



EPA

**UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY**

"...TO PROTECT HUMAN HEALTH AND SAFEGUARD THE NATURAL ENVIRONMENT..."

BIOLOGICAL SAMPLING PROCEDURES

**FOR
REGIONAL COUNTERTERRORISM RESPONSE PLANS**

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INTRODUCTION

Under Technical Direction Document (TDD) number S05-0212-009, the U.S. Environmental Protection Agency (EPA) tasked Tetra Tech EM Inc. (Tetra Tech) to purchase equipment kits for biological agent sampling for Region 5 and prepare a guidance manual for equipment use. Tetra Tech purchased the equipment, put the equipment into sampling kits and prepared this manual to provide a guidance on the equipment operation.

This manual provides detailed instructions on using the biological sampling equipment in the U.S. EPA Region 5 biological sampling kits. This manual does not provide health and safety recommendations, and assumes that all personnel using the sampling equipment will conduct any sampling in the appropriate personal protective equipment as established by a site-specific health and safety plan.

Perishable sampling supplies (such as petri dishes with the appropriate agar solutions) will either be provided by the laboratory contracted to perform the sample analyses, or they will be purchased from a laboratory supplier. The contracted biological laboratory should be contacted prior to sample collection to ensure that proper storage, shipping, and handling protocols are followed.

Some equipment (Quick Take 30 pump, mounting bracket, and sampling wand) is unavailable until September 2003. The Quick Take 30 pump is a battery-operated pump capable of 30 liters per minute. The mounting bracket allows the single stage or six-stage impactor to be mounted directly on the pump. The sampling wand is a telescoping wand for the Air-O-Cell cassettes that allow for aggressive sampling of duct work and behind furniture.

The information presented in this guide was either obtained from manufacture's instruction or from the National Response Team's Guide "Technical Assistance for Anthrax Response" Interim-Final Draft for Review, June 2002 and the Tetra Tech START "Region 5 Biological Sampling Procedures", draft date January 2003. Refer to Tetra Tech's START "Region 5 Biological Sampling Procedures", January 2003, for more detailed sampling protocols and custody considerations.

SIX-STAGE IMPACTOR

1.0 INTRODUCTION

The Andersen Six-Stage Viable Particle Sampler is a multi-orifice, cascade impactor that is normally used to measure the concentrations and particle size distributions of aerobic bacteria and fungi in the intramural or ambient air (Figure 1). This instrument has been widely used as a standard for enumerating the viable particles in a microbial aerosol. Viable particles can be collected on a variety of prepared glass agar plates (15 by 100 millimeters) (Figure 2) and incubated in situ for counting and identification.

This sampler has been factory-calibrated so that all particles, regardless of physical size, shape, or density, are sized aerodynamically and can be directly related to human lung deposition. Particles as small as 0.65 micron are captured on Stage 6, and particles greater than 7.0 microns are captured on Stage 1 of the Andersen Sampler.

2.0 DESCRIPTION

The Andersen Sampler is constructed with six aluminum stages that are held together by three spring clamps (Figure 3) and sealed with O-ring gaskets. Each impactor stage contains multiple precision-drilled orifices (Figure 4). The size of the orifice is reduced on each succeeding stage. Any particle not collected on the first stage follows the air stream around the edge of the petri dish to the next stage.

3.0 COLLECTION OPERATION

Assembly

- The sampler should be cleaned and disinfected before each use. First, all jet holes in each stage should be inspected for material, and if found, blown out using a jet blast of dry air. Just before use, all stages should be wiped down with a sterile gauze pad wetted with a 70 percent isopropyl alcohol solution.
- **PETRI DISHES PREPARED WITH THE LABORATORY-SPECIFIED AGAR SOLUTION SHOULD BE OBTAINED FROM THE CONTRACTED LABORATORY OR OBTAINED FROM A LABORATORY SUPPLIER. DO NOT USE PETRI DISHES SUPPLIED WITH THE UNIT UNLESS THEY HAVE BEEN PROPERLY PREPARED BY THE ANALYTICAL LABORATORY.**
- While wearing sterile gloves, remove the cover from the petri dish and place the cover in a re-sealable plastic bag. Mark the bag according to the stage number (Stage 1, 2), as well as routine sample information.
- Place the petri dish on the base of the unit, resting on the three metal pins (Figure 5). Place Stage 6 over the petri dish. Place another petri dish on top of Stage 6 and then place Stage 5 over the petri dish. Continue in this manner until all six petri dishes and stages have been assembled.
- Place the inlet cone on top of Stage 1.

- Place the spring clamps on top of the inlet cone.
- Connect the sampler to a vacuum pump using Tygon tubing.

Calibration

- **IF USING THE GAST PUMP SUPPLIED WITH THE SAMPLER (THERMO ANDERSEN MODEL NUMBER 10-709), CALIBRATION IS UNNECESSARY SINCE IT HAS BEEN FACTORY-CALIBRATED TO OPERATE AT 28.3 LITERS PER MINUTE.**
- If using a pump other than that supplied with the kit, calibrate the pump to 28.3 liters per minute using a dry cell calibrator.
 - Attach a hose from the inlet cone of the sampler with a calibration adaptor to the outlet port on the dry cell calibrator (Figure 6).
 - Turn the pump on and adjust the pump flow rate until three consecutive readings of 28.3 liters per minute (+/- 10 percent) are obtained.

Operation

- Place the sampler in the sampling area, either at floor level or sitting on another stable object (desk, cabinet, sample stand), plug in the sample pump (Figure 7), and turn on the pump.
- **DO NOT OPERATE THE PUMP FOR MORE THAN 30 MINUTES (IDEALLY 10 MINUTES) SINCE THE AGAR WILL DEHYDRATE AND VIABLE PARTICLES WILL BE DAMAGED.**
- After the sampling period is over, turn off the pump.

Sample Collection

Leave the sampler in the sampling area until all petri dishes have been removed.

- To avoid cross-contamination, don a new pair of sterile gloves for each petri dish.
- Remove the spring clamps from the inlet cone and Stage 1 of the sampler.
- Remove the petri dish from the sampler and place the correct lid on top of the petri dish.
- Write the sample number on the bottom of the petri dish.
- Seal the petri dish and lid with Parafilm[®] tape.
- Place the sample upside down (agar oriented up) in a re-sealable plastic bag. Double bag the sample.
- Repeat for all remaining petri dishes.

- Place all six double-bagged samples into a larger re-sealable plastic bag. **MAKE SURE ALL PETRI DISHES ARE STORED UPSIDE DOWN.**

4.0 EQUIPMENT NEEDED

- Andersen Sampler
- Petri dishes with appropriate agar solution (to be supplied)
- Tygon tubing
- Pump capable of 28.3 liters per minute
- Rotometer or dry cell calibrator
- Sterile sample gloves
- Re-sealable plastic bags
- Parafilm[®]
- Isopropyl alcohol (70 percent)
- Sterile gauze pads
- Detergent, water, and paper towels
- Sharpie
- Sample paperwork

Andersen Six Stage Impactor Kit

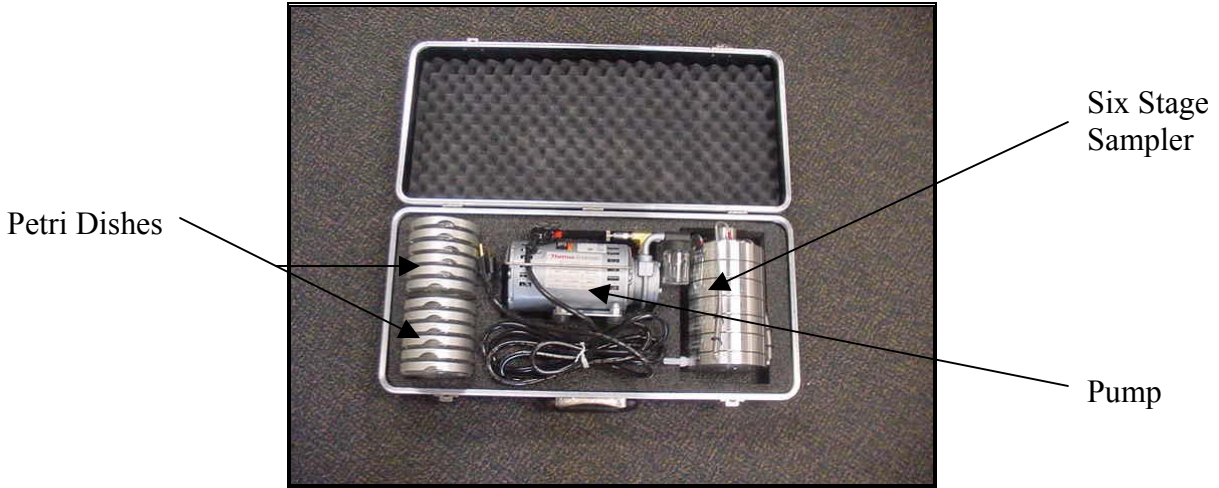


Figure 1

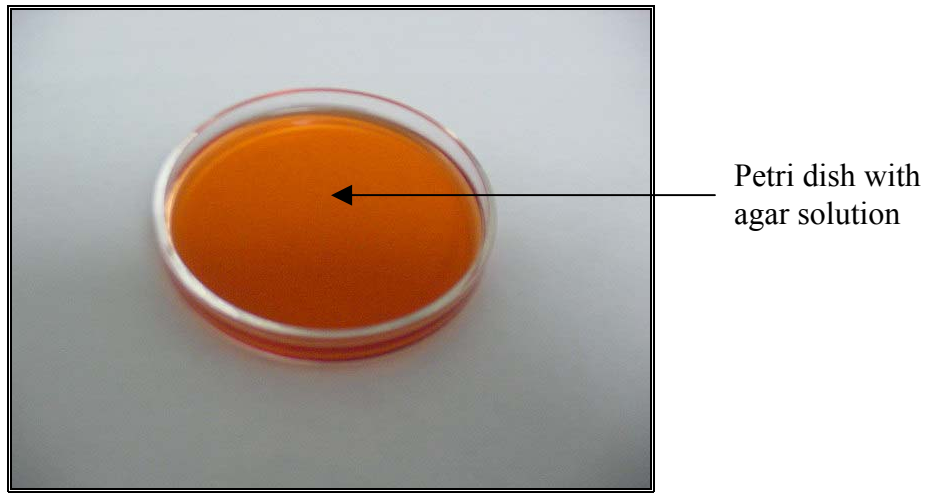


Figure 2

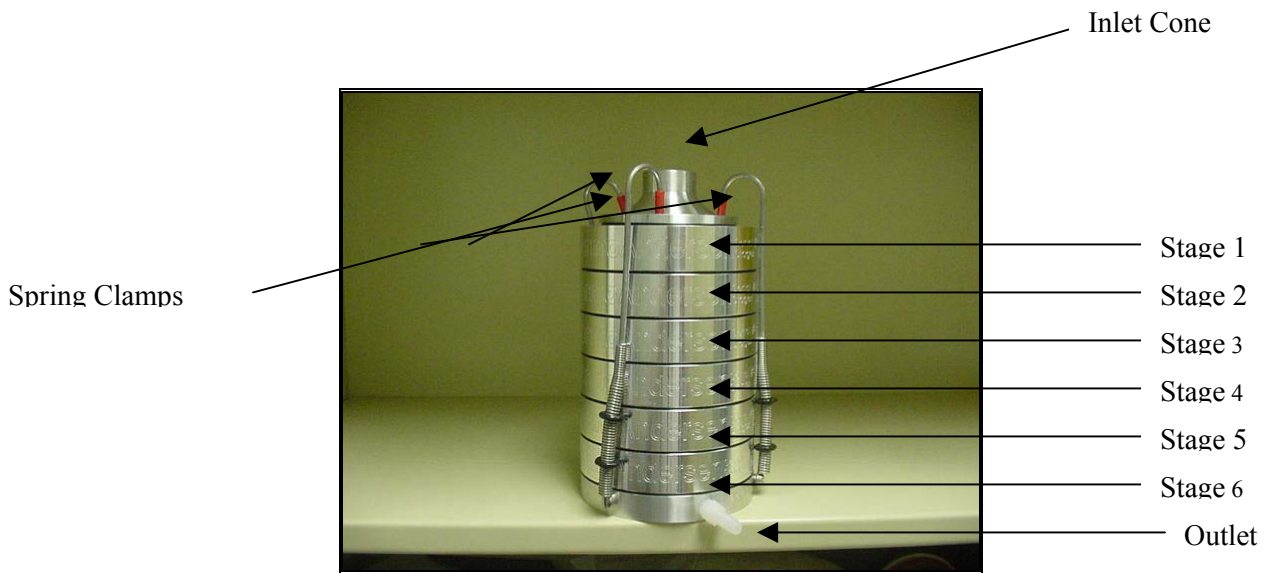
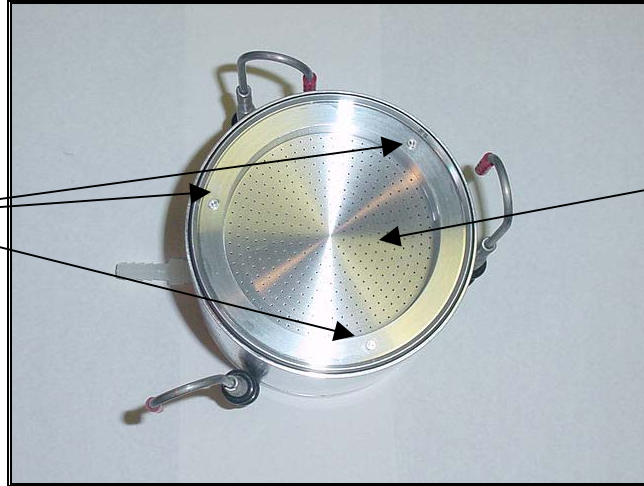


Figure 3

Metal Pins. Keeps the petri dish the correct distance from the jet orifices to allow for air flow around the petri dish.



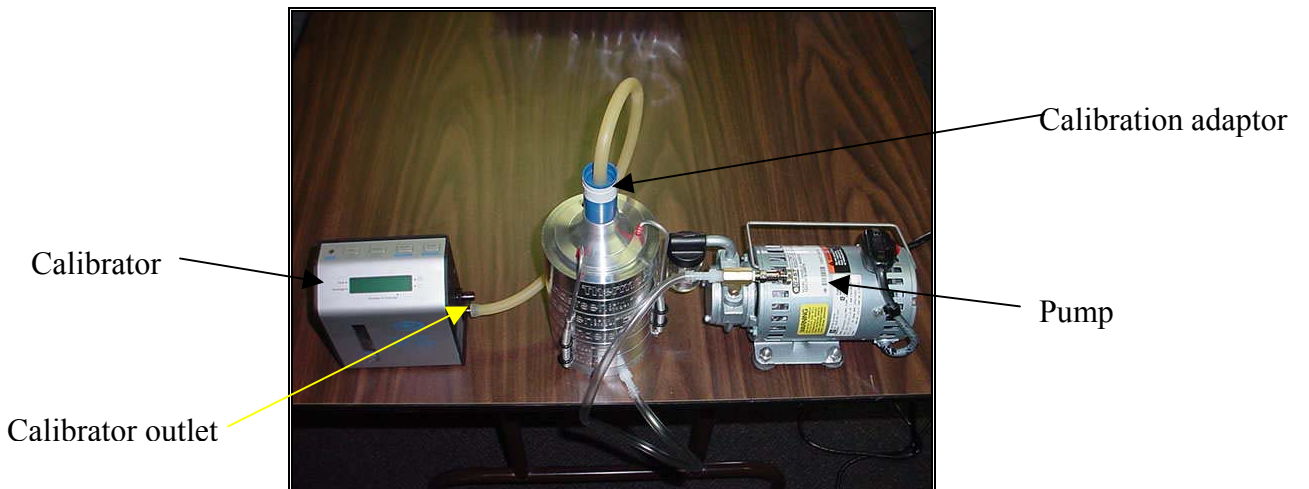
Jet orifices

Figure 4



Andersen Six Stage Sampler with a petri dish on Stage 3

Figure 5



Calibrator

Calibration adaptor

Calibrator outlet

Pump

Figure 6

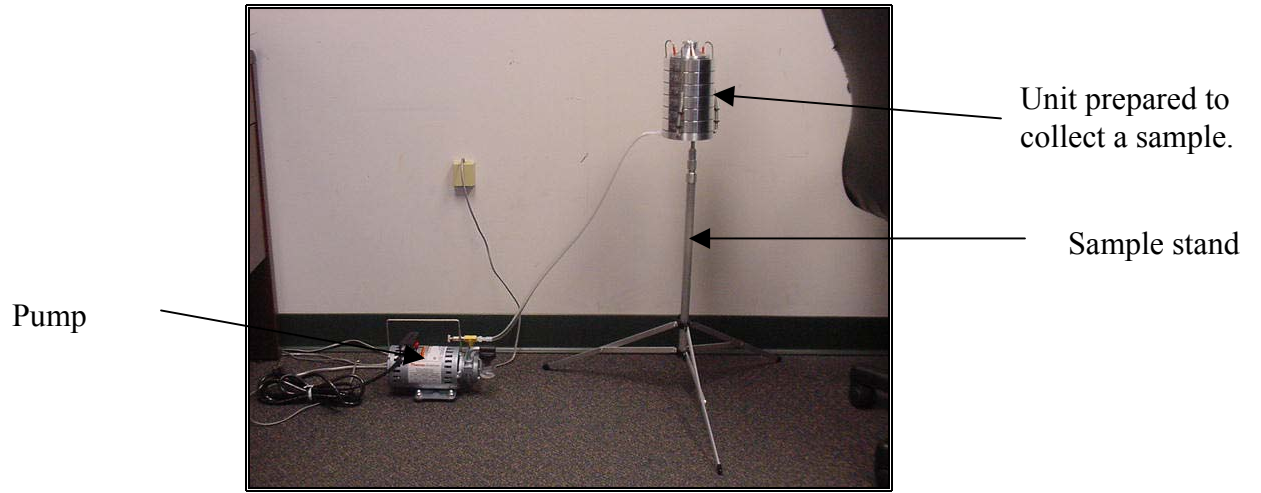


Figure 7

SINGLE-STAGE IMPACTOR

1.0 INTRODUCTION

The BioStage[®]-1 single-stage impactor is a replica of the Andersen Sampler, although it consists only of a single stage (Figures 8 and 9).

2.0 DESCRIPTION

The BioStage[®]-1 consists of one aluminum stage, base plate, and inlet cone that are held together by three spring clamps and sealed with O-ring gaskets. The single impactor stage contains multiple precision-drilled orifices (0.25 millimeter in diameter). The stage mimics Stage 6 of the Andersen Sampler by collecting particles in the 0.65 to 1.1 micron size range.

3.0 COLLECTION OPERATION

Assembly

- The sampler should be cleaned and disinfected before each use. First, all jet holes on each stage should be inspected for material, and if found, blown out using a jet blast of dry air. Just before use, all stages should be wiped down with a sterile gauze pad wetted with a 70 percent isopropyl alcohol solution.
- **A PETRI DISH WITH THE LABORATORY-SPECIFIED AGAR SOLUTION SHOULD BE OBTAINED FROM THE CONTRACTED LABORATORY OR OBTAINED FROM A LABORATORY SUPPLIER.**
- While wearing sterile gloves, remove the cover from the petri dish and place the cover in a re-sealable plastic bag.
- Place the petri dish on the base of the unit, resting on the three metal pins. Place the impactor stage over the petri dish.
- Place the inlet cone on top of the sampler.
- Place the spring clamps on top of the inlet cone.
- Connect the sampler to a vacuum pump using Tygon tubing.

Calibration

- **IF USING THE GAST PUMP (SKC MODEL NUMBER 228-505) SUPPLIED WITH THE SAMPLER, ADJUST THE AIR FLOW UNTIL THE ATTACHED ROTOMETER READS 28 LITERS PER MINUTE (Figure 10).**
- If using a pump other than that supplied with the kit, calibrate the pump to 28.3 liters per minute using a dry cell calibrator.
 - Attach a hose from the inlet cone of the sampler with a calibration adaptor (Figure 6) to the outlet port on the dry cell calibrator.

- Turn the pump on and adjust the pump speed until three consecutive readings of 28.3 liters per minute (+/- 10 percent) are obtained.

Operation

- Place the sampler in the sampling area, either at floor level or sitting on another stable object (desk, cabinet, sample stand) (Figure 7) and turn on the pump.
- **DO NOT OPERATE THE PUMP FOR MORE THAN 30 MINUTES (IDEALLY 10 MINUTES) SINCE THE AGAR WILL DEHYDRATE AND DAMAGEVIABLE PARTICLES.**
- After the sampling period is over, turn off the pump.

Sample Collection

Leave the sampler in the sample area until the petri dish has been removed.

- To avoid cross-contamination, don a new pair of sterile gloves to remove the petri dish.
- Remove the spring clamps from the inlet cone.
- Remove the inlet cone from the sampler.
- Remove the impactor stage from the sampler.
- Remove the petri dish from the sampler and place the correct lid on top of the petri dish.
- Write the sample number on the bottom of the petri dish.
- Secure the petri dish and lid with Parafilm[®] tape.
- Place the sample upside down (agar oriented up) in a re-sealable plastic bag. Double bag the sample.
- **MAKE SURE THE PETRI DISH IS KEPT UPSIDE DOWN DURING SAMPLE STORAGE AND TRANSPORTATION.**

4.0 EQUIPMENT NEEDED

- BioStage[®]-1 single stage sampler
- Petri dish with appropriate agar solution (to be supplied)
- Tygon tubing
- Pump capable of 28.3 liters per minute
- Rotometer or dry cell calibrator
- Sterile sample gloves
- Re-sealable plastic bags
- Parafilm[®]
- 70 percent isopropyl alcohol
- Sterile gauze pads
- Detergent, water, and paper towels
- Sharpie
- Sample paperwork

Single-Stage Impactor



Six-Stage Impactor

Figure 8

SKC BioStage-1 Impactor Kit

Single-Stage Impactor



Sample Stand

Pump

Figure 9

Pump



Flow adjust

Rotometer

Figure 10

AIR-O-CELL SAMPLING CASSETTES

1.0 INTRODUCTION

The 37-mm Air-O-Cell, manufactured by Zefon International, is designed for rapid collection of airborne aerosols. The design of the airflow pathway creates a deposition of airborne particulates on the media in the center of the special glass slide contained in the cassette housing.

2.0 DESCRIPTION

The Air-O-Cell cassette is a 37-mm-diameter plastic cassette with a glass slide and collection media in the center. A high volume pump capable of 15 liters per minute is used for sampling purposes. The cassette can either be left stationary or used for aggressive sampling in tight locations (duct work, behind furniture) using the sampling wand available with the Quick Take 30 pump.

3.0 OPERATING INSTRUCTIONS

Calibration

- **NO ADAPTER IS AVAILABLE TO CALIBRATE THE AIR-O-CELL SAMPLING CASSETTE. FOR THIS REASON, USE THE HIGH VOLUME PUMP SUPPLIED IN THE SINGLE-STAGE IMPACTOR KIT SINCE IT HAS AN INLINE ROTOMETER ATTACHED. IF THIS PUMP IS UNAVAILABLE, CALIBRATE THE PUMP TO 15 LITERS PER MINUTE PRIOR TO INSERTING THE CASSETTE.**
- Attach a dry cell calibrator to a high volume pump.
- Turn on the pump and adjust the flow rate until three consecutive readings of 15 liters per minute (+/- 10 percent) are obtained.

Stationary Operation

- Don sterile sample gloves.
- Remove and retain the tape seal covering the Air-O-Cell inlet and outlet.
- Adjust the sample stand to the desired sample height and thread the tubing from the pump through the top of the stand.
- Attach the outlet of the cassette to the tubing leading from the high volume pump (set at 15 liters per minute) (Figure 11).
- Turn on the pump and sample according to the following suggested time schedule:
 - Clean (no visible dust) room or outdoors – 10 minutes
 - Indoors with high activity and personnel – 5 minutes
 - Indoors with heavy dust – 1 minute

Aggressive Sampling

- Don sterile sample gloves.
- Attach the sampling wand to the high volume pump (set at 15 liters per minute).
- Connect the Air-O-Cell cassette to the sampling wand.
- Sweep the cassette over the desired area (carpeting, behind furniture, ductwork) using the same time schedule as above.

Sample Collection

- Turn off pump after the designated sampling time.
- With a sterile pair of sample gloves, remove the Air-O-Cell cassette from the sample tubing.
- Replace the tape seal over the inlet and outlet of the cassette.
- Label the sample accordingly.
- Place sample in a re-sealable plastic bag.

4.0 EQUIPMENT NEEDED

- High Volume pump
- Air-O-Cell cassette
- Tygon tubing
- Rotometer or dry cell calibrator
- Sample stand
- Sample label
- Re-sealable plastic bags
- Sample wand
- Sharpie
- Sterile sample gloves
- Sample paperwork

Air-O-Cell cassette set up for sampling

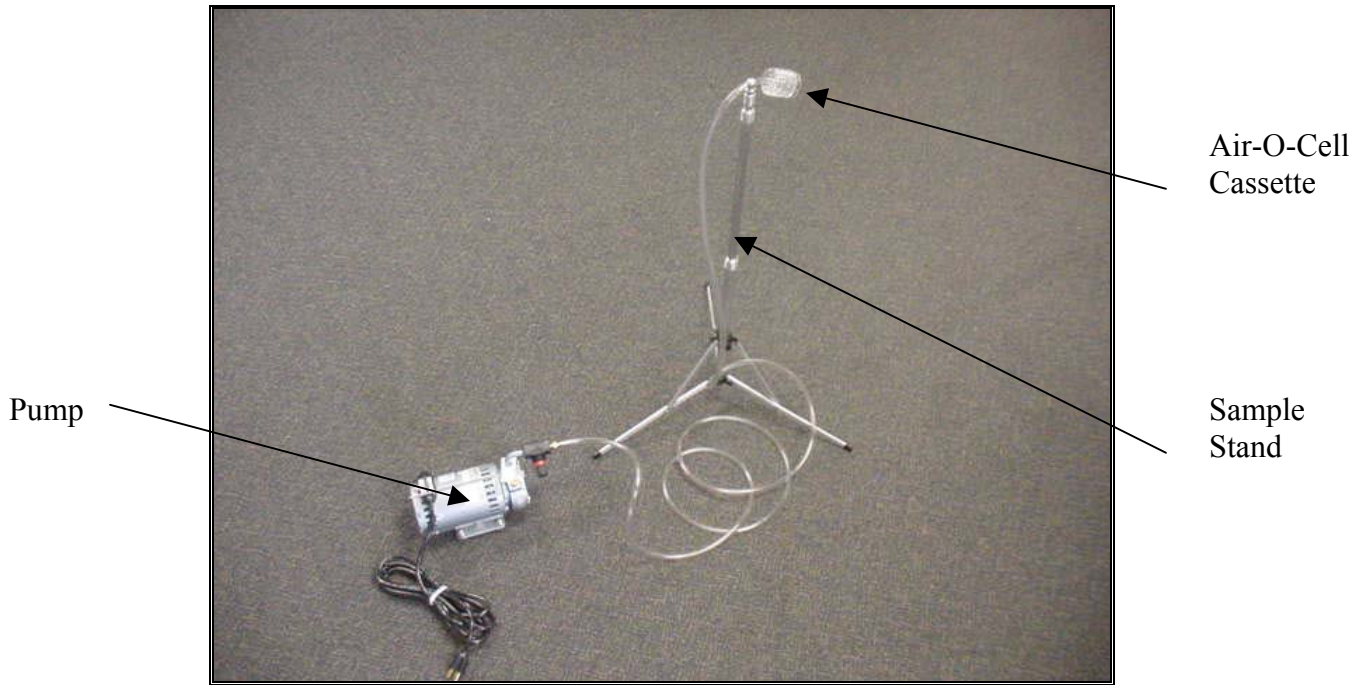


Figure 11

GELATIN FILTERS

1.0 INTRODUCTION

SKC Gelatin Filters are designed for the collection and analysis of airborne microbes. Gelatin filters not only retain bacteria and molds, but are also effective for the collection of viruses. Gelatin filters have an inherently high moisture content that helps to prevent desiccation of captured microbes, and maintains viability of stress-sensitive microorganisms during sampling.

2.0 DESCRIPTION

A clear plastic three-stage cassette will be used to hold the filter in place. The top portion of the cassette (marked inlet) is removed during sampling to allow for greater airflow across the gelatin filter. The SKC Gelatin Filter will remain in the cellulose package, separate from the cassette until ready to sample (Figure 12). The gelatin filter is 37 mm in diameter and contains a water-soluble gelatin material.

3.0 OPERATING INSTRUCTIONS

Assembly

- Don a clean pair of sterile gloves.
- Cut open the bag of gelatin filters (each bag has five filters in cellulose pockets).
- Remove one filter packet by hand. Avoid touching the filter with your hand.
- Let the gelatin filter slide out of its packet onto the outlet portion of the 37-mm plastic cassette.
- Use forceps to position the gelatin filter. **DO NOT BEND THE FILTER.**
- Place the middle stage of the cassette on top of the outlet stage.

Calibration

- **SINCE THE CASSETTES WILL BE RUN OPEN-FACED, CALIBRATION IS IMPOSSIBLE SINCE THE CASSETTE OPENING IS 37 mm IN DIAMETER.**
- Calibrate the pump to 2 liters per minute using a dry cell calibrator prior to attaching the gelatin filter.
 - Attach a dry cell calibrator to the sample pump.
 - Turn on the pump and adjust the flow rate until three consecutive readings of 2 liters per minute (+/- 10 percent) are obtained.

Operation

- Set the sampler in the area to be sampled.
- Connect the 37-mm cassette to the low-flow pump using flexible tubing.
- Turn on the pump and sample according to the following suggested time schedule:
 - Dry conditions – 20 minutes
 - Normal Conditions – 30 minutes
 - Humid conditions – 45 minutes

UNDER NO CIRCUMSTANCES SHOULD THE FILTER COMPLETELY DRY OUT. IF THIS HAPPENS, A NEW SAMPLE WILL HAVE TO BE COLLECTED, WHICH WILL SHORTEN THE SAMPLE TIME.

Sample Collection

- Turn off the pump.
- Remove the cassette from the tubing.
- Attach the top portion of the cassette.
- Insert the inlet and outlet plugs into the sample cassette.
- Label the sample cassette and place in a re-sealable plastic bag (double-bag the sample).

4.0 EQUIPMENT NEEDED

- Gelatin filters
- Forceps
- Empty 37-mm sample cassettes
- Flexible tubing
- Low flow pump
- Labels
- Sharpie
- Sterile sample gloves
- Re-sealable plastic bags
- Sample paperwork

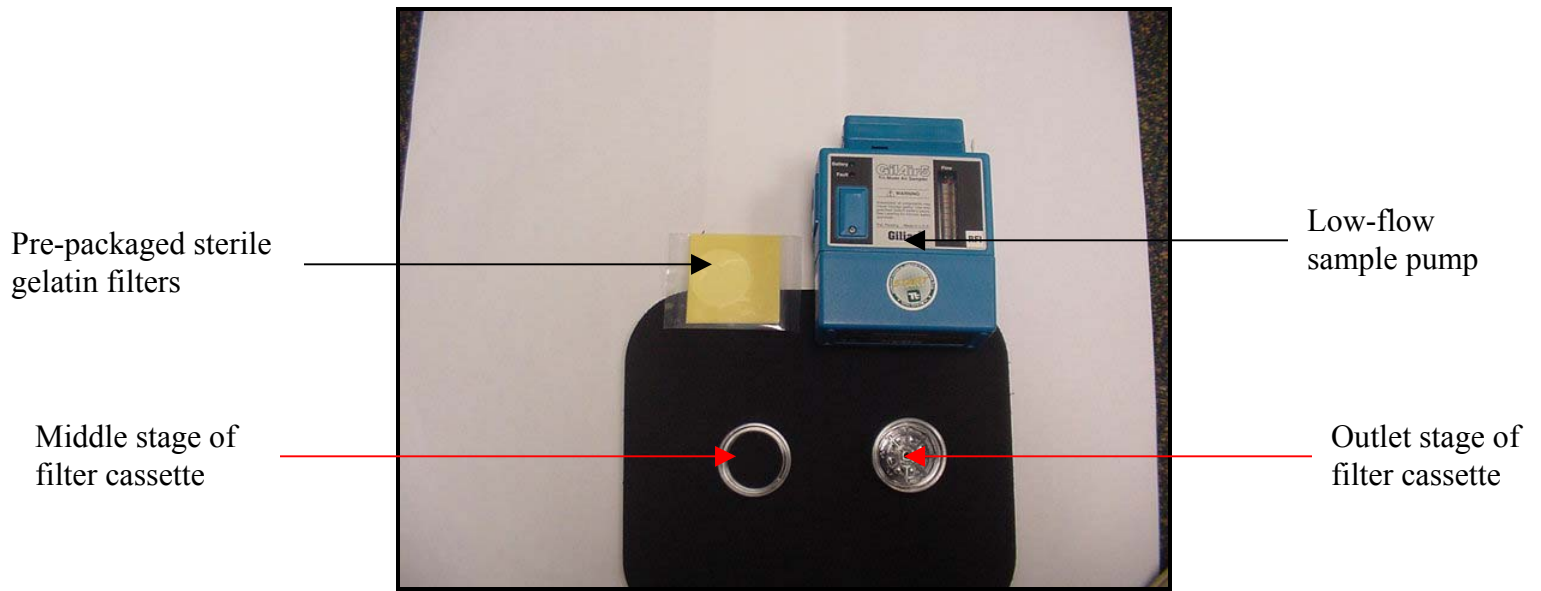


Figure 12

MIXED CELLULOSE FIBER (MCE) CASSETTES

1.0 INTRODUCTION

Since MCE filter cassettes are dry, they can be operated for a longer period of time. The MCE filter cassettes are biologically inert and are used for trapping microbes for colony counting, microscopic analysis, or DNA identification.

2.0 DESCRIPTION

The MCE filter cassettes are 37-mm plastic, three-stage cassettes. The MCE filter is preloaded into the cassette (Figure 13) and has a white background with black gridlines (Figure 14) that allows for easy colony counting. The top portion of the cassette is removed for sampling and a low-flow pump is used.

3.0 OPERATION

Assembly

The MCE filter cassette is pre-loaded in a three-stage plastic cassette. Remove the top portion of the cassette prior to sampling to allow for greater airflow across the sample medium.

Calibration

Calibration cannot be performed on an open-faced cassette. No calibration is necessary for an MCE filter since it is used for qualification (present/not present) purposes only.

Pump should be set at greater than 2 liters per minute for maximum collection efficiency.

Operation

- Don a pair of sterile sample gloves.
- Remove the inlet and outlet plugs from the sample cassette and retain for use at the end of the sample period. Remove the top portion of the sample cassette.
- Set the sampler in the area to be sampled.
- Connect the 37-mm cassette to the low-flow pump using flexible tubing.
- Turn on the pump.

Sample Collection

- After the predetermined sample period, turn off the pump.
- Disconnect the cassette from the flexible tubing.
- Reattach the top portion of the sample cassette.

- Replace the inlet and outlet plugs into the cassette.
- Properly label the sample.
- Place sample in a re-sealable plastic bag. Double-bag the sample.

4.0 EQUIPMENT NEEDED

- MCE filter cassette
- Low-flow pump
- Flexible tubing
- Sample stand
- Sample label
- Sharpie
- Sterile sample gloves
- Sample paperwork
- Re-sealable plastic bag



Figure 13

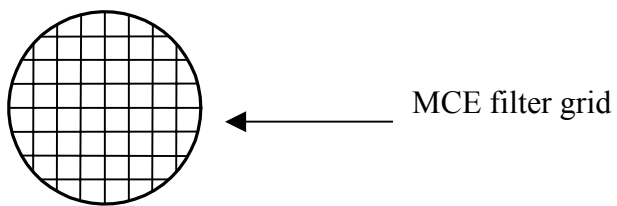


Figure 14

HEPA VACUUM SAMPLING

1.0 INTRODUCTION

The EUROCLEAN Hip Vac Sr. is used to aggressively sample for biological spores.

2.0 DESCRIPTION

The Euroclean Hip Vac Sr. is a hip-mounted, canister-style vacuum cleaner with an installed HEPA filter (Figures 15 and 16). A “filter sock” is placed in the nozzle of the hose attachment (Figure 18) to capture small particulates. HEPA vacuum sampling can cover large areas; however, a new filter sock should be used for each different sampling area.

3.0 OPERATIONS

Assembly

The Euroclean Hip Vac Sr. has a HEPA filter already installed. Remove the side cover with the hose inlet to check that there is a vacuum bag present and that the HEPA filter has not been removed. **DO NOT USE IF THERE IS NOT A HEPA FILTER AND VACUUM BAG PRESENT.**

- Insert the hose attachment into the vacuum.
- Don a sterile pair of gloves.
- Insert a “filter sock” into the end of the hose attachment.
 - Insert the white portion of the filter sock into the end of the hose.
 - Roll the blue portion of the filter sock over top of the end of the hose.

Operation

Belt the vacuum around the operator’s waist.

- Determine the area to be sampled.
- **HOLD ON TO THE BLUE PORTION OF THE FILTER SOCK DURING SAMPLING** (Figure 19). Failure to do so will result in the entire sock being lost in the vacuum with subsequent loss of the sample.
- Turn on the vacuum.
- Vacuum the desired sampling area, keeping the inlet nozzle as close to the surface that is being sampled as possible.

Sample Collection

- Turn off the vacuum after the area has been sampled.

- Don a new pair of sterile sample gloves.
- Remove the filter sock from vacuum hose, touching only the blue portion.
- Fold the filter sock with the blue portion out until it is small enough to fit into a sterile sample container (Figure 20).
- Label the sample container

IT IS NOT NECESSARY TO CHANGE VACUUM BAGS OR DECONTAMINATE THE VACUUM BETWEEN SAMPLES. IF COLLECTING MORE THAN ONE SAMPLE, INSERT A NEW FILTER SOCK INTO THE VACUUM HOSE AND CONTINUE AS ABOVE.

4.0 EQUIPMENT NEEDED

- Hip-style HEPA vacuum
- Filter socks
- Sterile sample vial
- Sterile sample gloves
- Labels
- Sharpie
- Sample paperwork

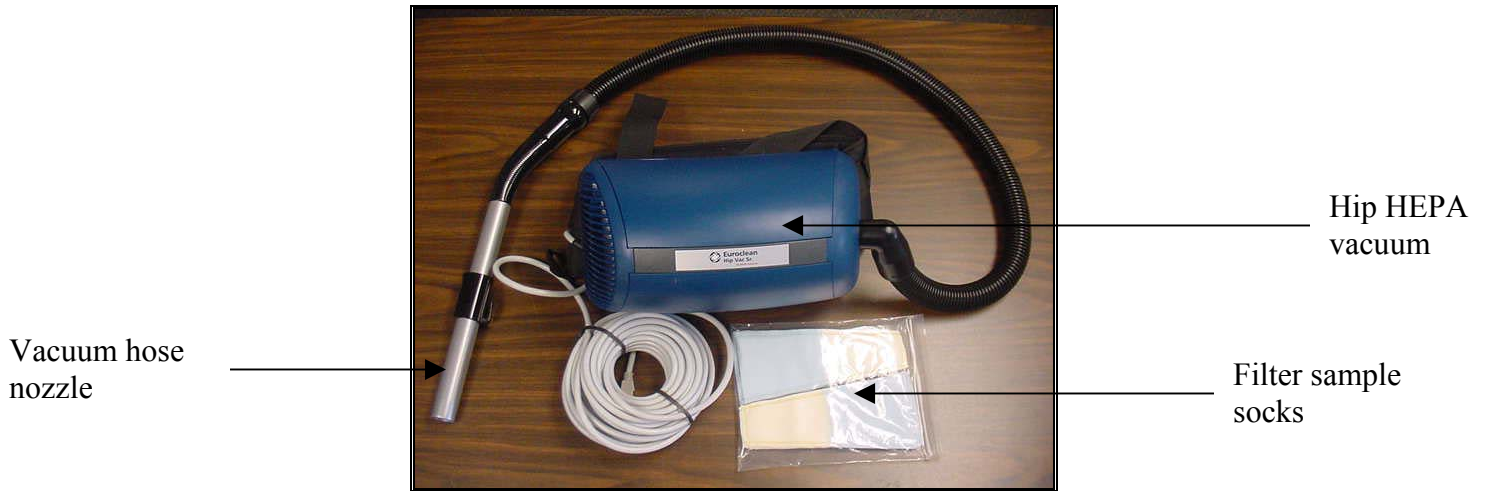


Figure 15



Figure 16

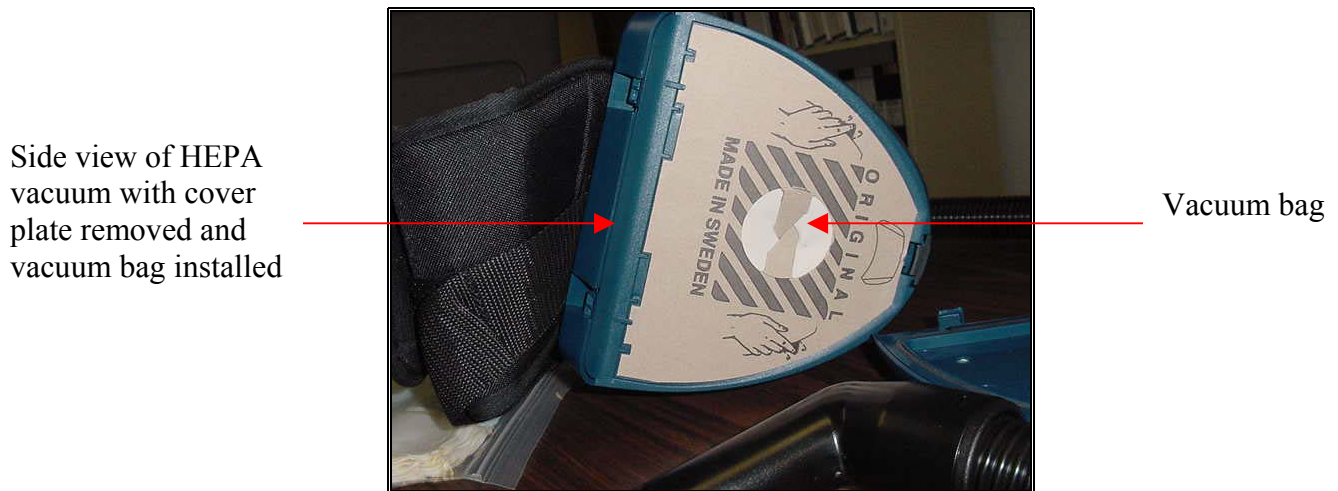


Figure 17

Filter sock installed on the end of the vacuum hose nozzle



Figure 18

Collecting a sample and holding on to the filter sock.

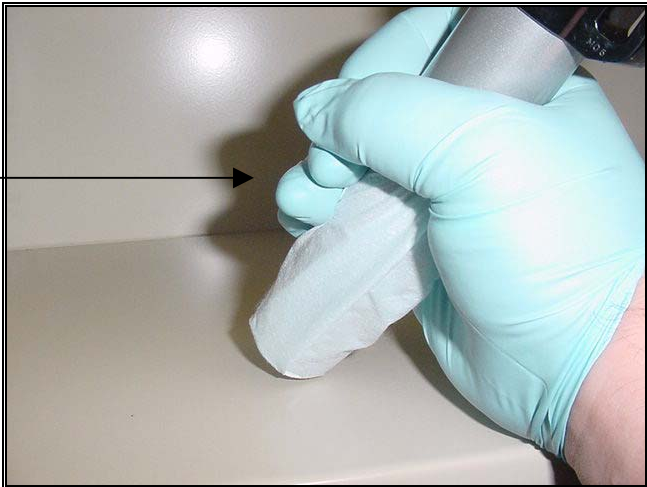


Figure 19

Filter sock inside a sample container



Figure 20

WIPE SAMPLING

1.0 INTRODUCTION

Wipe samples are collected from smooth surfaces to indicate surficial contamination. Different solvents are used and are contaminant- and/or laboratory-dependent. Wipe samples can be collected from an area as small as 10 square centimeters for specific information, or as large as necessary (desktop, cabinets, computer parts).

2.0 DESCRIPTION

Sampling equipment can either be dry (sterile synthetic gauze pads or swabs) or pre-moistened (SpongeSicle™, Hydra Sponge™, or swab) (Figures 21 and 22). Swabs are packaged individually with their own sample container, and the swab is usually attached to the lid of the container (Figure 23). Pre-moistened sponges (HydraSponge™/SpongeSicles™) are packaged individually inside a whirl-pac bag. Swabs are used for small areas and sponges/gauze pads are used for larger areas. The solution that will be used with the dry materials is primarily dependant on the laboratory performing the analysis or the suspected contaminant. Sterile water can be used as the solution.

3.0 SAMPLE COLLECTION

- Swabs
 - Don a sterile pair of sample gloves
 - A 10 square centimeter plastic template should be used with swab sampling. If no template is available, measure a 10-square-centimeter area and mark the desired area to be sampled with duct tape.
 - The pre-moistened swabs have the solution in the container. Remove the lid from the container and dip the swab in, squeezing the bottom of the tube to wet the swab. If using a dry swab, pour a small amount of the appropriate solution into the swab container and moisten the swab.
 - Place the template over the area to be sampled.
 - Using an “S” motion wipe the inside the template area from top to bottom and then from side to side (Figure 24).
 - Place the swab back into the container.
 - Label the container.
 - Place the container in a whirl-pac bag.
 - Place that bag inside a re-sealable plastic bag.
 - If collecting more than one sample, don a new pair of sterile gloves.

- Sponges/Gauze Pads
 - Don a sterile pair of nitrile gloves.
 - The pre-moistened sponges have the solution in a whirl-pac bag. If using a dry sponge/gauze pad, moisten the media with the appropriate solution by pouring a small amount of the solution into a sample collection bag (whirl-pac).
 - Wipe the item to be sampled making sure that the whole surface is sampled.
 - Do not sample multiple items or areas with the same sponge/gauze pad. Use a new sponge/gauze pad to sample multiple shelves in a bookcase, different parts of a desk, which will help ensure that the sponge/gauze pad does not dry out and that the contaminated sample area could be identified.
 - Place the used sponge/gauze pad in a whirl-pac bag.
 - Label the bag.
 - Place the sample bag inside another re-sealable plastic bag.
 - If collecting more than one sample, don a new pair of sterile gloves.

4.0 EQUIPMENT NEEDED

- Sample media (sponges, swabs, gauze pads)
- Solvent
- Whirl-pac bags
- Re-sealable plastic bags
- Labels
- Sharpie
- Sample template (or duct tape and ruler)
- Sterile sample gloves

Wipe Sampling Materials

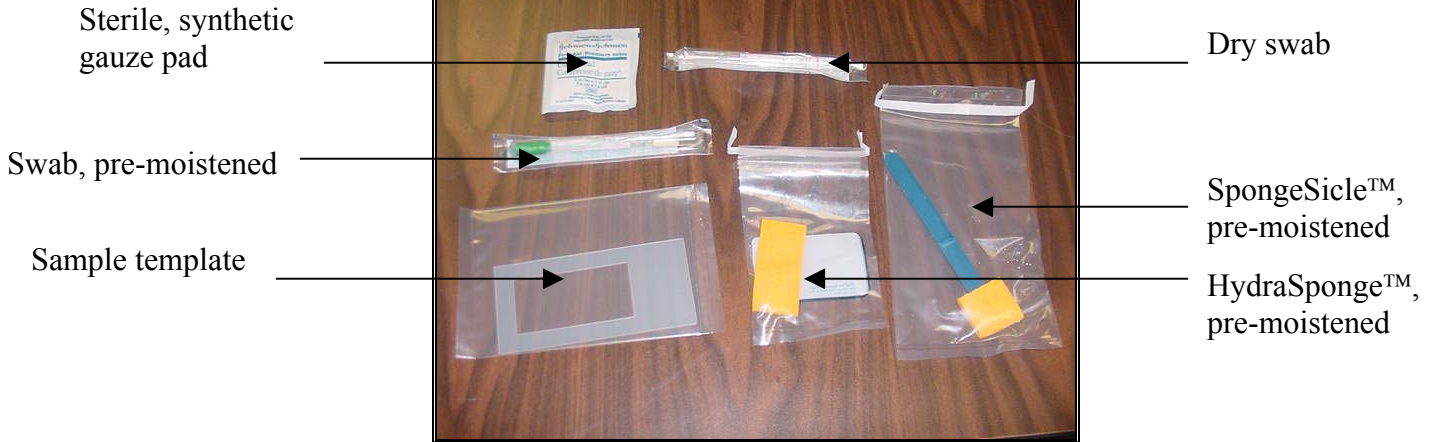
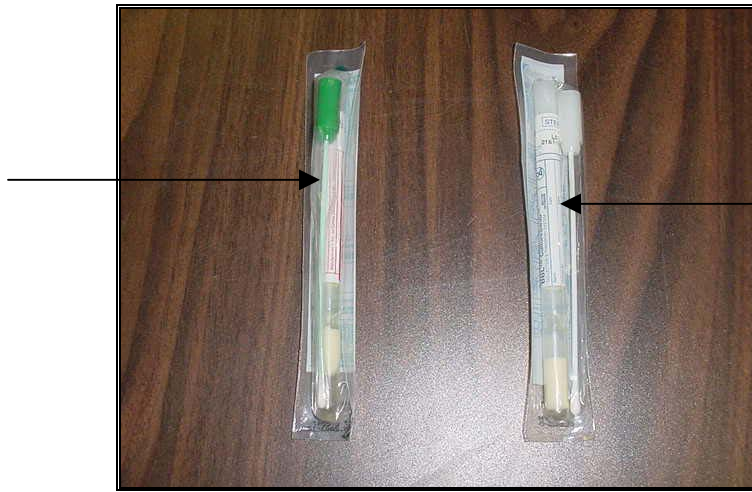


Figure 21

Swab, green or colored cap indicates pre-moistened



Swab, white cap indicates dry

Figure 22

Swab container



Swab attached to container cap

Figure 23

Sample Wiping technique using a template

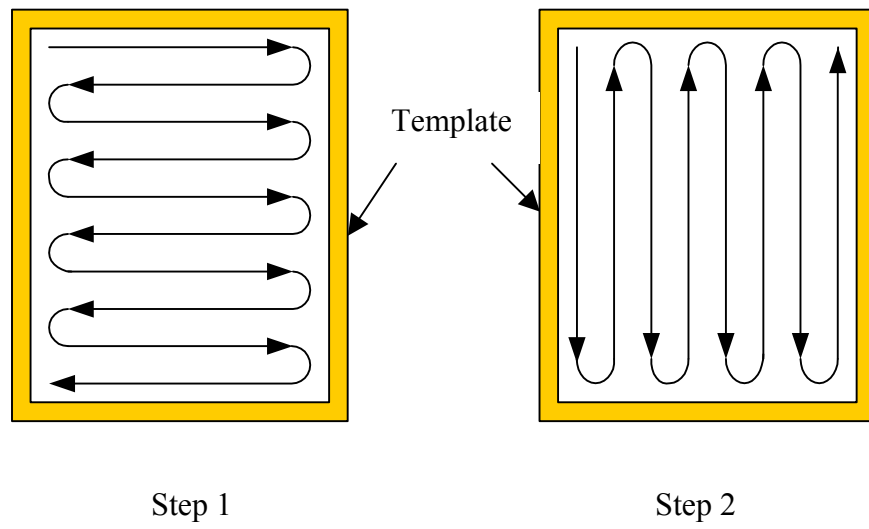


Figure 24

Appendix A

Comparison of Surface and Airborne Sampling Methods for Select Biological Agents

Sample Type	Description	Effectiveness	Uses	Biological Agents
Porous and Non-porous Surface and Bulk Sampling				
Wet Wipe	Sterile synthetic gauze pad moistened with sterile deionized water or other sterile laboratory-determined solution is used to wipe area of specified size (10 x 10 cm, or other).	Area sampled is small; effective on non-porous surfaces; laboratories may prefer this sample medium	Screening small surfaces; discriminate sampling location; extent of contamination; decontamination effectiveness.	Bacteria; virus; retroviruses; and biological toxins.
Wet Swab/Swube	Sterile synthetic swab or swube moistened with sterile deionized water or other laboratory-determined solution. Swab/swube in transport tube with screw top or sterile conical vial.	Area sample is very small; effective on non-porous surfaces; corners and small crevices; routine laboratory medium.	Screening very small locations, crevices and corners. Often collected with other sample media. Extent of contamination and critical item screening.	Bacteria; viruses; retroviruses; and biological toxins.
HEPA Vacuum Collection Sock	Sample collected into HEPA collection sock that will fit into the inlet nozzle of a small portable HEPA vacuum hose.	Can use in large porous areas; works best with spore-forming bacteria, must have significant volume of dust and/or small particles; can disturb other sample locations with circulating air. Best for agents with vegetative forms and/or persistence in environment.	Screening large porous surfaces and objects. Often collected with other sample media. Extent of contamination and critical item screening.	Bacteria; viruses; retroviruses; and biological toxins
Bulk Sampling	Visible bulk material collected as standard solid sample. Samples also include HVAC filters, letters, devices, or containers.	Laboratories may not accept concentrated sample. Check with laboratory prior to sending	Discriminate sampling; HVAC systems screening; evidence screening; disposal acceptance samples.	Bacteria; viruses; retroviruses; and biological toxins.

Sample Type	Description	Effectiveness	Uses	Biological Agents
Air Sampling				
Gel Filters	Low flow sampling used with personal sample pumps or other low flow pump. Used with 37-mm open faced cassettes or button samplers.	Reduced laboratory preparation required for culture. Very short sample time. Must prevent gel from drying out.	Used for airborne biological agents; personnel monitoring in the breathing zone; aggressive sampling to determine extent of contamination.	Bacteria; viruses; and retroviruses.
Mixed Cellulose Ester Filters	Low-flow sampling used with personal sample pumps or other low flow pump. Utilized with 37 mm open faced cassettes.	Can desiccate and/or kill biological agents. Limited use with live culture. Sample collection times can be greater than gel filters.	Used for airborne biological agents; personnel monitoring in the breathing zone; aggressive sampling to determine extent of contamination. Best use for toxins.	Bacteria; viruses, retroviruses; and biological toxins.
Single- and Six-Stage Impactor Samplers	Single-stage impactor captures airborne particulates on a prepared agar plate. Six-stage impactor size selectivity captures airborne particulates onto agar plate.	Limited sample times and sample volume; reduced laboratory preparation time; best used as a supplemental sampling method; impactor units must be cleaned between uses.	Collection of airborne agents only; use with desiccation sensitive agents.	Bacteria; viruses; and retroviruses.
Dry Filter Units	Operational units consist of high-flow sampling pump and 1-micron dry filters. Filters would be removed and sent as a bulk sample to the laboratory.	Not available commercially; lower extraction efficiency than wet methods; laboratories not familiar with media; evaluation of effectiveness of method not complete.	Initial stages of release when concentrations are highest.	Bacteria; viruses; retroviruses; and biological toxins.
Liquid Impingers	Sterile impinger contains liquid (nutrient broth or mineral oil) into which the air sample is drawn, capturing any agent. The liquid can be cultured and/or analyzed.	Allows long sample time. Very high extraction efficiency with correct collection media. Not effective with statically charged particles. Impingers are fragile and difficult to use.	Use during post-decontamination sampling or as a supplement to surface sampling.	Bacteria; viruses; and retroviruses

SOURCES:

SKC Inc.
Thermo-Andersen

Center for Disease Control
National Response Team Guide

Appendix B

Environmental Sample Selection

Disease/ Agent	Sample Selection		Time and Temp		Laboratory Specimen Plating and Processing						
			Transport	Storage	S B A	C A	M A C	T - M	T S B	Stain	Other
Anthrax	Wipe, Swab, and Swube	Collect routine wipe, swab or swube with sterile water into a sterile, leakproof container and seal.	#1 h 4E C	#24 h RT	X	X	X		X	Gram stain	India ink for capsule
	Bulk, solid or HEPA Vacuum	Place collect of approximately 50 to 100 grams of material into sterile container.	#2 h RT	#24 h RT	X	X	X		X	Gram stain	India ink for capsule
	Air	Collect routine air sample with sterile MCE, gel filter, petri dishes and/or impactor.	#2 h RT	#24 h RT 4E C	X	X	X		X	Gram stain	Minimal recovery
	Water	Collect a minimum of 100 milliliters into a sterile container with leak-proof lid.	#1 h RT	#24 h RT 4E C	X	X	X		X	Gram stain	Minimal recovery
Brucellosis	Wipe, Swab, and Swube	Collect with sterile wipe, swab or swube with sterile water into a sterile, leakproof container and seal of an area of > 100 square centimeters. Specimen should be stored and shipped frozen at -20EC.	~2 h RT	-20EC	X	X		X		Gram stain	Oxidase and Urease test positive
	Bulk, solid or HEPA Vacuum	Place collect approximately 50 to 100 grams of material into sterile container. Specimen should be stored and shipped frozen at -20EC.	#2 h RT	-20EC	X	X		X		Gram stain	Oxidase and Urease test positive
	Air	Collect routine air sample with sterile gel filter, petri dishes and/or impactor.	#15 min RT	#24 h RT	X	X		X		Gram stain	Minimal recovery
	Water	Collect a minimum of 100 milliliters into a sterile container with leak-proof lid.			X	X		X		Gram stain	Minimal recovery

Disease/ Agent	Sample Selection		Time and Temp		Laboratory Specimen Plating and Processing						
			Transport	Storage	S B A	C A	M A C	T - M	T S B	Stain	Other
Botulism	Wipe, Swab, and Swube	Collect routine wipe, swab or swube with sterile water into a sterile, anaerobic transport, leakproof container and seal.	#1 h 4E C	#24 h RT						Isolate C. botulinum	Utilize anaerobic media, Port-a-Cul vials.
	Bulk, solid or HEPA Vacuum	Collect approximately 50 to 100 grams of material into sterile container.	#2 h RT	#24 h RT						Isolate C. botulinum	Utilize anaerobic media, Port-a-Cul vials. India ink for capsules.
	Air	Collect routine air sample with sterile gel filter, petri dishes and/or impactor.	#2 h RT	#24 h RT 4E C						Isolate C. botulinum	Utilize anaerobic media, Port-a-Cul vials. Minimal recovery.
	Water	Collect a minimum of 100 milliliters into a sterile container with leak-proof lid.	#1 h RT	#24 h RT 4E C						Isolate C. botulinum	Utilize anaerobic media, Port-a-Cul vials.
Plague	Wipe, Swab, and Swube	Collect routine wipe, swab or swube with sterile water into a sterile, leakproof container and seal.	#2 h RT	#24 h RT 4E C	X		X			Gram Stain	Alternate preparation is Wayson stain
	Bulk, solid or HEPA Vacuum	Collect approximately 50 to 100 grams of material into sterile container.	#2 h RT	#24 h RT 4E C	X		X			Gram Stain	Alternate preparation is Wayson stain

Disease/ Agent	Sample Selection		Time and Temp		Laboratory Specimen Plating and Processing						
			Transport	Storage	S B A	C A	M A C	T - M	T S B	Stain	Other
	Air	Collect routine air sample with sterile gel filter, petri dishes and/or impactor.	#2 h RT	#24 h RT 4E C	X		X			Gram Stain	Alternate preparation is Wayson stain
	Water	Collect a minimum of 100 milliliters into a sterile container with leak-proof lid.	#2 h RT	#24 h RT 4E C	X	X	X			Gram Stain	Alternate preparation is Wayson stain
Tulereima	Wipe, Swab, and Swube	Collect routine wipe, swab or swube with sterile water into a sterile, leakproof container and seal.	#1 h 4E C	#24 h RT 4E C	X	X	X	X		Gram Stain	
	Bulk, solid or HEPA Vacuum	Collect approximately 50 to 100 grams of material into sterile container.	#2 h RT	#24 h RT 4E C	X	X	X	X		Gram Stain	.
	Air	Collect routine air sample with sterile gel filter, petri dishes and/or impactor.	#2 h RT	#24 h RT 4E C	X	X	X	X		Gram Stain	.
	Water	Collect a minimum of 100 milliliters into a sterile container with leak-proof lid.	#1 h RT	#24 h RT 4E C	X	X	X	X		Gram Stain	
Small Pox	Wipe, Swab, and Swube	Contact laboratory. Collect wipe, swab or swube with sterile water into a sterile, leakproof container and seal. May place on dry ice.	~6 h 4E C	-20EC to -70EC	X		X			Gram Stain	Alt. prep:Wright stain. Call Health Dept
	Bulk, solid or HEPA Vacuum	Contact laboratory. Collect approximately 10 to 12 cc of material into sterile container. May need to place on dry ice.	~6 h 4E C	-20EC to -70EC	X		X			Gram Stain	Alt. prep:Wright stain. Call Health Dept.
	Air	Contact laboratory. Collect routine air sample with sterile gel filter, petri dishes and/or impactor. May need to place on dry ice.	~6 h 4E C	-20EC to -70EC	X		X			Gram Stain	Alt. prep:Wright stain. Call Health Dept.

Disease/ Agent	Sample Selection		Time and Temp		Laboratory Specimen Plating and Processing							
			Transport	Storage	S B A	C A	M A C	T - M	T S B	Stain	Other	
	Water	Contact laboratory. Collect a minimum of 100 milliliters into a sterile container with leak-proof lid. May need to place on dry ice.	~6 h 4E C	-20EC to -70EC	X		X				Gram Stain	Alt prep: Wright stain. Call Health Dept.
Venezuelan Hemorrhagic Fever (VHF)	Wipe, Swab, and Swube	Collect routine wipe, swab or swube with sterile water into a sterile, leakproof container and seal.	#2 h RT	#24 h 4E C							NA	Specific handling conditions are currently under development.
	Bulk	Collect approximately 50 to 100 grams of material into sterile container.	#2 h RT	#24 h 4E C							NA	
	Air	Collect routine air sample with sterile gel filter, petri dishes and/or impactor.	#2 h RT	#24 h 4E C							NA	
	Water	Collect a minimum of 100 milliliters into a sterile container with leak-proof lid.	#2 h RT	#24 h 4E C							NA	

Select Sources: Center for Disease Control, 2000

Key:

BCYE buffered charcoal-yeast extract agar
C centigrade
CA Chocolate agar
cc cubic centimeter
h hours
MAC MacConkey agar
RT room temperature
T-M Thayer-Martin Agar
SBA Sheep Blood Agar
TSB Tryptic Soy Agar
ELISA Enzyme Linked Immunosorbent Assay
PCR Polymerase Chain Reaction

Appendix C

DOT Regulations for Shipping Biological Agents

DOT Regulations for Shipping Biological Agents

Proper Shipping Name _____ Infectious Substance, affecting humans (*Substance Name*)

DOT Hazardous Classification _____ 6.2

DOT Label _____ Infectious Substance

DOT Marking _____ Infectious Substance, (*Substance Name*) UN 2814

DOT Placard _____ Infectious Substance

Precautions: Motor vehicles will be placarded regardless of quantity. Drivers will carry full information regarding shipment and procedures in case of an emergency.

Packaging: Use triple packaging that is capable of meeting the performance requirements in § 178.609 of the HMR (e.g. drop tests, water immersion test).

Source: International Air Transport Association, Dangerous Goods Regulations 42nd Edition, Montreal, OT, 2001

Appendix D

Where to Buy

WHERE TO BUY

ITEM	COMPANY	PART NUMBER	COST
Air Sampling			
Six Stage Impactor kit	Thermo Andersen	10-800, 10-709, 10-830	\$5,911
Biostage-1 Single Stage Impactor Kit	SKC	225-BS-1K	\$1,000
High volume pump Kit (2 pack kit)	SKC	228-505K	\$665
Quick Take 30 sample pump	SKC	228-9530	\$1,195
Mounting bracket for Quick Take 30 and Biostage-1	SKC	228-9531	\$75
Sampling Wand	SKC	228-9521	\$75
Air-O-Cell Cassettes (10 pack)	SKC	225-9501	\$71.25
Gel filters (50 pack)	SKC	225-9552	\$155
37mm cassettes (50 pack)	SKC	225-3050	\$41
MCE filter cassettes (50 pack)	SKC	225-513	\$92
Dry-cell calibrator	SKC	225-9535K	\$1,139
Vacuum Sampling			
Euroclean Hip Vac UZ 964 SR	Parish Maintenance Supply	1250964	\$373
HEPA filter for UZ 964 SR	Parish Maintenance Supply	1257160	\$68
HEPA filter socks (100 pack)	Stopallergy	000516	\$375
Wipe Sampling			
Sterile swab kit, premoistened (10 pack)	SKC	225-2402	\$36
SpongeSicle (100 pack)	International BioProducts	SSL-10NB	\$128
HydraSponge (100 pack)	International BioProducts	HS-10NB	\$104.77
Sterile Swab, dry (100 pack)	VWR International	BB44320115	\$26.20
Miscellaneous			
Whirl-pac bags (500 pack)	VWR International	11216-781	\$68.76
Glass sample bottle 32 ml (144 pack)	VWR International	66015-100	\$107.52
Falcon tube, plastic 50ml, (1 case)	VWR International	21008-938	\$218.86
Parafilm 4in X 250 ft	VWR International	52858-032	\$21.07
Sterile water 1 liter	VWR International	EM-9610	\$24.00

Appendix E

Supplier Contact Information

International BioProducts

P.O. Box 0746
Bothell, Washington 98041
PH (425) 398-7993
FAX (425) 487-6574
www.intlbioproducts.com

Parish Maintenance Supply

1015 E. Hiawatha Blvd.
Syracuse, New York 13208
PH (315-422-1495

SKC

863 Valley View Road
Eighty Four Pennsylvania 15330
PH (800) 752-8472
FAX (724) 941-1369
www.skcinc.com

Stopallergy

9525 Kenwood Road
Suite 7
Cincinnati, Ohio 45242
PH (513) 936-0224

Thermo Andersen

500 Technology Court
Smyrna Georgia 30082
PH (800) 241-6898
FAX (770) 319-0336
www.thermoandersen.com

VWR International

405 Heron Drive
Bridgeport, New Jersey 08014
PH (800) 932-5000
FAX (856) 467-3336
www.vwr.com