PLATE COUNT PROTOCOL (YEAST OR \textit{E. coli}) 42 a & b

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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 \textit{E. coli}. It requires these steps:

1) suspension of the yeast in a liquid (*or a fresh overnight culture of \textit{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 \textit{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 repipetts with sterile dH\textsubscript{2}O, 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 pipetters set to 100 \textmu L & sterile tips
   - 1 flame
   - 1 yeast suspension on Magnet-o-stir to keep suspended and/or \textit{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 pipetters, 0.1 and 0.2 mL, sterile yellow tips
   - 1 flame
   - 1 used pipet container with 1 inch deep diluted Lysol

**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 \(10^2\), \(10^4\) and \(10^6\) dilutions). Repipet 9.9 mL sterile dH\textsubscript{2}O into each tube.

3. **Perform a \(10^6\) 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 \(A_{660}\) of the \(10^2\) 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 (\(10^6\)).

6. **Plate out samples:** 0.1 and 0.2 mL (100 and 200 \textmu L) of the \(10^6\) 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:
   
   \[
   \text{CFU/plate} \times \text{dil factor} \times \frac{1}{\text{aliquot}} \times 100 \text{ mL/pkg}
   \]

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 \textit{E. coli}, dilute a stationary overnight culture 1:10 (1.0 + 9.0). Read its \(A_{660}\). Then serially dilute to \(10^3\), \(10^4\), \(10^6\) (0.1 mL into 9.9 diluent each time). Plate out 0.1 and 0.2 mL of the \(10^7\) dilution on NA plates. Incubate as with yeast.