

Culturable organisms: bacteria, fungi, thermophilic actinomycetes

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**PURPOSE:** Identification of culturable microorganisms and assessment of possible proliferation and dissemination of bacteria or fungi from building reservoirs.

**FIELD EQUIPMENT:**

1. Samplers:
  - a. Andersen 2-stage cascade impactor, or equivalent, for fungi and mesophilic bacteria.
  - b. Andersen N-6 single-stage sampler, or equivalent, for thermophilic actinomycetes.
2. Sampling media, in plates prepared according to sampler manufacturer's recommendations:
  - a. Malt extract agar (MEA) for fungi.
  - b. Trypticase soy agar (TSA) for mesophilic bacteria and thermophilic actinomycetes.

NOTE: Other media may be used, if appropriate, e.g., dichloran glycerol agar (DG18) for xerophilic molds, R2A agar for heterotrophic bacteria, and rose bengal agar for slow-growing fungi such as *Stachybotrys*.
3. Sampling pump capable of meeting sampler manufacturer's flow specification (e.g., 28.3 L/min), with flexible connecting tubing.
4. Cotton gauze pad, e.g., 4" x 4".
5. Rubbing alcohol, 70% isopropanol.
6. Refrigerant packs, if necessary for keeping samples cool during shipment.

NOTE: Keep samples cool, but protect from freezing.

**SAMPLING STRATEGY:**

1. Select at least three sites, one each to represent complaint area, a noncomplaint area (otherwise as similar as possible to complaint area), and outdoors.
2. In turn at each site, sample simultaneously for fungi, mesophilic bacteria, and thermophilic actinomycetes. Typical sampling time is ten minutes. Before moving to the next site, repeat twice to obtain triplicate, consecutive samples.
3. Load and immediately unload one set of sampling media in each sampler to serve as field blanks.
4. Collect another complete set of samples and blanks on the next day.

**SAMPLING:**

1. Calibrate each sampling pump with a representative sampler in line.
2. Before each run, carefully and thoroughly wipe each sampler stage with rubbing alcohol. Allow to dry. Make sure air passages are not blocked.
3. Load sampling media into sampler, remove covers from media, and attach sampler to pump with flexible tubing.

NOTE: Take special care to prevent contamination of media during loading and unloading. Do not touch agar surface.
4. Sample at known preset flow for an accurately known time, e.g., 10 min. (In heavily contaminated areas, a shorter sampling time may be necessary.)
5. Replace covers on sampling media, unload, and pack securely for shipment (plates should be media side up).

**SHIPPING:** Keep collected samples and blanks cool (not necessarily ice-cold) and ship as quickly as possible to a laboratory for enumeration and identification.

**ANALYSIS:** Mesophilic bacteria and thermophilic actinomycetes are usually identified to species and fungi usually identified to genus. Interpretation is subjective and based on total numbers and rank order of taxa in complaint area compared with control areas (noncomplaint and outdoors).

**METHOD**

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