

SAMPLE COLLECTION GUIDELINES

Note: Sample integrity must be maintained during the entire sampling process, from the initial sample collection to the completed final report. Chain of Custody forms are provided by Environmental Safety Technologies, Inc. (EST) for all clients to use in order to document sample collection, identification, location, conditions, and sample traceability.

Legionella Analysis

When sampling, avoid generating aerosols that may be contaminated with the bacteria. Observe safety precautions when sampling. Wear a disposable N-95 mask and disposable gloves compatible with chemicals that may be present in treated water.

Sample Site <i>All samples on individual reports with Ph.D. Interpretations and Recommendations</i>	Test Code	Sample Type	Limit of Detection
COOLING TOWER	L001	Bulk Liquid / Water	Limit of Detection = 10 CFU/ml
	L003	Swab	Limit of Detection = 20 CFU/swab
AHU, COILS, CONDENSATE ♦ with presence/absence of mold growth	L004	Bulk Liquid / Water	Limit of Detection = 10 CFU/ml
	L006	Swab	Limit of Detection = 20 CFU/swab
CLOSED LOOP SYSTEM ♦ with presence/absence of mold growth	L007	Bulk Liquid / Water	Limit of Detection = 10 CFU/ml
	L009	Swab	Limit of Detection = 20 CFU/swab
POTABLE WATER ♦ shower, faucet, hot water heater, drinking fountain, etc.	L010	Bulk Liquid / Water	Limit of Detection = < 1 CFU/ml
	L011	Swab	Limit of Detection = 20 CFU/swab
	L012	Bulk Liquid / Water	Limit of Detection = < 1 CFU/ml
WHIRLPOOL, Jacuzzi, Spa, Hot tub, Swimming Pool ♦ with <i>Pseudomonas aeruginosa</i> screen	L013	Swab	Limit of Detection = 20 CFU/swab
	L014	Bulk Solid / Sand from filter	Limit of Detection = calculated CFU/g
	L015	Bulk Liquid / Water	Limit of Detection = 10 CFU/ml
DECORATIVE FOUNTAIN ♦ with <i>Pseudomonas aeruginosa</i> screen	L016	Swab	Limit of Detection = 20 CFU/swab

LEGIONELLA DFA ONLY - <i>L.p.</i> SG 1-14 + species ("a-w")	L025	Bulk Liquid / Water	Limit of Detection = 35 CFU/ml
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INDUSTRIAL FACILITY REPORT <i>All samples on one custom report with Ph.D. Interpretations and Recommendations</i>	Test Code	Sample Type	Limit of Detection
Cooling Tower	L002	Bulk Liquid / Water	Limit of Detection = < 1 CFU/ml
AHU, Coils, Condensate	L005	Bulk Liquid / Water	Limit of Detection = < 1 CFU/ml
Closed Loop System	L008	Bulk Liquid / Water	Limit of Detection = < 1 CFU/ml
Eyewash ♦ with <i>Pseudomonas aeruginosa</i> screen	L017	Bulk Liquid / Water	Limit of Detection = < 1 CFU/ml
Potable Water	L018	Bulk Liquid / Water	Limit of Detection = < 1 CFU/ml
Metal Working/Cutting Fluid	L019	Bulk Liquid / Water	Limit of Detection = < 1 CFU/ml
Swab – any source	L027	Swab	Limit of Detection = 20 CFU/swab

Note: Extra processing fees apply – please call for details; ALL samples must be 500 ml volume.

- **Sampling Device for Water Sample:** Use a clean, dry sample bottle (do NOT use a recycled/used beverage bottle). EST recommends using a high density polyethylene bottle containing one 30 mg sodium thiosulfate tablet.

(*Legionella* analysis from samples taken from chlorinated samples (potable water, swimming pools, jacuzzi, etc.) may result in a false negative test due to the residual halogen biocides. Therefore, it is recommended by the CDC (US) that water samples contain sodium thiosulfate at a concentration of 0.5 ml of 0.1 N solution per liter of sample water to neutralize the disinfectant. This concentration may be achieved by collecting 500 ml of samples in a bottle containing one 30 mg sodium thiosulfate tablet, a neutralizing agent, to inactivate the chlorine.)

- **Sampling Device for Swab Sample:** EST recommends using a Copan™ Swab 114C – swab, Aimes with charcoal, rayon tip (black top). * **Note: DFA only analysis (L002) is not available for swab samples.**
- **Sample Transport:** Samples should be shipped on the day of collection Monday through Thursday for overnight delivery to the laboratory before noon the following day. Call to arrange for rush weekend processing/analysis; extra charges will apply.

(Guidelines established by the U. S. Department of Health and Human Services Centers for Disease Control and Prevention (CDC) in the document, “Procedures for the Recovery of *Legionella* from the Environment.” The directions for specimen collection states: “Samples that will not reach the laboratory within 72 hours should be refrigerated before shipping. Samples should be shipped so that they are protected from extreme heat or cold.” The validity of microbial counts beyond recommended shipping times becomes questionable since the environmental conditions during shipping could either artificially increase or decrease total viable bacteria and viable *Legionella* counts in a sample. Therefore, in order to obtain the most accurate analysis possible, samples should be shipped within 72 hours, preferably overnight. Results obtained from samples arriving later will be processed with customer approval, but may not accurately reflect the bacteria in the site at the time of sampling. Samples delayed beyond 72 hours will have a delayed transit comment on the sample report).

- **Sampling Method for Cooling Towers, Evaporative Condensers, and Air Handling Units, and Decorative Fountains**

Water Sample – **A minimum of 100 milliliters of water is required.** The Limit of Sensitivity for this assay is 10 colony forming units per milliliter of sample (CFU/ml). Depending on the accessibility of the tower, collect samples from the tower basin at the furthest most point from the source of the make-up water. Take caution to prevent losing the tablet from the bottle during sampling. Shake each sample for thirty seconds to help dissolve the tablet. Securely tighten each sample bottle top to prevent leaking during shipping (electrical or Teflon tape around the bottle top works well).

- If possible, samples should be free of sediment. Please do not fill the containers to maximum capacity, leave approximately one inch of air space in the neck of the container. If there is not enough water available at the sample location, a swab sample may be collected from any biofilm and/or moisture present on a drain, condensate pan, or coils.
- **IMPORTANT:** 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 sooner than 48 hours after the addition of any disinfectants/biocides to the system.
- Sample Decorative Fountains while operational.

Swab Sample - If there is not enough water available at a sample location, a swab sample may be collected from any biofilm and/or moisture present on the drain, condensate pans, or coils.

- **Sampling Method for Potable Water Systems (Hot Water Tanks, Faucets and Showerheads, City Water, and Whirlpool Spas, etc.)**

Water Samples - **A minimum of 500 milliliters of water is required.** The Limit of Sensitivity for this assay is less than one CFU/ml of sample.

- **Hot Water Tanks** – Water samples should be collected from the bottom of the tank immediately after opening the drain valve (if necessary, let water run for approximately 10 seconds to purge the drain line). Please do not fill the containers to maximum capacity, leave approximately one inch of air space in the neck of the container. The water temperature of this sample should be recorded on the Chain of Custody (it is unlikely to be the same as at the top of the tank). Take caution to prevent losing the tablet from the bottle during sampling. Shake each sample for thirty seconds to help dissolve the tablet. Securely tighten each sample bottle top to prevent leaking during shipping (electrical or Teflon tape around the bottle top works well).
- **Faucets and Showerheads** - 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. Because *Legionella* bacteria may multiply inside a faucet, the concentrations will be highest immediately after turning on the water. Thus, the best sample will be the first liter of water out of the faucet, preferably a faucet that has not been used for several hours. Please do not fill the containers to maximum capacity, leave approximately one inch of air space in the neck of the container. After obtaining the sample, allow the hot water to run until it has reached its maximum temperature (approximately one to two minutes). Record this temperature on the Chain of Custody. Take caution to prevent losing the tablet from the bottle during sampling. Shake each sample for thirty seconds to help dissolve the tablet. Securely tighten each sample bottle top to prevent leaking during shipping (electrical or Teflon tape around the bottle top works well).

Swab samples - Samples should be collected with an Aimes swabs with charcoal prior to collection of bulk water samples collected at the same site. The faucet and/or showerhead should be extensively swabbed inside as far as possible. If the faucet has an aerator, it should be removed and swabbed on the inside, particularly the rubber gasket. Insert the swab sample into the transport tube and securely push together to close.

- **Sampling Method for City Water** - 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 as close as possible 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 30 seconds before collection of sample. Please do not fill the containers to maximum capacity, leave approximately one inch of air space in the neck of the container. Take caution to prevent losing the tablet from the bottle during sampling. Shake each sample for thirty seconds to help dissolve the tablet. Securely tighten each sample bottle top to prevent leaking during shipping (electrical or Teflon tape around the bottle top works well).
- **Sampling Method for Whirlpool, Jacuzzi, Swimming Pool** - The number of bacteria in pools, particularly whirlpool spas, often varies greatly during the day and from day to day. Thus, any one sample is only a spot check of the water at these sites. 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), although other times are also acceptable. The sample 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, although it is not absolutely necessary. Take caution to prevent losing the tablet from the bottle during sampling. Shake each sample for thirty seconds to help dissolve the tablet. Securely tighten each sample bottle top to prevent leaking during shipping (electrical or Teflon tape around the bottle top works well).

Bacteria Analysis

Airborne Culturable Bacteria – Aerobic, Heterotrophic

- **Test Codes:** **B002 Bacterial Air Culture Count Only - Aerobic**
B001 Bacterial Air Culture Count & Identification - Aerobic
- **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).
- **Suggested Sample Time for Airborne Culturable Bacteria:** 1-3 minutes (*Journal of Applied Microbiology*, Volume 102 Issue 6 Page 1479-1484, June 2007).
- **Sample Media:** Trypticase Soy Agar (TSA) or other commonly accepted bacterial media as chosen by sampler (e.g., 5% Sheep Blood, R2A, 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 samples overnight to be received by lab within 24 hours.
- **Limit of Sensitivity for Assay:** Bacterial concentrations are expressed as CFU/m³, calculated from sample duration and flow rate.

Surface Culturable Bacteria - Swab – Heterotrophic

- **Test Codes:** **B004 Bacterial Surface Count Only – Aerobic**
B005 Bacterial Surface Count Only – Anaerobic (extra fee)
B003 Bacterial Bulk Count and Identification - Aerobic
- **Sampling Device:** Copan™ Swab 4108 – Aimes (blue top)
- **Sampling Method:** Gently roll swab over suspected bacterial growth.
- **Sample Transport:** Samples should be received by lab within 24 hours, otherwise store sealed samples refrigerated. In warm weather, ship samples with wrapped ice packs. Ice packs should not be placed directly on sample.
- **Limit of Sensitivity for Assay:** 10 CFU/swab
- **Processing Media:** Samples will be plated on TSA agar unless otherwise stated by sampler.

Surface Culturable Bacteria - Contact Plate

- **Test Code:** **B027 Bacteria Contact Plate Count Only - Aerobic**
B028 Bacteria Contact Plate Count & Identification - Aerobic
- **Sampling Device:** Contact plate
- **Sampling Method:** Firmly press agar surface of contact plate onto suspected mold 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. Slightly curved surfaces may be sampled with a rolling motion.
- **Sample Transport:** Transport to laboratory for processing.
- **Limit of Sensitivity for Assay:** 1 CFU/plate
- **Processing Media:** Samples will be directly incubated according to media type.

Bulk Culturable Bacteria* – Heterotrophic

- **Test Codes:** **B007 Bacteria Bulk-Solid Count Only – Aerobic**
B008 Bacteria Bulk-Solid Count Only – Anaerobic (extra fee)
B006 Bacteria Bulk-Solid Count & Identification – Aerobic
B010 Bacteria Bulk-Liquid Count Only - Aerobic
B011 Bacteria Bulk-Liquid Count Only – Anaerobic (extra fee)
B009 Bacteria Bulk-Liquid Count & Identification - Aerobic
- **Sampling Method:** Bulk liquid samples should be collected in clean, dry sample bottles (not recycled/used beverage bottles). Securely tighten each sample bottle top to prevent leaking during shipping (electrical or Teflon tape around the bottle top works well). Bulk solid samples should be collected by placing a representative sample into a sealed plastic bag.
- **Sample Transport:** Samples should be received by lab within 24 hours.

- **Limit of Sensitivity for Assay:** Bacterial concentrations are expressed as CFU/ml for bulk liquid/water samples or CFU/g for bulk solid samples, from sample weight and diluent volume.
- **Processing Media:** Samples will be plated on TSA agar unless otherwise requested by sampler.

*** For Collection of the Bacterium, Thermophilic Actinomycetes (bulk liquid/water, bulk solid, or swab**

- **Test Code:** BTAM
- **Sampling Device and Sampling Method:** Follow the instructions for routine bulk bacteria sample collection.
- **Sample Transport:** Samples MUST be shipped with wrapped ice packs. Ice packs should not be placed directly on sample.
- **Sample Shipping:** Samples MUST be shipped overnight to be received by the lab within 24 hours.
- **Processing Media:** Samples will be plated on TSA agar unless otherwise requested by sampler.

Bulk Water – Sewage Contamination: Presence/Absence of Total Coliforms and E. coli (Colisure®) and Enterococci (Enterolert™) in Wastewater (EST is not certified to perform drinking water analysis)

- **Test Codes:** B012 E. coli and Enterococci Presence/Absence Water
B022 Total Coliform/E. coli Presence/Absence Water
B024 Enterococci Presence/Absence Water
- **Sampling Device:** Collect water samples in a high clarity 100 ml polystyrene vessel containing one 30 g sodium thiosulfate tablet, which must be included in order to prevent interference by residual chlorine in water sample. Collect swab sample with a Copan™ Swab 4108 – Aimes (blue top).
- **Sampling Method:** Collect waste water or swab sample from water loss site.
- **Sample Transport:** Samples MUST be shipped overnight to be received by the lab within 24 hours.
- **Limit of Sensitivity for Assay:** 1 CFU/100 ml of sample

Fungal Analysis

Airborne Culturable Fungi

- **Test Codes:** F001 Fungal Air Culture Count & Identification
F002 Fungal Air Culture Count Only
F016 Fungal Settle Plate Count & Identification
- **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 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).
- **Suggested Sample Time for Airborne Culturable Mold:** 2 minutes for indoor residential samples, 5 minutes for hospital and clean settings, 1 minute for outdoor samples.
- **Sample Media:** Malt Extract with 0.1% Chloramphenicol or other commonly accepted fungal media chosen by sampler (e.g., Potato Dextrose, Malt Extract Agar, Dichloran Glycerol 18 Agar, 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 samples overnight to be received by lab within 24 hours.
- **Limit of Sensitivity for Assay:** Fungal concentrations are expressed as CFU/m³, calculated from sample duration and flow rate.

Surface Culturable Fungi - Swab

- **Test Code:** F003 Fungal Surface Culture Count & Identification
F004 Fungal Surface Count Only
- **Sampling Device:** Copan™ Swab 159C – sterile, polyester tip (red top)
- **Sampling Method:** Gently roll swab over suspected mold growth.
- **Sample Transport:** Transport to laboratory for processing.
- **Limit of Sensitivity for Assay:** 10 CFU/swab
- **Processing Media:** Samples will be plated on Malt Extract Agar with 0.1% Chloramphenicol unless otherwise requested by sampler.

Surface Culturable Fungi - Contact Plate

- **Test Code:** F013 Fungal Contact Plate Count Only
F014 Fungal Contact Plate Count & Identification
- **Sampling Device:** Contact plate
- **Sampling Method:** Firmly press agar surface of contact plate onto suspected mold 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. Slightly curved surfaces may be sampled with a rolling motion.
- **Sample Transport:** Transport to laboratory for processing.
- **Limit of Sensitivity for Assay:** 1 CFU/plate
- **Processing Media:** Samples will be directly incubated according to media type.

Bulk Culturable Fungi

- **Test Code:** F005 Fungal Bulk-Solid Count & Identification
F006 Fungal Bulk-Solid Count Only
F007 Fungal Bulk-Liquid Count & Identification
F008 Fungal Bulk-Liquid Count Only
- **Sampling Method:** Bulk liquid samples should be collected in clean, dry sample bottles (not recycled/used beverage bottles). Bulk solid samples should be collected by placing a representative sample into a sealed plastic bag.
- **Sample Transport:** Deliver to laboratory
- **Limit of Sensitivity for Assay:** Fungal concentrations are expressed as CFU/ml for bulk liquid/water samples or CFU/g for bulk solid samples, from sample weight and diluent volume.
- **Processing Media:** Samples will be plated on Malt Extract Agar with 0.1% Chloramphenicol unless otherwise requested by sampler.

Fungal Air – Direct Exam (Air-O-Cell, Spore Trap)

- **Test Code:** F009 Fungal Direct Exam – Air – Spore Trap: Spore Count, ID, Debris Rating
- **Sample Environment:** Samples should be collected from complaint and noncompliant areas. An outdoor sample should be collected and submitted to the laboratory along with a control sample for comparison purposes.
- **Sampling Device:** Spore Trap (e.g., Air-O-Cell Cassette, Micro 5, etc.)
- **Sampling Method:** 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., SKC Quick Take 15).
- **Suggested Sample Time for Airborne Direct Exam:** 5 minutes for indoor residential samples, 10 minutes for hospital and clean settings, 5 minute for outdoor samples.
- **Sample Transport:** Deliver to laboratory.
- **Limit of Detection for Assay:** Expressed as spores/m³, calculated from sample duration and flow rate.

Surface – Direct Exam (Tape Lift)

- **Test Code:** F012 Fungal Direct Exam – Surface Tape Lift: Spore ID, Detection Quantity, Debris Rating
- **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
- **Limit of Sensitivity for Assay:** N/A – The detection quantities of fungal spores, mycelial fragments, and pollen are reported as very low, low, moderate, or high.

Cleanroom Assessment

Cleanroom Assessment USP <797> Pharmaceutical Compounding – Sterile Preparations

- **Test Codes:** F029 Fungal Air Culture – Count Only
F029 Fungal Air Culture Identification Follow-up for Assessment
F032 Fungal Fingertip Surface Count
F033 Fungal Contact Surface Count
B029 Bacterial Air Culture – Count Only
B030 – Bacterial Air Culture Identification Follow-up for Assessment
B032 Bacterial Fingertip Surface Count
B033 Bacterial Contact Surface Count
- **Sampling:** Samples should be collected according to guidelines and recommendations of USP <797> Pharmaceutical Compounding – Sterile Preparations
- **Sample Media:** General microbiological growth medium such as TSA that supports the growth of bacteria and malt extract that supports the growth of fungi should be used. Media used for surface sampling must contain neutralizing agents such as lecithin and polysorbate 80.
- **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 lab within 24 hours.
- **Limit of Sensitivity for Assay:** Fungal concentrations are expressed as CFU/m³, calculated from sample duration and flow rate for air samples and 1 CFU/plate for contact plate samples.