**Video Conference on Air Sampling Methods**

March 11, 2013

*Attendees:* Adam Altrichter, Rachel Adams, Seema Bhangar, Brandon Bubba Brooks, Andrew Hoisington, Denina Hospodsky, Marzia Miletto, Ann Womack

*Sound*

* Denina Hospodsky: No problem in classrooms, placed pumps just outside and ran lines through the window. White noise audible inside but activity levels sufficiently high to drown them out. In homes the sound is a problem even with pumps placed outside. Is working with SB to make noise absorbing sound cases (with highly sound-absorbing egg-crate foam from McMaster Carr).
* Adam Altrichter and Ann Womack: Show and tell. Plastic box lined extensively with foam, with SKC button sampler pump placed inside. No fan needed. Have also worked with bench-top pumps but those did release heat.
* Seema Bhangar: Used two approaches for classroom sampling. Pump in a nearby custodial closet with ~60 ft of tubing directed from it to the Andersen sampler. Fan-cooled, foam-lined box for UVAPS. The box reduced the sound from >80 to about 60 db, and dampened the high pitch edge so it was tolerable in a functioning classroom. Pictures and various design details available upon request.
* Andrew Hoisington: Retail store. Played with placing pumps in a back room with ~10 ft lines to the sampler. But had to stand near any samplers so abandoned this approach and used HVAC filters instead.

*Use of standards (i.e. spiking a known quantity of bacteria or fungi on to a filter) to evaluate extraction efficiencies:*

* Denina Hospodsky: Methods and findings in Hospodsky et al., 2010, Appl. Environ. Microbio. 76, 7004.
* Marzia Miletto: Yes, checks periodically
* Andrew Hoisington: Inhibiting effects are not the same from one filter to the next. For qPCR suggests using internal standards. Also concerned about loss of DNA “viability” over days and weeks of being exposed to airflow (e.g., for HVAC filters)

*Additional comments*

* New sampler developed by a researcher at NIOSH offers size-selection and samples particles directly into an eppendorf tube, so no need for filters and loss in the extraction process (<http://www.cdc.gov/niosh/topics/aerosols/biosampler.html>)
* Marzia Miletto: Samples diluted 1:10 for PCR to avoid inhibition. (Comments from others on any problems with inhibition and required dilutions are invited)
* Andy Hoisington: The devices we are using were designed for things slightly different than what we are using them for. So, it would be very helpful is each of us using a device did a little validation and included the findings in a supplement to the paper. For example, include data on extraction efficiency of filter material type or the particular extraction protocols we used.

**Table 1.** Air sampling approaches used by researchers in attendance

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Researcher or research group | Instrument | Flow rate (L/min) | Size-resolution? | Sampled environment | Typical hours to get amplification | Filter material | Extraction efficiency | Cells dislodged before processing?8 |
| Denina Hospodsky | Nonviable cascade impactor1 | 28  | Eight size bins from 0.43 to >9 m. Two stages removed to collect more mass per stage | Classrooms (going into homes) | Depends on environment. Typically runs OPCs side by side and now by looking at large particle levels can roughly judge how long a sampling time will be needed.6 | Quartz7 (New Star Environmental) and PCTE3 (Whatman, cut by hand to 81 mm for cascade impactor) | 10% with MoBio and Qiagen. More harsh method that includes lysis and STS can get 50% |  |
|  | SKC single-stage impactors | 10 | PM2.52 and PM10 |  |
|  | Liquid impinger4 | 10 | None | Experimenting with this now | None | Better than filters |  |
| Andrew Hoisington | HVAC filters |  | None |  |  |  | ~10% |  |
|  | SKC PEMs |  |  |  |  |  |  |  |
| Ann Womack and Adam Altrichter | PEMs |  |  |  | 1 m3 of air seems to be a magic number | MCE  |  |  |
|  | Open-face filter holder5 | 6-12 samplers in series so flow per filter is 10 or 16  | None | Has used it outdoors | 0.25 m Cellulose nitrate |  |  |
| Marzia Miletto | Open-face filter holder | 20 (Millipore pump) | None | Indoors and outdoors | 1-1.25 h | Cellulose nitrate | 50% (MoBio kit) | No |

Table Notes:

1 Has also been used by SB based on training from DH

2 Did not work for PCR, typically only enough material on it for qPCR, so DH has mostly given up on the PM2.5 sampler

3 Has the advantage of dissolving in phenol chloroform; also DH found it to be better for bacteria in terms of extraction efficiency

4 DH comments on limitations with the impinger: Needs to be sterilized each time; buffer evaporates and must be refilled from the top; not great for fungi, better for bacteria. AW and AA (Green lab) noted they use plastic pipettes to refill without opening the lid

5 RA suggests (based on advice from Dr. Mark Nicas) operating these upside down to avoid a bias from particles that passively settle on the collector. AH noted he routinely operates his samplers inverted

6 Has seen a factor of 5 or order of magnitude range in number concentrations of large (>5 or >10 m) particles. What % particles is biological is unknown, hopes to publish these data but it will take a few more months. Rule of thumb: <10/L “large” particles is a clean environment, needs longer. >100/L, even if these are only occasional peaks and not average values, greater success.

7 RA: Size of Andersen filters is a problem, they have to be cut into quarters to be analyzed, and pooled. Especially a problem with bulky quartz material. Suggested ideas: Could extract DNA from them using PBS with Tween in a 15 ml falcon tube as a first step.

8 BB is interested in knowing how many people disoldge cells from filters (saturate in PBS, agitate, etc.) before further processing. Please indicate with a Y or N if you routinely include this step.