

NEBULIZER CHARACTERISTICS FOR CERTIFICATION TESTS OF BIOSAFETY CABINETS WITH BACTERIA AND SIMULANTS

Melvin W. First¹, Janet Macher², Robert Gussman³, David Stuart⁴, and Terence Webb⁵

¹Harvard School of Public Health, Boston, Massachusetts, ²California Department of Health Services, Berkeley, California, ³BGI Inc., Waltham, Massachusetts, ⁴The Baker Company, Sanford, Maine, and ⁵Microzone Corporation, Ontario

ABSTRACT

NSF International Standard 49-1992, that covers certification of biological safety cabinets, makes "special note" that a "stainless steel 6-jet Collison refluxing nebulizer will deliver the [required] bacterial spore aerosol" when certain stated conditions are met and "need not be retested for performance before use" (Appendix-C, page C1) (1). The basis on which this nebulizer was vetted was presented at the XXV Biological Safety Conference (Boston, MA, 1984) but never published in the open literature. In view of the importance of this device for the procedures used to certify the performance of biological safety cabinets, the authors are of the opinion that the test protocols and test data on which the selection was based should be made a matter of record.

Collison nozzle studies were conducted to determine (a) whether all 6 Collison nozzles manufactured by BGI give the same spore output when operated at 140 kPa (20 psig) with an equal number spore suspension in the flask (b) whether spore delivery by the 6-jet Collison nozzle equals or exceeds the minimum number specified by NSF 49 when charged with the recommended spore suspension (c) whether performance of Collison nozzles with a bacterial spore aerosol can be predicted accurately with a monodisperse 1.1 μm polystyrene latex spherical simulant, and (d) the optimum nebulizer flask geometry.

INTRODUCTION

The Collison Nebulizer

K.R. May published a definitive paper on this instrument in 1973 (2) that should be consulted for a thorough knowledge of the device and all its performance factors. May worked only with 1-jet

and 3-jet nozzles ("nozzle" refers to the active tip of the nebulizer that contains the spray-making jets) whereas all the devices used in the present study contained 6-jet nozzles obtained from BGI, Inc. (3). In all respects, (other than the presence of 6 jets rather than 3) the BGI nozzle conforms exactly to the Collison nozzle described by May. For the BGI 6-jet nozzle, air-flow rates and liquid consumption rates at equal air pressure correspond to double the values reported by May for the 3-jet nozzle. At 140 kPa (20 psig) air pressure, free air consumption for the 6-jet nozzle is 14.2 L/min and liquid loss with dry compressed air is 0.3 mL/min.

For the experiments reported here, a variety of flasks was employed (a) a small screwtop jar having an ID of 3.8 cm (1.5 in.), a 1.3 cm (0.5 in.) diameter spout, and containing 15 mL of suspension (b) a larger screwtop jar having an ID of 5 cm (2 in.), a 1.3 cm (0.5 in.) diameter spout, and containing 25 mL of suspension (c) a graduated series of straight-sided metal flasks having inside diameters of 2.0 cm (0.79 in.), 2.5 cm (1.02 in.), 3.9 cm (1.54 in.), 5 cm (2.00 in.), and 7.5 cm (2.94 in.), all with an outlet spout of 2.1 cm (13/16 in.) diameter, and containing an AGI-type sampling flask used routinely at the Baker Co. (4) having a spout diameter of 2.1 cm (13/16 in.) and containing 55 mL of suspension. All tests were conducted with an air pressure of 140 kPa (20 psig). Seven different 6-jet nozzles were used in various combinations with the several flasks in the experimental series that was conducted. The nozzles were designated A, B, C, D, E, F, and Baker, and the identity of each nozzle preserved throughout. The Baker nozzle was one that had been carefully calibrated by D. Stuart (4) for satisfactory spore delivery and one that he had been using successfully in biosafety cabinet tests for several months. Data from 32 trials using the Baker nozzle

showed that with a starting suspension of between 5 and 8×10^8 *Bacillus subtilis*, var. *niger* (BG) (formerly *Bacillus globigii*) spores per mL this nebulizer consistently delivered 1- 4×10^8 spores during 5 min. of continuous operation, as called for in NSF 49. Its performance characteristics were considered the standard against which the characteristics of the other nebulizers were compared.

TEST PROTOCOLS

BG Tests

As the preparation of the spore suspension and the methods used for generation, sampling, plating, and counting the BG aerosols followed standard NSF 49 procedures, they are adequately identified by reference (1).

PSL Tests

Monodisperse polystyrene latex spheres (PSL) of 1.1 μm diameter closely match BG suspensions in size, shape, and specific gravity and therefore were assumed to be a reliable simulant when comparing the number output of similar nebulizers. The manufacturer's label showed that the PSL suspension contained 10% spheres by volume in water. As one drop of suspension is 1/25mL, each drop of the stock suspension contained approximately 6×10^9 particles. For all PSL tests, 3 drops of stock suspension were added to each 10 mL of dust-free water plus antifoam to prepare the suspension placed in the nebulizer flask. The PSL suspension contained 17×10^8 PSL particles per mL. Aerosolized PSL particle counts were performed with a laser spectrometer after dilution with 0.0243m³ s (50 cfm) of

HEPA-filtered air using pooled data within the following size intervals.

0.76 - 1.24 μm (singlets)

1.24 - 1.88 μm (doublets and triplets)

As the counts were intended only to compare various combinations of nozzles and flasks under identical operating conditions, no attempt was made to translate the count data taken from the spectrometer readout device into particles generated per 5 min. intervals, as is customarily done for the BG tests. For reference and comparison purposes, the 5 min. output was approximately 5×10^8 particles.

RESULTS

Effect of Varying the Flask Diameter of the Nebulizer

Tests were conducted with Nozzle C in metal flasks of graduated diameter. The distance between the bottom of the nozzle and the bottom of the flask was held constant and the immersion of the nozzle was maintained at 1.9 cm (0.75 in.) by varying the volume of the suspension placed in the flask. Comparative counts are shown in Table 1. A flask diameter of 5 cm (2 in.) appeared to be optimum and coincides with the dimensions of the M unit (2). A larger flask diameter of 7.5 cm (2.94 in.) gave almost as high delivery rates. The disadvantage of a larger flask is that it requires a larger volume of suspension, but it could also be considered an advantage because each spore passes through the nozzle fewer times during a run and the kill rate from trauma may

TABLE 1
Effect of flask diameter on particle delivery using a suspension of PSL and a laser spectrometer particle counter

Particle size - (μm)	No. of Particles Counted in 100 s.				
	Flask Inside Diameter - cm (in.)				
	2 cm (0.79)	2.5 cm (1.02)	3.9 cm (1.54)	5 cm (2.0)	7.5 cm. (2.94)
0.76 - 1.24	540	1,100	1,500	2,400	2,100
1.24 - 1.88	27	77	95	220	190
% doublets and triplets	4.8	6.7	6.0	8.6	8.2

be less. However, the same effect could be achieved in a smaller diameter flask by raising the height of the nozzle to accommodate a larger liquid volume without changing the nozzle-to-liquid geometry. There does not seem to be any question that flask diameters less than 5 cm (2 in.) resulted in progressively fewer particles in the discharge. The conclusion reached is that a flask of 5 cm (2 in.) diameter is about the optimum dimension. Two factors interact in the selection of flask diameter: (a) as the diameter increases, the upward air velocity declines for the same airflow rate of 18 L/min and, as a consequence, larger drops settle back into the pool and (b) as the flask diameter decreases, the horizontal liquid jets strike the walls of the flask at higher velocity, and this tends to decrease the number of droplets that become airborne, perhaps decreasing the larger droplets at a greater rate because of their greater inertia. It is reasonable to conclude, therefore, that there should be a diameter that delivers a maximum number of droplets to the aerosol discharge spout.

PSL as a Simulant for BG

Four nozzles were tested with both PSL and BG. Some of the nozzles had metal chips in some of the

jets when they were first tested so that the output was not uniform; but this made an analysis of comparative numbers between the two types much more valuable as it provided an opportunity to compare the BG/PSL ratio over a range of nozzle deliveries. Table 2 summarizes BG and PSL data for nozzles that originally contained chips and repeat tests after the chips were removed. It may be seen from Table 2 that the trend of both BG and PSL counts was the same before the nozzles were cleaned. After the nozzles were cleaned the counts were indistinguishable taking into account experimental variability. On the basis of the two series of tests, it was concluded that when appropriate quality control measures are observed during manufacture, 6-jet Collison nebulizers can be depended upon to deliver essentially identical numbers of spores when operated under standardized conditions of air pressure and liquid suspension numbers.

DISCUSSION

An open question was what the discharge velocity from the spout should be because the "throw" of a discharge nozzle depends not only on nozzle diameter but also on droplet size and discharge

TABLE 2
Comparative particle* delivery of BG and PSL

Nebulizer Combination	5 min. BG spore delivery no.s	PSL laser spectrometer counts	
		Before Jet Cleaning	After Jet Cleaning
Baker Nozzle in Baker Flask	3.3×10^8	2.4×10^4	-
Baker Nozzle in 3.9 cm (1.5-in.) diameter screwtop jar with 1.25 cm (0.5-in.) diameter spout	-	4.6×10^4	5.1×10^4
Nozzle A in screwtop jar	-	3.7×10^4	5.0×10^4
Nozzle B in screwtop jar	-	3.3×10^4	-
Nozzle C in screwtop jar	0.46×10^8	2.1×10^4	4.9×10^4
Nozzle D in screwtop jar	0.60×10^8	2.4×10^4	-
Nozzle E in screwtop jar	0.70×10^8	3.6×10^4	-
Nozzle F in Baker Flask	3×10^8	-	-

*BG numbers are 5 min. delivery counts. PSL numbers are counting machine output figures that have not been translated into 5 min. delivery values.

velocity. The discharge velocity from the DeVilbiss 40 nebulizer is 1.5 m/s (300 fpm). From Collison's 3-jet nebulizer, the discharge velocity is 1-m/s (200 fpm), and from the 6-jet nebulizer at 140 kPa (20 psig), using a 1.25 cm (0.5 in.) diameter spout, the discharge velocity is about 2 m/s (400 fpm). What made this an issue was that it was feared that a discharge velocity of 2 m/s (400 fpm) would prove to be excessive for cabinet testing, i.e., give a false indication of failure, and that the discharge spout of the 6-jet Collison nebulizer assembly should be made to give a discharge velocity identical with that of the DeVilbiss 40 nebulizer that has been used for prior testing. Fortunately, the discharge velocity from the 6-jet nebulizer could be modified simply by using a spout of a different diameter and it was enlarged to give a discharge velocity of 1.5 m/s (300 fpm).

At the time the experiments reported here were conducted, NSF Standard No. 49 had only one requirement for nebulizers used to certify biosafety cabinets, namely, spore delivery numbers. The standard called upon the certifier to verify that the delivery from the chosen nebulizer met the spore delivery standard prior to conducting cabinet certification procedures. A number of different nebulizers were in use at that time that met the delivery requirement (some are reported to be still in use). The difficulties that were being experienced with these nebulizers were that the glass units have thin glass jets that are subject to erosion, chipping, and breakage, and the plastic units often experience distortion as a result of sterilization and ageing. This means that these nebulizers must be retested frequently for the verification of spore delivery numbers, a time consuming and onerous task. The advantage of using a standard, machined, stainless steel nebulizer is that it does not have to be verified initially or retested periodically for spore delivery. It does, however, have to be cleaned carefully after each use as solid deposits left in the capillary-scale passages will alter the delivery characteristics of the nebulizer.

The discharge air volume and discharge velocity from the nebulizer were not specified in NSF Standard No. 49 but it was considered prudent to duplicate the discharge characteristics of nebulizers in current use for biosafety cabinet certification lest cabinets already certified might not meet recertifi-

cation requirements, or unsafe cabinets might be able to qualify for certification. This consideration was the motivation for the extended search for a stainless steel nozzle, glass containment jar, and discharge spout combination that would duplicate all the operating characteristics of glass and plastic units in then-current use for biosafety cabinet certification with BG spores.

SUMMARY

The successful search for and validation of a nebulizer that can meet all requirements for conducting biological testing of biosafety cabinets for safety certification and that requires neither initial nor repeated verification of its performance characteristics is described. On the basis of these test results the BGI 6-jet Collison nebulizer is now authorized by NSF Standard 49-1992 for use when conducting biosafety cabinet certification tests with microorganisms. A picture of the unit is shown in Figure 1.



FIGURE 1

REFERENCES

- NSF International Standard 49-1992, Class II (Laminar Flow) Biohazard Cabinetry, NSF International, Ann Arbor, MI.
- May, K.R. The Collison Nebulizer; Description, Performance and Application. *Aerosol Science*, Vol. 4, pp. 235-243, 1973. BGI, Inc., Waltham, MA.
- David Stuart, The Baker Co., Inc. Personal Communication, 1994.
- Dow Chemical Co., Midland. MI.