ENUMERATION OF BACTERIA, YEASTS AND MOLDS: STANDARD PLATE COUNT

The standard plate count method consists of diluting a sample with sterile saline or phosphate buffer diluent until the bacteria are dilute enough to count accurately. That is, the final plates in the series should have between 30 and 300 colonies. Fewer than 30 colonies are not acceptable for statistical reasons (too few may not be representative of the sample), and more than 300 colonies on a plate are likely to produce colonies too close to each other to be distinguished as distinct colony-forming units(CFUs).

MATERIALS NEEDED

1) 24-hour 10 ml nutrient broth culture of *Escherichia coli*
2) 4 sterile 99-ml saline bottles
3) 1ml and 5ml pipettes
4) 6 Petri plates
5) 6 agar pour tubes of nutrient agar (plate count agar)

PROCEDURE

1. Using aseptic technique, the initial dilution is made by transferring 1 ml of *E. coli* sample to a 99ml sterile saline blank. This is a 1/100 or 10^{-2} dilution.

2. Immediately after the 10^{-2} dilution has been shaken, uncap it and aseptically transfer 1ml to a second 99 ml saline blank. This second blank represents a 10^{-4} dilution of the original sample.

3. Shake the 10^{-4} dilution vigorously and transfer 1ml to the third 99 ml blank. This third dilution represents a 10^{-6} dilution of the original sample. Repeat the process once more to produce a 10^{-8} dilution.

4. Shake the 10^{-4} dilution again and aseptically transfer 1.0 ml to one Petri plate and 0.1 ml to another Petri plate. Do the same for the 10^{-6} and the 10^{-8} dilutions.

5. Remove one agar pour tube from the 48 to 50^0 C water bath. Carefully remove the cover from the 10^{-4} Petri plate and aseptically pour the agar into it. The agar and sample are immediately mixed gently moving the plate in a figure-eight motion or a circular motion while it rests on the tabletop. Repeat this process for the remaining five plates.

6. After the pour plates have cooled and the agar has hardened, they are inverted and incubated at 25^0 C for 48 hours or 37^0 C for 24 hours.

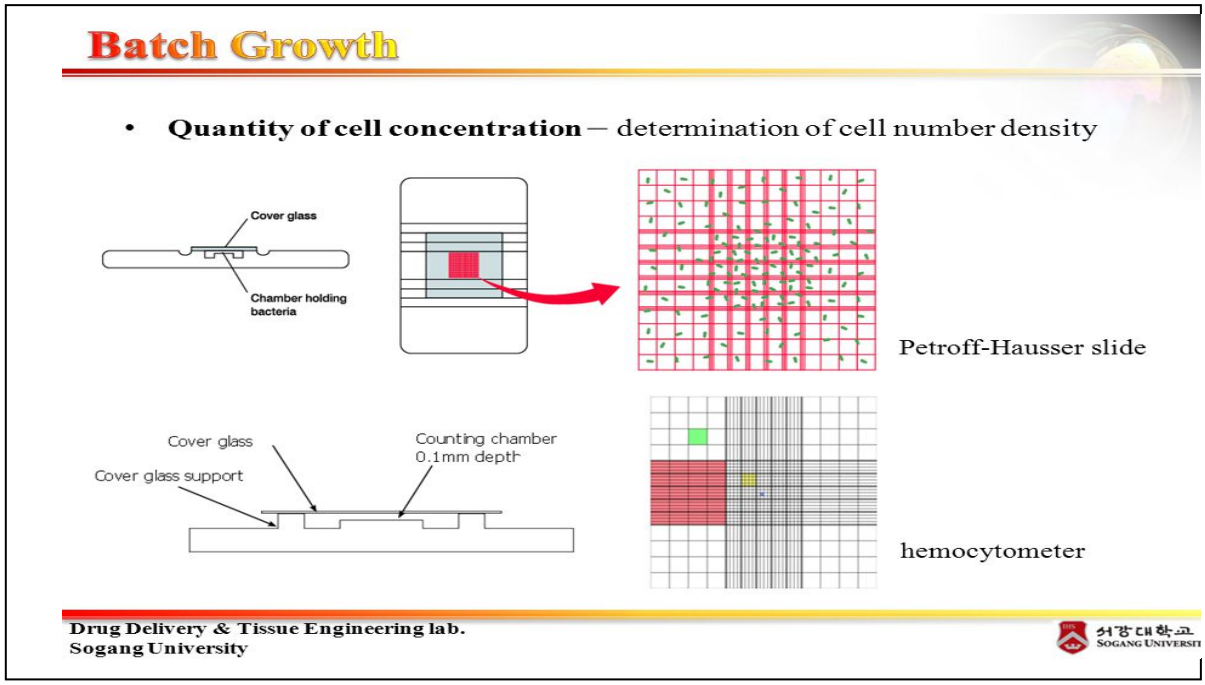
7. At the end of the incubation period, select all of the Petri plates containing between 30 and 300 colonies. Plates with more than 300 colonies cannot be counted and are designated too many to count (TMTC). Plates with fewer than 30 colonies are designated too few to count (TFTC). Count the colonies on each plate. A Quebec colony counter is used to count the colonies in the culture plate.

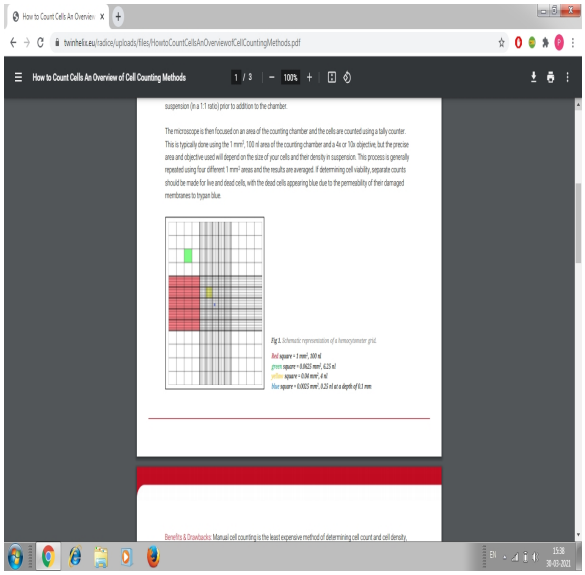
8. Calculate the number of bacteria (CFU) per millilitre or gram of sample by dividing the number of colonies by the dilution factor multiplied by the amount of sample added to liquefied agar.

Number of No. of bacteria = No of colonies (CFUs) / dilution x amount plated

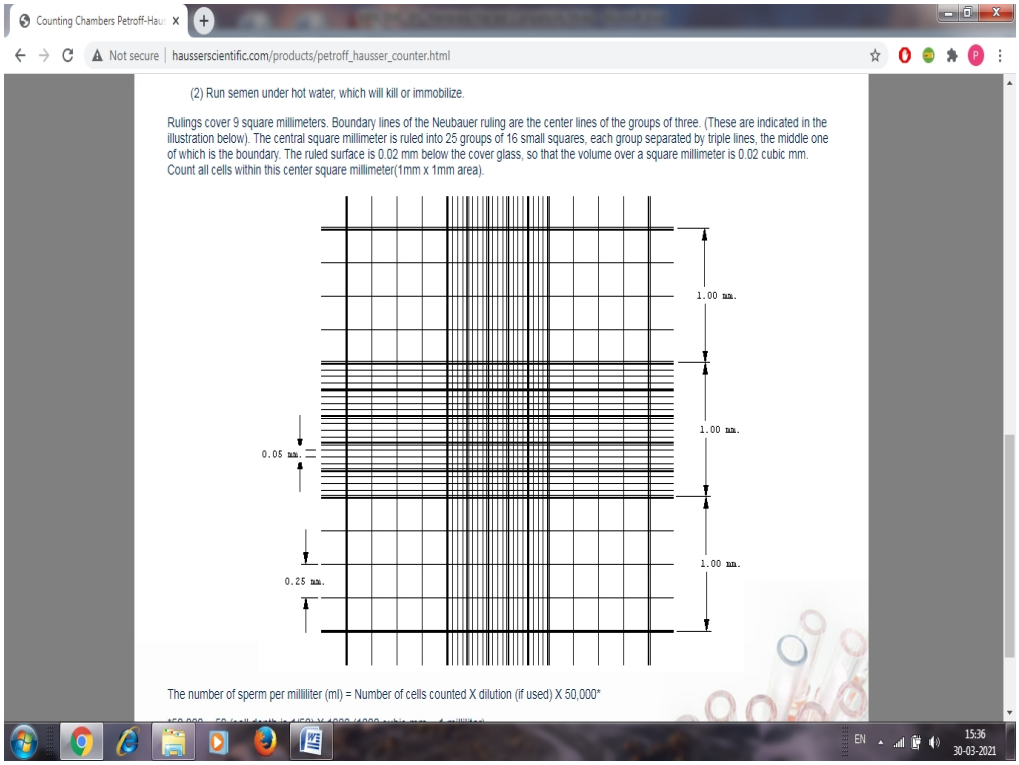
Direct microscopic count

This method requires a haemocytometer (Petroff-Hausser chamber)





Depth between counting chamber and cover slip is mostly 0.1 mm



0.2 mm

Large square dimensions:

Area: 1 x 1 mm^2 Volume: 1 x 1 x 0.1mm^3 or (1 x 1 x 0.1)/1000 cm^3

Calculation: n x 10^4

Smaller square dimensions:

Area: (1 x 1)/(5 x 5) mm^2 Volume: [(1 x 1)/(5 x 5)] x 0.1mm^3 or

[(1 x 1)/(5 x 5)] x 0.1/1000 cm^{3w}

Calculation: n x (25 x10^4)

Smallest square dimensions:

Area: (1 x 1)/(20 x 20) mm^2 Volume: [(1 x 1)/(20 x 20)] x 0.1mm^3 or

[(1 x 1)/(20 x 20)] x 0.1/1000 cm^3

Calculation: n x (400 x 10^4) or n x (4 x 10^6)

Procedure

Methylene blue is added to the sample of bacterial suspension.

Petroff Hausser chamber's grid is covered with a cover slip and a drop of sample is placed in the chamber with a Pasteur pipette.

Sample observed under microscope.

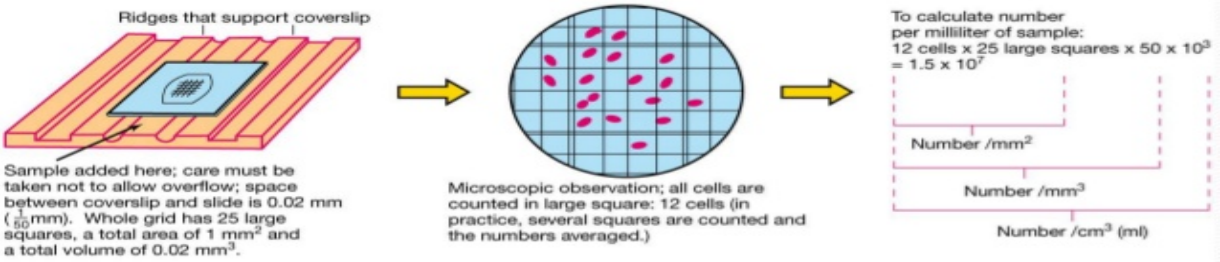
Count the number of cells in five smaller squares.

Calculate the average number of cells for five squares.

Estimate the number of cells (cfu) for one ml by the formula

Cfu/ml = average number of cells in smaller square X (25 X 10^4) X dilution factor

Direct microscopic count



In direct microscopic counting: 1) dead cells are not distinguished from living cells; 2) small cells are difficult to see under the microscope; 3) precision is difficult to achieve; 4) we need a phase contrast microscope; 5) not a good method for cell suspensions of low density.