Methods of Measuring Microbial Growth

There are different methods of counting microbial growth:

1. **Wet-weight measurement**
2. **Dry-weight measurement**
3. **Absorbance**
4. **Total Cell Count**
5. **Viable Count**
6. **Spread Count Method**
7. **Pour Plate Method**
8. **Flow cytometry**

1. Wet Weight Measurement:

- Measuring **cell mass** is an easy step of cell growth measurement.
- A **known volume** of sample from liquid culture is withdrawn and **centrifuged.**
- Wet weight of pellets is measured by using **pre-weighed filter paper.**
- Thus **wet-weight of cells** is calculated.

2. Dry Weight Measurement:

- Dry weight measurement of cell material is **similar** to that of wet weight.
- Here dry weight of **pre-weighed filter paper** containing pellets of microbial cells is measured.
- Dry weight of filter paper is **nullified** by subtracting the dry weight of filter paper only of similar size.
- Thus dry weight of microbial cells can be obtained.

- For example dry weight of **about one million cells of *E. coli* is equal to 150 mg.**

- Dry weight of bacterial cells is usually **10-20%** of wet weight.

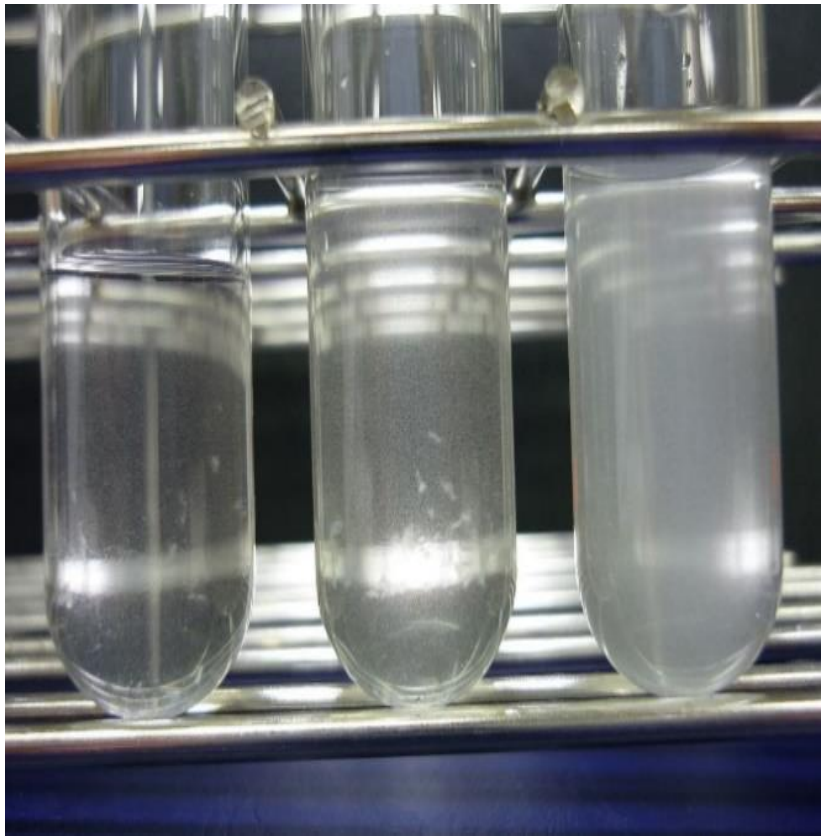
3. Absorbance:

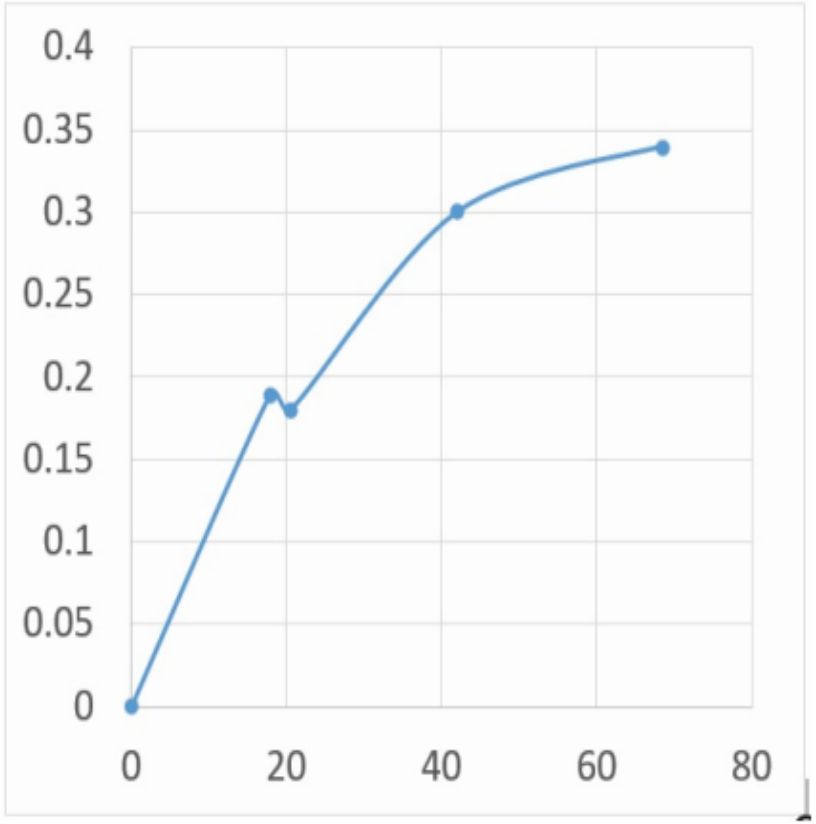
- **Absorbance** is a measure of the capacity of a substance to absorb light of a specified wavelength.
- Absorbance is measured by using a **spectrophotometer** or a **colorimeter.**
- When light is passed through bacterial cell suspension, light is **scattered by the cells**.
- Scattering of light **increases** with increase in cell number.
- Therefore, **transmission of light declines** and **absorbance increases.**

Left: No turbidity, no absorbance, all light passes
Right: Turbidity, high absorbance, only a part of light passes

- At a particular wavelength absorbance of light is **proportional** to the cell concentration of bacteria present in the suspension.

- Thus cell growth of any bacterial suspension at a particular wavelength at different intervals can be measured in terms of **absorbance and a standard graph (between absorbance and cell concentration).**




0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
20
40
60
80

4. Total Cell Count:

- Cell growth is also measured by counting **total cell number** of the microbes present in that sample.

- Total cells **(both live and dead)** of liquid sample are counted by using a special microscope glass slide called **Petroff-Hausser Counting Chamber.**

- In this chamber a **grid** is marked on the surface of the glass slide with **squares of known area.**

- The whole grid has **25 large squares**, a total area of **1 mm^2** and a total volume of **0.02 mm^3 .**

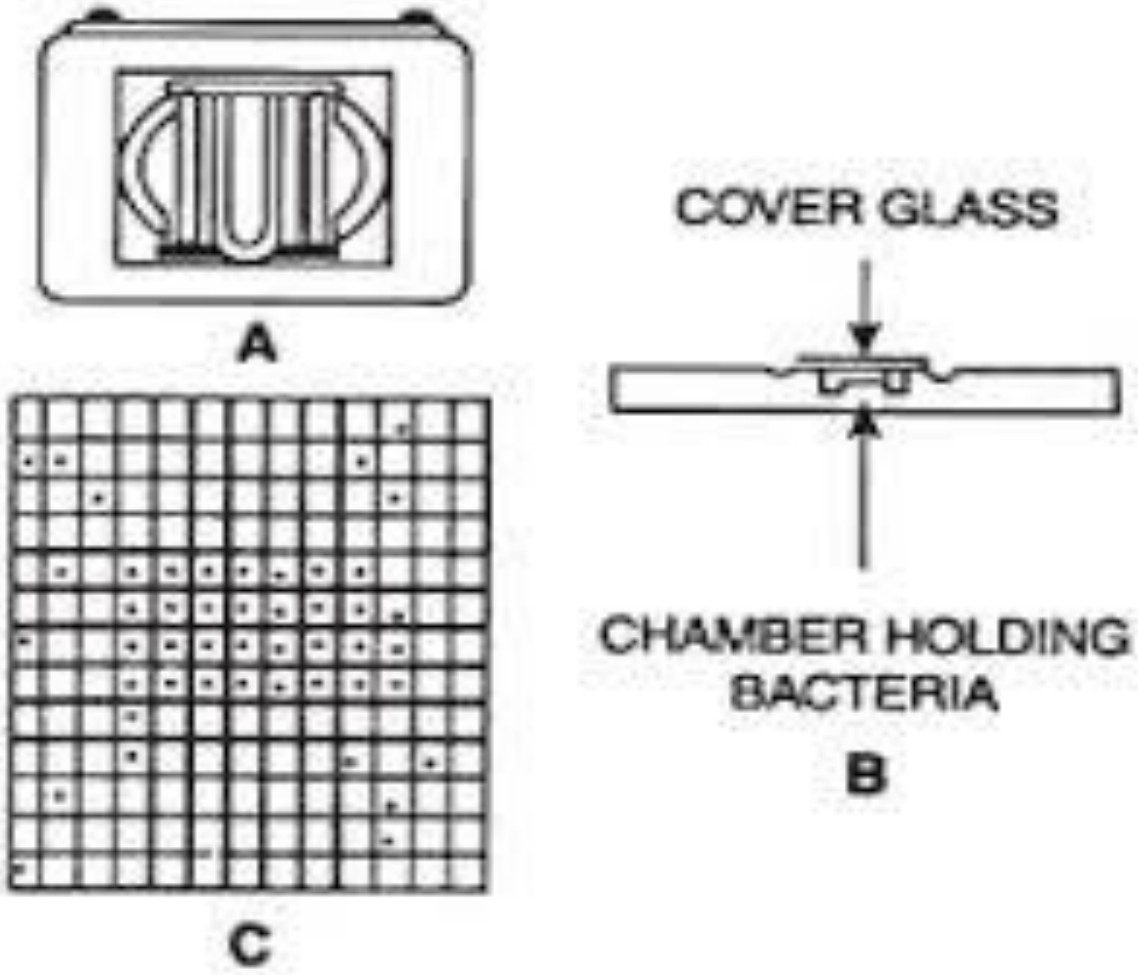


FIG. 19.9. Petroff-Hausser counting chamber used for counting of bacteria.

- All cells in **large square** are counted and total number per ml sample is measured.

- If there is a **low growth** culture, direct cell counting can be done **directly**.

- However, the cell culture of high density has to be **diluted**. Otherwise **clumps** of cells would be formed which would create problem in exact counting of bacterial cells.

5. Viable Count:

- A viable cell is **defined as a cell which is able to divide** and increase cell numbers.
- The normal way to perform a viable count is to determine the number of cells in the sample which is **capable of forming colonies** on a suitable medium.
- Here it is assumed that **each viable cell will form one colony**.
- Therefore, viable count is often called **plate count** or **colony count.**

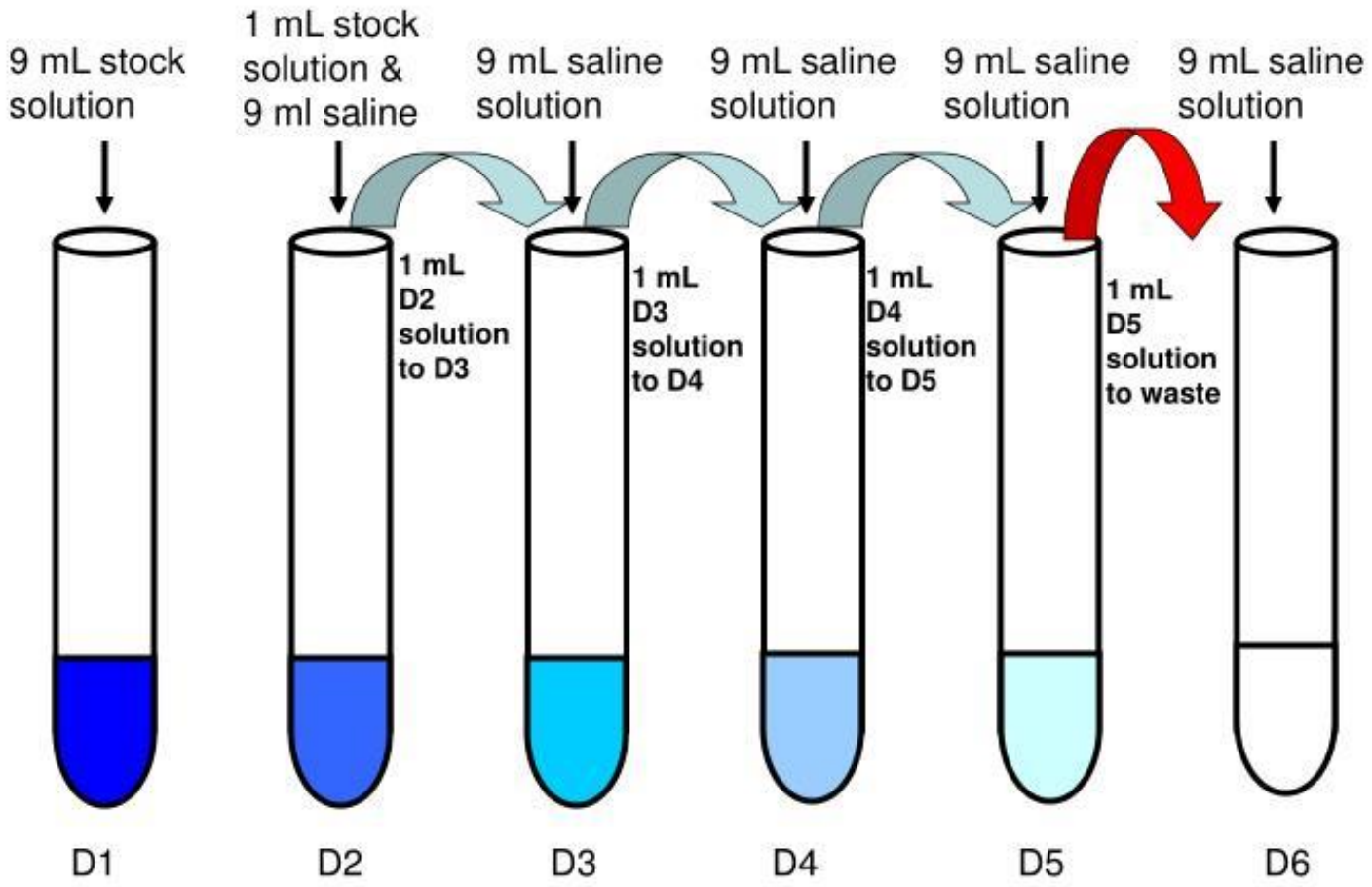
- In practice, it is **never possible** to count **whole populations** of microorganisms.

- Instead, the cells in a **very small sample** of culture are counted, and the **result multiplied** up to give a **population density** in **organisms per cm^3 of culture**.

- Even then, the population density is likely to be so high that **cell counts** are usually **made in known dilutions of the culture**, usually in **10-fold steps**.

- This is known as **serial dilution.**

Serial Dilution



The dilution plating technique:

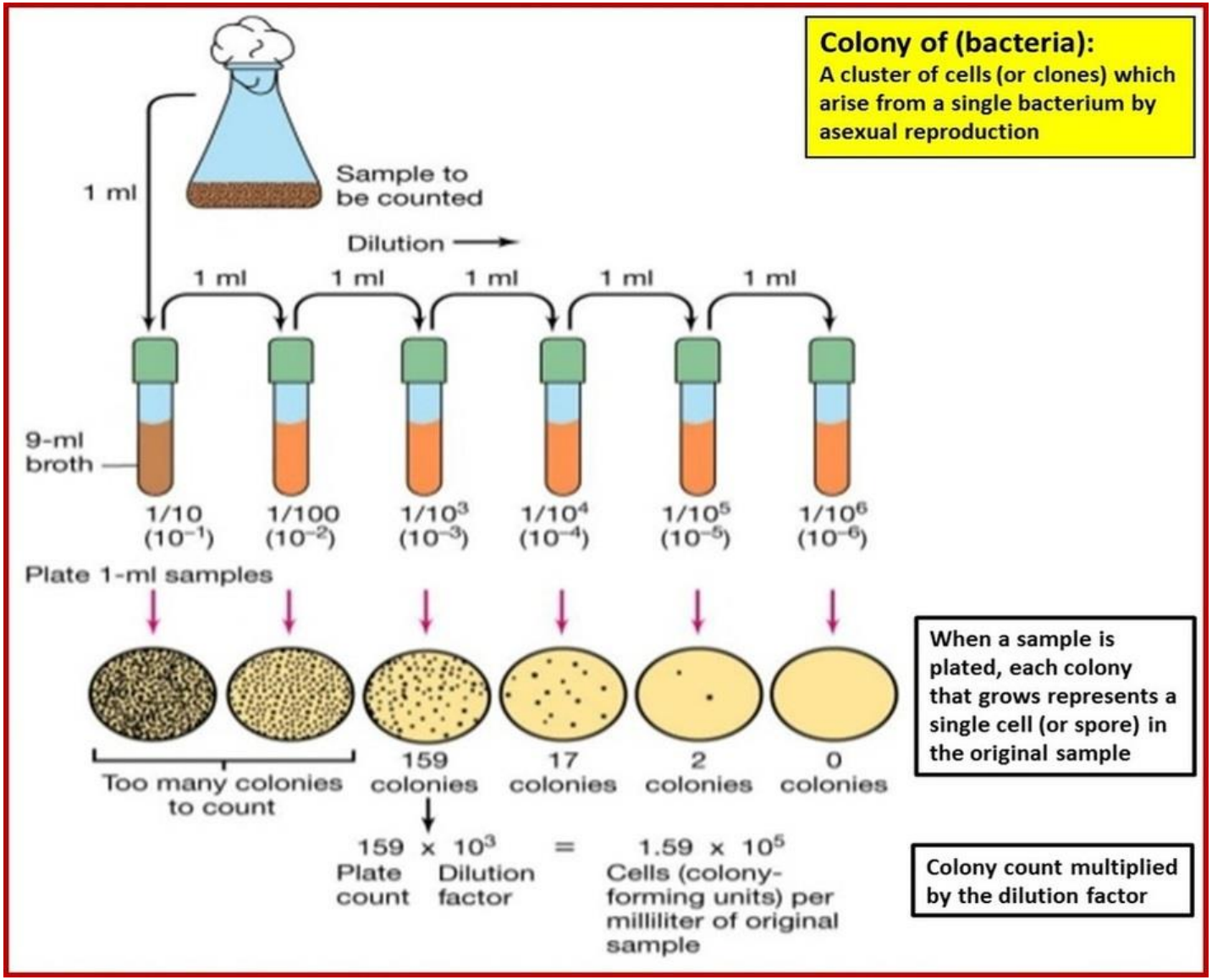
A sample, such as milk or a water sample, is made into a **series of dilutions** using the **serial dilution technique**.

- **1 cm^3** (almost same as 1ml) of each of the **diluted samples**, are individually streaked onto **sterile agar plates.**

- The plates are then placed in an incubator at **25°C** for **1-2 days** to allow time for the **bacteria to grow**.

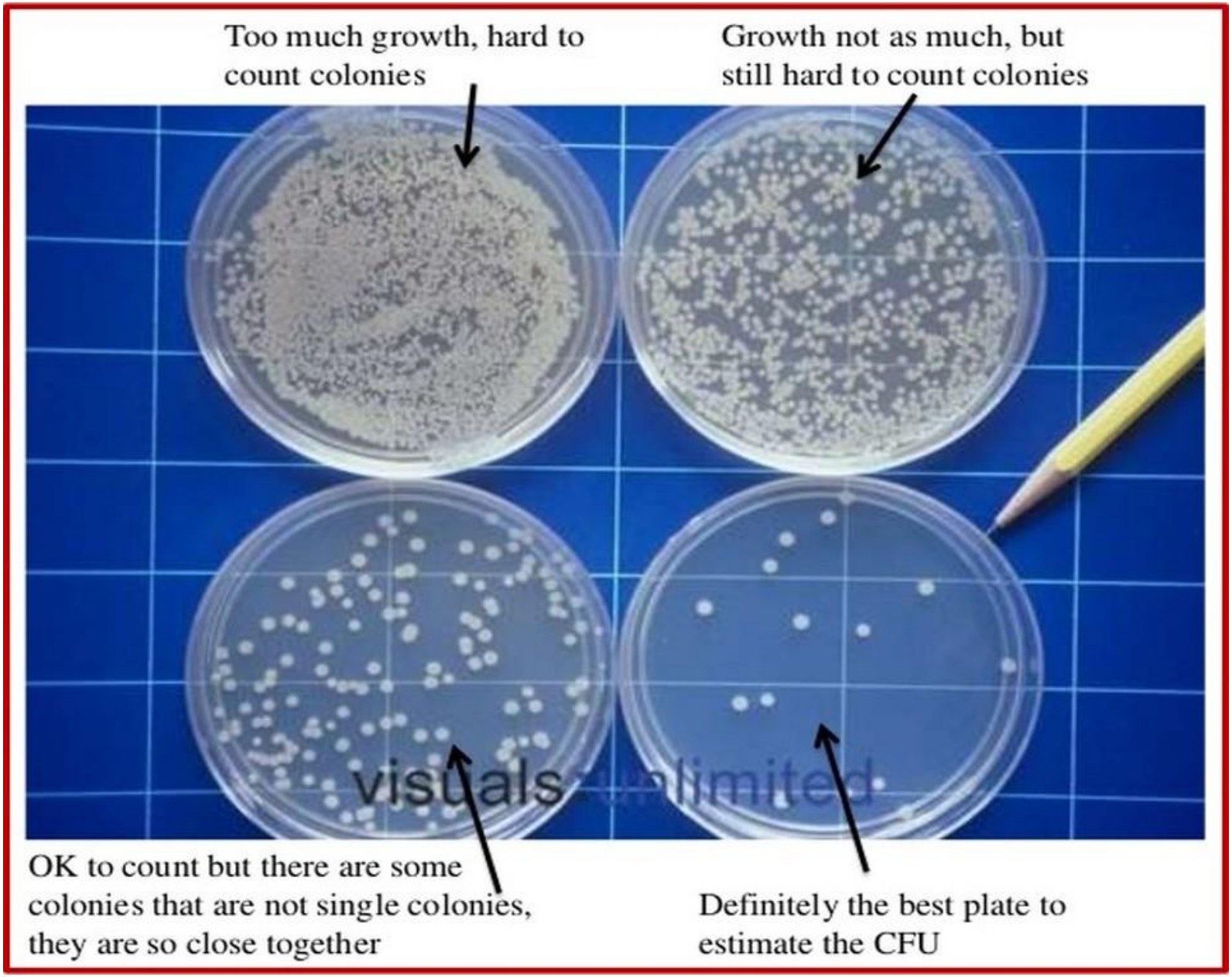
- The dilution at which the **colonies** are **distinct and separate** is noted down (this might be the 1/1000 dilution or more from the example above).

- If the dilution is insufficient then **colonies will merge**, referred to as ‘clumping’, and counting is **inaccurate** (the example above would be the 1/10 and the 1/100 dilution).


Colony of (bacteria):
A cluster of cells (or clones) which arise from a single bacterium by asexual reproduction
1 ml
Sample to be counted
Dilution
1 ml
1 ml
1 ml
1 ml
1 ml
9-ml broth
1/10 (10^{-1})
1/100 (10^{-2})
1/10^3 (10^{-3})
1/10^4 (10^{-4})
1/10^5 (10^{-5})
1/10^6 (10^{-6})
Plate 1-ml samples
Too many colonies to count
159 colonies
17 colonies
2 colonies
0 colonies
When a sample is plated, each colony that grows represents a single cell (or spore) in the original sample
159 x 10^3 = 1.59 x 10^5
Plate count
Dilution factor
Cells (colony-forming units) per milliliter of original sample
Colony count multiplied by the dilution factor

- The **distinct and separate** colonies of bacteria from the agar plate are counted.

- To find the **total viable cell count** the **number of colonies** is **multiplied** by the **appropriate dilution factor**.


Too much growth, hard to count colonies
Growth not as much, but still hard to count colonies
visuals unlimited
OK to count but there are some colonies that are not single colonies, they are so close together
Definitely the best plate to estimate the CFU

6. Spread Count Method:

- A **known volume** of culture (e.g. 0.1 ml) is taken in a **calibrated loop** and spread over the surface of an agar plate by using a **sterile glass spreader**.
- The plate is incubated to develop **colonies**.
- Then the number of colonies is counted as previously mentioned.

7. Pour Plate Method:

- In this method a **known volume** (0.1-1.0 ml) of the culture is poured into the sterile Petri dishes.
- Then **melted agar medium** is poured and mixed gently.
- The plate is incubated.
- Colonies growing **on the surface** or **within the agar** are counted.
- By this technique colonies formed by **microaerophilic** and **partially anaerobic bacteria** can be counted.

8. Flow cytometry:

- Flow cytometry is a technique used to **detect and measure** a population of cells (animal or microbial) or particles.

- A sample containing cells or particles is **suspended** in a fluid and **injected** into the flow cytometer instrument.

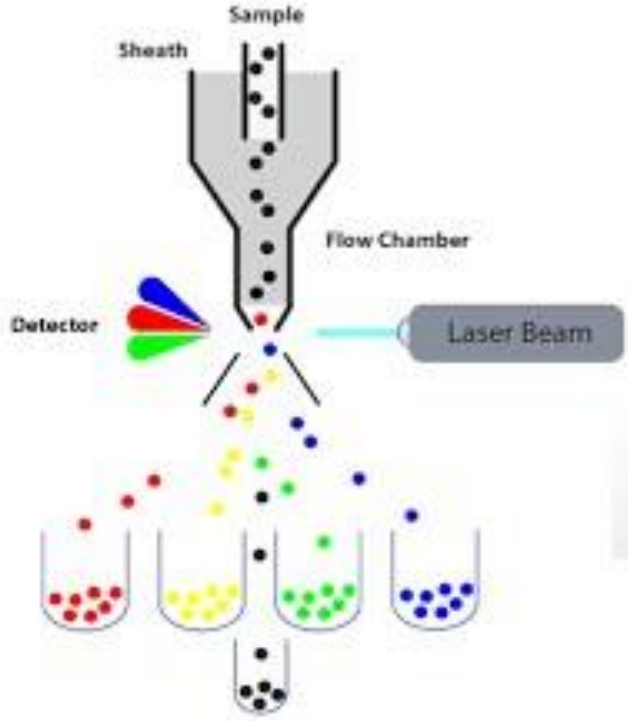
- The sample is focused to ideally flow **one cell at a time** through a **laser beam** and the light scattered is characteristic to the cells and their components.

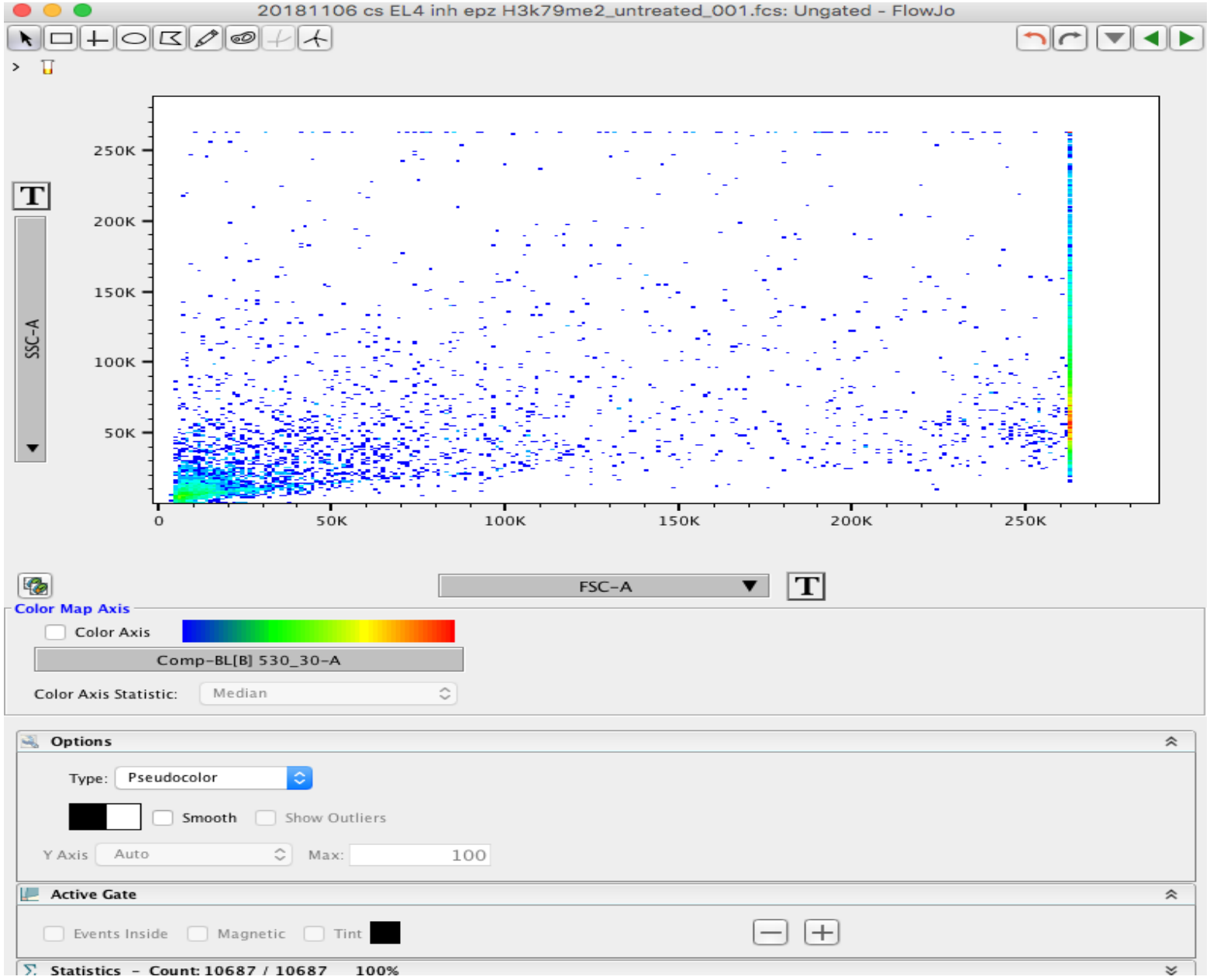
- Cells are often labeled with **fluorescent markers** so that light is first absorbed and then emitted in a band of wavelengths.

- Tens of thousands of cells can be **quickly examined** and the data gathered are processed by a **computer.**

- The machine used is called **Fluorescence-activated cell sorting (FACS)** machine**.**

Flow cytometry

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SSC-A
250K
200K
150K
100K
50K
0
50K
100K
150K
200K
250K
FSC-A
Color Map Axis
Color Axis
Comp-BL[B] 530_30-A
Color Axis Statistic:
Median
Options
Type:
Pseudocolor
Smooth
Show Outliers
Y Axis
Auto
Max:
100
Active Gate
Events Inside
Magnetic
Tint
Statistics - Count: 10687 / 10687 100%


Thank You!