

Kirby-Bauer test

Materials:

Chemical

- Antibacterial extract
(0.5% iodine¹, sodium hypochlorite², H₂O₂ and Sodium bicarbonate³), (1.5% *C. longa*, *S. aromaticum*⁴, *A. tinctoria*⁵ and dried lime³) and (10% *M. alternifolia*⁶ essential oil and *C. limon*⁷)
- Agar plate

Equipment and Glassware

Microcentrifuge tube, thick filter paper, sterilized swaps, forceps and beaker with Dettol and water.

Protocol:

- Mark five fifths on plate and label each fifth with the extract used. (**Figure 1**).
- Apply a bacterial inoculum of approximately $1-2 \times 10^8$ CFU/mL to the surface of a large (150 mm diameter) Mueller-Hinton agar plate. *leave the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes.*
- Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry.
- Apply ~1 drop of each extract on each disc.
- Partially remove the lid of the petri dish. Place the disk on the plate and gently press the disk with the forceps to ensure complete contact with the agar surface (**Figure 2**). (*Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location*)
- Plates are inverted and incubated for 16–24 h at 37°C prior to determination of results.
- The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter.

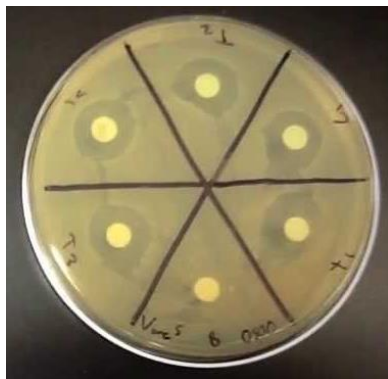


Fig. 1. Plate labelling

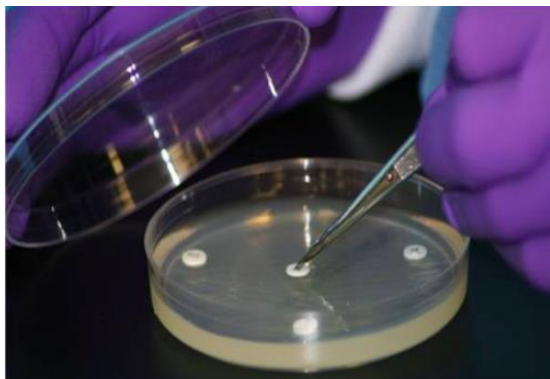


Fig. 2. Disc placement on agar plate

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