

PLATE COUNT PROTOCOL (YEAST OR *E. coli*) 42 a & b

29 June 1988, rvsd 5 July '93, 28 July '95, 21 July 1996, 20 July '97, 17 July 98, 18 July 01, 24 June 03, 23 July 07, 25 June 09
http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Yeast_Plate_Count/Yeast_Plate_Count.htm

This experiment employs the fundamental skills of microbiology to determine the number of viable cells or colony forming units (CFU) in a package of baker's yeast or culture of *E. coli*. It requires these steps:

- 1) suspension of the yeast in a liquid (*or a fresh overnight culture of *E. coli*).
- 2) sterile serial dilution of the suspension to approximately 300- 3000 CFU/ mL
- 3) sterile spreading of aliquots of the diluted sample over the surface of sterile nutrient medium
- 4) incubation of the plates to induce each cell to develop into a colony
- 5) counting of the resulting visible colonies
- 6) calculation of the number of CFU in the original package or suspension from the number of colonies on the plates.

*If you have previously done a yeast plate count, you will use this procedure with minor adjustments to assay the CFU/mL in a bacterial culture.

REQUIRED SUPPLIES: fresh package of baker's yeast or fresh culture of *E. coli**

FOR EACH STUDENT: two nutrient agar + 4% glucose plates

THREE PROGRESSIVE APPROPRIATELY EQUIPPED WORK STATIONS

1. **One Repipet station:** (one per class)
 - sterile capped 16 x 150 mm test tubes, three per student
 - test tube rack, one per student
 - 2 repipets with sterile dH₂O, set to deliver 9.9 mL
 - flame
2. **Four Serial dilution stations** (arranged as 2 pairs of adjacent stations, sharing equipment):
 - Per table: 2 displacement pipettors set to 100 µL & sterile tips
 - 1 flame
 - 1 yeast suspension on Magnet-o-stir to keep suspended and/or *E. coli* culture
 - 1 vortex
 - 1 plastic used pipet container with 1 inch deep diluted Lysol
3. **Four Plating out stations** with sterilized fields, two tables each with adjacent stations:
 - per table: 2 turntables
 - 1 vortex
 - 2 spreaders in:
 - 2 250 mL beakers, 1/2 filled with 95% EtOH
 - 2 displacement pipettors, 0.1 and 0.2 mL, sterile yellow tips
 - 1 flame
 - 1 used pipet container with 1 inch deep diluted Lysol

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PROTOCOL:

- 1 **Prepare yeast suspension:** For the class: weigh full package of yeast, then suspend contents in 100 mL water. Mix thoroughly (magnet-o-stir works well) for 5-10 minutes. Weigh empty package, determine the dry weight of the yeast added.
2. **Prepare dilution tubes:** Each student labels three sterile 16 x 150 mm capped tubes: 2, 4, 6 (for 10², 10⁴ and 10⁶ dilutions). Repipet 9.9 mL sterile dH₂O into each tube.
3. **Perform a 10⁶ serial dilution of the suspension** as follows, using a fresh tip on a displacement pipetter for each stage: (See page 37 on dilution principles)
 - a. Deliver 0.1 mL of original yeast suspension into first tube (it is numbered 2), vortex to mix.
 - b. Deliver 0.1 from tube 2 into tube 4, vortex to mix
 - c. Deliver 0.1 from tube 4 into tube 6, vortex to mix
4. **Determine the A₆₆₀ of the 10² dilution**, record in your book and on the computer spread sheet.
5. **Label two 4% glucose nutrient agar plates** on their *bottoms* with these four bits of data: date, your initials, mL aliquot plated (0.1 or 0.2), and dilution factor (10⁶).
6. **Plate out samples:** 0.1 and 0.2 mL (100 and 200 µL) of the 10⁶ dilution onto appropriate plates.
7. **Incubate** the plates inverted at 37°C for 48 hours.
8. **Count the number of colonies**, record raw data, and calculate the original number of colony forming units (CFU) in the package:
CFU/plate x dil factor x 1/aliquot x 100 mL/pkg
9. **Smear and stain examples of a yeast colony and a contaminate**, staining with methylene blue, illustrate at 1000x.

* to perform a plate count of *E. coli*, dilute a stationary overnight culture 1:10 (1.0 +9.0). Read its A₆₆₀. Then serially dilute to 10³, 10⁵, 10⁷ (0.1 mL into 9.9 diluent each time). Plate out 0.1 and 0.2 mL of the 10⁷ dilution on NA plates. Incubate as with yeast.