Unit - 1 Air Microbiology

I) Composition of air:-

The atmosphere consists of a mixture of Permanent gases & variable quantities of water and solid particles. Its gaseous content, vapour pressure and suspended matter are not constant in composition.

According to Landsberg, air has the following composition

	Elements	Percentage
1)	Nitrogen	78
2)	Oxygen	20
3)	Argon	0.9
4)	Carbon Dioxide	0.03
5)	Hydrogen	0.01
6)	Other gases like Neon Krypton, Helium	0.005
7)	Ozone	Variable
8)	Water Vapour	Variable
9)	Dust	Variable

Various layers can be recognized in the atmosphere up to a height of about 1000km. The layer nearest to the earth is called as troposphere. In temperate regions, troposphere extends up to about 11 km whereas in tropics up to about 16km. This troposphere is characterized by a heavy load of microorganisms.

The temperature of the atmosphere varies near the earth's surface. However, there is a steady decrease of about 1^0C / 150 meter until the top of the troposphere. Above the troposphere, the temperature starts to increase.

The atmosphere as a habitat is characterized by high light intensities, extreme temperature variations, low amount of organic matter and insufficiency of available water making it a non hospitable environment for microorganisms and generally unsuitable habitat for their growth. Nevertheless, considerable numbers of microbes are found in the lower regions of the atmosphere.

II) Distribution of air borne microorganisms

Air does not have its own microbes; they are transported from aquatic and terrestrial habitats into the atmosphere. Microbes of air within 300-1,000 or more feet of the earth's surface are the organisms of soil that have become attached to fragments of dried leaves, straw or dust particles, being blown away by the wind. Relative humidity, temperature and radiation exposures affect the kind and number of microbes in air.

More microbes are found in air over land masses than far at sea. Spores of fungi, especially *Alternaria*, *Cladosporium*, *Penicillium* and *Aspergillus* are more numerous than other forms over sea within about 400 miles of land in both polar and tropical air masses at all altitudes up to about 10,000 feet.

Microbes found in air over populated land areas below altitude of 500 feet in clear weather include spores of *Bacillus* and *Clostridium*, ascospores of Yeasts, fragments of myceilium and spores of Molds and Streptomycetaceae, Pollen, Protozoan cysts, Algae, Micrococcus, Corynebacterium etc.

In the dust and air of schools and hospital wards or the rooms of persons suffering from infectious diseases, microbes such as *tubercle bacilli, streptococci, pneumococci and staphylococci* have been demonstrated.

These respiratory bacteria are dispersed in air in the droplets of saliva and mucus produced by coughing, sneezing, talking and laughing. Viruses of respiratory tract and some enteric tract are also transmitted by dust and air. Pathogens in dust are primarily derived from the objects contaminated with infectious secretions that after drying become infectious dust.

Droplets are usually formed by sneezing, coughing and talking. Each droplet consists of saliva and mucus and each may contain thousands of microbes. It has been estimated that the number of bacteria in a single sneeze may be between 10,000 and 100,000. Small droplets in a warm, dry atmosphere are dry before they reach the floor and thus quickly become droplet nuclei.

III) Sources of air borne microorganisms

Although a number of microorganisms are present in air, it doesn't have an indigenous flora. Air is not a natural environment for microorganisms as it doesn't contain enough moisture and nutrients to support their growth and reproduction.

Numbers of sources have been studied in this connection and almost all of them have been found to be responsible for the air micro flora. One of the most common sources of air micro flora is the soil.

Soil microorganisms when disturbed by the wind blow, liberated into the air and remain suspended there for a long period of time. Man made actions like digging or ploughing the soil may also release soil borne microbes into the air. Similarly microorganisms found in water may also be released into the air in the form of water droplets or aerosols. Splashing of water by wind action or tidal action may also produce droplets or aerosols.

Air as a carrier of microorganisms.

Air currents may bring the microorganisms from plant or animal surfaces into air. These organisms may be either commensals or plant or animal pathogens. Studies show that plant pathogenic microorganisms are spread over very long distances through air. For example, spores of *Puccinia graminis* travel over a thousand kilometers. However, the transmission of animal diseases is not usually important in outside air.

The main source of airborne microorganisms is human beings. Their surface flora may be shed at times and may be dispersed into the air. Similarly, the commensal as well as pathogenic flora of the upper respiratory tract and the mouth are constantly discharged into the air by activities like coughing, sneezing, talking and laughing.

IV) Number and kinds of microorganisms in air

The kinds & members of microbes in air vary & dependent up on the sources of contamination in the environment & locality. However, certain

microorganisms are commonly found in air & in some localities. E.g. Spores of molds & yeasts are quite resistant to light, which is lethal to bacterial spores. Also, fungal spores are resistant to drying than bacteria.

The numbers of organisms in the air depends upon the activity in the environment of the amount of dust stirred up. Microorganisms survive in the air for varying period of time. This is controlled by

➔ Atmospheric conditions

E.g. Speed of the air current, humidity, sunlight, temp, size of the particles on which they are attached & the nature of the organisms i. e. ability to survive in a new physical environment.

 # Microorganisms generally present in air

- **Spores of fungi: -**

e.g. of *Alternaria, Aspergillus, Cladosporium, Homodendrum, Penicillium,*

- *Ascospores of yeasts, conidia of Streptomycetes.*

- **Endospores of bacteria: -**

e.g. *Bacillus & clostridium*

- **Bacteria: -**

e.g. species of the genera *Alcaligenes, Chromobacterium, Micrococcus, Sarcina,* etc.

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❖ **Droplets, Droplet nuclei and Bioaerosols:-**

- **Droplets**

Droplets are usually formed by sneezing, coughing or talking. Each consists of saliva and mucus. Droplets may also contain hundreds of microorganisms which may be pathogenic if discharged from diseased persons. Pathogens will be mostly of respiratory tract origin. The size of the droplet determines the time period during which they can remain suspended.

Most droplets are relatively large, and they tend to settle rapidly in still air. When inhaled these droplets are trapped on the moist surfaces of the

respiratory tract. Thus, the droplets containing pathogenic microorganisms may be a source of infectious disease.

- **Droplet Nuclei**

Small droplets in a warm, dry atmosphere tend to evaporate rapidly and become droplet nuclei. Thus, the residue of solid material left after drying up of a droplet is known as droplet nuclei. These are small, 1-4µm, and light. They can remain suspended in air for hours or days, traveling long distances.

They may serve as a continuing source of infection if the bacteria remain viable when dry. Viability is determined by a set of complex factors including, the atmospheric conditions like humidity, sunlight, temperature, the size of the particles bearing the organisms, and the degree of susceptibility or resistance of the particular microbial species to the new physical environment.

If inhaled droplet nuclei tend to escape the mechanical traps of the upper respiratory tract, enter the lungs. Thus, droplet nuclei may act as more potential agents of infectious diseases than droplets.

- **Bioaerosols**

Airborne biological particles /droplets containing biological material, dead or alive usually 0.5-30 micrometer are called as bioaerosols.

VI) Techniques for microbiological analysis of air / Enumeration of microorganisms in Air (Sampling of Air-born microorganisms): -

There are several methods, which require special devices used for the enumeration of microorganisms in air. Following are the methods—

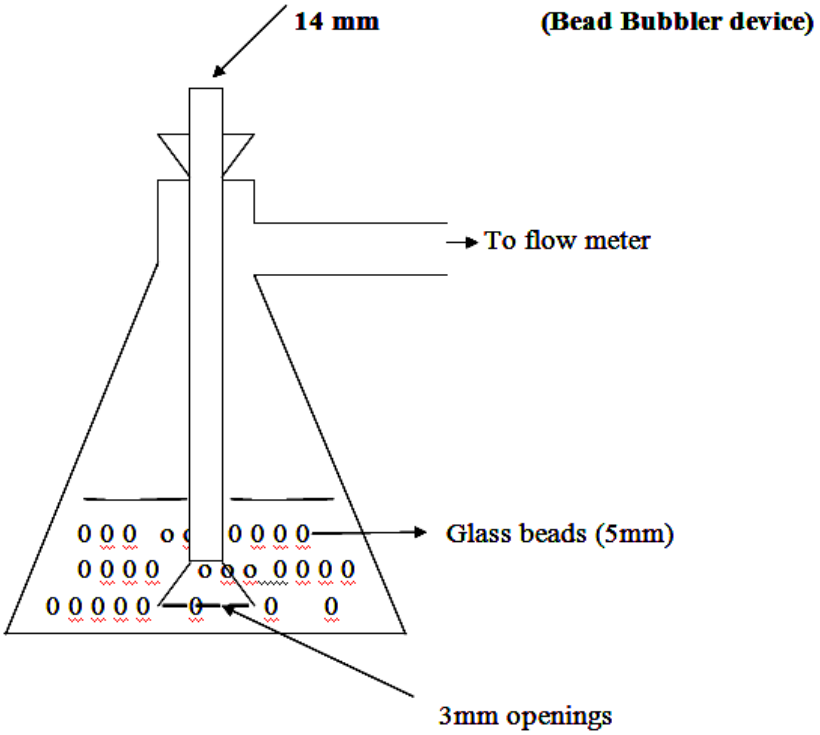
1) Impingement in liquids
2) Impingement (Impaction) on solids.
3) Filtration
4) Sedimentation
5) Centrifugation
6) Electrostatic precipitation
7) Thermal precipitation

1) Impingement in liquids: -

In this method, the air is drawn though a very small opening or a capillary tube & bubbled through the liquid. The organisms get trapped in the liquid medium.

Aliquots of the liquid are then plated to determine the number.

The commonly used device is the bead bubbler device developed by Lemon (1943).



It consists of

- ➔ A suction flask containing broth
- ➔ Glass beads
- ➔ Glass bubbler

Glass aeration tube with a bulb at one end perforated by 6 holes is passed through a hole in rubber stopper & the bulb is centered near the bottom of the flask. A flow meter measures the rate of airflow entering the upper open end of the tube. An air pump is attached to the exhaust end.

The entire bubbler should be sterilized by autoclaving or by rinsing with 70 % alcohol & drying. Air is drawn in at the rate of 25 to 30 liters per minute & is dispersed through 20 ml of broth containing 2 to3 drops of olive oil to prevent foaming. The glass beads also break the foaming & air particles.

An aliquot part of the water or medium is mixed with melted nutrient agar in a petri dish. After incubation the colonies are contend.

2) Impingement (Impaction) on solids: -

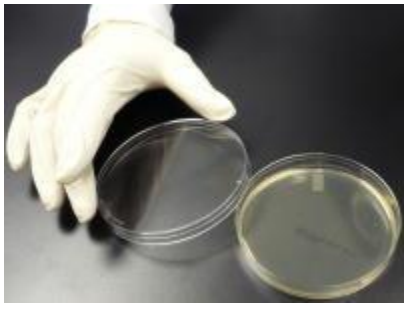
In this method, the microorganisms are collected or impinged directly on the solid surface of agar medium. Colonies develop on the medium where the organism impinges. Following are the methods of impingement on solid medium.

A. Settling Plate technique
B. Sieve device
C. Slit device
D. Tilak sampler
E. Aircraft sampling

A) Settling Plate Technique: -

This is the simplest & often used method. In this method, the cover of the petri dish containing sterile nutrient agar medium is removed & the surface of medium is exposed to the air for some minutes. Then cover is kept & plate is incubated at room temperature for particular time to develop colonies. Each colony represents a particle carrying microorganisms.

This technique gives information about the kind of microorganisms in a particular area but does not give the information about the volume of air actually sampled. It gives only a rough estimate.



B) Sieve Device: -

This device consists of a large number of small holes in a metal cover, under which a petri dish containing an agar medium. A measured volume of air is drawn through these small holes.

Airborne particles impinge upon the agar surface. The plate is incubated & then colonies are counted.

C) Slit Device:-

This is a special apparatus used for air sampling. A known volume of air is directed on to a medium in a plate through a slit of 0.25 mm wide. The plate is mechanically rotated so that microorganisms are evenly deposited over it. One cubic foot of air per minute is allowed to pass through the slit.

The slit is approximately of length of the petridish. The petridish is rotated at a particular speed under the slit. One complete turn is made during the sampling operation.

Then plate is removed, incubated, colonies are counted & studied. This method is generally used in examining the amount of microbial contamination in the hospitals, schools, industries, hours etc.

D) Tilak Sampler:-

This sampler is designed by Tilak & Kulkarni in 1970 by modifying panzer's slide spore collector. The sampler gums on electric power supply & results in a continuous air sampling for 8 days. The air is sucked through a projecting tube at the rate of 5 liters/minute & passes through a transparent cello tape (1.5 cm in breadth) which is stuck on the slowly rotating drum. The drum completes one circle in 8 days & hence provides the trace of microbial catches within 8 days.

E) Air craft Sampling:-

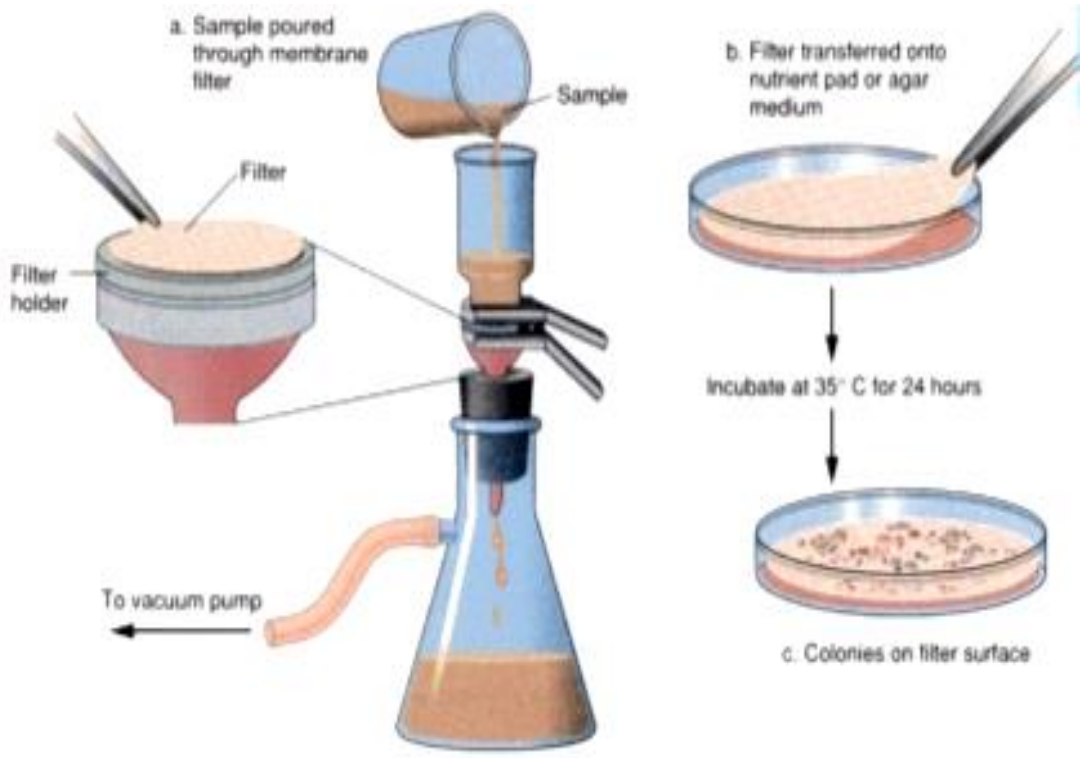
Air crafts furnished with sticky microscope slide or Petri dish are used for the study of atmosphere. This method has been considered the simplest & widely used method for collecting aerosol particles.

3) Filtration:-

In filtration polypore & millipore type membranes are used for the removal of organisms from air.

The diameters of the collected particles are laser than the pores of the membranes and are retained on or within a few microns of the filter surface. After filtration the membrane is agitated in a suitable liquid to disperse the

particles, then aliquots of the suspension are mixed with melted agar & incubated & counts are determined.



4) Sedimentation:-

Air microorganisms may be collected by exposing agar plates for definite periods of time. Then the covers are replaced, plates are incubated & counts are determined.

This is one of the simplest procedures used but is of no value from a quantitative standpoint. It does not indicate the number of organisms present in a given volume of air. However this method & gives relative results & is commonly used for that purpose.

Air movement may influence the results by causing the deposition of the larger – sized particles. When aerosols are collected in a closed room free from air movements, a quantitative measure of particle size distribution may be obtained by microscopic examination.

5) Centrifugation:-

In this type of samples centrifugal force is used to collect air particles on to the collecting surface when air moves in a circular direction at high speed, the suspended aerosols are impacted on the collecting surface by a force proportional to the particle velocity & mars.

In one type of centrifuge the sampler remain stationary while the aerosol travels in a circular path, the larger particles are collected at the bottom. In another type the collecting vessel and aerosol rotate at high velocity resulting in the impaction of particles on the walls.

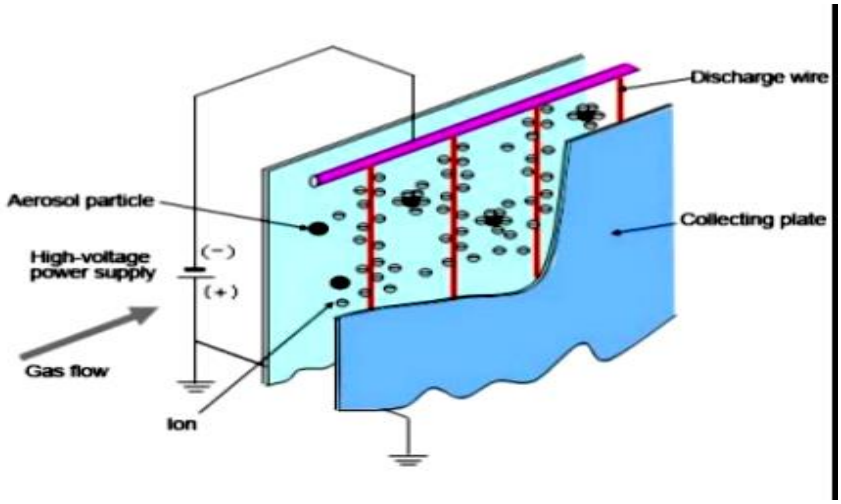
The centrifuge consists of a head assembly with exhaust fan, air inlet tube & chamber to contain the sample tube. The variable speed motor provides speeds from 2000 to 4500 rpm. Air flow is measured by a manometer tube with reservoir bottle & rubber connecting tube. The head assembly fan & sample chamber are driven by the motor the sample tube. The air inlet tube serves as the upper shaft.

6) Electrostatic precipitation:-

Samplers using this method collect particles by drawing air over an electrically charged surface.

Luckiesh et. Al (1946) devised a sampler holding 2 petri dishes in separate plastic units with removal covers. A small electrically operated blower draws air at equal rates through the 2 units. One unit has the lower electrode negative & the upper electrode a fairly flat metal cone positive. In the other unit the electrical conditions are reversed.

Both positively & negatively charged organisms exist in the air simultaneously. There having a positive charge are collected on the petri dish placed over the negative electrode, these negatively charged are collected on the petri dish placed over the positive electrode.



7) Thermal precipitation:-

Samplers of this type collect particles on surfaces by means of thermal gradients. Air borne particles are repelled by hot surfaces & deposited on colder surfaces by forces proportional to the temperature gradient. Since such samplers

require careful adjustment & the air sampling rates are quite low, they are not commonly used.

VII) Significance of Air Micro flora in Human Health / Hospitals / Industries

Human Health

The significance of air micro flora in human health relies on the fact that air acts as a medium for the transmission of infectious agents. An adult man inhales about 7 to 8 liters of air per day. Although most of the microorganisms present in air are harmless saprophytes and commensals, less than 1 % of the airborne bacteria are pathogens.

Even though the contamination level is very low, the probability of a person becoming infected will be greatest if he is exposed to a high concentration of airborne pathogens. Carriers, either with the manifestation of corresponding symptoms or without any apparent symptoms, may continuously release respiratory pathogens in the exhaled air.

Staphylococcus aureus is the most commonly found pathogen in air since the carriers are commonly present. The number of S. aureus in air may vary between 0-l/m3 and 50/m3.

Practically speaking, outdoor air doesn't contain disease causing pathogen in a significant number to cause any infection. The purity of outdoor air, however, is an essential part of man's environment. Dispersion and dilution by large volume of air is an inherent mechanism of air sanitation in outside air.

In the case of indoor air chance for the spread of infectious disease is more, especially in areas where people gather in large numbers. For example, in theatres, schools etc.

Hospitals

Although hospitals are the war fields for combating against diseases, there are certain occasions in which additional new infectious diseases can be

acquired during hospitalization. Air within the hospital may act as a reservoir of pathogenic microorganisms which are transmitted by the patients.

Infections acquired during the hospitalization are called nosocomial infections and the pathogens involved are called as nosocomial pathogens.

Nosocomial infection may arise in a hospital unit or may be brought in by the staff or patients admitted to the hospital. The common microorganisms associated with hospital infection are *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus, Pseudomonas aeruginosa*, members of *Enterobacteriaceae* and respiratory viruses.

Development of high antibiotic resistance is a potential problem among nosocomial pathogens. For example, Methicillin Resistant *Staphylococcus aureus* (MRSA) and gentamicin resistant Gram-negative bacilli are of common occurrence. Even antiseptic liquids used would contain bacteria, for example Pseudomonas, due to their natural resistance to certain disinfectants and antiseptics and too many antibiotics.

Nosocomial pathogens may cause or spread hospital outbreaks. Nosocomial pneumonia is becoming a serious problem nowadays and a number of pathogens have been associated with it.

Frequent agents are *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa, Enterobacter, Klebsiella, Escherichia coli and Haemophilus influenzae*. There are two main routes of transmission for nosocomial pathogens, contact (either direct or indirect) and airborne spread.

Industries

The significance of air flora is well understood in industries in which sterile products are prepared, processed, packaged or stored aseptically. These include food industries, pharmaceutical industries and other fermentation industries.

In food industries, contamination of the food products by microorganisms may lead to spoilage of the products and may cause food borne illness in consumers who take the spoiled foods. This can be prevented by providing aseptic atmosphere for the processing and packaging of food products.

In pharmaceutical and allied industries where aseptic filling is done or aseptic assembly of sterile products is done, sterile atmosphere is required which can be met by providing filtered sterile air. In fermentation industries,

where microorganisms are used for the production of antibiotics, enzymes or organic acids, the significance of air micro flora has been well recognized.

IX) Air Sanitation / Control of airborne Micro-organisms.

For the removal or destruction of microorganisms, various methods are used. Airborne microorganisms are controlled through the application of Physical techniques or Chemical agents**.**

Under certain conditions disinfection or sterilization of air, is desirable. Following are the methods generally used for the control of microorganisms in the air of rooms & buildings.

1) Dust control
2) Ultraviolet radiation.
3) Bactericidal vapours.
4) Laminar air flow system.

1) Dust Control.

Dust found in homes offices, schools, factories and hospitals arises from airborne sand, are and soot, soil and lint from bedding, clothing and carpets. Most dust particles microorganisms. There microorganisms are studied in relation to infections of respiratory tract & skin and secondary infections of burns & wood. Therefore suppression of dust in room cleaning operations is extremely important.

For the control of dust following methods are generally applied.

a) Oiling –
b) Use of dry-vacuum Pick up.
c) Disinfectant detergent solution.

a) Oiling- oiling of floors, bedclothes & other tektites is a highly effective method.

b) Use of dry-Vacuum pick up-

Vacuum pumps are used to pick up dust, then dust then disinfectant detergent solution is used.

c) Disintectant detergent solution

Disinfectants like phenol, dettol; & detergents are used to work the floor & the material to control dust.

Where vacuum cleaning facilities are not available sooner material such as oiled saw dust should be applied before sweeping this prevents the scattering of dust.

2) Ultraviolet radiation –

The ultraviolet light of wavelength 254 mm is microbicidal by affecting nucleic acid of microorganisms this wavelength interfere the multiplication of microorganisms & thus they are killed.

Application of this killing effect has been made in the irradiation of air with u.v. light using a wavelength are effective only when they make direct contact with the particles carrying the microorganisms there rays have title penetrating power & are irritating to human eyes & skin there fore care should be taken during application.

Lamps or tubes of U.V. light are used. In occupied rooms indirect irradiations are used & the occupants are protected from direct exposure to the rays. In non-occupied rooms rays are left on.

In some situations, air can be treated apart from the room or space. In air circulating systems, air is first frittered then passed through a tube where it is irradiated by powerful U.V. sources.

3) Bactericidal Vapours.

Many airborne microorganisms are killed when certain chemical substances are vaporized or sprayed into the air of a room. Germicidal substances are dispersed as aerosols. Vapours of Formaldehyde, β-Propiolactone, Ethylene oxide, propylene glycol and triethylene glycol are strongly germicidal. These are colourless, tasteless, non-irritating, nontoxic, and not explosive or corrosive.

These are extremely reactive chemicals which combine readily with enzymes, proteins and nucleic acids of microorganisms and inactivate them.

Formaldehyde

Both in gaseous and in solution state has high anti microbial activity which kills all types of microorganisms. It is used to sterilize closed rooms, cabins and operation theaters.

β- Propiolactone

It is sporicidal, fungicidal and viricidal. Only 2 to5 mg per liter is required to kill all types of microorganisms.

Ethylene oxide

It exists in liquid state below 10.8 ^{o} C and vaporizes above this temperature. It can be applied at room temperature. It is highly inflammable therefore it is generally mixed with CO_2 or Freon to decrease inflammability. 400 to 800 mg per liter is the usual concentration to be used. It is used to sterilize large packages of materials, bundles of cloths and plastics.

Propylene glycol

The vapour from as little as 0.5 mg of propylene glycol can kill nearly all the microorganisms in a liter of heavily contaminated air within 15 seconds.

Triethylene glycol

Triethylene glycol is nearly 10 times as germicidal.

4) Laminar air flow system

In this system air passes through high efficiency particulate air, (HEPA) filters. These consist of cellulose acetate (filter medium) pleated around aluminium foil. Particles as small as 0.3 µm are removed by this filter system. Air is passed through a bank of these filters and into the enclosure, so that the entire body of air moves with uniform velocity along parallel flow lines. Many other methods and practices are useful in controlling microorganisms in air.

Ventilation is one such method which is very effective in controlling airborne diseases indoors. With extensive development in space technology,

electronics, and the aerospace industry an extremely high degree of cleanliness is required.