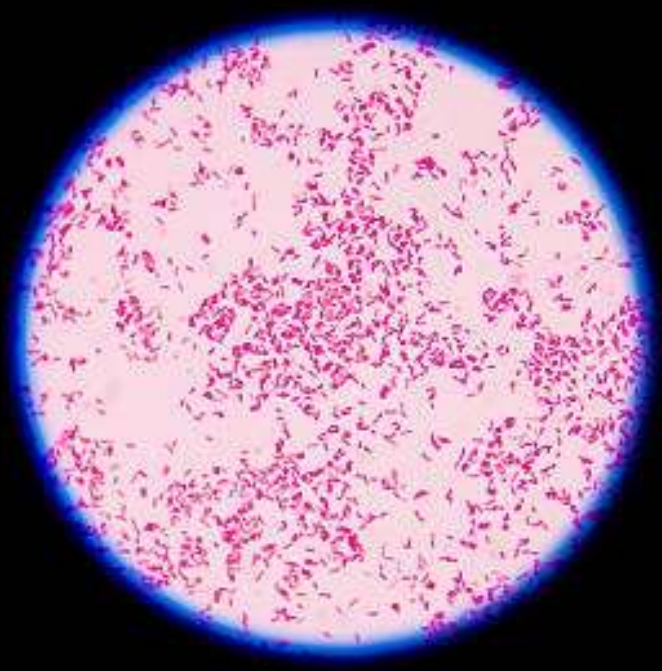
~ fixation : smear made on slide- air dried & then heat fixed.
~ keep fixed slide on staining rack
Step 1: cover the smear with methyl violet for 1 min
~ pour off excess stain with water.

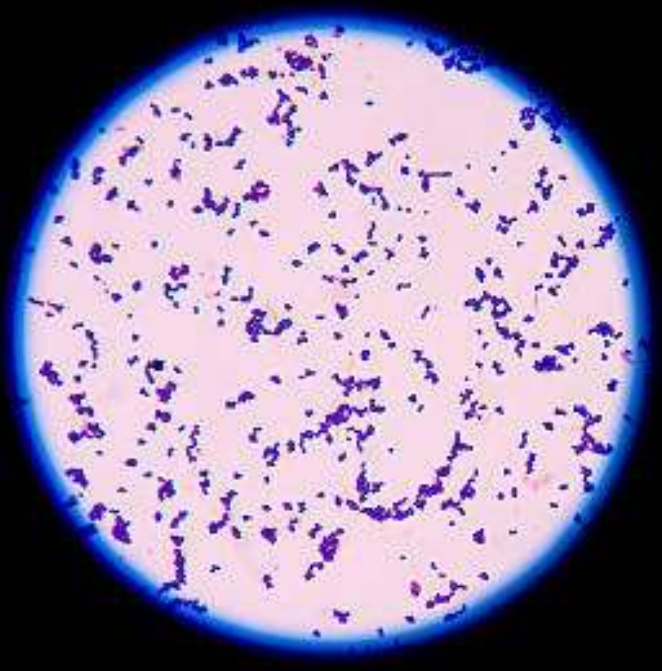
Step 2: cover smear with Gram's iodine & keep for 1 min.
~ wash smear with water.

Step 3: 2-3 drops of acetone & immediately wash off with water.

Step 4: add dilute carbol fuchsin for 30 sec
~ wash off
~blot dry /air dry
~examine under oil immersion objective.

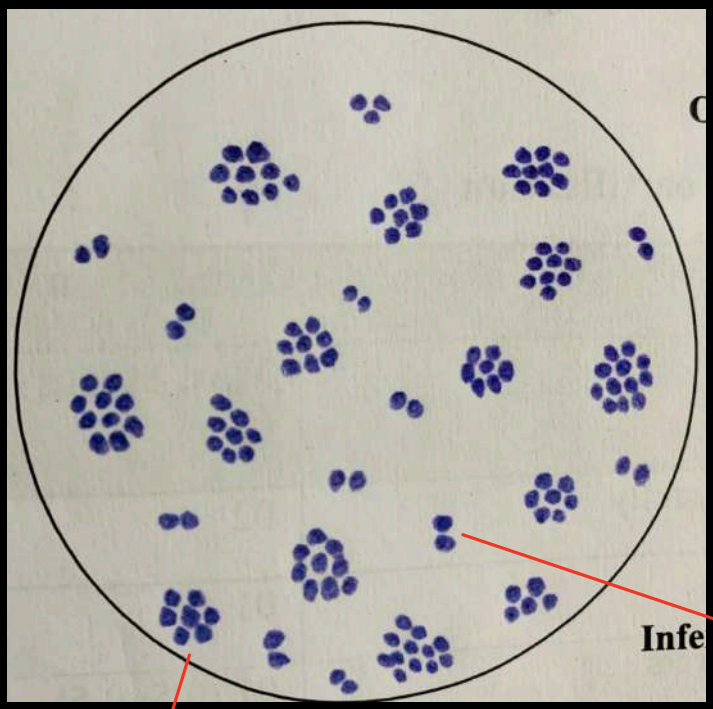






Gram -ve

Gram +ve



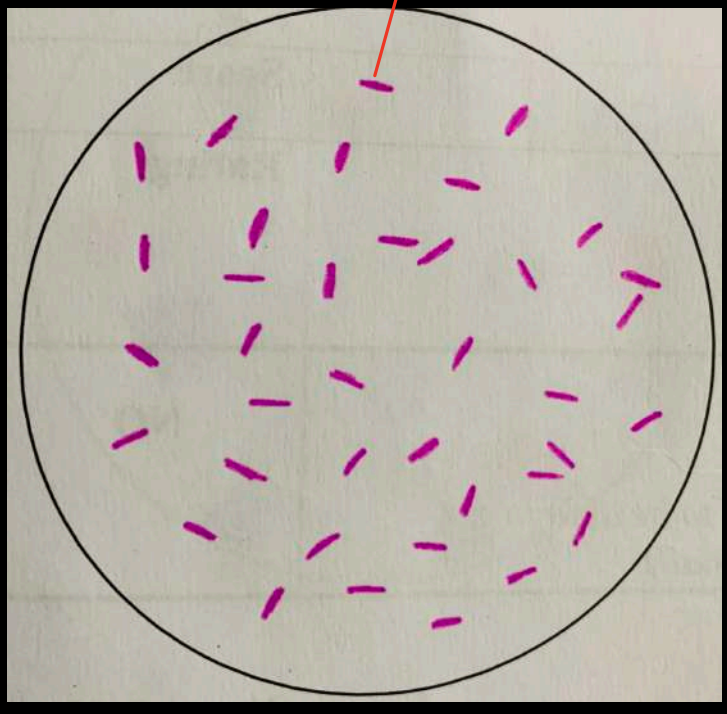
Observation:
- Colour: purple
- Shape: spherical
- Arrangement: pairs & clusters

Inference:
Given smear contains gram positive cocci probably staphylococcus.

gram positive cocci in pairs

gram positive cocci in clusters

Gram negative bacilli

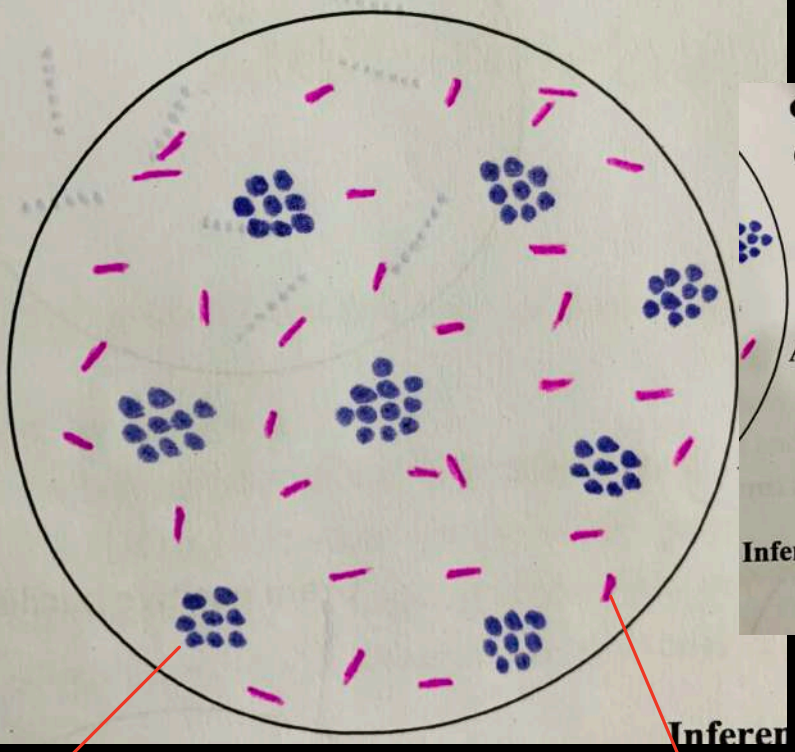


Observation:
- Colour: pink
- Shape: rod shaped
- Arrangement: scattered

Inference:
Given smear contains Gram negative bacilli.

Specimen: Swab from diabetic ulcer

1.



Observation

Colour- organism - I → violete
organism - II → pink

Shape- organism - I → Spherical
organism - II → rod shaped.

Arrangement- organism - I → cluster
organism - II → scattered.

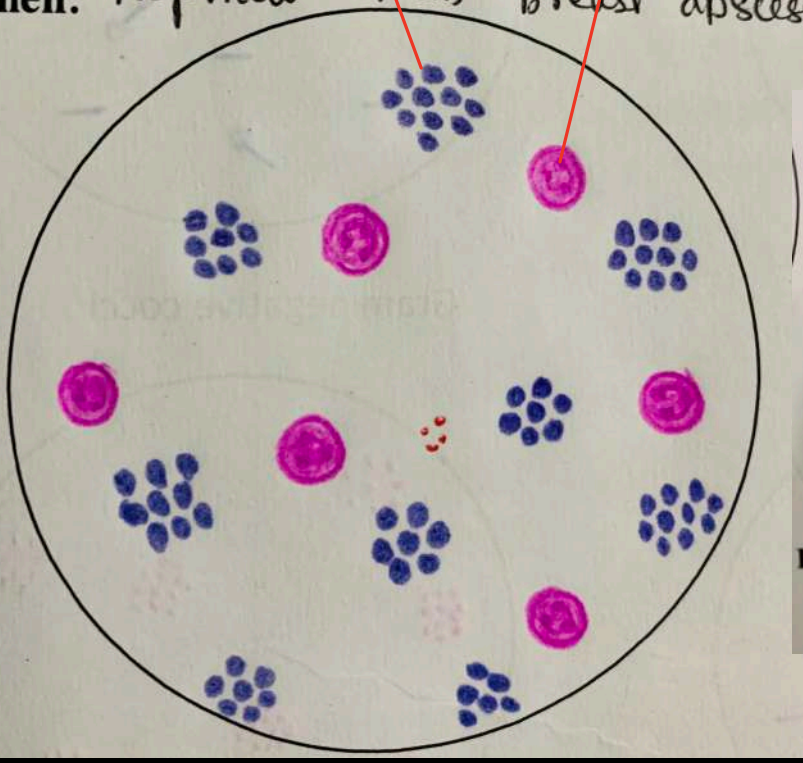
Inference Given smear contain a mixture of Gram positive cocci & Gram negative bacilli

gram positive cocci in clusters

gram negative bacilli

Pus cells

cimen: Aspirate from breast abscess.



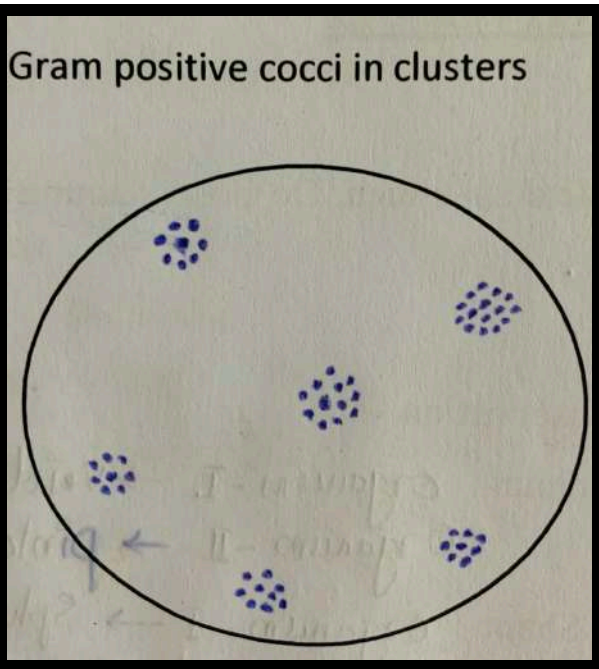
Observation

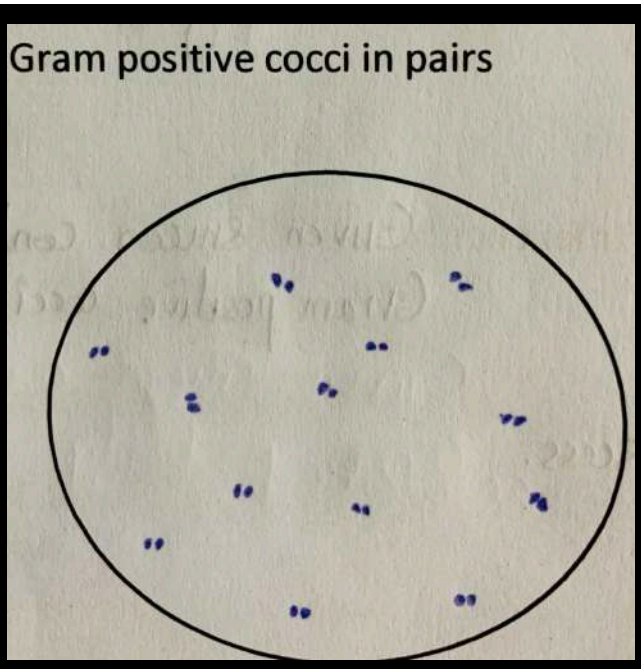
Colour- violet

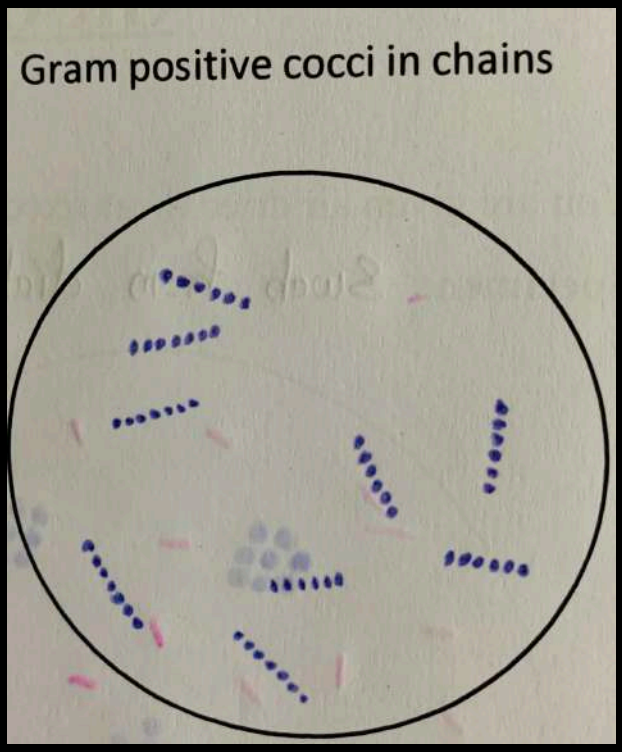
Shape- Spherical

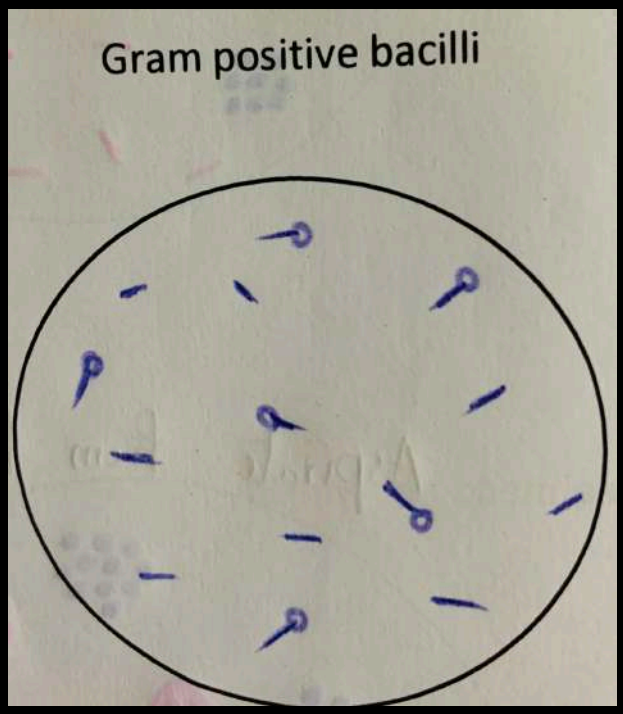
Arrangement- Clusters.

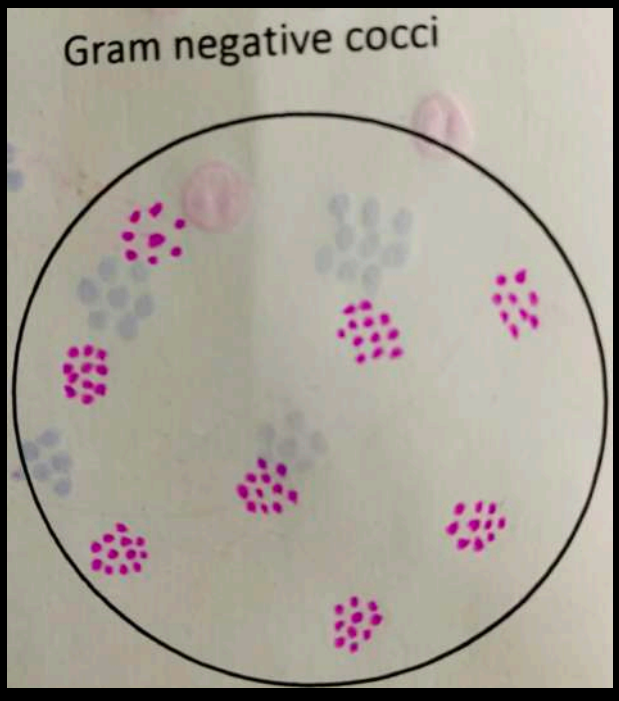
Inference The given smear contains Gram positive cocci ~~and~~ with pus cells

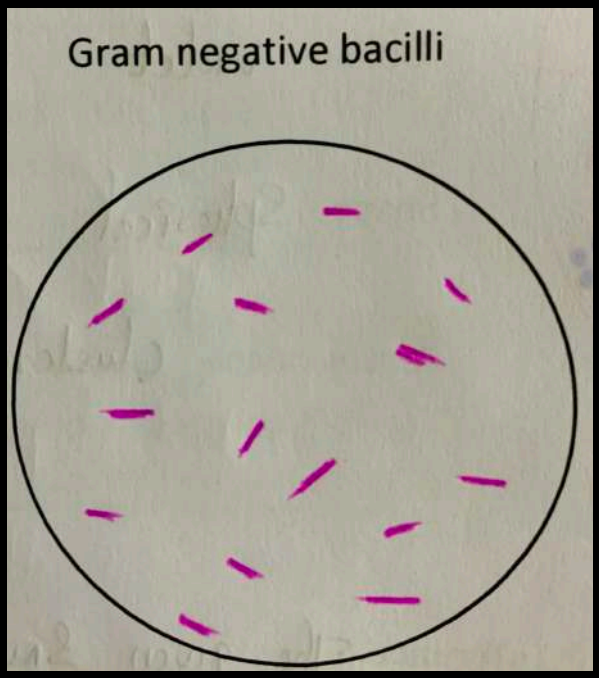

Gram positive cocci in clusters


Gram positive cocci in pairs


Gram positive cocci in chains


Gram positive bacilli


Gram negative cocci


Gram negative bacilli

- It is a differential staining method
- Other eg: acid fast staining, Albert staining.
- Stains used:
  1) primary stain - methyl violet
  2) counter stain - dilute carbol fuchsin / safranin
- Mordant - Gram's iodine-it bind to dye & form bigger dye- iodine complex.
- Decoloriser - acetone -it remove primary stain from gram -ve bacteria.
- Counter stain - give pink colour to gram -ve bacteria.
- Priciple : 2 theories

1)pH theory : cytoplasm of gram +ve bacteria is more acidic, hence retain the basic dye.

2) cell wall theory: gram +ve bacteria have thick peptidoglycan layer & strong cross linkages, hence prevent wash off of primary stain.

But gram -ve bacteria have thin peptidoglycan layer, hence dye easily wash off.

- Crucial step in gram staining:

~decolourization

~if there is over decolourization- gram +ve bacteria loss primary stain.

~ if there is under decolourization- gram -ve bacteria retain the primary stain.

Bacteria	Example
Gram-positive cocci arranged in	
Cluster	*Staphylococcus*
Chain	*Streptococcus*
Pairs, lanceolate shaped	Pneumococcus
Pair or in short chain, spectacle shaped	*Enterococcus*
Tetrads	*Micrococcus*
Octate	*Sarcina*
Gram-negative cocci arranged in	
Pairs, lens shaped	Meningococcus
Pairs, kidney shaped	Gonococcus
Gram-positive bacilli arranged in	
Chain (bamboo stick appearance)	*Bacillus anthracis*
Chinese letter or cuneiform pattern	*Corynebacterium diphtheriae*
Palisade pattern	Diphtheroids
Branched and filamentous form	*Actinomyces* and *Nocardia*
Gram-negative bacilli arranged in	
Pleomorphic (various shapes)	*Haemophilus, Proteus*
Thumb print appearance	*Bordetella pertussis*
Comma shaped (fish in stream appearance)	*Vibrio cholerae*
Curved	*Campylobacter* and *Helicobacter*

List the differences between Gram positive and Gram negative cell walls

Gram positive cell walls	Gram negative cell walls
Have thick peptidoglycan layer	Have thin peptidoglycan layer
Teichoic acid is present	Teichoic acid is absent
Lipopolysaccharide absent	Lipopolysaccharide present.
Variety of amino acids are few	Variety of amino acid are abundant
Aromatic amino acid absent	Aromatic amino acid present.

Uses of Gram Stain

- **To differentiate bacteria into gram-positive and gram-negative:** It is the first step towards identification of bacteria
- **For identification:** Gram staining from bacterial culture gives an idea to put the corresponding biochemical tests for further identification of bacteria
- **To start empirical treatment:** Gram stain from the specimen gives a preliminary clue about the bacteria present (based on the shape and Gram staining property of the bacteria) so that the empirical treatment with broad-spectrum antibiotics can be started early before the culture report is available
- **For fastidious organisms**, such as *Haemophilus* which takes time to grow in culture; Gram stain helps in early presumptive identification

- **Anaerobic organisms**, such as *Clostridium* do not grow in routine culture. Therefore, organisms detected in Gram stain, but aerobic culture-negative gives a preliminary clue to perform an anaerobic culture of the specimen
- **Yeasts:** In addition to stain the bacteria, Gram stain is useful for staining certain fungi such as *Candida* and *Cryptococcus* (appear gram-positive)
- **Quality of specimen:** Gram stain helps in screening the quality of the **sputum specimen** before processing it for culture. Presence of more pus cells and less epithelial cells indicates good quality specimen.

Table 3.2.4: Types of motility shown by different bacteria.

Types of motility	Bacteria
Tumbling motility	*Listeria*
Gliding motility	*Mycoplasma*
Stately motility	*Clostridium*
Darting motility	*Vibrio cholerae, Campylobacter*
Swarming on agar plate	*Proteus, Clostridium tetani*
Corkscrew, lashing, flexion extension motility	Spirochete

Common staining techniques:

~ simple staining:
methylene blue, basic fuschin

~ negative staining:
India ink, nigrosin - demonstration of capsule

~ impregnation method:
Fontana, Levaditi- silver impregnation for spirochete

~ differential stain:
Gram stain, Albert stain , acid fast stain

Modifications of Gram Staining

There are a few minor modifications of Gram stain which vary slightly from the method described earlier.

- **Kopeloff and Beerman's modification:** Primary stain and counter stain used are methyl violet and basic fuchsin respectively
- **Jensen's modification:** This method involves use of absolute alcohol as decolorizer and neutral red as counter stain. It is useful for meningococci and gonococci
- **Brown and Brenn modification:** This is used for Actinomycetes.