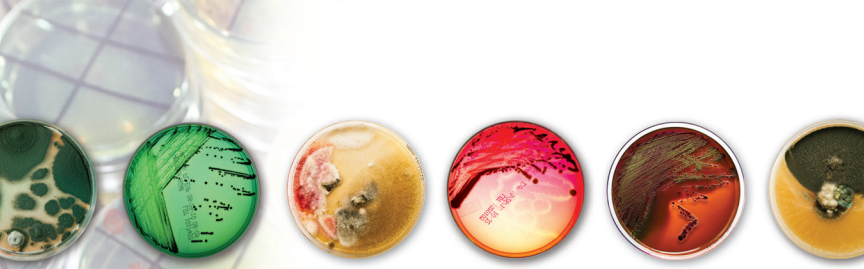

Microbiology Sampling

POCKET GUIDE

EMSL

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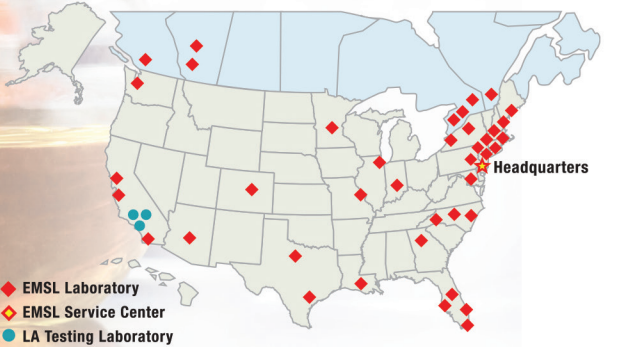

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Air-O-Cell Test Code M001

- The Air-O-Cell™ cassette (Product ID 8715301B) is a single-use sampling device designed for the rapid collection and analysis of a wide range of airborne particles. These include fungal spores, pollen, insect parts, skin cell fragments, fibers, and other inorganic particulates.
- The cassette is designed to operate at a flow rate of 15 LPM. Lower flow rates may result in a collection loss of some spores and the accumulation of others in a non-uniform manner. Therefore, it is critical to run the sampling pumps at the manufacturer's recommended air flow rate.

Benefits

- Useful for initial site testing, especially if fungal growth is not visible.
- Quick and simple procedure.
- Fast turn around times available.
- Low chance of sample contamination.
- Necessary for determining allergic mold spore potential. Mold spores can cause allergies whether they are viable or non-viable.

Air-O-Cell
50 Pack, #8715301B
10 Pack, #8715302

Disadvantages

- Fungi cannot be identified to species with this method.
- Due to the similarities in spore morphology, some spores will be grouped together, i.e., *Aspergillus* sp. and *Penicillium* sp.
- Spore viability cannot be assessed.

Sampling Procedure

1. Prior to sampling, calibrate your pump to 15 liters per minute via a rotameter. It is also recommended that the rotameter or sampling pump be periodically calibrated to a NIST primary standard. If using the Zefon Bio-Pump (Product ID 8706002) use the specifically designed Air-O-Cell flow indicator. It cannot be used with any other type of pump.
2. Remove and retain tape seal covering inlet and outlet on the cassette.
3. Attach the outlet (round hole) to a standard 1/2" PVC tubing (for use with high volume pumps only).

4. Start the sampling pump and sample for an appropriate period of time (see recommendations below).
5. Remove Air-O-Cell from tubing and reseal with the original seals. Label sample.
6. Complete an EMSL Chain of Custody (COC), available at www.emsl.com, detailing client name and information, project name or number, sample #, description of sampling area, and volume of air collected.
7. To reduce shipping damage, it is recommended that the Air-O-Cell be placed in a corrugated box with padding to ensure safe arrival at the laboratory.

Sampling Duration

- The sampling time is dependent on the density of particulate in the environment. It is important not to overload the sample, otherwise it will be impossible to accurately count the types of spores, pollen or other particulates that are present. The following list represents typical sample times to attain a sharply defined trace with good dispersion of the spores:
  1. Clean "office" or outdoors (no visible dust) = 10 minutes
  2. Indoor environment, high activity & personnel = 5 minutes
  3. Indoor environment, drywall renovation or heavy industrial dust = 1 minute.

Quality Control Recommendations

- An effective interpretation is based on the comparison of indoor and outdoor samples. Outdoor samples will help determine whether spore amplification is occurring indoors.
- Obtain a control sample from a non-complaint area for comparison.
- Sending a blank cassette for analysis per project is a good practice.
- Flow rate is critical for accurate results. Remember to calibrate and recalibrate the pump prior to all sampling. (15 liters/minute)
- Never use cassettes that are damaged or expired.

Bio-Pump Plus
#8706002

Contact for Sampling Supplies and Cassettes, www.emsl.com, 1-800-220-3675
Air-O-Cell™ is a registered trademark of Zefon International.

MoldSnap Test Code M174
Micro5 Test Code M030

- The Zefon MoldSnap (Product ID 8715304) and the Micro5 MicroCell (Product ID 8715306) were designed to operate at a flow rate of 5 liters per minute for optimal collection efficiency. Cassettes should be stored at 50° - 80° F.
- Do not use in temperatures below 37° F!

Mold Snap
50 Pack, #8715304B

Sampling Procedure

1. Remove pin from the bottom of the MoldSnap or Micro5.
2. If using a conventional high volume pump: Simply connect one end of tubing (5 feet or less) to the bottom of the cassette.
3. If using a low volume pump such as a GilAir5: Simply attach a 1" piece of tubing to the bottom of the cassette.
4. Connect the other end of tubing to a pump pre-calibrated to a flow rate of 5LPM.
5. Remove cap from the top of the cassette.
6. Turn pump on and take a sample for an appropriate amount of time depending on environment. (See "Sampling Duration" below.)
7. When sampling is completed, replace the pin & cap to the bottom and top of the cassette.

Gilian GilAir5R
#8706209

Sampling Duration

- Outdoor / Indoor Clean Environments = 8-10 minutes
- Indoor (Normal Activity) = 5 minutes
- Indoor (Heavy Particulates) = 1-3 minutes
- Inner wall cavity using the Micro5 only (with optional Inner Wall Adapter, Product ID 8715901) = 1-2 minutes

E-Lite Pump w/Rotameter
Adjustable Rotameter flow control
3 - 30 L/min
#8706004

Allergenco-D Test Code M032

- The Allergenco-D (Product ID 8715307) was designed to operate at a flow rate of 15 liters per minute for optimal collection efficiency.

Sampling Procedure

1. Remove seal from the bottom of the Allergenco-D.
2. Simply connect one end of tubing (5 feet or less) to the bottom of the Allergenco-D.
3. Connect the other end of tubing to a pump pre-calibrated to a flow rate of 15 LPM.
4. Remove seal from the top of the Allergenco-D.
5. Turn pump on and collect a sample for up to 10 minutes, depending on environment (See "Sampling Duration").
6. When sampling is complete, replace seals to the bottom and top of the cassette.

Sampling Duration

- Outdoor sample 1-10 minutes
- Dust-free environment (clean office) 5-8 minutes
- Indoor environment (occupied space) 3-5 minutes
- Indoor environment (excess visible dust) 1-3 minutes
- Inner wall sample 1-5 minutes
  (using optional wall adapter, Product ID 8715908)

***Tech Tip:* Basic Mold Cleanup**

The key to mold control is moisture control. It is important to dry water damaged areas and items within 24-48 hours to prevent mold growth. If mold is a problem in your home, clean up the mold and get rid of the excess water or moisture. Fix leaky plumbing or other sources of water. Wash mold off hard surfaces with detergent and water, and dry completely. Absorbent materials (such as ceiling tiles & carpet) that become moldy may have to be replaced. (Source: EPA http://www.epa.gov/mold/moldresources.html)

TSI 4146
Primary Calibrator
.01 - 20 L/min
#8703915

Culturable Air Sampling (Fungi or Bacteria)

Test Code M005 - Fungi Genus ID
Test Code M006 - Fungi Species ID
Test Code M433 - Aspergillus Nosocomial Panel
Test Code M434 - Aspergillus Comprehensive Panel
Test Code M370 - Comprehensive Culture for Potential Mycotoxin Producing Fungi
Test Code M009 - Bacteria Gram Stain
Test Code M010 - Bacteria 3 MPT
Test Code M011 - Bacterial 5 MPT

Particle Impactors (Andersen-type Samplers)

- This method of air sampling involves drawing a measured volume of air over culture media in Petri dishes. The Petri dishes are incubated in the laboratory so the organisms impacted on the plate can grow. The fungi or bacteria are counted and identified. This method commonly uses an Andersen N-6 type impactor (e.g. EMSL VP-400 Microbial Sampler Product ID 8709001). Different agar plates are available from EMSL Analytical, Inc., depending on the types of fungi or bacteria to be identified.

Benefits

- Fungal cultures can determine whether the fungus is viable (alive), and allows for genus and species identification.
- Bacterial cultures provide enumeration and identification of culturable bacteria present in the air.

Disadvantages

- Cultures take 6-10 days for the microorganisms to grow and be identified.
- Since most environmental samples contain a large number of organisms, each has to compete with others to grow on the media. As a result, fungi and bacteria present in the air may not be as well represented in culture.
- Some microbes do not grow well or at all on the culture media (viable but non-culturable, VBNC).
- Some microorganisms are unable to be identified, as they fail to produce key characteristics such as spores or they may not be described in the scientific literature.

General Media Recommendations Fungi and Bacteria

- For fungal sampling, in general, we recommend Malt Extract Agar (MEA).
- If you are sampling in dry areas, the use of DG18 will help select for the growth of dry-loving fungi that may not grow on MEA agar.
- Sampling specifically for *Stachybotrys* sp. can be achieved with either Cellulose Agar (CA) or Cornmeal Agar (CMA).

- For bacterial sampling, in general, we recommend Tryptic Soy Agar (TSA) or TSA w 5% blood.
- For sampling Gram negative bacteria, we recommend MacConkey Agar (MAC).
- For all other situations, the Microbiology Department will be happy to make recommendations based on your individual sampling situation.
- Sampling supplies may be ordered at www.emsl.com or by calling Customer Service: 800-220-3675.

How to Handle Microbiological Media (Agar plates)

- Agar plates must be kept refrigerated or on freezer packs until ready to use.
- The plates must be allowed to warm up to room temperature before taking a sample (approx. 15 minutes).
- Do not remove the lid from the plate at anytime except during sampling.
- Seal the lid to the plate after sample collection with Parafilm or tape.
- The plates must be shipped back to EMSL with freezer packs by OVERNIGHT PRIORITY. Refreeze and reuse the original freezer pack (this type of freezer pack is stable for 24 hours).
- Adequate packing material must be sent to protect the plates.
- Plates must not come into direct contact with the freezer pack, as the media may freeze, invalidating the tests.
- If there is any delay in sending the agar plates to EMSL, they should be refrigerated until ready for overnight delivery.

EMSL VP-400 Single Stage Microbial Sampler #8709001

Recommendations

- Wear latex or nitrile gloves during sampling.
- Use 70% isopropyl alcohol to disinfect sampling device between each sample.
- Place Petri dish lid in a clean bag during sampling to reduce any cross contamination.
- Include outside samples and a field blank for control.

Sampling Procedure

1. Allow agar plates to reach room temperature before use.
2. Attach one end of tubing to the intake of the vacuum pump and the other end to the inlet of the sampler.
3. Calibrate the flow rate of the vacuum pump:
   A. Place an uncovered Petri dish into sampler (Do not submit this dish as a sample, discard after calibration).

EMSL VP-400 Basic Kit w/E-Lite Pump #8709003

B. Turn on pump and adjust flow until the rotameter is at 28.3 LPM (flow rate is read from the middle of bearing on the rotameter).

4. Wipe all exposed surfaces of sampler with a 70% isopropyl alcohol pad and allow to air dry.
5. Place the agar plate on the sampler base so that the Petri dish rests on the three raised metal pins.
6. Remove the cover of the Petri dish and place into a clean sample bag to minimize contamination (available upon request).
7. Assemble the jet classification stage on the sampler and secure the inlet cone with the three attached clips.
8. Set timer to appropriate time depending on environmental conditions (sampling time is usually between 2-5 minutes).
9. Turn on the pump and start the timer simultaneously.
10. When the time is up, turn off the pump and disassemble sampler and place cover back onto agar plate.
11. Secure lid onto Petri dish with masking tape or Parafilm (avoid using electrical, packing, transparent and duct tape).
12. Write the sample number on the bottom of the Petri dish.
13. Record all appropriate information on the Chain of Custody.
14. Return samples with an ice pack to EMSL Analytical for analysis.

Tech Tip: ***Can mold cause health problems?***

Molds are usually not a problem indoors, unless mold spores land on a wet or damp spot and begin growing. Molds have the potential to cause health problems. Molds produce allergens (substances that can cause allergic reactions), irritants, and in some cases, potentially toxic substances (mycotoxins). Inhaling or touching mold or mold spores may cause allergic reactions in sensitive individuals. Allergic responses include hay fever-type symptoms, such as sneezing, runny nose, red eyes, and skin rash (dermatitis). Allergic reactions to mold are common. They can be immediate or delayed. Molds can also cause asthma attacks in people with asthma who are allergic to mold. In addition, mold exposure can irritate the eyes, skin, nose, throat, and lungs of both mold-allergic and non-allergic people. Symptoms other than the allergic and irritant types are not commonly reported as a result of inhaling mold. Research on mold and health effects is ongoing. This brochure provides a brief overview; it does not describe all potential health effects related to mold exposure. For more detailed information consult a health professional. You may also wish to consult your state or local health department. (Source EPA Document # 402-K-02-003)

Surface Sampling Test Code M041

Direct Examination (Tape Lift, Bulk, Swab)

- A direct exam allows for the rapid determination of the presence of fungal spores as well as identifies the types of fungi.
- Direct examinations should only be used to sample visible mold growth in a contaminated area since most surfaces will have a deposit of fungal spores that are normally present in the environment.

Bio-Tape
25 Pack, #8708325

Benefits

- The direct exam is inexpensive and can be performed quickly.
- A useful test for determining if there is mold amplification.
- Direct sampling may reveal indoor reservoirs of spores that have not become airborne yet.

Disadvantages

- Areas of fungal growth are often small and scattered, so they may not all be picked up. Choosing multiple sampling locations will help overcome this problem.
- Health problems related to indoor microbial growth are generally caused by the inhalation of substantial numbers of airborne spores, sometimes over a long period of time. The presence of biological materials on a particular surface may not be a direct indication of what is in the air.
- This method detects both viable and non-viable spores but cannot distinguish between them. It is advisable to combine direct exam samples with culture methods if knowing viability is important to your project.
- Tape lifts are not able to be cultured.
- If a direct examination of a swab sample is taken, a follow up by culture is possible.
- Direct examinations of dirt/soil and dust samples cannot be performed reliably because of preparation limitations.
- Fungi usually cannot be identified to species and sometimes not even to genus with this method. For example, *Aspergillus* sp. and *Penicillium* sp. are normally reported together due to the similarities in spore morphology, unless fruiting structures are present that allows for a better identification.

Materials

For Tape Lift

- We recommend using EMSL Tape kits or Bio-Tapes (Zefon Intl) otherwise clear (transparent) Scotch or other brand tape (frosted tape obscures the spores).
- New plastic bag to hold sample(s) (provided in a tape lift kit available from EMSL).
- Only use tape lifts on hard, dry, flat surfaces

For Bulk

- Sterile container or new Ziplock-type plastic bag (provided by EMSL) to hold and transport samples.

For Swab

- Sterile TransPorter swab to collect and transport samples (provided by EMSL).

For all Matrices

- Latex/nitrile gloves (also can be provided at your request).

Sampling Procedure

Tape-lift

1. Take a few inches of clear tape. Avoid touching the sticky side, especially the part to be used to collect the mold.
2. Wearing gloves, apply the central inch of tape to the suspect area (choose one that is free of extraneous debris). Apply light pressure to the non-adhesive side.
3. Pull tape off surface with slow, steady pressure, holding the tape edges only.
4. Apply sticky side of tape to the inside of the plastic bag (ziplock).
5. Ensure there are no folds or creases in the tape.
6. Close bag and label appropriately. (Put only one sample in each bag.)

Bulk

1. Wearing latex gloves remove a small piece of the suspect material (1 x 1 inch piece is more than sufficient).
2. Place piece inside clean sterile container or new plastic bag (ziplock).
3. Close bag or cap container and label appropriately.

Swab

1. Wearing gloves, remove swab from packaging material.
2. Remove plug from media tube.
3. Swab the desired area thoroughly, rolling the swab lightly back and forth over sampling area.

4. Insert the swab in the tube, firmly close cap, and label appropriately.
5. Complete an EMSL Chain of Custody (COC), available on our website (www.emsl.com), detailing client name and information, project name or number, sample #, and a description of the area.

Quality Control Recommendations

For Tape Lift

- Use clear tape--not frosted, electrical, duct, or packing tape.
- Do not fold tape onto itself.
- Stick tape on the inside of the plastic bag only.
- Please do not send tape on slides or cover slips. They may arrive broken making the sample difficult to analyze.

For Bulk

- Send a representative portion of the sample, if large. This prevents over-handling of the sample and contamination. If analysis of a specific portion of sample is required, please note area(s) or take a tape lift of the area.

For Swab

- For quantitative culture reporting, the area swabbed needs to be entered on the chain of custody.

Culturable Surface Sampling (Bulk or Swab)

Test Code M007 - Fungi Genus ID
Test Code M008 - Fungi Species ID
Test Code M370 - Comprehensive Culture for Potential Mycotoxin Producing Fungi
Test Code M009 - Bacteria Gram Stain
Test Code M010 - Bacteria 3 MPT
Test Code M011 - Bacteria 5 MPT
Test Code M028 - Cryptococcus neoformans

Benefits

- The sampling method is inexpensive and surfaces can be quickly sampled.
- A useful test for initial site sampling.
- Species level identification possible.
- Viability of fungi is determined.

Disadvantages

- Areas of fungal growth are often small and scattered, so they may not all be picked up. Multiple sample locations will help overcome this problem.
- Health problems related to indoor microbial growth are generally caused by the inhalation of substantial numbers of airborne spores, sometimes over a long period of time. The presence of biological materials on a particular surface may not be a direct indication of what is in the air.
- This method detects only viable spores and hyphae but cannot detect nonviable or difficult to culture fungi. It is advisable to combine direct exam samples with culture methods if knowing the presence of non-viable fungi is important to your project.
- Cultures cannot distinguish between spores, hyphae and other fungal cells; the results are reported as colony forming units.

Nitrile Gloves
Small #8705400
Medium #8705401
Large #8705402
X-Large #8705403

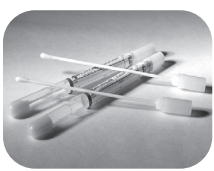
Materials Required

- Latex/nitrile gloves

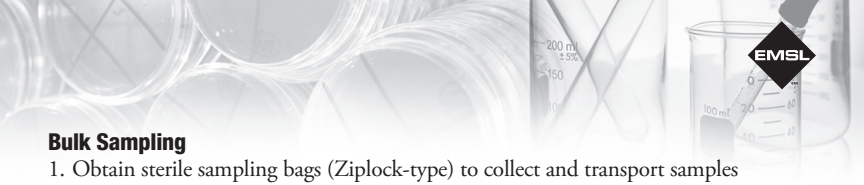
Sampling Procedure

Swab Sampling

1. Obtain sterile 1 mL Butterfield's Solution swab to collect and transport samples (provided by EMSL).
2. Wearing gloves, remove swab from packaging material.
3. Remove plug from media tube.
4. Swab the desired area thoroughly, rolling the swab lightly back and forth over sampling area.
5. Insert the swab in the tube, firmly close cap, and label appropriately.
6. For quantitative culture reporting, the area swabbed needs to be entered on the chain of custody.



Sterile Swab
#8708301
FREE to EMSL Customers
Call for Details



Bulk Sampling

1. Obtain sterile sampling bags (Ziplock-type) to collect and transport samples (provided at your request by EMSL).
2. Wearing gloves and using clean tools remove a representative area of growth along with the building material (sheetrock, wood, etc). 1 inch square is sufficient.
3. Place bulk material into sampling bag and label the outside of the bag with sampling location or description.

Sample Shipment

- Complete an EMSL Chain of Custody (COC), available on our website (www.emsl.com), detailing client name and information, project name or number, sample #, and a description of the area.
- Place samples in a cooler with reusable freezer packs.
- Overnight shipping recommended.

Tech Tip: ***General Media Recommendations For Air Sampling of Culturable Fungi and Bacteria***

Fungal sampling in general, we recommend MEA. Malt Extract Agar is a general isolating media for culturing a wide-spectrum of fungi. A good media for most of your IAQ projects. Sampling for cellulose degrading microfungi in water damaged buildings (e.g. *Stachybotrys* sp.) either Cellulose agar or Corn Meal Agar. Bacterial sampling in general, we recommend TSA or TSA w 5% Blood Sampling for Gram negative bacteria, we recommend MacConkey Agar. All other situations, the Microbiology Department will be happy to make recommendation based on your individual sampling situation or project.

USP <797> Environmental Sampling

Test Code M401 - Fungal Count **Test Code M403 - Bacterial Count**
Test Code M407 - Microbial Counts **Test Code M406 - Microbial Identification**

USP <797> provides minimum practice and quality standards for compounded sterile preparations (CSPs) of drugs and nutrients based on current scientific information and best sterile compounding practices. Environmental sampling in compounding facilities includes viable airborne particle testing, contact plate or surface swab testing, and compounding personnel gloved fingertip testing. Regardless of the number of colony forming units (CFUs) observed within these samples, immediate corrective actions are required if any highly pathogenic organisms are identified. The following sampling guidelines are offered as per USP <797> official from November 1, 2023.

Viable Airborne Particle Sampling

As per USP <797>, an appropriate sampling plan shall be developed for viable airborne particles. Review of the viable airborne particle data may detect elevated amounts of viable airborne particles and these changes may be indicative of adverse changes within the same environment. See Culturable Air Sampling on page 8 as the sampling protocol is similar with the following changes:

- This sampling method commonly uses a high volume air impactor (e.g. EMSL SAS 100 Sampler Product ID 8709100; also available for rent Product ID 87RD021).
- Collect 400-1000 Liters of air sampled on 100 mm or 60 mm sized agar plates, depending upon the sampling equipment used.
- For fungi use Sabouraud Dextrose (SAB DEX) agar with lecithin & tween (EMSL Product # 8714103), for bacteria use Tryptic Soy Agar (TSA) with lecithin and tween (EMSL Product # 8714101).

Surface Sampling with Contact Plate

Surface sampling is useful for evaluating facility and work surface cleaning and disinfecting procedures as well as employee competency in cleaning and disinfection activities. Contact plates can be used on regular or flat surfaces to establish and monitor the efficacy of disinfectants, cleaning techniques, and microbial bioburden on hard surfaces. Obtain sterile 60 mm contact plates also known as RODAC plates; for bacteria use TSA with Lecithin & Tween (EMSL Product # 8714100) and for fungi use SAB DEX (EMSL Product # 8714102).

1. Wearing gloves, remove contact plate from packaging.
2. Remove the lid and gently roll the agar surface across the area to be sampled. This will transfer microbes on the sampled surface to the agar.
3. Replace the lid and clean the sampled area with an alcohol wipe in order to remove any agar residue left by the contact plate.
4. Label the plate and return to EMSL for incubation.

Surface Sampling with Swab

Swabs are best suited for sampling on irregular surfaces or equipment such as switches and dials, where contact plates cannot be used effectively. If an area is sampled using the swab method, use appropriate procedures that will result in the surface location equivalent to that of a contact plate.

1. Obtain sterile 1 mL HiCap Neutralizing Broth swab to collect and transport samples (EMSL Product # 8708942).
2. Wearing gloves, remove swab while allowing excess liquid to remain in the tube.
3. Swab the desired area thoroughly, rolling the swab lightly back and forth over sampling area.
4. Insert the swab back into the tube, firmly closing cap, then label appropriately.
5. For quantitative culture reporting, the area swabbed needs to be entered on the chain of custody.

Gloved Fingertip Sampling

As per USP <797>, the sampling of compounding personnel glove fingertips shall be performed for all CSP risk level compounding because direct touch contamination is the most likely source of introducing contaminants into CSPs prepared by humans.

1. Operators should exit the ISO Class 5 area to complete this sampling and do not disinfect gloves before sampling.
2. Aseptically remove the lid from two TSA with lecithin & tween plates (EMSL Product #8714100 or #8714101).
3. Lightly press the four fingers and thumb for each hand on separate plates (one plates is to be used to sample each hand). When done correctly a visible impression of each finger will be seen on the agar surface.
4. Carefully replace each lid when finished.
5. Label each plate and submit to EMSL for incubation.

ERMI Dust Sampling Test Code M180

- The ERMI© is an acronym for Environmental Relative Moldiness Index.
- It was developed by scientists at the Environmental Protection Agency (EPA) to provide a straightforward, objective, and standardized way to obtain results for indoor air quality investigations.
- The EPA has developed an ERMI ranking system based on dust samples collected from homes across the United States.
- The ERMI helps predict the moldiness of homes. Homes with high ERMI values have a greater chance of having a mold problem than homes with a low ERMI.
- 36 different fungi make up the ERMI and are designated as Group I (those found in a typical, water damaged homes) and Group II (those commonly found in all homes).

Sampling Locations

- For residential sampling, EPA recommends taking a living room and bed room sample as a composite using the same vacuum dust collector for both rooms. Other areas should be sampled separately.
- In the Common Living Area (family room or living room), select the sofa. In the absence of a sofa, select another commonly used chair.
- Using the tape measure and the roll of tape, mark the corners of a 3 foot x 6 foot rectangular sampling area on the floor immediately against the sofa. Place the long side of the rectangle against the long side of the sofa. If the area cannot accommodate the recommended sampling area, adjust the dimensions accordingly Sample a total of 18 square feet.
- For the bedroom, select the most frequently used bedroom. Using the tape measure and the roll of tape, mark the corners of a 3 foot x 6 foot rectangular sampling area on the floor immediately against the side of the bed where the resident is most likely to get in and out of the bed. If possible try to have the rectangular sampling area extend under the bed by 3 or 4 inches so that part of the sample goes under the bed. Place the long side of the rectangle against the long side of the bed. If the area cannot accommodate the recommended sampling area, adjust the dimensions accordingly. Sample a total of 18 square feet.
- Record the final sampling area you marked with tape on the lines next to the bedroom you sampled on this data sheet.
- Areas other than the Living Room, Family Rooms, or Bedrooms may be sampled. If you choose to sample other areas, a separate vacuum dust collector should be used for each area. Please call the lab if you have any questions.

Sampling Procedure

1. Make sure the hose attachment is connected to your vacuum cleaner properly. Turn on the vacuum cleaner to make sure the hose attachment is pulling air, and then turn off the vacuum cleaner. Sometimes the dust collection device will not fit correctly onto the hose attachment. If this is the case, you may use duct tape or electrical tape to tape the dust collector to the hose attachment.
2. Use the extension cord as needed to reach the marked area with the vacuum hose.
3. Remove both caps from sampling device. Place the caps in a location so you can find them after the test is completed. Attach the flat, round end of the sampler device to the end of the hose attachment of your vacuum cleaner.
4. Use the slanted end of the sampling device to collect your sample. Keep the slant end of the sampling device flush with the surface to be sampled.
5. Turn on the vacuum cleaner and start the watch or timer. Start timing the vacuuming procedure using the stopwatch. Try not to disturb the tape. Do not exceed the 5 minute sampling period.
6. Vacuum the area contained within the duct or electrical tape. Do this by passing the sampling device over slightly overlapping, imaginary parallel lines within the sampling area for about 5 minutes. If necessary, adjust your rate of movement so that a total of 5 minutes is used to vacuum the entire 18 square foot sampling area.
7. Move to the second room and repeat the vacuuming of the target area. After the sampling is completed, hold the sampling device upward toward the ceiling and turn off the vacuum cleaner. Re-cap the slant end of the sampling device so as not to lose the dust collected.
8. Avoid vacuuming up any large debris that is not dust. If you accidentally suck up the tape, point the sampling device toward the ceiling and turn off the vacuum cleaner. Pick the tape out of the sampling device. Turn the vacuum cleaner back on and return to vacuuming the sampling area. Be sure to account for lost sample time when you do this so you get a total of 5 minutes of sampling time.
9. Separate the sampling device from the hose of the vacuum cleaner and re-cap the flat end of the device.
10. After the small caps are secured on the dust sampling device, make sure there is dust in the sampling container before you send it to the lab. If no visible dust is noticed, repeat the sampling procedure in both rooms in different locations until visible dust is present in the device.
11. If you lose the small caps, seal the openings completely and securely with duct or electrical tape.

Real-Time PCR

Test Code M233 - EPA 36 Panel
Test Code M181 - Water Damage 20 Panel
Test Code M186 - Aspergillus 15 Panel
Test Code M189 - Penicillium 13 Panel
Test Code M208 - Histoplasma capsulatum (swab or air)
Test Code M143 - Cryptococcus neoformans (swab or air)
Test Code M236 - Raccoon Roundworms (swab, dust, bulk)
Test Code M146 - Bed Bug (swab)

PCR Analysis Individual Cassette
#8715309

- EMSL offers state-of-the-art fungal detection and enumeration using US EPA-licensed PCR technology. Real-Time PCR is an excellent complement to your current sampling strategies.
- Use PCR testing to rapidly detect microorganisms of interest.

EMSL Rotary Vane Pump
(Stand not included)
#8706102

Sampling Procedure

Air Samples

1. Obtain a 3-piece PCR air/dust-sampling cassette from EMSL.
2. Remove the upper (blue) and lower (red) plugs of the cassette.
3. Attach a vacuum pump to the cassette through the lower opening.
4. Sample as much air as desired through the upper opening. There is no upper limit to sampling time.
5. Record the VOLUME of air sampled and ship the cassette to EMSL. No refrigeration is needed.

Water Testing Bottles
FREE to EMSL Customers
Call for Details

Dust Samples

1. Obtain a 3-piece PCR air/dust-sampling cassette from EMSL.
2. Remove the upper (blue) and lower (red) plugs of the cassette.
3. Attach a small piece of tubing to the upper opening. Cut a 45-degree angle at the end of the tubing.
4. Attach a vacuum pump to the cassette through the lower opening.
5. Begin collecting dust through the upper tubing. There is no upper limit to sampling time.
6. Ship the cassette to EMSL. No refrigeration is needed.

Swab Sampling

1. Obtain sterile 1 mL Butterfield's Solution swab to collect and transport samples (provided by EMSL).
2. Wearing gloves, remove swab from packaging material.
3. Remove plug from media tube.
4. Swab the desired area thoroughly where there is suspected contaminant.
5. Insert the swab in the tube, firmly close cap, and label appropriately.

Allergen Sampling

Sampling Procedure

Dust Sampling with a Dust Collector Kit

(Kit available from EMSL, Product ID 8715600)

1. Insert the white filter tube securely into the dust collector, through the opening at the angled end.
2. Attach the dust collector to the vacuum cleaner hose or tube.
3. Turn on the vacuum cleaner and vacuum four separate areas for 30 seconds each, where each area is about 1/4 square meter. Total sampling time is 2 minutes and total area sampled is about 1 square meter.
4. A minimum of 100 mg of dust is required for allergen analysis.
5. Remove the filter tube containing the dust sample and place it in a small Ziploc-type bag or equivalent. Place entire device in the bag.
6. Label the bag with your sample name or code and ship to EMSL Analytical, Inc. for allergen analysis.

Dust Sampling with a Filter Cassette

(Cassettes available from EMSL, Product ID 8715314)

1. 25mm 0.45m MCE filter cassettes can be attached to a vacuum pump using PVC tubing.
2. A flow rate of 5-10 LPM is sufficient to collect dust into the cassette.
3. We recommend using a template to establish and standardize sampling areas (carpet, furniture, bedding, etc.).
4. Check clear window at inlet end of cassette to determine that an appreciable amount of dust has collected.
5. A minimum of 100 mg of dust is required for allergen analysis.

EMSL Carpet Sampling Kit
25mm Kit, #8715314

Allergen Sampler
#8715600

Indoor Allergen Analyses Available

Test Code and Description:

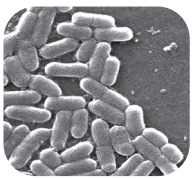
- M034 Cat Dander (Fel d 1) by MARIA
- M035 Dog Dander (Can f 1) by MARIA
- M036 Cockroach (Bla g 1) by MARIA
- M037 Dust Mites (Der p 1 & Der f 1) by MARIA
- M038 Mouse (Mus m 1) by MARIA
- M039 Rat (Rat n 1) by MARIA
- M044 Indoor Allergen Group: Cat, Dog, Cockroach, and Dust Mites by MARIA (Multiplex Array for Indoor Allergens)
- M254 Rat & Mouse Combo by MARIA

Note: Multiple allergens can be analyzed from a single dust sample.

Dust Mite

Water Sampling for Total Coliform, *E. coli*, & Fecal Coliform

Sampling Collection



- All water samples must be taken in approved, sterile sampling containers, which our laboratory can provide upon request. Treated (municipality/distribution systems) water samples must be de-chlorinated with sodium thiosulfate which is provided in the 100-mL lab issued bottles.
- Keep sampling bottle closed until it is to be filled.

Sampling Procedure

Distribution or Sink Faucet Sample

1. Remove aerator from faucet.
2. Rinse faucet with a bleach solution.
3. Flush line by turning tap fully on and letting it run for 2 to 3 minutes, or for a time sufficient to permit clearing the service line.
4. Reduce water flow to permit filling bottle without splashing.
5. Collect a 100-mL sample to submit for analysis.

Well Samples

1. If a sample is taken from a well, fitted with a hand pump, pump the water for about 5 minutes before collecting sample.
2. If the well is equipped with a mechanical pump, collect sample from a tap on the discharge.
3. If there is no pumping machinery, collect sample directly from the well by means of a sterilized bottle attached to a rope or stick of appropriate length. Fit a weight to the base of the bottle. Lower the bottle into the well. Take care to avoid contaminating sample by any surface scum.
4. After the sample has been collected, tighten the lid securely. Using an alcohol wipe, clean the outside of the bottle.

Hold Times

- If the sample is taken for compliance purposes or for a discharge permit, Fecal Coliforms, Fecal Streptococcus, and Enterococci samples must be run within 6 hours of sample collection.

- If the sample is taken for compliance purposes or for a discharge permit, Total Coliforms and *E. coli* need to be run within 24 hours of sample collection, and Heterotrophic Plate Count samples within 8 hours of collection.
- If client is aware that hold times are going to be exceeded but would like analysis anyway, please indicate this on the Chain of Custody.
- If hold times are exceeded, this may impact the results. This will be noted on the report.

Chain of Custody (COC)

- A COC must contain sampling date, time sampled, and sample source. This is needed to determine whether or not samples are within hold times and correct analysis procedure.
- If looking for Total Coliforms please inform us if you would like Presence/Absence P/A or Enumeration (MFT-Membrane filtration technique).
- Test Code M015 - Heterotrophic Plate Count
  Test Code M017 - Total Coliform & *E. coli* P/A
  Test Code M129 - Enterococci P/A
  Test Code M251 - Enterococci MPN
  Test Code M012 - *Pseudomonas aeruginosa* P/A
  Test Code M167 - *Pseudomonas aeruginosa* MPN
- Drinking water samples for compliance or regulatory testing need to have free chlorine tested in the field and included on the chain of custody.

Turn Around Times

- Total Coliform-Presence/Absence (Colilert) results can be given in 24-48 hours.
- Total Coliform enumeration (MFT), Fecal Coliform, Fecal *Streptococcus* and Enterococci will take 6-10 days.

Sample Shipping

- All samples must be shipped cold, not frozen and should remain upright. The best method of shipment for these samples is to use freezer packs, not soda bottles filled with frozen water, bags of ice or loose ice. Samples that are not shipped properly can become contaminated or may be rejected by the lab.

Sewage Contamination in Buildings

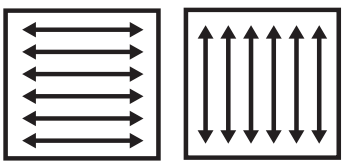
- Methods available to test for sewage contamination in buildings include:

M117 – a culture-based method detecting Total Coliforms, *E. coli,* and Enterocci (48 hr TAT)
M013 – a culture-based method detecting Total Coliforms, Fecal Coliforms, *E. coli,* and Fecal Streptococcus (6-10 day TAT)
M095 – a PCR-based method for detecting Total *Bacteroides* (24 hr TAT available)
M199 – PCR-based method for Human *Bacteroides*
M410 – Bovine Bacteroidales (cow)
M411 – Ruminant Bacteroidales (deer, goat, sheep)
M412 – Swine Bacteroidales (pig)
M415 – Avian Helicobacter (bird)
M414 – Canine *Bacteroides* (dog)
M416 – Comprehensive Source Tracking Panel

- The culture-based methods have the advantage of looking for only living bacterial contaminants which may be the only ones of interest if disease is of concern. Endotoxin testing can also be of value to measure the total amount of Gram negative bacteria in the building (see page 31).
- The *Bacteroides* test has some advantages over the traditional culture-based tests including:
  - Total *Bacteroides* is specific for fecal contamination from all sources – animals, birds, and human
  - Human *Bacteroides* is specific for human sources of fecal contamination.
  - Coliforms, *E. coli,* Fecal Streptococci, and Enterococci can grow in water, soil, sediments and on vegetation in uncontaminated environments (*E. coli* is found in pristine tropical environments).
  - Coliforms, *E. coli*, Fecal Streptococci, and Enterococci can not distinguish between animal and human sources of contamination.
  - *Bacteroides* will not multiply in the environment.
  - *Bacteroides* outnumber coliforms by 1,000:1 and outnumber *E. coli* by 10,000:1; therefore, the chance of finding *Bacteroides* is greatly enhanced.
  - Traditional culture-based tests rely on the presence of live bacteria. These bacteria often will not be viable in indoor environments. **THIS MEANS THAT A NEGATIVE COLIFORM or *E. COLI* RESULT DOES NOT MEAN THE ABSENCE OF FECAL CONTAMINATION.** The new *Bacteroides* test overcomes these culture limitations. The laboratory can detect live, nonviable, or viable but not culturable bacteria.

Sampling Procedure for all Sewage Contamination Tests

1. Obtain a sterile 1 mL Butterfield's Solution swab (EMSL Product ID 8708935) to collect and transport samples (provided at your request by EMSL).
2. With gloves on, remove swab from sterile packaging.
3. Carefully unscrew cap of sampling device – swab is attached to the lid of the cap.
4. Gently press out excess solution from sampling swab by pressing the swab against the inside wall of the tube with a rolling motion.
5. Hold swab at an approximate 30° angle from the sampling surface, taking care not to contaminate any part of the swab or the sampling site.
6. Using firm, even pressure move the swab slowly and thoroughly over an entire 4" x 4" sampling area, rewetting the swab tip with the transport solution as needed. First horizontally, then vertically:



Horizontally *Vertically*

7. After sampling is complete, carefully put swab back into vial and close cap tight.
8. Label the sample using a permanent ink marker.
9. **Background samples in non-fecally contaminated areas should be taken.**

Sample Shipping

- Samples that will be submitted for culture-based analysis must be shipped overnight cold, not frozen, within 24 hours from the sample collection. The best method of shipment for these samples is to use a cooler with freezer packs, not bags of ice or loose ice.
- There are no special shipping requirements or hold times for *Bacteroides* since they will not multiply outside of the intestine. However, we recommend sending them to the laboratory as soon as possible.

Legionella - Sampling Instructions

While a 1000mL sample for potable water and a 250mL sample for non-potable water are recommended, any size sample is acceptable. Even a 100mL sample can provide a limit of detection of 0.5 CFU/mL when fully-filtered. Be sure to use sterile bottles with a chlorine neutralizing agent. Since biofilms are the actual reservoirs for the bacteria it is also recommended to take sterile swab samples of biofilm in areas where it is present. Samples should be shipped overnight to the lab on freezer packs. Culturable analysis either by the US Center for Disease Control or the International Standard Organization is the "gold standard" and requires 10-14 days. Testing by Polymerase Chain Reaction (PCR) takes 2-3 days and may be very useful for providing fast, presumptive results to reduce liability during an outbreak. Testing by Next Generation DNA Sequencing can provide evidence that compares the molecular fingerprint of an environmental isolate to a clinical isolate. Isolating Legionella from environmental samples is difficult. Make sure to use an experienced lab that is either CDC ELITE or HPA (United Kingdom) proficient.

1. Personal safety and precautions should be observed during sampling. Avoid breathing aerosols that may be contaminated with *Legionella* bacteria. Avoid generating aerosols or water mists during sampling of the water system. Wear a respirator equipped with an N95 respirator, a HEPA cartridge, goggles, and sterile nitrile gloves.
2. Prepare or obtain sterile, screw-capped plastic bottles for sampling. Sodium thiosulfate is routinely added to the bottle as a preservative and halogen (chlorine or bromine)-neutralizing agent.
3. For drinking or potable water, such as water fountains, faucets, and shower heads, obtain a "first draw" sample by collecting the first water that comes out of the hot faucet into a bottle. This sample will be more likely to contain any biofilm associated bacteria that may be present. Leave a one-inch space on top of the water sample. Take cold water samples from drinking water fountains and areas that have dead legs. Also take ice samples, allowing them to melt.
4. When sampling faucet aerators and showerheads, remove the aerators asceptically. Take swabs of the inside the faucet and showerheads as far as you can reach with the swab. Swirl the swab on the inside of the pipe three times. Your swabbing procedure should be consistent between sampling locations. When sampling cooling towers, whirlpool spas or fountains, look for areas of biofilm and take a swab sample of the biofilm. We will provide sterile swabs for this purpose.

5. For non-drinking or non-potable samples from such sources as cooling towers, chillers, condensate pans, surface water in reservoirs, sprinklers, hot tubs, water walls etc., collect water from the bottom or side of the vessel or reservoir. Leave a one inch space on top of the sample. Record any biocide used in water treatment when collecting non-drinking water. If sampling whirlpool spas, consider taking a swab sample of any biofilm as well as a sample of the sand filter.

6. Label sample number on the bottle and record on the sample data sheet. Use a distinctive number for each sample. Complete all sample information on a sample data sheet for your own record. Send a copy with the samples to the laboratory.

7. Tightly cap the bottles. Make sure that water does not leak during shipping and transporting. Taping of bottle around the cap and neck with electric vinyl tape is recommended. Place taped bottles in a clean plastic bag.

Place the samples in insulated boxes with freezer packs to protect specimens from extreme temperature fluctuations in the summer months. **NEVER USE ICE OR DRY ICE.** Stuff the box with foam chips to cushion, and seal the box securely for shipping. Send samples by overnight express carrier. Schedule sampling between Monday and Friday so that samples can be delivered to the laboratory no later than Saturday. (Consider holidays)

Contact EMSL for the shipping address to your nearest

CDC ELITE LABORATORY

Phone: 800-220-3675 or Email: info@emsl.com

Where to Look

This is a list of water systems that have been demonstrated to harbor *Legionella* bacteria and have been associated with outbreaks. Their investigation should be included in any *Legionella* Risk Assessment program:

- Potable water systems
- Cooling towers
- Aerosol generation during the biological treatment of some industrial process wastewater streams. For example, pulp and paper manufacturing, food and beverage manufacturing and pharmaceutical manufacturing.
- Aerosol generation during municipal water and wastewater treatment
- Raw, utility or fire water
- Ornamental outdoor and indoor water fountains and ponds
- Heated swimming pools
- CPAP water
- Potting soil
- Hot tubs
- Humidifiers
- Metal working fluids
- Medical therapy equipment like dialysis units, nasogastric tubes, respiratory equipment and nebulizers, whirlpool baths
- Commercial car wash facilities particularly those using recycled water
- Outdoor body misters at ballparks and amusement parks
- Supermarket vegetable misters
- Ice machines in hotels and hospitals

Cooling Tower Sampling Locations

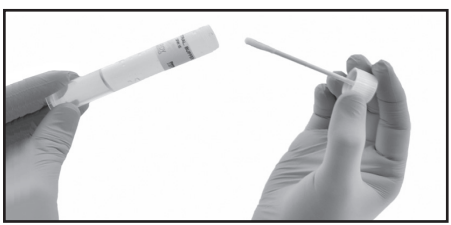
Sample Quarterly

- Tower Makeup
- Tower Sump away from makeup*
- Inlet to Heat Exchanger
- Outlet from Heat Exchanger
- Distribution Pack
- Tower Pack*

** Test Routinely. Test all locations to establish a baseline. Test all locations during an outbreak.*

Legionella - Swab Sampling Instructions

1. Ask Facilities Management to remove aerators from shower heads and faucets where you will be sampling.
2. Insert the EMSL swab deep into the faucet/pipe. Try to get beyond the bend and swab around the inside surface firmly without breaking the swab stem. (If there is visible biofilm on the inside of the showerhead or faucet aerator when these are removed, they can also be swabbed.)
3. Place the swab into the EMSL sterile plastic tube prefilled with 5 mL of buffer to keep the swab tip moist during transport. Tighten the tube top to prevent leakage.
4. Label the tube with a unique identifier. Record the type and location of the sample on a Sample Data Sheet, and place the tube into a cooler. Take bulk water samples:
5. After the biofilm swab is collected, turn on the water and let it run for a few minutes until the water is warm but not hot. The goal is to obtain water currently in the distribution system along with any material shed from biofilm. Avoid heating water excessively (approximately 122°F or higher) since free-floating Legionella will die quickly at elevated temperatures. Collect up to 1 L of water from the faucet into a sterile bottle, leaving a 1 in. space at the top. Tighten the top to prevent leakage.



***Legionella* Swab, ID# 8708322**

Call 1-800-220-3675 or 1-866-798-1089

ORDER TODAY!

Healthcare-Associated Infections (HAIs) Sampling Instructions

EMSL accepts environmental samples for HAI investigations for the following bacteria. Quantitative or Presence/Absence test methods are available options:

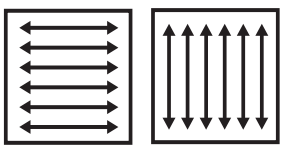
- VRE
  - Test Code M701 P/A
  - Test Code M702 Quantitative
- VRSA
  - Test Code M703 P/A
  - Test Code M704 Quantitative
- CRE
  - Test Code M705 P/A
  - Test Code M706 Quantitative
- *Staphylococcus aureus*
  - Test Code M707 P/A
  - Test Code M708 Quantitative
- *Streptococcus* spp.
  - Test Code M709 P/A
  - Test Code M710 Quantitative
- *Acinetobacter baumannii*
  - Test Code M711 P/A
  - Test Code M712 Quantitative
- *B. cepacia* complex
  - Test Code M713 P/A
  - Test Code M714 Quantitative
- *Listeria monocytogenes*
  - Test Code M715 P/A
  - Test Code M716 Quantitative
- *Salmonella*
  - Test Code M717 P/A
  - Test Code M718 Quantitative
- *Campylobacter*
  - Test Code M719 P/A
  - Test Code M720 Quantitative
- *C. difficile*
  - Test Code M721 P/A
  - Test Code M722 Quantitative
- *Enterococcus faecalis*
  - Test Code M723 P/A
  - Test Code M724 Quantitative
- *Klebsiella pneumoniae*
  - Test Code M725 P/A
  - Test Code M726 Quantitative
- *Stenotrophomonas maltophilia*
  - Test Code M727 P/A
  - Test Code M728 Quantitative
- MRSA
  - Test Code M729 P/A
  - Test Code M730 Quantitative
- ESBL
  - Test Code M731 P/A
  - Test Code M732 Quantitative

Please note: we are not able to accept clinical specimens for testing.

Swab Sampling Procedure

Caution must be taken by wearing gloves when collecting samples from contaminated items; put on clean gloves immediately before collection. Remove gloves promptly after use, before touching non-contaminated items and environmental surfaces and wash hands immediately to avoid transfer of microorganisms to other environments.

1. Obtain a sterile swab in 1 mL HiCap neutralizing broth (EMSL Product ID 8708942) to collect and transport samples.
2. With gloves on, remove swab from container.
3. Carefully unscrew cap of sampling device – swab is attached to the lid of the cap.
4. Gently press out excess solution from sampling swab by pressing the swab against the inside wall of the tube with a rolling motion.
5. Hold swab at an approximate 30° angle from the sampling surface, taking care not to contaminate any part of the swab or the sampling site.
6. Using firm, even pressure move the swab slowly and thoroughly over an area suspected of contamination. Ideally an entire 4" x 4" area is sampled while rewetting the swab tip with the neutralizing broth as needed:



Horizontally *Vertically*

7. After sampling is complete, carefully put swab back into vial and close cap tight.
8. Label the sample using a permanent ink marker.

Sample Shipping

All samples should be shipped overnight cold, not frozen, within 24 hours from the sample collection. The best method of shipment for these samples is to use freezer packs, not bags of ice or loose ice. Samples that are not shipped properly could get contaminated and may be rejected by the lab.

Endotoxin Sampling Test Code M014

- Endotoxins are compounds that are found in the cell walls of Gram negative bacteria. Endotoxins can cause flu-like symptoms when inhaled and may aggravate asthma.
- High levels have been reported from a variety of environments, such as in recycled water, cotton mills, agriculture or wastewater treatment facilities, industrial wastewater mists, and contaminated room humidifiers. High levels of endotoxins are found in any recycled fluid.

Summary of Procedures

Air samples are collected on endotoxin-free membrane filters, stored desiccated wherever possible, or stored at 4°C. Endotoxin analysis can be done on air, water, or bulk samples.

Caution

- There are no accepted threshold values, so relative value comparisons have to be made between the suspect area, and a non-suspect one under similar environmental conditions.
- Since endotoxin levels may be significant in outside air, the latter may not be suitable for background levels; especially in environments known to have high levels of Gram-negative bacteria (see above, 1st paragraph).
- All sampling utensils must be certified endotoxin-free. PVC and polypropylene materials cannot be used due to their affinity for binding with endotoxin.

Materials

- Fluids and Bulk samples: Collect in endotoxin-free (glass or polystyrene) containers (EMSL Product # 8715711).
- For air sampling: low-flow pump, rotameter flexible tubing endotoxin-free polystyrene cassettes (EMSL Product # 8715700) with 37mm diameter 0.4 µm polycarbonate membrane filters and AP40 glass-fiber backing pads. These were specially treated to remove all endotoxins by the manufacturer.
- Sterile surgical gloves should be worn for all samples.

Sampling Procedure

Endotoxin Individual Cassette
#8715700

Air Sampling

1. Caution must be taken to avoid breathing on, touching, or otherwise exposing the sampling containers to human contamination.
2. For indoor sampling, ensure that all doors and windows are closed.
3. Calibrate pump to 1.5 liters per minute.
4. Using gloves, connect cassette to pump with the red-capped end toward the pump. Collect air samples for 4 hours per sample.
5. Disconnect cassette and replace the protective covering back on both the inlet and outlet of the cassette. Wrap entire cassette in original packing and seal with tape.

Liquid or Bulk Sampling

1. Liquid samples must be collected in endotoxin-free vials only. These bottles can be purchased from the lab.

Sample Shipping

- Complete an EMSL Chain of Custody (COC), available on the website (www.emsl.com), detailing client name and information, project name or number, sample #, and a description of the area.
- Ship samples to EMSL Analytical, Inc. as soon as possible. Liquid samples should be shipped on freezer packs.

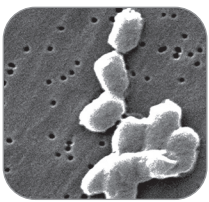
Quality Control Recommendations

- Multiple samples need to be collected at a site in order to compare a possibly affected area with an unaffected one.

Sampling in the Food Industry

Food Contact Surfaces

Wash hands thoroughly with soap and water before and after sample collection.

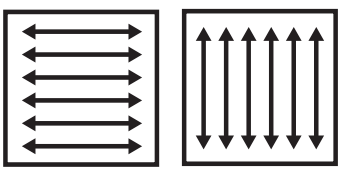


Materials

- Environmental swab with 10-mL Buffer Solution (1 per sampling site)
- Permanent Ink Marker
- Predetermined sampling site of approximately 4" x 4" (~ 100 cm^2), or for more intricate surface areas, an equivalent area estimation or a "per part" sample is acceptable.
- Gloves (non-sterile)

Sample Procedure

1. With gloves on, remove swab from sterile packaging.
2. Carefully unscrew cap of sampling device – swab is attached to the lid of the cap.
3. Gently press out excess solution from sampling swab by pressing the swab against the inside wall of the tube with a rolling motion.
4. Hold swab at an approximate 30° angle from the sampling surface, taking care not to contaminate any part of the swab or the sampling site.
5. Using firm, even pressure move the swab slowly and thoroughly over the entire sampling area, rewetting the swab tip with the solution as needed. First horizontally, then vertically:



Horizontally *Vertically*

6. After sampling is complete, carefully put swab back into vial and close cap tight.
7. Label the sample using a permanent ink marker.
8. Keep sample(s) at a refrigerated temperature (35° – 40° F) until it is submitted to the laboratory for analysis. Submit within 24 hours of sampling.

Sampling Non-Food Contact Surfaces

Wash hands thoroughly with soap and water before and after sample collection.

Materials

- Whirl-Pak Bag, Sterile Sponge (pre-wetted), Sterile Gloves (Pre-packaged) 1 per sampling site
- Predetermined sampling site of approximately 10" x 10" (100 in^2), for more intricate surface areas, an equivalent area estimation or a "per part" sample is acceptable.
- Permanent Ink Marker

Sample Procedure

1. Separate the glove/sponge portion from the sponge Whirl-Pak at the perforation.
2. Tear off the clear, perforated strip at the top of the Whirl-Pak bag.
3. Put on the sterile glove.
   a. Remove the sterile glove from the pouch by the top edge with out contaminating (touching, breathing on, contacting etc.) the glove.
   b. Remove a glove by holding it from the wrist-side opening. Avoid any contact with the outer surface of the glove. Insert clean hand into glove, taking care not to puncture the glove.
4. Open the bag containing the sponge, wearing sterile gloves, being careful not to touch sponge to anything but the gloved hand.
5. With gloved hand, remove sponge from the bag.
6. Using firm, even pressure move sponge slowly and thoroughly over sampling area. First horizontally, then vertically.
7. Return sponge back to Whirl-Pak bag taking care not to contaminate the sponge or the bag with the ungloved hand.
8. Close the bag by folding the top down three times and bending the wire ends over onto the bag.
9. Label the sample using a permanent ink marker.
10. Keep sample(s) at a refrigerated temperature (35° - 40° F) until it is submitted to the laboratory for analysis.

Whirl-Pak Bags

2oz Write-On Bags
500 qty, #8708908

4oz Write-On Bags
500 qty, #8708909

Limiting Conditions for Pathogen Growth

Guidance provided by EMSL Analytical, Inc.

Pathogens	Min. a_w	Min.pH	Max.pH	Max%Salt	Min.Temp. (°C)	Max.Temp.	Oxygen Requirement
Campylobacter jejuni	0.99	4.9-5.5	8.0	1.5-2.0	30-32	42-45	Micro-aerophilic
Clostridium botulinum type A, proteolytic B & F	0.93-0.96	4.7	9.0	10.0	10.0	48-50	Anaerobe
Clostridium botulinum type E, nonproteolytic B and F	0.93-0.96	4.7-4.8	9.0	4.5-6.0	3.0	45	Anaerobe
E. coli	0.93-0.95	3.6-4.7	9.5	7.5-8.0	0.6-3.0	45	Facultative anaerobe
Listeria monocytogenes	0.92-0.95	4.8	9.6	8-12	0-2.0	45	Facultative anaerobe
Salmonella spp.	0.92	4.0	9.0	8.0	5.0	46-47	Facultative anaerobe
Shigella spp.	0.96	6.0	10.0	6.0	7.0	46	Facultative anaerobe
Staphylococcus aureus	0.85-0.86	4.0	10.0	18-20	5-6*	45-45	Facultative anaerobe
Vibrio cholerae	0.95	3.6-6.0	9.6	6-8	8.0	42-46	Facultative anaerobe
Vibrio parahaemolyticus	0.94	4.8-5.0	9.6	8-10	5.0	43	Facultative anaerobe
Vibrio vulnificus	0.95	6.3	9.0	6.0	13.0	44	Facultative anaerobe
Yersinia enterocolitica	0.95-0.96	4.1-4.4	9.0	6-7	-1.0 – 1	44	Facultative anaerobe

*Minimum temperature for toxin formation is 10° C
Micro-aerophilic: requires limited levels of oxygen
Anaerobe: requires the absence of oxygen
Facultative anaerobe: grows either with or without oxygen

Microbiology Culture Media Information

EMSL Analytical, Inc. is able to provide a large variety of sampling media to our customers. The following information is designed to help aid in the selection of culture media, providing the most representative sample of the environment possible. The media listed below are not exhaustive; if you have specific media requirements, please let us know.

Bacterial Media

- TSA (Tryptic Soy Agar)
- TSA w/ 5% Blood
- MAC (MacConkey)
- HE Agar (Hekton Enteric)
- BCYE agar (Buffered Charcoal Yeast Agar)
- MSA (Mannitol Salt Agar)
- EMB (Eosin Methylene Blue)
- BE (Bile Esculin Agar)
- Chocolate Agar
- BHI (Brain Heart Infusion)
- Xylose lysine deoxycholate agar (XLD agar)

Fungal Media

- MEA (Malt Extract Agar)
- PDA (Potato Dextrose Agar)
- Cellulose Agar
- SAB (Sabouraud Dextrose Agar)
- Corn Meal Agar
- DG18 (Dichloran-Glycerol Agar 18)
- Bird Seed Agar
- Mycobiotic Agar

General Media Recommendations for Air Sampling of Culturable Bacteria and Fungi

- For fungal sampling, in most cases, we recommend MEA.
- For fungal species identification use MEA and DG18 plates
- For bacterial sampling, in most cases, we recommend TSA or TSA w 5% Blood.
- When sampling specifically for Gram negative bacteria, we recommend MacConkey Agar.
- The Microbiology Department will be happy to make recommendations based on your individual sampling situation.

Fungal Culture Media

- MEA (Malt Extract Agar) — Gold Standard media for the speciation of fungi.
- PDA (Potato Dextrose Agar) — General-purpose media used for the isolation of fungi and molds.
- Cellulose Agar — Multi-purpose microbial media used for isolating hydrophilic fungi able to utilize complex carbohydrates. Most often recommended in IAQ investigations of *Stachybotrys* sp. contamination.
- Corn Meal Agar — General purpose media for the cultivation of fungi from the environment, commonly used in IAQ investigation of *Stachybotrys chartarum* contamination.
- DG18 (Dichloran-Glycerol Agar 18) — Media containing 18% glycerol, used for the recovery of xerophilic yeast and molds. Recommended for recovery in dry locations such as dry powder storage and manufacturing areas.
- Bird Seed Agar — For the isolation of *Cryptococcus neoformans.*
- Mycobiotic Agar — For the isolation of *Histoplasma capsulatum.*

Bacterial Culture Media

- TSA (Tryptic Soy Agar) — General purpose media for isolation and culture of bacteria (Gram positive and Gram negative).
- TSA w/ 5% Blood — General purpose media for cultivation, isolation and determination of hemolytic activity of bacteria.
- MAC (MacConkey) — For quantitative procedures for the isolation of Gram negative bacteria and for the differentiation of those microorganisms based on fermentation of lactose.
- HE Agar (Hekton Enteric) — For the isolation and differentiation of Gram negative enteric microorganisms.
- BCYE agar (Buffered Charcoal Yeast Agar) — For the isolation, selection and differentiation of *Legionella* sp. form environmental samples.
- MSA (Manitol Salt Agar) — For the selective isolation and differentiation of *Staphylococcus* sp.
- EMB (Eosin Methylene Blue) — For the isolation and differentiation of Gram negative bacteria.
- BE (Bile Esculin Agar) — For the isolation and identification of Group D *Streptococcus* sp.
- Xylose lysine deoxycholate agar (XLD agar) — For the isolation and differentiation of *Salmonella* sp.

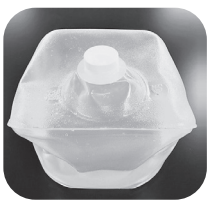
Cryptosporidium and *Giardia* Test Code M640

All public water systems using surface water or groundwater under the direct influence of surface water are required to monitor *Cryptosporidium* ("crypto") under EPA LT2 (Long Term 2 Enhanced Surface Water Treatment Rule).

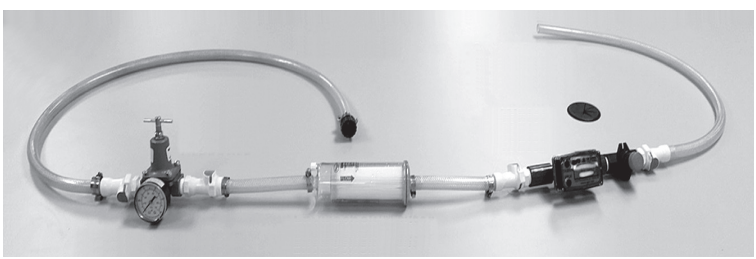
EMSL has been certified in 38 states to analyze *Cryptosporidium* and *Giardia* using EPA 1623.1. Results can be delivered as fast as 3 days. **Sample pick up service is available in certain NJ, NY, and PA areas.**

Sampling Procedure for Bulk Water Samples

1. Fill the 10 L cubitainer to the neck and store under refrigeration (1-10°C).
2. Indicate sampling date, time, location, and name of sampler on the cubitainer using waterproof pen.
3. Attach temperature strips (ColdMark and WarmMark) on the cubitainer. Samples should be shipped with enough ice on the day collected.



Sampling Procedure for Field Filtered Samples



1. Connect the filtration unit, without the Envirochek™ filter, to the water source.
2. Flush the sampling unit. Adjust the pressure < 60 PSI and a flow rate ≤ 2.0 L/min.

3. Insert the filter into the filtration unit. Ensure the directional flow arrow is pointing toward the water meter.
4. Filter 10 L or 50 L of water sample or use two filters, whichever comes first.
5. Record date, time, initial/final flow totalizer reading, sample ID, sampling location, and the operator's name on the label of the filter using a waterproof pen.
6. Replace the blue vinyl caps on filter(s). Seal each filter in a Ziploc style bag.
7. Store filter(s) under refrigeration (1-10°C) as soon as possible until shipment.
8. Attach temperature strips (ColdMark and WarmMark) to the filter(s).
9. Ship filter(s) with enough ice on the day collected.

Notes:

- A matrix spike sample must be submitted for every 20 samples. The procedure is the same as for the bulk water.
- Accept temperature on receipt is 0-20°C.
- Try to avoid sample delivery on Fridays and before holidays.

Sampling Procedure for Swimming Pool

- Collect 1 liter back wash and ship with enough ice.
- Collect 10 liters pool water as a bulk water sample.

Water Quality Taste & Odor Testing

Test Code M617

Taste and odor test includes geosmin and 2-Methylisoborneol (2-MIB).

Sampling Procedure

1. Fill two vials (such as 40 mL TOC bottles) for each sample.
2. After tightening the cap, the vial should be inverted and tapped to check for air bubbles. If bubbles are present, slowly add several additional drops of water until the air is eliminated.
3. Record the sampling date, time, site, and name of sampler on both the bottle labels and the enclosed Chain of Custody.

Algal Sampling

EMSL offers comprehensive algal analyses for various algae, such as blue green algae (cyanobacteria), green algae, diatom, red algae, dinoflagellate, etc.

Sampling Procedure for Algal Identification and Enumeration

1. Collect 1 liter water for each sample and add 10 mL of Lugol's solution within 2 hours to obtain a final concentration of 1%.
2. Samples are stored in the dark and under refrigeration and shipped on ice as soon as possible.

Sampling Procedure for Algal Toxins

Collect at least 40 mL of water sample and store in clear or amber glass vials or polyethylene terephthalate (PETG) containers.

- **Drinking Water:** Add sodium thiosulfate for a concentration of 1 mg/mL.
- **Ambient Water:** No sodium thiosulfate is needed.

Sampling Procedure for Chlorophyll a, b, c and Pheophytin a

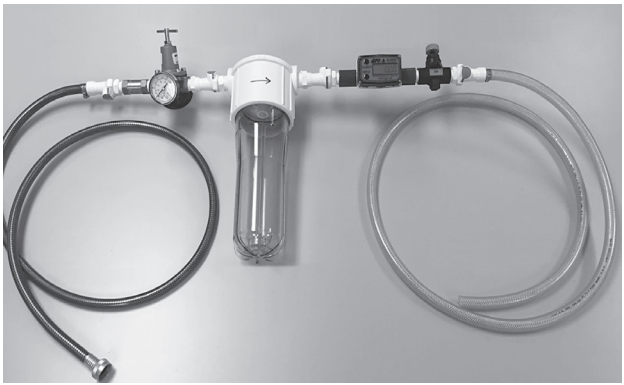
Collect 2 liters water in a clean glass or a plastic bottle. Store samples under refrigeration (1-10°C) in the dark and ship on ice as soon as possible.

Comprehensive Blue Green Algal Analysis

To help our clients to better understand the water contamination caused by blue green algae, EMSL recently started to offer comprehensive blue green algal analysis (M623), which includes algal identification, chlorophyll a analysis, and the most common algal toxin - microcystin. Please contact your sales rep for details.

Microscopic Particulate Analysis (MPA)

Test Code M653



Sampling Procedure

1. Connect the filtration unit, without the cartridge filter, to the water source.
2. Flush the unit, and adjust the flow rate to 1 gpm and the pressure to 10 psi.
3. Insert filter into the housing. Turn water on slowly. Record the date, time of day, and gallon reading from the water meter before sampling.
4. Run the unit for an 8-24 hour period to collect at least 500 gallons of water.
5. After filtering sample, pour residual water from filter housing into a Ziploc bag and place filter in a second heavy duty quality ziploc bag and seal.
6. Record stop time, final meter reading, and the total volume collected.
7. Record the sample identification, gallons sampled, collection dates and times, collector's name directly on the bag or on a waterproof label.
8. Double pack the bags containing filter and residual water with Ziploc bags. Make sure all bags are sealed to prevent leakage.
9. Deliver samples on ice to the laboratory within 48 hours after sample collection. Samples that arrive at the laboratory frozen will be rejected.

EMSL Aquatic Microbiology Test Codes

Test Code	Description
M600	Phytoplankton identification (division level)
M601	Phytoplankton identification (genus level)
M602	Phytoplankton identification (species level)
M603	Phytoplankton identification and enumeration (genus level)
M604	Phytoplankton identification and enumeration (species level)
M605	Phytoplankton identification, enumeration, and biovolume (genus level)
M606	Phytoplankton identification, enumeration, and biovolume (species level)
M607	Periphyton (attached algae) identification and enumeration (division level)
M608	Periphyton (attached algae) identification and enumeration (genus level)
M609	Periphyton (attached algae) identification and enumeration (species level)
M610	Algae permanent slide HPMA (3 slides per sample)
M611	Algae permanent slide Naprax diatom (3 slides per sample)
M612	Biomass-Dry weight
M613	Biomass-Ash-free dry weight
M614	Chlorophyll *a*
M615	Chlorophyll *a* & Pheophytin *a*
M616	Chlorophyll *a, b, c* and Pheophytin *a*
M617	Taste and Odor Test (Geosmin and 2-MIB)
M618	Microcystin (Blue green algae toxin)
M619	Cylindrospermopsin (Blue green algae toxin)

EMSL Aquatic Microbiology Test Codes

Test Code	Description
M620	Saxitoxin (Blue green algae toxin)
M622	Brevetoxin (Red Tide Toxin)
M623	Comprehensive Blue green algae analysis (M601, M615, M618)
M640	*Cryptosporidium* and *Giardia* (EPA 1623.1)
M641	Matrix Spike for *Crypto* & *Giardia* testing
M642	Additional filter analysis for *Crypto* & *Giardia* testing
M643	Additional subsamples for *Crypto* & *Giardia* testing
M644	Bacterial abundance (DAPI or AO)
M645	Zooplankton identification to major taxa
M646	Zooplankton total count (major taxa)
M647	Zooplankton identification and enumeration (major taxa)
M648	Zooplankton identification and enumeration (Genus)
M649	Zooplankton identification and enumeration (Species)
M650	Zebra Mussel Veligers count
M651	Permanent slide for zooplankton
M652	Invertebrates Identification to major taxa
M653	Microscopic Particulate Analysis (MPA)
M660	Aerobic endospore (SM9218B)
M669	Special project (custom protocols)

Notes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