Laboratory Instruments and Safety Cabinets:

Instruments which are used in virology lab are the followings

1- Autoclave: it is steam sterilization; all living microorganisms including bacterial and fungal spores are killed at 121 ^{o}C within 15-20 min.

2- Dry heat (oven): It is done in a special instrument (dry oven), which is heated electrically to a temperature at 160 ^{o}C for 1-2 hrs.

3- Centrifugation: balancing the buckets of the centrifuge is needed for preventing breaking of centrifuge tubes; glasses tubes can be closed by folding the cotton plug and screwing with a rubber band or rubber stopper or with parafilm.

A- **Normal centrifuge** (ordinary centrifuge)

B- **Ultra centrifuge**: it is used at high speeds 45000-60000 rpm

C- **Micro centrifuge**

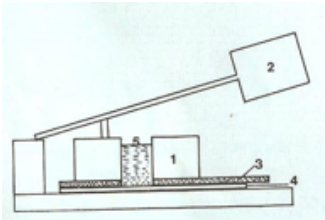
4- Refrigerator

5- Filtration

a. Filtering small amount: It is done through small disposable filtering unit, which can be connected to a disposable syringe.

b. Filtering large amount: Large amount like medium, serum and buffers can be sterilized by filtration, up to 100ml is filtrated in disposable units, all these filters let viruses pass.

6- Sayk sedimentation chamber. When cells from fluids are be examined for the presence of virus or virus inclusions slides have to be made. An elegant way to do this is to use this instrument in which the cells from the sediment by the natural gravity, added by filter-paper.



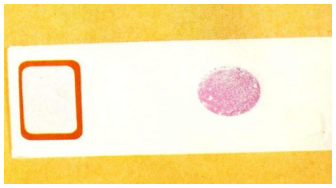
This instrument is consist of the following parts

1. A plastic ring
2. Weight pressing down the plastic ring
3. Filter paper perforated

4. Microscope slide

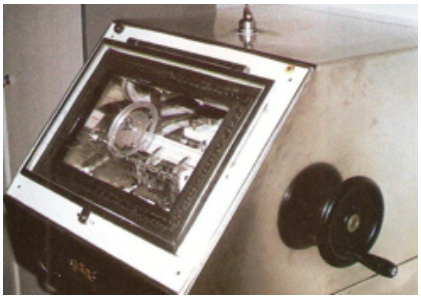
5. The fluid to be examined

The fluid is sucked by the filter paper and cells from sediment on the glass. The time taken to make a slide is about half an hour. The cell sediment on the microscope slide is fixed before staining with dye or fluorescent antibody technique.



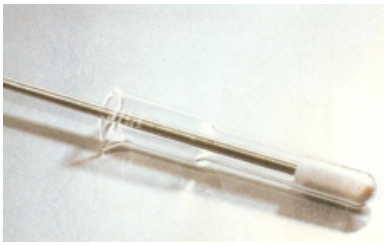
7- Cytocentrifuge. The cytocentrifuge enables us to make cell sediments from fluids on microscope slide. The cells are deposited on the glass by centrifugal force and the fluid is absorbed into filter paper.

8- Refrigerated microtome. To make sections the frozen piece of tissue is placed on the chuck of the refrigerated microtome and cut into sections of the desired thickness.

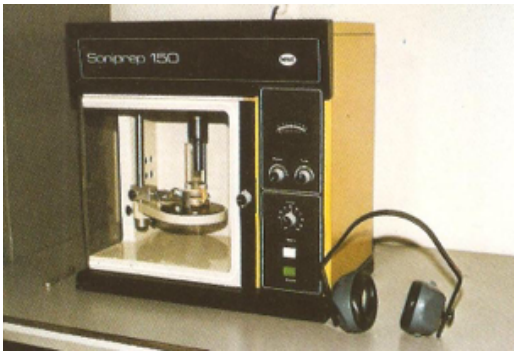


9- Grinding tissue in a mortar and pestle: Tissues from man and animal must be ground and extracted before inoculating into tissue culture, eggs, or animals. Grinding is aided by adding acid washed sterile sand. The work must be done in a safety cabinet as aerosols are produced by this method.

10- Grinding in homogenizer: the speed of the homogenizer can be as a high as 1600 rpm. This will lead to considerable heating of the material. At higher speeds the beaker has to be placed in ice to prevent loss of viability of the virus.



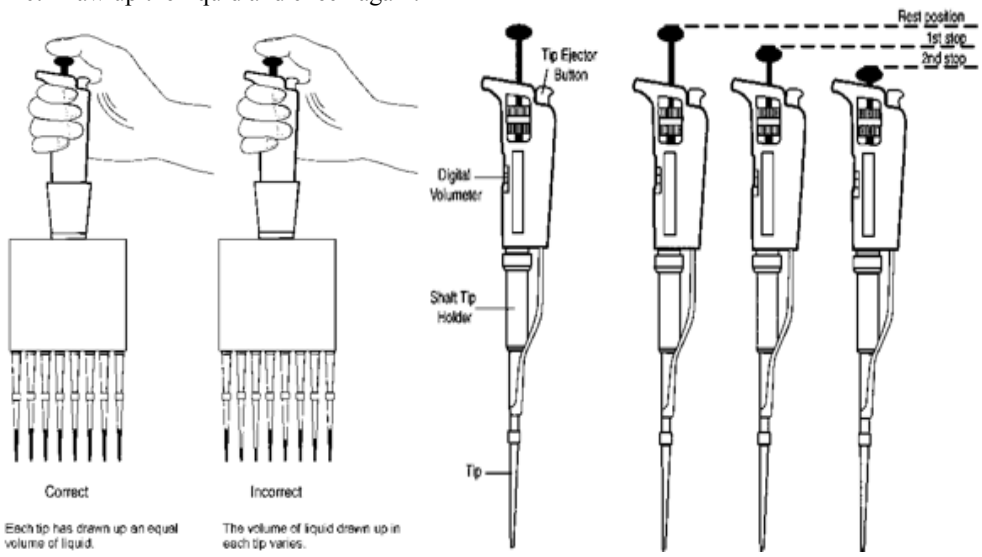
11- Ultrasonic disrupting of cells: Sonification of the cells can be used for preparing virus antigen, especially for freeing virus from the material. Ultrasonic disruption causes rise in temperature of the material, so adequate cooling is needed. Prevent damage to your ear by wearing protector.



12. The single and multichannel micropipettes

Micropipettes use plastic disposable tips. For ease of use, tips are usually packed into plastic boxes that can be autoclaved. Make sure the tips you are using will fit tightly onto the end of the pipette.

1. Treat micropipettes very gently as they are precision instruments.
2. Keep upright when in use to prevent liquids running inside the shaft of the pipette.
3. Do not leave pipettes lying on the workbench where they can be knocked off and damaged.
4. Do not allow pipettes to come into contact with corrosive chemicals.
5. Before use, make sure the volume has been correctly set. Adjust the volume before use. Most pipettes have a digital display of volume.
6. Check all tips are securely fitted to pipette.
7. Draw liquid up.
8. Check that the liquid drawn up has reached the expected level in the tip and there are no air bubbles in the tip. When using multichannel pipettes, check that the volume of liquid is the same in each tip.
9. If necessary, expel the liquid and manually tighten the tips onto the pipette.
10. Draw up the liquid and check again.



Instructions for pipetting liquids using a micropipette

1. Micropipettes have 3 positions:
   a. Rest position
   b. First stop
   c. Second stop
2. Fit the tip to the end of the shaft. Press down and twist slightly to ensure an airtight seal.
3. Hold the pipette in a vertical position. Depress the plunger to the first stop. Air equal to the volume of the setting (e.g. 100 mL) is displaced.
4. Immerse the tip into the liquid. Release the plunger back to the rest position. Wait a second for liquid to be sucked up into the tip. The volume of liquid in the tip will equal the volume of the setting of the micropipette.
5. Place the tip at an angle (10° to 45°) against the wall of the vessel receiving the liquid, for example a well of a microwell plate. Depress the plunger to the first stop, wait one second, press the plunger to the second stop to expel all the liquid
6. Move the end of the tip away from the liquid. Release the plunger to the rest position.

Graduated pipettes

Graduated pipettes are calibrated and marked with graduation lines that allow the measurement of more than one volume. The volume is read by eye by reading the value indicated on the scale at the bottom of the meniscus.

Disposable plastic graduated pipettes are available and are useful for pipetting toxic or viscous

substances.
Graduated pipettes made of glass can be washed and reused. The pipettes should be plugged with cotton wool on the top and sterilized before use to minimize contamination of fluids being measured.

Pasteur pipettes
Pasteur pipettes are glass pipettes used to transfer fluids from one place to another. They are not graduated and are therefore not used to measure volumes. Like graduated pipettes they should be plugged with cotton wool and sterilized before use.

Pipette fillers
Pipetting by mouth is not an acceptable laboratory practice. Fluids are drawn up into pipettes using pipette fillers. There are several options. A simple rubber bulb is suitable for a 1 mL pipette. For 10 mL pipettes, use a triple valve rubber bulb, a hand operated pump or an electronic pipette filler.

Safety Manual:

Every laboratory should have a safety manual.
A list of some of the safety issues to be addressed in a laboratory safety manual

1. Fire
2. First aid
3. Safe use of laboratory glassware
4. Safety with handling boiling liquids
5. Cleaning up spills and broken glass
6. Decontamination of benches and waste
7. Recycling of infected materials for example, needles and syringes
8. Safe use of chemicals

A list of basic laboratory rules:

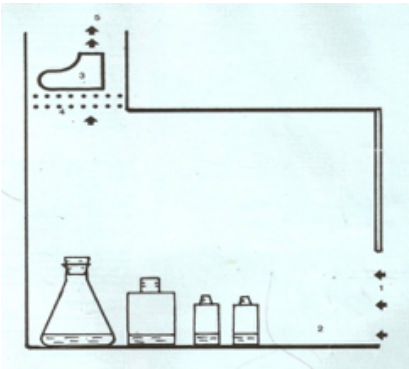
1. Always wash hands thoroughly with soap on entering and leaving the laboratory.
2. Do not eat, drink or smoke in the laboratory.
3. Always wear a laboratory coat.
4. Label all reagents clearly with contents, date and the initials of the person who prepared the reagent.
5. Do not pipette by mouth.

All works with contagious (air born infection) material has to be done in a safety cabinet to prevent laboratory acquired infections. Safety cabinets for virological purposes are constructed so that all aerosols are carried away over a filter before the air is disposed of. The worker is protected by a curtain of sterile air.

There are four (4) types of Safety Cabinets:

1- Laminar flow clean air cabinet: This protects the work against microbial contamination by a laminar flow of sterile air. This type of cabinet is suitable for making media and handling of non-infected tissue cultures. It is not suitable for infectious work as personnel are exposed to the exhaust air.

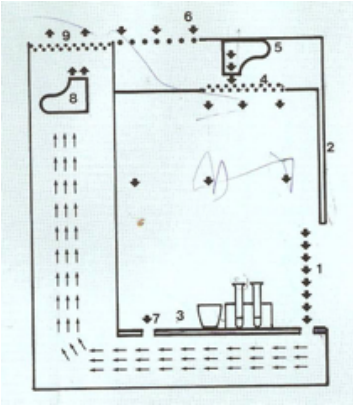
2- Class I microbiological safety cabinet: This protects the worker. Air is drawn from the room, with the air steam away from the worker thus protecting him against infection. The incoming air is not sterile and may contaminate the work. The exhausted air is filtered to protect the environment.



1- Open front with inward airflow
2- Working space
3- Exhaust fan
4- Exhaust hepafilter
5- Sterile exhaust air

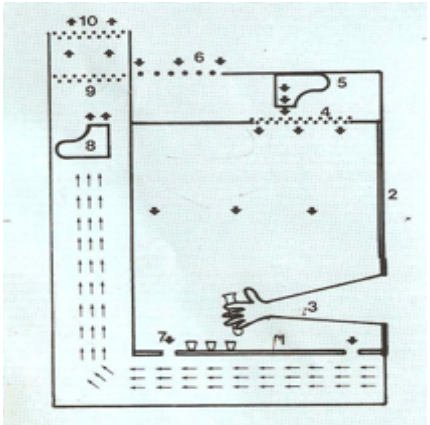
3- Class II microbiological safety cabinet: This protects both the work and the worker. Incoming and exhaust air is filtered. The open front is protected by an air current, which acts as a curtain.

Highly contagious material must be handled in a class III cabinet as air and liquid spilling through the front are not absolutely prevented.



1- Open front with air curtain
2- Window
3-Working space
4- Inlet with hepafilter
5- Inlet fan
6- Air inlet with prefilter
7- Air vents in the bottom
8- Exhaust fan
9- Exhaust hepafilter

4- Class III bacteriological safety cabinet: This type gives maximal protection for the test, the worker and the environment. Regular inspection of the filter and the fan motors is necessary for maintaining the maximal safety level.



1- Working space
2- Window
3-Gloves, making the cabinet front totally closed
4- Air inlet with prefilter
5- Air inlet fan
6- Air inlet prefilter
7- Air vents to the duct in the bottom
8- Exhaust fan
9 and 10- A double set of hepafilters in the exhaust