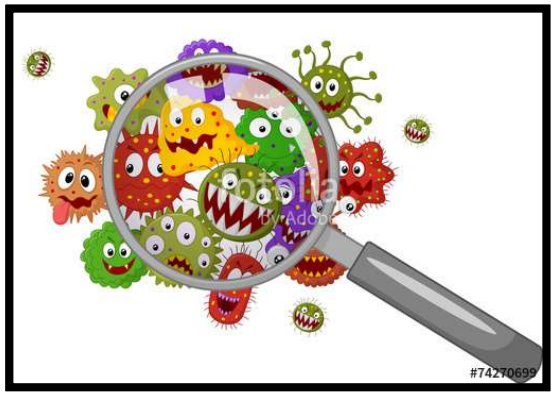
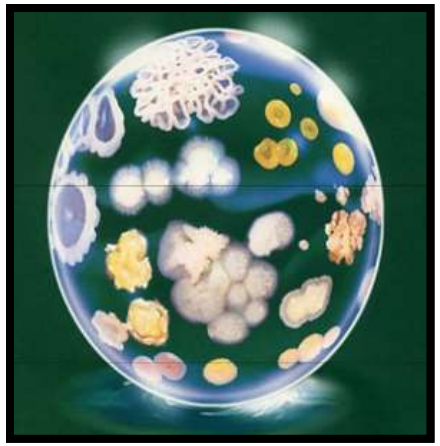
Culture media

Pure culture techniques

&

Bacterial colony





Microbiological culture media

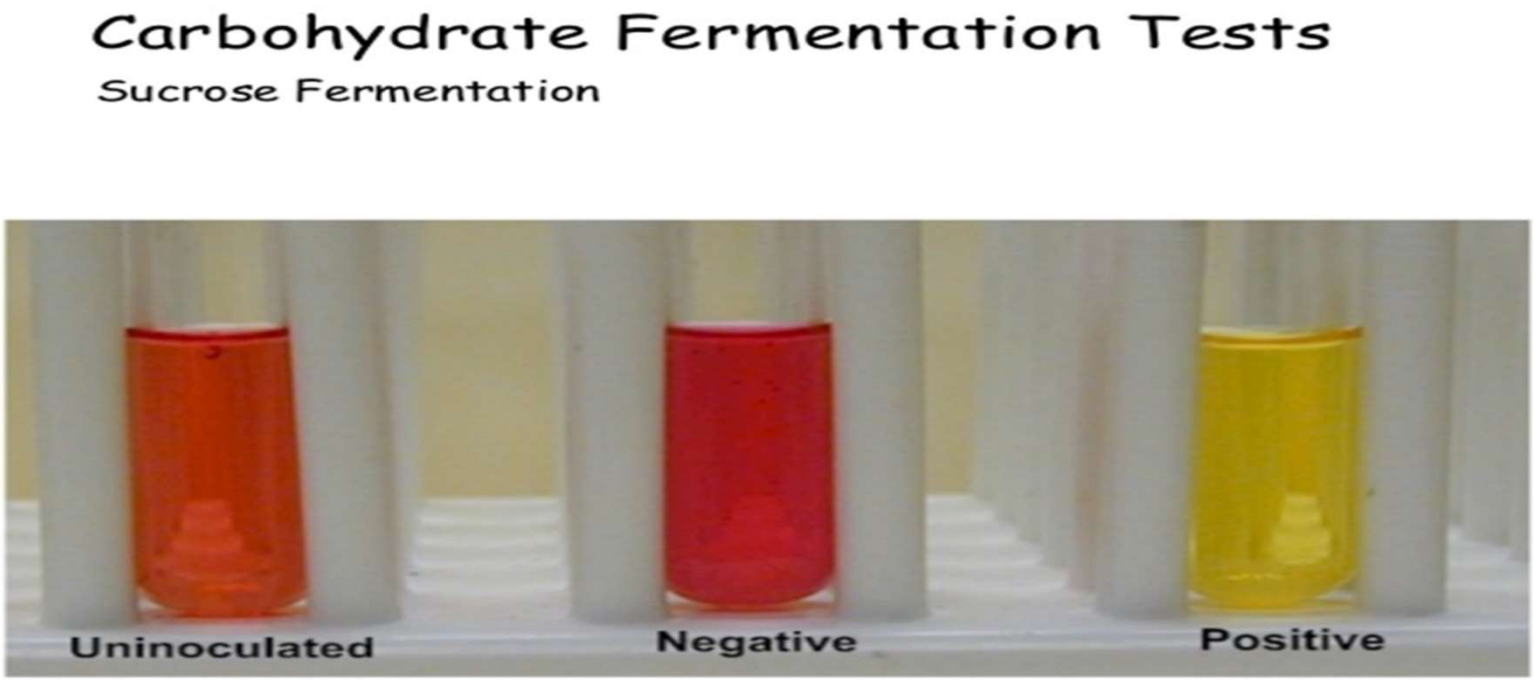
The survival and growth of microorganisms depend on a available nutrients and favorable growth environment. In the laboratory, the nutrient preparations that are used for culturing microorganisms are called media.

Three physical forms are used

- Liquid media (broth)
- Semi solid media
- Solid media

1- **liquid media (or broth)** : such as nutrient broth , tryptic soy broth , brain heart infusion broth , can be used to propagate large numbers microorganisms in fermentation studies and for various biochemical tests.

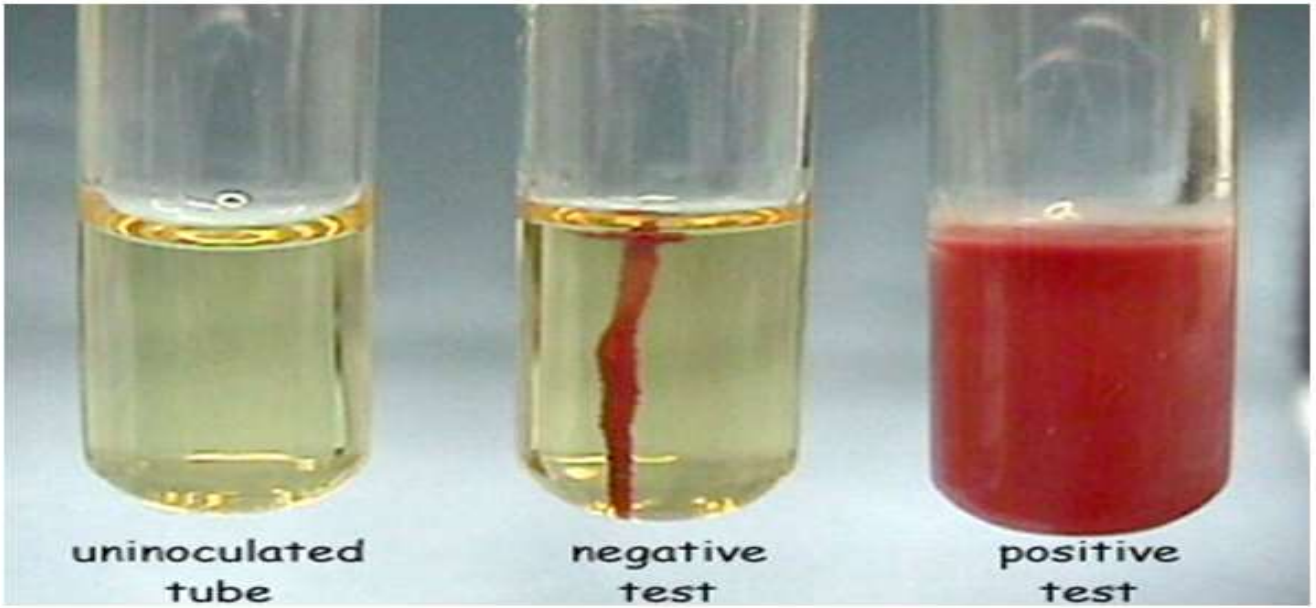
For example:



2- semi solid media : can be used in fermentation studies , in determine bacterial motility , and in promoting an aerobic growth .

For example:

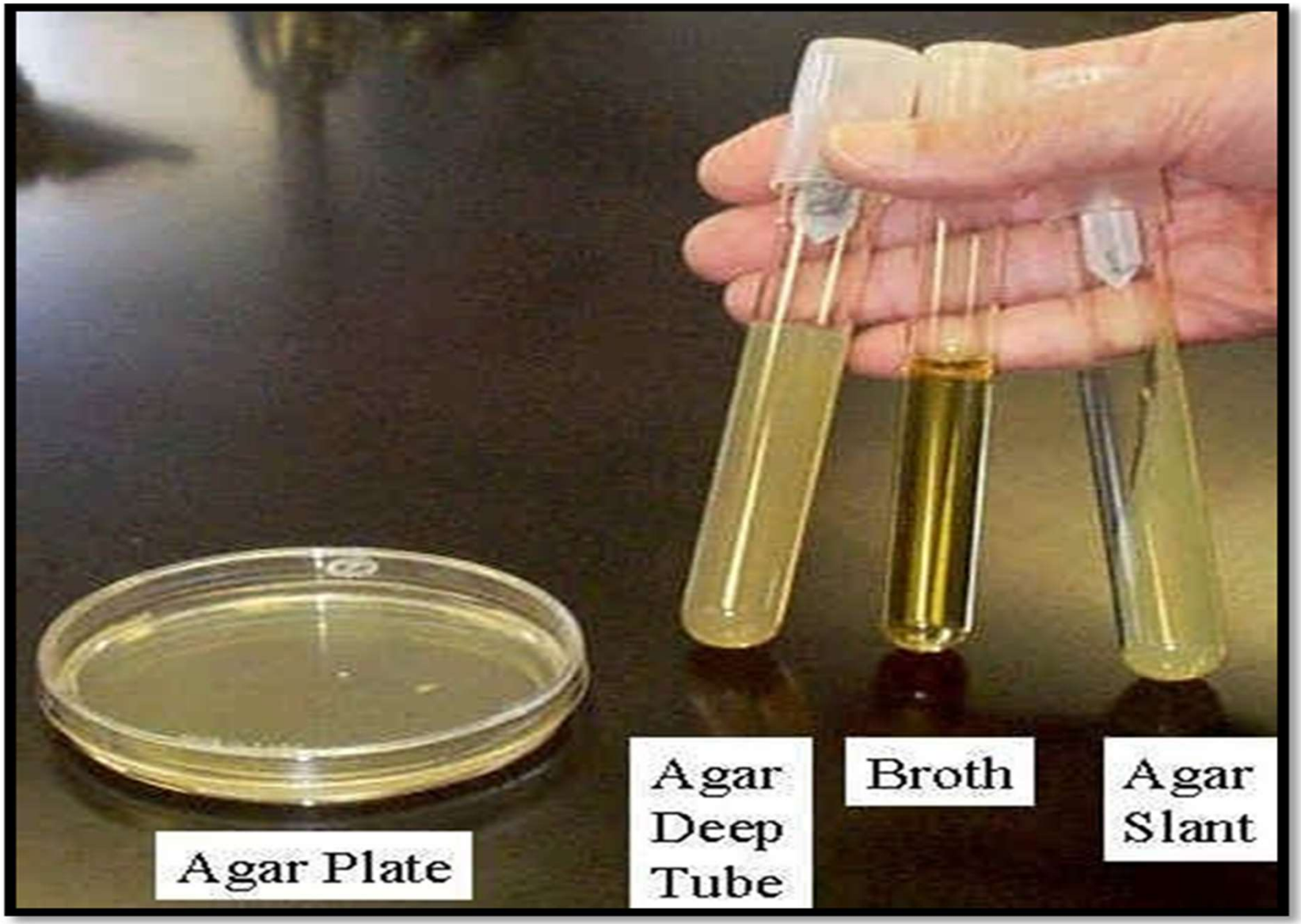
- Motile bacteria appear as diffused growth (with red color)
- Non-Motile bacteria appear as single line of growth (the original stabbed line with pin color)



3- **solid media**: such as nutrient agar or blood agar are used:

- For pure culture isolates.
- For storage of cultures.
- To observe specific biochemical reactions.
- For the surface growth of microorganisms in order to observe colony appearance.

- Solid media can be poured in to either a test tube or petriplate(dish).
- If a medium in test tube is allowed to harden in a slanted position , the tube is designated an agar slant.
- If the tube allowed to harden in an upright position , the tube is designated an agar deep tube.
- If the agar is poured in to petri plate , the plate is designated an agar plae.


Agar Plate
Agar Deep Tube
Broth
Agar Slant

Nutrient Broth

Component	Composition
Beef extract	10 g
NaCl	5 g
Peptone	10 g
Double distilled water	1 L

Nutrient Agar

TABLE 6.4 Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

Pure culture techniques

Spread – plate technique

Streak – plate technique

Pour – plate technique

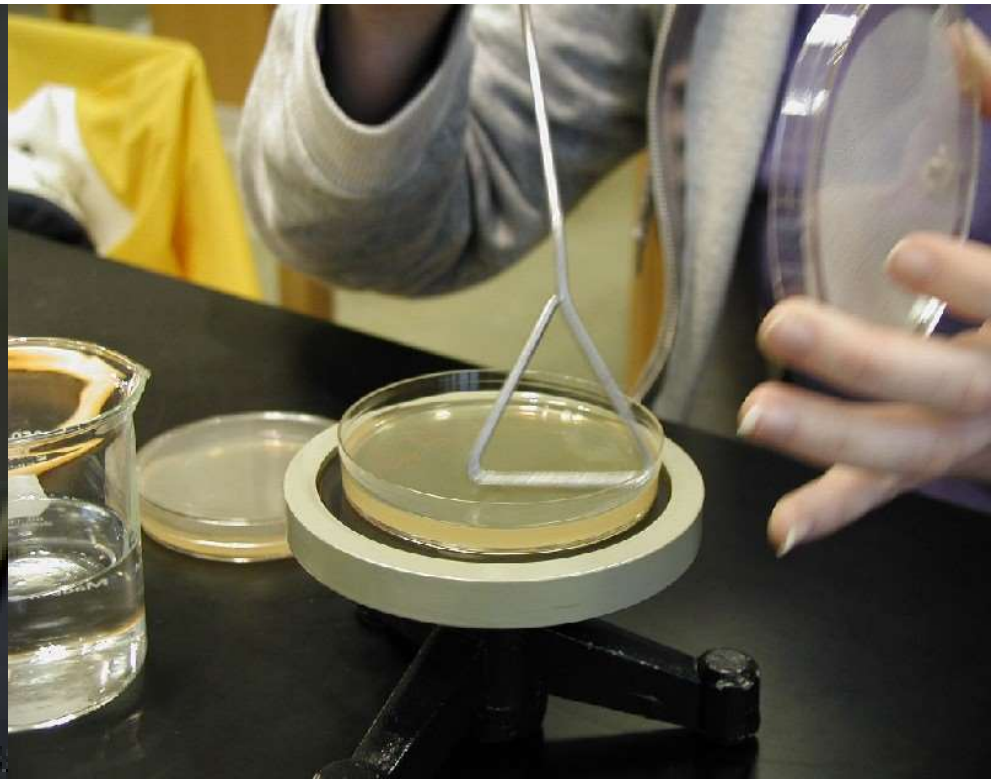
❑Spread – plate technique

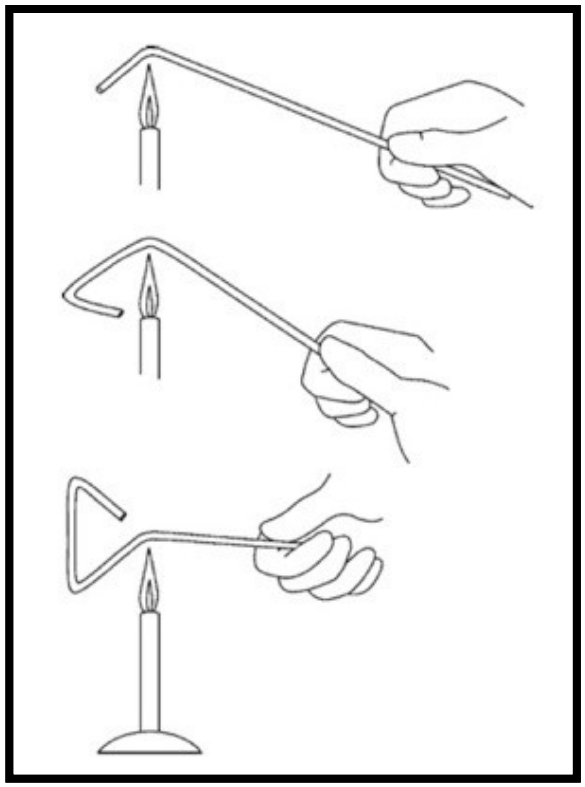
Is an easy , direct way of achieving a pure culture. In this technique a small volume of dilute bacterial mixture is transferred to the center of an agar plate and is spread evenly over the surface with sterile , L-shaped glass rod.

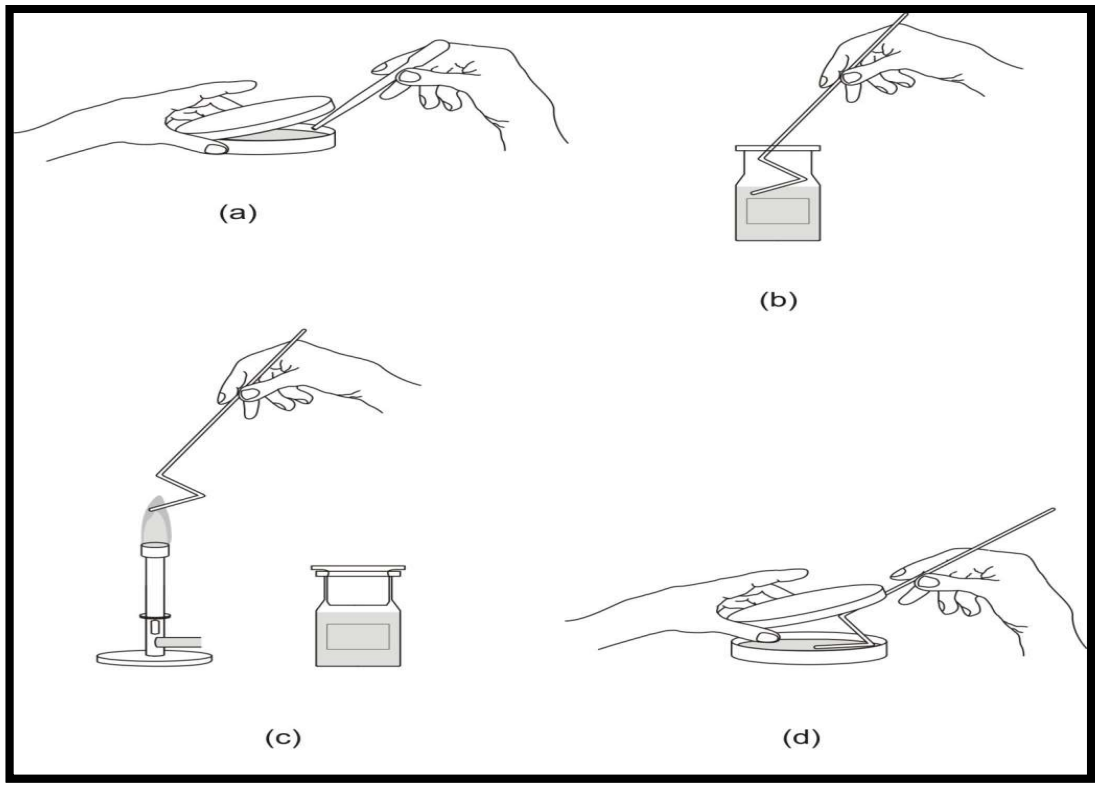
The glass rod is normally sterilized by dipping in alcohol and flamed to burn off the alcohol.

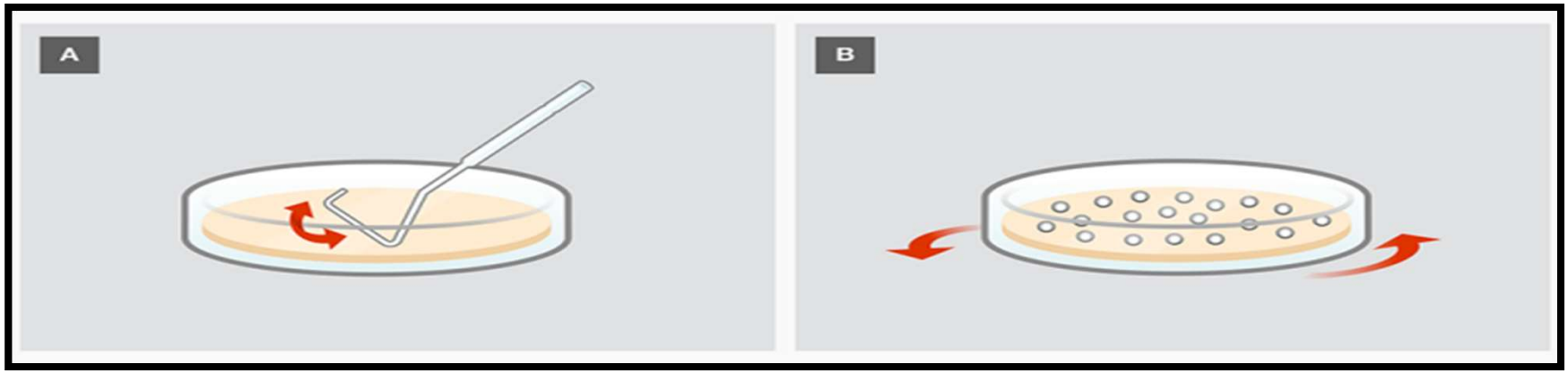
After incubation some of the dispersed cells developed in to isolated colonies.

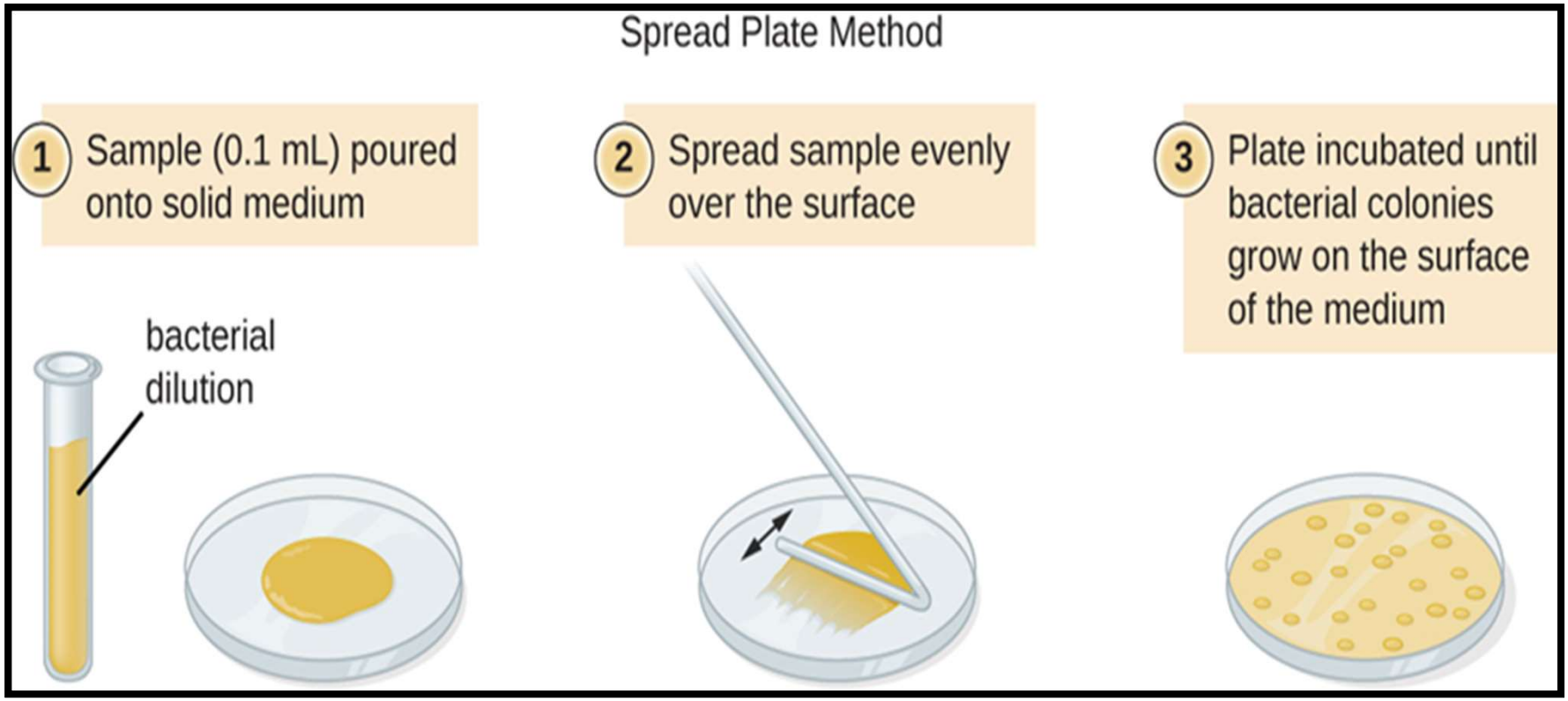
spread plate technique






(a)
(b)
(c)
(d)


A
B


Spread Plate Method
1 Sample (0.1 mL) poured onto solid medium
2 Spread sample evenly over the surface
3 Plate incubated until bacterial colonies grow on the surface of the medium
bacterial dilution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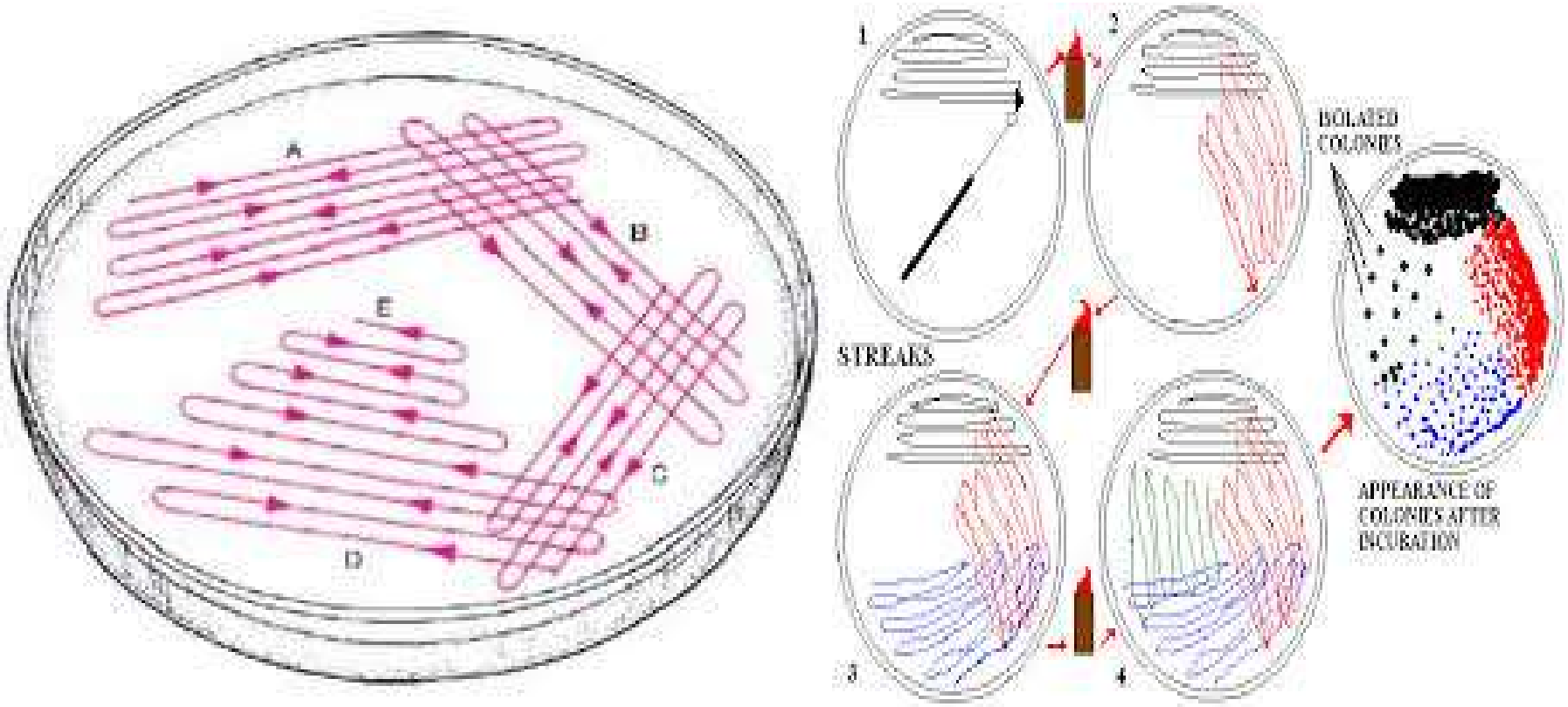
❑Streak – plate technique

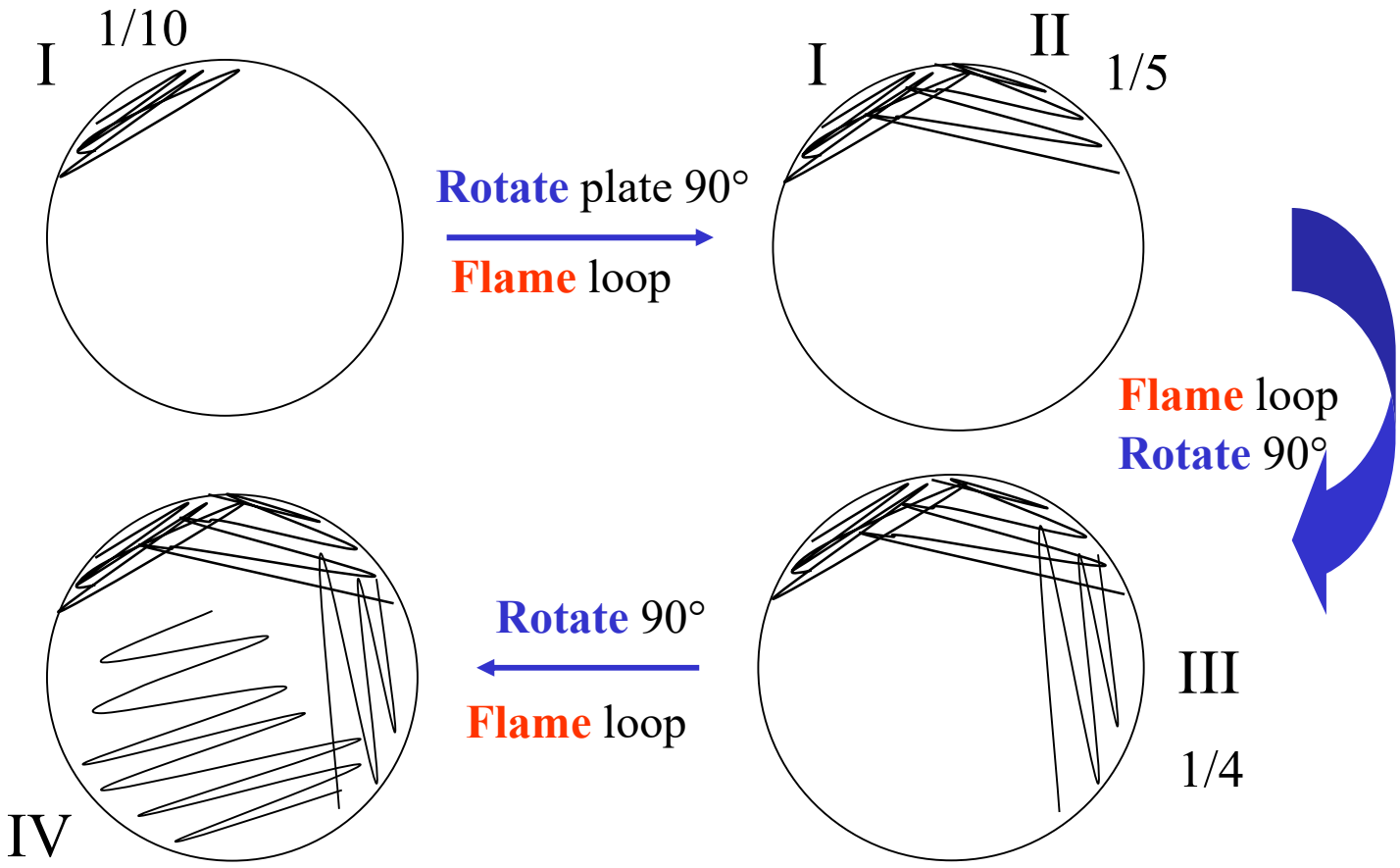
- Isolated , pure colonies can also be obtained by the streak plate technique .
- In this technique , the bacterial mixture is transferred to the edge of an agar plate with an inoculating loop and then streaked out over the surface in one of several patterns .
- At some point on the streaks , individual cells will be removed from the loop as it glides along the agar surface and will give rise to separate colonies.
- Again one assumes that one colony comes from one cell.

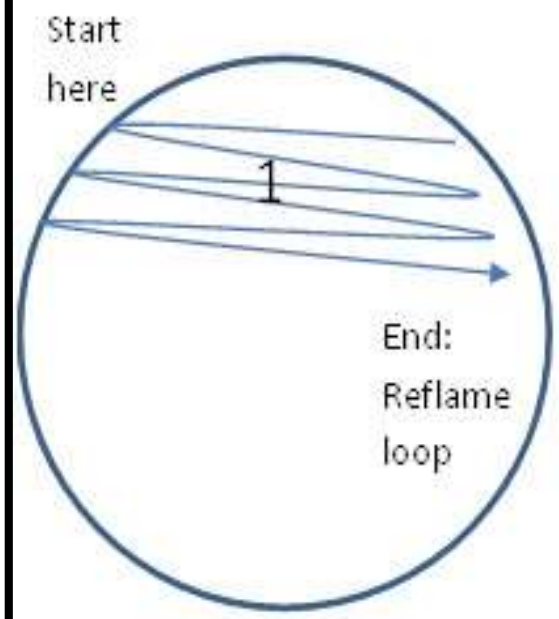
streak – plate technique



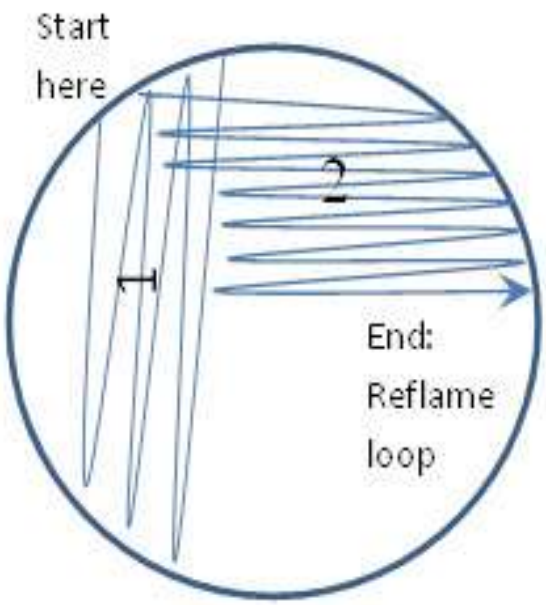
Streak-plate technique

four-area streak plate technique

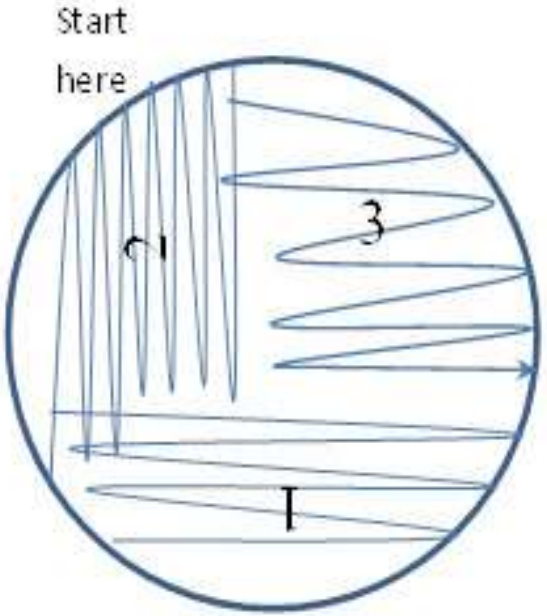




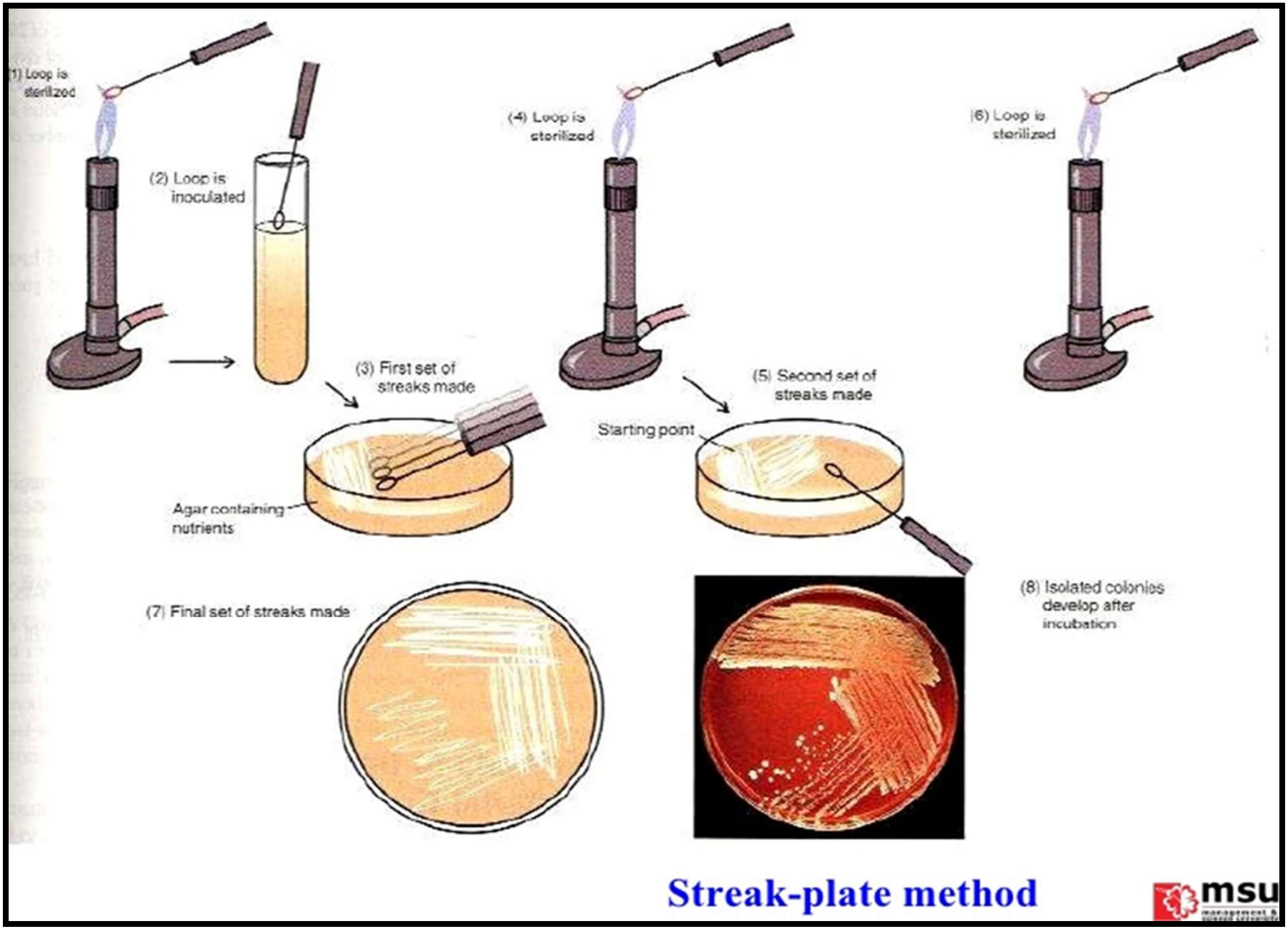
Step 1: Streak plate across the top sector, using continuous motion, but do not cross lines.



Step 2: Turn plate 90 degrees. Streak plate across the second sector, using continuous motion, making sure to cross into the first sector during the first streak or two.



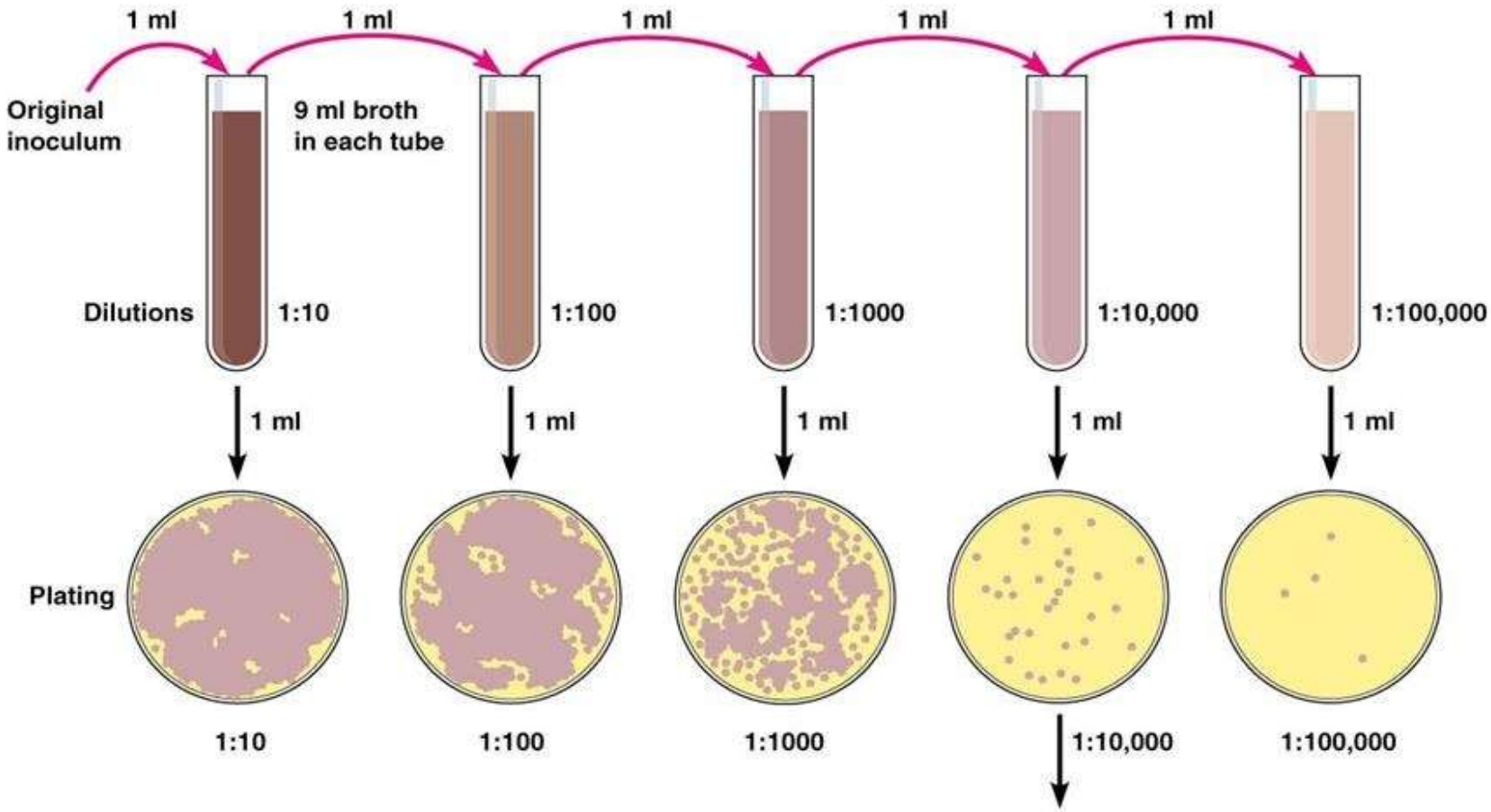
Step 3: Repeat step two, but streak across sector 3.



Streak-plate method

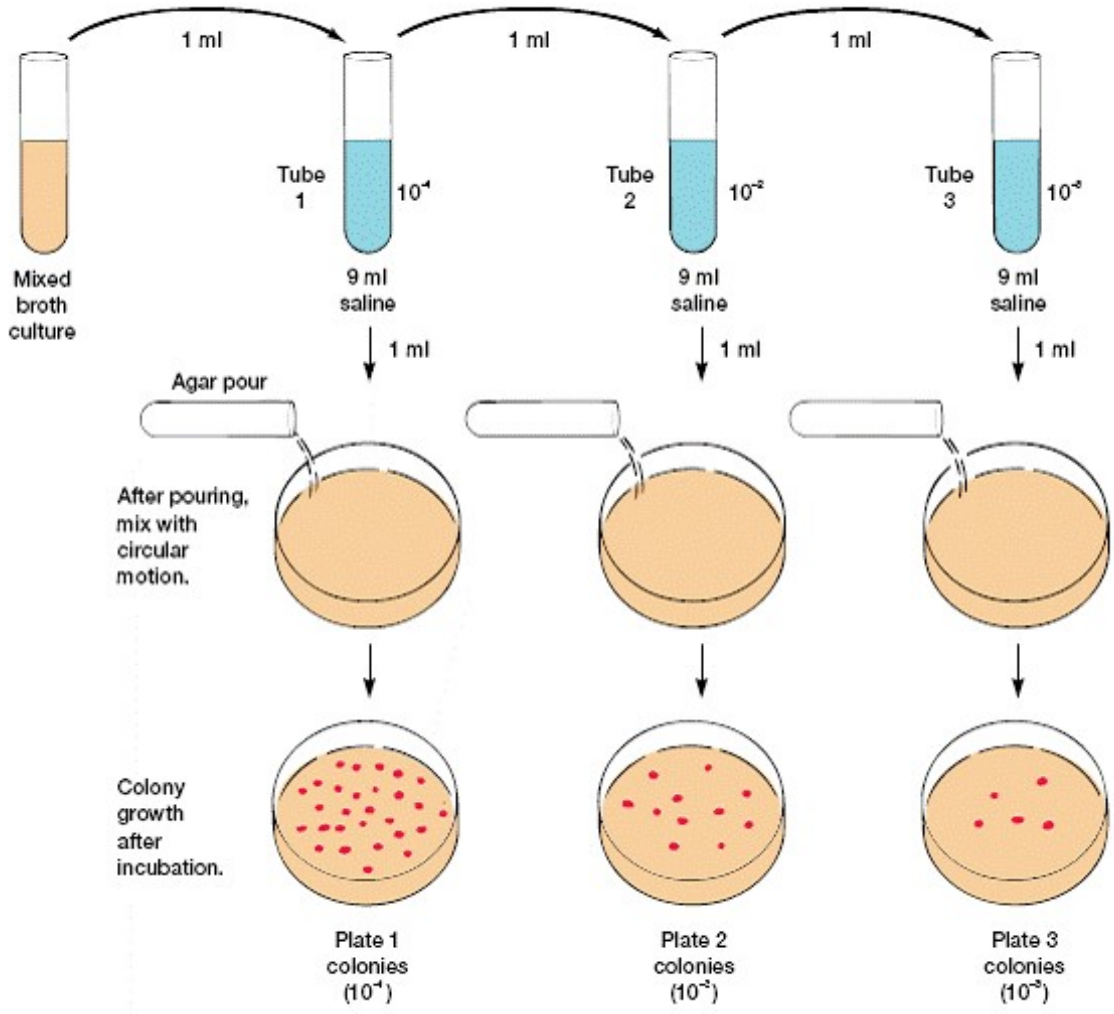
❑Pour – plate technique

- The original sample is diluted several time to reduce the microbial population sufficienty to obtain separated colonies upon plating.
- The small volume of several diluted samples are added to sterile petridishes and mixed with liquid tryptic soy agar that has been cooled to about 48-50C°
- After agar has hardened each cell is fixed in place and will form an individual colony if the sample is dilute enough
- To prepare pure culture , colonies growing on the surface or sub-surface can be inoculated in to fresh medium.



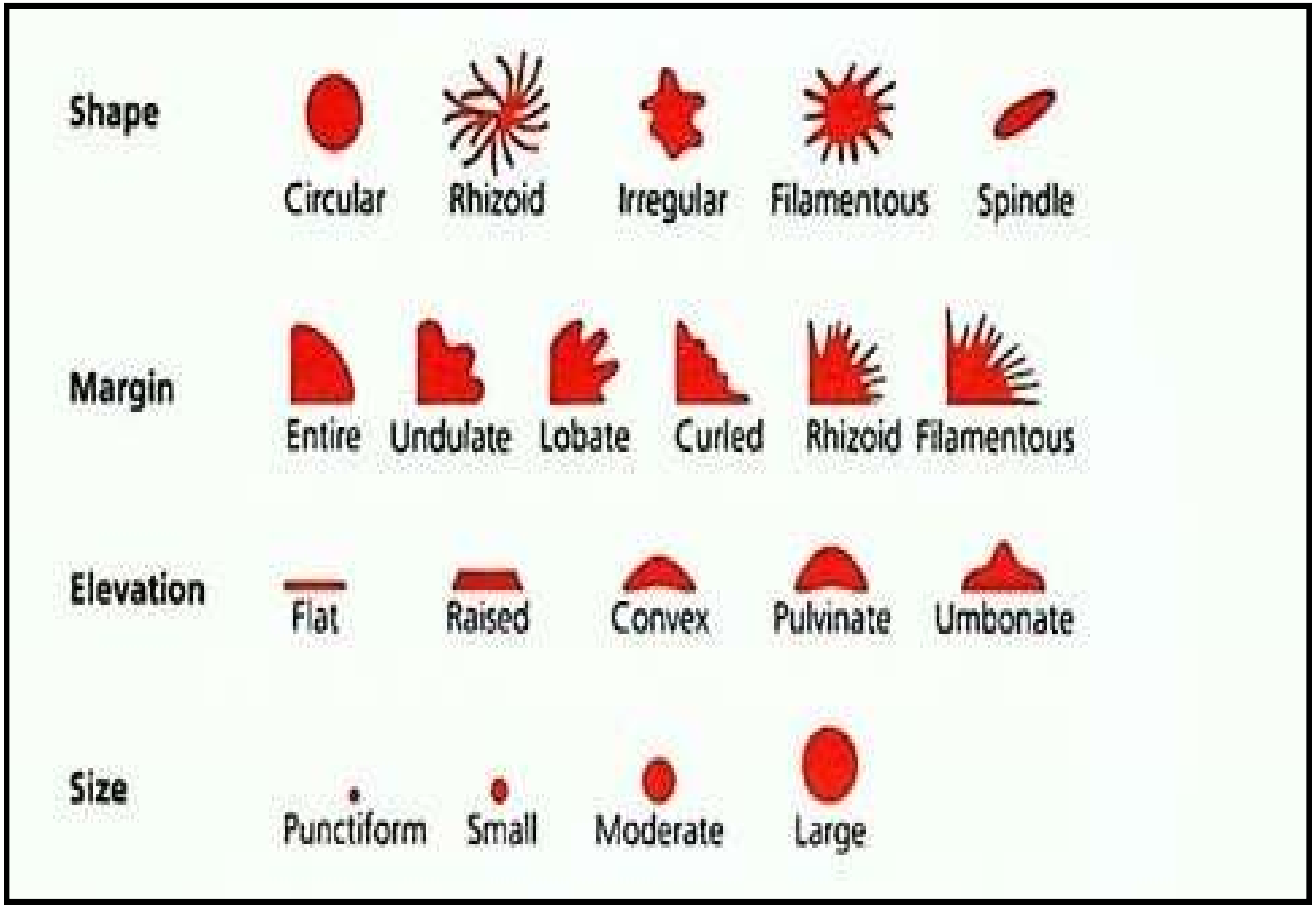
Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000 bacteria/ml in sample.)

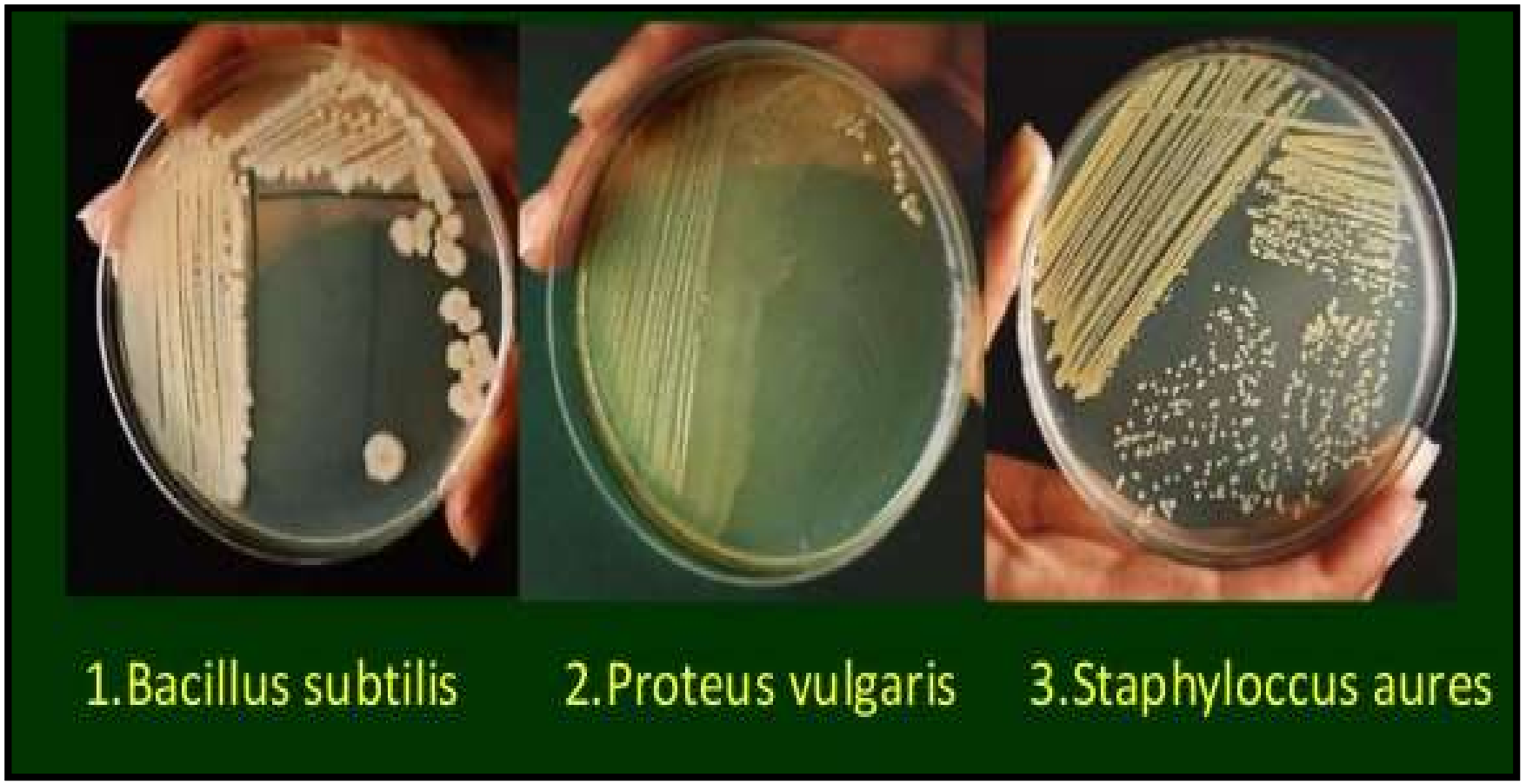
pour plate technique

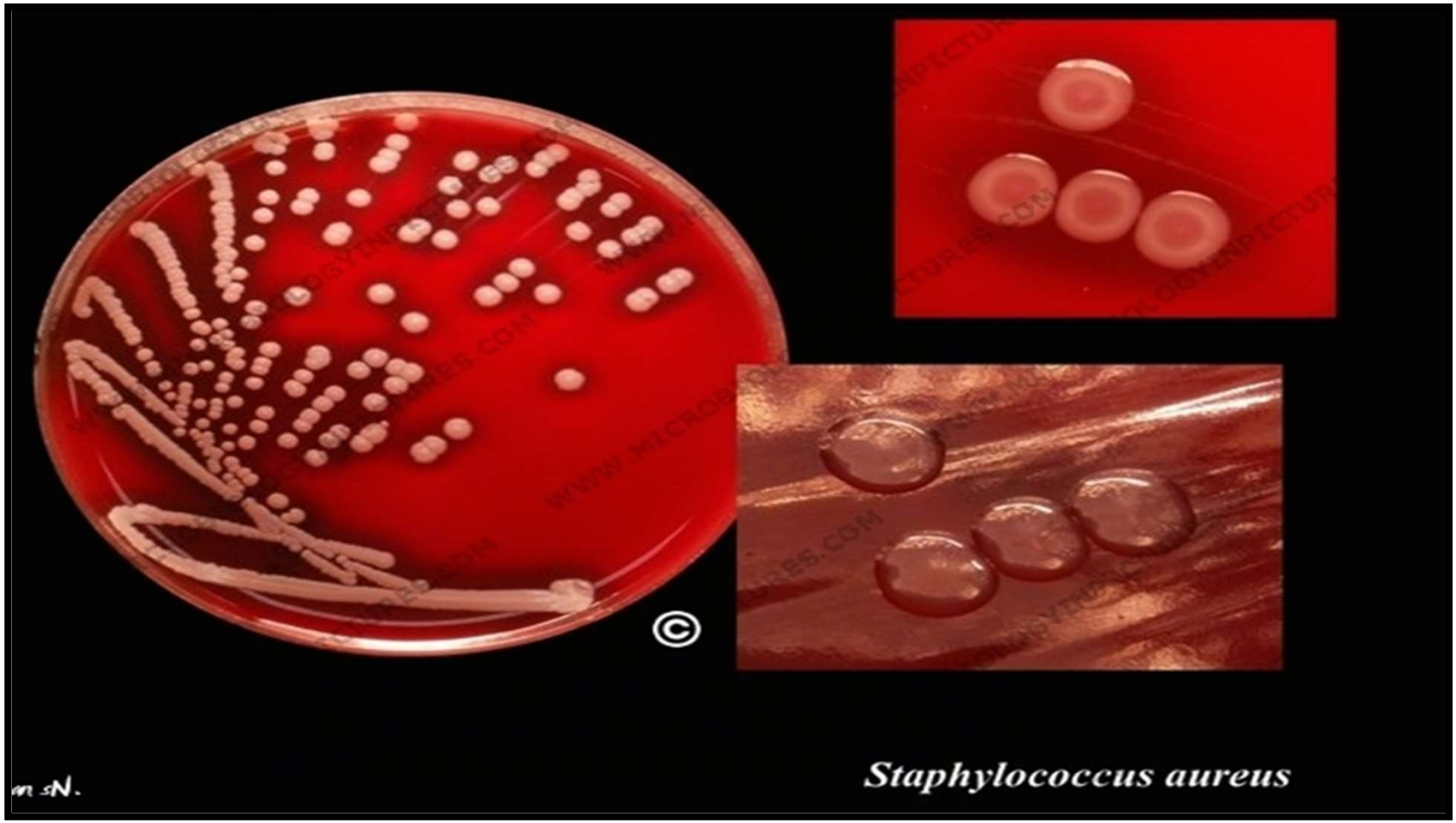


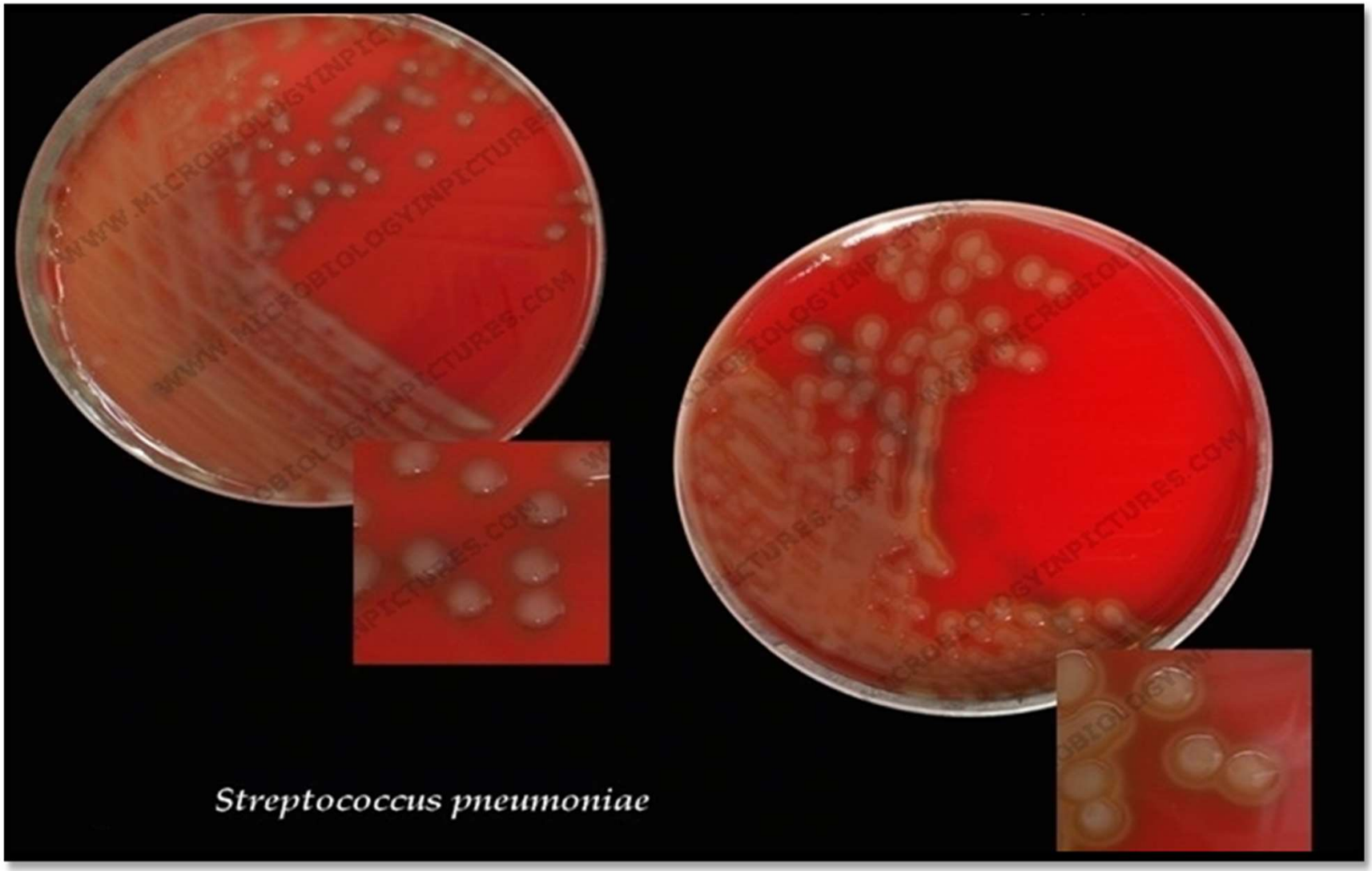
Bacterial Colony

- Is a large number of bacterial cells on solid medium , which is visible to the naked eye as a discrete entity .
- After incubation , the general form of the colony and the shape of the edge or margin can be determined by looking down at the top of the colony , the nature of the colony elevation is apparent when viewed from the side as the plate is held at eye level .


Shape
Circular
Rhizoid
Irregular
Filamentous
Spindle
Margin
Entire
Undulate
Lobate
Curled
Rhizoid
Filamentous
Elevation
Flat
Raised
Convex
Pulvinate
Umbonate
Size
Punctiform
Small
Moderate
Large


1.Bacillus subtilis
2.Proteus vulgaris
3.Staphyloccus aures


Staphylococcus aureus


Streptococcus pneumoniae