ABSTRACT

Title of Dissertation:	ANALYSIS, QUANTIFICATION AND SIMULATION OF THE RISK FROM AIRBORNE INFLUENZA
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Despite the development of effective vaccines, influenza still remains as a global concern. For appropriate public health intervention, it is crucial to accurately determine the routes of transmission. Influenza is believed to have three primary modes of transmission: big droplet, direct contact and aerosol particles. Considerable evidence points to both aerosol and droplet transmission routes as being significant. Because of the limitation of sampling and analysis, the quantitative dynamics of the aerosol mode of transmission are not completely understood. In this dissertation I have characterized the physical and biological collection efficiency of a novel exhaled breath aerosol collector named Gesundheit II (G-II). The device was proven to successfully collect and preserve infectivity with different types of influenza virus. I have also been involved in epidemiological data analysis, experimental quantification and numerical modeling. On experimental quantification, I have been

part of a multi-member team that has conducted a study of characterization of respiratory droplets from influenza infected individuals at the University of Maryland campus during the flu seasons of 2012-2013. The exhaled breath was collected with the G-II for accurate quantification of the influenza virus. 218 pairs of fine (< 5 μm) and coarse (≥ 5μm) exhaled breath samples were obtained from 142 subjects and analyzed. The relationship between culturability, coughing frequency, and symptoms were investigated. The high rate of RNA detection and the frequent recovery of influenza virus by culture from fine aerosol samples suggest a contribution of fine particle aerosols in the transmission of influenza. Given these new findings, to understand the risk of influenza infection from these finer droplets, we have modified an existing mathematical risk analysis model and studied the effect of these droplets on subjects in presence or absence of a respiratory protective device (RPD). Two of the major enhancements in our model are (1) the ability to account for subject-to-subject variability over a wide range of age groups and (2) the heterogeneous population was introduced into the model with some infectees or susceptibles not wearing RPDs.

ANALYSIS, QUANTIFICATION AND SIMULATION OF THE RISK FROM AIRBORNE INFLUENZA

by

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Abbreviations

RPD	Respiratory protection devices
WHO	World Health Organization
G-II	Gesundheit II
EMIT	Evaluating Mechanisms of Influenza Transmission
UML	University of Massachusetts, Lowell
UHK	University of Hong Kong
UMD	University of Maryland – College Park
NUS	National University of Singapore
PCR	Polymerase chain reaction
RT-qPCR	Reverse transcript-quantitative polymer chain reaction
SIR	Susceptible-infected-recovered model
SEIR	Susceptible-exposed-infected-recovered model
HA	Hemagglutinin
NA	Neuraminidase
AGIs	All glass impingers
BS	Biosampler
SARS	Severe acute respiratory syndrome
NIOSH	National Institute for Occupational Safety and Health
CDC	Centers for Disease Control and Prevention
SD model	Model developed by Stilianakis and Drossinos
FDA	Food and Drug Administration of the United States
$TCID_{50}$	Tissue culture infective dose

PR8	Puerto Rico influenza A virus
EBA	Exhaled breath aerosol
RH	Relative humidity
AH	Absolute humidity
PBS	Phosphate-buffered saline
DBPS	Dulbecco's phosphate-buffered saline
BSA	Bovine serum albumin
FFU	Fluorescent focus units
DMEM	Dulbecco’s modified eagle medium
MDCK	Madin-Darby canine kidney cells
T/I	Ratio of total virus particle number to the number of infectious particles
ILI	Influenza like illness
NP	Nasopharyngeal
BMI	Body mass index
95% CI	95% confident interval
LOD	Lower limit of detection
ARI	Acute respiratory illness
UHC	University health center
ODE	Ordinary differential equations
PF	Protection factor
PSL	Polystyrene latex
HEPA	High-efficiency particulate arrestance

Nomenclature

η	Collection efficiency
N_{CF}	Total number of viruses released in the coarse fraction
N_{FF}	Total number of viruses released in the fine fraction
V_{FF}	Total number of viable virus particles in the fine fraction
S	Susceptible population
I	Infected population
N	Total population
R	Recovered population
t	Exposure time (days)
$D(t)$	Total number of droplet
$\tilde{\beta}$	Transmission rate per inhaled droplet
κ_w	Respirable-droplet production rate at specific size
υ^{-1}	Droplet decay at specific size
C	Contact rate between a susceptible and an infected person
B	Breathing rate (m^3/day)
V_{cl}	Volume of the personal cloud of an infected person (m^3)
τ	Characteristic breathing time (days)
p	Probability of infection by an inhaled pathogen
d_i	Droplet diameter (μm)
q_i	Inhaled-droplet deposition probability
N_{pi}	Number of pathogens per droplet

T_{in}	The fraction of incoming pathogens transmitted by the RPD
T_{out}	The fraction of outward pathogens (expelled by the infected person) for the barrier
f_i	The fraction of infected persons deploying RPDs
μ_d	Inactivation rate of airborne pathogens (per day)
θ_i	Gravitational settling rate (per day)
μ_I	Infection recovery rate (per day)

CHAPTER 1: INTRODUCTION AND OVERVIEW

This dissertation is focused on the study of the airborne transmission of influenza virus, and the development of a mathematical modeling approach to predict airborne transmission in scenarios involving use of respiratory protection devices (RPDs). In this chapter, I will address the overall problems and research approaches.

1.1 Problem Description and Motivation

Human influenza is an acute respiratory disease and is considered as one of the most important infectious diseases of mankind. The World Health Organization (WHO) estimated that annual epidemics account for an estimated three to five million severe illnesses and up to 500,000 deaths worldwide (Brankston et al., 2007; Elovainio, 2008). The infection mainly occurred in the epithelial cells of the upper and lower respiratory tract. An accurately defined route of transmission is important for public health policy and practice (Atkinson and Wein, 2008; Cowling, 2012). Transmission of bioaerosols resulting in the spread of disease can take place via various routes. The modes of transmission have been well documented in the literature, but the relative importance of these modes has been debated in recent years. Influenza is believed to have three main modes of transmission: (1) by direct contact with secretions (2) by transport of large droplets, >5 μm in diameter, that land in the mouths, eyes, and noses of people nearby and (3) by aerosol transmission, breathing in smaller droplets suspended in the air (Weber and Stilianakis, 2008). Literature work supported that mechanisms such as coughing and sneezing will produce a ‘respiratory spray’ of

different sizes containing infectious virus. No strong evidence has been shown for the importance of simple tidal breathing in airborne transmission.

At present, the available literature is insufficiently clear to determine which of these three routes of transmission plays a role in the human-human spread of influenza (probably all three are possible), nor the relative importance of each. Because of the limitation of sampling and analysis, the quantitative dynamics of the aerosol mode of transmission are not completely understood. Clear guidance on the prevention of influenza transmission in homes, schools, workplaces and healthcare settings cannot easily be provided while the evidence base remains unclear. For example, if transmission via droplet nuclei (aerosols) was shown to predominate, control measures in healthcare settings might include ventilation and ultraviolet upper room irradiation in addition to respiratory protection.

It has been a great challenge to characterize the airborne transmission route due to the difficulty in collecting and analyzing micrometer-sized particles. Studies to date have rarely included quantitative analyses of the total viral load. Most sampling methods affect the viral viability and result in lower detection of the concentration of infectious airborne virus (Huynh et al., 2008). The presence of contaminants could also inhibit the laboratory assays. Previous reports on the generation of fine particle influenza aerosols by infected persons used instruments that require the subject to breathe through a mouthpiece or face mask and/or required unnatural or forced natural breathing pattern and do not give an accurate picture of virus shedding by a subject breathing normally and coughing spontaneously. Use of a high physical and biological collection efficiency human exhaled breath sampler, which allows for natural

breathing, could answer questions about transmission. Some clinical trial studies have used experimentally infected volunteers to simulate the naturally infected cases, but it is still unclear if these experimentally infected cases will be able to simulate the natural ones in all aspects.

RPDs such as respirators play an important role in the control strategy against the transmission of influenza. Experiments to validate the protective device effect on influenza transmission require deliberate infection of a susceptible population and controlled use of protective equipment. It is extremely difficult and complicated, and the feasibility of attaching protective devices to animals is likewise low. Mathematical modeling will be a valid tool to evaluate the reduction of risk associated with the deployment of a given RPD. Numerous models of various types have been developing to simulate the spread of epidemics (Chen and Liao, 2008; Coburn et al., 2009; Furuya, 2007; Stilianakis and Drossinos, 2010), but no systematic treatment of the effect of RPDs has been incorporated into the models. In determining the type of RPD to deploy in the epidemic, it is important to have a model which shows the reduction in the infection rate that the RPD enables for the given pathogen and population.

1.2 Research Approaches and Objectives

The dissertation consists of four major projects, plus a review of relevant literature (Chapter 2). The first project is to characterize a human bioaerosol sampler named the Gesundheit II, or G-II, for both physical and biological collection efficiency before performing on real human subjects (Chapter 3). This is followed by using the device to study the shedding of influenza virus into respiratory droplets of volunteers with

community-acquired influenza virus infection (Chapter 4). The third project is to explore the climate effects on infectious influenza virus in human exhaled breath (Chapter 5). The fourth project focuses on developing numerical risk assessment models from exposure to influenza bioaerosols. The model was developed from the existing literature work and modified to include protection factors of the RPDs (Chapter 6). Finally, I will give overall conclusions and implications of my results, limitations of my work, remaining research questions and possible further work (Chapter 7).

1.2.1 Device Characterization

The novel exhaled breath aerosol collector named G-II was designed allowing natural breathing, coughing, speaking, and singing during sampling. The optimal G-II operating parameters were established through a series of experiments. The parameters that were taken into consideration are condenser supersaturation ratio, coolant supply temperature, and different mass flow rate of the saturated steam. These parameters will affect the ability of G-II to collect aerosols in the airstream and the ability to preserve virus viability during the collection process. The biological collection efficiency comparison between BioSampler and G-II was performed with four types of influenza virus. My role in this project was to conduct the characterization experiments and interpret the lab results.

1.2.2 Evaluating Airborne Transmission Mode from Naturally Infected Cases

An understanding of influenza virus transmission is crucial for public health interventions. The objective of our CDC funded Evaluating Mechanisms of Influenza

Transmission (EMIT) clinical study is to determine the contribution of aerosols to transmission of human influenza virus. Volunteers with influenza-like illness were recruited on the College Park campus. As part of a multi-member team, we have successfully conducted a study of characterization of respiratory droplets from influenza-infected individuals at the University of Maryland campus during the flu seasons of 2012-2013. The exhaled breath was collected with the G-II for accurate quantification of the influenza virus. 218 pairs of fine (< 5 μm) and coarse (⩾ 5μm) exhaled breath samples were obtained from 142 subjects and analyzed. My role in this project was sampling human subjects' exhaled breath, and conducting statistical analysis and interpretation of the different resulting datasets.

1.2.3 Influenza Virus in Respiratory Droplets Produced by Infection Cases from Different Climates

We performed a nested validation study comparing aerosol shedding by infection cases from three locations, University of Massachusetts (UML), University of Hong Kong (UHK) and National University of Singapore (NUS). These three locations represent three types of climates, temperate, subtropical and tropical. The effects of outside environmental parameters on human viral shedding are discussed in this chapter. We have 7 confirmed H3 influenza infection cases from UHK, 23 confirmed H3 and 8 confirmed B cases from NUS, 21 confirmed H3 cases and 16 confirmed B cases from UHK. All the exhaled breath and cough aerosols were collected using G-II sampler and all samples analyzed by RT-qPCR. The difference in viral aerosol

shedding from all three locations was evaluated. My role in this project was performing a thorough statistical analysis and interpretation of results.

1.2.4 Infection Risk Assessment Model

In order to ascertain a holistic understanding of reduction of risk of influenza transmission by using protective measures, we modified the susceptible-infected-recovered (SIR) epidemic model as presented Stilianakis and Drossinos, 2010 to account for the influence of RPDs. We accounted for the fact that only a fraction of the population will likely deploy protective measures by dividing the susceptible population into two groups, one of which deploys RPDs and one which does not. Similarly, a fraction of the infected population utilizes RPDs, thereby reducing the source of pathogens. We first implement our modifications under the idealized assumption of a monodisperse aerosol distribution, and then extend the formulation to the more realistic case of a polydisperse size distribution. The mathematical risk assessment model for influenza transmission will provide a means for improving pre-clinical assessments of safety and efficacy of personal protection devices used for prevention and source control. My role in this project was to conduct the literature review and interpret and modify the SIR model.

CHAPTER 2: LITERATURE REVIEW

2.1 Virology of Influenza

Influenza viruses are enveloped, single negative-stranded RNA viruses, and divided into types A, B, and C according to their antigenic differences. The viruses are capable of infecting different species of birds and mammals. Only influenza A and B are capable of causing annual epidemics in humans. Influenza A viruses are subdivided into subtypes according to the types of surface protein hemagglutinin (HA) and neuraminidase (NA) (Tamura et al., 2005), e.g. influenza A (H1N1), A (H1N2) and A (H3N2) are common subtypes in annual human influenza epidemics. The viruses in each subtype undergo gradual changes in genetic makeup through point mutations in the HA and NA (Tamura and Kurata, 2004). An "antigenic shift" between avian flu and human influenza could happen due to such change, and since no immunity has been prepared against the modified virus, a global pandemic will arise.

2.2 Identification of Influenza Virus Infections

Recently, as reported by Gralton et al. (2013), samples of exhaled breath aerosols from both adults and children with symptomatic respiratory infections were collected using a 6 stage Anderson impactor and were found to contain viral RNA. In this study, during tidal mouth breathing through a mouthpiece, 58% (31/53) of participants produced coarse particles containing viral RNA and 80% (42/53) of participants produced fine particles with viral RNA (Gralton et al., 2013). The processes by which pathogens spread, deposit and initiate infection are highly influenced by the size of the airborne particles (Weatherall, 2004). The mechanisms behind the size distribution

dynamics of such naturally generated bio-aerosols include diffusion, impaction, interception, or electrostatic attraction (Verreault et al., 2008). The human respiratory tract can be divided into three parts, nasopharynx, tracheobronchial region and alveolar region. Once the infectious influenza virus aerosol particles reach the respiratory tract, the virus will enter the airway epithelium through specific target cells. The viruses will attach to host cells that are located in the respiratory tract by binding of the hemagglutinin to sialosaccharides on the host cell surface and initiate infection (Baigent and McCauley, 2003). If the virus binding occurs in the lower airway and alveoli, it leads to more severe illness (Baigent and McCauley, 2003).

The transmissibility of influenza is also highly influenced by the exposure environmental conditions, such as, humidity, temperature, seasonality, settings (indoor or outdoor), solar irradiation and air exchange in which the pathogen and host meet (Pica and Bouvier, 2012; Tamerius et al., 2013). These factors strongly affect the production of influenza-laden particles and also the viability of the virus particles, which is linked to risk of infection. Lowen et al., (2007) used guinea pigs to show that the infected hosts shed significantly higher quantities of viral particles when exposed to lower ambient temperature than those were exposed to higher temperature. Having an ultraviolet light in the room could denature the virus, and increasing ventilation could dilute the virus in the air. Social distancing through quarantine, isolation of ill persons can reduce the rate of transmission successfully. It is believed that airborne route is dominant in the temperate climate region due to distinct seasonality. The relative lack of seasonality in tropical regions with less variability in temperature and humidity suggests dominance by the other transmission route.

A respiratory infection occurs when an infected person spreads the respiratory pathogens to a susceptible person. The pathogen carrying respiratory secretions can be transferred by making direct physical contact (e.g. shaking hands) or indirectly, when the susceptible person makes physical contact with contaminated objects. The transmission can also happen by inhaling the respiratory secretions, which are released by the infected person in air during breathing, coughing, sneezing or talking. The large ballistic droplets can only travel a short distance from the infected person before falling to the ground quickly. Small particles with an aerodynamic diameter ≤ 5 μm that are generated either from respiratory tract or the desiccation of large droplets, can stay and remain suspended in air for a relatively longer period of time. Nicas et al. (2005) and Yang et al. (2007) have confirmed that coughing or sneezing can generate a large number of aerosol particles with size greater than 2 μm. Edwards et al. (2004) also confirmed that exhalation during normal breathing can also generating a large amount of aerosols and the majority of them with a size around 1 μm or less. It is believed that the aerosol generated from tidal breathing are from the lower respiratory tract (LRT), and heterogeneity exists among individuals in aerosol production. Even though per cough or sneeze can put out more aerosols comparing with per normal breathing, but normal breathing is long term and continuous which should count as a more significant fraction in aerosol transmission (Tellier, 2006, 2007).

Once a person inhales the infectious particles that were exhaled by the infected host, there is a chance that the virus may deposit on the receptor of a susceptible cell and initiate infection. The air becomes a medium for the transmission of respiratory influenza virus between infectious source and new host. The literature work has shown

evidence supporting the presence of aerosol transmission in both animals and people. Animal studies in ferrets, guinea pigs, mice and monkeys have shown that disease developed after exposure to aerosolized influenza virus. The aerosol route of transmission has been observed among ferrets placed in adjacent cages in 1941 (Andrewes and Glover, 1941). In human volunteer studies (Knight, 1980), a lower infection dose was required to cause infection by aerosol than intranasal inoculation. In both animal and human studies, evidence showed that infection caused by aerosolized virus can lead to more severe disease than intranasal inoculation (Little et al., 1979; Mumford et al., 1990). When the virus is deposited in the alveolar region, only a small dose can lead to a greater risk for infection.

2.3 Current Sampling and Analyzing Techniques and Remaining Challenges

It has been a great challenge to characterize the airborne transmission route due to the difficulty in collecting and analyzing micrometer-sized particles. Studies to date have rarely included quantitative analyses of total viral load. Most sampling methods affect the viral viability and result in lower detection of the concentration of infectious airborne virus (Verreault et al., 2008). During the sampling, the presence of contaminants could also inhibit the laboratory assays.

The impaction mechanism is considered as the most common one for particle collection. The Stokes number (inertial impaction parameter) is the key parameter in impactor design, which influences the efficiency of impaction collector. The air sampling technologies also depend on Brownian motion, thermal gradients, inertia of the particles, adhesion properties of the airborne particles and the aerodynamic

diameter of the particles (Verreault et al., 2008). Aerosol measurement requires that an aerosol sample be transferred to a collecting medium by withdrawing the sample from the environment. The surface tension will act on the medium in the sampler and affect the collection efficiency. Particles on the order of micrometer or more have greater inertia and gravitational attraction than the smaller ones but are less influenced by Brownian motion. Because of that, the bigger particles are more easily diverted from a gas streamline and impact on the surface, especially at higher velocities and altered angle of the airflow, and the smaller particles easily follow the airflow. The sampling techniques have improved greatly over the years, but the issue of lack of efficiency still remains because of the wide range of aerodynamic properties of airborne virus.

Currently, there are a variety of samplers available for bioaersol detection. All glass impingers (AGIs) and SKC BioSampler are two commonly used liquid impingers for airborne virus sampling. The liquid impingers are efficient for collection of submicrometer particles (Fabian et al., 2009). AGIs were designed to accelerate particles in the air through the narrow orifice and leading to turbulent deposition of particles. It mimics the respiratory tract in terms of deposition of the particles. The major difference between SKC and AGIs is that SKC splits the airflow into three tangential nozzles and creates a swirling motion in the sampling liquid. Comparing with AGIs, SKC has significantly minimized the reaersolization of the collected particles and reduced the particle bouncing. The sampling process for liquid impingers is gentle, and the particles impacting on a liquid media can easily maintain their integrity and viability. Studies have demonstrated that SKC has a high collection efficiency of almost 100% for particles larger than 2 μm and 80% for those larger than

300 nm (Willeke et al., 1998). The flow rates are low for the AGIs and SKC, around 12 lpm.

Anderson impactors and slit impactors are also common types of impactors used for virus air sampling. Anderson impactors can contain up to 6 stages to collect aerosol particles with size selection. Bischoff et al. (2013) has used Anderson impactors to detect the influenza virus up to six feet from an infected patient's head. A slit impactor was used during the SARS outbreak in 2003 with great success. It operates by impacting particles on a rotating dish and can recover the viruses with a liquid layer on top of the culture medium. The culture media on the Anderson impactor and slit impactor can introduce contamination and drying over time. Several filters operate with basic mechanism including interception, inertial impaction, diffusion, gravitation settling and electrostatic attraction (Hinds, 1999). These filters are made out of different materials such as cellulose, polycarbonate and gelatin with the ability to collect airborne particles. Fabian et al. (2009) showed that influenza viruses quickly lose infectivity on filters.

The National Institute for Occupational Safety and Health (NIOSH) has developed a cyclone BioSampler that operates by using centrifugal force to push airborne particles into a solid surface using their inertia. This NIOSH sampler operates at 3.5 L/min and can be used as a personal or area sampler. The particles were collected in three stages, 4μm and above on the first stage, 1-4 μm in the second stage and the remaining were collected on a backup filter. Filed and laboratory studies have confirmed the influenza virus recovery ability of NIOSH (Blachere et al., 2007, 2009; Cao et al., 2011; Lindsley et al., 2010). A wetted wall cyclone developed in the McFarland group is capable of

delivering a small liquid effluent flow rate of highly-concentrated hydrosol. The collection efficiency was proved to be 85% for particle sizes larger than 2 μm at the 0.1 mL/min liquid effluent flow rate for 100 L/min air flow rate (McFarland and Burroughs, 2011; McFarland et al., 2010). The submicron collection efficiency has been tested to be low for wetted wall cyclone (Kesavan et al., 2008).

Although there are many different samplers designed to collect airborne influenza virus, the sampling method is still remaining challenging in the field study due to variety effects. The dry samplers have been shown to result in damage and desiccation of the viruses. Cao et al. (2011) showed that NIOSH BioSampler performed significantly worse at maintaining infectious airborne virus than the SKC BioSampler due to the desiccation and degradation of the virus over time. For prolonged sampling, it is important to preserve the activity of the virus and also maintain a high collection efficiency. The liquid media filled samplers can easily dry out over time under low humidity and the gel filters can also likely to dissolve under high humidity. The evaporation during sampling can alter the characteristic of the media. The bubbling liquid during sampling can easily reaerosolized the viral particles. In summary, the limitations of current samplers include the inability to separate particles based on size, limited sampling time, and low biological collection efficiency. In order to accurately collect and detect an individual's virus aerosol shedding, a long-term sampling strategy needs to be explored.

Infective influenza virus can be quantified using a fluorescent focus assay. The fluorescent focus assay binds fluorescently labeled antibody to viral proteins produced by infected cells (Mentel et al., 1996) and enables the identification of individually

infected cells, but the detected sensitivity is too low and many viable virus particles could be missed. When viral concentration in the samples is low, technology such as polymerase chain reaction (PCR) and real-time quantitative polymerase chain reaction (RT-qPCR) are popular methods to detect the presence of viruses in collected samples. Comparing with other enzyme-linked immunosorbent assays, PCR has been considered as the most effective, sensitive and specific for detecting samples with low viral concentrations (Elden et al., 2001). Using this technology, Fabian's group has successfully detected influenza virus RNA in aerosol particles generated by the patient wearing a facemask (Fabian et al., 2008). The limitation of PCR is it cannot it cannot determine if the detected viruses are infectious or not.

2.4 Mathematical Modeling of Influenza Epidemics and Non-Pharmaceutical Intervention

Mathematical models have made considerable contributions to the understanding of influenza infection. In planning the response to a future or ongoing epidemic, development of an infection risk assessment model is essential. Quantitative infection risk assessment will incorporate both demographical and epidemiological effects during an outbreak to estimate the infection risk of a population (Sze To and Chao, 2010). The model will provide a means for improving pre-clinical assessments of safety and efficacy of personal protection devices used for prevention and source control.

Several influenza transmission models have been constructed in the literature combining both physical dynamics and the biological processes to estimate the risk. Most of them support the concept that all transmission routes can be important towards

the risk of infection (Atkinson and Wein, 2008). One of the most used models is Wells-Riley exponential model, derived from the Reed-Frost Equation (an early stochastic model) (Sze To and Chao, 2010). It is a predictive model that quantitatively evaluates airborne infection risk in a single generation of an outbreak. The Wells-Riley model incorporates the source strength in terms of a quantum of infection (Sze To and Chao, 2010). It does not give the direct access to identify the number of pathogens that constitute the source strength. The Wells-Riley model also requires measurement of outdoor air supply rates, which is hard to define as it often varies with time.

Deterministic models have been most widely used for respiratory disease transmission models, in which SIR (susceptible-infected-removed), SEIR (susceptible-exposed-infected-removed) and Carrier State Models are the most notable ones (Keeling and Rohani, 2008). The choice of which model to use depends on the characteristics of the disease and the purpose of the model.

The classic compartment SIR model is an epidemiological model which was developed by Ronald, et al. in the early twentieth century (Anderson, 1991). The model, which has been widely applied, consists of several differential equations coupling the changes in the population of susceptibles (S), the exposed population (E), the population of infection cases (I), and the population of formerly infected, now recovered to an immune state (R). The SIR model can quantify risk by considering source strength in terms of pathogen numbers, assuming the 50% infectious dose is known (Furuya, 2007). The model has been proven to be effective at fitting epidemic curves.

Control of influenza transmission during an epidemic is a major issue. Both immunization and the proper use of respiratory protective equipment (RPD) are important in preventing transmission of communicable respiratory illness. Vaccine use can cut down some of the risks of the transmission but not completely. In a recent study by the CDC, it was shown that flu vaccination was responsible for a 70% reduction in infections among all population groups during the 2011-2012 flu season (Centers for Disease Control and Prevention (CDC), 2013). During normal seasons with gradual revolution of influenza virus, vaccine can be effective. But vaccine is normally not available in time for a new virus strain to prevent virus spread and wearing RPD could reduce the risk of influenza transmission further. Thus, RPDs such as respirators may constitute an important element of the control strategy against the transmission of infectious diseases. In determining whether to deploy a given RPD to protect a specific population against a certain pathogen, including whether to allocate substantial resources to stockpile RPDs against a possible future threat, realistic estimates of the reduction of risk associated with the use of the RPD in the scenario of interest is important. In evaluating the reduction of risk associated with deployment of a given RPD, important parts of the assessment include determination of the intrinsic penetration of the device (Technologies et al., 2010) which is governed by the microscopic properties of the barrier, and the amount of leakage due to imperfect fit (Coffey et al., 2004; Lee et al., 2004) between a particular facial profile and the RPD. Another important part of the risk assessment for RPDs is an estimate of the change in the infection rate associated with the change in pathogen transport introduced by the presence of the barrier. The change in pathogen transport could apply to either an

uninfected person attempting to reduce the intake of pathogens or an infected person attempting to reduce the output. While numerous models of various types have been developed to simulate the spread of epidemics, no systematic treatment of the effect of RPDs has been incorporated into the models.

Of particular interest for RPD evaluation is the transmission of disease by inhalable respiratory droplets. To serve the purpose of monitoring the source strength in the influenza airborne transmission, a model that is well suited to estimate the spread of disease by inhalable respiratory droplets is the modified deterministic SIR epidemic model. This model was developed by Stilianakis and Drossinos (2010) (SD) and takes the inhalable droplets as the transmission vector, assuming a closed, homogeneously mixed population. The time course for the susceptible, infected, and recovered populations was derived in the SD model using parameters from experimental evidence. This model did not address the effect of any type of prevention in the influenza infection.

Myers et al. (2016) take the effect of different types of RPDs into the SIR model by SD, and when the growth of the infected population will occur is evaluated for a given protection strategy for adult and child populations separately. The model also indicates how aerosol size distribution in the polydisperse exposure affects the growth of the infected population. In the SIR models, the influence of RPDs can accounted for in a systematic manner in the parameters within the system of differential equations. Relevant parameters of the model include the droplet production rate, gravitational settling rate, deposition probability, and a number of pathogens per droplet, all of which are a function of droplet size. This risk assessment model will be essential to help with

making decisions for a) quantifying the risk reduction from use of RPD and b) help FDA in the decision making of stockpiling of RPDs for influenza pandemics.

CHAPTER 3: EXHALED BREATH COLLECTION AND ANALYSIS OF VIRUS SHEDDING RATES USING G-II BIOAEROSOL COLLECTOR

3.1 Abstract

We performed a series of experiments to characterize the ability of the exhaled-breath bioaerosol collector (G-II) to collect and preserve culturability of four biologically different influenza viruses. We used those data to calculate the strength of the infectious source. Experiments were designed to characterize physical collection efficiency and ability to preserve infectivity. Optimal collection efficiency was achieved when the G-II was operated with a supersaturation ratio of 2.6 or higher. Infectivity was increased when the inlet air temperature was 24˚C or higher and the relative humidity of the inlet air was 65% or higher. The G-II preserved infectivity as well or better than the commercial SKC BioSampler. Therefore, the G-II can be successfully used to collect samples from subjects infected with influenza without prior knowledge of influenza subtype. Using the data collected from laboratory samples and samples collected from three infected subjects, we demonstrate a calculation of the quantity of virus released into aerosols by influenza-infected individuals.

3.1.1 Practical Implications

This study describes the instruments and process necessary for quantification of aerosolized influenza virus from infected individuals. The G-II can be used to study source strength in studies of influenza transmission by aerosols, providing useful

information, should airborne transmission be found a significant contributor to propagation. These results will have implications on determining correct ventilation rates, air distribution strategies, and deployment and effectiveness of air cleaning technologies. The most important application would be in the design of heating, ventilation and air conditioning systems in hospitals and indoor environments with high occupant densities.

3.2 Introduction

Pandemic influenza remains an important global threat. The most recent influenza pandemic of 2009 showed how rapidly the virus can spread worldwide. Influenza transmits by contact, droplet and airborne modes. Aerosol transmission of influenza was first reported between ferrets in 1941 (Andrewes and Glover, 1941). Alford et al., 1966 is the only study that addressed the question of the minimum infectious aerosol dose in humans (0.6-3 $TCID_{50}$). In natural settings it is difficult to identify the route of transmission of individual cases and the relative importance of each of the modes is still a topic of disagreement. Several authors have concluded that the airborne mode is the key pathway of influenza transmission (Fabian et al., 2008; Gralton et al., 2013; Tellier, 2009; Weber and Stilianakis, 2008). Reanalysis by Cowling et al., 2013 of previously collected data suggested that airborne transmission might be a major factor, especially in causing more severe infections. In contrast to these studies, a review by Brankston et al., 2007 and study by Tang et al., 2014 suggest that airborne transmission does not contribute significantly to influenza transmission. Knowledge of the infectious influenza virus aerosol is critical to 1) understand the role of aerosols in influenza virus transmission, and 2) design and evaluate non-pharmaceutical control measures.

However, there are few studies that examine this critical issue.

The initial description and characterization of the Gesundheit-II (G-II) human exhaled aerosol collector, by McDevitt et al., 2013, described the efficiency of collecting a virus known to have a spherical morphology (A/Puerto Rico/8/1934 (H1N1), PR8). The objectives of the present study were: 1) to quantify all the losses encountered during the sample collection, processing and analysis to enable more accurate determination of virus shedding rates from infected volunteers, 2) to extend the analysis to virus strains previously shown to have a filamentous morphology, and 3) to extend the analysis to influenza B virus.

During the quantification of losses, we examined the impact of the G-II operating parameters on the biological and physical collection efficiency, and quantified losses that take place during sample processing. Because human clinical strains exhibit filamentous morphology, we used two strains previously shown to produce filamentous virions. We compared the results to those obtained using viruses known to have a spherical morphology. In the final part of the present study, we used data obtained in the experiments and information from the literature to calculate viable virus shedding rates by three influenza infected volunteers.

3.3 Methods

3.3.1 Equipment

A schematic of the G-II is presented in Figure A1. In this study we examined different sets of parameters to find optimal collection conditions of the G-II. Then we

quantified loses encountered during the sample preparation and analysis, and finally we introduced a method to calculate the amount of virus shed by infected volunteers. Pilot collection of exhaled breath samples from influenza-infected subjects was conducted after collection parameters were optimized.

A P-Trak ultrafine condensation particle counter (TSI Inc, Shoreview, MN) was used to measure aerosol concentration at various locations in the G-II. The P-Trak can measure cumulative particle concentrations in the particle size range from 0.02 μm to 1 μm and concentration up to 5×10^5 particles/cm^3. The sample flow rate of the P-Trak was 1 L/min. A pump with a sampling flow rate of up to 30 L/min (SKC, Eighty Four, PA) was used to draw air samples from the G-II airstream. Since the sampling pump draws much higher airflow rate then necessary for P-Track measurement, the airstream coming out of the pump was connected to the 50 mL sterile vial. The sterile vial served as an air reservoir with one inflow stream from the sampling pump and two outflow streams. One outflow stream was connected to the P-Trak and the other served as a relief for the excess air.

In order to maintain conditions necessary for sub-micron exhaled breath aerosol (EBA) sampling, a booth was used to segregate the sampling environment from the immediate surroundings. The air in the booth was heated (26.7 ^oC) and humidified (RH_{booth} = 80 %). Air from the booth was pulled into the cone (aerosol-sampling inlet) with the flow rate of 125 L/min and passed through an inertial impactor designed to have 50 % sampling efficiency for particles with aerodynamic diameter of 5 μm (McDevitt et al., 2013). After the inertial impactor, saturated steam was injected into

the air stream. Adding saturated steam increased temperature of the mixture to 27.2 °C. Subsequently, the air stream with suspended aerosols was then rapidly cooled down to 8 °C in the condenser. This produced supersaturation conditions in the airstream and allowed the submicron particles in the aerosol to serve (point 3 on Figure A1 and A2). This rapid cooling induced aerosol growth and allows for submicron particle collection using a 1 μm inertial impactor.

The collection efficiency of the G-II was compared with the SKC BioSampler (SKC, Eighty Four, PA) for several influenza strains. The SKC BioSampler was filled with 20 mL PBS/0.1% BSA. The sampling flow rate was 12.5 L/min. Sampling was performed for 10 min to avoid evaporation of the liquid collection medium (Lin et al., 1999) at the flow rate of 12.5 L/min.

A six-jet, Collison nebulizer (BGI Inc., Waltham, MA, USA) filled with 20 ml liquid buffer with suspended virus and pressurized with N_2 at 1.38×10^5 Pa (20 psi) was used to create aerosols containing virus particles. Aerosols suspended in the airstream moved through the pipe connected to the nebulizer on one side and open on the other. The open end of the pipe was positioned in the center of the cone allowing aerosols to be discharged into the cone at a supply flow rate of 2.5 L/min.

3.3.2 Viruses

Influenza viruses A/California/04/2009 (pandemic H1N1), A/Puerto Rico/8/1934 (H1N1), A/Udorn/1972 (H3N2) and B/Lee/1940 were used to test whether the G-II collection process is capable of preserving infectivity of different viruses. The following abbreviations will be used for virus names: A/Puerto Rico/8/1934/ (H1N1)

will be abbreviated PR8, A/Udorn/1972/ (H3N2) will be abbreviated A/Udorn, A/California/04/2009 (pandemic H1N1) will be abbreviated A/California, and B/Lee/1940 will be abbreviated B/Lee. These viruses were chosen because they represent a range of properties that could be encountered by the sampler. PR8 is a laboratory strain originally isolated in 1934. This virus grows exclusively as spherical particles in tissue culture, and has been used previously to test collection devices (Singer et al., 1972). A/California and A/Udorn grow as a mixture of spherical and filamentous particles in tissue culture, and A/California is within 4 passages of clinical isolation. B/Lee is an influenza B laboratory strain isolated in 1940. For each virus, stocks were diluted to 20 ml in PBS/ 0.1% BSA. Final virus concentrations were 5.5×10^5 fluorescent focus units (FFU) per ml.

3.3.3 Laboratory Analysis

At the end of an EBA collection session, the Teflon substrate in the 5 μm inertial impactor was removed and placed in a sterile vial. The surface of the substrate was scrubbed with a Copan flocked nylon swab wetted with phosphate buffered saline supplemented with 0.1% bovine serum albumin (PBS/0.1% BSA). The tip of the swab was cut off in 1 ml PBS/0.1% BSA and the tube was vortexed for 1 minute at full speed prior to removal of the swab tip. The amount of virus in resulting sample was quantitated by real-time RT-PCR. The 1 μm inertial impactor collected approximately 125 ml of condensate sample in the reservoir placed below the impaction plate. After collection was completed, sample was removed from the reservoir with a sterile syringe and placed in sterile 50 ml conical tubes. The sample was then concentrated using a

Centricon Plus-70 centrifugal ultrafiltration device (Millipore, Billerica, MA) and analyzed by RT-PCR and focus assay.

Real-time Reverse Transcriptase Quantitative Polymerase Chain Reaction (Real-time qRT-PCR) measures the total number of copies of virus genome per sample. RNA from 200 µl of each sample was extracted using the Minelute Virus Spin kit (Qiagen) and eluted in 50 µl sample buffer. 10 µl was analyzed by real-time RT-qPCR using primer/probe sequences designed at the US Centers for Disease Control and Prevention. Standard curves were constructed using a dilution series of a PR8 stock that had been quantitated by electron microscopy (Advanced Biotechnologies Inc.).

In order to quantify the amount of infectious virus in each sample, fluorescent focus assays were used. 10-fold dilutions of the sample were made in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1 µg/ml N-acetyl trypsin and 0.1% BSA. Madin-Darby canine kidney (MDCK) cells were grown in 24-well plates, and after washing thoroughly, 150 µl of each virus dilution was incubated with the cells in triplicate at room temperature for 1 h. After incubation, 300 µl DMEM supplemented with 10% fetal bovine serum was added to each well. This serves two purposes. It nourishes the cells to try to maintain cell morphology, and it limits infections to a single round by inactivating the trypsin in the inoculum. Cells were then incubated at 37 °C for 8 h prior to fixation with ice-cold 80% acetone. Following rehydration, cells were stained for immunofluorescence microscopy with anti-NP antibodies (AA5h-Abcam and sc-57885-Santa Cruz Biotechnologies) and an AlexaFluor488-conjugated goat anti-mouse secondary antibody (Life Technologies). Positive cells were counted on an

Olympus inverted microscope fitted with an X-cite 120 LED fluorescence light source (EXFO Photonic Solutions). The amount of virus in the original sample was calculated based on the dilution plated and the number of positive cells in the well or in the area of the well examined.

3.3.4 Experimental Design

This study contains 3 sets of experiments, designed to quantify shedding rates of airborne virus released by the influenza-infected person. The first set of experiments was performed to determine optimal operating parameters for maximizing physical and biological collection efficiency. The second set of experiments investigated collection performance for four different types of influenza viruses. The third set of experiments was designed to characterize losses during the sample preparation and analysis, to develop a method of calculating virus shedding rates based on the measured results.

The G-II operates on the concept of using water condensation to grow sub-micron particles (which may contain virus particles) to a size that can be easily and efficiently collected (McDevitt et al., 2013). In order to optimize collection by the G-II, we performed two types of experiments. The objective of the first experiment was to determine what quantity of water vapor is required to be in the air to grow particles via condensation to large enough size to reach high collection efficiency. The objective of the second experiment was to determine the optimal set of operating conditions (booth temperature, humidity, and steam production) to reach sufficient quantities of water vapor in airstream inside the G-II.

Supersaturation ratio is defined as the actual water vapor pressure divided by the equilibrium water vapor pressure. In the G-II condenser, supersaturation ratio is effectively the ratio of the water vapor pressure of the mixture of air and saturated steam immediately before stream enters the condenser divided by the water vapor pressure immediately after it exits the condenser (Sioutas et al., 1995). This parameter is influenced by conditions coming from the booth where the subject sits, mass flow of saturated steam injected into the airflow, and amount of cooling that takes place in the condenser. The supersaturation ratio determines the increase in diameter of the particles from their initial size when they enter the condenser until they exit the condenser.

The G-II was operated at a flow rate of 125 L/min. Aerosols with diameters from 0.02 μm to 1 μm, present in the ambient air, were used to investigate the relationship between supersaturation ratio and the physical collection efficiency. Different air – steam mixture conditions above the condenser were achieved by varying the booth temperature (T_{booth}), the relative humidity in the booth (RH_{booth}) and mass flow rate of saturated steam injected in the G-II. Each measurement was performed for 15 min after steady state was reached. Particle concentration was measured with a P-Trak at two locations. The upstream measuring point was at the aerosol collector inlet (cone) and the downstream measuring point was after the fine fraction collector. The ratio of particle concentrations after the 1 μm impactor and at the cone is used to calculate collection efficiency η of the G-II fine fraction collection

$$\eta = 1 - \frac{C_{downstream\ 1\mu m\ impactor}}{C_{cone}} \qquad (1)$$

During an exhaled-breath collection session, subjects must sit in the booth and breathe into the cone for 30 minutes in order to collect enough exhaled breath to reliably detect exhaled influenza virus using the current laboratory detection methods. To maintain some level of thermal comfort for subjects, we decided to reduce heating and humidification of the booth and substitute injection of a larger quantity of saturated steam to reach the appropriate supersaturation ratio. The mixture of the booth air and saturated steam must have a temperature of 27 ℃ and humidity ratio of 0.02735 kg_{water}/kg_{air} to achieve the required supersaturation ratio (≥ 2.6) for physical collection efficiency above 90%. Based on the ideal gas mixing law, required mass flow rates of saturated steam were calculated for a range of booth air conditions. The experiment described below is designed to test whether changes of saturated steam mass flow rate have an impact on virus viability.

G-II collection efficiency for coarse (≥ 5 μm) and fine (< 5 μm) aerosol fractions for one set of operating parameters was described in McDevitt et al. (2013). In this chapter, we selected the conditions in the booth range from surrounding ambient conditions to warm and humid environment conditions (eg. T_{booth} = 20 ℃, RH_{booth} = 50% to T_{booth} = 27 ℃ and RH_{booth} = 70%). The booth conditions were chosen to maintain a supersaturation ratio of 2.6 or higher with the changing of saturated steam flow rates. Three saturated steam mass flow rates were explored in this experiment: 3.1×10^{-5} kg/s, 4×10^{-5} kg/s and 5.4×10^{-5} kg/s. Virus was aerosolized with the collison nebulizer for 10 min and released in the cone with the flow rate of 2.5 L/min. Virus was collected in PBS/0.1% BSA. After 10 min aerosolization was stopped, and the

condensate was collected from the reservoir. Each experiment was repeated 3 times for each of the conditions tested.

A/California, PR8, A/Udorn and B/Lee influenza virus were aerosolized with a Collison nebulizer and released into G-II cone as described in the previous section. The G-II was operated under conditions that produced physical collection efficiency above 90%. Virus was collected in PBS/0.1% BSA. After 10 min, aerosolization was stopped and the condensate was collected from the reservoir. Experiments were repeated 3 times for each virus. Between each of the experiments each of the G-II parts with the exception of the condenser was decontaminated with 10% bleach followed by thorough rinsing with deionized water. Since the condenser could not be submerged in the bleach solution, it was decontaminated with a 70% ethanol spray instead.

To quantify the losses on the Teflon impactor, the 5 μm Teflon substrate was spiked with a known amount of influenza virus for each strain used (between 1.0×10^5 to 8.6×10^5 virus particles per impactor) in a level 2 biological safety cabinet. The Teflon substrate was allowed to air dry until all of the liquid had evaporated. The Teflon substrate was then placed in the G-II for 30 minutes to simulate conditions during the EBA collection. After 30 minutes, the Teflon substrate was removed the surface of the substrate was scraped with a Copan flocked nylon swab wetted with PBS/0.1% BSA. The tip of the swab was cut off in 1 ml PBS/0.1% BSA and the tube was vortexed for 1 minute at full speed prior to removal of the swab tip. The amount of virus in the resulting sample was quantitated by real-time RT-PCR. That number was then compared to the total number of virus particles present in the solution used to coat the plate. This experiment was repeated 4 times for each virus type.

The total number of influenza virus particles present in the coarse fraction of exhaled breath can be calculated based on the number of virus particles detected by RT-PCR method and Teflon substrate recovery efficiency:

$$N_{CF} = \frac{n_{qPCRcf}}{\eta_{cf} \cdot \eta_{recovery}} \quad (2)$$

N_{CF} represents the total number of viruses released in the coarse fraction by the infected person; n_{qPCRcf} is the number of virus particles detected with RT-PCR method; η_{cf} is the collection efficiency of coarse fraction; $\eta_{recovery}$ is the experimentally determined Teflon substrate virus recovery efficiency.

During a 30 min sample collection from a human volunteer, 120 mL to 150 mL of condensate gets collected from the bottom reservoir. This volume of liquid is unwieldy, and likely to be too dilute to detect infectious virus particles shed in exhaled breath. Therefore, it was deemed necessary to concentrate the condensate sample prior to analysis.

In order to quantify possible losses during the concentration process 2.2×10^7 real-time PCR units of A/California were suspended in 120 mL of PBS/0.1% BSA. This solution was concentrated as described and the amount of virus in the concentrated sample was measured by real-time RT-PCR.

Similar to the coarse fraction, total number of virus particles in the fine fraction can be calculated based on the real-time RT-PCR results of the collected sample.

$$N_{FF} = \frac{n_{qPCRff}}{\eta_{ff} \cdot \eta_{sc}} \quad (3)$$

N_{FF} represents the total number of virus particles released in the fine fraction by the infected person; n_{qPCRff} is the number of virus particles detected in the sample; η_{ff} is the experimentally determined fine fraction collection efficiency; η_{sc} is the experimentally determined efficiency of condensate concentration.

The number of viable virus particles can be calculated using

$$V_{FF} = \frac{N_{FF}}{T/_I} \qquad (4)$$

V_{FF} represents number of viable virus particles in the fine fraction. Quantification of these processes was conducted to properly account for the viable virus particles, when we calculated amount of viruses shedded in aerosols by influenza infected people based on the measured virus quantities.

$T/_I$ is the experimentally determined ratio of the total virus particle number to the number of infectious particles in the collected sample, which equals to the PCR results vs. focus assay results. Pan et al., 2016 showed that BioSampler has viable collection efficiency of 6.5 ± 2.8%. McDevitt et al., 2013 showed that G-II viable collection

efficiency is similar to BioSampler. Results from Pan et al., 2016 were also used to calculate the amount of viable virus released from the source of infectious particles.

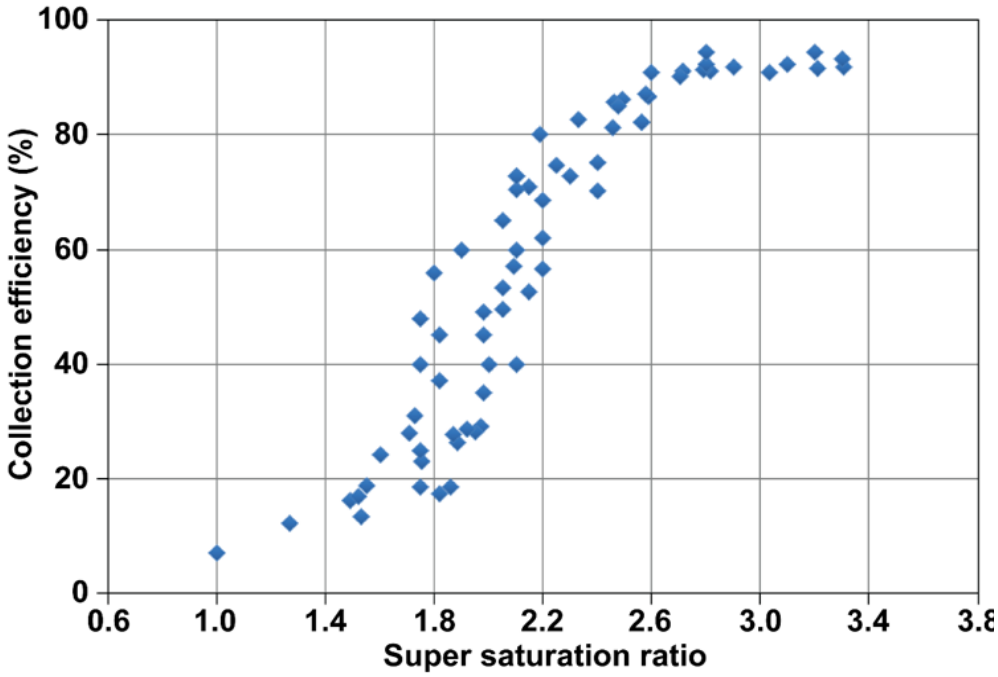

Figure 3.1. Physical collection efficiency of G-II dependency on supersaturation ratio.

3.4 Results and Analysis

3.4.1 Optimization of The G-II Collection Parameters

The relationship between fine fraction collection efficiency and supersaturation ratio follows the "S" shape curve (Figure 3.1) analogous to the inertial impactor collection curve plotted against particle diameter. The number of particles removed from the air stream with the fine fraction collector depends on the particle growth achieved by rapid cooling in the condenser.

The results in Figure 3.1 showed that 50% physical collection efficiency is achieved with supersaturation ratio of 2. This result suggests that 50% of the ambient aerosols in the range of 0.02 µm – 1 µm (measurement range of P-Track) were able to

grow beyond the size collected with fine fraction collector. Physical collection efficiency above 90% is achieved for supersaturation ratio of 2.6 and above. Results also suggested that increasing supersaturation ratio beyond this point does not increase collection efficiency. These conditions should be maintained during the influenza infected participants exhaled breath collection.

Calculations presented in Figure 3.2 are based on the ideal gas mixing law. RH_{booth} is depicted on the x-axis and T_{booth} are presented with individual lines. The required saturated steam mass flow rates corresponding to the fine fraction collection efficiency above 90% are plotted on the y-axis. We performed several experiments (results are superimposed on the Figure 3.2) to validate these calculations.

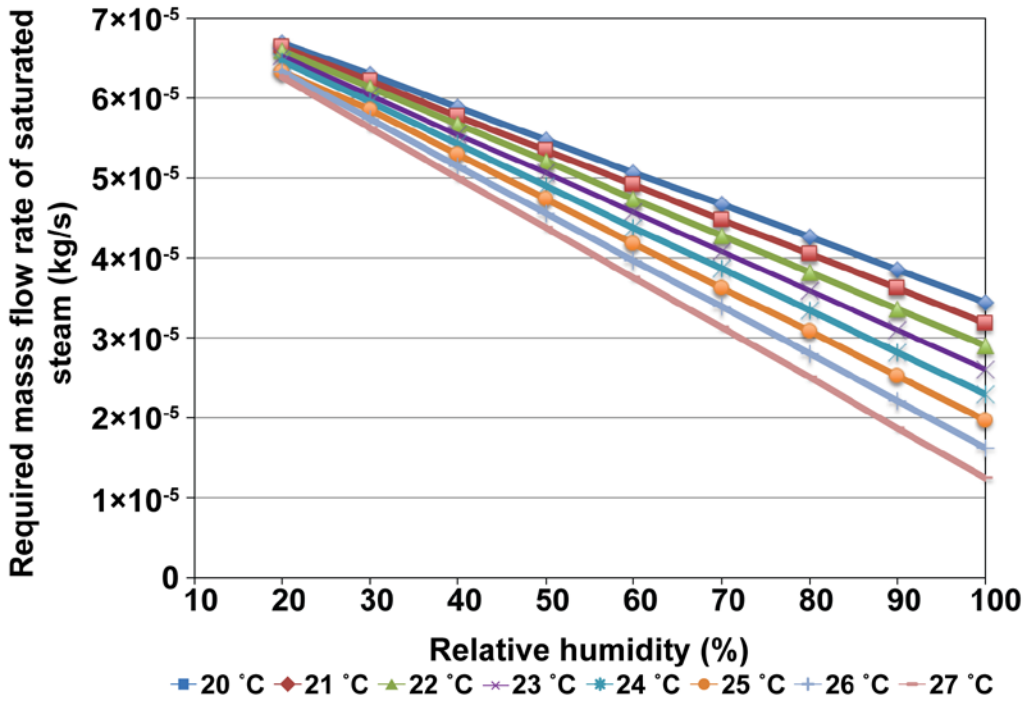

Figure 3.2. Required saturated steam mass flow rate for maintaining physical collection efficiency above 90% for a given booth condition.

Results depicted in Figure 3.3 represent the ratio between total number of virus particles in the sample and number of viable viruses in that sample (T/I ratio) for A/California virus at different injected steam flow rate. When this type of representation (T/I ratio) is used, a larger T/I ratio indicates reduction of virus viability. Results on Figure 3.3 show that when steam was injected with mass flow rates of 3.1×10^{-5} kg/s and 4×10^{-5} kg/s T/I ratios were very similar, but when 5.4×10^{-5} kg/s was injected, the viability of collected aerosolized influenza virus decreased between 2.4 to 3.6 times. When 5.4×10^{-5} kg/s of steam was injected, most probably virus particles had longer contact time with the hot steam before they mixed with the air stream and this caused virus inactivation. This issue requires further investigation. The results presented in Figure 3.3 suggest that during sample collection, the saturated steam should not be injected at a mass flow rate higher then 4×10^{-5} kg/s. Because of this finding, the booth should be maintained at 24 ℃ or higher and RH of 65% or higher based upon the supersaturation ratio calculation.

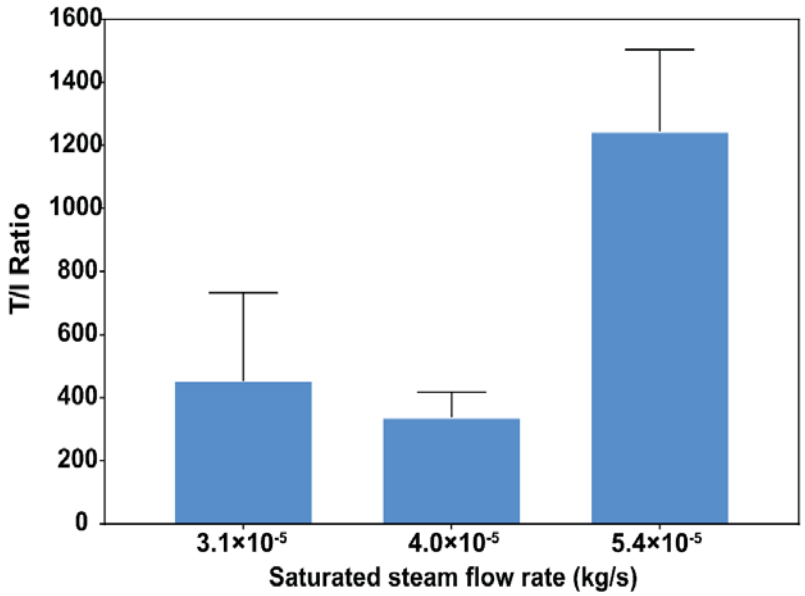

Figure 3.3. T/I ratios for different saturated steam mass flow rates.

Conditions of air leaving the condenser depend on the amount of cooling that takes place in the condenser. Coolant flow rate was constant; hence cooling condenser-cooling capacity was determined by coolant supply temperature. This suggest that supersaturation ratio will depend only on the coolant supply temperature.

During the expiratory droplets collection chiller that regulated coolant temperature rejected heat into the surrounding ambient air. This can cause increase of ambient air temperature and subsequently can causes reduction of chiller cooling capacity. Reduction of cooling capacity causes increase of the coolant supply temperature and change in collection efficiency. Increase of ambient temperature and subsequent reduction of cooling capacity represents limitation of the experimental setup used in the present study, hence it is very important to characterize collection efficiency that can occur during suboptimal operation. Knowledge of collection efficiency during suboptimal conditions is important in order to properly calculate initial viral shedding rates based on the measure amount.

Process occurring in the condenser is depicted with point 2 and point 3 on Figure A1 and Figure A2. In order to quantify changes in the physical collection efficiency caused by the variation of the cooling fluid supply temperature collection efficiency measurements (as described in the previous section) were performed while coolant supply temperature was varied between -2.2 ℃ and 1.1 ℃.

G-II physical collection efficiency was tested for cooling fluid supply temperatures between -2.2 ℃ and 1.1 ℃. Results of Figure 3.4 show that physical collection efficiency reduces linearly with the increase of the supply coolant temperature. This is

very important result because it indicates that in order to keep physical collection efficiency high during the collection of expiratory aerosols from the subjects infected with Influenza cooling fluid supply temperature should not be increased above -1.5 ℃.

Results also indicate that during collection of the expiratory samples, cooling fluid

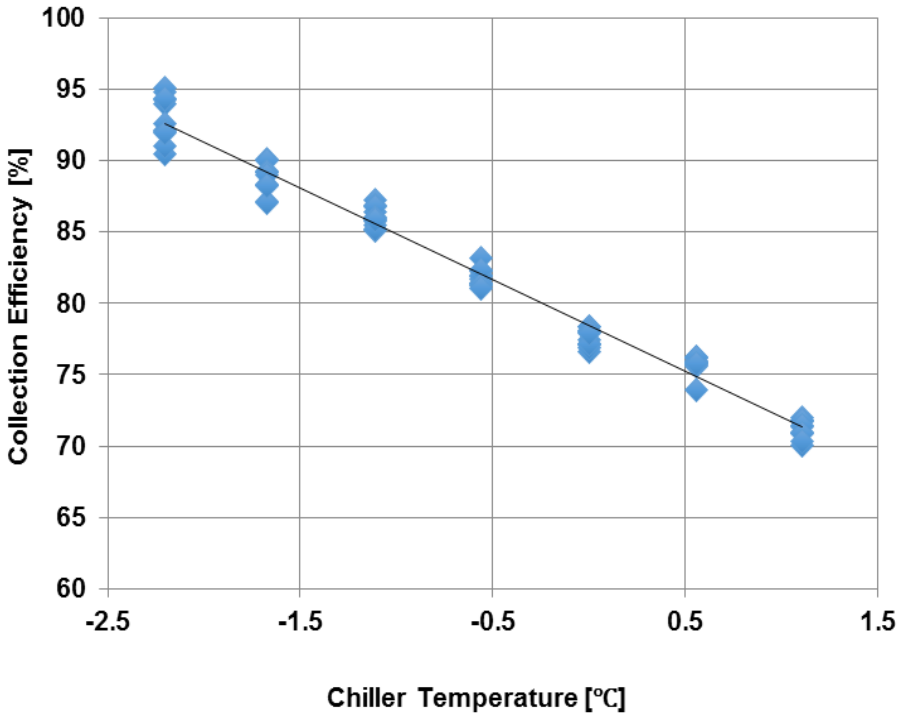

Figure 3.4. Physical collection efficiency dependence on coolant supply temperature.

supply temperature should be recorded because physical collection efficiency can be reduced up to 20% if cooling fluid supply temperature is increased for 2 ℃. This is very important especially when viral shedding rates are calculated based on the amount of virus detected in the collected sample.

3.4.2 Biological Collection Efficiency for Four Types of Influenza Viruses

The results presented in Figure 3.5 show that G-II collected 77% ± 6%, 93% ± 24%, 91% ± 14%, and 117% ± 15% compared to BioSampler for A/California, PR8, A/Udorn and B/Lee respectively. These results suggested that G-II can be effectively used for sampling different types of influenza viruses, and can be used effectively for collection of human exhaled breath from the influenza infected subjects.

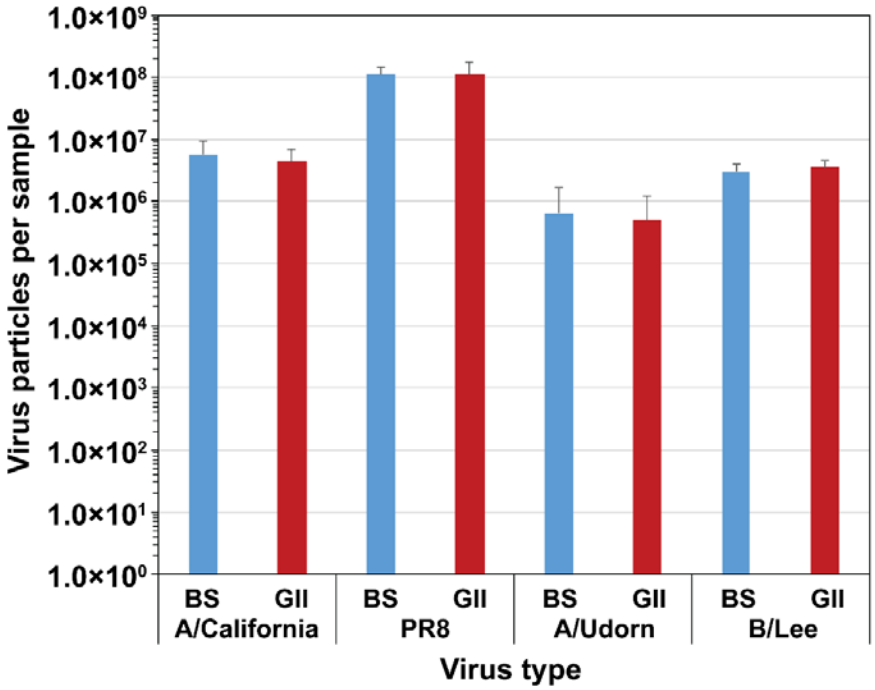

Figure 3.5. Collection efficiency comparison between BioSampler (BS) and G-II for A/California/04/2009, A/Puerto Rico/8/1934, A/Udorn/1972 and B/Lee/40.

T/I ratios presented in Table 3.1 showed that the G-II can preserve virus viability for A/California, PR8 and A/Udorn. T/I ratios for G-II collected sample were compared to those collected by the SKC BioSampler, and the ratios were very close. G-II performed even better than the BioSampler with PR8. T/I ratio was not available for B/Lee, since aerosolizing B/Lee with the Collison nebulizer cannot preserve the

Table 3.1. T/I ratios for A/California/04/2009, A/Puerto Rico/8/1934, A/Udorn/1972 and B/Lee/40 with both G-II and BioSampler (BS)

	Virus types			
	A/California	**PR8**	**A/Udorn**	**B/Lee**
BS T/I ratio	$(1.39 \pm 0.70) \times 10^3$	$(4.17 \pm 1.63) \times 10^5$	2.71 ± 0.44	NA*
G-II T/I ratio	$(3.85 \pm 1.81) \times 10^3$	$(3.71 \pm 1.31) \times 10^2$	3.53 ± 0.49	NA*

*Lee B has no infectious particles detected by focus assay

viability of the virus. The results for influenza A viruses showed that sampling with the G-II preserves viability of collected virus particles.

3.4.3 Quantifying Amount of Virus Released by Influenza-Infected Person

When viruses deposited on the Teflon substrate were removed with Copan swabs, the RT-PCR results showed variability in recovery among viruses used in the experiment. A/California was recovered with 16% efficiency, PR8 with 31% efficiency, A/Udorn 33% efficiency and B/Lee with 41% efficiency (Figure 3.6). The average recovery was 30%. This result indicates that amount of virus impacted on the Teflon substrate was 3.3 times greater than that measured with the RT-PCR method.

Real-time RT-PCR results showed that the ratio of the amount of viruses detected in the concentrated sample and the amount originally placed in the 120 mL of 1% PBS-BSA was between 0.98 and 1.02. Taking into account measurement uncertainty, the results show that sample concentration did not introduce additional losses. Similarly,

focus assay results show that virus infectivity is not influenced by the process of sample concentration.

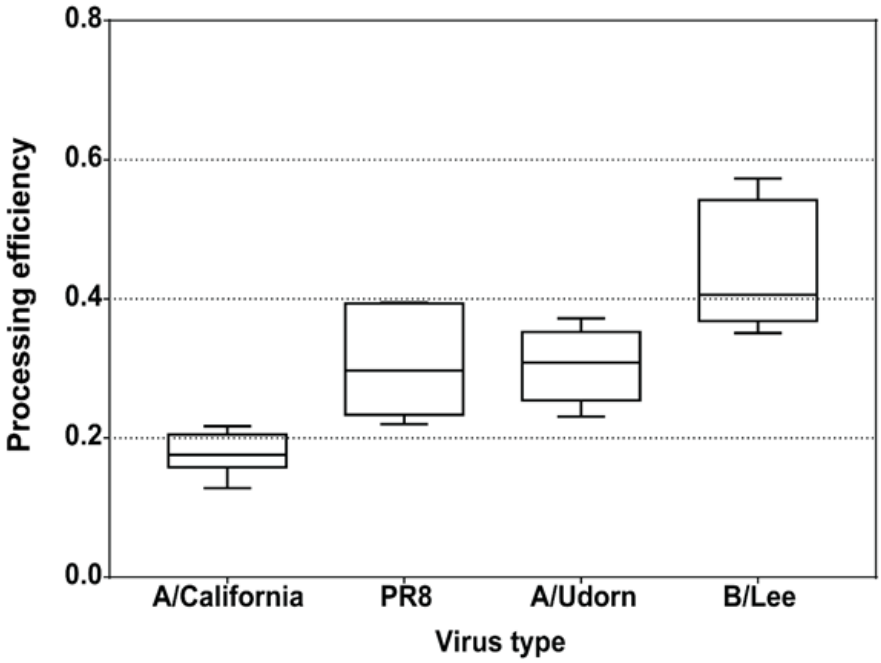

Figure 3.6. Virus recovery efficiency for Teflon substrate with A/California/04/2009, A/Puerto Rico/8/1934, A/Udorn/1972 and B/Lee/40.

Average G-II operating parameters during sample collection are presented in Table A1 in the Appendix A. Based on the results from previously described studies, experimental values used for calculation of viral shedding rates are:

$$\eta_{recovery} = 0.30;\ \eta_{sc} = 1;\ \eta_{cf} = 0.5;\ \eta_{ff} = 0.92;\ {}^{T}/_{I} = 350$$

Equation 2 was used to calculate the total number of viruses collected on the 5 µm impactor. This represents the coarse fraction in the collected sample. Equation 3 was used to calculate total number of viruses collected in the fine fraction condensate. The

number of live viruses collected in condensate sample was calculated using Equation 4. Measured quantities and calculated source strengths are presented in Figure 3.7.

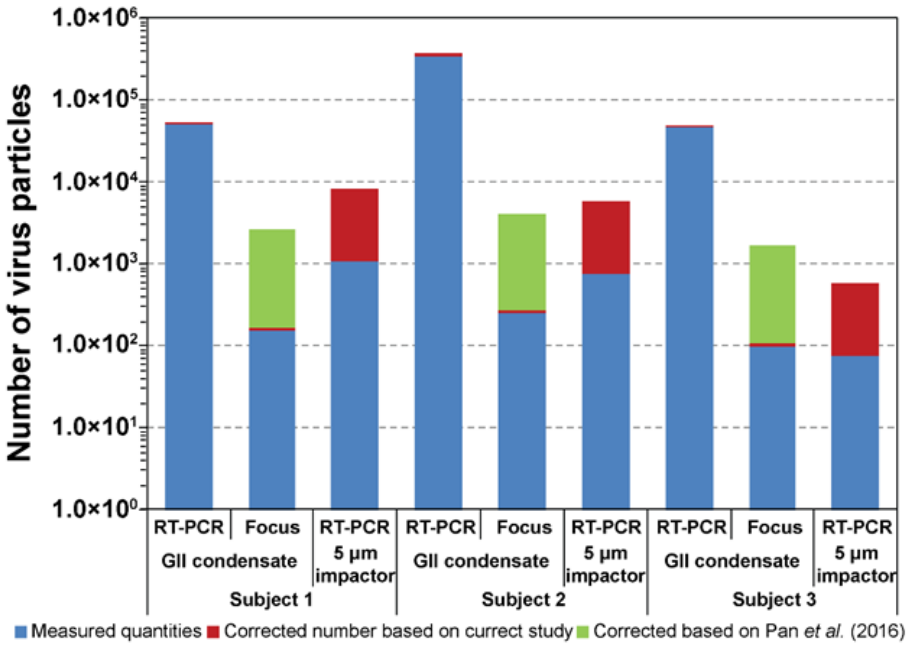

Figure 3.7. RT-PCR, Focus Assay results for 3 samples collected from the influenza infected subjects in 30 min and calculated initially released virus particles from the exhaled breath.

The results presented in Figure 3.7 showed that, after collection of EBA from three subjects, their fine fraction samples could be corrected based on our experiments. Up to 3.7×10^5 viruses can be release into the indoor environment in 30 minutes, and up to 2.7×10^2 of the released viruses could be viable influenza viruses.

3.5 Discussion

Based on the three samples from influenza-infected subjects, we knew that the G-II can quantify viral shedding rates efficiently when an infected person acts as a source of viruses in the indoor environment, and the virus viability can still be preserved during the sampling. From the literature, quantification of the virus content in the

exhaled air has been done by several groups. (Fabian et al., 2008; Gralton et al., 2013; Hatagishi et al., 2014; Huynh et al., 2008; Lindsley et al., 2010; Milton et al., 2013; Stelzer-Braid et al., 2009). The NIOSH BioSampler has been characterized for study of influenza aerosols (Blachere et al., 2007; Cao et al., 2011). That sampler with its low flow and small size is well suited to personal exposure sampling, but requires use of artificial means, such as breathing or coughing into a volume spirometer, to capture exhaled breath for direct analysis. The application of a six-stage Andersen sampler by (Gralton et al., 2013) similarly required subjects to exhale or cough through a mouthpiece. The electret mask system developed by Stelzer-Braid et al., 2009 gives an excellent measure of overall virus shedding, but does not distinguish the aerosol component of the shed virus.

Cough released aerosols were collected in a 10-liter piston style accumulation chamber and then sampled by NIOSH BioSampler and SKC BioSampler in studies by Lindsley et al. (2010) and Lindsley et al. (2015). Forcing cough through a mouthpiece is a limitation of the collection method used in two studies by Lindsley et al. The commercially available RTube® exhaled breath condensate sampler was used by Houspie et al. (2011) to collect exhaled aerosols through a mouthpiece from subjects with influenza illness like symptoms. Hatagishi et al. (2014) used a single stage Sartorius MD8 portable sampler with gelatin filter and cone shaped collection nozzle to collect forced cough aerosols from influenza-infected patients. Limitations of collection equipment used by Hatagishi et al. (2014) were the inability to distinguish viral content in ballistic droplets versus aerosols, low overall viral gene recovery rate and backflow in the conical collection nozzle.

Valuable information has been obtained with all of these sampling methods. However, to accurately estimate the quantity and size distribution of virus aerosol shed into an indoor environment, a device that does not restrict respiratory activities, collects aerosol with high efficiency and preserves viability is needed. Influenza studies so far have not reported quantities of virus released into the indoor air by infected building occupants through expiratory activities. Reported studies have only used epidemiological data to calculate viral load in terms of quanta (rate infectors generate infectious doses) (Rudnick and Milton, 2003; Sze To et al., 2008; Zhu et al., 2012) and then based on quanta, calculated effectiveness of engineering and non-engineering control measures. Development of aerosol sampling technology and methods for quantification of source strength presented in this chapter will allow more realistic estimation of the effectiveness of engineering and non-engineering methods used in indoor environment for mitigation. Based the limited data presented in this chapter it can be observed that more than 10^5 virus particles can be released by infected person during 30 minutes' collection. In all three samples $1.5 – 2.5\times10^2$ viruses were viable, or even one order of magnitude higher $2.4 – 3.9\times10^3$ if correction from Pan et al. (2016) (Figure 3.7) are included. This will have significant impact on evaluation of ventilation rates, total air exchange rates and use of upper room ultra violet germicidal irradiation in hospitals, health clinics and other densely occupied environments with high risk of influenza transmission.

It is difficult to mitigate the infectious disease spread, since it lies in mismatch between detectible symptoms and the onset of infectiousness. Onset of infectiousness precedes the onset of detectible symptoms (Fraser et al., 2004). When infection is

diagnosed and public health measures like face masks (Milton et al., 2013) can be effectively deployed, virus particles exhaled by infected occupant have already been polluting indoor environment for several days. This suggests that deploying only non-engineering control strategies may not be sufficiently effective to mitigate disease outbreaks (Cheng and Liao, 2013). Previous studies demonstrated that proper ventilation could reduce exposure to simulated expiratory droplets (Cermak and Melikov, 2007; Licina et al., 2015; Pantelic et al., 2009, 2015). Although these studies showed promising results in occupant exposure reduction, it is still unclear if reduced exposure is sufficient to reduce number of secondary cases. In order to evaluate how effective different measures are or how effective they need to be to mitigate airborne disease spread, knowledge of the source strength represent the starting point. The technology and methods described in this study could be used to effectively quantify the source strength, and act as a basis for further prevention analysis.

One limitation of the present study is the inability to perform focus assay analysis of the coarse fraction of the aerosol. Further development of coarse fraction collection and enabling more advance biological analysis will be discussed in the future work section of the dissertation. Aerosolization of B/Lee virus with Collison nebulizer caused virus inactivation, another limitation of our study. Even though influenza B is less common, it still can cause outbreaks of seasonal flu. It is important to know if our G-II can sample the influenza B and preserve the viability efficiency. In our future work we will test different methods of aerosolization of B type influenza viruses to perform further investigation of G-II aerosol collector operation.

T/I ratios measured in our study refer to the virus after aerosolization took place; hence any reduction of virus viability due to the aerosolization method is embedded in the result. We used Pan et al. (2016) T/I results to compensate for the losses during aerosolization. Besides viability decay due to aerosolization, T/I ratio will vary from virus to virus, will depend on the method of virus preparation and probably will have person to person variability.

3.6 Conclusions

The G-II can be used to effectively collect different strains of influenza. Viability of different strains was preserved during the collection process. This suggests that the G-II can be successfully used to collect samples from subjects infected with influenza without prior knowledge of influenza subtype. During sample collection, the G-II should be operated with supersaturation ratio of 2.6 or higher. Viability of collected sample can be increased if booth air temperature is 24 ℃ or higher and RH of booth air is 65% or higher. These settings are recommended during sample collection. Temperature of coolant should be recorded during the sampling. Analysis presented in this study showed that between 16% and 41% of the virus captured on the Teflon substrate could be recovered via RT-PCR analysis. Based on the three samples collected from influenza-infected subjects we showed how the quantity of virus influenza that infected persons release into the ambient air could be estimated.

CHAPTER 4: ROLE OF TIDAL BREATH, COUGH, AND SNEEZE IN GENERATION OF INFECTIOUS AEROSOLS BY 142 CASES OF COMMUNITY ACQUIRED INFLUENZA

4.1 Abstract

Understanding of the relative importance of the modes of influenza virus transmission is key to the design of effective public health intervention strategies. Previous reports characterized influenza aerosols from small numbers of subjects; none have characterized large numbers or examined the role of cough.

We screened volunteers with influenza like illess (ILI) and recruited those meeting the following criteria: (1) positive rapid test, or (2) T >37.8 °C plus cough or sore throat, and (3) within the first 3 days of symptom onset. We collected NP swabs and exhaled breath samples from each subject on enrollment and for up to 3 consecutive days. Each NP swab and fine (< 5 μm) aerosol sample was assayed by culture passage and fluorescent focus assay (FFU) on MDCK cells. Influenza RNA copies in all samples were quantified by RT-qPCR.

We screened 355 individuals and enrolled 177 (87 females and 90 males, mean age 23) for 178 illness episodes. Of the 178 episodes, we confirmed influenza infection in 156 cases, and identified 89 influenza A infections, 50 influenza B infections and 3 dual infections.

Among the confirmed cases: We obtained valid culture results (passage and/or focus assay) from 169 NP swabs and 134 fine aerosol samples; 150 (89%) of NP swabs

and 52 (39%) of fine aerosol samples were positive. RT-qPCR was positive in 88 of 218 (40%) coarse and 166 of 218 (83%) fine 30-min aerosol samples. We observed significant correlations of cough with viral RNA copies in coarse ($p = 0.0083$) and fine ($p < 0.0001$); some cases without cough shed fine aerosols with up to $2.3*10^5$ RNA copies and 140 FFU/ 30-min.

The presence of culturable influenza virus in nearly half of the fine aerosol samples demonstrates that influenza cases shed infectious virus as well as RNA into airborne droplets and contributes to the biological plausibility and likely importance of airborne influenza transmission. However, cough was not a strong predictor of infectious aerosol generation suggesting an important role for other mechanisms of aerosol generation.

4.2 Introduction

Influenza remains a global threat; The World Health Organization (WHO) estimated that annual epidemics account for an estimated 3 to 5 million cases of severe illness and 250,000 to 500,000 deaths each year worldwide (Elovainio, 2008). Non-pharmaceutical interventions have been employed to control and reduce the impact of influenza epidemics and pandemics. However, to design effective non-pharmaceutical interventions, it is necessary to accurately define the contribution of each route of transmission (Atkinson and Wein, 2008) and implement interventions that impede the important routes.

Influenza is thought to have three main routes of transmission: (1) by direct and indirect contact with secretions, (2) by large droplet spray (droplets >5 to 10 μm in diameter) that land in the mouths, eyes, and noses of people nearby, and (3) by aerosol

transmission with increasing probability for smaller droplets that can remain suspended in the air for minutes to hours (Alford et al., 1966; Atkinson and Wein, 2008; Duguid, 1946; Gralton et al., 2011; Tellier, 2006, 2009). Due to limitations inherent to sampling virus shedding via various routes from infected individuals and the difficulty of distinguishing routes of transmission in observational studies, the quantitative dynamics and relative contributions of each route are not well understood (Atkinson and Wein, 2008; Tellier, 2009). Yet, accurate quantitation is needed to develop models to predict the impact of non-pharmaceutical interventions. Recent reports have shown that, at least with forced coughs or forced vital capacity maneuvers, infectious influenza virus can be recovered from exhaled aerosols (Lindsley et al., 2010, 2016; Milton et al., 2013). These studies do not provide sufficient data to quantify the extent of aerosol shedding during natural breathing or identify the contributions of spontaneous coughs and sneezes commonly thought to be the most important mechanism for viral shedding. This chapter addressed these key knowledge gaps by characterizing influenza virus in exhaled breath from community acquired cases during natural breathing, coughing, and sneezing, and assessing the infectivity of naturally occurring influenza aerosols.

4.3 Methods

4.3.1 Study Population and Procedures

We recruited volunteers with acute respiratory illness on the University of Maryland-College Park campus (UMD) and surrounding community from December 2012 through March 2013. The UMD Institutional Review Board approved the study,

and we obtained a signed consent (or assent and parental verbal assent) from volunteers who reported fever with a cough or sore throat (Appendix B Figure B1).

During the initial visit, we administered a brief screening questionnaire, measured oral temperature, height, weight, and collected two nasopharyngeal (NP) swabs [Copan, Murrieta, CA] for each volunteer screened. One swab was used to perform QuickVue A/B rapid tests for influenza (except when results of a rapid test performed by medical provider were available). The second swab was used for viral culture and PCR for those meeting enrollment criteria and for PCR in a random sample of 24 of those not enrolled. Volunteers were enrolled in exhaled breath collection if they met the following criteria: (1) positive QuickVue rapid test, or oral temperature $\geq$ 37.8 °C plus cough or sore throat, and (2) presented within the first 3 days of symptom onset.

The screening questionnaire asked about sex, antipyretic use, vaccination status, and current symptoms rated on a 4-point scale [none, mild, moderate, severe]. We defined symptoms as upper respiratory (runny nose, stuffy nose, sneezing, sore throat, and earache), lower respiratory (chest tightness, shortness of breath, and cough), and systemic (malaise, headache, muscle/joint ache, fever/sweats/chills, and swollen lymph nodes). Participants who met eligibility criteria for exhaled breath testing were asked, at the time of enrollment, to rate the worst symptoms during the illness thus far, and about respiratory symptoms, use of steroid medications, and medical and smoking history.

We collected exhaled breath for 30 min while the participant was seated with their face inside of the large open end of a cone shaped inlet for the G-II human-source

bioaerosol sampler as previously described (Fabian et al., 2008; McDevitt et al., 2013; Milton et al., 2013) (Appendix B Figure B3). The cone shaped inlet act as a capture hood with a 130 L/min flow allowed participants to breathe, talk, cough, and sneeze naturally throughout sample collection while maintaining >100% collection efficiency for exhaled and coughed droplets ≤ 100 μm. Subjects were asked to breathe normally and to recite the alphabet once at 5, 15, and 25 min). We collected "coarse" (>5 μm) aerosol droplets by impaction on a Teflon® surface and "fine" droplets (≤5 μm and >0.05 μm) by condensation growth and impaction on a steel surface constantly rinsed into a buffer containing (phosphate buffered saline with 0.1% bovine serum albumin) liquid reservoir. Audible spontaneous coughs and sneezes during breath collection were counted by direct observation in real-time (59) or by playback of digital recordings (159).

Participants enrolled prior to the third day after symptom onset were asked to come in for up to three consecutive daily follow-up visits (Figure B1) with repeat questionnaire, NP swab and exhaled breath collections. Final analyses included only visits for enrolled cases occurring on days 1 to 3 post onset with complete data on cough and sneeze, symptoms, PCR results for swab and aerosol samples.

4.3.2 Laboratory Tests

NP swabs were eluted in 1 mL elution medium, and Teflon® impactors were scrubbed with a nylon swab saturated with phosphate buffered saline supplemented with 0.1% bovine serum albumin (PBS/0.1% BSA). The swab was eluted in 1 ml

PBS/0.1% BSA. Fine aerosol samples were concentrated to 1 mL using centrifugal ultrafiltration.

RNA was extracted from NP swab, fine and course aerosol samples, and whole-virion standards using, viral RNA was quantified by one-step real-time RT-PCR. Standard curves were calibrated for virus copy number using plasmids containing a cDNA copy of the RT-qPCR target amplicon. For influenza A, the limit of detection (LOD) of the RT-qPCR assay was 20 copies per reaction and the limit of quantification (LOQ) was 80 copies per reaction. For influenza B the LOD was 20 copies per reaction, and the LOQ was 360 copies per reaction. After accounting for dilution factors, the LOQs for NP swabs were 8,000 and 36,000 copies and for aerosol samples were 2000 and 9,000 copies for influenza A and B respectively.

Virus culture on Madin-Darby canine kidney (MDCK) cells was used to detect infectious virus in NP swab and fine aerosol samples. Infectious virus was not measured on the Teflon® impactor samples, since the method of collection is expected to have affected infectivity of those samples. Infectious influenza virus was quantified using an immunofluorescence assay for influenza nucleoprotein, and positive cells were counted by fluorescence microscopy. Details of laboratory methods can be found in the Appendix B.

4.3.3 Statistical Analysis

We entered and cleaned data using locally hosted REDCap data capture tools (Harris et al., 2009) and performed data management and analyses in R (version 3.2.3 R Development Core Team, Vienna, Austria) and SAS (version 9.4, Cary, NC, USA),

and produced graphics with Prism Software (PRISM software version 7.0; GraphPad). We used Spearman correlation, generalized linear models (SAS Proc GENMOD), and Tobit regression (Twisk and Rijmen, 2009) with nested random effects of subject and sample ID in SAS (Proc NLMIXED) to analyze FFU counts and RNA copy numbers and compute geometric mean virus concentrations. Tobit regression accounted for uncertainty and censoring of the observations by the limit of quantification. We included all independent variables with unadjusted $p < 0.10$ in initial adjusted models and selected final models using the Akaike information criterion.

4.4 Results

We screened 355 volunteers with acute respiratory illness; 178 met enrollment criteria and provided 278 visits for sample collection. We confirmed influenza infection in 156 of the enrolled using RT-qPCR; 152 had at least one positive NP swab and 4 (3%) were confirmed on the basis of positive aerosol samples. NP swab analysis was positive for 8 (33%) of 24 randomly selected volunteers who did not meet enrollment criteria; thus, sensitivity and specificity of our enrollment criteria, during the 2012-13 season, were 57% and 73% respectively. We excluded from analysis 8 visits made on the day of symptom onset, 10 made >3 days after onset, 7 with missing data for cough, and 3 visits with incomplete RT-qPCR data resulting in complete data on RNA copies, cough, and symptoms for 218 visits by 142 cases including 89 influenza A, 50 influenza B, and 3 dual influenza infections (Appendix B Figure B2).

Our study population (Table 4.1) consisted mostly of young adults with high asthma prevalence, normal body mass index (BMI), and a low influenza vaccination rate. We observed at least one cough during 195 (89%) and at least one sneeze during 11 (5%) of the 218 visits. Cough varied considerably from 5/30 min at the 25th percentile to 39/30 min at the 75th. Most volunteers rated their upper respiratory symptoms as mild to moderate, systemic symptoms as moderate to severe and lower respiratory symptoms as mild (Appendix B Figure B6).

Table 4.1. Characteristics of Study Participants

	Screened Only	**Enrolled**	**Complete Data**
N participants	177	178	142
Breath collection visits	–	278	218
Male (%)	89 (50)	91 (51)	69 (49)
Flu shot this season (%)	53 (30)	42 (24)	31 (22)
Asthma, self reported (%)	–	38 (21)	30 (21)
Smoker, current (%)	–	30 (17)	21 (15)
Anti-viral medication last 24 hr (%)	2	11 (6)	7 (5)
Age (IQR)*	20 (19-22)	21 (19-22)	20 (19 – 21)
BMI (IQR)	23.6 (21.3-26.2)	23.2 (21.0-25.7)	22.7 (20.9-25.5)
Body temperature measured onsite	36.9 (36.8-37.1)	37.2(36.9-37.7)	37.2(36.9-37.6)
Median Coughs/30 minutes (IQR)†	–	17 (6-39)	18 (5 – 39)
Median Sneezes/30 minutes (IQR)	–	0 (0-0)	0 (0 – 0)
Median Upper respiratory symptoms (IQR)‡	7 (4-9)	6 (5-8)	7 (5 – 8)
Median Lower respiratory symptoms (IQR)	2 (1-4)	3 (2-5)	3 (2 – 6)
Median Systemic symptoms (IQR)	6 (3-9)	8 (5-11)	8 (5 – 11)

* IQR denotes innerquartile range.
† Cough, sneeze, and symptom scores are reported per visit
‡ Twelve symptoms were rated from 0 to 3 with maximum possible composite score of 15 for upper respiratory, 9 for lower respiratory, and 15 for systemic symptoms.

Infectious virus was recovered from 52 (39%) of 134 fine aerosol samples and 150 (89%) of 169 NP swabs from which we obtained valid cultures. Quantitative cultures

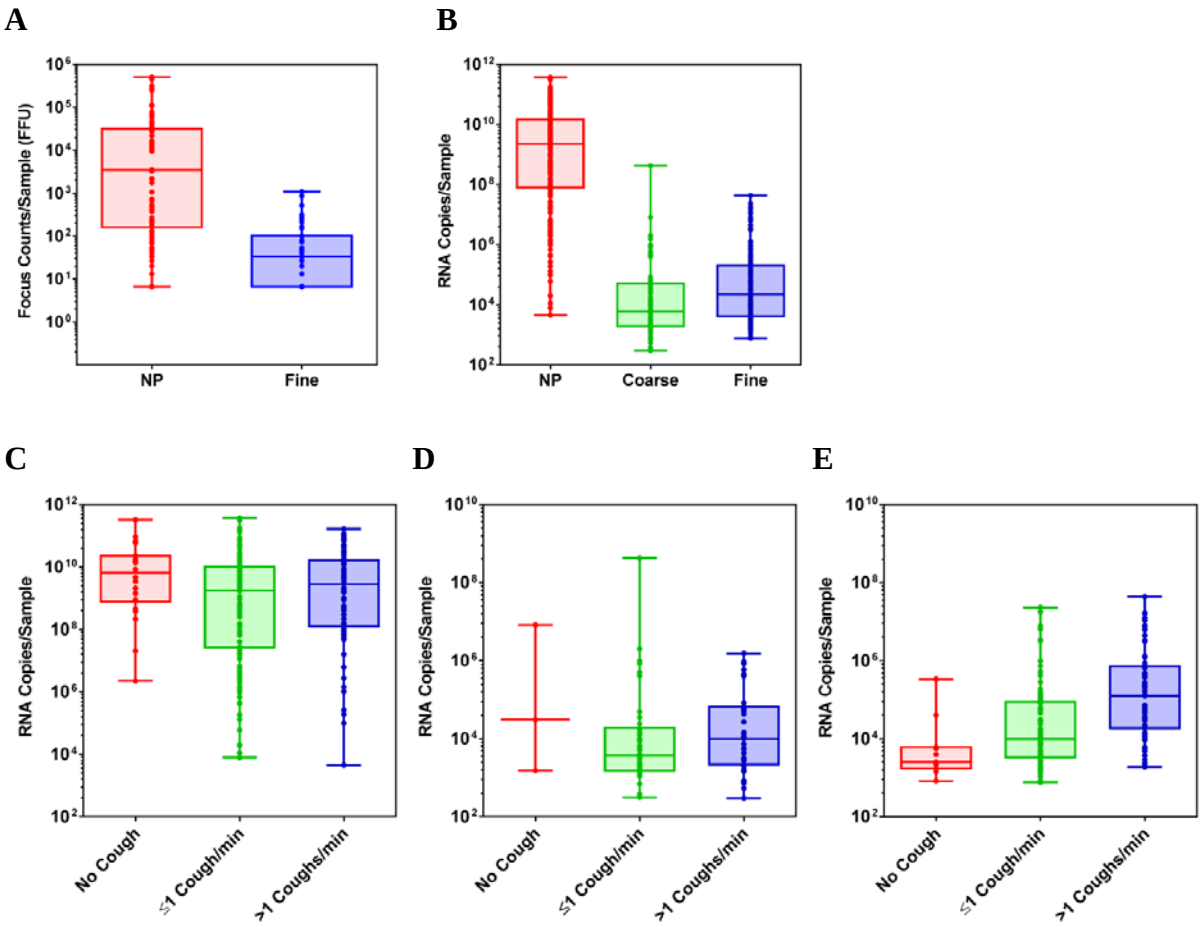

Figure 4.1. Viral shedding in swabs and aerosol samples and the effect of cough: A) infectious influenza virus in NP swabs and fine aerosols; B) RNA copies in NP swabs, coarse, and fine aerosols; C) RNA copies stratified by observed number of coughs in NP swabs, D) coarse aerosols, and E) in fine aerosols. NP = nasopharyngeal swab, Coarse Aerosol = droplets >5 µm and Fine Aerosol = droplets ≤ 5µm in aerodynamic diameter.

Table 4.2. Viral Shedding*

Sample type	RNA Copies positive/total (median, IQR)	Quantitative Culture positive/total (GM, max)	Culture Passage positive/total (%)
NP swab	211/218 (2.2×10^9, 8.3×10^7–1.5×10^{10})	98/159 (2.5×10^3, 5×10^5)	150/169 (89)
Coarse aerosol	88/218 (6.0×10^3, 1.9×10^3–5.0×10^4)	–	–
Fine aerosol	166/218 (2.2×10^4, 4.2×10^3–2.0×10^5)	41/136 (37, 1×10^3)	52/134 (39)

* IQR = innerquartile range, GM = Geometric mean, only positive samples were included in computation of GM and GSD. ND = not detected.

were positive for 98 (62%) of 159 NP swabs with geometric mean of positive samples $2.5x10^3$ (95%Cl 1.3×10^3 to 4.7×10^3); 41 (30%) of 136 fine aerosol quantitative cultures with GM 37 (95%Cl 23.4 to 60.0) FFU in 30-min aerosol samples (Table 4.2 and Figure 4.1A). Using Tobit analysis to adjust the estimate of the geometric mean for samples below the limit of detection we obtain GM 60.6 (95% CI 22.7 to 1.6×10^2) and GM 1.6 (95% CI 0.7 to 3.5) for NP swabs and fine aerosols respectively.

Influenza virus RNA was detected in 211 (95%) of the NP swabs, 88 (40%) of the coarse, and 166 (76%) of the fine aerosol samples from the 218 visits included in the final analysis. For the positive samples, we observed geometric mean viral RNA content of NP swabs was 8.2×10^8 (95%Cl 4.8×10^8 to 1.4×10^9), that of coarse aerosols 1.2×10^4 (95% CI 7.1×10^3 to 2.1×10^4) and for fine aerosols 3.8×10^4 (95% CI 2.5×10^4 to 5.7×10^4), Figure 4.1B. The adjusted geometric means for coarse aerosols were 6.0×10^2 (95% CI 3.0×10^2 to 1.2×10^3) and for fine aerosols 1.2×10^4 (95% CI 7.0×10^3 to 1.9×10^4).

We observed a moderately strong correlation between the number of viral RNA copies and quantitative culture (r = 0.58) for NP swabs and a weak but significant correlation (r = 0.34) for fine aerosols (Figure 4.2 A, B). We observed 16 fold (95% CI 10 to 27) greater viral content in fine compared with coarse aerosols. Cough frequency was not associated with viral RNA in NP swabs (r = 0.02). But, cough was associated viral RNA copies in coarse (r = 0.24) and fine (r = 0.45) aerosols (Figures 4.2 C-E). However, cough was not a requirement for shedding into aerosols; among the 23 (11%)

of cases who never coughed RNA copies ranged from <LOD to 3.7×10^5 (adjusted GM 1.5×10^3, 95% CI 4.2×10^2 to 5.3×10^3) and from 0 to 1.3×10^2 FFU.

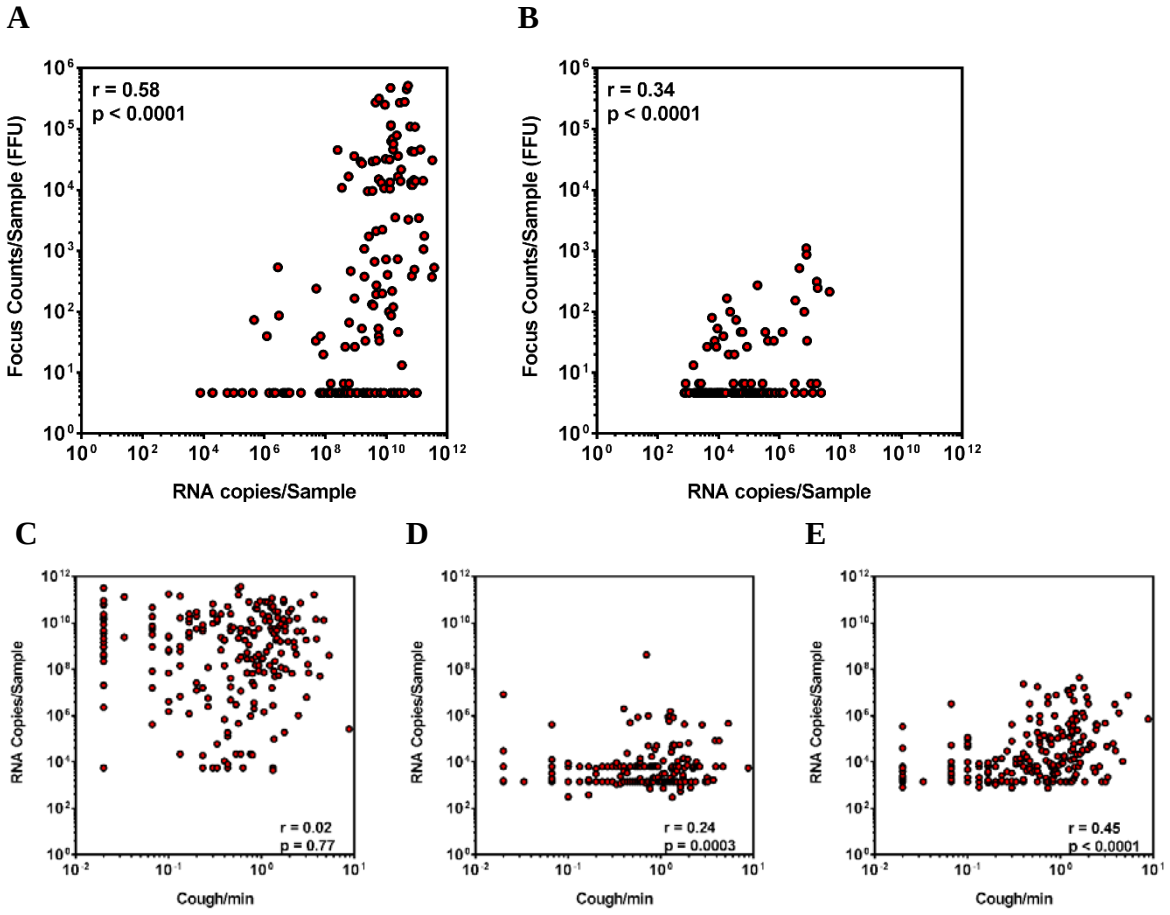

Figure 4.2. Correlation of Influenza Virus Load with Cough: A-B) Numbers of culturable influenza virus and cough, C-E) RNA copies and cough.

The detailed Tobit models are shown in Table 4.3 for NP, coarse and fine respectively. The detailed Tobit model SAS codes were attached in Appendix C. In single predictor models, NP fraction RNA virus copy number is neither associated with the fine ($p = 0.16$) fraction RNA copies nor the coarse ($p = 0.48$) fraction. Cough number is associated significantly with coarse ($p = 0.0083$) and fine ($p< 0.0001$) fraction RNA virus copy numbers. Reported feverishness is associated with a non-significant trend toward higher virus copy numbers in NP ($p = 0.11$) and fine ($p =$

0.29) fractions, and toward lower virus copy number in coarse fraction sample ($p = 0.60$). Among the 31 subjects who received the current year vaccine, 22 of them also got the previous year's vaccine. Vaccination in both years was associated with a significant trend toward higher copy numbers in fine ($p = 0.04$), and is neither associated with coarse ($p = 0.89$) nor NP fractions ($p = 0.80$). There were too few cases with anti-flu medication in our data collection to analyze. Smoking is not a significant predictor for any of the three sample types. Virus copy number in fine samples declined with time since onset of symptoms. On day 2 the amount of virus observed by RT-qPCR in fine fraction aerosol was 44% (95% CI 18% to 105%) of that observed on day one. Similar to day 2, observed virus on day 3 was 20% (95% CI 8% to 52%) the number of RNA copies detected on day one. This trend in RNA copies detected on day post symptom onset in fine aerosols was similarly observed for the coarse aerosol fraction. Compared with day 1, RNA copies quantified in coarse aerosols on days 2 and 3 post symptom_onset were 34% (95% CI 10% to 112%) and 29% (95% CI 8.1% to 103%) respectively. NP swab viral content was not associated with day post onset.

The influenza infection type, either flu A or B, is associated with a non-significant trend toward lower virus copy number in NP ($p = 0.62$) coarse ($p = 0.84$) and fine ($p = 0.23$) samples. Upper, lower and systemic symptoms are significant predictors for NP swab viral content. An increase from 25th to 75th percentile in upper symptom score produced a 3.4-fold increase in RT-qPCR quantified copy number in the NP swab. Lower and systemic symptom scores increase from 25th to 75th percentile produced 3.2 and 5.3-fold increase in RNA copy number respectively. Symptoms are not correlated with the fine and coarse fraction aerosol. BMI is associated with a significant

trend toward higher virus copy number in coarse ($p = 0.08$) and fine ($p = 0.06$) samples. An increase from 25^{th} to 75^{th} percentile in BMI produced around 2-fold increase in both coarse and fine fraction aerosols.

In multiple predictor models (Table 4.3), NP swab viral content is significantly associated with age and upper respiratory symptom score. With a 25^{th} to 75^{th} percentile change in upper respiratory symptom score producing a 3.3-fold increase, and subject who are older will shed less RNA copies in NP swab. In the full model, coarse and fine aerosol viral contents are significantly associated with BMI, cough and day post symptom onset, with similar trends observed in the single predictor model. Vaccination of both seasons is a significant predictor in fine aerosol, with people who were vaccine in two seasons in a row will shed about 4-fold more viral content in fine aerosol. The interaction between gender and cough is also a significant predictor in the fine aerosol full regression model, with males showing almost a 4-fold increase from 25th to 75th percentile in cough counts compared with female.

Table 4.3 Predictors of Viral Shedding

Parameter	NP Swab		Coarse Aerosol		Fine Aerosol	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Age*	**0.85**† **(0.72 –1.0)**	**0.77**‡ **(0.65 –0.92)**	1.0 (0.90 – 1.2)	-	0.99 (0.88 – 1.1)	-
Male	0.34 (0.14 – 2.5)	-	1.7 (0.52 – 5.6)	-	**2.4*** **(0.88 – 6.5)**	-
Asthma	3.8 (0.63 –22.9)	-	1.38 (0.32 – 6.04)	-	2.6 (0.77 –8.95)	-
Smoker	2.3 (0.30 –18.2)	-	0.48 (0.09 –2.7)	-	1.5 (0.36 – 6.1)	-
BMI	1.3 (0.58 – 2.7)	-	**1.9*** **(0.92 – 3.98)**	**1.9*** **(0.91 –3.91)**	**1.7*** **(0.98 – 2.9)**	**1.7**† **(1.0 – 2.7)**
Current year influenza vaccination	1.3 (0.22 – 7.6)	-	1.4 (0.34 –5.9)	-	**2.8*** **(0.85 –9.3)**	-
Previous year influenza vaccination	1.55 (0.45 – 5.5)	-	0.71 (0.15 – 3.3)	-	2.3 (0.79 - 6.6)	-
Both current and previous year influenza vaccination	0.77 (0.10 – 5.7)	-	1.13 (0.22 – 5.7)	-	**2.1**‡ **(1.3 –3.2)**	**3.9**‡ **(1.2 –12.7)**
Antiviral medication	1.1 (0.03 –33.9)	-	0.64 (0.04 –11.5)	-	1.04 (0.09 –12.0)	-
Influenza A	0.67 (0.13 – 3.3)	-	1.0 (0.18 – 5.5)	-	0.44 (0.13 –1.5)	-
Log (NP Swab)	-	-	1.3 (0.91 – 1.82)	-	1.2 (0.76 –1.78)	-
Day 2 post onset	2.13 (0.48 –9.5)	-	**0.34*** **(0.10 – 1.02)**	**0.33*** **(0.10 –1.08)**	**0.44*** **(0.18 –1.05)**	**0.41**† **(0.18 –0.94)**
Day 3 post onset	0.55 (0.11 – 2.8)	-	**0.29*** **(0.08 –1.03)**	**0.29*** **(0.08 –1.01)**	**0.20**§ **(0.08 –0.52)**	**0.23**‡ **(0.09 –0.55)**
Fever (T≥ 37.8 measured at visit)	3.6 (0.76 –17.2)	-	0.70 (0.19 –2.7)	-	1.7 (0.64 – 4.5)	-
Number of coughs	1.3 (0.65 – 2.8)	-	**2.1**‡ **(1.2 – 3.8)**	**2.2**‡ **(1.3–3.8)**	**2.9**¶ **(1.9 –4.6)**	**2.3**§ **(1.5 – 3.6)**
Upper respiratory symptoms	**3.4**§ **(1.8 –6.6)**	**3.3**‡ **(1.61 – 6.7)**	0.76 (0.44– 1.3)	-	0.88 (0.58 – 1.4)	-
Lower respiratory symptoms	**3.2*** **(0.96 – 10.8)**	1.3 (0.32 – 5.1)	0.94 (0.36 – 2.5)	-	1.9 (0.86 – 4.1)	-
Systemic symptoms	**5.3**‡ **(1.6 – 17.7)**	1.2 (0.58 – 9.3)	0.86 (0.33 –2.3)	-	1.8 (0.82 – 3.7)	-
Male × Number of Coughs	-	-	-	-	-	**3.9**§ **(1.8 – 8.4)**

* Effect estimates are shown as the ratio of male to female, Day 2 or Day 3 to Day 1, type A to B, or fold increase for an inner quartile range (IQR) change in age, the number of coughs, symptom reports, or BMI, or ratio of male number of coughs to female coughs over the IQR.

* p< 0.10, † p< 0.05, ‡ p< 0.01, § p< 0.001, ¶ p< 0.0001 from Tobit regression models with random effect of subject and sample within subject. Adjusted models were selected using the Akaike information criterion from initial models with all unadjusted parameters having p< 0.10.

4.5 Discussion

We have successfully cultured 52 aerosol samples. The presence of culturable influenza virus in nearly 40% of the fine aerosol samples demonstrates that influenza cases shed infectious virus as well as RNA into airborne droplets and contributes to the biological plausibility and likely importance of airborne influenza transmission. Analysis of exhaled breath particles from the naturally influenza infected cases indicates that the fine particle fraction of exhaled breath contains more RNA copies than the coarse particle fraction suggests that fine particle aerosols contribute to transmission of influenza. These results have implications on the prevention of influenza virus transmission.

We found that flu cases do not sneeze, despite having just undergone two NP swab collections (a procedure that generally makes one want to sneeze) and subsequent 30-minute observation. People with flu do cough (Roy and Milton, 2004; Tang and Settles, 2008; Yang et al., 2007), and taken together with the sneeze data could suggest that sneezing is more characteristic of other respiratory infections but not flu (Appendix B Figure B4). Cough did have an impact on how much virus observed cases were shedding (Lindsley et al., 2010), but without cough, cases still shed large quantities of culturable, contagious virus into fine aerosols, which suggests that other aerosol generating mechanisms are going on in the lung. The aerosol generation without cough may probably due to airway closure and opening (Almstrand et al., 2010). This has been extensively studied in the pulmonary literature in recent years in studies attempting to identify early biomarkers of pulmonary pathology in exhaled breath (Gralton et al., 2013; Kastelik et al., 2002).

When we look at predictors of viral content in NP swab specimens, we saw upper respiratory symptom score as a significant one, which supports the notion that symptomatic viral nasopharyngeal infections are indicative of upper respiratory infection, as opposed to lower respiratory or overall symptoms. The amount of viral content in the NP swab does not predict how much we observed in either coarse or fine aerosols suggesting that these are two are different phenomena and viral content in aerosols is probably representing infection of the lower respiratory tract. Another explanation is that there is not enough turbulence during breathing to generate aerosols in the upper respiratory tract, and most of the aerosols are generated from the lower airway in which we did not see a correlation between NP swab and aerosol samples driven by different symptoms (Gralton et al., 2013; Shinya et al., 2006). There was no difference in viral shedding between female and male when they don't cough, however among cases who coughed, males generated more aerosols than females. This may be due to a larger lung capacity in male volunteers, since the sex effect of our data is on total virus output, not corrected for lung size of individual and tidal breath of the case. The influence of height and pulmonary function may have resulted from the sex difference (Kastelik et al., 2002). If the data were corrected in that way, the results might be slightly different, with significant effect of sex resolving to non-significance.

People who received both current and previous year vaccination shed more viral content in fine aerosol based on our Tobit model results. It could possibly be due to the subjects having received mismatched vaccine for the 2012-2013 flu season. The current year vaccination is a significant predictor only in flu A infection, and CDC reported that the effectiveness of the 2012-2013 vaccine for flu A is 47% which is much lower

than the 67% of flu B (Centers for Disease Control and Prevention, 2013). The majority of the group received vaccine two seasons in a row, which may have reduced their opportunity to build naturally acquired immunity (immunity from having the disease itself), which is considered much stronger than the immunity provided by vaccine (Centers for Disease Control and Prevention, 2013). When they are exposed to influenza viruses not included in the vaccine, their immunity is not strong enough and they may have a higher chance of getting sick, leading to a more severe response towards the infections.

Not all cases with confirmed influenza infection had symptomatic illness, and it was hard to identify the transmissivity of these people. Based on our results, people without severe symptoms can still shed a large quantity of virus into the air. Cases who did not complain of any upper respiratory symptoms still shed 10^4 RNA copies. Cases who did not have any systemic symptoms can shed up to 10^7 RNA copies for fine fraction aerosols. The asymptomatic fraction of infected individuals requires further attention given the potential for them to transmit "silently" (Lau et al., 2010).

CHAPTER 5: CLIMATE EFFECTS ON INFECTIOUS INFLUENZA VIRUS IN HUMAN EXHALED BREATH

5.1 Abstract

Influenza transmission is often associated with climatic factors. The survival of influenza virus virions within the particles is affected by environmental factors such as temperature and humidity. The rate of evaporation of particles, which affects the final particle size distribution also highly influenced by the climate factors. In this study, we screened volunteers from three locations, University of Massachusetts (UML), University of Hong Kong (UHK) and National University of Singapore (NUS). These three locations represent three types of climates, temperate, subtropical and tropical. The volunteers that are invited for the study from all three sites have either a positive test with the rapid test or reported symptoms and who had a body temperature of $\geqslant$ 37.8 ℃. All three locations measured exhaled influenza viral particle copy number RT-PCR in two particle size fractions, $\geqslant$5 μm (coarse) and <5 μm (fine). In all three locations, the fine particles had more viral copy number than in the coarse fraction which have suggested an important role for aerosols in seasonal influenza transmission. NUS and UHK have relatively higher virus copies reading from RT-PCR for the collected exhaled breath samples compared to UML, which suggests airborne transmission route cannot be negligible in tropical regions even with less variability in temperature and humidity.

5.2 Introduction

Influenza respiratory infection still remains as a public health importance with substantial burden of morbidity and mortality (Belser et al., 2010; Elovainio, 2008). The ability of influenza viruses to spread through susceptible populations to cause annual epidemics and occasional pandemics is well documented. Whether the infection leads to disease depends on various factors. Once the infection occurs, the host will have the ability to release the pathogen within respiratory secretions into the air during breathing, coughing, sneezing or talking. There are three primary modes of transmission: droplet, contact and airborne. Droplet and contact modes involve large particles (>100 μm). The large respiratory droplets can travel only for a short distance before landing on the ground. Airborne transmission involves small aerosols with size < 10 μm that will stay and remain suspended in air for a relatively longer period of time and more likely to pass into the lower respiratory tract.

The transmissibility is highly influenced by the exposure environmental conditions, such as, humidity, temperature, seasonality, settings (indoor or outdoor), solar irradiation and air exchange in which the pathogen and host meet (Pica and Bouvier, 2012). The seasonality of influenza epidemics has been confounded by climate factors such as temperature and humidity. These factors strongly affect the production of influenza-laden particles and also the viability of the virus particles, which is linked to risk of infection. It is believed that airborne route is dominant in the temperate climate region due to distinct seasonality. The relative lack of seasonality in tropical regions with less variability in temperature and humidity are suggested to be dominant by the other transmission route.

There have been many published papers with a lot of experimental work in the literature to study the effect of environmental parameters on the survival of airborne influenza virus. To date, there is still debate over how the environmental impacts influence the infected host viral shedding. Lowen et al., 2007 used guinea pigs to show that the infected hosts shed significantly higher quantities of viral particles when exposed to lower ambient temperature than those were exposed to higher temperature. There have not been any human subjects studies on testing the climate effects on the naturally infected cases.

Our study fits this knowledge gap by screening naturally influenza infected volunteers from three locations, University of Massachusetts Lowell (UML), University of Hong Kong (UHK) and National University of Singapore (NUS). These three locations represent three types of climates, temperate, subtropical and tropical. The effects of outside environmental parameters on human viral shedding are discussed in this chapter.

5.3 Methods

5.3.1 Subject Recruitment

In the study that was done at the UML, the volunteers were mostly students and staff who were recruited with influenza-like illness from the Lowell, MA community, beginning January 29 and ending March 12, 2009. The study protocol was approved by the Institutional Review Boards and the detailed recruiting protocol can be found in Milton, et al., 2013. A nasopharyngeal specimen (NP swab) using a flocked swab was collected and temperature was taken with a digital ear thermometer. All volunteers with

a body temperature⩾37.8 °C and volunteers without fever who provided a NP swab positive for influenza by QuikVue influenza A/B were invited to provide exhaled breath samples, answer a questionnaire, and provide a second NP swab for analysis by real-time reverse transcription polymerase chain reaction (RT-PCR).

UHK conducted their study in a local outpatient clinic in a private hospital. The volunteers that were invited to the study were at least 11 years old and with at least two symptoms of acute respiratory illness (ARI) within 3 days of the onset of symptoms. The study was explained to the volunteers before they gave the signed paper consent. If the volunteer was between 11-18 years old, a signed consent form from both the subject and his guardian was obtained. The study protocol was also approved by the Institutional Review Boards. After obtaining the consent, a 5-minute questionnaire was administered by the research staff. A nasopharyngeal specimen using a flocked swab was collected to test for whether the subject is influenza A/B positive by the Quidel Sofia influenza A+B rapid test and temperature was taken with a Tympanic thermometer (Cat #TH-809, OTO Bodycare, Hong Kong). If the rapid test proved to be positive, a separate nasal swab and throat swab was obtained for further PCR testing and then subjected to exhaled breath collection for 30 minutes.

NUS performed the experiments in a similar way like UHK and UML. Patients were recruited at the University Health Centre (UHC) at the National University of Singapore (NUS). When patients registered at the clinic, those with 2 or more of any of the ARI symptoms were recruited for the study. The patients were only considered if they were within three days of the onset date. The first phase of the study was

screening for influenza cases. A nasopharyngeal specimen was taken to run the rapid test (quick NaviFlu). If the rapid test showed positive, the patient was asked to the follow up G-II collection. If patients had a fever, even if the rapid test is negative they were still asked to participate in exhaled breath sampling. If the patient did not have fever and two of the other symptoms, they were not invited to the follow up study.

5.3.2 Exhaled Breath Collection

The exhaled breath was collected with the subject seated in front of the inlet of novel exhaled breath aerosol collector named G-II. The device is capable of providing information of the total and viable virus counts in the exhaled breath. The detail description can be found in McDevitt et al. (2013) and is also discussed briefly in the Milton et al. (2013). The G-II inlet was cone shaped so that the subject's face was situated inside the large end of an open cone with intake air (125 L/min) drawn continuously around the subject and into the sampler. The subject could breathe normally while sitting awake in the cone. The cone captured the exhaled breath with minimal leakage even with redirection of the flow. Air flowed through a Teflon surface conventional slit impactor that collects $\geqslant$5 μm particles. After that, all the remaining fine particles were grown bigger by condensation and were captured by a 1.0 μm slit impactor and drained into a buffer containing liquid in the bottom of the reservoir. Concentrated buffer (phosphate buffered saline with 0.1% bovine serum albumin) was constantly pumped into the reservoir to preserve the virus viability. The collected sample was concentrated and extracted for future RT-PCR assay test.

5.3.3 Sample Analysis

For all three locations, the Teflon impactor surface was scraped with a flocked swab after collection and eluted in Dulbecco's phosphate buffered saline with calcium and magnesium with 0.1% bovine serum albumin (DPBS++BSA) for 1 minute with vortexing. The resulting sample was stored at −80 °C. The fine particle fraction collected in DPBS++BSA buffer (100 to 150 ml volume) was maintained at 4 °C and concentrated by ultrafiltration to a volume of approximately 400 µl. Following ultrafiltration, the filter was washed with 200 µl of DPBS++BSA, and the wash solution was combined with the retentate. Samples were stored at −80 °C.

For UML, quantitative PCR was performed and the limits of detection were 6 and 11 viral RNA copies per qPCR well for influenza A and B respectively. The detailed sample analysis for the UML was described in the Milton et al. (2013). For UHK and NUS, sample analysis was similar.

From UHK, 250 µl of sampled specimen was added to 2 ml lysis buffer provided by the manufacturer for nucleic acid extraction. The specimen was incubated for 10 minutes at room temperature to ensure complete lysis for the release nucleic acids. The lysate was mixed with 100 µl magnetic silica and preceded to automatic extraction by the NucliSENS® easyMAG®. 55 µl of RNase-free elution buffer was used for the recovery of nucleic acid. The extracted nucleic acids were kept frozen at -80^{o}C until processing. If the specimen result was outside the upper limit of the expected range, the extract of the sample was repeated with suitable dilution.

Since UHK brought the sample to 2 ml, extracted 250 µl of 2 ml, and then eluted to 55 µl, then used 5 µl of that for PCR, the dilution factor is 88. So the limit of detection for PCR is 880 virus copies /sample (highest detection limit among three locations).

Two samples of UHK data were analyzed at the University of Maryland (UMD) laboratory. The UHK sample was 2 ml original, and UMD extracted 200 µl of the 2 ml sample. The sample was then eluted to 50 µl, and 10 µl was used for PCR, and the dilution factor is 50. The limit of detection is 250 copies/sample.

In NUS, for NP swab samples, the original volume was 1.2 ml. 200 µl from the original volume was used for RNA extraction and the sample was eluted in 50 µl, then 10 µl from this 50 µl was used for qRT-PCR. Therefore, amount of viral copies in the original sample has a dilution factor of 30. For fine and coarse particles, the original volume was 1 ml; 200 µl from the original volume was used for RNA extraction and then eluted in 50 µl, 10 µl from this 50 µl was used for qRT-PCR. Therefore, the dilution factor is 25. The detection limit is 150 virus copies/ sample for NP samples and 125 for condensation samples.

5.3.4 Statistical Analysis

The data analysis was performed in both R and SAS. These two software systems are mostly used in epidemiology studies for data analysis and graphics. ANOVA is normally used to perform the comparison among multiple groups, but since the data do not follow a normal distribution, the Kruskal-Wallis test was used, which can be applied when we cannot make the assumption if the groups follow a Gaussian distribution. In our study, the Kruskal–Wallis (K-W) test is used for fine, coarse, and

NP particle virus copy counts to see the difference among three locations. Following with the K-W test, a post-hoc test (a priori statistical methods) is used to confirm the result.

The Chi-square test is used to see if the difference in limit of detection affects the comparison of data among all three locations. To test the effect of other influencing factors such as medical history, race, and age, the Tobit model was performed. The Tobit model analyzed log copy number with a random effect to account for variability among different climates. We also used Spearman's correlation coefficient to examine the relationship between the viral load in the nasopharyngeal swab and aerosol fractions.

5.4 Results

At all three locations, we picked the subjects with a positive nasal or nasopharyngeal specimen PCR data. The data that were excluded in this study were either with a negative swab RT-PCR data or due to laboratory error in sample processing. In UHK, 7 subjects with complete data were picked, 31 subjects from NUS and 37 subjects from UML. Exhaled breath samples were obtained for all the selected subjects. Table 5.1 shows the sex, symptoms and influenza virus type for all the three locations, and Table 5.2 shows descriptive statistics for age, swabs and exhaled aerosol fractions of viral RNA copy number for all the 78 volunteers from all three locations.

Table 5.1. Volunteer's sex, symptoms, temperature, and influenza virus type for all three locations

	UHK		NUS		UML	
	N	**Percent**	**N**	**Percent**	**N**	**Percent**
Number of subjects with complete data	7	100	31	100	37	100
Male	3	43	20	65	30	81
On antiviral medicine within past 24 hours	0	0	0	0	0	0
Asthmatic	1	14	7	23	5	14
Flu shot this season	0	0	0	0	1	3
Flu shot previous season	0	0	3	10	12	32
Smoker	1	14	3	10	9	24
Breathing difficulty	1	14	3	10	16	43
Temperature≥37.8 C	2	29	28	90	10	27
Influenza A	7	100	23	74	21	57

Among all the samples with positive swab data, 57% (4 of 7) of fine particle samples from UHK had detectable virus copies from PCR, 42% (13 of 31) of fine particle samples from NUS, and 92% (34 of 37) of fine particles samples from UML. For the coarse particle samples, UHK showed 29% (2 of 7) of detected virus copies from PCR, NUS showed 29% (9 of 31), and UML showed 43% (16 of 37) detected virus copies. Combing the coarse and fine fractions, the detected RNA virus copies were above 50% of the total samples, which demonstrates the potential importance of the airborne particles transmission route.

Table 5.2. Descriptive Statistics

		Percentiles				
		Min	**25th**	**Median**	**75th**	**Max**
UHK	**Age**	26	36	42	53	56
	Days since onset	1	1	2	2	3
	Nose swab copy number	1.82×10^5	2.26×10^6	5.48×10^6	1.42×10^7	9.16×10^7
	Coarse particle copy number	<LOD*	<LOD	<LOD	1.39×10^2	4.08×10^3
	Fine particle copy number	<LOD	<LOD	2.96×10^2	3.60×10^3	5.66×10^4
NUS	**Age**	19	22	23	27	54
	Days since onset	1	1	1	2	3
	Nasopharyngeal swab copy number	1.58×10^6	1.70×10^7	1.36×10^8	1.03×10^9	5.52×10^9
	Coarse particle copy number	<LOD	<LOD	<LOD	3.62×10^2	3.73×10^5
	Fine particle copy number	<LOD	<LOD	<LOD	9.08×10^3	5.00×10^6
UML	**Age**	18	18	19	20	54
	Days since onset	1	1	2	3	5
	Nasopharyngeal swab copy number	1.70×10^3	8.30×10^4	4.20×10^5	1.80×10^6	3.40×10^7
	Coarse particle copy number	<LOD	<LOD	<LOD	3.7	2.90×10^4
	Fine particle copy number	<LOD	1.1	1.10×10^2	5.60×10^2	1.30×10^5

*Lower limit of detection

Table 5.3. Chi-square test table for coarse PCR sample results

Coarse	**UHK**	**NUS**	**UML**	**Total**
Positive	1	5	2	8
Negative	6	26	35	67
Total	7	31	37	75

Table 5.4. Chi-square test table for fine PCR sample results

Fine	**UHK**	**NUS**	**UML**	**Total**
Positive	3	12	6	21
Negative	4	19	31	55
Total	7	31	37	75

Fine fraction copy numbers were on average 15 times greater than coarse fraction copy numbers for UHK, and 14 folds greater for NUS, and 5 folds greater for UML.

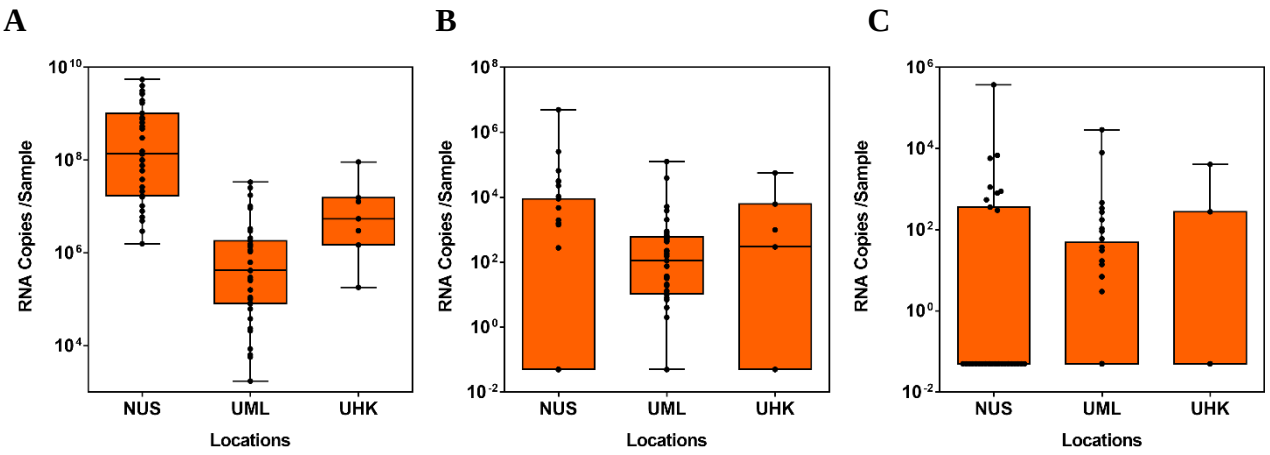

Figure 5.1. PCR virus copies per sample for A) NP; B) Fine; C) Coarse.

The coarse and fine fraction copy numbers were correlated ($r = 0.60$, $p < 0.0001$) only for UML, the other two locations had no such significant correlations. There was no significant difference in copy number between influenza A and B for NUS and UML. Fine fraction copy numbers were significantly related with the body temperature measured at the time of testing in NUS ($p=0.003$) and UML sites ($p = 0.014$), but coarse fraction copy numbers were only significantly impacted by the feverishness in the NUS site ($p = 0.002$) not UML. UHK site had limited data to do the analysis. Vaccination in any prior year was not significantly associated with copy numbers in both fine and coarse fractions for all three locations; too few individuals received the current season's vaccine to analyze for all three locations. Self-reported symptoms like breathing difficulty and smoking were not associated with significant shifts in aerosol viral load. From the boxplots in Figure 5.1, NUS and UHK have relatively higher virus copies reading from RT-PCR for all three types of samples compared to UML.

Since all three locations performed the sample analysis differently with different resulting detection limits, the differences in the data sets may mainly result from the variance of the limit of detection. In order to generalize the samples data and study if

the detection limit plays an important role in the data analysis, we picked the highest limit detection among all three locations, which is 880 virus copies/sample from UHK and did the Chi-square analysis. We categorized the data as above 880 or below 880 for all three locations. As shown in Table 5.3, the Chi-square test statistic was equal to 2.14 when degree of freedom (df) is 2 ($p = 0.34$). Since $p > 0.05$, the null hypothesis cannot be rejected, in which the amount of detectable virus copies in the coarse samples are independent of the difference in limit of detection among all three locations. For fine particle counts (Table 5.4), Chi-square test statistic was equal to 5.1, with $p = 0.08$ and $df = 2$. Since $p > 0.05$, the null hypothesis cannot be rejected, in which the amount of detectable virus copies in the fine particles is independent of the difference in limit of detection among all three locations.

From the Kruskal-Wallis rank sum test and post-hoc test, the results appear that the viral load of all three samples (NP, fine, coarse) from NUS was significant higher than the samples from UML ($p << 0.05$). The viral load of aerosol samples from UHK appears to have no significant difference with that from either NUS or UML.

5.5 Discussion

All three locations measured exhaled influenza viral particle copy number RT-PCR in two particle size fractions, ≥5 μm (coarse) and < 5 μm (fine). In all three locations, the fine particle fraction had more viral copy number than in the coarse fraction, suggesting an important role for aerosols in seasonal influenza transmission.

Influenza viruses circulate year round in tropical Singapore. In our collected cases the influenza infected subjects were observed mostly in July−August and

November−March. The climate in Singapore is characterized as uniform temperatures of minimum 23 °C – 26 °C and maximum 31 °C – 34 °C and a relative humidity of 84% (National Environment Agency, 2016). In temperate countries, seasonal influenza epidemics occur during colder months and remain above baseline levels for six to eight weeks (S. Monto and G. Webster, 2013). Hong Kong has a subtropical climate and an influenza seasonality lying approximately midway (March–June) of the year (Chan et al., 1999).

Singapore surveillance results suggest that influenza outbreaks can persist above baseline levels for more than 12 weeks. The influencing factors in determining local spread including weather, travel and population dynamics (S. Monto and G. Webster, 2013). Tropical and subtropical regions with mild winters are subjected to seasonal oscillations like rainy seasons in influenza incidence (Alonso et al., 2007). The seasonal patterns are generally more pronounced in temperate areas, since there is normally more than one period of viral activity occurring in a given year in tropical areas and it brings up more complicated mechanisms underlying seasonal patterns observation.

The results that were presented in this chapter are RT-qPCR measurements of the quantity of virus copies in the exhaled breath, not the survivability of the virus particles. In general, the airborne survival of the lipid-enveloped influenza virus is affected by the local environmental factors. Relative humidity (RH) describes the amount of water vapor in the air at a specific temperature at any time which could affect biological response (Ehrlich et al., 1970). In Shaman and Kohn, 2009 absolute humidity (AH), i.e., the actual water vapor content of air irrespective of temperature has a greater biological significance for influenza virus survivability (Shaman et al., 2010). The

lower temperature and lower relative humidity lead to higher viral survival and the higher temperatures and higher relative humidity cause lower survival (Lowen et al., 2007; Shaman and Kohn, 2009; Tellier, 2009). According to the trend, the survivability of the influenza virus particles in the tropical countries should be lower than the temperate and subtropical countries, which should probably lead to a lower infection rate among populations.

The annual all-cause death rate from seasonal influenza in Singapore has been estimated at 14.8/100,000 population per year (Chow et al., 2006); In subtropical Hong Kong, death rate from underlying pneumonia and influenza attributable to influenza were estimated to be 4.1/100,000 population per year, higher than the rate (3.1/100,000) reported in the United States (Chow et al., 2006). It appears that the influenza–related excess deaths in Singapore are higher than those in temperate and subtropical countries. There is a demonstration of increased airborne transmission indoors which has no extreme external environmental factors involved in the virus survival (Rudnick and Milton, 2003). The success of aerosol transmission may not solely depend on the absolute humidity or temperature effect of the outdoor environment.

The limited amount of data we have obtained may be difficult to interpret conclusively to determine the relationship between climate parameters and the amount of infectious particles generated by infected human beings. PCR-based methods cannot distinguish between viable and non-viable virus, which is another limitation of our study. Many social factors will also be involved with the seasons in different countries. People who spend most of their active life indoors in an air-conditioned environment in an over-crowded place like Singapore may easily get infected despite the outside

environmental factors. On the other hand, the shedding also varies with the individual, and the theory of the “super spreader” has been proved in several papers (Glass and Glass, 2008; Lloyd-Smith et al., 2005; Stein, 2011).

CHAPTER 6: MODELING THE EFFECTIVENESS OF RESPIRATORY PROTECTIVE DEVICES IN REDUCING INFLUENZA OUTBREAK

6.1 Abstract

Outbreaks of influenza represent an important health concern worldwide. In many cases vaccines are only partially successful in reducing the infection rate, and respiratory protective devices (RPDs) are used as a complementary countermeasure. In devising a protection strategy against influenza for a given population, estimates of the level of protection afforded by different RPDs are valuable. In this chapter, a risk-assessment model previously developed in general form was used to estimate the effectiveness of different types of protective equipment in reducing the rate of infection in an influenza outbreak. It was found that a 50% compliance in donning the device resulted in a significant (at least 50% prevalence and 20% cumulative incidence) reduction in risk for fitted and unfitted N95 respirators, high-filtration surgical masks, and both low-filtration and high-filtration pediatric masks. An 80% compliance rate essentially eliminated the influenza outbreak. The results of the present study, as well as the application of the model to related influenza scenarios, are potentially useful to public-health officials in decisions involving resource allocation or education strategies.

6.2 Introduction

Influenza remains a global public-health concern; the World Health Organization (WHO) estimates show that annual epidemics may cause up to five million severe illnesses and 500,000 deaths worldwide (Elovainio, 2008). An increasing number of studies suggest that influenza respiratory droplets generated by an expiratory event play an important role in transmission (Cowling et al., 2013). It has been demonstrated in the work of Milton et al. (Milton et al., 2013) and Lindsley et al. (Lindsley et al., 2010, 2015) that aerosol particles released from the human respiratory tract contain significant amount of infectious virus. The aerosol fraction that is less than 5 μm in diameter (the "respirable fraction") is of particular concern, because it can remain airborne for long periods of time (Gralton et al., 2011, 2013). The Lindsley et al. study (Lindsley et al., 2010) showed that 65% of the influenza viral RNA was contained in particles in the respirable size fraction. These particles are small enough to reach the lower respiratory tract through inhalation and cause severe infections (Cowling et al., 2013; Gralton et al., 2011).

Traditionally, proactive interventions such as seasonal vaccines are used to help prevent influenza infection (Elovainio, 2008). A 2014 CDC meta-analysis study showed that flu vaccination was responsible for up to 70% reduction in infections among all population groups during the 2010-2012 flu seasons (Centers for Disease Control and Prevention (CDC), 2013). This statistic represents a normal season when the strain has been predicted accurately a priori. In case of a new strain, a vaccine may not be available in time to prevent the virus from becoming a pandemic (Centers for Disease Control and Prevention (CDC), 2013). In such cases, wearing Respiratory

Protective Devices (RPD), such as respirators, surgical masks and pediatric masks for pediatric population, can provide protection to uninfected individuals, further reducing the risk of influenza transmission (Elovainio, 2008).

To have a better understanding of how influenza spreads, and to evaluate the possible influence of different strategies of interventions, many risk assessment models in the literature have been implemented (Beauchemin and Handel, 2011; Canini and Carrat, 2011; Chen and Liao, 2008; Furuya, 2007; Guo et al., 2015; Keeling and Rohani, 2008). Stochastic (probabilistic) and deterministic (compartmental) models are the two types of epidemiological modelling techniques (Neyman, 1956). Deterministic models have been most widely used for respiratory disease transmission models, in which SIR (susceptible-infected-removed), SEIR (Susceptible-Exposed-Infected-Removed) and Carrier state Models are the most notable ones (Keeling and Rohani, 2008). The first SIR model was proposed by Kermack and McKendrick (1927) (Kermack and McKendrick, 1927) and has been interpreted by Stilianakis and Drossinos (SD) (Stilianakis and Drossinos, 2010) to account for the dynamics of inhalable respiratory droplets. The SIR model consists of several ordinary differential equations (ODEs) coupling the changes in the population of susceptible (S), the population of infection cases (I), and the population of recovery to an immune state (R) (Stilianakis and Drossinos, 2010). Although a number of papers in the literature have addressed how influenza transmission can be prevented using the SIR model (Laguzet and Turinici, 2015; Levin et al., 2004; Nichol et al., 2010), major knowledge gaps still exist in considering the effect of respiratory protective devices in the epidemic model.

In particular, tools have not been available to quantify the reduction in risk associated with the deployment of different choices of RPDs, for different populations.

Recently, Myers et al. (Myers et al., 2016) presented a mathematical formulation for evaluating the effect of RPDs in reducing the risk of disease transmission by inhalable droplets. The model systematically extended previous SIR models to account for the change in pathogen generation and transmission introduced by RPDs. The primary purpose of the work was to provide a general mathematical formulation applicable to any infection scenario involving inhalable droplets, and to derive a "reproduction number" specifying a threshold separating growing and decaying infected populations.

In this chapter, we apply the formulation of Myers et al. (2016) (Myers et al., 2016) to model seasonal influenza outbreak and the effect of RPD intervention, in a closed community setting. We assess the effectiveness of a variety of different RPDs that might be used during such an outbreak, including fitted N95 respirators, unfitted N95 respirators, and facemasks. We evaluate the effect on both the adult and child populations. The models are informed by recently acquired data on the penetration factors for the different barriers. We also performed sensitivity analysis to demonstrate how polydispersity of aerosol size distribution, contact rates between susceptible and host, and the number of initial infected cases affects the level of influenza transmission.

6.3 Methods

6.3.1 Mathematical formulation

In the SIR model, susceptible individuals can become infectious, and then subsequently recover. Once a person has recovered, we assume that the person will remain immune for the rest of that influenza season. The susceptible group (*S*) has no new members added; the only way an individual leaves the S group is by becoming infected. The infected population (*I*) grows due to conversion from the susceptible population and decays due to conversion of individuals into the recovered (*R*) population. In the SIR model modified for RPDs, the susceptible population is divided into two groups: the S_r population that deploys RPDs, and the S_{nr} population that does not. For the infected population, a fraction of the population is assumed to deploy RPDs, but that population is assumed to convert to a recovered state at the same rate as the fraction not deploying RPDs, so it is not necessary to distinguish two populations. The equations governing the movement of the population between the different groups, due to infection by inhalable droplets and in the presence of RPDs are:

$$\frac{dS_r}{dt} = -\tilde{\beta}_r D \frac{S_r}{N} \quad (1a)$$

$$\frac{dS_{nr}}{dt} = -\tilde{\beta}_{nr} D \frac{S_{nr}}{N} \quad (1b)$$

$$\frac{dI}{dt} = -\frac{d}{dt}(S_r + S_{nr}) - \mu_I I \quad (1c)$$

$$\frac{dD}{dt} = \kappa_w I - \frac{1}{\nu} D \quad (1d)$$

$$\frac{dR}{dt} = \mu_I I \quad (1e)$$

Here N is the total population, equal to $S + I + R$. D is the total number of droplets, and $\tilde{\beta}_r$ and $\tilde{\beta}_{nr}$ are the transmission rates for protected and unprotected susceptible populations, respectively. Both $\tilde{\beta}_r$ and $\tilde{\beta}_{nr}$ are proportional to the breathing rate, denoted by B in (Myers et al., 2016). κ_w represents the infectious droplet production rate, μ_I the infection recovery rate, and $1/\nu$ the droplet removal rate $1/\nu$. Equations (1a) – (1e) apply for a monodisperse droplet size distribution. To account for multiple droplet sizes, differential equations of the form (1a) – (1e) apply for each size bin. In general, the droplet production rate and removal rate depend upon droplet size.

In Myers et al. 2016 (Myers et al., 2016), the relationship between the transmission rates for the protected and unprotected populations was derived:

$$\tilde{\beta}_r = T_{in}\,\tilde{\beta}_{nr} \qquad (2a)$$

Here T_{in} is the fraction of incoming pathogens transmitted by the RPD when the susceptible person breathes in. The transmission rate in the absence of RPDs can be written in terms of more fundamental quantities as (Myers et al., 2016)

$$\tilde{\beta}_{nr} = c\frac{B}{V_{cl}}\tau p q N_p \qquad (2b)$$

where c is the contact rate between a susceptible person and an infected person, B is the breathing rate, V_{cl} is the volume of the personal cloud of an infected person, τ the characteristic breathing time, p the probability of infection by an inhaled pathogen, q the inhaled-droplet deposition probability, N_p the number of pathogens per droplet. Of the quantities in Eqs. (2a) and (2b), q, N_p, and T_{in} are functions of droplet size.

The relationship between the droplet production rate in the presence of RPDs, κ_w, and the produced rate in the absence of RPDs, κ_{nr}, was also derived in (Myers et al., 2016):

$$\kappa_w = \kappa_{nr}[T_{out}\, f_i + (1 - f_i)] \quad (2c)$$

The quantity T_{out}, also a function of droplet size, is the outward transmission rate (expelled by the infected person) for the barrier, and f_i is the fraction of infected persons deploying RPDs.

The initial conditions for the governing differential equations are:

$$S_r(0) = f_s\ S_0 \quad (3a)$$

$$S_{nr}(0) = (\ 1 - f_s\ )\ S_0 \quad (3b)$$

$$I(0) = I_0 \quad (3c)$$

$$D(0) = 0 \quad (3d)$$

$$R(0) = 0 \quad (3e),$$

where S_0 is the initial number of susceptibles, f_s the initial fraction of susceptibles deploying RPDs, I_0 the initial number of infecteds.

6.3.2 Model Parameters

In the simulations, parameter values specific to influenza outbreaks were used where possible. Influenza specific parameter include the recovery rate μ_I (0.20 per day (Carrat et al., 2008)), the probability p of infection by an inhaled pathogen (0.5 (Li et

al., 2009)), the droplet removal rate $1/\nu$ and the size distribution (described below). The breathing rate B was taken to be 24 m^3d^{-1} for an adult and 7.2 m^3d^{-1} for a five-year-old child (Hinds, 2012). The contact rate c was assumed to be the 13 times per day (Mossong et al., 2008) and the characteristic breathing time was equal to 35 min. The average deposition probability q for a droplet was determined by the ICRP's Lung Deposition Model (Guha et al., 2014; Hinds, 2012), assuming both the adults and children to be nose breathers, and an exposure time of 8 hours. The estimated deposition probabilities are shown in Appendix D (Table D1).

Characteristics of a variety of RPDs were recently measured and published by Guha et al. (Guha et al., 2016). In Guha et al. (2016), the inward transmission rate T_{in} and outward transmission rate T_{out} were written in terms of the protection factor PF commonly used to characterize barriers. The relations are:

$$T_{in} = 1/(Receiver\ PF) \qquad (4a)$$

$$T_{out} = 1/(Source\ PF) \qquad (4b)$$

The source and reciever PF's for different droplet sizes are provided in Table 6.1. In our simulations, the following 6 RPDs were featured: N95 respirators fitted for the user (adult), N95 respirators not fitted (adult user), low-filtration surgical mask (adult), adult high-filtration surgical mask (adult), high-filtration pediatric mask (child), and low-filtration pediatric mask (child). The protection-factors for the masks and unfitted respirator are lower than that for the fitted respirator due to the presence of gaps between the device and the face (Brosseau, 2010; Brosseau and Harriman, 2007; Diaz and Smaldone, 2010; Mansour and Smaldone, 2013). While some of the devices were

not designed to protect against the spread of an epidemic, it was felt that in the event of a large-scale emergency, all the devices could potentially be used.

6.3.3 Scenarios

In the base case scenario, 20% of the susceptible and infected population wore the RPDs from the onset of the influenza season. Both the susceptible and infected populations were in a closed community in which a single symptomatic case introduced influenza virus and initiated infection. The initial susceptible population was 1000. The droplet size distributions were bimodal, with bins centered at 0.5μm and 5μm.

Other scenarios were constructed by changing the percentage of different RPDs deployed for both adult and child populations. Additional sensitivity analyses on values of key model parameters, such as the number of initial infected cases and the infectious contact rate were also explored. Additionally, to address the fact that in reality the distribution of airborne particles is polydisperse (Chao et al., 2009; Han et al., 2013; Holmgren et al., 2010; Nicas et al., 2005), different exposure particle size bins were also studied. It is time consuming to incorporate all the size bins characterizing the particle distribution; hundreds of differential equations can result. We considered 2, 3, and 4-size bins to evaluate the convergence rate of the solution as a function of the number of inhaled particle size bins. The characteristic sizes for the 2, 3, and 4 size bins were: (0.5 μm, 5 μm); (0.5 μm, 2.0 μm, 5 μm); (0.3 μm, 0.7 μm, 2.5 μm, 5 μm). The amount of respirable droplets within each size bin was determined based on Nicas.et al (Nicas et al., 2005). The number of pathogens per droplet, transmission rate per inhaled droplet, respiratory-droplet production rate, gravitational settling rate, and droplet removal rate were varying with different sizes.

Equations (1) – (3) were solved using a time-step 4th order Runge-Kutta method, as implemented in MATLAB (Version 8.6 (R2015b), Natick, Massachusetts: The MathWorks Inc., 2015). The estimated epidemic curves, time to the peak day of the outbreak and the cumulative incidence rate (total percent of infection, CIR) are reported in the next section.

Table 6.1. Protection factors for both source and receiver wearing different types of RPDs and exposed to different particle sizes

RPD Type	**Source**	**Receiver**					
		0.3 µm	**0.5 µm**	**0.7 µm**	**2 µm**	**2.5 µm**	**5.0 µm**
Fitted N95	10	20	20	20	40	40	40
Unfitted N95	7	2	2	2	2	2	2
Low filtration surgical mask	3	1.1	1.1	1.1	1.1	1.1	1.1
High filtration surgical mask	3	7	7	7	14	14	14
Low filtration pediatric mask	3	2	2	2	2	2	2
High filtration pediatric mask	3	7	7	7	7	7	7

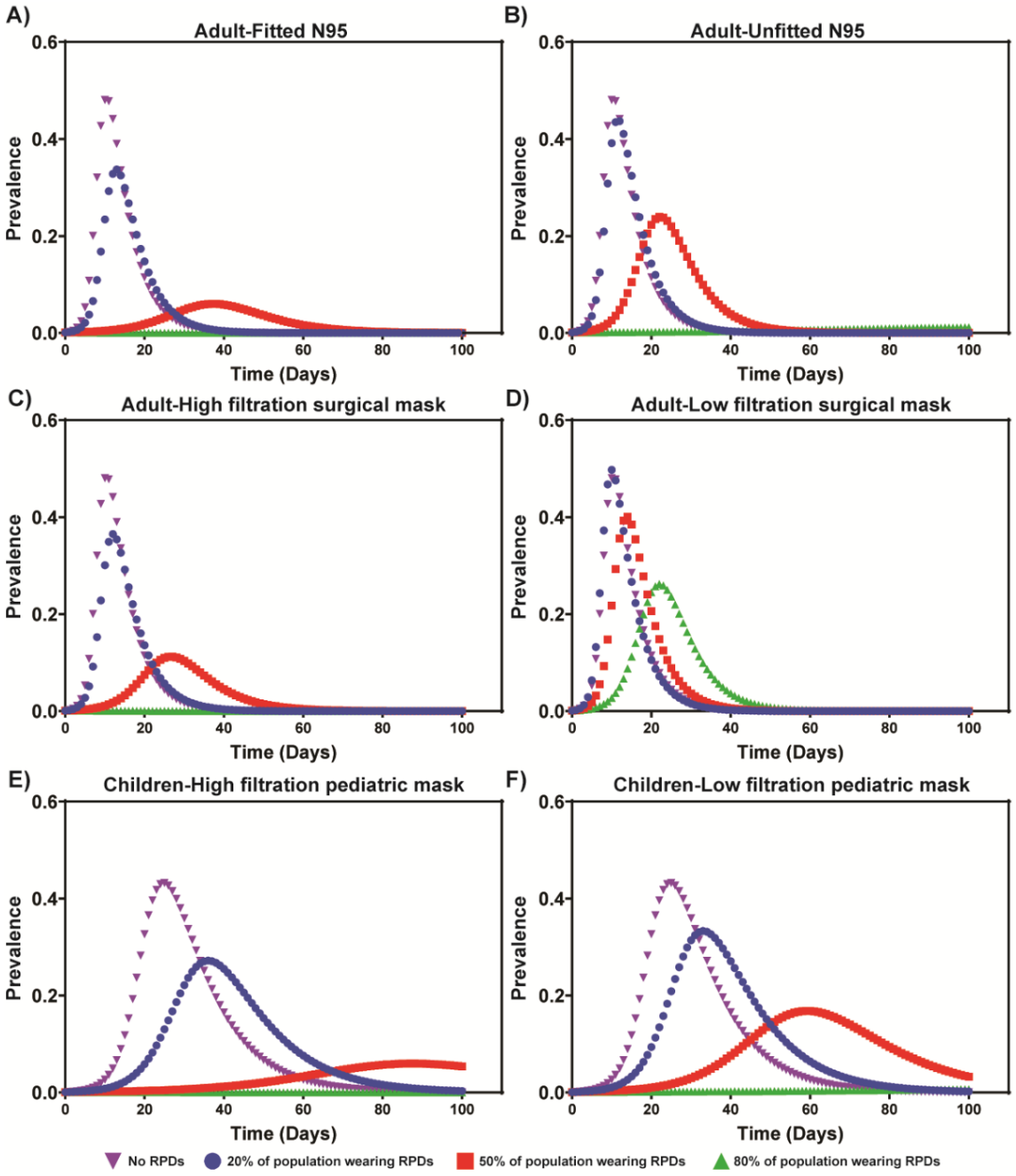

Figure 6.1. Influenza outbreak curves with varying RPDs and with 0%, 20%, 50% and 80% percentage of RPDs deploying on both adults and children.

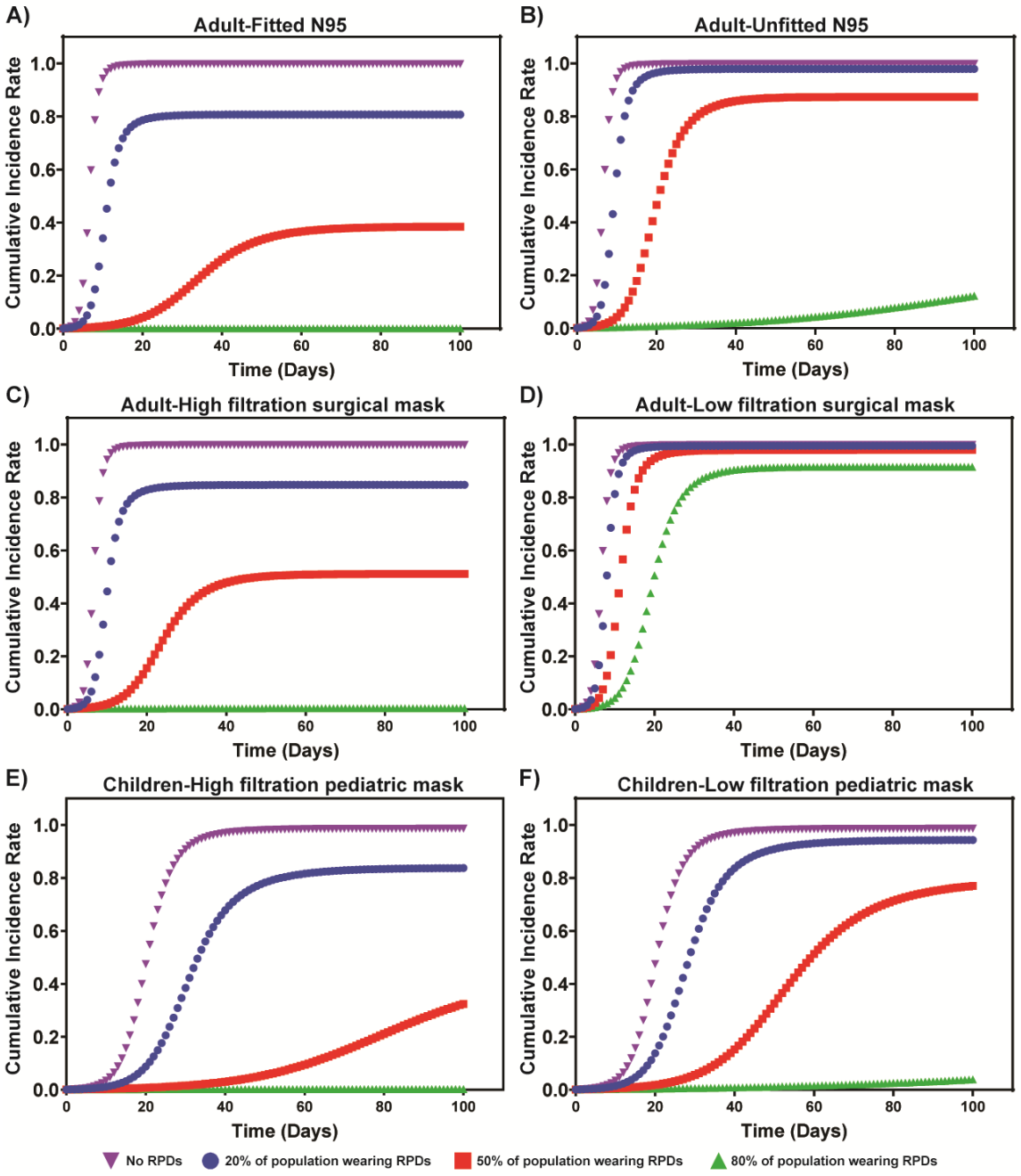

Figure 6.2. Influenza cumulative incidence rates with varying RPDs and with 0%, 20%, 50% and 80% percentage of RPDs deploying on both adults and children.

6.4 Results

The prevalence of infection is plotted in Fig. 6.1 for the 6 different types of RPDs, assuming 4 levels of compliance in donning the RPDs: 0%, 20%, 50%, and 80%. The level of compliance was assumed to be the same for the susceptible and infected populations. The cumulative incidence is plotted in Fig. 6.2.

In the case of adults with no protection (0% curves in Figs 6.1 and 6.2), the number of infected individuals at a given time reached a maximum of around 50% of the adult population and slightly over 40% of the pediatric population. The maximum number of infections at a given time occurred at around day 10 for the adults and day 25 for the pediatric population (Fig. 6.1). For both adults and children with no protection, 100% of the population was eventually infected (Fig. 6.2). For the pediatric population, the infection spread at a slower rate, as evidenced by the broader distributions of the prevalence curves (Fig. 6.1) and the slower climb of the cumulative incidence curves (Fig. 6.2).

At a 20% compliance rate, the decrease in the prevalence relative to no protection was about the same – roughly 30% - for the fitted N95 and the high-filtration surgical mask (Fig. 6.1). The time for onset of the outbreak was not changed relative to the no-protection scenario for either of these cases. For the unfitted N95 and the low-filtration surgical mask, there was very little change in the onset time, infection prevalence, or cumulative incidence rate for 20% compliance compared with no use of RPDs. For children, a decrease in prevalence of about 30% (low filtration pediatric mask) to 40% (high-filtration pediatric mask) relative to no protection was predicted by the model at

the 20% compliance rate. The onset time increased by about 10 days for both pediatric masks. The cumulative incidence rate decreased slightly – to about 80% for high-filtration pediatric masks and 90% for low filtration pediatric masks (Fig. 6.2), compared to the no-protection scenario.

At the 50% compliance rate, some significant protective effects can be observed. The prevalence dropped to about 10% for the high-filtration surgical mask and less than 10% for the fitted respirator. The unfitted N95 respirator exhibited approximately a 25% infection prevalence, roughly half the value for case of no protection. In terms of cumulative incidence, the fitted N95 results in enough protection that less than half (Fig 6.2A) of the population becomes infected. Approximately half of the population ultimately becomes infected with the high-filtration surgical mask (Fig. 6.2C). For the unfitted N95 and the low-filtration surgical mask, nearly all of the population still ultimately becomes infected at the 50% compliance rate. For children, both types of masks reduce the prevalence of infection significantly in the case of 50% compliance, by more than half relative to no protection for the low-filtration mask (Fig. 6.1F) and an order-of-magnitude for the high-filtration mask (Fig. 6.1E). The time of maximum prevalence increases to around 60 days for the low-filtration pediatric mask and 90 days for the high-filtration pediatric mask. Regarding cumulative incidence for the 50% compliance rate, by the end of 100 days approximately 30% of the pediatric population has been infected at some time for the high-filtration pediatric mask, and 75% for the low-filtration pediatric mask.

When 80% of the population deploys RPDs, an epidemic can be prevented with the use of many RPDs. The prevalence of infection, and cumulative incidence of

infection, was essentially zero for all adult and children's forms of protection considered, except the low-filtration adult surgical mask (Figs. 6.1A, B, C, E, F, 2A, B, C, E, F). For the low-protection adult surgical mask, the prevalence was reduced to about half of the no-protection value (Fig. 6.1D), and the cumulative incidence rate asymptotes at roughly 90%.

The number of bins used in the analysis appeared to affect primarily the peak day (Table 6.2). The cumulative incidence rate and duration of the outbreak were not significantly affected when 2, 3, or 4 bins were used.

Table 6.2. Impact of different number of size bins, with 20% percentage of high filtration surgical masks wearing on influenza outbreaks

	Types of aerosol distribution		
	Two size bins (0.5 μm, 5 μm)	Three size bins (0.5 μm, 2.0 μm, 5.0 μm)	Four size bins (0.3 μm, 0.7 μm, 2.5 μm, 5 um)
20% wearing fitted N95			
Peak day	13	10	9
Cumulative incidence rate, %	81%	82%	83%
Outbreak duration	48	43	42
20% wearing unfitted N95			
Peak day	12	9	8
Cumulative incidence rate, %	98%	99%	99%
Outbreak duration	47	44	42
20% wearing high filtration surgical mask			
Peak day	12	10	8
Cumulative incidence rate, %	85%	87%	89%
Outbreak duration	48	45	44
20% wearing low filtration surgical masks			
Peak day	10	8	7
Cumulative incidence rate, %	99%	99%	99%
Outbreak duration	45	42	41

The fraction of the population ultimately infected was not sensitive to the number of individuals initially infected (Table 6.3). The outbreak of the infection decreased from 48 days to 43 days, and the day of maximum prevalence decreased from day 12 to day 7, as the number of initial infecteds increased from 1 to 30.

An increase in the average of number contacts between a susceptible and infecteds from 13 to 20 per day increased the cumulative incidence rate from 85% to 88% of the population (Table 6.3). This increase was accompanied by a decrease in outbreak duration from 48 days to 43. A decrease in the average number of contacts from 13 to 10 per day reduced the cumulative incidence rate from 85% to 82%, and increased the outbreak duration from 48 days to 54.

Table 6.3. Sensitivity analyses exploring the impact of changing selected parameter values on the model results with 20% population wearing high filtration surgical masks

	Peak day	**Cumulative incidence rate, %**	**Outbreak duration (days)**
No. initial infected case			
1 symptomatic case (base case)	12	85%	48
10 symptomatic cases	9	85%	45
20 symptomatic cases	8	85%	44
30 symptomatic cases	7	85%	43
Contact rate between a susceptible and an infected			
13 contacts/day (base case)	12	85%	48
+25% from base case	9	87%	44
+50% from base case	8	88%	43
-25% from base case	16	82%	54

6.5 Discussion

Simulations revealed that a 20% compliance rate for people wearing RPDs showed some utility in reducing the spread of infection if the highest protection-factor devices (e.g. PF > 7) were deployed, but overall did have a big impact on the spread of infection due to the influenza virus. At a 50% compliance rate, however, the effect of the influenza outbreak was significantly reduced (prevalence cut by at least half) by all barriers except the low-filtration adult surgical mask. At 80% compliance, an influenza outbreak is essentially prevented by all of the RPDs except the low-filtration surgical mask. We conclude on the basis of the simulations that a roughly 50% compliance rate is recommended in order for RPDs that are likely to be used on an emergency basis for to constitute an effective countermeasure. We also conclude that low-filtration surgical masks (PF $\leq$ 2) for adults would not provide an effective countermeasure even as a high rate of compliance, consistent with the fact that the masks were not designed for that purpose.

The compliance rate for both the susceptible and infected populations was taken to be the same (20%). That doesn't necessarily imply that attention to the source and receiver played equally important roles, because the protection factor for a given barrier can be different in the incoming and outgoing directions. A fitted N95 respirator, for example, is roughly 4 times more effective in limiting the influx of influenza virus of size 2μm than it is in limiting the outgoing flux of that pathogen. The difference between source and receiver protection factors can be taken into account in devising a protection strategy. A higher protection factor can compensate for a lower degree of compliance by either the susceptible or infected populations. Increase the initial

infected cases and contract rate could result in an early outbreak and early peak day, since susceptibles will have a higher chance to inhale infectious droplets and initiate infection earlier.

The rate of infection for the pediatric population was lower than that for the adult population, owing to the lower breathing rate. The differences in immune response between adults and children was not accounted for in the model. A weaker immune system in children could be accounted for in a lower value of the infection recovery rate μ_I.

The results of the present model are potentially useful in designing a countermeasure strategy against an influenza outbreak. Providing all adults fitted N95 respirators clearly provides the highest level of protection. However, the availability of N95 respirators may be limited by financial constraints, or there may not be sufficient time to perform fitting. The high pressure differential across N95 respirators (Guha et al., 2016) may make them an infeasible choice for extended wear by individuals with difficulty breathing. The present model can be used to compute the increase in risk associated with other choices of protection. Another important application of the model is the determination of the level of compliance required for a certain level of reduction in the risk of infection by influenza. Knowing the level of compliance required, public-health officials can devise education strategies. Finally, the model described in this chapter can be used to evaluate new types of protective equipment, or existing equipment against new pathogens, in a manner that provides actual estimates of infection rate rather than just a measure of the transmission rate through the device.

Ideally, the results of the model should be validated against experimental data. Acquiring validation data is very difficult, due the inability to deliberately infect a control population. In the future, it is hoped that the model can be partially validated in a classroom or dormitory situation, with naturally infected individuals willing to commit to a regimen involving RPDs. For the present, we note that while the accuracy of the absolute predictions of infection rate is unclear, we expect that relative predictions of infection prevalence, e.g. between different levels of compliance, is likely to be more reliable.

6.6 Conclusion

A risk-assessment model previously developed in general form was used to estimate the effectiveness of different types of protective equipment in reducing the rate of infection in an influenza outbreak. It was found that a 50% compliance in donning the device resulted in a significant (at least 50% prevalence and 20% cumulative incidence) reduction in risk for fitted and unfitted N95 respirators, high-filtration surgical masks, and both low-filtration and high-filtration pediatric masks. An 80% compliance rate essentially eliminated the influenza outbreak. The results of the present study, as well as the application of the model to related influenza scenarios, are potentially useful to public-health officials in decisions involving resource allocation or education strategies.

CHAPTER 7: CONCLUSIONS AND FUTURE WORK

7.1 Conclusions

The G-II has been characterized for the ability to maintain virus infectivity and to efficiently collect submicron particles while operating at high flow rate. In the UMD EMIT study, the presence of culturable influenza virus in nearly 40% of the fine aerosol samples demonstrates that influenza cases shed infectious virus as well as RNA into airborne droplets and contributes to the biological plausibility and likely importance of airborne influenza transmission. However, cough was not a strong predictor of infectious aerosol generation suggesting an important role for other mechanisms of aerosol generation. The nasal shedding and aerosol shedding are independent, and only the viral load in NP swab but not the aerosol sample is associated with upper respiratory symptoms. The results suggest that aerosol particle samples are generated from the deep lung and are not correlated with the viral content in the upper respiratory tract. From the three climates study, environmental effects showed no influence on shedding of virus into exhaled breath, but fine aerosol fraction has significantly higher viral content than in the coarse fraction samples across different climates, which has suggested an important role for airborne transmission in temperate, subtropical and tropical areas.

To understand the spread of airborne disease and how the RPDs play an effect on epidemics, the mathematical model discussed in this dissertation deployed protective measures by dividing the susceptible population into two groups, one of which deploys RPDs and one which does not. Similarly, a fraction of the infected population utilizes

RPDs, thereby reducing the source of pathogens. The model also has the ability to track the initial dynamics of the infected population. This ability could prove useful, for example, in identifying the time interval available for medical assistance to arrive before the infected population reaches a certain size, as a function of the properties of the pathogen, the level of protection, and the characteristics of the susceptible population.

7.2 Future Work

7.2.1 Development of A New Device

The G-II collects samples in around 135 ml liquid during 30 minutes of sampling. The goal of designing an improved device, the G-III, is to collect the exhaled droplets with liquid volumes much smaller than the existing G-II device. It will alleviate the losses during cell-culture and improve the testing sensitivity. The current G-III design is the combination of a 100 L/min wetted wall cyclone and the G-II with standpipes built in (Figure 7.1). The wetted wall cyclone as the first stage will collects droplets larger than 5 μm in diameter. The cyclone collected droplets into around 3mL of aqueous fluid for 15 min and make the liquid immediately available for analysis. The peristaltic pump that is connected to the condenser drain could constantly pull out the condensing liquid on G-II over the sampling time and reduce the amount of collected liquid down to 25 ml over 15 min of collection. The collection efficiency (with reference to SKC BioSampler) of the combined collectors was evaluated with three sizes of fluorescent PSL particles, 1 μm, 3.1 μm, and 9.9 μm, respectively. Three samples were collected for each size, and the sampling time for each sample was 15 minutes. I recovered 85% of 1 μm from the collectors, for d = 3.1 μm PSL spheres, I recovered 128% from the collectors, and 115% recovery from the collectors with 9.9 μm PSL particles. The experimental results showed that the design can be used to effectively collect particles with different size.

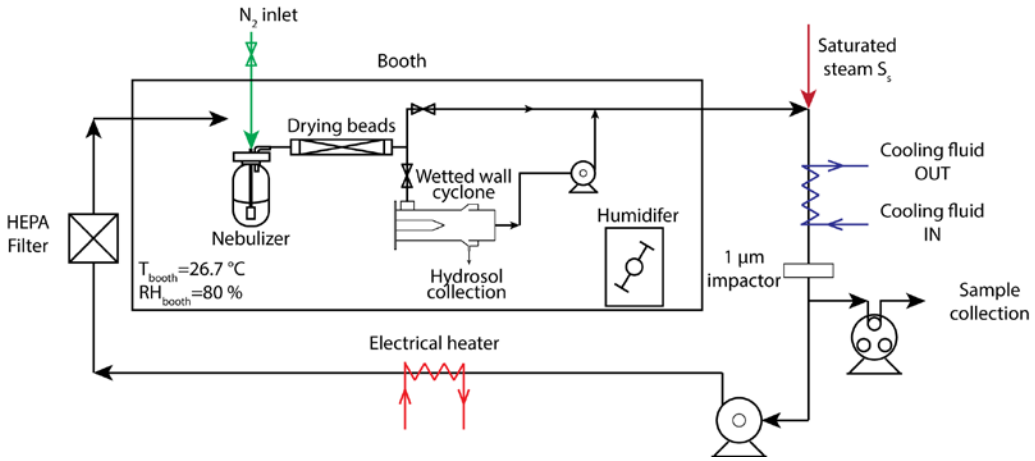

Figure 7.1 Schematic plot of the experimental design.

Future work can be done by designing a more portable device, which combines the features of both wetted wall cyclone and G-II and collects the particles in an even smaller volume of liquid. Two flow rate devices can be developed to meet different study purposes. The low flow rate (30 L/min) device can be used for biomarker discovery and testing for specific antibodies in exhaled breath air. The low flow rate design will require a mouthpiece supplied with filtered air and nose clip to specifically collect and isolate the exhaled breath air from background aerosols. The subjects will be asked to use special breathing maneuvers to increase airway closure and particle generation. Another high flow (130 L/min) version can incorporate a HEPA filtered air supplied booth in which a subject will sit facing the inlet of the collector and breath normally and shed respiratory particles and his/her personal aerosol into the booth. This high flow version will be used with microfluidic lab on a chip system and perform real-time bio-surveillance of individuals. The new designs could also be used at sites of emerging infection outbreaks to prevent the commuting issue for volunteers who participate in the flu study during their illness to give samples of their exhaled breath.

Experiments need to be conducted to test if the new designs could efficiently maintain the viability of different virus strains.

7.2.2 Modeling of Influenza Epidemics

Our study, by analyzing the modified SIR model, could give a theoretical framework for public health interventions. The results support the idea that RPD protection significantly reduces the spread of influenza via airborne transmission. The model could play an important role in planning initial intervention strategies and predicting the growth rate of an epidemic. However, the study findings were based on a deterministic model and need to be interpreted with caution. The parameter estimates were taken from the published literature and the sensitive analysis showed how the results were affected by changing the key parameter values. The model did not take into consideration pharmaceutical interventions and the air quality control in a closed environment. Vaccinating susceptible individuals could remove them from the susceptible group in the SIR model (Guha et al., 2016), and improving air quality could reduce the transmission rate by weakening the source strength (Tuomi, 1985). Future mathematical models can be performed to implement these factors in the influenza transmission mechanism. Clinical studies with human volunteers could be conducted within a university or elementary schools by recruiting naturally infected influenza cases and have them wearing protective devices to validate the results generated in this model. The process of conducting such experiments is challenging. It is hard to monitor the susceptible population and control the use of protective equipment.

In future work, use of models with further immunological details of infectious mechanisms will be a key approach (Nicas et al., 2005). It will help rationalize the criteria for effective control of disease transmission with a better understanding of the pathogenesis mechanism (Zambon, 1999). Empirical data linking the interactions of disease transmission and control is needed for further model validation. To be better prepared for an imminent influenza pandemic or the emergence of new viral infection, it is extremely important to understand the dynamics of diseases in population and communities. Reproducing epidemiological observations from public health data by translating biological, medical and social processes into mathematical models will be a critical step.

Appendix A

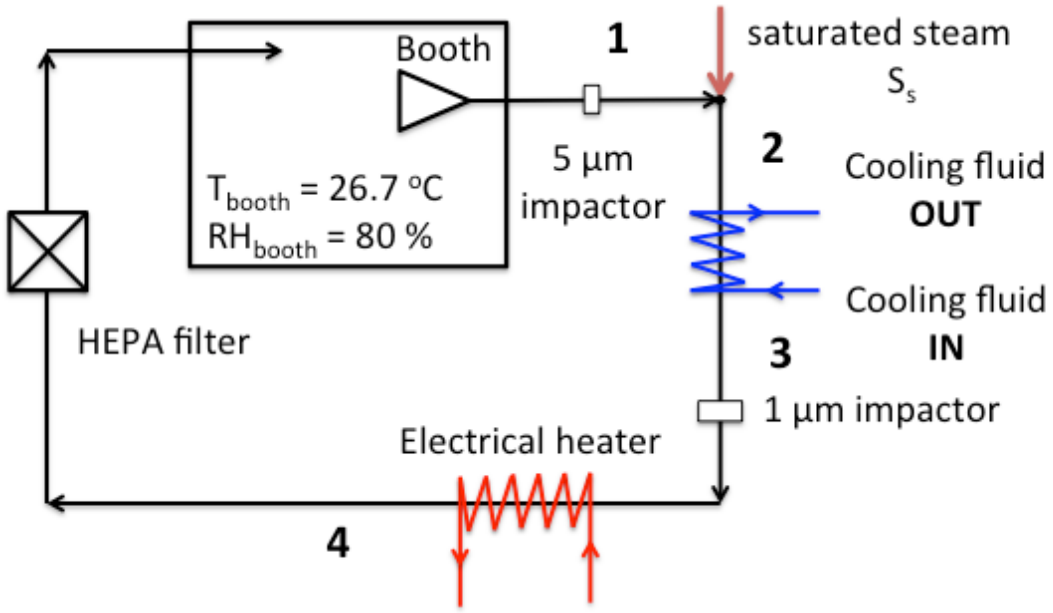

Figure A1. Schematics of the G-II.

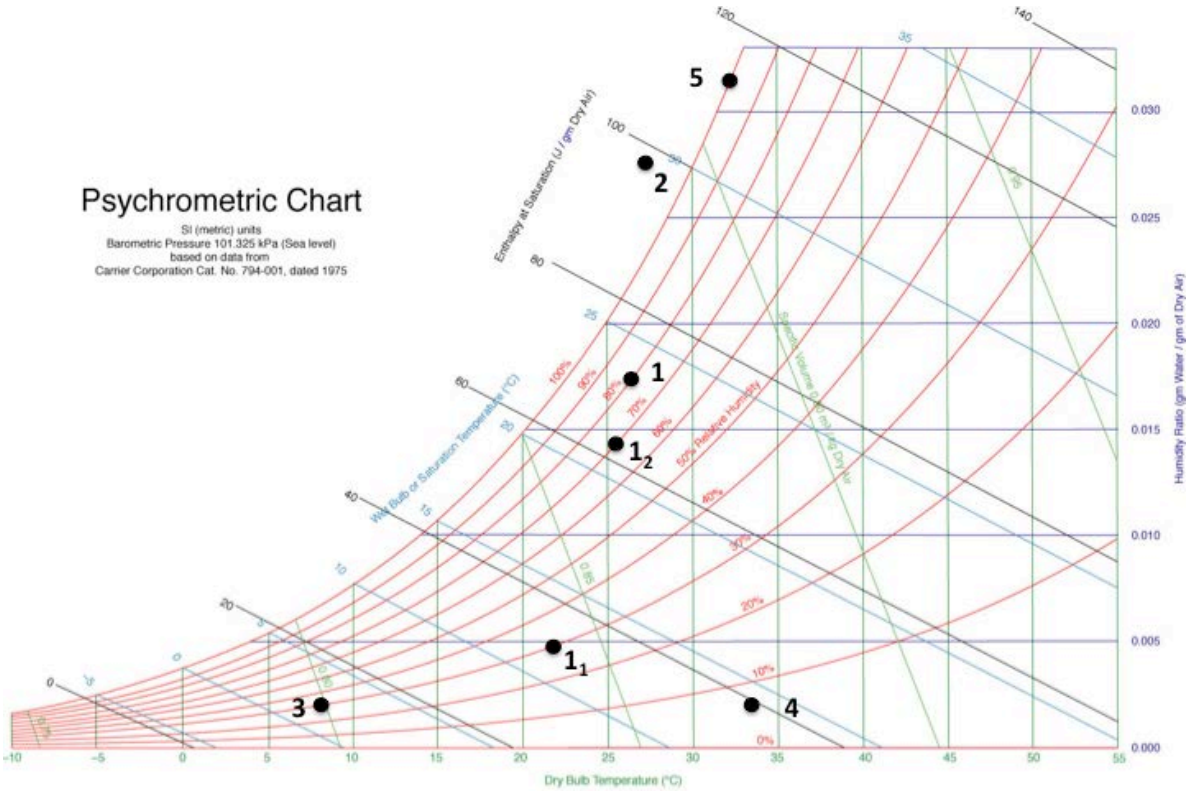

Figure A2. Psychometric states of the conditions achieved in different the G-II processes.

Table A1. Average G-II operating parameters during sample collection

	Subject 1	Subject 2	Subject 3
Booth air temperature [^{o}C]	26.6	26.7	27
Booth air humidity [%]	80	80	77
Mass flow rate of steam [kg/s]	0.000031	0.000031	0.000033
Air temperature after the condenser [^{o}C]	7.2	7.2	7.4
Condenser temperature [^{o}C]	-1.1	-1.1	-1.0

Appendix B

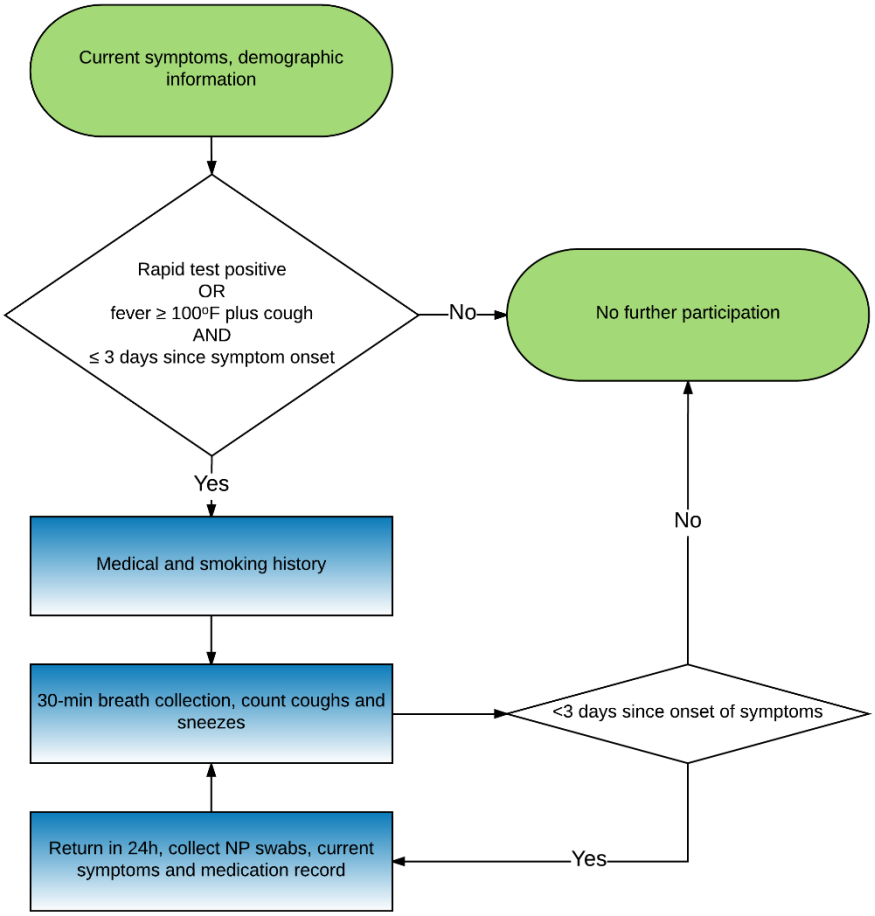

Figure B1. Screening and Sampling Protocol.

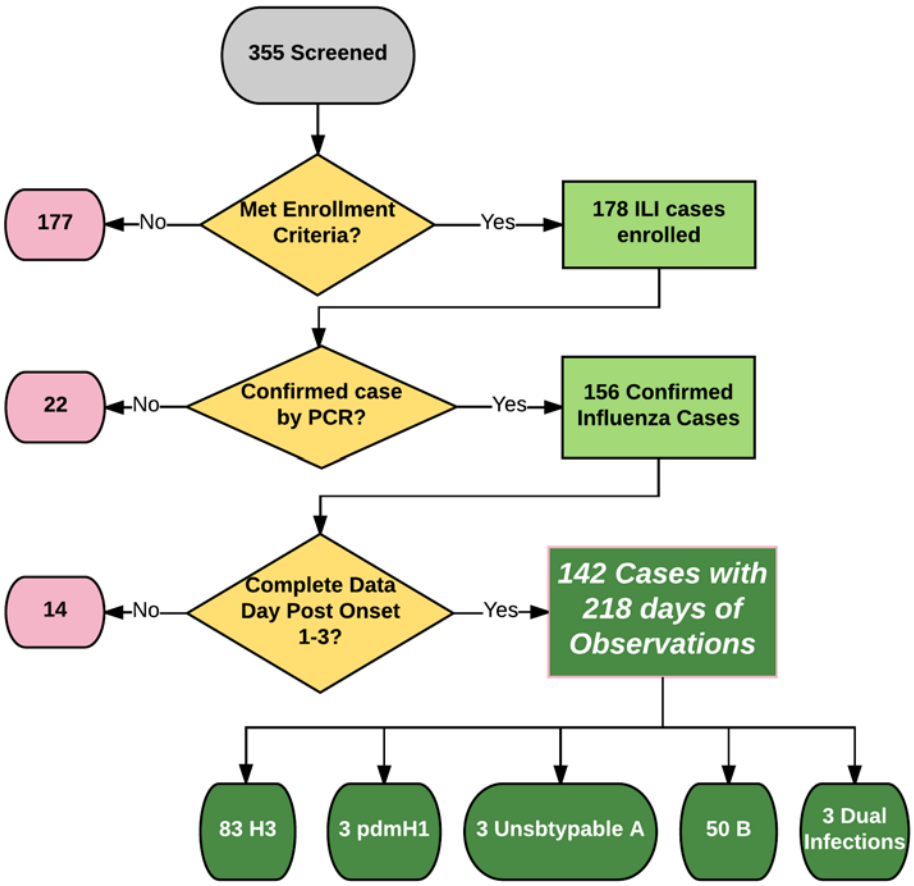

Figure B2. Screening, enrollment, exclusions, and composition of final study population.

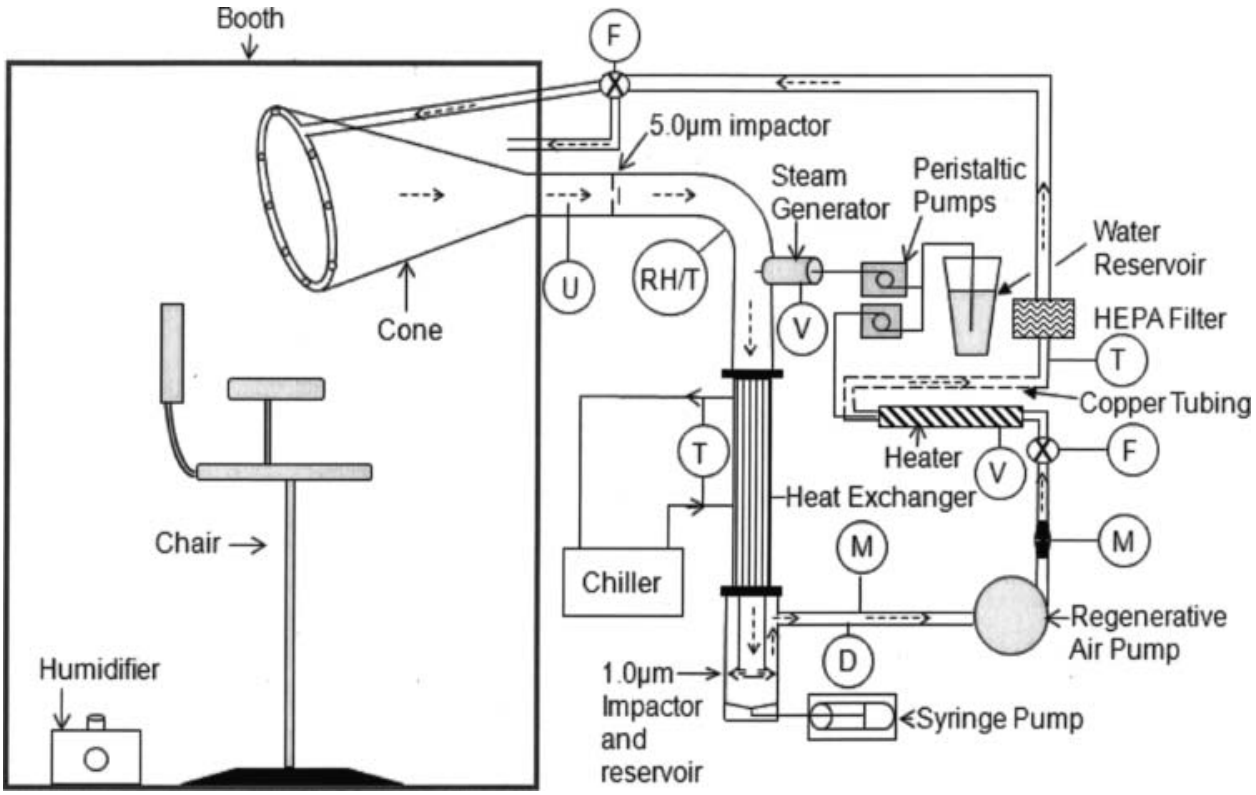

Figure B3. G-II human-source bioaerosol sampler**.**

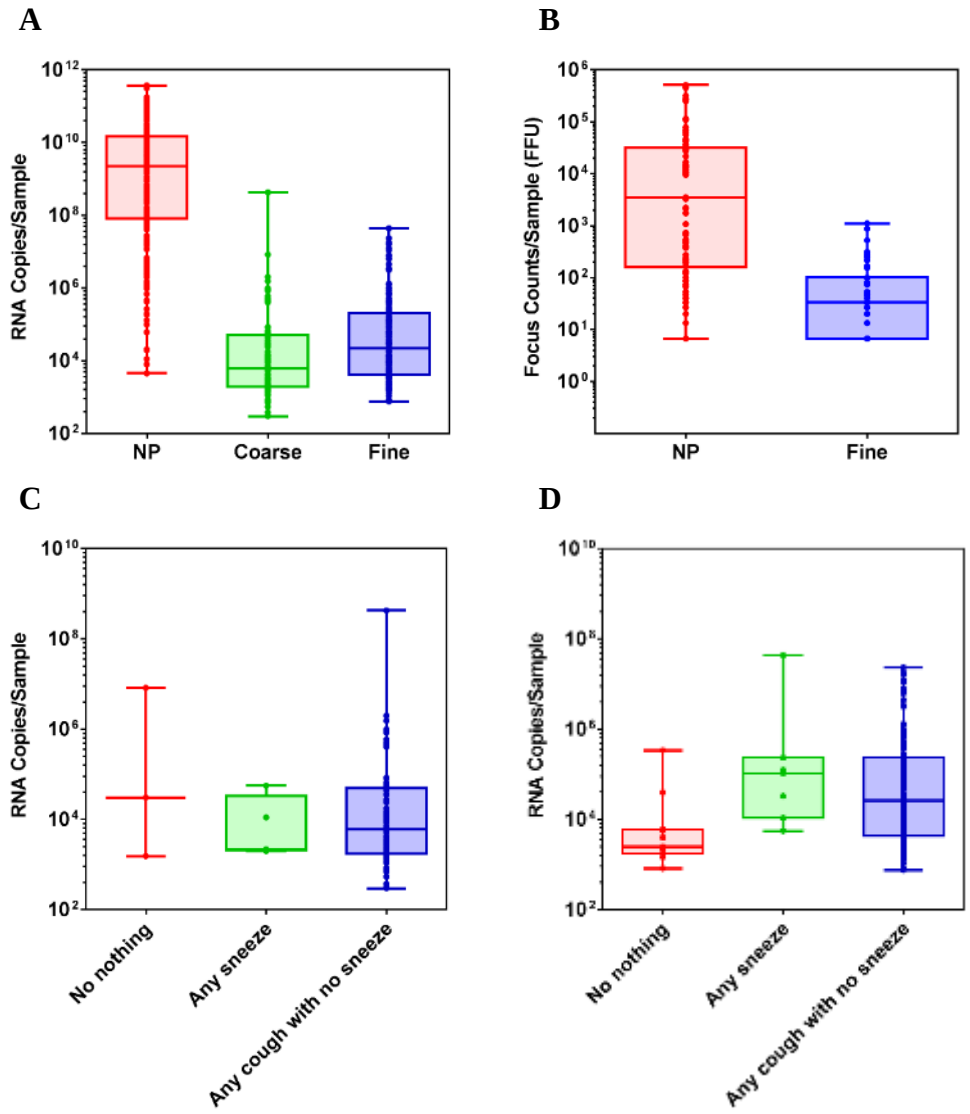

Figure B4. Viral shedding in swabs and aerosol samples and the effect of sneeze: A) RNA copies in NP swabs, coarse, and fine aerosols; B) infectious influenza virus in NP swabs and fine aerosols; C) RNA copies stratified by observed number of sneezes in coarse aerosols, and D) in fine aerosols. NP = nasopharyngeal swab, Coarse Aerosol = droplets > 5 µm and Fine Aerosol = droplets ≤ 5 µm in aerodynamic diameter.

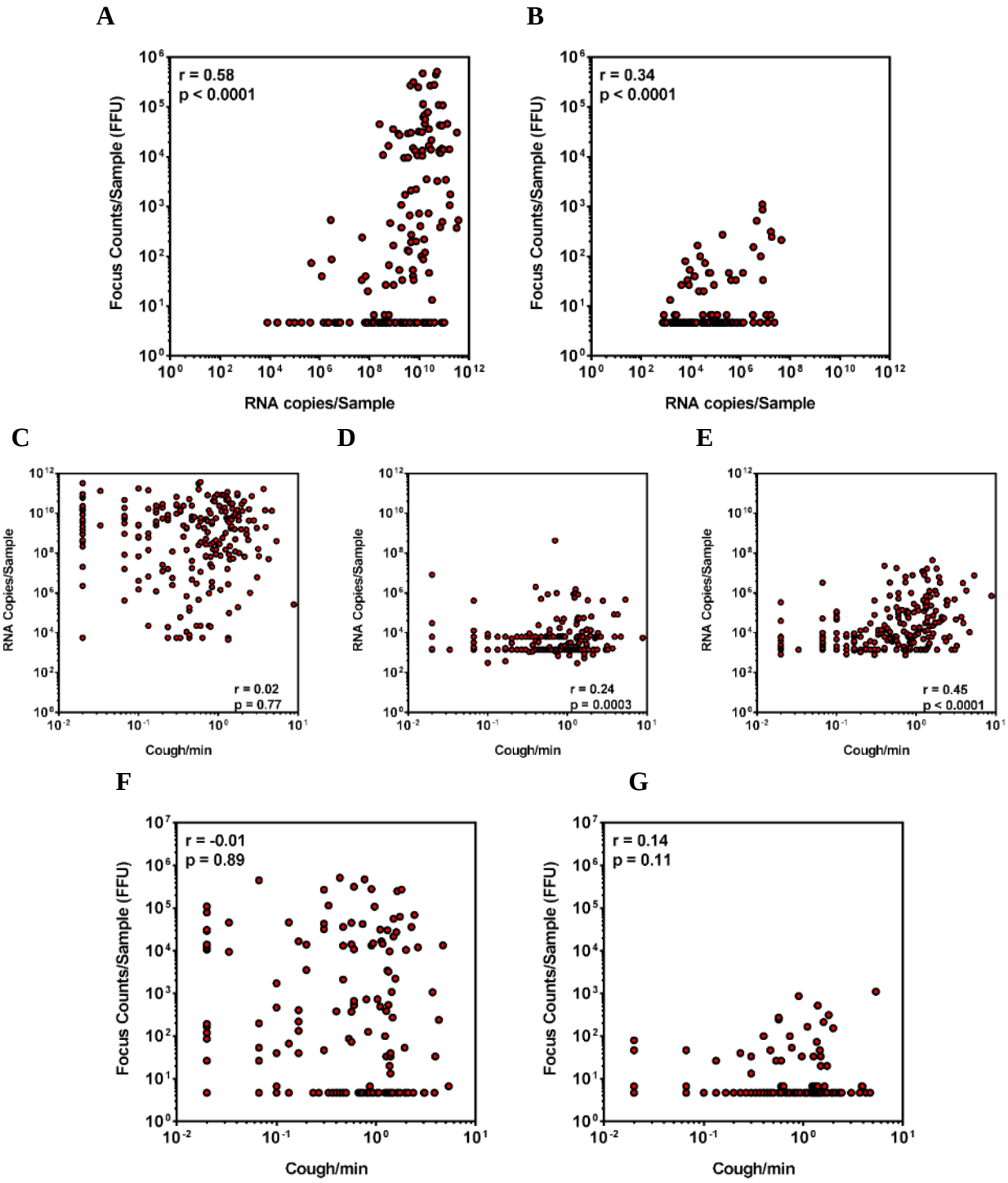

Figure B5. Scatter plots and Spearman correlation coefficients of focus counts versus RNA copies for NP (A) and fine (B) samples. Scatter plots and Spearman correlation coefficients of RNA copies versus cough/min for NP (C), coarse (D) and fine (E) samples. Scatter plots and Spearman correlation coefficients of focus counts versus cough/min for NP (F) and fine (G) samples.

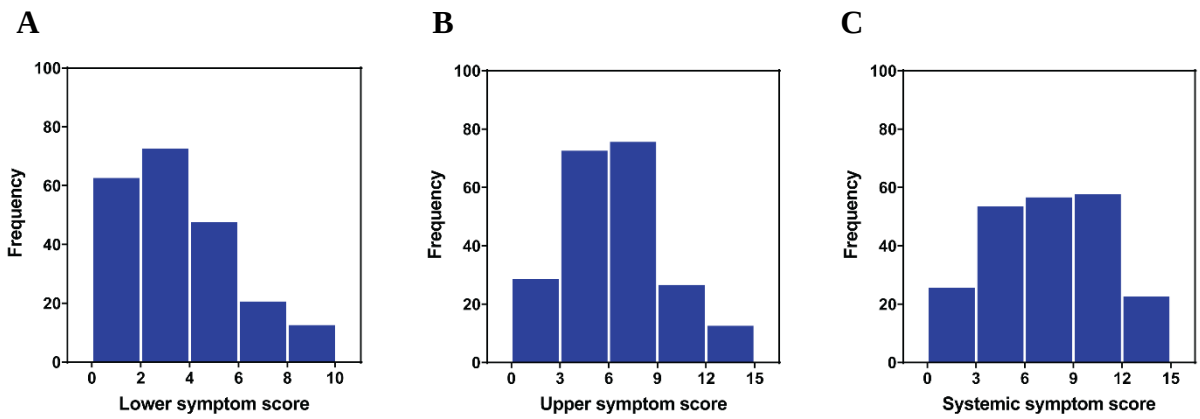

Figure B6. Histogram of symptom score A) Lower symptom B) Upper symptom C) Systemic symptom.

NP swabs were eluted in 1 mL of elution medium consisting of either phosphate buffered saline supplemented with 0.1% bovine serum albumin (PBS/0.1% BSA) or universal transport medium [UTM, Copan, Murrieta, CA]. Teflon® impactors were scrubbed using nylon Floq'd swab [Copan, Murrieta, CA] saturated with PBS/0.1% BSA. The end of the swab was cut off and placed in a tube containing 1 ml PBS/0.1% BSA. The tube was vortexed for 1 minute at full speed, to elute material from the swab, and the swab head was removed. Fine aerosol buffer samples were concentrated to 1 mL using a CentriconPlus-70 centrifugal ultrafiltration device with a nominal molecular weight cut-off of 100 KDa. All processed samples were stored either at 4 degrees until they could be analyzed for infectious virus or they were stored at –80 C until they could be analyzed for viral RNA.

RNA was extracted from each sample type using a QIAamp Minelute virus spin kit (Qiagen, Hilden, Germany) executed on a Qiacube liquid handling device. Taqman chemistry was used for the RT-qPCR assays, and Primer/probe sets designed at the US Center for Disease Control and Prevention for the detection of influenza B

and for the detection and subtyping of influenza A. Whole virion standards that had been quantitated by electron microscopy were used to generate standard curves, and those standard curves were calibrated against plasmid DNA containing the targets of either the influenza A or influenza B RT-qPCR reaction.

Virus culture of the NP swabs and fine aerosol fractions on Madin-Darby Canine kidney (MDCK) cells was used to identify samples with infectious virus. Culture was performed within 12 hours of sample collection, and cell monolayers were observed on the 4th day post-inoculation. Samples that did not exhibit cytopathic effect on the 4th day post-inoculation were transferred to fresh cell monolayers and incubated for an additional 4 days. Monolayers that exhibited cytopathic effect on either the 4th or the 8th day post-inoculation were considered infectious virus-positive. Samples with bacterial or fungal contaminants or not processed within 12 hours were rejected. Samples from the course aerosol fraction were not cultured, as they were not expected to contain infectious virus given the collection conditions of that aerosol fraction.

Infectious virus in the NP swab and the concentrated fine-particle aerosol was quantified using an immunofluorescence assay. MDCK cells were incubated with the samples at room temperature for 1 h, and then the temperature was shifted to 37 C to allow for virus entry to occur. After an hour at 37 C, medium containing fetal bovine serum was added to the culture and the cells were incubated for an additional 8 hours. The addition of the serum serves two purposes. First it helps to maintain integrity and morphology of the cells, and it limits infection to a single round of replication by inactivating extracellular trypsin in the culture that is required for the cleavage of influenza's hemagglutinin. In the absence of that cleavage event, virus particles are

not infectious. Cells were fixed with ice-cold 80% acetone, and were stained for influenza A and B nucleoprotein using primary antibodies AA5h and sc-57885 [Abcam, Cambridge UK, and Santa Cruz Biotechnoloy, Santa Cruz, CA, respectively] followed by a goat anti-mouse secondary antibody conjugated with Alexa-Fluor488 [ThermoFisher Scientific, Waltham MA]. Positive cells were counted by fluorescence microscopy and reported as fluorescent focus units in the original sample.

Appendix C

Tobit Model SAS code

```
proc import out= pcrtotal datafile = 'C:\Users\Jing\Box Sync\Box
Sync\EMIT\EMIT_Data_Analysis\UMD_SAS_input\Coarsefinenpflatform.csv'
dbms=csv replace;
GETNAMES=YES;
run;
data  pcrtotal;
set  pcrtotal ;
if fine_final_copies ~=. then logfine=log10(fine_final_copies);
if fine_final_copies  =. and typeAB='A' then logfine=log10(2000);
if fine_final_copies  =. and typeAB='B' then logfine=log10(9000);
run;quit;
data pcrtotal;
set pcrtotal ;
  if np_final_copies ~=. then lognp=log10(np_final_copies);
if np_final_copies  =. and typeAB='A' then lognp=log10(8000);
if np_final_copies  =. and typeAB='B' then lognp=log10(36000);
 run;quit;
data pcrtotal;
set pcrtotal;
if coarse_final_copies ~=. then
logcoarse=log10(coarse_final_copies);
if coarse_final_copies  =. and typeAB='A' then
logcoarse=log10(2000);
if coarse_final_copies  =. and typeAB='B' then
logcoarse=log10(9000);
 run;quit;

/* Effect of np swab PCR results on the fine and coarse samples,
Does the NP results reflect the amount of RNA copies in the exhaled
breath samples ? */
proc genmod data = pcrtotal;
  class  subject_id finesampleid ;
  model logfine= lognp/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
run;
proc nlmixed data=pcrtotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of logNP";
```

```
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&unfBeta0
beta1=&unfBeta1 ;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*lognp;
      if fine_final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2)))
* exp( -(logfine-mu)**2 / (2*sigma2) );
      if fine_final_copies = .  then ll = probnorm( (logfine - mu) /
sqrt(sigma2) );
      L=log(ll);
      model logfine~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=finesampleid(subject_id);
      run;
quit;
  proc genmod data = pcrtotal;
  class  subject_id coarsesampleid;
  model logcoarse= lognp/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('uncBeta0', COL1 );
 call symput('uncBeta1', COL2 );
run;
proc nlmixed data=pcrtotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of coarse Particles: Effect of logNP";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&uncBeta0
beta1=&uncBeta1;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j + b_1j+ beta1*lognp;
      if coarse_final_copies ne . then ll = (1 /
(sqrt(2*pi*sigma2))) * exp( -(logcoarse-mu)**2 / (2*sigma2) );
      if coarse_final_copies = .  then ll = probnorm( (logcoarse -
mu) / sqrt(sigma2) );
      L=log(ll);
      model logcoarse~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=coarsesampleid(subject_id);
      run;
quit;

proc import out= pcrtotal datafile = 'C:\Users\Jing\Box Sync\Box
Sync\EMIT\EMIT_Data_Analysis\UMD_SAS_input\finaldatasetrepeat.csv'
dbms=csv replace;
GETNAMES=YES;
```

```
run;
data  pcrtotal;
set  pcrtotal ;
if final_copies ~=. and Fine=1  then
logfinalcopies=log10(final_copies);
if final_copies  =. and Fine=1 and typeAB='A' then
logfinalcopies=log10(2000);
if final_copies  =. and Fine=1 and typeAB='B' then
logfinalcopies=log10(9000);
run;quit;
data pcrtotal;
set pcrtotal ;
  if final_copies ~=. and NPswab=1 then
logfinalcopies=log10(final_copies);
if final_copies  =. and NPswab=1 and typeAB='A' then
logfinalcopies=log10(8000);
if final_copies  =. and NPswab=1 and typeAB='B' then
logfinalcopies=log10(36000);
 run;quit;
 data pcrtotal;
set pcrtotal;
if final_copies ~=. and Coarse=1 then
logfinalcopies=log10(final_copies);
if final_copies  =. and Coarse=1 and typeAB='A' then
logfinalcopies=log10(2000);
if final_copies  =. and Coarse=1 and typeAB='B' then
logfinalcopies=log10(9000);
 run;quit;

data finetotal;
set pcrtotal;
if Fine= 0 then delete;
run;quit;
data coarsetotal;
set pcrtotal;
if Coarse= 0 then delete;
run;quit;
data nptotal;
set pcrtotal;
if NPswab= 0 then delete;
run;quit;
proc nlmixed data= finetotal XTOL=1E-12 method=GAUSS qpoints=100;
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 1 beta0=4 ;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j;
      if  final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if  final_copies = .  then ll = probnorm( (logfinalcopies -
mu) / sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
```

```
proc nlmixed data= coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 1 beta0=4 ;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j;
      if  final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if  final_copies = .  then ll = probnorm( (logfinalcopies -
mu) / sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc nlmixed data= nptotal XTOL=1E-12 method=GAUSS qpoints=100;
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 1 beta0=4 ;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j;
      if  final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if  final_copies = .  then ll = probnorm( (logfinalcopies -
mu) / sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
/*Age as continuous*/

 proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= age/ dist=poisson ;
  repeated subject = subject_id/printmle;
  ods OUTPUT  parameterestimates=params;
run;

data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('cfBeta1', COL2 );
 call symput('cfBeta0', COL1 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of age";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&cfBeta0
beta1=&cfBeta1;
```

```
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*age;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
   proc genmod data = coarsetotal ;
  class  subject_id sampleid;
  model logfinalcopies= age/dist=poisson;
  repeated subject =  subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ccBeta1', COL2 );
 call symput('ccBeta0', COL1 );
run;

proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of age";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ccBeta0
beta1=&ccBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j+ + beta1*age;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

  proc genmod data = nptotal ;
  class  subject_id sampleid;
  model logfinalcopies= age/dist=poisson;
  repeated subject =  subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
```

```
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ccBeta1', COL2 );
 call symput('ccBeta0', COL1 );
run;

proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP swab Particles: Effect of Age";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ccBeta0
beta1=&ccBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j+ + beta1*age;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
/*cough as continuous*/
 proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= cough_number/ dist=poisson ;
  repeated subject = subject_id/printmle;
  ods OUTPUT  parameterestimates=params;
run;

data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('cfBeta1', COL2 );
 call symput('cfBeta0', COL1 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of Cough";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&cfBeta0
beta1=&cfBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
```

```
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*cough_number;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
   proc genmod data = coarsetotal ;
  class  subject_id sampleid;
  model logfinalcopies= cough_number/dist=poisson;
  repeated subject =  subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ccBeta1', COL2 );
 call symput('ccBeta0', COL1 );
run;

proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of Cough";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ccBeta0
beta1=&ccBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j+ + beta1* cough_number;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

  proc genmod data = nptotal ;
  class  subject_id sampleid;
  model logfinalcopies= cough_number/dist=poisson;
  repeated subject =  subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
```

```
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ccBeta1', COL2 );
 call symput('ccBeta0', COL1 );
run;

proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP swab Particles: Effect of Cough";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ccBeta0
beta1=&ccBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j+ + beta1* cough_number;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
/*cough as categorical */
data finetotal;
set finetotal;
if 0< cough_number <30 then X1 = 1; else X1 = 0;
if cough_number GE 30 then X2 = 1; else X2 = 0;
 run;quit;
proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2/ dist=poisson ;
  repeated subject = subject_id/printmle;
  ods OUTPUT  parameterestimates=params;
run;

data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('cfBeta1', COL2 );
 call symput('cfBeta0', COL1 );
  call symput('cfBeta2', COL3 );
run;
```

```
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of Cough";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&cfBeta0
beta1=&cfBeta1 beta2=&cfBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1 +  beta2*X2 ;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data coarsetotal;
set coarsetotal;
if 0< cough_number <30 then X1 = 1; else X1 = 0;
if cough_number GE 30 then X2 = 1; else X2 = 0;
 run;quit;
   proc genmod data =coarsetotal;
  class  subject_id sampleid;
  model logfinalcopies=  X1 X2/dist=poisson;
  repeated subject =  subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('cfBeta1', COL2 );
 call symput('cfBeta0', COL1 );
  call symput('cfBeta2', COL3 );
run;

proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of Cough";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&cfBeta0
beta1=&cfBeta1 beta2=&cfBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j+ + beta1*X1 +  beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
```

```
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data nptotal;
set nptotal;
if 0< cough_number <30 then X1 = 1; else X1 = 0;
if cough_number GE 30 then X2 = 1; else X2 = 0;
 run;quit;
   proc genmod data =nptotal;
  class  subject_id sampleid;
  model logfinalcopies=  X1 X2/dist=poisson;
  repeated subject =  subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('cfBeta1', COL2 );
 call symput('cfBeta0', COL1 );
  call symput('cfBeta2', COL3 );
run;

proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of Cough";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&cfBeta0
beta1=&cfBeta1 beta2=&cfBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j+ + beta1*X1 +  beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
/* Day post onset,for fine and coarse samples */
data finetotal;
set finetotal;
 if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

 proc genmod data = finetotal ;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2/dist=poisson;
```

```
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of
day_post_onset";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1+ beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data coarsetotal;
set coarsetotal;
 if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;
  proc genmod data = coarsetotal;
  class  subject_id sampleid;
  model  logfinalcopies= X1 X2/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('cdpoBeta0', COL1 );
 call symput('cdpoBeta1', COL2 );
```

```
 call symput('cdpoBeta2', COL3 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of day post
onset";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&cdpoBeta0
beta1=&cdpoBeta1 beta2=&cdpoBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1+ beta2*X2 ;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model  logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data nptotal;
set nptotal;
 if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

 proc genmod data = nptotal ;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of
day_post_onset";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1+ beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
```

```
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
/* Effect of Asthma for NP, Fine and coarse */
 proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= asthma/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('faBeta1', COL2 );
 call symput('faBeta0', COL1 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of Asthma";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&faBeta0
beta1=&faBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*asthma;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
 proc genmod data = coarsetotal;
  class  subject_id sampleid;
  model logfinalcopies= asthma/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
```

```
run;

data _null_;
 set Paramst;
 call symput('caBeta1', COL2 );
 call symput('caBeta0', COL1 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of Asthma";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&caBeta0
beta1=&caBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*asthma;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

 proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies= asthma/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('naBeta1', COL2 );
 call symput('naBeta0', COL1 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of Asthma";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&naBeta0
beta1=&naBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*asthma;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
```

```
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
/* Effect of subtype for NP,Fine and coarse */
data finetotal;
set finetotal;
 If typeAB='A' then fineA=1;else fineA=0;
 run;quit;
 proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= fineA/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fsBeta1', COL2 );
 call symput('fsBeta0', COL1 );
run;

proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of subtypes";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fsBeta0
beta1=&fsBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*fineA;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data coarsetotal;
set coarsetotal;
 If typeAB='A' then coarseA=1;else coarseA=0;
 run;quit;
 proc genmod data = coarsetotal;
  class  subject_id sampleid;
  model logfinalcopies= coarseA/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
```

```
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('csBeta1', COL2 );
 call symput('csBeta0', COL1 );
run;

proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of subtype";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&csBeta0
beta1=&csBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*coarseA;;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data nptotal;
set nptotal;
 If typeAB='A' then npA=1;else npA=0;
 run;quit;
 proc genmod data =nptotal;
  class  subject_id sampleid;
  model logfinalcopies= npA/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('nsBeta1', COL2 );
 call symput('nsBeta0', COL1 );
run;

proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of subtypes";
```

```
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&nsBeta0
beta1=&nsBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*npA;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
/* Effect of feverishness for NP, Fine and coarse */
data finetotal;
set finetotal;
 If body_temp GE 37.8 then fever = 1; else fever = 0;
 run;quit;
 proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= fever/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ftBeta1', COL2 );
 call symput('ftBeta0', COL1 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of fever";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ftBeta0
beta1=&ftBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*fever;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
```

```
data coarsetotal;
set coarsetotal;
 If body_temp GE 37.8 then fever = 1; else fever = 0;
 run;quit;
 proc genmod data = coarsetotal ;
  class  subject_id sampleid;
  model logfinalcopies= fever/dist=poisson;
  repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ctBeta1', COL2 );
 call symput('ctBeta0', COL1 );
run;

proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of fever";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ctBeta0
beta1=&ctBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*fever;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data nptotal;
set nptotal;
 If body_temp GE 37.8 then fever = 1; else fever = 0;
 run;quit;
 proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies= fever/dist=poisson;
repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;
```

```
data _null_;
 set Paramst;
 call symput('ntBeta1', COL2 );
 call symput('ntBeta0', COL1 );
run;

proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of fever";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ntBeta0
beta1=&ntBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*fever;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;


/* Effect of body temperature as three groups for NP,Fine and
coarse*/
data finetotal;
set finetotal;
 If 37<body_temp <38 then X1 = 1; else X1 = 0;
 if body_temp GE 38   then X2=1; else X2=0;
 run;quit;
proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies=X1 X2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ftBeta1', COL2 );
 call symput('ftBeta0', COL1 );
  call symput('ftBeta2', COL3);
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of body_temp as
three catogories";
```

```
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ftBeta0
beta1=&ftBeta1 beta2=&ftBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1 + beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data coarsetotal;
set coarsetotal;
 If 37<body_temp <38 then X1 = 1; else X1 = 0;
 if body_temp GE 38   then X2=1; else X2=0;
 run;quit;
proc genmod data = coarsetotal;
  class  subject_id sampleid;
  model logfinalcopies=X1 X2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ftBeta1', COL2 );
 call symput('ftBeta0', COL1 );
  call symput('ftBeta2', COL3);
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of body_temp as
three catogories";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ftBeta0
beta1=&ftBeta1 beta2=&ftBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1 + beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
```

```
      run;
quit;

data nptotal;
set nptotal;
 If 37<body_temp <38 then X1 = 1; else X1 = 0;
 if body_temp GE 38   then X2=1; else X2=0;
 run;quit;
proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies=X1 X2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ftBeta1', COL2 );
 call symput('ftBeta0', COL1 );
  call symput('ftBeta2', COL3);
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of body_temp as
three catogories";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ftBeta0
beta1=&ftBeta1 beta2=&ftBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1 + beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/* Effect of vaccination for NP,Fine and coarse */
 proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= fluvac_cur/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;
```

```
proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fvBeta1', COL2 );
 call symput('fvBeta0', COL1 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of Vaccination";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fvBeta0
beta1=&fvBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*fluvac_cur;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
 proc genmod data = coarsetotal;
  class  subject_id sampleid;
  model logfinalcopies= fluvac_cur/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('cvBeta1', COL2 );
 call symput('cvBeta0', COL1 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of Vaccination";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&cvBeta0
beta1=&cvBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*fluvac_cur;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
```

```
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
 proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies= fluvac_cur/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('nvBeta1', COL2 );
 call symput('nvBeta0', COL1 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of Vaccination";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&nvBeta0
beta1=&nvBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*fluvac_cur;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model  logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*Study the three categories for three groups of symptom score with
the PCR results (NP, Fine, Coarse)
Set one category in each group as the reference( ß0)
Upper: x<=7, 7<x<10, x>=10
Lower: y<=3,3<y<5, x>=5
Systemic: z<=4, 4<z<7, z>=7*/

data finetotal;
set finetotal;
 If 7<upper_sym<10 then X1 = 1; else X1 = 0;
If upper_sym GE 10 then X2 = 1; else X2 = 0;
If 3<lower_sym<5 then Y1 = 1; else Y1 = 0;
If lower_sym GE 5 then Y2 = 1; else Y2 = 0;
If 4<systemic_sym<7 then Z1 = 1; else Z1 = 0;
```

```
If systemic_sym GE 7 then Z2 = 1; else Z2 = 0;
 run;quit;
proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
 call symput('fuperBeta2', COL3 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of three
catogories of upper respiratory symptoms";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1 beta2=&fuperBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1 + beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= Y1 Y2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('flowerBeta0', COL1 );
 call symput('flowerBeta1', COL2 );
```

```
 call symput('flowerBeta2', COL3 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of three
catogories of lower respiratory symptoms";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&flowerBeta0
beta1=&flowerBeta1 beta2=&flowerBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Y1 + beta2*Y2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= Z1 Z2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fsystemicBeta0', COL1 );
 call symput('fsystemicBeta1', COL2 );
 call symput('fsystemicBeta2', COL3 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of three
catogories of Systemic symptoms";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2
beta0=&fsystemicBeta0 beta1=&fsystemicBeta1 beta2=&fsystemicBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Z1 + beta2*Z2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
```

```
      run;
quit;


data coarsetotal;
set coarsetotal;
 If 7<upper_sym<10 then X1 = 1; else X1 = 0;
If upper_sym GE 10 then X2 = 1; else X2 = 0;
If 3<lower_sym<5 then Y1 = 1; else Y1 = 0;
If lower_sym GE 5 then Y2 = 1; else Y2 = 0;
If 4<systemic_sym<7 then Z1 = 1; else Z1 = 0;
If systemic_sym GE 7 then Z2 = 1; else Z2 = 0;
 run;quit;
proc genmod data = coarsetotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('cuperBeta0', COL1 );
 call symput('cuperBeta1', COL2 );
 call symput('cuperBeta2', COL3 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of coarse Particles: Effect of three
catogories of upper respiratory symptoms";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&cuperBeta0
beta1=&cuperBeta1 beta2=&cuperBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1 + beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = coarsetotal;
  class  subject_id sampleid;
  model logfinalcopies= Y1 Y2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
```

```
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('clowerBeta0', COL1 );
 call symput('clowerBeta1', COL2 );
 call symput('clowerBeta2', COL3 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of coarse Particles: Effect of three
catogories of lower respiratory symptoms";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&clowerBeta0
beta1=&clowerBeta1 beta2=&clowerBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Y1 + beta2*Y2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = coarsetotal;
  class  subject_id sampleid;
  model logfinalcopies= Z1 Z2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('csystemicBeta0', COL1 );
 call symput('csystemicBeta1', COL2 );
 call symput('csystemicBeta2', COL3 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of three
catogories of Systemic symptoms";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2
beta0=&csystemicBeta0 beta1=&csystemicBeta1 beta2=&csystemicBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
```

```
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Z1 + beta2*Z2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data nptotal;
set nptotal;
 If 7<upper_sym<10 then X1 = 1; else X1 = 0;
If upper_sym GE 10 then X2 = 1; else X2 = 0;
If 3<lower_sym<5 then Y1 = 1; else Y1 = 0;
If lower_sym GE 5 then Y2 = 1; else Y2 = 0;
If 4<systemic_sym<7 then Z1 = 1; else Z1 = 0;
If systemic_sym GE 7 then Z2 = 1; else Z2 = 0;
 run;quit;
proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('nuperBeta0', COL1 );
 call symput('nuperBeta1', COL2 );
 call symput('nuperBeta2', COL3 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of three catogories
of upper respiratory symptoms";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&nuperBeta0
beta1=&nuperBeta1 beta2=&nuperBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1 + beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
```

```
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies= Y1 Y2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('nlowerBeta0', COL1 );
 call symput('nlowerBeta1', COL2 );
 call symput('nlowerBeta2', COL3 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of three catogories
of lower respiratory symptoms";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&nlowerBeta0
beta1=&nlowerBeta1 beta2=&nlowerBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Y1 + beta2*Y2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies= Z1 Z2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
```

```
 call symput('nsystemicBeta0', COL1 );
 call symput('nsystemicBeta1', COL2 );
 call symput('nsystemicBeta2', COL3 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of three catogories
of Systemic symptoms";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2
beta0=&nsystemicBeta0 beta1=&nsystemicBeta1 beta2=&nsystemicBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Z1 + beta2*Z2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/* Effect of chiller Temperature on PCR reading*/

data finetotal;
set finetotal;
  if chiller_t GE 30 then X1=1 ; else X1 = 0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= X1 /dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of chillertemp";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*X1 ;
```

```
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*Effect of cough_number, day post onset, systemic symptom on fine
aerosol*/
data finetotal;
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies=cough_number X1 X2 systemic_sym/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of  cough_num dpo2
dpo3, systemic";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*systemic_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
```

```
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/* Effect of cough number, day post symptom on coarse fraction
samples*/
data coarsetotal;
set coarsetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = coarsetotal;
  class  subject_id sampleid ;
  model logfinalcopies=cough_number X1 X2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of  cough_num
dpo2 dpo3";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 ;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
/* effect of chiller temperature and condenser in and out
temperature for fine fraction samples*/
data finetotal;
set finetotal;
```

```
  if chiller_t GE 32 then X1=1 ; else X1 = 0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= X1 cond_tin cond_tout/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
run;
proc nlmixed data= finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of chillertemp
cond_Tin cond_Tout";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*X1 + beta2*cond_tin +
beta3*cond_tout;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
/*Effect of chiller temperature, elbow relative humidity and elbow
temperature on fine fraction samples*/
data finetotal;
set  finetotal;
  if chiller_t GE 32 then X1=1 ; else X1 = 0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= X1 elbow_rh elbow_t/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
```

```
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of chillertemp
elbow_rh elbow_t";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*X1 + beta2*elbow_rh +
beta3*elbow_t;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;


/*Study the symptom score as contineous with the PCR results (NP,
Fine, Coarse)
*/

proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= upper_sym/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
```

```
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of upper symptoms
as continuous";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*upper_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= lower_sym/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of lower symptoms
as continuous";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*lower_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = finetotal;
```

```
  class  subject_id sampleid;
  model logfinalcopies= systemic_sym/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of Systemic
symptoms as continuous";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*systemic_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

proc genmod data = coarsetotal;
  class  subject_id sampleid;
  model logfinalcopies= upper_sym/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
```

```
Title "Tobit Regression of Coarse Particles: Effect of upper
symptoms as continuous";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*upper_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = coarsetotal;
  class  subject_id sampleid;
  model logfinalcopies= lower_sym/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of lower
symptoms as continuous";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*lower_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = coarsetotal;
  class  subject_id sampleid;
```

```
  model logfinalcopies= systemic_sym/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of Systemic
symptoms as continuous";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*systemic_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies= upper_sym/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of upper symptoms as
continuous";
```

```
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*upper_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies= lower_sym/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of lower symptoms as
continuous";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*lower_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies= systemic_sym/dist=poisson;
 repeated subject = subject_id/ printmle;
```

```
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of Systemic symptoms
as continuous";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*systemic_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data finecoarse;
set pcrtotal;
if NPswab= 1 then delete;
run;quit;
proc genmod data = finecoarse;
  class  subject_id sampleid;
  model logfinalcopies= Coarse/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=finecoarse XTOL=1E-12 method=GAUSS qpoints=100;
```

```
Title "Tobit Regression of Fine and Coarse logviruscopies
comparison";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Coarse;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data finecoarse;
set  finecoarse;
  if Coarse=1 then X1=1 ; else X1 = 0;
 run;quit;
proc genmod data = finecoarse;
  class  subject_id sampleid;
  model logfinalcopies= X1/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=finecoarse XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine and Coarse logviruscopies
comparison";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
```

```
      run;
quit;

/*Within flu A, compare virus copy number of fine and coarse*/
data finecoarseA;
set pcrtotal;
if NPswab= 1 | typeAB='B'  then delete;
run;quit;
proc genmod data = finecoarseA;
  class  subject_id sampleid;
  model logfinalcopies= Coarse/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=finecoarseA XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine and Coarse logviruscopies
comparison";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Coarse;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*Within flu B, compare virus copy number of fine and coarse*/

data finecoarseB;
set pcrtotal;
if NPswab= 1 | typeAB='A'  then delete;
run;quit;
proc genmod data = finecoarseB;
  class  subject_id sampleid;
  model logfinalcopies= Coarse/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
```

```
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fuperBeta0', COL1 );
 call symput('fuperBeta1', COL2 );
run;
proc nlmixed data=finecoarseB XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine and Coarse logviruscopies
comparison";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1.2 beta0=&fuperBeta0
beta1=&fuperBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Coarse;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*Effect of BMI as continuous*/

 proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= BMI/ dist=poisson ;
  repeated subject = subject_id/printmle;
  ods OUTPUT  parameterestimates=params;
run;

data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('cfBeta1', COL2 );
 call symput('cfBeta0', COL1 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of BMI";
```

```
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&cfBeta0
beta1=&cfBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*BMI;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
   proc genmod data = coarsetotal ;
  class  subject_id sampleid;
  model logfinalcopies= BMI/dist=poisson;
  repeated subject =  subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ccBeta1', COL2 );
 call symput('ccBeta0', COL1 );
run;

proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of BMI";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ccBeta0
beta1=&ccBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j+ + beta1* BMI;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

  proc genmod data = nptotal ;
  class  subject_id sampleid;
  model logfinalcopies= BMI/dist=poisson;
```

```
  repeated subject =  subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ccBeta1', COL2 );
 call symput('ccBeta0', COL1 );
run;

proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP swab Particles: Effect of BMI";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ccBeta0
beta1=&ccBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j+ + beta1* BMI;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*BMI as catogorical*/
data finetotal;
set finetotal;
if BMI>25 then X1 = 1; else X1 = 0;
 run;quit;
proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= X1/ dist=poisson ;
  repeated subject = subject_id/printmle;
  ods OUTPUT  parameterestimates=params;
run;

data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('cfBeta1', COL2 );
```

```
 call symput('cfBeta0', COL1 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of BMI";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&cfBeta0
beta1=&cfBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data coarsetotal;
set coarsetotal;
if BMI>25 then X1 = 1; else X1 = 0;
 run;quit;
   proc genmod data = coarsetotal ;
  class  subject_id sampleid;
  model logfinalcopies= X1/dist=poisson;
  repeated subject =  subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ccBeta1', COL2 );
 call symput('ccBeta0', COL1 );
run;

proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of BMI";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ccBeta0
beta1=&ccBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j+ + beta1* X1;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
```

```
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data nptotal;
set nptotal;
if BMI>25 then X1 = 1; else X1 = 0;
 run;quit;
  proc genmod data = nptotal ;
  class  subject_id sampleid;
  model logfinalcopies= X1/dist=poisson;
  repeated subject =  subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ccBeta1', COL2 );
 call symput('ccBeta0', COL1 );
run;

proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP swab Particles:Effect of BMI ";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ccBeta0
beta1=&ccBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j+ + beta1* X1;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;


 /*Within NP swab, study the effect of lower upper and systemic
symptoms*/

 proc genmod data = nptotal ;
  class  subject_id sampleid;
  model logfinalcopies= lower_sym upper_sym
systemic_sym/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
```

```
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
  call symput('fdpoBeta3', COL4 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of lower upper and
systemic";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2 beta3=&fdpoBeta3;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*lower_sym + beta2*upper_sym +
beta3*systemic_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*Study the effect of gender, Male is 1*/
proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= sex/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ftBeta1', COL2 );
 call symput('ftBeta0', COL1 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: sex";
```

```
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ftBeta0
beta1=&ftBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*sex;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
 proc genmod data = coarsetotal ;
  class  subject_id sampleid;
  model logfinalcopies= sex/dist=poisson;
  repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ctBeta1', COL2 );
 call symput('ctBeta0', COL1 );
run;

proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: sex";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ctBeta0
beta1=&ctBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*sex;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
 proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies=sex/dist=poisson;
repeated subject = subject_id/ printmle;
```

```
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ntBeta1', COL2 );
 call symput('ntBeta0', COL1 );
run;

proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: sex";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ntBeta0
beta1=&ntBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*sex;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*Study the effect of smoking*/
proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= Smoker/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ftBeta1', COL2 );
 call symput('ftBeta0', COL1 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Smoker";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ftBeta0
beta1=&ftBeta1;
```

```
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Smoker;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
 proc genmod data = coarsetotal ;
  class  subject_id sampleid;
  model logfinalcopies= Smoker/dist=poisson;
  repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ctBeta1', COL2 );
 call symput('ctBeta0', COL1 );
run;

proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Smoker";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ctBeta0
beta1=&ctBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Smoker;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
 proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies=Smoker/dist=poisson;
repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
```

```
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ntBeta1', COL2 );
 call symput('ntBeta0', COL1 );
run;

proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Smoker";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ntBeta0
beta1=&ntBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*Smoker;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*Study the effect of anitiviral medication taken within 24 hours*/

proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= anitviral_24h/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ftBeta1', COL2 );
 call symput('ftBeta0', COL1 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Antiviral medication";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ftBeta0
beta1=&ftBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
```

```
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*anitviral_24h;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
 proc genmod data = coarsetotal ;
  class  subject_id sampleid;
  model logfinalcopies= anitviral_24h/dist=poisson;
  repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ctBeta1', COL2 );
 call symput('ctBeta0', COL1 );
run;

proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: anitviral_24h";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ctBeta0
beta1=&ctBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*anitviral_24h;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
 proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies=anitviral_24h/dist=poisson;
repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
```

```
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ntBeta1', COL2 );
 call symput('ntBeta0', COL1 );
run;

proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: anitviral_24h";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ntBeta0
beta1=&ntBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*anitviral_24h;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;


/*For fine model, take cough_number day post symptom and systemic
symptom and gender */

data finetotal;
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 systemic_sym
sex/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
```

```
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
  call symput('unfBeta5', COL6 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 Systemic_sym SEX ";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4
beta5=&unfBeta5;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*systemic_sym + beta5*sex;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*For coarse model, take cough_number day post onset and gender*/

data coarsetotal;
set coarsetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = coarsetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 sex/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
```

```
  call symput('unfBeta5', COL6 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of  cough_num
dpo2 dpo3 SEX";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*For NP model, take lower upper and systemic symptom score and
gender*/

proc genmod data = nptotal ;
  class  subject_id sampleid;
  model logfinalcopies= lower_sym upper_sym systemic_sym
sex/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
 call symput('fdpoBeta3', COL4 );
 call symput('fdpoBeta4', COL5 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of lower upper
systemic sex";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2 beta3=&fdpoBeta3 beta4=&fdpoBeta4;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*lower_sym + beta2*upper_sym +
beta3*systemic_sym + beta4*sex;
```

```
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*For fine model, take cough_number day post onset and gender*/

data finetotal;
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 sex/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 SEX ";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
```

```
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*For fine model, take cough_number day post onset,gender and
interaction between gender and cough number*/
data finetotal;
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 sex
sex*cough_number/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
 call symput('unfBeta5', COL6 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 SEX SEX*COUGH";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4
beta5=&unfBeta5;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex +beta5*sex*cough_number;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
```

```
/*For fine model, take cough_number day post onset, gender,systemic
symptom and interaction between gender and cough number*/

data finetotal;
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 sex systemic_sym
sex*cough_number/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
 call symput('unfBeta5', COL6 );
  call symput('unfBeta6', COL7 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 SEX systemic SEX*COUGH";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4
beta5=&unfBeta5 beta6=&unfBeta6;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex + beta5*systemic_sym + beta6*sex*cough_number;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;


data finetotal;
```

```
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 systemic_sym
sex*cough_number/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
 call symput('unfBeta5', COL6 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 systemic SEX*COUGH";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4
beta5=&unfBeta5;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*systemic_sym + beta5*sex*cough_number;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;



/*For fine model, take cough_number, day post onset, gender, and
interaction between gender and day post onset*/


data finetotal;
```

```
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 sex sex*X1
sex*X2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
 call symput('unfBeta5', COL6 );
  call symput('unfBeta6', COL7 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 SEX SEX*dpo2 sex*dpo3";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4
beta5=&unfBeta5 beta6=&unfBeta6;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex +beta5*sex*X1 +beta6*sex*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*For fine model, take cough_number, day post onset, gender,
interaction between gender and day post onset and interaction
between gender and cough number*/

data finetotal;
set finetotal;
```

```
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 sex*cough_number sex*X1
sex*X2/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
 call symput('unfBeta5', COL6 );
  call symput('unfBeta6', COL7 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 SEX*cough SEX*dpo2 sex*dpo3";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4
beta5=&unfBeta5 beta6=&unfBeta6;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex*cough_number +beta5*sex*X1 +beta6*sex*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data finetotal;
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = finetotal;
```

```
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 sex*cough_number sex*X1
sex*X2 sex /dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
 call symput('unfBeta5', COL6 );
  call symput('unfBeta6', COL7 );
    call symput('unfBeta7', COL8 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 SEX*cough SEX*dpo2 sex*dpo3 sex";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4
beta5=&unfBeta5 beta6=&unfBeta6 beta7=&unfBeta7;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex*cough_number +beta5*sex*X1 +beta6*sex*X2 +
beta7*sex;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*For coarse model, take cough_number, day post onset, gender, and
interaction between gender and cough number*/

data coarsetotal;
set coarsetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

 proc genmod data = coarsetotal;
```

```
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 sex
sex*cough_number/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
 call symput('unfBeta5', COL6 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of cough_num
dpo2 dpo3 SEX  SEX*COUGH";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4
beta5=&unfBeta5;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex + beta5*sex*cough_number;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*For Coarse model, take cough_number, day post onset, gender, and
interaction between gender and day post onset*/

data coarsetotal;
set coarsetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

 proc genmod data = coarsetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 sex sex*X1
sex*X2/dist=poisson;
```

```
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
 call symput('unfBeta5', COL6 );
  call symput('unfBeta6', COL7 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of cough_num
dpo2 dpo3 SEX  SEX*dpo2 SEX*dpo3";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4
beta5=&unfBeta5 beta6=&unfBeta6;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex + beta5*sex*X1 + beta6*sex*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/*For fine model, take cough_number, day post onset, gender, and
interaction between gender and day post onset*/


data finetotal;
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2
sex*cough_number/dist=poisson;
 repeated subject = subject_id/ printmle;
```

```
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 SEX*cough";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex*cough_number;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
data finetotal;
set finetotal;
 If body_temp GE 37.8 then fever = 1; else fever = 0;
 run;quit;

 proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= fever upper_sym fever*upper_sym
/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
```

```
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of fever upper_sym
fever*upper_sym";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*fever + beta2*upper_sym +
beta3*fever*upper_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data nptotal;
set nptotal;
 If body_temp GE 37.8 then fever = 1; else fever = 0;
 run;quit;

 proc genmod data = nptotal;
  class  subject_id sampleid ;
  model logfinalcopies= fever upper_sym fever*upper_sym
/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of fever upper_sym
fever*upper_sym";
```

```
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*fever + beta2*upper_sym +
beta3*fever*upper_sym;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

/* test for A/B if the day post onset play an effect*/
data fineAtotal;
set finetotal;
if typeAB= 'B' then delete;
run;quit;

data fineAtotal;
set fineAtotal;
 if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

 proc genmod data = fineAtotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
run;
proc nlmixed data=fineAtotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles with flu A infection:
Effect of day_post_onset";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1+ beta2*X2;
```

```
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data fineBtotal;
set finetotal;
if typeAB= 'A' then delete;
run;quit;

data fineBtotal;
set fineBtotal;
 if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

 proc genmod data = fineBtotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
run;
proc nlmixed data=fineBtotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles with flu B infection:
Effect of day_post_onset";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1+ beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
```

```
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;


data coarseAtotal;
set coarsetotal;
if typeAB= 'B' then delete;
run;quit;

data coarseAtotal;
set coarseAtotal;
 if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

 proc genmod data = coarseAtotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
run;
proc nlmixed data=coarseAtotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of coarse Particles with flu A infection:
Effect of day_post_onset";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1+ beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data coarseBtotal;
```

```
set coarsetotal;
if typeAB= 'A' then delete;
run;quit;

data coarseBtotal;
set coarseBtotal;
 if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

 proc genmod data = coarseBtotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
run;
proc nlmixed data=coarseBtotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of coarse Particles with flu B infection:
Effect of day_post_onset";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1+ beta2*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;



data finetotal;
set finetotal;
 if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
if typeAB='A' then X3=1; else X3=0;
 run;quit;
```

```
 proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2 X3/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
 call symput('fdpoBeta3', COL4 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles : Effect of day_post_onset
AND TYPE AB";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2 beta3=&fdpoBeta3;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1+ beta2*X2 + beta3*X3;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data coarsetotal;
set coarsetotal;
 if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
if typeAB='A' then X3=1; else X3=0;
 run;quit;

 proc genmod data = coarsetotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2 X3/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;
```

```
proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
 call symput('fdpoBeta3', COL4 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles : Effect of
day_post_onset AND TYPE AB";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2 beta3=&fdpoBeta3;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1+ beta2*X2 + beta3*X3;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data finetotal;
set finetotal;
 if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
if typeAB='A' then X3=1; else X3=0;
 run;quit;

 proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2 X3 X3*X1 X3*X2/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
 call symput('fdpoBeta3', COL4 );
```

```
 call symput('fdpoBeta4', COL5 );
  call symput('fdpoBeta5', COL6 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles : Effect of day_post_onset
AND TYPE AB";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2 beta3=&fdpoBeta3 beta4=&fdpoBeta4
beta5=&fdpoBeta5;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1+ beta2*X2 + beta3*X3
+beta4*X3*X1 + beta5*X3*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data coarsetotal;
set coarsetotal;
 if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
if typeAB='A' then X3=1; else X3=0;
 run;quit;

 proc genmod data = coarseftotal;
  class  subject_id sampleid;
  model logfinalcopies= X1 X2 X3 X3*X1 X3*X2/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
 call symput('fdpoBeta3', COL4 );
 call symput('fdpoBeta4', COL5 );
  call symput('fdpoBeta5', COL6 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles : Effect of
day_post_onset AND TYPE AB";
```

```
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2 beta3=&fdpoBeta3 beta4=&fdpoBeta4
beta5=&fdpoBeta5;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*X1+ beta2*X2 + beta3*X3
+beta4*X3*X1 + beta5*X3*X2;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;



data finetotal;
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;
 proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 BMI/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 BMI";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*BMI;
```

```
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data coarsetotal;
set coarsetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = coarsetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 BMI/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
  call symput('unfBeta5', COL6 );
run;
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: Effect of  cough_num
dpo2 dpo3 BMI";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*BMI;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
```

```
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data finetotal;
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies= cough_number X1 X2 sex*cough_number
BMI/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
 call symput('unfBeta5', COL6 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 SEX*cough BMI";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4
beta5=&unfBeta5;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex*cough_number +beta5*BMI;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
```

```
/* Effect of body temperature as continuous for NP,Fine and coarse
*/
 proc genmod data = finetotal;
  class  subject_id sampleid;
  model logfinalcopies= body_temp/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ftBeta1', COL2 );
 call symput('ftBeta0', COL1 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Fine Particles: Effect of fever";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ftBeta0
beta1=&ftBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*body_temp;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
 proc genmod data = coarsetotal ;
  class  subject_id sampleid;
  model logfinalcopies= body_temp/dist=poisson;
  repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ctBeta1', COL2 );
 call symput('ctBeta0', COL1 );
run;
```

```
proc nlmixed data=coarsetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of Coarse Particles: body_temp as
continuous";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ctBeta0
beta1=&ctBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*body_temp;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
 proc genmod data = nptotal;
  class  subject_id sampleid;
  model logfinalcopies=body_temp/dist=poisson;
repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('ntBeta1', COL2 );
 call symput('ntBeta0', COL1 );
run;

proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: body_temp as continuous";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&ntBeta0
beta1=&ntBeta1;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*body_temp;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
```

```
proc genmod data = nptotal ;
  class  subject_id sampleid;
  model logfinalcopies= lower_sym upper_sym systemic_sym
age/dist=poisson;
   repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('fdpoBeta0', COL1 );
 call symput('fdpoBeta1', COL2 );
 call symput('fdpoBeta2', COL3 );
 call symput('fdpoBeta3', COL4 );
 call symput('fdpoBeta4', COL5 );
run;
proc nlmixed data=nptotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of NP Particles: Effect of lower upper
systemic age";
      parms sigma2_u= 1 sigma2_u1= 1 sigma2= 0.1 beta0=&fdpoBeta0
beta1=&fdpoBeta1 beta2=&fdpoBeta2 beta3=&fdpoBeta3 beta4=&fdpoBeta4;
      bounds sigma2_u sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j +b_1j + beta1*lower_sym + beta2*upper_sym +
beta3*systemic_sym + beta4*age;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies-mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;

data finetotal;
set finetotal;
   if dpo=2 then X1=1;else X1=0;
if dpo=3 then X2=1; else X2=0;
 If typeAB='A' then fineA=1;else fineA=0;
 if typeAB='B' then fineB=1;else fineB=0;
 run;quit;

  proc genmod data = finetotal;
  class  subject_id sampleid ;
  model logfinalcopies=  cough_number X1 X2 sex*cough_number BMI
fineA*fluvac_cur fineB*fluvac_cur/dist=poisson;
 repeated subject = subject_id/ printmle;
  ods OUTPUT  parameterestimates=params;
```

```
run;
data Params;
set Params (keep=Parameter Estimate);
run;

proc transpose data=Params out=Paramst;
run;

data _null_;
 set Paramst;
 call symput('unfBeta0', COL1 );
 call symput('unfBeta1', COL2 );
 call symput('unfBeta2', COL3 );
 call symput('unfBeta3', COL4 );
 call symput('unfBeta4', COL5 );
 call symput('unfBeta5', COL6 );
 call symput('unfBeta6', COL7 );
  call symput('unfBeta7', COL8 );
run;
proc nlmixed data=finetotal XTOL=1E-12 method=GAUSS qpoints=100;
Title "Tobit Regression of fine Particles: Effect of cough_num dpo2
dpo3 SEX*cough BMI subtype*vaccination";
      parms sigma2_u= 1 sigma2_u1=1 sigma2= 1 beta0=&unfBeta0
beta1=&unfBeta1 beta2=&unfBeta2 beta3=&unfBeta3 beta4=&unfBeta4
beta5=&unfBeta5 beta6=&unfBeta6 beta7=&unfBeta7;
      bounds sigma2_u  sigma2_u1 sigma2 >= 0;
      pi = constant('pi');
      mu = beta0 + b_0j+ b_1j + beta1*cough_number + beta2*X1 +
beta3*X2 + beta4*sex*cough_number +beta5*BMI +
beta6*fineA*fluvac_cur + beta7*fineB*fluvac_cur;
      if final_copies ne . then ll = (1 / (sqrt(2*pi*sigma2))) *
exp( -(logfinalcopies - mu)**2 / (2*sigma2) );
      if final_copies = .  then ll = probnorm( (logfinalcopies - mu)
/ sqrt(sigma2) );
      L=log(ll);
      model logfinalcopies~ general(L);
      random b_0j ~ normal(0,sigma2_u) subject=subject_id;
      random b_1j~ normal(0, sigma2_u1)
subject=sampleid(subject_id);
      run;
quit;
```

Appendix D

Bimodal Distribution Analysis

$$\frac{d}{dt}\hat{S}_r = -(\tilde{\beta}_{r,1}\widehat{D}_1 + \tilde{\beta}_{r,2}\widehat{D}_2)\hat{S}_r \quad \text{A.(1a)}$$

$$\frac{d}{dt}\hat{S}_{nr} = -(\tilde{\beta}_{nr,1}\widehat{D}_1 + \tilde{\beta}_{nr,2}\widehat{D}_2)\hat{S}_{nr} \quad \text{A.(1b)}$$

$$\frac{d}{dt}\hat{I} = -\frac{d}{dt}(\hat{S}_r + \hat{S}_{nr}) - \mu_I\hat{I} \quad \text{A.(1c)}$$

$$\frac{d}{dt}\widehat{D}_1 = \kappa_{w,1}\hat{I} - \frac{1}{\nu_1}\widehat{D}_1 \ , \ \frac{d}{dt}\widehat{D}_2 = \kappa_{w,2}\hat{I} - \frac{1}{\nu_2}\widehat{D}_2 \quad \text{A.(1d)}$$

where

$$\hat{S}_r = S_r/N \quad \text{A.(1e)}$$

$$\hat{S}_{nr} = S_{nr}/N \quad \text{A.(1f)}$$

$$\hat{I} = I/N \quad \text{A.(1g)}$$

$$\widehat{D} = D/N \quad \text{A.(1h)}$$

The boundary conditions are:

$$\hat{S}_r(0) = \hat{S}_{r,0} = S_{r,0}/N \quad \text{A.(1i)}$$

$$\hat{S}_{nr}(0) = \hat{S}_{nr,0} = S_{nr,0}/N$$ A.(1j)

$$\hat{I}(0) = \hat{I}_0 = I_0/N$$ A. (1k)

$$\hat{D}(0) = 0$$ A.(1l)

Polydispersity Analysis Equations

$$\frac{d}{dt}\hat{S}_r = -(\tilde{\beta}_{r,1}\hat{D}_1 + \tilde{\beta}_{r,2}\hat{D}_2 + \cdots \tilde{\beta}_{r,j}\hat{D}_j)\,\hat{S}_r$$ B.(1a)

$$\frac{d}{dt}\hat{S}_{nr} = -(\tilde{\beta}_{nr,1}\hat{D}_1 + \tilde{\beta}_{nr,2}\hat{D}_2 + \cdots \tilde{\beta}_{nr,j}\hat{D}_j)\,\hat{S}_{nr}$$ B. (2b)

$$\frac{d}{dt}\hat{I} = -\frac{d}{dt}(\hat{S}_r + \hat{S}_{nr}) - \mu_I\hat{I}$$ B. (2c)

$$\frac{d}{dt}\hat{D}_1 = \kappa_{w,1}\,\hat{I} - \frac{1}{\nu_1}\hat{D}_1 \;,\; \frac{d}{dt}\hat{D}_2 = \kappa_{w,2}\,\hat{I} - \frac{1}{\nu_2}\hat{D}_2 \;,\cdots, \frac{d}{dt}\hat{D}_j = \kappa_{w,j}\,\hat{I} - \frac{1}{\nu_j}\hat{D}_j$$ B. (2d)

where

$$\tilde{\beta}_{r,j} = T_{in,j}\,c\,\frac{B}{V_{cl}}\,\tau p q_i N_{p,j}$$ B. (2e)

$$\tilde{\beta}_{nr,j} = c\,\frac{B}{V_{cl}}\,\tau p q_j N_{p,j}$$ B. (2f)

$$\kappa_{w,j} = \kappa_{r,j}\,f_i + \kappa_{nr,j}\,(1 - f_i)$$ B. (2g)

$$v^{-1}{}_j = (1 + c\tau)\frac{B}{V_{cl}}q_j + \mu_d + \theta_j \qquad \text{B. (2h)}$$

We consider boundary conditions analogous to those for the monodisperse case:

$$\hat{S}_r(0) = \hat{S}_{r0} \qquad \text{B. (2i)}$$

$$\hat{S}_{nr}(0) = \hat{S}_{nr,0} \qquad \text{B. (2j)}$$

$$\hat{I}(0) = \hat{I}_0 \qquad \text{B. (2k)}$$

$$\widehat{D}_j\,(0) = 0, j = 1,2,\cdots,M \qquad \text{B. (2l)}$$

Table D1. Deposition fraction in the respiratory tract of different inhaled particle sizes

Particle size (µm)	**Deposition fraction of inhaled particles in the respiratory tract**	
	Adult	Children
0.3	0.11	0.11
0.5	0.14	0.14
0.7	0.26	0.32
2.0	0.66	0.86
2.5	0.71	0.78
5.0	0.91	0.96

Smaldone *et al*. (2015) performed an *in vitro* study to determine the respiratory source control using RPDs. The results depend on the room type, the RPD type and whether the subject is coughing or breathing. The values can range from 2 to 4 for breathing and 5 to 17 for coughing. Since it is likely that a person spends their time breathing rather than coughing over a 4 to 8 hours' period, hence we assume average values that hold for breathing conditions. We also assume that for RPDs the protection factor is not a strong function of the brand used or fits. We arbitrarily assume a slightly lower value for unfitted respirators comparing with fitted respirators.

Regarding the respiratory receiver control using RPDs, with fitted N95, the results from bench top and subject experiments are mixed. While the former suggests no dependency on size, the later demonstrates at least some dependency. For fitted N95, we combine the findings and assume a size specific risk reduction: we assume a lower PF below 1 micron and then assume a relatively higher PF at larger values but then that does not increase with size. For the unfitted N95, Brosseau et al, 2010 has demonstrated that without fit-testing, at least a PF of 2 would be expected for two brands of N95s. The low filtration surgical mask PF was determined by Guha et al. (2016) and, the lab work demonstrated the low filtration can make the PF close to 1.1 which is the worst case. The high filtration surgical masks are likely to provide PF close to 7 as tested in Brosseau et al. (2008). the article does not provide any size dependency of leakage but the filtration is expected to strongly decay with increasing size. To be consistent with N95 data, we assume the PF doubles at size greater than or equal to 2 µm. Guha et al. (2015) and Guha et al. (2016) determined the PF values are

2 and 7 respectively for low and high filtration pediatric masks and are independent of exposure particle size.

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