**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)
* Denina Hospodsky to confirm PCR inhibition for small sized particles <2µm on filters. Either particle/ DNA ratios unfavorable or chemical composition in smaller sized particles inhibitory (silica based in larger sizes vs carbon based in smaller sizes).
* 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? 9 | Sampled environment | Typical hours to get amplification | Filter material | Extraction efficiency | Cells dislodged before processing?8 |
| Denina Hospodsky | Nonviable cascade impactor1 | 28.4 | Eight size bins from 0.43 to >9 m. Two stages removed to collect more mass per stage | Classrooms indoors and outdoors | 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, 0.8µm pore sized, cut by hand to 81 mm for cascade impactor) | ~10% with MoBio and Qiagen standard kits. More harsh method that includes enzymatic and chemical lysis (lysozyme, SDS, phenol chloroform, CTAB buffer) can get 50% | No |
|  | SKC single-stage impactors | 10 | PM2.52 and PM10 | same as above | same as above | PCTE (SKC) 0.8µm pore size | same as above | No |
|  | Liquid impinger4 | 12.5 | None, not efficient for collection of larger particles |  | Experimenting with this now | None | Not tested | Liquid from impinger is filtered through Pall filter on filter gallery and filter directly processed. |
| Andrew Hoisington | HVAC filters |  | None |  |  |  | ~10% |  |
|  | SKC PEMs |  |  |  |  |  |  |  |
| Marzia Miletto |  |  |  |  |  | Mixed cellulose esters (MCE) |  |  |
| UO: Ann Womack and Adam Altrichter | SKC Button Sampler | 4 | None | Indoors | ~8hrs in our study, 1 m3 of air seems to be a magic number | MCE, 1.2 um | Untested | No |
|  | Open-face filter holder5 | 12 samplers fed by a 1 horse pump, ea. ~10 l/min | None | Indoors and outdoors | Depends on flow rate, 2 hrs | 0.2 um Cellulose nitrate | Untested | Yes |
|  | Liquid impinger (SKC Biosampler) | 12.5 L/min | None | Indoors and outdoors | 2 hrs | Liquid filtered onto 0.2 um cellulose nitrate filters | Untested | No |
| Marzia Miletto | Open-face filter holder | 20 (Millipore pump) | None | Indoors and outdoors | 1-1.25 h | Cellulose nitrate | 50% (MoBio kit) | No |
| Siegel, Stephens | HVAC filter media | ~400 cfm or 11,300 lpm9 | Depends on filter efficiency curve10 | Hospital patient room | Not sure (refer to Dan Smith, ANL); we leave media up for ~1 week | Unsure; polymer | unknown |  |

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 sterile packed plastic pipettes to refill without opening the lid

5 RA suggests (based on advice from Dr. Mark Nicas) operating these inverted 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 to order of magnitude range in number concentrations of large (>2.5, >5, or >10 µm) particles from one place to another indoors. What % particles is biological is unknown and still widely discussed, hopes to summarize and publish particle number: DNA ratios in air for fungi and bacteria from these observation. Rule of thumb: <10/L >10µm particles is a clean environment, needs longer (aim for collecting at least 20m3 for one stage (better more) >100/L of >10µm is high, even if these are only occasional peaks and not average values, there is greater success in PCR amplifying those. Number concentration for >5µm<10µm: <20/L is low, >200/L is high, for >2.5µm<5µm: 50/L is low, 200/L is high.

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 0.01% Tween 20 or Tween 80 in a 15 ml falcon tube as a first step.

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

9 DH suggested that for inverted samplers the effective inlet size cutoff (i.e., largest particle size sampled) can be calculated by assuming a particle density (start off with assuming all particles are purely silica), and calculating at which size the gravitational settling is in balance with the suction from the sampler (for a particular flow rate).