

# Factors Influencing the Transmission of Influenza

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*Request for Office of Management and Budget Review and Approval for Federally Sponsored Data Collection*

## *Section B. Data Collection Procedures*

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## Section B. Data Collection Procedures

### B1. Respondent Universe and Sampling Methods

Statistical methods will not be used to select respondents for this study. The population for this study is adult patients presenting with influenza-like illness at outpatient healthcare clinics, and healthy adult patients to serve as controls. In our previous study, we found culturable influenza virus in 10% of the cough aerosol samples from patients of this type [1]. Similar results were found by Milton et al. [2]. We believe that, with improvements in our collection and detection methods, we can increase this detection rate to about 33%. In order to characterize the production of infectious aerosols by influenza patients, we will need about 15 participants each year with detectable viable influenza in their cough aerosols (because this is an observational study and because so little data is available on the presence of influenza in exhaled and coughed aerosols, it is not possible to perform a statistical power calculation). For this reason, 45 participants with influenza will be needed in each year of the study. A matched number of control participants who are not ill will also be needed to provide a basis for comparison of aerosol content and blood biomarkers. Thus, 90 participants will be needed each year, for a total of 270 participants over 3 years. During previous similar studies, we found that about 50% of the potential participants declined to participate or weren't eligible for the study. Thus, we estimate that we will need to verbally screen about 540 potential participants to reach our goal of 270. In the previous studies, no participants dropped out of the study once they had decided to participate.

We anticipate that the entire study will be conducted at the outpatient clinics of West Virginia University. Participation in the study is voluntary. Any person presenting at the clinic who meets the following criteria is eligible to participate in the study:

- Male or female adult ages 18 or older.
- Symptoms of influenza-like illness (fever with one or more of the following symptoms: headache, fatigue, cough, sore throat, and/or muscle aches).
- Symptoms present for 72 hours or less.
- Not vaccinated against influenza during the current season.
- No other respiratory illness such as severe asthma, COPD or tuberculosis.
- Otherwise good health with no underlying illnesses.
- Not pregnant.
- No medical condition or illness that would make it difficult or uncomfortable for them to perform the test procedure.

Control subjects must meet the same conditions as infected subjects, except that they must be free of symptoms and in good health. Volunteer adult participants will be recruited by distributing flyers at the clinic. Enlarged versions of the flyer will also be posted in the clinic. Interested potential participants will be screened verbally by a test coordinator to verify that they have influenza-like symptoms and that they do not have any medical conditions that would preclude their participation. This study will not interfere with the normal operation of the clinics.

## **B2. Procedures for the Collection of Information**

This project is intended to be primarily a descriptive study of the amount of viable influenza virus expelled by patients during normal breathing and coughing and of any correlations between the amount of virus expelled and the levels of biomarkers in the blood.

In order to maintain the quality of the collected data, study personnel will verify that the participant reads and signs the consent form, and the health questionnaire will be checked by study personnel after completion by the participant. Respondents will not be re-interviewed or re-contacted for data validation after their participation. The apparatus used in the experiments is calibrated as specified by the manufacturers.

### **Informed consent form and health questionnaire**

At the beginning of a test session, the study will be explained to the participant and any questions will be answered. The participant will be asked to read and sign a written informed consent form (Attachment 3). They will then be asked to fill out a brief health questionnaire (Attachment 4). Both of these forms will be on paper.

### **Nasopharyngeal mucus collection**

Nasopharyngeal mucus will be collected from both nasopharyngeal regions using a long nylon-flocked swab as shown in Figure 1. A dry swab is inserted into one nostril straight back (not upwards), along the floor of the nasal passage until reaching the posterior wall of the

nasopharynx. The distance from the nose to the ear gives an estimate of the distance the swab should be inserted. The swab is rotated gently and left in place for up to 10 seconds. The swab is then removed and placed in a container of transport media. This procedure is repeated with a fresh swab for the second nostril.

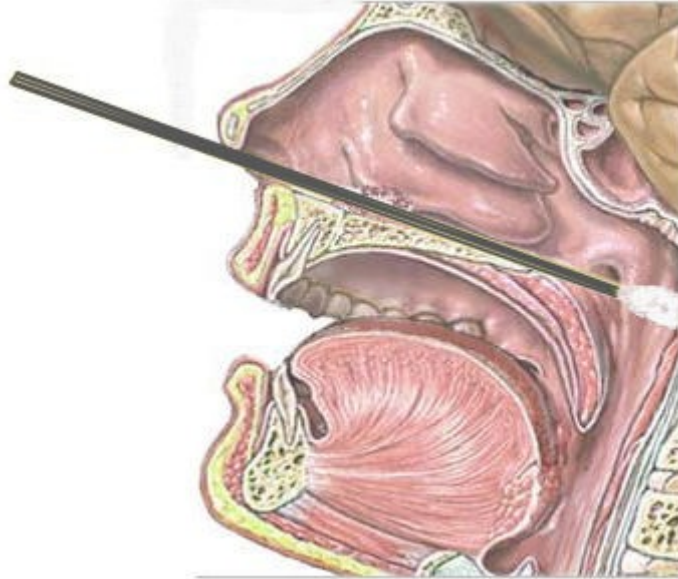


Figure 1: Nasopharyngeal mucus collection procedure. Illustration is from Utah Department of Health.

### **Aerosol particle collection apparatus**

This project will use the apparatus shown in Figure 2 to collect and characterize the airborne particles produced by human volunteers with influenza and by healthy controls. It consists of an elastomeric mask, an ultrasonic spirometer (Easy On PC, NDD Medical Technologies) a medical spirometer (SensorMedics), and an aerosol sampler (BioSampler, SKC). The aerosol particles produced by the subject as they breathe and cough will be collected by the aerosol sampler and analyzed using PCR and viability assays to measure the amount of influenza virus released during each cough.

To collect the aerosol particles produced by the test subject, the following procedure will be used: The subject will be asked to sit in front of the device and the device will be adjusted to a comfortable height. The subject will don a mask that is attached to the collection device, and the mask straps will be tightened to form a seal around the subject's face. The subject will then be asked to sit quietly and breathe and cough normally. The subject is free to read, listen to music, or use a phone or computer, so long as they do not move their head excessively or break the face seal of the mask. The subject will be asked to wear the collection mask for 40 minutes.

The aerosol particles produced by the participant will be collected using an SKC BioSampler and analyzed for influenza virus using PCR and culture-based viability assays.

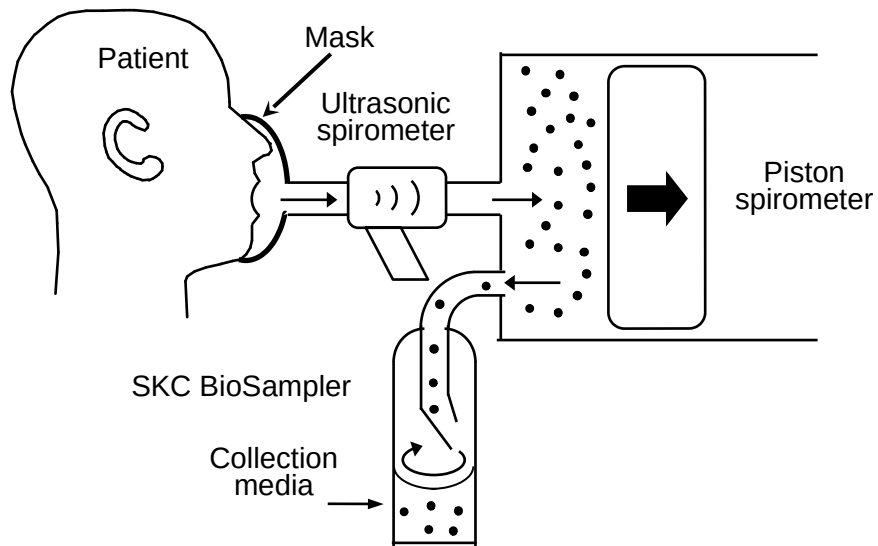


Figure 2: System for collecting aerosols produced by human volunteers during breathing and coughing. When the subject exhales, their breath flows through the ultrasonic spirometer, which measures the volume of the breath, and into a piston spirometer. The breath is then drawn into the aerosol sampler for collection into the collection media. The mask is removed and sanitized after each subject.

### Blood collection

Five ml of blood will be drawn into a Vacutainer blood collection tube (BD SST II Advance Tubes with Gel and Clot Activator, Becton Dickson) via peripheral venipuncture by a nurse or phlebotomist present at the clinical site. The specimen will be centrifuged and then kept on ice until it is transported to the laboratory for analysis. Blood samples will be examined for changes in the levels of cellular microRNA molecules, which are small regulatory molecules of 20-22 nucleotides that influence protein expression through mediation of posttranscriptional silencing of target genes.

### B3. Methods to Maximize Response Rates and Deal with No Response

We will continue to recruit until the study has been completed. As described in Section A9, to encourage participation, respondents will receive a \$40 gift card as a token of appreciation. Previous experience with recruiting volunteers suggests that this amount will result in a participation rate of about 50%.

### B4. Tests of Procedures or Methods to be Undertaken

The health questionnaire and informed consent forms used in this study are the same as those used in the previous approval (OMB 0920-0888). The collection of nasopharyngeal mucus samples and blood are commonly-used clinical procedures. The aerosol collection system is similar to that used in the previous study and has been adapted for use in the present work.

## **B5. Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data**

The data collection procedures were designed by the project officer, who will also perform the data collection and analysis:

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