Candida Killing Assay Protocol

1. Start overnight cultures in yeast extract peptone dextrose (YPD) at 30ºC on roller drum
2. Prepare cultures
    a. Wash 1 ml cultures in dH_2O
    b. Measure OD_{600}
    c. Adjust OD_{600} to 0.25 in RPMI (100 ul will contain ~500,000 cells)
3. Isolate healthy human neutrophils from fresh whole blood using protocol "Isolating human PolyMorphoNuclear Cells (PMNs) Protocol"
4. Resuspend PMNs in RPMI – 250,000 cells/100ul RPMI (MOI 2)
5. Add 100ul fungi and 100ul PMNs to a 1.5ml Eppendorf tube
    a. Include a "no PMN" control for each strain
6. Incubate 4 hours @ 37ºC
7. Centrifuge samples for 5min @ 4,000rpm
8. Aspirate off supernatant
9. Lyse neutrophils by resuspending pellet in sterile-filtered 0.1% Triton-X in PBS
10. Dilute each sample 1:10, 1:100, 1:1000
11. Plate 10ul of each dilution on YPD plates
12. Incubate at 37C for 24-48 hours – check to see if colonies are large enough to count. Leave them at room temp if you want them to grow a little more or put them at 4ºC if you want to count them later