

8 - Making Plates

Introduction

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Materials

- › Antibiotic Liquid- Should be in freezer or may need to make some up with:
 - › Antibiotic Powder - Above the bench in the middle
 - › Absolute Alcohol - This can be found in one of the solvent cupboards under the fume hood in the central support room (Labelled Armitage)
 - › Dissolve powder in alcohol (34mg/ml for Chloramphenicol, 100mg/ml for Ampicillin)
- › Lysogeny broth (LB) agar
- › Sterile plates
- › (Optional) A 25ml glass pipette and electronic pipette filler

Procedure

Making chloramphenicol broth

1. Melt bottle of LB agar (takes about 25-30 ml per plate) in microwave on defrost for 30 minutes
 - Melt agar on defrost in 15 minute intervals afterwards until all the agar is molten
2. Leave to cool to 55°C in water bath, or on the bench (about 20 minutes)
3. **CRITICAL** Move to laminar flow hood!
 - Ensure the laminar flow is on; otherwise, run the flow for a few minutes before proceeding
4. Add the antibiotic
 - Antibiotic to agar with ratio of 1/1000 to dilute further from stock (Ex. 100 µl for 100 ml of agar)
 - Ensure that you keep everything sterile at this point, don't put lid down or move hand over the top of the bottle
5. Mix
 - Swill bottle to mix antibiotic in without forming bubbles

Pour Plates

6. Place plates in hood

In such a way that when you open and close the lid your hand won't move over the top of the plates and potentially drop contaminants

7. EITHER Pour agar

Do this in such a way that you almost cover the bottom of the plate, then you should be able to swirl the plate to get the agar to cover the rest

8. OR Pipette Agar

Suck up 25ml of agar per plate and pipette smoothly into plate
Back suck a little bit just before the end to avoid spraying bubbles out

9. Leave to dry

In the lamilar flow hood for around 15/20 mins with lid off

10. Leave in cold room

Bag and tape, and make sure you label what antibiotic is in it! and the date made
-> red for chloramphenicol and blue for ampicillin