Methods for Aeroallergen Sampling

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Current Allergy and Asthma Reports 2004, **4**:376–383
Current Science Inc. ISSN 1529-7322

Air sampling provides information about the bioaerosol composition of the atmosphere. Principal methods of volumetric sample collection include impaction, impingement, and filtration. Many instruments have been developed based on these collection methods. The most widely used devices are slit impactors, rotating arm impactors, and sieve impactors. Samples can be analyzed by various methods, with microscopy and culturing the most important approaches; however, immunoassays, molecular methods such as polymerase chain reaction, and other new techniques are becoming more widely used to analyze samples.

Introduction

Air sampling has been used since the 19th century to examine the bioaerosol composition of the atmosphere. The early history of aerobiology and the development of air samplers have been thoroughly reviewed in several publications [1–5]. Aerobiological data have been used by physicians as well as scientists in many disciplines. Allergists have used aeroallergen information to aid in the diagnosis and treatment of patients and to determine pollen calendars for their geographic areas. The air sampling data have also been used in the medical community to determine the effects of allergen exposure on patient symptoms and to evaluate clinical trials. Plant pathologists utilize air sampling to study the dispersal of agriculturally important pathogens, and epidemiologists study human or animal pathogens. Air sampling is used to monitor the spread of genetically engineered microbes and pollen from genetically engineered crops in the natural environment. Paleoecologists, geologists, and archeologists use air sampling to understand the relationship between modern pollen deposition and modern plant communities as a guide to interpreting former plant communities using fossil pollen records. Recently, mycologists, industrial hygienists, and other indoor investigators have used air sampling data to evaluate exposure where indoor fungal amplification is evident or suspect.

A wide variety of sampling devices are in use today, and new methods and instruments are continually being developed. No single method is appropriate for all bioaerosols or for all applications. In addition, no standard protocols are available for many investigations. In this review, we describe several widely used samplers along with some new instruments and the analytical techniques used to study aeroallergens in both outdoor and indoor environments.

Principal Collection Methods

Airborne particles can be collected passively by gravity as well as with specific instruments that actively sample the atmosphere through impaction, impingement, filtration, or other methods that provide volumetric samples [2,6,7•,8–11]. The simplest, least expensive, but least accurate method of collecting airborne biological samples is through the use of gravity. This method often consists of exposing a coated microscope slide or open Petri dish containing agar (often called a settle plate) to the outdoor atmosphere or indoor air for a set period of time. Gravity sampling is nonquantitative for atmospheric concentrations of aeroallergens and is affected by particle size and shape and also by air movement. Gravity samples are biased toward larger and, therefore, heavier pollen and spore types. Consequently, small pollen, such as *Morus* and *Urtica*, and small spores, such as *Penicillium* and *Aspergillus*, will be underrepresented in the sample, although these four taxa are important allergens. This bias is well recognized, and gravity sampling is not recommended. Nevertheless, a mold test kit using a settle plate is sold commercially at home-improvement stores around the country. In addition, similar services are offered on the Internet by dozens of vendors.

The most widely used instruments for air sampling are impaction samplers. These samplers separate particles from the air stream by using the inertia of the particles; this causes deposition of the particles onto a solid or agar surface as the air stream bends to bypass the surface. The deflection of the air stream is achieved in both suction impactors and rotating arm impactors. A wide variety of impaction samplers are available for both outdoor and indoor sampling, including slit samplers for total spores and pollen, rotating arm impactors for total spores and pollen, and sieve samplers for culturable fungi (Table 1). Several of these are discussed in detail later. Analysis of the samples is generally done by microscopy or culturing.

Like impactors, impingement samplers separate particles from the air stream using inertia; however, the particles are deposited into a liquid collecting medium. Air is drawn in by a vacuum pump and bubbles through water or a dilute buffer. Particles from the air stream are dispersed into the

Table 1. Characteristics of commonly used impaction samplers

Collection method	Sampler	d_{50} (μm)	Comments
Slit impaction	Burkard spore traps (Burkard Manufacturing, Ltd, Rickmansworth, UK)	3.7	One-day and 7-day sampling heads available. Collects pollen and total spores.
	Lanzoni VPPS 2000 (Lanzoni, S.r.L., Bologna, Italy)		Wind oriented and allows time-discriminate sampling.
	Kraemer-Collins sampler (G R Electric, Manhattan , KS)		
	Burkard continuous recording sampler (Burkard Manufacturing, Ltd, Rickmansworth, UK)	5	Allows for time discrimination. Not wind oriented, so more suitable for indoor use.
	Allergenco MK-3 (Environmental Monitoring Systems, Charleston, SC)	2	Not wind oriented, so more suitable for indoor use. Programmable sampling and small particle efficiency.
	Burkard personal sampler (Burkard Manufacturing, Ltd, Rickmansworth, UK)	2.5	Single-grab sample for total spores and pollen. Battery operated.
	Air-O-Cell cassette (Zefon, St. Petersburg, FL)	2.6	Single-grab sample for total spores and pollen. Easy to sample and analyze.
	Cyclex-d (Environmental Monitoring Systems, Charleston, SC)	2	Single-grab sample for total spores and pollen.
Rotating-arm impaction	Rotorod sampler (Sampling Technologies/ Multidata, St. Louis Park, MN)	10	Independent of wind speed and direction. Easy to use and efficient for pollen sampling.
Culture plate impaction	Andersen 6 stage (Thermo Andersen, Franklin, MA)	7.0 for stage 1 to 0.65 for stage 6	Sieve impactor. Only for culturable fungi able to grow on medium used. Biocassette sampler is disposable N-6 sampler.
	Andersen 2 stage (Thermo Andersen, Franklin, MA)		
	Andersen 1 stage (N-6) (Thermo Andersen, Franklin, MA)		
	Aerotech 6 (Aerotech Laboratories, Phoenix, AZ)		
	Biocassette (Environmental Microbiology Labs, San Bruno, CA)		
	Burkard sampler for agar plates (Burkard Manufacturing, Rickmansworth, UK)	4	Sieve impactor. Only for culturable fungi able to grow on the medium used. Battery operated.
	SAS (Biotest Diagnostic Corp, Denville, NJ)	1.5 to 2.0	Sieve impactor. Only for culturable fungi able to grow on medium used.
	Biotest RCS (Bioscience International, Rockville, MD)	7.5	Centrifugal sampler with collection onto agar strips. Only for culturable fungi able to grow on medium used.

collecting fluid. Because the liquid will evaporate during prolonged sampling, and thus reduce sampling efficiency, most impingement samplers, such as the AGI-30, are only useful for short sampling periods of 1 hour or less [12]. However, the BioSampler (SKC, Eighty Four, PA) permits sampling for longer periods into nonevaporating fluids. Samples collected by impingers can be analyzed by a variety of methods, including microscopy, culture, biochemistry, immunochemistry, and molecular biology.

Filtration separates particles from the air stream by trapping them in a fibrous or porous substrate. Filtration samplers range from small, personal-cassette samplers worn by individuals to determine personal exposure to large, high-volume samplers that can process thousands of liters of air per hour [9]. Most applications use disposable plastic cassettes that hold filters from 25 to 47 mm in diameter. A wide range of filter material is available for use, depending on the type of bioaerosol to be trapped and the type of analysis desired. Collection efficiency is generally high but depends on filter pore size and flow rate. Also, loss of viability may occur during sampling due to dehydration [7•]. Like impingement samples, several analytical methods can be used for samples collected by filtration.

Other collection methods include electrostatic precipitation, thermal precipitation, and cyclone sampling [10], although these are not widely used compared with the methods previously described. In electrostatic precipitators or ionizers, particles are first charged and then attracted to an opposite charge on a collector plate in the sampler. This device is best suited for small particles. In a recent study, an ionizer was used for monitoring Fel d 1 cat allergen in homes and daycare centers [13]. The drawback pointed out by the authors of this study was the inability to quantify the amount of allergen per volume of air; results were expressed as allergen collected per 24 hours.

Sampler Performance

Sampler efficiency is based on the ability to capture the particles onto a collection surface or into a collection medium. Both physical and biological aspects are involved in the efficiency. Physical aspects include the size and shape of sampler inlet and the airflow rate, which are used to determine the d_{50}, often referred to as the cut size. This is the particle diameter at which 50% of the particles are collected. Because of a sharp cut-off, it is generally accepted that all larger particles are collected [7•]. For example, a d_{50} of 5 μm means that sampler efficiency drops significantly for particles smaller than 5 μm. The d_{50} values for several samplers are listed in Table 1. Wind velocity and direction also effect sampler performance. Biological aspects of efficiency relate to loss of viability due to sampling stress and are only important for samples analyzed by culture.

Widely Used Sampling Instruments

No single sampler is appropriate for all applications, and investigators must select the sampler type and method of analysis carefully, based on the type of data to be collected. Various samplers and methods have been reviewed and compared in previous publications [2,5,6,7•,8,10,11,14,15•], and these should be consulted in conjunction with the information herein. The major emphasis here is on impaction samplers.

Spore trap slit impactors

The Burkard spore trap (Burkard, Hertfordshire, England) is a suction slit impactor used for pollen and spore sampling. The first sampler of this type was designed by Hirst in 1952 [16]. In addition to the Burkard spore trap, other samplers based on the Hirst trap design include the Lanzoni (S.r.l., Bologna, Italy) sampler and the Kramer-Collins (G R Electric, Manhattan, KS) sampler (Table 1). Also, the slit orifice based on the Hirst sampler is the basis for the orifice design in the Burkard personal sampler, the Allergenco MK-3 (Environmental Monitoring Systems, Charleston, SC), Air-O-Cell sampling cassettes (Zefon, St. Petersburg, FL), and others.

In the Burkard spore trap, air is drawn into the 14 mm × 2 mm orifice at 10 L/min, and airborne particles with sufficient inertia are impacted on either tape or a microscope slide beneath the orifice. The impaction surface moves past the orifice at 2 mm/hr, permitting time-discriminate sampling. A wind vane is attached to the sampler head, which is able to rotate. This ensures that the orifice is always oriented into the wind. The standard orifice on the Burkard sampler is efficient for particles down to 3.7 μm; this means that all but the smallest spores will be efficiently trapped. An interchangeable orifice is available from the manufacturer for increasing trapping efficiencies for spores as small as 1 μm. The alternative orifice is 14 mm × 2 mm at the intake but tapers down to 14 mm × 0.5 mm.

Two sampling lids are available for the Burkard spore trap—the standard 7-day lid and an alternate 24-hour lid. In the 7-day lid, a metal drum is mounted on a clock attached to the lid. The clock causes the drum to make a complete revolution in 7 days (at 2 mm/hr). A strip of clear cellophane tape is fixed on the drum and held in place with a small piece of double-stick tape. The cellophane tape is lightly coated with an adhesive such as Lubriseal (Thomas, Swedesboro, NJ), silicone grease, petroleum jelly, or high-vacuum grease. The drum is changed weekly, and the tape is removed and cut into seven 48-mm pieces, representing the previous 7 days. The daily tape segments are affixed to microscope slides; a mounting medium, such as glycerin jelly containing basic fuchsin, and a cover slip are added.

In the alternate lid assembly, a slide-holding carriage is attached to the clock. A standard 25 mm × 75 mm microscope slide is coated with an adhesive and placed in the carriage that moves past the orifice. The slide is changed daily and carriage re-oriented at start position. The exposed slide is stained as described earlier for the daily tape segments. This lid assembly is widely used by allergists and other scientists who need bioaerosol data on a daily basis. Slides from either lid are examined with a compound microscope for spore identification and enumeration as described later.

Portable spore traps

Various types of portable spore traps are used for indoor sampling as well as for some outdoor applications. These include the Allergenco (Samplair) MK-3 and the Burkard continuous recording air sampler, which allow for time-discriminate sampling. The Allergenco was initially designed for outdoor use; however, the lack of wind orientation makes this instrument more suitable for indoor sampling. A programmable step mechanism allows the sampler to be programmed to take up to 24 discrete samples on a single microscope slide. The Burkard continuous air sampler is similar in operation, although the programming is not as versatile.

Several spore trap impactors collect a single air sample over a 1- to 15-minute period; these are often called grab samplers. They are commonly used for indoor air sampling because of their portability and ease of use. The Burkard personal sampler impacts airborne particles onto a standard coated microscope slide. The sampler flow rate is 10 L/min, and the orifice is 14 mm × 1 mm. This sampler is efficient for spores down to 2.52 μm in diameter [7•]. The Air-O-Cell cassette is a disposable spore trap manufactured by Zefon International. The intake orifice is similar in design to the Burkard sampler, tapering to a 14.4 mm × 1.1 mm slit. The cassette can be attached to any pump capable of drawing 15 L/min and has an efficiency down to 2.3 μm [7•]. Particles impact on a small adhesive-coated piece of glass within the cassette. Although the cassettes are individually expensive, they are convenient to use and easy to analyze. Cyclex-d (WSLH, Madison, WI) sampling cassettes are similar; however, instead of a slit orifice, there is a single, round-jet orifice 4 mm in diameter. The cassettes are attached to a pump drawing 20 L/min. All of the portable spore traps are analyzed by microscopy.

Rotating arm impactors

Rotorod samplers (Sampling Technologies, Minnetonka, MN) have been widely used by investigators in the allergy community. The first rotating impactor of this type was developed in the 1950s, and within the next decade various modifications of this instrument were developed [17]. These samplers contained a small battery-driven motor that rotated the sampling head at 2400 rpm; airborne particles are collected by adhesive-coated rods, bars, slides, or tape attached to the rotating component. On current and previous models, sampling rate is approximately 120 L of air per minute. As a result, these samplers are volumetric, and the average atmospheric concentration of the pollen and spores can be determined. The samplers can be run continuously for short periods of time or intermittently for longer periods. The Rotorod samplers that are currently available are intermittent samplers usually run for 30 to 60 seconds out of every 10 minutes (5% to 10% sampling time). Airborne particulates are collected onto two small plastic retractable "I" rods; the exposed area of each rod is 1.52 mm by 23 mm. When the instrument cycles on, the rods drop down as the arm begins rotating. The leading edge of the rods is coated with an adhesive, usually silicon grease. The rods are changed each day, placed in a specially designed plastic microscope slide with grooves to hold the rods, and stained with Calberla's stain. The rods are then examined with a compound microscope for pollen and spore identification and enumeration. This sampler is easy to use and is relatively efficient for pollen and large fungal spores. Unfortunately, sampler efficiency drops for particles below 10 μm [18]. This means that many small spores, especially basidiospores and small ascospores, will be significantly underrepresented in the total catch. Also, sampler efficiency decreases over time, with increasing numbers of particles, causing overloading of the exposed side of the rods. For areas where high concentrations of pollen and spores are common, 5% sampling times, or even less, should be used to avoid overload.

Sieve impactors

Culture-plate samplers impact airborne particles directly onto the surface of culture medium in a Petri dish. These samplers are used for airborne fungi and bacteria in both outdoor and indoor environments. These are often sieve impactors, with multiple holes that deposit the catch over the surface of the plate. The original sampler of this type is the Andersen six-stage cascade impactor (Thermo Andersen, Franklin, MA). Each stage has a perforated plate composed of 400 holes with decreasing diameter. The holes in the first stage have a diameter of 1.18 mm, whereas the holes in the bottom plate are 0.25 mm in diameter [10]. Size discrimination is possible as the air velocity increases through the smaller holes. One-stage and two-stage models of the Andersen sampler are widely used. In the two-stage sampler, only the second and fifth stages are used, and each stage has 200 holes. In the single-stage model, only the sixth stage (N-6) is used with 400 holes in the plate. The single-stage sampler is extensively used in indoor air investigations. Other manufacturers offer very similar samplers. Recently, a disposable sieve impactor, the Biocassette (Environmental Microbiology Labs, San Bruno, CA), has been developed. Studies showed no statistical difference between the mean of samples collected with the Biocassette and with the single-stage Andersen sampler [19]. The Burkard culture plate sampler is a portable sieve impactor with 100 holes.

Sampling times for sieve impactors are normally for 1 to 5 minutes at a flow rate of 28.3 L/min, although the Burkard model uses a lower flow rate. Following sampling, the Petri dishes are incubated, and the resulting colonies are counted and identified. Concentrations are expressed as colony forming units (CFU)/m^3 of air. With any sieve impactor, there is a possibility of multiple impactions on the agar beneath a single hole; however, these would appear and be counted as a single colony. The possibility of multiple impactions increases with increasing concentration of culturable organisms.

Personal samplers

Personal samplers are used to determine more precise levels of exposure to aeroallergens or other airborne particles. These samplers are also widely used in the workplace for testing compliance with permissible exposure limits. Although some personal samplers are passive samplers, they often consist of a small, disposable filter cassette worn in the breathing zone (usually on a lapel) attached to a lightweight, battery-powered pump worn at the waist. The flow rate is usually approximately 2 L/min, and it is worn for many hours. Several types of filter membranes can be used along with various methods of analysis [20]. The Button aerosol sampler is a reusable filter sampler with a porous, curved inlet that improves particle collection over the surface of the filter [21]. Because of the spherical inlet, this sampler has been shown to be wind insensitive. As a result, it is suitable for both indoor and outdoor environments for a variety of applications [22].

The nasal air sampler was recently developed to obtain a more precise personal exposure than current filters [23–25]. This novel personal sampler is an impaction sampler worn just inside the nose and requires no outside power source because it uses normal human respiration to impact airborne particles onto a pressure-sensitive adhesive tape. Particles that are 5 μm and larger are collected with minimal discomfort to subjects. This sampler has been used to measure inhaled pollen and spores, dust mite allergen, and other allergens with analysis by microscopy or immunochemistry [23–25].

Methods of Analysis

Air samples collected by the instruments described here can be analyzed by various methods, based on the type of sample and the information desired. The main methods of analysis for air samples include culture, direct microscopy, biochem-

Table 2. Methods of analysis for air samples

Method	Sampler types used with	Comments
Microscopy	Slit impactors, liquid impingers, filter samples	Pollen and total spores identification. Does not permit species identification for similar spore or pollen types.
Culturing	Sieve impactors, liquid impingers, filter samples	Allows for species identification. Only for viable organisms able to grow on culture medium used.
Biochemistry	Slit impactors, liquid impingers, filter samples	Estimate of total fungal biomass or identification of specific mycotoxins.
Immunochemistry	Slit impactors, liquid impingers, filter samples	Specific assays for allergens. Limited number of allergen assays commercially available.
Molecular biology (PCR)	Slit impactors, liquid impingers, filter samples	Detects specific DNA sequences. Eliminates the need for culturing or microscopy. Thus far, limited use in aeroallergen sampling.
Flow cytometry	Liquid impingers	Rapid analysis for quantifying total spores or pollen. Limited use in aerobiology.
Image analysis	Slit impactors, liquid impingers	Rapid analysis of spore and pollen identification. Eliminates the need for microscopy. Early stage of development for this methodology.

istry, immunochemistry, and molecular biology (Table 2). Flow cytometry and image analysis are also finding applications in air sample analysis. Culturing and microscopy are the most commonly used methods.

Culturing

Culturing is, of course, required for samples from Andersen samplers, other sieve impactors, and slit samplers used to collect fungal spores directly onto a culture plate. Samples collected by impingers and filter samplers can also be analyzed by culturing. Regardless of the type of sample, culturing is only useful for fungal spores that can germinate and grow on the culture medium utilized. A broad-based culture medium, such as malt-extract agar, is often suggested for mesophilic fungi. DG-18 agar, which contains dichloran and 18% glycerol, is recommended for xerophilic fungi. Both media are considered acceptable for environmental sampling [11,26]. Incubation is normally at room temperature for 5 to 10 days, although the incubation temperature and time, as well as the type of culture medium, may vary for different applications. Identification of fungal colonies usually depends on microscopic characteristics of reproductive structures and methods of spore development. As indicated earlier, multiple impactions at one spot can occur when using sieve impactors, and corrections to the count should be made to account for this possibility. Correction tables are usually available from the manufacturer and can be found in other publications [14]. The advantage of culture analysis is that fungi can be accurately identified to the species level, provided personnel are trained in fungal taxonomy. The disadvantage is that only a fraction of the airborne fungal spores will grow on the culture medium used. An air sample will normally contain a heterogeneous mixture of viable spores, spores that have lost viability, spores that cannot be grown in culture, and fastidious spores that have specific nutrient requirements. Although many of these spores are not culturable, they may still have allergenic properties.

Microscopy

Samples collected by spore-trap samplers or Rotorod samplers are usually analyzed by direct microscopy. Microscopy can also be used for the analysis of impingement and filter samples. This is the most important method of analysis for outdoor samples that contain a mixture of pollen and fungal spores. Outdoor samples are typically stained with basic fuchsin or phenosafranin to aid in pollen identification. Samples can be examined almost immediately without lengthy incubation periods. Another advantage is that microscopy permits the enumeration of all spores, culturable as well as nonculturable. However, there are several disadvantages. Some spores cannot be identified, especially small, spherical spores that lack distinctive morphologic features. Also, species identification is not possible, and, therefore, spores are usually identified to the generic level. Other spores can only be identified to a general group. For example, species of *Penicillium* and *Aspergillus* cannot be distinguished from each other and are usually categorized as *Penicillium*/*Aspergillus*–type spores. This is especially a disadvantage for indoor samples because *Penicillium* and *Aspergillus* are common indoor contaminants. Analysis by microscopy takes considerable time and requires trained personnel to identify the many types of spores and pollen common in air samples.

A magnification of 400× is normally used for pollen identification and enumeration. Fungal spores are generally analyzed with an oil immersion objective at a total magnification of 1000×. As a result, each sample is analyzed twice. For Rotorod samples, the entire sample on each rod is usually

analyzed. The exposed portion of each slide from a Burkard spore-trap sample is 14 mm × 48 mm, and the most accurate analysis would involve counting the entire sample. However, this is usually not possible because of the time required. As a result, a subset of the sample is analyzed. There are several methods in use for the microscopic analysis of slides from a Burkard spore trap. The most common methods involve one to four longitudinal traverses (down the 48 mm long axis of the sample) or 12 transverse traverses (across the 14 mm short axis of the sample). Because the slide carriage or sampler-drum with attached tape moves at 2 mm/hr by the intake orifice, analysis of the slide at 4-mm intervals (12 transverse traverses) can provide information on the concentrations every 2 hours during the day. The resulting data show the diurnal rhythm of airborne pollen and spores. The longitudinal traverses provide information on the average daily concentration. The accuracy of these counting methods has been reviewed in several studies [27–29]. None of the methods was equivalent to counting the entire slide, but the 12 transverse traverses gave slightly better approximations. These studies indicate that the usual methods of analysis for Burkard slides provide good indicators of the aeroallergen concentrations, but these should not be considered as absolute values.

Biochemistry

Biochemical analyses have been used as indicators of microbial presence as well as for the identification of specific compounds, usually from samples collected by an impingement sampler or by filtration. General compounds, such as ergosterol, β-glucans, or endotoxin, are detected by biochemistry. Ergosterol is sterol occurring in fungal cell membranes, and β-glucan is a carbohydrate that occurs in fungal wall. Both assays provide an estimate of total fungal biomass but are not specific for any genus or species. Ergosterol assays have been recently used to measure exposure to indoor mold in several studies [30,31]. β-glucan assays are less specific because other sources of β-glucan may be present [31,32]. Endotoxins are lipopolysaccharides found in the cell walls of gram-negative bacteria. Specific mycotoxins have also been identified by biochemical analyses from air samples. Mycotoxins from *Stachybotrys chartarum* have been detected on filter samples in both experimental conditions and in contaminated environments [33,34]. Also, ochratoxin A was detected from airborne conidia of *Penicillium verrucosum* isolated from a cowshed [35]. Different methods have been used from filter washings for identifying these compounds. Ergosterol and mycotoxins have been identified through HPLC, whereas *Limulus* amoebocyte lysate assay is usually used for endotoxin and β-glucan analysis. However, β-glucans can also be identified through an enzyme-linked immunoassay.

Immunochemistry

Air samples can also be analyzed by immunoassays for specific allergen molecules. As a result, these assays are not useful for routine analysis of air samples. These techniques are usually applied to filter or impingement samples; however, they can also be used with spore-trap samples. Immunoassays involve the binding of antibodies to the allergen of interest; therefore, they require the prior development of antibodies. Because of their greater specificity, monoclonal antibodies are usually preferred. Antibody binding is usually detected by linking a fluorescent dye, an enzyme, or radioactive label to the antibody.

A number of commercial immunoassays are available for several dust mite allergens, two cockroach allergens, and single allergens for cat, dog, mouse, rat, and horse. Although applicable for air samples, many of these have greater application for dust samples. For fungal analyses, immunoassays are currently available for *Alternaria alternata* (Alt a 1 allergen) and *Aspergillus fumigatus* (Asp f 1 allergen). Although several investigators have developed Alt a 1 assays, these have largely been used to quantify the amount of Alt a 1 allergen in commercial allergy extracts and in dust samples [36]. The advantages of immunoassays are their reported specificity, sensitivity, and ease of use, once the antibodies are available. The disadvantages involve the expense and time for the initial research effort required in developing the assay and equipment costs. In addition, recent studies showed the limitations of this technique. Low levels of Asp f 1 allergen were detected in only two out of 120 air samples from office environments, including areas where culturable *A. fumigatus* colonies were isolated from dust. Also, no Asp f 1 allergen was isolated from dust samples in these locations [37]. It is possible that Asp f 1 is not expressed in dormant spores collected in air or dust samples; spore germination may be necessary before this allergen can be detected. In another study, monoclonal antibodies were produced to detect *P. brevicompactum* spores from air samples in an experimental setting. Five monoclonal antibodies were produced, but all of them cross-reacted with several species of *Aspergillus*, *Penicillium*, and *Eurotium*. This would indicate that positive results obtained from an air sample with a cross-reacting monoclonal antibody could be due to various combinations of fungi in the sample [38]. Similar problems were found in attempting to develop air sample immunoassays for plant pathogenic fungi [39]. Clearly, for these immunoassays to work, greater focus must be placed on developing species-specific antibodies.

Molecular biology

Air samples from spore traps, rotating arm impactors, impingers, and filtration have also been analyzed by polymerase chain reaction (PCR). PCR is a method used to rapidly produce multiple copies of specific DNA sequences; this technique has been applied to many areas of research to improve detection of various organisms, including airborne fungal spores. Wakefield [40] identified *Pneumocystis carinii* from air samples collected in an orchard by three different types of air samplers, including a Burkard spore trap, a liquid impinger, and a cascade impactor. PCR was also used to identify plant pathogenic fungal spores from two

different species collected by a spore trap [41•]. *Penicillium roqueforti* spores were identified using samples from a spore trap, a rotating arm impactor, and a cyclone sampler [39,42]. Haugland *et al.* [43] described the detection of *Stachybotrys* spores with PCR from samples collected by a liquid impinger. Also, a system using fungus-specific primers and PCR has been developed to estimate total fungal biomass in an environment [44]. The expanding use of this technique suggests that PCR may one day become a standard method for routine monitoring of aeroallergens. The advantage of this method is the ability to detect organisms that cannot be easily grown in culture and lack distinctive spores for microscopic identification. The speed of identification with this method is also an advantage, even for organisms that can be grown in culture. However, it is known that allergens can also be carried on small particles released from pollen grains or on fungal fragments from the break-up of fungal hyphae [45]. These allergens may occur in the atmosphere in the absence of DNA and would not be detected by this technique.

Flow cytometry

Flow cytometry involves the analysis of a suspension of cells that are autofluorescent or that have been treated with fluorescent probes. A large number of cells can be rapidly detected and enumerated with a flow cytometer. This technique has been widely used in research and has been applied to environmental detection of organisms from aquatic samples. However, there has been limited use of flow cytometry for bioaerosols. In one study, it was shown that flow cytometry could differentiate *Phytophthora infestans* sporangia from most other airborne spores or pollen [46]. This study was conducted to develop methods for late blight detection and forecasting, and limited air sampling data were presented. Prigione *et al.* [47] recently developed a method to improve fluorescent staining of fungal spores prior to flow cytometry. This permitted the detection of accurate counts for total fungal spores in air samples collected with a liquid impinger. However, the fluorescent stain used, propidium iodide, is not specific for fungi, and small pollen might not be differentiated from spores. These studies suggest that flow cytometry may have practical applications in aeroallergen analysis, but some difficulties still need to be overcome. In addition, instrument expense suggests it will never replace current methods.

Image analysis

Direct microscopy of air samples is time consuming and requires highly skilled technicians. A fully automated system able to sample, identify, and quantify airborne pollen and spores is still far in the future. Image analysis, an important component of a fully automated system, is making some progress in recognizing specific pollen and spores. Boucher *et al.* [48] described the methods being used in the development of an automated system for the Advanced System of Teledetection for Healthcare Management of Asthma (ASTHMA). The project is focused on pollen in the families Cupressaceae, Poaceae, Urticaeae, and *Olea* pollen. Although still in early stages of development, the results show the recognition of 77% of pollen grains on reference images of 30 pollen types. Ronneberger *et al.* [49] describe a system based on the use of 3D fluorescent images of pollen taken with a confocal laser scanning microscope. This method achieved a 92% recognition rate for reference specimens of the 26 most important pollen taxa in Germany. Benyon *et al.* [50] described the detection of fungal spores by image analysis. Using seven spore parameters, this program was able to discriminate seven out of 11 genera of spores with an accuracy of 82%. Like the pollen analysis projects, the recognition was based on reference specimens, not on air samples.

Conclusions

Air sampling is widely used to identify aeroallergens in the environment. This can be a valuable tool to estimate exposure, but it is essential that a volumetric sampler be used. Currently, spore traps, rotating-arm impactors, and sieve impactors are the most widely used type of sampling equipment for routine monitoring of the outdoor atmosphere and the indoor environment. Direct microscopy and culture are the most important analytical methods for these sampling instruments. New instruments are constantly being developed, and analysis methods are constantly improved. Techniques on the horizon include expanded use of PCR and immunoassays for specific allergens and image analysis for real-time identification of bioaerosols in the atmosphere.

References and Recommended Reading

Papers of particular interest, published recently,
have been highlighted as:

- • Of importance
- •• Of major importance

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