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Single-Stage Impaction Device for Particle Sizing Biological Aerosols

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ABSTRACT

MALLIGO, JOHN E. (U.S. Army Biological Laboratories, Fort Detrick, Frederick, Md.), AND LEON S. IDOINE. Single-stage impaction device for particle sizing biological aerosols. Appl. Microbiol. **12**:32-36. 1964.—A single-stage impactor device, capable of fractionating a bacterial aerosol into its component mass size classes while preserving the viability of sensitive vegetative cells against the deleterious effects of desiccation and impaction, is described. Among the valuable features of the impactor are its negligible wall loss, ease of fabrication and use, and relative inexpensiveness.

Progress in the technology of biological aerosols has demanded increasingly sophisticated instrumentation and experimental procedures. In the fields of epidemiology and public health, an ever more prominent role is being taken by particle size studies of infectious aerosols. This general appreciation of the importance of size of inhaled particulates has resulted in the design and fabrication of numerous devices to fractionate biological aerosol samples into their component size classes. The variety and number of such devices were indicated by Wolf et al. (1959).

It is apparent that, with most biological materials, preservation of viability is a difficult problem in sampling devices involving high air-flow rates, such as those encountered with the cascade impactors (May, 1945; Mitchell and Pilcher, 1958), cyclones (Harris and Eisenbud, 1953), and air centrifuges (Wells, 1933). Death of cells results from a combination of desiccation and impaction effects, with the former perhaps the more important. One approach to preservation of dehydration-sensitive biological material under these adverse conditions has been to provide for impaction on collection surfaces coated with agar, petroleum jelly, or other similar materials, to diminish impaction losses and to maintain a degree of moisture compatible with viability of the collected sample. Limited success has been achieved by use of this procedure with available laboratory devices used for sizing concentrated bacterial aerosols.

Because of the limitations of existing devices, it was necessary to design an instrument to provide accurate viable size-fractionation of biological aerosols under somewhat specialized conditions. The restrictions on the desired design were numerous. (i) The device must be able to fractionate a concentrated aerosol of viable organisms with reasonable sharpness and without appreciable killing effect. (ii) Assay of the samples must be rapid and simple. (iii) Fabrication, manipulation, and maintenance of the device must be fairly economical. (iv) Size of the device and amount of necessary equipment must be kept to a minimum consistent with large-scale operations.

MATERIALS AND METHODS

Single-stage impactors. Consideration of the various aerodynamic systems available led to the choice of that of Ranz and Wong (1952) as a point of departure in developing the desired instrument. This work is well known, so only a brief resume of it will be given here. Ranz and Wong were able to relate particle stopping distance and jet diameter by the dimensionless inertial parameter, ψ, according to the following formula:

$$\psi = \frac{C\rho_p V_0 D_p^2}{18\mu D_c}$$

where C is the empirical correction factor for air resistance to small particle travel, ρ_p is particle density in grams per cubic centimeter, V_0 is jet velocity in centimeters per second, D_p is particle diameter in centimeters, μ is viscosity of air (poise), and D_c is jet diameter in centimeters.

Various physical configurations were fabricated with dimensional relationships calculated to extend the particle size classification system of Ranz and Wong (1952) into the region of 1 to 19 μ by means of an array of devices, with graduated jet sizes, run in parallel. The final design (Fig. 1) comprises a cylindrical housing 43 mm long and 57 mm in diameter fashioned from aluminum bar stock and threaded to receive an insert with a jet and impaction plate. The same housing is used with all inserts, which differ according to their particle size cutoff value. Shop drawings of the insert are shown in Fig. 2. Table 1 gives the critical insert dimensions, i.e., jet diameter and jet-to-impaction plate clearance for the particle size cutoffs found most useful in our work. In use, the impactor is attached to a modified all-glass impinger (May and Harper, 1957; Tyler, Shipe, and Painter, 1959), whose orifice meters air through the impactor at the rate of 12.5 liters per min. The impactor and impinger assembly are shown in Fig. 3. When sampling vegetative or other labile organisms, 2 ml of a suitable agar are used on the impaction plate to provide a collection surface for the larger, heavier particles of any given sample, while an appropriate collecting fluid in the impinger allows the lighter, smaller particles to be collected without losing

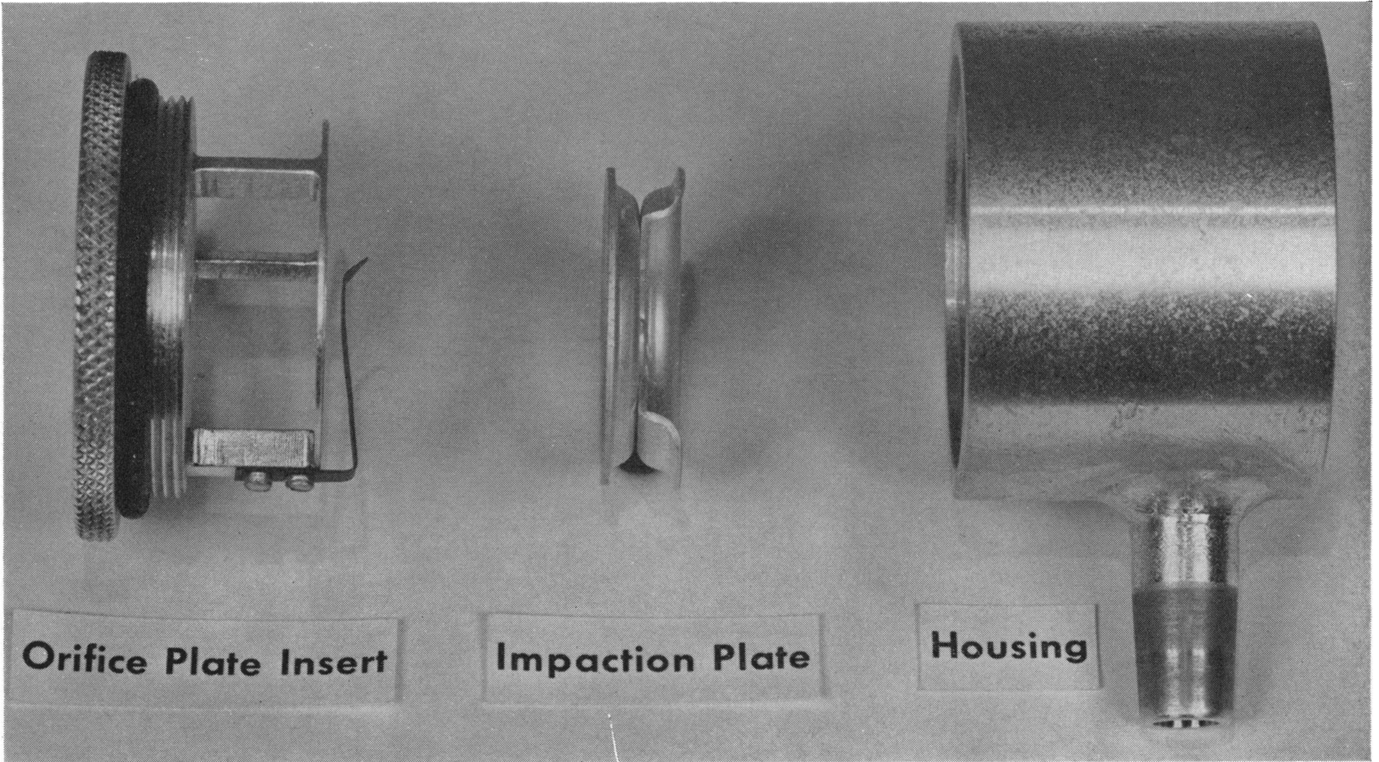

FIG. 1. *Components of the single-stage impactor.*

1/64 × 1/64 CHAMFER

COARSE DIAMOND KNURL

120°

"A"

"B"

"C"

60°

1 7/8 20 NS-2
MINOR DIA. - 1.8137 MAX.
PITCH DIA. - 1.8425 -.0056
MAJOR DIA. - 1.8750 -.0072

#2-64 NF-2 - 2 HOLES

MATL - ALUMINUM

FIG. 2. *Shop drawings of orifice plate insert.*

viability. It is possible to construct a bacterial viable-mass distribution curve for the aerosol by use of the formula:

$$X = \frac{\text{number of cells collected in impinger fluid}}{\text{number of cells collected on impaction plate plus number of cells collected in impinger fluid}} \times 100$$

and plotting the respective points for each impactor-impinger combination on logarithmic probability paper.

Culture and reagents. The bacterial cultures used were *Bacillus subtilis* var. *niger* grown in casein acid digest medium and autolyzed to eliminate vegetative cells, and *Serratia marcescens* suspensions grown in Tryptose, phosphate, and cerelose medium. The uranine dye solutions used were prepared from water-soluble uranine dye (Lot A823; Fisher Scientific Co., Washington, D.C.), and mixed with the bacterial suspension at a concentration of 0.025%. Assays were made by means of a fluorometer (Photovolt Corp., New York, N.Y.).

Aerosolization. Aerosols of the various bacterial and dye mixtures were set up in chambers similar to those described by Ray (1959).

TABLE 1. *Dimensions of insert*

Particle cutoff	Jet-to-plate clearance (dimension B)	Jet diameter (dimension C)
μ	*cm*	*cm*
1	0.396	0.185
3	0.713	0.374
5	1.031	0.523
7	1.270	0.653
9	1.508	0.770
11	1.747	0.879
19	2.540	1.264

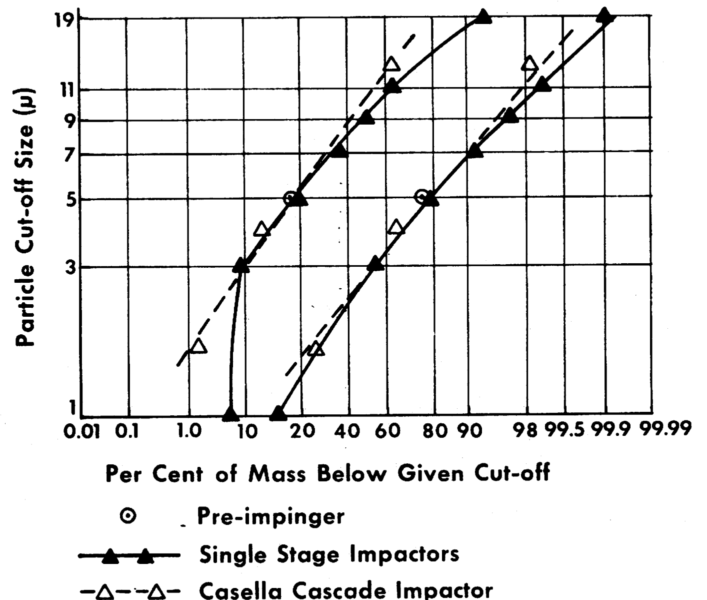

FIG. 4. *Particle size distribution of coarse and fine uranine aerosols as determined by three sizing devices.*

FIG. 3. *Single-stage impactor with impinger.*

Experimental design. All observations were replicated at least eight times to give adequate estimates of the error properties of the systems.

Results and Discussion

The difficulties involved in calibrating devices such as these single-stage impactors in absolute terms are obvious from the fact that, at the flow rates used, overloading of the impaction plates for purposes of microscopic examination is almost unavoidable.

In addition, microscopic sizing of impacted materials is subject to many hazards owing to flattening and fragmentation of liquid droplets, and fracture in the case of dry materials.

In view of their established calibrations and the long experience with the Casella cascade impactor and the preimpinger of May and Druett (1953), it was considered appropriate to use these two devices as references for evaluating the performance of the newly designed and fabricated single-stage impactors. Since aerosols from bacterial spore and vegetative cultures behave differently, it was decided to use a resistant spore, *B. subtilis*, and the vegetative form, *S. marcescens*.

The physical performance of the range of impactors was determined by sampling the same uranine dye aerosols with the single-stage impactors, a Casella impactor, and a preimpinger, and comparing the results. Figure 4 shows the agreement among the three sizing systems. The set of curves to the left is from large-particle aerosols (mass median diameter approximately 9μ), while that to the right is from small-size particle aerosols (mass median diameter approximately 3 μ). Since the systems divide the aerosol by quite different increments, the mass size distributions obtained each have a somewhat different validity over their respective extents. However, it should be noted that with the portion of aerosolized particles less than 5 μ, a fraction of much interest from an epidemiological viewpoint, the three devices did not give statistically different data. Having established the adequacy of the physical performance of the single-stage impactors, it was necessary next to determine their behavior with biological aerosols. To do this, a mixture of *S. marcescens* and *B. subtilis* spores was aerosolized and sampled by an array of the impactors. Previous experience (e.g., Miller et al., 1960) showed that the spores used suffer little or no loss of viability due to impaction, impingement, desiccation, or similar treatments, so that *B. subtilis* recovery is considered in the same manner as that of an inert tracer. Accordingly, the recovery of the vegetative *S. marcescens* with respect to that of *B. subtilis* is used as an index of the killing effect of the single-stage impaction systems. There was no consistent deleterious effect of impaction on the *S. marcescens* aerosols, using the *B. subtilis* recoveries as a physical standard, with either large or small particle size aerosols. Statistical evaluation of the ratios of *S. marcescens* to *B. subtilis* recoveries at each cutoff size has shown them to be not significantly different from each other in almost every case. As an exception, vegetative cells appear to be adversely affected on the impaction plate of the smaller cutoff size impactors, notably the 1-μ device, where the incident air stream reaches a velocity about one-third sonic. Apparently, the agar matrix gives only imperfect protection to labile cells under these conditions. In addition, in the 1-μ size range, the region of Cunningham's correction is approached, and impaction of the small particles is at best semiquantitative as discussed by Wilcox (1953).

During the proving of the impactors, it was found that over a reasonable particle size range (e.g., 1 to 11 μ) the total recovery of the individual systems (i.e., recovery from impactor plate plus that from the impinger) was relatively constant, which led to the attractive possibility of having only to assay the impinger fluid. Consequently, appropriate mass size distributions can be constructed from plots of recovery from the impinger portions of a range of impactors only, resulting in a saving in assay work of about one-half.

Wall loss, considered here as cells or tracer found anywhere except on the impaction plate or in the impinger fluid, was considered to be negligible after extensive testing. Even with large particle aerosols (mass median diameter of approximately 9 μ) of *B. subtilis* with the larger particle size cutoff impactors, wall loss seldom exceeded 3% of the total system recoveries.

Acknowledgments

The single-stage impactors described resulted from a continuing program of development. Important contributors were A. J. Shanahan, B. M. Roberts, and D. N. Lapedes. Design and proving of the final model were due to R. B. Johnson, then serving with the Army of the United States.

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