A Systematic Review of Large Particle Aerosol Generation and its Effects on the Clinical Manifestation of Pathogens

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Abstract

Large particle aerosol generation is defined as the generation of particles that are at least 10 μm in diameter. Large particle aerosol generation can be used in many kinds of research. Perhaps the most important aspect of aerosol research is in biodefense. Understanding the impact of particle size on subsequent infection and disease can lead to important insights into disease progression as well as potential vaccine and therapeutics. There are a variety of aerosol generators that specialize in making different sizes of particles as well as ways to measure the size of aerosol particles. Studies into large particle generation as it pertains to disease progression and severity have generally shown that infection via large particle aerosol typically results in that pathogen becoming less infective and less severe. This is likely due to the pattern of deposition in the respiratory tract that is observed based on particle size. Large particles tend to deposit in the upper respiratory tract whereas small particles tend to deposit in the deep lung. This review aims to emphasize the link between particle size and disease severity and go over the different aerosol generators and models for large particle generation.

Table of Contents

List of Tables

List of Figures

Preface

I thoroughly enjoyed my time at Pitt Public Health. My time here gave me excellent opportunities to learn and truly engage with material. I came to Pitt Public Health with an interest in Infectious Diseases and pathogen research and my interest has only grown since I have been here.

I would first like to thank my Essay Chair and Principal Investigator, Dr. Douglas Reed. Dr. Reed has been a great boss and mentor to me throughout my time working on this essay. He has continued to support me, and I am very grateful for the chance to work in his lab. I would also like to thank the great people in the Reed Lab. They have been very helpful to me, and I am lucky to work with such a great group of people.

I would also like to thank my advisor and Essay Reader, Dr. David Givens. Dr. Givens has helped me navigate through my time here at Pitt Public Health. He has also given me good advice and has always been there to answer my questions. I very much appreciate his guidance.

1.0 Introduction

People generate aerosols every minute of every day. Everyday activities like speaking, breathing, and coughing generate aerosol droplets expelled into the air. Some of these particles are small and, therefore, can travel great distances in the air after being expelled from the lungs and airway. These particles are defined as being less than five microns (Fennelly et al., 2014). On the other hand, some of these droplets are large, and they will only travel a short distance before they fall and land on whatever surface they end up on. These droplets are defined as being larger than ten microns. Most droplets contain harmless materials from the airway and the lungs. However, certain pathogens can hijack these droplets, and use them as a mode to spread from host to host.

Aerosol particles that are produced from an infected host can infect another host through inhalation. In the case of Influenza A virus, the primary mode of transmission is when respiratory droplets are created when an infectious person coughs or sneezes. These droplets then land in a person's mouth or can be inhaled through the nose (Cowling et al., 2013). During the COVID-19 pandemic, aerobiology became vital to the efforts to stop the spread of disease. The reason that the social distancing measures of staying six feet apart from people were put in place were to stay out of the range of a potentially infected person's respiratory droplets and fomites. However, due to aerobiology research, it was soon determined that COVID-19 was transmitted via airborne transmission. This was an important distinction because, had airborne transmission been recognized earlier, many more lives could have been saved through earlier masking precautions and enforced isolation. Aerosol pathogens are typically more infectious than pathogens that spread through other means because the infected host does not necessarily have to touch someone to infect them. Also, one host can infect several other hosts just by being in proximity with them. For

example, SARS viruses have documented “super-spreader” events where one-person infected dozens or even hundreds of people (Small et al., 2006). This makes aerosol pathogens difficult to track because it is harder to do contact tracing for an outbreak.

Aerobiology can also be used for defensive biowarfare research. There are two main aerosol pathogens that are severe bioterrorism threats due to their airborne transmission: smallpox and pneumonic plague (Dennis, 2009; Riedel, 2005). However, other pathogens such as Eastern Equine Encephalitis are bioterrorism threats because they can be aerosolized despite airborne transmission not being their natural form of transmission. Because of the infectivity and difficulty to track, deadly aerosol pathogens can an attractive option for a state or terrorist group that is looking to cause maximum damage with an attack (Leffel & Reed, 2004). These pathogens are typically highly infectious and can spread quickly. That maximizes the fear factor on a population which is an important factor in bioterror attacks. For example, Eastern Equine Encephalitis Virus is an arbovirus that is typically spread via mosquitos. However, it has the capability to be aerosolized and is a lethal virus, so it is considered a Biological Select Agent and Toxin (BSAT) by the United States Government due to its potential for aerosolization. Aerobiology can serve as defensive biowarfare research as well. Studying these pathogens can help scientists to gain insights into what makes them infectious as well as how to stop them. Many vaccines and therapeutics are tested in aerosol challenge studies as well. Identifying the pathogens most likely to be used in an attack and researching them is the best way to both prevent an attack and minimize its effects.

All of these factors make the field of aerobiology very important. In order to study the pathogenesis of different pathogens, animal aerosol challenge studies are done. These are studies that challenge the animal with some pathogen as an aerosol. The purpose of these studies can range from testing a vaccine or antibody to studying how the disease progresses in an animal model.

Particle size can affect the different characteristics of a pathogen such as its pathogenesis and infectivity. Humans generate different sizes of aerosols, so it is important to understand how these characteristics are affected by particle size for different pathogens. While it is easy to generate smaller particles in aerosol challenge experiments, it is more complicated to generate larger particles. This is due to various reasons outlined in this paper. A small particle is defined as any particle smaller than 5 μm MMAD. A large particle is defined as any particle larger than 10 μm MMAD (Fennelly, 2020). Any particles that fall in between are considered to be intermediate size particles.

This review will focus on three aspects of aerobiology as it relates to pathogen delivery for aerosol challenge studies. The first section will review three different aerosol generators and their strengths and weaknesses in aerosol delivery. Choosing the correct aerosol generator is vital for the success of any challenge study. The next section will focus on large particle generation specifically. This will review the limited literature that is published about the best ways to generate large particles, and why large particle generation is important. Finally, the last section will review papers on the effects of particle size on the clinical manifestation of select diseases.

2.0 Methods

In order to cultivate a relevant list of sources to review for large particle generation and pathogenesis, a literature search was done using different key words. To begin the literature search, the phrases, “large particle aerosol generation”, “effects of large particle on disease pathogenesis” and “aerosol generators” were searched in Google Scholar. To be included in this review article, the paper must be focused on aerosol generation as it relates to pathogens. Articles that included drug delivery were only included to provide definitions or context to certain phenomenon observed in pathogen aerobiology. For example, they were used to provide a definition and context for Mass Median Aerodynamic Diameter. In addition, some publications were pulled from the citation list of review articles focused on aerosol generation and disease pathogenesis. A summary of the overall search strategy used for this review can be found in Table 1 of Appendix A.

The pathogens chosen to be included in the review were chosen because of the abundance of literature on large particle research that surrounds them. Three of the pathogens chosen, Anthrax, Tularemia, and Eastern Equine Encephalitis Virus, are bioterrorism threats. Influenza A on the other hand was chosen because of its potential as a pandemic threat. There are other pathogens that large particle research has been done. Two examples of these are Ricin and Nipah Virus.

Similarly, the aerosol generators that were chosen are not the only available aerosol generators. The goal was to choose an aerosol generator that was proficient at generating small particles (the Collison), one that is proficient at generating a variety of particle sizes (the Ultrasonic), and one that is proficient at generating larger particles (the CenTAG).

3.0 Aerosol Generators

There are many different aerosol generators that are available. The basic function of an aerosol generator is to generate an aerosol from a liquid or dry powder. In infectious aerobiology studies, this is typically pushed into an exposure chamber to expose an animal to a pathogen. The three aerosol generators that will be discussed are the Collison nebulizer, the Ultrasonic nebulizer, and the Centered flow Tangential Aerosol Generator (CenTAG). Each of these nebulizers has its own pros and cons. The Collison nebulizer will be discussed first because it is considered the gold standard in aerobiology.

Before discussing the different nebulizers, it is important to speak to the way that particle sizes are measured. Particle sizes are measured using a unit known as Mass Median Aerodynamic Diameter (MMAD). The reason that this unit is needed is because not all particles are perfect spheres. It is impossible to get an accurate diameter reading, so MMAD takes into account the mass of the particle. It means that 50% of the particles in the aerodynamic size distribution, which is based on mass, lie above and below that diameter (Muralidharan et al., 2015). For example, an MMAD of 5 micron means that 50% of the total sample mass will be present in particles having aerodynamic diameters less than 5 micron, and that 50% of the total sample mass will be present in particles having an aerodynamic diameter larger than 5 micron.

3.1 Collison Nebulizers

The Collison nebulizer has been the dominant aerosol generator in aerobiology since its discovery in 1932 (Ibrahim et al., 2015). It is a type of jet nebulizer which means that it uses an air jet to aerosolize a liquid. It was developed by W.E Collison for inhalation therapy. The Collison Nebulizer works by using a high-speed jet of compressed air to create a negative pressure in the jet expansion channel. This negative pressure can siphon the liquid from the reservoir to the jet stream (Feng et al., 2021; Ibrahim et al., 2015). The Collison nebulizer creates droplets that have a wide range of sizes. The ideal range of sizes for these particles is 1-5 microns MMAD, which are ideal for inhalation therapy because they deposit themselves in the deep lung (Darquenne, 2012; Morawska & Buonanno, 2021; Thomas, 2013). In order to separate the smaller particles from the larger droplets, the jet flow carries the droplets towards the wall of the nebulizer. Inertia then causes the larger particles to deposit on the walls of the Collison. Meanwhile the smaller particles continue on the jet stream and are expelled from the Collison (Feng et al., 2021). This separation is not perfect, and larger particles also may be expelled from the generator. However, most researchers will use a ‘mixing tube’ with dilution air between the aerosol generator and the exposure chamber. That ensures that the larger particles that are expelled from the Collison will end up stuck in the mixing tube rather than making it all the way to the exposure chamber. In the end, only a small amount (about 0.1%) of the liquid enters the jet stream (May, 1973). Because of this mechanism, the Collison nebulizer is very good at making small particles.

Figure 1: Collison Nebulizer

There are several benefits of the Collison Nebulizer. First, the mechanism is relatively simple and is therefore user friendly. It requires a relatively small material volume and has a high particle output for a jet nebulizer (Alsved et al., 2020). Also, it excels at creating small aerosol particles. These small aerosol particles deposit in the deep lung, so the Collison nebulizer is a good option for studies that require penetration to the deep lung of a host. Finally, another important benefit of the Collison is its widespread use (Alsved et al., 2020; Ibrahim et al., 2015). Because the Collison is considered the gold standard, it is used by many aerobiologists worldwide. This makes it easy to compare between studies that use the Collison nebulizer. Despite the pros of the Collison, several researchers have hypothesized that the Collison has one flaw. The Collison

recirculates the liquid throughout the system and during this recirculation, it is possible that some of the biological material is damaged or loses viability (Alsved et al., 2020; Bowling et al., 2019; Brown et al., 2015; Fennelly et al., 2014). This can cause a time dependent degradation of the material and means that the Collison may not be the best option for aerosols that require longer exposure times. The Collison has acted as a de facto standard for aerosol generators for years. Despite that, the Collison is not able to generate a relatively homogenous population of large particles which makes it a poor candidate for large particle studies. Recently, new aerosol generators have become more relevant as alternatives for the Collison. One of these is the ultrasonic nebulizer.

3.2 Ultrasonic Nebulizers

The ultrasonic nebulizer relies on electrical fields and heat rather than air to create aerosol droplets. The basic mechanism for the ultrasonic nebulizer is a piezoelectric crystal vibration that is driven by alternating electric fields (Flament et al., 2001). The ultrasonic makes use of the piezoelectric effect that converts high frequency oscillations into mechanical vibrations. These vibrations then generate aerosol particles (Sidler-Moix et al., 2015). As the vibration reaches a critical value, droplets are generated, and an air vent allows them to be expelled. The ultrasonic nebulizer can create a distinct range of particle sizes. For example, Kooij et al. reports that ultrasonic nebulizers can create particles with median diameters ranging from 1.1 μm to 9.6 μm MMAD depending on the type of ultrasonic nebulizer used (Kooij et al., 2019). However, some Ultrasonic nebulizers can generate particles up to 35 microns. The capability of creating larger particles already separates the Ultrasonic from the Collison.

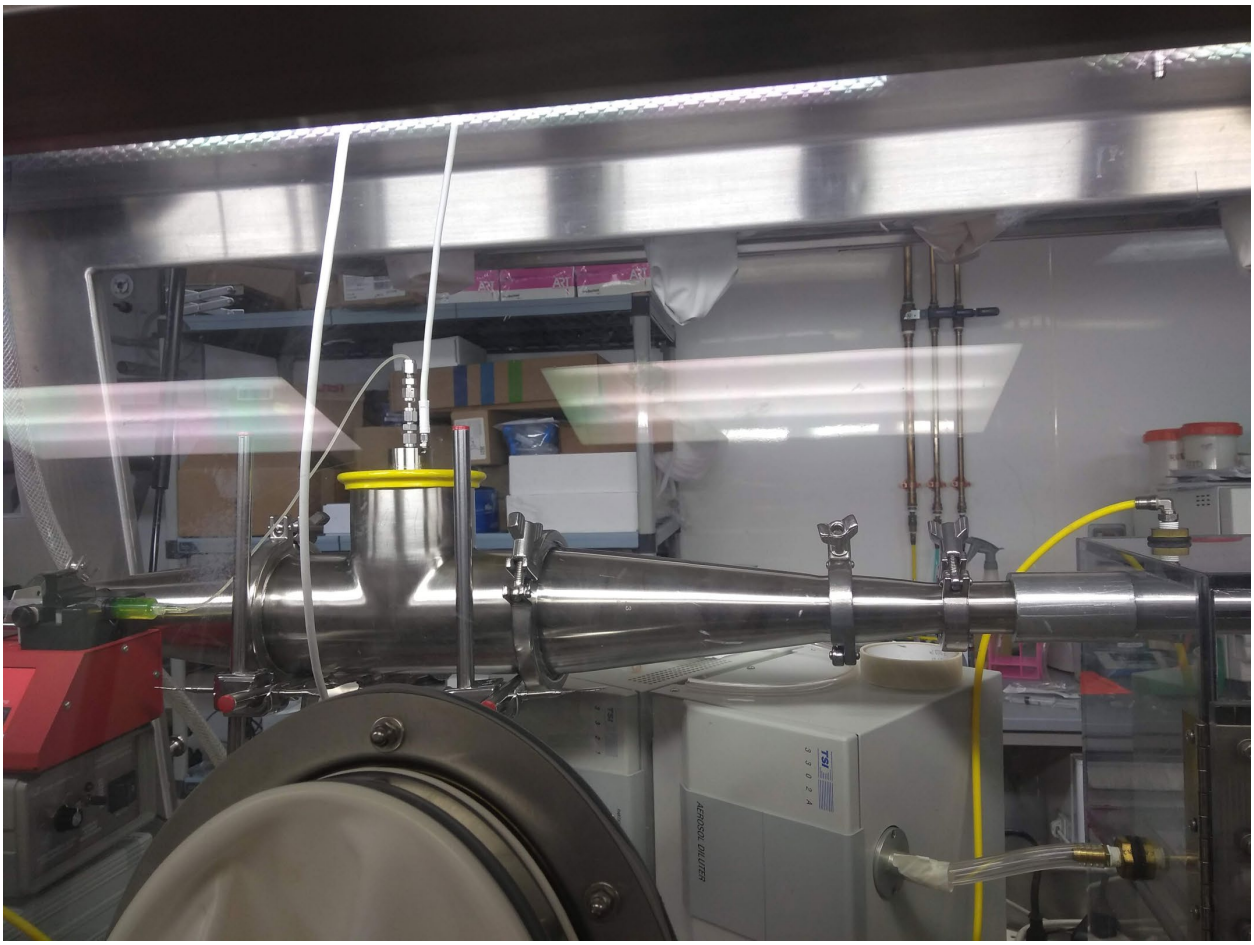

Figure 2: Ultrasonic Nebulizer

The major benefit associated with the ultrasonic nebulizer is that it will have a higher output rate as compared with the jet nebulizers (Rau, 2002). Aerosol output rate is the amount of drug (or pathogen) emitted in one minute of nebulization (Adorni et al., 2019). That means the ultrasonic nebulizer emits more material per minute than the jet nebulizers. This means that aerosols done with the ultrasonic nebulizers will be completed faster than aerosols done with the jet nebulizers. Also, the ultrasonic nebulizer will typically produce larger particles than the jet nebulizers (Rau, 2002). This can be either an advantage or disadvantage depending on the context of the study. If large particles are needed, then the ultrasonic is a fairly good option to generate them. The main

disadvantage of the ultrasonic nebulizer is the heat created during the nebulization process. Because the ultrasonic uses an electric field to create vibrations in the crystal, it will generate heat at the site of nebulization. This heat is due to the vibrations that are created. Unfortunately, this heat can also degrade heat-sensitive materials (Arı, 2014). For that reason, the ultrasonic nebulizer cannot be used to nebulize proteins. It is also hypothesized that the heat generated could degrade pathogens in aerosol challenge studies. However, there are not enough studies done to test this theory. Some other disadvantages of the ultrasonic nebulizer include large residual volumes and an inability to nebulize viscous solutions (Arı, 2014). The ultrasonic nebulizer is a good choice in specific situations. The Collison would be a good choice for smaller particles, but the Ultrasonic would be a better choice for larger particle studies that are not necessarily pathogens. However, if larger particles are needed, then the best choice of aerosol generator is the Center Flow Tangential Aerosol Generator (CenTAG).

3.3 Center Flow Tangential Aerosol Generator

The CenTAG is a spinning top generator that uses tangential force to create aerosol droplets. Bohannon et. al gives a good description of the mechanism of the CenTAG. To summarize, a syringe pump delivers the liquid onto the spinning top through a small nozzle. The tangential force will create aerosol droplets. The flow rate can be controlled and monitored through observation ports on the side of the CenTAG. Also, the speed of the spinning top is controlled via an electronic control panel on the side. The CenTAG is good at producing large particles and, to do this, a vacuum that is constructed around the top pulls the small particles away from the larger

ones (Bohannon et al., 2015). This is one of the main benefits of using the CenTAG for larger particle studies.

Figure 3: CenTAG

As stated above, the CenTAG can produce larger particles than most jet nebulizers. Spinning top aerosol generators like the CenTAG are typically used to create larger particles while Collison nebulizers are the standard for smaller particles (Cheah & Davies, 1984; May, 1973; Roy et al., 2003). The CenTAG can produce particles between 5-12 µm MMAD (Bohannon et al., 2015). This makes it useful for larger particle studies. That will be explained further in the following section. The CenTAG is also designed for use in a Class III Biosafety Cabinet (BSC) (Bohannon et al., 2015). Class III BSCs are used in Biosafety Level III and Level IV (BSL-III, BSL-IV) spaces. Level III labs are certified for work with pathogens that can cause serious or potentially lethal disease through respiratory transmission (Services). Level IV labs are certified

to work with the same kinds of pathogens as level III, but the pathogens in Level IV typically do not have any treatments available. As most respiratory pathogens are worked with in BSL-III space, this makes the CenTAG a great candidate for use in aerosol challenges as opposed to the ultrasonic nebulizer which is larger and harder to fit into a Class III BSC. Finally, whereas pathogen viability was a concern with the Collison and Ultrasonic Nebulizers, CenTAGs can improve pathogen viability as the aerosol progresses. This is due to the diluent used, which reduces desiccation and increases particle size. (Bohannon et al., 2015) All of these advantages make the CenTAG very good at producing large particles and the next section will discuss exactly how the CenTAG is able to do that.

The three aerosol generators discussed each have their own limitations and advantages. The easiest way to think of them is to imagine them on the spectrum. The Collison nebulizer is best at producing small particles to have them deposit in the deep lung. The ultrasonic nebulizer can create both large and small particles to give a mixture of particle sizes. Finally, the CenTAG is designed to create large particles by using a vacuum to separate out the smaller particles. It is also the best option to use if one was worried about pathogen viability because it does not produce heat as the ultrasonic does or reuse the liquid as the Collison does.

4.0 Large Particle Aerosol Generation

There are limited articles available that describe exactly how to generate large particles. In 2015, J. Kyle Bohannon et. al. developed a model for large particle generation that has largely become the standard model for experimenters who need to generate large particles. For example, Reed F. Johnson et al. cites Bohannon in their paper which involved generating large particles to expose rhesus monkeys to cowpox. This paper generated large particles of cowpox and exposed the Rhesus monkeys to them to follow up with an earlier paper that showed that small particle exposure resulted in severe, lethal respiratory disease (Johnson et al., 2015). This paper showed that a similar outcome is observed with large particle exposure. The only slight difference is that this exposure resulted in upper respiratory disease (Johnson et al., 2016). Also, Julia Port and colleagues cites Bohannon's article in their paper which concludes that SARS-CoV-2 disease severity and transmission efficacy increases for airborne rather than fomite exposure in hamsters. (Port et al., 2020). Both papers cite the work done by Bohannon and colleagues as their model for generating the large particles. Because Bohannon et al. provides a standard large particle generation model, this section will focus on their paper.

4.1 A Large Particle Aerosol Generation Model

Bohannon and colleagues describe a way to generate large particles using the CenTAG aerosol generator that was described above. Instead of using pathogens, they used nonpathogenic test suspension liquids such as Dulbecco's Modified Eagle Medium (DMEM). This was done for

two main reasons. First, not using pathogens meant that the experimenters would not need to work in a closed BSC. This makes it easier to conduct the experiments and eliminates all risks that are associated with working with pathogens. Also, by generating aerosols using suspension media, they could be reasonably sure that the results obtained would be the same when a pathogen was used. When a virus is aerosolized, the droplets contain the virus particles and the test suspension media. According to the researchers, previous experiments showed that adding virions to the suspension media would not change the particle size or distribution (Bohannon et al., 2015). Then, they added varying amounts of glycerol and changed the rotor speed of the CenTAG as experimental parameters to determine what the ideal set of conditions were for large particle generation.

The researchers found that adding glycerol to the test suspension **increased** the particle size. They also found that decreasing the rotor speed of the CenTAG **increased** particle size (Bohannon et al., 2015). They reported that running the CenTAG at 6960 rpm and using a mixture of DMEM and 20% glycerol gave the largest particle size at 10.97 μm MMAD (Bohannon et al., 2015). These findings are of great importance because they essentially provide an instruction model for other researchers to follow when they need to generate large particles.

4.2 Measuring the Size of Particles

4.2.1 Aerodynamic Particle Sizer

There are multiple different ways to measure the size of aerosol particles. Bohannon, et al used an Aerodynamic Particle Sizer (APS) in order to measure the particles. The APS uses the

velocity of the particles to measure their size. The velocity of the particles corresponds to a certain aerodynamic diameter. The APS measures the velocity of particles as they pass between two lasers. As the particle passes through the laser it produces two separate beams of light. The time delay between the two beams of light being emitted is used by the APS to measure the particle velocity and size (Manchester; Mitchell et al., 2003). The APS reports the particle size in real-time and does not require additional assays. However, if the lasers are not functioning correctly, the output for the APS may be incorrect or misleading. In cases such as those, it is sometimes necessary to use a cascade impactor.

4.2.2 Cascade Impactor

A cascade impactor is simply a series of impactors with smaller cut off diameters (Nichols et al., 2013). Impactors separate aerosol particles using particle sizes as the cut off. The particles flow into the cascade impactor and then are separated into little pans based on the particle size. Following the aerosol run, one can perform a protein assay to determine how many particles are in each pan, which will give an idea of the particle size. The disadvantage of the cascade impactor is that cannot give real time measurements. Also, it is more work and time intensive because of the assay that is required following the run. However, an impactor can be a good option as a quality control check of the APS. It can help an experimenter to know if the readings of the APS are accurate or not.

While there are limited articles discussing how to generate large particles, there are a number of articles that research the differences in pathogenesis between large and small particle exposures to diseases. That is the most important aspect of large particle research.

5.0 The Effects of Large Particle Exposure on Disease Pathogenesis

There are a variety of reasons that generating models for large particle aerosol infections are useful. Some particle size can affect the course of disease and pathogenicity of a disease. Also, small particle infection can be more lethal at times, but producing a homogenous population of small particles may not give a complete picture of infection. The challenge with generating large particles is that they will break down into smaller particles over time. That is something that Kyle Bohannon and colleagues addressed by adding glycerol to slow down the process of particle breakdown. Finally, natural infection is a mix of large and small particles, and therefore generating large particles for challenge studies is the only way to ensure that the model mimics human infection.

5.1 Particle Size and Lung Deposition

The reason that particle size can affect the infectivity and pathogenicity of a pathogen is due to where the particle deposits in the respiratory system. The respiratory system starts at the nose and mouth with the nasal and oral cavity. As a human takes a breath, the air travels from the oral/nasal cavities, past the pharynx and into the trachea. From the trachea, the air passes through the bronchial tree before reaching the alveolar sacs in the lungs where the gas exchange occurs. Gas exchange in the lungs is when the blood in the pulmonary arteries is oxygenated from the oxygen entering the lungs. At the same time, carbon dioxide is taken out of the blood and removed during exhalation.

The air that is breathed in is not just purely air. It often contains particles that are floating around in the outside air. The size of these particles will determine how far into the respiratory system they get before depositing. Larger particles will deposit in the upper respiratory system (Dabisch et al., 2017). This is around the nasal and oral cavities as well as the upper trachea. Intermediate size particles will deposit in the upper lung and lower trachea. Finally smaller particles will make it all the way into the deep lung where they will deposit (Cheng et al., 2008; Darquenne, 2012; Morawska & Buonanno, 2021; Thomas, 2013). Figure 4 illustrates the deposition patterns of particles. It is also worth noting that this is simply a general rule. Small particles are able to deposit at any point along the respiratory tract, but it is unlikely that large particles will make it all the way to the deep lung. This rule is simply saying that most of the particles will end up in a certain region based on their size. There are three forces that govern where the particle will end up in the respiratory tract: impaction, sedimentation, and diffusion (Roy et al., 2010). Impaction refers to particles getting stuck on the walls of the airway tract depending on their size and it is the major force that governs particle deposition in the upper respiratory tract. Larger particles will impact the walls more easily than smaller particles and therefore will not make it into the deep lung. Sedimentation and diffusion are the major forces that govern deposition in the deep lungs. Sedimentation refers to gravitational force pulling smaller particles down into the deep lung to deposit while diffusion is important in the small bronchioles and alveoli. The velocity of the air also plays a role in deposition. In the upper respiratory tract, air flow is highest and therefore impaction is more relevant as a force, but, in the small bronchioles and alveoli, air velocity is slow and diffusion becomes the more relevant force.

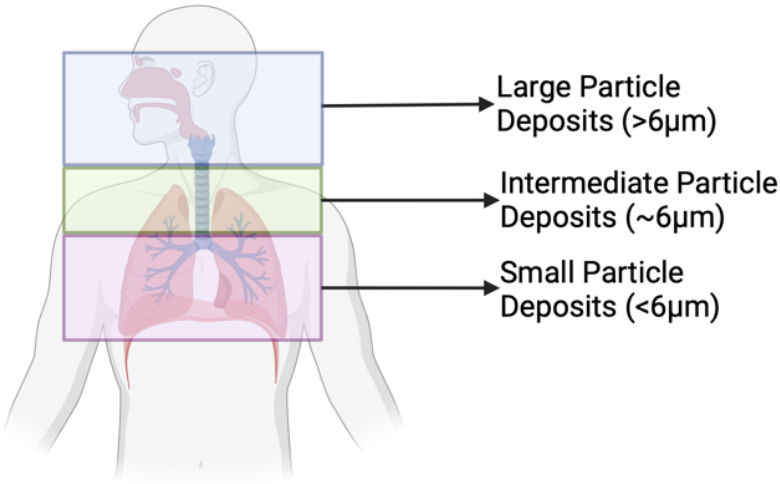

Figure 4: Particle Deposition

5.2 Disease Progression and Severity

There are many studies that study the infectivity or course of disease in animals that are infected with large particles. Most of these studies share a similar structure. First, they challenge an animal with a pathogen that is aerosolized into small particle aerosols. This is because small particle aerosols are easier to accomplish and most research done into an aerosol pathogen is done using small particles or a range of particle sizes. Small particles will reach the alveolar regions in the deep lung which facilitates the dissemination of the pathogen into the blood stream. Also, the deep lung has less lymphocytes than the rest of the respiratory tract, so there is less of an immune response there. Generating larger particles can influence the lethal dose of a pathogen as well as the pathogenesis of the disease.

5.2.1 Anthrax

Bacillus anthracis is a bacterium that causes the disease known as anthrax. Anthrax is a threat as a bioterrorism agent because of its aerosol capability and severe disease. Pulmonary anthrax occurs when the anthrax spores are inhaled and it is considered the most severe form of anthrax ("FactSheet: Anthrax," 2001). Because of its severity and aerosolization capability, researchers such as H.A. Druett and Richard Thomas have examined whether the size of the aerosol particle can affect the severity of anthrax disease.

The study done by Druett and colleagues was examining the relationship between particle size and infectivity of *Bacillus anthracis*. The study generated anthrax spores in aerosol particles varying sizes and exposed guinea pigs and monkeys to the spores. They found that as the particle size increased the infectivity decreased (Druett et al., 1953). The reason that the authors gave for that phenomenon is that anthrax needs to be deposited in the deep lung to be infective. Increasing the particle size changed the region of deposition and therefore made the anthrax strain less virulent (Druett et al., 1953).

A later study done by Richard Thomas et al. confirmed the findings of Druett and his colleagues. They exposed mice to endospores of anthrax with particles of 1μm and 12μm. They found that infectivity decreased with the larger aerosol particles and that a larger dose was required for lethality when the mice were exposed to the 12μm particles (Thomas et al., 2010). This relationship is likely because anthrax needs to deposit into the deep lung in order to disseminate throughout the body effectively. Without deep penetration, the anthrax will not spread effectively and therefore larger aerosol particles of anthrax spores will not be as lethal.

5.2.2 Tularemia

Similarly to the anthrax studies., William Day and Richard Berendt conducted a study where they tested the effect of different aerosol particle sizes on the infectivity and disease caused by *Francisella tularensis,* the bacteria that causes tularemia, in rhesus macaques. Similarly to anthrax, Tularemia is another bioterrorism threat. Inhalational Tularemia is also the most severe form of the disease. It has a 30-60% mortality rate if left untreated.

Day and Berendt found that animals exposed to the smaller particles of tularemia became infected and died within four to eight days of exposure. However, the animals infected with larger particles needed a larger dose to produce a lethal infection. According to them, that is the most significant finding in their study (Day & Berendt, 1972). They also found that aerosol particle size changes the pathology of the disease and the time to death in the macaques. Larger particle infection was associated with a longer time to death than smaller particle infection (Day & Berendt, 1972). Day and Berendt did not provide reasoning for this relationship, but it is plausible that the region of deposition also plays a role in the infectivity of the bacteria.

5.2.3 Type A Influenza

Type A Influenza is a virus that affects mainly the upper respiratory organs (Moghadami, 2017). It is commonly referred to as "flu" and has been responsible for significant morbidity and mortality worldwide. Influenza has caused epidemics and pandemics and has also been responsible for seasonal infections. Most recently, in 2009, the WHO declared a worldwide outbreak of A/H1N1 which is one of the seasonal strains of influenza. The two common seasonal strains are A/H1N1 and A/H3N2. These are typically milder strains that infect people during the "flu

season". However, there are also more severe strains of Influenza known as the highly pathogenic strains. The common strains of highly pathogenic avian influenza (HPAI) are H5N1 and H7N9 (Boktor et al., 2024). Just as with the other pathogens reviewed, there is a relationship between particle size and lethality for Type A Influenza.

For influenza A viruses, studies have shown that the seasonal strains bind to the α2-6 sialic acid receptors (de Graaf & Fouchier, 2014). These 2-6 receptors are located mostly in the upper respiratory system so there was a hypothesis that larger aerosol particle infections could have made certain seasonal flu strains more severe by depositing more virus in the region with more receptors for that virus. On the other hand, studies have shown that the highly pathogenic strains bind to the α2-3 sialic acid receptors that are located mostly in the deep lung (de Graaf & Fouchier, 2014). The highly pathogenic strains have been shown to be more severe in small aerosol particle infections (Wonderlich et al., 2017). However, interestingly, studies have shown that the seasonal strains are also more severe in small aerosol particle infections (Larson et al., 1976; Scott & Sydiskis, 1976).

Studies into influenza have shown a similar theme, large particle infections of Influenza cause less severe disease and cause a less potent antibody response than small particle infections. This relationship has been shown for both seasonal and highly pathogenic strains of influenza. For example, George Scott and Robert Sydiskis infected mice with A/H3N2 virus. They compared the antibody responses of mice that were immunized with H3N2 virus via small and large particle aerosols. The small particle aerosols were generated using the Collison nebulizer and the large particle aerosols were generated using a Spinning Top Aerosol Generator (STAG). The CenTAG is a descendent of the STAG. The mice were then challenged with the virus following immunization and they measured the immune response. They also compared intraperitoneal and

subcutaneous infection, but for the article's purposes, the review will only discuss their results as they pertain to large and small particle aerosols. They found that there was a higher dose of virus necessary to stimulate infection in the mice challenged with large particles than with the small particles (Scott & Sydiskis, 1976). They also found that there was a stronger immune response among mice immunized via the small particle aerosol route than the large particle aerosol route (Scott & Sydiskis, 1976).

Larson et al. conducted a similar study, but with different aims. They also challenged mice with H3N2. Their aim was to compare the virus population in the lungs, nasopharynx, and trachea after large particle aerosol challenge, small particle aerosol challenge, and intranasal challenge. Their study showed that higher populations of the virus existed in the lungs after a small particle aerosol challenge than with a large particle aerosol challenge (Larson et al., 1976). This validates the deposition patterns for large and small aerosol particle sizes.

5.2.4 Eastern Equine Encephalitis Virus

Similarly to the other pathogens discussed, research into the Eastern Equine Encephalitis Virus (EEEV) has also shown that there is a higher lethal dose associated with larger particles. Chad Roy and colleagues exposed guinea pigs with EEEV to test a hypothesis that infectivity and potentially severity would be increased with larger particle aerosol exposure. Encephalitic alphaviruses are believed to reach the brain through the olfactory nerve. Studies in rodents and macaques have suggested that aerosols containing encephalitic alphaviruses will deposit in the olfactory region, infecting the olfactory bulb and traveling up the olfactory nerve to the brain. Larger aerosol particles containing EEEV might provide a faster route into the brain through the olfactory bulb (Thomas, 2013). However, Roy and colleagues found that larger aerosol

distributions (>6μm) were associated with lower lethality and longer times to death than the smaller particle aerosol distributions (Roy et al., 2009). As stated above, this was a surprising result because it was believed that larger particles would result in more virus being deposited in the olfactory region and traveling up the olfactory nerve to the brain, shortening the time to disease and death as well as lowering the dose required. However, the authors found that the virus entered the brain at the same time regardless of the particle size. Despite that, the authors do not specify exactly what size the large particles were they just say that they were greater than 6μm. If the particles were only slightly greater, then it is possible that they were not large enough to follow a deposition pattern normally expected for large particles. Further research into the subject has shown that EEEV can reach the brain through the blood or the olfactory nerve rather than one or another. Further research may be warranted to identify a link between particle size and disease severity for Eastern Equine Encephalitis Virus.

6.0 Discussion

Based on the studies presented in this review, there is a link between the size of particles and disease severity and infectivity. The studies included in this review have shown that larger particles tend to be associated with less severe disease and less infectivity. They have also highlighted the CenTAG as the best aerosol generator to be used for the generation of large particles.

The link between the size of particles and disease severity seems to be caused by the patterns of deposition based on the particle size. Larger particles deposit in the upper respiratory system and smaller particles deposit throughout the respiratory tract including the lung. This link is present regardless of the identity of the pathogen. That said, some questions still exist about the link pertaining to Eastern Equine Encephalitis Virus. There may be a different relationship between particle size and severity that exists for those viruses due to their preferential targeting of the nervous system. Anthrax, Tularemia, and Type A Influenza are all pathogens that are most severe when they make their way into the deep lungs. Therefore, it makes sense that smaller particle aerosols of those diseases would result in more serious disease because the small particles deposit in the deep lung.

Also reviewed were three aerosol generators that were each proficient in generating certain sizes of particles. The Collison nebulizer was developed for drug delivery and is therefore proficient at creating smaller particles. The ultrasonic nebulizer is able to create a range of aerosol particle sizes but cannot be used with heat sensitive pathogens. Finally, the CenTAG can generate both large and small aerosol particle sizes but is proficient at generating larger aerosol particles using the model published by Bohannon and colleagues.

There are not many reviews available that studies the link between aerosol particle size and disease severity across multiple different pathogens and also discuss how one generates different particle sizes. Aerosol particle size and pathogenesis needs to be studied more thoroughly in the future. Infection via large aerosol particles can alter the pathogenesis of the disease in an animal. On top of that, large particle infection models can provide a more complete model of infection in animals. Since the FDA's "Animal Rule" that was established after the Amerithrax attacks established that animals can be used to test therapeutics and vaccines for diseases that are too dangerous or sporadic to do human challenge studies, animal models have taken on an increased importance in pathogen research. Therefore, developing accurate models of infection in these animals by generating both large and small particles is paramount to the development of these vaccines and therapeutics. The work done by Kyle Bohannon to develop a model for producing large particles will, hopefully, lead to more studies being done on this subject.

7.0 Implications

This topic has huge implications for public health and biomedical research. Aerosol pathogens remain a major concern because of their potential for use in a bioterrorist attack. (Reed book chapter; Reed and Lassell) The aerosol route of transmission is the fastest and most infective way to spread a pathogen in an offensive bioterrorism attack. The field of aerobiology is paramount to combating these attacks by developing vaccines and therapeutics as well as an understanding of how secondary infection can occur. In terms of public health, this research can help frame policies and suggestions for different viruses. For example, knowing that tularemia is more infective and severe when transmitted as a smaller particle can lead public health officials to making recommendations that people stay further apart from each other or lockdown completely to keep from being infected. While the United States no longer does offensive bioweapon research, there is still work being done studying pathogens for defensive purposes. Hopefully, more research will be done on establishing the link between particle size and disease severity even further.

Another major implication of this research is to help create a better model of human infection for certain pathogens. Because challenge studies with Biological Select Agents and Toxins (BSATs) are not able to be done on humans, aerobiology studies with animals remain the next best model to learn about these pathogens. While small aerosol particle studies are more likely to be lethal, studies with larger aerosol particles would provide additional information regarding dose and pathogenesis and would be useful in constructing a more complete model of infection that is more accurate to human infection. These studies would also further ensure efficacy of vaccines and therapeutics when they are used in a challenge study.

8.0 Future Directions

As emphasized in this review, more research is needed in the field of both large particle generation as well as its effects on the severity of diseases. There are not enough studies on this subject despite the huge implications it could have for public health and research. Even studies that generate large particles are often not able to generate truly large particles. More money and research would go a long way to answering the questions that are still left in large particle research.

Appendix A

Appendix A.1 Tables

Table 1: Medline Search Strategy

Literature Review Search Summary	
Provider/Interface	Ovid
Database	Medline® ALL
Date searched	March 25th, 2024
Database update	1946 to March 22, 2024
Search developer(s)	Christopher Katyal and Helena VonVille
Limit to English	Yes
Date Range	No dates specified
Publication Types	All publication types included
Search filter source	No search filter used

Table 2: Medline Search Strategy (Continued)

Key Search Terminology	
1.	Aerosols/ or aerosol*.ti,ab,kf.
2.	Particle Size/
3.	((large or size) adj3 particle*).ti,ab,kf.
4.	2 or 3
5.	1 and 4
6.	5 not (("Humans"/ or exp "Plants"/) not "Animals"/)
7.	exp Viruses/
8.	(viral or virus or viruses).ti,ab,kf.
9.	7 or 8
10.	exp Bacteria/
11.	bacteria*.ti,ab,kf.
12.	10 or 11
13.	9 or 12
14.	6 and 13
15.	limit 14 to english language
16.	exp Animals, Laboratory/
17.	6 and 16

Bibliography

Adorni, G., Seifert, G., Buttini, F., Colombo, G., Stecanella, L. A., Krämer, I., & Rossi, A. (2019). Aerosolization Performance of Jet Nebulizers and Biopharmaceutical Aspects. *Pharmaceutics*, *11*(8). https://doi.org/10.3390/pharmaceutics11080406

Alsved, M., Bourouiba, L., Duchaine, C., Löndahl, J., Marr, L. C., Parker, S. T., Prussin, A. J., & Thomas, R. J. (2020). Natural sources and experimental generation of bioaerosols: challenges and perspectives. *Aerosol Science and Technology*, *54*(5), 547-571.

Arı, A. (2014). Jet, Ultrasonic, and Mesh Nebulizers: An Evaluation of Nebulizers for Better Clinical Outcomes. *Eurasian Journal of Pulmonology*, *16*(1).

Bohannon, J. K., Lackemeyer, M. G., Kuhn, J. H., Wada, J., Bollinger, L., Jahrling, P. B., & Johnson, R. F. (2015). Generation and characterization of large-particle aerosols using a center flow tangential aerosol generator with a non-human-primate, head-only aerosol chamber. *Inhal Toxicol*, *27*(5), 247-253. https://doi.org/10.3109/08958378.2015.1033570

Boktor, S. W., Hafner, J. W., & Doerr, C. (2024). Influenza (Nursing). In *StatPearls*. StatPearls Publishing
Copyright © 2024, StatPearls Publishing LLC.

Bowling, J. D., O'Malley, K. J., Klimstra, W. B., Hartman, A. L., & Reed, D. S. (2019). A vibrating mesh nebulizer as an alternative to the collison three-jet nebulizer for infectious disease aerobiology. *Applied and environmental microbiology*, *85*(17), e00747-00719.

Brown, J. R., Tang, J. W., Pankhurst, L., Klein, N., Gant, V., Lai, K. M., McCauley, J., & Breuer, J. (2015). Influenza virus survival in aerosols and estimates of viable virus loss resulting from aerosolization and air-sampling. *Journal of Hospital Infection*, *91*(3), 278-281. https://doi.org/https://doi.org/10.1016/j.jhin.2015.08.004

Cheah, P. K. P., & Davies, C. N. (1984). The spinning-top aerosol generator—Improving the performance. *Journal of Aerosol Science*, *15*(6), 741-751. https://doi.org/https://doi.org/10.1016/0021-8502(84)90010-7

Cheng, Y. S., Irshad, H., Kuehl, P., Holmes, T. D., Sherwood, R., & Hobbs, C. H. (2008). Lung deposition of droplet aerosols in monkeys. *Inhal Toxicol*, *20*(11), 1029-1036. https://doi.org/10.1080/08958370802105413

Cowling, B. J., Ip, D. K. M., Fang, V. J., Suntarattiwong, P., Olsen, S. J., Levy, J., Uyeki, T. M., Leung, G. M., Malik Peiris, J. S., Chotpitayasunondh, T., Nishiura, H., & Mark Simmerman, J. (2013). Aerosol transmission is an important mode of influenza A virus spread. *Nature Communications*, *4*(1), 1935. https://doi.org/10.1038/ncomms2922

Dabisch, P. A., Xu, Z., Boydston, J. A., Solomon, J., Bohannon, J. K., Yeager, J. J., Taylor, J. R., Reeder, R. J., Sayre, P., Seidel, J., Mollura, D. J., Hevey, M. C., Jahrling, P. B., & Lackemeyer, M. G. (2017). Quantification of regional aerosol deposition patterns as a function of aerodynamic particle size in rhesus macaques using PET/CT imaging. *Inhalation Toxicology*, *29*(11), 506-515. https://doi.org/10.1080/08958378.2017.1409848

Darquenne, C. (2012). Aerosol deposition in health and disease. *J Aerosol Med Pulm Drug Deliv*, *25*(3), 140-147. https://doi.org/10.1089/jamp.2011.0916

Day, W. C., & Berendt, R. F. (1972). Experimental tularemia in Macaca mulatta: relationship of aerosol particle size to the infectivity of airborne Pasteurella tularensis. *Infection and immunity*, *5*(1), 77-82.

de Graaf, M., & Fouchier, R. A. (2014). Role of receptor binding specificity in influenza A virus transmission and pathogenesis. *Embo j*, *33*(8), 823-841. https://doi.org/10.1002/embj.201387442

Dennis, D. (2009). Plague as a Biological Weapon. *Bioterrorism and Infectious Agents: A New Dilemma for the 21st Century*, 1266-1264_1262.

Druett, H. A., Henderson, D. W., Packman, L., & Peacock, S. (1953). Studies on respiratory infection. I. The influence of particle size on respiratory infection with anthrax spores. *J Hyg (Lond)*, *51*(3), 359-371. https://doi.org/10.1017/s0022172400015795

FactSheet: Anthrax. (2001). *New South Wales Public Health Bulletin*, *12*(12), 338-338. https://doi.org/https://doi.org/10.1071/NB01114

Feng, J. Q., Go, L.-S., Calubayan, J., & Tomaska, R. (2021). Working mechanism and behavior of Collison nebulizer. *Aerosol Science and Engineering*, *5*(3), 285-291.

Fennelly, K. P. (2020). Particle sizes of infectious aerosols: implications for infection control. *Lancet Respir Med*, *8*(9), 914-924. https://doi.org/10.1016/s2213-2600(20)30323-4

Fennelly, K. P., Tribby, M. D., Wu, C.-Y., Heil, G. L., Radonovich, L. J., Loeb, J. C., & Lednicky, J. A. (2014). Collection and measurement of aerosols of viable influenza virus in liquid media in an Andersen cascade impactor. *Virus Adaptation and Treatment*, *7*(null), 1-9. https://doi.org/10.2147/VAAT.S74789

Flament, M. P., Leterme, P., & Gayot, A. (2001). Study of the technological parameters of ultrasonic nebulization. *Drug Dev Ind Pharm*, *27*(7), 643-649. https://doi.org/10.1081/ddc-100107320

Ibrahim, E., Harnish, D., Kinney, K., Heimbuch, B., & Wander, J. (2015). An experimental investigation of the performance of a Collison nebulizer generating H1N1 influenza aerosols. *Biotechnology & Biotechnological Equipment*, *29*(6), 1142-1148. https://doi.org/10.1080/13102818.2015.1059736

Johnson, R. F., Hammoud, D. A., Lackemeyer, M. G., Yellayi, S., Solomon, J., Bohannon, J. K., Janosko, K. B., Jett, C., Cooper, K., Blaney, J. E., & Jahrling, P. B. (2015). Small particle aerosol inoculation of cowpox Brighton Red in rhesus monkeys results in a severe respiratory disease. *Virology*, *481*, 124-135. https://doi.org/10.1016/j.virol.2015.02.044

Johnson, R. F., Hammoud, D. A., Perry, D. L., Solomon, J., Moore, I. N., Lackemeyer, M. G., Bohannon, J. K., Sayre, P. J., Minai, M., Papaneri, A. B., Hagen, K. R., Janosko, K. B., Jett, C., Cooper, K., Blaney, J. E., & Jahrling, P. B. (2016). Exposure of rhesus monkeys to cowpox virus Brighton Red by large-particle aerosol droplets results in an upper respiratory tract disease. *J Gen Virol*, *97*(8), 1942-1954. https://doi.org/10.1099/jgv.0.000501

Kooij, S., Astefanei, A., Corthals, G. L., & Bonn, D. (2019). Size distributions of droplets produced by ultrasonic nebulizers. *Scientific Reports*, *9*(1), 6128. https://doi.org/10.1038/s41598-019-42599-8

Larson, E. W., Dominik, J. W., Rowberg, A. H., & Higbee, G. A. (1976). Influenza virus population dynamics in the respiratory tract of experimentally infected mice. *Infect Immun*, *13*(2), 438-447. https://doi.org/10.1128/iai.13.2.438-447.1976

Leffel, E. K., & Reed, D. S. (2004). Marburg and Ebola viruses as aerosol threats. *Biosecur Bioterror*, *2*(3), 186-191. https://doi.org/10.1089/bsp.2004.2.186

Manchester, T. U. o. *Aerodynamic Particle Sizer*. Retrieved April 2024

May, K. (1973). The Collison nebulizer: description, performance and application. *Journal of Aerosol Science*, *4*(3), 235-243.

Mitchell, J. P., Nagel, M. W., Wiersema, K. J., & Doyle, C. C. (2003). Aerodynamic particle size analysis of aerosols from pressurized metered-dose inhalers: comparison of Andersen 8-stage cascade impactor, next generation pharmaceutical impactor, and model 3321 Aerodynamic Particle Sizer aerosol spectrometer. *AAPS PharmSciTech*, *4*(4), E54. https://doi.org/10.1208/pt040454

Moghadami, M. (2017). A Narrative Review of Influenza: A Seasonal and Pandemic Disease. *Iran J Med Sci*, *42*(1), 2-13.

Morawska, L., & Buonanno, G. (2021). The physics of particle formation and deposition during breathing. *Nature Reviews Physics*, *3*(5), 300-301. https://doi.org/10.1038/s42254-021-00307-4

Muralidharan, P., Malapit, M., Mallory, E., Hayes, D., & Mansour, H. M. (2015). Inhalable nanoparticulate powders for respiratory delivery. *Nanomedicine: Nanotechnology, Biology and Medicine*, *11*(5), 1189-1199. https://doi.org/https://doi.org/10.1016/j.nano.2015.01.007

Nichols, S., Mitchell, J., Shelton, C., & Roberts, D. (2013). Good Cascade Impactor Practice (GCIP) and Considerations for "In-Use" Specifications. *AAPS PharmSciTech*, *14*. https://doi.org/10.1208/s12249-012-9905-1

Port, J. R., Yinda, C. K., Owusu, I. O., Holbrook, M., Fischer, R., Bushmaker, T., Avanzato, V. A., Schulz, J. E., van Doremalen, N., Clancy, C. S., & Munster, V. J. (2020). SARS-CoV-2 disease severity and transmission efficiency is increased for airborne but not fomite exposure in Syrian hamsters. *bioRxiv*. https://doi.org/10.1101/2020.12.28.424565

Rau, J. L. (2002). Design principles of liquid nebulization devices currently in use. *Respir Care*, *47*(11), 1257-1275; discussion 1275-1258.

Riedel, S. (2005). Smallpox and biological warfare: a disease revisited. *Proc (Bayl Univ Med Cent)*, *18*(1), 13-20. https://doi.org/10.1080/08998280.2005.11928026

Roy, C. J., Hale, M., Hartings, J. M., Pitt, L., & Duniho, S. (2003). Impact of inhalation exposure modality and particle size on the respiratory deposition of ricin in BALB/c mice. *Inhal Toxicol*, *15*(6), 619-638. https://doi.org/10.1080/08958370390205092

Roy, C. J., Reed, D. S., & Hutt, J. A. (2010). Aerobiology and Inhalation Exposure to Biological Select Agents and Toxins. *Veterinary Pathology*, *47*(5), 779-789. https://doi.org/10.1177/0300985810378650

Roy, C. J., Reed, D. S., Wilhelmsen, C. L., Hartings, J., Norris, S., & Steele, K. E. (2009). Pathogenesis of aerosolized Eastern Equine Encephalitis virus infection in guinea pigs. *Virol J*, *6*, 170. https://doi.org/10.1186/1743-422x-6-170

Scott, G. H., & Sydiskis, R. J. (1976). Responses of mice immunized with influenza virus by serosol and parenteral routes. *Infect Immun*, *13*(3), 696-703. https://doi.org/10.1128/iai.13.3.696-703.1976

Services, U. S. D. o. H. a. H. *Science Safe Securities- Finding the Balance together*. ASPR. Retrieved April from

Sidler-Moix, A.-L., Paolo, E. R. D., Dolci, U., Berger-Gryllaki, M., Cotting, J., & Pannatier, A. (2015). Physicochemical Aspects and Efficiency of Albuterol Nebulization: Comparison of Three Aerosol Types in an In Vitro Pediatric Model. *Respiratory Care*, *60*(1), 38-46. https://doi.org/10.4187/respcare.02490

Thomas, R., Davies, C., Nunez, A., Hibbs, S., Flick-Smith, H., Eastaugh, L., Smither, S., Gates, A., Oyston, P., Atkins, T., & Eley, S. (2010). Influence of particle size on the pathology and efficacy of vaccination in a murine model of inhalational anthrax. *J Med Microbiol*, *59*(Pt 12), 1415-1427. https://doi.org/10.1099/jmm.0.024117-0

Thomas, R. J. (2013). Particle size and pathogenicity in the respiratory tract. *Virulence*, *4*(8), 847-858. https://doi.org/10.4161/viru.27172

Wonderlich, E. R., Swan, Z. D., Bissel, S. J., Hartman, A. L., Carney, J. P., O'Malley, K. J., Obadan, A. O., Santos, J., Walker, R., Sturgeon, T. J., Frye, L. J., Jr., Maiello, P., Scanga, C. A., Bowling, J. D., Bouwer, A. L., Duangkhae, P. A., Wiley, C. A., Flynn, J. L., Wang, J., . . . Barratt-Boyes, S. M. (2017). Widespread Virus Replication in Alveoli Drives Acute Respiratory Distress Syndrome in Aerosolized H5N1 Influenza Infection of Macaques. *J Immunol*, *198*(4), 1616-1626. https://doi.org/10.4049/jimmunol.1601770