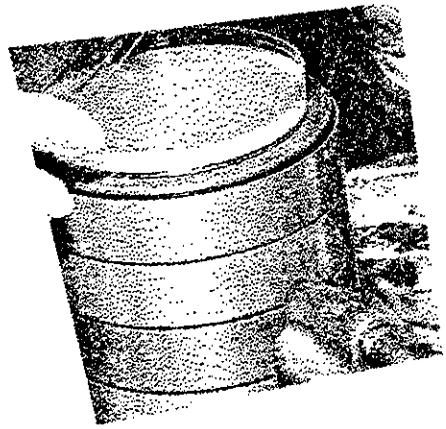
Sampling for microorganisms in occupational settings



BILANS DE CONNAISSANCES

Jacques Lavoie

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REPORT

IRSST
Institut de recherche
en santé et en sécurité
du travail du Québec

The Institut de recherche en santé et en sécurité du travail du Quebec (IRSST, Quebec Occupational Health and Safety Institute) is a scientific research agency committed to the identification and elimination at the source of occupational hazards, and the rehabilitation of workers who have suffered occupational injuries. With funding provided by the Commission pour la santé et la sécurité au travail du Quebec (CSST, Quebec Occupational Health and Safety Commission), the IRSST conducts, funds and contracts research aimed at reducing the human and financial costs of occupational accidents and diseases.

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Sampling for microorganisms in occupational settings

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ETUDES ET RECHERCHES
RAPPORT

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INTRODUCTION

In Québec, as in other industrialized countries, occupational health and safety issues are of deep concern. Occupational health and safety specialists have not been widely involved in the monitoring of biological contaminants, despite their recognized expertise in identifying and evaluating exposure to chemical and physical agents. The term "biological contaminants", as it is used here, includes infectious bacteria, fungi, viruses, pollen, insects, metabolites produced by non-infectious bacteria, and allergenic plant and animal fragments.

Exposure to biological contaminants, particularly those that are aerosolized, places several occupational groups at risk of infection or other health effects. Groups at risk include: hospital, laboratory, and dental personnel; teachers; social workers; excavation workers; garbage collectors; workers at waste water treatment facilities; workers in the agriculture, animal and animal byproducts, food, and textile sectors; and other workers whose job may bring them into contact with animals.

The identification, evaluation, and control of microorganisms in the work environment requires a multidisciplinary approach. Diagnosis and control of infectious and allergic diseases is the preserve of doctors, epidemiologists, pathologists, physiologists, and microbiologists. The industrial hygiene strategies for the evaluation and control of exposure to biological contaminants are similar to those developed for aerosol exposures, for which sampling apparatus and strategies are well-established. Culture, identification and enumeration of biological samples should be carried out by bacteriologists, virologists, and mycologists.

This document has been specifically prepared for occupational health and safety specialists, and is primarily concerned with microorganism sampling considerations.

1. BIOLOGICAL CONTAMINANTS

1.1 Definition of occupational biological contaminants

Biological contaminants are substances which are or arise from living organisms, and which are capable of producing harmful effects and/or disease in other organisms, particularly humans.

Organisms or substances capable of causing occupational illness include infectious and parasitic organisms, non-infectious microorganisms such as fungi and algae, plants and their by-products, and animals and their by-products (1).

1.2 Classification of biological contaminants

Biological contaminants commonly encountered in occupational settings fall into the following categories:

A) Bacteria
   1 - Pathogenic bacteria
   2 - Drug-resistant bacteria
B) Fungi
C) Viruses
   1 - Oncogenic viruses
   2 - Other viruses affecting animals
D) Rickettsia
E) Chlamydia
F) Parasites
G) Recombinant DNA
H) Allergens
I) Cultured animal cells, and potentially infectious agents associated with them
J) Infected clinical specimens
K) Tissues from experimental animals
L) Plant viruses, fungi, and bacteria
M) Toxins, e.g. mycotoxins, endotoxins

1.2.1 Microorganisms

The focus of this document is on microorganisms such as bacteria, viruses, fungi, and parasites which are associated with occupational illness.

Microorganisms are living organisms which are too small to be seen with the naked eye (2). They may exist in a variety of environments, including soil, air, water, plants, food, and both inside and outside animals, including humans, without causing any untoward effects (3).

Microorganisms which cause disease are known as pathogenic microorganisms or pathogens. Those which only cause disease under optimal conditions are known as opportunistic pathogens. Pathogenic microorganisms often produce substances called toxins which may cause ill-effects in their host organism. In many cases, the symptoms produced by the toxin differ from those produced by the microorganism itself (3).

1.2.1.1 Bacteria

Bacteria are unicellular, microscopic, living organisms with a relatively simple internal structure. They are probably responsible for the majority of occupational health problems caused by biological contaminants (3).

Bacterial infections are contracted in the work environment through contact with the respiratory or digestive tract, or through inoculation resulting from punctures, scratches, scrapes, or cuts.

Bacteria may become airborne in water aerosols from humidifiers, faucets, showers, etc., or may arise from human or animal sources (4).

Saprophytic pathogenic bacteria (microorganisms which live off of dead organic matter) are dispersed in aerosols generated by humans during sneezing, coughing, and talking. The survival time of these organisms depends on the size of the droplets in which they are dispersed, temperature, relative humidity, and the presence of a suitable transport substrate.

While it is generally known that disease may be spread from person to person through contact with contaminated aerosols, it is less widely appreciated that waterborne bacteria present in the general environment may infiltrate buildings and cause disease. "Humidifier fever", for example, is caused by airborne allergens, including endotoxins produced by a variety of Gram-negative bacteria present as contaminants in ventilation system

humidifiers. Symptoms include fever and either an elevated or depressed white blood cell count (4). Dermal contact with certain bacteria, e.g. those present in cutting oils, may cause dermatitis.

1.2.1.2 Viruses

Viruses differ from other microorganisms by their inability to reproduce outside of their host. Since human viruses are almost entirely species-specific, their presence and distribution is largely dependent on the level of human activity, and only partially affected by environmental factors (4).

The survival and transport of viruses in ambient air depends on the relative humidity, temperature, presence of other contaminants, and other, unknown, factors. Pathogenic human viruses number in the hundreds. The non-specific symptoms produced by these viruses, e.g. fatigue, headache, resemble those observed in individuals suffering from occupational stress.

1.2.1.3 Fungi

Fungi are plants which feed off of other plants or animals, either living or dead. Although responsible for some diseases in humans, fungi (including moulds and yeasts) may also be beneficial (4). They may attain quite respectable sizes (e.g. edible mushrooms), and may be either visible (e.g. bread mould) or invisible and composed of fine filaments or fibres (e.g. yeast).

Fungi reproduce by fission, budding, or sporulation from distinctive fruiting bodies typical of some species (4). This document will focus on airborne spores no larger than approximately 5 µm in diameter, as particles of this size may be inhaled, and consequently deposited in the lungs (4).

The effects of inhalation of fungi or spores on the immune system have been abundantly documented (4). Allergic reactions to airborne fungi include rhinitis, asthma, and extrinsic allergic alveolitis (hypersensitivity pneumonitis). The incidence of asthmatic symptoms is not always strongly correlated to spore concentration, however (4).

Some fungi directly infect the lungs, and produce more severe symptoms. Aspergillus fumigatus, for example may cause allergic bronchopulmonary aspergillosis, with fungal metabolites, particularly mycotoxins, the probable etiologic agents. Mycotoxins may also cause chronic, delayed-onset symptoms resembling those of infection (4). Because they are nonvolatile, the majority of inhaled mycotoxins are inhaled in spores or spore cases. Mycotoxins which affect the immune system, such as the trichotecenes, gliotoxins, and aflatoxins, are of particular interest, since decreased immunocompetence may alter the ability to react to allergens, and to infections which increase the oncogenicity of spores and spore cases (4). Only 30-70% of fungi found in natural settings have been found to produce mycotoxins (4).

Inhaled spores or spore cases may produce effects other than allergic reactions or direct infections. These conditions, of which atypical farmer's lung is an example, are known collectively as pulmonary mycotoxicoses (4).

Dermal contact with spore cases and mycotoxins may be direct, or mediated by contaminated surfaces or airborne particles. While contact with spore cases results in dermal irritation or weak dermal allergic reactions, severe dermal reactions may result from contact with mycotoxin-contaminated surfaces (4).

1.2.1.4 Parasites

Many parasitic diseases are transmissible from animals to humans under normal conditions. The majority of occupational parasitic infections are caused by protozoans, arthropods, and helminths. Exposure to parasite vectors, or direct contact with the infectious form of the parasite is associated with a high risk of developing parasitic disease.

Despite the fact that Dermatophagoides pternys sinus, an Acarid which lives in dust, is the second most important cause of allergic bronchial asthma, the allergenic properties of the Acaridae have often been overlooked (4). Xerophilic fungi are always found in association with Acaridae in house dust, and appear to have a symbiotic relationship with them (4).

1.3 Routes of exposure to microorganisms

The most common routes of exposure to biological contaminants capable of causing allergic or infectious reactions are:

1. ingestion
2. inhalation
3. punctures, scrapes, scratches, cuts
4. absorption across the skin and mucous membranes

Of these, inhalation accounts for 65-75% of all infections (1). Deposition and retention of aerosols in the airways is a prerequisite for the induction of respiratory infection. Particles smaller than 5 µm in diameter are the most likely to be deposited and retained, since they may remain suspended in the air long enough to cross rooms or be dispersed by ventilation systems. Given the potential of microorganisms attached to such particles to remain infectious for some time, contact with these particles poses a clear infection hazard. Obviously, not all exposed individuals will develop illness: the infectious dose in humans varies as a function of the microorganism, the route of exposure, and individual resistance.

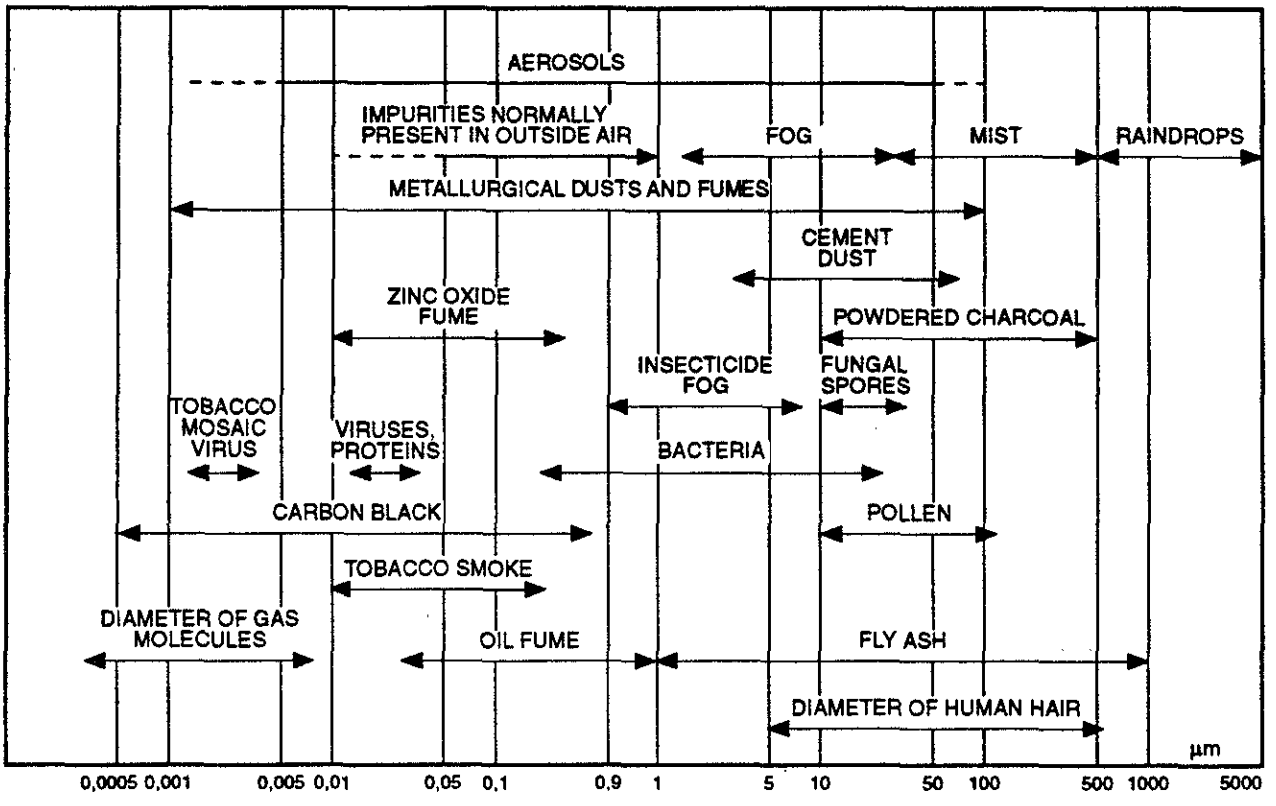
In the case of allergies, i.e. acquired hypersensitivity to inhaled microbial matter, the minimum effective dose may be very low. Individuals who are hypersensitive to biological or chemical contaminants in ambient air may be found in virtually all office buildings, for example. The contaminants to which this hypersensitivity has developed are present at concentrations which are difficult to measure using common industrial hygiene techniques. These levels are however well below maximum allowable levels, and do not pose a problem to the majority of building occupants.

1.4 Size of microorganisms

Bacterial diameters vary between 1 micrometer (um) and 10 um, and exhibit both inter-specific and individual variation. Yeasts typically have a diameter of 10 um, although the diameter of most fungal spores is less than 5 um. A typical virus, such as the influenza virus, measures 0.1 um. These small dimensions facilitate rapid reproduction, growth, and environmental dispersion (3).

Figure 1 illustrates the range of diameters of microorganisms and of aerosol particles typically encountered in the work environment (7).

FIGURE 1: TYPICAL AEROSOL PARTICLE SIZES (µm)



1.5 THE QUÉBEC CONTEXT

According to article 29 of the Law concerning occupational accidents and diseases (1985, Chapter 6), workers suffering from a disease appearing in the first column of Table 1 are considered to be victims of an occupational disease if they have been employed in an occupation appearing in the second column of the corresponding row (8).

Workers at increased risk of exposure to these microorganisms include:

- Workers in the agricultural, animal and animal by-products, and food sectors
- Other workers whose job exposes them to animals
- Hospital personnel
- Laboratory personnel

According to the database maintained by the Commission de la santé et de la sécurité du travail du Québec (CSST, Québec Occupational Health and Safety Commission), 17,100 compensation claims for occupational illness were filed in Québec in the four years between 1981 and 1984; of these, 14,580 were accepted and 2,520 rejected. The distribution of the causes of accepted claims is presented in Table 2. As this table indicates, infectious and parasitic diseases (i.e. occupational diseases caused by biological contaminants) are ranked fifth of all causes.

The number of cases, number of lost work days, and costs associated with infectious and parasitic disease for each of the years between 1981 and 1984 are presented in Table 3, while

Table 4 summarizes the distribution of cases of infectious and parasitic disease by economic sector.

These tables indicate that:

- The CSST paid a total of $2,628,619 in compensation for infectious and parasitic disease during the four-year period in question.

- While the medical and social services sector accounts for most of the cases, the food and drink sector incurs the highest costs and number of lost work days.

- The incidence of infectious and parasitic occupational disease in some sectors may be under-estimated. For example, not all workers in the agriculture sector pay CSST premiums, and infectious and parasitic disease in these workers is presumably under-reported

TABLE 1: INFECTIOUS DISEASES (8)

Disease	Type of Work
1. Bacterial or fungal dermal infection (pyodermatitis, bacterial folliculitis, pinares, dermato-mycosis, dermal Candida infection	Work involving contact with material contaminated by bacteria or fungi
2. Parasitosis	Work involving contact with humans, animals, or material contaminated by parasites such as Sarcoptes scabei or Pediculus humanis
3. Anthrax	Work involving the use, han dling, or other form of exposure to contaminated wool, hair, leather, or skins
4. Brucellosis	Work involving care, slaughter, dismem bering, or transporta tion of animals, or labora tory work involving contact with Brucella
5. Viral hepatitis	Work involving contaminated humans, animals, human prod ucts, animal products, or other products
6. Hand warts	Work performed in a slaughter house, or involving the han dling, in a humid environment, of animals or animal products

TABLE 2: CAUSES OF OCCUPATIONAL DISEASE COMPENSATED BY THE CSST BETWEEN 1981 AND 1984

Occupational Disease	Number of Cases	Percentage of Cases
Deafness	4,301	29
Dermatitis	2,642	18
Musculoskeletal problems	1,498	10
Systemic intoxication	1,335	9
Infectious and parasitic disease	1,097	8
Pneumoconiosis	563	4
Respiratory tract allergy	391	3
Other	434	3
Not coded	2,319	16
Total	14,580	100

TABLE 3: CASES, LOST WORK DAYS, AND COSTS ASSOCIATED WITH INFECTIOUS AND PARASITIC OCCUPATIONAL DISEASE

Year	Cases	Lost Work Days	Costs ($)
1981	230	8,373	973,406
1982	240	10,623	1,277,199
1983	331	3,472	197,080
1984	291	2,343	180,933
Total	1,097	24,811	2,628,619

TABLE 4 DISTRIBUTION OF CASES OF INFECTIOUS AND PARASITIC OCCUPATIONAL DISEASE BY ECONOMIC SECTOR (1981-1984)

Economic Sector	Cases	Lost Work Days	Costs ($)
Medical and social services	852	7,156	499,175
Food and drink	54	11,207	1,378,405
Teaching and related activities	38	641	38,586
Other commercial and personnel services	29	472	16,043
Not coded	28	169	21,978
Commercial	16	2,186	143,172

2. SAMPLING FOR MICROORGANISMS

The industrial hygiene methods typically used for the sampling and evaluation of chemical and physical agents are inappropriate for use in surveys of microbial contamination, in which adequate evaluation of contamination depends on the maintenance of sample viability.

2.1 Sampling considerations

The prior, accurate, identification or estimation of causal agent(s) is a prerequisite for effective sampling. This step is most appropriately performed in collaboration with physicians having observed clinical signs in exposed individuals, and with microbiologists. Once the identity of the causal agent has been adequately identified, sampling proceeds using the techniques prescribed by the local community health department or by specialised laboratories.

Should identification of the causal agent prove impossible, it is necessary to perform a wide-spectrum analysis (2).

The intrinsic properties of microorganisms - particularly their ability to reproduce rapidly - renders sampling and quantitative analysis difficult. In contrast to chemical and physical agents, for which safe exposure levels have been identified, there is no safe level of exposure to pathogens.

2.2 Sampling techniques

The choice of sampling method depends on the nature of the suspected biological contaminant, its dispersal characteristics, and its means of transmission. Sampling techniques for microorganisms fall into three main categories:

1- liquid sampling
2- surface sampling
3- air sampling

2.2.1 Liquid sampling

A wide variety of liquid media may harbour microorganisms. Typical media to be sampled include municipal and industrial wastewater, and metal coolants.

In most cases, the simplest method is to obtain a sterile, representative sample of the medium. Commercially-available equipment may be used to obtain samples at any required depth. Constant-flow streams may be dammed with rubber diaphragms, and samples taken using a sterile syringe (1).

Membrane filters may also be used to sample liquid media. This is a particularly effective technique when low microorganism density requires the sampling of large volumes of liquid (1). The membrane filter may be directly cultured.

Dip-slide sampling may also be performed. In this technique, a slide coated with culture medium is dipped into the liquid to be sampled, and subsequently incubated in its container, at the same temperature as the sampled liquid, for a standard period. Reference tables are available to facilitate the interpretation of the results. Multi-slide equipment capable of furnishing counts of aerobic bacteria, yeast, mould, coliform bacteria, and anaerobic sulphur-producing bacteria is available (1).

As is the case for chemical or physical agents, microbiological samples must be kept at optimal conditions during transportation and analysis. Variations in lighting or temperature, and long shipping delays may adversely affect the viability of sampled microorganisms (1).

2.2.2 Surface sampling

Many surface sampling techniques are available. The selection of the most suitable method for a given set of conditions must take into account several factors, including the nature and chemical composition of the surface to be sampled, the type and predicted levels of expected contaminants, and the specific objectives of the sampling survey. The five principal sampling methods for surfaces are:

1. swab rinse
2. rinse
3. agar-contact sampling
4. direct surface-agar plating
5. vacuum-probe surface plating

The many other techniques reported in the scientific literature are adaptations of these basic techniques.

2.2.2.1 Swab rinse

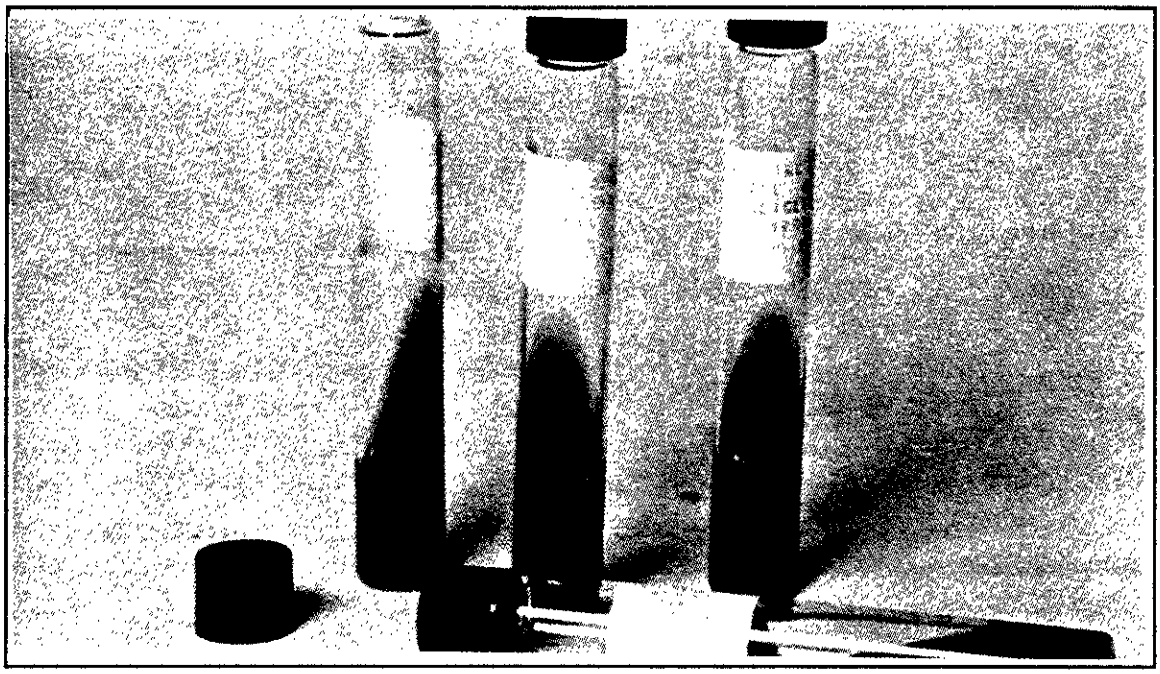
The first step in this technique involves wiping the surface to be sampled with a moistened cotton swab. The swab is then placed in a tube containing a sterile buffer solution, and the adhering microorganisms suspended by agitation (2) (Figure 2). Prior to shipment, the tube is stored in a refrigerator. Shipment to a laboratory should be undertaken as soon as possible. At the laboratory, the rinsate is cultured on an appropriate culture medium.

Although simple, this technique has a number of limitations, of which the most important is low sampling efficiency. In addition, significant interpersonal variations in sampling technique - such as swab pressure and wipe speed - exist. Lastly, complete recovery of microorganisms from the swab is not possible. Attempts to improve the technique by replacing the swab material with other materials (most notably calcium alginate) have not been encouraging.

When only a rough estimate of contamination is required, the swab may be applied directly to the appropriate culture medium. This is also a useful expedient when attempting to determine the presence of specific microorganisms.

The swab tube method remains a gross analytical technique, suitable for field use only, and does not accurately quantify the microbial population present on a surface.

FIGURE 2: SWAB TUBES (2)

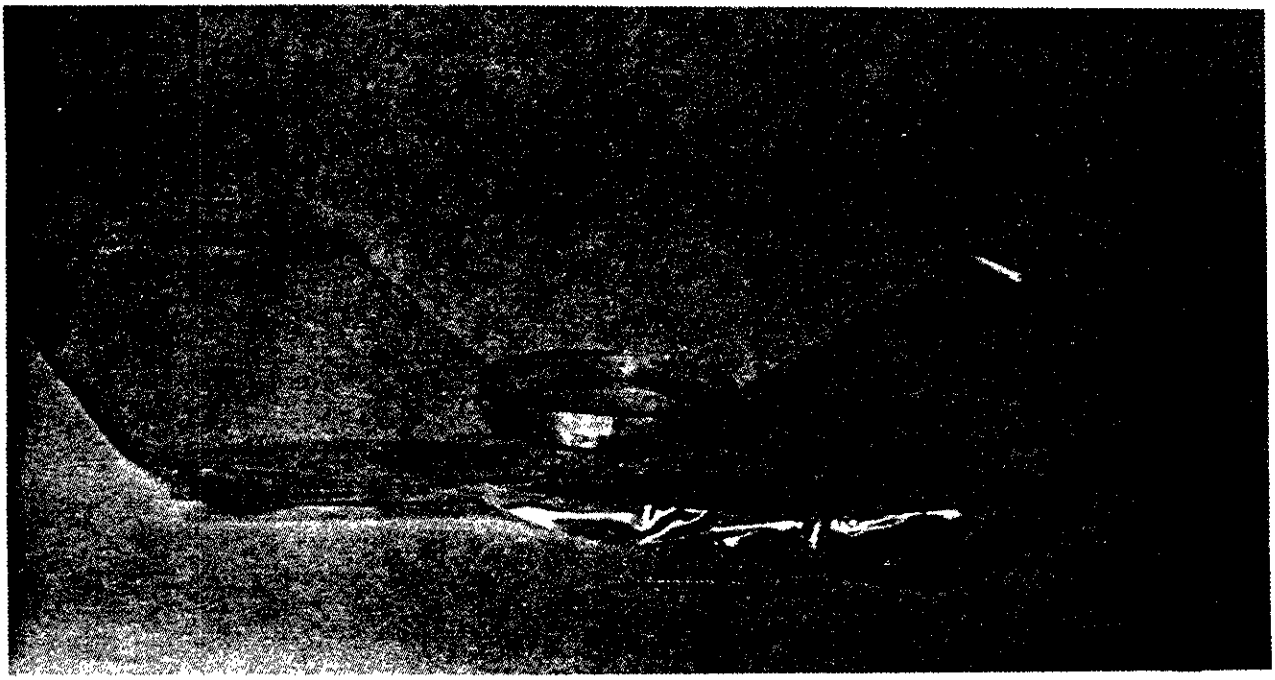


2.2.2.2 Rinses

In the rinse technique, the surface to be sampled is immersed in a sterile solution, and attached microorganisms dislodged by manual or mechanical agitation. Because the entire target surface is sampled, this technique is more accurate than the swab rinse technique. However, it is clearly not applicable to large surfaces.

The biological contamination of the hands is most effectively determined using a variant of this technique. In this variant, the hands are immersed to wrist-level in a sterilized polyethylene bag (Ziploc™) containing a known volume of sterile sampling medium, and microorganisms dislodged into the medium by gripping the bag at wrist-level and rubbing the hand against the bag wall.

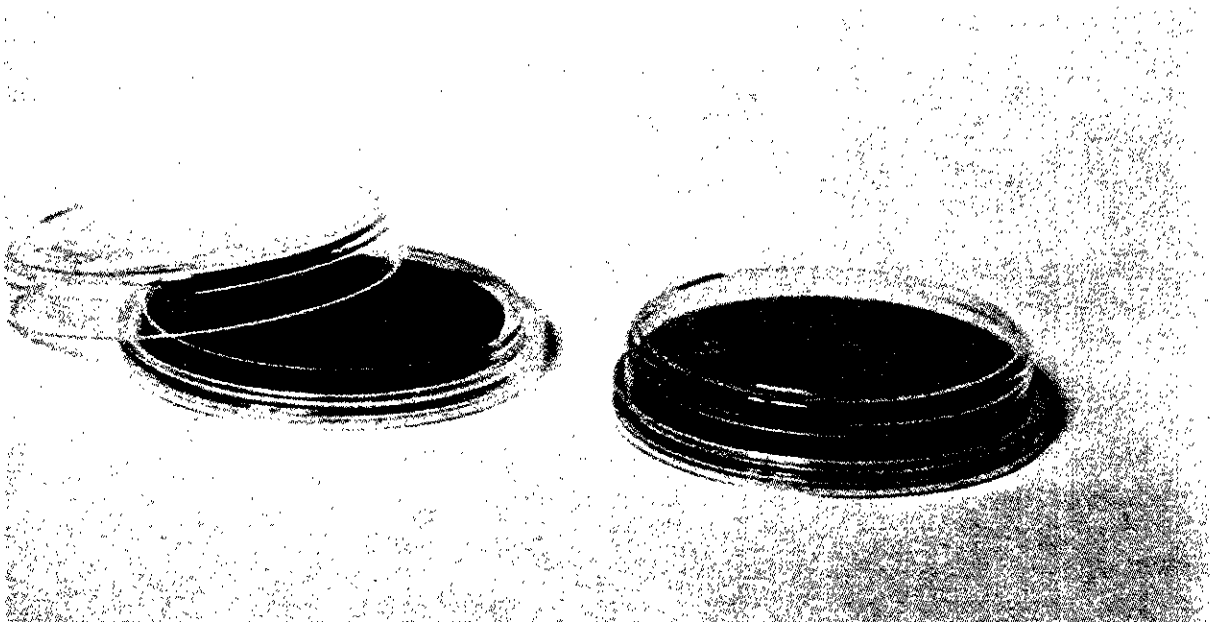
FIGURE 3: STERILE CONTAINERS FOR THE RINSE TECHNIQUE (2)



2.2.2.3 Agar-contact sampling method

In this technique, a slightly convex agar medium is drawn over a flat sample surface (e.g. walls, floors, ceilings, equipment) and subsequently incubated (2). Rodac medium is commonly used, in Petri dishes with a surface area of 25 cm^2. This technique is inappropriate for the sampling of high contaminant concentrations, as the medium is rapidly saturated. Quantification of contamination is poor.

FIGURE 4: RODAC MEDIUM (2)



2.2.2.4 Direct surface-agar plating

In direct surface-agar plating, a sterile agar medium is poured over the surface to be sampled, allowed to solidify under a sterile cover (in order to prevent further contamination), and incubated. After an appropriate incubation period, the colonies present at the agar-surface interface are counted. Alternatively, a portion of the surface to be sampled may be placed in a Petri dish, and covered with culture medium (Figure 5).

This technique yields only rough estimates of microbiological contamination. Since the majority of surfaces likely to be sampled are immovable, and thus difficult to maintain at optimal incubation temperatures, this technique is not widely applicable. In addition, direct surface-agar plating cannot be used where bactericides or bacteriostatic agents are present on the sample surfaces (1).

2.2.2.5 Vacuum-probe surface plating

The vacuum probe used in this technique consists of a Teflon™ tube equipped with a critical orifice and connected to a conical aluminum chamber fitted with a sterile membrane filter at the far end. Particles aspirated from the surface are deposited on the filter, which is subsequently incubated. At the end of the incubation period, microbial colonies are counted. Studies have demonstrated the vacuum-probe technique to be very efficient, with observed rates of microbial surface contamination removal and recovery of 98% and 88%, respectively (Figure 6). In direct comparisons with the rinse technique, vacuum-probe surface plating recovers twice as many microorganisms. This technique is useful for the sampling of large surfaces with relatively low levels of microbial contamination (1).

FIGURE 5: SPECIFIC AGAR MEDIA (2)

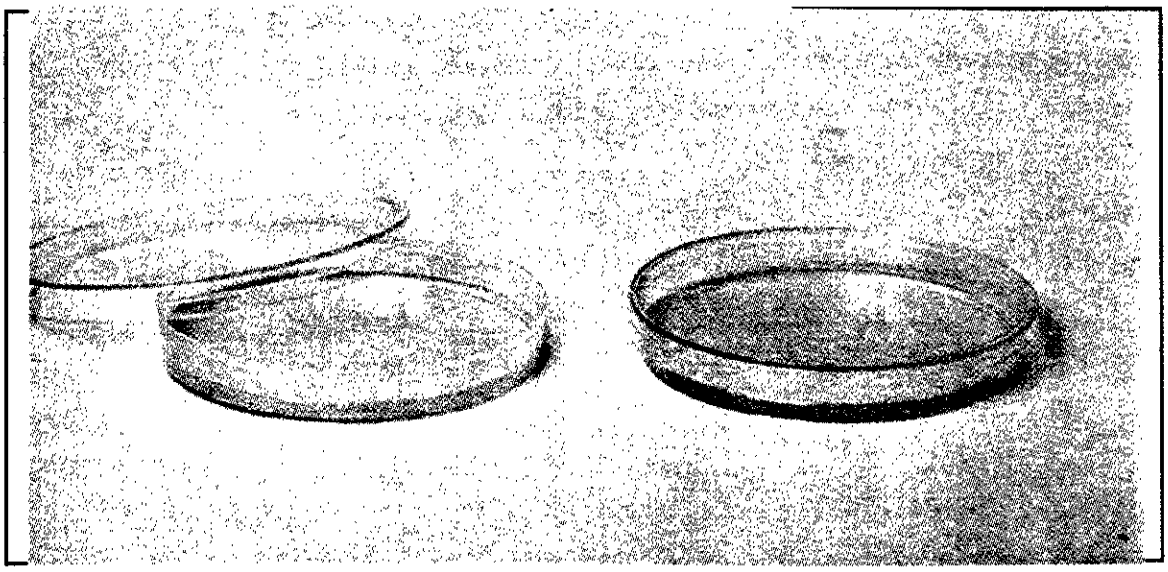
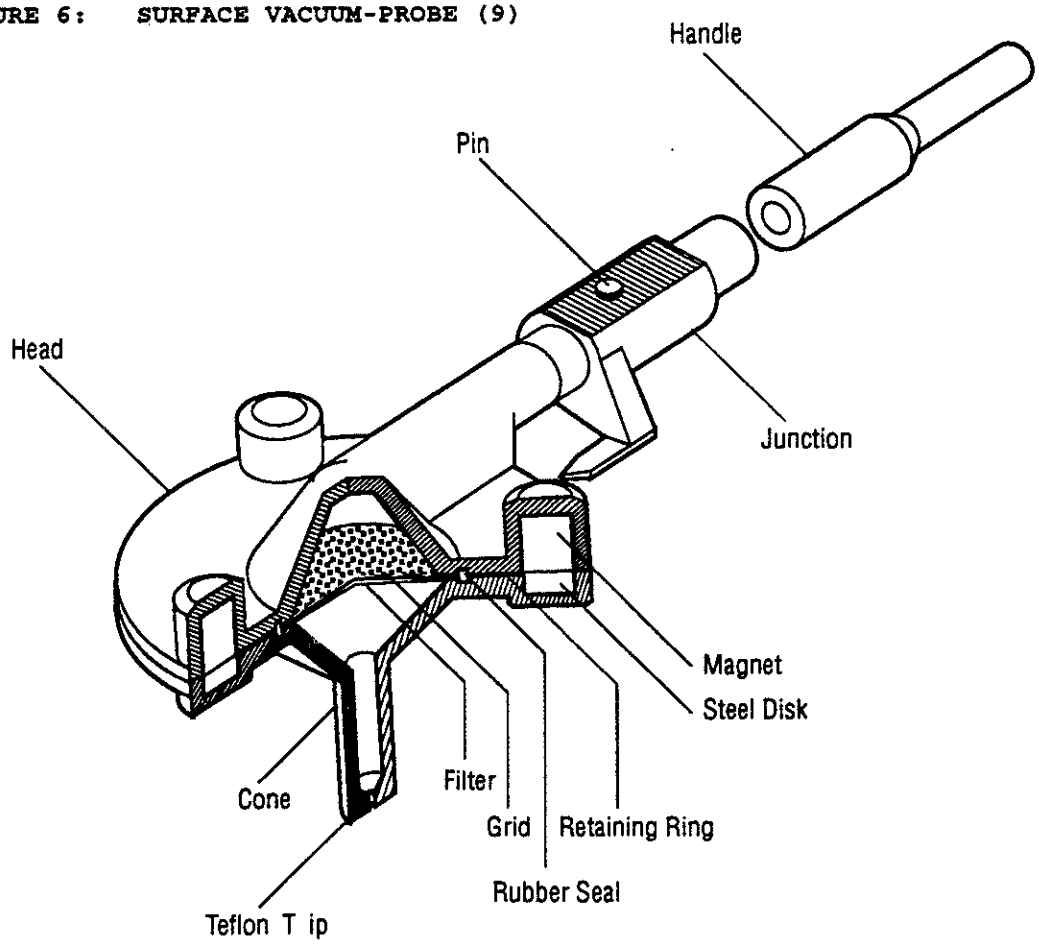


FIGURE 6: SURFACE VACUUM-PROBE (9)

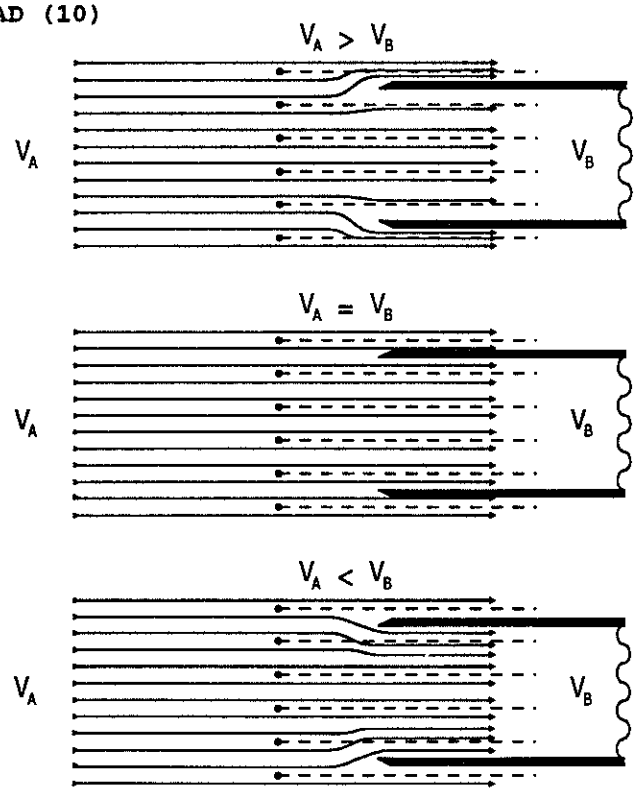


The principal factors to be considered in selecting a microbial sampling technique are the number and type of microorganisms likely to be present on the sample surface, the nature of the sample surface, and the presence of bactericides. No agar medium exists on which all microorganisms will grow sufficiently rapidly to allow their quantification after relatively short incubation periods, and none of the sampling techniques described can completely characterise microbial surface contamination. The development of appropriate sampling strategies is therefore dependent on the judicious consideration of all relevant factors.

2.2.3 Air sampling

In order to ensure that particulate aerosol samples are representative, sampling must be isokinetic, regardless of the specific characteristics of the particles. Sampling should be performed with the head of the sampling equipment placed in the air-stream, and at an aspiration velocity equal to the air-flow velocity (Figure 7). Errors due to non-isokinetic sampling are not excessive for particles of 1-5 _ in diameter, but increase with increasing granulometric heterogeneity.

FIGURE 7: AIR (___) AND PARTICLE (__.__) TRAJECTORIES AT THE SAMPLING HEAD (10)



Since it is not possible to apply correction factors to the results of non-isokinetic sampling, it is important to adapt flow rates to sampling conditions. In the discussion which follows, it is assumed that sampling is isokinetic.

Airborne microorganisms are rarely found in a free-floating state, but are more commonly attached to passive transportation vectors such as droplets or solid particles. Respirable particles and droplets are responsible for adverse health effects in humans.

Airborne particles vary enormously in shape. Solid aerosols may be long and slender (e.g. asbestos and fibreglass), spherical (e.g. pollen) or platelet-like (e.g. graphite). Liquid aerosols are almost always quasi-spherical. Because irregularly-shaped particles have an irregular diameter, it is necessary to devise a size index which is independent of particle shape. The *equivalent aerodynamic diameter* is such an index and is equal to the diameter of a particle with a specific gravity of 1 having the same terminal velocity as the irregularly-shaped particle in question. In practice, it is determined by measuring the terminal velocity of test particles in a test chamber of calm air, and comparing this velocity to standard tables of terminal velocity for spherical particles with a specific gravity of 1. Particle deposition in the respiratory tract is largely a function of equivalent aerodynamic diameter, and air-sampling equipment must therefore select for particles or droplets of specific equivalent aerodynamic diameter (7,11).

Respirable particles vary in size from 2 to 8 um (11). Penetration into the airways is a function of size, with particles with equivalent aerodynamic diameters of 3-5 um being deposited in the alveoli and the narrow, non-ciliated airways, where they may cause infections, allergic reactions, or other adverse health effects (11).

The three most important factors to be considered when planning an air sampling strategy for microorganisms are (1):

1. the concentration of microorganisms in the air
2. the size of the particles to which the microorganisms are at tached
3. temporal variations in the concentration of microorganisms

In cases where the microorganisms suspected to be present can cause disease at low concentrations, it may be necessary to perform high-volume sampling, in order to ensure collection of a sufficient number of microorganisms for analysis.

The size of the particles to which the microorganisms are attached plays an important role in determining airway penetration and retention, as well as sample viability during collection and transportation.

Monitoring temporal variations in microorganism concentration or type may facilitate the identification of causal relationships, and the evaluation of the effectiveness of filtration systems.

The specific objectives of microbial air sampling programmes will determine the type of equipment used. Application of the above principles will facilitate the selection of the most appropriate sampling equipment from the wide selection of available equipment (1).

Microbial air sampling equipment may be classified into the following three categories, defined by the capture principles involved:

1. inertial-capture devices
2. filters
3. thermal and electrostatic precipitators

2.2.3.1 Inertial-capture devices

The cyclical nature of respiration and the geometry of the airways results in respired air changing direction several times within the respiratory tract. Sampling devices in this group utilize similar principles to change the direction of air flow so that suspended particles may be captured by impaction on solid, or, in the cases of impingers, liquid surfaces.

The biological effects of particles depends on their site of deposition within the respiratory tract, itself dependent on the equivalent aerodynamic diameter of the particles. Estimates of aerosol deposition in each section of the respiratory tract may be obtained using particle-sizing equipment

which furnishes particles counts as a function of equivalent aerodynamic diameter.

2.2.3.1.1 Sedimentation equipment

Sedimentation equipment, in which microorganisms are collected by gravitational sedimentation onto culture media, are the simplest type of microbial samplers (9). The colonies appearing on the media following incubation reflect the microorganisms originally present (1). Specific microbiological contaminants may be rapidly identified by using organism-specific media.

This sampling technique is most effective under calm air conditions. Only large particles are sampled. Smaller particles, particularly those smaller than 3 µm, sediment very slowly, and are therefore difficult to sample. This technique furnishes qualitative and approximate results only.

The advantages of the technique are (9):

- low cost
- good commercial availability
- efficient sampling of surface contamination

Its disadvantages are:

- poor quantification
- susceptibility to air currents
- suitability only for particles or droplets larger than 3 um
- sampling bias for small particles

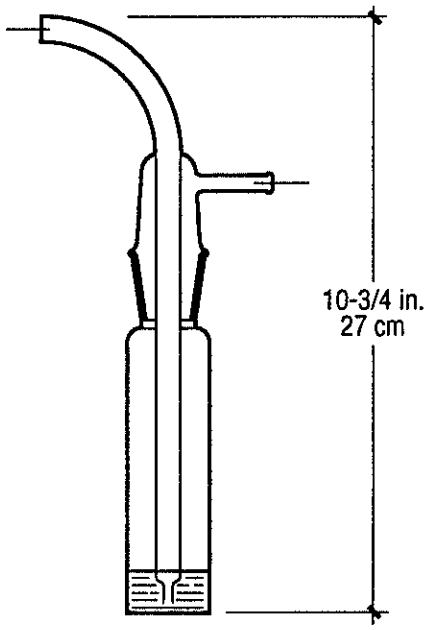
2.2.3.1.2 Impingers

With this technique, air is bubbled through a sterile isotonic liquid which is subsequently diluted and cultured. In glass impingers, air velocities may be as high as 760 mph (12). Impingers are equipped with critical orifices which, in the presence of a pressure drop of 41 cm of mercury, maintain a flow rate of 12.5 litres per minute, i.e. a rate approximately equal to that of normal human respiration.

Impingers are used to determine the number of viable microorganisms in an aerosol, rather than the total number of particles (13). The calculation of the air concentration of microorganisms (microorganisms/ft^3 or m^3) is simple if the flow rate, sampling time, and number of organisms in the sample is known.

The AGI-30 glass impinger (overall length: 30 cm) is recommended for general-purpose sampling. When used in conjunction with pre-impingers and multi-stage impingers, size-specific aerosol sampling may also be performed (13). With judiciously chosen culture media and dilution factors, this technique may be used under a wide range of circumstances. A further advantage of glass impingers is the ease with which they may be cleaned and sterilised (Figure 8).

FIGURE 8: ALL-GLASS IMPINGER (AGI) AG GLASS INC., VINELAND NJ



The precision with which impingers measure the concentration of large microorganisms is not as high as that obtained with impactors (13). Impingers generally overestimate the concentration of microorganisms which aggregate in airborne clumps, since the clumps tend to fragment during sampling. The handling errors which arise during the inoculation of culture media result in liquid media sampling techniques generally exhibiting higher variability than solid media techniques.

Sampling times must be kept short when using all-glass impingers, in order to avoid evaporation of the sampling medium. While the normal volume of sampling medium is 25 ml, volumes as low as 5 ml may be used. Sampling may be carried out for 1, 5, or 10 minutes, with 1 minute the most common sampling period. Longer sampling periods result in larger evaporative losses of sampling medium (9). Some sensitive microorganisms may not survive the effects of high sampling flow rates (1).

Summarizing, the advantages associated with impinger sampling are:

- high sampling efficiency
- low cost
- good commercial availability
- high selectivity: < 17 um when used alone, 5-17 um when used in conjunction with a May pre-impinger (12)
- flow rates similar to human respiratory rate
- existence of a standard sampling procedure

The disadvantages of this technique include:

- difficulty in sampling low concentrations
- fragility of glass impingers
- necessity of using sampling and culture media

In the sampling equipment discussed below, particles are accelerated through one or more openings, to impact upon an agar- or filter- based medium. The number of particles containing microorganisms in the air sample is determined by counting the number of colonies appearing during the incubation period.

2.2.3.1.3 Slit samplers

In slit samplers, air is aspirated through a slit and directed onto a Petri dish which is rotated to ensure even contaminant deposition. The recommended sampling rate is 28.3 litres per minute (LPM), although this rate may be varied in most samplers to accommodate the sampling of high or low microbial concentrations (1).

At the end of the sampling period, the Petri dish is removed and incubated. Following incubation, the number of colonies is counted.

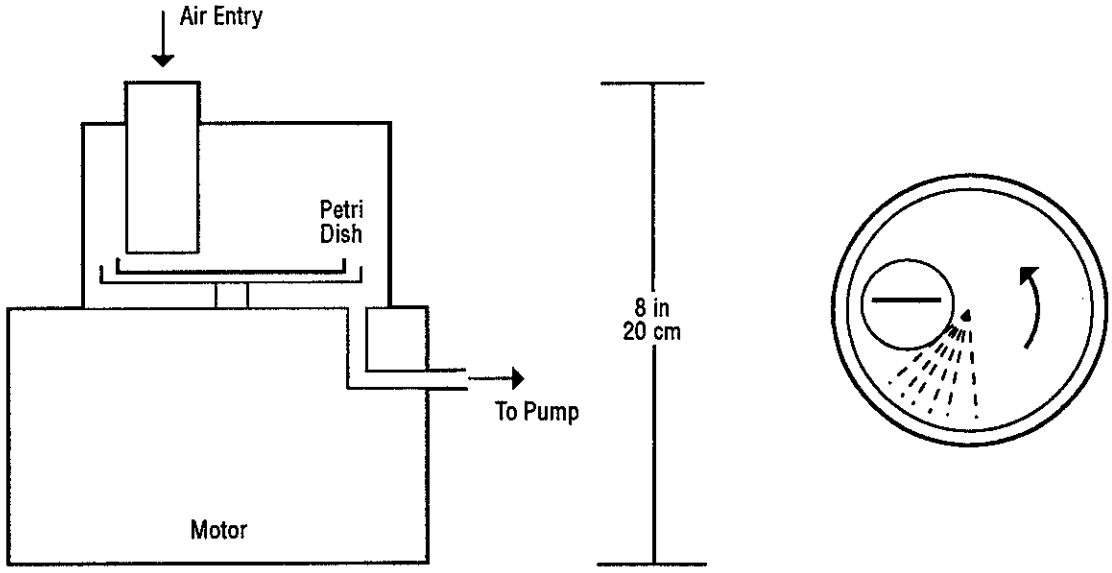
This sampling technique possesses the advantage of providing information on the temporal variation of microorganism concentration.

High-volume slit samplers, capable of sampling rates of 1 000 LPM, are useful for sampling very low concentrations.

Slit samplers have high capture efficiencies for particles larger than 2.3 _. Capture efficiency for 0.5 um particles is 70% of that of Anderson samplers (1) (Figure 9).

Culture media used with slit samplers measure 150 x 20 mm, a surface area approximately 22 times larger than standard Petri dishes (14). A maximum of 2 500 colonies can be distinguished on the culture medium. Sampling periods may be longer with slit samplers than with other equipment, as culture medium evaporation is much lower.

FIGURE 9 AND 10: SLIT SAMPLER



a) Side View b) Top View

Slit samplers have the following advantages:

- ability to furnish information on the temporal variation of microorganism concentration
- relatively high capture efficiency (85-95%)
- simple operation
- elimination of the need for laboratory dilutions

Their disadvantages are:

- inability to autoclave the equipment
- necessity for gaseous sterilisation
- relatively high purchase price

2.2.3.1.4 Cascade impactors

Cascade impactors are used to produce samples of ambient air particulates directly on microscope slides. Air entering the impactor passes through a series of plates with different size holes, and the changes of velocity produced by passing through these holes results in differential deposition of suspended particles. Since air flow through the impactor is constant, air velocity increases at each stage (Figure 10). The slides bearing the particles can either be stained and directly examined under the microscope, or rinsed, and incubated in culture medium for later identification and counting. Only xerophilic microorganisms such as fungal spores can be treated in this latter fashion (1).

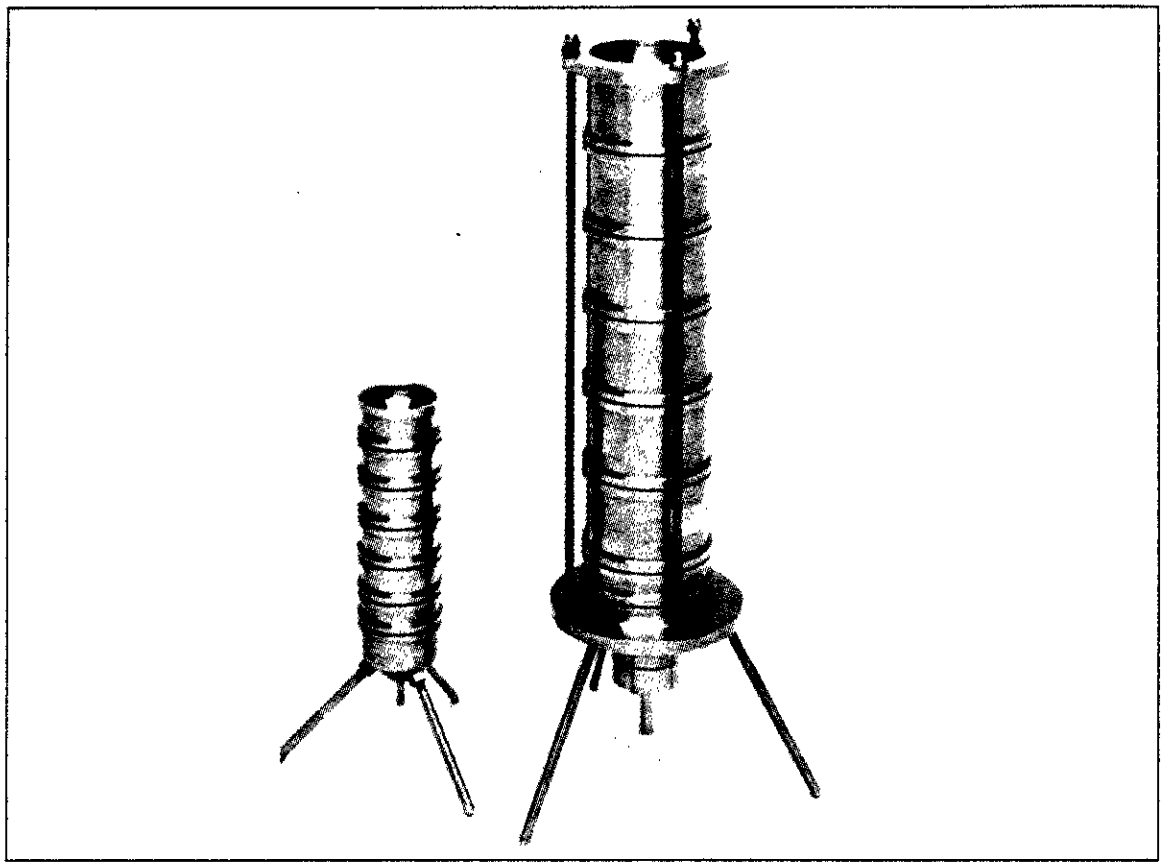
The advantages of cascade impactors are:

- ability to determine particle granulometry
- low cost
- good commercial availability

Disadvantages of this technique are:

- non-isokinetic sampling
- limited applicability (xerophilic microorganisms only)

FIGURE 11: MODIFIED CASCADE IMPACTOR



2.2.3.1.5 Andersen sampler (Sequential impaction cascade sieve volumetric sampler)

This type of device is more commonly known as an Andersen sampler, although many different brands, all based on the same principles, are available (e.g. Andersen Microbial Air Sampler, Microbial Air Sampler [Flow Sensor]). Andersen samplers capture viable microorganisms from ambient air and segregate them on the basis of size, using cascade impaction through a series of filtration grids. The more common forms of the device are composed of 2-6 aluminum stages, although 8-stage models are also available (Figure 11). Air enters each stage through a plate pierced by 400 equidistant holes, and particles are deposited on appropriate culture media placed beneath each stage. The diameter of the holes decreases with each stage, from top to bottom (1) (Figure 12). The sampling rate is 28.3 LPM, and sampling time varies from 1-20 minutes, depending on the concentration of airborne microorganisms. Six-stage samplers capture particles with diameters of 0.65-7.0 um. A 2-stage sampler which separates respirable from non-respirable particles is also available (Figure 13).

FIGURE 12: ANDERSEN MICROBIAL SAMPLER. LEFT: 6-STAGE MODEL. RIGHT: 2-STAGE MODEL.(11)

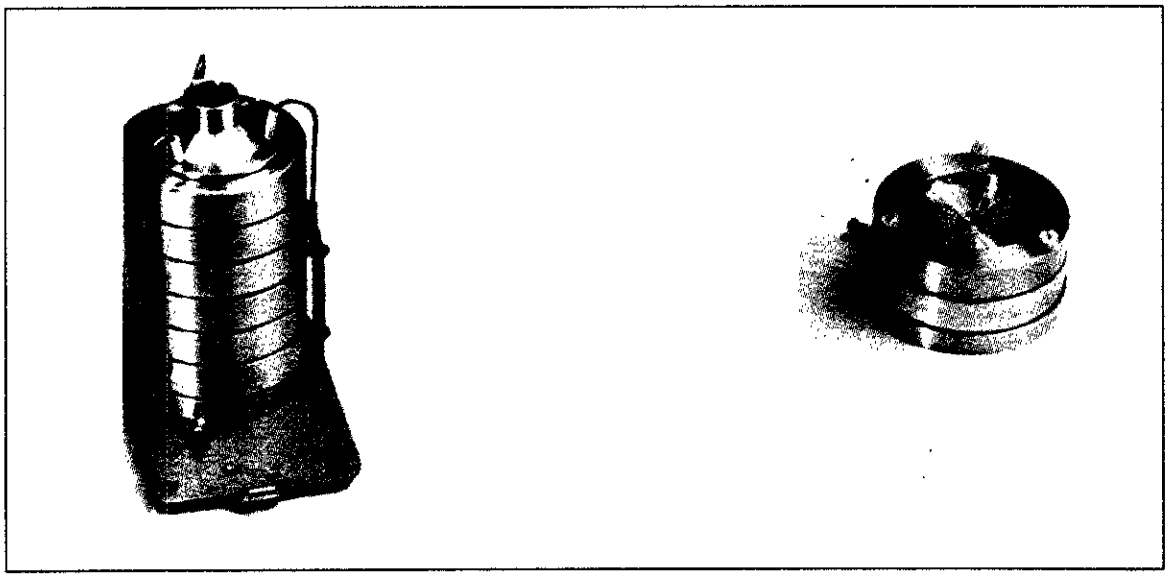
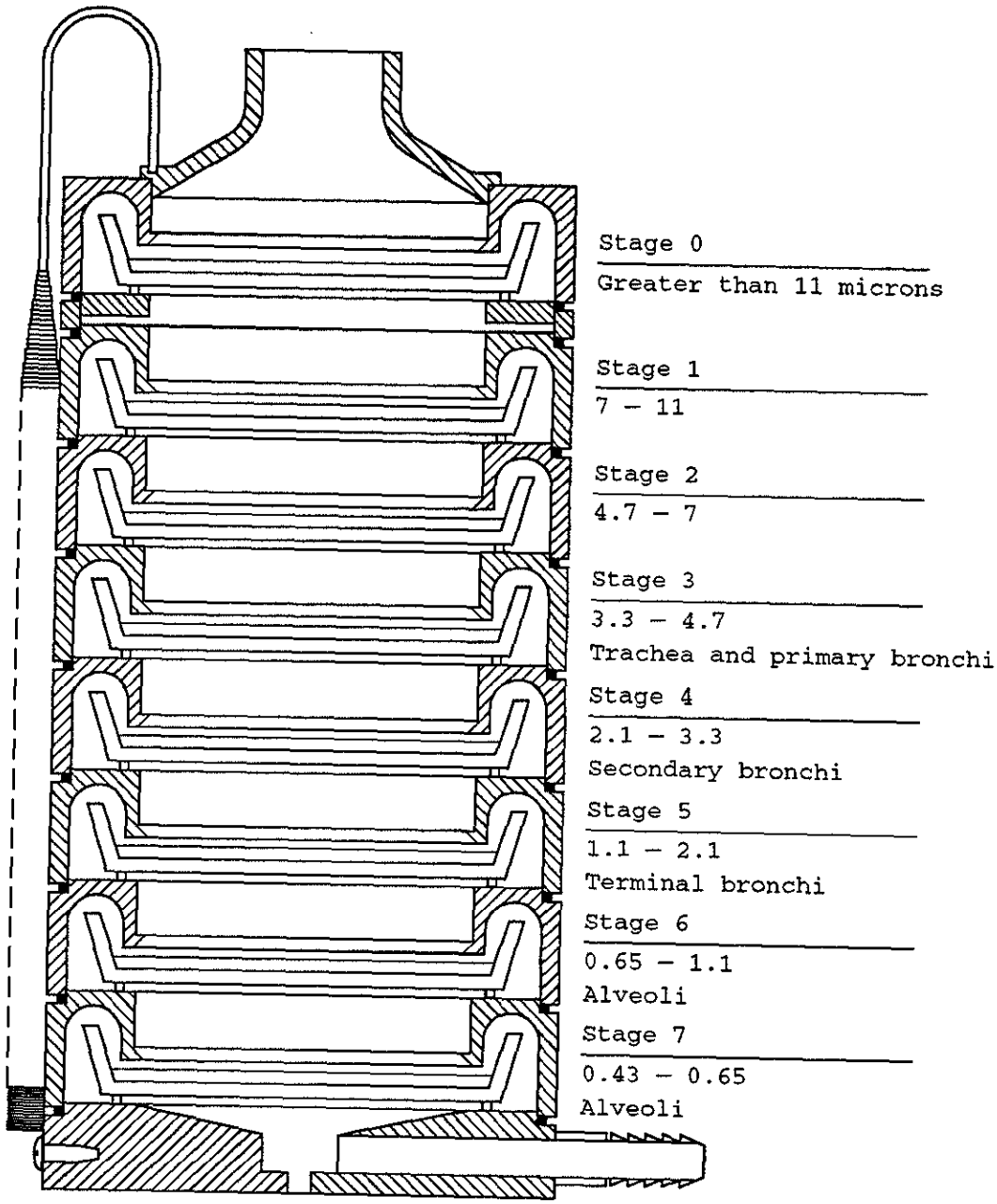


FIGURE 13: THE ANDERSEN SAMPLER (COURTESY OF ANDERSEN 2000, ATLANTA GA)



Andersen samplers were adopted as standard sampling equipment, particularly for low levels of microbial contamination, some 20 years ago (14). Today, Andersen samplers are used to determine the concentration of viable airborne microorganisms, and attain capture efficiencies of almost 100% for respirable particles.

Andersen reported that the accumulation of electrostatic charges on plastic Petri dishes resulted in the underestimation of true levels, while glass and aluminum Petri dishes gave acceptable results (15).

Andersen samplers possess the following advantages:

- granulometric fractionation of sampled particles
- commercial availability
- ability to sample aerosols in their natural state
- elimination of the need for laboratory dilutions
- existence of a standardised sampling protocol, with a capture efficiency of almost 100%
- ability to sample a wide spectrum of microbial concentrations

Its disadvantages include:

- limited sampling period, due to sampling medium dehydration
- necessity of using correction tables when counting high microbial concentrations (15)

2.2.3.1.6 Centrifugal air samplers

Centrifugal air samplers, based on cyclones used for industrial dust control, capture particles on the internal surface of a cone rinsed by a liquid. Microbial contamination is evaluated on the basis of analysis of the rinsate. Recently-developed versions of this equipment combine centrifugal sampling principles with agar impaction techniques (Figure 13). Here, air passes through a shallow cylindrical opening where it is centrifugally accelerated and projected onto a culture medium applied to a plastic strip with a surface area of 34 cm^2. Following sampling, the plastic strip is removed and incubated, and colony counting performed directly on the strip. The capture efficiency of this equipment is greater than that of slit samplers and significantly greater than that of impingers (16).

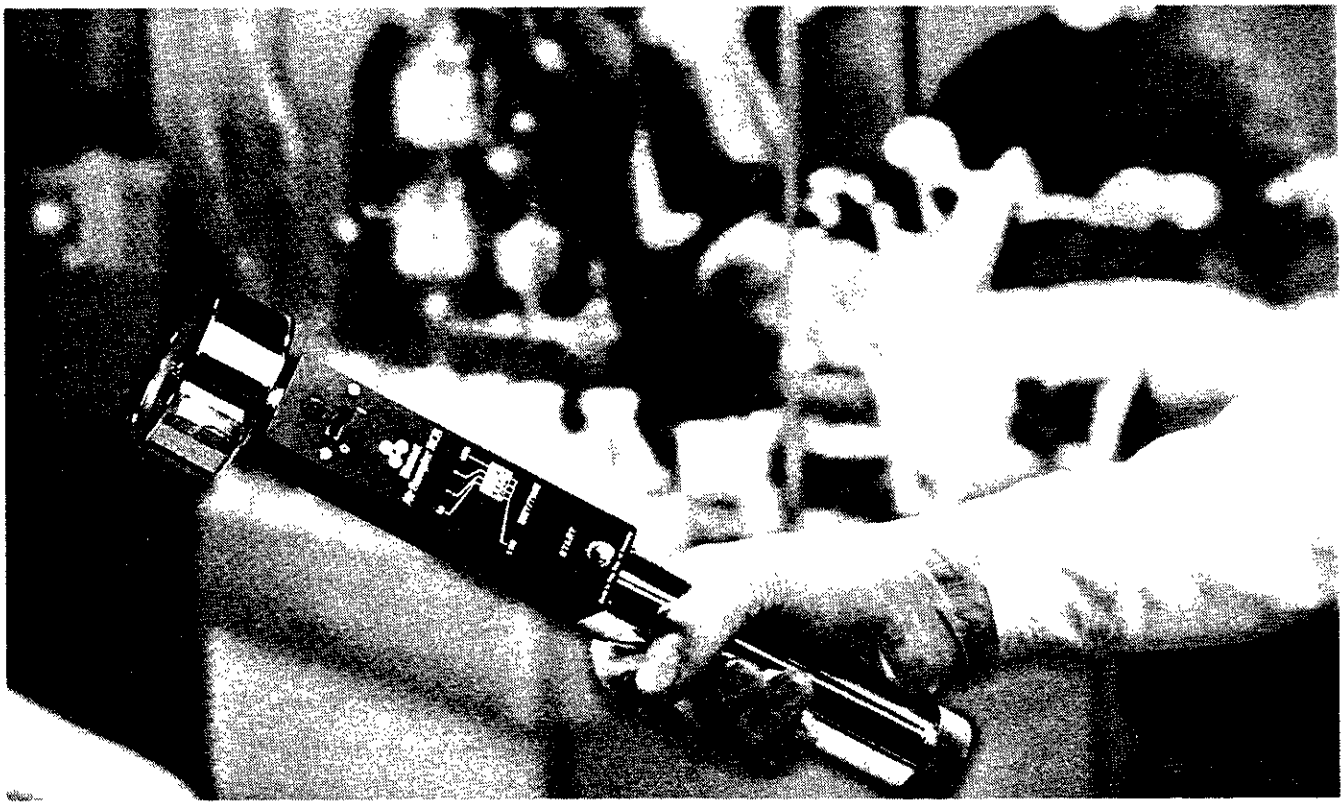
The advantages of centrifugal air samplers are:

- ease of sterilisation and use
- relatively short sampling periods
- commercial availability
- ability to sample aerosols in their natural state
- elimination of the need for laboratory dilutions
- rapid counting, if a colony-counter is used

Itµs disadvantages are:

- low capture efficiency for particles smaller than 3 um (17)

FIGURE 14: CENTRIFUGAL AIR SAMPLER



2.2.3.2 Filter techniques

The basis of filter techniques is the capture of suspended microorganisms by a porous material through which a known volume of air is passed. Two types of filters exist: fritted-glass filters, and membrane filters.

In fritted-glass filters, the interstitial spaces are larger than the diameter of the particles, and capture is effected through particle impaction on filter fibres (Figure 14).

Membrane filters capture particles directly, through retention of particles whose diameter is larger than the pore size of the filter. Membrane filters are available in a wide variety of plastic polymers. Pore sizes vary from 3 um to 8 um, depending on filter type (Figure 15).

Sampling rates may vary from 5-20 LPM. Sampling period is limited by the possible dehydration of microorganisms, particularly bacteria. At the end of sampling, the filter is removed and incubated. Particles may be extracted from the filter and incubated, or the entire filter may be incubated.

Because of its dehydrating effect, this technique is only appropriate for the sampling of spores and resistant vegetative cells (1). This is well-illustrated by a comparison of the high efficiency with which Bacillus subtilus Var. niger is captured, and the efficiencies as low as 0.1-0.2% with which vegetative cells of Serratia marcensens are captured under certain circumstances (13).

FIGURE 15: MEMBRANE FILTER (13)

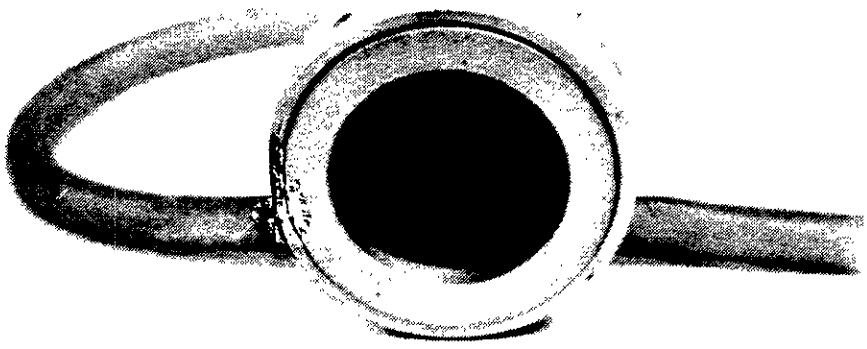


FIGURE 15: MEMBRANE FILTER (13)

FIGURE 16: FRITTED-GLASS FILTER (9)

Filter techniques are inappropriate for use in situations where long sampling periods or large sampling volumes are necessary, since these conditions adversely affect the viability of dehydration-sensitive microorganisms (13).

The advantages of filter techniques are:

- estimation of total suspended particle count
- ability to sample spores
- ability to sample non-viable particles
- low cost

Their disadvantage is:

- the limiting influence of dehydration on sampling period

2.2.3.3 Electrostatic and thermal precipitators

Although electrostatic forces operate to some extent in all high-volume sampling devices (Figure 16), they are the sole capture mechanism involved in electrostatic precipitators. In this equipment, suspended

particles receive an electrostatic charge and are subsequently collected by attraction to an oppositely-charged electrode (Figure 17).

Since all electrostatic precipitators generate ozone (13), their use is inappropriate in cases where viable microorganisms must be collected (1).

Generally, precipitators possess the following advantages:

- capacity for high-volume sampling
- relatively high (70-95%) capture efficiencies

Their disadvantages are:

- high purchase price
- complexity of operation and maintenance (requiring specialized personnel)
- suitability for laboratory, rather than field, work

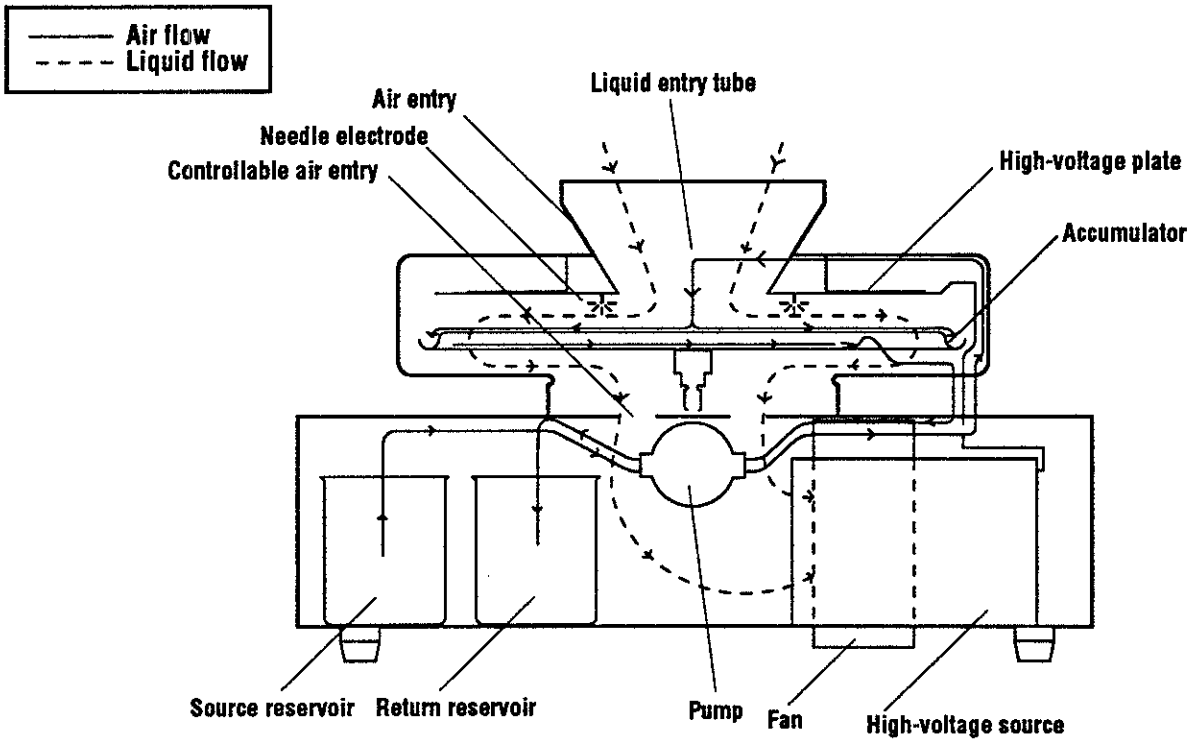


FIGURE 17: HIGH-VOLUME SAMPLER (9)

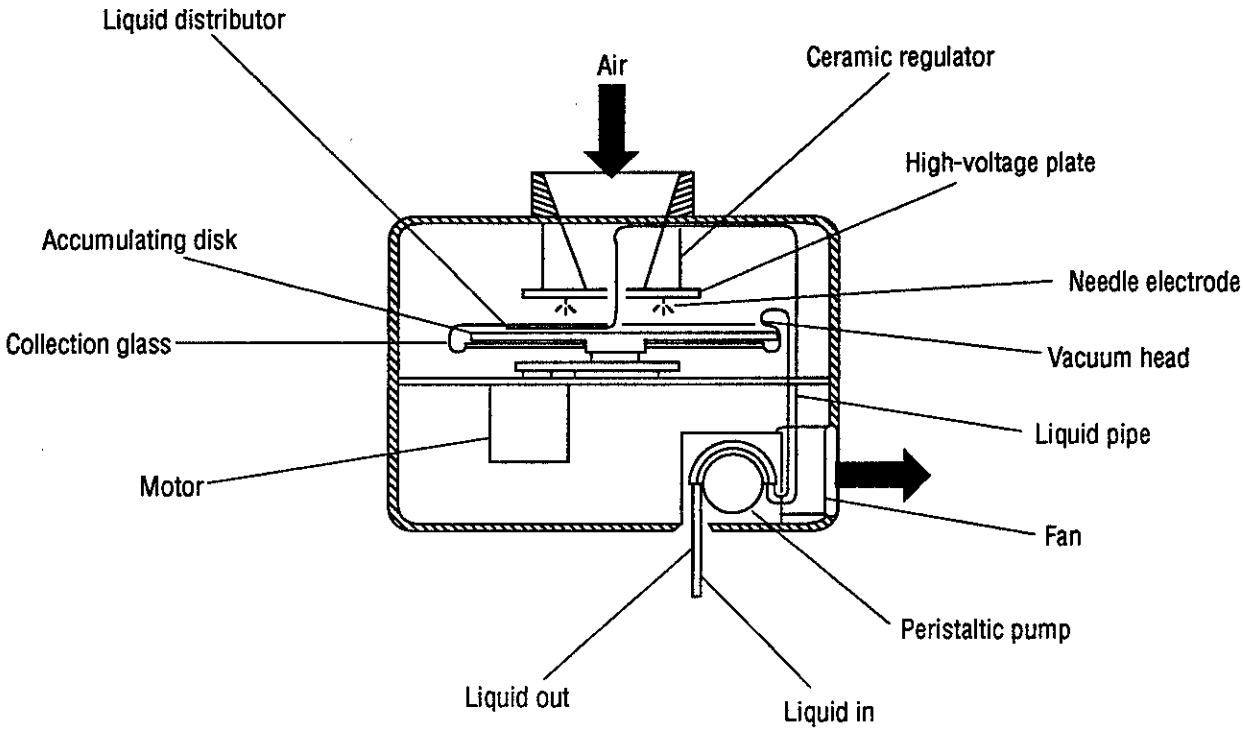


FIGURE 18: ELECTROSTATIC PRECIPITATOR (9)

2.3 Selection of sampling equipment

A list of the most frequently-recommended sampling equipment appears in Table 5. The selection of the most appropriate sampling equipment for a given situation must take several factors into account. These include (15):

1) capture efficiency
2) sensitivity
3) reliability
4) ease of equipment sterilisation
5) viability of sampled microorganisms
6) ease of use
7) ability to characterise the sample granulometry
8) cost

In addition, it is necessary to consider the specific objectives of

the sampling programme. If for example, one of the objectives is the evaluation of the risk of respiratory tract infection, it is essential to choose a technique capable of separating aerosols on the basis of particle size (13). Other considerations include the ability of the sampling technique to measure all airborne microorganisms, the level of identification (genus vs species) required, the airborne microorganism concentration, and the relative importance of total particle and cell counts (1). Equipment selection will also depend on whether the goal of the sampling programme is to enumerate all colonies or, as in the case of allergenic microorganism, to identify the microorganisms sampled.

High-volume samplers are useful when dealing with low concentrations of microorganisms. Multi-stage samplers provide additional valuable information on particle size.

Slit samplers are useful in cases where temporal variations in microorganism concentrations are of interest.

Some sampling techniques have very specific applications. Thus, filter techniques are useful for sampling resistant fungal spores.

Impaction samplers, using either solid or liquid media, have higher capture efficiencies. Filter techniques, although inherently efficient, have poor viable microorganism capture efficiencies, due to desiccation of sampled microorganisms.

Sampler	**Sampling Principle**	**Sampling Rate (LPM)**	**Sampling Period (minutes)**	**Applications**
Andersen (6-stage)	*Impact on solid media	28.3	1-20	Low and medium levels of bacteria and viruses**. Samples viable organisms. Yields granulometric information.
Andersen (2-stage)	*Impact on solid media	14-28.3	> 1	as above
AGI-30 glass impinger*	Capture in liquid media	12.5	15-30	Bacteria, viruses**, etc. Effective over a wide concentration range.
Electrostatic precipitator	Combination of electrostatic forces and capture in liquid media	500 - 10,000	unlimited (media may be recirculated)	Bacteria and viruses**. Particles are captured in liquid media and the number of viable organisms counted. Capture efficiency is 45-90% of that of the AGI-30.

TABLE 5: FREQUENTLY-RECOMMENDED SAMPLERS FOR VIABLE MICROORGANISMS (SUITE)

Sampler	Principle	Sampling Rate (LPM)	Sampling Period (minutes)	Applications
Membrane filters	—	5-50	Bacteria, viruses: several minutes; Spores, fungi: longer	Primarily for resistant spores, but may also be used for bacteria and viruses**. Typical spore diameter is 0.45 µm.
Slit samplers	Impact	28.3	1-60	Furnishes information on temporal variations. Samples viable microorganisms. Useful over a narrow concentration range.
Petri dish with agar medium	Gravity sedimentation	—	0-240	Biased toward overestimation of large particles. Samples viable organisms.
Direct surface-agar plating	Gravity sedimentation	—	unlimited	Biased toward overestimation of large particles. Samples viable organisms and resistant spores.
RCS centrifugal sampler	Impact	40	1-8	Bacteria and viruses** > 3 µm. Samples viable microorganisms.
Multi-stage liquid impinger	Impact	55	variable	Samples individual cells at moderate rates. Granulometric selection similar to that of the human respiratory tract.

Ref:(11)

* Recommended standard method

** Theability to sample viruses is largely theoretical. Sampling is only possible when:1) the specific virus in question is known, and it is possible to find a laboratory prepared to cultivate the virus for subsequent counting

2.4 Selection of culture media

Theoretical considerations dictate that culture media used in agar-impact or sedimentation techniques be chosen after consultation with the analytical laboratory and a microbiologist. In general, recommended media adequately support microbial growth. However, satisfactory culture or identification of certain fragile microorganisms may require specific media.

All culture media must be used fresh, i.e. within days of preparation. Prepared Petri dishes should be stored upside down, and condensation which has formed on the covers wiped off. Media should be sterilised by autoclaving for no longer than 20 minutes at 120 °C (18). Special precautions should be taken in transporting media to the analytical laboratory. Culture media should not be exposed to excessive heat or to sunlight, and should be shipped as soon as possible (1).

2.4.1 Recommended fungal culture media

Malt-extract agars of pH 4.5-5.0 are recommended (18), as these yield results at least equal to other fungal media such as Sabourand dextrose agar (SDA), Rose Bengal streptomycin (RBS), and potato agar. They do not support bacterial growth, and are diagnostic for Aspergillus sp.

Although there is no practical difference between RBS and SDA media in terms of colony count results, RBS media remain the media of choice for wide-spectrum incubation of fungi found in ambient air (19).

Fungal media should be incubated at 22-30 °C for a period of 3-7 days.

2.4.2 Recommended culture media for bacteria, including the thermophilic Actinomyces

Tryptase-soya agars (TSA) of pH 7.0-7.2 are recommended (18). Incubation is at room temperature, in the case of microorganisms sampled from ambient air, or at 35 °C in the case of microorganisms from human sources. Incubation periods are typically 48 hours, but may vary between analytical laboratories.

3. CONTROL MEASURES

3.1 Decontamination

The goal of decontamination is the elimination of the effects of chemical, biological, or radioactive contaminants on or in objects. Biological decontamination involves the elimination, killing, or inhibition of growth of undesirable microorganisms. The effect is transient and affects only microorganisms present at the time.

Sterilisation and disinfection are the two most common biological decontamination techniques. Sterilisation is the more radical of the two, and destroys all microorganisms, including viruses, spores, fungi, and protozoans, and microorganisms present in vegetative or spore form. Disinfection, on the other hand, is undertaken to eliminate pathogens present in liquids or on objects or surfaces, and to prevent contamination (20). Of the two processes, only sterilisation is completely effective: while sterilisation completely eliminates the risk of infection, disinfection merely diminishes it. The few disinfectants with activities comparable to sterilizing agents require contact times of several hours to be effective.

Mandated exposure levels must not be exceeded during decontamination processes. Specific preventive measures will be required for decontamination processes employing liquids, gases and vapours, and radiation.

3.1.1 Decontamination methods

Decontamination is effected through the use of the following chemical and physical agents: heat; liquid disinfectants; gases and vapours; radiation.

3.1.1.1 Heat

3.1.1.1.1 Moist heat

Decontamination with moist heat is the most reliable method of destroying all microorganisms. The technique involves the application of saturated steam in an autoclave at a pressure of 15 psi and a temperature of at least 121 °C (250 °F). Sterilisation must be performed for at least

15 minutes, measured from the time the material to be sterilised reaches a temperature of 121 °C, with 20-minute exposures most common (1). The survival of a test microorganism is considered the most reliable index of sterilisation effectiveness. The microorganism most commonly used for this purpose is the spore form of Bacillus stearothermophilus (1).

3.1.1.1.2 Dry heat

Dry heat is a less effective sterilising agent than moist heat, and requires either longer exposure times or higher temperatures. Exposure times and temperatures should be determined for each material to be sterilised; a safety factor should then be applied, to allow for the effects of other factors capable of influencing the sterilisation process (1).

Dry-heat sterilisation is performed at 160-170 °C (320-338 °F) over a period of 2-4 hours (1). Heat resistant materials may be sterilised using higher temperatures and shorter exposure periods.

3.1.1.2 Liquid disinfectants

The many commercially-available liquid disinfectants generally fall into one of the following categories: halogens, acids, alkalis, salts of heavy metals, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols, and amines.

Unfortunately, the most effective liquid disinfectants also possess undesirable properties such as corrosiveness. No universally applicable liquid disinfectant exists.

Disinfection with liquid chemicals is necessary in situations where the application of pressurised steam, the most effective sterilisation technique, is not feasible, e.g. sterilisation of large areas or surfaces, or of immovable equipment. In addition, pressurised steam is not an appropriate sterilizing agent for delicate instrumentation (e.g. optical and electronic componetry) which is damaged by excessive heat and humidity.

Halogens are the most reactive group of liquid disinfectants, and retain their effectiveness over a wide variety of temperatures. The actual

active chemical species in this group of disinfectants is the free halogen. Chlorine, iodine, bromine, and fluorine all rapidly kill spores, viruses, rickettsia, and fungi. However, halogens also possess a number of undesirable properties, including rapid reaction with proteins, instability at low pH, and corrosiveness towards metals.

3.1.1.2.1 Alcohols

Ethanol and isopropanol, at concentrations of 70-85% (p/p), are used. Alcohols denature proteins but are slow-acting germicides (1). They may be applied to all materials, including metal surfaces. They do not stain, leave no residue, and are non-corrosive. A 70% ethanol solution is most effective.

These disinfectants are flammable. They do not kill spores, and are of limited effectiveness against viruses. Organic matter may neutralise them (20).

3.1.1.2.2. Formaldehyde

Formaldehyde may be used as a disinfectant, and is available as a 37% aqueous solution, known as formalin, or as paraformaldehyde, a solid polymer (1). Solutions containing 5% formaldehyde are effective liquid disinfectants. Concentrations of 0.2-0.4% are used in the preparation of vaccines to inactivate viruses (1).

Formaldehyde loses much of its disinfectant properties at low temperatures. Because of its irritating odour, special precautions must be followed when using it in laboratories.

A solution of 70% ethanol and 8% formaldehyde is a highly effective disinfectant (20) suitable for use on all surfaces, including metal ones. This rapid, wide-spectrum germicide should be used whenever the identity of the pathogen is unknown, or when handling highly pathogenic fungi such as Coccidiodes immitis or Histoplasma capsulatum (20).

3.1.1.2.3 Phenolic compounds

Phenol itself is not used as a disinfectant, since it has an oppressive odour and it leaves a gummy residue on surfaces. However, several popular disinfectants are based on phenol derivatives. Phenolic compounds are effective against certain viruses, rickettsia, fungi, and vegetative bacteria (1).

3.1.1.2.4 Quaternary ammonium compounds

Even after 40 years of use, the effectiveness of quaternary ammonium disinfectants remains the subject of controversy. Cationic detergents of this class are powerful surface-active agents. Quaternary ammonium compounds bind to proteins. They are odourless, non-corrosive to metals, stable, economical, and relatively non-toxic.

3.1.1.2.5 Chlorine

This universal disinfectant is effective against all microorganisms, including bacterial spores. Chlorine combines with proteins, and this reaction reduces its concentration as combination proceeds. Available free chlorine is the active species. Chlorine is a powerful oxidizer, and corrodes metals. Since chlorine solutions rapidly lose their disinfectant properties, fresh batches must be prepared frequently (1).

Sodium hypochlorite (Javel water) is recommended for general laboratory use. Its rapid, wide-spectrum action, availability, and low cost, have rendered it one of the most frequently used disinfectants. Sodium hypochlorite solutions must not be mixed with concentrated acids or bases, since this results in the rapid evolution of chlorine gas.

3.1.1.2.6 Iodine

Iodine possesses similar properties to chlorine. Wescodyne solutions are commonly used in laboratories, at a dilution of 1 ounce of solution to 5 gallons of water. This yields an available iodine concentration of 25 ppm. A 75 ppm solution (3 ounces to 5 gallons of water) yields a free iodine concentration of 0.0075%. These low concentrations react rapidly with proteins (1).

Iodine solutions in 70% ethanol may be used when disinfecting metal surfaces, and in other situations where application of sodium hypochlorite would be inappropriate, and are recommended for virological disinfection (20). The major disadvantage of these rapid-acting germicides is the stain they leave on certain materials (20).

3.1.1.2.7 Glutaraldehyde

Alkaline solutions of 2% glutaraldehyde are powerful, wide-spectrum disinfectants. Glutaraldehyde solutions require a long contact period to be effective: disinfection times are of the order of 10-30 minutes, while sterilisation may require contact for 10-12 hours. They are, however rapid-acting bactericides against the vegetative form of bacteria.

Fragile equipment which cannot be autoclaved or sterilised in a Pasteur oven, such as centrifuge components and rubber parts, may be disinfected by immersion in glutaraldehyde solutions (20).

t periods required. It retains its germicidal properties well, even in the presence of organic matter, and is considered a chemical sterilizing agent (20).

TABLE 6: LISTS THE CHARACTERISTICS OF SOME COMMON BIOCIDES (21).

Characteristics of Some Common Biocides

Biocide	Sporicidal Activity	Mechanism	Human Health Effects (inhalation/contact)
Hypochlorites	yes	enzyme inactivation	irritation; corrosion
Hydrogen peroxide solutions	?	free hydroxyl radicals	none, for 3% solutions
Quaternary ammonium compounds	?	increases permeability of cell membrane	toxicity; irritation
Alcohols	no	protein denaturation	none reported
Phenolic compounds	no	protein denaturation	toxicity; irritation; corrosion
Glutaralde-hyde	yes	protein cross-linking	toxicity; irritation
Iodine, iodophores	yes	protein iodination and oxidation	irritation of skin and mucous membranes
Formaldehyde	?	binding to DNA and cell proteins	toxicity; irritation; possibly carcinogenic

3.1.1.3 Gases and vapours

Of the many gases and vapours possessing germicidal properties, the most commonly-used are ethylene oxide and formaldehyde. If applied in closed systems under controlled conditions of temperature and humidity, they act as sterilizing agents. They are used for the sterilization or disinfection of (1):

- biological safety cupboards and their components
- immoveable or large equipment for which surface application of liquid disinfectants is not possible
- optical equipment liable to damage by other sterilisation techniques
- apartments and other buildings, and their ventilation systems

Other chemical disinfectants such as peroxyacetic acid, betapropriolactone (BPL), methyl bromide, and glutaraldehyde may also be used in closed systems under controlled conditions of temperature and humidity. All of these are excellent disinfectants.

3.1.1.3.1 Formaldehyde

Formaldehyde is generally the disinfectant of choice for safety cupboards (laminar flow hoods), incubators, laboratory suites, buildings, and other confined spaces. It may be generated by heating or vaporising aqueous solutions containing 37-40% of formaldehyde (formalin), or by heating paraformaldehyde, a solid polymer containing 91-99% formaldehyde (1).

Formaldehyde is a toxic substance with a threshold limit value (TLV) of 2 ppm. Special precautions must be followed when handling, storing, or using it. Repeated exposure is a recognized cause of allergic reactions in some people (1).

Formaldehyde is explosive in concentrations of 7.0-7.3% in air. However, concentrations of this order are not generated when standard operating procedures are followed (1).

3.1.1.3.2 Ethylene oxide

Gaseous ethylene oxide kills spores, viruses, pathogenic fungi, moulds, and highly resistant thermophilic bacteria (1).

The following parameters must be strictly controlled during ethylene oxide sterilisation (1):

1. **Temperature.** Penetration of ethylene oxide into microorganisms is highly temperature-dependent. Ethylene oxide activity increases approximately 27-fold for every 10 °C increase in temperature. Normal usage temperatures for ethylene oxide vary between 49 °C and 60 °C.

2. **Concentration.** Ethylene oxide sterilisation may be performed more rapidly if the concentration is increased. Concentrations of 500-1,000 mg/l are recommended.

3. **Humidity.** Relative humidity levels of 30-60% are frequently maintained when performing ethylene oxide sterilisation.
4. **Exposure period.** In the majority of cases, the exposure period required to achieve the necessary degree of sterilisation is experimentally determined, using test organisms such as Bacillus subtilus var. niger.

All clothing, shoes, masks, tapes, and other items liable to come into contact with the skin must be aerated for at least 24 hours following ethylene oxide sterilisation. Aeration times should be extended if the air temperature is low.

Mixtures of 3-10% ethylene oxide in air are explosive. Commercial mixtures, contained in Freon™ or CO_2, are not explosive.

3.1.1.4 Radiation

The germicidal effect of ionizing radiation such as X-rays has been known for 80 years. Gamma radiation is used to destroy microorganisms in food products and in some medical products. Ionizing radiation is not, however, of practical laboratory use.

Ultraviolet radiation is a practical disinfectant for viruses, mycoplasmae, bacteria, and fungi. This non-ionizing radiation is particularly useful for killing airborne microorganisms, inactivating surface microorganisms, and treating unstable products which cannot be otherwise disinfected. However, the practical utility of ultraviolet radiation is limited by its poor penetration (1).

3.2 Protective clothing and equipment

Protective clothing and equipment provides protection against infectious, toxic, or corrosive agents, fire, excessive heat, and other physical hazards. The safety, practicality, and comfort offered by different types of protective clothing and equipment varies widely.

3.2.1 Protective clothing for laboratory work

Protective clothing worn in the laboratory protects the worker, the experiment, and the environment against contamination. Maximal protection depends on use of the clothing in the recommended manner.

Microorganisms may survive on wool or cotton fabric, and be carried out of the laboratory to other workplaces, the home, or other sites. Protective clothing prevents this from occurring.

Both reusable and disposable clothing is available. Reusable clothing, although initially more expensive, lasts longer. It must, however, be sufficiently durable to resist several washing-decontamination cycles. Disposable clothing does not offer this durability, and is worn by visitors to the laboratory, or when decontamination equipment such as autoclaves or ethylene oxide sterilizers are unavailable.

Factors to be considered when selecting protective clothing include comfort, water-resistance, seam quality, appearance, type and effectiveness of closings, shrinkage (which should not exceed 1%), antistatic properties, style, colour, and ability to survive multiple autoclavings at 250 °F. Fabric made of a 65% polyester, 34% cotton, 1% stainless steel fibre blend is recommended (1).

Disposable clothing made of polyethylene or cellulose fibres is popular. In polyethylene fibre clothing, knitted fibres are bonded together by heat and pressure. The resultant material is solid, resistant to particle penetration, and possesses breathing properties which render it comfortable. Under reasonable conditions, polyethylene clothing is solid enough to be worn for a number of days. Cellulose fibre clothing is made by applying the fibres in layers and attaching the layers to a nylon mesh support. The normally absorbent fibres may be treated to render them non-absorbent. The porosity of the fabric results in good breathing properties. Polyethylene fibre fabrics are resistant to a wider range of solvents than fabrics made of cellulose fibre.

Contaminated clothing should be sprayed with disinfectant and immediately autoclaved.

3.2.2 Gloves, shoes, and aprons

Gloves, shoes, and aprons are important components of a protective clothing programme. Gloves should be comfortable and long enough to protect the wrist and fore-arm. While the specific context in which they are to be used will determine exact glove type, gloves should generally afford good dexterity, be of solid construction, have low permeability, resist penetration by sharp objects, and provide protection against excessive cold or heat.

Protective footwear is required where there is a risk of foot injury. Shoes with splash-guards which are resistant to hot or corrosive liquids are available. It is good practice to change footwear when working in a microbiology laboratory, since this reduces the introduction of external contamination into the laboratory, and of laboratory contamination into the home.

Aprons are worn with other protective clothing in order to reduce liquid or particle penetration. They are particularly useful in laboratories where chemicals are handled. The best protection is offered by long, solvent-resistant aprons.

3.2.2.1 Gloves

No glove is effective under all conditions. Gloves may be made of fabric, leather, and natural (latex) or synthetic (e.g. neoprene) rubber or plastic. New rubber and plastic formulations are continually being developed in response to new protective requirements.

Glove selection will depend on the specific application (1). Delicate work requires thin gloves. Heat-resistant gloves (e.g. pevlar gloves) are necessary in biomedical laboratories for handling of hot glassware or dry ice.

Gloves should be worn whenever toxic substances, concentrated solvents, acids, and bases are handled. They must limit liquid penetration and be solid enough to maintain a barrier at points where stress is applied. Glove decontamination will vary from one setting to another. Ethylene oxide or gaseous formaldehyde is used in the many applications where sterilisation is necessary. Following sterilisation, gloves must be aerated at a temperature of at least 21 °C for at least 24 hours. This prevents residual disinfectant from causing skin irritation and burns (1).

3.2.2.2 Shoes

Personnel handling animals, heavy equipment, or corrosive chemicals should wear protective boots or shoes equipped with splash-guards. Typical heavy equipment found in laboratories includes cage supports, cages, and gas cylinders. Foot injury can be painful and can result in work absence.

All protective or special-use footwear to be used in controlled-access areas should be clearly marked, to allow easy identification. Paint marks may be used, for example, to identify shoes to be worn in areas in which biological contaminants are present.

Contaminated shoes should be rapidly sterilised with ethylene oxide or formaldehyde. A standard sterilisation schedule should be established for all shoes.

Shoes may also be sterilised using ultraviolet light or liquid disinfectants such as 8% solutions of formalin or 2% solutions of phenolic disinfectants. All trace of disinfectant must be removed from the shoes following sterilisation, to avoid causing burns and allergic skin reactions (1).

3.2.2.3 Aprons

The penetration of clothing by toxic liquids and particles can be reduced through the use of long, solvent-resistant aprons. Rubber or plastic aprons worn over protective clothing furnishes a further barrier. The use of aprons is recommended when handling equipment in the presence of steam or hot water (1).

3.2.3 Eye and face protection

Ensuring eye and face protection is a crucial element of any prevention programme, and is particularly important in laboratories, where head, face, and eye contact with liquid or solid foreign bodies is always of concern. A wide variety of visors, hoods, safety goggles, and safety glasses are available. The factors to be considered when selecting appropriate equipment includes construction material, comfort, compatibility with disinfectants, and extent of protection offered.

3.2.3.1 Eye protection

It is essential to provide adequate eye protection, since some of the products used by microbiologists and virologists can cause blindness if splashed into the eyes. Personnel should be informed of the dangers present and should be instructed in the use of protective equipment for the eyes, face, and hands. This equipment also protects against conjunctival infections caused by pathogens splashed in the eyes.

Accidents leading to blindness may be prevented by following certain simple work practices. Caustic solutions should be kept in small quantities corresponding to daily needs, thus reducing the hazard presented by breakages. Personnel handling explosive, corrosive, or caustic chemicals should always wear eye protection. A good rule of thumb is that eye protection should be worn at all times by all individuals entering or working in a work area where eye hazards exist. Eye-wash stations should also be present.

Contact lenses furnish no eye protection (1). Foreign bodies may infiltrate the capillary space between the eye and the lens, and if the foreign body causes pain, the resultant muscular spasms may make it extremely difficult to remove the lens. The wearing of contact lenses is not recommended in areas where chemicals, fumes, particles, or other dangerous substances are present. If they must be worn, protective equipment completely covering the eyes must also be worn.

3.2.3.2 Facial protection

Visors and hoods provide facial protection against impacts and splashes, and protect both the face and neck against dangerous particles and aerosols.

Visors should completely cover the face, and should be easily removable in case of accidents. Hoods are uncomfortable unless cooled by a source of outside air.

3.2.4 Respiratory protection

Many different types of respiratory protection equipment exists. Although varying in design, application, and level of protection, they may be broadly separated into two categories: air-purifying equipment, and supplied-air equipment.

3.2.4.1 Purifying respiratory protection equipment

This equipment is characterised by the presence of a mechanical filter and a chemical cartridge. The mechanical filter provides protection against biological aerosols, but is ineffective against gases and vapours. Chemical cartridges filter out gases and vapours present at concentrations as low 0.1% (v/v). Different chemical cartridges exist for use against specific chemicals.

Purifying respiratory protection equipment may be either full-face or half-face. Half-face masks protect only the mouth and nose, while the more sophisticated full-face equipment protects most of the face. Full-face equipment is more effective against biological contaminants, gases, and vapours (1).

3.2.4.2 Supplied-air respiratory protection equipment

Three types of supplied-air respiratory protection equipment are available:

1. compressed air equipment
2. supplied-air hoods
3. self-contained breathing apparatus

3.2.4.2.1 Compressed air equipment

In this type of equipment, filtered air is supplied to a half-face mask. Respirators of this kind are useful in situations requiring maximal protection. They do, however, have limitations, most notably their total dependence on an external air supply. Should the airline fail, the worker must leave the work area immediately, since the system has only a 30-minute reserve supply. The length of the airline may present further limitations.

Three types of compressed air equipment exists: demand-supply equipment, positive-pressure equipment, and continuous-flow equipment (22).

Demand-supply respirators are used when air cylinders are available. Because of the negative pressure created during inhalation, this equipment is not recommended for laboratory use.

Positive-pressure equipment furnishes a constant flow of air under positive pressure, using air volumes comparable to those used by continuous-flow equipment. Continuous-flow equipment is used when a compressor is available to supply large volumes of air (1).

3.2.4.2.2 Air-supply hoods

This equipment isolates the worker from the external atmosphere, since it incorporates an independent air supply. Air-supply hoods are used for short periods (11-30 minutes), and are rarely used in laboratories except in emergencies (1).

3.2.4.3 Decontamination of respiratory protection equipment

Masks used in areas known to be biologically contaminated should be decontaminated with ethylene oxide. The portion of the equipment in contact with the face should be aerated for 24 hours, to avoid irritation and burns.

The decontamination process degrades carbon filters, and filters of this type must be replaced after decontamination. This equipment should never be autoclaved (1).

At the end of each day, respirators should be washed with a chemical disinfectant, rinsed in hot water, and left to air-dry for at least 30 minutes. Half-face masks should then be stored in plastic bags, and full-face masks and other respirators stored in boxes or specially-designed cases (1).

3.3 Maintenance and housekeeping

To reduce health hazards arising from biological contaminants, maintenance and housekeeping programmes should be clearly defined and thoroughly implemented.

Typical objectives of such programmes are:

1. to maintain clean research areas
2. to maintain the work area free of biological hazards
3. to prevent the accumulation of material from previous experiments
4. to prevent the generation of dangerous aerosols during maintenance operations

The principal function of a maintenance and housekeeping program is the prevention of the accumulation of waste which may:

1. harbour microorganisms which may be harmful to the biological systems under study
2. favour the survival of accidentally-released microorganisms
3. hinder disinfectant penetration
4. be transported between areas on clothes
5. if allowed to multiply sufficiently, become a biological hazard
6. cause allergic sensitization

3.4 Ventilation

Before discussing methods for the control of occupational exposure to microorganisms, it is necessary to describe the manner in which microbial contamination of building ventilation systems occurs.

3.4.1 Microbial respiratory hazards associated with air-conditioning and ventilation systems

Ventilation, heating, and air-conditioning systems, in fulfilling their primary function of distributing heated or cooled air throughout a building, are also effective distribution systems for airborne contaminants. Should the contaminants be pathogenic microorganisms, respiratory illness will occur in building occupants.

Respiratory illness associated with ventilation systems are classified into two groups (23):

1. **Allergies.** Development of hypersensitivity to respirable microbial matter (e.g. humidifier fever)
2. **Infections.** Invasive growth of microorganisms in the respiratory tract (e.g. Legionnaire's disease)

These illnesses are caused by microorganisms which multiply in humidifier or cooling-equipment water, and are subsequently distributed throughout the building by the ventilation system. The common element in all cases of allergy or infection is the presence of contaminated water.

Immunological disorders of this type were first recognised in the 1950s. In most cases, an association was observed between the onset of symptoms and the operation of heating, ventilation, or air-conditioning equipment. Outbreaks are more common in the winter, due to the higher air-recirculation rate typically practised to reduce heat losses.

Health effects take the form of hypersensitivity pneumonitis (extrinsic allergic alveolitis), humidifier fever, tight-building syndrome, and nasal conjunctivitis. Symptoms include headache, fatigue, muscle soreness, fever, chills, eye, nose, and throat irritation, erythema, cough, chest tightness, hypersensitivity, and allergies (asthma) (23). Symptoms appear during consecutive work-days and disappear over the week-end (Monday fever). Although not normally serious, the symptoms, if untreated, may develop into irreversible lung damage.

Strong evidence exists indicating that the causal agents are thermophilic Actinomyces, non-pathogenic amoebae, yeasts, moulds, protozoans, and toxins (22).

The use of the protocol described in "Guidelines For the Assessment of Bioaerosols in the Indoor Environment" (21), published by the Bioaerosol Committee of the American Conference of Governmental American Hygienists (ACGIH), is recommended for all microflora.

3.4.2 Preventive measures

Little information is available on techniques of reducing high levels of airborne microbial contamination in office buildings. The goal of the preventive measures presented below is the reduction of microbial contamination and of illness associated with office buildings (24):

1. Reduce humidity levels in occupied spaces and in the heating, ventilation, and air-conditioning (HVAC) systems
2. Remove stagnant water and slime from building mechanical systems
3. Use steam-based humidifiers
4. Eliminate water vaporizers from HVAC systems
5. Maintain relative humidity below 70%
6. Use filters with efficiencies of 50-70% in air-supply systems
7. Remove all equipment contaminated by microorganisms
8. Institute a comprehensive maintenance program for all HVAC systems
9. Clean and disinfect all ventilation ducts

In addition, the following practices should be followed to reduce the risk of epidemics in industrial settings such as hospitals (25):

- Siting of air-conditioning towers at ground level rather than on the roof, to reduce their contamination
- Installation of safety valves between air-conditioning towers and the rest of the water system
- Cleaning of air-conditioning towers at least once a month, especially during the summer
- In proven cases of hospital epidemics: Short-term hyperchlorination of water, and elevation of water temperature to 70 °C for 24-48 hours

3.5 Personal hygiene

3.5.1 Washing of hands

Washing of hands is the most important means of preventing the spread of infection, and should be practised following any contact with a source of infection. Microorganisms collected through contact with the normal environment can be eliminated with soap and water. In the case of more hazardous contacts, e.g. with carriers of harmful microorganisms, the use of disinfectant soap is recommended (26).

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