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How ill the wind? Issues in aeroallergen sampling

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The effective size of bioaerosols is the principal determinant of their behavior during takeoff, transport, and deposition. Collection methods involving impaction, impingement, and filtration allow recovery of increasingly small aerosol units and do so "per unit volume of processed air" with more or less well-defined efficiency. These principles offer direction in choosing sampling devices for specific applications; similarly, a particle's appearance, growth potential, and assayability define the scope of relevant analyses. Where a variety of aerosol types occur, multiple collection and/or analytic approaches might be required. The development of a sampling grid in North America makes possible studies of distribution, transport, and climatic effects in ways never previously possible; however, some directive planning and oversight are essential to realize these goals. Methods sensitive to paucimicronic and submicronic particles should be included increasingly in new and ongoing survey protocols. Similarly, available means now facilitate study of aerosols at specific sites, both indoors and outdoors, with estimates of personal exposure during defined events. Resulting data describing airborne prevalence gain special value in light of competent inspection of implicated venues and consideration of alternative sources, such as incursion of bioaerosols into enclosed spaces. (*J Allergy Clin Immunol* 2003;112:3-8.)

Key words: Aeroallergen sampling, aerobiology, pollen, allergens

Recognition that windborne agents could determine allergic morbidity led to prompt and sustained investigations of their nature and prevalence that continue to expand in scope.¹ The enduring goals have been, at the very least, to recognize candidate offenders and, at best, to perceive still-elusive dose-response relationships for groups and symptom determinants for individuals. Although an array of investigative methods vie for attention,² on close scrutiny, they reflect a few basic principles of collection and analysis. Rather, it is in the realm of application strategy and data interpretation that informed opinions conflict, and truly enduring questions remain.

Abbreviation used

AGI: All-glass impinger

That said, it seems only fair to emphasize that the opinions expressed in this article are those of the author and reflect no higher intelligence or authority.

Surveys at many sites emphasize the diversity of immunogenic (bio)aerosols (ie, aeroallergens) regarding origin, dispersion, and size-related transport. The last of these commands special attention in view of highly credible clinical effects^{3,4} mediated by small and often derivative allergenic aerosols. Early hopes for a single universally rewarding sampling strategy have now embraced the practical reality of several best choices for distinctive aerosol groups.

WHEN SIZE REALLY MATTERS

During their essential travels, bioaerosols respond predictably to familiar physical forces (Fig 1).¹ The earthward-directed pull of gravity is partially opposed by particle buoyancy, a competition operating especially in the vertical. Accelerations might be imparted in any direction by moving air currents, rebound from obstructions, or forceful primary release (eg, of fungal ballistospores and pollens of some urticaceous plants).⁵ The tendency of aerosol units to remain in linear motion is opposed by frictional interaction with air molecules, a property termed *drag*. Drag varies (minimally) with the density of air and with the comparative roughness of aerosol surfaces. Nonspherical particles that yaw or tumble through air incur increased drag compared with smooth, radially symmetric units of equivalent mass.

Acceleration-induced particle motion tends to proceed in straight lines but can be opposed and deviated by drag effects. These conflicting vector forces determine an entrained particle's path and its ability to strike or avoid (ie, to flow around) obstructions. At equilibrium, a particle's fall speed, as well as its tendency to follow a linear nonvertical path, is a function of its effective size* (largely its diameter squared). As a result, under equivalent con-

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*Effective diameter is the diameter of a unit density sphere that moves with the same rate characteristics as the aerosol unit in question.

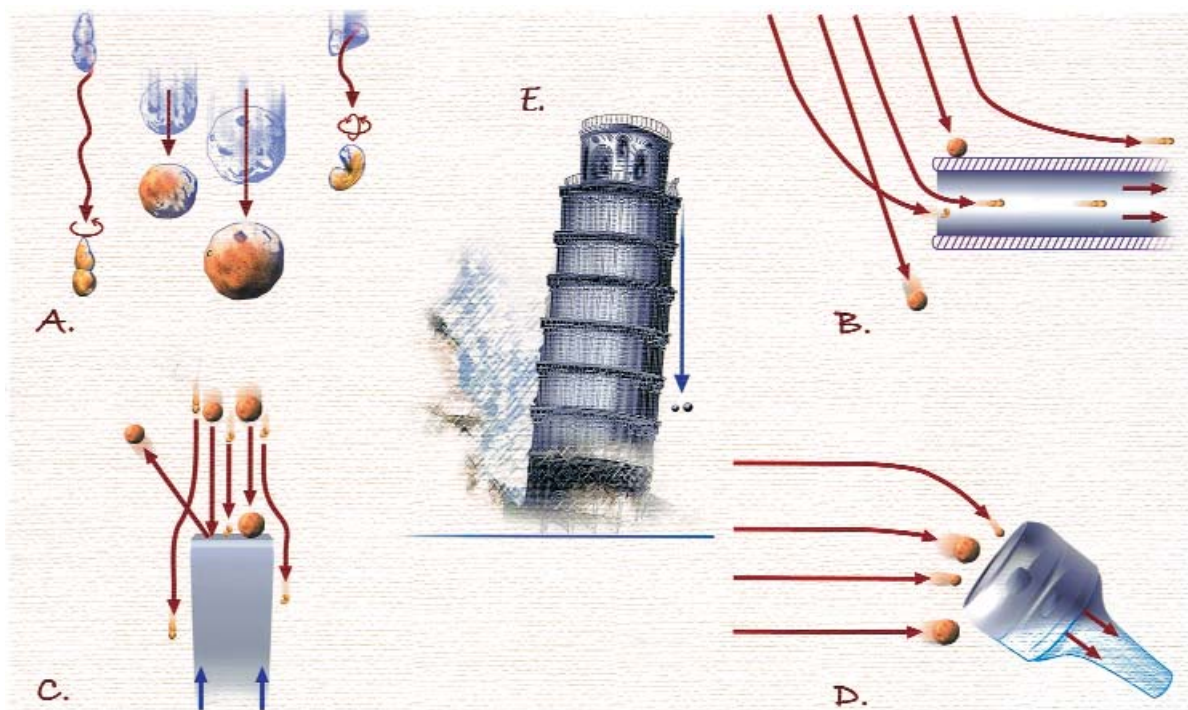


FIG 1. A, Fall regimens in air of 4 different particle configurations. The center 2 solid spheres differ in size only, which determines a faster rate for the larger unit; in a vacuum their fall rates would be equal. At right is an irregular shape that will tumble, increasing drag. Particles such as that at the left, although not spherical, appear to align themselves to present their smallest dimension and effectively limit drag. In each case the uppermost arrow is the vector sum of gravity, buoyancy, and drag. B, Particle behavior at a flow channel varies with its size and accelerations applied to it. Both above and below, large particles proceed in relatively straight lines, regardless of impending obstructions. Small aerosol units (above) tend to flow around a passive obstruction in response to drag forces. When suction is applied (the situation for the 2 particles below), small units will cross stream lines in response to this stronger acceleration and enter the flow channel. C, Particle behavior at the moving surface of a rotorod. The primary loss of small aerosol units that follow airflow is shown. Although efficiency for large particle capture in the cylinder of air swept out is good, loss by rebound after impact does occur, reducing adhesive efficiency. D, Air and particle behavior at the surface of a filter cassette with active suction applied. Again, capture of small particles will exceed that of large particles, but without wind orientation, all losses are increased. E, A depiction of the experiment, attributed to Galileo, in which large and small cannon balls, dropped from the bell tower in the Piazza dei Miracoli (Plaza of Miracles) in Pisa, allegedly struck the ground at the same instant. This was probably not an accurate observation because of differential drag effects, but the difference was too small for human senses to recognize. That a basic law of physics should have come from this misperception is miraculous indeed.

ditions, a 20- μm spherical particle's tendency to fall onto a surface is 100 times that of an equally smooth 2- μm unit.[†] Similar differences result when airborne particles of unequal size encounter an obstruction (eg, a collection surface) around which stream lines of air must flow. Large, high-inertia aerosol units, affected comparatively little by the drag of diverging air currents, proceed in relatively straight lines that favor impaction (Fig 1). By contrast, smaller particles, responding primarily to drag forces, often elude collision. These same differences that favor capture of larger particles by rotating-arm impactors (Fig 1, C) determine the relatively greater efficiency of suction devices (Fig 1, B and D) for smaller particles.⁶ Unless a suction collector is perfectly aligned with and has an entrance air speed equal to ambient flow, some deviation in stream lines must occur at the orifice.¹

[†]Studies to determine bioaerosol density have suggested that most types approximate 1.0, and therefore this is usually assumed.

Minute aerosols, strongly influenced by drag, readily follow these trajectories into collector intakes. By contrast, large particles tend to cross streamlines in faithfully pursuing linear paths. There is, of course, no point of sharp behavioral shift between large and small aerosols; graded interplay of momentum and drag effects is continuous. However, at larger than 20 μm , drag forces are comparatively minor, whereas at less than 10 μm , their influence increasingly determines particle paths.

WAYFINDING IN THE SAMPLING HARDWARE LABYRINTH

On casual contact, bioaerosol samplers present an intimidating array of whirling, whining, and hissing small appliances, often grievously overpriced and with no immediate clue to their application or merit. So confronted, it is well to recall that all such devices exemplify the following: (1) a collection function, by which

aerosol units are recovered from air, and (2) an analysis function, by which recoveries are quantitated. Currently useful devices include rotating-arm impactors (eg, rotorod units) with highest efficiency for larger particles.⁶ The other major groups are suction collectors, including impingers and filters. Impingers (eg, Burkard-type traps and the Andersen Sampler) aspirate particle-bearing air at high speed and then force a sharp directional change in which all but the smallest particles are thrown against an adhesive-coated or other sticky collection surface. Additional cyclone collectors are usually vertical tubes, tapering toward one end. Air is aspirated tangentially to the inner wall at the wider end, and complex bidirectional vortices are created with particle deposition on the internal surface. Airflow and cyclone configuration determine the particle size scavenged with 50% efficiency (50% cutpoint). These provide small bulk samples for analysis; however, their absolute retentive efficiency remains uncertain.⁷

Although often overlooked, filters can subserve many functions, from personal collectors in minute cassettes worn on clothing and powered by small DC pumps to filtration surfaces of a square foot or more in immobile “high vols,” processing thousands of liters per hour. The commercial availability of various filter media, each in a range of effective pore sizes, allows choice of a lower (size) limit to recovered aerosol. However, this is an approximation in that some aerosol units at and below the pore rating in micrometers also will be retained. The diversity of filter materials facilitates subsequent immunologic or chemical assay of the catch, as well as direct microscopy or culture on nutritive liquids if desired. Because binding of complex molecules to certain filters can be extremely avid, media require careful selection for desorptive properties by using the target aerosol of interest. The percentage of material recoverable by means of elution from filters spiked with a specific substance must be determined (and judged appropriate) before choosing that filter medium to collect the aerosol for assay.

Applications in which aerosol is to be handled in suspension or solution can exploit the simplicity and low cost of all-glass impingers (AGIs). In these devices air is aspirated, accelerated, and impinged at a nozzle on a small glass target immersed in a prescribed volume of collecting fluid. Several sizes of AGIs are available, and within limits, throughput can be selected.² Foaming should be prevented. Allergen recoveries can be increased by adding diluted serum albumin to the collection fluid to block glass surface absorption sites.⁸ Tween addition also might be helpful.

All suction collectors require orientation of intakes toward airflow, especially where wind speed exceeds 8.3 kph (5 mph; ie, meteorologic calm). Recent experience with a multiply perforated hemispheric sampler intake suggests that it might increase particle capture in rapidly changing air streams⁹; however, its potential remains to be defined. In essentially calm conditions (eg, many enclosed situations), fixed lateral or upward-directed intakes still provide nominally unbiased collections; however, air

motion from human activity (including that of technical personnel), HVAC blower units, or vacuum pump backwash should be minimized both indoors and outdoors.¹⁰

EVALUATING THE CATCH

The analysis function of aerometry remains well served by traditional light microscopy, culture with laboratory media, or both, despite their limitations. Microscopy can limit losses because of (non) “viability” and is classically preferred for pollens and unambiguous spores. For the once and future candidate microscopist, a brief review of proper lighting, condenser function, and focusing strategies is usually in order.^{11,12} However, even for significant pollens, important distinctions (as between box elder and certain oak grains and among smaller urticaceous pollens) remain obscure, and data sets lacking an “unidentified” category must be considered incomplete.

Culture methods must contend with, for example, vagaries of media selectivity, competition, contact inhibition, and mite and other contamination; however, there is no other current choice for taxa with small spherical spores (or for hyphal fragments alone). Stains such as cotton blue will color these anonymous units for rapid estimates of the category. Unstained, however, they are not confidently distinguished from variably colored ash spheres by most observers. Much confusion has resulted because ascospores and basidiospores typically produce no growth, only sterile growth, or their imperfect (asexual) stages (eg, form species of *Fusarium*, *Geotrichum*, and *Aspergillus*) on media, such as malt extract or Sabouraud glucose agar. Despite these annoyances, indoor studies, often dominated by small-spored taxa, usually require a substantial cultural component, and outdoor work also might be enhanced thereby.

Even precise, volumetric,* particle-based data constitute surrogate variables for total suspended allergen and even more distantly for relevant exposure of specific persons, a still elusive goal.

The lack of enumerable units for mammalian and arthropod allergens plus such materials as sericin, seed pomaces, food emanations, and immunogenetic simple chemicals is established fact.¹³ The recent recognition of airborne pollen allergens in diverse small aerosol size fractions has underscored established needs for a complementary allergen-based assay.⁷ Although details of the immunoassay procedure cannot be addressed here, the ability to relate small allergen exposure with epidemic clinical events compels intense interest.^{4,13,14}

The increasing availability of site-specific mAbs has transformed assay procedures for paucimicronic and submicronic allergen-bearing aerosol. A suitable substrate (eg, glass, polystyrene, or cellulose) is coated with antibody to which a known or potential allergen is added. After incubation and washing of that conjugate, tagged antibody to a second site is added, and the tag (ie, color,

*On the basis of index volumes of processed air (usually cubic meters).

radioactivity, fluorescence, and binding capacity to a ligand) is assayed and compared with known values separately determined; lacking mAbs, reliance has been placed on using aerosol eluates to inhibit immune interactions, such as ELISA and Western blotting.^{14,15} Although not without value, these approaches are more difficult to standardize and risk errors from nonspecific binding and inhibition.

Certain defined agents have been assayed as indicators of more complex immunoreactive components. Among the earliest of such approaches was the estimate of guanine in bulk dust as an estimator of dust mite feces and, *pari passu*, the population density of normally functioning mites.¹⁶ The usual lack of other indoor guanine sources provides a measure of specificity, and correlations between guanine content classes and levels of Der p 1 and Der f 1 are recognized.^{16,17} However, with low allergen content samples, this colorimetric test loses its precision.¹⁵ It seems most helpful in detection of high point sources in areas of low or modest mite frequency; alternatively, strong positive results might prompt action in homes in which remediation is overdue.¹⁸ Measures of total fungus burden have been proposed by using ergosterol, which is present in essentially all airborne fungus cell walls, as an indicator.^{19,20} Suitable material is readily acquired by using filters or fluid-filled impingers. However, the assay still requires specialized hardware and standards, mandating reliance on a few central facilities if this is to reach clinical application. β -D (1-3) glucan is an analogous candidate marker of fungal biomass, with independent, direct, pro-inflammatory effects on mammalian tissue.²¹⁻²³ Strongly suggested efforts to use this and related carbohydrate moieties as broad or restricted (eg, to *Penicillium-Aspergillus* form species)²² biomarkers have languished for want of funding. By contrast, analysis of distinctive volatile hydrocarbons produced by fungi is an established tool of food microbiology with possible potential for aerobiologic application.

Endotoxin, once merely a surrogate for gram-negative bacterial prevalence, has taken on new allure as a pluripotent arbiter of immune function and pro-inflammatory factor; measurement efforts have burgeoned accordingly.^{23,24} Again, as with fungal structural sugars, analysis mandates highly selected techniques and reagents.²⁵ Although decades old, the lysis of *Limulus* amebocytes by endotoxin is still the current assay's core event*; however, a kinetic adaptation is widely preferred at present.²⁶ Gas chromatography-mass spectroscopy have not given superior results in direct comparisons, but all such assays require well-honed technical competence. Resulting data also can be affected by collection venues and techniques,²⁷ properties of different endotoxins, sample storage, quality of reagents, and interference with *Limulus* amebocytes because of materials other than endotoxin.^{25,28}

**Limulus* species, the horseshoe or king crab, is the primitive source of amebocytes. Harvest of these creatures has accelerated recently so as to render them endangered where previously they were considered an abundant nuisance.

ANSWERS BLOWIN' IN THE WIND? WHAT ABOUT QUESTIONS . . . ?

Easily obtainable equipment allows the clinician-researcher to explore several clinically relevant aspects of bioaerosol exposure. Broad values for regional pollen and spore prevalence remain the principal focus but are only one of the available opportunities. Where a full range of micronic particles is the goal, reliable spore traps, such as the Burkard volumetric trap, offer high collection efficiency and excellent hardware durability.¹ All recoveries are time specific, with models providing 24-hour traces on 1 × 3-inch microslides or 7-day records on a greased tape-wound drum 33.6 cm (approximately 13.2 inches) in circumference. For pollen, staining with fuchsin or phenosafranin in an aqueous base, such as hard glycerine jelly, is helpful, whereas fungus spores are best viewed unstained. Ringing cover slips with colorless nail polish allows extended storage and opportunities for re-examination.

Where pollens and pollen-sized spores (>10-12 μ m) are the focus, intermittent rotorod devices also serve well. However, impaction efficiency for aerosols of less than 10 μ m decreases rapidly toward single figures.¹ Furthermore, the 1.2-mm-wide rotorod collection surfaces overload measurably in smog and bioaerosol clouds, even during total operating periods of 30 to 60 minutes.²⁹ Because of this, sampling intervals of 10 seconds or less per 10-minute timer cycle are often essential. Rods might be cleaned and reused but do not lend themselves to permanent preparations in the manner of slide-mounted, flat, Burkard tape segments. Counting uses a microstage support holding 2 rods in a stained aqueous medium; dismantling this variably disrupts the particle trace. The Burkard unit and similar traps are gust responsive, whereas the circular path of the rotorod confers partial wind independence. Both devices are affected by wind speed,³⁰ and this varies with particle size,³¹ suggesting that comparisons of data require strict sharing of defined sampling protocols. However, efforts to operate fixed suction devices out of doors usually results in poor particle recoveries unless wind action is nil. Occasional reports of equivalent recoveries by fixed and wind-oriented outdoor collectors are not easily explained but offer scant assurance that wind effects might be ignored.

The proper siting of collection points continues to stir controversy in the notable absence of directive data. Whatever the location chosen, the aerosol content of ambient air often is equated with exposure; however, a 100% aerosol capture efficiency by the downward-directed human nares should not be assumed with (normally) horizontal airflow. Studies of mixed outdoor air warrant a site lacking significant obstructions and, preferably, little influenced by emissions from the substrate or surrounding sources. However, because secure and open surface sites are rare, especially in cities, rooftop surfaces or masts are routinely chosen. Here, similar needs for power, easy access, security, and unhindered airflow exist. Placement height is a secondary concern. Raynor et al³² showed relatively uniform bioaerosol levels in the

lowermost several hundred feet at a rural site with unidirectional upwind sources, but other, especially urban, settings still are unstudied. Locations with obstructions producing stagnation points are poor choices,* as are roof margins at which vertical airflow often predominates. Where photochemical smog or other particles are dense, choice of a cleaner site should be carefully considered to prevent obscuration of material for microscopy.

Sampling efforts have helped define the importance of some dominant particles in seasonal morbidity, whereas the importance of many of the less abundant types remains unclear, most notably when exposures overlap.³³ The clinical effect of many demonstrably abundant airborne fungi (eg, ascospores of *Leptosphaeria* and *Gibberella* species) especially defies evaluation by current measures.

Even where weather and land use do not change, several annual collection cycles are needed to validly characterize outdoor aerosol prevalence at a point; thereafter, replicate efforts might progressively yield diminishing returns. Longer series are necessary for correlation of prevalence data with weather variables, land use, or effects of specific sources, including adventive species. Efforts to model these effects demand especially extended data sets. With the accepted collection methods in use, valid longitudinal comparisons at single sites are possible (even without maintaining a continuous series). Sampling data at alternative local sites can add useful dimension to concepts of regional allergen prevalence, even if few differences appear; often, this provides better use of hardware and technical effort than nurturing an unbroken series at one point.

The achievement of a North American network of outdoor stations, each with defined analytic competence, offers a potential tool with power far exceeding the sum of its components. Issues as basic as the changes, with latitude, in appearance and peak prevalence dates for short ragweed pollen invite attention. Because extreme weather conditions often are regional in scope, several affected stations might coordinate data during outlier periods of temperature, rainfall, solar input, and wind extremes, with basic insight resulting. However, premature attribution of bioaerosol changes to specific weather variables, when these often change in concert, is a lingering and seductive pitfall to be actively avoided. The full regional aerobiologic effect of large passing weather systems in specific seasons might also emerge by means of analysis of time-defined synoptic collections from a series of affected stations and correlation with National Weather Service network data. Gauging possible extended transport of native bioaerosols is a task also well adapted to present network resources, as demonstrated by findings of the mature European aerometry network.³⁴ The addition of means to assess small particle-associated allergen regionally might reveal effects with comparable or even greater clinical importance.

*The angle of elevation of a structure's roof edge estimated from the collection point offers a crude index of its tendency to restrict access to airflow; of course, its girth is also critical. Smaller angles are preferred, and those of greater than 30° are avoided if at all possible.

Reports of symptoms at home or work occurring seasonally or with specific activities suggest that offenders might be identified by short periods of appropriate air sampling. In fact, the equipment required is simple, acceptably priced, and easily deployed. Yet few clinicians elect this often practical approach, which can be implemented by a trained technician and should be reimbursable. Large wind-responsive devices are seldom available for discrete, short-period collections and might produce limiting noise levels indoors. However, several wearable personal filter cassette units³⁵ (DC pump powered) will operate for extended periods. (The most direct approach might be the nasally adapted filters developed by Renström et al,³⁶ which require no power sources.) Each of these units can be used sequentially if the recovery surface is renewed as events change. Samples can be taken singly or in sequence, also by using the personal Burkard device (DC or AC models available), which impinges aerosol in a narrow band on a greased glass slide; staining this with cotton blue allows excessive (stained) spores to be determined, and later, that venue can be studied with culture by using single petri plate sieve impingers from the line of Andersen samplers or Burkard, Ltd. The Simplair device provides a timed series of such impingement bands, when correlation with ambient events is feasible. With modest effort, glass impingers and filter cassettes can also provide samples for microscopy, culture, and immunochemical (or other) assay. Interposition of an upstream filter will partition aerosol by withholding particles above a given size. Direct light microscopy of cleared* filters is feasible, and aliquots from AGIs might be plated directly on agar media. Some loss of growth potential will occur for filter collections that spend several days awaiting processing; however, tight 24- to 48-hour transport should be quite acceptable.³⁷

Evaluation of air quality in domestic situations remains a principal unmet need of patient care, especially with fungal toxicity as a growing public health concern. Yet the essentials comprise only simple equipment and supplies, motivated technical assistance, and a modest specialized knowledge base grounded in experience. Once again, filters and AGIs serve many purposes, whereas the personal Burkard collector or simple rotorod devices provide collections for light microscopy. Of several candidate devices, the single-plate Andersen impinger is perhaps most helpful,³⁸ although overloading can obscure the catch; usually 150 seconds is a safe initial collection interval. Steadfast discretion should oppose the seductive attraction of open or gravity plates. These collect principally large spores from outdoor sources and the occasional spore aggregate from indoor growth. However, even when small single spores are abundant, they remain grossly underrepresented.

Aeroallergen sampling, despite its intrinsic attractions, is but one part of the environmental awareness that helps individualize patient care. This might be clearest in outdoor examples, in which field observations complement aerometric findings regarding pollen source activity and,

*Using immersion oils or other fluids.

incidentally, provide known material as analytic aids. Weather observations and recordings offer useful correlates because outdoor conditions, especially, can affect both current prevalence and indoor recoveries (with latencies of ≥ 1 day). Similarly, even vague inferences regarding indoor sources are guesswork without indoor versus outdoor counts and, preferably, consistent indoor preponderance documented.

Domestic sampling generally is contemplated in situations of lingering diagnostic uncertainty despite ample recorded details. The home visitor can then validate points regarding, for example, antimitic measures, pets, vermin infestations, odors, general cleanliness, and neighborhood irritant sources. Direct determination of relative humidity (by using a hygrometer or sling psychrometer) is essential. In addition, direct observations of water intrusion, mold growth, HVAC components, supplementary moisture sources, and indoor plantings and aquaria are possible. Relative humidity strongly predicts levels of microbial growth indoors.³⁹ However, central relative humidity might be quite modest despite high water activity at some surfaces because of leakage, condensation, or other adventitious moisture sources (eg, vaporizers, fish tank aerators, sterilizers, or poorly vented clothes dryers). Although visible fungus growth indoors implies an environmental problem, neither significant exposure of occupants nor resulting adverse effects on their health can be assumed. Airflow patterns might largely exclude such sites, maintaining high humidity but limiting passive spore dispersion. By contrast, a contaminated, well-ventilated source might feed microbial products into a living space displaying only dry, spotless surfaces. Controlled studies of aerosol, suitably performed and interpreted, offer an effective reality check in these settings and direction for patient and physician alike.

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