**Plating of Swine Manure Slurry**

Decisions to make

* What are you plating for?
  + This will dictate the agar you will use
* Are you plating for antibiotic resistance?
  + This can be done via disk diffusion assays or by infusing the agar with antibiotics at an MIC breakpoint
  + Breakpoints for specific bacteria can be found here (<https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf>)

Summary

This SOP is meant to provide general recommendations when plating swine manure slurry. Detailed instructions will depend on the intent of the plating based on decisions described above.

General materials

* Appropriate plates
* 95% Ethanol
* Serologic pipette
* Serologic pipette tips
* Pipettes
* Wide-bore pipette tips
* Pipette tips
* PBS
* 15 mL conical tubes
* Appropriate plating equipment (filtration setup, L-shaped cell spreaders, turntable, loop, alcohol burner, etc)

Preparation of Wide-bore pipette tips

1. Take a non-barrier 1,000 µL or 1,250 µL pipette tip
2. Wipe a razor blade clean using 70% ethanol
3. Cut the tip using the clean razor blade for an opening ~ 4 mm
   1. Opening just needs to be large enough that the tip will not get clogged by any solids present in manure
4. Re-box the tips like normal pipette tips with a label indicating these are wide-bore tips
5. Autoclave to sterilize (use normal autoclave cycle for tips)

Tips:

* Swine manure slurry often has small solids that can clog pipette tips, if your manure is >99% moisture you may be able to pipette with regular pipette tips, if not you may have better luck pipetting with wide-bore tips
* If you still cannot pipette using wide-bore tips you may have to measure your volume via added weight based on the density of your manure

Serially Diluting Swine Manure Slurry and Plating

1. Thoroughly mix/vortex/homogenize swine manure slurry
2. Pipette 9 mL of PBS into a 15 mL conical tube (labeled 10-1)
   1. Can serologic pipette/pipette tips
3. Pipette 1 mL of homogenized swine manure slurry into 9 mL of PBS
   1. Use pipette and wide-bore pipette tips
   2. You can pipette up and down a couple of times to ensure all manure is into PBS solution
4. Create appropriate serial dilutions (see figure below)
   1. Typical the dilutions we will use range from 10-3 – 10-7
   2. You can likely use regular pipette tips after the first dilution
5. Plate 1 mL of appropriate serial dilutions using either spreader or filtration apparatus
   1. You can likely use regular pipette tips to plate the appropriate dilutions
6. Appropriately incubate your plates

Tips:

* We use PBS to dilute as the swine manure is a highly saline/hypotonic solution and we have found that diluting with water can cause cell lysis
* General manure will range from ~ 1,000,000 – 1,000,000,000 bacteria/mL, you can choose to adjust your serial dilution methodology or the volume you are plating based on this estimation
* Make sure you are flame sterilizing whenever appropriate

Serial Dilution Process

Icon

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