

## Sample Collection Guidelines

### Chain of Custody (COC) Records

- Environmental Safety Technologies, Inc. (EST) Chain of Custody (COC) record forms are provided to document sample collection information including company and job site info (name/address/city/state/zip), sample collection information (sampling date/time/name of person sampling), description/location for each sample, rate/time duration for air samples, optional collection information documented by sampler (pH/temperature/retest/etc.), and relinquishment to the laboratory (person submitting/date/time).
- EST will provide a custom COC with your company's information filled out and saved in a printable PDF format for routine sampling use upon request.
- Only fully completed and signed COCs qualify to serve as evidence in legal proceedings.
- Each submitted COC record also serves as the request for laboratory processing and analyses.
- All samples listed together on the same COC will be collectively grouped as a batch of samples and results printed together on the same report. Submit multiple COCs to receive separately issued reports as needed.
- Please use a separate COC for separate batches of samples requiring individual reports.
- Call (502) 893-6080 or send an email to [est@estechlab.com](mailto:est@estechlab.com) if you have any questions or concerns.

### Contact Info for Customer Assistance & Sampling Supply Requests

- Laboratory business hours are Monday through Friday, 8:00 AM to 5:00 PM.
- Routine sampling and bottle requests can be made on EST's website at [www.estechlab.com](http://www.estechlab.com) emailing the office staff at [est@estechlab.com](mailto:est@estechlab.com), or by calling 502-893-6080 during regular office hours.
- For emergency project management consultation, urgent sample scheduling, or sample collection questions outside normal business hours, please call George Young at 502-550-4207.
- Please call to arrange for weekend processing/analysis; extra charges will apply.

### Shipping Instructions & Transit Delay Information

- See the following sections below for test specific sample collection and recommended shipping guidelines for different laboratory analyses.
- Customers will be notified by EST if the duration of sample transit exceeds recommended shipping times.
- Samples received that exceed recommended transit shipping times shall be held until EST receives acknowledgement of the delay from the submitting representative.
- Sample processing shall proceed despite the delay only after authorization is relayed to EST by the customer.
- Transit delay samples rejected from processing by customers shall be destroyed upon notice or if notice is not given with five business days.
- Customer authorizations shall be documented by EST personnel on the COC record accompanying samples and electronically archived.

### Submitting Samples to the Laboratory: EST's Shipping Address

Environmental Safety Technologies, Inc.  
1815 Brownsboro Road  
Suite 200  
Louisville, KY 40206


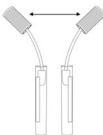


## General Sample Collection Supplies

### Bottles for Water/Bulk-Liquid Sample Collection

- Upon request, EST provides free sterile 250 ml, 500 ml (wide-mouth for ice collection), or one liter (1000 ml) sample bottles pre-dosed with sodium thiosulfate to neutralize up to 15 ppm of free chlorine or other halogens in accordance with Standard Methods for water/bulk-liquid sample collection.
- Sample dichlorination with sodium thiosulfate is recommended for most microbiological water analyses.
- Do not rinse sample bottles prior to filling.
- Take care to prevent tablets or sodium thiosulfate powder from washing out of bottles during sampling.
- Fill sample containers leaving sufficient headspace ( $\approx 1$  inch) for mixing prior to sample processing.
- Secure the lid on each water sample and shake to help dissolve the sodium thiosulfate tablet or powder.
- Ensure each sample bottle top is tightened to prevent leaking during shipping; electrical or Teflon tape may be used around the bottle cap to secure.
- If you provide your own sample collection bottles, sterile high-density polyethylene bottles are recommended as they have minimal breakage during transport. Polycarbonate bottles and whirl-pack bags are not recommended due to frequently observed transport damage. DO NOT use recycled drinking water or beverage bottles for sampling.

### Swabs for Sample Collection

- Upon request, EST provides free sterile polyurethane tip swabs containing broth for the neutralization of free chlorine and residual sanitizers (e.g., quaternary ammonium chloride).
- Only use provided swabs with neutralizing broth to collect samples for bacteria (including *Legionella*) and fungi culture analysis to ensure organism viability for recovery, do not use dry swabs.
- Most microorganisms will not survive during transport to the laboratory when dry swabs are used for collection.
- Dry swabs without broth may be used for *Legionella* biofilm sampling only if they are used and then are placed inside an approved sample bottle along with water/bulk-liquid from the same sample site. Aseptically collect and break-off swab tip so only untouched parts of swab drop into the collected water sample bottle containing sodium thiosulfate. Note that the CDC recommends to not use cotton-tipped swabs as they inhibit *Legionella* growth (<https://www.cdc.gov/legionella/downloads/cdc-sampling-procedure.pdf>).

			
Swabs with neutralizing broth: Write sample number on tube	Unscrew cap from swab tube. Press each side of the swab tip against the inside of the tube to remove media. Do not touch swab tip or shaft. The tube contains neutralizing broth.	Press down firmly against surface so that the swab tip makes full contact with the sample surface.	Return the swab to the tube and tighten cap.

- To prevent spillage of broth from tube, maintain tube upright during sample collection.
- Do not invert.
- Securely tighten cap after sampling.

### Containers for Bulk-Solid Sample Collection

- Collect sample and place in a sterile container.

## Legionella General Sample Instructions (Culture or PCR)

### Legionella Sample Shipping & Transport:

- Transport samples to the laboratory within 24 hours (recommended) but not exceeding 72 hours; see section above named Shipping Instructions & Transit Delay Information for additional information.
- If samples require collection on a Friday and the transit delay is pre-approved, refrigerate samples over the weekend at 2-8°C (36-47°F) and ship on the following Monday for Tuesday morning delivery.
- Record your acknowledgement of transit delay and approval for processing on the Chain of Custody record in the Notes section at the bottom of the form when submitting samples.
- Never freeze samples prior to shipping or ship samples directly on ice.
- Place EST supplied bottles back inside the provided shipping materials.
- Return using the original shipping box, if possible.
- If shipping yourself, ship using a sturdy box with adequate packing material to minimize transport breakage.
- Place signed EST COC record in a ziplock bag to protect against water damage and place inside the shipment.

### Legionella Safety Notice

- When sampling, avoid breathing in generated aerosols/mists that may be contaminated with the bacteria.
- EST recommends that samplers wear a disposable N-95 mask and disposable gloves compatible with chemicals in treated water.

### Legionella Collection Instructions: "Non-Potable" Sites

#### Legionella Analysis - Non-Potable Water Sample Locations

Examples: cooling towers, evaporative condensers, air handling units (AHU), fan rooms, loop, chilled water loop, technical water tank, well water, decorative fountains, bulk sand filters (no available water). Note that non-potable whirlpool/spa samples are addressed separately on page 4.

Test Code	Test Description	Detection Limit
L001	<i>Legionella</i> Culture <i>L. pneumophila</i> SG1, <i>L. pneumophila</i> SG2-15, and <i>Legionella non-pneumophila</i> - Bulk-liquid (water)	10 CFU/ml
L002	<i>Legionella</i> Culture <i>L. pneumophila</i> SG1, <i>L. pneumophila</i> SG2-15, and <i>Legionella non-pneumophila</i> - Swab Surface Samples	10 CFU/swab
L003	<i>Legionella</i> Culture <i>L. pneumophila</i> SG1, <i>L. pneumophila</i> SG2-15, and <i>Legionella non-pneumophila</i> - Bulk-Solid Samples	CFU/g calculated from diluent volume and weight of analyzed sample
P001	<i>Legionella</i> Viable qPCR (vPCR) – <i>L. pneumophila</i> SG1 and <i>L. pneumophila</i> SG2-15 - Bulk-liquid (water)	10 CFU/ml

### **Non-Potable Water Collection Instructions:**

- See General Sample Collection Supplies, **Bottles for Water/Bulk-Liquid Sample Collection**, page 2.
- Samples should not be collected immediately following the addition of disinfectants/biocides to the cooling tower. It is strongly recommended that samples be collected no earlier than 48 hours after the addition of any disinfectants/biocides to the system.
- Cooling tower samples be collected from the tower basin at the furthest point from the source of the make-up water and biocide feeds or tablets.
- Samples should be free of sediment.
- Sample Decorative Fountains while operational at the furthest point from the source of the make-up water and biocide feeds or tablets.
- Do not fill the sample bottles to maximum capacity, leave approximately one inch of air space in the top of the container to allow for adequate mixing of the sodium thiosulfate to neutralize free chlorine or other halogens.

### **Non-Potable Swab Sampling Instructions:**

- See General Sample Collection Supplies, **Swabs for Sample Collection**, page 2.
- Evaporative Condensers and AHUs may require swab sampling of the condensate pan when no water is present for bulk sampling.

### **Non-Potable Bulk-Solid Sampling Instructions:**

- See General Sample Collection Supplies: **Containers for Bulk-Solid Sample Collection**, page 2.
- Use aseptic technique to collect sample; only a minimum amount of sample (<5 grams) is required.

## *Legionella* Collection Instructions: "Whirlpool Spa" Sites

### **Whirlpool Spa Water Sampling Instructions:**

- See General Sample Collection Supplies, **Bottles for Water/Bulk-Liquid Sample Collection**, page 2.
- The number of bacteria in whirlpool spas (Jacuzzis™), often varies during the day, and day to day. Ideally these samples should be taken when the disinfection conditions are at their weakest (i.e., generally toward the end of the day or after a period of heavy usage).
- For water samples, bottles should be filled by collecting from the surface of the water. It is also a good idea to collect these samples while pool pumps, blowers, etc. are operating.

### **Whirlpool Spa Swab Sampling Instructions**

- See General Sample Collection Supplies: **Swabs for Sample Collection**, page 2.
- Collect from tub surfaces, jets, or filters while the tub is operational.

## Legionella Collection Instructions: "Potable" Sites

### Legionella Analysis - Potable Water Samples

- ANSI/ASHRAE Standard 188-2021 outlines a plan for the risk management of *Legionella* in building water systems. All healthcare facilities and many commercial buildings are required to comply with this standard. Water Management Programs (WMP) require verification and validation that control procedures are working to reduce the risk of *Legionella*.
- The following list of potable water sample types are typical sampling points in WMPs: faucets, showerheads, city water, hot water tanks, drinking fountains, and Reverse Osmosis (RO) water. Please contact EST if you have questions regarding fixtures not detailed in this list.

Test Code	Test Description	Detection Limit
L011	<i>Legionella</i> Culture <i>L. pneumophila</i> SG1, <i>L. pneumophila</i> SG2-15, and <i>Legionella</i> non- <i>pneumophila</i> - Bulk-liquid (water)	0.1 CFU/ml
L002	<i>Legionella</i> Culture <i>L. pneumophila</i> SG1, <i>L. pneumophila</i> SG2-15, and <i>Legionella</i> non- <i>pneumophila</i> - Swab Surface Samples	10 CFU/swab

L003	<i>Legionella</i> Culture <i>L. pneumophila</i> SG1, <i>L. pneumophila</i> SG2-15, and <i>Legionella</i> non- <i>pneumophila</i> - Bulk-Solid Samples	CFU/g calculated from diluent volume and weight of analyzed sample
P011	<i>Legionella</i> Viable qPCR (vPCR) – <i>L. pneumophila</i> SG1 and <i>L. pneumophila</i> SG2-15 - Bulk-liquid (water)	0.1 CFU/ml <i>Note that if other analyses are requested, an additional bottle of water sample is required for processing.</i>

### Potable Water Sample Collection Instructions:

- See General Sample Collection Supplies, **Bottles for Water/Bulk-Liquid Sample Collection**, page 2.
- Do not fill the sample bottles to maximum capacity, leave approximately one inch of air space in the top of the container.
- Refer to ASHRAE 188-2021 to develop a WMP specific to the building or facility and follow any sampling strategy designated by that WMP.
- First draw or Pre-Flush samples can help to capture the biofilms and related microbial activity and are recommended to assess risk of exposure.

### Hot Water Tank Sampling Instructions:

- Post-flush samples are recommended to assess the risk associated with hot water tanks and/or loops.
- Water samples should be collected from the bottom of the tank at the drain valve. Let the water run for approximately 15-30 seconds to purge the drain line of any sediment.

### **City Water Sampling Instructions:**

- This sample is intended to monitor the *Legionella* bacteria entering the facility from the city water supply. As such, this sample should be taken from a point closest to where the water enters the facility. Since this sample is intended to analyze the incoming water, the faucet or drain valve should be opened and flushed for at least 30-60 seconds before collection of the sample.

### **Faucet and Showerhead Water Sampling Instructions:**

- Water samples should be collected at various sites throughout the facility, depending on the type of exposure that may be encountered and the distance from the hot water heaters, in accordance with any WMP in place for that facility. Because *Legionella* bacteria may multiply inside a faucet, the concentrations may be highest immediately after turning on the water.
- First draw or Pre-Flush samples, preferably a faucet that has not been used for several hours which can help to capture the biofilms and related microbial activity, are recommended to assess risk of exposure.

### **Faucet and Showerhead Swab Sampling Instructions:**

- See General Sample Collection Supplies, **Swabs for Sample Collection**, page 2.
- The faucet and/or showerhead may also be extensively swabbed inside to collect any biofilm in fixture. If the faucet has an aerator or the shower head that can be removed, it should be swabbed on the inside, particularly any available rubber gaskets.
- Insert the swab sample into the transport tube or placed in the bulk water sample from that same fixture.

### **Potable Bulk-Solid Sampling Instructions:**

- See General Sample Collection Supplies, **Containers for Bulk-Solid Sample Collection**, page 2.
- Use aseptic technique to collect sample; only a minimum amount of sample (<5 g) is required.

## Legionella Collection Instructions: Air Samples

### Legionella Culture - Air Samples

Test Code	Test Description	Detection Limit
L099	<i>Legionella</i> Air	Calculated as colony forming units (CFU/m <sup>3</sup> ) per cubic meter of air based on sample time (minutes) and sample rate (Liters/minute)

- **Sample Environment:** Samples should be collected from complaint and noncompliant areas. An outdoor and control sample should also be collected for comparison purposes.
- **Sampling Device:** A calibrated sampling pump is recommended to be used to collect these samples. The pump must be adjusted according to the type of air sample being collected as recommended by manufacturer (e.g., QuickTake® 30 with Andersen N-6 sample head or a SAS™ Microbial Air Sampler).
- **Sample Media:** Buffered charcoal yeast extract (BCYE) with Glycine, Polymyxin, Vancomycin, and Cycloheximide (GVPC) selective agar. These specific agars must be utilized; *Legionella* will not grow on standard agars (e.g., TSA, Standard Methods Agar, or 5% Sheep Blood Agar).
- **Sample Transport:** Place parafilm or Teflon tape around each individual microbial plate. If parafilm or Teflon tape is not available, securely tape each lid to its corresponding petri dish and place in a sealed plastic bag. Surround sample with bubble wrap, paper, or packing peanuts for insulation and protection.
- **Sample Shipping:** Ship to the laboratory within 24 hours of collection to prevent desiccation of agar.
- **Sample Blanks:** Submit field blanks from the same media lot with samples where appropriate.

# Bacterial Analysis

## Heterotrophic Aerobic Bacteria Culturable – Air Samples

Test Code	Test Description
B002	Bacterial Air Culture Count Only - Aerobic
B040	Bacterial Air Culture Count & Characterization – Aerobic

- Limit of Sensitivity for Assay: Bacterial concentrations are expressed as CFU/m<sup>3</sup>, calculated from sample duration and flow rate.
- Sample Environment: Samples should be collected from complaint and noncompliant areas. An outdoor and control sample should also be collected for comparison.
- Sampling Device: A calibrated sampling pump is used to collect these samples. The pump must be adjusted according to the type of air sample being collected as recommended by manufacturer (e.g., Quick Take 30 with Andersen N-6 sample head).
- Sample Media: Trypticase Soy Agar (TSA), Standard Methods Agar (SMA) or other commonly accepted bacterial media as chosen by sampler (e.g., 5% Sheep Blood, R2A).
- Sample Transport: Place parafilm or Teflon tape around each individual microbial plate after sampling. If parafilm or Teflon tape is not available, securely tape each lid to its corresponding petri dish and place in a sealed plastic bag. Surround sample with bubble wrap, paper, or packing peanuts for insulation and protection.
- Sample Shipping: Ship samples overnight to be received by laboratory within 24 hours of collection; no ice packs are necessary/do not freeze. Note that agar is prone to transport damage and cross-contamination issues due to condensation during shipping.
- Sample Blanks: Submit field blanks with samples where appropriate.

## Heterotrophic Bacteria Surface Culturable - Swabs

Test Code	Test Description
B004	Bacterial Surface Count Only – Aerobic
B005	Bacterial Surface Count Only – Anaerobic
B039	Bacterial Surface Count & Characterization - Aerobic

- Limit of Sensitivity for Assay: 10 CFU/swab
- Sample Device: See General Sample Collection Supplies, **Swabs for Sample Collection**, page 2.
- Sampling Method: Gently roll swab over the surface with suspected bacterial growth.
- Sample Transport: Samples should be received by laboratory within 24 hours of collection.
- Processing Media: Samples will be plated on Standard Methods Agar (SMA) agar unless otherwise specified by sampler.



### Heterotrophic Bacteria Surface Culturable – Contact Plates

Test Code	Test Description
B027	Bacteria Contact Plate Count Only - Aerobic
B047	Bacteria Contact Plate Count & Characterization - Aerobic

- Limit of Sensitivity for Assay: 1 CFU/plate
- Sampling Device: Contact plate
- Sampling Method: Firmly press agar surface of contact plate onto the surface with suspected growth. Hold the plate with the thumb and second finger and use the index finger to press the plate bottom firmly against the surface; do not touch the agar surface with your fingers. Apply the same pressure to each sample. Do not move the plate laterally across the surface. Slightly curved surfaces may be sampled with a gentle rolling motion.
- Sample Transport: Ship samples overnight to be received by laboratory within 24 hours of collection. Note that agar is prone to transport damage and cross-contamination issues due to condensation during shipping.
- Processing Media: Samples will be directly incubated according to media type.

### Heterotrophic Bacteria Culturable – Bulk-Liquid (Water)

Test Code	Test Description
B010	Bacteria Bulk-Liquid Count Only – Aerobic
B011	Bacteria Bulk-Liquid Count Only – Anaerobic
B045	Bacteria Bulk-Liquid Count and Characterization - Aerobic

- See General Sample Collection Supplies, **Bottles for Water/Bulk-Liquid Sample Collection**, page 2.
- Limit of Sensitivity for Assay: 10 CFU/ml
- Sample Transport: Ship samples overnight to be received by laboratory within 24 hours of collection.
- Processing Media: Samples will be plated on Standard Methods Agar (SMA) agar unless otherwise requested by sampler.

### Heterotrophic Bacteria Culturable – Bulk-Solid

Test Code	Test Description
B007	Bacteria Bulk-Solid Count Only – Aerobic
B008	Bacteria Bulk-Solid Count Only – Anaerobic
B046	Bacteria Bulk-Solid Count and Characterization – Aerobic

- See General Sample Collection Supplies, **Containers for Bulk-Solid Sample Collection**, page 2.
- Limit of Sensitivity for Assay: Bacterial concentrations are expressed as CFU/g for bulk solid samples, calculated from diluent volume and weight of analyzed sample.
- Sample Transport: Ship samples overnight to be received by laboratory within 24 hours of collection.
- Processing Media: Samples will be plated on Standard Methods Agar (SMA) agar unless otherwise requested by sampler.

### Heterotrophic Bacteria Culturable – Identifications

- See Environmental Pathogen Screen section for routine laboratory analyses.
- Contact EST for bacterial identification consultation and pricing for aerobic organisms not listed.

**Test Codes: Wastewater - Sewage Contamination Screen for Environmental Samples: Presence/Absence of Total Coliforms and *E. coli* & Enterococci**

Test Code	Test Description	Required Sample Volume or Number of Swabs Required Per Sampling Location
B022	Total Coliform/ <i>E. coli</i> - Water	> 100 ml
B012	<i>E. coli</i> and <i>Enterococci</i> - Water	> 200 ml
B024	<i>Enterococci</i> - Water	> 100 ml
B023	Total Coliform/ <i>E. coli</i> - Swab	One swab
B013	<i>E. coli</i> and <i>Enterococci</i> - Swab	Two swabs
B025	<i>Enterococci</i> - Swab	One Swab

**Note:** EST provides Coliform and *E. coli* analyses for environmental samples collected for noncompliance purposes such as sewage contamination screening only, not for drinking water certification.

- **Sample Transport:** Ship to the laboratory so as to arrive within 24 hours of collection. The time from sample collection to the initiation of total coliform and *E. coli* analysis of water samples for noncompliance purposes should not exceed 30 hours.
- **Ship samples along with ice packs but do not ship directly on ice to prevent freezing.**
- **Limit of Sensitivity for Assay:** 1 CFU/100 ml of sample or 1 CFU/swab.
- See General Sample Collection Supplies, **Bottles for Water/Bulk-Liquid Sample Collection**, page 2.
- See General Sample Collection Supplies, **Swabs for Sample Collection**, page 2.

**Endotoxin Screen for Environmental Samples:**

Test Code	Test Description	Required Sample Volume Per Sampling Location
E010	Endotoxin Gel-clot Assay Presence/Absence Endotoxin limit: 10 EU/ml	> 100 ml
E020	Endotoxin Gel-clot Assay Presence/Absence Endotoxin limit: 20 EU/ml	> 100 ml

**Note:** EST provides Endotoxin analyses for environmental samples collected for noncompliance purposes, not for dialysis water certification.

- **Sample Transport:** Ship to the laboratory within 24 hours of collection.
- **Ship samples along with ice packs but do not ship directly on ice to prevent freezing.**
- **Limit of Sensitivity for Assay:** Presence  $\geq$  endotoxin limit, Absence  $\leq$  endotoxin limit
- See General Sample Collection Supplies, **Bottles for Water/Bulk-Liquid Sample Collection**, page 2.
- Contact EST prior to testing if other endotoxin detection limits is required.

# Fungal Analysis

## Fungi Culturable – Air Samples

Test Code	Test Description
F001	Fungal Air Culture Count & Identification
F002	Fungal Air Culture Count Only

- Limit of Sensitivity for Assay: Fungal concentrations are expressed as CFU/m<sup>3</sup>, calculated from sample duration and flow rate.
- Sample Environment: Samples should be collected from complaint and noncompliant areas. An outdoor and control sample should also be collected for comparison purposes.
- Sampling Device: A calibrated sampling pump is recommended to collect these samples. The pump must be adjusted according to the type of air sample being collected as recommended by manufacturer (e.g., Quick Take 30 with Andersen N-6 sample head).
- Suggested Sample Air Volume for Airborne Culturable Mold: Sample volumes should be between 50-500 liters depending on the environment and anticipated fungal activity; cleanroom regulations may require as much as 1000 liters of air for those environments.
- Suggested Sampling Time for Airborne Culturable Mold: Two (2) minutes for indoor residential/commercial samples, five to ten (5-10) minutes for hospital and clean settings, and one (1) minute for outdoor samples.
- Sample Media: Malt Extract with 0.01% Chloramphenicol or other commonly accepted fungal media chosen by sampler (e.g., Potato Dextrose, Malt Extract Agar, Dichloran Glycerol 18 Agar, Rose Bengal, etc.).
- Sample Transport: Place parafilm or Teflon tape around each individual microbial plate. If parafilm or Teflon tape is not available, securely tape each lid to its corresponding petri dish and place in a sealed plastic bag. Surround sample with bubble wrap, paper, or packing peanuts for insulation and protection.
- Sample Shipping: Ship to the laboratory within 24 hours of collection.
- Sample Blanks: Submit field blanks from the same media lot with samples where appropriate.

## Fungi Surface Culturable - Swabs

Test Code	Test Description
F003	Fungal Surface Culture Count & Identification
F004	Fungal Surface Count Only

- Limit of Sensitivity for Assay: 10 CFU/swab
- Sampling Device: See General Sample Collection Supplies, **Swabs for Sample Collection**, page 2.
- Sampling Method: Gently roll swab over suspected mold growth.
- Sample Transport: Ship to the laboratory within 24 hours of collection.
- Processing Media: Samples will be plated on Malt Extract Agar with 0.01% Chloramphenicol unless otherwise requested by sampler.

### Fungi Surface Culturable - Contact Plates

Test Code	Test Description
F013	Fungal Contact Plate Count Only
F014	Fungal Contact Plate Count & Identification

- Limit of Sensitivity for Assay: 1 CFU/plate
- Sampling Device: Contact plate
- Sampling Method: Firmly press agar surface of contact plate onto suspected growth/test area. Hold the plate with the thumb and second finger and use the index finger to press the plate bottom firmly against the surface; do not touch the agar surface with your fingers. Apply the same pressure to each sample. Do not move the plate laterally across the surface. Slightly curved surfaces may be sampled with a gentle rolling motion.
- Sampling Method: Firmly press agar surface of contact plate onto suspected mold growth/test area.
- Sample Transport: Ship to the laboratory within 24 hours of collection.
- Processing Media: Samples will be directly incubated according to media type.

### Fungi Culturable Bulk-Liquid (Water) Samples

Test Code	Test Description
F007	Fungal Bulk-Liquid Count & Identification
F008	Fungal Bulk-Liquid Count Only

- Limit of Sensitivity for Assay: Fungal concentrations are expressed as CFU/ml for bulk liquid/water samples.
- Sampling Method: Bulk liquid samples should be collected in a sample container that is a clean unused or sterile high density polyethylene bottle. Do not fill the sample bottles to maximum capacity, leave approximately one inch of air space in the top of the container.
- Sample Transport: Ship to the laboratory within 24 hours of collection.
- Processing Media: Samples will be plated on Malt Extract Agar with 0.01% Chloramphenicol unless otherwise requested by sampler.

### Fungi Culturable Bulk-Solid Samples

Test Code	Test Description
F005	Fungal Bulk-Solid Count & Identification
F006	Fungal Bulk-Solid Count Only

- Limit of Sensitivity for Assay: Fungal concentrations are expressed as CFU/g for bulk solid samples, calculated from diluent volume and weight of analyzed sample.
- Sampling Method: Bulk solid samples should be collected by placing a representative sample into a sealed plastic bag or sterile container.
- Sample Transport: Ship to the laboratory within 24 hours of collection.
- Processing Media: Samples will be plated on Malt Extract Agar with 0.01% Chloramphenicol unless otherwise requested by sampler.

### Fungi Surface Direct Exam (Tape Lift)

Test Code	Test Description
F012	Fungal Direct Exam – Surface Tape Lift: Spore ID, Detection Quantity, Debris Rating

- Limit of Sensitivity for Assay: 1 spore/slide.
- Detection quantity: The detection quantities of fungal spores, mycelial fragments, and pollen are reported as very low, low, moderate, or high.
- Sampling Device: Copan™ Swab 159C – sterile, polyester tip (red top)
- Sampling Method: Gently roll swab over suspected mold growth, secure swab into transport tube.
- Sample Transport: Deliver to laboratory.

## Cleanroom Assessment – Bacteria and/or Fungi

### Cleanroom Assessment USP <797> Pharmaceutical Compounding – Sterile Preparations

Test Code	Test Description	Detection Limit
B061	Personal Aseptic Technique Example sampler: GroMed™ Bag GM7030 (customer supplied) Duration/Incubation: 14-day growth at room temp.	Presence/Absence
B066	<b>Bacteria Culturable Count - Water Sample</b> (e.g., dental lines & pharmacy water) EMD Millipore™ Cat. No. MHPC10025 HPC Red Test Sampler with m-HPC medium (customer supplied) Duration/Incubation: 48 -72 hours at 35°C; 90% humidity	1 CFU/ml
C001	<b>Bacteria &amp; Fungi Air Culturable Count – One Plate Method</b> Sampling Media: Standard Methods Agar or TSA Duration/Incubation Bacteria: 48-72 hours at 30°C Duration/Incubation Fungi: 7 days at 30°C	Calculated CFU/m <sup>3</sup> calculated from sample duration and flow rate
C005	<b>Bacteria &amp; Fungi Air Culturable Count – Two Plate Method</b> Bacteria Count Sampling Media/Duration/Incubation: Standard Methods Agar or TSA for 48-72 hours at 30°C Fungal Count Sampling Media/Duration/Incubation: Malt Extract Agar with 0.01% Chloramphenicol for 7 days at 30°C	Calculated CFU/m <sup>3</sup> calculated from sample duration and flow rate
C002	<b>Bacteria Count &amp; Characterization &amp; Fungi Culturable Surface Count – One Plate</b> Sampling Media: Contact Plate (e.g., Hardy Diagnostics™ P34 TSA with lecithin & Tween® 80, USP, with Lok-Tight™ lid). Duration/ Incubation Bacteria: 48-72 hours at 30°C Duration/ Incubation Fungi: 7 days at 30°C	1 CFU/plate
C006	<b>Bacteria &amp; Fungi Culturable Surface Count – One Plate</b> Sampling Media: Contact Plate (e.g., Hardy Diagnostics™ P34 TSA with lecithin & Tween® 80, USP, with Lok-Tight™ lid). Incubation/Duration: ≥48 hours for bacteria count at 35°C then move to 30° ≥5 days for fungal count	1 CFU/plate
C003	<b>Bacteria Surface Count – Fingertip Dab</b> Sampling Media: Contact Plate (e.g., Hardy Diagnostics™ P34 TSA with lecithin & Tween® 80, USP, with Lok-Tight™ lid). Incubation/Duration: 48-72 hours at 35°C	1 CFU/plate

- **Cleanroom Assessment Sampling:** Samples should be collected according to guidelines and recommendations. For more information, refer to the 2019 USP General Chapter <797> Pharmaceutical Compounding – Sterile Preparations: [https://www.techsafety.com/sites/default/files/blog-related/2019%20Revisions%20USP\\_GC\\_797\\_2SUSP42%20\(1\).pdf](https://www.techsafety.com/sites/default/files/blog-related/2019%20Revisions%20USP_GC_797_2SUSP42%20(1).pdf)
- **Cleanroom Assessment Sample Media:** General microbiological growth medium such as Standard Methods Agar (SMA) that supports the growth of bacteria and malt extract that supports the growth of fungi should be used. If separate fungal media plates are used, incubate at room temperature for no less than 5 days. Media used for surface sampling must contain neutralizing agents such as lecithin and polysorbate 80.
- **Cleanroom Assessment Sample Transport:** Place parafilm or Teflon tape around each individual microbial plate. If parafilm or Teflon tape is not available, securely tape each lid to its corresponding petri dish and place in a sealed plastic bag. Surround sample with bubble wrap, paper, or packing peanuts for insulation and protection.
- **Cleanroom Assessment Sample Shipping:** Ship to the laboratory within 24 hours of collection.
- **Cleanroom Assessment Sample Blanks:** Submit field blanks from the same media lot with samples where appropriate.

## Environmental Pathogen Screens

Test Code	Sample Type	Organism(s)
Waterborne (CMS Pathogen) Screen Combo CMS1	Bacteria & Fungi Bulk-Liquid Culturable	Total Aerobic HPC Bacteria Count Bacteria: <i>Acinetobacter baumannii</i> , <i>Burkholderia cepacia</i> , <i>Pseudomonas aeruginosa</i> , <i>Stenotrophomonas maltophilia</i> , Rapidly growing non-tuberculosis mycobacteria (NTM), Fungi: Yeast & Mold Count and Mold Identification (Genus with common <i>Aspergillus</i> speciation)
B016 B017	Bacteria Bulk-Liquid Culturable Bacteria Surface Culturable	<i>Acinetobacter baumannii</i>
B064 B065	Bacteria Bulk-Liquid Culturable Bacteria Surface Culturable	<i>Burkholderia cepacia</i>
F036 F037 F038	Bacteria Bulk-Liquid Culturable Bacteria Surface Culturable Bacteria Air Culturable	<i>Candida</i> species
B057	Bacteria Surface Culturable	<i>Clostridium difficile</i> (Presence/Absence)
B044 B006	Bacteria Bulk- Liquid Culturable Bacteria Surface Culturable	<i>Elizabethkingia meningoseptica</i> (EKM)
B071 B072	Bacteria Bulk-liquid Culturable Bacteria Surface Culturable	Enterobacteriaceae
B009 B021	Bacteria Bulk-Liquid Culturable Bacteria Surface Culturable	Enterobacteriaceae, Carbapenem Resistant: E. coli and KES group ( <i>Klebsiella aerogenes</i> , <i>Klebsiella oxytoca</i> , <i>Klebsiella pneumoniae</i> , <i>Enterobacter cloacae</i> complex, and <i>Serratia marcescens</i> )
B055	Bacteria Air Culturable	<i>Escherichia coli</i>
B056	Bacteria Surface Culturable	<i>Klebsiella</i>
B041	Bacteria Surface Culturable	<i>Listeria</i> (Presence/Absence)
B018 B019	Bacteria Bulk-Liquid Culturable Bacteria Surface Culturable	<i>Pseudomonas aeruginosa</i>
B063 B062	Bacteria Bulk-Liquid Culturable Bacteria Surface Culturable	<i>Pseudomonas</i> (Genus only)
B067 B068	Bacteria Bulk-Liquid Culturable Bacteria Surface Culturable	<i>Ralstonia pickettii</i>
B043 B054 B028	Bacteria Bulk-Liquid Culturable Bacteria Air Culturable Bacteria Surface Culturable	Rapidly growing non-tuberculosis mycobacteria
B042 B029	Bacteria Bulk-Liquid Culturable Bacteria Surface Culturable	<i>Salmonella</i> (Presence/Absence)
B051 B030	Bacteria Bulk-Liquid Culturable Bacteria Surface Culturable	<i>Serratia marcescens</i>
B014 B015	Bacteria Air Culturable Bacteria Surface Culturable	<i>Staphylococcus aureus</i>
B059 B060	Bacteria Air Culturable Bacteria Surface Culturable	<i>Staphylococcus aureus</i> Methicillin Resistant (MRSA)
B050 B070	Bacteria Bulk-Liquid Culturable Bacteria Surface Culturable	<i>Stenotrophomonas maltophilia</i>
B031 B049	Bacteria Bulk-Liquid Culturable Bacteria Surface Culturable	Vancomycin resistant Enterococci (VRE)



### **Pathogen Screen: Bulk-Liquid (Water) Culturable Samples**

- See General Sample Collection Supplies, **Bottles for Water/Bulk-Liquid Sample Collection**, page 2.
- Limit of Sensitivity for Assay: Bacterial concentrations are expressed as 10 CFU/ml for bulk liquid/water samples.
- Sample Transport: Ship samples overnight to be received by laboratory within 24 hours of collection.
- Processing Media: Samples will be plated on media selective for the chosen pathogen.

### **Pathogen Screen: Surface Contact (Swab) Culturable Samples**

- See General Sample Collection Supplies, **Swabs for Sample Collection**, page 2.
- Limit of Sensitivity for Assay: 10 CFU/swab
- Sample Transport: Samples should be received by laboratory within 24 hours of collection. In warm weather, ship samples with wrapped ice packs; ice packs should not be placed directly on sample.
- Processing Media: Samples will be plated on media selective for the chosen pathogen.

### **Pathogen Screen: Air Culturable Samples**

- Limit of Sensitivity for Assay: Bacterial concentrations are expressed as CFU/m<sup>3</sup>, calculated from sample duration and flow rate.
- Sampling Device: A calibrated sampling pump is used to collect these samples. The pump must be adjusted according to the type of air sample being collected as recommended by manufacturer (e.g., Quick Take 30 with Andersen N-6 sample head).
- Sample Media: Trypticase Soy Agar, Standard Methods Agar (SMA) or other commonly accepted bacterial media as chosen by sampler (e.g., 5% Sheep Blood, R2A).
- Sample Transport: Place parafilm or Teflon tape around each individual microbial plate. If parafilm or Teflon tape is not available, securely tape each lid to its corresponding petri dish and place in a sealed plastic bag. Surround sample with bubble wrap, paper, or packing peanuts for insulation and protection.
- Sample Shipping: Ship samples overnight to be received by laboratory within 24 hours of collection; no ice packs are necessary/do not freeze.
- Sample Blanks: Submit field blanks with samples where appropriate

## Microbial Corrosion Screens

Test Description – Microbial Population	Test Code Bulk-liquid/water	Test Code Bulk-solid	Test Code Swab
Acid Producing Bacteria	M001	M011	M021
Denitrifying Bacteria	M002	M012	M022
Iron Related Bacteria	M003	M013	M023
Slime-Forming Bacteria	M004	M014	M024
Sulfate Reducing Bacteria	M005	M015	M055
Fluorescent Pseudomonads	M006	M016	M026
Nitrifying Bacteria	M007	M017	M027
Algae	M008	M018	M028

- Limit of Sensitivity for Assay:  
 Acid Producing Bacteria – 75 CFU/ml  
 Denitrifying Bacteria – 1 CFU/ml  
 Iron Related Bacteria – 25 CFU/ml  
 Slime-Forming Bacteria – 500 CFU/ml  
 Sulfate Reducing Bacteria – 75 CFU/ml  
 Fluorescent Pseudomonads – 35 CFU/ml  
 Nitrifying Bacteria – 1 CFU/ml  
 Algae - <100 CFU/ml
- Sample Devices – Use aseptic technique to collect samples:  
 Bulk-liquid (water) – See *Legionella* Sampling Supplies: Bottles for Bulk-Liquid (Water) Samples  
 Surface Swab – See *Legionella* Sampling Supplies: Swabs for Surface Sample  
 Bulk-solid –See *Legionella* Sampling Supplies: Container Requirements for *Legionella* Bulk-Solid Samples;  
 only a minimum amount of sample (<5 g) is required.
- Sample Transport: Deliver to laboratory; follow same guidelines as for *Legionella* samples.
- Processing Media: See BART™ Tester listed for each microbial population listed in the above table.