Salahaddin University \Erbil
College of Agriculture
Plant protection Department
4th class

Phytobacteriology\Practical

Medium for isolation of bacteria

2nd lab.

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outlines

- Types of media.
- Components of some media.
- Isolation from plant materials and others.

Types of media

- General media
- Semi-selective (differential)media
- Selective (specific) media

- **General-Purpose Media**

- Basal media also called general-purpose media are basically simple media that support the growth of most non-fastidious bacteria.
- Peptone water, Nutrient broth, and Nutrient agar (NA) are considered basal mediums.
- These media are generally used for the primary isolation of microorganisms.

- **Differential media** contain compounds that allow groups of microorganisms to be visually distinguished by the appearance of the colony or the surrounding media
- **Selective or specific media** contain ingredients that inhibit the growth of some organisms but allow others to grow.

Some examples of different media :

a)Nutrient agar **(NA)**

b) Nutrient gelatin **(NG)**

c) Nutrient glucose agar **(NGA)**

d)Yeast extract dextrose CaCo3 **(YDC)**

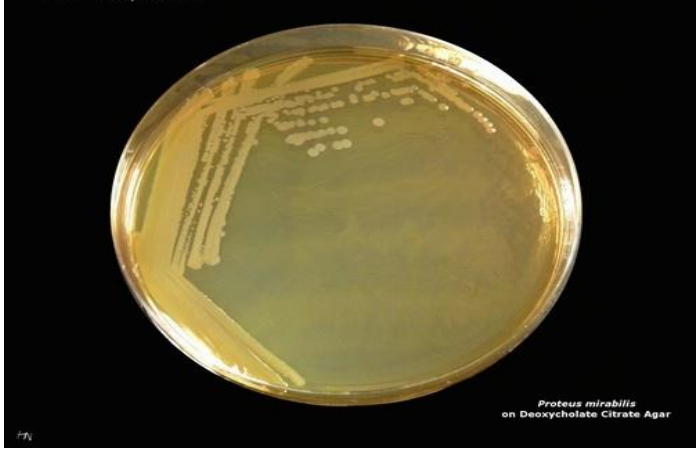
e) Nutrient broth yeast extract agar **(NBY)**

f)) King's B medium **(KB)**

g) MacConkey Agar

- All media should be autoclaved at 121ºC for 15 min, at 15 bar, and adjusted to pH 7.2.

Nutrient agar (NA)



- Beef extract 3.0 g
- Peptone 5.0 g
- Agar 15.0 g
- Distilled water 1.0 L
- Prepared formulations of NA are commercially available and usually preferred.
- **Nutrient broth** is NA without **agar.**

Nutrient gelatin (NG)

- Beef Extract 3.0 g
- Tryptone 5.0 g
- Gelatin 40 g
- dH2O 1 L
- pH 7.0

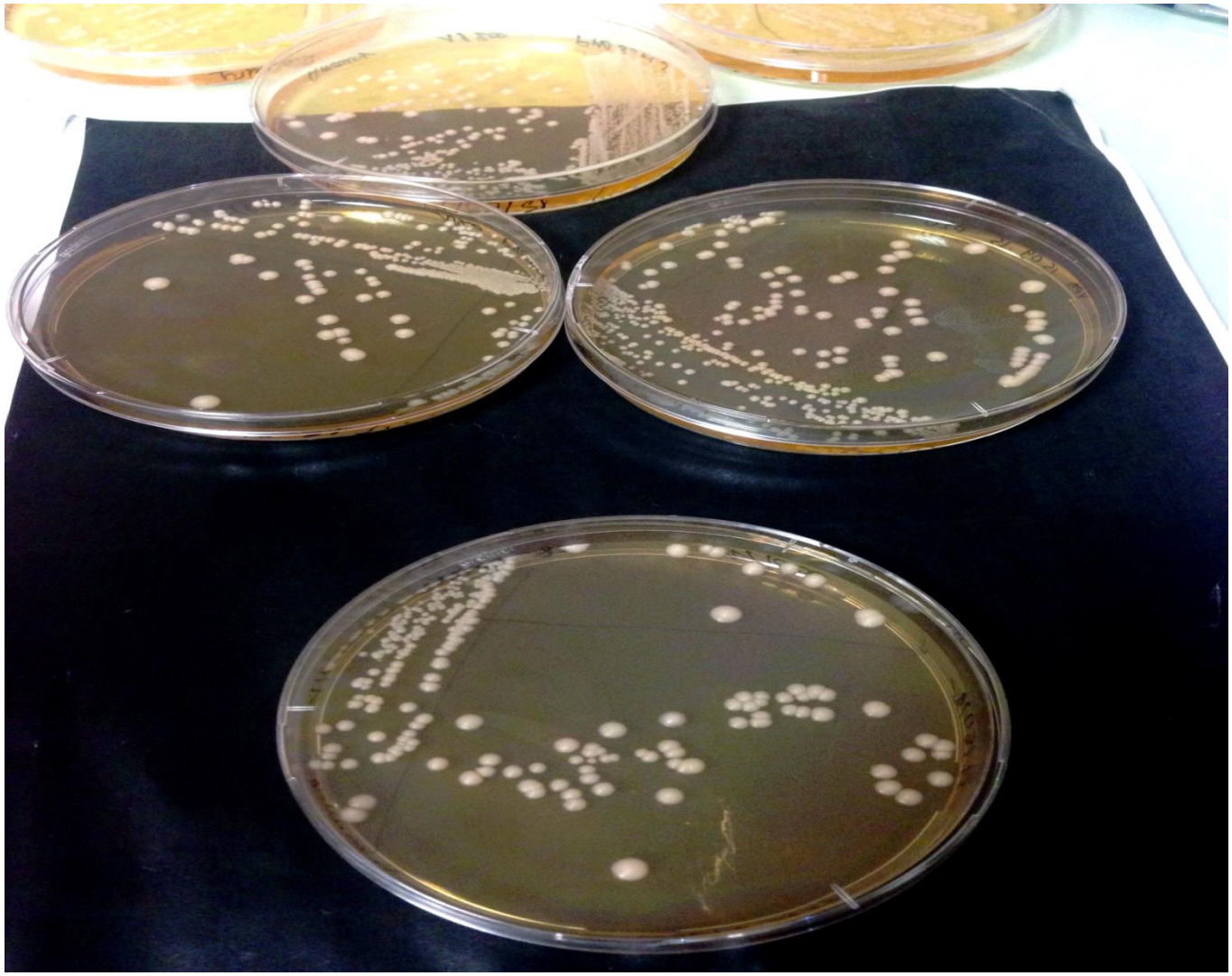
Nutrient glucose agar (NGA)

	g\L
Beef extract	3.0
Peptone	5.0
Glucose	2.5
Agar	15.0

- Or adding glucose to commercial nutrient agar

Yeast extract-dextrose-calcium carbonate agar (YDC)

- Yeast extract 10.0 g
- Dextrose 20.0 g
- Calcium carbonate (light powder) 20.0 g
- Agar 15.0 g
- Distilled water 1.0 L
- Yeast Extract Calcium Carbonate Glucose Agar is used for the isolation and cultivation of ***Erwinia*** **species**

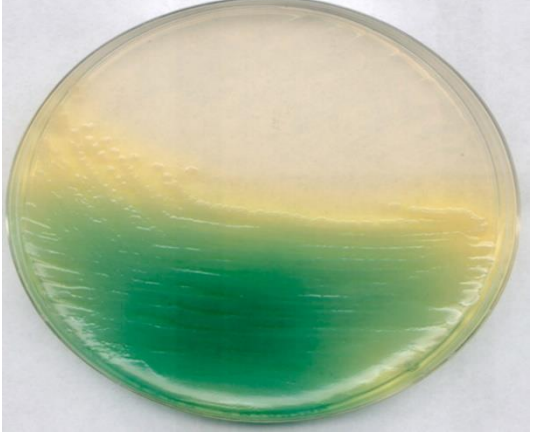


Yeast extract-dextrose-calcium carbonate agar (YDC)

Nutrient broth yeast extract agar (NBY)

	g\L
Nutrient broth	8.0
Yeast extract	2.0
K2HPO4	2.0
KH2HPO4	0.5
Glucose	2.5
Agar	15.0

King’s B medium (KB)



- Proteose peptone (Difco No.3/Oxoid L46) 20.0 g
- K2HPO4 1.5 g
- MgSO4. 7H2O 1.5 g
- Agar 15.0 g
- Glycerol 10.0 ml
- Distilled water 1.0 L
- This general purpose medium is particularly **useful for detecting fluorescent Pseudomonads**, but other bacteria also grow easily on it.

- **Kings B Broth (KBB)** these components without agar

MacConkey Agar (selevtive media)

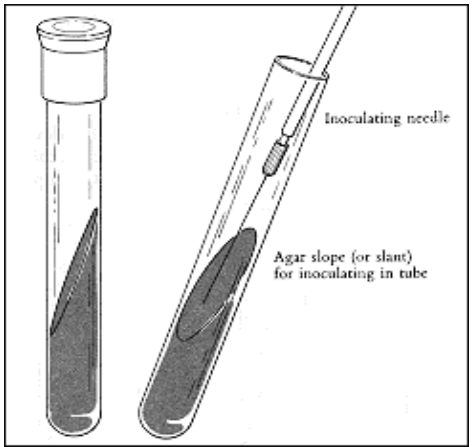
- Pepton 17gm
- Proteose peptone (meat and casein) 3gm
- Lactose monohydrate 10gm
- Bile salts 1.5gm
- Sodium chloride 5gm
- Neutral red 0.03gm

PREPARATION OF SLANTS

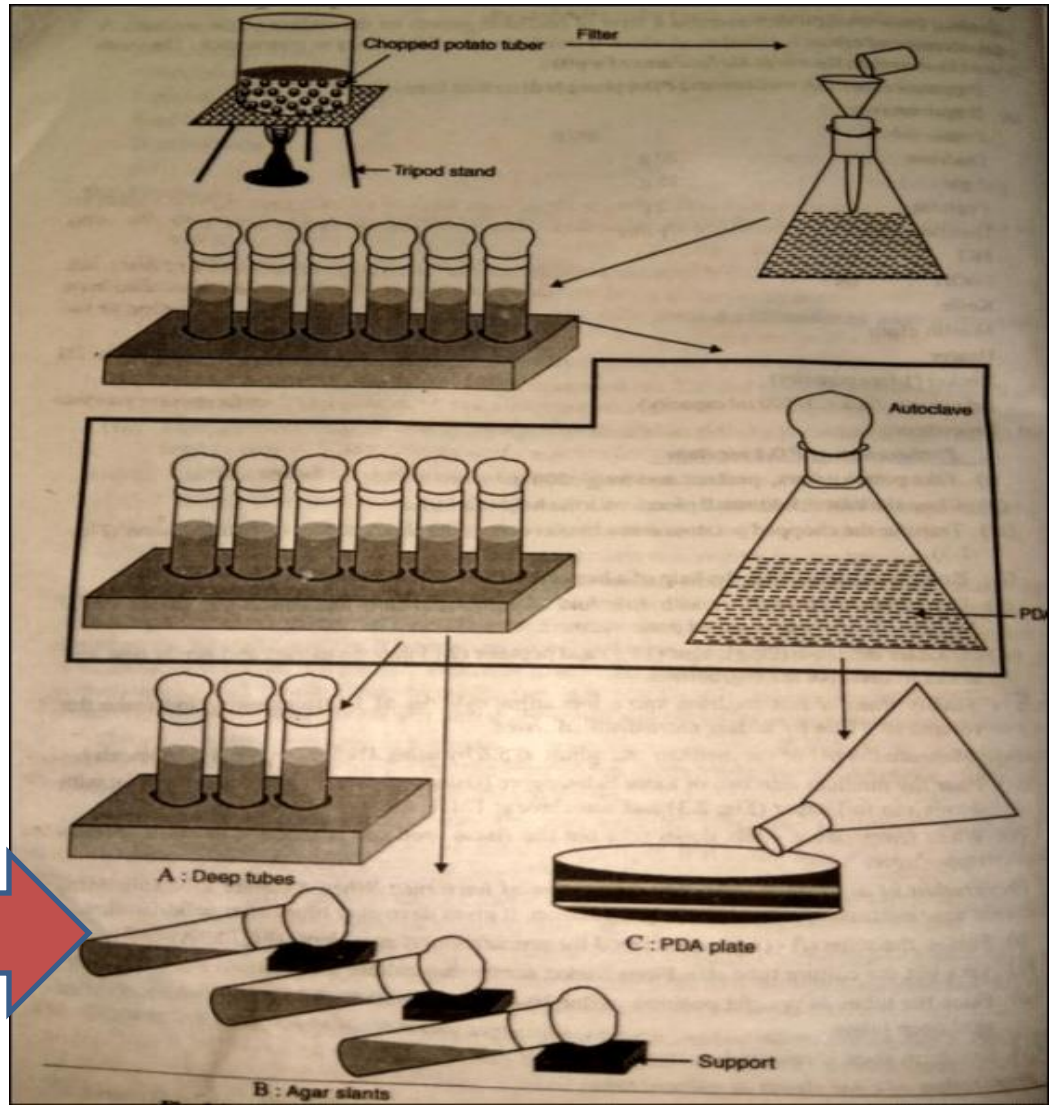
- The media slants are used to subculture and maintain the bacterial cultures. It is always easy and safe to maintain the bacterial cultures on media slants.

Procedure

- Pour the media in test tube at one-third of test tube length or volume and plug with a cotton plug.
- Put the media test tubes in a sterilization basket in an upright position and sterilize in the autoclave under pressure at 15 pounds for 30 minutes.
- Remove the test tubes after sterilization and keep them in a slanted position by using a wooden bar so as to get slants. When the media is solidified in the slanted positions, collect the slants and store in freezer or use as per requirements.



PREPARATION OF SLANTS



slants

Isolation

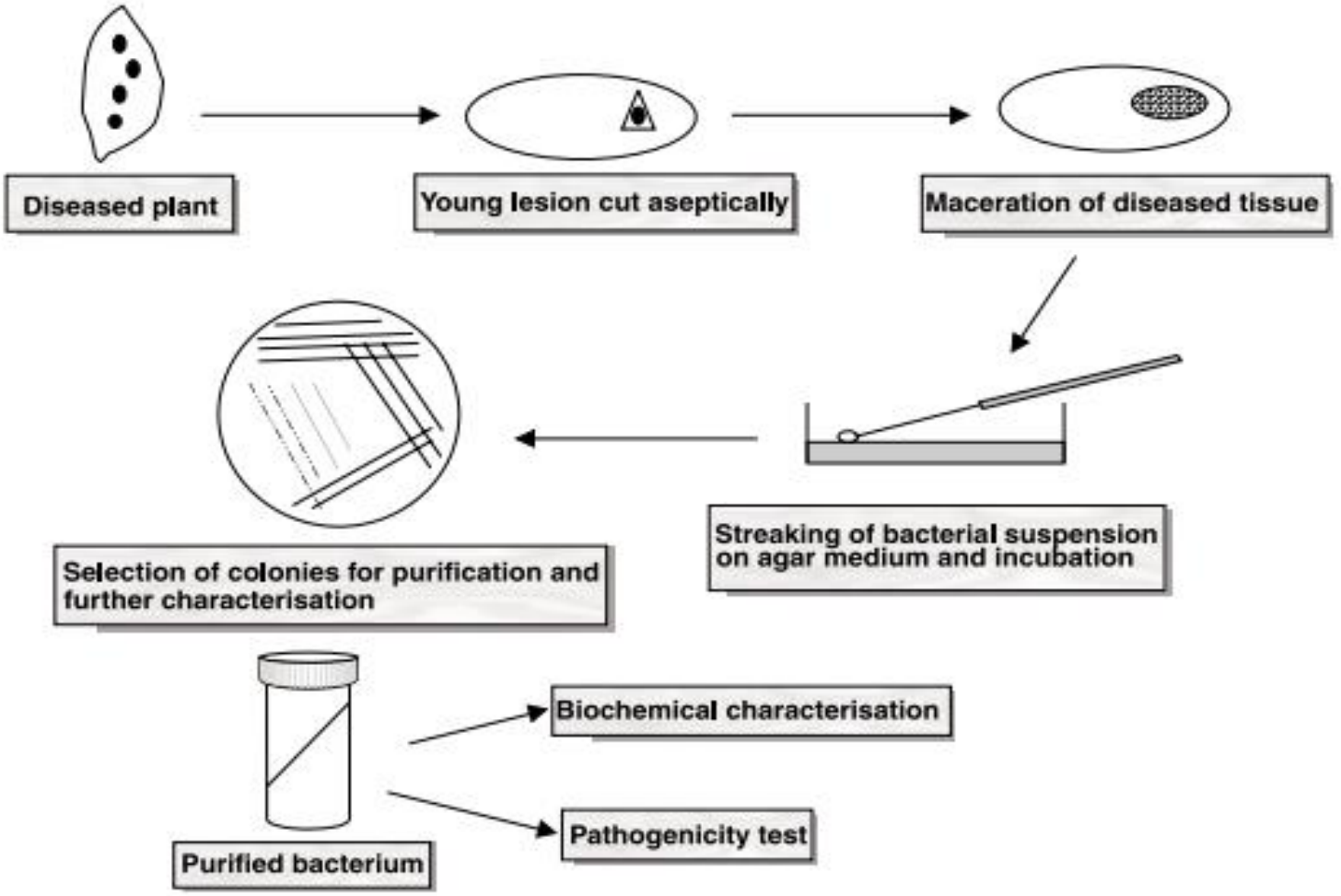
1- From diseased plant sample

2- From disease field soil sample

3 -From field water sample

4- From infected seed material

Isolation



Steps to isolate and identify phytopathogenic bacteria.

Isolation

- Sterilize your loop and put the loop to the agar surface against the far end of your first streak. Repeat by dragging back and forth.
  - Do not drag into the center of your plate.
  - You should be able to see the faint indentations of your streaking line on the agar surface.
- Using a sterile loop, repeat the procedure on your second streak.
- Using a sterile loop, repeat the procedure on your third streak. Zigzag the last part into the center of the plate.
  - You should end up with isolated colonies somewhere in your last streak.
- Place your completed plates agar side up on the incubation rack on the front desk in the incubate section.

Streak plate method

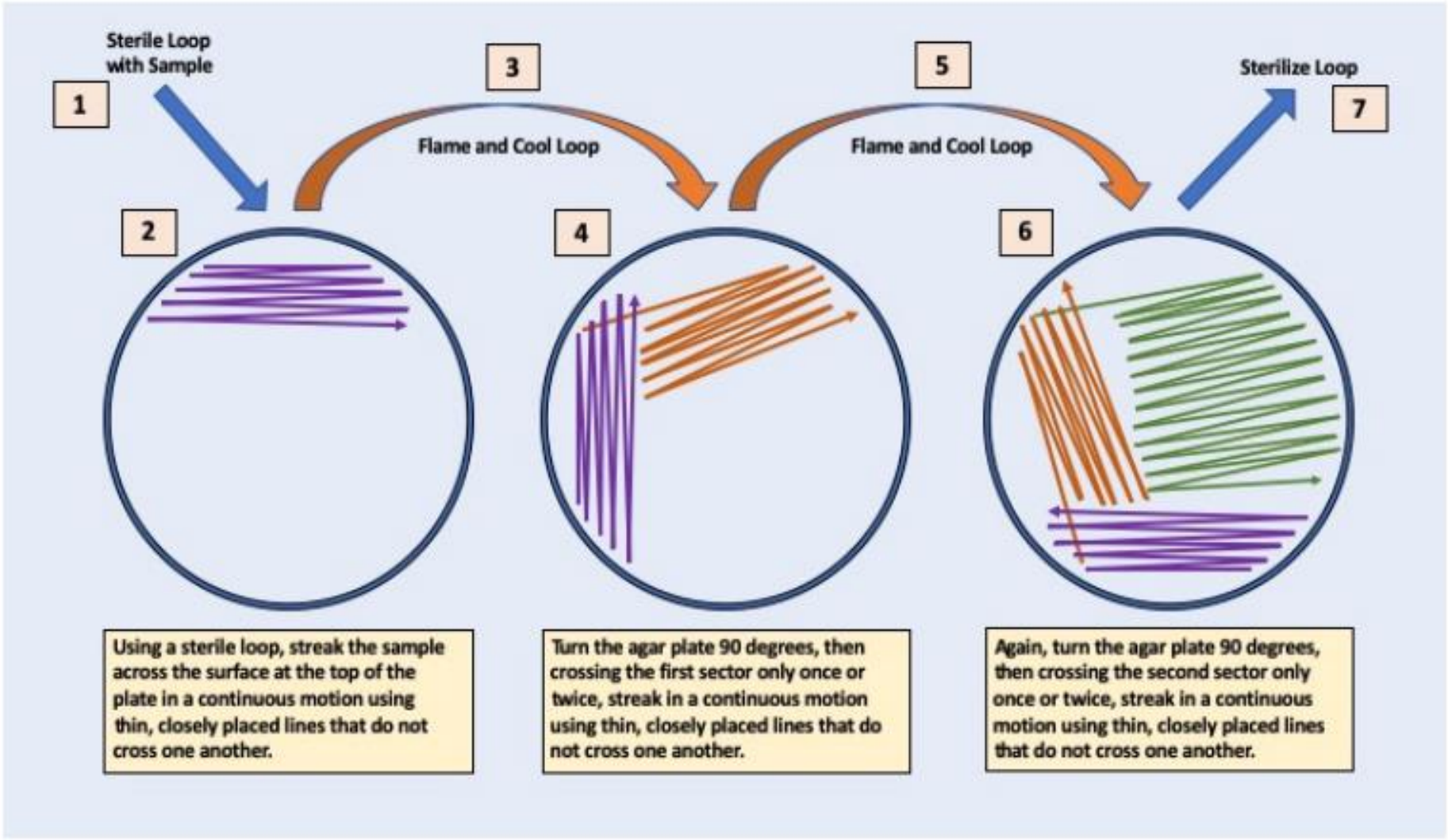
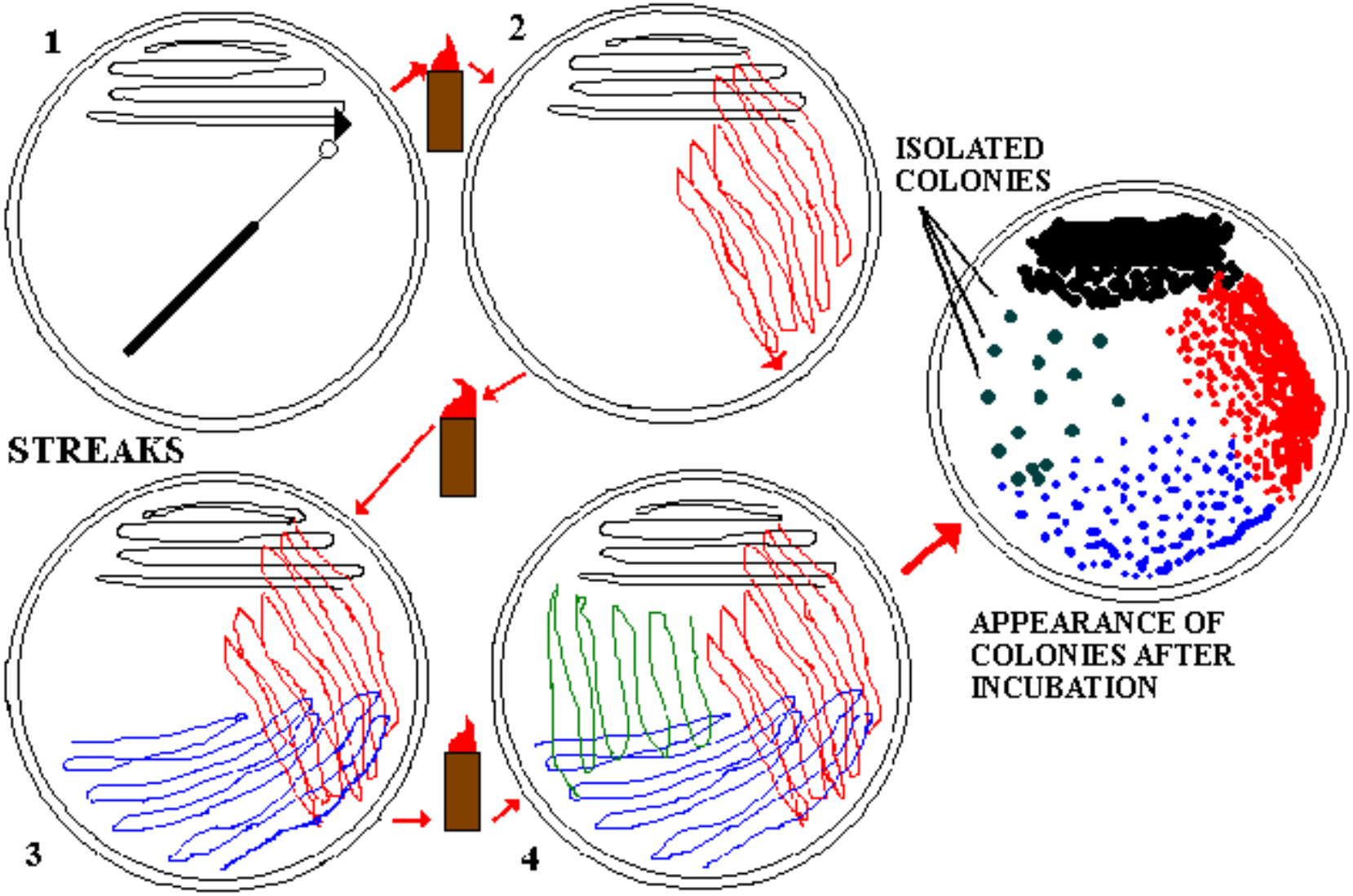
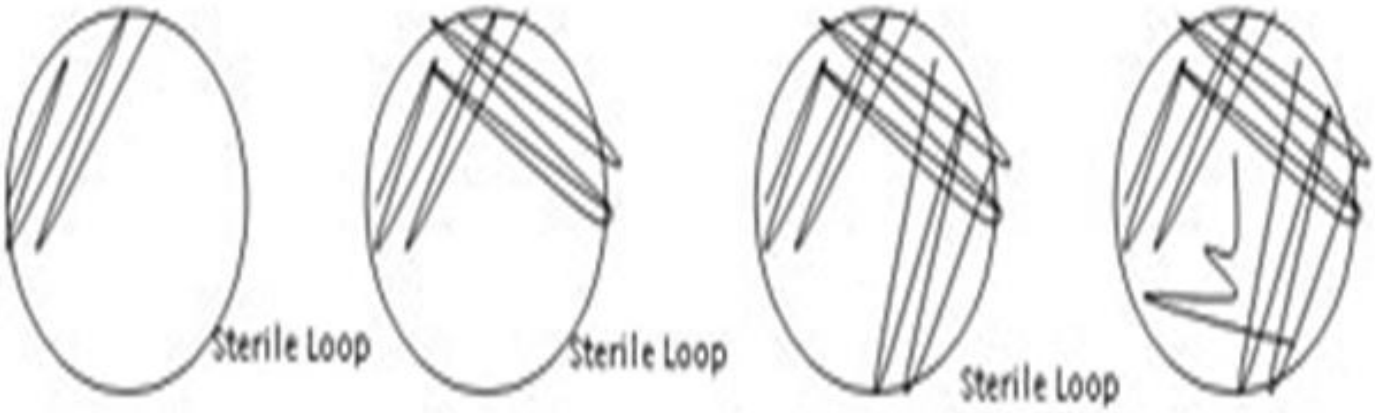


Figure 1. A diagrammatic depiction of the three-phase streak plate method. The individual steps noted above correspond with the directions given elsewhere in this exercise.

Streak plate method





The purpose: to isolate individual colonies of bacteria from large mixed populations.