**Proposed protocol for ampicillin quantification**

## **1-Preparation of the turbidity calibration 0.5 McFarland**(1)**:**

1. we add 0.5 mL of a 0.048 mol/L solution of BaCl2 (1.175% w/v BaCl2 2H2O) to 99.5 mL of a 0.18 mol/L solution (0.36 N) of H2SO4 (1% v/v) and we shook vigorously
2. We check the density of the suspension using a spectrophotometer with a 1 cm beam and matching cuvettes. The absorbance at 625 nm should be between 0.08 and 0.13
3. We distribute the suspension in tubes of the same size as those used to adjust the inoculum and then we seal the tubes
4. Once sealed, we store these tubes at room temperature and protect from light. Before use, we mix the tube vigorously using a Vortex (6 months’ storage)

**2- The three bacterial strains which can be used:**

E.*coli* / S.*aureus* / S.*pneumonia*

**3- Quantification of the produced ampicillin using the disk diffusion method:**

**Preparation of the inoculum:**

1. We took 3 to 5 colonies of the isolated colonies with a loop, and we added them in 2ml sterile saline (NaCl 0.9%)
2. We Vortex the saline tube to create a smooth suspension.
3. We adjust the turbidity of this suspension to a 0.5 McFarland standard

by adding more organism if the suspension is too light or diluting with

sterile saline if the suspension is too heavy.

1. Use this suspension within 15 minutes of preparation.
2. We inoculate the surface of Mueller Hinton agar plate by streaking the swab 3 times over the entire agar surface, we rotated the plate approximately 60֯ each time to ensure an even distribution of the inoculum
3. We allow the plate to sit at room temperature at least 3 to 5 minutes (but no more than 15 minutes) for the surface of the agar plate to dry before proceeding to the next step

**Preparation of the disks:**

1. We dilute the standard ampicillin 10 times (Concentrations: 1.5; 1.4; 1.3; 1.2; 1.1; 1.0; 0.9; 0.8; 0.7; 0.6) to obtain different concentrations
2. We dip each of the 10 discs in one of the 10 concentrations of ampicillin
3. We dip another one disk in the unknown produced ampicillin
4. We distribute the 11 disks in plates at a distance of (26) mm apart
5. Once all disks are in place, we replaced the lid, inverted the plate and placed it at 37oC for 18 to 24 hours

**Quantification of the produced ampicillin:**

1. After the growth time, we measured the zone of inhibition that had appeared using a ruler
2. We drew a graph showing the concentration of ampicillin as a function of the diameter in order to be able to quantify the produced ampicillin (Log C as a function of diameter)