Bacteriology Lab-1

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Unit-10

MSC-II

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Bacteriology Lab-1

- Two ways to identify bacteria:

1. Microscopy

2. Culture

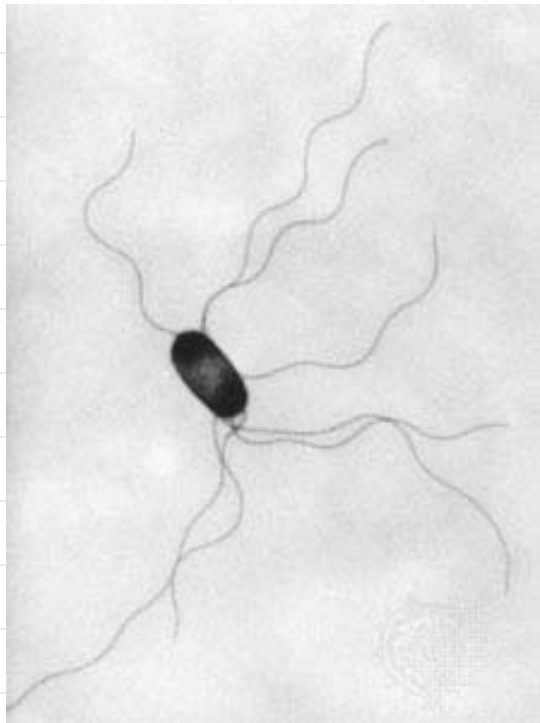
Microscopy

- Types of microscopy:

1. Native
2. Stained
   - Simple
   - Differential
3. Phase contrast
4. Dark field microscopy

1. Native Microscopy

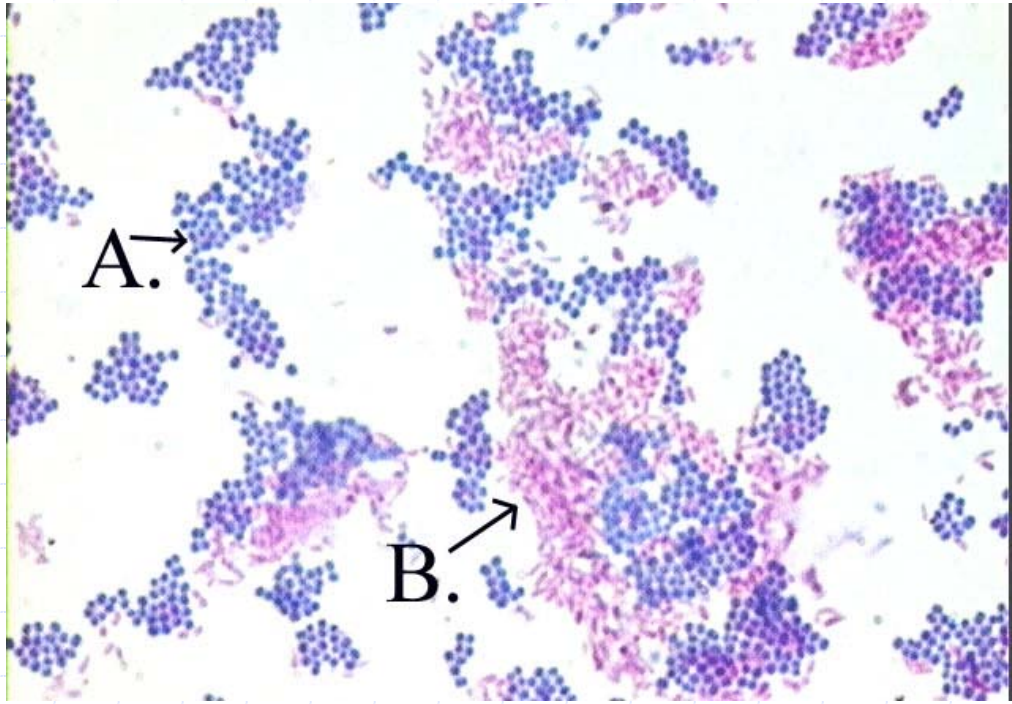
- Used to check motility of bacteria.
  - E.g. *Proteus;* very motile bacteria



2.Stained Microscopy

i. **Simple:** used to make bacteria visible.

- **Methylene Blue:** commonly used to observe shape and arrangement of bacteria



2.Stained Microscopy

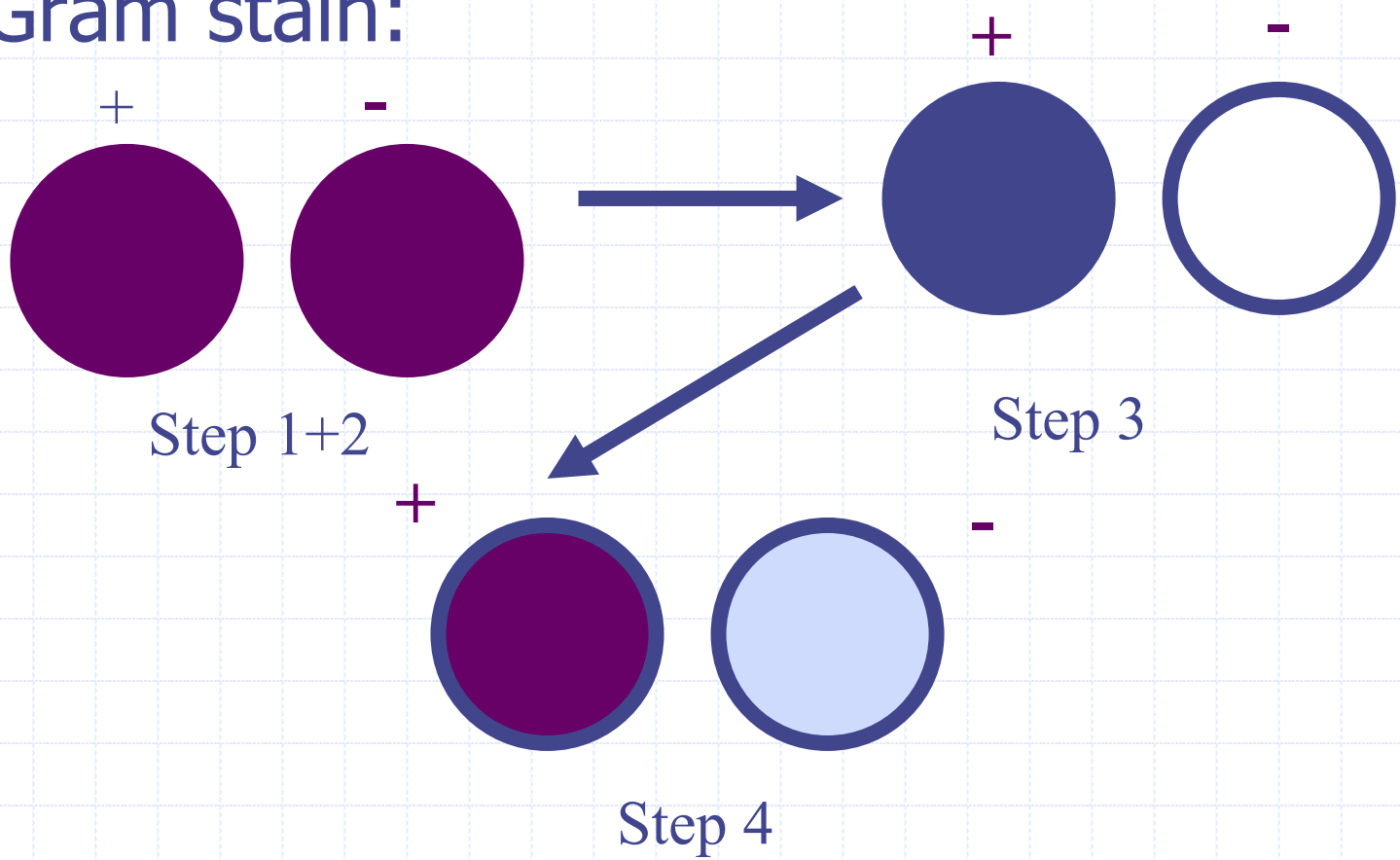
ii. Differential ~ Gram Stain

- 4 Steps:
  1. Add crystal Violet
  2. Add iodine

  Will give dark blue color for both Gram neg. & pos.
  3. Wash with ethanol ~ in this step, Gram negative bacteria will appear colorless since the stain will be washed away.
  4. Safranin: will stain Gram negative bacteria pink..

2.Stained Microscopy

- Gram stain:



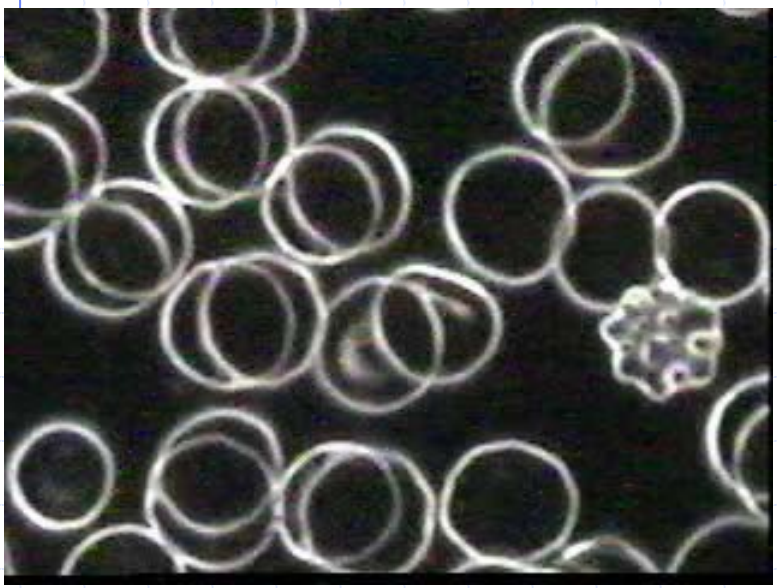
3. Phase Contrast

- Microscope that makes visible details of colorless transparent objects. It employs a method of illumination such that small differences of refractive index of the materials in the object cause differences of luminous intensity by interference.
- For direct observation of unstained material; used to observe living organisms.
- Not commonly used.

Phase Contrast

4.Dark Field

- A specialized technique which enhances the contrast of specimens, forming a bright image of the specimen superimposed onto a dark background. Small details such as flagella which cannot be seen under bright field illumination become visible.



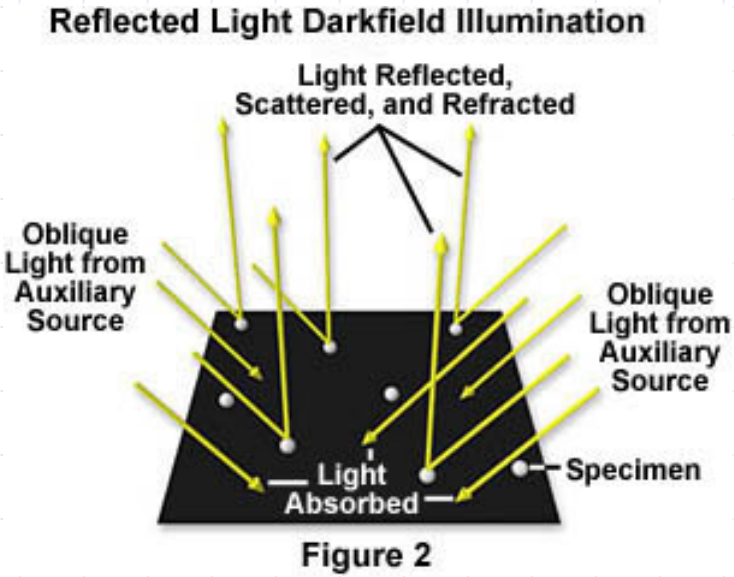


Figure 2

Culture

- It is process of growing cells in vitro ~ artificial environment.
- Types of Culture:
  - Liquid Medium
    1. Broth
  - Solid Medium
    1. Agar-agar
    2. Blood-Agar
    3. Chocolate Agar
    4. MacConkey -Agar
    5. Selective Plates

Liquid Medium

- Broth: is made by boiling food and then isolating the liquid. It contains nutrients needed for bacterial growth.
- In liquid media, bacterial growth goes through four stages.
- At a certain stage, when bacteria number reaches approx. 10^6 it can be visible.
- Used only to detect presence of bacteria. Not for Differentiation.

Liquid Medium

- Several types of broths:
  - **Nutrient broth:** rich in nutrients, used for bacteria that demand a lot of nutrients to grow.
  - Addition of 10 ml of blood in medium will further increase nutrient content.

Solid Medium

- First discovered by Robert Koch. Gelatin was added to liquid media in order to solidify it. Was not very successful since gelatin liquidified again after a while.
- Now a days, agar-agar is used instead. It does not have nutrients; only solidifies.
- Solid medium: usually contains ***1.5 – 1.7% agar.***

Advantages of Solid Medium

1. **Colony Morphology:** allows differentiation of species since different bacteria will form different colonies.

2. **Isolation of Cells:** allows isolation of cells from different colonies ~ pure cultures.

1.Agar-agar

- Agar is a gelatinous material, extracted from red algae (seaweeds), is most frequently used as a culture medium, especially for bacteria.

2. Blood-Agar

- General purpose non-selective medium is used in almost all routine culture procedures. It support the growth of most medically significant organisms (notable exceptions being *Haemophilus* and *Neisseria* species). It is a differential medium in the sense that the type of hemolysis produced by some species can be used for preliminary identification.

Blood-Agar

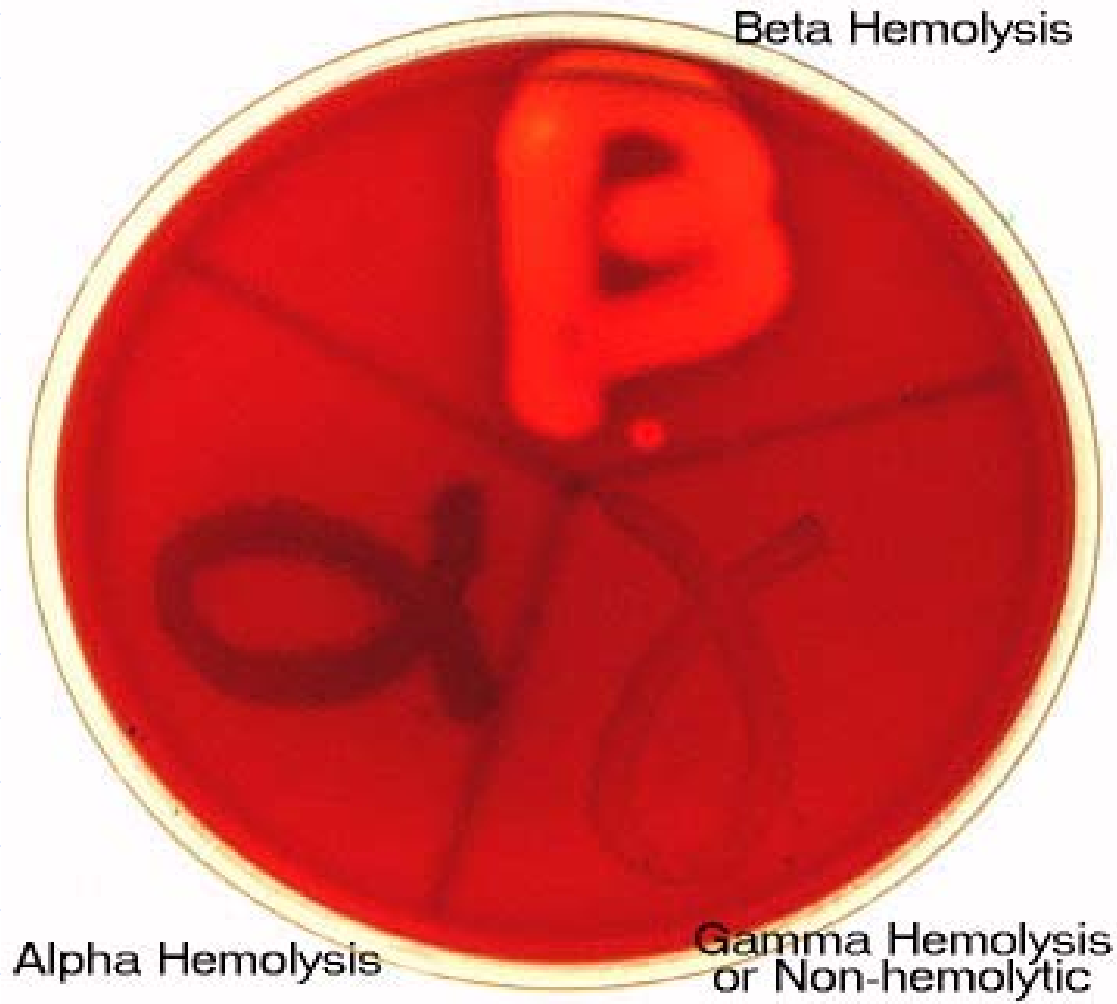
- Hemolysis types:

1. α : incomplete hemolysis
   - Green appearance.

2. β: complete hemolysis
   - Removes RBC's around the colonies

3. γ: no hemolysis

Hemolysis Appearance



3.Chocolate-Agar

- This medium is blood agar which has been **enriched** by lysing the red blood cells and making their contents available to organisms growing on the medium. Two important species: *Haemophilus influenza* and *Neisseria gonorrheae* will grow on chocolate agar but not on ordinary agar.

Chocolate-Agar

4.MacConkey-agar

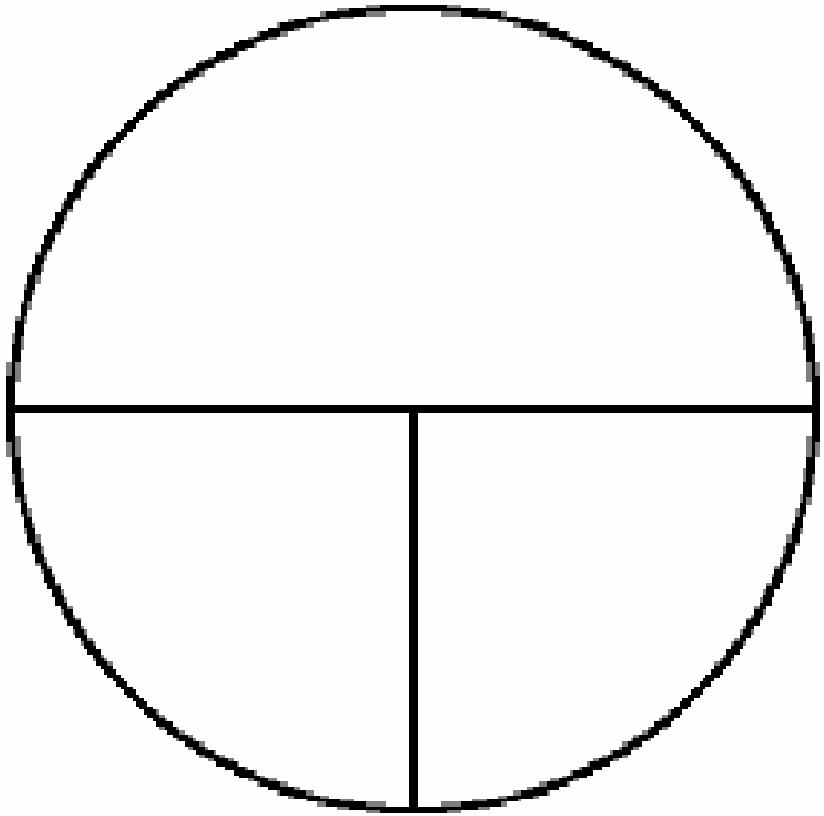
- This is a selective and differential medium for the cultivation of non-fastidious (easy to culture) gram negative bacilli. It lacks specific growth factors needed by some organisms and contains inhibitory substances which prevent the growth of others. Its purplish color is provided by neutral red, a pH indicator, which causes organisms which grow on the medium and ferment lactose to appear as red colonies. Rarely, MacConkey agar is used with a sugar other than lactose (e.g., MacConkey Sorbitol agar to identify *E. coli* O157:H7).

MacConkey-agar

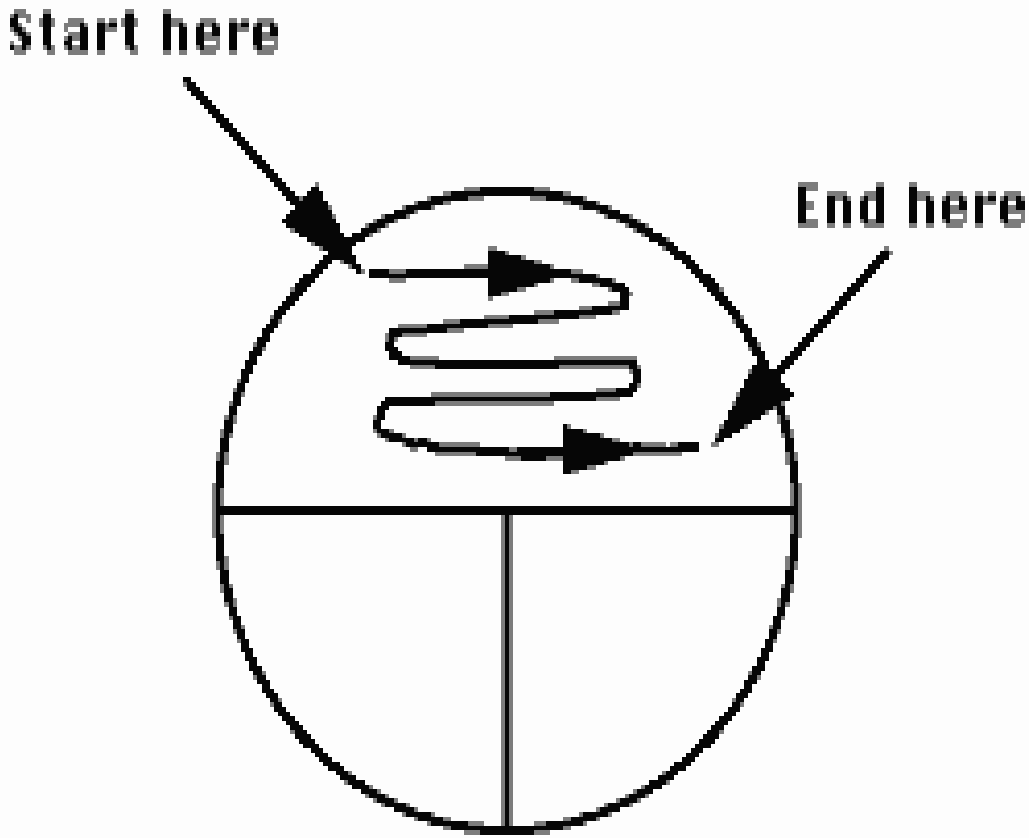
5. Selective Plates

- Used when the percentage of pathogen is low. E.g. Feces.
- In this technique, the sample is cultured on desoxycholate citrate agar (DCA), which suppress the growth of normal flora while permits the growth of pathogens. Thereby enabling identification of pathogen.

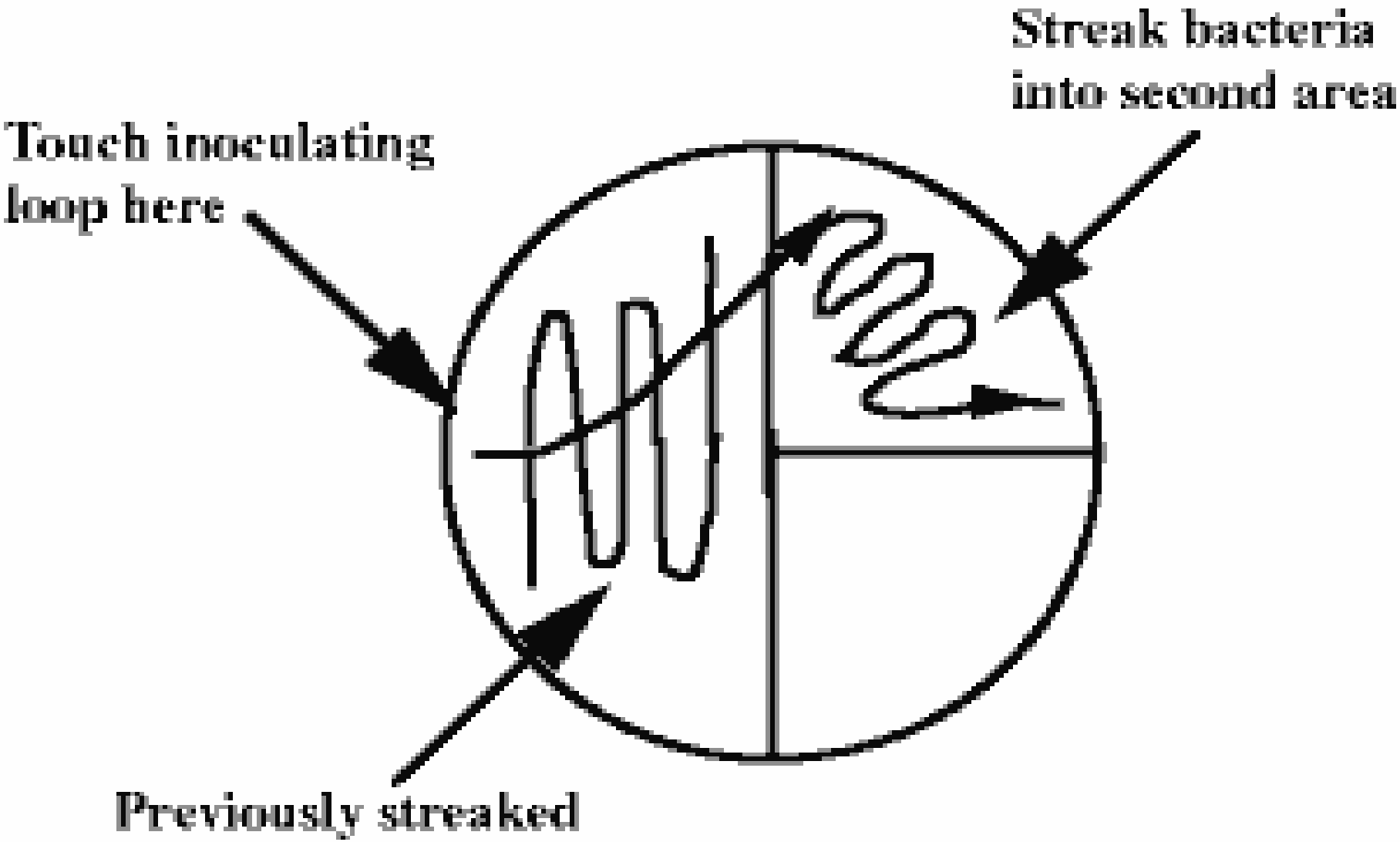
Inoculation Technique



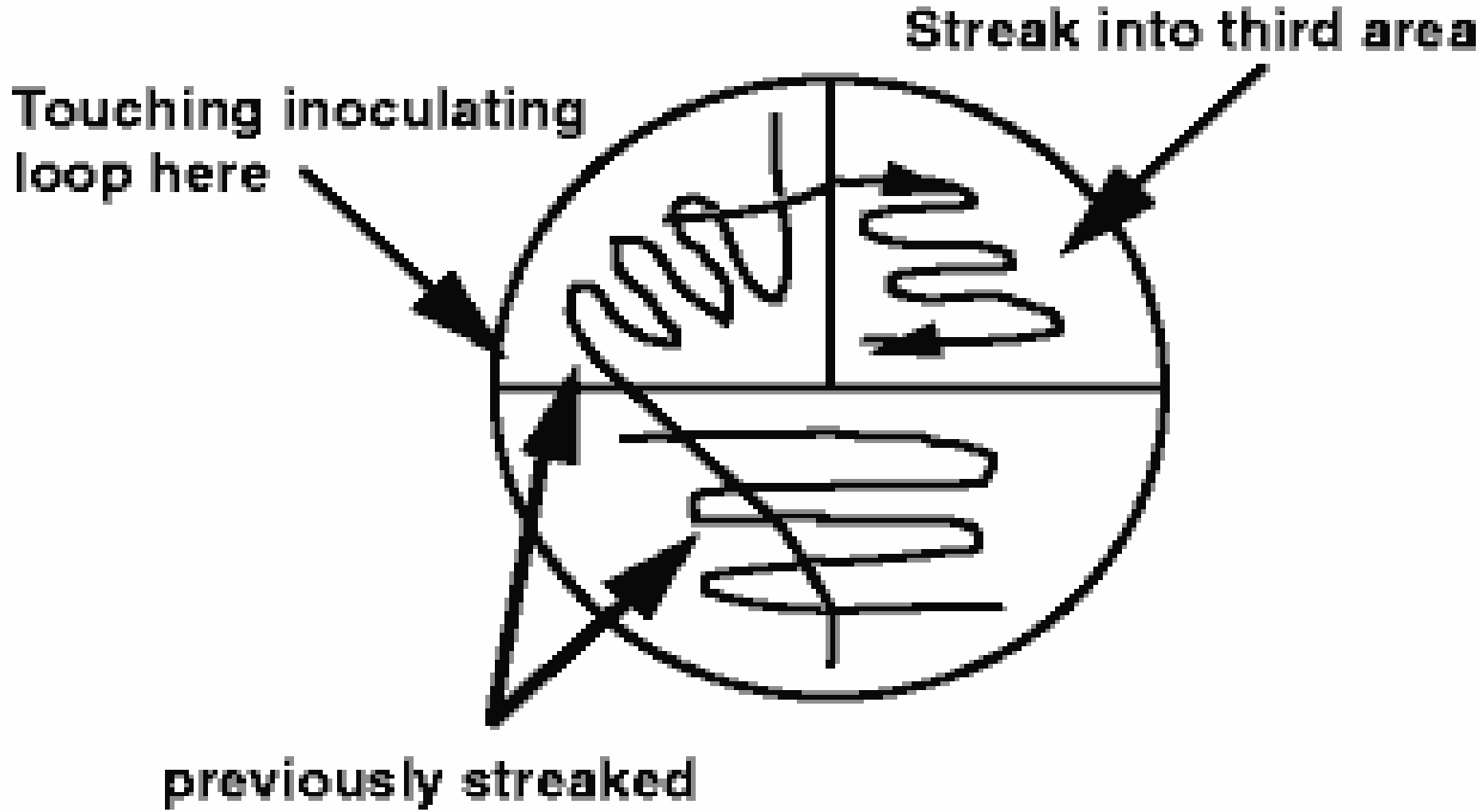
Step 1. Draw a "T" on the bottom of your petri dish as shown.



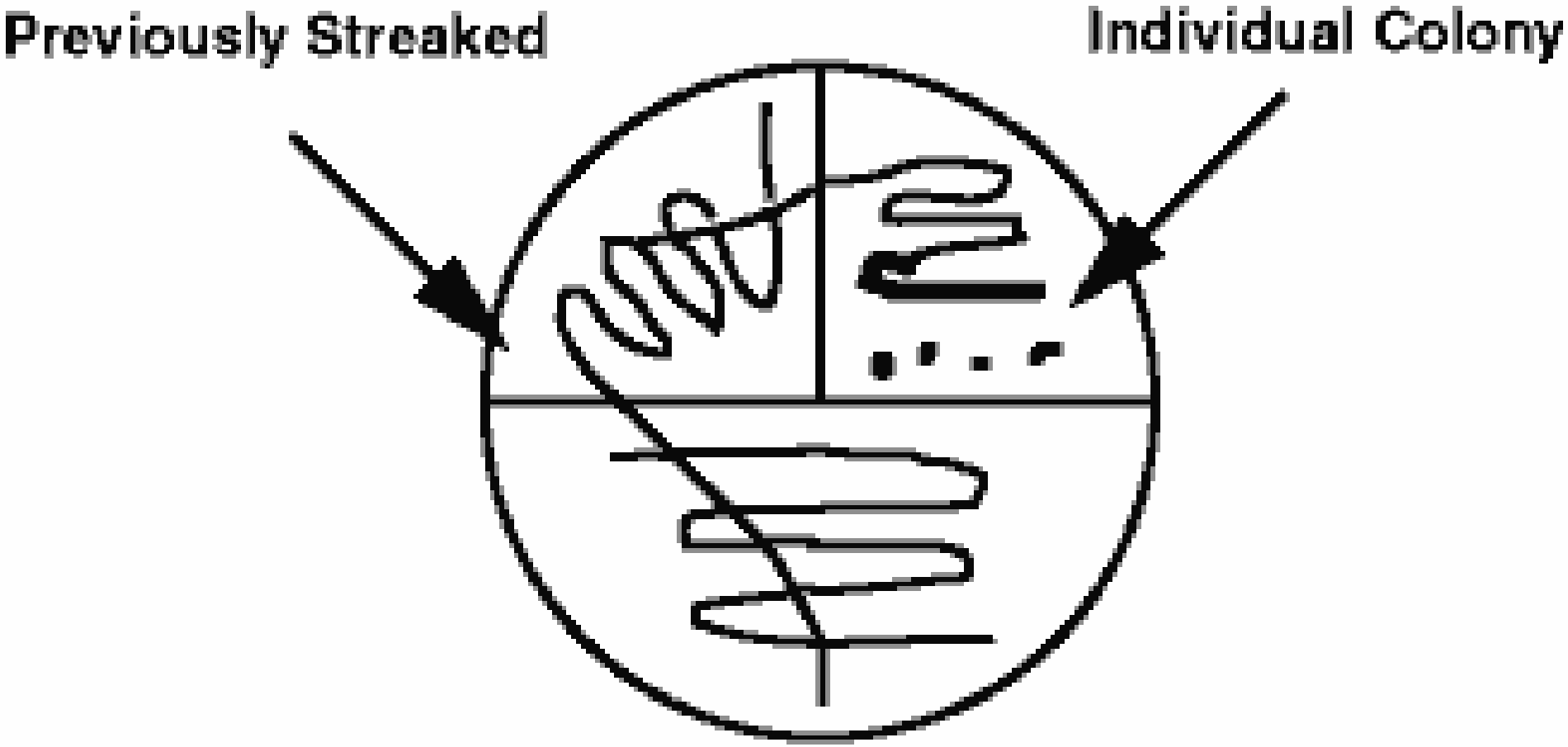
Step 2. Touch the inoculating loop to the upper left hand corner and then move it across the agar from left to right as shown.



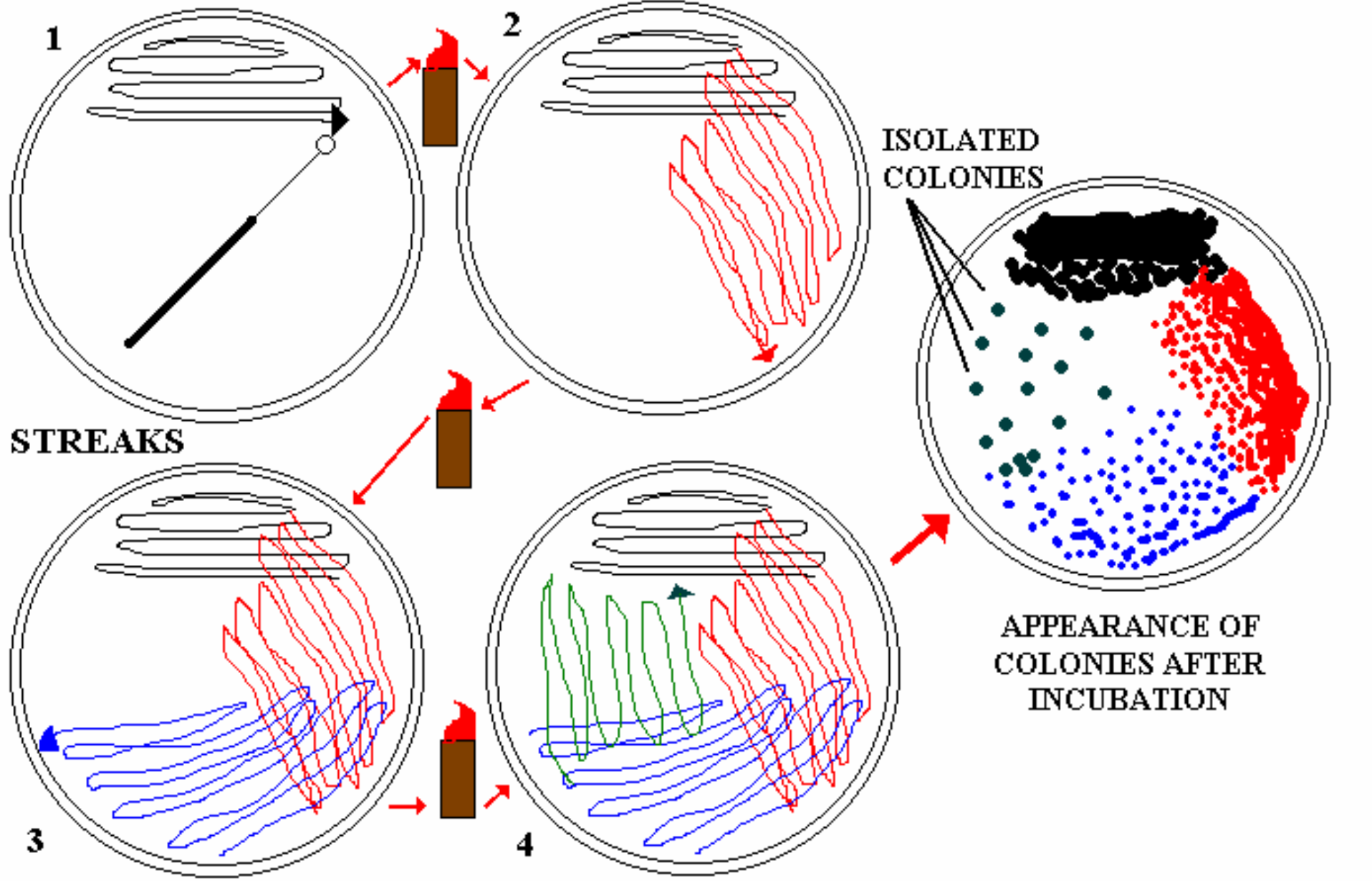
Step 3. Touch the loop to the area previously streaked and then move the loop across the agar as shown.



Step 4. Touch the loop on the previously streaked area. Then move the loop across the agar onto the third area as shown.



Step 5. Incubate the streak plate until you can see individual colonies.


1
2
ISOLATED
COLONIES
STREAKS
3
4
APPEARANCE OF
COLONIES AFTER
INCUBATION