

## POUR PLATE TECHNIQUE FOR BACTERIAL ENUMERATION

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David B. Fankhauser, PhD

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[http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Meat\\_Milk/Pour\\_Plate.htm](http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Meat_Milk/Pour_Plate.htm)

**RELATED PROTOCOLS:**      *Commonly Used Media in This Course*  
   *Bacterial Contamination of Milk and Meat*  
   *Yeast Plate Count*

### PURPOSE:

The pour plate technique can be used to determine the number of microbes/mL in a specimen. It has the advantage of not requiring previously prepared plates, and is often used to assay bacterial contamination of foodstuffs. The principle steps are to:

- 1)      prepare and/or dilute the sample
- 2)      place an aliquot of the prepared sample in a labeled empty sterile plate
- 3)      pour 15 mL of melted agar, cooled to 45°C, into the plate, swirl to mix well
- 4)      let cool to solidify (without disturbing)
- 5)      invert and incubate to develop colonies (24-48 hours).

Each colony represents a "colony forming unit" (CFU). As usual, for accurate counts, the optimum count should be within the range of 30 to 300 colonies/plate.

One disadvantage of pour plates is that embedded colonies are much smaller than those which happen to be on the surface. Thus, one must be careful to score these so that none are overlooked. Also, obligate aerobes may grow poorly if deeply imbedded in the agar.

### EQUIPMENT:

15 mL sterile Plate Count Agar (PCA)	sterile capped 16 x 150 mm test tubes
in capped 16 x 150 mm test tubes*	0.1, 1.0 and 2.0 mL pipets, sterile
Hot Block, 45° C (or water bath)	petri dishes, empty and sterile
3" deep to equal agar depth	flame
	colony counter with magnifying glass

### POUR PLATE TECHNIQUE:

1. **Construct a table in your notebook: details of preparing and plating your specimen(s):** with a line for each plate which describes:
  - a: the detailed identity or source of the specimen
  - b: the dilution of the specimen used (prepare the dilution expected to contain between 30-300 CFU/aliquot. Describe the method for its dilution/preparation.
  - c: the volume of diluted specimen (aliquot) you will plate (usually 0.1 to 1.0 mL)
2. **Label the bottom of the plate** with the above data plus initials, seat number and date.
3. **Dilute specimen** as written out in 1.b.
4. **Inoculate labeled empty petri dish** with specified mL diluted specimen (from 1.c.)
5. **Pour 15 mL of melted, 45°C Plate Count Agar** into the inoculated petri dish.
6. **Mix thoroughly by tilting and swirling the dish.** *Do not slop the agar* over the edge of the petri dish.
7. **Allow the agar to completely gel without disturbing it.** (about 10 minutes).
8. **Invert and incubate** at 37° C for 24-48 hours.
9. **Count, record, calculate:** Count all colonies (again: note that the embedded colonies will be much smaller than those which happen to form on the surface). A magnifying colony counter can aid in counting small embedded colonies. Record the data. Calculate CFU/mL or CFU/g. Enter results in your table.

$$[\text{CFU} \times \text{dilution factor} \times 1/\text{aliquot} = \text{CFU/mL}]$$

\* For 600 mL of NA + 1% glu: 9 g agar, 4.8 g nutrient broth, 6 g dextrose. Dissolve ingredients at 95°C, repipet into 16 x 150 mm tubes, cap, autoclave, 15 lb, 15 min. Cool to 45 °C before using. Plate Count Agar may also be used.