

Procedure for Broth Microdilution MIC Testing According to NCCLS H7-A5 (2000)

- 1) Desired strains are grown to $OD_{625} = 0.08 - 0.1$ (McFarland 0.5)
 - 2) Concentrations tested should encompass entire range, but can be any number of the breakpoints:
1, 2, 4, 8, 16, 32, 64, 128, 256, 512 $\mu\text{g/ml}$
 - 3) Mueller Hinton Broth containing 2x desired final peptide concentration is prepared.
 - 4) 50ul MHB + Peptide is added to appropriate wells.
 - 5) 50ul freshly adjusted inoculum is added to each well. (1:2 dilution makes final peptide concentration)
 - 6) Incubation: 35C for 16 to 20 hours in ambient air incubator. (Do not stack >4 high)
- 24 hours incubation: Haemophilus, Streptococci and determining Oxacillin & Methicillin for Staph and Enterococcus.

MIC = lowest concentration that completely inhibits visible growth.