Invasion/Replication Assay
(from Anthea Lee, Falkow Lab) modified by Igor Brodsky 11/12/98

Day 1:
1. Seed macrophages at 2.5x105 cells/well (24-well dishes)
   Use 3 wells per data point (triplicate)
   Hemocytometer: count cells in grid. #cells x 10^4 = cells/ml of preparation used to count
2. Inoculate standing O/N (s. typhimurium) cultures into high salt LB

Day2:
1. Subculture O/N cultures (1:20-1:100) again standing – allow to grow up (optional – may give greater invasiveness)
   Calculate cfu/ml from OD_{600}: OD_{600} of 1.0 = $1.2x10^9$ cfu/ml
2. Aspirate medium from cells, wash 1X w/ PBS prewarmed to 37 °C. Aspirate PBS, Replace with 200µl of prewarmed fresh medium.
3. Infect cells with appropriate MOI (usually 10) in 10-50 µl
   Plate input (use as standard for % invasion)
4. Incubate abcteria with cells for 30-60' to allow invasion
5. Aspirate DMEM, wash 2x with DMEM, aspirate washes, add 1ml DMEM containing 100µg gentamyic /ml to each well.
6. Incubate with gentamycin for 90-120' to allow killing of extracellular bacteria
7. Wash 3x w/ DMEM

For Invasion Assay:
8. Aspirate medium, add 200µl 1% Triton X-100. Incubate RT 5' to allow host-cell lysis
9. Pipette vigorously up&down to ensure complete/uniform lysis
10. Add 800 ml DMEM
11. Make 10-fold serial dilutions, plate 10^{-3} and 10^{-4} (other dilutions if desired)

For Replication Assay:
8. Aspirate medium, add back DMEM containing 10µg/ml gentamycin to each well
9. At each desired time point, wash 1-2X with DMEM, follow steps 8-11 of *Invasion Assay*