

# BACTERIOLOGIC PROCEDURES IN SANITARY AIR ANALYSIS

## WITH SPECIAL REFERENCE TO AIR DISINFECTION

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Evidence, amassed from diverse sources (Wells, Wells and Mudd, 1939) proves the importance of air-borne infection. The classic work of Laidlaw and his associates (Dunkin and Laidlaw, 1926; Andrewes and Glover, 1941) upon air-borne spread over considerable distance, of the viruses of dog distemper and influenza, Lurie's (1930) experiments on tuberculosis, dust-borne between animals in separate cages, observations on air-borne surgical infection by Hunt (1933) and Meleney, (1935), Cruickshank's (1935) studies of air-borne infection of burns, the Colebrooks' (1935, 1936) analysis of the rôle of nasopharyngeal organisms in puerperal infection, and the work of Allison (Brown and Allison; Allison and Brown 1937) on streptococcal infection in fever wards and of McKhann (1938) on nosocomial infection in children's wards, are examples of the variety of evidence accumulated in recent years. Conversely, the effect of radiant disinfection of air in reducing surgical infection (Hart, 1936; Overholt and Betts, 1940) and cross-infection in pediatric wards (del Mundo and McKhann, 1941; Robertson, Doyle and Tisdale, 1943), a nursery (Rosenstern, 1942) and an orphanage (Barenberg, *et al.* 1942), and in the environmental control of epidemic spread of contagion in schools (Wells, Wells, and Wilder, 1942), now provides experimental evidence of the importance of air-borne infection. Improved bacteriologic procedures in sanitary air analysis have also reinterpreted Flugge's theory of droplet infection (Wells, 1934; Wells and Stone, 1934), proved quantitative inhalation of droplet nuclei infection to the lung (Wells and Lurie, 1941), demonstrated habitual exchange during winter months of respiratory flora among aggregations occupying enclosed atmospheres (Wells and Wells, 1936), and measured the sanitary inadequacy of present ventilation practice and the potentiality of air disinfection in control of dynamic spread of air-borne infection (Wells and Wells, 1943). Elements of three types of procedure presented to the Committee on Ventilation and Atmospheric Pollution of the Industrial Hygiene Section of the American Public Health Association by the Subcommittee on Bacteriologic Procedure (1937-1943) can now be integrated into a consistent system. (References to these reports will be indicated by year only under the heading "APHA" and original references given in the reports will be repeated only when cited for a different purpose.)

1. *Sanitary survey of inhabited atmospheres.* Rapid methods of collecting

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particles from large volumes of air enable the routine sampling necessary to obtain statistically significant indices of sanitary ventilation.

2. *Experimental studies of bacteria suspended in controlled atmospheres.* The mechanics of air-borne infection and control by ventilation have been studied bacteriologically in experimentally controlled atmospheres.

3. *Measurement of sanitary ventilation.* By quantitative sampling of test organisms added to atmospheres under different ventilating conditions, the hygienic importance of air disinfection has been demonstrated.

*Settling rate.* The density of bacteria laden particles ( $D$  = number per cubic foot) multiplied by settling velocity ( $V_s$  = feet per minute) determines settling rate ( $A$  = count per sq. foot per minute). If, for example,  $V_s = 1$ , then  $D$  particles will settle upon a Petri dish (1/15th sq. ft.) in 15 minutes and the settling rate of larger particles ( $V_s > 1$ ) or smaller particles ( $V_s < 1$ ) will be proportionately greater or less. The atmospheric density of particles of given size depends upon the rate at which they are lifted into or removed from the air. Since greater effort is required to lift larger particles which settle faster, and since smaller particles which remain longer in air are produced with greater difficulty,<sup>3</sup> actual size distribution under uniform conditions tends toward a dynamic equilibrium where mean settling velocity becomes relatively constant (table 1). Within normal limits of variation, then, the Petri dish count, the simplest and for some purposes the most significant determination, is proportional to bacterial concentration ( $D = AV_s$ ) (APHA 1942).

*Volume settling.* Particles settle rapidly from small confined volumes (Winslow, 1908), but in a closed room the density is progressively reduced by settling, while the rate of deposit remains constantly proportional to the residual density, a relation leading to the rate of decrease in both density and rate of deposit,

$$\log_e D/D_0 = -V_s t/H$$

The logarithm of the residual density, expressed as a proportion of the original density, is a linear function of time ( $t$ ) when settling velocity ( $V_s$ ) and room height ( $H$ ) are constant; or of settling velocity when room height and settling time are constant.

If  $A_t$  represents the number of particles which settle on unit area in a given time, then

$$A_t H = D_0 - D = D_0 - D_0 e^{-V_s t/H}$$

$$\text{or } A_t H \doteq D_0$$

as settling velocity and time increase and chamber height decreases. Thus  $D_0 e^{-V_s t/H}$  becomes a small correction term where time and settling velocity are large and chamber height is small (A.P.H.A., 1942).

<sup>3</sup> Thus the work done in grinding powders increases out of all proportion to their fineness, and the velocity of air required to atomize liquids similarly increases without limit as droplets become very small. Just as deposits of water-borne sand tend toward uniformity, so does the equilibrium between carrying power of air and rate of deposition tend to classify air-borne particles.

TABLE 1  
*Mean settling velocity (Vg) of bacteria laden dust*

SOURCE	NUMBER OF SAMPLES	Vg (FEET PER MINUTE) <sup>a</sup>
Outside air <sup>b</sup>		
Near laboratory.....	14	1.67
Near Textile Mills.....	14	25
Textile mill air <sup>b</sup>		
Dusty (carding, etc.).....	17	2.43
Settled (spinning, etc.).....	17	0.91
Humidified (weaving, etc.).....	14	0.42
Hospital air		
Clinic (children, Boston) <sup>c</sup> .....	23	1.66
Cubicle Wards (infants, Philadelphia) <sup>c</sup> .....	27	1.14
Operating rooms		
Boston <sup>c</sup> .....	8	2.04
Pittsburgh <sup>d</sup>		
Air-conditioned.....	76	1.56
Not air-conditioned.....	76	1.32
Iowa City <sup>e</sup>		
General surgery.....	108-64 <sup>(f)</sup>	1.59
Head surgery.....	80-46 <sup>(f)</sup>	0.83
Orthopedic surgery.....	69-36 <sup>(f)</sup>	1.47
Delivery rooms, Iowa City <sup>e</sup> .....	41-28 <sup>(f)</sup>	1.41
Halls		
Philadelphia <sup>e</sup> .....	3	1.33
Iowa City <sup>e</sup> .....	38-16 <sup>(i)</sup>	2.22
Orphanage air (Philadelphia) <sup>e</sup>		
Nursery.....	6	1.71
Play-room (1-2 year children).....	6	5.26
Dormitory		
Army barracks used as ward <sup>f</sup>		
Morning.....	3	2.93
Evening.....	3	2.00
Sneeze infected air <sup>g</sup> .....	11-8 <sup>(i)</sup>	1.06
Droplet nuclei from atomizer <sup>h</sup> .....	150	0.03

<sup>a</sup> Area count (per sq. ft. per min.)/volume count (per cu. ft.).

<sup>b</sup> Wells, W. F., and Riley, E. C. An investigation of the bacterial contamination of the air of textile mills with special reference to the influence of artificial humidification. *J. Indust. Hyg. and Toxicol.*, **19**, 513, 1937.

<sup>c</sup> Wells, W. F. Sanitary Air Analysis. Unpublished paper read before A.P.H.A., Detroit, Oct. 11, 1940.

<sup>d</sup> Cook, W. L. Report on Air-conditioning in Surgery. Univ. Pittsburgh, 1940.

<sup>e</sup> Macdonald, K. Quantitative bacterial analysis of the air of operating rooms of a general hospital. *Am. J. Hyg.*, **31**, 74, 1940.

<sup>f</sup> These figures were obtained from investigations carried out by the Commission on Cross Infections in Hospitals of the Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, and are cited by permission of the Surgeon General.

<sup>g</sup> Bourdillon, R. B., Lidwell, O. M., and Lovelock, J. W. Sneezing, and disinfection by hypochlorites. *Brit. Med. J.*, **1**, 42, January 10, 1942.

<sup>h</sup> Phelps, E. B., and Buchbinder, L. Studies on microorganisms in room environments. I. A study of the performance of the Wells air centrifuge and of the settling rates of bacteria through the air. *J. Bact.*, **42**, 321, 1941.

Number of volume and area samples, respectively.

*Air centrifuge.* The air centrifuge is essentially a sedimentation chamber within which settling velocity is increased by centrifugal force, fixed height

(radius) and settling time (flow) determining an operating constant for the machine. This constant, experimentally determined for smallest bacteria-bearing nuclei gives

$$D = B/(1 - e^{-12v_s}),$$

as the formula of normal machine performance (Phelps and Buchbinder, 1941; Wells, 1942), where ( $B$ ) is the centrifuge count per cubic foot.

For particles settling faster than one-half a foot per minute, the correction term ( $e^{-12v_s}$ ) becomes insignificant, and the formula of performance reduces to  $D = B$ . The centrifuge count thus approximates true density for most particles encountered in surveys summarized in table 1, and if the correction term cancels in ratios used to determine bacterial changes under experimental conditions, it would seldom be required in practice (A.P.H.A., 1942).

*Operation.* The air centrifuge combines three functions in one operation: pumps a measured quantity of air through a collecting chamber; collects particles in measured quantities of media; or plants them directly on solid nutrient media ready for incubation and enumeration. Solid media must be stiffened by addition of 7-10 grams of agar per liter to the ordinary formula, and in removal from the machine and in incubation, the tubes should be kept horizontal. Dehumidification of the incubator air (by calcium chloride) retards growth of troublesome spreaders and counting may be facilitated by special apparatus (A.P.H.A., 1939). Dilution methods also may be adapted with measured quantities of liquid, the centrifuge serving as a pipette for collecting bacteria from measured quantities of air (A.P.H.A., 1941).

*Impingement.* The principle of the Owens dust counter has recently been applied to bacterial air analysis (Bourdillon, Lidwell and Thomas, 1941). Particles from a high velocity jet impinge upon a moving agar surface, 2 mm. from the nozzle. The narrow slit nozzle radial to a rotating Petri plate delivers one cubic foot a minute under negative pressure of eleven inches of water. The efficiency of the slit sampler, as it is called, depends upon scrupulous adherence to the dimensions given by the authors, and, if so designed and operated, will remove particles of bacterial dimensions. Fulfillment of these exacting requirements is facilitated by ingenious contrivances neatly built into the apparatus. Where adequate suction is available, this provides a simple, compact and accurate collecting device. Auxiliary equipment required for operation, however, adds to the weight of equipment carried into the field.

Arbitrary combinations of impingement with gravity settlement and with electrical precipitation have also been proposed (Hollaender and DallaValle, 1939; Berry, 1941). Neither settling time nor velocity of approach are adequate to remove small particles, and interpretation depends upon empirical calibration of each device.

*State of suspension.* Since state of atmospheric suspension reflects the conditions responsible for the presence of particulate matter in air, settling velocity may distinguish sources of bacterial pollution. The sanitary behavior of coarse dust particles also differs significantly from that of aerosols. Mechanical removal of particulate matter by purification devices or by the filtering mechanisms of

the nasal passage is more effective against larger particles, but chemical or radiant disinfection may be more effective against smaller particles which penetrate more readily to the lung. Settling velocity, therefore, as measured by the ratio of

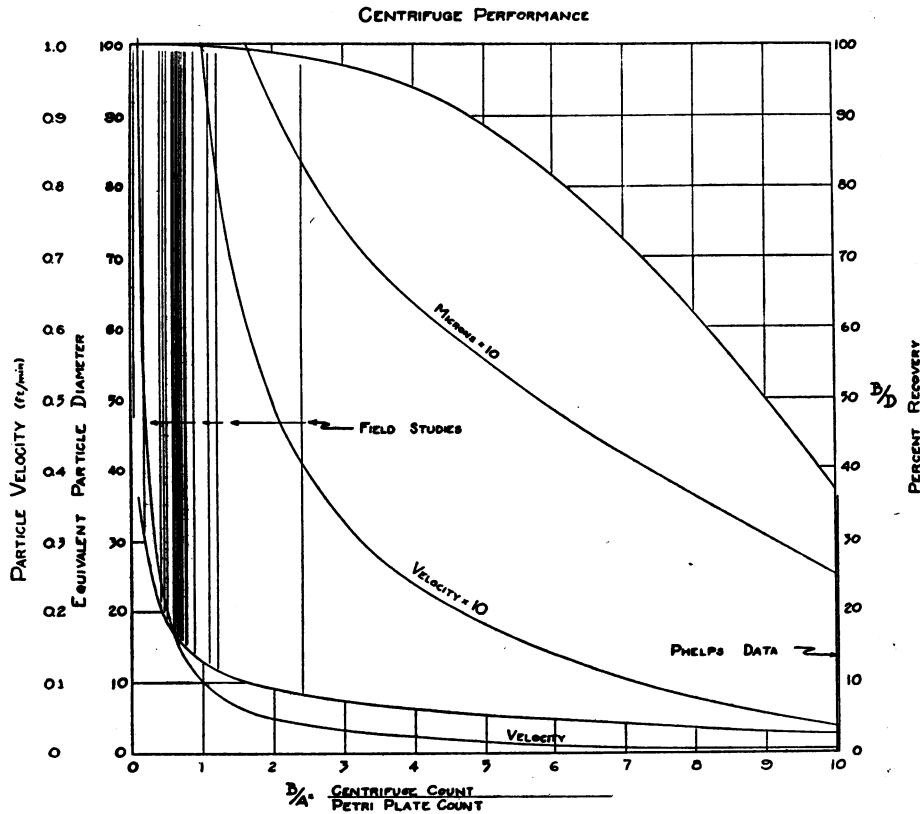


CHART I. AIR CENTRIFUGE PERFORMANCE

$$\text{Per cent recovery} = 100 B/D = 1 - e^{-12V_0}$$

where the constant 12 is derived from Phelps data (Phelps and Buchbinder, 1941) assuming turbulent flow (Wells, 1942).

$$\text{Particle velocity} = V_0 = A/D$$

Field study data taken from Table I; equivalent particle diameter calculated from Stokes law (curve "velocity  $\times 10$ " magnifying ordinates tenfold).

$$\text{Equivalent diameter (microns)} = 13.3 \sqrt{V_0}$$

(Curve "Microns  $\times 10$ " magnifying ordinates tenfold).

- A = Area count (per sq. ft. per min.)
- B = Centrifuge count (per cu. ft.)
- D = Density (particles per cu. ft.)
- $V_0$  = Settling velocity under gravity (ft. per min.)

volume to area count, serves in the interpretation of bacterial analysis of pollution and purification of air. The ratios of centrifuge tube to plate count, under various ventilating conditions are shown on chart 1, together with indicated particle size and percentage recovery.

It is apparent that dust particles rather than droplet nuclei usually dominate bacterial air counts. In normal respiration these generally harmless saprophytic organisms from decomposing organic matter are filtered out in the nasal passages. Though not proportionately represented in the count, and numerically insignificant, nuclei from evaporated droplets derived from tissues which may be infected are of major hygienic importance. Their detection provides a more serious problem of bacteriologic procedure than the measurement of air cleansing from dust.

*Bacteria laden particles.* Rarely under field conditions will the number of bacteria-laden particles represent the total number of bacteria present in air. The chance that dust fragments bear single organisms is remote, and particles of pulverized dirt are more likely to carry hundreds of bacteria. If bacterial clumps of decomposing matter collected in liquids are shaken apart before planting, the number of colonies will greatly exceed the number obtained by direct precipitation on agar. The ratio of counts from solid and liquid media collections thus become a bacterial index of air dirtiness.

Dust may, on the other hand, have greater sanitary significance in wards where the sick are gathered together, and where cross infection of the nose and throat by hemolytic streptococci is not uncommon. Thus, the liquid method of collection may provide a sensitive index of air cleanliness in hospitals. Bacteria collected by the Petri filter method, adopted by the American Public Health Association (Committee on Standards for the Examination of Air, 1917), are washed into sterile water preliminary to planting and several direct methods of air washing have also been proposed (Rettger, 1910; Palmer, 1916; McConnell and Thomas, 1925; Robertson, Bigg, Miller and Baker, 1941; Wheeler, Foley and Jones, 1941; Moulton, Puck and Lemon, 1943).

*Sanitary air analysis.* The total count, therefore, offers little direct evidence of air-borne infection, nor can bacteriologic procedures hope to recover the few pathogenic organisms in the huge volumes of indoor air breathed per winter, which account for the universal spread of epidemic respiratory disease. Sanitary interest in such procedures rests upon ability to evaluate the defensive barriers against the environmental spread of air-borne infection. Just as the sanitary hazard from drinking water is judged by the number of coliform bacteria of intestinal origin, so does the number of respiratory streptococci in the air offer a basis for estimating hazard of respiratory contagion. Parallel procedures of analysis can be applied to air bacteria collected in liquids, if media adaptable for identification of alpha streptococci are substituted.

Bacteria from 10 cubic feet of air are equally distributed into ten tubes of lactose proteose No. 3 broth containing an indicator (brom-thymol blue) and incubated at 37° for twenty-four hours. Laboratory routine may be simplified by use of circular batteries of Wasserman tubes dispensing with manipulation of cotton plugs (Wells, 1942). Streptococci of the *S. salivarius* type form acid in this medium, but so also do staphylococci which are abundant in inhabited atmospheres. Acid-forming organisms are therefore transferred to gentian-violet blood agar (0.00005 per cent gentian violet in protease No. 3 blood agar)

which inhibits staphylococci. Tubes showing acid after twenty-four hours, and those which do not yield streptococci on the plates, are streaked after forty-eight hours. Recovery of streptococci from a majority of cubic foot volumes tentatively indicates inadequate ventilation (APHA 1942). The dilution method requires less skill than isolation of alpha hemolytic streptococci collected directly on blood agar (2 per cent blood in protease No. 3 agar), but with experience the rusty halo can be recognized even among the large numbers of other organisms obtained in heavily contaminated atmospheres.

*Measurement of sanitary ventilation.* The principal sanitary contribution of bacteriologic procedures has so far been experimental. Study of the behavior of air-borne pathogenic microorganisms in controlled atmospheres has unveiled a mechanism of spread of contagion and disclosed effective means of control. The effect of physical and chemical agents upon the viability of air-suspended microorganisms and the effect of state of suspension upon the invasion of the respiratory tract can be determined by quantitative laboratory techniques for infecting and disinfecting air. By amplification of tests under ventilating dimensions with a standardized index organism the practical performance of sanitary ventilation can then be interpreted through these basic data.

Bacteriologic procedures for measuring sanitary ventilation follow the principles laid down by Pettenkofer in determining ventilation load by measuring the equilibrium concentration of carbon dioxide constantly expired per occupant. Lethal equivalents by air disinfection, however, can be determined only by bacteriological methods. Test organisms are atomized at a constant rate into the atmospheres and their rate of elimination determined by the air centrifuge (A.P.H.A., 1938, 1942).

The technique is adapted to the form of the space and the ventilating conditions to be measured. In ordinary rectangular rooms up to 10,000 cubic feet capacity, the following setup should yield satisfactory results under normal winter conditions. An air centrifuge is centrally placed at working level and four atomizers are placed at the same height about half way (about 10 feet) between the centrifuge and corners of the room. An atomizer<sup>4</sup> containing a liter of water to which has been added 10-15 ml. of a 24-hour culture of *Escherichia coli* in lactose broth, delivers a constant amount (about 1 ml. per minute) over test periods.

The concentration of bacteria in a room usually reaches equilibrium in twenty minutes, depending upon the ventilating rate. Since equilibria are reached more quickly at higher rates of ventilation, it is better to commence a series with maximum ventilating rate and progress to minimum air change. After each equilibrium concentration following a ventilation change has been reached, a series of three 5-minute samples at minute intervals are collected on eosin methylene-blue agar. Atomizers are then turned off and a similar series immediately collected to compute the die-away.

The working rule for obtaining equivalent sanitary ventilation from this

<sup>4</sup>The "Fragrant Mist" atomizer, manufactured by Walton Laboratories, Inc., Irvington, N. J., meets these specifications.

die-away becomes: *the number of lethals<sup>5</sup> or overturns per hour is equal to 138 times the difference in the logarithms of two counts divided by the elapsed time in minutes between the two counts.* Equilibrium concentration being proportional to rate of elimination, if rate of addition of infection remains constant, equivalent air change can be readily computed for each ventilating condition.

*Concentration gradients.* Pollution gradients from infection to disinfection zones (A.P.H.A., 1942) can be studied by simultaneous sampling of a single source of infection at different points. Gradients are inherent in ventilation, and also result from the limited viability of some organisms. They depend upon the relative rates of mixing and disappearance of the organisms, the measurement of which may be required to interpret the pattern of spread of infection among members of an aggregation.

*Interpretation.* Ultimate interpretation must be based upon broad sanitary principles and epidemiologic experience. Their meaning in terms of air-borne infection and disinfection will become apparent as data accumulated by these procedures are correlated with hygienic indices of the spread of contagion.

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<sup>5</sup> The disposal of bacteria by air replacement and by disinfection can strictly be equated only for the particular organism tested but the vulnerability of different microorganisms to ultraviolet radiation is sufficiently uniform to justify the interchangeability of these units in ventilating practice.



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