Automated Aerosol Collection and Nucleic Acid Purification Using the SASS 2300 Air Sampler

Introduction

Research International has recently demonstrated the simultaneous collection of aerosol particulates and the performance of *in situ* nucleic acid purification on captured microbes. Such a capability has obvious application to the front-end processing of samples for biowarfare agent identification systems. Incorporating such an automated capability should reduce overall assay time; improve the quality and uniformity of nucleic acid samples; reduce processing costs; and be an enabling technology that defines new conops for field applications.

Figure 1: SASS 2300 cyclonic air sampler.

The system would be designed around a custom version of the SASS 2300 air sampler (Figure 1), and could be used with any existing PCR-based detection system selected for portability and low power consumption. Our initial goal would be to design, develop and build several copies of a fieldable prototype system for customer evaluation. However, there is no reason that collection, purification and detection couldn't eventually be implemented within one automated system.

The SASS 2300 and its predecessor SASS 2000 have been used for diverse purposes ranging from human and agricultural aerosol pathogen collection, to subway and mailroom biothreat monitoring (see www.resrchintl.com). SASS 2000 subsystems are for example an integral part of the APDS bio-monitoring systems that have been collecting aerosols in the subways of New York and Washington D.C. for over three years. Agricultural researchers have also found the systems to be particularly effective for pen-side virus collection. They have been successfully used to collect and detect the viruses that cause hoof-and-mouth disease, Exotic Newcastle disease (1), and strains of avian influenza. *The SASS 2000 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).

Technical Discussion:

Background

In the SASS series of aerosol collectors, air is drawn into the sampler at the rate of 325 liters per minute (Figure 2). Proprietary fluidic components and electronic circuitry maintain a fluid sample volume of 4 to 5 cc in the sampler that is 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 and bacteria that are present at very low aerosol concentrations.

As mentioned in the Introduction, nucleic acid assay methods have already been successfully used to detect and identify bacterial and viral agents captured by SASS samplers. However, these assay techniques are typically designed to work with small concentrated samples in the 10-100 microliter range, instead of the 4 to 5 cc samples created by the SASS samplers. In addition, nucleic acid assays require, for reliable results, a multi-step sample cleaning and preparation protocol that is laborious, costly, and prone to error. Through certain modifications of the SASS 2300 discussed below, we believe it is possible to continuously collect bio-aerosols, extract nucleic acids and to periodically transfer concentrated, purified and virtually PCR-ready samples of RNA and DNA to a nucleic acid-based detection system either automatically, or with minimal human intervention.

The Concept

Briefly, Research International proposes to do simultaneous aerosol collection and nucleic acid processing by incorporating the following modifications into the sampler.

- The distilled water now used to capture and store organisms will be replaced by a chaotropic salt solution. Chaotropic salt solutions (such as guanidine thiocyanate) are known to be very effective at lysing bacterial cells within 1 to 2 minutes, inactivating nucleases, and stabilizing both RNA and DNA. Since such a salt kills the organism, sample handling would also be rendered much safer. As an added bonus, the salt depresses the sample solution's freezing point, perhaps by 20C, allowing sampler operation at significantly lower temperatures.

- Nucleic acids released by the chaotropic salt solution will be captured and concentrated onto silica-coated magnetic beads that are either allowed to circulate with the sample water, or are bound magnetically in a zone that is subjected to high fluid velocities. The chaotropic salts are doubly-desirable working fluids because they also significantly enhance the binding of nucleic acids to silica.

- Using new microprocessor-controlled pumps and valves, the beads and/or nucleic acids would be cleaned and periodically pumped out, sans the salt solution.

These proposed materials and processing steps require comparatively modest changes to the existing microprocessor-controlled SASS2300.

Feasibility Demonstration/Design Implications

A proof-of-principle test was defined to show a) that a chaotropic salt solution circulating in the SASS air sampler could lyse cells; b) that released nucleic acids would not be lost through the sampler's exhaust or by attachment to its walls after a prolonged operating period; and c) that the nucleic acids could be collected and affinity purified by attachment to a small aliquot of circulating silica-coated magnetic microbeads.

That is, if a small quantity of silica-coated magnetic microspheres are added to the collection fluid, then over time, nucleic acids in solution should bind to these particles. This, in effect, defines a highly efficient in situ sample preparation process that is ongoing during sample collection. The magnetic particles with bound nucleic acid can then be magnetically captured and washed to remove all other non-magnetic particles and PCR inhibitors. The nucleic acids can then be eluted with a small volume (100 microliters) of water to provide a purified nucleic acid concentrate ready for analysis. Depending on the subsequent analytical technique, the magnetic particles may or may not be carried along in the concentrate.

Figure 3 illustrates the results of some DNA purification experiments in which Salmonella typhimurium cells were introduced into a SASS 2000 containing the chaotrope guanidinium thiocyanate, and magnetic silica coated particles. Following 30 minutes of circulation in the SASS (vide infra), the particle solution was pumped out, washed twice with buffer, and any DNA attached to the beads eluted from them. An aliquot of this purified DNA was then PCR amplified with a portable thermal cycler using primers specific for the Salmonella rRNA gene. A fluorescent intercalating dye was used to measure the extent of DNA amplification with a handheld fluorescent reader. Gel electrophoresis confirmed the correct size of the amplified DNA. A positive control of purified Salmonella DNA was used in this experiment that came from about 10,000 cells.

The chaotrope produced PCR amplifiable DNA detectable by both fluorescence and gel electrophoresis. In the most sensitive test of the concept, 10,000 cells were initially placed in the SASS, and 40% of the total extracted DNA sample was subsequently used in a PCR reaction. This provided a small but reproducible signal, implying an overall detection limit of 4000 cells or less for the SASS and particular PCR protocol used. It must be emphasized that this was only an initial feasibility test and no attempt was made to optimize any parameters.

How the magnetic beads are circulated within the SASS (or not) may have a significant impact on sensitivity. While it may initially appear advantageous to simply mix the beads into the sample fluid, they are so small that in a viscous medium such as water, these micron-sized particles will simply be carried along with the fluid and may not be effective at impacting and binding the nucleic acids that are also suspended in the water. Sample water in the SASS continuously re-circulates through the aerosol collection zone about 6 to 20 times per minute (see Figure 2), returning to the start of the collection zone through a 3 mm ID gravity-driven feedback tube. This water recirculation tube may be an ideal place to magnetically 'lock' the magnetic particles in place to maximize mass transfer coefficients. Alternatively, the beads may be allowed to circulate a fraction of the time, and be immobilized for the remainder, depending on empirical findings.

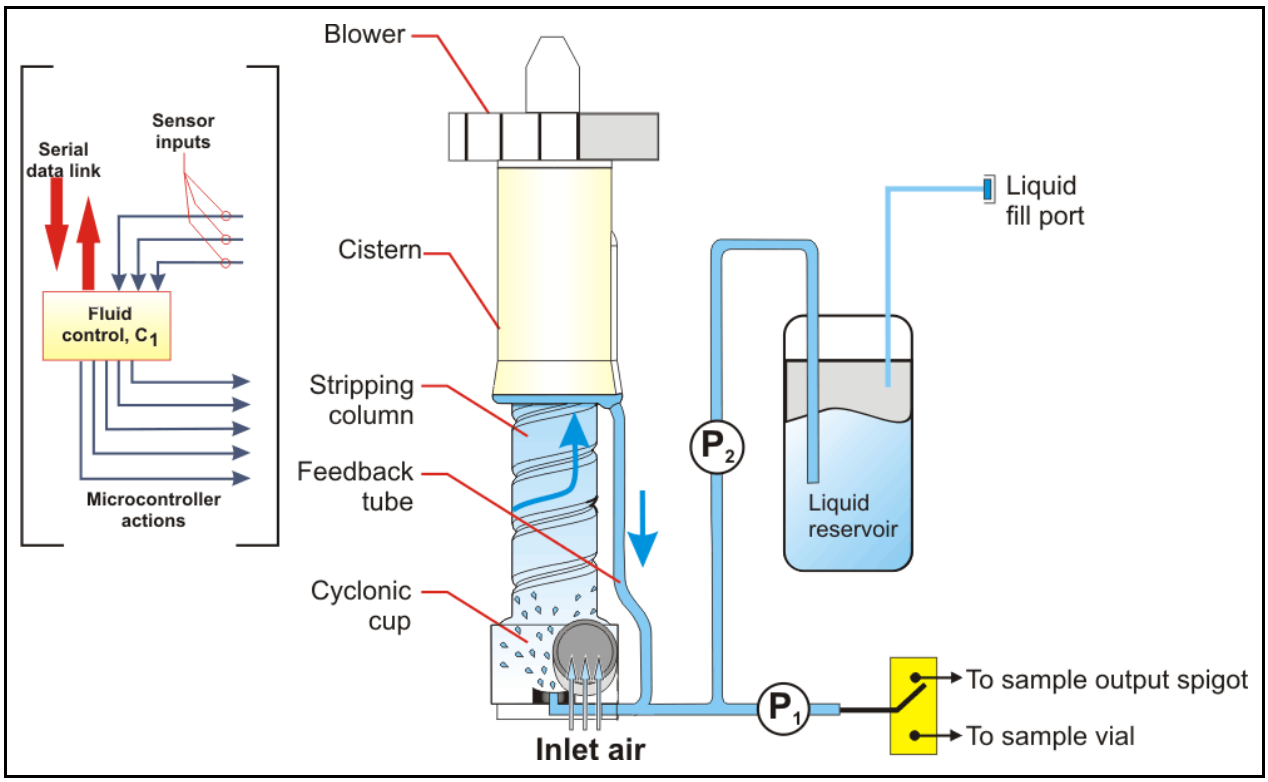

Figure 2: Schematic diagram of the fluidic circuit used in a SASS 2300.

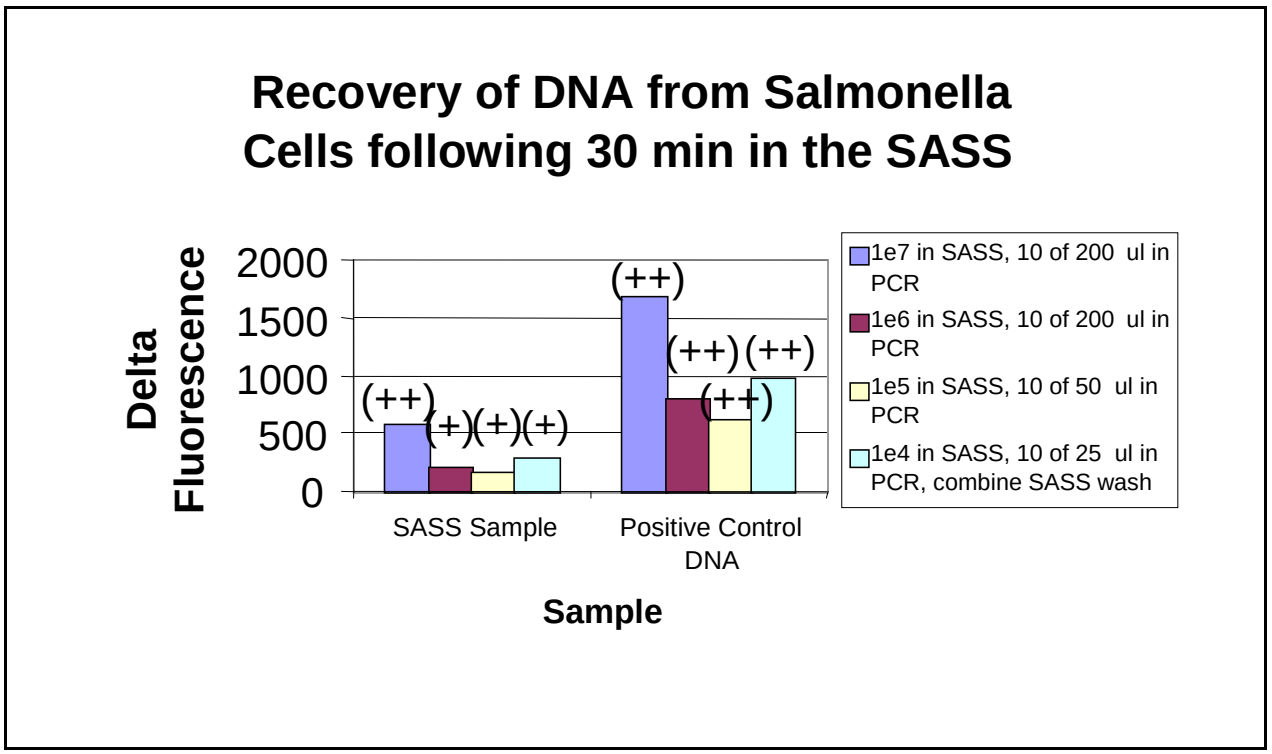

Figure 3: PCR fluorescence from SASS 2000 purified *Salmonella* DNA.

In a baseline operating scenario for the SASS with PCR sample-prep capability, whenever an analytical sample was required, the beads would be captured magnetically, air sampling halted, and the chaotropic salt solution pumped out. The beads would then be washed to remove contaminants. Finally, the nucleic acids would be desorbed from the beads with 100 to 300 ul of distilled water. An internal standard of DNA or RNA virus could of course be added to ensure the effectiveness of the nucleic acid isolation protocol.

Figure 4 shows a fluidic circuit and the fluidic components that could accomplish the tasks outlined above, while Table 1 provides a possible sequence of operations. The additional hardware required is not excessive or complex in function. The equivalent of five separate peristaltic pumps are needed. However, Research International can perform the desired functions with three drive motors. We have perfected a novel and compact pump design that allows one motor to drive two separate pumps.

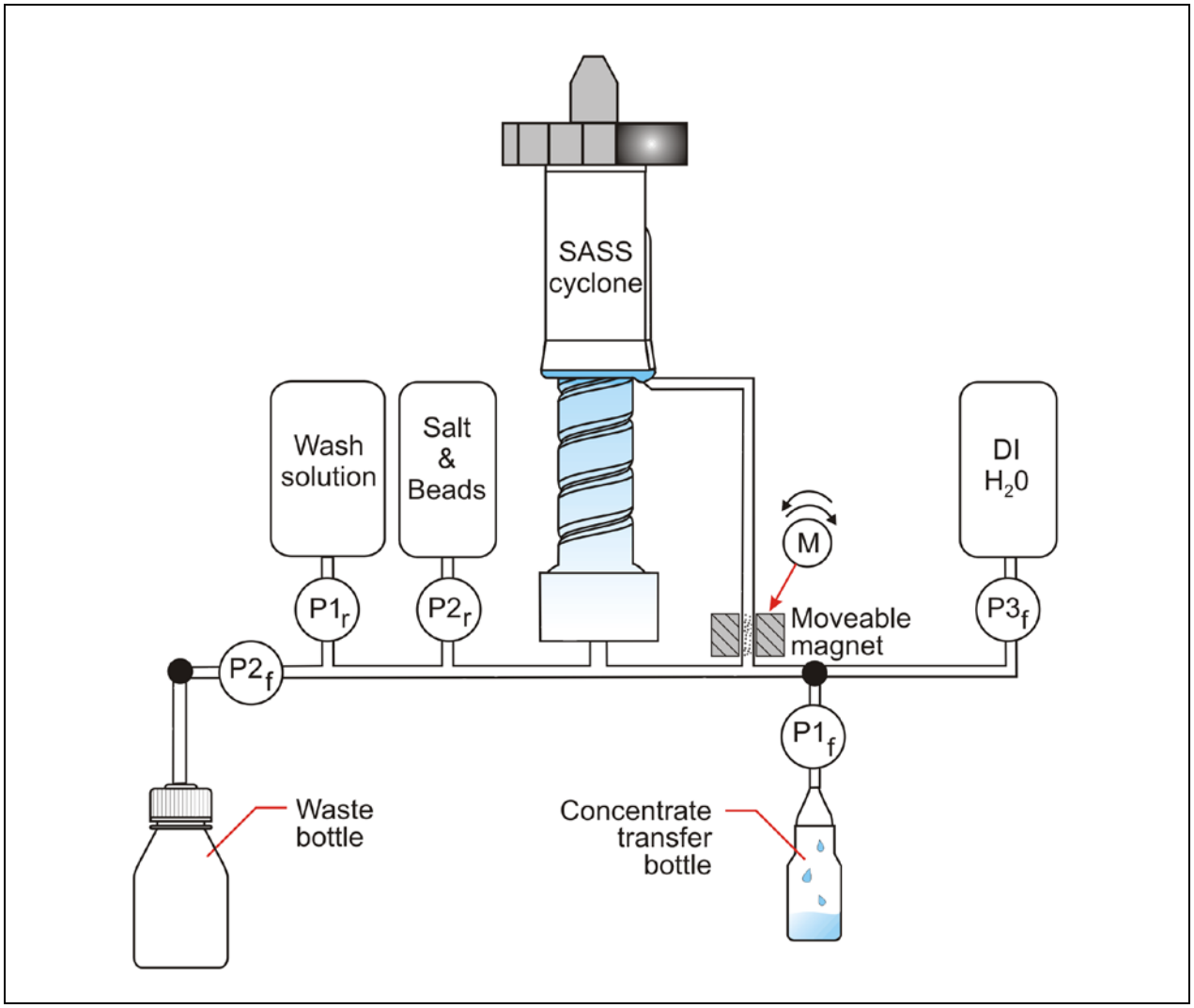

Figure 4: SASS 2300 sampler fluidics suitable for augmented nucleic acid processing.

To perform this protocol, the following hardware would need to be added to the baseline SASS2300:

- One new peristaltic pump assembly and driver electronics;
- One magnet assembly and magnet driver electronics;
- One chaotropic salt reservoir; and
- One wash solution reservoir.

Table 1: Operating Steps to Perform a DNA/RNA Extraction During Air Sampling with the SASS 2300

1. Add 4.0 cc concentrated salt solution w/beads ($P2_r$).
2. Increase airflow to nominal and sample air for predetermined period (beads rapidly become a coating on the feedback tube interior at the magnet point).
3. Add water as needed ($P3_f$) to counter evaporation. Dl water misses beads and salt content of circulating fluid normalizes before contacting beads. Move beads slightly up and down tube with moveable magnet or occasionally release beads and recapture with magnet to keep bead surfaces clean and exposed.
4. Stop airflow for sample removal.
5. Pump salt solution to waste ($P2_f$).
6. Add wash solution ($P1_r$), run fan briefly to flush cyclone tube and wash beads. Let drain and pump to waste ($P2_f$). Repeat as necessary.
7. Add I00-300 ul of DI water until beads are submerged ($P3_f$); soak; move magnet up and down to release nucleic acid.
8. Disengage magnet if beads are to be in sample.
9. Pump Dl water and any beads in that solution into the sample vial ($P1_f$).
10. Flush system with Dl water to prepare for next sample.

The preceding protocol will collect and isolate all the nucleic acids from biological particulates captured by the SASS 2300. This may lead to capture of immense quantities of nucleic acids from non-targeted but populous species in the environment that may interfere with analysis.

In an alternative protocol that addresses this concern, the targeted agent could be selectively captured prior to nucleic acid isolation. DI water or a non-foaming buffer solution, as is presently used in the SASS, can be employed along with a mixture of antibody-coated magnetic beads. These antibody-coated beads would be capable of capturing the entire panoply of agents, from toxins to DNA/RNA-containing viruses, bacteria, and spores. In addition, we have previously shown that immunocapture can occur in the recirculation tube of the SASS.

Summary

A field-portable aerosol monitoring system has been outlined that allows DNA and RNA extraction and processing to proceed in parallel with aerosol collection. Such an approach could significantly help to streamline the overall nucleic acid assay process while improving assay reliability and reducing sample processing costs.

Research International believes that with the hardware and electronic modifications to the SASS 2300 described here, and the use of silica coated microspheres and non-traditional collection fluids, real-time processing of nucleic acid samples within the SASS is feasible, and nucleic acid concentrates can be delivered virtually PCR-ready. We anticipate this system to be able to maintain the composition of the sample fluid continuously for sampling periods of up to several hours, thereby providing time integration of targeted species that are present at very low aerosol concentrations. Feasibility experiments at Research International have established a detection limit of 4000 cells or less, and system optimization can be expected to improve on this result.

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