

Guidance Document on Microbial Sampling



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Microbial Investigations: Art and Science

The American Council for Accredited Certification (ACAC) recognizes the many factors involved with conducting microbial investigations. There are few laws and no established exposure limits for microbials because there are too many variables that can influence the investigation and sampling required to prove or disprove hypotheses. These variables include regional and seasonal conditions as well as characteristics of individual human receptors.

Regional differences involving mountains, high deserts, low deserts, coastal, agricultural, large cities and sprawling suburban areas influence the microbial composition of indoor and outdoor air. Seasonal changes introduce variations involving wind, humidity, and rain. Examples of these variations are easy to imagine. Wind-borne microbial flora coming from an ocean area differ from flora borne by inland breezes. Wind-borne microbial flora coming from agricultural areas differ from flora coming from urban areas. Fall breezes carrying particles from decaying plant life differ from spring breezes at the beginning of the growing season. Variations in the microbial world create an unseen world that is seldom constant and never repeatable.

Differences among human receptors affect microbial investigations as profoundly as they affect everything else. It is commonly understood, for example, that the young, the old and the immunocompromised are more at risk from microbial exposure. Additionally, however, it seems that every individual has a unique dose-response relationship to various substances, from shellfish to strawberries.

Given the variables described above, it is difficult to imagine the development of useful standard exposure limits for microbials.

ACAC certifiants are expected to conduct sampling – not for exposure criteria, but to prove or disprove a hypothesis. Experienced microbial investigators realize that snapshots gained from sampling are just that – snapshots. They also realize there are many ways to gather those snapshots, and the results will differ with each method. Last, but not least, understanding the protocols used for analysis and how the analyst arrived at his/her extrapolations are paramount to the interpretation of the snapshot.

In summary, effective microbial investigation is an art as well as a science.

Microbial Sampling

All laboratories have their own standard operating policies guiding their clients on sampling.

ACAC would like to thank the limited guidance provided by Aerobiology Laboratory Associates, Inc., EMSL Analytical, Inc. and Joe Spurgeon, Ph.D. This publication is not intended to represent their individual guidance or opinions nor to create or represent a Standard or a Standard of Care.

Special laboratory guidance may be necessary when conducting sampling for specific analyses; i.e., Environmental Relative Moldiness Index (ERMI), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Legionella*, Polymerase chain reaction (PCR), allergens, endotoxins, *E. coli*, etc.

The focus of this section is to acquaint the reader with general microbial sampling principles.



Each reader should consult with their laboratory of choice before conducting sampling.

This document is neither a standard of care nor a field manual for microbial sampling. ACAC disclaims all responsibility for actions taken by individuals based on statements contained herein.

Sampling methodologies discussed in this document:

1. Direct Examination of Air Samples (Non-culturable)
2. Direct Examination of Surface Samples (Non-culturable)
3. Culturable Air Sampling (Fungi or Bacteria)
4. Culturable Surface Sampling (Fungi or Bacteria)
5. Water

Direct Examination of Air Samples (Non-culturable)

Air-O-Cell, MoldSnap, Micro5 Microcell, Allergenco-D, Filter Cassettes, etc.

These are single-use sampling devices designed for the collection and analysis of a wide range of airborne particles, including fungal spores, pollen, insect parts, skin cell fragments, fibers and other particulates.

Each sampling cassette was designed to be used with flow rates ranging from 5 to 20 liters per minute (LPM). Lower or higher flow rates than manufacturer's specifications may result in a collection loss and/or the accumulation in a non-uniform manner. Do not deviate from the manufacturer's specifications.

Potential Benefits:

- Quick, simple procedure
- Useful for initial site testing
- Fast analysis reporting

Potential Disadvantages:

- Fungal genera may be reported in groups, example Pen/Asp
- Fungal species cannot be identified
- Spore viability cannot be determined.

Example of a Typical Sampling Procedure:

1. Prior to sampling, calibrate your pump to the cassette's specified flow rate with a device that has been periodically calibrated to a primary standard.
2. Remove and retain the seals or pins covering the inlet and outlet on the cassette.
3. Attach the sampling pump tubing to the outlet of the device and start sampling for a specific time.
4. Stop the sampling pump, detach tubing and reseal using the original seals.
5. Label the sample with a unique identifier and complete all sections of the chain of custody (COC).
6. Carefully package and ship cassette(s) to the laboratory using a trackable service.
7. Consult your laboratory of choice for detailed instructions and recommendations.

Tips:

- Report the sampling rate and time or area sampled in the COC.
- Laboratories should state their analytical procedures; i.e., ASTM- D7391-09.
- Outdoor and comparison areas should be considered in the sampling regimen.
- Observe temperature limitations for use and storage.
- Comparison samples are a must.

- Field blank cassettes may be meaningful in litigious cases.
- Damaged and/or expired cassettes should never be used.
- Direct examination for bacterial content isn't possible.
- PCR sampling requires a 3-piece sampling cassette with filter.
- Chain of custody must include a signature at each handoff.
- Microbial volatile organic compounds (mVOCs) cannot be detected with these methods.
- Standard flow rates are 15 liters per minute (LPM) for 5 – 10 minutes.

Direct Examination of Surface Samples (Non-culturable)

Tape lift, Swab and Bulk Samples

Direct Examination usually includes using tape lift, swab and bulk methodologies. Laboratory technicians then conduct a visual analysis of the sample without culturing. Fungal results may include spores, hyphae and/or mycelia. Examinations may include general non-fungal content and specific non-fungal content when requested.

Potential Benefits:

- Quick, simple, inexpensive procedure
- Useful for initial site testing and/or visible growth identification
- Fast analysis and reporting

Potential Disadvantages:

- Fungal genera may be reported in groups, example Pen/Asp
- Fungal species cannot be identified
- Spore viability cannot be determined.

Examples of Typical Direct Examination Sampling Procedures:

Surface Sample – Tape lift

1. First, create a half-inch tab on one end of the tape so the lab technician can easily lift it from the adhered surface.
2. Holding clean, clear single-sided tape by the edges, press the center of the adhesive side against the surface.
3. Afterwards, adhere the sample side of the tape to a glass slide and place in a slide box or alternatively adhere the tape to the inside of a new Ziploc bag.
4. Label the slide box or Ziploc bag, complete the chain of custody and ship in a trackable manner.
5. Consult your laboratory of choice for detailed instructions and recommendations.

Tips:

- Do not fold tape onto itself.
- Apply only one tape per slide or new Ziploc bag.
- Protect against breakage and/or damage during trackable shipping.
- Comparison samples may delineate contaminated areas.
- Chain of custody must include a signature at each handoff.
- Microbial volatile organic compounds (mVOCs) cannot be detected.

Vacuum – PCM Cassette

1. Remove the inlet and outlet caps from the Phase Contrast Microscopy (PCM) cassette and attach a vacuum pump to the outlet end.
2. Use the inlet end to vacuum enough area to cover the filter with $\frac{1}{4}$ to $\frac{1}{2}$ teaspoon of sample material.
3. Turn off the vacuum only after orienting the open-face cassette vertically, then replace the caps.
4. Label the cassette, complete the chain of custody and ship in a trackable manner.
5. Consult your laboratory of choice for detailed instructions and recommendations

Tips:

- Area size must be reported to laboratory for any extrapolations.
- Filtered pump is recommended.
- Personal protection is recommended.
- Dust samples for allergens should contain a minimum of 100 mg – no refrigeration needed.
- Comparison samples may delineate contaminated areas.
- PCR (Polymerase Chain Reaction) sampling requires a 3-piece sampling cassette.

Bulk Sample (Contaminated material)

1. Wearing clean, new gloves, remove bulk sample material and place inside a new Ziploc bag or similar container.
2. Label the bag or container, complete the chain of custody and ship in a trackable manner.
3. Consult your laboratory of choice for detailed instructions and recommendations

Tips:

- Do not send the laboratory large bulk samples like whole HVAC filters.
- Sample sizes of 1 inch by 1 inch are usually sufficient for analysis.
- Personal protection is recommended when sampling.
- Chain of custody must include a signature at each handoff.
- No refrigeration is needed.

Culturable Air Sampling (Fungi or Bacteria)

Andersen N6, BioStage, BioCassette, A6, VP-400 and Other Impactor Samplers

These single-stage viable impactors are designed to impact air into an inserted Petri dish containing various agars. Agars contain nutrients for growth of viable microbes and can be designed and selected for specific microbes. Air, which is drawn into the impaction device via an attached pump, impacts the nutrient-rich agar material and “seeds” the impaction area.

Potential Benefits:

- Culturable air sampling can enumerate and identify viable airborne fungal content to the genus level.
- Culturable air sampling can enumerate and identify viable airborne bacterial content.
- Culturable air samples can be used to further identify content to the species level.

Potential Disadvantages:

- Culturing takes 6-10 days for growth and identification to the genus level.
- Additional culturing and time is typically necessary for identification to the species level.
- Some microbes grow better on specific culture media than others.
- Some fungi that grow in culture are still not identifiable because they don't sporulate in culture.

Example of a Typical Sampling Procedure:

1. Check air passages to ensure there are no blockages and Insert a disposable agar dish in the impactor device.
2. Prior to sampling, calibrate an air pump to the manufacturer's recommended flow rate with a device such as a rotameter that has been periodically calibrated to a primary standard.
3. Connect an air pump to the impactor device and calibrate air flow – afterwards, the agar plate used for calibration can be discarded.
4. Clean impactor device prior to collecting each sample with isopropyl alcohol (IPA) wipes and allow it to dry.
5. Insert an ambient temperature agar dish into the impactor device
6. Run the pump for a specific period and then remove the agar dish.
7. Cover the dish and check the agar for impaction indentions on its surface.
8. Secure the lid, label the sample and refrigerate inverted.

9. Fill out chain of custody (COC) and ship the sample refrigerated and trackable overnight.
10. Consult your laboratory of choice for detailed instructions and recommendations.

Tips:

- Viable culture results do not include dormant or dead microbes.
- Actual airborne fungal and bacterial results can be skewed by competition on the agar plate.
- Analysts may find viewing the bottom of the Petri dishes beneficial.
- Agar plates must be kept refrigerated both before and after use
- Agar plates should be warmed to ambient temperature before use.
- Different agar plates may be selected to target growth of a specific microbe of interest.
- Agar plates should be sealed with Parafilm after use.
- Refrigerated agar plates should be stored upside down to prevent condensation.
- Never use Petri dishes with degraded agar (outdated, discolored, dry, etc.).
- 70% isopropyl alcohol (IPA) should be used to clean sampling equipment between samples.
- A clean Ziploc bag should be used for each Petri dish sample.
- Always use overnight, trackable shipping.
- Don't ship on Fridays – refrigerate and ship on Monday.
- Comparison samples and field blanks are a must.
- Chain of custody must include a signature at each handoff.
- Standard flow rates are 28.3 liters per minute (LPM)

Culturable Surface Sampling (Fungi or Bacteria)

Culturable Surface Sample - Wet Swab

Potential Benefits:

- Quick, simple and inexpensive procedure.
- Useful for initial site contamination mapping
- Cultured samples can be identified to the genus level.
- Further culturing can identify to the species level.

Potential Disadvantages:

- Culturing to the genus level takes 6 to 10 days.
- Culturing to the species level typically takes additional time for fungi.

Example of a Typical Sampling Procedure:

1. Using a sterile swab, moisten it by pressing into the liquid at the base of the container.
2. Lightly rub or roll the moistened swab on the surface to be sampled.
3. Return the swab to the container, seal and label the container.
4. Complete the chain of custody indicating size of area and type of analysis desired.
5. Ship via a cool, trackable overnight method.
6. Consult your laboratory of choice for detailed instructions and recommendations.

Tips:

- A light rolling motion should be used to pick up fragile content in growth areas.
- Only touch the handle of the swab – never the swab itself.
- Comparison samples may delineate contaminated areas.
- Chain of custody must include a signature at each handoff.

Culturable Surface Sample - Contact Plate Agar

Potential Benefits:

- Quick, simple and inexpensive
- Useful for initial site contamination mapping
- Cultured samples can be identified to the genus level.
- Further culturing can identify to the species level.

Potential Disadvantages:

- Culturing to the genus level takes 6 to 10 days.
- Culturing to the species level typically takes additional time.

Example of a Typical Sampling Procedure:

1. Remove lid and place the agar onto the surface to be sampled.
2. Replace the lid and seal with Parafilm or tape.
3. Label the sample and complete the chain of custody.
4. Ship via a cool, trackable overnight method.
5. Consult your laboratory of choice for detailed instructions and recommendations.

Tips:

- Wear gloves to prevent contamination.
- Comparison samples may delineate contaminated areas.
- Chain of custody must include a signature at each handoff.

Water Samples

Potential Benefits:

- Quick, simple and inexpensive
- Useful for identifying contaminated water issues
- Reasonably fast

Potential Disadvantages:

- Limited sample hold times
- Glass sampling vessels are subject to breakage (request plastic from the laboratory).
- Analysis may take up to 10 days or longer depending on the target microorganisms.

Example of a Typical Sampling Procedure:

1. Don gloves and remove cap from the sample bottle.
2. Collect at least 100 mL of water in a sterile container.
3. Replace cap and label the sample.
4. Keep water samples cold in a cooler using freezer packs after collection.
5. Complete chain of custody and ship via a trackable method.
6. Consult your laboratory of choice for detailed instructions and recommendations.

Tips:

- All water samples must be taken in approved, sterile sampling containers.
- All potable water samples must contain sodium thiosulfate (de-chlorinate agent).
- Avoid collecting sediment in water samples.
- Specific hold times may apply to the testing for the results to be accurate – consult with your laboratory for any hold time requirements.
- The outside of well samples should be cleaned with an alcohol wipe.
- Legionella water samples should contain 1 liter of water.
- Most water samples do not need comparison samples.
- Chain of custody must include a signature at each handoff.