

Factors Influencing the Transmission of Influenza

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

Section B. Data Collection Procedures

Project Officer:

William G. Lindsley, PhD

National Institute for Occupational Safety and Health

1095 Willowdale Rd. M/S 4020

Morgantown, WV 26505

wlindsley@cdc.gov

304-285-6336

304-285-6394 (fax)

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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 out-patient healthcare clinics. 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]. In order to characterize the production of infectious aerosols by influenza patients, we will need about 18 participants with 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 cough aerosols, it is not possible to perform a statistical power calculation). For this reason, 60 participants will be needed in each year of the study for a total of 180 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 360 potential participants to reach our goal of 180. 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 West Virginia University Health Sciences Center. 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.

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. Because it is not possible to accurately screen participants for influenza at the clinic in a timely manner, all eligible subjects with febrile respiratory illness who volunteer will be allowed to participate in the study.

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 coughing and of any correlations between the amount of virus expelled and the different features of the cough sounds. Based on our previous work in this area [1], we anticipate that the most difficult aspect of this project will be the detection of live influenza virus in airborne particles. The concentration of infectious particles appears to be low, and the current detection methods are not very sensitive. In addition, volunteers can only be recruited and tested during influenza season, which typically lasts 1 to 3 months each year but is unpredictable and can vary significantly in time and intensity. Based on our previous studies, we estimate we will need at least 18 positive results from the assays of viable influenza of the cough aerosol samples, and that this will require about 180 total subjects. Because of time, space and personnel constraints and the need to refine the methodology for the detection of viable influenza in cough aerosol particles, a maximum of about 60 subjects can be tested each year. Thus, we estimate that three years will be required to complete the study.

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.

Apparatus

This project will use the apparatus shown in to collect and characterize the aerosols and record the sounds produced by human volunteers during coughing. It consists of a disposable mouthpiece, an ultrasonic spirometer (Easy On PC, NDD Medical Technologies) a medical spirometer (SensorMedics), a microphone to record cough sounds (Model 4136, Bruel & Kjaer), and an aerosol sampler (BioSampler, SKC). The cough-generated aerosol particles 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 perform a cough-generated aerosol measurement, the following procedure is used: The subject is asked to sit in front of the device, exhale completely, inhale as much as possible, seal their lips around the mouthpiece and cough. Subjects are asked to cough forcefully using as much of the air in their lungs as possible. After coughing, the subject sits back and waits a few minutes while the cough aerosol is collected. This procedure is then repeated for a total of six coughs.

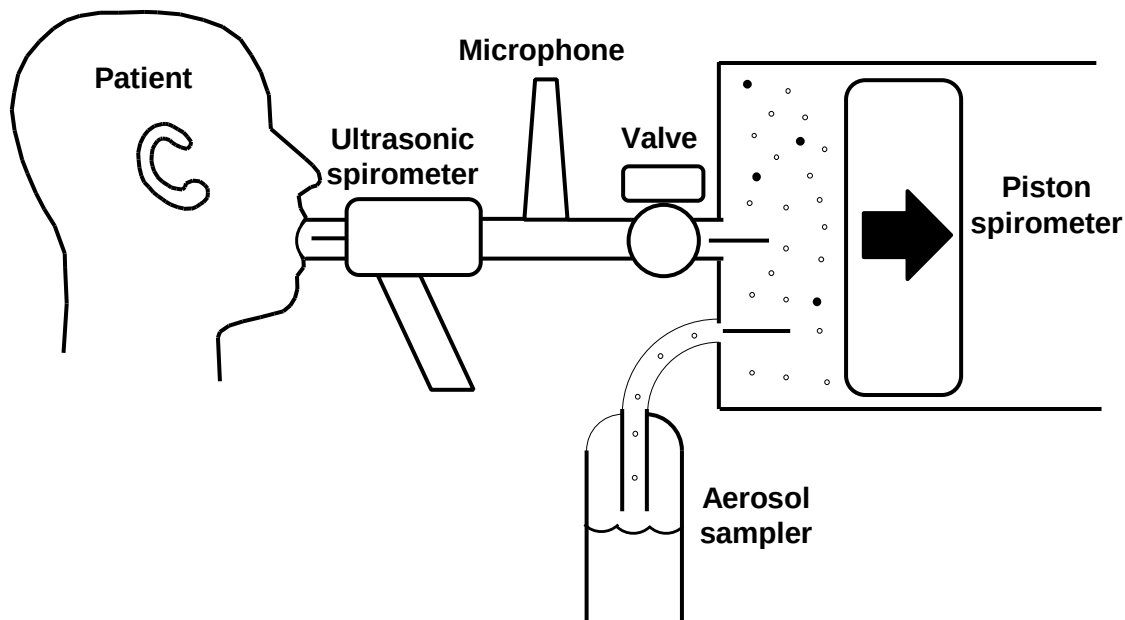


Figure 1: System for measuring aerosols produced by human volunteers during coughing. When the subject coughs into the mouthpiece, the cough first flows through the ultrasonic spirometer, which measures the volume of the cough. Next, the cough flows past the microphone, through the valve, and into a piston spirometer. After the cough is completed, the valve is closed and the aerosol from the cough is drawn into the aerosol sampler for collection. The mouthpiece (which extends through the ultrasonic spirometer) is removed and discarded after each subject.

Analysis of cough aerosols, cough sounds and environmental air samples

After each cough, the cough-generated aerosol from the patient will be collected using an SKC BioSampler and analyzed using PCR and culture-based viability assays. Our studies conducted during two previous influenza seasons have shown that assays such as the standard viral plaque assay to detect infectious virus are limited in sensitivity, and low viral numbers may go undetected. The low quantity of viable virus in cough aerosols thus makes detection and quantitation difficult. The severity of illness and the strain of the influenza virus also appear to play a role. Using PCR, we were able to readily detect influenza in the cough aerosols from subjects recruited during the 2009 H1N1 pandemic, but viral concentrations appeared to be much lower in cough aerosols collected from patients during the seasonal influenza outbreak in early 2011. Using standard viral plaque assays, we only were able to detect viable influenza virus in the cough aerosol from 2 of 20 subjects in 2009 and 2 of 32 subjects in 2011.

To improve detection of viable influenza virus, the collected coughed aerosols will be assayed for the presence of infectious influenza using two improved technologies for detection referred

to as the Viral Replication Assay (VRA) and the Luciferase Reporter Assay (LRA). Both technologies increase sensitivity by first amplifying the amount of infectious virus in an aerosol sample several hundred-fold in cell cultures of canine cells. With the VRA, the viral RNA genes are then detected using a standard Polymerase Chain Reaction protocol [3]. With the LRA, the amplified virus is infected into canine cells engineered to contain the Luciferase gene that, when activated by the virus, results in light emission that can be detected by a luminometer [4].

Cough sounds will be analyzed as described previously [5]. A custom-written computer program will be used to capture the sound pressure and flow signals generated as a subject coughs through the mouthpiece. This system is capable of performing spectral analysis of cough sound signals in the frequency domain between 50 Hz and 25 kHz. The recorded signals will be analyzed in the time and frequency domain to determine components that correlate with the amount of influenza virus expelled by the patients while coughing.

B3. Methods to Maximize Response Rates and Deal with Nonresponse

We will continue to recruit until the study has been completed. As described in Section A9, respondents will receive \$25 as a token of appreciation for their participation. Previous experience with recruiting volunteers has indicated that this token results 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 very similar to those used in a previous NIOSH study, "Experimental and Theoretical Study of Early Detection and Isolation of Influenza" (OMB No. 0920-0777). The cough aerosol collection system was also 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:

William G. Lindsley, PhD
National Institute for Occupational Safety and Health
1095 Willowdale Rd. M/S 4020
Morgantown, WV 26505
WLindsley@cdc.gov
304-285-6336

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