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Influenza Virus Collection and Detection from Electret Filters

A White Paper

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Influenza virus collection and detection from electret filters

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A recent paper by Fabian, et al., (1) has demonstrated the collection of influenza virus on Teflon filters from human exhaled breath. The RNA was successfully isolated and identified via RT-PCR. Two recent reports describe difficulty in detecting the influenza virus recovered from electret filters, but not other respiratory viruses.

The first published by Huynh, et al., (2) described the successful collection, elution, and RT-PCR detection of rhinovirus and parainfluenza virus in nine symptomatic patients based on a) an electret filter mask that collected the virus in aerosol form during either coughing, talking, or breathing, or b) from nasal mucus samples. The results are as follows:

- Of the 9 patients, 6 tested positive for rhinovirus from the nasal mucus samples, two for influenza A, and one patient for parainfluenza virus 3.
- All six nasal-positive rhinovirus patients tested positive on the electret filters after either coughing, talking or breathing (4 of them when only talking or breathing).
- One patient who tested nasally positive also tested positive for both coughing and talking;
- One patient who tested positive for rhinovirus and negative for parainfluenza tested positive for rhinovirus in all three aerosol modes, but also positive for parainfluenza for coughing and talking.
- Neither of the two who tested positive for Influenza A in their nasal mucus tested positive for Influenza A with any of the aerosol collection methods.

This brief report is encouraging that RNA viruses can be collected, eluted, and RT-PCR amplified using the Qiagen RNA lysis buffer and RNA isolation kits from electret filters. The reason that the influenza test failed is not known. Filters were not spiked with influenza virus to examine the sample recovery process, nor was any data presented regarding the presence or absence of any RT-PCR inhibitors in the extracted samples. It is possible the two patients were not shedding virus at the time of the test. No data was presented regarding the relative sensitivity of the various RT-PCR assays.

The second report is the Masters Thesis of Anja Valen of the Norwegian University of Science and Technology. This report describes the use of Research International's SASS 3100 air sampler and SASS 3010 filter extractor to collect and elute bacteria and influenza virus from electret filters. The air samples were collected in a subway station during the flu season. Bacterial DNA was isolated and amplified successfully. Influenza RNA amplification was unsuccessful.

The RT-PCR protocol was verified using commercial whole influenza viral particles. Electret filters were eluted with 20 ml of extraction buffer (PBS + 0.05% Triton X-100), centrifuged at 6000 G for 45 min, and the supernatant pelleted at 136,000 G for 90 min. The RNA in the pellet was extracted using the trizole-chloroform method and RT-PCR amplified. No viral RNA was detected. Moreover, when positive control RNA was spiked into the extracted sample, amplification was unsuccessful.

Clearly a RT-PCR inhibitor was present in the spiked extracted sample. The assay was sensitive enough to detect 1,600 copies of viral RNA per microliter of sample. The nature of the inhibitor is unknown. Further work is required to determine the problem. Does the inhibitor come from the extraction buffer, the electret filter, or the air sample? It is not likely that the filter media is the problem, as it is polypropylene that has been ETO sterilized and is relatively inert. An experiment using spotted known amounts of virus onto the filter followed by the elution, extraction, and amplification procedure would have identified if the inhibitor comes from the air sample or the extraction materials. If the inhibitor does come from the air sample, other RNA extraction procedures should be examined.

In summary, influenza viruses have been successfully collected on Teflon filters and other RNA viruses have been collected by electret filters and the viral RNA successfully amplified using RT-PCR. However, the two reports found that mention influenza A were not successful with it when using electret filters and neither report determined the source of the problem. It is unlikely that the filter itself is the problem as it is made of comparatively inert polypropylene fibers, but neither researcher tracked down the problem. All three reports describe different methods for recovering the RNA. As the work of Fabian successfully collects and amplifies the RNA, the protocol represents a logical starting place, substituting an electret filter for the Teflon filter.

1) Patricia Fabian, et al., “Influenza Virus in Human Exhaled Breath: An Observational Study,” PloS ONE, **3** (7) July 2008, pg. e2691.

http://www.plosone.org/article/info:doi%2F10.1371%2Fjournal.pone.0002691

2) Karrianne N. Huynh, et al., “A New Method for Sampling and Detection of Exhaled Respiratory Virus Aerosols,” Clin. Infect. Dis., **46**, 2008, pp93-95.

3) Anja Valen, “Characterization of Airborne Microorganisms at Nationaltheatret Subway Station, Masters Thesis submitted to Norwegian University of Science and Technology, May 2011.

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