SAMPLING MICROBIAL AEROSOLS

Mark A. Chatigny

INTRODUCTION

Collecting microbial aerosols is not substantially different from collecting any other airborne particulates. After collection, however, the processing of the sample is all important. These particles have life and the capacity to grow, multiply, and—as parasites—cause undesirable effects in a multiplicity of hosts. No chemical or physical measurement(s) available today can assess all these characteristics. Even detection of their presence often requires the bio-amplification provided by their growth characteristics. Many toxic materials are effective in the ppm ($1/10^6$) or even ppb ($1/10^9$) ranges; microbes may be active in the $1/10^{12}$ to $1/10^{14}$ concentrations. (For example, inhalation of a single tubercle bacillus (10^{-12} to 10^{13} gm) can initiate an active tuberculosis lesion.)

Both indoor and outdoor air are seas of microbial particles. Depending on local conditions, concentrations of viable particles will range from a few per ft^3 to many thousands or even millions. Particles are nearly indistinguishable so that detecting a specific viable and infective type is a little like selecting a specific raindrop in a rainstorm. Only by careful choice of growth and assay precedures, can the microbes of interest be selected out of the collectate.

Some description of the important sources, receptors, and transport mechanisms in the transfer of infectious agents is useful in understanding how infections occur. People, the major subjects of our concern, can be targets, carriers, sources, or vectors. As such, they range from the "Typhoid Mary" carrier, or the person with a cold shedding virus, to the dairy worker whose boots are laden with foot and mouth virus which he spreads through a susceptible animal population. The sources of aerosolized material can include growth sites such as sewage treatment plants, infected surgical wounds, animals, soil, people, and "other warm, moist and nutritive locations"(3). Microbial aerosols can also be dispersed directly from animate carriers or by activities disturbing an infected but normally passive source. For example, many respiratory infections of construction workers have been caused by soil fungi aerosolized at excavation sites.

Table I-42 lists various occupations and some of the diseases workers may acquire through exposure to microbial aerosols. The route of infection may be oral, or through the respiratory system, conjunctiva, or open wounds, etc. Disease descriptions are general and limited to those resulting from infection with viable organisms. Exposure to nonviable organisms can also cause disease (primarily allergies or hypersensitization phenomena). The indication of routes of infection by "contact" includes all other routes. The frequent occurrence of alternate routes is at least one indication as to why it is difficult to establish a direct cause and effect relationship between microbial aerosols and infection.

By and large, with the exception of fungal infections, the airborne route of infection is not the predominant mode. Occupational diseases due to aerogenic exposure to microorganisms or their toxic products may not be the most frequent hazards in work areas, but they are so widespread and the severity so great that they must be given close attention. A variety of occupations provide opportunity for aerogenic exposure. In the case of anthrax infections of goat hair pickers and sorters, most infections were through skin breaks, but an estimated 3% were by the respiratory route (1).

Special emphasis is placed on sampling viral aerosols because sampling for these agents is difficult. The problem is not only the mechanics

Table I-42

Occupation	Infection	Agents	Possible Contact Infections?
Hospital workers (and patients)	Conjunctivitis, otitis, sinusitis, diarrhea, etc.	Staphylococcus sp., E coli	Yes
		Proteus sp., pseudomonads,	Yes
		viruses	Yes
	Urinary tract infection	E. coli, Klebsiella sp. (bacteria)	
		Staphylococcus, aureus	Yes
	Surgical wound infection	Staphylococcus, aureus	Yes
	Skin infection	Staphylococcus, aureus	Yes
		Pseudomonads	Yes
		Staphylococcus albus	Yes
	Respiratory infections	M. tuberculosis bacteria	Some
		Influenza virus	Few
		Rhino virus, Adeno virus	Yes
	Hepatitis	Hepatitis virus	?
Microbiology laboratory workers (clinical and research)	Every infectious disease worked on including animal diseases	Bacteria	Yes
		Virus	Yes
		Rickettsiae	Yes
		Chlamydia	Yes
Stock handler	Glanders	A. mallei (bacteria)	Yes
	Brucellosis	Brucella sp. (bacteria)	Yes
	Tularemia (rabbit fever)	Francisella tularense (bacteria)	Yes
	Encephalitis	Equine encephalitis virus	Yes
Hair and hides handler	Anthrax	Anthrax spores	Yes
	Tetanus	Clostridium, Tetanii (spores)	Yes
Rendering plant worker	Q-fever	Cox. Burnetii	Yes
Lab animal care	Almost all agents studied	Bacteria, virus, fungi	Yes
Pet shops operator	Psittacosis		Yes
Meat packing plant workers	Brucellosis	Brucella sp. (bacteria)	Yes
Poultry packers	Ornithosis, psittacosis	Various psittacine chlamydia	Yes
Construction site prep. workers, ventilation system repair men	Histoplasmosis	Histoplasma capsulatum (fungi)	No
	Blastomycosis	Cryptoccosis (fungi)	No
	Aspergillosis	A. fumigatus	No

Table I-42 (Continued)

Occupation	Infection	Agents	Possible Contact Infections?
Farmers	Farmers' lung	Microspora faeni (fungi)	No
	Ornithosis	Various psitticine, chlamydia	Yes
	Coccidioidomycosis	Coccidioides immitis (fungi)	No
	Brucellosis	Brucella sp.	Yes
	Erysipelas	Fungi	Yes
	Newcastle disease	Newcastle virus	Yes
	Rocky Mountain spotted fever	Rickettsia	?
	Q-fever	C. burnetii	?
	Anthrax	B. anthracis spores	Yes
	Plague (bubonic and pneumonic)	Yersinia pestis (bacteria)	?

of particle collection; the most difficult part of the operation is handling the catch. Viruses are the smallest entities said to be "living" and require sites within our living cells to propagate. Most viruses are fastidious and require specific host cells in which to multiply. The process of multiplication is necessary if they are to be detected from the "sea" of other particulates always present.

Detection (sampling and assay) of viral aerosols is perhaps the most difficult aspect of sampling microbial aerosols. However, many of the problems in sampling for viruses are common to sampling other microbes and a listing of needed improvements is applicable to most microbial sampling work. Research on sampling is needed in the following general areas:

1. Development of samplers that will concentrate the aerosol, provide some particle size discrimination ranging from 0.1 to 50 μm, will work with minimal energy input and and noise output, and will utilize a variety of collection media.
2. Development of collection media broadly useful in the collection of bacteria, fungi, rickettsia, and viruses with minimal loss of viability. The need is for material that will retain its physical characteristics for prolonged sampling periods and will, with some adjustment, provide the needed nutrients or stabilizers for optimal survival and recovery of viable particles.
3. Developrnent of assay (and collection in some cases) and growth media or additives for a basic substrate that will facilitate the selection of the agents of interest. Currently available formulations do this to some extent but usually provide less than optimal growth conditions for the agent to be selected.

The very nature of these requirements points out that although we have some hardware and technology as described in the section following, we are not yet able to sample the air of a workplace and define hazardous conditions except in a few exceptional circumstances. There are deficiencies in instrumentation and sample processing procedures. Worse, there are no standards for allowable or tolerable burdens of airborne microbes. The presence of low concentrations of measles virus in the air of classrooms was detected by W. F. Wells in 1942 (11). The presence of tubercle bacilli in a tuberculosis ward was demonstrated by R. L. Riley in 1961 by the use of sentinel guinea pigs exposed to the ward exhaust air for prolonged periods (8). There have been a few other examples of known pathogens collected from spaces with infected workers or materials (packing plants, goat hair sorting, etc.). In no case has the recovery of airborne pathogens been linked quantitatively with the incidence of disease. At this time, the Communicable Disease Center does not recommend prospective sampling of hospital environments

but relies on maintenance of clean environments and retrospective epidemiological data for confirmation of control efficacy (6). The FDA in their Good Manufacturing Practice Guides for Pharmaceuticals and Parenteral Solution Preparation does require air sampling in the work place. Their concern is with *all* particulate contamination that may enter the product. Microbial sampling alone as described by Kraidman (5) and Fincher (2) may not meet this requirement.

We do have many of the tools for monitoring a work place where the type and approximate concentration of a pathogen is known or suspected. Improvement is needed, but this should not be a total deterrent to monitoring aerosols and developing needed information on observed, expected, and, as the data base permits, a rational expression for an "allowable" microbial concentration.

Since publication of the following (extracted) material, two new samplers have become available. The first is a small portable battery operated sampler that can be useful in a variety of areas where the noise of an air mover is undesirable or it is necessary to move the sampler often. The "RCS" unit is essentially a straight vane centrifugal blower wheel about four inches in diameter with the fan scroll case totally surrounding the rotor. A special flexible strip of plastic containing pockets of nutrient agar is slipped into a slot in the scroll case to provide a liner for the housing. Thus, air is drawn through the rotor center at about 40 liters per minute and the particles are swirled out by centrifugal force to impinge on the agar surface lining the housing. The rotor is battery driven by four D cells and the entire device is approximately the size and appearance of a four cell flashlight. Although there are some limited data on efficiency, most of it indicates the samplers yield results comparable to the slit-impinger or the sieve sampler. Some reports show overall higher collection efficiencies than are seen with these well-known samplers. The strip bearing the collecting medium (agar with special nutrients added to meet unique sampling requirements) is available from the suppliers in sterile packaging. A colony counting device is also available. The sampler is simple in concept and should be reliable in operation. Its simplicity and lack of need for pumps and external power enhance its appeal for sampling in relatively inaccessible locations. It does not yield data on particle size of the aerosols collected as does the Andersen sampler, nor does it provide time-concentration data as does the slit impinger. In concept it might be considered an advanced modification of the centrifugal sampler developed by W. F. Wells in 1933 (9)(10). Although not advertised as such, it can be used for sampling particulates onto surfaces for morphologic or chemical analysis. The limitations cited above suggest that further research be applied to this sampler.*

The second microbial aerosol sampler, only recently available, is the "Microban" Air Sampler (Model AS-101). This is a pump, single stage sieve collector and timing device package in a small baggage type container. It uses a standard 100 x 15 mm plastic petri dish and requires only connection to 100 VAC power for operation. The device most simply resembles a single stage Andersen sampler with sieve holes of 0.014″ diameter closely approximating the 5th stage of the Andersen (0.0135 D holes). At the stated sampling rate of .01 M^3/minute the sampler should impinge 1.5 to 2.0 μm and larger samples directly onto the nutrient agar. The sampler is simple, small, light, and quiet. It would appear to be most useful in sampling air in relatively clean intramural environments. Although performance data are not available, the sampler should have the efficiency and characteristics of the Andersen sampler or the "sieve" sampler described in PHS Monograph No. 60.**

*This device is available from Folex-Biotest-Schlussner Inc., 60 Commercial Avenue, Moonachie, New Jersey 07074.

**It is available from Ross Industries, Inc., Midland, Virginia 22738.

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SAMPLING AIRBORNE MICROORGANISMS

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The authors consider Mark Chatigny's chapter on Sampling Airborne Microorganisms—from the 5th Edition of Air Sampling Instruments, 1978, M. Lippman ed.—an excellent state-of-the art treatise. Accordingly, and with Dr. Chatigny's kind permission, we herewith include his chapter. We have edited the introduction he authored especially for this book, but have not altered the excerpted section.

INTRODUCTION

In response to a rapidly increasing awareness of problems in air pollution and air hygiene, considerable emphasis has been placed on sampling of gaseous and particulate contaminants. Although included in the latter category, airborne microbes have not been considered major air pollutants as have chemical aerosols. They have been of some concern in extramural environments (e.g., plant diseases) and of considerable interest in intramural (e.g., hospital surgical theaters) environments.

The intrinsic characteristics of microbes make them difficult to collect and assay quantitatively. The collection instrumentation available tends to be less sophisticated, though no less diverse, than that for other particulates and to require more processing after collection. There are few standard devices for sampling and virtually no standards for allowable or desirable microbial burden of the air. The most frequent practice in sampler selection is to review the literature in a particular area, select a system shown to work in circumstances similar to those expected and modify it as deemed necessary. There is nothing wrong with this approach, although it is a bit laborious for the air hygienist who may be more concerned with defining an ambient condition than in developing new techniques. The general purpose of this section is to point out some of the major problems to be expected, to provide leads to the work of others in the field and to develop a rationale for selection of samplers requiring minimal modification. Public Health Monograph No. 60 (1) covers a great deal of basic information and equipment description which is almost a prerequisite for selecting a sampling system, although many of the equipment descriptions are now somewhat dated. Items F1, F2, F3, and F4 contain excellent detailed data and basic principles of more recent devices used for sampling airborne microbes. Chapter 4, by Akers and Won in *An Introduction to Experimental Aerobiology,* one of the selected references listed in "Selected Reviews and Monographs" below, contains an excellent review of the subject, comparisons of efficiency of equipment and methodology for assay of data collected.

Table I-43 provides some suggestions for selection of samplers, considering only a few of the most widely used samplers and a limited set of "typical" sampling problems. Many more of the particles collection devices discussed in this volume will also be usable and are discussed further below.

Background

Sampling for airborne microorganisms does not differ from ordinary particulate sampling except for the added necessity of assessing viability of the microbes of interest. Although sampling for allergenic materials, usually proteinaceous, is similar in many respects, discussion in this section will be limited to the requirements for sampling atmospheres for living microbes.

Much of the technology of sampling of airborne microbes has been developed by medical researchers concerned with both the viability and infectivity of the airborne microorganisms in

Table I-43

SAMPLERS MOST FREQUENTLY RECOMMENDED FOR USE IN SAMPLING MICROBIAL AEROSOLS

Sampler	Principle	Sampling Rate lpm	Sampling Time	Application
Andersen multi-stage sieve-type[A,1]	Impact on nutrients	28.3	1 min Min.* 20 min Max.	Bacteria and viruses. Low to medium concentration aerosols
Andersen 2-stage disposable sieve-type.	Impact on nutrients	14-28.5	1 min Min.* 20 min Max.	Collect CFU unless surfaces are washed into medium. Provides particle-size data.
AGI-30 raised jet all-glass impinger [A,2]	Impinge into fluid	12.5	ca. 15-60 min. Max.	Bacteria, viruses, etc. will work on wide range of concentrations.
Large volume electrostatic sampler (LVS/2K) (LVS/10K)[3], (LEAP)[4]	Combination electrostatic and impaction into fluid	500-10,000	Unlimited (Fluid may be recirculated w/some makeup)	Bacteria and viruses. Collects into fluids and counts total viable unit. Efficiency 60-95 of ACI-30.
Multiple slit impinger (MSI)[4]	Impaction	1,000	Same	Same
Membrane filter[5]	—	5-50	"Minutes" for bacteria and virus. Longer for spores fungi.	Primarily hardy spores but can be used for bacteria and viruses.
Slit sampler[6]	Impaction	28.3	1 min. to 1 hr.	Provides time-concentration. Collects CFU. Limited concentration range.
Open Petri dish with nutrient agar		—	0-4 hrs.*	Biased to collect large particles (CFU)
Open settling surface uncoated.		—	Unlimited	Same as above, collects hardy spores.
Hirst spore trap[7]	Impaction	10	24 hrs.	Spores and pollen collected outdoors.

Table I-43

SAMPLERS MOST FREQUENTLY RECOMMENDED FOR USE IN SAMPLING MICROBIAL AEROSOLS (Continued)

Sampler	Principle	Sampling Rate lpm	Sampling Time	Application
Multi-stage liquid impinger[8]	Impingement	55	Varies	Collects individual cells gently at moderate flow rates with size selection similar to respiratory tree.

* May be extended by use of OED wash. See Reference 52.
A Recommended as "laboratory standard" samplers.
(1) 2000 Inc., 5899 South State Street, Salt Lake City, Utah 94017.
(2) Ace Glass, Inc., Vineland, New Jersey 08360.
(3) Litton Systems, Inc., Applied Science Division, 2003 East Hennepin Ave., Minneapolis, Minnesota 55413.
(4) Environmental Research Corp., 3725 North Dunlap Street, St. Paul, Minnesota 55112.
(5) Gelman Instrument Co., 600 South Wagner Road, Ann Arbor, Michigan 48106; Millipore Filter Corp., Bedford, Massachusetts 01730.
(6) New Brunswick Scientific Co., Inc., 1130 Somerset St., New Brunswick, New Jersey 08903.
(7) C.F. Casella & Co., Ltd., Regent House, Britannia Walk, London N.1, England.
(8) A.W. Dixon & Co., 30 Anerly Station Road, London S.E. 20, England.

growth media, cell culture, or in suitable *in vivo* host systems. Infectivity may lead to pathogenic response, and this parameter has been of interest in studies of intramural air hygiene which have ranged from contagion of such diseases as measles (2) to more recent studies of methods for control of airborne infection in surgical theaters (3) and wards, and even nuclear submarines (4). Air hygiene studies in laboratories have gained some interest based largely on the demonstration that virtually every operation with a suspension of microbes in the research or clinical microbiology laboratory can produce an aerosol (5) with particles in the respirable size range (6), and laboratory infections with every pathogenic agent studied have been recorded. The obvious connection is not believed to be mere coincidence.

Extramural air hygiene studies have been done. Workers have studied dispersal of microbes from sewage plants (7), airborne Q-fever virus from rendering plants (8), *Coccidioides immitis* from open ground (9), and rabies virus in bat caves (10). More frequently, extramural air sampling studies are of importance to workers concerned with transmission of animal diseases as recently reviewed by Hugh-Jones (11) or with plant infections (12). In almost every case, the strategy is to collect viable organisms by optimal means and to demonstrate their presence by appropriate culture methods. Viability will be the parameter of primary concern in this section, and infectivity will be considered a response dependent on the host/parasite system used.

The major difference between indoor and outdoor microbial aerosols and their sampling requirements is that the outdoor aerosol particles collected will be of a wide variety from ill-defined sources, tend to be the hardy fractions of cell populations that have undergone relative humidity (RH) stress, ultraviolet (UV) irradiation and exposure to air pollutants, be heterodispersed, and usually require collection during an unknown variety of meterological conditions. On the other hand, the indoor environment will have fewer types of infection sources and variety of microbes but more favorable environments for survival of the airborne microbes. It usually will have more airborne flora from human activity sources and will require sampling in still or low velocity air masses.

Factors to be Considered in Selection of a Microbial Aerosol Sampler

1. *The entire sampling system should be considered.* One should consider the objective of the work, the sampling plan, the proposed location(s) of samplers, the number of samples and the time period during which the samples are to be taken,

the effect of time variation on the cloud from which the samples will be collected, the techniques and logistics of the assay system to be used and what quantitation is required. The last two factors are of great importance since the biological characteristics of the sample can be far more variable than any of the physical or instrumentation factors involved. These factors, considered below in further detail with other important parameters, should be considered in a systematic manner.

2. *The sampler used should permit assay of the microorganism-bearing particles in a manner related to the end objective of the study.* For many purposes it is adequate to assume that one or more microorganisms exist per particle collected and, accordingly, one can relate the particles collected directly to colony-forming units (CFU). This is perhaps the simplest method of collection and it is usually done by impaction or settling deposition onto solid nutrient on which the microbes grow directly. In some cases it is desirable to have data useful for projecting total infective dosages, and it is necessary to evaluate the total number of viable organisms in a given volume of air. This type of sample is best collected into a liquid and dispersed in various dilutions onto growth medium for quantitative assay. Most microbe-bearing aerosol particles are readily dispersed in water with wetting agents, and individual cells will be counted. The latter technique is particularly useful if it is expected that the concentration of aerosol may vary widely. As examples of the above; if one is examining the intramural air of a hospital, it would be expected that a large fraction of the viable microbial organisms of interest would be of a limited variety of species borne at low concentrations on particles of dust, skin flakes, hair, or other detritus which would be deposited on surfaces of wounds or other susceptible areas. Sampling relatively large volumes of air for colony-forming particles would provide a reasonable assay of the infectious potential of the aerosol. On the other hand, particles generated from some microbiology laboratory operations (e.g., centrifugation) have been shown to be within the respirable size range and frequently to contain several microorganisms as have those generated in sewage treatment plants. In these cases one might wish to sample at relatively low volumetric rates into liquids for assay of total viable organisms in the aerosol.
3. *The selected should meet the physical requirements of the application.* Sampling in a surgery may require a quiet-operating, large-volume sampler; sampling spores for plant pathogens in the extramural environment will require a low-power, robust sampler suitable for exposure to adverse weather conditions (e.g., the Hirst spore trap (13), see page O-12 of the 4th Edition), while sampling in a clean-room or spacecraft may require use of many sampling points using simple filtration devices (14). These are mentioned because too often one finds that workers attempt to apply laboratory devices to field situations, with attendant complications.
4. *The sampler should collect (and present) particles in the size ranges of interest.* More precise definitions of particle size and methods for measuring this parameter are given in sections F and G (4th Edition) of this text. With respect to humans and air hygiene problems, the respirable size range of 2-8 μm is perhaps the most important (15). These particles penetrate deeply into the lung and are retained in the nonciliated small passages and the alveoli for a sufficient period of time to initiate infection. The size is, of course, an aerodynamic particle size with an assumption of unity density. Less dense particles which may act aerodynamically the same, e.g., mycelia, dust particles, etc. ranging up to 30 microns, may be of equal interest for this reason. In the extramural environment, and frequently in the intramural environments with mechanical ventilation, the relatively high velocity of winds and turbulence of the atmosphere can keep rather large particles airborne for sustained periods of time. The protective effect of large par-

ticles against UV radiation may permit otherwise sensitive microorganisms to be carried great distances (16). Such protective effect may be a strong factor in reported incidents of extramural aerial transmission of Newcastle disease virus (17) and foot-and-mouth disease virus (18, 19). Other environmental factors (e.g., RH and air pollutants) were also considered in these epidemiological studies (11). Relative humidity, in particular, affects viability of airborne microorganisms markedly.

Various methods of fractionating sampled particulates by particle size are available and range from the simple liquid impinger used with a Porton pre-impinger or the size selective Andersen sampler to a variety of classifying devices employing impaction principles, charge-mass ratios, mass-area ratios (thermal precipitation), etc., (described further in other sections). The most practical method is that which is simplest, provides the needed data, and is consistent with other recovery requirements described here. Settling plates do not provide good overall size representation since they preferentially collect large particles, but they can be useful in many applications requiring knowledge of surface contamination from aerosols. The data collected do not represent aerosol concentration because it is related to specific particle sizes, air velocity and turbulence, sampling time, and other factors which must all be defined. If one knows or has good reason to postulate a particle size distribution (e.g., outdoor aerosols are most frequently log-normal as described by Junge (20)) one can infer an estimate of total concentration.

5. *It is desirable to know the expected aerosol concentration.* If one has a reasonable estimate of the range of concentration to be found, one can select a sampler that will have the necessary sensitivity and will facilitate the assay procedure. Concentration effects are most critical in direct impingement type samplers; e.g., the Andersen, sieve, or slit-type sampler where CFU counts greater than 200 or 250 per plate are difficult to count and in the case of the former sampler, prohibit the use of sampler "correction" table which correct the measured count in consideration of the possibility of multiple particles per hole area. Within a limited range (ca. 10:1), one can dilute the incoming aerosol with clean air, concentrate the aerosol if necessary (ERC collector-concentrator, Environmental Research Co., St. Paul, Minn.), or vary the time of sampling to control the quantity deposited. In the last case, one should be careful that the time is not made so short that the air clearance rate through the sampler becomes a significant fraction of the sampling period. Collection into liquid has the obvious advantage of readily permitting serial dilution for assay and accommodates a very wide range of aerosol concentration. Bubblers and impingers should not usually be used for prolonged periods (>30 min.) because of evaporation of aqueous collecting fluid, but continuous flow samplers can be used for longer periods.
6. *The wind velocity (and direction).* This parameter probably marks the single largest difference between intramural and extramural air sampling other than UV radiation. Sampling intramurally, one can expect low velocity airflow, rarely exceeding 100 lfpm (31 m/min) whereas extramural sampling may find the velocity ranging from 0 to 50 km/hr and direction changing radically. Intramural sampling of large size particles may be done with such simple devices as settling plates, and considerable variation is tolerable in the design of inlet configurations of samplers for particles of sizes below approximately 5 μm that are not greatly affected by anisokinetic conditions (21). On the other hand, sampling of particulates from a medium velocity airstream requires at least nominal isokinetic sampling, as discussed in Section L (4th Edition) and by others (22), if a representative sample is to be collected. Isokinetic sampling may be achieved by utilizing a sharp-edged nozzle pointing into the wind with natural wind through-

put, or pumps, as is done with the Hirst spore trap (See Section O-12, 4th Edition), by using air movers with filters in the stream, or by building an "isokinetic wind tunnel" from which the sample can be drawn (23). It may also be done by using a baffle to provide a "stagnation point" immediately above the sampler entry from which a sample can be collected (20)(22). May (24) has suggested that the stagnation point baffle, if used, should be as large as possible; since it then subtends a wide arc, it is relatively insensitive to direction.

In the extramural environment, directional control of the sampler may be as important as sampling isokinetically. Wind direction can change radically and rapidly, and the motion of a prevailing wind is somewhat misleading. Yaw losses are greatest when sampling large particles and when the diameter of the inlet nozzle is small (21). Particles greater than 10 microns cannot navigate sharp turns without heavy losses and must be sampled directly. For example, the AGI-30 sampler has a curved neck with a cut-off in this size range, and particles larger than 8 to 10 μm are recovered by washing out the inlet tube. Sampling of particles smaller than those from air velocities less than five MPH is probably not significantly affected by airflow direction.

7. *The biological characteristics of the agents sampled.* After the physical characteristics of the particulate matter have been considered in the sampler selection, the biological characteristics of the organisms and the collectate must be considered in the selection of the sampler, the media, the collecting time, and the storage of the collectate. It is the biological area wherein the most variability exists, and the considerations of relative sampling efficiency differences of 10 to 50% among samplers can be meaningless if one considers the not-infrequent 3 to 5 log biological variation. Numerically this may not be important if a qualitative measure of the aerosol is sufficient (i.e., simple detection of the presence of specific microbes). This may require collection conditions optimized for the particular organisms. On the other hand, quantitative collection and recovery may require use of several types of samplers and processing techniques. Collection on filters or water-free surfaces is probably best limited to hardy bacterial spores that can withstand desiccation. This technique has been used in the detection of hardy spores of concern as models of contaminants on interplanetary vehicles (14). Fungal spores, similarly, can be collected on impaction plates, sticky surfaces, or electrodes, because they are so robust; (if viability/infectivity is not to be measured, the sample can be put onto nutrient medium or fluids with little damage, but it is important that the trauma associated with sampling (e.g., desiccation, osmotic shock, etc.) be minimized. When collecting directly onto nutrient, a rich medium is perhaps more effective than one which will permit differentiation by species through limitation of growth of non-desirable organisms. The use of rich collection media will permit growth of undesirable material, and for most bacterial sampling a compromise using such additives as a fungicide (amphotericin B), or similar materials will usually be required to prevent overgrowth by molds and fungi. Subsequent transfer of viable colonies to selective media will aid in classification and identification. If spores are sought, the collection may be heat-shocked before adding further nutrient materials, and the irrelevant nonsporing bacterial burden will be reduced substantially. Noble (25) has discussed assay techniques at some length. Viruses pose some rather unique problems in sampling. The interest in sampling aerosols of viruses has increased rapidly in recent years, and the technology accordingly has become more diverse. Many of the methods for sampling bacteria are useful but are complicated by the requirement for a viable substrate for virus replication. Because sampling for viruses is a relatively recent development it will be discussed in further detail below.

Infectivity has been noted above in its

broadest terms and related directly to viability. In most cases, infectivity must be related to an *in vivo* host system. Most simply, it can be measured by using sentinel animals, and this technique has been successful where others have failed (26). If this is not desirable or possible, perhaps due to requirements for extended collection times, then inoculation of suitable host animals with a concentrate of a liquid collectate may be done. However, there is no substitute for aerogenic infection procedures, and if the objective of the sampling is to evaluate respiratory infection potential, the resultant "aerogenic" infectious dose should be evaluated with some caution. Intranasal instillation has been shown to be an excellent substitute for direct aerosol challenge in some cases and abysmally poor in others but is perhaps the nearest approach to aerosol challenge in determining dose response.

8. The *"efficiency" of the sampler.* This is discussed as a parameter only to emphasize its ephemeral character. The below extract from remarks by Gregory (27) serve well to emphasize some of the problem areas.

"Under simple conditions it is not difficult to define a standard for air sampling. With nonaggregated spores of one species liberated in a wind tunnel, isokinetic sampling through a feathered orifice facing up-wind collecting into a suitable membrane filter with precautions against overloading should give a reliable estimate of the number of particles in a measured volume of the air. The cascade impactor, catching on a thick layer or soft adhesive, tends to reveal spore clumps intact; and if this feature is undesirable, the liquid impinger should be used to break up aggregates. The more varied the population in species, particle size, state of aggregation, the harder it becomes to measure the concentration in the air."

In addition, this warning succinctly advises against "comparison of results broadly." Efficiency of particle collection should be maintained but not at the cost of changing the particle characteristics or if viable loss of viability or infectivity is the characteristic of interest. As an example, the 0.4 μm Millipore filter as an air sampler of small particles may be somewhat more efficient than the liquid impinger (AGI-30), but viable recovery will usually be lower except in the case of hardy spores or fungi. Similarly, the Litton (LVS) high volume sampler is from 40 to 70 percent as efficient as the AGI-30, but it has a sampling rate approximately 100 times the AGI. Selection of a sampling device must include consideration of these efficiency factors. Considering the range of biological variations discussed previously, the absolute efficiency of the sampler as a particle collector is not usually the most important parameter and comparison of overall efficiencies of collecting living microbes is probably valid only for single species and strains of microorganisms and defined growth media and conditions.

SAMPLING VIRAL AEROSOLS

Background

For the most part, virus aerosols originating from natural sources, i.e., humans, hospital activities, animals, etc., tend to consist of relatively large particles (28)(29)(30). Those from laboratory operations have been shown to be in the respirable size range (5)(6)(31) and to contain some particles with single virions. A great deal of energy coupled with a very high titer virus suspension at the source (ca. 10^{12}-10^{16}/ml.) is usually required to generate concentrated aerosols of viral particles in the submicron particle size ranges. While the liklihood of single virion particles is not great, there are occasions when they will be found. For example, in the operation of zonal centrifuges where virion particle counts as high as 10^{12} (32) or more per ml are being concentrated, a leakage may create an aerosol with a mass median diameter (mmd) of 1-3 μm but with large numbers of single virus particles. The same has been shown to occur to a much lesser degree in the output from a cough or a sneeze (28). Since the electron microscope count of virus particles is frequently four or more logs higher than that of plaque-forming (or infective) units (this may be an artifact of

the infectivity assay system), multivirion particles in the respirable size range or larger should be considered of primary interest. For the most part this relieves one of the difficult tasks of sampling submicron particles. Accordingly, most of the physical factors discussed previously with respect of sampling bacteria are applicable to viral aerosols, and most of the devices useful for collecting bacterial aerosol samples will also be useful for collecting virus-bearing particles. This was amply demonstrated by the early work of Meiklejohn, et al. (33) who sampled large volumes of air in a smallpox hospital and recovered virus on very few occasions using the impinger sampler. When, in other experiments, settling plate samplers were added, the virus was recovered in particles of large equivalent diameter (34).

The biological response of virus particles to sampling can vary widely and be quite different from that of bacteria. It has been demonstrated (35) that humidifying the air immediately before collection into an impinger can yield recovery of T_3 coliphage increased by as much as three logs over than from an impinger alone. On the other hand, other studies (36) showed that presampling humidification decreased the recovery of mengovirus 37A and vesicular stomatitis virus. The latter is a lipid-containing virus which has been reported to be inactivated rapidly at high RH. Although generalizations are hazardous, the work of many aerobiologists shows that airborne viruses are at least as sensitive to different relative humidities as are bacteria. DeJong and Winkler (cited by Benbough (37)) concluded that viruses with structural lipids generally survived best in aerosols at low humidities while those without structural lipids generally survived best at high RH's. These conclusions have been confirmed by Benbough (37) who attemped to isolate this effect from that of composition of suspending fluids and sampling methods.

The selection of the air sampling technique will be affected by the desired observations. This may include infectivity for animals or tissue cultures as opposed to morphological observation by electron microscopy. In the former case one would be concerned with micron-size or larger particles while in the latter case, it may be necessary to collect submicron size particles. In the final analysis, the overall probability of infection can only be assessed by the viable or infective dose recovery, as well described by Noble (25) and by Akers and Won (38).

Review of Sampling and Assay Methods

A simple settling chamber technique was devised by Hankings and Hearn (39). They sampled VEE virus from aerosols containing as few as one plaque-forming unit (PFU) per liter of air by drawing the sampled air through serial cell culture flasks at rates up to 1.0 liter/min.

Impingers using tissue culture nutrient with added serum, antibiotics and antifoam agents (Tween 80, Dow Corning, etc.) and a variety of other fluids have been used successfully in collection of Coxsackie A_{21} (40), Simian virus 40 (41), vaccinia, influenza, VEE and poliomyelitis (42). Some of the hardy viruses (e.g. coliphages) have been successfully collected on paper, Millipore, and Nucleopore filters and subsequently transferred to suitable growth medium (43)(44).

The slit sampler has been used (45)(46) with a 12% gelatin collecting medium which was subsequently liquified by heating to 37 °C and poured onto cell mats. It was also used with agar and the collectate washed off the agar onto the cells (42). These media were employed to avoid the damage to sensitive cell culture mats from drying in the sampling airstream. Recovery from such media is often hampered by retention of the virus particles in the agar (43)(47). Jensen (43) found improved recovery with this technique if he coated the agar with skim milk.

The Andersen sampler has been used for sampling viral aerosols using similar collecting techniques. Guerin and Mitchell (48) used a collection medium of 3% gelatin with added antibiotics and melted this (37 °C) onto cell culture mats. Thornley (49), on the other hand, simply cut out a disc of agar after sampling and placed this on the cell monolayers. This may be an oversimplification for sampling-sensitive viruses. In an improvement on such techniques, Thomas (50) used a mixture of sucrose, glycerol and bovine serum albumin on raised discs in the Andersen sampler (and in a slit sampler). By his technique, Thomas was able to sample for periods up to one hour with this modified Andersen unit. This sticky surface provided good recovery in the laboratory of polio, vaccinia and Semliki Forest viruses. In the field he recovered rabbit pox virus (51). Incidentally, it was observed that

laboratory-generated aerosols showed a preponderance of particles collected on the fifth and sixth stages of the Andersen sampler, while the field-collected aerosol appeared upon the first three stages. Although these adaptations of the Andersen sampler provide increased total sample volumes over impingers and impactor-samples used with plain agar (usual flow 12 to 30 lpm with 30 min max. sampling time), there is frequently need for much larger sample volumes in air hygiene studies to evaluate very low aerosol concentrations. The Andersen type sampler can be used for only limited times due to agar drying. May (52) has suggested that the use of 0.2% oxyethylene docosnol (OED) emulsion poured over the dry agar surface and allowed to soak in for a few second retards evaporation by as much as fivefold in two hours and almost twofold in six hours. Colony counts on tryptone agar with 0.2% OED in the Andersen samplers run as long as 7-1/2 hours showed increased counts of the first two stages.

The LVS (Litton) sampler (1000-2000 lpm) has received increased attention in recent years. Gerone, et al. (28) used this sampler with Eagle's Basal Medium with added calf serum and antibiotics to collect Coxsackie A-21 virus. Artenstein, et al. (53) recycled the collecting medium through the LVS in sampling for human respiratory disease pathogens. Hugh-Jones, et al. (17) used the LVS with peptone water containing pencillin G, (5,000 units/ml) in conjunction with a fungicide for recirculating through the sampler during a 60-min. sampling period. They demonstrated that the Herts, 33/56 strain of Newcastle disease virus (NDV), recirculated for a 60-minute period, suffered no significant viable loss. Larson, *et al.* (54) used the LVS in laboratory studies of small particle Rauscher leukemia virus (RLV) aerosols and in their studies of natural aerosols. The collecting medium was tissue culture broth medium of "Hanks' balanced salt solution" with 10% fetal calf serum added. Laboratory studies have shown the LVS to be comparable in efficiency to the AGI sampler in collection of viruses from deliberately generated aerosols, although in sampling animal rooms in which RLV-infected animals were held, no virus was recovered from the LVS samplers. Winkler (10) used this sampler to recover rabies virus in bat caves after being unsuccessful with several other techniques. In recent work, Chatigny and Biermann (55) have used a steam-injection modification of the cyclone separator described by Errington and Powell (56) for collection of aerosols deliberately generated to produce single virion-bearing particles in the submicron size range. The T_3 bacteriophage used was plated directly with *Escherichia coli* from the water collectate.

SAMPLER SELECTION

Only two samplers have been suggested as standards (by a learned committee (57)); the all-glass impinger, with or without the Druett-May pre-impinger, and the Andersen sampler (58). Although the six-stage sampler has been described, the two-stage, "disposable" Andersen sampler which fractionates the sample into respirable (2-8 μm) and nonrespirable (>8 μm) sizes can also be considered a "standard," particularly since it incorporates the hole-spacing components. Although it suffers from the same limitation of concentration range that affects the six-stage unit, it is economical and can provide adequate data for many studies. The samplers listed in Section O (4th Edition) of this volume are, almost without exception, usable for sampling microbial aerosols. Some will require adaptation to meet specific needs. The Milipore filters described in Section N (4th Edition) and the precipitators listed in Section P (4th Edition) can be used. The LEAP sampler (Environmental Research Co.), the Electrostatic Bacterial Air Sampler (Gardner Associates) and the abovementioned LVS sampler made by Litton Systems, Inc., are all usable in particular applications. Although references have been made above to the application and use of the LVS, which has an electrostatic charge principle as its major mode of collection, the multiple slit impinger (MSI) sampler (59) (see page O-13 of 4th Edition) is being used more frequently and should be considered equally acceptable. It is considerably less subject to electrical and mechanical failures than the electrostatic charge-based devices. The electrostatic charge units do not function well in outdoor environments of high humidity conditions and must be considered primarily laboratory tools; further, the effect of corona-discharge on sensitive microorganisms is not well defined, and the MSI sampler may yield a higher viable recovery in some cases.

There are cases wherein a novel method may be necessary. Without fully reviewing the sampler development literature, it can be stated

that in recent years developments have been directed toward large volume sampling and some classification of the particles on the basis of size or density to facilitate assay after collection. The simple cyclone, similar to those described in Section O-31 (4th Edition), has been made up in a size to sample 80 to 150 lpm (56) and modified (55) to collect submicron particles. A similar liquid scrubber device had been reported (60). Modifications of the May three-stage sampler and of the Andersen sampler (50) have been described. These extend the size selection range to more than 20 μm in recognition of the needs of intramural sampling. A simple "man-operated" filter-type sampler has been reported (61) as have variations of the "rotorod" (62) collector modified to collect a wide spectrum of particle sizes (63). An even simpler electrostatic rod collector, described some years ago (64), permitted collection of particles and subsequent deposition on a nutrient surface. No quantitative comparison date are available for these devices.

Recovery and growth on suitable media, as described previously, is the usual procedure for sample assay but other methods, usually more complicated, can work as well or better to meet special requirements. Analysis of the collectate by such techniques as fluorescent antibody staining (65), chemiluminescence (66), protein content, or a wide variety of techniques described by Strange (67) can permit use of the best features of particle selection equipment, electrostatic collection, sticky-strip collection, settling plate samplers, or even the simple charged glass rod. Most of these methods are discussed in one or more of the several books or monographs listed below. However, whenever possible, well-callibrated commercial equipment and well tested assay procedures should be used, if for no other reason than that there is usually a good body of data on the expected performance. Table I-43 and selected references listed below in conjunction with catalogue sections of this book, should provide the worker with adequate information, either to conduct a sampling program or to become sufficiently aware of research areas and apparatus availability to define the needs for special techniques.

Equipment catalogued and commercially available has grown mightily in 15 years. Nevertheless, there is still room for the use of simple self-devised techniques to meet special requirements. The reader is advised to look not only at those devices listed as "microbial aerosol samplers" but to examine any of the particulate aerosol samplers with an eye toward his own application. It is the writer's opinion that most should be afforded some method of calibration, usually with laboratory-generated aerosols closely simulating those to be sought.

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SELECTED REVIEWS AND MONOGRAPHS

Material in each of the texts listed below will be found helpful. They are listed by title and author/editor, then chapters of particular interest.

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   K. R. May: Physical aspects of sampling.
   W. C. Noble: Sampling airborne microbes; handling the catch.
   R. E. O. Williams: Spread of airborne bacteria pathogenic for man.
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2. The Microbiology of the Atmosphere, 2nd Edition. P. H. Gregrory. In particular, Chaps. IX, XI XIV, XV and XVIII. John Wiley and Sons, New York (1973).
3. An Introduction to Experimental Aerobiology. R. L. Dimmick and A. Akers (eds.), Chaps. 4, 11, 12, 17. John Wiley and Sons, New York (1969).
4. Particulate Clouds: Dusts, Smokes and Mists. H. L. Green and W. R. Lane. In particular Chaps. 7, 9, 10, E. and F. N. Spon, Ltd., London (1964).
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