Middlebrook Media Prep Protocol

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Equipment Needed:
Middlebrook powdered medium (broth or agar base)
Glycerol
Water
OADC
Kanamycin (if necessary)
Erlenmeyer flask
Water bath
Hot plate/stirrer
Autoclave

Setup: Before beginning or before autoclaving, set *the water bath to 45-55 C (check with a thermometer for accuracy). Use a water bath with sufficient capacity for your media. This will be used to equilibrate agar media to ~55C prior to the addition of OADC and antibiotics.*

Note: Typically, Middlebrook medium is made in volumes in multiples of 200ml. This is because OADC is the limiting ingredient in this procedure, and it comes in 20 mL vials. It is ideal that a whole vial would be used (to avoid partial vials of OADC left in fridge, and subject to contamination). The following will yield 200ml (or 10 standard plates), and the recipe can be scaled up as needed.

PROCEDURE

1. In a 500 ml flask add the following:
   180 mL of distilled water

3.8 g of Middlebrook 7H10 Agar Base powder
   1 mL of 100% glycerol (or 2 ml of 50% glycerol)
(*Notes:*
-the above will produce 200 mL of media, so a flask must be used that can hold a volume much greater than this, for example a 500 mL flask.
-Water is added first because if it spills, it can be poured out and measured again. If it spills and media is partially mixed, both steps are needed again.
-due to the viscous nature of glycerol, it may be difficult to accurately pipette, a trick is to draw up a little less than intended, and give it a second or two to "catch up". This will help ensure you measure out the intended amount. <u>Alternatively, a stock of 50% glycerol can be used, and 2ml would be necessary.</u>

2. Insert a stir bar into flask, cover with a small piece of foil, and place flask on hotplate stirrer. Turn hotplate on to about 280 C (or medium-high heat depending on plate) and stir. Wait for powdered medium to completely dissolve as the liquid becomes clear, and bubbles may begin to form.

Notes:
-do not leave flask unattended on the hot plate. The media will boil over quite quickly if left to boil much longer than a minute.
-The liquid does not need to boil. Once it is clear and bubbles form, it can be autoclaved.

3. Ensure the foil on top of the flask is loose fitting, and place a small piece of autoclave tape on aluminum foil, and autoclave on the "liquid ten" setting. Leave stir bar in flask.
Notes:
It is important to autoclave immediately after heating to avoid media from cooling and solidifying. If autoclave is not immediately available, place flask in the hot water bath while waiting (~55C) to avoid solidification.
-Time in the autoclave depends on volume. A rule of thumb is 20min per liter of media. For small volumes, 10 minutes is sufficient. Scale up as necessary.
-It is critical that a 'liquid' cycle be used in the autoclave. This is the slow exhaust setting. A 'dry' cycle will exhaust quickly (fast exhaust), and pressure will decrease before the medium has cooled below 100C and therefore boil over.
-Remember to bring autoclave gloves with you to autoclave to be available when finished.
-Set a timer for when autoclave will be finished. Letting media set for too long after autoclaving may cause it to solidify. People waiting to autoclave have a tendency to take your stuff out and set it on the counter, so it is good to be there when autoclave finishes its cycle.

4. Place autoclaved media in preheated water bath (45-55 C) and allow to equilibrate to the bath temperature (about 20-30 min).
Notes:
-The water level in the bath should be deep enough to cover the flask beyond the level of the medium inside, but not so deep the flask floats and tips over. A lead ring can stabilize the flask.

5. While the medium cools, arrange plates on bench top, into which you will be dispensing the medium. Also take the OADC out of the fridge to equilibrate to room temperature. If antibiotics are required, have these ready, but they can be kept cool in the fridge.

6. Remove autoclaved medium from water bath and place on a hot plate set to a very low temperature. Activate the stirring mechanism on the plate. Add 1 vial (20 mL) OADC (and 200 μL of 50μg/ml kanamycin if necessary) to flask.
Notes:

-It is important that OADC and kanamycin (or other antibiotic) are added after autoclaving, and to a flask that has been cooled. If media is too hot, this will destroy these ingredients
- Hot plate should just barely be on, just enough to keep from solidifying, while dispensing. ~15 C is fine. The hot plate is used to stir in final ingredients (remember, stir bar should still be in flask)
-The amount of antibiotic added depends on the required final concentration, and the starting stock solution. Check your specific protocol.

7. Using a 25 mL serological pipette, dispense roughly 18 mL of media into plates. Avoid bubbles as much as possible. To achieve this, draw up 19 mL of media, and dispense 18 mL into plate, leaving the last 1 mL or so in pipette (much of this last 1ml contains bubbles).
Notes:
-If a bubble forms or is dispensed onto the plate, they can be picked up with the pipette.
-As the medium is dispensed, it may not cover the entire plate. Cover and slide the plate gently on the table and the medium will spread. Avoid aggressively shaking the plates, because the media can spill or contact the lid.

7. Allow plates to cool on bench top until condensation dissipates. This can take anywhere from 4-8 hours. It is not advised to leave plates overnight because they can dry out. Label a Ziplock bag, put plates in the bag seal partially, and place in lab fridge.
Notes
-Label should indicate type of medium made, and if it contains additives like an antibiotic. Also include date and your name.