# Bacterial Staining

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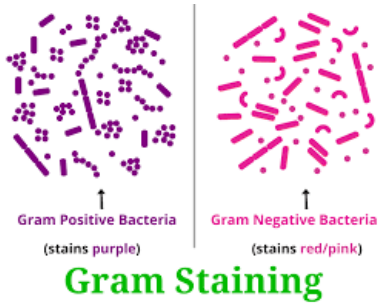


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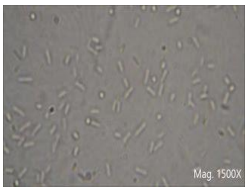
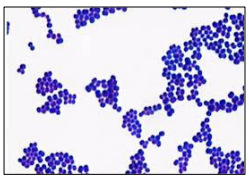
## Bacterial Staining

- Most types of bacterial cells are difficult to see under the light microscope unless they are stained.




- This information can help in classify microorganisms, and also can be used to diagnose the cause of a bacterial infection.



## Preparation of bacterial smear

Bacterial smears must be prepared prior to performing any of the staining techniques.

This include the following:

**1. Preparation of the glass microscopic slides**

Clean slides by 95% alcohol to remove grease on slides.

**2. Labelling of slides**

The initials of the organism can be written on either end of the slide.

**3. Smear preparation**

Avoid thick smears due to heavy inoculum of the culture used.

**4. Heat fixation**

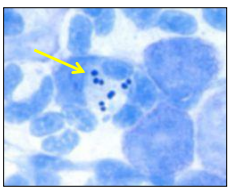
Heat fixation allows the bacteria to adhere on the slide by the rapid passage of the air-dried smear **2 or 3 times** over the flame of the Bunsen burner..



## Types of stains
## 1- Simple stain

**Methylene Blue Stain**

It consists of one type of stain reagent. After staining, all types of bacteria appear to have one color (Blue) when examied under the microscope e.g.



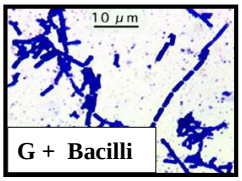
Simple stains can be used to determine

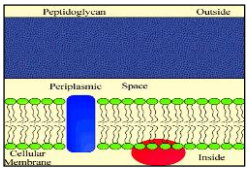
1. Bacterial species morphology.
2. Arrangement.
3. *Pasteurella* spp. or *Mannheimia haemolytica* (Bipolar staining).

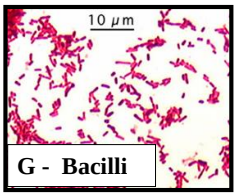
# 2- Differential stains

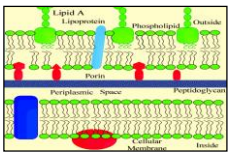
## Gram stain

Generally bacteria can be divided into **Gram positive (+) bacteria** and **Gram negative (-) bacteria** based on difference in chemical composition of bacterial cell walls. Gram positive cells stain purple/violet, while Gram-negative cells stain pink.






# Gram stain Solutions

**1. Primary stain:** It gives a color to all cells e.g. crystal violate (purple).

**2. Mordant:** Used to intensify the color of the primary stain e.g. iodine.

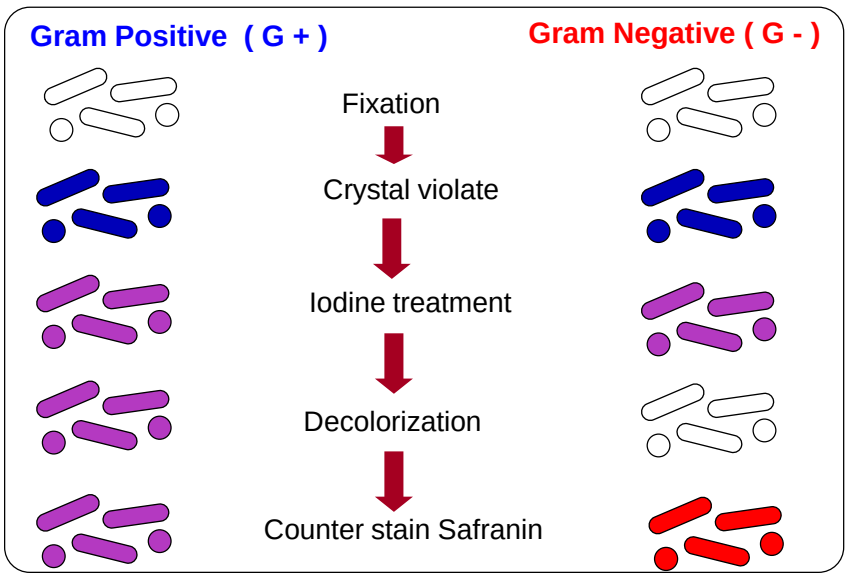
**3. Decolorizing agent:** e.g. 95% ethanol alcohol. The decolorizing agent may or may not remove the primary stain from the bacterial cell based on the chemical composition of the bacteria.

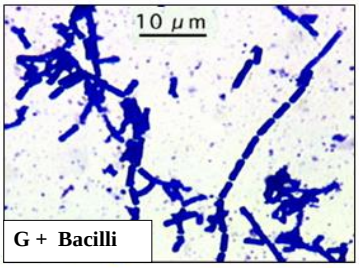
**4. Counterstain:** It has a contrasting color (different color to that of the primary stain) e.g. safranin (red).

## Solution of Gram's stain

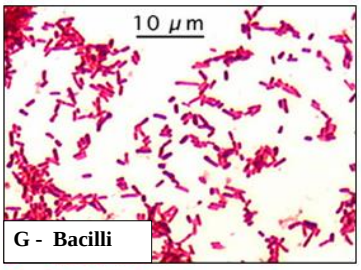



## Steps of Gram's stain

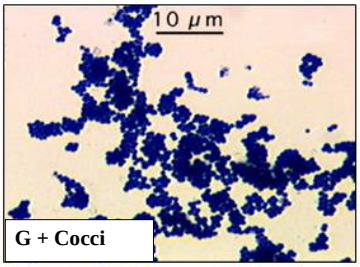




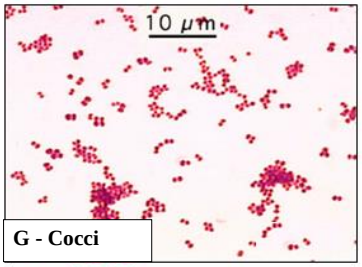

G + Bacilli




G - Bacilli



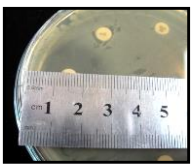

G + Cocci




G - Cocci



# Antibiotic Sensitivity

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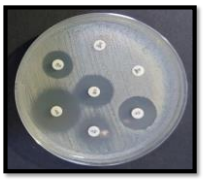

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# Antibiotic Sensitivity

- **Chemotherapeutic** are chemicals that have been artificially synthesized in the laboratory with antimicrobial activity that can inhibit the growth of bacteria such as the sulphonamides, quinolones, and isoniazid.

- **Antibiotics** are chemicals produced by some bacteria e,g. *Streptomyces* and fungi e.g. *Penicillium* with antimicrobial activity that can inhibit the growth of bacteria.

- **Narrow spectrum antibiotics**
  Antibiotics that are only effective against Gram positive or only effective against Gram-negative bacteria have a narrow spectrum of activity.

- **Broad spectrum antibiotics**
  Antibiotics that are effective against many different types of bacteria are called broad spectrum antibiotics.



# Disc diffusion method (Kirby Bauer method) (qualitative method)

This method is commonly used for determination of susceptibility using disc diffusion test.

**Kirby Bauer method procedure**

- Prepare a bacterial suspension in Muller Hinton broth for 3-4 hours until its turbidity reaches 0.5 Mc Farland Standard which is equivalent to $10^5$ CFU/ml.

- Using a cotton swab culture the Mueller Hinton agar at least 3 time and rotating the plate 60° after each streaking. Leave the plate for 5 min. to absorb.

- Using sterile forceps distribute the discs on the agar surface, then let Petri dish stand for 15 min.
- Incubate the plates for 16 to 18 hours at 37°C. (Do not invert the plates).

- Measure the zone of inhibition diameter in mm using a ruler and interpret the results as resistance ( R ) , sensitive ( S ) , intermediate (M) according to the disc manual.

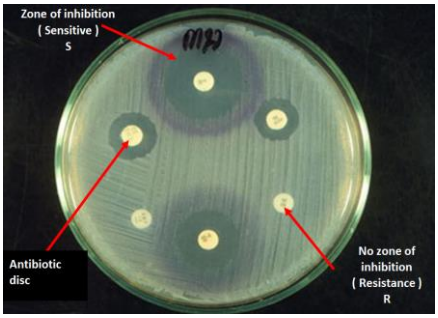
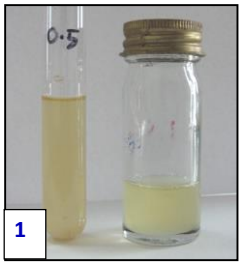



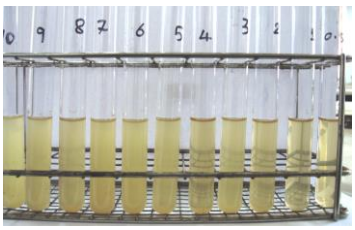

**Figure 11.2 Disc diffusion method, determination the zone of inhibition**

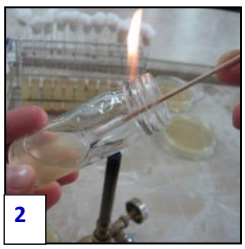


## Procedure

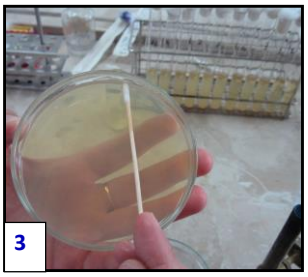
- Prepare saturated cotton swab with bacterial culture broth prepared by comparing with McFarland turbidity standard (0.5McFarland tubes).





- Inoculate the surface of the plate by streaking the swab over the surface of the plate.
- Repeat this procedure two more times, rotating the plate 60 degrees each time.
- Let petri dish stand for 15 min to absorbed the inoculum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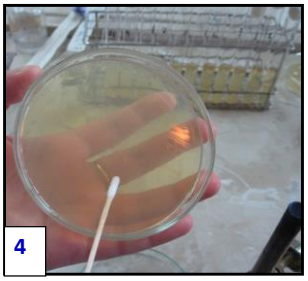
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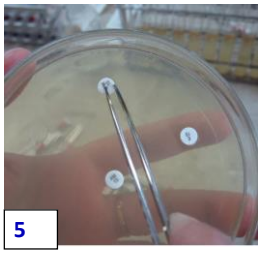
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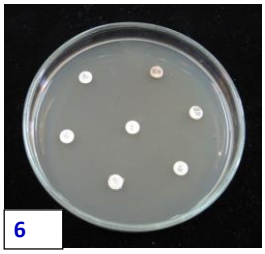
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- Sterile forceps (alcohol and flaming) was used to put uni disc or multi disc on agar surface.
- By using a forceps, press on disc to be sure that it is fixed on the agar surface properly and let the petri dish stand for 15 min.
- Incubate petri dish at 37 °C for 18 h.

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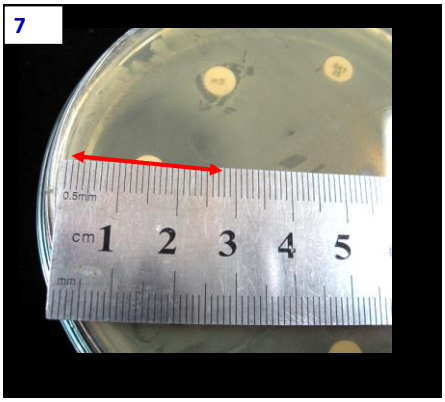
## Determination the zone of inhibition




Determine the zone of inhibition according the diameter.

**26 mm** = **Inhibition zone**

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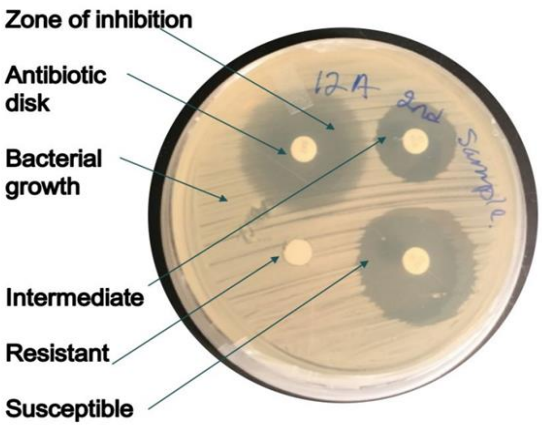
## Disc diffusion method – result explanation

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| Name of antibiotics (dose) | Inhibitory zone diameter to nearest millimeter (mm) | | |
|---|---|---|---|
| | Sensitive (S) | Moderately sensitive (MS) | Resistant (R) |
| Amoxicillin (30 μg/disk) | ≥18 | 14–17 | ≤13 |
| Cloxacillin (5 μg/disk) | ≥25 | 22–24 | ≤21 |
| Cephalothin (30 μg/disk) | ≥18 | 15–17 | ≤14 |
| Cephradine (25 μg/disk) | ≥18 | 13–17 | ≤12 |
| Cefuroxime (30 μg/disk) | ≥23 | 15–22 | ≤14 |
| Cefixime (5 μg/disk) | ≥19 | 16–18 | ≤15 |
| Kanamycin (30 μg/disk) | ≥18 | 14–17 | ≤13 |
| Streptomycin (10 μg/disk) | ≥15 | 12–14 | ≤11 |
| Neomycin (30 μg/disk) | ≥17 | 13–16 | ≤12 |
| Vancomycin (30 μg/disk) | ≥12 | 10–11 | ≤9 |
| Erythromycin (15 μg/disk) | ≥23 | 14–22 | ≤13 |
| Azithromycin (15 μg/disk) | ≥18 | 14–17 | ≤13 |
| Ciprofloxacin (15 μg/disk) | ≥21 | 16–20 | ≤15 |
| Levofloxacin (5 μg/disk) | ≥17 | 14–16 | ≤13 |
| Tetracycline (30 μg/disk) | ≥15 | 12–14 | ≤11 |
| Doxycycline (30 μg/disk) | ≥14 | 11–13 | ≤10 |
| Cotrimoxazole (25 μg/disk) | ≥16 | 11–15 | ≤10 |
| Chloramphenicol (30 μg/disk) | ≥18 | 13–17 | ≤12 |



# Thank You