

## Isolating a Potato Killer: How to Create a Potato “Sandwich”: Day One

Selection of Good Samples: Good samples show both good *signs* (white mycelia growth) and *symptoms* (brown/black damage to the tuber.) See Figure 1.

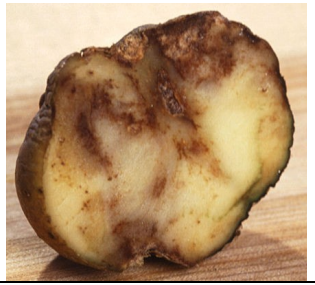


Figure 1: A Late Blight Infected Potato Tuber

Preparation: Procure materials for Day One from laboratory sheet.

### Laboratory Procedure:

1. Procure a plastic container with a fitting lid. Clean the entire container with rubbing alcohol and wipe clean. Place a folded paper towel at the bottom with the wire mesh on top. Replace the lid immediately. If wire mesh and plastic containers are not available, you may substitute container with a **reseal able plastic bag on moist paper towel**.
2. Set up 3 petri dishes with their lids removed. Using your 10% bleach solution, pour the bleach solution into 2 of the petri dishes (column A). Pour 100% distilled water into the remaining four dishes (columns B and C). See Figure 2.

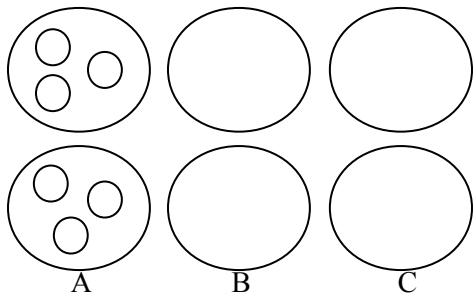
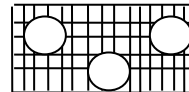


Figure 2: Potato Wash Setup ○ = Potato Slices in wash

3. Using a sterilized knife/scalpel, slice a small potato into six 0.5 cm to 1.0 cm slices (width.) Use a ruler to help you.
4. Place the slices into the bleach solution Column A (3 pieces in each dish) for five minutes using the timer or classroom clock. While you are waiting, dip your forceps into the rubbing alcohol solution (or heat source) to sterilize. See Figure 2.
5. After five minutes, using sterilized forceps, place the potatoes into the distilled water wash, Column B. Again, wait an additional five minutes. Then place them into the final distilled water wash, Column C. Again, wait for 5 more minutes.
6. After the total 15 minute wash, carefully blot dry your potato slices onto a piece of paper towel.

Figure 3: The top view of the Potato “Sandwich” from above.



7. From your instructor, you will receive 3 small squares (no larger than a 1 cm<sup>2</sup>) placed on top of your potato slices in the center. Using your forceps, place the other potato slices onto the slices containing the infection section of the diseased potato. It will resemble a “sandwich” where the infected section is located in-between two potato slices.
8. Add a small amount (1 ml) of distilled water drop wise via a sterilized pipette to paper towel underneath the mesh and quickly replace the lid. *If no mesh*, then drop water directly onto the paper towel.
9. Cut parafilm into 15 cm strips (using the ruler if needed) and stretch the parafilm to seal the container along the perimeter. *If using plastic bags*, simply allow some air into the bag and seal. Label your container with **date, location, group name(s), and class period** on the lid with a sharpie pen.
10. Place in a cool, undisturbed location for at least 7 days to then observe its growth underneath the dissection microscope. Wipe up your lab station with the bleach solution.