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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, viruses	Yes
			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. Development 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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