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Use of the Next Generation Pharmaceutical Impactor for Particle Size Distribution Measurements of Live Viral Aerosol Vaccines

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ABSTRACT

Aerosol administration of live measles vaccine virus has proven to be extremely efficacious in field trials using an industrial compressor coupled to a disposable nebulizer (IPI). To develop a new system for administration, it is necessary to characterize the operating characteristics of the old system. There are no standardized techniques for measuring particle size of live biological agents. This study evaluated the Next Generation Pharmaceutical Impactor's (NGI) ability to particle size wet aerosols in an effort to measure the particle size distribution of live measles vaccine from the IPI nebulizer. As a control albuterol was aerosolized using a Pari LC Star, since the soluble albuterol is evenly distributed throughout the droplets and laser diffraction measurements should agree with those from the NGI, as long as the NGI is cooled to prevent heat transfer to the aerosol. Albuterol was also used as a control for the IPI using quantitative ultraviolet spectrophotometry. There was close agreement in MMD (mean ± 95% CI) for the LC Star, measured by laser diffraction (3.24 ± 0.06 μm) and the NGI (2.93 ± 0.22 μm) and the IPI (4.26 ± 0.17 and 4.26 ± 0.24 μm, respectively). For the measles vaccine assayed for plaque forming units, there were significant differences between the NGI MMD (6.14 ± 0.39 μm) compared to laser diffraction (4.95 ± 0.16 μm) indicating that the vaccine is not evenly distributed among the droplets of various sizes. This is likely clumping of the virus due to gelatin in the formulation. These data indicate that the NGI is capable of particle sizing live biological agents.

Key words: next generation pharmaceutical impactor (NGI), particle size distribution measurement techniques, laser diffraction, measles vaccine virus

INTRODUCTION

MEASLES, a preventable disease through immunization, still kills in excess of 750,000 children a year world wide.[1,2] Field trials with live aerosolized measles vaccine suggest both efficacy and a wide safety margin,[3,4] as well as being easier and safer for relatively untrained personnel to administer. The original system developed in Mexico, the "classical Mexican device," was a ¾ horsepower (HP) industrial compressor driving an IPI disposable nebulizer, and was de-

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veloped on a purely empirical basis. The classical Mexican system proved to be a very effective form of immunization, and the World Health Organization wants to develop a more practical and licensable system. In order to develop a system as efficacious as the classical Mexican device (CMD), the technical specification of this device must be known. This would include the rate of output of live measles vaccine particles, particle size distribution (PSD) of these particles, and viral potency studies. The particle size of an inhaled droplet is the most important determinant of the probability of deposition in the lungs and for the likely site of deposition.[5,6] This study will focus on particle size distribution.

The vaccine, the Edmonston-Zagreb strain prepared by the Serum Institute of India, is intended for subcutaneous injection. It contains gelatin and other agents that could result in viral clumping. While the virus particles themselves are very small, for example, $<0.5\ \mu$m, clumping could easily give rise to an aerosol where the distribution of virus within particles of different sizes was not uniform, with many small droplets having a very low concentration of virus particles and larger ones having much higher concentrations. If the vaccine acted in such a non-uniform manner, light scattering based particle size measuring techniques that measure only droplet size and assume a uniform distribution of the agent of interest within the droplets would not be appropriate.[7,8] In such a situation, particle sizing the "active" aerosol requires an inertial impaction technique such as a cascade impactor[8] with quantification of drug mass within the size range of the impactor. Liquid impinger techniques would be a possibility, but the dilution of the virus particles by the relatively large amount of liquid required by each stage would make viral assaying a major challenge. Cascade impaction techniques are an option as long as desiccation is not necessary since the virus particle would be killed by the drying process. This would not be a problem if the viruses were not destroyed during nebulization, since desiccated viral RNA could be eluted from the plates and assayed using Northern blot techniques. However, this assumption may not be valid since jet nebulization has been shown to disrupt complex molecules such as those proposed for lung gene therapy.[9,10] Even when desiccation is prevented, Griffiths et al.[11] have pointed out that some types of inertial impactors can render indoor bioaerosols non-viable. The Next Generation Pharmaceutical Impactor (MSP Corp., Minneapolis, MN) (NGI), an inertial technique for separating particles of various sizes and collecting them in size specific cups could be the solution.

The NGI was developed for the pharmaceutical industry[12,13] as a device to particle size dry powder aerosols that is simpler and less labour intensive than the Andersen Cascade Impactor (ACI), which is the industry standard. Compared with the ACI, the NGI has been shown to have virtually identical values for particle size determination from metered dose inhalers for Intal™ and Qvar™ and less agreement for Flovent™. This discrepancy was attributed to the failure of complete evaporation of the propellant coupled with problems due to particles bouncing off the plates after impact.[14] The NGI has been used successfully to size analyze wet aerosols providing the device is cooled to the temperature of the aerosol to prevent heat transfer and evaporative losses.[15] Based on a flow of 30 Lpm into the device, the seven-stage NGI has nominal cut points for dry powder at 11.719, 6.395, 3.988, 2.299, 1.357, 0.833, 0.541 (μm, and a micro-orifice collector (MOC) to capture any particles too small to impact on the last stage.[12] Since droplets of aerosol coalesce on cups specific to the size of the droplet, viral assays from the cups of the NGI should theoretically yield the aerodynamic particle size distribution of the measles vaccine aerosol.[12,13] If the measles vaccine is uniformly distributed, it is hypothesized that particle size measurements made by laser diffraction would be comparable if not equal to those made on the NGI both for the measles vaccine as well as albuterol, a solution known to have uniform drug distribution throughout droplets irrespective of size.[16] The unique assembly of the CMD had the nebulizer in a cup of ice to maintain vaccine viability, and the aerosol exited via 15 cm corrugated tubing. The aerosol warmed between generation of the droplets and where they exited the device, resulting in a change in concentration of the agent being aerosolized, especially within the smallest droplets that have a high surface area to volume ratio. In the present study, the Pari LC Star nebulizer was also evaluated as a control with both particle sizing methods for two reasons. The NGI draws the aerosol through the device using 30 Lpm, but the output for the IPI nebulizer when used with the Evans compressor is 10.5 Lpm. In

this arrangement, supplemental flow must be added to the aerosol output to meet the demands of the NGI, and any difference between the temperature and humidity of the added flow will distort the aerosol output and particle size distribution. The Pari LC Star allows additional flow not supplied by the compressor driving the nebulizer to be "entrained air" through the inspiratory valve at the top of the nebulizer, instead of adding flow to the already generated aerosol to meet the requirement of the NGI. Second, if there is a direct connection between the nebulizer and the NGI (i.e., no corrugated tubing at outlet like the IPI) there is insufficient time for warming of the aerosol before entering the NGI. The particle size distribution measured by laser diffraction for a solution of albuterol from the Pari LC Star should be identical to that measured by the NGI since it is a uniformly distributed solution and the setup is such that there would be minimal evaporation post aerosol generation.

MATERIALS AND METHODS

Particle size and distribution measurements

The Mexican system consisted of an IPI nebulizer (Medex Medical, Product number C4107, Chicago, IL) driven by the Evans compressor (model T407, DeVilbiss Air Power Company, Jackson, TN). The compressor drives the nebulizer at 10.5 L/min at a pressure of 40 psi. These values are approximate because of the very pulsatile pressure generated by the compressor which makes precise measurements very difficult. One side of the nebulizer T-piece was sealed, and the other led to 15 cm of corrugated tubing which was attached to a plastic funnel with a paper face mask insert (Fig. 1). The nebulizer was kept in a container of ice as per the field trials, and the temperature of the aerosol was measured within the well and at the end of the corrugated tubing.

The nebulizer, T-piece, and tubing were weighed, and then charged with 10 mL of either albuterol (0.625 mg/mL) or measles vaccine virus. The 10 mL of vaccine was made up from two multi-dose vials of live measles vaccine (Edmonston Zagreb strain, Serum Institute of India, Hadaspar, Pune, India)—certified minimum of 1000 $CCID_{50}$ (Cell Culture Infective Dose) with 0.5 mL of sterile water per dose (5 mL for the 10 dose vial). Particle size measurements were made using two independent techniques, laser diffraction using a Malvern Mastersizer X (Malvern Instruments Inc., Worcestershire, UK), and cascade impaction using the NGI (MSP Corp.) (Fig. 2). The following were special considerations:

- At steady state conditions the temperature of the aerosol in the well was approximately 12°C and climbed to 16°C at the end of corrugated tubing, at which point it entered the NGI. It was recognized that changes in particle size would occur due to evaporation, and subsequently increase

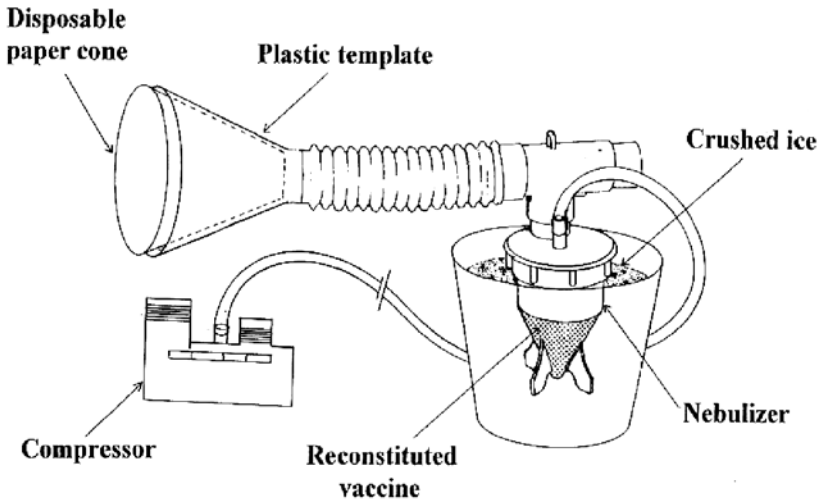

FIG. 1. Classical Mexican device used during field trials. The figure was modified[22] to reflect the removal of the plastic template and disposable paper cone for particle sizing due to concerns of vignetting.[17]

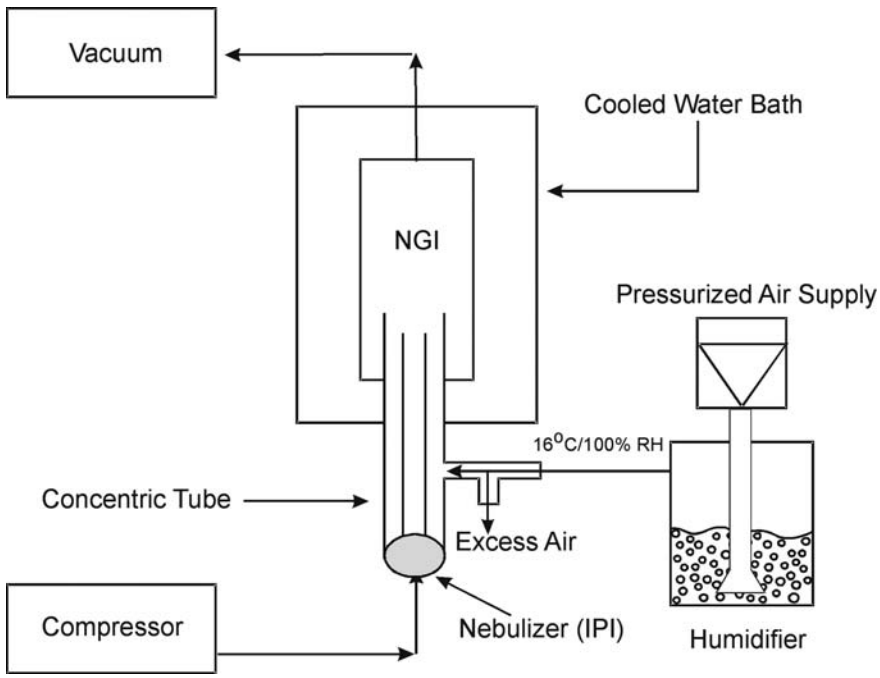

FIG. 2. Set-up used for the NGI with the classical Mexican device. The IPI nebulizer was kept in a cup of ice to mimic field trials.

the concentration of the agent in question within the droplets. By calculating the water loss from 12°C to 16°C, an adjustment was made to laser diffraction data and the particle size distribution (see Appendix). This was important for the interpretation of the data from laser diffraction, since the aerosol changes from time of generation to the moment of actual particle sizing.

- The funnel interfered with both particle sizing devices and was therefore removed for testing.

Initially, the NGI cups with appropriately sized pieces of laboratory film were weighed. The laboratory film was used to cover the cups upon removal from the NGI post run, to prevent evaporation of the sample when exposed to air. The weighed cups were put back into the NGI, which was closed and placed in a water bath cooled to the temperature of the aerosol leaving the corrugated tubing of the IPI nebulizer (16°C).[15] The induction port of the NGI protruded above the bath and was cooled by pouring water from the bath over it periodically during nebulization. The aerosol was drawn through the NGI using the calibrated pump (Gast, Andersen Instruments) at exactly 30 Lpm. For the IPI nebulizer with a flow of 10.5 Lpm out of the device, approximately 19.5 Lpm of supplemental air needed to be added. This supplemental air needed by the NGI was provided using 25 Lpm of 100% humidified air at 14–16°C, which was generated by bubbling compressed dry air through a distilled water bath minimally cooled with a small amount of ice added. A Hudson RCI (Temecula, CA) 1-L medical gas humidifier was used, and the result was steady-state conditions where the air above the water was fully saturated at 14–16°C with evaporative heat losses maintaining a constant water temperature. The connection was made between the humidifier and the NGI with standard oxygen tubing (70 cm), and the flow was sufficiently high to prevent significant ambient heat transfer. This flow was directed to a Y-connector open to atmosphere, therefore any air flow greater than that being drawn through the NGI by the Gast pump could escape at atmospheric pressure. Temperature and humidity of this added gas was verified by an electronic hygrometer/thermometer (Radio Shack, 63-1032). To minimize turbulence and potential increased impaction at the right angle induction port of the NGI, the supplemental air entered a concentric tube encircling the output corrugated tubing of the nebulizer, and both streamed into the NGI with the aerosol being in the centre. Since the temperature was identical to that of the aerosol and there was 100% RH at this point, there should have been no alteration in particle size due to heat

transfer at the exit of the corrugated tubing,[16] although warming and hence loss of mass of the droplets through evaporation had occurred between generation and exiting of the droplets. Work with measles vaccine involved the use of sterile water and ice, and the equipment was rinsed in ethanol and allowed to air dry to ensure absence of bacterial contamination that could overgrow the viral cultures.

As a control, a Pari LC Star nebulizer driven by a Pari ProNeb Ultra compressor was charged with 10 mL of 0.625 mg/mL albuterol. For the Pari LC Star, the entire flow for the Gast pump was supplied by the Pari ProNeb compressor at 5 Lpm with the balance being drawn through the nebulizer as entrained flow (Fig. 3). This entrained flow takes part in the nebulizing process and the resulting aerosol is 100% saturated with water at 17°C.[16] Previous work[16] has demonstrated that as long as heat transfer to the aerosol is prevented, inertial particle sizing by the Andersen Cascade Impactor is virtually identical to that from the laser diffraction for the Pari LC Star nebulizing albuterol. Hence, any difference between the laser diffraction and NGI would be due to differences in the device (NGI compared to Andersen Cascade Impactor) rather than the type of droplet size measurement technique.

Both nebulizers were run for 20 min in order to generate sufficient volume of droplets in the cups representing both the upper and lower extremes of particle size. The NGI was removed from the water bath and carefully dried to prevent water contamination of the cups. The water was "wicked" from the area between the rubber sealing gasket and the edge of the NGI. Once the maximum possible water was removed, it was opened and water wiped from the bottom and sides of the cups. As soon as the outside of each cup was dried it was immediately covered with the pre-weighed laboratory film. The cup and film were reweighed in order to calculate the volume collected. After the cups with the live measles vaccine were weighed, they were placed on ice to maintain viability of the virus until assaying occurred. Obtaining the weight change of each cup allowed for the determination of the distribution of volume, which should agree with the laser diffraction analysis and act as an additional control. Samples were then taken from the well of the nebulizer for post viral assays or ultraviolet (UV) spectrophotometry for albuterol. During

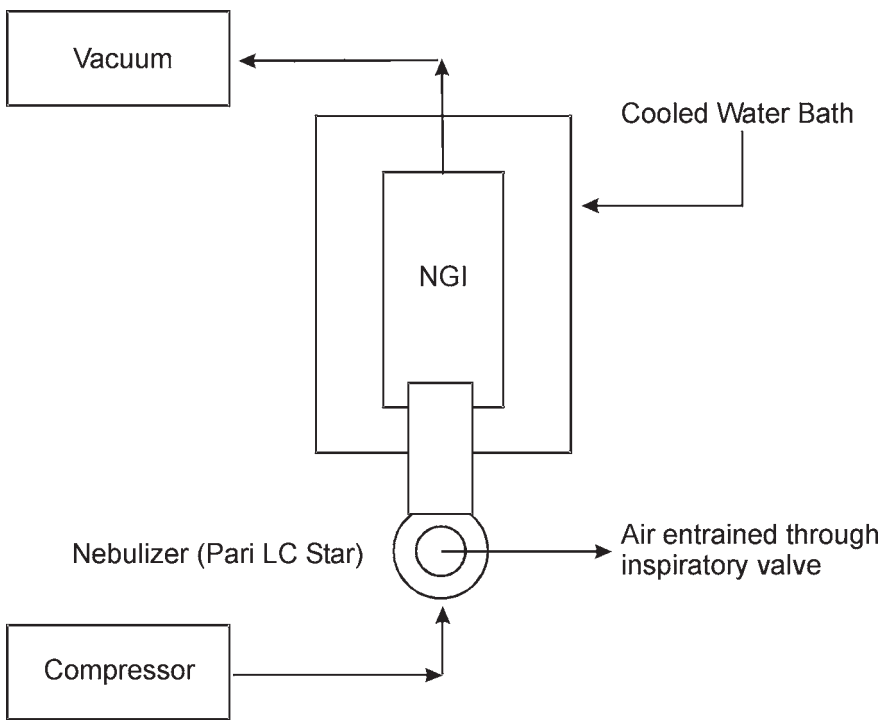

FIG. 3. Set-up used for the NGI with the Pari LC Star experiments.

the drying process, which took 5–10 min, the NGI was out of the water bath and was subject to warming. Furthermore, once the NGI was opened the droplets in the cups were exposed to the ambient air in the viral protection fume hood at 28°C and virtually zero relative humidity, some evaporative losses were unavoidable. To calculate these losses, the difference between the output of the nebulizer in terms of both volume (mL) and mass (mg) of albuterol that left the nebulizer was compared to the amount of both volume and mass of albuterol that was captured in the NGI. For this calculation to be valid, the assumption was made that albuterol is not damaged during nebulization, an assumption that may not be valid for measles vaccine virus.

For the albuterol assay, a 20-fold dilution was made to the liquid in each cup, after which they were continuously rocked for 15 min. Ultraviolet spectrophotometric techniques were used to assay the amount of albuterol[16] within each cup. For the live viral assay, droplets from each cup were removed by pipette. Plaque assays was the method used to establish the number of infectious units in a given viral suspension from each impaction plate.

The technique for measuring droplet size used a helium neon laser particle sizer (Malvern Mastersizer X) based on Lorenz-Mie theory of low angle light scattering by transparent spheres.[17] The details and validation of this technique have been previously described.[16] For the Pari LC Star, approximately 25 Lpm of medical air at 40% RH was used as the entrained flow, which was supplied in place of the inspiratory valve.

Quantitative viral assay

Fukuda et al.[18] have described the optimization of a plaque assay for titrating infectious measles virus in Japanese measles vaccines. The Fukuda procedure, with some minor modifications, was used for the titration of infectious measles virus for this aerosolized measles vaccine study. The samples in this study were aliquots of solutions collected from the different impaction plates of the NGI. Multiple replicates of pre-aerosolized reconstituted vaccine as well as the corresponding post-aerosolized NGI samples were tested. The outline for the measles plaque assay was as follows: Vero cells from standardized identical frozen stocks were seeded into 6-well tissue culture plates (4×150 cells/well) so that cells were approximately 80–90% confluent. Dilutions of 1:10 of the samples in EMEM (Earle's Minimal Essential Medium) media (no FBS (Fetal Bovine Serum) or antibiotics) were made. Cells were infected with 100 μL of diluted virus and allowed to adsorb for 1 h at 35°C with continuous rocking of the plates, after which 3.0 mL overlay media (EMEM, 0.5% agarose, and 2% calf serum, 1% antibiotic/antimyotic) was added to the 6-well dishes and then incubated for 7 days at 35°C (5% CO_2, humidified). A 1.0% neutral red solution (in overlay media) was added to the wells on the second to final day of incubation. The cells were incubated overnight. The plaques were counted on a light box, which allowed for the calculation of the infectivity titter, expressed as plaque forming units per mL (pfu/mL), and was calculated as follows:

$$(\text{Plaque number}) \times (\text{reciprocal of the dilution}) \times (\text{reciprocal of volume in mL})$$

Quality control issues for the virology testing

Vero cell stocks were prepared in an ISO17025 accredited cell culture production facility at the Canadian Science Centre for Human and Animal Health (CSCHAH). All Vero cell stocks were from the same lot and were tested for contamination (e.g., mycoplasma testing). Seeding of cells for the plaque assays was completely standardized. The same lot number for other reagents including culture media and cell culture plates were used. An incubator for the plaque assay incubations was dedicated for this purpose, and was monitored using both a datalogger (for hourly monitoring of the temperature), and an electronic alarm system in place at the CSCHAH.

Data analysis

Triplicate runs using one example of each nebulizer were used to compare particle sizing techniques. Laser diffraction and NGI data were used to construct linear and logarithm-probability (log-probit) plots[19] from which the mass median diameter (MMD) and the geometric standard deviation (σSD) were derived.[16] It was assumed that the particle size distribution was unimodal and normally distributed. This would be confirmed by a linear log-probit plot. Also plotted were the cumulative volume distribution from the plates of the NGI for both the live virus assay and albuterol. Since there were evaporative

TABLE 1. COMPARISON OF THE MMD OBTAINED FROM THE PARI LC STAR AND IPI WHEN NEBULIZING ALBUTEROL

	Pari LC Star		*IPI*	
Albuterol (mean ±95% CI)	*MMD (μm)*	*σSD (μm)*	*MMD (μm)*	*σSD (μm)*
Laser diffraction (raw data)	3.24 ± 0.06	1.80 ± 0.06	4.49 ± 0.15	2.47 ± 0.23
Laser diffraction (adjusted data to 16°C)	—	—	4.26 ± 0.17	2.96 ± 0.15
NGI (volume)	3.22 ± 0.25	1.84 ± 0.03	5.02 ± 0.14	2.08 ± 0.09
NGI (UV spectrophotometry)	2.93 ± 0.22	1.88 ± 0.03	4.26 ± 0.24	2.41 ± 0.08

Values presented in this table were obtained from three runs by a single LC Star and IPI nebulizer. The LC Star did not require adjustment of laser diffraction data since evaporative losses were not an issue. Values are mean ± 95% CI. MMD, mass median diameter; σ_{SD}, geometric standard deviation.

losses with the IPI, it was anticipated that the quantification of albuterol from the NGI would result in a smaller particle size distribution compared to laser diffraction. If the volume of liquid water that went evaporated with this warming is subtracted from the droplets proportional to the surface area of the droplets (since heat transfer is a function of liquid surface area exposed to vapor) the concentration of albuterol in each droplet size can be calculated (see Appendix) and a new plot constructed for comparison to data from the NGI. Data were expressed as mean ± 95% confidence limits (95% CI) for comparison purposes.

RESULTS

The particle size distribution for the Pari LC Star nebulizing albuterol measured with the NGI was similar to that measured with laser diffrac-

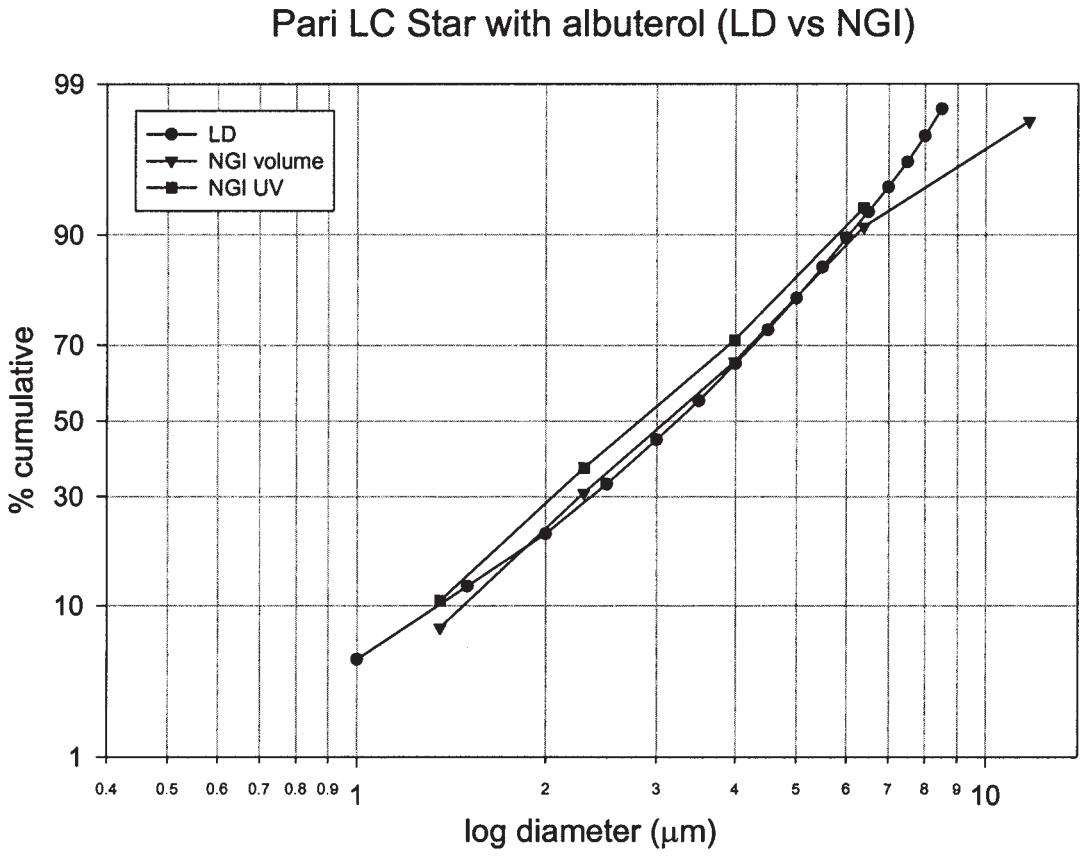

FIG. 4. Particle size distribution obtained from the Pari LC Star nebulizing albuterol when measured with laser diffraction (LD), volume collection with the NGI, and UV spectrophotometry with the NGI.

tion (Table 1 and Fig. 4). Both curves are reasonably linear suggesting that the true size distribution was both log normal[19] and unimodal. Whether the results were plotted for the amount of albuterol (UV spectrophotometry) in each cup of the NGI or the volume of material collected, the results were the same indicating that the concentration of albuterol was evenly distributed throughout all the droplets. For the IPI nebulizer, there was a half micron difference between laser diffraction and the NGI for volume collected in each cup (Fig. 5). There was no difference between the laser diffraction particle size distribution adjusted to 12°C and when the amount of albuterol in each cup is quantified (Table 1). This was in keeping with warming of the aerosol between when it was generated in the well of the nebulizer until it entered the NGI, resulting in evaporation which changed the drug content of the particles proportional to the surface area of the droplets so that the concentration was higher in the smaller droplets compared to the larger. Once the raw data was adjusted to reflect the change in particle size distribution due to heat transfer, there was good agreement between the calculated albuterol concentration and laser diffraction (Fig. 6). If distribution of drug was the goal, it was the NGI that was correct not laser diffraction, since the albuterol was not uniformly distributed in the droplets by the time the droplets left the corrugated tubing at the end of the IPI nebulizer.

When live measles vaccine was aerosolized using the IPI nebulizer, there were marked differences in particle size distribution based on the quantitative plaque assay from the NGI and laser diffraction measurements of droplet size (Fig. 7). In this case, the particle size distribution from the NGI was significantly greater than the droplet size distribution measured by laser diffraction

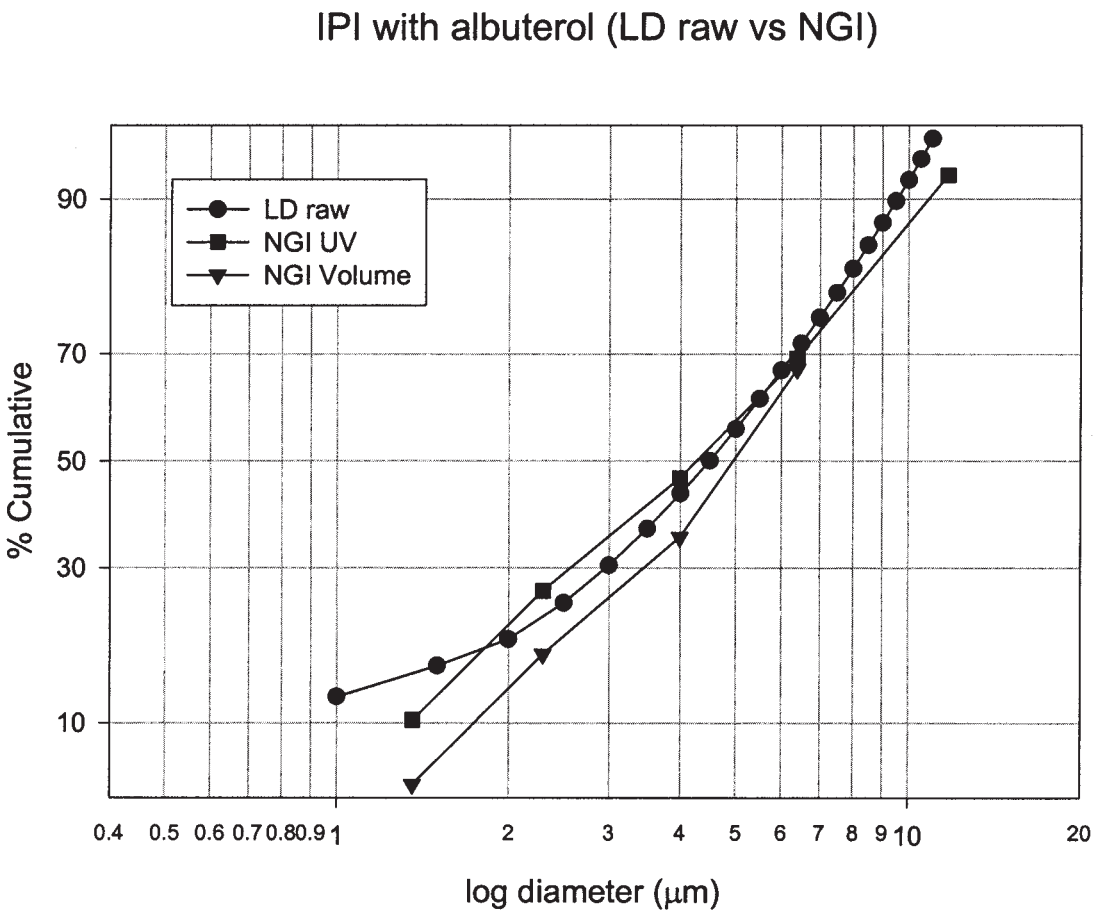

FIG. 5. Particle size distribution obtained from the IPI nebulizing albuterol when measured with laser diffraction (LD), volume collection with the NGI, and UV spectrophotometry with the NGI.

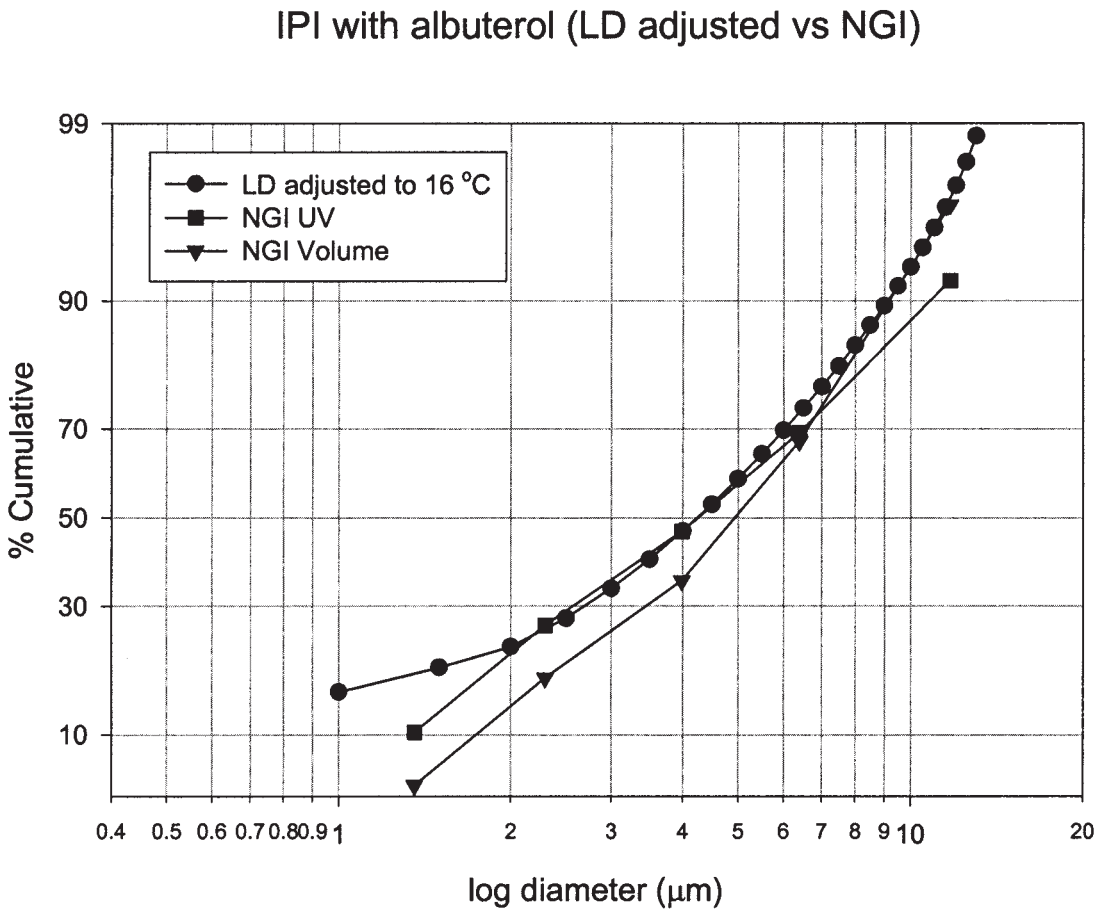

FIG. 6. Particle size distribution obtained from the IPI nebulizing albuterol when measured with laser diffraction (LD), volume collection with the NGI, and UV spectrophotometry with the NGI. The LD data has been adjusted to reflect warming and evaporative water loss from 12°C to 16°C.

(Table 2 and Fig. 7). This suggests that there must have been a significant amount of viral clumping which resulted in the virus being under-represented in the smaller droplets and over represented in the large droplets. This was confirmed comparing volume (weight distribution) in the NGI to droplet size measured by the laser diffraction (Fig. 7) which shows an agreement much like that shown in Table 1 for albuterol.

DISCUSSION

These results would suggest that the NGI can be used to determine active particle size distribution of a live biological agent. The particle size distribution is that of the biological agent itself rather than just droplets as would be determined by laser diffraction. The distinction is important where the distribution of the agent may not be uniform. An advantage of the NGI based method is that the biological agent is not desiccated for analysis, and sufficient volume may be collected to facilitate quantitative viral assay. Furthermore, the NGI is quite appropriate for particle size determination of any liquid aerosol, even if changes have occurred so that the distribution of the drug is not uniform within all the droplets.

Jauernig et al.[15] compared the particle size distribution of the NGI to the ACI and laser diffraction. Their results comparing the ACI with laser diffraction are in agreement with the results of Kwong et al.,[16] who found very good agreement between the two devices. Jauernig et al.[15] were also able to demonstrate good agreement between laser diffraction and the NGI using albuterol, but the NGI had a consistently smaller MMD by 0.3 μm. In the present study, the results using albuterol and the LC Star echo this difference, therefore the question remains why the ACI

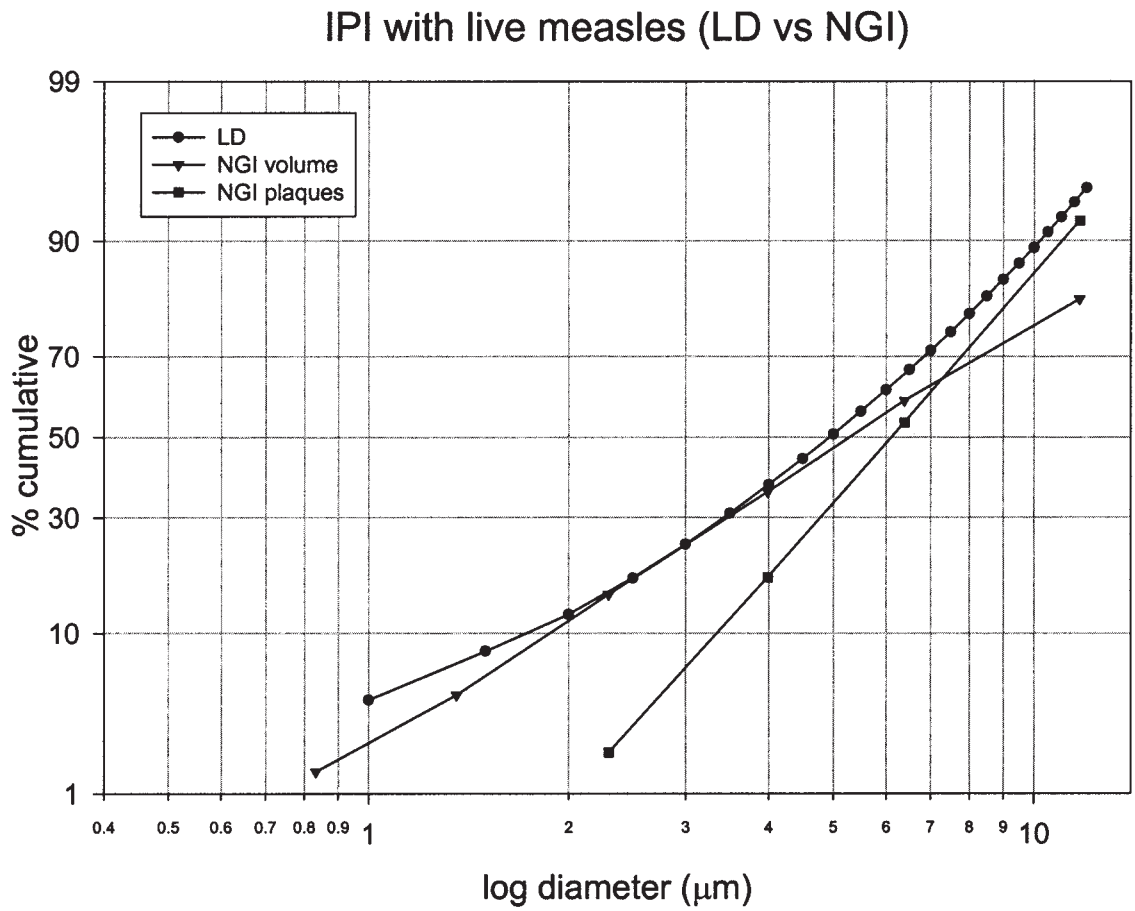

FIG. 7. Comparison of the particle size distributions obtained from the IPI nebulizing live measles vaccine. Particle size distributions obtained by LD, volume accumulated in the NGI, and plaque assay results obtained from the cups of the NGI. Evaporation of measles vaccine is not as predictable as albuterol; therefore, LD data is raw and not adjusted as in previous figures.

has better agreement than the NGI to laser diffraction. It is important to realize that the log probit plot greatly exaggerates the upper and lower end of the y-axis and the slightest of differences in these regions appear large, whereas it is the data in the middle of the graph between 20% and 80% of the data which should be used for comparisons.

The purpose of this paper is to define any variation in particle size distribution between techniques (laser diffraction vs. NGI), and the appropriateness of using the NGI to particle size live measles vaccine, not the differences and variations between the nebulizers themselves. The decision was therefore made to complete triplicate testing of one nebulizer to prevent the introduction of variation due to differences within nebulizer functioning. One sample of the LC Star and IPI were used for all runs with albuterol, and differences in mean values were implied when there was no overlap of the 95% CIs.

At first glance, it is apparent that the agreement with laser diffraction and the NGI for the Pari LC

TABLE 2. RESULTS OF THE IPI NEBULIZING MEASLES VACCINE

Measles (mean ±95% CI)	*IPI*	
	MMD (μm)	*σ_{SD} (μm)*
Laser diffraction	4.95 ± 0.16	1.96 ± 0.08
NGI (volume)	5.43 ± 0.26	2.31 ± 0.02
NGI (plaques)	6.14 ± 0.39	1.69 ± 0.02

Values presented in this table were obtained from three runs of a single IPI nebulizer. Values are mean ± 95% CI. Laser diffraction data was not corrected for possible evaporative losses, since the assumptions for albuterol can not be made regarding the measles vaccine virus. MMD, mass median diameter; σ_{SD} geometric standard deviation.

Star was much better than that obtained by the IPI when nebulizing albuterol. There are two major concerns when characterizing the IPI with the NGI. The first is the minimization of heat transfer; the second was altering the characteristics of the aerosol due to the addition of supplemental flow. The NGI and supplemental flow were both cooled to avoid heat transfer within the NGI, but heat transfer had already occurred before the aerosol either entered the NGI or was presented to the Malvern due to evaporation that occurred from the well of the IPI to the end of the corrugated tubing. In this situation, it is evident that the laser diffraction measurements will be in error as this technique measures only droplet size and assumes uniform distribution of drug within the droplets. As described in the methods and Appendix, the particle size distribution for the IPI obtained by laser diffraction can be adjusted to reflect evaporative water loss and the concentration of albuterol in each particle size range can be calculated. When this operation was carried out, the results between the NGI and laser diffraction agreed. Note that there was little difference between the particle size distributions measured by volume or quantification of albuterol for the Pari LC Star where the concentration of albuterol was distributed evenly throughout all the droplets. Furthermore, the calculated concentration of albuterol for the IPI nebulizer in each size range seen by the NGI agreed with that measured. All of this would imply that there was no significant heat transfer within the NGI, or that there was a distortion of the particle size distribution by the additional conditioned air added to meet the flow requirements of the NGI. In other words, in this particular case, the accurate measurement of particle size distribution was the NGI, not laser diffraction. The difference between laser diffraction at 16°C and the NGI volume collections for the IPI could be due to small errors in the "condition" of the added flow to meet NGI requirements, since it is not seen with the LC Star nebulizer where no added flow is required. These errors would include some turbulence where the flow is added, small differences in humidity, temperature or a combination of all three. It is impossible to go through similar calculations to correct for evaporative losses for the live measles vaccine, because viral clumping is not as predictable as increases in drug concentration due to evaporation. There is nothing to suggest that the particle size distribution from NGI was less accurate with measles vaccine virus as it was with albuterol. We can speculate that since evaporative losses affect the smallest particles most and viral clumping resulted in many empty small particles, the results are a true reflection of reality. As all the droplets were liquid, it was assumed that once they impacted on a cup, they remained there, in other words, "particle bounce"[20] that may be seen with metered dose inhalers was not considered a factor in this situation. At the time of the study there was only one calibration available for the NGI at 30 Lpm, which necessitated much extra work to ensure the supplemental flow was not distorting the aerosol output. There is now a second calibration for the NGI at 15 Lpm,[21] which will make future work less intensive.

In summary, the Next Generation Pharmaceutical Impactor offers accurate inertial particle sizing possibilities for wet aerosols that do not require desiccation, and hence are very appropriate for particle sizing live or active biological agents. The NGI also overcomes the inevitable problems with laser particle sizing when heat transfer to the aerosol has allowed a change in droplet size between the generation of the aerosol and the measurement.

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APPENDIX

The following calculations were performed for particles from 0.5 to 20.0 μm in 0.5 μm increments. The data was obtained from laser diffraction measurements that were generated at 12°C, but due to warming during passage through the corrugated tubing of the CMD, the aerosol was measured at 16°C. The calculations show how the data was adjusted to reflect evaporative losses.

Known variables:

1. 22.4 L Air weights 29 g at STPD (Standard Temperature and Pressure, Dry)

Using Charles Law,

a. At 12°C the density of air = 29/(22.4*(285/273)) = 1.2401 g/L. At 16°C the density of air = 29/(22.4*(289/273)) = 1.2230 g/L

2. Rate of Output of the IPI nebulizer = 0.45 ml/min

Flow driving the nebulizer = 10.2 L/min

b. Aqueous output per L of air at 16°C = (0.45)/(10.2) = 0.04412 ml/L

3. To calculate the water loss from 12°C to 16°C, there is 8.93 mg of water in one liter of air at 12°C at 100% RH and 11.67 mg/L at 16°C at 100% RH,

c. Water loss = 8.93 – 11.67
= −2.74 mg/L

To continue with the calculations, the following assumptions were made:

- Loss of water vapor is proportional to the surface area of the particle
- No particles disappeared through evaporation or added rain out
- Relative humidity in all situations is 100%
- There was no damage to the albuterol during nebulization

The following example calculations are for 5 μm diameter particles measured at 16°C by laser diffraction (LD) for the IPI nebulizing salbutamol (0.625 mg/ml):

From LD data – 6.35% of volume was found in particles between 4.5-5 μm diameter

d. Total volume of H_2O in 5 μm particles per L of air
= 0.4412 ml/L × 0.0635
= 0.00280 ml

Volume of a sphere = $(4/3)\pi r^3$, where r = radius (μm)

e. Volume of water per 5 μm particle
= $(4/3)(3.1416)(5/20000)^3$
= 6.545×10^{-11} ml/particle

f. Total number of 5 μm particles
= (d) / (e)
= 4.2803×10^7 particles

Surface area (SA) of a sphere = $4\pi r^2$

g. Surface area per 5 μm particle
= $4(3.1416)(5/20000)^2$
= 7.854×10^{-7} cm^2

h. Total cumulative SA for 5 μm particles
= (g) / (f)
= 33.6167 cm^2

i. Fraction of total SA for 5 μm particles
= (h) / Total cumulative SA for all particles
= 33.6176/804.7059
= 0.04178

j. Water vapour loss for 5 μm particles
= ((c) × 1/1000) / (i)
= 1.1445×10^{-4} ml

k. Concentration of salbutamol in 5 μm particles
= 0.625 mg/ml × ((d) / ((d) − (j)))
= 0.6516 mg/ml

l. Mass of salbutamol in 5 μm particles
= (k) / (d)
= 0.001825 mg

m. Percentage of salbutamol in 5 μm particles
= (l)/Total cumulative mass of salbutamol
= 6.1897%

Once the percentage of salbutamol was calculated for each particle size, the % cumulative salbutamol was calculated and used in the log-probit plot (Fig. 4). Confirmation that this analysis was correct comes from agreement in particle size distribution between the calculated NGI particle size distribution and that measured by UV spectrophotometry quantification of the amount of albuterol in each cup at each cut point, as well as the agreement between the concentration of albuterol in the particle size ranges measured by laser diffraction (summed over the larger intervals of the NGI). These calculations cannot be adapted to the measles vaccine since there is some degradation to the virus during nebulization.

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