

## Sterility Test

### Materials:

- LB media, LB plates
- Sterile spreaders
- Positive control: E.coli DH5 $\alpha$ 
  - Inoculate into 2 ml of LB, grow at 37°C shaker overnight
  - Transfer 2 ml to 18 ml LB/15% glycerol ( $10^{-1}$ )
  - Aliquot 100 ul/tube, kept frozen at  $-80^{\circ}\text{C}$

### 1. Inoculation of test samples (Day 1):

- In the hood, label the 14 ml-falcon tubes with sample lot #, do duplicates for each sample. Add 2 ml of LB to each tube. Set up two additional tubes for negative control.
- Add 10 ul of test sample to the corresponding tube.
- Grow at 37°C shaker overnight

### 2. Inoculation of positive control (Day 1)

Do **NOT** bring the positive controls into tissue culture room, do it on a clean lab bench.

- Thaw a positive control tube (DH5 $\alpha$ ,  $10^{-1}$ ) from  $-80^{\circ}\text{C}$
- Add 900 ul of LB to the tube (this is  $10^{-2}$ ), then do 1:10 serial dilution using LB to  $10^{-6}$
- Set up two 14 ml-falcon tubes, label with '-6', add 2ml of LB medium to each tube
- Transfer 10ul from tube  $10^{-6}$  to the corresponding falcon tube
- Grow at 37°C shaker overnight

### 3. Plating (Day 2):

- At a clean bench, turn on the flame, use a sterile spreader to plate 100 ul of each sample on a LB plate

### 4. Incubation

- Incubate the plates at a 37°C incubator for 48 hrs. Check for colony presence. Record the result on the batch record form.

