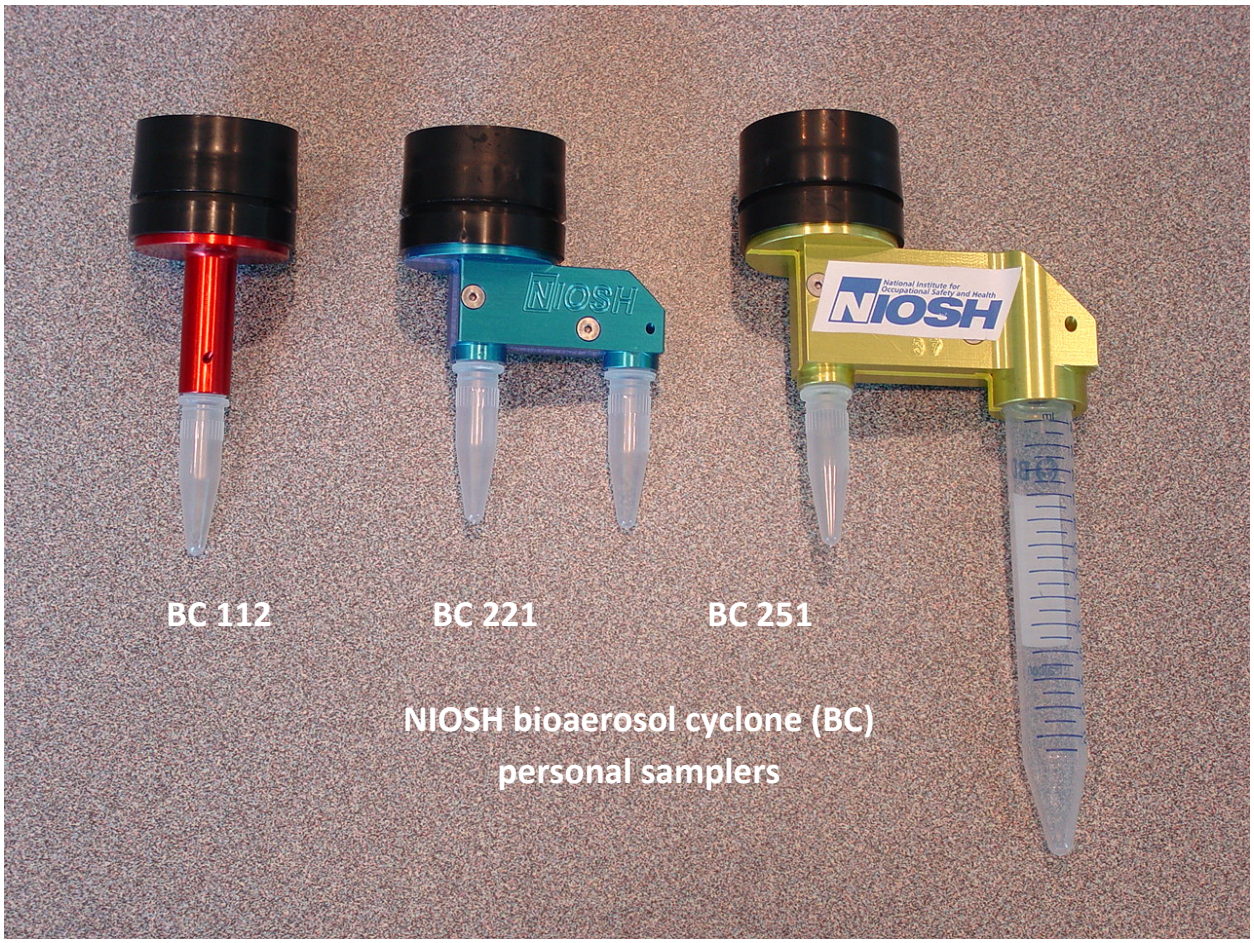
Notes on using the NIOSH one- and two-stage bioaerosol cyclone (BC) personal samplers



Contents

Bioaerosol sampling

A review of many aspects of sampling and characterizing bioaerosols can be found in the NIOSH Manual of Analytical Methods, Chapter BA, "Sampling and Characterization of Bioaerosols", available online at https://www.cdc.gov/niosh/nmam/chapters.html. Information about filter pore sizes and filter selection can be found in Chapter FP, "Filter pore size and aerosol sample collection", available online at http://www.cdc.gov/niosh/nmam/chapters.html.

Video demonstration

A short video demonstrating the use of the NIOSH BC 251 sampler was published in the *Journal of Visualized Experiments* (JoVE). It can be found at: https://www.jove.com/video/56730/collection-extraction-occupational-air-samples-for-analysis-fungal (Citation: Lemons, A. R., Lindsley, W. G., Green, B. J. Collection and Extraction of Occupational Air Samples for Analysis of Fungal DNA. *J. Vis. Exp.* (135), e56730, doi:10.3791/56730 (2018)).

Collection efficiencies for the NIOSH bioaerosol samplers

Sampler	*flow rate (liters/min)*	*1st stage 50% cut-off size (μm)*	*1st stage sharpness (geometric standard deviation)*	*2nd stage 50% cut-off size (μm)*	*2nd stage sharpness (geometric standard deviation)*
one-stage BC 112	1	3.7	1.45	--	--
	1.5	3.1	1.47	--	--
	2	2.5	1.67	--	--
	4	1.5	1.42	--	--
two-stage BC 212	2	2.6	1.45	1.6	1.75
	3.5	1.8	1.42	1.0	1.55
two-stage BC 221	2	2.7	1.43	1.5	1.74
	3.5	1.9	1.37	1.0	1.56
two-stage BC 251	2	4.9	1.48	1.7	1.68
	3.5	4.1	1.51	1.0	1.59
	10	2.1	1.44	0.41	1.56

Assembly & disassembly of sampler bodies

The two-stage samplers are machined in two matching pieces and screwed together. Final machining is then done on the assembled halves. Each half is stamped with a serial number. If the samplers are taken apart, be sure to rejoin the matching pieces. The screws holding the sampler together require either a 1/16" Allen head (hex-head) wrench or a #1 Phillips head (cross-head) screwdriver. **Sealing the seam is important because otherwise the sampler will pull air in through the seams.** Tape is used to seal the seam where the samplers join together. I use color-coding tape from McMaster-Carr (http://www.mcmaster.com/), P/N 8581T47 for 1/8" width tape or 8581T34 for 1/4" tape. Silicone adhesive or epoxy adhesive can be used on the outside of the sampler if a solvent-resistant seal is needed. However, **do not** place sealant, adhesive or grease between the halves of the sampler. This can ooze into the internal passage and clog it, and also will change the dimensions of the nozzle leading into the second stage.

Collection tubes

The BC 112 uses one threaded 1.5 ml centrifuge tube (also called an Eppendorf tube). These can be ordered from Fisher Scientific (non-sterile tubes are part number 02-681-339; caps are part # 02-681-358; sterile DNase-free RNase-free tubes with caps are part # 02-681-373) or PGC Scientifics (http://www.pgcsci.com, P/N 506-624). The BC 212 and 221 sampler uses two threaded 1.5 ml centrifuge tubes. The BC 251 uses one of the 1.5 ml tubes and one Falcon 35-2096 15 ml centrifuge tube (Fisher Scientific part # 14-959-49B). Other tubes may work but have not been checked.

The 1.5 ml centrifuge tubes can have a significant amount of variability in their dimensions. Some tubes screw in easily while others (even from the same lot) are very difficult to get in. If screwing in the tube is

difficult, make sure it is going in straight and press it in while turning. It also helps to turn the tube in the reverse direction until the threads seat and then screwing it in. Practice helps.

Sealing tubes to samplers

The tubes don't form an airtight seal with the sampler. For the one-stage sampler, seal the tube to the sampler by wrapping a piece of tape around them (see photos at end of notes). For the older BC 212 two-stage samplers, seal around the top of the sampler tubes with rope caulk (a type of removable sealing putty). The BC 221 and 251 samplers have a collar so that tape can be used to seal the tubes. I use 3/4" vinyl electrical tape to seal the 15 ml tubes and 1/4" color-coding tape to seal the 1.5 ml tubes. Heat-shrink tubing can also be used to seal the tubes and filter cassettes if done carefully.

O-rings

O-rings should be very lightly lubricated with silicone grease. EPDM (Ethylene Propylene) O-rings work very well (McMaster-Carr P/N 9557K122). Silicone rubber O-rings are OK but are more prone to tearing and abrasion damage. Black neoprene O-rings are OK. BUNA rubber O-rings degrade relatively quickly in Morgantown due to the high local ozone levels and thus are not recommended. The O-rings for the samplers are size AS568A Dash Number 027 (the 027 is the actual size specification; the first part refers to the standard used to size O-rings).

Filters & filter cassettes

I use black conductive polypropylene filter cassettes (SKC #225-309) because polystyrene cassettes sometimes crack when pressed onto the tapered mount on the sampler. The conductive cassettes have slightly lower wall losses compared to the white non-conductive cassettes. I wrap electrical tape around the cassette and the top of the sampler to make sure the cassette doesn't come off while in use and to provide an extra seal against leaks. One disadvantage of the polypropylene cassettes is that they do tend to creep (stretch) over time if they are left assembled. I have found that autoclaving the polypropylene cassettes after use reverses some of the stretching and helps to improve the sealing of the cassettes.

Filter cassettes should be assembled using a press. Closing them by hand only will allow air to leak around the filter and reduce the collection efficiency (see PA Baron et al. (2002), *Aerosol Sci Technol* 36(8): 857-865 for a discussion of this). The filter cassette comes with three pieces: (1) a piece with a gridded surface on the inside, (2) a ring-shaped piece, and (3) the piece labeled "inlet" which has a smooth surface inside. To assemble the filter cassette, put a filter support pad on the gridded surface. Put the filter on top of the filter support pad. Insert the ring-shaped piece. Use a press to tightly and evenly press down the ring-shaped piece. The ring-shaped piece needs to press down into the filter tightly enough that air doesn't leak around the filter. Check the sampler O-ring for nicks and tears and replace it if any are seen. Make sure the O-ring has a very light coating of silicone grease. Press the assembled cassette onto the top of sampler. Wrap a piece of tape around the outside of the filter cassette and the sampler to hold the filter cassette in place and to act as a back-up seal to block leaks. The inlet piece is not used on the sampler, but it can be used to cover the filter cassette to protect the filter after a sample has been collected.

One of the important considerations when choosing a filter is the pressure drop across the filter (i.e., the flow resistance of the filter). If the flow resistance is too high, the pump won't be able to pull enough air through the sampler. This is especially important with personal sampling pumps. We most commonly use a Millipore 37 mm PTFE filter with a 3 µm pore size (FSLW03700, Millipore). The following types of filters work OK at 3.5 liters/min with the two-stage samplers and the personal sampling pumps we have (i.e., the total pressure drop is acceptable): Glass A/E, PTFE with 2 µm or greater pore size, PVC with 5 µm pores, Nucleopore polycarbonate filters with 0.8 µm pore size or larger, Millipore mixed cellulose membranes with 0.8 µm pore size or larger. Gelatin filters have an acceptable pressure drop but tend to crack in use.

The following types of filters do not work with the two-stage samplers and our portable sampling pumps at 3.5 liters/min because the total pressure drop is too high. They will work OK at a lower flow rate, with more powerful pumps or using a lab vacuum supply: Nucleopore polycarbonate filters with a 0.4 µm pore size or smaller, PTFE filters with a 0.45 µm pore size or smaller.

It is important to note that filters will retain particles much smaller than the nominal "pore size" given by the manufacturer. "Pore size" is not the physical size of the opening in the filters. It comes from a common test procedure used for filters (called the "bubble point test") and refers to an equivalent size of a capillary tube. A 3 µm PTFE filter, for example, has been shown to retain >96% of aerosolized MS2 virions, which are <80 nm in diameter (NC Burton et al. 2007, *Ann Occup Hyg* 51(2): 143-51). For more information on this, see the NIOSH Manual of Analytical Methods, Chapter FP, "Filter pore size and aerosol sample collection", available for free download at http://www.cdc.gov/niosh/nmam/chapters.html.

Luer fittings

The samplers are connected to the sampling pumps via vinyl tubing and a Luer fitting (also called a slip fitting) that plugs into the top of the filter cassette. Metal Luer fittings are available from Cadence, Inc. (http:// www.cadenceinc.com, P/N 6154, Hose end for 1/8" to 3/16" ID tubing to male Luer, large bore). *Note that the Luer fitting should not protrude past the base of gridded surface on the inside of the filter cassette (see photos below).* Air needs to flow through the filter and filter support path, through the channels in the gridded surface, and then into the fitting, tubing and pump. If the Luer fitting protrudes too far into the cassette, its opening can be obstructed by the support pad (see the picture below). This will dramatically increase the pressure drop and reduce the flow rate through the filter. If sampling pumps are used, this typically prevents you from reaching the needed flow rate and causes the pumps to shut down (flow fault). If this occurs, check the fit of the Luer fittings in the cassette and grind or cut the fittings shorter if necessary.

This metal Luer fitting in the center of the cassette fits properly. The tip of the fitting is at the bottom of the channels in the gridded surface, which allows air to flow through the channel and into the fitting.

This plastic Luer fitting in the center of the cassette does not fit properly. The tip of the fitting is protruding above the top of the channels in the gridded surface. The filter support pad will press against the tip of the fitting, obstructing the flow.

Pumps & pressure drop

The samplers require high-volume personal sampling pumps to get the maximum flow rates. The SKC Universal pumps (Model 224-44XR, 224-PCXR4 and 224-PCXR8) and the Gilian Model HFS 513A Hi Flow Sampler work fine. With these sampling pumps, the maximum flow rate is about 3.5 liters/min for the two-stage samplers and 4 liters/min for the one-stage BC 112. The Gilian Aircon-2 high volume air sampler can be used to draw 10 liters/min through the BC 251.

In the paper, the pressure drop across the BC 212 samplers is given as 44.5 cm H_2O at 3.5 liters/min. However, subsequent tests with newer batches of BC 212 samplers have found that the pressure drop is typically 50-65 cm H_2O at 3.5 liters/min depending on the sampler and the filter. The pressure drop across the BC 221 is about 45 to 50 cm H_2O at 3.5 liters/min using a glass A/E filter. The pressure drop across the BC 251 is about 35 to 40 cm H_2O at 3.5 liters/min and about 209 cm H_2O at 10 liters/min using a glass A/E filter.

Calibration flow rate

Note that the samplers should be calibrated using volumetric liters/min, **not** standard liters/minute.

Wearing the samplers

To allow people to wear the sampler, the BC 212 samplers have stainless steel L-shaped brackets with alligator clips. These brackets screw in the back of the sampler underneath the filter holder. The bracket has four holes into which alligator clips can be screwed as desired.

The BC 221 and BC 251 samplers have brackets held to the back of the samplers with strips of 3M Dual Lock mushroom-head fastener (similar to Velcro, but stronger; McMaster-Carr part # 96055K21 for 1/2”

wide, # 96055K23 for 1" wide). To mount the sampler to the bracket, simply press the fastener strips together. Pull them apart to remove the sampler.

The sampling pumps are fairly loud when running at 3.5 liters/min. We often place them in use black nylon noise-reducing cases (SKC) or backpacks with acoustical foam placed around the pumps.

Sample collection & recovery

Most of the material in the sampler tubes accumulates near the top of the tube. Because of this, it is very important to make sure that the top region of the tube wall is washed during processing. The tubes should be capped and vortexed both upright (right side-up) and inverted (upside-down).

During experiments with influenza virus, if the collected sample will be analyzed using qPCR only, we add Lysis/Binding solution concentrate (Life Technologies) to each tube and vortex them thoroughly, both upright and inverted. We typically add 0.5 ml to the 1.5 ml tube and 15 ml tube to minimize the volume, although 1 ml works better in the 15 ml tube if possible. If the samples are to be analyzed using viral plaque assays or other viability assessment methods, we add 1 ml of modified Hank's Balanced Salt Solution (HBSS containing 0.1% bovine serum albumin (BSA), 100 units/ml penicillin G and 100 units/ml streptomycin) to each tube and vortex thoroughly.

During the processing of fungal spore samples, we found that a 0.2% solution of the detergent Tween 20 was required to reliably remove the spores from the tube surface for analysis.

Tiina Reponen, Sergey Grinshpun and Sung-Chul Seo at the University of Cincinnati tested the BC 212 sampler at 3.5 lpm with fungal spores. For *Stachybotrys chartarum,* they found that if the number of collected spores exceeded about 8×10^6, some spores began to bounce off the tube and collect on the filter. For *Aspergillus versicolor*, spore bounce began when the total collected spore count exceeded 2×10^7.

Ann Miguel and Rick Flagan at Cal Tech spiked Falcon 15 ml and Fisher 1.5 ml tubes with a recombinant grass allergen protein, at low concentrations (25 ng/ml). Their preliminary tests found that while about 80% of the protein was recovered from the Fisher 1.5 ml tubes, less than 50% was recovered from the Falcon 15 ml tubes, probably due to protein binding to the tubes. They found that coating tubes with the surfactant Pluronic L-121 (BASF) significantly improves recovery. To do this, prepare 0.5% Pluronic L-121 (w/v) in H_2O: isopropanol (90:10, w/v). Add to the tubes, cap the tubes and incubate for 30 min with slow agitation at room temperature. Remove surfactant solution and allow the tubes to dry before use.

Chemical resistance

The samplers are made of aluminum with stainless steel outlet tubes, screws and alignment pins. The aluminum is anodized after machining, which provides some protection against corrosion from things like salt particles. Do not soak the samplers in strong acid or base solutions; this will destroy the anodizing and render the sampler unusable.

Cleaning

For many applications, the samplers can simply be rinsed with water and/or alcohol between uses. If water only is used for rinsing, use lab-grade distilled water and blow out the samplers with air after rinsing to avoid build-up of mineral residue from water in the internal passages, particularly the 2nd stage inlet. The samplers can also be rinsed with ethyl acetate (this is done during calibration tests using fluorescent microspheres), but the seam tape and labels may become damaged and need to be replaced.

The following protocol is used by Sung-Chul Seo at NIOSH to prepare the two-stage samplers for β-glucan assays of airborne fungi and fungal fragments. A similar protocol is used at the University of Cincinnati:

1) Remove the O-ring and disassemble the sampler.
2) Wash with soap
3) Place in ultrasonic cleaner with soap solution for 10 minutes.
4) Rinse sampler with running tap-water.
5) Put the sampler into a baked beaker (1000 ml) with ethanol.
6) Do 1 hour ultrasonic cleaning.
7) Dry the sampler in the biosafety hood for 10 minutes.
8) Cover the metal parts with aluminum foil.
9) Heat all metal parts in an oven at 210 °C for 1 hour.
10) Put parts in the biosafety hood and allow to cool for 30 minutes.
11) Assemble sampler with O-ring and 1.5-ml tubes.

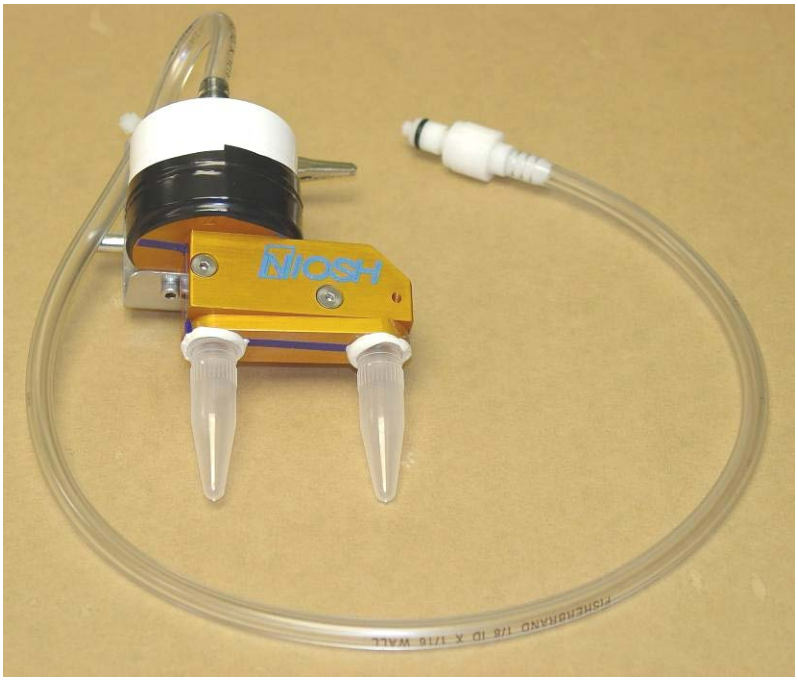
Preparing samplers for sampling for airborne endotoxin

Duquenne et al. (2018) recommended the following protocol to clean the BC 112 sampler before using it to collect airborne endotoxin:

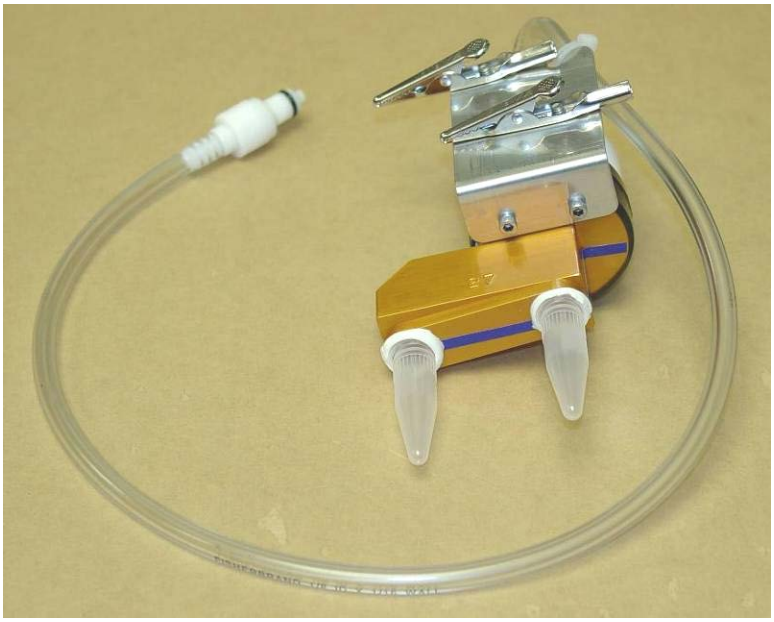
1) Disassemble the cyclone (gaskets, collection tube and backup cassette).
2) Place the sampler in an ultrasonic cleaner with detergent solution for 10 min.
3) Rinse with tap water.
4) Place the sampler in an ultrasonic cleaner with ethanol for 60 min.
5) Dry under a laminar flow cabinet for 10 min.
6) Pack the body of the sampler in aluminum foil and submit to dry heat at 210 °C for 60 min.
7) Assemble the cyclone (gaskets, collection tube and backup cassette).
8) Store the mounted cyclone in a clean and dry area until use.

Duquenne, P, C Coulais, S Bau and X Simon (2018). Performances of the BC-112 NIOSH cyclone for the measurement of endotoxins in bioaerosols: A study in laboratory conditions. *J Aerosol Sci* 116: 92-105.

Assembled single-stage BC 112 sampler: The tubes and filter cassette on the BC 221 are sealed with tape as shown. Note that the sampler inlet must face forward.



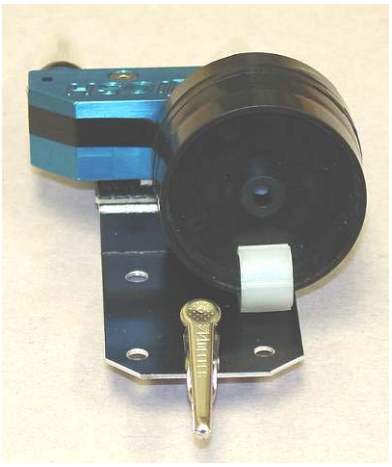
Assembled two-stage BC 212 sampler: White material at top of Eppendorf tubes is rope caulk for sealing. Blue tape on sampler seals the seam where the two halves join together. Black tape around the filter cassette helps keep the cassette in place and seals any possible leaks. A Luer fitting plugs into the top of the filter cassette. 1/8" ID tubing connects the Luer fitting to a CPC quick-disconnect fitting



Rear view of BC 212 sampler: The stainless-steel L-bracket allows the sampler to be clipped onto a worker. This bracket attaches with two socket-head screws as seen. The bracket can be removed if it is not needed. The bracket has four holes for alligator clips (two clips are shown). The clips can be rotated or moved to different holes as desired.

BC 221 sampler: The tubes and filter cassette on the BC 221 are sealed with tape as shown rather than caulk. The seam around the sampler is sealed with tape to prevent air leakage during use. The filter cassette is black conductive polypropylene.

Side view of BC 221: Clothing clips are mounted on aluminum holders. The holders have black Dual Lock strips for fastening to the back of the sampler. A split round nylon retainer is also used to help hold the sampler more securely.



Top view of BC 221: To mount the sampler on the holder, slide the nylon retainer onto the filter cassette and press the sections of Dual Lock strip together. Pull them apart to remove the sampler.

Two-stage BC 251 sampler attached to personal sampling pump: Note that the white CPC quick-disconnect fittings contain valves that shut off the fitting when they are disconnected. Because of this, do not turn on the pump while the sampler is not connected; the pump will not be able to draw air and will shut down.

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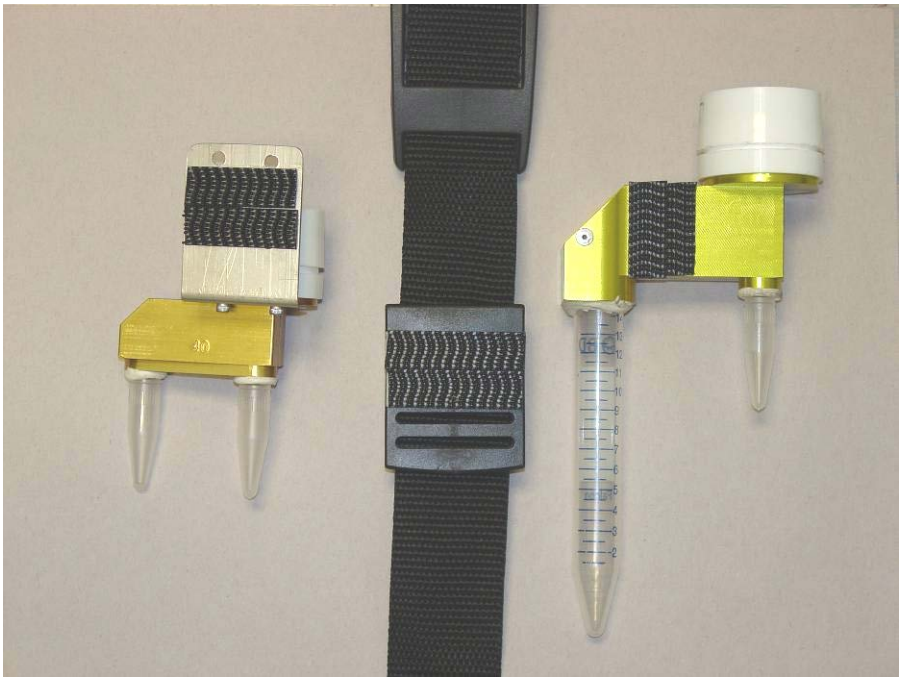
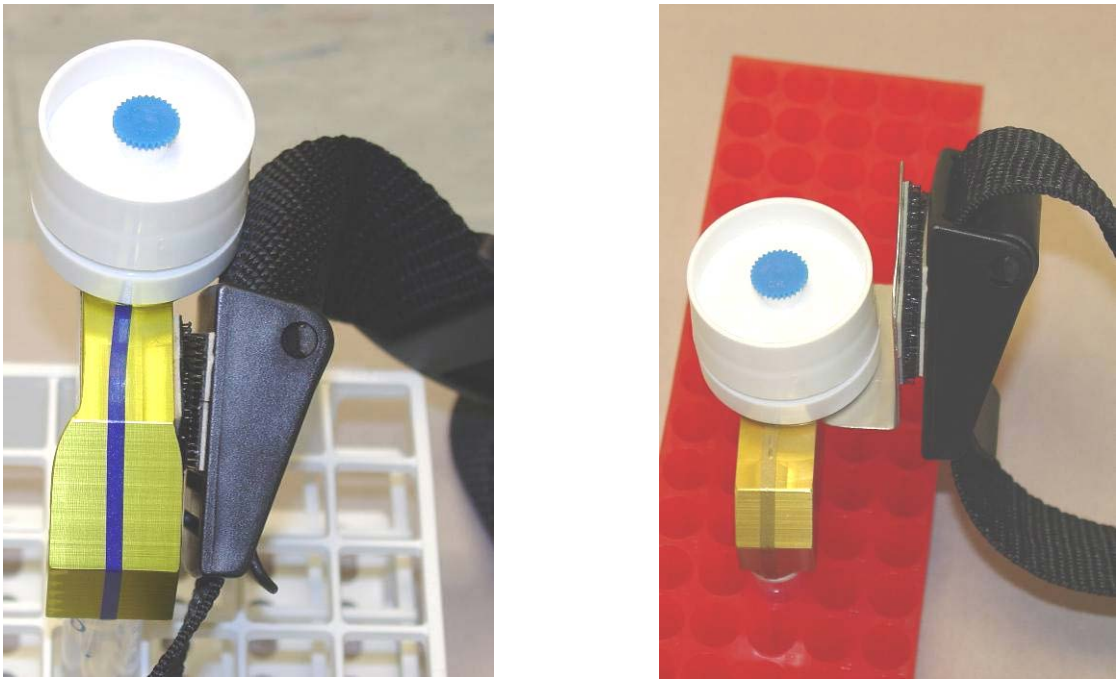
Clothing clips and lanyard for BC 251 sampler. The sampler can be clipped to clothing or worn on a lanyard around the neck. The plastic clips are easier on clothing but do not hold as well as the metal alligator clips. The metal alligator clips hold better, but their teeth can damage some types of clothing. An optional extra clip mounted on a cable retainer can be placed on the 15 ml tube as shown to steady the sampler and keep it close to the body. The sampler can also be worn using an adjustable lanyard. Note that, for safety, the lanyard must have a breakaway clasp.

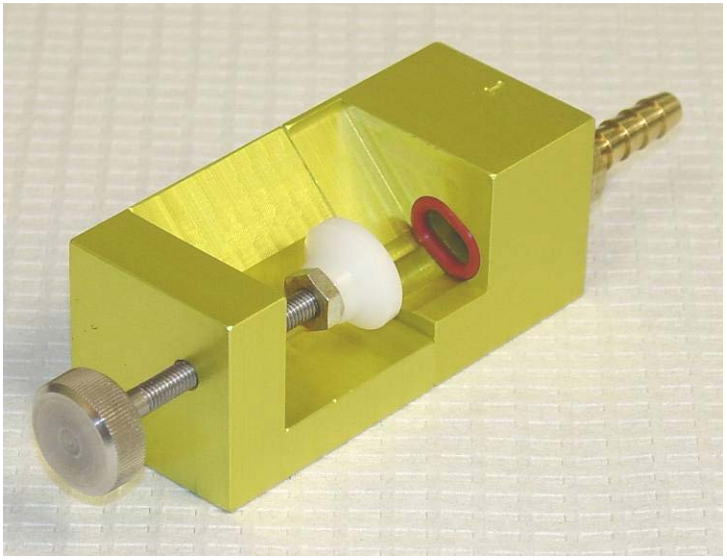


Samplers and buckle showing black Dual Lock strips. When the noise-reducing cases are used, the sampler is mounted on a buckle on the case shoulder strap. To mount the sampler on the buckle, press the sections of Dual Lock strip together. Pull them apart to remove the sampler. The buckle is on the shoulder strap for a noise-reducing case. It can be moved along the strap as needed, and can be pried apart and moved to a different part of the strap if necessary.

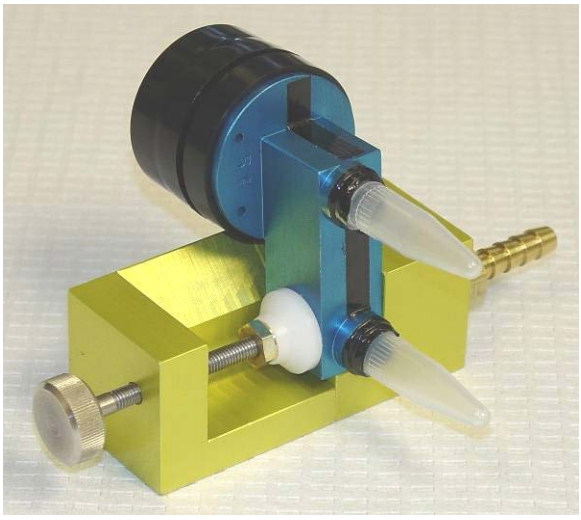


BC 251 (left) and BC 212 (right) mounted on buckle on shoulder strap.

Flow calibration. The airflow through the sampler must be calibrated before each use. A calibration jar may be used for this purpose. SKC makes a large calibration jar (#225-112) that will fit all of the samplers, including the BC 251. Attach the sampler to the fitting as shown and place it in the jar. Connect the pump and flow meter. Turn the pump on and allow it to warm up for 5 minutes before setting the flow rate.

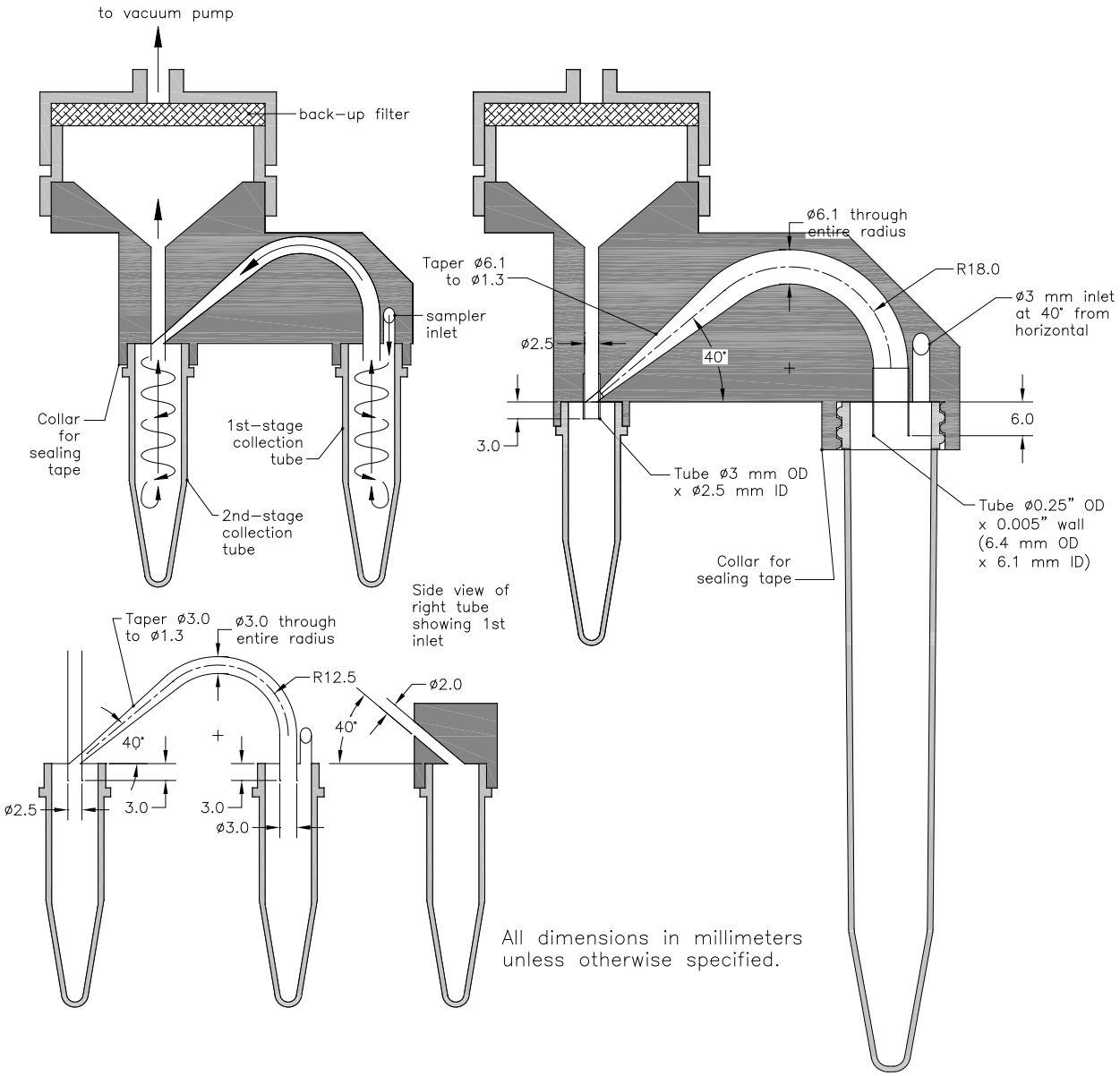


The two-stage also can be calibrated using a custom flow calibration adaptor. To use the adaptor, insert the sampler fully so that the top and side surfaces of the sampler are pressed against the inner sides of the adaptor. The sampler inlet must fall completely within the internal O-ring of the adaptor. Tighten the thumbscrew to seal the sampler against the adaptor O-ring. While tightening the thumbscrew, hold the sampler firmly to keep it from shifting position. The brass fitting of the adaptor should be connected to a flow meter.



The calibration adaptor is shown here with a BC 221 sampler (left) and a BC 251 sampler (right) properly inserted.

Versions of all three samplers have been made that allow direct connection to a sampling port. Shown is a BC 253 sampler, which is a BC 251 with the first inlet counterbored to hold a stainless steel tube with the same inner diameter as the inlet. It is very important that any lines or tubes leading to this sampler must be straight; any bend will collect particles by the same process that causes particles to collect on the curved surface of the centrifuge tube. For the red BC 253 samplers, the stainless steel tube is pressed into place and held by friction. For the green BC 253 samplers, the stainless steel tube is glued in place using Loctite 480 Prism Impact Resistant Super Glue. The glue bond is not disrupted by autoclaving the sampler.



Inlet, outlet and internal dimensions of two-stage BC samplers.

If you have questions about any of the samplers or would like more information, please contact:

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