SPREADING TECHNIQUE FOR PLATING BACTERIA

David B. Fankhauser, PhD.

21 February 1983, rvsd 5 July '93, 10 July '95, 21 July 1996, 20 July '97, 16 July 98, 14 July 01, 24 June 03, 25June09
http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Yeast_Plate_Count/Spreading_Tech.htm

See also: *Sterile Technique: Delivery of Liquids by Pipet*

EQUIPMENT:

- Notebook and pen
- 70% EtOH in squirt bottle, wipettes
- Wax pencil
- Labeled samples in test tube rack
- agar plates, labeled on bottoms
- Sterile pipets or displacement pipets. (usually 0.1, 0.2 or 1.0 mL)
- Pipet bulb
- Turntable
- 95% EtOH in 250 mL beaker (1/2 full)
- Spreader, made from bent glass rod
- Flame (Bunsen burner or alcohol lamp)
- Plastic bucket for used pipets
- Incubator set previously for correct temperature, usually 37 C.

1. **Construct a table** in your notebook with vertical columns for the following information for each plate in your series: plate number, plate type, sample source, dilution factor (if appropriate), volume plated, and blank columns to enter colony counts and calculations.
2. **Sterilize the field** by wiping with 70% alcohol
3. **Label plate bottoms** at edge in small print with wax pencil: date, initials, sample source, dilution factor (if any), volume plated.
4. **Assemble all specimens and equipment** in an orderly array on your sterile work surface.
5. Flame the sterile tip of the pipet and, holding the lid in the crook of little finger of pipetting hand, also flame **the mouth of culture tube**. Do *not* place lid on desk.) (See the protocol on sterile technique: delivery of fluids)
6. With the tip of the sterile pipet just below the surface of the liquid, **draw up the desired volume**.
7. Flame lip of culture tube, replace cap, set in rack. Keep pipet horizontal so that fluid does not dribble out.
8. **Deliver desired aliquot of sample to the surface of the plate**(s) without gouging plate. Gently blow out to ensure total transfer to the plate. (If you are plating out duplicate samples, you may use the same tip again.)
9. Place pipet or contaminated tip into used pipet receptacle containing dilute Lysol.
10. **Pick up the spreader from the EtOH beaker, shake off excess EtOH, briefly pass through flame to burn off the rest.**
11. Hold spreader *away from EtOH* beaker as it flames off. Touch spreader to inside of the top of petri dish to cool (if necessary), then, **turning the turntable *slowly* with one hand, hold spreader like a rake in the other hand and rotate on surface of agar in the *opposite direction*.** NOTE THE ANGLE OF THE SPREADER bends toward the agar. Do not press hard enough to damage agar surface. Continue this action until the fluid has been absorbed (the spreader will begin to drag slightly).
12. Replace the spreader into 95% EtOH, invert plates, **incubate at desired temperature** for one to two days.
13. Count the colonies on the plates, record the raw numbers in the table in your notebook. Calculate the number of colony forming units/mL or /100 mL in the original sample, enter in notebook.