J. SAMPLING AND CHARACTERIZATION OF BIOAEROSOLS[‡]

by Paul A. Jensen, Ph.D., PE, CIH and Millie P. Schafer, Ph.D., NIOSH/DPSE

CONTENTS: PAGE

1. INTRODUCTION 84
   a. General 84
   b. Indoor and Outdoor Bioaerosol Sampling 84
   c. Viable and Nonviable Bioaerosol Sampling 84

2. PRINCIPLES OF OPERATION 85
   a. General Principles 86
   b. Impaction 87
   c. Filtration 87
   d. Impingement 88
   e. Characteristics of Several Bioaerosol Samplers 89

3. SAMPLING CONSIDERATIONS 93
   a. Safety 93
   b. Environmental Conditions 93
   c. Flow Calibration 94
   d. Culture Media 94
      (1) Fungi 94
      (2) Bacteria 95
      (3) Thermophilic *Actinomycetes* 95
      (4) Additional Media 95
   e. Blanks 96
      (1) Laboratory Media Blanks 96
      (2) Field Blanks 96

4. PREPARATION, IDENTIFICATION, AND ENUMERATION PROCEDURES FOR CULTURABLE BIOAEROSOLS 96
   a. Sample Preparation 96
   b. Enumeration 97
      (1) Total Concentration (Colony Forming Units per cubic meter) 97
      (2) Adjusted Concentration (CFU/m^3) 97
      (3) Limitations 98
   c. Identification of Culturable Bioaerosols 98
      (1) Classical or General Microbiology 98
      (2) Clinical and Environmental Microbiology 99
   d. Interpretation of Data 100

5. ADDITIONAL IDENTIFICATION AND ENUMERATION PROCEDURES FOR NONVIABLE OR VIABLE BIOAEROSOLS 101
- a. Microscopy 101
  - (1) Bright-Field or Light 101
  - (2) Phase Contrast 102
  - (3) Fluorescence 102
  - (4) Electron 102
- b. Endotoxin Assay 102
- c. Immunoassays 103
  - (1) Radioimmunoassays (RIA) 103
  - (2) Fluorescent Immunoassays (FIA) 104
  - (3) Enzyme Immunoassays (EIA) 104
- d. Gene Probes 104
  - (1) Nick-translated DNA Probes 105
  - (2) Synthetic Oligomer DNA Probes 105
  - (3) Polymerase Chain Reaction (PCR) 105
  - (4) Restriction Fragment Length Polymorphic (RFLP) Analysis 105

6. MANUFACTURERS 107

7. REFERENCES 109

---

‡Revision of Jensen PA, Lighthart B, Mohr AJ, Shaffer BT [1994]. Instrumentation used with microbial bioaerosol. In: Lighthart B, Mohr AJ, eds. Atmospheric microbial aerosols: theory and applications. New York, NY: Chapman & Hall, pp. 226-284.

1. INTRODUCTION

a. General

Bioaerosol monitoring is a rapidly emerging area of industrial hygiene. Bioaerosol monitoring includes the measurement of viable (culturable and nonculturable) and nonviable microorganisms in both indoor (e.g., industrial, office or residential) and outdoor (e.g., agricultural and general air quality) environments. In general, indoor bioaerosol sampling need not be performed if visible growth is observed. Monitoring for bioaerosols in the occupational environment is one of the many tools the industrial hygienist uses in the assessment of indoor environmental quality, infectious disease outbreaks, agricultural health, and clean rooms. Contamination (microbial growth on floors, walls, or ceilings, or in the HVAC system) should be remedied. If personnel remain symptomatic after remediation, air sampling may be appropriate, but the industrial hygienist should keep in mind that false negative results are quite possible and should be interpreted with caution. Other exceptions for which bioaerosol sampling may be appropriate include epidemiological investigations, research studies, or if situations indicated by an occupational physician and/or immunologist.

Sampling for fungi and bacteria (including *Actinomycetes*) is included in this chapter. Less developed methods for bioaerosols such as viruses, protozoa, antigenic fragments, algae, arthropods, and mycoplasmas are not addressed at this time.

b. Indoor and Outdoor Bioaerosols

In general, indoor microflora concentrations of a healthy work environment are lower than outdoor concentrations at the same location [ACGIH 1989, Step two; Macher et al. 1995]. If one or more genera are found indoors, in concentrations greater than outdoor concentrations, then the source of amplification must be found and remedied. Bioaerosol sampling is often performed out of doors for pollen and fungi to assist allergists in their treatment of patients by identifying taxa distribution and concentration in air over time. On occasion, outdoor bioaerosol sampling is conducted in an occupational environment (e.g., agricultural investigations and sewage treatment plants). Indoor bioaerosol sampling is often conducted in occupational (industrial and office environments) and nonoccupational (residential and educational buildings) settings. When sampling is indicated, it is advisable to sample before, during, and after the sampling area is occupied, including times when the heating, ventilating, and air conditioning system is activated and inactivated.

c. Viable and Nonviable Bioaerosols

Viable microorganisms are metabolically active (living) organisms with the potential to reproduce. Viable microorganisms may be defined in two subgroups: culturable and nonculturable. Culturable organisms reproduce under controlled conditions. Information regarding environmental conditions and media to culture microorganisms is shown in Sections 3.a. and 3.c. Nonculturable organisms do not reproduce in the laboratory because of intracellular stress or because the conditions (e.g., culture medium or incubation temperature) are not conducive to growth. As the name implies, viable bioaerosol sampling involves

collecting a bioaerosol and culturing the collected particulate. Only culturable microorganisms are enumerated and identified, thus leading to an underestimation of bioaerosol concentration.

Nonviable microorganisms are not living organisms; as such, they are not capable of reproduction. The bioaerosol is collected on a "greased" surface or a membrane filter. The microorganisms are then enumerated and identified using microscopy, classical microbiology, molecular biological, or immunochemical techniques. When sampling for culturable bacteria and fungi, the bioaerosol is generally collected by impaction onto the surface of a broad spectrum solid medium (agar), filtration through a membrane filter, or impingement into an isotonic liquid medium (water-based). Organisms collected by impaction onto an agar surface may be incubated for a short time, replica-plated (transferred) onto selective or differential media, and incubated at different temperatures for identification and enumeration of microorganisms [Tortora et al. 1989]. Impingement collection fluids are plated directly on agar, serially diluted and plated, or the entire volume of fluid is filtered through a membrane filter. The membrane filter is then placed on an agar surface and all colonies may be replica-plated. Culturable microorganisms may be identified or classified by using microscopy, classical microbiology, or molecular biology techniques such as restriction fragment length polymorphic (RFLP) analysis. Classical microbiology techniques include observation of growth characteristics; cellular or spore morphology; simple and differential staining; and biochemical, physiological, and nutritional testing for culturable bacteria. Analytical techniques which may be applied to both nonviable and viable microorganisms, but not distinguish between them, include polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). Such methods may be used to identify specific microorganisms and to locate areas of contamination. Though these latter methods are generally qualitative, current research efforts involve modifying the methods to obtain semi-quantitative or quantitative results.

2. PRINCIPLES OF BIOAEROSOL COLLECTION

a. General Principles

Most aerosol sampling devices involve techniques that separate particles from the air stream and collect them in or on a preselected medium. Impaction, filtration, and impingement are three common sampling techniques used to separate and collect the bioaerosol.

b. Impaction

Impaction is used to separate a particle from a gas stream based on the inertia of the particle. An impactor consists of a series of nozzles (circular- or slot-shaped) and a target. Perfect impactors have a "sharp cutoff" or step-function efficiency curve. Particles larger than a particular aerodynamic size will be impacted onto a collection surface while smaller particles proceed through the sampler [Hinds 1982]. Marple and Willeke [1976] have reported that high velocity, inlet losses, interstage losses, and particle reentrainment affect the performance characteristics of an impactor. The cut-diameter (d_{50}) is a function of the Stk_{50}. In other words, the mass of the particles smaller than the d_{50} that are collected equals the mass of the particles larger than the d_{50} that pass through the impactor. The collection efficiency of the

impactor approaches 100% when the aerodynamic diameter is greater than the d_{50} [Hinds 1982]. Aerodynamic diameter (d_{ae}) is defined as the diameter of a hypothetical sphere of unit density ($_p$ = 1 g/cm^3) that has the same settling velocity as the particle [Hinds 1982]. Impactors are selected so that the desired size particles will be collected. For the same aerosol sample, the mass and count particle distributions will have distinctive means and medians; however, they share the identical geometric standard deviation. The mass median aerodynamic diameter (MMAD) is descriptive of the mass distribution. In other words, the MMAD equals the diameter where particles larger than MMAD contribute half the collected mass; and those particles smaller than MMAD contribute the other half. The count median aerodynamic diameter (CMAD) is the median of the number of particles in a given particle distribution.

Investigators are likely to employ stationary cascade impactors or individual impactors used in survey instruments, either as the primary collection mechanism, or as a preclassifier (for example to remove nonrespirable particles from the sampled air stream). Cascade impactors consist of a stack of impaction stages: each stage consists of one or more nozzles and a target or substrate. The nozzles may take the form of holes or slots. The target may consist of a greased plate, filter material, or growth media (agar) contained in petri dishes. Each succeeding stage collects smaller particles. A filter may be used as the final stage so that all particles not impacted on the previous stages are collected. The target may be weighed to determine the collected mass, or it may be washed and the wash solution analyzed. Filters may induce more particle bounce than greased or oiled plates. Although personal cascade impactors are available, these devices are not as widely used in personal sampling for bioaerosols as are filters [Macher and Hansson 1987].

Impactors used for the collection of airborne microorganisms may have range from a single slit to more than 400 holes per stage. The particles impact onto growth medium with one or more bacterial or fungal colonies forming at some impaction sites. Multiple particles, each containing one or more organisms, passing through a single hole may be inaccurately counted as a single colony. As the number of organism-containing particles deposited onto the growth medium increases, the probability that the next organism-containing particle will impact a "clean" hole decreases. The basic formula for the coincidence correction follows:

$$P_r \qquad N \left[\frac{1}{N} \quad \frac{1}{(N\ 1)} \quad \frac{1}{(N\ 2)} \qquad\qquad \frac{1}{(N\ r\ 1)} \right]$$

Andersen [1958] and Leopold [1988] stated that $\mathbf{P_r}$ is the estimated culturable particle count given **r** culturable particles are observed on the sample plate, and **N** is the total number of holes per impactor stage. For example, if 75% of the holes have received one particle, the chance that the next particle will impact a "clean" hole is one in four (25%) [Andersen 1958; Andersen 1984; Leopold 1988; Macher 1989].

c. Filtration

Collection of particles from a nonbiological aerosol sample is most commonly achieved by filtration. Filter media are available in both fibrous (typically glass) and membranous forms. Deposition occurs when particles impact and are intercepted by the fibers or surface of filter

membranes [Hinds 1982]. Thus, particles smaller than the pore size may be efficiently collected. Sampling filter media may have pore sizes of 0.01 to 10 µm. The efficiency of removing particles from the air depends on the face velocity (i.e., the cross sectional air velocity of the filter holder). For particles less than 1 µm, the overall efficiency decreases with increasing face velocity [Liu et al. 1983; Lippmann 1995]. For particles greater than approximately 1 µm, the filter collection efficiency is greater than 99%. The overall efficiency of membrane filters is approximately 100% for particles larger than the pore size [Lippmann 1995].

Membrane filters are manufactured in a variety of pore sizes from polymers such as cellulose ester, polyvinyl chloride, and polycarbonate. Polymeric membrane filters lack rigidity and must be used with a support pad. The choice of a filter medium depends on the contaminant of interest and the requirements of the analytical technique. For gravimetric analysis, nonhygroscopic materials such as glass fibers, silver, or polyvinyl chloride membranes are selected. For analysis by microscopy, cellulose ester or polycarbonate membranes are the usual choices.

Filters are often held in disposable plastic filter cassettes during bioaerosol sampling. The three-piece cassette may be used either in open- or closed-face modes. Open-face sampling is performed by removing the end plug and the plastic cover from the three-piece cassette and is used when the particulate must be uniformly deposited (i.e., for microscopic analysis). If a three-piece cassette is used in the open-face arrangement, the plastic cover is retained to protect the filter after sampling is concluded. All plastic cassettes are securely assembled and sealed with a cellulose shrink band or tape around the seams of the cassette to prevent leakage past the filter.

Membrane filters for use in sampling are usually supplied as disks of 37- or 47-mm diameter. Because the pressure drop across a filter increases with the air velocity through the filter, the use of a larger (47-mm) filter results in a lower pressure drop for a given volumetric flow rate. The use of the smaller (37-mm) filter concentrates the deposit of the contaminant onto a smaller total area, thus increasing the density of particles per unit area of filter. This may be helpful for direct microscopic examination of low concentrations of organisms. In areas of high concentration, the microorganisms may have to be eluted, diluted, and then refiltered for microscopic analysis.

Filtration techniques are used for the collection of certain fungi and endospore-forming bacteria that are desiccation-resistant. The sampled organisms are washed from the surface of smooth-surface polycarbonate filters. The microorganisms in the wash solution are either cultured or refiltered to distribute the microorganisms uniformly on the membrane filter. In the latter case, the microorganisms are stained and examined microscopically [Wolf et al. 1959; Fields et al. 1974; Lundholm 1982; Palmgren et al. 1986a]. To culture the organisms, the membrane filter from each sampling cassette is washed with a 0.02% Tween™ 20 (J.T. Baker Chemical Co., Phillipsburg, NJ) in aqueous solution (three 2-mL washes), with agitation. Some of the recovered wash volume is serially diluted from full strength (1:10, 1:100, 1:1000) and 0.1 mL of each dilution is inoculated onto duplicate 100-mm x 15-mm petri dishes containing the appropriate medium. Residual culturable microorganisms on the membrane filter from each sampling cassette are counted by placing the filter on a medium

in a Petri dish to allow the microorganisms to colonize. The Petri dishes are incubated and the colonies are identified and enumerated [Muilenberg et al. 1992]. This method of serial dilution allows flexibility in dealing with unpredictable levels of spores by permitting a count of the spores collected on the filter either directly or by serial dilution of the wash solution. An inherent weakness in this procedure is that high analytical dilutions can statistically exclude taxa present in the air sample at low concentrations. This dilution technique favors the predominant fungi populations at the expense of minor populations.

d. Impingement

The liquid impingers are a special type of impactor. Impingers are useful for the collection of culturable aerosols [White et al. 1975; Lembke et al. 1981; Henningson et al. 1988]. Impingers such as the Greenberg-Smith impinger or the AGI-30 use a liquid (e.g., a simple salt solution such as 0.3 mM phosphate-buffered dilution water) as the collection medium. Additives to the collection medium such as proteins, antifoam, or antifreeze aid in resuscitation of bacterial cells, prevent foaming and loss of the collection fluid, and minimize injury to the cells. The jet is positioned a set distance above the impinger base and consists of a short piece of capillary tube designed to reduce cell injury when the air is dispersed through the liquid and the particles are entrapped. The Greenberg-Smith and AGI-30 samplers operate by drawing aerosols at nominal flow rates of 28.3 and 12.5 L/min, respectively, through an inlet tube [Macher et al. 1995]. The d_{50} of these samplers is approximately 0.3 µm [Wolf et al. 1959; Cown et al. 1957]. The AGI-30 inlet tube is curved to simulate particle collection in the nasal passage [Cox 1987]. This makes it especially useful for studying infectious airborne microorganisms by separating respirable (collection fluid) and nonrespirable (inlet tube) microorganisms. When the AGI-30 is used to recover total airborne organisms from the environment, the curved inlet tube is washed with a known amount of collecting fluid after sampling because larger particles (i.e., over 15 µm) are collected on the tube wall by inertial force. After sampling for the appropriate amount of time, 10 mL of the full-strength collection fluid is filtered through a 0.45-µm pore size membrane filter. In addition, serial dilutions of the remaining collection fluid are handled similarly [Greenberg et al. 1992]. The membrane filter is placed in a 100-mm by 15-mm sterile plastic petri plate filled with the appropriate medium and incubated for later identification and enumeration.

e. Characteristics of Several Bioaerosol Samplers

Once the purpose or the goal of bioaerosol sampling is determined, the appropriate sampling method(s) may be chosen. The selected bioaerosol sampler(s) must be capable of high efficiency particle collection within the physical and biological conditions required by the microorganism(s) to be sampled. Experimental, theoretical, and physical characteristics of several commonly used bioaerosol samplers are shown in Table I. The physical characteristics (flow rate, diameter of hole or width of slit, area of nozzle, and velocity of air through the nozzle) were used to calculate the theoretical cut-diameters of the listed samplers. The theoretical characteristics were discussed in the preceding subsections.

The particle size distribution of the bioaerosol is very important in the evaluation of the data obtained using the selected sampler. If the selected sampler does not provide particle size

distribution data, then a cascade impactor such as the Andersen 6-stage sampler also should be used. For example, if an SAS-Compact sampler was the selected sampler for collection of culturable *Escherichia coli*, an Andersen 6-Stage sampler should be used to determine the particle size distribution at each location sampled. However, a membrane filter sampler is not appropriate for sampling culturable *E. coli* because the cells would desiccate and become either nonviable or viable but not culturable under these conditions [Jensen et al. 1992]. In another example, an impactor with a d_{50} of 4 µm should not be used to collect *Aspergillus niger* spores (d_{ae} 1-3 µm) because most spores would remain entrained in the air passing through the instrument.

General guidelines for matching the appropriate sampler with the bioaerosol of interest are shown in Table II. The bioaerosol of interest categories include culturable bioaerosol sampling, and nonculturable and nonviable bioaerosol sampling. Subcategories include free bacteria (i.e., mostly single cells), free fungi (i.e., mostly single spores), and clumped bacteria and fungi with MMAD 4 µm. Culturable bioaerosol sampling instruments must minimize injury during the collection process and maintain the culturability of the collected microorganisms. Free bacteria and fungi are the bioaerosols of interest in some environmental investigations, and the sampler must collect these small aerosols [Wright et al. 1969; Lee et al. 1973]. Often, however, the bioaerosols will be clumps of microorganisms or microorganisms attached to another particle such as a skin scale or piece of lint. When using any culturable bioaerosol sampler, the investigator must select sampling time, considering estimated concentration, such that 30-100 colonies (up to 300 in some situations) develop per plate [Tortora et al. 1989]. The lower limit (30 colonies) is necessary to obtain sufficient statistical power for comparison purposes. However, when a clean room or other environment having extremely low levels of culturable bioaerosols is sampled, the lower limit of 30 colonies may not be achievable. In such a situation, a qualitative representation must be used without accommodation of statistical validity. The upper limit (100-300 colonies) is the maximum range in which one can easily count and differentiate colonies. When nonviable microorganisms are sampled or when culturability is not of concern, collection efficiency is the overriding concern. Table II is not an all inclusive listing of bioaerosol samplers. Investigators should take special note of the limitations listed at the bottom of the table.

Table I. Experimental, theoretical, and physical characteristics of several commonly used bioaerosol samplers.

Sampler		d_{50} True µm	d_{50} Theoretical µm	# holes	Q L/min	D_j or W_j mm	A_j mm²	U_j m/s
Andersen 6-Stage [Andersen 1958, 1984]								
	Stage 1	7.0	6.24	400	28.3	1.18	1.10	1.08
	Stage 2	4.7	4.21	400	28.3	0.914	0.656	1.80
	Stage 3	3.3	2.86	400	28.3	0.711	0.397	2.97
	Stage 4	2.1	1.84	400	28.3	0.533	0.223	5.28
	Stage 5	1.1	0.94	400	28.3	0.343	0.092	12.8
	Stage 6	0.65	0.58	400	28.3	0.254	0.051	23.3
Andersen 2-Stage [Phillips 1990]								
	Stage 0	8.0	6.28	200	28.3	1.50	1.77	1.33
	Stage 1	0.95	0.83	200	28.3	0.400	0.126	18.8
Andersen 1-Stage [Andersen 1958; Phillips 1990]								
	Stage N6	0.65	0.58	400	28.3	0.254	0.051	23.3
Mattson-Garvin Slit-to-Agar			0.53	1	28.3	0.152	6.23	75.7
Ace Glass All-Glass Impinger-30								
			0.30	1	12.5	1.00	0.785	265.
PBI Surface Air Sampler [Lach 1985]								
	Compact	2.0	1.97	219	90	1.00	0.785	8.72
	Standard	2.0	1.52	260	180	1.00	0.785	14.7
BIOTEST Reuter Centrifugal Sampler [BIOTEST undated; Macher and First 1983]								
	Standard	3.8	7.5		280			
	RCS-Plus							
Membrane Filter Samplers								
Burkard Spore Trap (1,7-Day)								
	Standard Nozzle		3.70	1	10	2.00	28.0	5.95
	High Efficiency Nozzle		2.17	1	10	2.00	10.0	16.7
Burkard (Personal) Sampler								
	Slit			1	10			
	Seive		4.18	100	20	1.00	0.785	1.94
Burkard May-Type Multi-Stage Impinger								
	Stage 1	10		1	20			
	Stage 2	4		1	20			
	Stage 3			1	20			
Allergenco MK-2				1				

Where:

d_{50} = Cut-diameter or aerodynamic diameter above which the collection efficiency of the impactor approaches 100%, both the true and the theoretical d_{50}s are shown;
Q = Airflow rate;
D_j or W_j = Diameter of seive or hole ***j***/Width of slit ***j***;
A_j = Area of hole ***j*** or slit ***j***; and
U_j = Velocity of air through hole ***j*** or slit ***j***.

Table II. General guidelines for matching the appropriate sampler with the bioaerosol of interest.

Sampler	**Culturable Bioaerosol Sampling**			**Non-Viable Bioaerosol Sampling**	
	Free Bacteria MMAD < 4 µm	Free Fungi MMAD < 4 µm	Clumped MMAD 4 µm	Bioaerosols MMAD < 4 µm	Bioaerosols MMAD 4 µm
Andersen 6-Stage	A	A	A		
Andersen 2-Stage	A	A	A		
Andersen 1-Stage	A	A	A,H		
Mattson-Garvin Slit-to-Agar	A	A	A		
Ace Glass All-Glass Impinger-30	C,D	C,D	D,E		
PBI Surface Air System					
Compact	G	G	B		
Standard	G	G	B		
BIOTEST Reuter Centrifugal Sampler					
Standard			B		
RCS-Plus			B		
Membrane Filter Samplers	C,F	C	C	C	C
Burkard Spore Trap (1,7-Day)					
Standard Nozzle					I
High-Efficiency Nozzle					
Burkard (Personal) Sampler					
Slit					
Seive			B		
Burkard May-Type Multi-Stage Impinger	C,D	C,D	E		
Allergenco MK-2					

= Satisfactory for specified application.

A = Concentrations greater than 5,000-7,000 CFU/m^3 will overload sample;

B = Concentrations greater than 1,000-2,000 CFU/m^3 will overload sample;

C = Good for very low to very high concentrations;

D = Bioaerosols may be reaerosolized and drawn out of the impingers during sampling, resulting in an underestimation of concentration and a decrease in precision;

E = May overestimate concentration due to breaking up of clumps;

F = For desiccation-resistant bacteria only;

G = May be acceptable with new sampling head being evaluated by PBI/Spiral Biotech;

H = May underestimate conc. of large bioaerosols (MMAD > 10 µm) due to impactor entry losses;

I = May underestimate concentration of small bioaerosols (MMAD 5 µm).

3. SAMPLING CONSIDERATIONS

a. Safety

Investigators should use appropriate personal protective equipment (PPE) and practice good personal hygiene when conducting indoor environmental quality, disease outbreaks, and agricultural health investigations that have resulted in medically diagnosed symptoms. PPE may include respiratory protection to prevent inhalation of microbes and microorganism-resistant clothing to prevent the transmission (bodily contact with microorganisms) to investigators. Good personal hygiene practices include washing exposed skin and clothing thoroughly and refraining from eating, drinking, or smoking in a contaminated area. These simple steps will help minimize the ingestion, inhalation, or uptake of microorganisms.

All samplers, culture plates, equipment, etc. should be handled aseptically to prevent contamination of the samplers and, more importantly, to prevent the spread of potential human pathogens to the worker or the work environment [CDC/NIH 1992; McKinney et al. 1991]. All surfaces, including washed hands, harbor microorganisms or spores unless they are specifically sterilized. Practically speaking, however, not all objects may be sterilized. While disinfection with an oxidizing chemical or alcohol destroys most vegetative cells, these agents do not destroy all spores. Samplers should be disinfected or, if possible, sterilized after each sample collection. Special care should be given to samplers with convoluted inlets or air pathways where microorganisms may accumulate.

b. Environmental Conditions

Temperature and relative humidity (RH) should be recorded over the sampling period. Airborne bacteria will dessicate (i.e., intracellular and extracellular water evaporate) when exposed to unstaurated air. The degree of cellular stress and rate of evaporation increase as relative humidity decreases and temperature increases [Marthi and Lighthart 1990]. In field experiments (greenhouse), survival of certain bacteria was 35- to 65-fold higher at 80% RH than at 40% [Walter et al. 1990]. In laboratory experiments, survival of certain bacteria was virtually complete at low RH but was reduced at RH values above 80% [Cox 1968]. Cox [1987] believes the potential for the movement of the solvent water is an important environmental criterion in assessing survivability of bacteria, viruses, and phages. Limited studies have been made of temperature effects. Temperature induces morphological changes in dimorphic fungi. For example, *Histoplasma capsulatum*, a pathogen, exists as a spore or mycelial form below 25 ^{o}C. However, higher temperatures have been shown to induce a transition from the mycelial form to the yeast form [Salvin 1949]. Like most particles, freshly generated microbial aerosols are nearly always electrostatically charged unless steps are taken to neutralize them. There is very little published information about electric charges on actual workplace aerosols, and even less on bioaerosols [Johnston et al. 1985]. In general, the effect of electrical charge has been overlooked, resulting in the possible bias of sampling results. At RH values above 70% RH, electrostatic phenomena are minimal [Hinds 1982; Cox 1987].

Sampling locations should be selected to assist in evaluation of your hypothesis. If you are evaluating worker exposure, then the samplers should be placed in inhabited areas where worker exposures may be measured. If you are evaluating contamination of a ventilation system, then sampling in the system and at the ventilation louvers would be appropriate. Care

must be exercised to ensure people do not tamper with the samplers and that you do not inadvertently aerosolize microorganisms on surfaces or in duct-work.

c. Flow Calibration

Accurate airflow rates are very important in calculating the concentration of microorganisms in the air. All samplers should be calibrated before and after sampling to ensure the flow rate is within the manufacturer's specifications and does not change from the initial calibration. Calibration may be performed using a primary standard such as a spirometer or bubble calibrator. Where it is not possible to calibrate using a primary standard, a secondary standard such as a dry gas meter may be used. The calibration of such a secondary standard should be traceable to a primary standard. See Chapter D. General Considerations for Sampling Airborne Contaminants of this manual for further details on calibration of airflow rates.

d. Culture Media

General detection and enumeration media are normally used in the collection of fungi, bacteria, and thermophilic *Actinomycetes*. Plates can be replicated on differential or selective media for identification after the organisms have been collected. In addition, it may be advisable to concurrently use more than one type of culture medium to collect aerosolized microorganisms, because of inherent biases caused by media selection. The following are some general guidelines for media:

(1) Fungi

Traditionally, malt extract agar (MEA) and rose bengal agar (RBA) have been recommended as broad spectrum media for the collection and enumeration of fungi [Morring et al. 1983; Burge et al. 1977; Smid et al. 1989]. MEA and RBA are generic terms and formulations vary from supplier to supplier and laboratory to laboratory. One MEA recipe is a less nutritious, unamended 2% MEA, which is reported to promote better sporulation than MEA amended with glucose and/or peptone [Hunter et al. 1988; Strachan et al. 1990]. With RBA, the colonies remain small; however, natural or artificial light may make the medium toxic to some fungi. In addition, pigmentation of the fungal growth on RBA complicates the identification process. Based on the work of researchers in Australia and Holland, dichloran glycerol 18 agar (DG-18) is recommended for identification and enumeration of fungi [Hocking and Pitt 1980; Pitt et al. 1983; Verhoeff et al. 1990]. This medium is adequate for most fungi, including xerophilic fungi. DG-18 does not have the disadvantages of RBA. To inhibit the growth of bacteria, antibiotics, such as streptomycin, may be added to the RBA medium [DIFCO 1984].

(2) Bacteria

Tryptic soy agar (TSA), casein soy peptone agar (CSPA) and nutrient agar (NA) are broad spectrum media for the collection and enumeration of bacteria. Special purpose media (i.e., selective media) are often used to select for specific microorganisms of interest. As with the media for fungi, chemicals may be added to the media which restrict growth of selected fungi and bacteria [DIFCO 1984].

(3) Thermophilic *Actinomycetes*

Thermophilic *Actinomycetes* is a special class of bacteria that has been associated with indoor environmental quality problems [Nevalainen 1989]. CSPA, TSA, and tryptone glucose yeast agar (also known as standard methods agar [SMA] and standard plate count agar [SPCA]) are broad spectrum media for the collection and enumeration of thermophilic *Actinomycetes* [DIFCO 1984; Amner et al. 1989; ACGIH 1989, Bacteria].

(4) Additional Media

Other media for the detection and enumeration of fungi and bacteria may be used. To discriminate for a general class of microorganisms by inhibiting or eliminating other microorganisms, a selective medium containing an antibiotic or other growth-restricting chemical may be used. To distinguish among species, a differential media may be used. Differential media contain indicators that permit the recognition of microorganisms with particular metabolic activities. Different incubation times or temperatures can also be used to get differential growth on the same medium. When a specific medium (i.e., selective or differential) is needed, the investigator(s) should refer to the most recent Manual of Clinical Microbiology published by the American Society for Microbiology, DIFCO Manual: Dehydrated Culture Media and Reagents for Microbiology published by DIFCO Laboratories, or the various catalogues published by the American *Type Culture* Collection (ATCC) [DIFCO 1984; Murray et al. 1995; Gherna et al. 1992; Jong and Edwards 1991].

e. Blanks

(1) Laboratory Media Blanks

Laboratory media blanks are unexposed, fresh media samples. These samples are generally not taken into the field. Before using any batch of media, incubate at least three culture plates under the same conditions as planned for the field samples, in order to check for sterility of the media. Approximately five media blanks should be included with each sample set. If the samples are to be analyzed by an outside

laboratory, consult the specific laboratory procedure for the number of blanks to be submitted.

(2) Field Blanks

Field blanks are simply unopened, fresh media samples that are handled in every way the same as field samples, including labeling, except that no air is drawn through the sampler. The recommended practice for the number of field blanks is to provide two field blanks for every 10 samples with a maximum of 10 field blanks for each sample set.

4. PREPARATION, IDENTIFICATION, AND ENUMERATION PROCEDURES FOR CULTURABLE BIOAEROSOLS

a. Sample Preparation

Inoculated agar plates are incubated at the appropriate temperature for times ranging from hours for a fast-growing bacterium to develop a microcolony; to days for a fungus to develop into a visible colony, and perhaps sporulate; to weeks for an organism such as drug resistant *Mycobacterium tuberculosis* to produce visible colonies [ATS 1990]. As a rule, plates are incubated at the temperatures shown in Table III [ACGIH 1989, Fungi and Bacteria; Baron and Finegold 1990].

Table III. Incubation temperatures and conditions for viable (culturable) microorganisms.

Fungi	25 °C or Room temperature with natural light
Bacteria, environmental	25 to 30 °C
Bacteria, human-source	35 to 37 °C
Bacteria, thermophilic *Actinomycetes*	50 to 56 °C

Laboratory media blanks and field media blanks must be handled in the same manner as samples.

b. Enumeration

(1) Total Concentration (Colony Forming Units Per Cubic Meter)

The total concentration of culturable microorganisms is calculated by dividing the volume of air sampled into the total number of colonies observed on the plate. A colony is a macroscopically visible growth of microorganisms on a solid culture medium. Concentrations of culturable bioaerosols normally are reported as colony forming units (CFU) per unit volume of air. CFU is the number of microorganisms that can replicate to form colonies, as determined by the number of colonies that develop.

(2) Adjusted Concentration (CFU/m^3)

Often, it is difficult to identify multiple colonies at one location on a plate because of the lack of differential colony morphology or because the chemicals secreted by one microorganism might inhibit the growth of other microorganisms at that same location [Burge et al. 1977]. In addition, some organisms produce large, spreading colonies while others produce microcolonies. Also, the morphology of the colony of one microorganism may completely obscure that of another, and a fast-grower might obscure a slow-grower. In these cases, a statistical adjustment of the observed number of colonies is needed to account for the probability that more than one particle impacted the same site [Andersen 1958; Leopold 1988; Macher 1989]. The adjusted concentration of culturable microorganisms is calculated by dividing the volume of

air sampled into the adjusted number of colonies observed on the plate (see section 2.b.).

(3) Limitations

Bioaerosol collection methods are "grab sample" techniques and, thus, represent only approximations of transient microbial concentrations in problematic atmospheres. Timely ascertainment of bioaerosol involvement is not possible, because of the time-dependent nature of the cultivation of samples and the subsequent enumeration of colonies.

The methods thus far pertain to culturable microorganisms. Microorganisms that are stressed or injured either by environmental conditions or bioaerosol sampling procedures may be viable, but not culturable [McFeters et al. 1982]. Certain species may be too fastidious to grow in laboratory culture. For instance, some bioaerosols (e.g., *Legionella pneumophila*, *Histoplasma capsulatum* or *Pneumocystis carinii*) are very difficult, if not impossible, to collect and culture [Ibach et al. 1954; Dennis 1990].

c. Identification of Culturable Bioaerosols

The science of classification, especially the classification of living forms, is called taxonomy. The objective of taxonomy is to classify living organisms to establish the relationship between one group of organisms and another, and to differentiate between them. Several criteria and methods for the classification of culturable microorganisms and the routine identification of some are discussed in the subsections that follow. Besides using these methods, the nonviable and nonculturable methods of identification discussed in Section 5 also may be used with culturable microorganisms.

(1) Classical or General Microbiology

Classical microbiology includes general methods for classifying or identifying microorganisms. The least specific of these is the observation of growth characteristics. Growth characteristics include the appearance of the microorganisms in liquid medium, colonial morphology on solid medium, and pigmentation.

On the cellular level of bacteria, cell shape, cell size, arrangement of cells, and presence (absence) of flagella, capsule, or endospores are characteristic of general classes of microorganisms. Simple and differential staining may be performed on bacteria. Simple staining is a method of staining microorganisms with a single basic dye that highlights cellular size, cellular shape, cellular arrangement, and presence (absence) of flagella, capsule, or endospore using a microscope. Stains such as methylene blue, carbolfuchsin, crystal violet, or safranin may be used for bacteria. A stain that distinguishes among structures or microorganisms based on reactions to the staining procedure is called a differential stain. Two examples of differential stains are the Gram stain and the acid-fast stain. The mechanism of the Gram stain may be explained on the basis of physical differences in the cell walls of these two general groups of bacteria (Gram-positive and Gram-negative). The Gram-positive bacteria possess a cell wall composed of a relatively

thick peptidoglycan layer and teichoic acids. Gram-negative bacteria possess a cell wall composed of a thin peptidoglycan layer and an outer membrane which consists of lipoproteins, lipopolysaccharides, and phospholipids [Tortora et al. 1989]. A few of the commercially available identification kits require a Gram-stain prescreening to assure that the correct reagents are used. Some species of bacteria, particularly those of the genus *Mycobacterium*, do not stain readily. In the acid-fast staining process, the application of heat facilitates the staining of the microorganism.

In general, fungi are classified by spore morphology or colonial morphology. Stains such as lactophenol cotton blue, periodic acid-Schiff stain, or potassium hydroxide (10% KOH) may be used.

Biochemical, physiological, and nutritional tests for bacteria evaluate cell wall constituents, pigment biochemicals, storage inclusions, antigens, temperature range and optimum, oxygen relationships, pH tolerance, osmotic tolerance, salt requirement and tolerance, antibiotic sensitivity, energy sources, carbon sources, nitrogen sources, fermentation products, and modes of metabolism (autotrophic, heterotrophic, fermentative, respiratory). As a rule, batteries of such tests, rather than any one individual test, are used to identify or classify microorganisms. A few commercially available test batteries are discussed in the following subsection.

Fungi are very difficult to classify [Smith 1990].

(2) Clinical and Environmental Microbiology

All identification systems should permit the efficient and reliable differentiation between microorganisms. Several modifications of classical biochemical procedures have been used in recent years to facilitate inoculation of media, to decrease the incubation time, to automate the procedure, and to systematize the determination of species based on reaction patterns. Historically, clinical microbiological techniques are used for analysis of environmental samples. However, clinical strains and environmental isolates may differ, requiring modification of clinically-based techniques.

(I) Biochemical Analyses

Several commercial multitest systems have been developed for identification of members of the family *Enterobacteriaceae* and other pathogenic microorganisms because of the high frequency of isolation of Gram-negative rods in clinical settings. These microorganisms are indistinguishable except for characteristics determined by detailed biochemical testing. These systems require that a pure culture be examined and characterized. Following is a listing of commercially available identification kits: API® 20E (Analytab Products, Plainview, NY); Enterotube II and R/B Enteric (Roche Diagnostics Systems, Nutley, NJ; Hoffmann-La Roche & Co., AG, Basel, Switzerland); Micro-ID (Organon Teknika-Cappel, Durham, NC); Minitek and Sceptor (BBL Microbiology Systems, Cockeysville, MD); and MicroScan (American Microscan, Inc., Sacramento, CA). Automated identification systems include Quantum II (Abbott Laboratories, North Chicago, IL), Autobac IDX (General

Diagnostics, Warner-Lambert Co., Morris Plains, NJ), and AutoMicrobic System (Vitek Systems, Inc., Hazelwood, MO). All ten of these multitest systems have documented accuracies greater than 90% in clinical settings [Baron and Finegold 1990; Koneman et al. 1988]. Biolog (Biolog, Inc., Hayward, CA) is one of the newest multitest systems on the market, but its application to environmental and clinical samples is not well documented [Amy et al. 1992; Miller and Rhoden 1991].

(ii) Cellular Fatty Acid Analysis

Cellular fatty acids (CFA) of bacteria are structural in nature, occurring in the cell membrane or cell wall of all bacteria. When the bacteria are grown under standardized growth conditions, the CFA profiles are reproducible within a genus, down to the subspecies or strain level in some microorganisms. The Microbial Identification System (MIS), developed by MIDI (Newark, DE), provides a chromatographic technique and software libraries capable of identifying various microorganisms based on their CFA composition [Sasser 1990a; Sasser 1990b]. The chromatographic technique is also known as gas chromatography fatty acid methyl ester analysis (GC-FAME). MIS has a database containing the analysis libraries for culturable Gram-negative and Gram-positive bacteria, and yeasts. In a comparison study [Amy et al. 1992], only 8 of 18 isolates, identified by either API multitest or MIDI MIS, were identified accurately using Biolog multitest. A prototype method for extracting and analyzing fungi is currently being distributed by MIDI.

d. Interpretation of Data

Generally speaking, the literature is divided on whether identification is necessary or recommended. If clinical or research aspects of the investigation would benefit by identification of the source of an etiologic agent, the following general guidelines are suggested:

Dose-response data are not available for most microorganism exposures. Indoor bioaerosol levels must be compared to outdoor levels or to an asymptomatic control area. In general, indoor levels are lower than outdoor levels, and the taxa are similar [ACGIH 1989, Step two, Fungi, and Bacteria; Solomon et al. 1980]. The Bioaerosol Committee of the American Conference of Governmental Industrial Hygienists (ACGIH) states that outdoor airborne fungi concentration "**routinely** exceeds 1000 CFU/m^3 and may average near 10,000 CFU/m^3 in summer months." No occupational exposure limit for bioaerosols has been promulgated by the Occupational Safety and Health Administration (OSHA). ACGIH [1989, Bacteria] also states that concentrations of less than 100 CFU/m^3 may be unhealthy to immunosuppressed people. However, the population of microorganisms must be evaluated for potential toxigenic microorganisms or microorganisms which emit volatile organic compounds. A low airborne concentration of microorganisms, in and of itself, does not indicate a clean and healthful environment.

Where local amplification and dissemination of bacteria have not occurred in an occupied, indoor environment, Gram-positive cocci (e.g., *Micrococcus* and *Staphylococcus*) are normally dominant [Morey et al. 1986]. ACGIH states that airborne human skin scales and respiratory secretions may contain Gram-positive cocci. Detection of high levels of these microorganisms are an indication of over-crowding and inadequate ventilation. Indoor air that tests high for Gram-negative bacteria

indicates a need to identify and eliminate the source of contamination. Concentrations ranging from 4,500-10,000 CFU/m^3 have been suggested as the upper limit for ubiquitous bacterial aerosols [Nevalainen 1989; ACGIH 1989, Bacteria]. These exposure limits, however, do not apply to pathogenic microorganisms.

Actinomycetes (mesophilic and thermophilic) are commonly found in agricultural areas. Their presence in indoor environments is an indicator of contamination [ACGIH 1989, Bacteria; Banaszak et al. 1970; Lacey and Crook 1988]. Thermophilic *Actinomycetes* at concentrations above 70 CFU/m^3 in an affected person's work area have been regarded as the threshold for triggering remedial action [Otten et al. 1986].

5. ADDITIONAL IDENTIFICATION AND ENUMERATION PROCEDURES FOR NONVIABLE OR VIABLE BIOAEROSOLS

Classifying nonviable and nonculturable microorganisms cannot be performed using the methods described in the previous section. Identification of nonviable or nonculturable microorganisms or components of microorganisms can be performed using microscopy and molecular biology techniques. In addition, microscopy techniques may be used for enumeration of suspensions of viable and nonviable microorganisms [McCrone 1973].

a. Microscopy

(1) Bright-Field or Light

In bright-field or light microscopy, an ordinary microscope is used for simple observation or sizing. Visible light from an incandescent source is used for illumination and the specimen appears against a bright backfield. Objects smaller than 0.2 µm cannot be resolved. The image contrast (visibility) decreases as the refractive index of the substance/microorganism under observation and the mounting medium become similar. To maximize the contrast, the mounting medium should have the same refractive index as glass or the immersion oil. Membrane filters are often "cleared" by using the appropriate immersion oil. This method is commonly used to observe various stained (killed) specimens and to count microorganisms. In addition, pollen grains and fungi spores are often identified and enumerated in this manner [Eduard et al. 1990].

(2) Phase Contrast

Phase-contrast microscopy is used when the microorganism under observation (e.g., *Escherichia coli*) is nearly invisible and an alternative mounting medium is not possible or permissible. A phase-contrast microscope uses a special condenser and diffraction plate to diffract light rays so that they are out of phase with one another. The specimen appears as different degrees of brightness and contrast. One cannot see an object exactly matching the refractive index of the mounting liquid; however, very slight differences produce visible images. This type of microscope is commonly used to provide detailed examination of the internal structures of living specimens; no staining is required.

(3) Fluorescence

Fluorescence microscopy uses ultraviolet or near-ultraviolet source of illumination that causes fluorescent compounds in a specimen to emit light. Fluorescence microscopy for the direct count of microorganisms has been described in a number of studies. Direct-count methods to enumerate microorganisms found in soil, aquatic, and food samples have been developed using acridine orange [Palmgren et al. 1986a, Palmgren et al. 1986b, Karlsson and Malmberg 1989]. More recently, this method was applied to airborne microorganisms and it was concluded that it is of the utmost importance to combine viable counts with total count enumeration in the study of microorganisms in work-related situations [Palmgren et al. 1986a].

(4) Electron

Electron microscopy uses a beam of electrons instead of light. Because of the shorter wavelength of electrons, structures smaller than 0.2 µm can be resolved. Scanning electron microscopy (SEM) is used to study the surface features of cells and viruses (usually magnified 1,000-10,000X); and the image produced appears three-dimensional. Also, SEM permits visualization of microorganisms and their structure (e.g., single spores or cells, clumps, chains, size, shape, or other morphological criteria). Viable microorganisms cannot be distinguished from nonviable microorganisms [Donham et al. 1986; Karlsson and Malmberg 1989]. Transmission electron microscopy is used to examine viruses or the internal ultrastructure in thin sections of cells (usually magnified 10,000-100,000X); and the image produced is not three dimensional.

b. Endotoxin Assay

A virulence factor possessed by all *Enterobacteriaceae* (as well as other Gram-negative bacteria) is the lipopolysaccharide, endotoxin, found in the outer membrane of the cell wall. Individuals may experience disseminated intravascular coagulopathy, respiratory tract problems, cellular and tissue injury, fever, and other debilitating problems. Amebocytes are carried "within the blood-like circulating fluid" of the *Limulus polyphemus* (horseshoe crab). After exposure to the lysed amebocyte cells, a liquid suspension containing trace levels of endotoxin (lipopolysaccharides) will gel. This test is called the *Limulus* amebocyte lysate (LAL) assay. Clinical microbiology laboratories use this assay to test for contamination by Gram-negative bacteria [Baron and Finegold 1990]. Airborne endotoxin has been found in high concentrations in agricultural, industrial, and office environments [Milton et al. 1990; Rylander and Vesterlund 1982]. Endotoxin aerosol measurement techniques lack comparability between results obtained in different laboratories because of differing sampling, extraction, and analytical methods (generically called the *Limulus* method) [Rylander and Vesterlund 1982; Olenchock et al. 1983; Jacobs 1989; Milton et al. 1990]. Concentrations of endotoxin, a lipopolysaccharide found in the cell wall of Gram-negative bacteria, determined using the LAL assay method, have been correlated with patient symptoms in very few studies [Rylander and Vesterlund 1982; Milton et al. 1990].

c. Immunoassays

The immunoassay is an analytical technique for measuring a targeted antigen, which is also referred to as an analyte. A critical component of the immunoassay is the antibody, which binds a specific antigen. The binding of antibody and targeted antigen forms the basis for immunoassay, and numerous formats have been devised which permit visual or instrumental measurements of this reaction. Antibodies are commonly employed to detect organisms by binding to antigens, usually proteins or polysaccharides, on the surface or "coats" of organisms. The analysis is usually performed in a complex matrix without the need for extensive sample cleanup. Many immunoassays are now readily available from commercial sources, permitting laboratories to rapidly develop in-house immunochemical analytical capability without lengthy antibody preparation. Some of the more widely used formats are as follows:

(1) Radioimmunoassays (RIA)

Radiolabelled antigen is quantitatively added to antibody along with various concentrations of unlabeled antigen. The unlabeled antigen competes with the radiolabelled antigen for binding to the antibody. Thus, the higher the concentration of unlabeled antigen in the sample, the lower the level of radiolabelled antigen-antibody complexes. The unbound antigens are removed prior to determining the amount of radiolabelled antigen-antibody formed. A standard curve is then constructed showing the effect of known amount of unlabeled antigen on the amount of radiolabelled antigen-antibody formed. It is now possible to determine the amount of an unknown, unlabeled antigen present in a sample by determining where the value is located on the standard curve [Garvey 1977]. Alternatively, radiolabelled antibody is employed.

(2) Fluorescent Immunoassays (FIA)

Utilization of fluorescent-labeled antibodies to detect bacterial antigens was introduced by Coons et al. [1941 and 1942]. Various FIA techniques have now evolved. These are referred to as: (1) direct FIA, to detect antigen (cell-bound) using fluorescent antibody; (2) indirect FIA, to detect antigen (cell-bound) using antibody and fluorescent antigamma globulin antibody; and (3) indirect FIA, to detect serum antibody using antigen, serum, and fluorescent antibody. Various fluorescent dyes, such as fluorescein, fluorescein isothiocyanate, and rhodamine isothiocyanate, may be employed. A fluorescent microscope is used to evaluate the samples and to count the number of fluorescent organisms [Garvey et al. 1977]. FIA is used to detect viruses and microorganisms.

(3) Enzyme Immunoassays (EIA)

The binding of an antibody or antigen to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase (AP), is the basis of EIA techniques. Enzymatic activity, in the presence of a chromogen, results in a colored end-product. Quantitation is performed using a spectrophotometer [Monroe 1984]. Many, if not most, commercially available EIAs are enzyme-linked immunosorbent assays (ELISAs). In this competitive-binding EIA, the antibody is coated onto the surfaces of test tubes, or wells of a microtiter plate, and antigen-containing samples and enzyme-linked antigen are added, resulting in a color change. The more intense the color, the less antigen or analyte are present in the sample. ELISAs can be qualitative or quantitative. A standard curve must be generated if quantitative results are

desired. ELISAs are now highly automated and efforts are underway to commercially develop well-standardized kits containing appropriate controls and materials.

d. Gene Probes

Diagnostic bacteriology, virology, and mycology are rapidly adapting molecular biology techniques in addition to classical identification methods to identify organisms. Thus, diagnostic assays utilizing nucleic acid or DNA probes have now been developed for the detection of numerous pathogenic organisms. Prior to employment of a DNA probe, it must first be demonstrated that the DNA probe is highly specific for the targeted organism. For example, a DNA probe for *Mycobacterium tuberculosis* (*Mtb*) should not detect other *Mycobacteria* species. However, it should detect all *Mtb* isolates. Described below are various types of DNA probes and formats used for the detection of organisms.

(1) Nick-translated DNA Probes

An isolated genomic DNA fragment is enzymatically disrupted and some of the DNA bases replaced with highly radioactive DNA bases [Sambrook et al. 1989]. The radioactive probe is now tested for its ability to bind (hybridize) the extracted DNA or RNA from the organism of interest. Prior to hybridization, the targeted DNA or RNA is either fixed to a membrane, microscope slide or resuspended in an aqueous buffer. After hybridization, the unbound DNA probe is then removed and the specimen DNA or RNA analyzed for bound DNA probe. Alternatively, nonradioactive labeling is often employed, but these probes, in general, are less sensitive than use of phosphorus-32 (^{32}P) labeled DNA [Goltz et al. 1990].

(2) Synthetic Oligomer DNA Probes

A short single-stranded DNA segment, usually 20-60 bases in length is designed and chemically synthesized. If the precise DNA sequence of the targeted organism is known, a complementary probe, representing a perfect match to the targeted DNA is designed. However, the precise sequence of the targeted DNA is often not known. Instead, the starting point for probe design is the amino acid sequence. In this situation, because of codon degeneracy, a single probe exhibiting exact complementarity cannot be designed. Thus, an educated guess, based on understanding the genetic code and codon usage, is used to design the probe. This type of probe usually exhibits a high degree of matching, although seldom is a perfect match achieved. Alternatively, a set or "family" of probes is synthesized. These are designed to cover all possible DNA sequences in the targeted organism. The probes are labeled, usually with ^{32}P, prior to hybridization experiments [Sambrook et al. 1989].

(3) Polymerase Chain Reaction (PCR)

First introduced in 1985, the PCR has revolutionized the way DNA analysis is conducted in clinical and research laboratories. Application of the PCR results in the amplification (the *in vitro* enzymatic synthesis of thousands of copies) of a targeted DNA [Saiki et al. 1985]. Two synthetic, single-stranded DNA segments, usually 18-25 bases in length, are bound to the targeted DNA. These serve as primers and permit the rapid enzymatic amplification of complementary DNA. The method is extremely sensitive and specific. Culturing of the

targeted organism prior to DNA extraction is often not necessary. This approach has been successfully utilized to detect various organisms including *Mtb* [Brisson-Noel et al. 1989; Wren et al. 1990; Eisenach et al. 1991].

(4) Restriction Fragment Length Polymorphic (RFLP) Analysis

RFLP is widely utilized to distinguish genetic changes within a species. A pure clone of each of the organisms of interest must be generated using standard culturing techniques. The genomic DNA is isolated and cut with a series of restriction enzymes. Each of these enzymes cut double-stranded DNA at a unique, short sequence of DNA bases, generating genomic DNA fragments of various sizes [Sambrook et al. 1989]. The DNA fragments are examined by sizing them on agarose gels. Eventually, the region(s) of altered DNA is detected. The fragment appears as a different size when compared to the other isolates. Confirmation may be accomplished by using gene probes as described above.

6. MANUFACTURERS

Impaction Samplers

Andersen 6-Stage, 2-Stage, and 1-Stage
Graseby Andersen
500 Technology Court
Smyrna, GA 30082-5211
(404) 319-9999
(800) 241-6898

SAS, and Compact SAS
Spiral Biotech, Inc.
7830 Old Georgetown Road
Bethesda, MD 20814
(301) 657-1620

Mattson-Garvin Slit-to-Agar
Barramundi Corporation
P.O. Drawer 4259
Homosassa Springs, FL 32647
(904) 628-0200

Cassella Slit Sampler
BGI Incorporated
58 Guinan Street
Waltham, MA 02154
(617) 891-9380

Reuter Centrifugal Sampler
BIOTEST Diagnostics Corp.
66 Ford Road, Suite 131
Denville, NJ 07834
(201) 625-1300
(800) 522-0090

Allergenco MK-2
Allergenco/Blewstone Press
P.O. Box 8571
Wainwright Station
San Antonio, TX 78208
(210) 822-4116

Aeroallergen Rotorod
Sampling Technologies, Inc.
26338 Esperanza Drive
Los Altos, CA 94022
(415) 941-1232

Volumetric Spore Traps (Indoor/Outdoor, 1- & 7-day; Personal)
Burkard Manufacturing Co. Ltd.
Woodcock Hill Industrial Estate
Rickmansworth, Hertfordshire WD3 1PJ
England
0923-773134

Filtration Samplers

Samplers and Supplies
Costar Nuclepore
One Alewife Center
Cambridge, MA 02140
(617) 868-6200
(800) 492-1110

Gelman Sciences Inc.
600 South Wagner Road
Ann Arbor, MI 48106
(313) 665-0651

Millipore Corporation
80 Ashby Road
Bedford, MA 01730
(617) 275-9200
(800) 225-1380

Impingement Samplers

All Glass Impinger-30 and -4 (AGI-30 & AGI-4)
Ace Glass Incorporated
P.O. Box 688
1430 Northwest Blvd.
Vineland, NJ 08360
(609) 692-3333

Multi-Stage Liquid Impinger (May)
Burkard Manufacturing Co. Ltd.
Woodcock Hill Industrial Estate
Rickmansworth, Hertfordshire WD3 1PJ
England
0923-773134

General Air Sampling Equipment Vendors

Industrial Hygiene News
Buyer's Guide
Circulation Department
8650 Babcock Blvd.
Pittsburgh, PA 15237
(412) 364-5366
(800) 245-3182

American Chemical Society
Environmental Buyer's Guide
1155 16th Street, NW
Washington, DC 20036
(202) 872-4600

General Molecular and Micro-Biology Equipment Vendors

American Biotechnology Laboratory
Buyers' Guide Edition and
American Laboratory
Buyers' Guide Edition
International Scientific Communications, Inc.
30 Controls Drive
P.O. Box 870
Shelton, CT 06484-0870
(203) 926-9310

BIO/TECHNOLOGY
International Buyers' Guide
Subscription Department
P.O. Box 1721
Riverton, NJ 08077-7321
(800) 524-0328

American Chemical Society
BIOTECH Buyers' Guide
Sales and Distribution Department
1155 16th Street, NW
Washington, DC 20036
(202) 872-4600

7. REFERENCES

ACGIH [1989]. Step two: On-site investigation, pp. 1-8; Fungi, pp. 1-10; Bacteria, pp. 1-7. Committee on Bioaerosols, eds. In: Guidelines for the assessment of bioaerosols in the indoor environment. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Amner W, Edwards C, McCarthy AJ [1989]. Improved medium for recovery and enumeration of the farmer's lung organism, *Saccharomonospora viridis*. Appl Environ Microbiol *55*(10):2669-2774.

Amy PS, Haldeman DL, Ringelberg D, Hall DH, Russell C [1992]. Comparison of identification systems for classification of bacteria isolated from water and endolithic habitats within the deep subsurface. Appl Environ Microbiol *58*(10):3367-3373.

Andersen AA [1958]. New sampler for the collection, sizing, and enumeration of viable airborne particles. J Bacteriol *76*:471-484.

Andersen Instruments, Inc. [1984]. Operating manual for Andersen sampler, Inc., viable (microbial) particle sizing sampler. Atlanta, GA: Andersen Instruments, Inc. (Currently Smyrna, GA: Graseby Andersen), TR# 76-00042, pp. 18-19.

American Thoracic Society (ATS) [1990]. Diagnostic standards and classification of tuberculosis. Am Rev Resp Dis *142*(3):725-735.

Banaszak EF, Thiede WH, Fink JN [1970]. Hypersensitivity pneumonitis due to contamination of an air conditioner. New Engl J Med *283*(6):271-276.

Baron EJ, Finegold SM [1990]. Bailey & Scott's diagnostic microbiology, 8th ed. St. Louis, MO: The C.V. Mosby Company, pp. 76, 79, 82, 100-126, 133.

Brisson-Noel A, Lecossier D, Nassif X, Gicquel B, Levy-Frebault V, Hance AJ [1989]. Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples. Lancet *No. 8671*:1069-1071.

Burge HP, Boise JR, Rutherford JA, Solomon WR [1977]. Comparative recoveries of airborne fungus spores by viable and non-viable modes of volumetric collection. Mycopathologia *61*(1):27-33.

CDC/NIH [1993]. Biosafety in microbiological and biomedical laboratories, 3rd ed. Washington, DC: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control; National Institutes of Health. HHS Publication No. (CDC) 93-8395.

Coons AH, Creech HJ, Jones RN [1941]. Immunological properties of an antigen containing a fluorescent group. Proc Soc Exp Biol Med *47*:200-202.

Coons AH, Creech HJ, Jones RN, Berliner E [1942]. The demonstration of pneumococcal antigen in tissues by use of fluorescent antibody. J Immunol *45*:159-170.

Cown WB, Kethley TW, Fincher EL [1957]. The critical-orifice liquid impinger as a sampler for bacterial aerosols. Appl Microbiol *5*:119-124.

Cox CS [1968]. The aerosol survival of *Escherichia coli B* in nitrogen, argon and helium atmospheres and the influence of relative humidity. J Gen Microbiol *50*(1):139-147.

Cox CS [1987]. The aerobiological pathway of microorganisms. New York, NY: John Wiley & Sons Ltd., pp. 4, 56, 73, 172-205.

Dennis PJL [1990]. An unnecessary risk: Legionnaires' disease. In: Morey PR, Feeley JC, Otten JA, eds. Biological contaminants in indoor environments. Philadelphia, PA: ASTM, STP1071, pp. 84-89.

DIFCO Laboratories [1984]. DIFCO manual: dehydrated culture media and reagents for microbiology, 10th ed. Detroit, MI: DIFCO Laboratories, Inc, pp. 9, 75, 565-566, 679-680, 749-752.

Donham KJ, Popendorf W, Palmgren U, Larsson L [1986]. Characterization of dusts collected from swine confinement buildings. Am J Ind Med *10*(3):294-297.

Eduard W, Lacey J, Karlsson K, Palmgren U, Ström G, Blomquist G [1990]. Evaluation of methods for enumerating microorganisms in filter samples from highly contaminated occupational environments. Am Ind Hyg Assoc J *51*(8):427-436.

Eisenach KD, Cave MD, Bates JH, Crawford JT [1990]. Polymerase chain reaction amplification of a repetitive DNA sequence specific for *Mycobacterium tuberculosis*. J Infect Dis *161*:977-981.

Fields ND, Oxborrow GS, Puleo JR, Herring CM [1974]. Evaluation of membrane filter field monitors for microbiological air sampling. Appl Microbiol *27*(3):517-520.

Garvey JS, Cremer NE, Sussdorf DH [1977]. Methods in immunology, 3rd ed. Reading, MA: W.A. Benjamin, Inc., pp. 87, 301-312, 431-434.

Gherna R, Pienta P, Cote R, eds. [1992]. Catalogue of bacteria and phages, 18th ed. Rockville, MD: American *Type Culture* Collection.

Goltz SP, Donegan JJ, Yang H-L, Pollice M, Todd JA, Molina MM, Victor J, Kelker N [1990]. The use of nonradioactive DNA probes for rapid diagnosis of sexually transmitted bacterial infections. In: Macario AJL, de Macario EC, eds. Gene probes for bacteria. San Diego, CA: Academic Press, Inc., pp. 1-44.

Greenberg AE, Clesceri LS, Eaton AD, eds. [1992]. Standard methods for the examination of water and waste water. 18th ed. Washington, DC: American Public Health Association. pp. 9-38 - 9-39.

Henningson EW, Fängmark I, Larsson E, Wikström LE [1988]. Collection efficiency of liquid samplers for microbiological aerosols. J Aerosol Sci *19*:911-914.

Hinds WC [1982]. Aerosol technology. New York, NY: John Wiley & Sons, Ltd., pp. 78, 104-126, 165.

Hocking AD, Pitt JI [1980]. Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods. Appl Environ Microbiol *39*:488-492.

Hunter CA, Grant C, Flannigan B, Bravery AF [1988]. Mould in buildings: the air spora of domestic dwellings. Int Biod *24*(2):81-101.

Ibach MJ, Larsh HW, Furcolow ML [1954]. Isolation of *Histoplasma capsulatum* from the air. Science *119*:71.

Jacobs RR [1989]. Airborne endotoxins: an association with occupational lung disease. Appl Ind Hyg *4*:50-56.

Jensen PA, Lighthart B, Mohr AJ, Shaffer BT [1994]. Instrumentation used with microbial bioaerosol. In: Lighthart B, Mohr AJ, eds. Atmospheric microbial aerosols: theory and applications. New York, NY: Chapman & Hall, pp. 226-284.

Jensen PA, Todd WF, Davis GN, Scarpino PV [1992]. Evaluation of eight bioaerosol samplers challenged with aerosols of free bacteria. Am Ind Hyg Assoc J *53*(10):660-667.

Johnston AM, Vincent JH, Jones AD [1985]. Measurements of electric charge for workplace aerosols. Ann Occup Hyg *29*(2):271-284.

Jong SC, Edwards MJ, eds. [1991]. Catalogue of filamentous fungi, 18th ed. Rockville, MD: American *Type Culture* Collection.

Karlsson K, Malmberg P [1989]. Characterization of exposure to molds and actinomycetes in agricultural dusts by scanning electron microscopy, fluorescence microscopy and the culture method. Scand J Work Environ Health *15*:353-359.

Koneman EW, Allen SD, Dowell VR, Janda WM, Sommers HM, Winn WC [1988]. Color atlas and textbook of diagnostic microbiology, 3rd ed. Philadelphia, PA: J.B. Lippincott Company, pp.136-143.

Lacey J, Crook B [1988]. Fungal and actinomycete spores as pollutants of the workplace and occupational allergens. Ann Occup Hyg *32*(4):515-533.

Lee RE, Harris K, Akland G [1973]. Relationship between viable bacteria and air pollution in an urban atmosphere. Am Ind Hyg Assoc J *34*(4):164-170.

Lembke LL, Kniseley RN, Nostrand RCV, Hale MD [1981]. Precision of the all-glass impinger and the Andersen microbial impactor for air sampling in solid-waste handling facilities. Appl Environ Microbiol *42*:222-225.

Leopold SS [1988]. "Positive hole" statistical adjustment for a two-stage, 200-hole-per-stage Andersen air sampler. Am Ind Hyg Assoc J *49*(2):A88-A90.

Lippmann M [1995]. Filters and filter holders. In: Cohen BS, Hering SV, eds. Air sampling instruments for evaluation of atmospheric contaminants, 8th ed. Cincinnati, OH: American Conference of Governmental Industrial Hygienists, Inc., pp. 247-279.

Liu BYH, Pui DYH, Rubow KL [1983]. Characteristics of air sampling filter media. In: Marple VA, Liu BYH, eds. Aerosols in the mining and industrial work environments, vol. 3, instrumentation. Ann Arbor, MI: Ann Arbor Science, pp. 989-1038.

Lundholm IM [1982]. Comparison of methods for quantitative determination of airborne bacteria and evaluation of total viable counts. Appl Environ Microbiol *44*:179-183.

Macher JM [1989]. Positive-hole correction of multiple-jet impactors for collecting viable microorganisms. Am Ind Hyg Assoc J *50*(11):561-568.

Macher JM, Chatigny MA, Burge HA [1995]. Sampling airborne microorganisms and aeroallergens. In: Cohen BS, Hering SV, eds. Air sampling instruments for evaluation of atmospheric contaminants, 8th ed. Cincinnati, OH: American Conference of Governmental Industrial Hygienists, Inc., pp. 589-617.

Macher JM, Hansson HC [1987]. Personal size-separating impactor for sampling microbiological aerosols. Am Ind Hyg Assoc J *48*(7):652-655.

Marple VA, Willeke K [1976]. Inertial impactors: theory, design and use. In: Liu BYH, ed. Fine particles: aerosol generation, measurement, sampling, and analysis. New York, NY: Academic Press, Inc., pp. 411-446.

Marthi B, Lighthart B [1990]. Effects of betaine on enumeration of airborne bacteria. Appl Environ Microbiol *56*(5):1286-1289.

McCrone WC, Delly JG [1973]. The particle atlas. Vol. II. 2nd ed. Ann Arbor, MI: Ann Arbor Science Publishers, pp. 342-345.

McFeters GA, Cameron SC, LeChevallier MW [1982]. Influence of diluents, media, and membrane filters on detection of injured waterborne coliform bacteria. Appl Environ Microbiol *43*(1):97-103.

McKinney RW, Barkley WE, Wedum AG [1991]. The hazard of infectious agents in microbiological laboratories. In: Block SS, ed. Disinfection, sterilization, and preservation, 4th ed. Philadelphia, PA: Lea and Febiger, pp. 749-756.

Miller JM, Rhoden DL [1991]. Preliminary evaluation of Biolog, a carbon source utilization method for bacterial identification. J Clin Microbiol *29*(6):1143-7.

Milton DK, Gere RJ, Feldman HA, Greaves IA [1990]. Endotoxin measurement: aerosol sampling and application of a new *Limulus* method. Am Ind Hyg Assoc J *51*(6):331-337.

Monroe D [1984]. Enzyme immunoassay. Anal Chem *56*(8):920A-931A.

Morey P, Otten J, Burge H, Chatigny M, Feeley J, LaForce FM, Peterson K [1986]. Airborne viable microorganisms in office environments: sampling protocol and analytical procedures. Appl Ind Hyg *1*:R19-R23.

Morring KL, Sorenson WG, Attfield MD [1983]. Sampling for airborne fungi: a statistical comparison of media. Am Ind Hyg Assoc J *44*(9):662-664.

Muilenberg ML, Sweet TM, Burge HA [1992]. A comparison of methods of estimating bacterial levels in machining oil mists (Abstract #20). In: Rogers C, Hall B, Ritchie J, eds. Aerobiology 1992. Toronto, Ontario, Canada: Symposium of the Pan-American Aerobiology Association.

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. [1995]. Manual of clinical microbiology, 6th ed. Washington, DC: American Society for Microbiology (ASM).

Nevalainen A [1989]. Bacterial aerosols in indoor air [dissertation]. Helsinki, Finland: National Public Health Institute, NPHI A3/1989, pp. 58, 66-69, 73.

Olenchock SA, Mull JC, Jones WG [1983]. Endotoxins in cotton: washing effects and size distribution. Am J Ind Med *4*:515-521.

Otten JA, Morey PR, Burge HA, Chatigny MA, Feeley JC, Peterson K [1986]. Airborne viable microorganisms in office environments: sampling protocol and analytical procedures. In: Proceedings of the 1986 EPA/APCA Symposium on Measurement of Toxic Air Pollutants, EPA Report No. 600/9-86-013. Raleigh, NC: U.S. EPA, Environmental Monitoring Systems Laboratory, Air Pollution Control Association, pp. 36-44.

Palmgren U, Ström G, Blomquist G, Malmberg P [1986a]. Collection of airborne micro-organisms on nuclepore filters, estimation and analysis - CAMNEA method. J Appl Bacteriol *61*:401-406.

Palmgren U, Ström G, Malmberg P, Blomquist G [1986b]. The nuclepore filter method: a technique for enumeration of viable and nonviable airborne microorganisms. Am J Ind Med *10*(3):325-327.

Pitt JI, Hocking AD, Glenn DR [1983]. An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. J Appl Bacteriol *54*:109-114.

Rylander R, Vesterlund J [1982]. Airborne endotoxins in various occupational environments. In: Back N, Brewer GJ, Eijsvoogel VP, Grover R, Hirschhorn L, Kety SS, Udenfriend S, Uhr JW, series eds. Progress in clinical and biological research. Vol. 93. New York, NY: Alan R. Liss, Inc., pp. 399-409.

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N [1985]. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science *230*:1350-1354.

Salvin SB [1949]. Cysteine and related compounds in the growth of the yeastlike phase of *Histoplasma capsulatum*. J Infect Dis *84*:275-283.

Sambrook J, Fritsch EF, Maniatis T [1989]. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp. 5.1-5.95, 10.1-10.70, 11.1-11.61, 14.1-14.35.

Sasser M [1990a]. Identification of bacteria through fatty acid analysis. In: Klement Z, Rudolph K, Sands DC, eds. Methods in phytobacteriology. Budapest, Hungary: Akadémiai Kiadó, pp. 199-204.

Sasser M [1990b]. Identification of bacteria by gas chromatography of cellular fatty acids. Newark, DE: Microbial Identification, Inc. (MIDI), Technical Note #101.

Smid T, Schokkin E, Boleij JSM, Heederik D [1989]. Enumeration of viable fungi in occupational environments: a comparison of samplers and media. Am Ind Hyg Assoc J *50*(5):235-239.

Solomon WR, Burge HP, Boise JR [1980]. Exclusion of particulate allergens by window air conditioners. J Allergy Clin Immunol *65*(4):305-308.

Smith EG [1990]. Sampling and identifying allergenic pollens and molds. San Antonio, TX: Blewstone Press, pp. 15, 41-49.

Strachan DP, Flannigan B, McCabe EM, McGarry F [1990]. Quantification of airborne moulds in the homes of children with and without wheeze. Thorax *45*(5):382-387.

Tortora GJ, Funke BR, Case CL [1989]. Microbiology - an introduction. 3rd ed. Redwood City, CA: The Benjamin/Cummings Publishing Company, Inc., pp. 72, 82-83, 162, 215-216.

Verhoeff AP, van Wijnen JH, Boleij JSM, Brunekreef B, van Reenen-Hoekstra ES, Samson RA [1990]. Enumeration and identification of airborne viable mould propagules in houses: a field comparison of selected techniques. Allergy *45*(4):275-284.

White LA, Hadley DJ, Davids DE, Naylor I [1975]. Improved large-volume sampler for the collection of bacterial cells from aerosol. Appl Microbiol *29*:335-339.

Wolf H, Skaliy P, Hall LB, Harris MM, Decker HM, Buchanan LM, Dahlgren CM [1959]. Public Health Monograph No. 60: sampling microbiological aerosols. U.S. Department of Health, Education, and Welfare, Public Health Service, pp. 2-3, 6, 18-19, 24.

Wren B, Clayton C, Tabaqchali S [1990]. Rapid identification of toxigenic *Clostridium difficile* by polymerase chain reaction (letters). Lancet *335*:423.

Walter MV, Marthi B, Fieland VP, Ganio LM [1990]. Effects of aerosolization on subsequent bacterial survival. Appl Environ Microbiol *56*(11):3468-3472.

Wright TJ, Greene VW, Paulus HJ [1969]. Viable microorganisms in an urban atmosphere. J Air Pollut Control Assoc *19*(5):337-341.