Lab. 3

Aseptic technique

Prepared by
Samira fattah
Assis. Lec.
College of health sciences-HMU

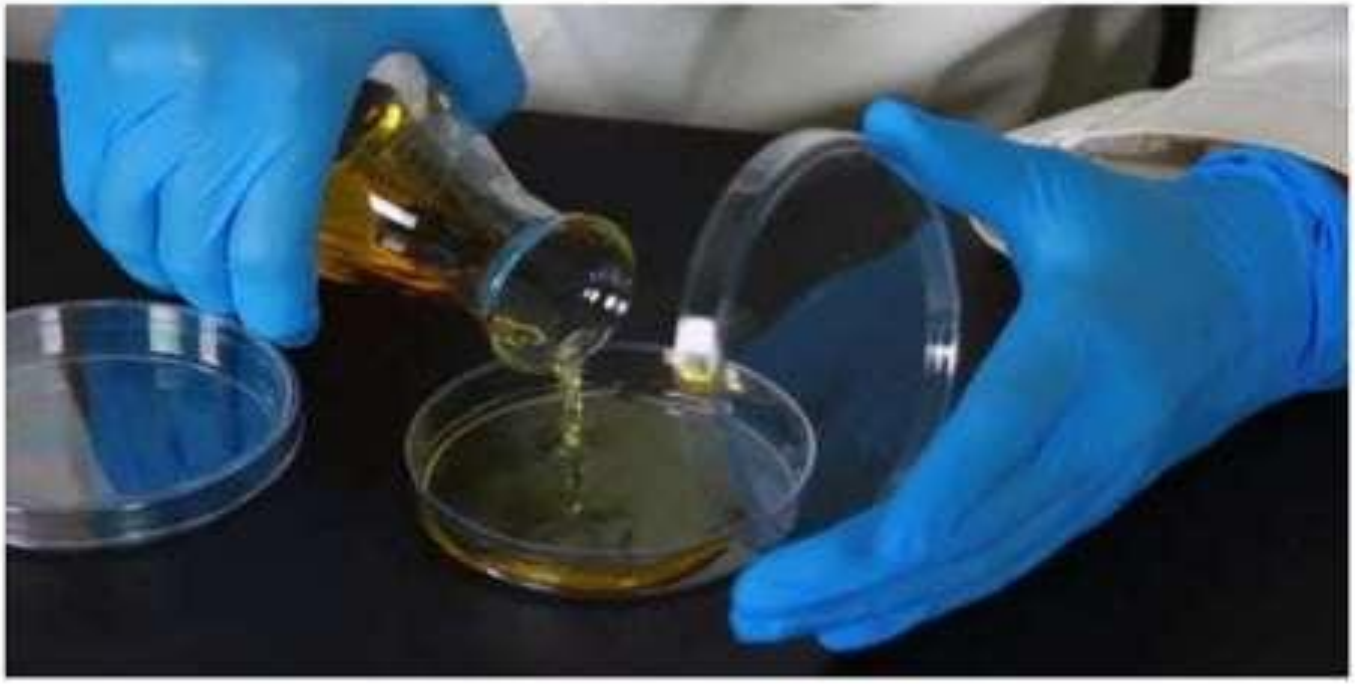
ASEPTIC TECHNIQUE

- Aseptic technique refers to a procedure that is performed under sterile conditions.

- This includes medical and laboratory techniques which deal with cultures and human cells and tissue for transplantation.

What is the Aim of Aseptic Technique?

- To prevent the access of micro-organisms during the preparation and testing.



In the microbiology lab we use aseptic technique to:

- Prevent contamination of the specific microorganism we are working with.
- Prevent contamination of the room and personnel with the microorganism we are working with.

SOURCES OF CONTAMINATION

1. The Atmosphere
2. The Breath
3. The Hands
4. . Clothing
5. The Hair
6. The Working Surface
7. Equipment

Some terms

- **Sterilization:**

  It is the process by which article, surface or medium is made free from all microorganisms either in the vegetative or spore state.

- **Disinfection:**

  It is the process by which an article, surface or medium is made free from all pathogenic microorganisms (that is organisms that are capable of giving rise to infection).

- **Antisepsis :**

  It is the process by which the growth of bacteria is inhibited but they are not killed.

What need to be sterilized in the process of aseptic technique?

1. Culture media
2. Fluids used in the labs
3. Reagents
4. Laboratory containers
5. Laboratory equipment

Methods of sterilization

A-Physical methods:

1. **Dry heat:**

   i-hot air oven ⟶ glassware

   ii-flaming ⟶ inoculation loop &needle

2-moist heat(steam under high pressure):

autoclave ⟶ culture media

3-**filteration**:

used for heat sensitive liquids like, antibiotic solutions and sera.

4- **radiation**:

used for enclosed area such as inoculation room and virus labs.

B-Chemical methods:

1. **Alcohols** ⟶ surfaces, skin
   eg. ethyl alcohol 70% conc.

2.aldehydes:

eg. formaldehyde ⟶ equipment such as centrifuge.

3. **Phenolics** ⟶ floors, walls and benches.

eg. Phenols(chloroxylenol)

General Principles of aseptic technique

1. Disinfect the work area before starting to reduce potential contaminants on the bench top, and after work is finished to protect others from possible contamination.

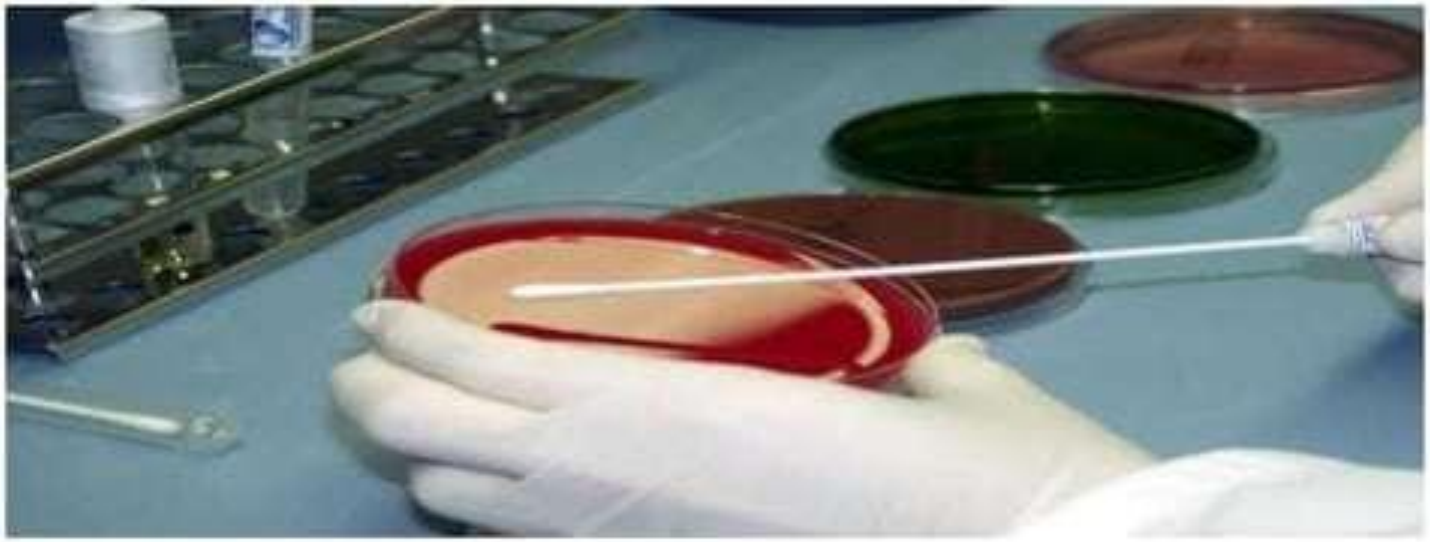
2. Flame the inoculating loop before and after making a transfer of bacteria from one container to another.

Never lay an inoculating loop on the bench top if you are not sure it has been flamed first. When in doubt, flame the loop again.

3. Flame the opening of glass containers before removing bacteria from them and again after bacteria have been removed. Likewise, flame the opening before transferring bacteria to a container and again after the transfer is completed.

4. Do not lay the cap of containers of bacteria on the bench top while bacteria are removed from or transferred to the container. The cap should remain under your control throughout the transfer.

5. Work quickly and efficiently to minimize the time the culture is exposed to the environment.



Example of a typical transfer

As an example, these are the steps required for good sterile technique in transferring bacteria from one capped test tube to another.

1. Flame the inoculating loop.
2. Flame the mouth of the tube while holding the cap.
3. Remove some bacteria with the loop.
4. Flame the mouth of the tube and replace the cap.
5. Flame the mouth of the tube to which the bacteria are being transferred.
6. Inoculate the tube with bacteria from the loop.
7. Flame the mouth of the tube and replace the cap.
8. Flame the inoculating loop.

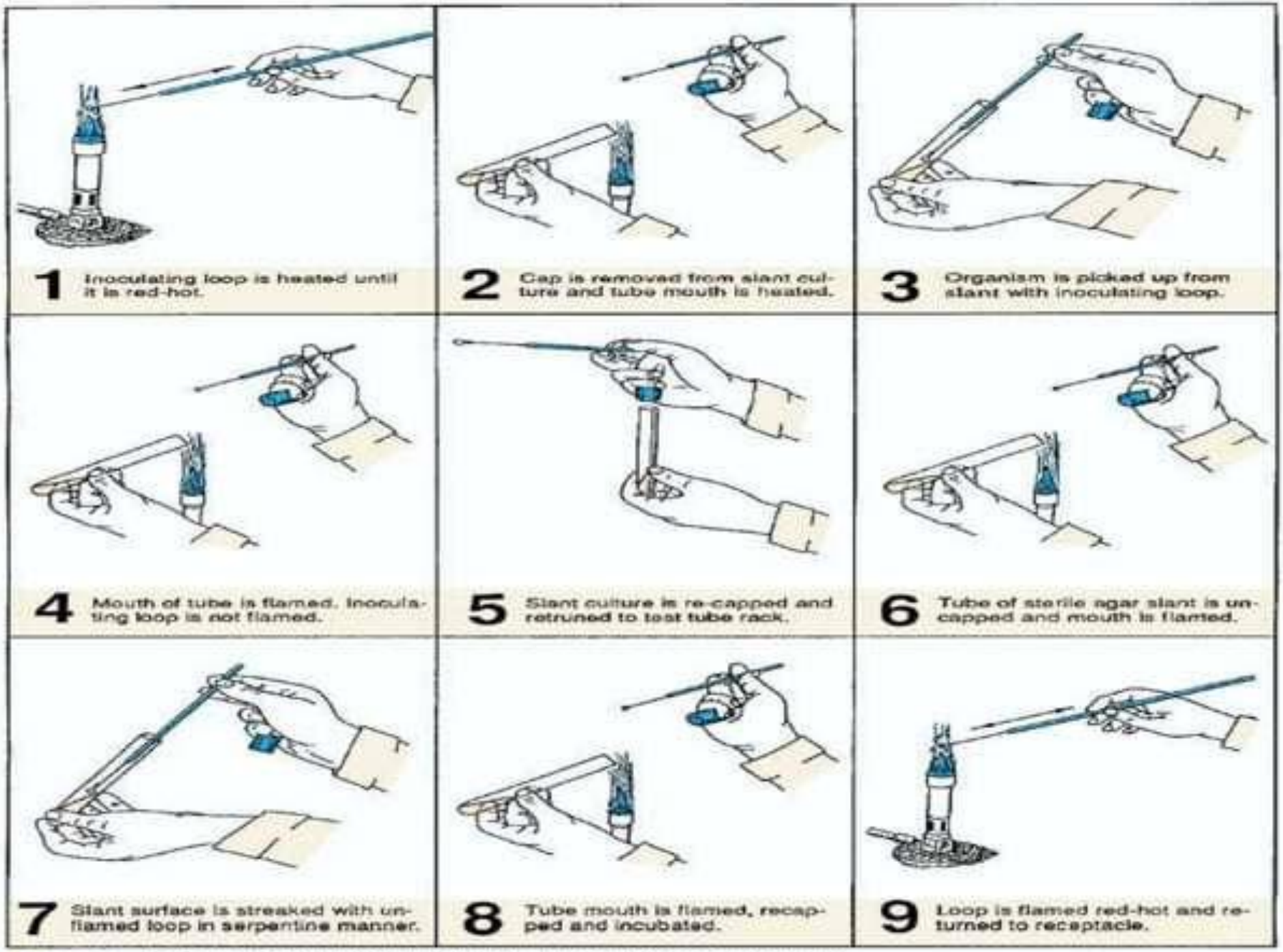


Figure 4 Procedure for inoculating a nutrient agar slant from a slant culture

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Lab. exercise

1- student will transfer bacteria from culture media to another by following aseptic technique.

2- student take samples to culture without following aseptic technique.