Pouring Agar Plates

The directions for pouring agar plates will be referenced in each experiment where this activity is required. Plates may be poured several days or weeks in advance if stored properly. Follow the detailed instructions provided below and watch the instructional video to learn how to properly prepare agar plates. The entire process takes approximately 1 hour, so please plan your time accordingly.

1. Gather and/or locate the following items: a large cooking pot (at least 8-inches deep), stove, access to tap water, test tube rack, roll of paper towels, 10% bleach solution or 70% isopropyl alcohol, oven mitt, permanent marker, pair of safety gloves, coffee mug, test tube clamp, agar tubes listed in experiment step, and Petri dishes listed in experiment step.

2. Use the permanent marker to label the bottom of each Petri dish with the type of agar it will hold. Do not open the Petri dishes. See Figure 1. Note that the label says NA, which stands for "nutrient agar."

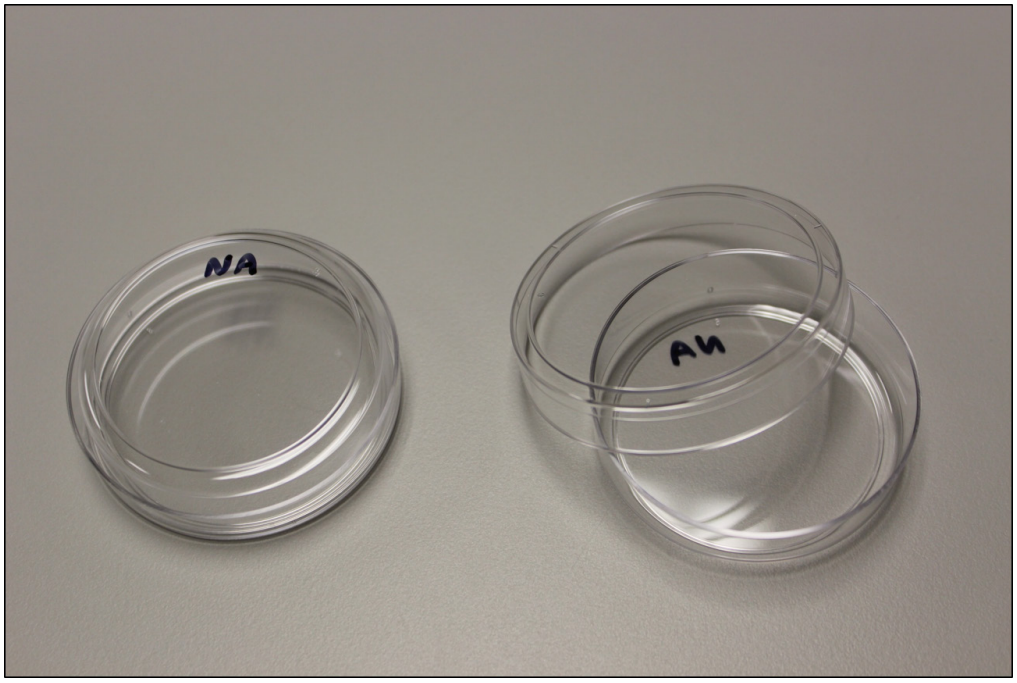

Figure 1. Labeled Petri dishes. The outside of the bottom (smaller) side of each Petri dish is labeled with the type of agar.

3. Place the test tube rack in the middle of a large cooking pot on a stovetop burner. Then carefully place the agar tubes into the test tube rack.

4. Add tap water into the large cooking pot until the water level is approximately 1 mm higher than the agar in the tube. The water level must be higher than the agar to ensure that the agar will melt evenly. See Figure 2.

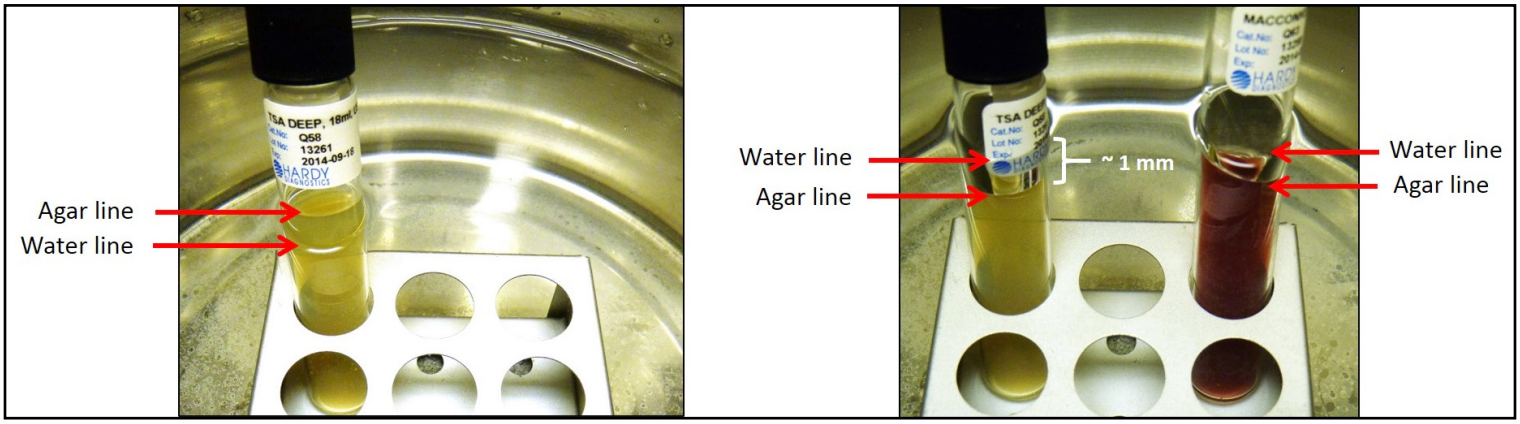

Figure 2. Water levels for melting agar. The picture on the left shows that the agar line is above the water line. There is not enough water. The picture on the right shows that the water line is approximately 1 mm higher than the agar in both tubes. This is the correct amount of water.

Note: If you are preparing tubes of different heights, the water line should be approximately 1 mm above the highest agar line.

5. Carefully loosen the caps of the agar tubes to allow air to escape from the tubes as they are heated. Gently tilt the caps on the tubes so they provide coverage while allowing air to escape. See Figure 3.

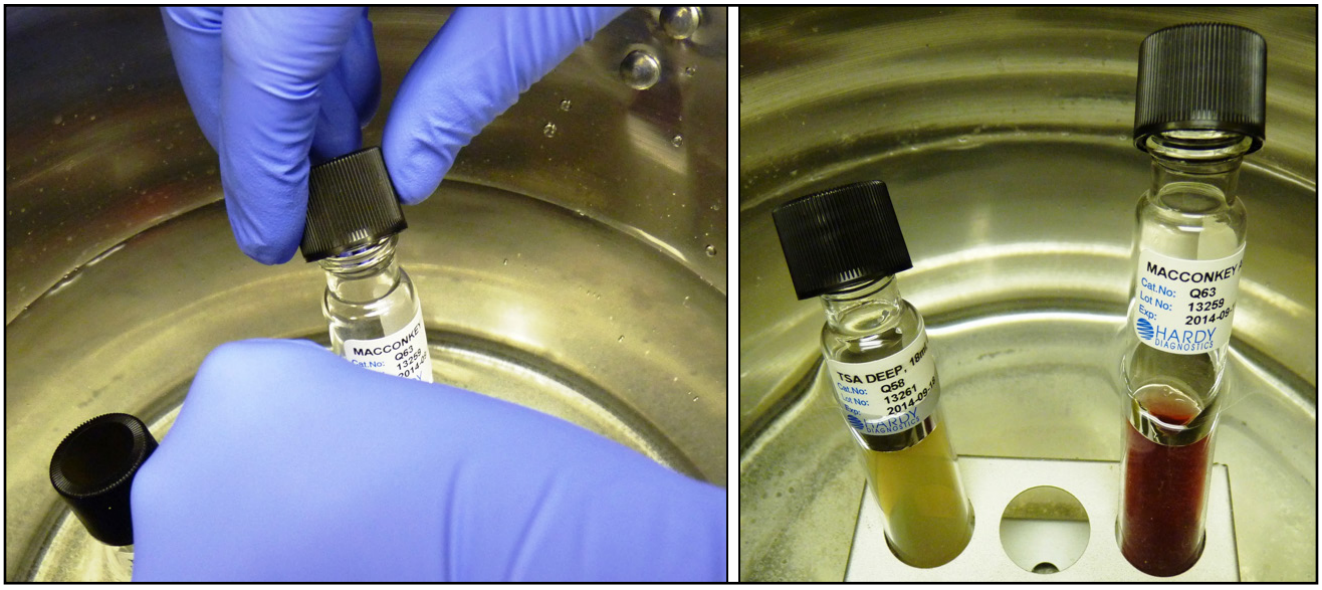

Figure 3. Loosening the caps on the tubes. Carefully loosening the cap of an agar tube as demonstrated in the left picture. The caps of each agar tube are tilted to cover the agar tube while allowing air to escape as demonstrated in the picture on the right. Note that the water level is above the agar line of each tube as shown.

6. Turn the stove on and allow the water to come to a rolling boil. The temperature of the water must be above 90°C in order for the agar to melt uniformly.

7. Monitor the agar tubes as the water comes to a rolling boil. Periodically lift the agar tubes from the water to monitor the melting process, without removing the caps. Use an oven mitt or paper towels as necessary to protect your hands from the heat of the glass agar tubes. See Figure 4.

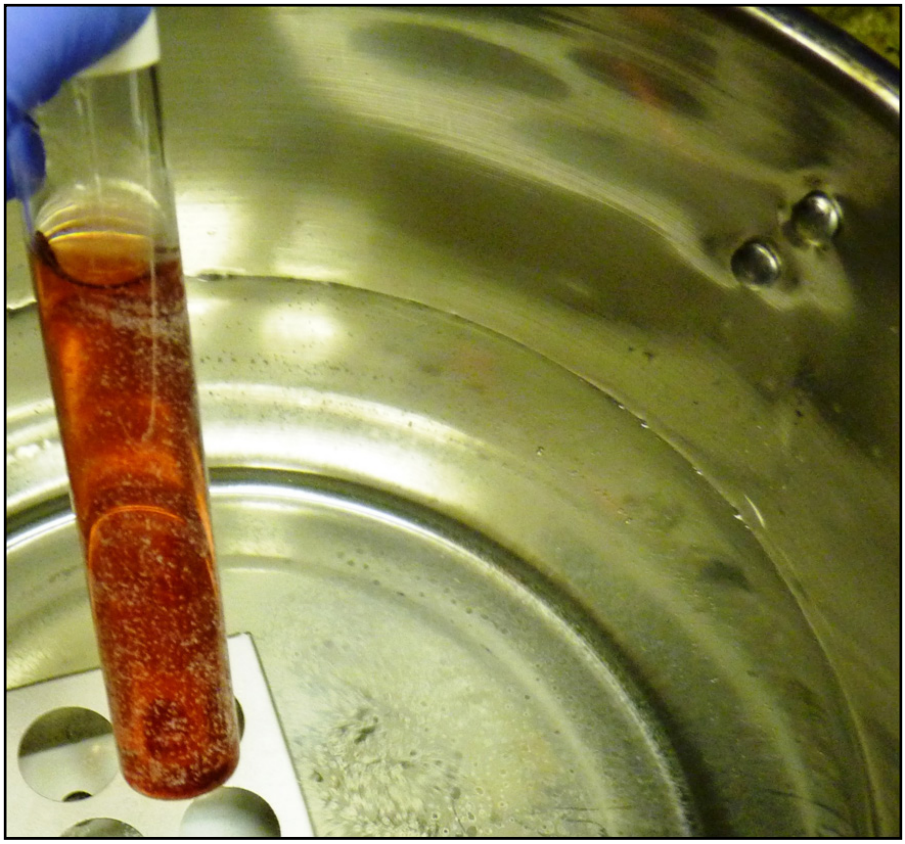

Figure 4. Carefully lifting the agar tubes from the hot water to monitor the melting process.

8. Continue monitoring the agar tubes until the agar is completely melted (in liquid form). The agar will appear translucent when it is completely melted. See Figure 5. If the agar has not melted after 10 minutes in boiling water, place a lid on the pot for 2 minutes before rechecking the agar. Continue to check at 2 minute intervals until the agar is completely melted.

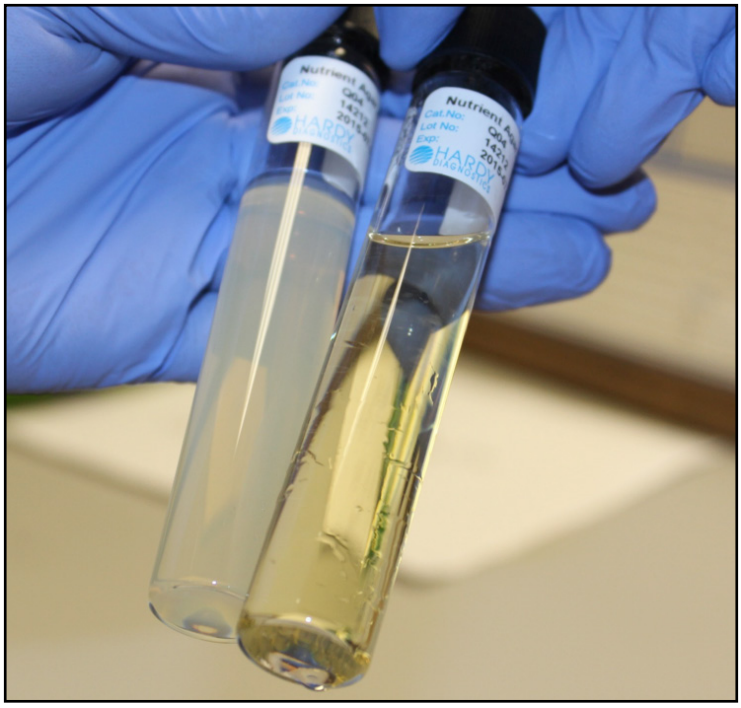

Figure 5. Melted agar is in liquid form on right and is "translucent" while the solid agar on the left is "cloudy".

9. When the agar is completely melted, turn off the burner, and carefully remove each agar tube. Place the tubes into a coffee mug filled with hot water. See Figure 6.

Figure 6. Melted agar tubes in a coffee mug of hot water.

10. Pour approximately 1 tablespoon of alcohol or bleach solution onto your work surface and use a paper towel to spread the liquid across the surface. This will create a sterile working surface to pour the plates.

11. Working quickly, place the labeled Petri dish onto the prepared working surface with the labeled side (bottom) of the plate touching the surface. Remove the corresponding melted agar tube from the coffee mug, unscrew the lid and place it to the side of the Petri dish. Lift the lid of the Petri dish up, just enough to allow for the pouring of the agar. Gently pour half the contents of the agar tube into a small Petri dish or all of the contents of the agar tube into a large Petri dish. The instructions for each experiment will notify you of the size of Petri dish to use. See Figure 7 for an example of pouring into a small Petri dish.

Note: Use the test tube clamp or a paper towel as necessary to hold the hot agar tube.

Note: If the agar from the tube has begun to solidify during pouring, simply hold the agar tube in the still hot water for approximately 1 minute until the agar has returned to a liquid form.

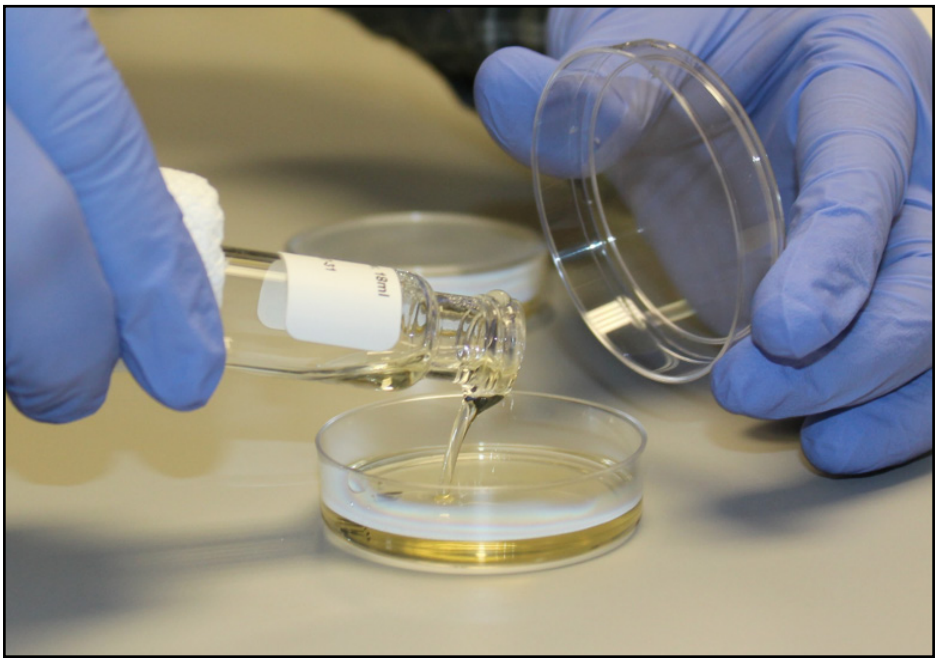

Figure 7. Quickly pouring the melted agar into the labeled Petri dish.

12. Once the agar is poured it should appear as a smooth surface in the Petri dish. If the agar appears slightly lumpy in the dish, it will still work for the experimentation. You will become more proficient with practice.

13. Place the lid of the Petri dish over the poured plate, and leave a slight opening to allow the heat to escape from the plate. See Figure 8.

Figure 8. Poured plate. Lid of Petri dish gently tented over the cooling agar.

14. Allow the poured agar plates to sit, untouched, until they are completely solidified in the Petri dishes. When the agar has completely solidified, the plates can be inverted and the agar will remain in the plates. Plates should remain in the inverted, agar side up, position until inoculated. See Figure 9.

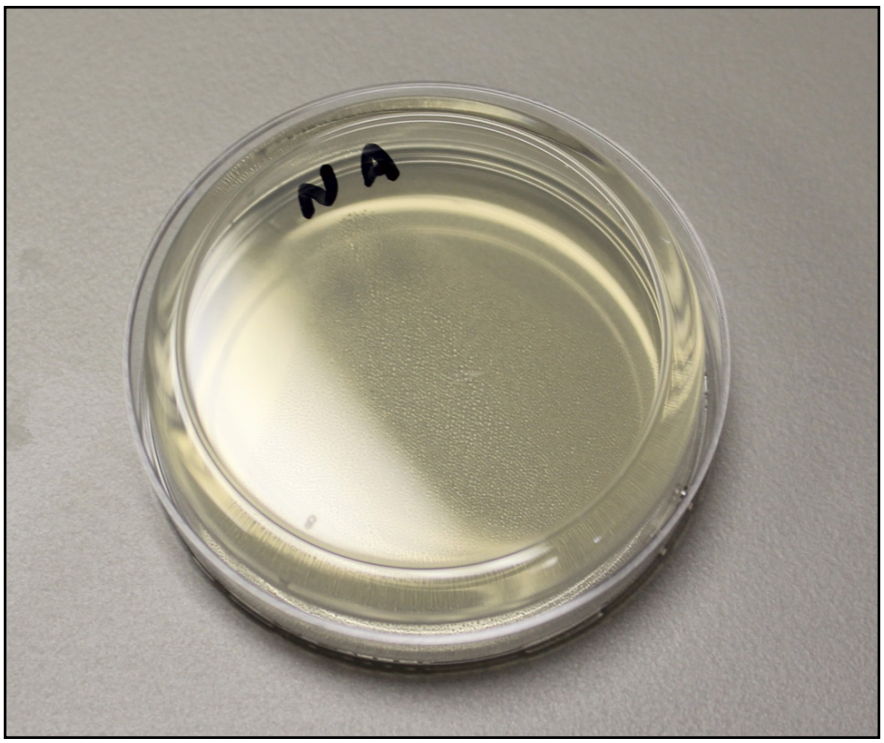

Figure 9. Poured, cooled, and inverted agar plate.

15. When the plates are cooled they are ready to be used in the experiment. The entire process takes approximately 1 hour.

Note: Poured plates may be stored in a sealed plastic bag and refrigerated for several weeks before use. Make sure to store the plates inverted (agar side up). See Figure 10.

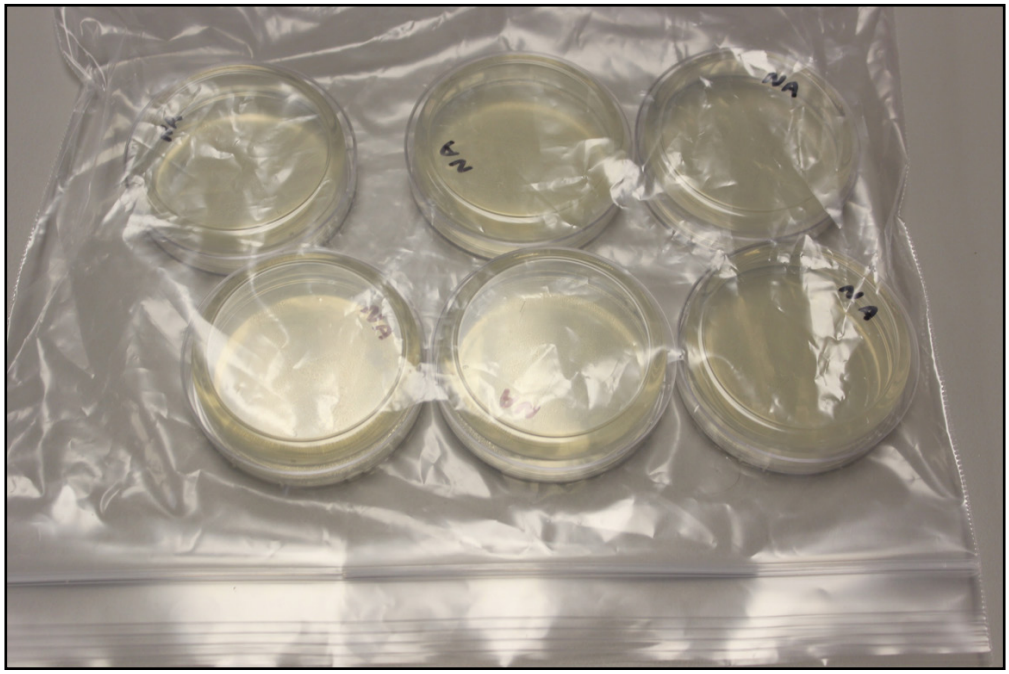

Figure 10. Plates ready for cold storage. The plates are inverted and sealed in a plastic bag.

