

Use of Pipettes and Pipetting Aids

Pipetting aids should be selected with care. Their design and use should not create an additional infectious hazard and they should be easy to sterilize and clean.

1. A pipetting aid should always be used. **Pipetting by mouth is prohibited.**
2. All pipettes should have cotton plugs to reduce contamination of pipetting devices.
3. Plugged (aerosol-resistant) pipette tips should be used when manipulating microorganisms and cell cultures.
4. Air should never be blown through a liquid containing infectious agent.
5. Infectious materials should not be mixed by alternate suction and expulsion through a pipette.
6. Liquids should not be forcibly expelled from pipettes.
7. Mark-to-mark pipettes are preferable to other types as they do not require expulsion of the last drop.
8. Contaminated pipettes should be completely submerged in a suitable disinfectant contained in an unbreakable container. They should be left in the disinfectant for 18-24 h before disposal.
9. A discard container for pipettes should be placed within the biological safety cabinet, not outside it.
10. Syringes fitted with hypodermic needles must not be used for pipetting. Blunt cannulas should be used instead of needles. There are devices for opening septum-capped bottles that allow pipettes to be used and avoid the use of hypodermic needles and syringes.
11. To avoid dispersion of infectious material accidentally dropped from a pipette, a disinfectant-soaked cloth or absorbent paper should be placed on the working surface; this should be autoclaved or discarded as infectious waste after use.
12. Pipettes with cracked or chipped suction ends should not be used as they damage the seating seals of pipetting aids and so create a hazard.

Section 9.4 Protection of Vacuum System when Filtering Biohazardous Materials

The aspiration of tissue culture media from monolayer cultures and of supernatants from centrifuged samples into primary collection flasks is a common laboratory procedure.

1. A HEPA filter provides an effective barrier to protect the vacuum system.
2. Flexible tubing is used of appropriate inside diameter for the flask and filter fittings and of sufficient wall thickness for the applied vacuum.
3. Flasks should be of appropriate size to contain the amount of fluid aspirated.
4. Flasks contain an appropriate disinfectant solution. Use an antifoam additive to prevent foam production, if allowed to reach the filter, foam will shut off the vacuum.
5. If the filter becomes contaminated or requires changing, the filter and flask can be safely removed by clamping the line between filter and vacuum source. The filter should be autoclaved before the filter is discarded.

The apparatus is shown in Figure 1:

- two suction flasks (A & B)
- HEPA filter (C)
- vacuum source (D)
- rubber stoppers
- flexible vacuum tubing
- glass tubing
- glass sparger (aerosol passing through the collection flask is dispersed in small bubbles so that adequate contact is made with the disinfectant solutions)

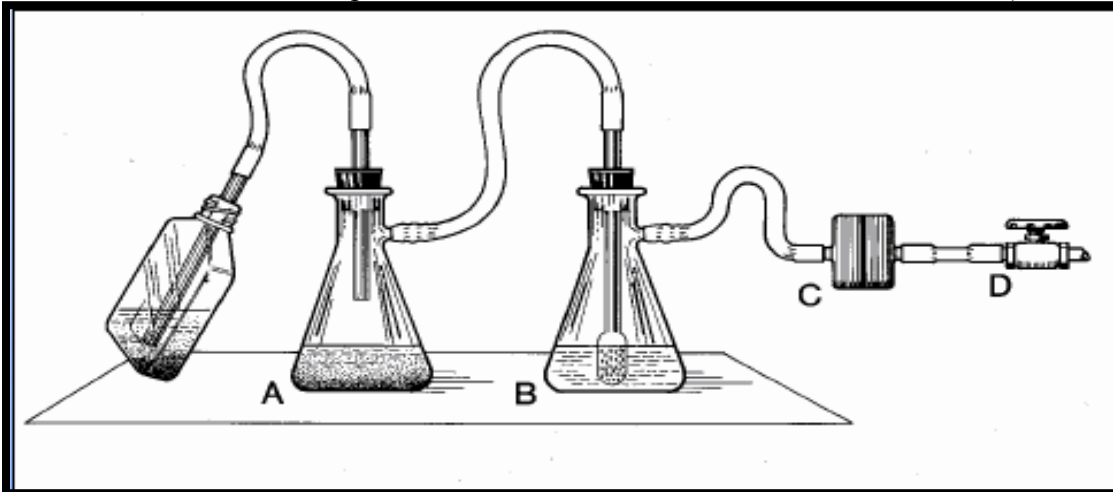


Figure 1 (Office of Health and Safety, Centers for Disease Control and Prevention, 1600 Clifton Road N.E., Mail Stop F05 Atlanta, Georgia 30333, USA Last Modified: 1/2/97)
 One method to protect a house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask serves as a fluid overflow collection vessel. A glass sparger in flask (B) minimizes splatter. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

Section 9.5 Autoclave Operating Procedures

The following procedures are recommended by the Biosafety Office.

What Materials Should Be Autoclaved?

The following materials are recommended to be autoclaved:

- Culture and stocks of infectious agents (bacteria, viruses, fungi, etc.)
- Reusable items to be sterilized: plastic pipette tips, pipettes, surgical instruments, and scrubs
- Animal tissue specimens and cages of potentially pathogenic animal carcass(es)

Autoclave Cycles

There are three basic autoclave cycles:

1. **Gravity** or "Fast Exhaust" Cycle: Used to sterilize dry goods, glassware, etc. This cycle charges the chamber with steam and holds it at a set temperature for a set period of time. At the end of the cycle a valve opens and the chamber rapidly