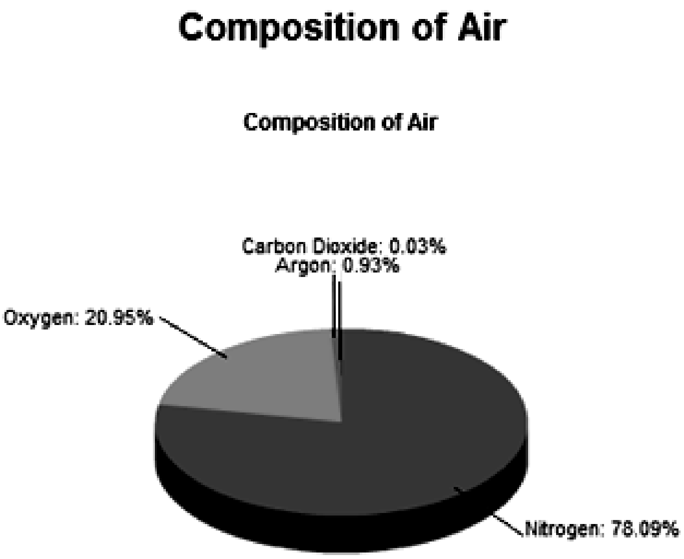
AIR MICROBIOLOGY



Component	Symbol	Volume	
Nitrogen	N_2	78.084%	99.998%
Oxygen	O_2	20.947%	
Argon	Ar	0.934%	
Carbon Dioxide	CO_2	0.033%	
Neon	Ne	18.2 parts per million	
Helium	He	5.2 parts per million	
Krypton	Kr	1.1 parts per million	
Sulfur dioxide	SO_2	1.0 parts per million	
Methane	CH_4	2.0 parts per million	
Hydrogen	H_2	0.5 parts per million	
Nitrous Oxide	N_2O	0.5 parts per million	
Xenon	Xe	0.09 parts per million	
Ozone	O_3	0.07 parts per million	
Nitrogen dioxide	NO_2	0.02 parts per million	
Iodine	I_2	0.01 parts per million	
Carbon monoxide	CO	trace	
Ammonia	NH_3	trace	

Microbes Found in Air

In addition to gases, dust particles and water vapour, air also contains microorganisms. There are vegetative cells and spores of bacteria, fungi and algae, viruses and protozoan cysts. Since air is often exposed to sunlight, it has a higher temperature and less moisture. So, if not protected from desiccation, most of these microbial forms will die. Air is mainly it transport or dispersal medium for microorganisms. They occur in relatively small numbers in air when compared with soil or water. The microflora of air can be studied under two headings outdoor and indoor microflora.

Outdoor microflora: The air in the atmosphere, which is found outside the buildings, is referred to as **outside air**. The dominant microflora of outside air are fungi. The two common genera of fungi are *Cladosporium* and *Sporobolomyces*. Besides these two genera, other genera found in air are *Aspergillus*, *Alternaria*, *Phytophthora* and *Erysiphe*. The outdoor air also contains basidispores, ascopores of yeast, fragments of mycelium and conidia of molds. Among the bacterial genera *Bacillus* and *Clostridium*, *Sarcina*, *Micrococcus*, *Corynebacterium* and *Achromobacter* are widely found in the outside air. The number and kind of microorganisms may vary from place to place, depending upon the human population densities.

Indoor microflora: The air found inside the building is referred to as Indoor air. The commonest genera of fungi in indoor air are *Penicillium*, *Aspergillus*. The commonest genera of bacteria found in indoor air are *Staphylococci*, *Bacillus* and *Clostridium*. In case of occupants being infected, The composition shows slight variations with latitude and to a lesser extent with altitude. The ozone owes its existence in the atmosphere to photosynthesis from oxygen under the influence of solar ultraviolet radiations.

Distrubution of Microorganisms in Air

No microbes are native to the atmosphere rather they represent allochthonous populations 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. Species vary greatly in their sensitivity to a given value of relative humidity, temperature and radiation exposures. 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,* ascos-pores 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.

Many plant pathogens are also transported from one field to another through air and the spread of many fungal diseases of plants can be predicted by measuring the concentration of airborne fungal spores. Human bacterial pathogens which cause important airborne diseases such as diphtheria, meningitis, pneumonia, tuberculosis and whooping cough are described in the chapter "Bacterial Diseases of Man".

Sources of Microorganisms in Air

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. Quite a number of sources have been studied in this connection and almost all of them have been found to be responsible for the air microflora. One of the most common source of air microflora 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 soilborne 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 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 disseminated 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. The microorganisms are discharged out in three different forms which are grouped on the basis of their relative size and moisture content. They are droplets, droplet nuclei and infectious dust. It was Wells, who described the formation of droplet nuclei. This initiated the studies on the significance of airborne transmission.

Droplet: 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 and 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 and enter the lungs. Thus, droplet nuclei may act as more potential agents of infectious diseases than droplets.

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Infectious Dust - Large aerosol droplets settle out rapidly from air on to various surfaces and get dried. Nasal and throat discharges from a patient can also contaminate surfaces and become dry. Disturbance of this dried material by bed making, handling a handkerchief having dried secretions or sweeping floors in the patient's room can generate dust particles which add microorganisms to the circulating air.

Most dust particles laden with microorganisms are relatively large and tend to settle rapidly. Droplets expelled during coughing, sneezing, etc consist of sativa and mucus, and each of them may contain thousands of microorganisms. Most droplets are large, and, like dust, tend to settle rapidly. Some droplets are of such size that complete evaporation occurs in a warm, dry climate, and before they reach the floor quickly become droplet nuclei. These are small and light, and may float about for a relatively long period. Airborne diseases are transmitted by two types of droplets, depending upon their size.

(1) Droplet infection proper applies to, droplets larger than 100 μm in diameter.

(2) The other type may be called airborne infection, and applies to dried residues of droplets. Droplet infection remains localized and concentrated, whereas airborne infection may be carried long distances arid is dilute.

Microorganisms can survive for relatively longer periods in dust. This creates a significant hazard, especially in hospital areas. Infective dust can also be produced during laboratory practices like opening the containers of freeze dried cultures or withdrawal of cotton plugs that have dried after being wetted by culture fluids. These pose a threat to the people working in laboratories.

Techniques for microbiological analysis of air

There are several methods, which require special devices, designed for the enumeration of microorganisms in air. The most important ones are solid and liquid impingement devices, filtration, sedimentation, centrifugation, electrostatic precipitation, etc. However, none of these devices collects and counts all the microorganisms in the air sample tested. Some microbial cells are destroyed and some entirely pass through in all the processes. Some of the methods are described below.

Impingement in liquids: In this method, the air drawn is through a very small opening or a capillary tube and bubbled through the liquid. The organisms get trapped in the liquid medium. Aliquots of the liquid are then plated to determine its microbial content. Aliquots of the broth are then plated to determine microbial content.

Impingement 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. Several devices are used, of which the **settling-plate technique** is the simplest, In this method the cover of the pertridish containing an. agar medium is removed, and the agar surface is exposed to the air for several minutes. A certain number of colonies develop on incubation of the petridish. Each colony represents particle carrying microorganisms. Since the technique does not record the volume of air actually sampled, it gives only a rough estimate. However, it does give information about the kind of microorganisms in a particular area. Techniques where in a measured volume of air is sampled have also been developed. These are sieve and slit type devices. A sieve device has a large number of small holes in a metal cover, under which is located a petridish containing an agar medium.

A measured volume of air is drawn, through these small holes. Airborne particles impinge upon the agar surface. The plates are incubated and the colonies counted. In a slit device the air is drawn through a very narrow slit onto a petridish containing agar medium. The slit is approximately the length of the petridish. The petridish is rotated at a particular speed under the slit One complete turn is made during the sampling operation.

Filtration: The membrane filter devices are adaptable to direct collection of microorganisms by filtration of air. The method is similar in principle to that described for water sampling.

Significance of Air Microflora

Although, when compared with the microorganisms of other environments, air microflora are very low in number, they playa very significant role. This is due to the fact that the air is in contact with almost all animate and inanimate objects.

The significance of air flora has been studied since 1799, in which year Lazaro Spallanzani attempted to disprove spontaneous generation. In t 837, Theodore Schwann, in his experiment to support the view of Spallanzani, introduced fresh heated air into a sterilized meat broth and demonstrated that microbial growth couldn't occur. This formed the basis of modern day forced aeration fermentations. It was Pasteur in 1861, which first showed that microorganisms could occur as airborne contaminants. He used special cotton in his air sampler onto which the microorganisms were deposited. He microscopically demonstrated the presence of microorganisms in the cotton. In his famous swan necked flask experiment, he showed that growth could not occur in sterile media unless airborne contamination had occurred.

Air Microflora Significance in Human Health - The significance of air microflora in human health relies on the fact that air acts as a medium for the transmission of infectious agents. An adult man inhales about '5m3 of air per day. Although most of the microorganisms present in air are harmless saprophytes and commensals, less than I % of the airborne bacteria are pathogens. Eventhough 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.

Air Microflora Significance in 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.

Infection acquired during the hospitalization is called nosocomial infections and the pathogens involved are called as nosocomial pathogens. Infections, manifested by the corresponding symptoms, after three days of hospitalization can be regarded as nosocomial infection. 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 to 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*. Other less frequent agents are enterococci, streptococci other than

S. pneumoniae, *Serratia marcescens*, *Citrobacter freundii*, *Acinetobacter sp.* and *Xanthomonas sp*.

In addition *Legionella, Chlamydia pneumoniae* and *Mycobacterium tuberculosis* have also been reported. Nosocomial transmissions of tuberculosis from patients to patients and from patients to health care workers have also been well documented. There are two main routes of transmission for nosocomial pathogens, contact (either direct or indirect) and airborne spread. Airborne spread is less common than the spread by direct or indirect contact. It occurs by the following mechanisms. The source may be either from persons or from inanimate objects.

In case of spread from persons the droplets from mouth, skin scales from nose, skin exudates and infected lesion transmit diseases such as measles, tuberculosis, pneumonia, staphylococcal sepsis and streptococcal sepsis. Talking, coughing and sneezing produce droplets. Skin scales are shed during wound dressing or bed making. In case of inanimate sources particles from respiratory equipment and air-conditioning plant may transmit diseases. These include Gram-negative respiratory infection, Legionnaire's disease and fungal infections.

Significance of Microorganisms in Industries - As long as microorganisms remain in the air they are of little importance. When they come to rest they may develop and become beneficial or harmful. Knowledge of the microorganisms in air is of importance in several aspects.

Food manufacture: Microorganisms that have been transported through the air and have settled on, or in, the material are involved in various fermentation products. Production of alcoholic beverages, vinegar, sauerkraut, ensilage, dairy products, etc., is often due to microbial activity.

Spoilage of foods and fermentation products: Microorganisms are often troublesome in the home and in industry where foods and other fermentation products are prepared. In industrial processes, where particular organisms are to be grown, to supply sterile air free from contaminating organisms is a considerable problem.

Airborne diseases

There are two main sources of microorganisms in air. These are saprophytic soil organisms raised as dust, and organisms from body tissues introduced into the air during coughing, sneezing talking, and singing. Most dust particles laden with microorganisms are relatively large and tend to settle rapidly. Droplets expelled during coughing, sneezing, etc consist of sativa and mucus, and each of them may contain thousands of microorganisms.

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Major Diseases Transmitted By Air: Among various modes of disease transmission, air is one of the important routes and a number of diseases have been shown to be transmitted through air. Since human beings and animals are continuously inhaling the external air, the chances for an airborne microorganism to find a host and cause infection are more. Most of the respiratory tract infections are acquired by inhaling the air containing the pathogen. Microorganisms in droplets and infectious dusts and spores can be easily disseminated through air. Some of the respiratory diseases, which have an airborne mode of transmission, are briefly described as follows. Since air enters the body through the respiratory tract and since such diseases frequently localize in the nose and throat, they are called respiratory diseases as a group.

Air Borne Bacterial Diseases

- **Brucellosis:** *Brucella suis* It is mainly an occupational disease among veterinarian, butcher and slaughter house workers.
- **Pulmonary Anthrax:** Bacillus anthracis is the causative agent. Transmission is mainly by inhaling the dust contaminated by animal products.
- **Diseases Caused by Streptococcus Pyogenes:** A number of diseases are caused by *Streptococcus pyogenes* which is mainly transmitted through air. Diseases Caused by *Streptococcus pyogenes* occur in the throat, skin, and systemically.
- **Rheumatic Fever**: This is upper respiratory tract infection by *S. pyogenes* characterized by inflammation and degeneration of heart valves.

- **Streptococcal Pneumonia:** It is of major occurrence among the bacterial pneumonia. Causative agent is *Streptococcus pneumonia*.
- **Meningitis :** *Haemophilus influenzae* causes meningitis in children between 6 weeks and 2 years of age.
- **Diptheria:** Diphtheria is mainly contracted by children. Infection of the tonsils, throat and nose and generalized toxemia are the symptoms. The causative agent is *Corynebacterium diphtheria*.
- **Tuberculosis:** Pulmonary tuberculosis is a severe respiratory disease. Loss of appetite, fatigue, weight loss, night sweats and persistent cough are some of the symptoms. Causative agent is *Mycobacterium tuberculosis*.
- **Legionellosis:** It is a type of branchopneumonia. *Legionella pneumophila* is the causative agent. It occurs in natural water. At times it enters and proliferates in cooling tower, air cooler and shower bath. Spraying and splashing of water containing pathogen may produce aerosols which are disseminated in air.

Air Borne Fungal Diseases: It consists of many types. They are following,

- **Cryptococcosis: L**eads to mild pneumonitis. Causative agent is the yeast *Cryptococcus neoformans*. It is a soil saprophyte. Infection is acquired by inhalation of soil particles containing the causative agent.
- **Blastomycosis:** Formation of suppurative and granulomatous lesions in any part of the body. *Blastomyces dermatitis* is the causative agent. It is a soil borne fungus and hence inhalation of soil particles containing the fungus produces the infection.
- **Coccidiodomycosis:** Infection may not be apparent but in severe cases it is fatal. Usually infection leads to self-limited influenza fever known as valley fever or desert rheumatism. Causative agent of the disease is a soil fungus, *Coccidioides immitis*. Inhalation of dust containing arthrospores of the fungus leads to infection.
- **Aspergillosis:** It is an opportunistic disease of human. Causative agent is *Aspergillus fumigatus*. Infection occurs through inhalation of spores.

5.9.3 Air Borne Viral Diseases: Air borne viral diseases are of different types. They are following,

- **Common Cold:** It is the most frequent of all human infections. Characteristic symptom includes running noses. *Rhinovirus* is the causative agent. Droplets with nose and throat discharges from infected persons are the source.

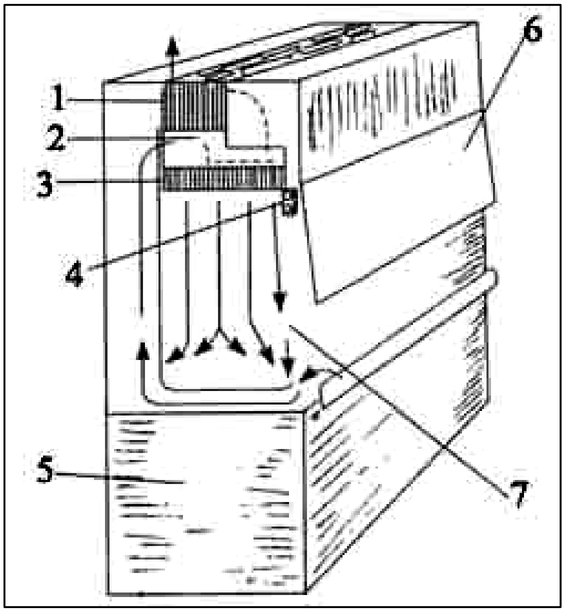
- **Influenza:** Symptoms of influenza are nasal discharge, head ache, muscle pains, sore throat and general weakness. Causative agents are *orthomyxovirus*.
- **Measles:** Measles is the most common communicable human disease mainly affecting children. Symptoms are fever, cough, cold and red, blotchy skin rash. Causative virus is *morbillivirus*. Source of infection is respiratory tract secretions in the form of droplets.
- **Mumps:** It is a communicable disease and is a common childhood disease. It is characterized by painful swelling of parotid glands and salivary glands. *Mumps virus* causes the disease. Droplets containing infected saliva are the main source.
- **Adeno Viral Diseases:** *Adenoviruses* cause acute self-limiting respiratory and eye infections. *Adenoviruses* are transmitted by airborne mode. Diseases include acute febrile pharyngitis, acute respiatory disease and adenovirus pneumonia.

Physical Techniques

(a) Dust Control: As stated earlier, the dust particles are laden with large number of microbial agents that cause diseases and allergies. Since dust occurs in home, schools, factories, laboratories and hospitals, its suppression to lower down microbial content is, therefore, extremely important. Use of dry-vacuum pickup followed by the application of suitable disinfectant detergents solution has strongly been recommended to control dust in indoor environments. In addition, oiling floors, bedclothes, and other textiles has proved a highly effective dust control device.

(b) Ultraviolet Radiation: This technique has great potential value for reducing indoor airborne microbial population. Various types of germicidal lamps are used for this purpose. These lamps emit radiations in 250-260 nm region, the most effective bactericidal region. These radiations prove effective only when they come in direct contact with the particles laden with microbes; it is because these radiation have little penetrating power. Since these radiations are irritating to human eyes and skin, skillful installation of the lamps is required to avoid injury to persons using those rooms.

(c) Laminar-airflow System: This is a new technique recommended for controlling indoor microbial population. This technique represents unidirectional airflow system in which the air passes **high-efficiency particulate air (HEPA)** filters and the entire body of air moves with uniform velocity along parallel flow lines. The HEPA filters consist of cellulose acetate plated around aluminium foil and can remove particles as small as 0.3 mm. The laminar airflow system is, therefore, suitable device in electronic and aerospace industries where an extremely high degree of cleanliness is required for product reliability.



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Thus, bacterial contamination is prevented. Coarse filters should be changed every 3-4 months depending upon the climatic conditions while HEPA filters should be changed every six months.

Bactericidal Vapours (Chemical Agents)

Indoor air-borne microbial population can be effectively reduced by vapourizing or spraying certain chemical substances into the air. Some of such chemical substances are propylene glycol, triethylene glycol, resorcinol, hypochlorous acid and b-propiolactone. Polyethylene glycol and triethylene glycol are colourless, tasteless, non-irritating, and non-toxic chemicals. Nearly all microorganisms present in a liter of heavily contaminated air can be killed by vapour from only 0.5 mg of polyethylene glycol; triethylene glycol is nearly ten times effective in comparison to polyethylene glycol. However great care is required for safe and efficient application of chemical agents and, therefore, they may be used only when rapid control of air-borne microbial content is essential.

Filtration

This is a simple method for collecting particles from air. The filter can be made of any fibrous or granular material like sand, glass fibre and alginate wool (in phosphate buffer). However, recovery of organisms for culture is not so easy.

Tube Sampler - This is one of the oldest devices for collecting and enumerating microorganisms in the air. It consists of a tube with an inlet at the top and an outlet at the bottom which is narrower than the top end. Near the bottom there is a filter of wet sand which is supported by a cotton plug below. The entire device can be sterilized. After sterilization the air to be sampled is allowed to pass through the sand and cotton. Microorganisms as well as dust particles containing microorganisms in the air are deposited in the sand filter as the air passes through it. Later the sand is washed with broth and a plate count is made from the broth by taking aliquotes of the broth.

Millipore Filter - This type of filters are made of pure and biologically inert cellulose ethers. They are prepared as thin porous, circular membranes of about 150 μm thickness. The filters have different porosity. Grades from 10nm to 811m. The assemblage contains a funnel shaped inlet and a tube like outlet. In between these two the filter is fitted. The outlet may be connected to a vacuum pump to suck known amount of air. After collecting required volume of air through the filter, it can directly be placed onto the surface of a solid medium. After incubation colonies formed can be counted.