Bacteriologic Procedures in the Evaluation of Methods for Control of Air-borne Infection

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MICROÖRGANISMS ordinarily invade the atmosphere on dust particles, in droplets, or in the nuclei of instantaneously evaporated droplets, normally differentiated as follows:

The differentiation by bacteriologic procedure of each of these three classes of air-borne infection has profoundly changed our concepts of disease transmission. Thus the demonstration, a

	Dust	*Droplets*	*Droplet Nuclei*
SOURCES OF MATERIAL	Solid matter; fabrics, etc.	Fluids from nose and throat	Solid residues of evaporated droplets
PRODUCTION	Attrition	Atomization of fluids	Evaporation of droplets
MODE OF SUSPENSION	Air wafted	Projected into air by sneezing, etc.	Caught in air by evaporation
PARTICLE DIAMETER	10 to 100 microns	Larger than 100 microns	2 to 10 microns
SETTLING VELOCITY	Foot per minute to foot per second	Greater than foot per second	Less than foot per minute
TIME OF SUSPENSION	Limited by settling velocity	Less than 3 seconds	Limited indoors by ventilation
FLIGHT RANGE	Hovers in clouds	Immediate in space	Dispersed throughout indoor atmosphere
CONCENTRATION	Locally high	Immediately intense	Diffuse and dilute
AGE OF INFECTION	Survivors of accumulation	Fresh	Limited by ventilation
TYPES OF ORGANISMS	Mostly saprophytic	Parasitic and pathogenic	Parasitic and pathogenic
NUMBER PER CU. FT.	Normally below 100	Indeterminate	Normally below one
BACTERIA/PARTICLE	Clumps	Indeterminate	Seldom more than one
INHALATION	Trapped in nose and throat	Indeterminate	Penetrate to lung

	Dust	*Droplets*	*Droplet Nuclei*
MODE OF INFECTION	Endemic infection of nose and throat	Contact infection	Epidemic contagion
PATTERN OF INFECTION	Static	Indeterminate	Dynamic
VULNERABILITY	Resistant	Indeterminate	Vulnerable to chemical and physical agents
REMOVAL	Filtration and electrostatic precipitation	Best by face mask	Electrostatic precipitation; difficult by filtration
CONTROL	Air cleanliness	"spacing out"	Sanitary Ventilation (air change and equivalent air disinfection)

century ago, that boiled fluids might not spoil if protected from floating germs led to the science of bacteriology and the germ theory of disease. Air-borne germs seemed to offer a rationalization of the generally accepted miasmic theory, and the study of air-borne organisms dominated the early development of bacteriology. But dust-borne bacteria were found to be mostly saprophytic; pathogenic organisms causing intestinal disease were found mostly to be ingested; and the most typical miasm of all—malaria—was found at last to be insect-borne. The validity of these newer concepts was abundantly confirmed by the success of sanitary control based on analytical procedures.

But respiratory infections, caught from persons sharing indoor atmospheres—i.e., contagious diseases—did not yield to sanitary control; for, almost fifty years ago, bacteriologists had concluded that these infections also were not air-borne, because droplets coughed or sneezed into the air did not settle upon Petri plates exposed beyond a few feet. Our generation, taught that contagious disease was transferred by immediate contact—for proximity in time and space required to infect by such droplets was construed as a form of contact—paid little heed to sanitary ventilation.

This fallacy was however refuted, a decade ago, by recovery of virulent organisms in the residues of instantaneously evaporating droplets; these nuclei settled so slowly that they had been missed on exposed Petri plates. If the nuclei of infected droplets, coughed or sneezed into the air, drift like cigarette smoke on air currents until breathed, vented by air replacement, or removed by purification processes, then sanitary control of ventilation must be guided by bacteriological procedures capable of distinguishing these droplet nuclei.

BACTERIOLOGIC PROCEDURES

Procedures developed in half a century of intensive study of dust-borne bacteria and droplet infection were reviewed by the Committee on Standard Methods for the Examination of Air (1917); adequate methods adopted by the Association are still in force: the exposed plate gave the number of bacteria-bearing particles; the filter or washer gave the number of dust-borne bacteria. But dust-borne organisms had already been acquitted by bacteriology; between the acceptance of the final report in 1917 and the description of a new apparatus for study of the bacterial behavior of air in 1933, practically no contributions to this subject appeared in the *American Journal of Public Health*.

Experimental procedures introduced by the new sampling method aroused

immediate interest in air-borne infection and disinfection of air, which led in 1936 to the creation of a subcommittee with the special task of recommending procedures capable of differentiating droplet nuclei infection from dust- or droplet-borne infection. Six progress reports have been submitted (1937–1942) and these were integrated into a consistent system of sanitary air analysis (Wells, 1943). The creation of another subcommittee for evaluating methods of control of air-borne infection indicates the need of routine bacteriological procedures in specifying sanitary ventilation.

Droplet nuclei are characterized by small size of particle—small weight compared to surface area. Air friction clutches the surface of small particles in accordance with Stokes' Law of Viscosity, the essential quality which makes air a vehicle of infection. Except for air viscosity, particles would quickly sediment in accordance with Newton's Law of Gravity. Settling velocity which, according to Stokes' Law, varies with the surface area or the square of particle diameter, governs suspension or flight range of particles; measures air drag which sweeps them into the respiratory tract; and is often correlated with sources of infection. Settling velocity thus becomes the most significant physical determination of air-borne infection relative to pollution and purification of air.

The settling velocity of nuclei of culture broth, atomized into a quiet chamber 8 ft. on a side, has been exhaustively studied by Phelps. Simultaneous plate exposures at different points (Phelps and Buchbinder, 1941) showed uniform density throughout the chamber, enabling computation of settling velocity from successive plate exposures. Bacteria-bearing nuclei, according to these computations, settled 0.03 ft. per min.—the value plotted on Chart 1. Few droplet nuclei would therefore be deposited in ventilated rooms. So slowly do they settle that their concentration in ventilated atmospheres is determined by rate of addition divided by ventilation rate, rather than upon room activities. As they do not accumulate on surfaces, the dispersion of infected droplet nuclei depends upon the presence within the atmosphere of a person in an infective stage—a characteristic of contagious disease recognized since early times.

Dust deposit depends upon settling velocity; in fact, the ratio of area count (rate of deposit) to volume count (number of bacteria-bearing particles rather than bacteria borne by the particles) defines settling velocity of particles. Values of mean settling velocity of bacteria-bearing particles in various atmospheres as determined by this simple procedure, are tabulated and plotted on Chart 1. Obviously most dust particles settle faster than 1 ft. per min. in ordinary atmospheres. Under conditions of exceptional activity, capable of retaining coarser matter in the air, the average value is greater; under quiet conditions, or where droplet nuclei have been added to the atmosphere (e), the average value may be less. In any case, accumulated dust can be stirred up by activities independent of the presence of an infective person. Resistant organisms in such accumulations, if not removed, are a continual threat of static air-borne infections in contrast to dynamic nuclei contagion. Dust particles, unlike droplet nuclei which readily penetrate to the lung, are more associated with endemic infections of the nose and throat than epidemic phenomena of contagion.

It follows, from the small size of atomized droplets, that seldom would more than one organism be included in a nucleus; this distinguishes droplet nuclei from dust-borne clumps of bacteria. Individual organisms in dust particles, collected in liquids, as by the accepted methods of the Association,

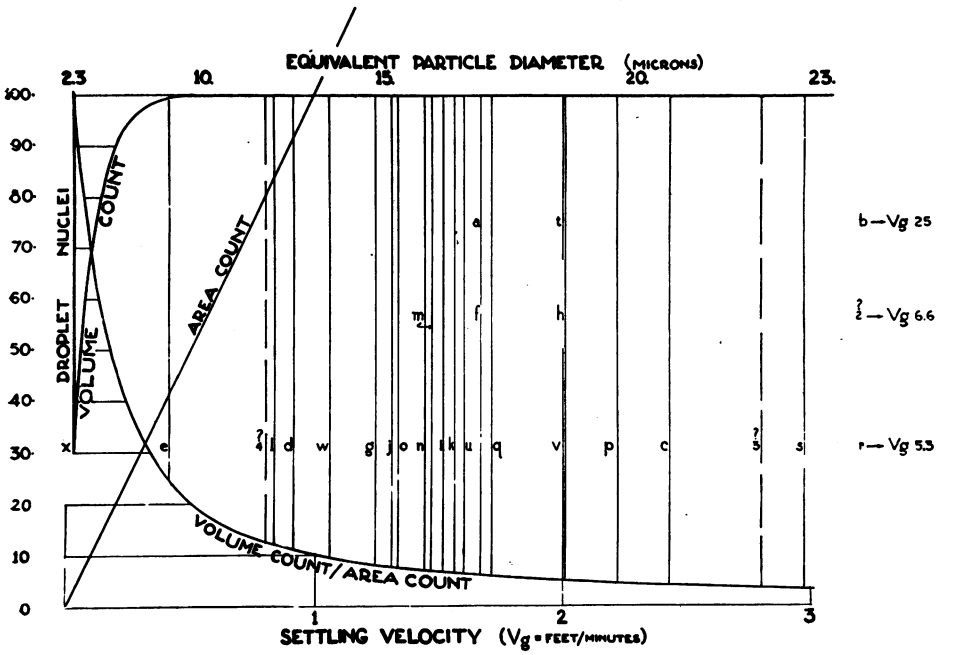

CHART 1. SETTLING VELOCITY of Air-borne Infection, Vg = A/D, where A = rate of deposit and D = density. AREA COUNT = rate of deposit per sq. ft. per min. (15 min. exposure of Petri dish). VOLUME COUNT = air centrifuge count per cu. ft. per density of 100 bacteria-bearing particles per cu. ft. VOLUME COUNT/AREA COUNT is multiplied by ten.

a. Outside air, Near laboratory	1.67
b. " " , Near Textile Mills	25
c. Textile mill air, Dusty (carding, etc.)	2.43
d. " " " , Settled (spinning, etc.)	0.91
e. " " " , Humidified (weaving, etc.)	0.42
f. Hospital air, Clinic (children, Boston)	1.66
g. " " , Cubicle Wards (infants, Philadelphia)	1.14
h. " " , Operating rooms, Boston	2.04
i. " " , " " , Pittsburgh, Air-conditioned	1.56
j. " " , " " , " , Not air-conditioned	1.32
k. " " , " " , Iowa City, General surgery	1.59
l. " " , " " , " " , Head surgery	0.83
m. " " , " " , " " , Orthopedic surgery	1.47
n. " " , Delivery rooms, Iowa City	1.41
o. " " , Halls, Philadelphia	1.33
p. " " , " , Iowa City	2.22
q. Orphanage air (Philadelphia), Nursery	1.71
r. " " " , Play-room (1–2 year children)	5.26
s. Dormitory, Army barracks used as ward, Morning	2.93
t. " " " " " " , Evening	2.00
u. Schools, Unirradiated	1.6
v. " , Irradiated	2.0
w. Sneeze infected air	1.06
x. Droplet nuclei from atomizer	0.03

References from (a) to (t), also (x) given on Table 1 (Wells, 1943).
(u) and (v), anonymous.
(w) from Bourdillon, Lidwell, and Lovelock, *Brit. M. J.*, 1, 42 (Jan. 10), 1942.

are separated and counted as colonies; whereas a droplet nucleus would probably register merely as a single colony. Thus a large number of bacteria per particle, or total bacteria count divided by the count of bacteria-laden particles, may be a useful index of dustiness rather than infectiousness of air.

These procedures merely characterize dust in routine analysis, for droplet nuclei seldom carry more than a negligible fraction of the total number of air-borne bacteria. Their genesis in the respiratory system, however, gives hygienic significance out of all proportion to their number, and necessitates means for distinguishing them from dust. Generally they contain organisms typical of the nasopharynx—lactose fermenting, Gram-positive, hemolytic streptococci. These organisms grow well on blood agar; but dilution methods analogous to those used for isolation of coliform organisms in drinking water are more sensitive. Air-borne organisms, collected in liquids, are distributed into enrichment broth tubes exactly as in testing drinking water, the presence of acid rather than gas in lactose being presumptive evidence of pollution. Hemolytic organisms are confirmed on blood agar containing enough gentian violet to inhibit staphylococci. The method of enumeration is similar to that applied in water analysis.

The growing recognition of air-borne infection as a basis for a theory of contagion has revived interest in sanitary ventilation. Just as bacteriologic procedures guided the development of water purification practice, so will they evaluate air disinfection processes. The rate of disposal of droplet nuclei infection can be measured by sampling standard suspensions of organisms atomized into air at a constant rate. Ventilation rates are proportional to the equilibrium concentrations, one dieaway rate being determined by successive samplings. Compared to the amount of pure air replacement required to effect equal removal of the organisms, results express disinfection in terms of equivalent ventilation. To estimate disinfection against pathogenic organisms, relative vulnerability is determined in the laboratory against the standard organism.

Interpretation of the data is also subject to statistical limitations. Single determinations have little significance; fluctuations in occupation load in ventilation are greater than in pollution of drinking water. The hygienic quality of air, as in water, is an abstraction derived from a statistical analysis of a large number of samples obtainable only with a practical routine.

SAMPLING

It is generally conceded that, " There is no simple method that is optimal for all circumstances and species, and some degree of compromise is necessary." For our purpose, an air sampler must be capable of concentrating small numbers of nuclei from large volumes of air (a person breathes several cubic feet of air per hour) upon solid culture media or in small volumes of liquid. Phelps, in a careful analysis of the mechanism of deposition in the centrifuge, " operating between 4,000 and 5,000 r.p.m. with an air flow of approximately 1.4 to 1.9 cu. ft. per min.," recovered approximately one-third of the nuclei settling " approximately 22 inches per hour." Substituting this value of centrifuge performance in his equation, or a formula simplified by assumption of turbulent flow (Wells, 1943), gives for rated flow of 1 cu. ft. per min. and density of 100 particles per cu. ft. the " Volume Count " shown on Chart 1. Approximately one-half of these smaller nuclei and practically all particles settling more than 2 in. a minute (probably more representative of nuclei coughed or sneezed into the

atmosphere) would be collected according to this formula.

Complete collection of the smallest bacteria-bearing nuclei, rarely required, can be accomplished by reducing the flow of air or increasing the speed of the centrifuge. The rated flow is a practical compromise between complete recovery of the smallest laboratory nuclei capable of bearing a bacterial cell and the distributed collection of dust-borne bacteria on solid media satisfactory for counting. The settling velocity of droplet nuclei encountered in ventilation practice lies between these values, and their recovery by the centrifuge is practically complete.

A careful study of impingement methods developed for collection of fine industrial dusts (Bourdillon, Lidwell, and Thomas, 1941) has established the necessary velocity of particles of bacterial dimensions (through slits at optimal distances from the agar surface) to be 334 ft. per min.; sampling efficiency falls rapidly at lower velocities—dropping to one-fifth as the velocity falls to one-fourth. The slit sampler, satisfying these conditions, recovered from three to five hundred times as many fine broth spray nuclei from 10 cu. ft. of air as settled on a Petri plate in 10 minutes. The authors conclude, "This range of over 200 to 1 on the slit Petri ratio is very striking proof of the selective action of open dishes when used for collecting bacteria. It is probably safe to say that such dishes are at least 200 times as effective for collecting large bacteria-carrying particles as for single washed air-borne bacteria."

All samplers except the centrifuge require auxiliary equipment to draw measured quantities of air through the instrument. Convenience is important in collecting large numbers of samples, and the convenience of the centrifuge—creating its own measured air flow and depositing both dust and droplet nuclei directly on solid or in fluid media—recommends it for field use. To obtain uniform results it is necessary to standardize on some method, even if it be acknowledged that no method can be most satisfactory for every purpose. The subcommittee, therefore, tentatively recommends the centrifuge, because of its simplicity, adaptability, and convenience, for practical sanitary air analysis.

CULTURE

It is also conceded that, "The efficiency of any sampling method is measurable only in terms of the optimum cultural technique for each bacterial species present and for each variation in the material under test." Most dust organisms are saprophytes which grow well on plain culture media. In determining the count either of bacteria-bearing particles or of total organisms, the subcommittee has adopted media used by the New York State Department of Health in sanitary analysis.

Though nasopharyngeal parasites—specifically hemolytic streptococci of the nose and throat—are more fastidious, they grow well in media containing proteose and tryptose. Blood agar containing these ingredients becomes selective with the addition of 2 p.p.m. of gentian violet, which inhibits most common staphylococci in air; it is hardly necessary to complicate the medium with substances for inhibition of Gram-negative organisms—most of which are short-lived in air. Organisms enriched in proteose-tryptose broth, containing lactose and brom thymol blue to indicate acid production, and also ¼ p.p.m. of gentian violet to inhibit staphylococci, may be transferred to selective media for confirmation. Tubes showing acid in 24 or 48 hours are streaked on the gentian violet blood agar, hemolytic organisms being further confirmed as Gram-positive.

SUMMARY OF STANDARD BACTERIOLOGIC PROCEDURES

I. Density of bacteria-bearing dust particles
  A. Area count per sq. ft. per min.
    (a) 15 min. Petri dish count (rough approximation of bacteria-bearing dust particles per cu. ft.).
  B. Volume count per cu. ft.
    (a) Slit sampler
    (b) Air centrifuge, solid media
II. Settling velocity (ft. per min.) Vg=A/B
    (a) Particle diameter in microns = $13\sqrt{Vg}$
III. Total bacteria per cu. ft.
  C. Amer. Public Health Assn. methods
    (a) Washings from filtration
    (b) Air washers
  D. Air centrifuge
    (a) Collection in liquid
IV. Average number of bacteria per particle = C/B or D/B
V. Dust-borne pathogens
    (a) Beta hemolytic streptococci on blood agar plates
VI. Median volume per bacteria-bearing droplet nucleus
    (a) Direct count of alpha hemolytic streptococci on blood agar
    (b) Median sample volume containing lactose-fermenting hemolytic streptococci by dilution method
VII. Measurement of sanitary ventilation by sampling at center of room test organisms atomized into four quadrants
    (a) Dieaway method
    (b) Equilibrium method

INTERPRETATION

Information obtained by items I to V characterizes bacteria-bearing dusts; the determination of volume and area counts (by I), and their ratio (by II), determines settling velocity and particle diameter. Coarse dust particles indicate atmospheric turbulence rather than dust accumulation, and the presence of particles with high settling velocity indicates activity in a room; so area and volume samples should be taken simultaneously.

The "total" bacteria per cu. ft. (by III) indicate on the other hand dust accumulation as well as air disturbance. Bacterial masses in dust fragments from contaminated fabrics, or dirt tracked into the room on shoes, are indicated by high average number of bacteria per particle (IV). Where dust arises from infected persons, clothing, or bedding, as in hospital wards and to a less degree in barracks where men are housed under crowded conditions, dust may have special significance. This index of air dirtiness may be useful in judging the efficiency of dust suppressive measures.

The determination of beta hemolytic streptococci on blood agar plates (by V) has received considerable attention in recent years. Compared with the total number of streptococci determined by dilution methods, beta hemolytic streptococci collected on plates may provide additional information upon sources of infection. Also the epidemiology of air-borne infection, in so far as the clinical pattern is determined by the penetration of particles into the respiratory system, depends upon particle size as indicated by settling velocity.

Breathing droplet nuclei constitutes prima facie evidence of the exchange of respiratory organisms—the principal hygienic hazard of confined air. This measurement of occupation load upon ventilation by special techniques is a major task of sanitary air analysis. The test for respiratory organisms (by VII) in breathing air will, as did the test for intestinal organisms in sanitary analysis of drinking water, therefore become the most useful bacteriological procedure in ventilation surveys of public gathering places—movies, schools, department stores, factories, and other places of work and recreation.

Evaluation of methods of control of air-borne contagion will ultimately be based on measurement of sanitary ventilation (by VII) after threshold sanitary ventilation has been established epidemiologically.

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New Levels of Professional Compensation Set in Veterans Administration

Public Law 293, now in effect in the United States, creates in the Veterans Administration, Washington, a Department of Medicine and Surgery under a chief medical director. Major General Paul R. Hawley, M.C., U.S.A., has been designated to serve as Chief Medical Director. Professional personnel is set up under the Chief Medical Director in an organization resembling the corps service of the Army, the Navy, and the Public Health Service. General Bradley, the chief of the Veterans Administration, has announced that there is an immediate need for 1,125 physicians, 1,200 nurses, and 100 dentists.

Public health workers will be specially interested in the salary levels set for these clinical specialists. The chief medical director will receive $12,000, a deputy medical director will receive $11,500 and eight assistant medical directors will be paid $11,000 each.

In the medical service the Chief grade carries salaries $8,750 to $9,800; Senior grade $7,175 to $8,225; Intermediate grade $6,230 to $7,070; and the "Full grade" $5,180 to $6,020, with Associate and Junior grades at lower levels.

Appointments of the executives will be for a four year term, subject to removal by the administrator for cause. Specialists certified by the Veterans Administration may be paid a salary up to a limit of $11,000. Promotions will be made on recommendation of special Veterans Administration boards, which will operate like the selection boards of the Army and Navy for higher ranking officers.

This plan will also involve setting up a series of residencies in Veterans Administration hospitals where younger physicians may qualify to become specialists. It is believed that this will serve to raise substantially the quality of medical care available to veterans and it will be similar to the system now in effect in the larger medical centers.