Making agar plates

1. Figure out the total number of plates you will need, then multiply x 25 to get the approx. amount of agar you will need (in ml). Its better to make more than you think you need ! Alternatively, just make up the agar in multiples of 400 ml, this a standard amount that will make ~16 plates (this is about right for one 'sleeve' of sterile petri dishes, which have 20 dishes in them).

2. Add all the required media ingredients (eg. for LB agar, this is 10 g tryptone, 5 g yeast extract and 5 g of NaCl per litre) – make sure you correct these ingredient weights for the amount of agar you are making (e.g. multiply by 0.4 if you are only making 400 ml).

DON'T ADD THE AGAR YET ! (this doesn't dissolve, it settles out)

DON'T ADD ANTIBIOTICS, TRACE METALS, or TWEEN yet, if these ingredients are needed.

3. Dispense the media into multiple lots of 400 ml in 500 ml media bottles. Make sure that the media bottles have a plastic pouring 'lip' on them, not just a naked glass top; the lip is important to be able to pour the agar neatly. You could also make multiples of 200 ml in 250 ml bottles, but don't make >400 ml in a 1 L bottle – this is too heavy to easily pour plates with.

4. Once you have dispensed the media into the bottles, now you can add the appropriate amount of agar to each bottle. This is 17 grams per litre (=6.8 grams per 400 ml). DON'T USE AGAROSE!

5. Add bottle caps. Don't screw these on all the way, leave them a bit loose. Hold the caps in place with a small piece of autoclave tape.

6. Arrange bottles of agar in a wire rack, add a piece of masking tape on one corner with your name and the lab number (566), then take to the autoclave. If the agar media will not be autoclaved on the same day, keep it in the cold room until the next day (microbes will start to grow within a few hours, especially in rich media like LB)

7. When the agar comes out of the autoclave, if its still molten and very hot, put it in the hot water bath (~60°C) until it cools down to pouring temperature (~15 min). If you don't want to use it immediately, leave on the bench until it solidifies (if it hasn't already), then tighten the cap and it can be stored indefinitely at room temp. If agar has solidified and you want to use it straight away, microwave it (lid loose!) for approx 10 min on 50% power (these settings are for 400 ml of agar at room temp, adjust accordingly!), then put in hot water bath for ~15 min.

8. Prepare laminar flow hood for plate pouring as follows: give it a blast of UV for ~15 min (switch in upper position, with the doors closed!), then turn off UV light (switch in middle position), take out the doors, then turn on the light and fan (switch in bottom position). Swab down with 80% ethanol.

9a (optional). If you are working with very slow-growing microbes and/or you are making rich medium non-selective plates, you can add an extra layer of sterility to the proceedings by wearing gloves, and rinsing the gloves thoroughly in 80% ethanol before you start pouring the plates.

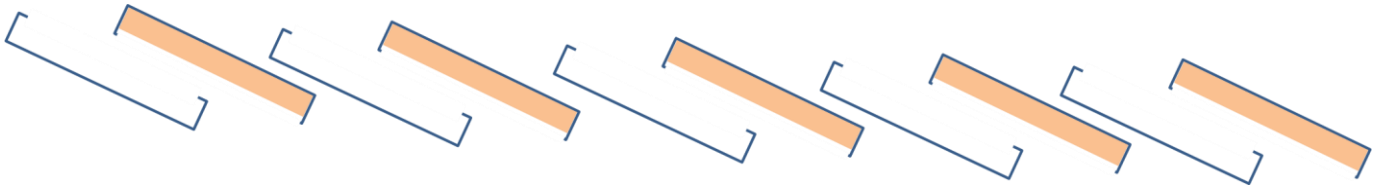
9b. (optional). If you don't have access to the laminar flow hood, you can make plates on your regular lab bench. This is usually fine, but swab down the bench with ethanol first, and work close to the Bunsen flame. (if you swab your gloves with ethanol, be careful to let this evaporate before turning on the Bunsen burner!)

10. Collect one bottle of molten agar from the waterbath, wipe down the outside with paper towel, and take to your bench. Add any “after-autoclaving” additions at this stage, from filter-sterilised stock solutions (this may be antibiotics, or trace metals solution, or Tween etc). Mix by swirling for 10-20 seconds, don’t shake the bottle as this will cause persistent bubbles. Its good practice to write all the additions on the bottle, then tick them off after each addition. Forgetting these is bad!

11. Take the final agar to the laminar flow hood. Open up one sleeve of plates (at the ‘base’ end), and slide them out. Keep the sleeve! Label all the plates with the type of medium (e.g LB-Cm25). Pour the agar into the first plate; use a single smooth pouring motion. Stop when it reaches all the edges. Put the lid on. Do the next plate. Repeat. Don’t stack up the plates in big towers, they will take longer to cool.

12. Leave the plates for about 10 min to alow the agar to solidify. Then invert the plates with the lids off to allow them to dry for 20-30 min. The setup should look like this:

or this:



If you don’t dry the plates, you won’t get nice spread-plates, and the risk of contamination is greater from other microbes ‘climbing’ into the plate over the edge.

13. Pack the plates back into the sleeve, then label the sleeve with the type of agar and the date of manufacture and your name. (don’t label the individual plates with the date, this is confusing, since the date written on an agar plate is usually the date it was inoculated).

14. Store plates in the cold room. They should stay good for >6 months if you have been careful !