

Well diffusion method

Typically, an antibiotic is applied to a well that is cut into the agar. Thus, the antibiotic will tend to move from this region of high concentration to the surrounding regions of lower antibiotic concentration. If more material is present in the well, then the zone of diffusion can be larger.

This diffusion was the basis of the agar diffusion assay devised in 1944. A bacterial suspension is spread onto the surface of the agar. Then, antibiotic is applied to a number of wells in the plate. There can be different concentrations of a single antibiotic or a number of different antibiotics present. Following a time to allow for growth of the **bacteria** then agar is examined. If **bacterial growth** is right up to the antibiotic containing well, then the bacterial strain is deemed to be resistant to the antibiotic.

If there is a clearing around the antibiotic well, then the bacteria have been adversely affected by the antibiotic. The size of the inhibition zone can be measured and related to standards, in order to determine whether the bacterial strain is sensitive to the antibiotic.



Procedure

1. Pour Muller Hinton Agar Medium in plates at depth 4 mm .
2. Spread a broth culture of an isolated bacterium in to an agar plates by spreader or by cotton swab .
3. After drying the plate at 37 °C for 30 minutes , make a well in the plate (several wells when used many concentration of a certain antibiotic or several antibiotic for the same bacteria) by using a sterile cork borer in appropriate diameter ranging from (5-10) mm under aseptic condition ,
4. Fill the well with attested antibiotic .
5. Incubation the plates at 37 °C for 18-24 hr.
6. Measure the inhibition zone around the wells .