A Field Portable Avian Flu Detection System Using the SASS 2300 Air Sampler and PCR Methods

Introduction

Research International is looking for parties who need a portable DNA-based bioassay system that can detect bacteria and viruses such as the potentially pandemic avian flu influenza A strains without direct contact with infected animals. The compact system designed for this purpose by Research International includes a custom version of the SASS 2000 air sampler (Figure 1), along with PCR-based detection equipment that has been selected for portability and low power consumption. This method can eliminate or complement random blood sampling, and allows monitoring of large areas where livestock or poultry are present.

Figure 1: SASS 2300 cyclonic air sampler.

The SASS 2000 has been previously used for diverse purposes ranging from human and agricultural aerosol pathogen collection, to subway and mailroom biothreat monitoring (see www.resrchintl.com). Agricultural researchers have found it to be particularly effective for pen-side virus collection. It has for example been successfully used to collect and detect the viruses that cause hoof-and-mouth disease, Exotic Newcastle disease (1), and strains of avian influenza. *It is currently the only portable air sampler certified by the U.S. Department of Homeland Security.*

(1) Hietala S.K., Hullinger P.J., Crossley B.M., Kinde H., Ardans A.A., "Environmental air sampling to detect exotic Newcastle disease virus in two California commercial poultry flocks," J. Vet Diagn Invest., 17, 198-200 (2005).

Operating Principals:

For this application, the basic SASS product has been modified to both efficiently collect respirable-size particles from sampled air, and to extract and pre-concentrate nucleic acids from pathogenic organisms within the sampled air. These nucleic acids are then amplified in number using PCR technology and detected, if present, using optical fluorescence methods. PCR-based assay methods are arguably the most sensitive and accurate means now available for detecting targeted organisms at low concentrations.

During the air sampling process, air is drawn into the sampler at the rate of 325 liters per minute. Proprietary fluidic components and electronic circuitry maintain the fluid sample volume at 4 to 5 cc independent of collection time, relative humidity, or temperature. Clean water is added from an onboard reservoir as needed to compensate for evaporation. This allows long-term unattended monitoring and the pre-concentration of airborne organisms present at low levels. After a 10 minute sample period, for example, the trace pathogen concentration in the fluid will be 750,000 times greater than its concentration in the sampled air. This is an example of a short collection. We have shown that this system is capable of maintaining the fluid sample for sampling periods of up to several hours, thereby providing time integration of targeted viruses that are present at very low aerosol concentrations.

After sampling is completed, nucleic acids are transferred to a small volume (50-100 microliters) of water using a simple extraction and concentration procedure, providing a purified nucleic acid concentrate ready for analysis.

Figure 2 illustrates the results of some preliminary experiments in which DNA from Salmonella typhimurium cells were introduced into the SASS 2000. These tests were performed to see if the DNA could be recovered after a long period of sampler operation. Tests were performed with two different collection fluid compositions. Following 30 minutes of circulation in the SASS, the procedure for extraction and concentration of DNA was performed. A portion of this purified DNA was then PCR amplified with a portable thermal cycler using primers specific for the Salmonella rRNA gene. A fluorescence-based detection method was then used to measure the extent of DNA amplification using a handheld fluorescent reader. Gel electrophoresis confirmed the correct size of the amplified DNA, and a 0.3 ng quantity of purified Salmonella DNA, equivalent to about 10,000 cells, was also analyzed as a positive control in this experiment. As shown in Figure 2, both collection fluid compositions produced PCR amplifiable DNA from as few as 2,000 cells. This is comparable sensitivity to a standard laboratory PCR analysis.

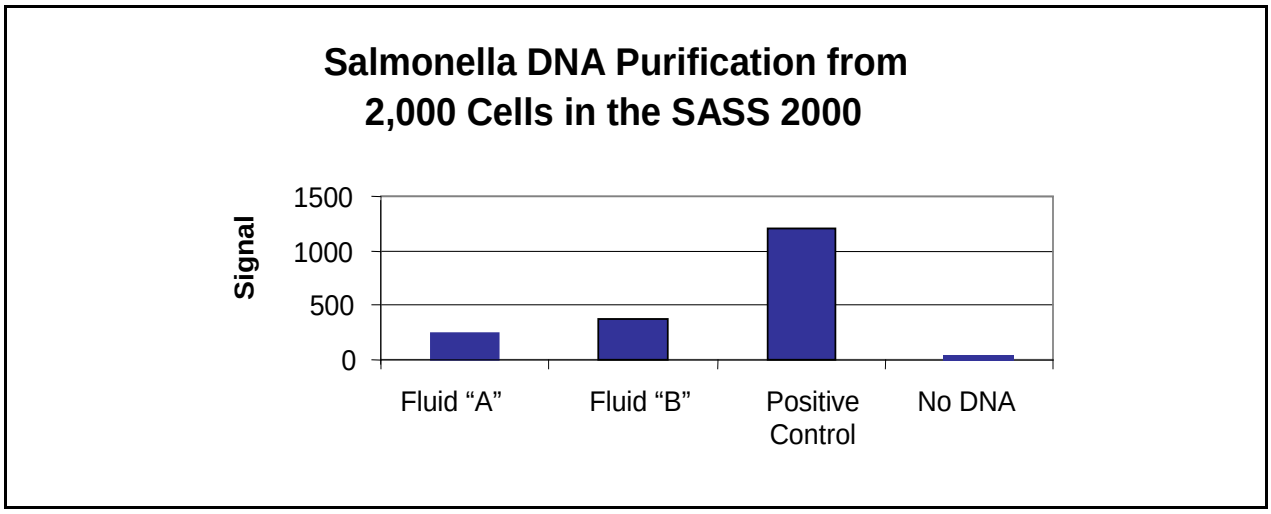

Figure 2: PCR fluorescence from SASS 2000 purified *Salmonella* DNA

This procedure can be easily performed in the field using hardware and simple consumables provided by Research International. The operating steps in performing such a DNA/RNA collection and purification during air sampling with the SASS 2000 are described in Table 1.

Table 1: Operating Steps to Perform a DNA/RNA Extraction During Air Sampling with the SASS 2000

1. Add 4 to 5 cc of collection solution into the cyclone through the air inlet using the syringe provided.
2. Operate the instrument for the desired collection period (10 mins to several hours, typ.). The instrument will add water as needed to counter evaporation.
3. Stop airflow for sample removal.
4. Transfer the sample fluid to a collection tube using the SASS's built-in peristaltic pump.
5. Perform a washing and DNA transfer protocol using reagents and consumables supplied by Research International. Total processing time is about 10 minutes.
6. Place the purified fluid in a PCR tube. Use 10 microliters of purified fluid per 50 microliter PCR or RT-PCR reaction. Read the PCR tube fluorescence after 35-40 cycles without opening the tube (to avoid contamination).
7. Flush SASS 2000 to prepare for next sample. Flush system twice with DI water to prepare for next sample.

All major hardware components and consumables necessary to perform the procedure outlined in Table 1 are available from Research International. There are three principal hardware components:

- SASS 2300 air sampler;
- Portable PCR thermal cycler; and
- Fluorescence reader (fluorometer).

The SASS 2000 and handheld fluorometer are battery powered. However, the thermal cycler requires wall plug power or an alternative power source. Research International can supply a

small gasoline generator, or an automobile equipped with a 500 watt DC to AC inverter would also be suitable. Table 2 provides specifications for the recommended system components, while Figure 3 illustrates the portable thermal cycler and handheld fluorescent reader.

Table 2: Low cost Portable PCR System Hardware Components

Component	Description	W x D x H cm (in.)	Power, Watts	Weight kg (lbs)	Other
Air sampler SASS 2300	325 LPM; 16 hr internal water supply	18.3x 21.3x34.3 (7.2 x 8.4 x 13.5)	10.8	4.6 (10)	58 dB @ 1 meter sound level
Thermal Cycler	24 x 200 or 20 x 500 Tubes/uL cap.	18.0x30x23.0 (7.1 x 11.81 x 9.06)	250	7.5 (16.53)	**Ramp:** 3C/min up 2C/min dwn
PCR Tube Reader	1 channel	8.9x18.4x4.5 (3.5 x 7.25 x 1.75)	4 x AAA cells	-	-
Portable Power Supplies					
Honda	8.3 hrs use @ 250W	23.9x45.0x38.1 (9.4 x 17.7 x 15)	1000 max.	13.2 (29)	53 dB @1/4 load
Yamaha	12.0 hrs use @ 225W	23.9x45.0x37.9 (9.4 x 17.7 x 14.9)	900 max.	12.7 (27.9)	47 dB @1/4 load
DC/AC invertor	-	13.0x20.1x6.1 (5.1 x 7.9 x 2.4)	500 W out, 556 W in	1.1 (2.4)	-

Figure 3: An Example of a Portable Thermal Cycler and a Handheld Fluorescence Reader Provided

For more information please contact
Research International directly at: