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Identifying and measuring indoor biologic agents

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CFU Colony-forming units

The investigation of allergens in the indoor environment requires laboratory analysis of air and dust samples. This permits quantitation of the level of exposure to specific allergens in an environment and assessment of allergen reduction efforts. Environmental levels can then be compared with levels known to be a risk for sensitization and development of symptoms.

The prevalence of health problems associated with exposure to indoor allergens from mites, pets, and insects is well documented unlike indoor fungi and bacteria, whose contribution is difficult to

estimate. This is partly because of the diversity of microorganisms that can induce human disease and the variety of exposures in residential, commercial, and public buildings. It is known that between 10% and 32% of all asthmatic persons are sensitive to fungal allergens present in both the indoor and outdoor environment.¹

Biologic Agents

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Domestic mites, pets, insects, mold, and bacteria produce the indoor allergens of major clinical importance. Threshold levels for sensitization and symptoms have now been established for the major allergens of mites (group I) and cat (*Fel d I*).² Threshold levels for cockroach allergen, mold, or bacterial colony-forming units (CFUs) in air and dust have been proposed but not yet accepted.²

Typically bacteria constitute one third of the airborne, viable organisms and fungi two thirds.³ Indoor microorganisms originate from outside air entering the building, from the building's inhabitants, and their occupations, and from contaminated structural materials and furnishings.³ Usually concentrations of fungi and bacteria in normally ventilated interiors are directly correlated with concentrations in outdoor air.⁴ Bacteria and most fungi can persist in the indoor environment long after the initial contamination has occurred and may cause infections and hypersensitivity diseases. Microorganisms can also produce volatile organic compounds that may be mucosal irritants or systemic toxins.⁴

Relative humidity in the range of 30% to 70% correlates with indoor mold spore levels.⁵ Most fungi are unable to propagate in the normally dry indoor environment, but in damp buildings they can grow on more hygroscopic materials and contribute to the patient's allergen load.¹ Data on typical indoor and outdoor airborne microbial species and concentrations can help investigators identify indoor areas with abnormally high counts or atypical microorganisms. Environmental sampling can identify the materials, furnishings, or processes that contribute to microbial contamination and what should be decontaminated or removed.³

Mycotoxins are produced as secondary metabolites by many fungi and are among the most carcinogenic substances known. Acute toxic effects from airborne mycotoxins are rarely identified, but low level chronic effects may be significant. Cancer, probably associated with low-level exposure, has been reported in peanut handlers, mycotoxin researchers, and farmers.⁴

Endotoxins are part of the outer membrane of gram-negative bacteria (e.g., *Pseudomonas* and *Flavobacterium*). Bacterial endotoxins are proinflammatory substances and are present in various domestic or occupational environments. These toxins may play a role in causing or aggravating asthma.⁶ Inhaled endotoxin has been implicated as a causal factor in both short- and long-term airflow limitation among workers in cotton mills, swine confinement facilities, and poultry barns. Symptoms include fever, chills, chest tightness, breathing difficulties, and itchy eyes.⁴

Dust collection sampling sites

Samples of settled dust should be collected with an external vacuum attachment because this improves the consistency and the reproducibility of the dust sample. Mite samples are obtained from the bedding, carpets, and furniture. All bed covers are sampled by layers including the mattress pad and the surface of the mattress. One square meter of bedroom and living room carpets are sampled for 2 minutes. One third of the sofa is sampled including seat back, seat cushion, under the cushion, arm rest, and particularly the seams and folds.

Dust samples for detection of cat allergen should be obtained from carpet, furniture, and bedding. Dust samples for cockroach analysis should be obtained from kitchens, bathrooms, basements, cabinets, attics, and interior wall spaces. Fungal spore and bacterial samples should be obtained from any moist area such as basements, bathrooms, air ducts, drop ceilings, air-conditioning drip pans, condensation coils, walls, and other building materials with visible mold.

Sample processing

The collected dust is sieved through a 250 μm screen onto waxed paper or aluminum foil. Greater than 99% of all allergenic material is contained in this sieved material.² The sample can then be extracted (1:20 to 1:100 weight to volume) in water or aqueous solution (phosphate-buffered saline, borate-buffered saline, or ammonium bicarbonate) with agitation for 1 hour. The suspension is subsequently centrifuged to pellet the insoluble material. Unpelleted fine dust can be removed by passage through a coarse filter. Dust extracts can be preserved with glycerin (50% by volume) or stored frozen.

Surface swabs should be taken from various locations such as desk tops or floors. The recovered sample can be transferred to a slide for microscopic analysis or streaked onto nutrient media. Although swab recoveries are not quantitative, the presence and identity of surface fungi or bacteria can be determined. Surface swabs do not correlate with airborne concentration of viable organisms.⁷

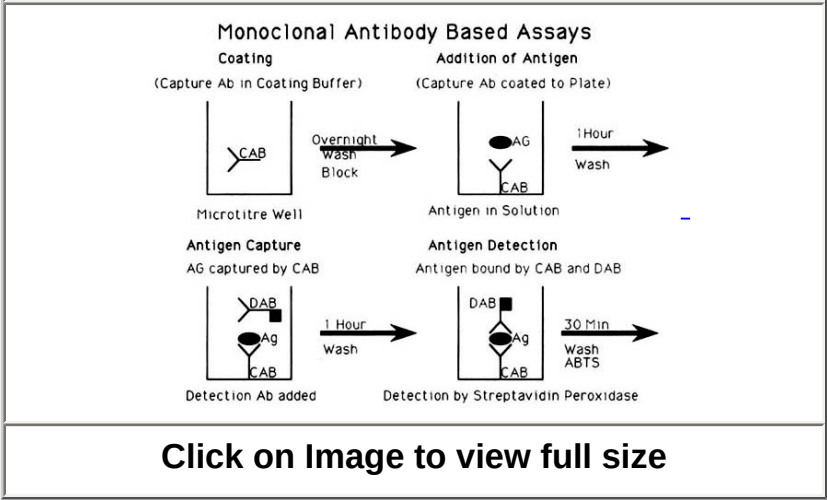
Samples such as small pieces of carpet can be thoroughly washed with extraction buffer and the resulting suspension diluted and cultured. Results are expressed as CFUs per cm^2 or per unit weight. Dusts or fine material can be weighed, extracted, serially diluted, and cultured. Recoveries are expressed as CFU/gm. Contaminated water or other fluids can be diluted serially and cultured. Results are expressed as CFU/ml of original fluid.⁷

Dust samples can be used to quantify and identify mite species. A known amount of sieved dust is suspended in a volume of physiologic buffer in a small plate. The plate is examined under a stereomicroscope, and the mites are removed to a slide where they can be stained, morphologically identified, and counted. Results are expressed as mites per gram of sieved dust.

Sample analysis

Monoclonal antibody immunoassays for quantitation of *Fel d I*, *Der p I*, *Der f I*, *Bla g I*, and *Bla g II* are based on the use of allergen-specific monoclonal antibodies as capture and detection antibodies (■Fig. 1). These antibodies are directed against different epitopes on the allergen molecule. The capture antibody is first attached to the microtiter plate and subsequently binds to the allergen in a solution. A second enzyme-labeled detection antibody binds to a different epitope on the allergen molecule and is quantitated by the degree of color development when substrate is added. Comparison is made to a series of known quantities of allergen for conversion of optical density readings to mass units. Correction is then made for dilution and the weight of dust extracted, and the result is expressed as nanograms per gram of sieved dust.

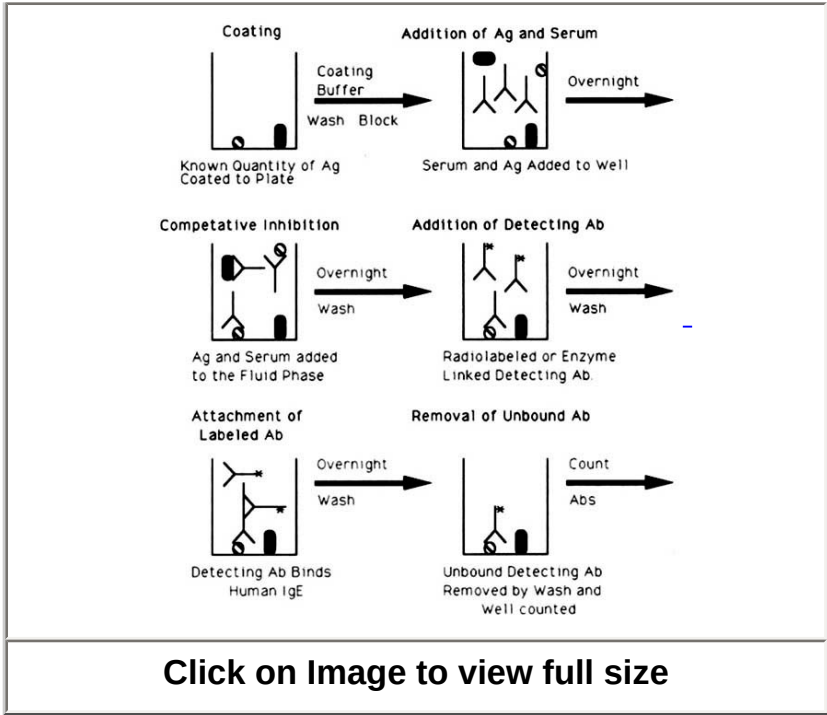
FIG. 1. Monoclonal antibody-based assays.



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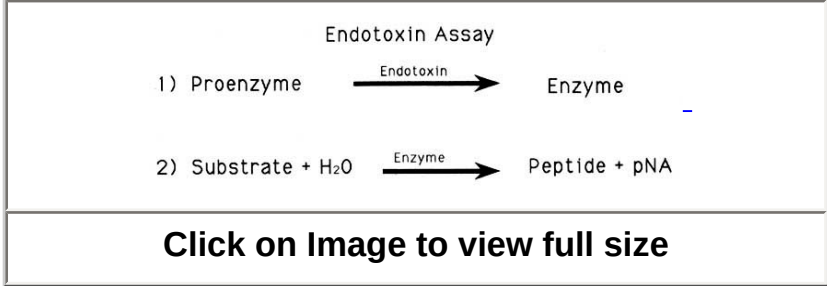
Total allergen determinations or RAST inhibition are used when monoclonal antibodies are not available or desired (■Fig. 2). This assay measures total and not specific allergen. Quantification is based on competitive inhibition of the binding of allergen-specific human IgE to allergen coated on the solid phase by the allergens to be quantified in solution. Calibration is by the optical density or counts per minute derived from a standard curve added to the fluid phase. Correction is then made for the dilution and the weight of dust extracted, and the results are expressed as nanograms per gram of sieved dust.

FIG. 2. Total allergen assays or RAST inhibition.



The bacterial endotoxin assay is based on the ability of the lipid A portion of endotoxin to activate the coagulation cascade of the amoebocyte lysate from the horseshoe crab (■Fig. 3). Endotoxin activates a proenzyme present in horseshoe crab lysate that catalyzes the cleavage of paranitroaniline from the colorless chromogenic substrate. The paranitroaniline is measured colorimetrically at 405 nm.⁶ Endotoxin levels in a sample are calculated from the absorbance value of solutions containing known amounts of endotoxin standard and expressed as nanograms per gram of house dust extract.

FIG. 3. Endotoxin assay.



Mold and bacterial analysis

Many microorganisms have specific growth requirements; therefore no single set of culture conditions will promote the growth of all possible dust constituents. If the presence of a specific organism is suspected, a defined culture medium that will promote growth of that organism should be used. Otherwise, a general-purpose media designed to recover microorganisms with shared growth requirement is the best choice (■Table I).

TABLE I. Common fungal and bacterial culture mediums

	Sabouraud's dextrose agar	
Dextrose		40 gm
Peptone		10 gm
Agar		20 gm
Distilled H ₂ O		1000 ml
Sterilize in the Autoclave at 120° C for 10 min and then adjust pH to 5.6		
	Neutral Sabouraud's Dextrose agar	
Dextrose		20 gm
Neopeptone		10 gm
Agar		20 gm
Distilled H ₂ O		1000 ml
Sterilize in the Autoclave at 120° C for 10 min and then adjust pH to 6.8-7.0; 2% dextrose is adequate and a neutral pH induces optimum growth of most pathogenic fungi		
	Sabouraud-cycloheximide-chloramphenicol agar	
Dextrose		20 gm
Neopeptone		10 gm
Agar		20 gm
Chloramphenicol		40 mg
Cycloheximide		500 mg
Distilled H ₂ O		1000 ml
Bacterial growth of a contaminated sample is easily controlled by addition of antibiotics such as chloramphenicol. Cycloheximide reduces growth rate of many saprobic fungi and other yeasts		
	Malt extract agar	
Peptone		1 gm
Dextrose		20 gm
Malt extract		20 gm
Agar		20 gm
Distilled H ₂ O		1000 ml

Mold spore assessment is based on the assumption that each viable spore will produce a separate colony from which mycelia will be visible. Fungi that grow well at room temperature (mesophilic) are most commonly cultured on Sabourauds's medium, malt extract agar, or rose bengal–streptomycin. Sabouraud's medium produces adequate colony counts. Although rose bengal–streptomycin medium is light sensitive, it is a very useful medium in that it restricts colony size and enhances early sporulation. Growth of identical inoculums on rose bengal–streptomycin and malt extract agar shows that the malt extract agar supported the same colony counts unDer fluorescent lights as did rose bengal–streptomycin cultured in the dark. Consequently, malt extract agar is recommended by

some reference laboratories.⁷

Quantitation of mold spores in dust begins with transferring weighed samples of sieved dust onto the surface of the culture medium. The plates are inverted and incubated at 21° C and the number of colonies are counted at 24 and 36 hours. Corrections are made for the amount of dust cultured, and the results are reported as the number of colonies per gram of sieved dust in 36 hours. If samples overgrow the plate at 24 hours, they are recultured with a smaller inoculum. A control dust sample with a known amount of mold spores should be assayed with each batch to ensure good interrater consistency.⁷ Species determinations are not performed routinely because of the inherent difficulty and questionable clinical value of such determinations.

Nutrient agar, blood agar, tryptic soy agar, and soybean casein digest agar are general-purpose bacterial media.⁷ Specialized media that meet the nutritional requirements of specific bacteria can be useful in the identification of bacterial taxa. Several reference laboratories recommend the use of nutrient agar for mesophilic bacteria and trypticase soy agar for thermophilic bacteria and actinomycetes. Inverted plates are usually incubated at 30° C, CFUs are counted at 24 and 48 hours, and the results are expressed as CFUs per gram of sieved dust.

Incubation temperatures are critical because growth optima vary for different organisms. Common airborne environmental microorganisms usually grow best at a temperature between 15° and 27° C. Incubation temperatures can be chosen to exclude certain taxa. For example, most environmental bacteria will grow well at 30° C, which inhibits the growth of most mesophilic fungi and eliminates the need for fungal growth suppressants.⁷ Most fungi and bacteria grow well in darkness or light.

Macroscopic and microscopic identification

Both macroscopic and microscopic characteristics contribute to the classification of fungi. Some colonies can be assigned to a genus based on the size, texture, and color of the colony; however, positive identification usually requires high-power (×1000) examination.

Bacterial colonies usually appear very wet and glossy with colors that are variable. An experienced bacteriologist is often required for the visible identification of bacterial colonies.

Counting of fungal spores requires a microscope with a magnification of ×100 to ×1000. Particle size is an important aid in identification; therefore the use of an eyepiece microscale calibrated with a stage micrometer is recommended. This will enable precise measurement of particles as small as 1 to 2 μm. Microscopy will identify many particles that are nonviable or fail to grow on available media; consequently, microscopic counts are higher than viable culture counters.

Spores range in size from 1 to 100 μm, but most are in the 3 to 12 μm range. Spores take up stain very poorly; therefore staining usually does not aid identification. Characteristics that may aid in spore identification are as follows: the shape, which can be spheric, ellipsoidal, fusiform, filamentous, etc.; the number, orientation, and thickness of septa; attachment structures; the scars and pores on asexual spores; the color, which varies from colorless to brown and black and may change as the spore matures; the spore form; and the mode of spore formation and the morphology of spore-forming structures.

Subculture of a colony may be necessary to prevent it from being overgrown before it is identified. This can also be useful when special media are used to elicit certain diagnostic characteristics that are not expressed in broad-spectrum media. These include fruiting bodies and arrangements of a simpler, spore-bearing structures. Culture analysis can result in genera and occasionally specific identifications of many microbes.

Spores can rarely be identified precisely with regard to species. Most are grouped by appearance, which does not always reflect taxonomic or allergenic relationships. A count will usually contain a large number of unknowns because of the spore orientation on the slide and the frequent lack of diagnostic characteristics.

Air sampling

Immunochemical quantitation of airborne allergens

Airborne allergens in the indoor environment can be quantitated by collection of appropriate air samples followed by immunochemical quantitation with use of the techniques described previously. We use the air sentinel manufactured by Quantec Air (Rochester, Minn.), which incorporates a vacuum device and polytetrafluoroethylene (PTFE) (Teflon) filters with a pore diameter of 0.2 μm . Twelve-hour samples are collected, the PTFE filter is delaminated from its fiberglass backing, and the collected allergens are eluted into 1 ml of aqueous extraction buffer. The air filter extract is then centrifuged to remove insoluble particulate matter. The extract is then used directly in a monoclonal antibody or total allergen assay. Results are expressed as nanograms per cubic meter of air sampled.

Volumetric devices

Open culture plates are a simple but a very inaccurate method of collection. Recoveries are biased toward heavier particles and should not be used to estimate suspended aeroallergens. Collection efficiency depends on particle inertia, which in turn depends on particle diameter and velocity. At constant speed inertia increases with particle size. Therefore small particles are less inclined to move in straight lines and will follow the air stream around the collecting surface. To overcome this most inertial samplers utilize a moving collection surface or vacuum source to increase particle velocity should be used. Both of these modifications increase the probability that the particle will strike the collecting surface.

Inertial samplers such as the Rotorod (Sampling Technologies, Los Altos Hills, Calif.) (■Table II) are commonly used for pollen collection, and like most aeroallergen samplers, are considered inertial impactors. Rotating arm impactors such as the Rotorod are not efficient collectors of particles less than 15 μm and are not adequate for quantification of most fungal spores.

TABLE II. Characteristics of common air samplers

Sampler type	Sampler	Particle size (μm)	Time
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Rotating arm impactor	Rotorod	>10	1 min-48 hr
Suction slit impactor	Burkard	>2-3	Up to 7 days
Suction sieve impactor	Anderson cascade	>1	1 to 20 min
Centrifugal impactor	RCS	>5	30 sec to 8 min
Liquid impinger	AGI	>2	10-30 min

Suction impactors use a pump or other vacuum source to accelerate air through a slit or sieve, increasing particle speed and particle momentum. The most common of these, the Burkard (Burkhard Manufacturing Co., Hetfordshire, England) has high efficiency with particle sizes greater than 3 μm . The sample is collected on a thin film of vacuum grease applied to a detachable tape mounted on a drum. After sampling the tape is removed, mounted, and stained for morphologic identification of mold spores or pollen. The drum is rotated by a clockwork mechanism, resulting in a time-differentiated sample. Indoor models are available.

Slit-type viable suction samplers draw air through a narrow opening onto the surface of agar medium in a culture dish. The dish is rotated mechanically under the slit at variable speed to obtain a time-differentiated sample. Collection efficiency for particle sizes down to 1 μm is greater than 95%.

Sieve-type viable samplers are suction impactors that use a sieve plate with multiple holes that allow impaction of particles onto agar medium in a standard Petri plate. For example, the Anderson microbial cascade (Anderson Sampler, Inc., Atlanta, Ga.) sampler sizes particles into six ranges using six sieve plates each with 400 holes. The diameter of the holes decreases from 1.14 mm on the top stage to 0.25 mm on the lowest stage. The air stream is accelerated by passing through each successive stage, resulting in impaction of smaller and smaller particles onto the culture medium. As the air stream moves through the device, the largest particles, greater than 7 μm in diameter, are collected by the top stage and the smallest particles, 0.65 to 1.1 μm in diameter, by the sixth and final stage. In theory each stage corresponds to deposition in different respiratory system levels, with the sixth stage corresponding to the alveoli.^{7 8} Anderson also manufactures two stage collectors, which size samples into respirable and nonrespirable particles.

Centrifugal impactors, such as the RCS (Biotest Diagnostics, Fairfield, N.J.) sampler, use air centrifugation to impact particles onto an agar-coated plastic strip lining the inside of the sampling cylinder. This type of sampler is not accurate quantitatively, especially for small particles.

Filtration samplers actively pull air through a barrier (filter cassettes) on which particles are trapped. The sample can then be analyzed by microscopy or culture of the filter or by washing the particles from the filter followed by microscopy or culture. Collection efficiency for a given particle size depends on the pore diameter of the filter. Most filters will retain a large percentage of particles larger than their rated pore size so a filter with a 1 to 2 μm pore size should retain almost all fungal spores and bacteria.

Liquid impingers, such as the AGI, suction air through a glass tube, with a narrow orifice immersed in a liquid. Particles are impinged within the liquid onto the container base or onto a glass platform suspended below the orifice. Because the orifice and intercepting surface are submerged, impinged

particles that leave the air stream are trapped in the reservoir fluid. Liquid impingers are commonly used for collecting aerosols. Many particles, because of their hydrophobicity, are not efficiently trapped in fluid media and this, along with the lack of proved methods for analyzing recoveries, has limited their use for aeroallergen determinations.

Interpretation of Results

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Interpretation of allergen levels in dust is based on the following two assumptions: (1) that the dust sample is representative of the dust in the relevant areas of the patient's building or house and (2) that a relationship exists between the allergen content in settled dust and the amount of airborne inhaled allergen.

The level of 2000 ng of group I dust mite allergen per gram of dust is equivalent to 100 mites per gram of dust. This amount of allergen has been determined to be clinically significant. Group I levels below 2000 ng are desirable and of low risk for allergen sensitization, whereas levels greater than 2000 ng place the occupants at risk for sensitization. Group I levels above 10,000 ng result in the occupant being at high risk for both sensitization and symptoms.^{[2](#)}

Eight thousand nanograms of *Fel d I* per gram of dust has been proposed as a significant level of cat allergen. *Fel d I* levels of 80,000 ng/gm are considered very high and place the occupants at risk of developing symptoms, such as asthma.^{[2](#)}

No guidelines have been established that relate cockroach levels to health effects. However, some investigators feel that any detectable level of cockroach allergen is clinically significant because its presence identifies a building in which persons who are cockroach allergic are at risk to develop symptoms because of exposure.^{[2](#)}

No guidelines have been established that relate indoor or outdoor bacterial or fungal levels with health effects, but some generalizations have been accepted. At present fungal spore or bacterial colony counts of greater than 10,000 per gram of dust should be considered sufficiently high to identify homes in which environmental intervention, such as mildew removal and/or humidity control, may be appropriate. Bacterial concentrations in standing water (such as drip pans) above 1 million per milliliter are excessive, and decontamination is recommended. Fungal concentrations on water-damaged materials (e.g., carpet, dry wall, or ceiling tile) above 1000 to 10,000 CFU/gm or 1000 to 10,000 CFU/cm² are undesirably high.^{[2](#) [9](#)}

An intrabronchial bacterial endotoxin level of 9 ng results in a significant decrease in forced expiratory volume in 1 second (FEV₁). Endotoxin levels greater than 18 ng/m³ in a building is statistically associated with clinical complaints of the building's occupants.^{[7](#)}

Typical indoor microbial findings

No significant differences exist in the microbial content of outside and inside air in naturally ventilated residences. Air-conditioned homes have fewer fungi but significantly greater amounts of

Aspergillus species.⁹ Hirsh et al.¹⁰ studied the effect of residential air conditioning on indoor spore counts and found that the counts in air-conditioned homes were significantly lower than in naturally ventilated homes.¹⁰ Twenty-seven different mold species were identified in eight major species groups. The relative percentages of species did not differ significantly between the air-conditioned and naturally ventilated homes. Strom et al.¹¹ compared the microbial flora of sick and healthy buildings and found no significant difference in the levels of both total and viable microorganisms.¹¹ Morey¹² measured very high levels of airborne fungi in buildings with occupant complaints and buildings without such problems.

Schrober¹ studied fungi in carpeting and furniture and found that the number of fungal species in carpet dust is higher than that from upholstered furniture. *Penicillium brevicompactum* was found in all furniture and *Aspergillus versicolor* and *Aspergillus restrictus* on all floors. The number of spores from xerophilic, mesohygrophilic, and hydrophilic species in dust from carpeting or from upholstered furniture was examined. The number of spores from the hydrophilic general per gram of dust was significantly higher on floors ($p < 0.05$), and mesohygrophilic spores were significantly higher in furniture dust ($p < 0.05$). The number of viable spores was significantly higher in floor dust than that in dust from carpeting ($p < 0.05$). Xerophilic fungi such as *Aspergillus* have been reported to appear at a constant level in dwellings throughout the year, whereas the mesohygrophilic and hydrophilic species are strongly dependent on seasonal conditions. Some mesohydrophilic species such as *Pencillilum* are also common in dwellings throughout the year. Regularly used furniture harbor primarily *Aspergillus* and *Penicillum*, suggesting that furniture should be regarded as a major source of fungal spores.¹

Gram-positive cocci and gram-negative rods predominate on damp surfaces such as the commode, kitchen faucet, and bathtub. Gram-positive cocci are frequently isolated from the bathtub. Gram-positive rods are found on both damp and dry surfaces. Bacteria are carried predominantly on particles larger than 7 μm and on particles of approximately 2 μm aerodynamic diameter.¹

Yeasts were the predominant fungi found on surfaces and were present in largest amounts on damp surfaces.³ Median particle diameters were 3 μm indoors. Although fungal spores are generally larger than bacterial cells, they are often disseminated individually, whereas bacterial cells are attached to debris.³

Conclusion

Because of advances in sampling technology and laboratory methods, we have now begun to define indoor exposure to biologic agents. Clinically relevant sensitization and symptom levels of exposure have now been proposed or established for several allergens. Consequently, we are now able to identify when intervention by application of allergen reduction methods is warranted for those species. Understanding of the clincial relevance of other insect and mammalian allergens and biologically active agents such as fungi and bacteria is in its infancy, and much remains to be elucidated.

1. Schrober G. Fungi in carpeting and furniture dust. *Allergy* 1991;46:639-43.

MEDLINE

2. Hamilton RG, Chapman MD, Platts-Mills TAE, Adkinson NF. House dust aeroallergen measurements in clinical practice: a guide to allergen-free home and work environments. *Immunol Allergy Practice* 1992;XIV:3:96-112.

3. Macher JM, Huang FY, Flores M. A two year study of microbial indoor air quality in a new apartment. *Arch Environ Health* 1991;46:25-9.

MEDLINE

4. Burge HA. Toxic potential of indoor microbiological aerosols: in the analysis of complex environmental mixtures. Sandhu SS, DeMarini DM, Mars MJ, Moore M, Mumford JL, eds. New York: Plenum Publishing, 1987:391-7.

5. Burge HA. Fungus allergens. *Clin Rev Allergy* 1985;3:319-29.

MEDLINE

6. Michel O, Ginanni R, Douchateau J, Vertongen B, Le Bon B, Sergysels R. Domestic endotoxin exposure and clinical severity of asthma. *Clin Exp Allergy* 1991;21:441-8.

MEDLINE

7. Muilenberg AL. Allergy assessment by microscopy and culture. *Immunol Allergy Clin North Am* 1989;9:2:245-67.

8. Verhoeff AP, van Wijnen JH, Boleij JSM, Brunkereef B, van Reenen-Hoekstra ES, Samson RA. Enumeration and identification of airborne viable mold propagules in houses. *Allergy* 1990;45:275-84.

MEDLINE

9. Harrison J, Pickering CAC, Faragher EB, Austwick PKC, Little SA, Lawton L. An investigation of the relationship between microbial and particulate indoor air pollution and the sick building syndrome. *Respir Med* 1992;86:225-35.

MEDLINE

10. Hirsch DJ, Hirsh SR, Kalbfreisch JH. Effect of central air conditioning and meteorologic factors on indoor spore counts. *J ALLERG CLIN IMMUNOL* 1978;62:22-6.

11. Strom G, Palmgren U, Wessen B, Hellstrom B, Kumlin A. The sick building syndrome: an effect of microbial growth in building construction? *Indoor air 90: proceedings of the 5th International Conference on Indoor Air Quality and Climate. Vol 1. Toronto, Canada, 1990:173-8.*

12. Morey PR. Case presentation: problems caused by moisture in occupied spaces of

office buildings. In: Evaluating office environmental problems. Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists, 1984:121-7.

Publishing and Reprint Information

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