Sterility Test

Materials:
- LB media, LB plates
- Sterile spreaders
- Positive control: E.coli DH5α
  - 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°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 (DH5α, 10⁻¹) from –80°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.
Sterility Test Batch Record Form  # ______

Incubation in LB medium from ______________ to _______________

Incubation on LB Plates from ______________ to _______________

LB Plates made on ___________ Positive control DH5α (10⁻¹) made on ___________

<table>
<thead>
<tr>
<th>Lot #</th>
<th>Vector Name</th>
<th>Colony #</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative Control</td>
<td></td>
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<tr>
<td></td>
<td>Positive Control 10⁻⁶</td>
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</tbody>
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Assay Date ___________ Performed by ___________

Reviewed by ___________ Date ___________