Design of Experimental Setup for Identification of Parameters for Optimal Aerosolization of Measles Vaccine

by

Laura A. Nicholson

SUBMITTED TO THE DEPARTMENT OF MECHANICAL ENGINEERING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE IN ENGINEERING AS RECOMMENDED BY THE DEPARTMENT OF MECHANICAL ENGINEERING
AT THE
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

JUNE 2009

Signature of Author: _____

Department of Mechanical Engineering
May 11, 2009

Certified by: _____

Professor John Kim Vandiver
Professor of Mechanical and Ocean Engineering
Thesis Supervisor

Accepted by: _____

Professor J. Lienhard V
Collins Professor of Mechanical Engineering
Chariman, Undergraduate Thesis Committee

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ABSTRACT

Given the pressing worldwide need for measles vaccination coverage, measles vaccine administration via inhalation is a viable option which overcomes many obstacles currently facing vaccine distribution. Although aerosolization is well understood, studies have focused on pharmaceuticals and very little data is available regarding vaccines. The proposed study analyzes the relationships between various pre- and post-aerosolization parameters in order to calculate the "aerosol fingerprint," or combination of pre-aerosolization parameters optimized to produce the most effective aerosol particle size distribution for measles vaccination. Relevant pre-aerosolization parameters are identified as relative humidity, nebulizer temperature, vaccine reconstitution, solution pH, surface tension, viscosity, air pressure, and nebulizer geometry. Relevant post-aerosolization parameters are identified particle size distribution, aerosolization endurance and bioavailability, and drug delivery rate. Sensing, actuation, automation and special concerns for each variable are considered.

Thesis Supervisor: J. Kim Vandiver
Title: Professor of Mechanical and Ocean Engineering

Acknowledgements

First I would like to thank Jose-Gomez Marquez for inviting me into his research and allowing me to define this thesis in the way I found most interesting and personally valuable. His technical advice made this paper possible, and I'm especially grateful for his desire to be supportive when work was the most stressful.

I would also like to thank Amit Srivastava for lending his expertise and knowledge in vaccines, and his patience in helping me remember chemistry lessons long forgotten.

I am indebted to Professor Vandiver for his support throughout my last year at MIT, especially in this thesis project.

I am deeply grateful to Professor Asada, my academic advisor, who supported me and remained on my side through my less-than-smooth path through MIT.

I would also like to mention Professors Eric Hudson and Haynes Miller, for the confidence they displayed in me early in my undergraduate career, when it was most needed. Their gestures were so small I doubt they remembered them by the end of the day, but I still remember clearly after more than three years.

And finally, I would like to thank my parents, family and friends, who have supported me throughout my whole life and especially during the past four years.

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1. Introduction

1.1 Need for and Justification of Measles Vaccine Inhalation Technology

The World Health Organization estimated that in 2002, 1.4 million children under five years of age died from vaccine-preventable diseases. Of this total, 510 000 of those deaths, or 36 percent, were caused by measles[1]. Although measles vaccine coverage has been improving in recent years, there were still 197 000 measles-related deaths worldwide in 2007 (WHO Measles)[2].

Despite this progress, measles vaccine administration remains a logistical challenge throughout the developing world. A large percentage of the world's poor live in rural and isolated areas, making vaccine distribution (and indeed any medical intervention) difficult or even impossible. Also, as vaccines are extremely heat-sensitive and must be kept within a narrow temperature range to prevent the denaturing of the essential proteins, delivery depends on the integrity of the cold chain. Given the unreliable or even nonexistent access to electricity in most of these resource-limited settings, the need for refrigeration is one of the most important obstacles to universal vaccine coverage. Additionally, the use of hypodermic needles for vaccine administration poses a large public health hazard. Needle misuse and reuse is common, as resources are scarce and medical personnel often lack appropriate training[3]. Lack of adequate biohazardous waste facilities further poses a problem with safe disposal of used materials.

These obstacles can be overcome by the adaptation of aerosolization technology to the administration of vaccines. Aerosolization, or nebulization, has long been used to administer medication directly to the lung in the treatment of respiratory illnesses such as asthma and chronic obstructive pulmonary disease. The effective use of the technique for the purposes of vaccination was demonstrated as early as 1960[4]. Further, a study done in 1984 demonstrated that measles vaccination by inhalation in fact produced a greater antibody response when compared to traditional subcutaneous

administration by injection[5]. This result was corroborated by further studies in 2000[6] and 2002[7]. The World Health Organization has recognized the potential benefits of this technology and is actively sponsoring research through the Measles Aerosol Project, which seeks to facilitate the development of "aerosol vaccination devices"[8].

One suitable device is Aerovax, which is currently under development at MIT. Aerovax is an appropriate technology device designed to allow local health care workers to administer aerosolized vaccines in extremely resource-limited settings with very little medical training. The device is needle-free (and therefore safe and painless), portable, and low-cost. The device's jet nebulizer can be operated on energy supplied by a battery, human, or compressed air, making it usable even in areas where the infrastructure necessary to operate developed-world medical devices is unreliable or non-existent. Initial development of the device is focused on aerosolization of commercially available injectable measles vaccine. However, more data is needed about the optimal aerosolization of reconstituted vaccines.

1.2 Need for Aerosol Fingerprinting

Although many studies have investigated the mechanics of aerosolization (both in general and in relation to specific drugs) and the mechanism of particle deposition in the lung tissue, very little work has been done which is directly useful to the development of an effective vaccine aerosolization device. Reconstituted vaccines have different physiochemical properties (which affect droplet formation) and endurance requirements (which relate to post-deposition efficacy) than drugs which have been tested. Additionally, previous studies have focused on simple relationships and have not attempted to optimize across multiple variables. Numerous parameters have been shown to affect droplet formation (which in turn affects lung deposition), but none of these parameters have been optimized to achieve the aerosolization with the ideal particle size distribution for that same purpose[9]. An experiment identifying the "aerosol

fingerprint," defined as the parameters for optimal aerosolization, has been proposed to further the development of the Aerovax device and ensure that the resulting technology incorporates the relationships between pre- and post-aerosolization variables in order to produce the most efficacious vaccine possible. This paper will focus on the identification of the variables relevant to optimization, and an overview of known relationships, the appropriate choice of sensor and actuator for the automated experiment, and discussion of important considerations for each.

1.3 Overview of Proposed Experiment

A high-throughput experiment with feedback will allow aerosol fingerprint mapping, whereby multiple variables can be controlled and measured at once. A baseline sample of buffer (normal or phosphate buffered saline, which are typical vaccine diluents) will be nebulized for 30 seconds, producing an aerosol cloud whose particle size distribution will be analyzed and recorded. A computer-controlled actuation system will then cycle through a range of input parameters, creating a full matrix mapping all combinations of these parameters to the corresponding particle size distribution and other output variables. A feedback loop will then focus on promising pre-aerosolization parameter regions, narrowing down the possible parameters of the aerosol fingerprint. This will be repeated with a measles vaccine simulant (such as an enzyme whose activity can be measured post-aerosolization to assess if the aerosolization process caused any protein degradation), and then with the commercially available live attenuated measles vaccine to verify the effectiveness of the deduced aerosol fingerprint. Plating of the aerosolized vaccine will then determine the effect that the process has its bioavailability. Finally, an Andersen Cascade Impactor will be used to simulate the filtering action of the human respiratory tract, verifying that the aerosolized particles are able to penetrate to the appropriate level of the respiratory tract and deposit live vaccine.

Important pre-aerosolization parameters are relative humidity, nebulizer temperature, vaccine reconstitution, solution pH, surface tension, viscosity, air pressure, and nebulizer geometry. Relevant post-aerosolization parameters are particle size distribution (PSD), aerosolization endurance and bioavailability, and drug delivery rate.

The results of this experiment will allow development of the Aerovax device to focus on the factors which will directly affect the successful administration of the vaccine.

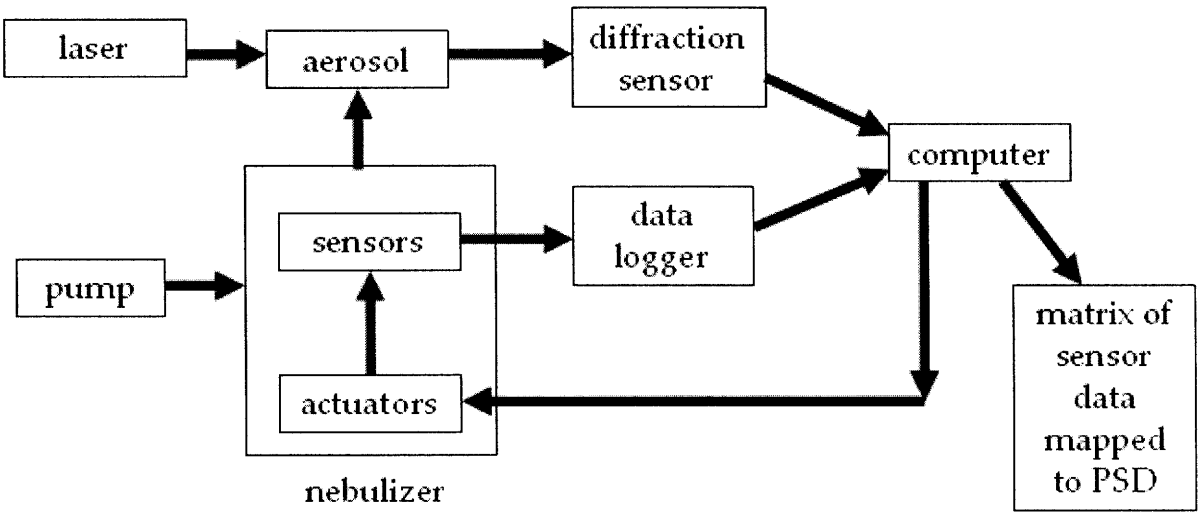

Figure 1. Schematic of the proposed experiment

2. Pre-Aerosolization Parameters

2.1 Relative Humidity

Since the Aerovax device is being developed for use all across the world, it must be functional in a wide variety of climates, from the deserts to the tropics. Therefore it is imperative to study the effect that ambient relative humidity has on droplet formation. Humidity is known to affect output particle size distribution indirectly through its effect on nebulizer temperature[10]. However, this is not an important relationship for our purposes because we will also be measuring temperature

independently of humidity. Relative humidity is important, however, because it can lead to post-aerosolization clumping[11].

We will measure humidity using a Honeywell HIH-4000 Series integrated circuit humidity sensor, which operates between 0% and 100% relative humidity. This sensor was chosen because it can operate within the temperature range of -40° C to 80° C, which is well within the temperature range which can be expected in the testing scenario. More importantly, however, the sensor output is an analog voltage which is nearly linear with relative humidity. This allows the signal from the sensor to be read, converted, and recorded by data logging software.

We will vary the humidity with a commercially available humidifier designed for home use, the Honeywell QuietCare Cool Moisture Humidifier (HCM650). This model was chosen because it features a digital control system which allows the user to set and maintain exact humidity values, eliminating the need for feedback control between the sensor and the actuator. Computer control will be accomplished by modifying the device to send and receive signals from the data logger. The relative humidity should be tested along the full range of 0% to 100% to represent the full array of conditions the device will encounter.

As relative humidity will not be possible to manipulate in the field, it must be treated separately from the other pre-aerosolization parameters. Ideally the humidity would not affect output parameters, but in the case that it does, the variable cannot be included in the optimization feedback loop. Instead, it should be considered as a local variable within the context of appropriate technology design. Optimized aerosol fingerprints can be identified at various values along the full range. With this information, various iterations of the device could be developed to operate properly in all climatic regions of the world.

2.2 Nebulizer Temperature

During the nebulization process, a well-documented decrease of about 10°C in the nebulizer temperature takes place[10]. Phipps, et al indicated that the falling temperature of the nebulizer led to a reduction in the particle size distribution[12].

The sensor to be used to measure temperature is a Flexible Hermetic Sealed PFA RTD Sensor Probe, which was chosen because the experimental temperatures will fall well within the range of the sensor (which is -60°C to 260°C) and because the probe has a standard four wire configuration which will allow for easy integration with data logging software. Also, the sensor simply consists of a thin, flexible wire which can be easily inserted into the nebulizer and remain operational throughout the length of the trials, allowing real time measurement.

The temperature will be controlled through a simple heating coil, the Amana Dryer Element Restring Kit. This was chosen because it is a simple heating coil designed as a replacement part for a commercial product. As a consequence, it has no additional features or circuits, allowing us to easily control the heat output by simply controlling the current flow through the coil. The exact relationship between applied voltage or current and temperature must be established experimentally to allow for the automation of the temperature control.

Because a change occurs during the process, the important factor to consider here is the length of time it takes for the system to reach a steady state temperature, and how that compares to the length of time necessary to dispense a single dose. This delivery time will depend on the values of other parameters in the final aerosol fingerprint (see Section 3.2), but should be on the range of a few minutes or less. If time to achieve a steady state temperature is not a small portion of the total drug delivery time, patients could receive different doses based on the order in which they were vaccinated. In this case, further study into the relationships involved will be necessary.

For the purposes of this experiment, this time-dependent relationship can be simplified by implementing a feedback loop between the temperature sensor and actuator. Each trial will be designed to take place at a certain temperature, and feedback from the sensor will adjust the output from the actuator to ensure that only small fluctuation in the nebulizer temperature occur over the trial.

2.3 Vaccine Reconstitution

Vaccine reconstitution comprises two variables: buffer solution and solute concentration. These two parameters will be the main factors affecting several chemical properties which are known to affect droplet formation, namely pH, surface tension, and viscosity. Although all five variables are closely interrelated, I will consider each separately because in most cases variation along one parameter is still possible without variation along all others. For example, surface tension will vary with choice of buffer solution and solute concentration, but it can still be varied independently of the other two. Also, even though complex interrelations exist between the variables, understanding the basic effect that each has on nebulization will be useful.

Vaccine, the solute, is dissolved in diluent for injection. A more dilute solution might be necessary for nebulization (compared to the injectable solution). This would necessitate a longer exposure time to the aerosol to achieve the same does.

Solute concentration of nebulized drugs is known to increase with time, as water evaporates from the gas-liquid surface at a rate faster than drug-saturated diluent is nebulized[9]. Concentration as a function of time can be expressed by the equation

$$C(t) = C_0 \left(\frac{V_0}{V_0 - (W+S)Ft} \right)^{(WW+S)}$$

where W and S are the output per liter of air (in mL/L) of the solution and solvent, respectively, F is the flow rate, and V_0 is the initial volume.

Since this relationship is a result of the jet nebulization technology it cannot be altered. Therefore our trials will investigate the effect that the initial concentration has on the output parameters. We can expect it to affect the drug delivery rate directly (see Section 3.2), but we hope to discover if it also affects particle size distribution.

The buffers to be used are tris-buffered saline and phosphate buffered saline, because these are commonly used in other vaccine reconstitutions[13]. The two will be tested separately to determine if the choice of buffer solution has any effect on the relevant output parameters. Solute concentration will be controlled by manually varying the vaccine formulation and recording the values used.

Since concentration increases over time, precipitation of the vaccine out of solution is a possible concern. The range of concentrations tested will be limited to those which prevent that danger.

2.4 Solution pH

No studies have attempted to determine the possible relationships between pH and aerosol generation, but as a wide range of pH values can be generated using buffer solutions, it merits investigation as a possible source of aerosol improvement.

The experiment will use a Lazar Ultra-M micro pH electrode, which was chosen because it operates on the full pH scale with a resolution greater than needed for the experiment, and because it has a digital output which can be read by a data logger. Its

small size allows it to be fixed in the device during the trial and provide real-time measurements.

If a significant change in pH is found to occur during the length of a single trial (which might occur because of the varying rates of aerosolization and evaporation as discussed in the previous section), further investigation will be necessary.

The pH will be controlled by the addition of the buffer solutions, prepared according to commonly available buffer solutions and not to exceed the range within which the vaccine remains viable. Automated titration is possible with a computer controlled actuator which would release prepared solution in response to an electrical signal. The pH should be varied along a wide range centered on the normal value (sterile water has a pH value around five). However, drastic changes in pH might damage the live vaccine. The actual safe range of pH values must be determined experimentally.

2.5 Surface Tension

Surface tension has been shown to affect droplet formation. Surface tension represents the tendency of a liquid to resist changes in surface area. Since droplet formation represents an increase in total surface area, the energy required for nebulization can be expressed by multiplying the surface tension by the increase in liquid surface area[14]. Therefore a higher surface tension relates to larger droplets which are formed more slowly[15].

We will measure surface tension using the pendant drop method. Because none of the methods available for measuring surface tension can be performed in the nebulizer, each reconstitution will be analyzed before aerosolization and manually recorded. The pendant drop method is more reliable than other available measurement methods, and can be performed with a device such as the Kruss machine, which uses a drop-fitting software package to calculate the surface tension.

Surface tension can be decreased by the addition of surface acting agents, or surfactants. Surfactants are molecules which consist of a hydrophilic head and a hydrophobic tail, enabling them to gather at the liquid-gas interface and reduce the surface tension of the liquid. Possible surfactants include cetyl alcohol and lecithin, both of which are non-toxic and are regarded as safe for human consumption. Values along the full range (from maximum to minimum surface tension for each solution) should be tested. The relationship between surfactant concentration and surface tension will be determined experimentally beforehand to allow for automation.

It is important to note that a study of the use of surfactants in aerosolization suggested a correlation of surfactant use with reduction in particle delivery to the lung[11]. Although the conditions in the study were different from those in this application (the study attempted to promote cetyl alcohol adsorption through prolonged contact post-aerosolization, while in our scenario contact will be shorter and occur pre-aerosolization), it is important to note that surface tension cannot be treated as a valid parameter in the aerosol fingerprint until aerosol endurance and bioavilability of the live attenuated vaccine after treatment with surfactant have been measured. Before the addition of surfactants can be automated and included in the aerosol fingerprint mapping process, surfactant-treated live vaccine must be aerosolized to determine the effect the agents have on lung deposition and bioavailability.

2.6 Viscosity

The effect that viscosity has on nebulizer output appears to be complex and not very well understood. Increased viscosity seems to be associated with reduced particle size, but the relationship is stronger in the extremes of the range (jet nebulizers are unable to aerosolize extremely viscous liquids, whereas changes in the middle range of viscosity values lead to relatively small changes in particle size distribution). Viscosity

also appears to affect the behavior of the liquid-gas interface in the nozzle region, resulting in decreased mass flow (and therefore increased nebulization time)[14].

However, we will not measure the viscosity directly, as the only factors which can affect the viscosity (vaccine reconstitution and temperature) are already being recorded. This simplifies the experiment somewhat by eliminating a variable which can be difficult to measure without actually ignoring any relevant information.

2.7 Air Pressure

Typically the air pressure required for nebulization is between 40 and 60 pounds per square inch (psi). This parameter has a direct effect on the output flow rate, but it is unclear how it also affects the particle size distribution.

The air pressure can be easily controlled and measured by the use of a pressure regulator attached to the Precision Medical EZ Neb PM7 air compressor used to operate the nebulizer. We will test pressures within the 30-70 psi range in order to the able to examine data outside the usual range.

2.8 Nebulizer Geometry

The effect that nebulizer geometry has on particle size distribution is not well understood. It is widely recognized that different nebulizers produce aerosols with different properties, but studies usually just keep track of different models, and not the variables which differentiate them. As a preliminary step, this study will associate different nebulizer models with their geometries in an attempt to discover possible relationships between nebulizer geometry and aerosol output.

Aerosolization within jet nebulizers occurs in two stages. First, the high pressure air from the compressor flows through the central tube, creating a pressure differential between the top and bottom of the liquid reservoir. This causes the liquid to rise up the liquid feed tube and come into contact with the flowing air just before the nozzle (see

Figure 2). Primary droplet formation occurs at this interface as the flowing gas breaks particles free from the liquid surface. These particles are still slightly too large for effective inhalation, so secondary droplet formation is induced by placing baffles between the jet nozzle and the final output stream. Particles crash against these baffles and are broken into smaller particles which are of an appropriate size for deposition in the lungs.

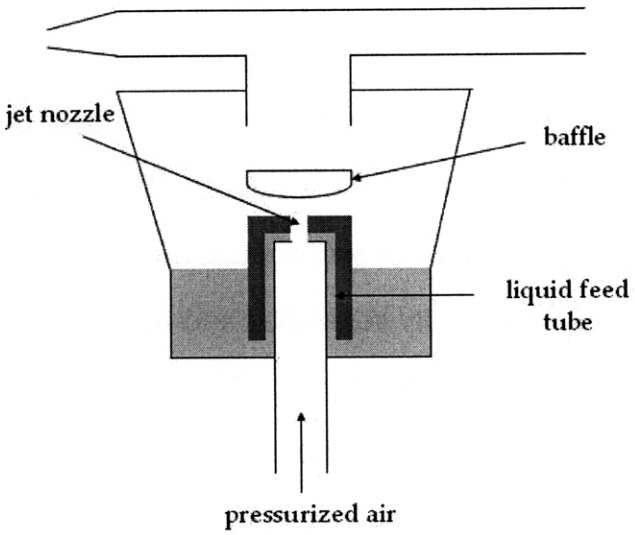

Figure 2. Schematic diagram of a typical jet nebulizer

The following three dimensions might be of interest in studying the effect that nebulizer design has on the resultant particle size distribution: the jet nozzle diameter, the baffle surface area, and the distance between the jet nozzle and the baffle[9].

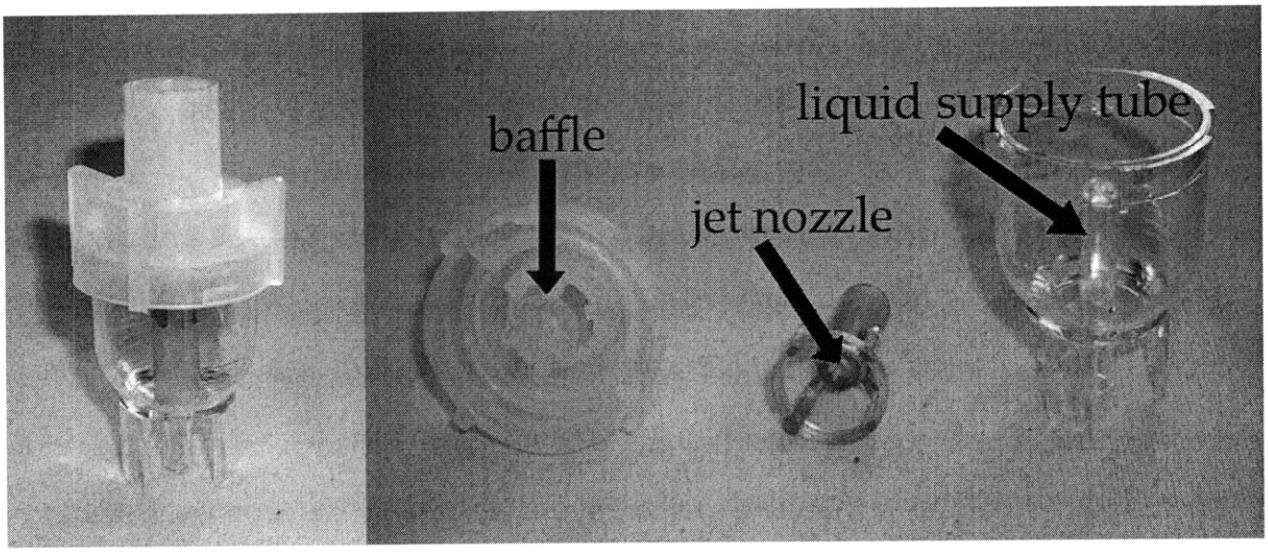

Figure 3. A typical jet nebulizer and its component parts.

Unfortunately, this experimental setup is limited to currently available nebulizer designs and whatever geometric parameters they happen to have. Ideally, these geometric parameters could be studied directly, by using a specially designed adjustable nebulizer. If a nebulizer with an adjustable nozzle diameter were used in several trails, a direct correlation between nozzle size and particle size distribution could be formulated if such a correlation existed. Likewise a more definitive understanding of the effects of the other nebulizer dimensions could be gained, potentially leading to a quality improvement in all nebulization. Based on current approximate values and a desire to examine data across a wide range centered on those values, the nozzle diameter would be varied from 2-6 mm, the baffle surface area would be varied from 1-10 mm^2, and the nozzle-to-baffle distance would be varied from 1-5 mm.

3. Post-Aerosolization Parameters

3.1 Particle Size Distribution

The deposition of inhaled particles in the lungs has been studied extensively, and particle sizes of about five to six microns are considered optimal for lung delivery. The characteristic droplet measurement is the mass median aerodynamic diameter (MMAD), which represents the droplet's effective size based on its behavior in fluid flow. A diffraction sensor can be used to measure the particle size distribution (PSD) of the aerosolized liquid. We will use a Malvern Spraytec Laser Diffraction instrument to measure this distribution.

The particle size distribution is the key factor in determining the success an aerosol will have in penetrating the respiratory system and depositing biologicals in the lung. For this reason, the PSD will represent the primary dependent variable of the experiment. The geometric standard deviation (GSD), which reflects the tightness of the distribution, will also be recorded. The optimization algorithm will seek to vary the pre-aerosolization parameters in order to create a PSD closely clustered around the ideal value of five microns.

3.2 Flow Rate and Drug Output

The pre-aerosolization parameters don't just have an effect on the particle size distribution, they also affect the flow and drug delivery rates. Nebulizer geometry and air pressure will have the largest effect on these variables. While the drug delivery rate is not related to the general effectiveness of the vaccine inhalation, it is important to know so that the vaccine can be properly administered in the field. If we measure the drug delivery rate and store the data, we will be able to use it later (after a final aerosol fingerprint is chosen).

The easiest and most common method of measuring drug delivery rate is to weigh the nebulizer before and after use to calculate the mass which was emitted over

time. This is usually expressed in units of grams per minute (g/min). Although this method does tend to overestimate the actual rate because it fails to take into account the changing concentration, it is useful for the purposes of comparison[16]. Considering the high throughput nature of the proposed experiment, this slightly inaccurate method can still be utilized to compare different aerosol fingerprints to determine which are feasible in the field. (For example, one potential aerosol fingerprint might have a much lower drug delivery rate and therefore a much longer nebulization time, which might make it a less appealing option despite its slightly better PSD.) However, we must keep in mind that this calculation is an overestimate, and a better understanding of the actual drug delivery rate of the chosen aerosol fingerprint must be developed before implementation with human subjects.

3.3 Aerosol Endurance and Bioavailability after Lung Deposition

The vaccine's aerosol fingerprint will define the "most effective" aerosolization, defined as the aerosol with the PSD most closely clustered around five microns. This is the single most important factor in ensuring that delivery to the lung occurs; however, it does not take into consideration the fact that the nebulized material is in fact biologically live. Several of the pre-aerosolization parameters (notably pH and temperature) could damage or kill the live attenuated vaccine, rendering it less effective. Therefore a biological test is necessary to ensure that the aerosol optimization did not disrupt the biological effectiveness of the vaccine.

Because measles vaccine is a controlled substance, we will avoid using it as much as possible. Once the aerosol fingerprint has been identified, a first simulation of the bioavailability test can be done with another agent, such as an enzyme or bovine serum albumin (BSA). The pre- and post- aerosolization activity of the enzyme can be compared to determine if any damage was done to the proteins during nebulization. If

this test indicates a drop in biological activity, the choice of aerosol fingerprint can be reevaluated and the simulation run again, all without exposing any measles vaccine.

When an aerosol fingerprint which seems biologically viable has been identified, live attenuated measles vaccine can be nebulized using those identified parameters. The resultant aerosol will be deposited in Vero cells and plaque forming units counted. Finally, to test both requirements (particle size and bioavailability) at once, the aerosolized vaccine will be passed through an Andersen cascade impactor (a device which simulates the increasingly fine filtration which occurs in the respiratory tract). Particles which travel the length of the tract will be plated and studied for evidence of colony forming units. This final step helps us to analyze the real world feasibility of the technique and estimate how well the aerosol fingerprint will actually deliver active vaccine to the human patient.

4. Data Logging and Analysis

We will acquire data logging hardware and software to record the variable changes. The Dataq Instruments DI-710 Data Logger will suit our needs because of its large number of channels (16) and flexible computer interface. The inputs and outputs can be controlled with any common programming language. Although various data logging software packages are available, writing our own will give us the flexibility we need to include a large number of inputs and outputs, capture and record the data in matrices we define, and formulate the optimization algorithm all within the same framework.

5. Future Work

5.1 Integration with Development of Aerovax Device

The results of this experiment will be directly incorporated into the further development of the Aerovax device. The current prototype will be modified to enable it

to produce effective aerosols of measles vaccine according to the aerosol fingerprint identified. The device will be insulated against parameter changes that were identified as detrimental to the vaccine aerosolization and incorporate features allowing the easy control of parameters that need to be manipulated in order to increase the vaccine's bioavailability post-nebulization. This might include thermal insulation, limited adjustments to the vaccine formulation, better pressure regulation, and/or nebulizer redesign to incorporate the optimal geometric parameters as identified by the experiment.

5.2 Expansion to Other Vaccines

Although this initial experimentation and device deployment is targeted to the measles vaccine, vaccination against other deadly diseases is also possible. Indeed, the scale-up of this technology to vaccinate against other diseases is a long-term goal of the project. Most of the aerosolized vaccine research has focused on measles given its high prevalence. Very little research has been done into the possible aerosolization of other vaccines.

The World Health Organization tracks "vaccine-preventable deaths" due to numerous diseases, most notably Hemophilus influenza, pertussis, tetanus (neonatal and nonneonatal), polio, diphtheria, and yellow fever. This proposed experimental setup could be quite easily modified for use in identifying the optimal aerosol fingerprint for each of these vaccines as well. Most sensors and actuators could remain the same, although special consideration should be given to the issue of vaccine reconstitution based on the specific biochemistry of the target vaccine. Also, although the relevant post-aerosolization parameters remain relevant, their target values might shift. The desired particle size distribution and drug delivery rate might be different than those of measles vaccine based on biochemical and pharmaceutical properties.

6. Conclusion

The high prevalence of measles and the obstacles to vaccination that plague much of the world pose serious challenges in the global health field. The development of a new vaccination strategy is necessary. Measles vaccine administration via inhalation has been demonstrated to be efficacious and safe, but little is known about the best way to produce the vaccine aerosol.

The identification of an aerosol fingerprint would elucidate the parameters necessary to optimize the nebulization process, which would in turn aid in the development of a portable, safe, low-cost device able to operate in extreme field conditions. The complex process of finding the optimization point can be automated with the use of sensors, computer controlled actuators, and feedback loops. Special consideration must be given to factors which might affect the bioavailability of the vaccine, a concern not usually present in nebulizer experiments. The process can be applied to the problem of identifying aerosol fingerprints for other important vaccines with little modification.

Ultimately, the automation of the process provides a step forward in understanding nebulizer technology as well as developing appropriate technology solutions for health problems in resource-limited settings.

Appendix A: Summary Table of Variables

Variable	Range of values to be tested	Actuator	Sensor	Important considerations
Relative Humidity	0-100%	QuietCare Cool Moisture Humidifier (HCM650)	Honeywell HIH-4000 Series	Not controllable in the field, not to be included in optimization algorithm
Nebulizer Temperature	To be determined	Amana Dryer Element Restring Kit	Flexible Hermetic Sealed PFA RTD Sensor Probe	Time dependency must be addressed
Buffer Solution	Tris-buffered saline; phosphate buffered-saline	Manually prepared	Manually recorded	May affect bioavailability
Solute Concentration	To be determined	Manually prepared	Manually recorded	Time dependent; precipitation may occur
Solution pH	To be determined	Manually prepared buffer solution, dispensed automatically	Lazar Ultra-M micro pH electrode	May affect bioavailability in addition to PSD
Surface Tension	full to minimum values for each solution	Automated addition of surfactant	Valued recorded from automated dropper	Only to be included in optimization process after bioavailability has been verified
Air Pressure	30-70 psi	Precision Medical EZ Neb PM7	Pressure regulator	None
Jet nozzle diameter	Available dimensions; or 2-6 mm	Multiple nebulizer models; or adjustable nebulizer	Manually recorded; or sensor to be built into adjustable design	None

Variable	Range of values to be tested	Actuator	Sensor	Important considerations
Baffle surface area	Available dimensions; or 1-10 mm^2	Use of multiple nebulizer models; or adjustable nebulizer	Manually recorded; or sensor to be built into adjustable design	None
Distance between jet nozzle and baffle	Available dimensions; or 1-5 mm	Use of multiple nebulizer models; or adjustable nebulizer	Manually recorded; or sensor to be built into adjustable design	None
Particle Size Distribution	n/a	n/a	Malvern Spraytec Laser Diffraction sensor	None
Drug Delivery Rate	n/a	n/a	Gravimetric measurement	Will determine vaccination time
Aerosol Endurance and Bioavailability	n/a	n/a	Post-plating CFU counts	None

Figure 4. Summary of measured variable measurement, control, and special considerations.

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