

FE 204 Experiment 2

Aseptic Transfer Techniques and
Colony selection

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- Definitions
- Transfer techniques
- Procedure
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Aseptic Techniques

Definitions:

Inoculation: The act of introducing microorganisms into surroundings suited to their growth, as a culture medium.

Inoculum: The substance used for inoculation.

Aseptic technique: is the collection of procedures and techniques designed to prevent the introduction of unwanted organisms into a pure culture.

Growth: Result of multiplication of microorganisms in a medium.

Colony: Microbial accumulation which can be seen by naked eye on an agar surface.

Culture: A medium containing one type of microorganism grew inside.

Aseptic
transfer
techniques

Broth → Broth

Broth → Slant

Broth → Deep

Slant → Broth

Petri Plate → Broth

Advantages of Transfer

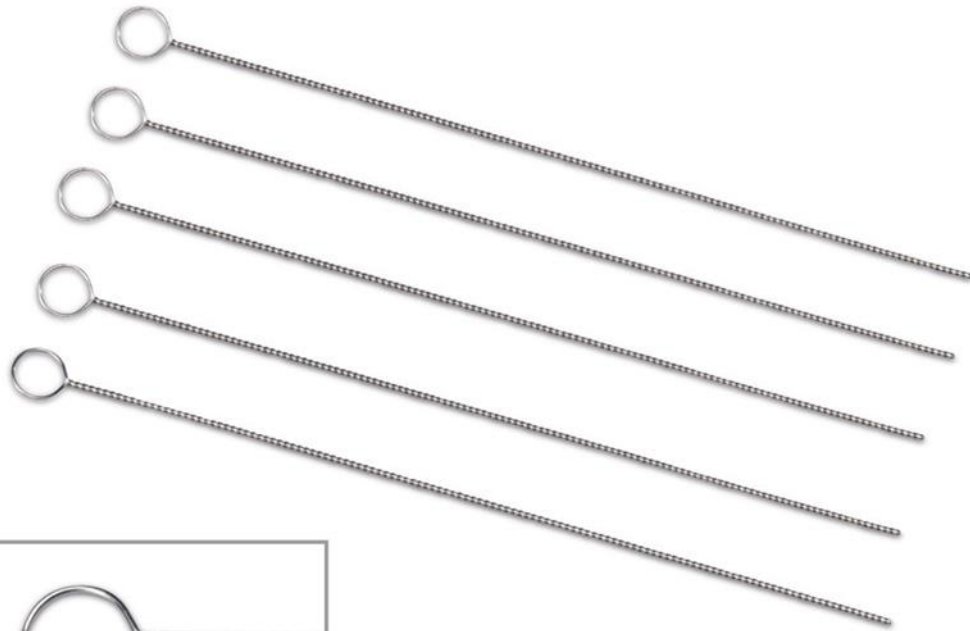
- Microorganisms use nutrients and deplete energy source in one medium. To keep them alive they should be transferred into a fresh medium containing nutrients.
- In order to store microorganisms longer time, slant and deep media is used for keeping culture fresh at freezing temperatures.
- By transferring, media format can be changed from agar to broth.
- For activation of freeze stored microorganisms, transfer methods are used.

Materials used in LAB

- Sterile nutrient broth
- Sterile nutrient agar slant
- Sterile nutrient agar deep
- *E. coli* broth culture
- *E. coli* slant culture
- *E. coli* petri plate culture
- Inoculating Loop
- Needle
- Bunsen burner
- 1 ml pipettes

Inoculating Loop and Needle

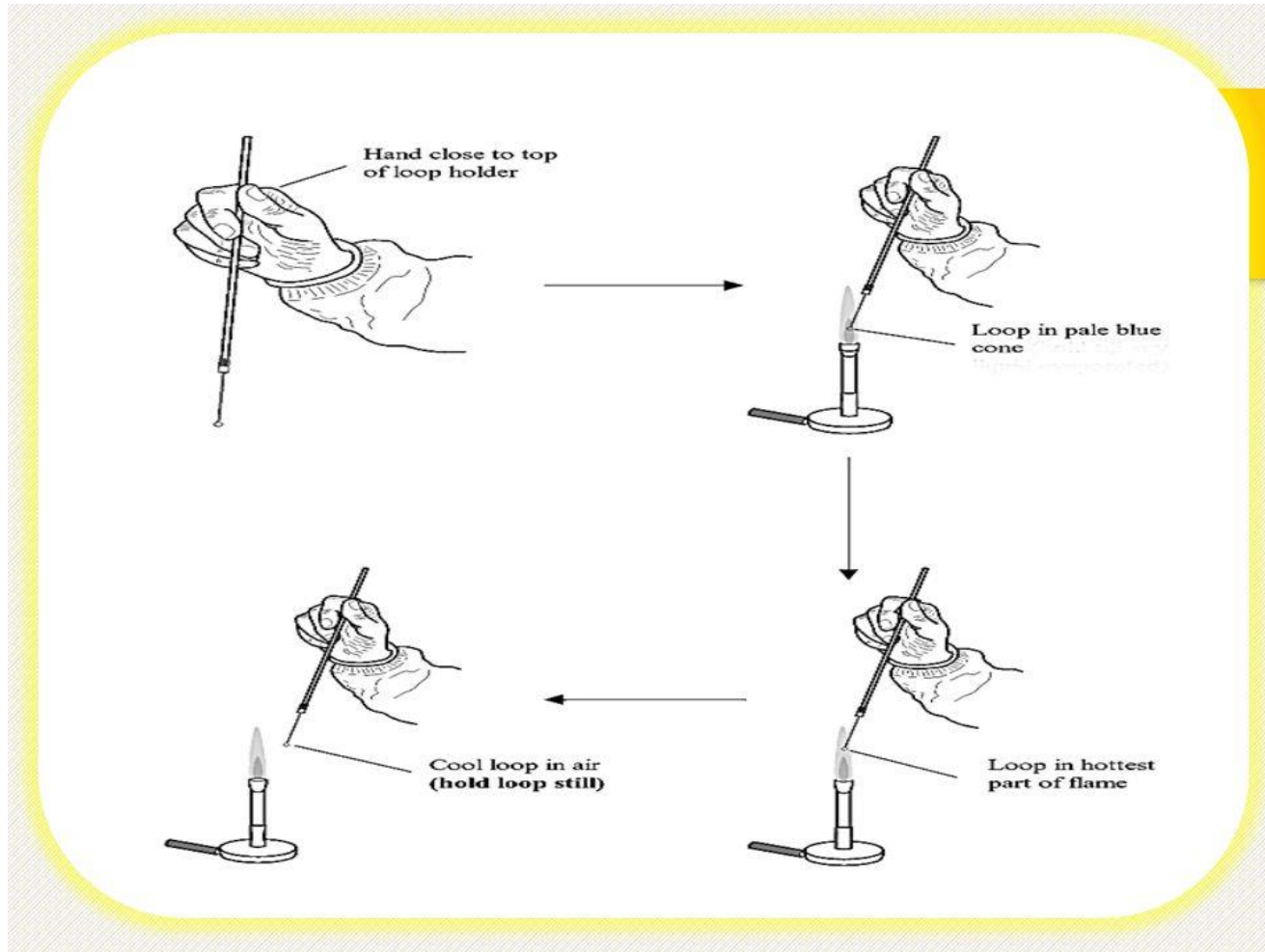
- Inoculating Loop



- Inoculating needle



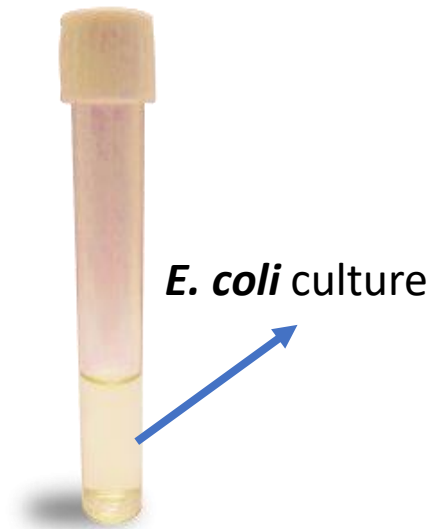
Sterilization of loop and needle



Broth → Broth Transfer

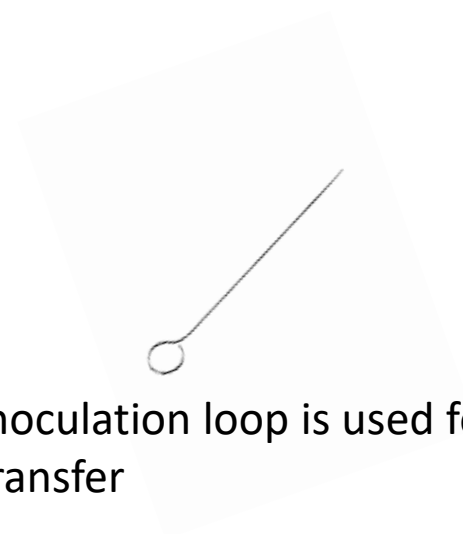
- ***E. coli* broth**

Nutrient broth containing ***E. coli*** bacteria growth inside.

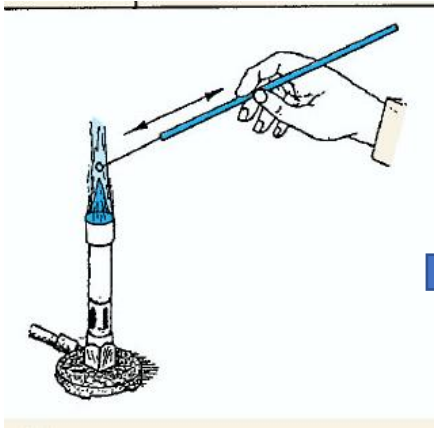


- **Sterile broth**

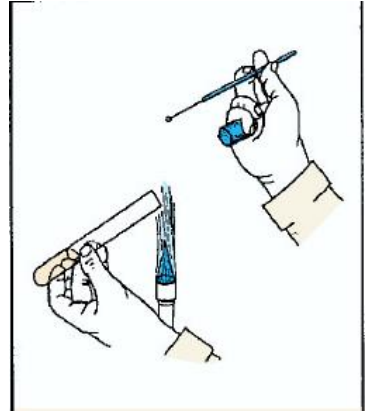
Nutrient broth containing ***no*** bacteria growth inside.



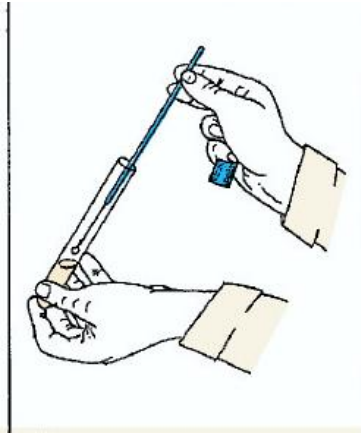
Broth → Broth Transfer



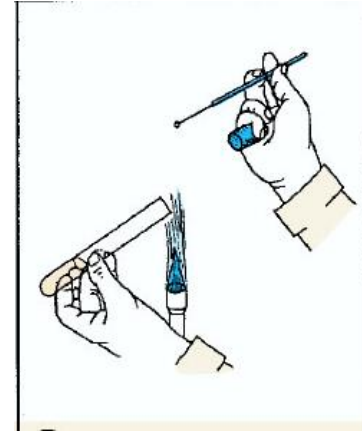
Sterilize loop



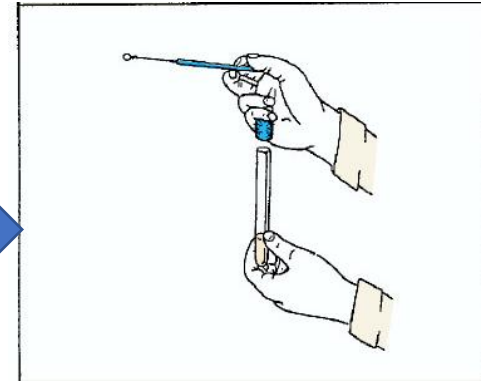
Open the cap of *E. coli* broth and pass the mouth through flame



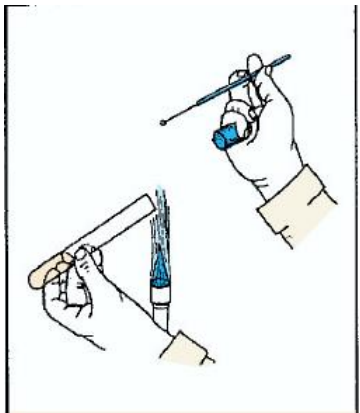
Hold the cap with little finger and take 1 loopful culture.



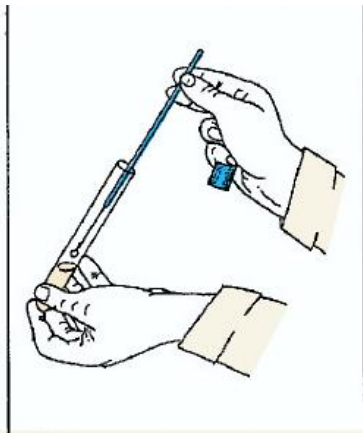
Flame the mouth of tube again and close the cap.



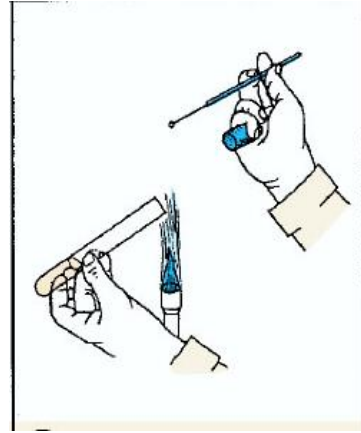
Take a sterile nutrient broth and open the cap.



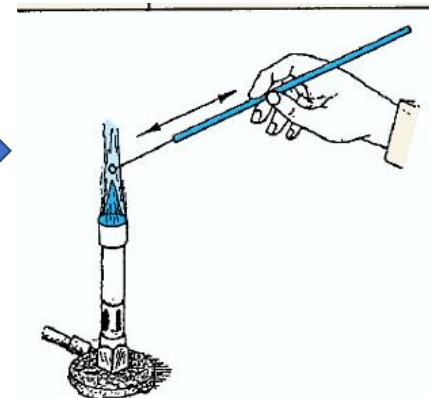
Flame the mouth of tube.



Inoculate 1 loopful *E. coli* sample into sterile Nutrient broth.



Flame the mouth of tube again and close the cap.

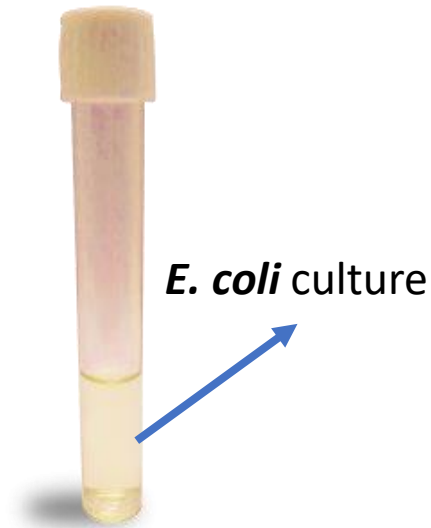


Sterilize loop

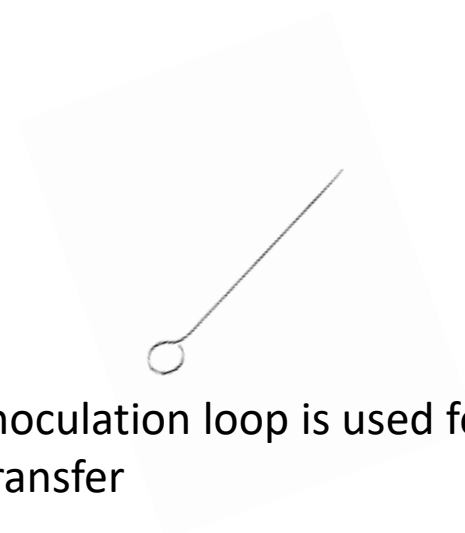
Inoculated broth is incubated at 37 C for 24 hours to obtain *E. coli* growth in broth.

Broth → Agar Slant Transfer

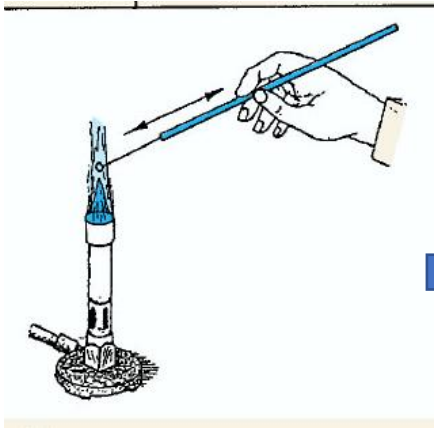
- ***E. coli* broth**
Nutrient broth containing ***E. coli*** bacteria growth inside.



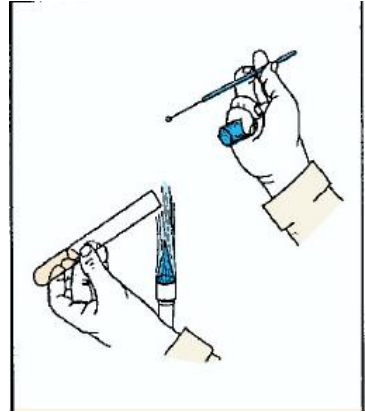
- **Sterile agar slant**
Nutrient agar slant containing ***no*** bacteria growth inside.



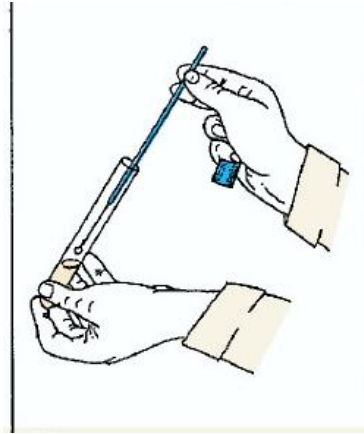
Broth → Agar Slant Transfer



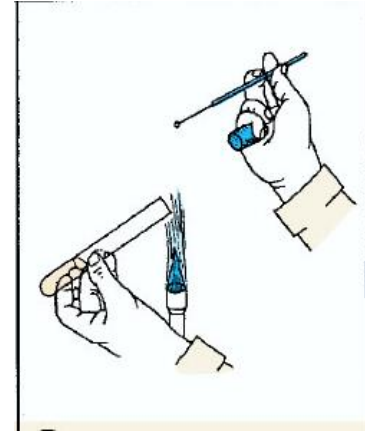
Sterilize loop



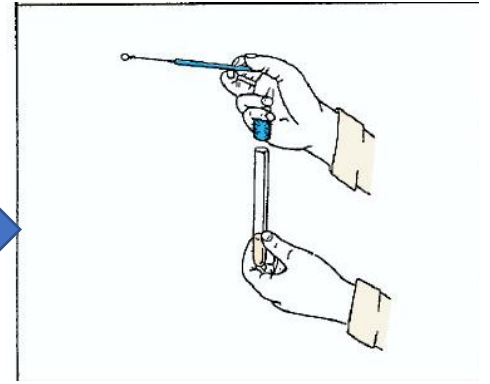
Open the cap of *E. coli* broth and pass the mouth through flame



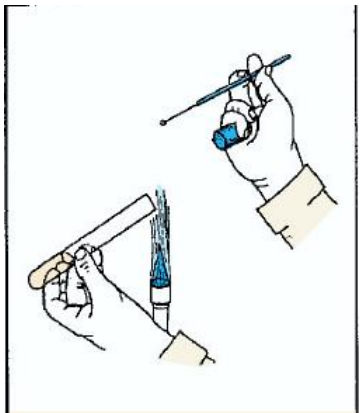
Hold the cap with little finger and take 1 loopful culture.



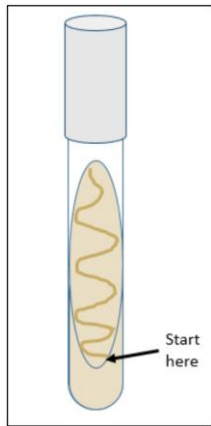
Flame the mouth of tube again and close the cap.



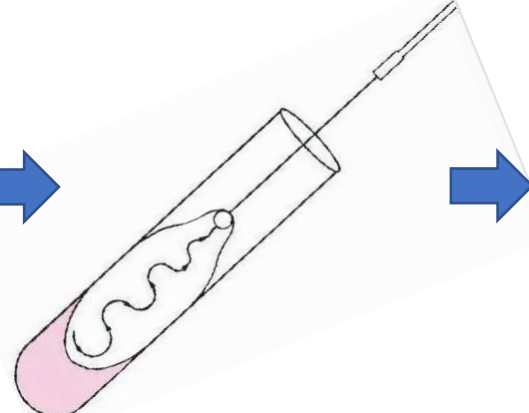
Take a sterile nutrient agar slant and open the cap.



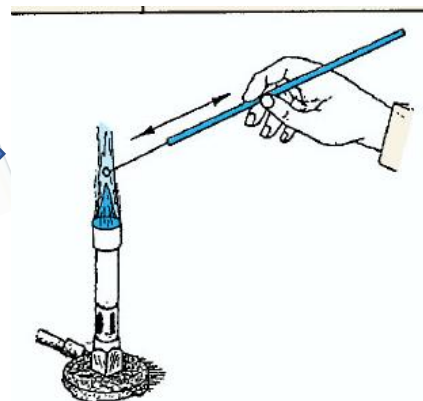
Flame the mouth of tube.



Inoculate 1 loopful *E. coli* sample into sterile Nutrient agar slant.



Draw a zig-zag line when streaking with loop and close the cap.



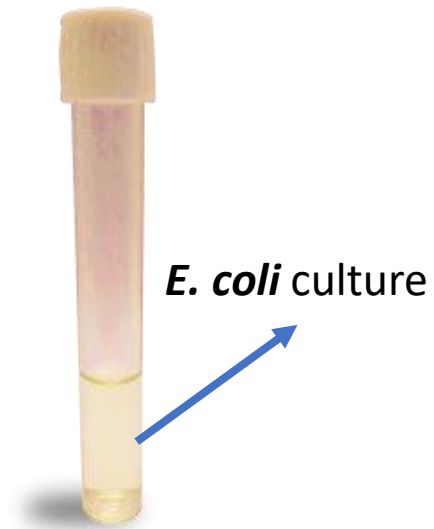
Sterilize loop

Inoculated agar slant is incubated at 37 C for 24 hours to obtain *E. coli* growth in broth.

Broth → Agar Deep Transfer

- ***E. coli* broth**

Nutrient broth containing ***E. coli*** bacteria growth inside.



- **Sterile agar deep**

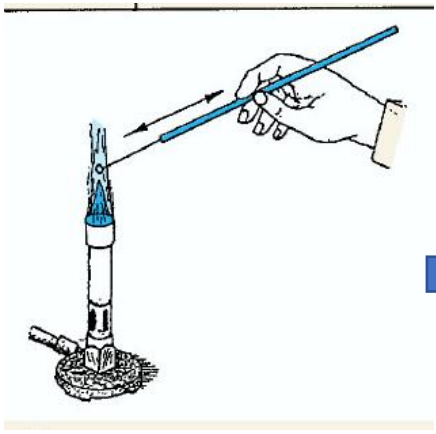
Nutrient agar deep containing ***no*** bacteria growth inside.



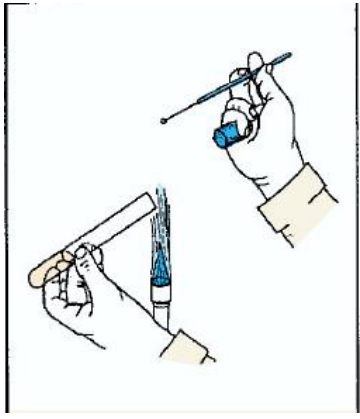
Inoculation needle is used for transfer



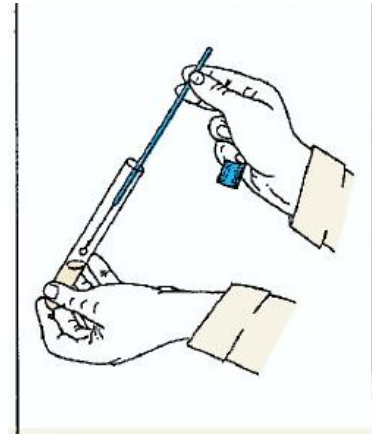
Broth → Agar Deep Transfer



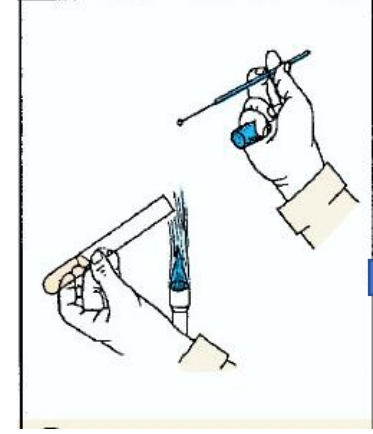
Sterilize needle



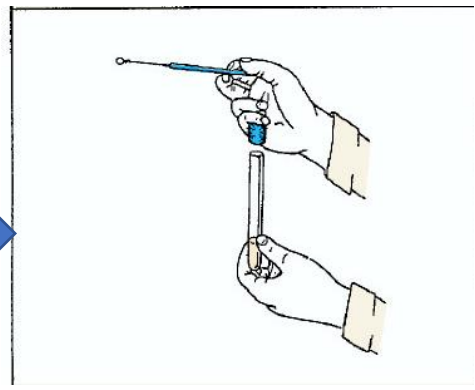
Open the cap of *E. coli* broth and pass the mouth through flame



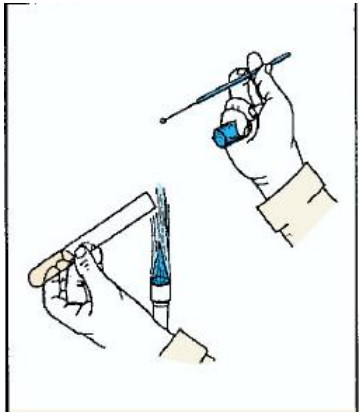
Hold the cap with little finger and take 1 needle culture.



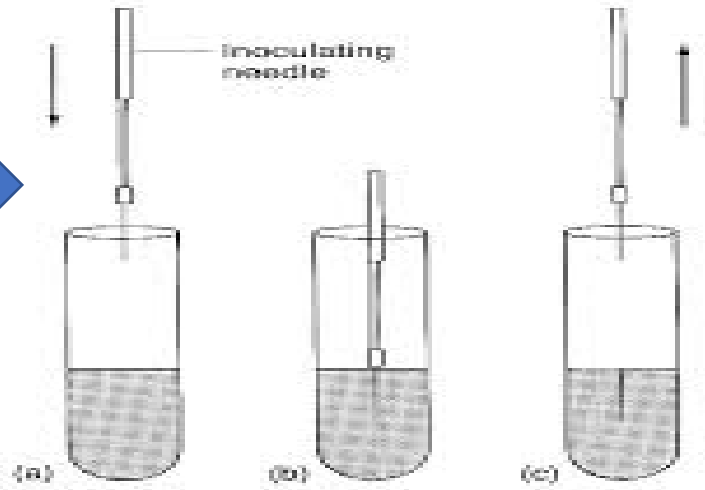
Flame the mouth of tube again and close the cap.



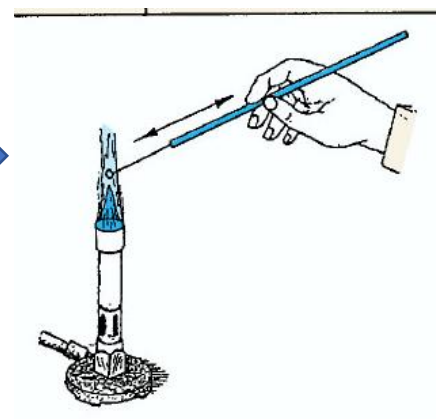
Take a sterile nutrient agar deep and open the cap.



Flame the mouth of tube.



Soak the needle into deep $\frac{3}{4}$ and pull back upwards.



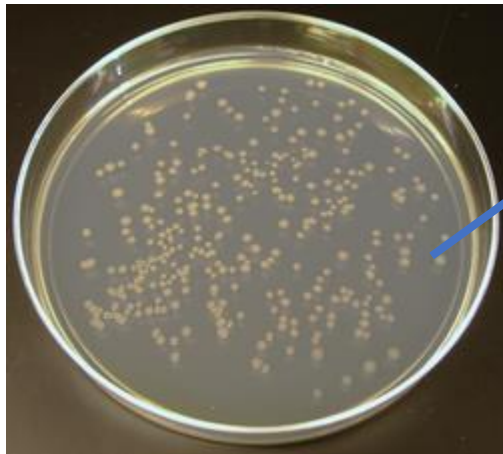
Sterilize needle

Inoculated agar deep is incubated at 37 C for 24 hours to obtain *E. coli* growth in broth.

Agar Petri Plate → Broth Transfer

- ***E. coli* agar slant**

Nutrient agar slant containing ***E. coli*** bacteria growth inside.



E. coli culture



- **Sterile broth**

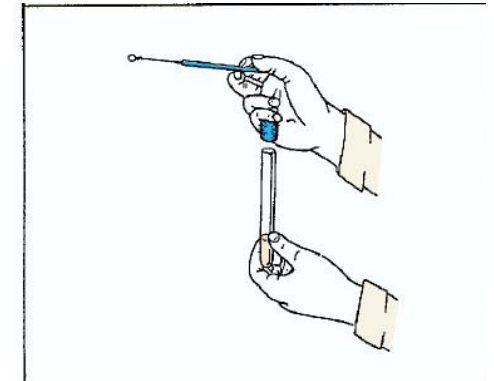
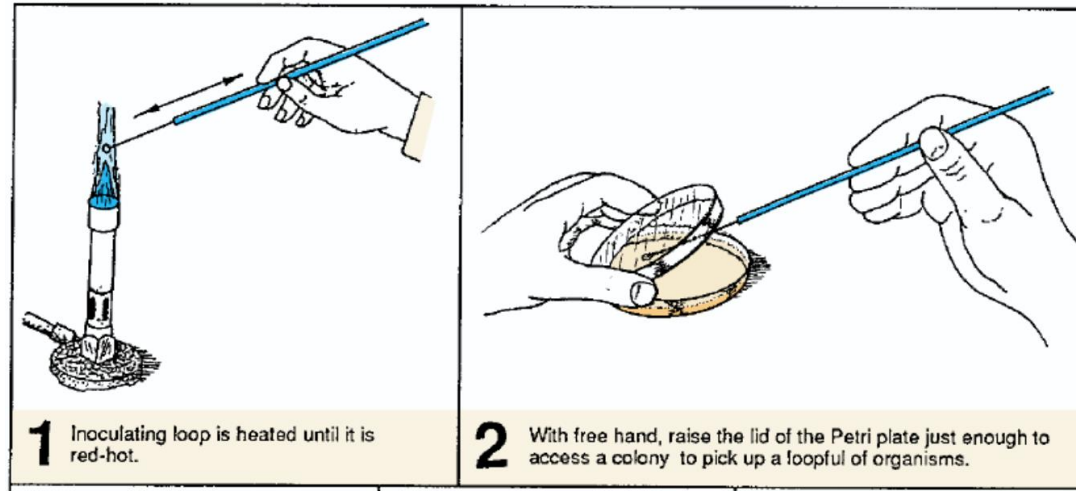
Nutrient broth slant containing ***no*** bacteria growth inside.



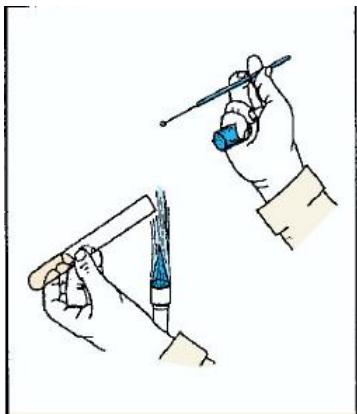
Inoculation loop is used for transfer



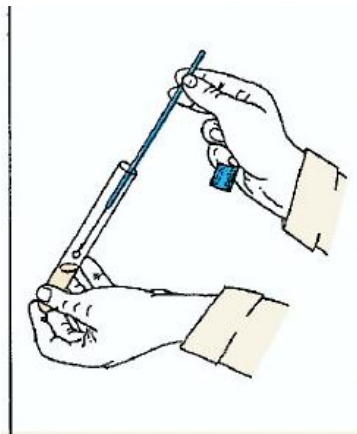
Agar Petri Plate → Broth Transfer



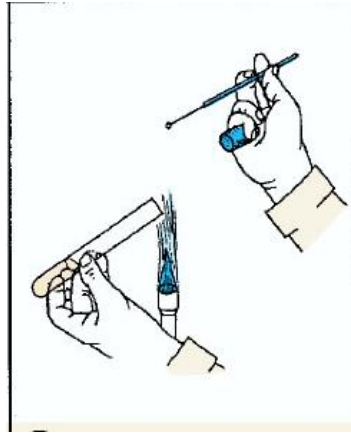
Take a sterile nutrient broth and open the cap.



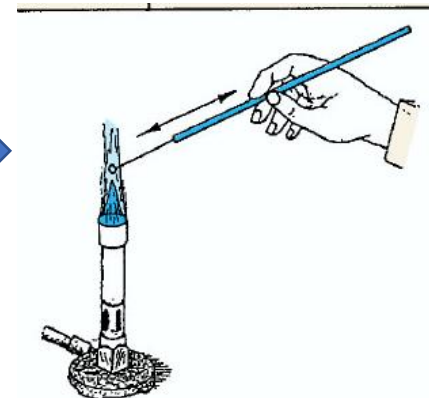
Flame the mouth of tube.



Inoculate 1 loopful *E. coli* sample into sterile Nutrient broth.



Flame the mouth of tube again and close the cap.

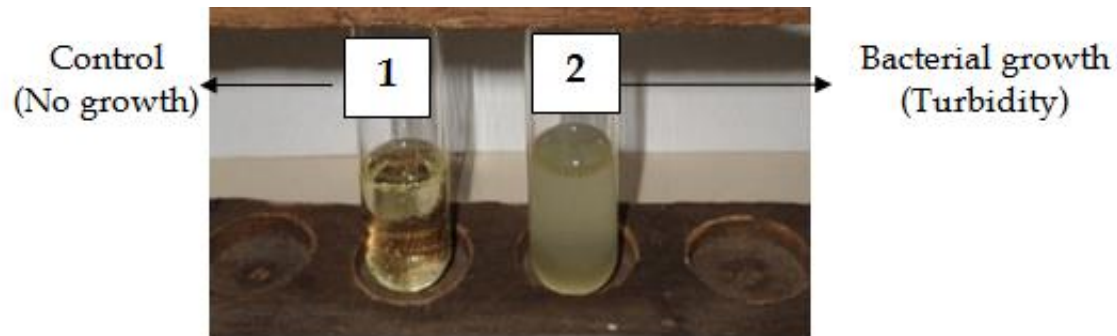


Sterilize loop

Inoculated broth is incubated at 37 C for 24 hours to obtain *E. coli* growth in broth.

Results Evaluation

Transfer Name	Growth indicator	Draw Results
a) Broth → Broth	Suspended materials in the broth and turbidity	Draw turbidity in test tubes and suspended materials
b) Broth → Agar Slant	Colonies formed on the surface of Slant	Draw the formed colonies on agar surface
c) Broth → Agar Deep	Colonies formed inside the agar	Draw the formation of growth inside the agar deep
d) Agar Petri Plates → Broth	Suspended materials in the broth and turbidity	Draw turbidity in test tubes and suspended materials



a) Bacterial growth in broth
d) Bacterial growth in broth

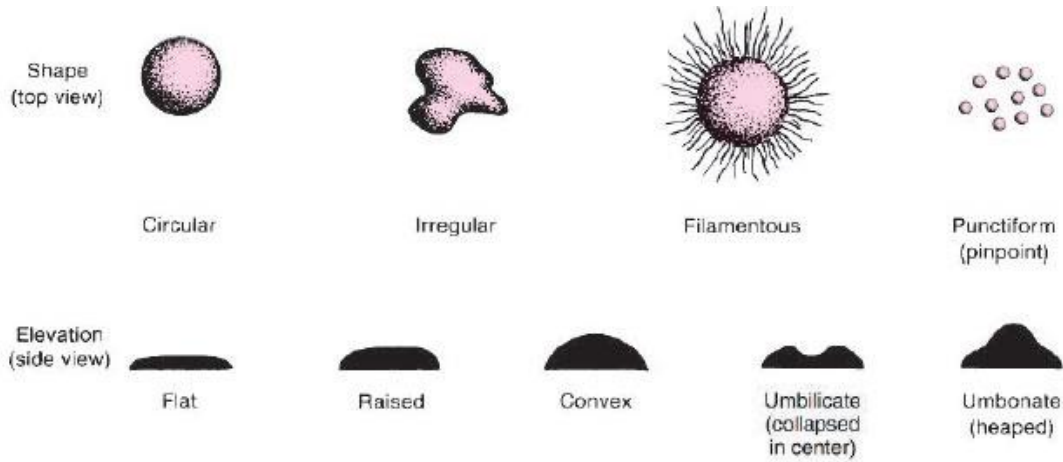


b) Bacterial growth on slant surface

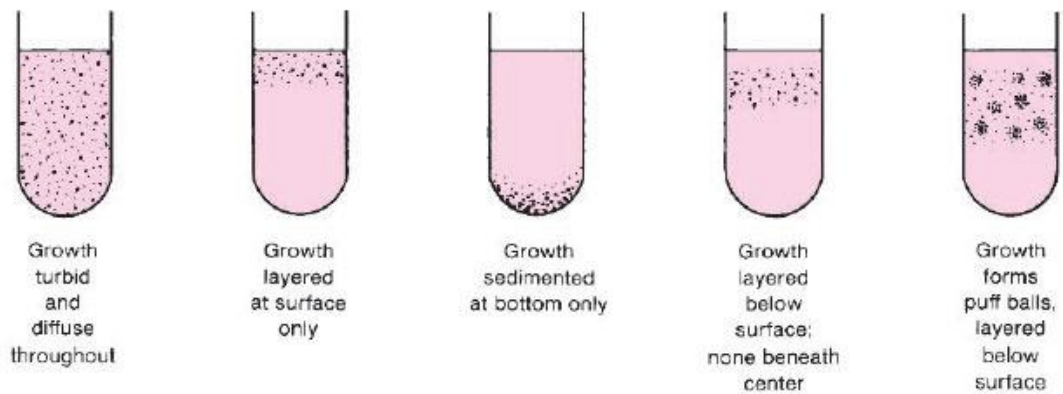


c) Bacterial growth in Agar deep

Additional Info about growth patterns

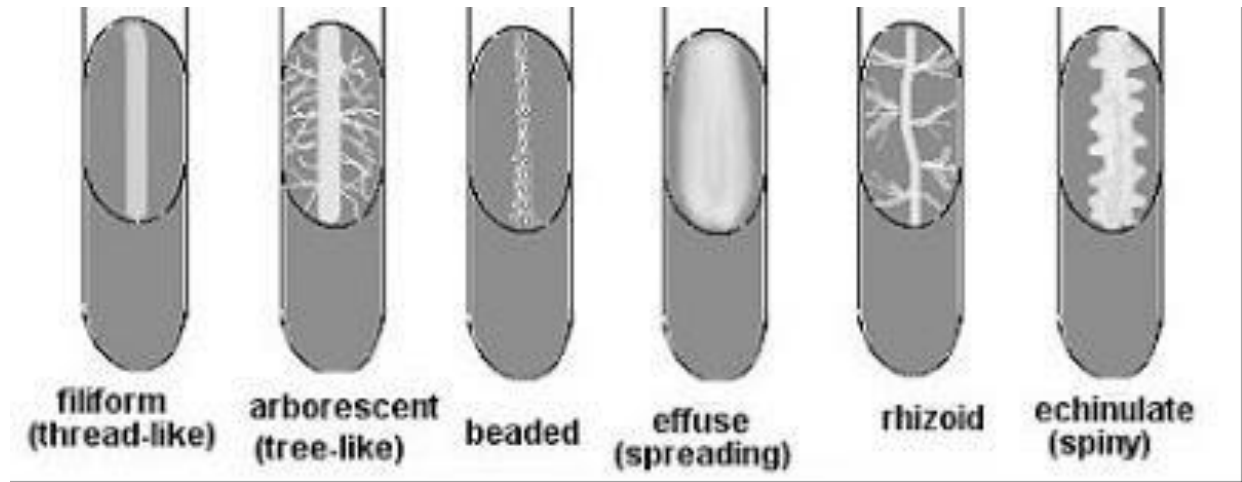


(a) Some colonial characteristics on agar media*



(b) Some growth patterns in broth media

*Note: Shapes and elevations shown in this diagram are not intended to be matched.



Growth patterns on agar slant surface