Aseptic Technique

Introduction

Aseptic technique is used to prevent unintentional contamination of cultures and reagents during transfer and inoculation steps. These are general guidelines that should be applied during such procedures as petri to petri transfer (LC-01-001-011), petri to flask transfer (LC-01-001-012), flask to carboy transfer (LC-01-001-020), and any other procedures that involve transfer or organisms from one vessel to another where contamination is unacceptable. Aseptic technique can be broken down into three main focus areas as follows:

1. **Personal hygiene and PPE**
2. **Maintaining a clean and organized work area**
3. **Work speed and handling techniques to minimize contamination opportunities**

Steps in Aseptic Technique

More detailed examples of steps within each area are listed below.

1. **Personal hygiene and PPE**
    a. Wash hands
    b. Where lab coat and gloves
2. **Maintain a clean and organized work area**
    a. Clean hood or work area before during and after procedures.
    b. Clean spills immediately.
    c. Ensure supplies used are clean and sterile (where appropriate). Regularly clean pipettes and other heavy use items.
    d. Organize and declutter work area to maintain airflow in the sterile field and to prevent crossing items over each other, especially when open/in use.
3. **Work speed and handling techniques to minimize contamination opportunities**
    a. Only open containers immediately before they are required and minimize the time that they are open for
    b. Hold lids rather than placing them on surface if possible
    c. Place lids right side up on surface if unable to hold
    d. Do not cough, sneeze, sing, whistle etc. that could disrupt air flow
    e. No sudden movements to disrupt airflow

The links below have more detailed descriptions of these procedures.

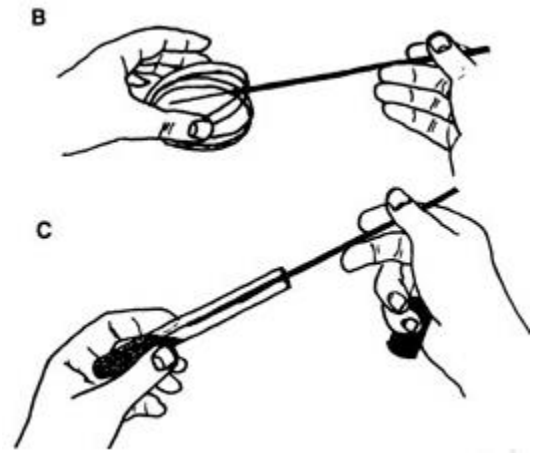
1) This is a nice introductory video from Gibco which also shows liquid transfer by serological pipette and also by pouring between containers. The second link has good summary text on procedures.

https://www.youtube.com/watch?v=cyJ2zUbLMMo

https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/aseptic-technique.html

2) The following website has some good images showing ways to open plates etc. and one example is pasted in below.

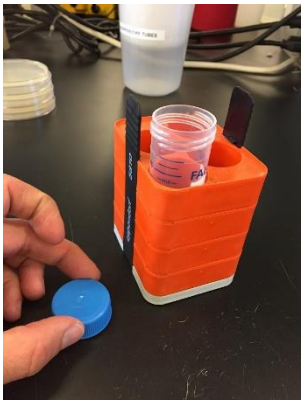
http://www.jlindquist.net/generalmicro/102asepticتechnique.html



Examples of sample handling techniques

Lids can be held open in such a way to minimize the opening where contamination can enter such as keeping the lid close and above the sample (B/upper), or holding vessels at an angle to minimize the effective surface area for contamination to fall into (C/lower). The lower picture also shows a method for removing and holding a container lid in your hands during a procedure instead of placing it on the work surface.





Examples of opening containers

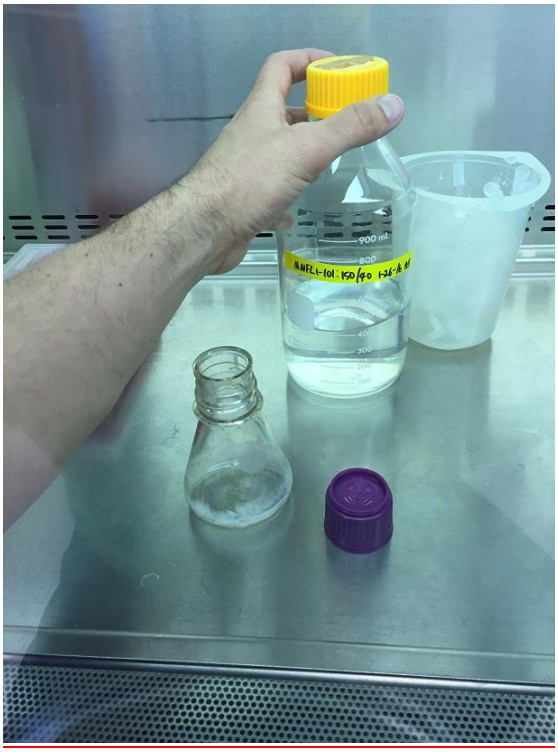
Ideally containers are opened with one hand with the lid and container being held in the same hand (Left). A common way for contamination to occur when using such a method is contact between the user's fingers/thumb and the opening of the container (Middle). If the user is unable to properly manipulate the container and lid with one hand, the lid may be removed and placed on the clean surface during the procedure. Ideally the open container would be held at an angle while open (reducing the effective surface area for contamination to fall into) but may be placed in a holder if this is not possible (Right).

Examples of pipetting with open containers

Ideally the open container would be held at an angle while open (reducing the effective surface area for contamination to fall into) but may be placed in a holder if this is not possible. If pipettes are to be inserted into sample containers care should be taken to not allow the pipette to touch the sides (Left). Contamination can easily occur when placing pipettes into sample containers (Middle). If possible only clean pipette tips should enter the sample and sample container (Right).

Examples of poor hood use

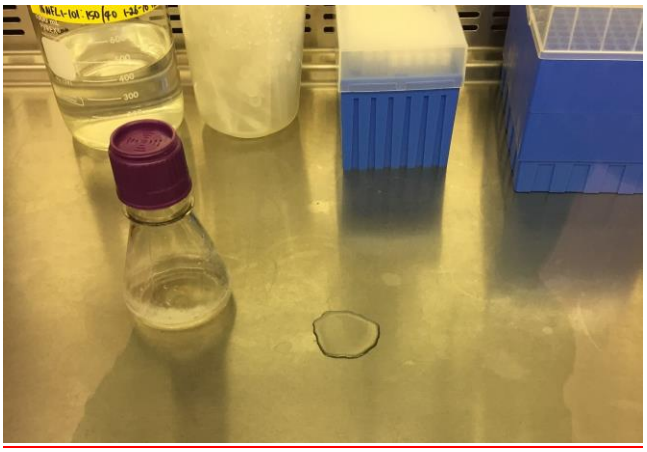
- The user's cell phone is blocking the airflow by covering part of the front vent of the hood.
- The cell phone is unlikely to be clean and thus is a potential source of contamination.
- Cell phones etc. should not be in use during aseptic procedures.



- Reaching over open containers is a potential source of contamination
- Set up the work area so that containers are not reached over during procedures

- Hood is cluttered and likely contains more items than are needed for the task at hand. Only items that are needed for the task in hand should be in the hood.

- Items in the hood are lined up along the back wall, blocking air flow to the working area of the hood. Any block of the air flow reduces the effectiveness of the hood to create a clean work environment.
- Many items are hanging up in the hood above the working area where containers may be opened. It is best practice to prevent arms or equipment from passing over open containers.
- Hood does not appear to have been cleaned recently. The hoos should be cleaned before and after each time it is used.



- Spills are a potential source of both chemical and biological hazard / contamination
- Spills should be cleaned up immediately.