Indian Institute of Technology Delhi
Department of Biochemical Engineering and Biotechnology

I SEMESTER
BBL132 – GENERAL MICROBIOLOGY
LABORATORY

EXPERIMENT # 4

AIM

To determine total microbial population count by Neubar's Slide method (Direct microscopic count)

BACKGROUND

The number of cells in a microbial population can be measured by counting under microscope. Population of cells can be known by counting number of cells or weight of cell mass. There are several methods for counting microbial cells or estimating cell mass, suited to different organisms or different problems. Wolford stain distinguishes between dead and viable cells. Viable cells do not take up the stain and appear colorless while dead cells appear colored.

APPARATUS AND MATERIALS REQUIRED

Microbial culture (*Saccharomyces cerevisiae*), Wolford stain, Neubar's Slide, pipettes, coverslips, microcentrifuge tubes, microscope etc.

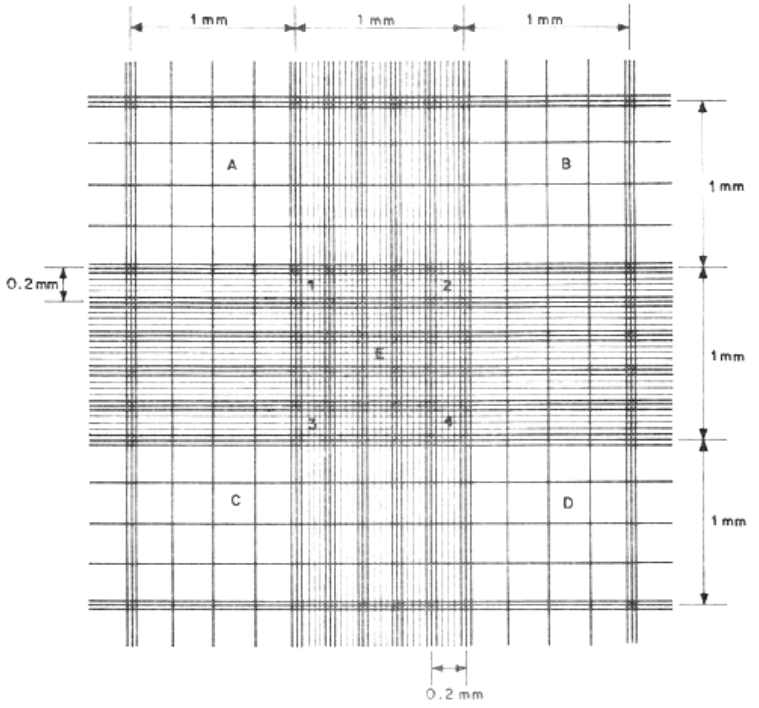
Details of Neubar's Slide: It is a thick glass slide (Fig. 1) on which there is an H-shaped trench enclosing two plateform A and B which is 1/10 mm deeper than rest of the slide.

PROCEDURE

1) Take 1 ml of microbial culture after shaking in a test tube.

2) Add 1 ml of Wolford stain and mix properly
3) In 9 ml of distilled water, add 0.1 ml of stained microbial solution and make it upto 10 ml.
4) Place a cover glass on the Neubar's counting chamber; charge the solution of cells inside from the pipette. Wait for one minute so that the cells should settle down in chamber.

5) Count the number of microbial cells in the four corners squares 1, 2, 3, and 4 (Fig. 2) on Neubar Chamber under high power in Bright field microscope.
6) Calculate the total microbial cell count.



CALCULATION

Each corner square has a side of 1 mm and as the depth is 1/10 mm.
Volume of each square = 1/10 cubic mm
Therefore volume of 4 corner square = 4/10 cubic mm.

a) Assume X be the number of microbial cells in four squares
b) In 4/10 cubic mm area of slide, the number of microbial cells = N
c) In 1 cubic mm of diluted mixture of cells = $\frac{N \times 10}{4}$

And as dilution of microbial culture is 1 x 200,

Total population of microbial cells in undiluted culture 1 cubic mm = $\frac{N \times 10 \times 200}{4}$ = 500 x N

DISADVANTAGES OF THE METHOD

1. Small cells are difficult to see under microscope and some cells may probably be missed.
2. Precision is difficult to achieve.
3. Unsuitable for low density culture.
4. It will be difficult to count highly motile cultures.