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TECHNICAL MANUSCRIPT 602

HOMOGENEOUS BACTERIAL AEROSOLS PRODUCED WITH A SPINNING DISK AEROSOL GENERATOR

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HOMOGENEOUS BACTERIAL AEROSOLS PRODUCED WITH A SPINNING DISK AEROSOL GENERATOR

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Physical Defense Division
COMMODITY DEVELOPMENT AND ENGINEERING LABORATORIES

Project 1B662706A072 May 1970

ABSTRACT

Homogeneous bacterial aerosols with median diameters between 1 and 4 microns and geometric standard deviations averaging 1.1 were produced with a commercial spinning disk aerosol generator from aqueous suspensions of *Bacillus subtilis* var. *niger* spores containing various amounts of dextran to regulate the aerosol particle size.

I. INTRODUCTION*

A large number of devices have been developed for sampling microbiological aerosols. Thirty-seven were described in 1959 in Public Health Monograph 60.[1] The most sensitive method of determining their collection efficiency has been to use laboratory aerosols of resistant microorganisms that could be assayed easily and with a high degree of accuracy. These studies have used aerosol generators such as the DeVilbiss and Vaponefrin nebulizers. However, these are limited in their ability to produce homogeneous aerosols because their droplet distributions are heterogeneous in size.[2,3] To produce homogeneous aerosols with these generators, the bacterial suspension must be free of impurities and sufficiently dilute so that the occupied droplets will contain a single bacterium. Non-biological aerosols of uniform particles such as polystyrene latex spheres have been widely used to evaluate air samplers and study aerosol behavior.[4-6] These spheres are available in several sizes, but their usefulness is limited by the difficulty of determining aerosol concentration. The biological method has been preferred because of its greater sensitivity in that each individual organism that is collected in a sample can be quantitated; however, one disadvantage is the lack of a suitable range of different particle sizes. One commonly used test organism has been Bacillus subtilis var. niger in spore form. These spores are nonpathogenic and highly resistant to biological decay (death). When they are nebulized from distilled water, a uniform aerosol consisting mainly of single spores about 1 micron in diameter[7] is produced. Studies with radioactive submicron coliphage aerosols of 0.1 to 0.2 micron diameter and B. subtilis spore aerosols showed the importance of particle size in the sampling and filtration of aerosols. Liquid impingers, which are almost complete collectors of 1-micron particles, allowed up to 50% of the submicron particles to pass through them.[8] Phage aerosol penetration through HEPA filters averaged 0.00095%; spore aerosol penetration was 0.00005%, which represents a 19-fold difference.[9] Naturally occurring aerosols seldom contain single microorganisms. Their particles are usually heterogeneous in size so that the microorganisms are often in clumps or associated with nonviable material. It therefore would be desirable to evaluate air samplers at different size ranges, particularly in sizes above 1 micron.

Spinning disk aerosol generators have been used to produce homogeneous non-biological aerosols. Unlike most other dissemination devices, they put out droplets of nearly uniform size. Walton and Prewett[10] and May[11] were among the first to develop a spinning disk type of aerosol generators. More recently, spinning disk generators have been used by Whitby et al.[12] to study the effect of particle size on aerosol behavior and air filtration

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and by Flesch et al.[4] and Knuth[13] to produce aerosols of methylene blue dye for calibrating air samplers. Lippmann et al.[14,15] designed one to study the effect of particle size on the deposition of inhaled iron oxide aerosols in the human respiratory tract. The vibrating reed also disseminates homogeneous droplets, but its droplet output is negligible compared with that of the spinning disk.[16]

Recently, a spinning disk aerosol generator* has become commercially available. This paper reports on the utilization of this generator to produce aerosols, both homogeneous and viable, over a wide range of sizes by using bacterial spores together with a controlled amount of inert material accompanying each spore. Such aerosols would be useful for calibrating air-sampling devices where particle collection efficiencies may be size-dependent and for evaluation of certain air filter systems.

II. MATERIALS AND METHODS

A. SPINNING DISK AEROSOL GENERATOR

A diagram of the generator is shown in Figure 1. The generator produces an aerosol by feeding a solution or suspension through a flowmeter onto the center of the disk (1 inch diameter) rotating at a fixed speed of 60,000 rpm. The liquid is spun off the disk in two groups of droplet sizes. The larger or primary droplets are used as the homogeneous test aerosol. The smaller or satellite droplets, which are about one-third the diameter of the primary droplets and several times more numerous, are heterogeneous in size. They are removed from the main air stream by a separate exhaust system.

The size of the primary droplet is a function of the size and speed of the disk and the density and surface tension of the liquid as described by the following formula used by Walton and Prewett:[10]

$$D_d = \frac{K}{W}\left[\frac{T}{PD}\right]^{\frac{1}{2}} \qquad (1)$$

Where D_d is the droplet diameter, K is a dimensionless proportionality constant, W is the angular velocity of the disk, D is the disk diameter, P is the liquid density, and T is the liquid surface tension. With a fixed size and speed of the disk, droplet diameter becomes dependent on the density and surface tension of the liquid.

* Environmental Research Corporation (ERC), St. Paul, Minnesota.

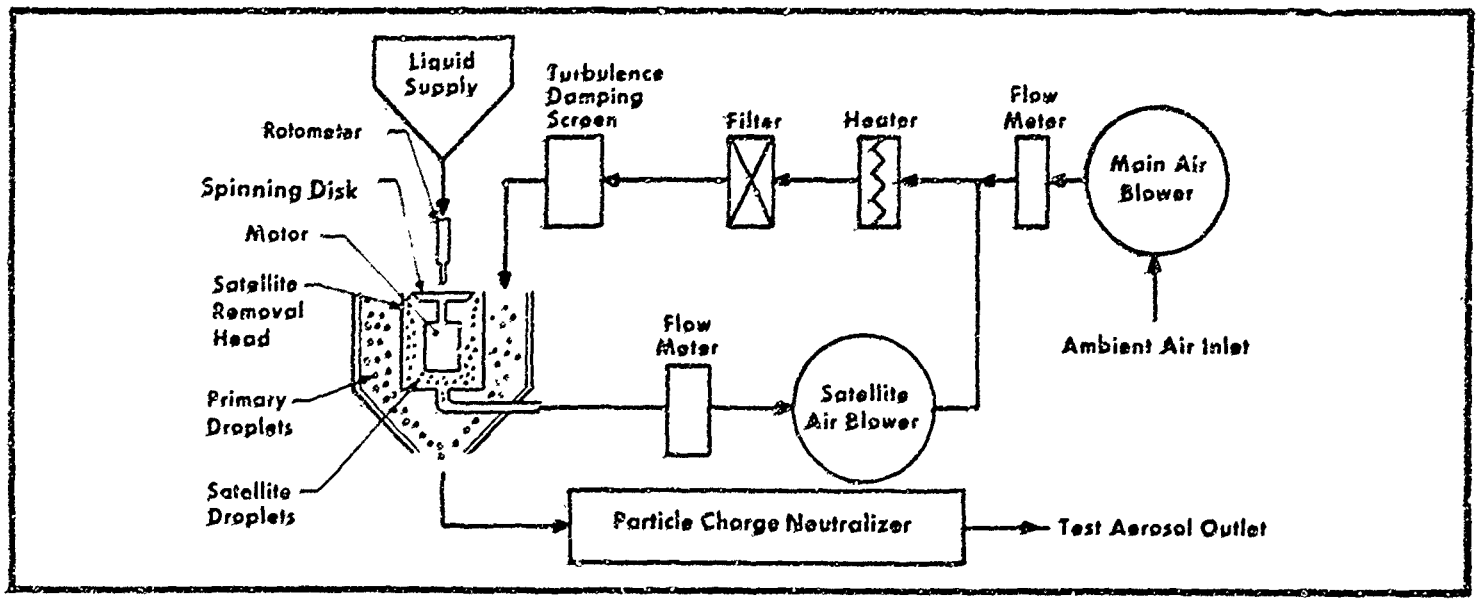

FIGURE 1. Flow Diagram of ERC Model 8320 Spinning Disk Aerosol Generator.

Aerosol particle size is directly proportional to the size of the primary droplets and the solute content of the liquid as described by the following formula, which assumes complete evaporation of the solvent in the droplet and the subsequent formation of a solid-core sphere of the dissolved solute.

$$\frac{\text{volume of particle}}{\text{volume of droplet}} = \frac{\text{volume of solute}}{\text{volume of solution}}$$

Converting to units used in the tests, the expression becomes:

$$\frac{D_p}{D_d} = \left[\frac{\text{wt. solute}}{\text{wt. solution}}\right]^{1/3} \qquad (2)$$

which assumes that the density of the solute is the same as that of the solution or, in the case of a dilute aqueous solution, not far from 1.0 g/ml. D_p is the diameter of the evaporated particle, and D_d is the diameter of the liquid droplet.

If the liquid droplet contains a spore, it can be assumed that the volume of the resultant evaporated particle equals the volume of an equivalent uninhabited particle plus the volume of the spore, since the spore detracts little volume from the liquid phase of the original droplet. Using diameters rather than volumes, this results in the formula:

$$D_{sp} = \left(D_p^3 + D_s^3\right)^{1/3} \tag{3}$$

Where D_{sp} is the equivalent spherical diameter of a spore-bearing particle, D_p is the diameter of an uninhabited particle, and D_s is the equivalent spherical diameter of a spore, 0.87 micron. If, for example, the solute content of the suspension is such that the diameter of an uninhabited particle is 1 micron after the solvent has evaporated, then the diameter of a particle containing a single spore should be 1.18 microns:

$$D_{sp} = (1^3 + 0.87^3)^{1/3} = 1.659^{1/3} = 1.18$$

With more concentrated suspensions that would result in larger evaporated particles, the difference between the size of the inhabited and uninhabited particles would be expected to be still smaller.

B. DESIGN OF EXPERIMENTS

Aerosols were produced with the spinning disk from aqueous suspensions of B. subtilis spores containing various amounts of dissolved dextran 2000* to regulate aerosol particle size. The size of a spore was determined from aerosols generated from spore suspensions without dextran. Average dimensions, based on the measurement of 652 spores, were 0.68 by 1.17 μ, which gives a volume of 0.34 μ^3 and an equivalent spherical diameter of 0.87 μ, assuming spore shape to be that of a cylinder with hemispherical ends. Dextran 2000 is an anhydroglucose polymer with an average molecular weight of 2×10^6. It is stable, inert, and soluble in water.

Preliminary tests with dextran-spore aerosols used spore concentration that theoretically would average about one spore in each of the primary droplets. However, examination of aerosol samples showed that the aerosol particle sizes were heterogeneous. The aerosols consisted mainly of particles containing a single spore, but there were also lesser numbers of uninhabited and multiply inhabited particles. Therefore, dilute spore suspensions were used so that the capabilities of the spinning disk generator and the particle size parameters of the aerosols could be more accurately defined. This procedure naturally results in aerosols composed of dextran particles with

* Pharmacia, Uppsala, Sweden.

and without spores, but almost all of the inhabited particles contained only a single spore. Aerosols were generated from spore suspensions in distilled water without dextran and from suspensions containing 0.001, 0.01, 0.1 and 1% dextran. The aerosols were sized with an electron microscope.

Spore concentration of the aerosols was determined from samples collected with cotton filters.[1] Spores were dislodged from the cotton by shaking in water containing 0.1% Tween 80* and assayed on pour plates of Bacto tryptose agar. Colonies were counted after 24 hours' incubation at 37 C.

C. AEROSOL PARTICLE SIZING

Particle size parameters of the aerosols were determined with an electron microscope from aerosol samples collected on a collodion-coated specimen grid mounted in a Greenburg-Smith Impinger as described by Flesch et al.[4] The grids were then shadowed with uranium and examined in an electron microscope at a magnification of 1870; random areas of the grid were photographed. For sizing, the electron micrographs were projected on a screen of translucent paper and the individual particles were measured perpendicular to the shadow. For each size interval, the number of particles, the percentage of the total number of particles, and the cumulative percentages were determined. The cumulative percentages, when plotted against particle diameter, gave a good straight-line fit on log probability paper. Therefore, the particle size distributions of the aerosols were log-normal. Populations of 25 to 150 particles were used to determine the following parameters: number median diameter (NMD), which is the 50% size, and geometric standard deviation (GSD), which indicates the degree of heterogeneity of the aerosol. A perfectly homogeneous aerosol has a GSD of 1.0, i.e., all of the particles are the same size. Aerosol GSD is determined by the following formula:

$$\text{GSD} = \frac{84.13\%\ \text{size}}{50\%\ \text{size}} = \frac{50\%\ \text{size}}{15.87\%\ \text{size}} \qquad (4)$$

Figure 2 shows the particle size distribution of a typical aerosol produced with the spinning disk from a dilute spore suspension containing 0.1% dextran. The cumulative percentages are plotted against particle diameter on log probability paper, the NMD (50% intercept) is 1.80 microns, and the GSD is 1.12. This is a good value because the closer the GSD is to unity the more nearly homogeneous is the aerosol.

Figure 3 shows some electron micrographs of the aerosols produced with the spinning disk generator. Frame a shows naked spores generated from a spore suspension without dextran. Frames b through e are aerosols

* Atlas Chemical Industries, Inc., Wilmington, Delaware.

produced from dilute spore suspensions containing 0.001 to 1% dextran. The great majority of the dextran particles are uninhabited because the spore concentration was 10^5 per ml, which would result in less than 0.1% of the aerosol particles being inhabited. Frame f illustrates a technique used to observe uninhabited particles and to determine the number of spores per dextran particle. The dextran is eluted from the particle by placing a drop of water on the electron microscope specimen grid and removing it with a blotter after approximately 30 seconds. If the contact time is correct the dextran particle partially disintegrates to reveal the spores inside. This technique also showed that the diameters of inhabited and uninhabited particles agreed with those predicted by equation (3) because particle diameter is indicated by the shadow.

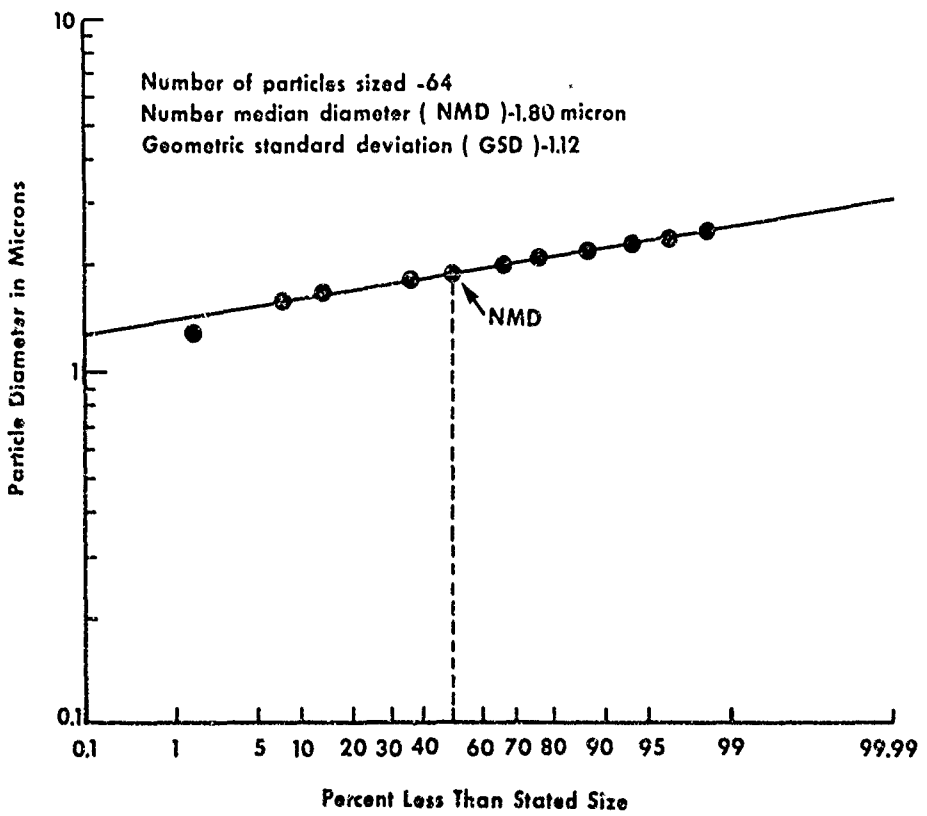

FIGURE 2. Size Distribution of Aerosol Produced with the Spinning Disk Generator from a Dilute Suspension of B. subtilis var. niger Spores Containing 0.1% Dextran.

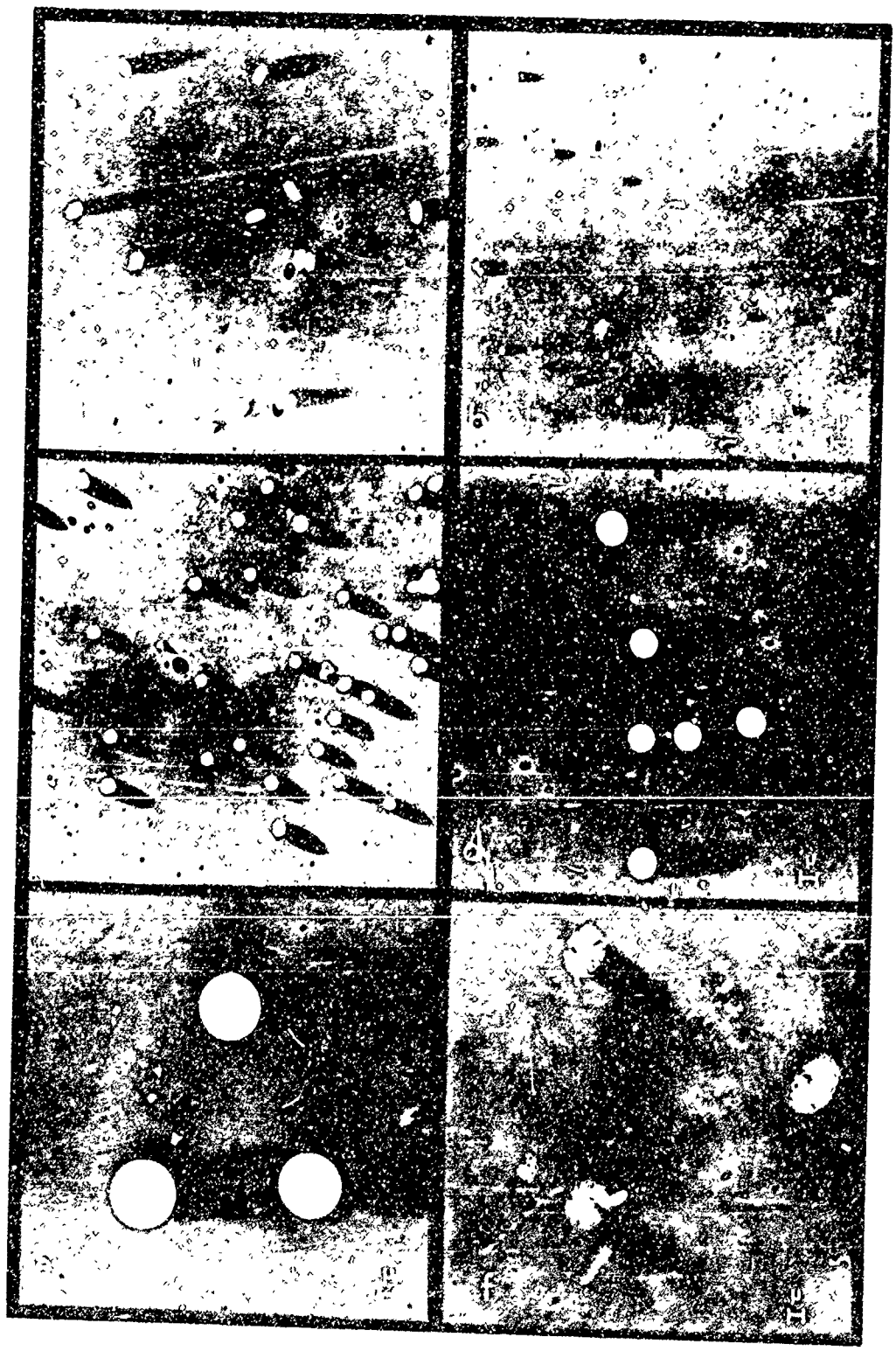

FIGURE 3. Electron Micrographs of Aerosol Particles Produced with the Spinning Disk Generator from Dilute Spore Suspensions. They contain the following dextran concentrations: (a) none; (b) 0.001%; (c) 0.01%; (d) 0.1%; (e) 1.0%; and (f) inhabited particles disseminated from 0.1% dextran and subsequently partially disintegrated.

III. RESULTS AND DISCUSSION

Results of 42 tests are shown in Table 1. The spinning disk routinely produced homogeneous aerosols as evidenced by the low GSD. The GSD of the 42 aerosols sized ranged from 1.05 to 1.16 (not shown in Table 1). This compares favorably with reports in the literature. Utilizing equation (2), the apparent diameters of the primary droplets were calculated, and utilizing equation (3), the diameters of the spore-bearing particles were calculated from the dextran particle diameters. It should be noted that increasing the dextran concentration apparently did not alter the diameter of the primary droplets. The primary droplet NMD of the 42 aerosols sized varied from 16.1 to 18.4 microns (not shown in Table 1). This is considered good reproducibility. However, other aerosols (not reported here) produced with the same generator but a different disk were also of good quality but had significantly larger droplet NMD (19 μ). Therefore, the aerosols should be sized with any change in the generator or its operating conditions.

TABLE 1. PARTICLE SIZE PARAMETERS OF AEROSOLS PRODUCED WITH THE SPINNING DISK GENERATOR FROM DILUTE *B. SUBTILIS* VAR. *NIGER* SPORE SUSPENSIONS CONTAINING VARIOUS AMOUNTS OF DEXTRAN[a]

Dextran Concentration by Weight, %	Number of Aerosols Sized	Mean NMD,[b] micron			Mean GSD
		p	sp	d	p
None	6	-[c]	0.87	-	-
0.001	2	0.39	0.90	17.9	1.09
0.01	4	0.78	1.04	16.7	1.13
0.1	10	1.73	1.80	17.3	1.11
1.0	20	3.60	3.62	16.7	1.10

a. Dextran-spore suspensions titered 2×10^7 spores/ml, suspensions were fed onto disk at rate of 2 to 5 ml/minute.

b. p is solid dextran particle as observed from electron micrographs.
sp is spore-bearing dextran particle as calculated from p, using equation (3).
d is primary liquid droplet as calculated from p, using equation (2).

c. Not applicable or no method of determining.

The ability of a commercially available spinning disk generator to produce homogeneous aerosols over a range of sizes using bacteria as a viable tracer has been established. This type of aerosol is especially useful for calibrating air-sampling devices, evaluating air filters, or wherever aerosol behavior may be size-dependent.

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13. ABSTRACT

Homogeneous bacterial aerosols with median diameters between 1 and 4 microns and geometric standard deviations averaging 1.1 were produced with a commercial spinning disk aerosol generator from aqueous suspensions of Bacillus subtilis var. niger spores containing various amounts of dextran to regulate the aerosol particle size.

14. Key Words

Spinning disk
Homogeneous aerosols
Spores
B. subtilis
Aerosol generator
Aerosol disseminator
Monodisperse aerosols
Particle size
Particle size distribution
Viable aerosols
Calibration
Dextran 2000

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