

### Bacterial Killing by Bactericidal Permeability Increasing Protein (BPI) Assay

1. Grow overnight culture of bacteria (PA14) in 5ml lysogeny broth (LB) at 37°C, shaking, for 16-18 hours
2. Sub-culture bacteria (1:30) in 3ml LB, grow for 3 hours at 37°C, shaking
3. Wash 1ml of subculture in PBS- centrifuge 3 min at 12,000rpm
4. Re-suspend pellet in 1ml LB
5. Measure OD<sub>600</sub> of a 1:10 dilution of bacteria (OD of 1 = 10<sup>9</sup> bacteria)
6. Pre-incubate 10<sup>4</sup> bacteria in 200ul LB with killing agent- BPI or antibiotic (gentamicin)
7. Grow for 1 hour at 37°C, shaking
8. Dilute samples (except antibiotic sample)
9. Plate 20ul of sample on 1.5% LB agar plates
10. Incubate plates overnight at 37°C
11. Count colony forming units (CFU)