Sampling for Airborne Influenza Virus Using RNAlater®: A New Approach

Elanie M. Girlando[1], Matthew W. Nonnenmann[1], Lucy Desjardin[2], Travis Henry[2]
[1]Department of Occupational and Environmental Health, The College of Public Health, and [2] State Hygienic Laboratory, The University of Iowa

Background

Influenza is a contagious respiratory illness caused by an infection of the nose, throat, and lungs with an influenza virus. Airborne transmission of influenza can result from aerosolized droplets being inhaled by a host.

Determining the airborne concentration of influenza virus is difficult due to the instability of the viral genetic material (RNA). Recently developed RNA preservation product (i.e., RNA*later*®) has been shown to stabilize even low levels of influenza virus and preserve infectivity for months at ambient conditions.

Further testing is needed to determine the extent to which RNA*later*® preserves viral RNA compared to currently used sampling matrices such as Hank's Balanced Salt Solution.

Objectives

- Determine the accuracy, precision and bias of using RNA*later*® as a sampling matrix to estimate concentrations of aerosolized influenza compared to using Hank's Balanced Salt Solution (HBSS).
- Use the sampling methodology in field-based applications to determine airborne influenza concentrations in settings where influenza viruses are likely to be present.

Methods

Sampling trials were performed using the SKC Biosampler and 20 ml of RNA*later*®.

Preliminary sampling was conducted in the lab to determine if problems such as foaming or excessive evaporative losses would occur while using RNA*later*® as a sampling matrix.

In addition, supplementary samples were spiked using H1N1 viral RNA to perform a side-by-side comparison of RNA*later*® and HBSS in terms of virus retention.

Field sampling was conducted using RNA*later*® at a swine farm (approximately 2000 pigs), which was experiencing confirmed H1N1 type influenza. Sampling was conducted for 15 minutes, each sampler drawing air at 12 Lpm. Samples were taken to the State Hygienic Lab for qPCR analysis to determine presence of viral RNA.

Results

Preliminary Lab Testing Results

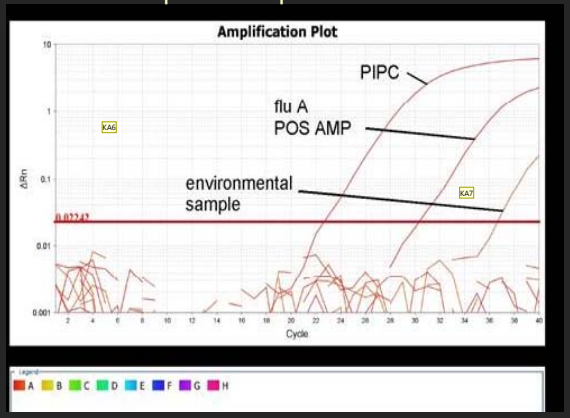
After 15 minutes of sampling, the volume of RNA*later*® remaining in the SKC Biosampler was above 15 ml, therefore less than a 25% loss of volume occurred during sampling. No foaming of the matrix resulted, and no additional logistical or sample handling problems were identified.

In side-by-side comparisons of virus retention, RNA*later*® and HBSS performed similarly over the course of one week.

Field Sampling Results

Preliminary analyses of the sampling with RNA*later*® resulted in the qPCR detection of influenza A in the swine building.

PCR amplification plot for Influenza A



"PIPC" stands for Pooled Influenza Positive Control, provided by the CDC. It consists of positive samples of known subtype that are pooled together and then extracted by the site lab as an RNA control. "Flu A POS AMP" is an extracted influenza A sample. The influenza A positive sample collected at the swine barn is labeled as "environmental sample."

The amplification plot lists the cycle number on the bottom. The lower the number, the more copies of the target were present in the starting sample. For qualitative analysis, the cycle threshold (Ct) is set as low as possible above amplification noise and away from strange slopes in the curves.

Images of SKC Biosampler and Hog Confinement Barn

Picture from University of Nebraska Medical Center

Conclusions

- RNA*later*® shows no signs of foaming or evaporation that would hinder its use as a sampling matrix.
- RNA*later*® and HBSS performed comparably in side-by-side virus retention experiments, thus there is no reason why RNA*later*® cannot be used instead of HBSS.
- One of seven field samples amplified.
- More samples might be positive with addition of more starting RNA for amplification.
- The successful detection and amplification of influenza A demonstrates the need to further evaluate the proposed methodology of using RNA*later*® as a sampling matrix for aerosols of influenza.

Future Research

Use time-plot and further sampling to determine ability of RNA*later*® to preserve viral RNA at ambient conditions

This research was funded by the National Institute for Environmental Health Sciences through the University of Iowa Environmental Health Sciences Research Center, NIEHS/NIH P30 ES05605.

UI COLLEGE OF PUBLIC HEALTH
DEPT OF OCCUPATIONAL & ENVIRONMENTAL HEALTH

Slide 1

KA6 I would increase the axis text size on this graph. I can't see it on the computer and the conference is full of very old people who will have problems seeing this!!
Kim Anderson, 5/14/2013

KA7 I would give this a title and a caption explaining what the colors (A B C, etc) mean and what the dark red straight line means.
Kim Anderson, 5/14/2013