Bacterial Count

Many bacteriological studies require that we be able to determine the number of organisms that are present in a given unit of volume. Several different methods are available to us for such population counts. The method one uses is determined by the purpose of the study.

In order to observe microbial reproduction ,it is necessary to determine numbers of M.O., the estimation of microbial population or count could be:


The electrode of a Coulter counter

- **Direct Count of cell number:**
  Under the microscope ,used in food microbiology to assess the sanitation level of product and it is also used for performing blood cell counts in hematology.
- **Indirect count :**
  The growing of M.O. on a suitable media and use the number. of the development M.O. as colonies to determine the number. of cells since each microbial cell multiply & from one colony , thus the number of colonies should give the number of lives bacteria that can grow under the incubation condition employed.

There are different methods that can be employ which include :

A)Total count ….(for counting living and nonliving cells)

1- **Breed method**

2- Haemocytometer (counting chamber)

3- **Turbidity measurements**: to determine the number of bacteria in a culture sample with Optical density (O.D) by spectrophotometer

B)Viable plate count …(counting living cellsonly)

1-Dilution to extinction

2- Pour plate method

3-Most probable number(MPN)

4-Spread plate method

5- Membrane filter method

A)Total count:

1- Breed method : It is possible to do a population count by diluting out the organisms and counting the organisms in a number of microscopic fields on a slide. Direct examination of milk samples with this technique can be performed very quickly with a minimum of equipment, and the results obtained are quite reliable. A technique similar to this can be performed on a Petrof-Hauser counting chamber.

1-Draw a square (1 cm^2) by wax pen on the slide

2-Put 0.01 ml (one loop full) from broth culture on the back of the square

3-Make a smear inside the lines of the square

4-Fix on the flame

5- Stain with crystal violet for 1 min

6-Wash with tap water

7- Examine under oil-immersion objective lenses

8-Count the cells in 10 fields

And then find the range of this count & use the following formula:

No.of cells /1ml = the average no. of cells in 10 fields×5000×100× inverase of dilution if used

2-Haemcytometer (Counting chamber)

1-Put the cover on the counting chamber .

2-Put 0.01 ml one loop full of the culture near the edge of the cover ,the drop will spread (under the cover)

3- Count the cells as the following :

Count the cells in squares (4 corners & one in the middle)if he chamber with 25 squares & used the formula:

No.of cells /1ml = No.of cells in 5 squares ×5×10×100×inverase of dilution if used

Count the cells in squares (4 corners & one in the middle)if he chamber

with 16 squares & used the formula:

No.of cells /1ml = No.of cells in 4 squares ×4×10×100×inverase of dilution if used

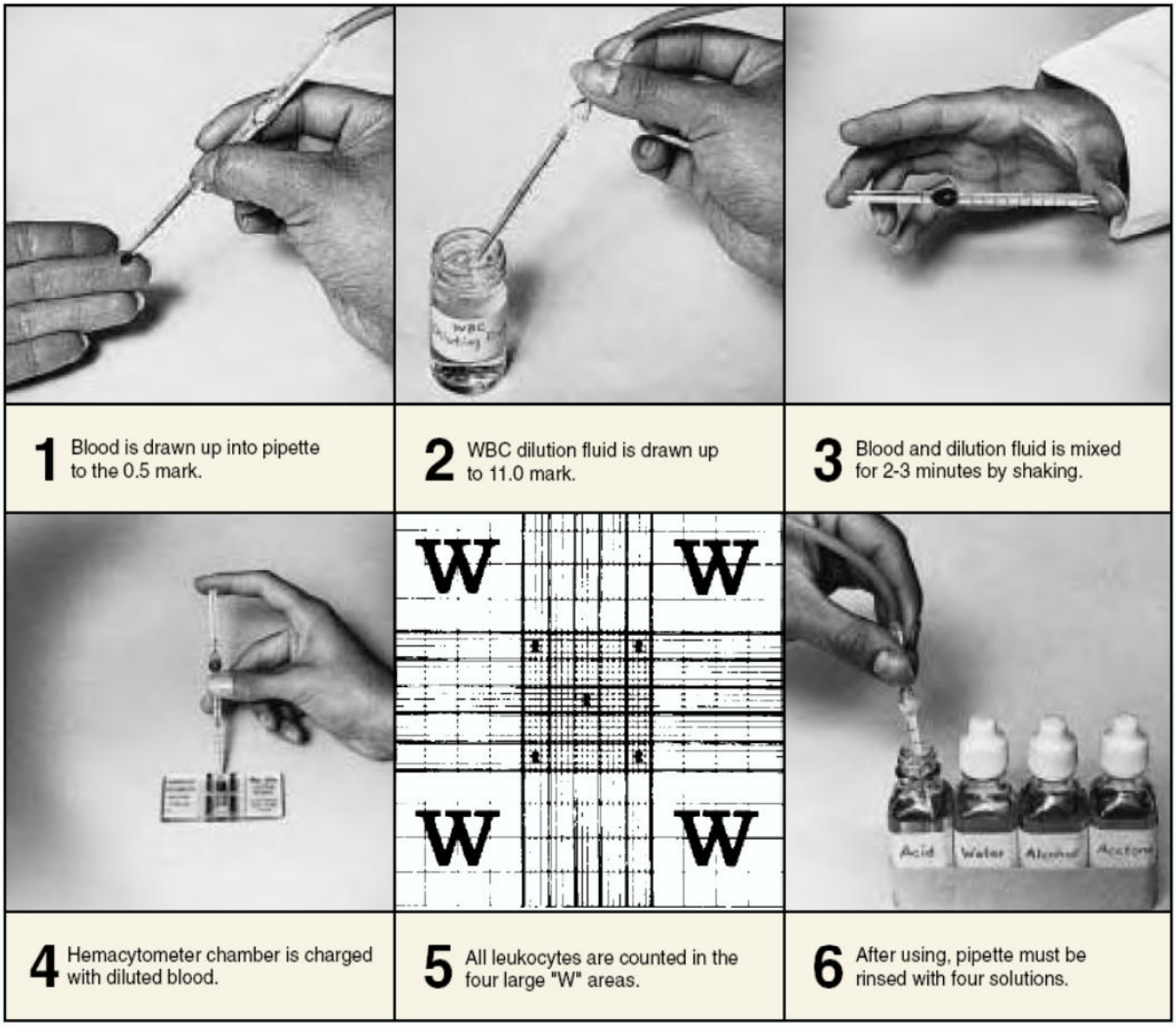

Figure 88.2 Procedure for charging a hemacytometer and doing a total white blood cell count