Protocol for Bacteria Susceptibility Testing in CellDirector® 3D

For example *E. Coli* or *S. aureus*

Day before the experiment:

1. Equilibrate media (e.g. BBL Mueller-Hinton 2 Broth from BD, Ref # 212322) overnight at 37 °C.
2. Start an overnight liquid culture of the bacteria strain to be tested in 2 ml Mueller-Hinton 2 broth (or inoculate bacterial colonies from agar plates directly into media before experimental start).

Day of the experiment:

3. Start with placing the unopened CellDirector blister package in a degassing chamber for 30 min before sample loading.
4. Before experimental start: Adjust the turbidity of your bacterial suspension to the same McFarland value that is recommended for an equivalent E-test inoculum preparation*. A recommended McFarland value of 0.5 applies to most strains. Use Mueller-Hinton 2 broth for dilution.
5. Mix the bacteria suspension 1:1 with 0.5% agarose (e.g. Top Vision Low Melting Point Agarose from Thermo Scientific, Ref #R0801).
6. Pipet 8 µl of the bacteria/agarose mixture into CellDirector 3D through the cross-shaped slit. Use reverse pipetting.
7. Be sure that the agarose has polymerized before starting the experiment.
8. Fill syringe 1 with Mueller-Hinton 2 broth. Fill syringe 2 with Mueller-Hinton containing your antimicrobial substance (e.g. 4 µg/ml Vancomycin).
9. Start the CellDirector 3D experiment as described in the manual.
10. Collect images using dark-field microscopy (2x objective) at 0.1 fpm (1 image every 10 min) during at least 3 hours.

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* Find tables of recommended E-test inoculums McFarland values here:
Link to recommended E-test inoculums McFarland values