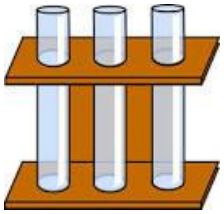
Neo-Rapid CARB Carbapenemase Detection

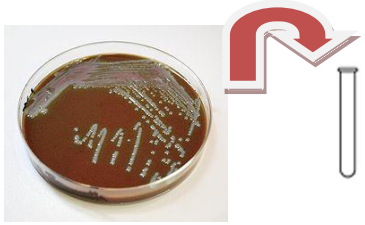
1. Prepare tubes, one tube for each unknown isolate and each control.
2. Pipette **150 µl of lysis buffer** per tube for each unknown/control.



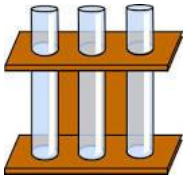
Add 150 µl Lysis buffer

3. Use a **pure culture**, (single colony growth) of each unknown/control And inoculate the corresponding **labeled tube**.

4. Using a sterile loop, **remove a loopful (5-10µl)** of growth and inoculate each labeled tube. Emulsify to **make a suspension, 3-4 McFarland**.



5. Once all tubes are inoculated **vortex each suspension** for 10-15 seconds to ensure an even suspension.



6. Let this suspension set at **Room temperature for 30 minutes**.

7. **Remove 50µl of this suspension** after the 30 minute incubation, and add to a micro-centrifuge tube containing 100µl of sterile saline. **Repeat this** by adding another 50µl of this suspension to a 2nd micro-centrifuge tube. Label each tube with the sample # or control type.

Tube 1 100µl of sterile saline + 50µl of bacterial suspension.

Add positive (imipenem) disc to tube 1.

Place a + on each cap after adding disc

Tube 2 100µl of sterile saline + 50µl of bacterial suspension.

Add negative (no imipenem) disc to tube 2.

Place a Minus (-) on each cap after adding disc.

8. Each unknown and control isolate will be a set/pair of micro-centrifuge tubes, **one with a positive imipenem disc**, **one a negative imipenem disc**.

9. Incubate all tubes and **observe at 30 minute intervals** for up to 2 hours. Once positive, no further incubation needed.

10. **Positive reaction** = yellow color appearance
11. **Negative reaction** = reddish color appearance
12. **Indeterminate reaction** = negative control tube appears yellow or yellow orange color, not reddish color.