The chance observation in 1881 of bacteria growing on the surface of a spoiling slice of boiled potato led Robert Koch to devise the technique called single colony isolation. He correctly deduced that each spot, or colony, of bacteria had grown as a clone from a single contaminating bacterium. He subsequently developed the use of agar, a polysaccharide derived from certain red algae, to solidify nutrient liquid media. This allowed a specimen to be spread across the surface of the medium and grown to produce single colonies, thus isolating the various microorganisms which might be present. The power and elegance of this technique will be explored in later exercises.

Agar is particularly suited as a solidifying agent because it will not melt until the medium is heated to near boiling, but will remain melted until cooled to around 42°C. It is not degraded by the vast majority of bacteria, and therefore maintains its structure during bacterial growth, and because it is not a nutrient for bacteria, it also allows strict control of growth factors.

There is a wide variety of media which are used in microbiology, but the procedures used in their preparation are generally the same. They include weighing out the dehydrated medium, dissolving it in dH₂O, sterilizing it (usually by autoclaving), pouring the plates, preincubation to check for contamination and to dry out the plates, and storage.

Because medium boils up during decompression from autoclaving, we autoclave only 600 mL of medium in a 1 liter bottle. You may need to calculate the amount of powder needed per 600 mL of medium since the directions on reagent bottles are for 1 full liter. Teams of 3-4 students prepare a medium assigned to them. On a page titled with the your specific medium, record the actual weights measured, and outline the steps you took. Have media in autoclave within 75 minutes of start of class.

**Equipment:**
- 1000 mL beaker
- Balance
- spirit thermometer, -10 to 110°C
- heat source, hot pads
- 1000 mL bottle with cap
- funnel
- autoclave

**Supplies:**
- dehydrated media
- 600 mL dH₂O
- 16-20 sterile petri dishes

**WEIGH OUT THE DRY MEDIA, ADD WATER:**
1. Tare out the 1000 mL beaker, use counterweights if necessary, record tare weight.
2. Sum the desired mass of the dehydrated medium + tare weight, set balance to read the sum.
3. Add dry reagent with care to beaker with a clean spatula. After each addition, gently tap the edge of beaker with spatula to judge progress. When close to the desired weight, tilt and roll the reagent bottle, tap its neck to sprinkle in the final amount until equal swings are achieved. Do not remove any excess back to the reagent bottle. Replace the cover immediately on the reagent bottle (it is hygroscopic). Record the actual amount added.
4. If there are more than a single substance in your medium, repeat steps 2 & 3 for other dry materials. (Use each new apparent weight as the new tare, repeat the sequence of steps.)
5. Add dH₂O to the beaker with stirring, break up clumps of powder, q.s. to 600 mL.

**HEAT TO NEAR BOILING TO DISSOLVE THE MEDIA:**
6. Stirring with a thermometer, heat to boiling: do not allow to boil over, nor to burn on bottom. Have hot pads available. (Microwave in cycles of heating and stirring, do not boil over.)
7. Pour into a 1000 mL bottle with a funnel. Cap loosely, label bottle with name of medium and your group’s name, place in autoclave.

**AUTOCLAVE:** (After all student groups have placed their loosely capped bottles in autoclave)
8. Set for slow exhaust, autoclave the medium at 15 lbs. pressure for 15 minutes (for most media).
9. Remove from autoclave (CAUTION: HOT STEAM), allow to cool to 50-60°C (feels hot, but possible to hold). If too hot, the water condenses, falls on agar, forms pits in the surface.*

**POURING PLATES:**
10. Label the petri dishes on bottom in small letters: plate type, date. On a sterile field, each student pours at least four plates using sterile technique as demonstrated. (Position stacks of four sterile plates near edge of desk, remove and hold cap with little finger, flame lip of bottle, fill bottom plate first, at least half full, repeat.) The last student pours all of the rest of the medium.
11. Rinse bottle immediately after pouring last plate before the remnant solidifies.
12. When plates have solidified, invert, place in 37°C incubator for 48 hours to check for sterility and to dry out excess moisture. Store in labeled plastic bag at 4°C. Pre-warm before using.

*The cooling process may be hastened by placing in front of fan, or immersion of the hot bottles of medium into a bucket of 50°C water. (Colder water may cause the bottles to break...)