



TITLE: INVASION ASSAY MDCK c

Name: Ilan Zemsky 27.7.2020

1. Grow cells in 10 cm plates to 100% confluency (MDCK II). 3 days culture after seeding of 1.5 ml cells $\sim 1.5 \times 10^6$ cells.
2. Grow bacteria overnight in LB static culture (not shaker) at 37°C (3 ml small starter).
3. Prepare 10 ml of plain DMEM/MEM Activation tubes in 37°C in CO₂-incubator a day prior infection.
4. Add bacteria from the overnight culture (at a ratio of 1:50) to warm plain DMEM and incubate for 3 hours at 37 °C in a CO₂ incubator. (WT, escV and LB without bacteria -as control activation medium)
5. Before infection wash the cells x 2 from the pen/strep media with plain DMEM/MEM. For WT infect with 10 ml Activation medium/plate. For escV infect with 5 ml escV- Activation medium+ 5 ml control activation medium.
6. Incubate 2 hrs 37°C in CO₂-incubator.
7. Remove the medium and add instead 10 ml plain DMEM with or without the supplementation of 100 µg/ml Gentamycin, add to each plate (+Genta) 20µl of 50mg/ml Gentamycin. One plate of WT and one plate of escV are not exposed to Gentamycin, so leave them with 10 ml plain DMEM alone.
8. Incubate 90 min 37°C in CO₂-incubator.
9. **A. without Gentamycin-** Wash cells x 4 with ~10ml PBS.
9. **B. With Gentamycin-** Wash cells x 2 with ~10ml PBS.
10. Lyse the cells with 1ml 1% Triton X-100 (10µl) in 10 mM Tris 7.4 (10µl), scrape the cells with Rubber policeman and repeated pipetting up & down for 5 times and transfer to an Eppendorf tube (this 1ml is typically containing few millions of live bacteria). Freeze these tubes after preparing the dilutions for BCA assay.
11. Make serial dilutions in PBS.
 - A. With Gentamycin: 10^{-2} - 10^{-6}
 - B. Without Gentamycin: 10^{-3} - 10^{-7}
12. From each dilution, 100µl were plated in LB-agar plates and incubated overnight at 37°C. (Prepare the LB-Agar plates a day or 2 before the experiment with the appropriate antibiotics).
13. Count the number of colonies in each plate with colony counter.