

**ENVIRONMENTAL LABORATORY APPROVAL PROGRAM
CERTIFICATION MANUAL**

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GENERAL

The US Environmental Protection Agency (US EPA) and the Federal Food and Drug Administration (FDA) are the federal regulatory entities for the protection of the environment and food including bottled water, respectively. The EPA's regulations fall under the Clean Water Act as detailed in Title 40, CFR 136 and the Safe Drinking Water Act as detailed in CFR 141. The FDA's bottled water regulations are detailed in Title 21, CFR 129 and CFR 165. All these federal regulations are available at the following link: <http://ecfr.gpoaccess.gov/>.

Sample preservation requirements related to microbiological testing vary among and between 40 CFR 136, 40 CFR 141, and 21 CFR 165 as well as, other federally-related environmental rules such as the following:

Clean Water Act

- National Pollutant Discharge Elimination System Rule (NPDES) - 40 CFR 136, Tables IA, IH, and II

Safe Drinking Water Act

- Total Coliform Rule (TCR) - 40 CFR 141.21(f)(3);
- Surface Water Treatment Rule (SWTR) - 40 CFR 141.74 (a)(1);
- Long Term 2 Enhanced SWTR (LT2) - 40 CFR 141.704(b);
- Ground Water Rule (GWR) - 40 CFR 141.402(c)(2)

Bottled Water Rule (BWR)

- Processing and Bottling of Drinking Water – 21 CFR 129.35
- Beverages - 21 CFR 165.110

With respect to microbiological testing as it relates to the quality of water used to prepare dialysate for chronic renal dialysis services, the applicable federal and state regulations and laws are as follows:

- NYS Public Health Law, Section 2803
- NYS Subpart 757.1 and 757.2 – Chronic Renal Dialysis Services and General Requirements
- Conditions for Coverage for End-Stage Renal Disease Facilities; Condition: Water and Dialysate Quality – 42 CFR 494.40, which incorporates by reference the Association for the Advancement of Medical Instrumentation (AAMI) publication, "Dialysate for hemodialysis".

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Monitoring and testing requirements for heterotrophic bacteria and Legionella in cooling towers, general hospitals and residential health care facilities is addressed in the proposed permanent regulation, Part 4 of Title 10 NYCRR, Protection Against Legionella.

A. Table – Summary of Sample Collection & Preservation Requirements

The table summarizes the sample collection and preservation requirements as noted in New York State and federal regulations and rules. Either a plastic or a glass (non-reactive borosilicate) container can be used for any samples.

Sample Type		Analyte	Max. Holding Time	Rule Reference	Temp. < 10 °C	0.008% Na₂S₂O₃
Dialysis Product Water		Standard (Heterotrophic) Plate Count	24 hours ⁶	42 CFR 494.40, AAMI	recommended/ encouraged ⁶	not required
Drinking/Potable Water		Legionella	24 hours ⁷	ISO 19458:2006(E)	5 +/- 3 °C ⁷	ISO 19458:2006(E) ₈
Cooling Tower Water		Standard (Heterotrophic) Plate Count	8 hours	n/a	recommended/ encouraged	not required
		Legionella	24 hours ⁷	ISO 19458:2006(E)	5 +/- 3 °C ⁷	ISO 19458:2006(E) ₈
Processed Drinking ¹ /Bottled Water		Coliform (Total) and <i>E. coli</i> (P/A)	30 hours	TCR, BWR	recommended/ encouraged	required if samples are chlorinated ⁵
		Standard (Heterotrophic) Plate Count	8 hours	SWTR, BWR		
Source for Drinking/ Bottled Water	Surface ² Water	Coliform (Total & Fecal) and <i>E. coli</i> (P/A)	8 hours	SWTR, BWR	required	not required
		<i>E. coli</i> (enumeration) ³	30 hours	LT2		
		Standard (Heterotrophic) Plate Count	8 hours	SWTR, BWR		
	Ground Water	<i>E. coli</i> (P/A)	30 hours	BWR, GWR	recommended/ encouraged	not required
		Enterococci	30 hours	GWR		
		Coliphage	30 hours	GWR		
		Standard (Heterotrophic) Plate Count	8 hours	BWR		
Waste and Ambient Water		Coliform (Total and Fecal), <i>E. coli</i> , and Enterococci	8 hours ⁴	NPDES	required	required if samples are chlorinated ⁵
		Standard (Heterotrophic) Plate Count	8 hours	NPDES		

¹ Includes drinking water collected from a system with a treatment waiver. There are no preservation and hold time requirements for sealed containers of bottled water (refer to Certification Manual Item 272). However, once the seal is broken, the water sample must be tested within the hold time noted above.

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- ² Surface water includes groundwater under the direct influence (GWUDI) of surface water.
- ³ The time from sample collection to initiation of analysis may not exceed 30 hours unless the State determines, on a case-by-case basis, that analyzing an *E. coli* sample within 30 hours is not feasible. *E. coli* samples held between 30 and 48 hours must be analyzed by the Colliert version of SM 9223 B.
- ⁴ Samples analysis should begin immediately, preferably within 2 hours of collection. The maximum transport time to the laboratory is 6 hours, and samples should be processed within 2 hours of receipt at the laboratory. For fecal coliform samples for sewage sludge (biosolids) only, the holding time is extended to 24 hours for the following sample types using either EPA Method 1680 (LTB-EC) or 1681 (A-1): Class A composted, Class B aerobically digested, and Class B anaerobically digested.
- ⁵ ASTM D7365-09a specifies treatment options for samples containing oxidants (e.g., chlorine). Also, Section 9060A of Standard Methods for the Examination of Water and Wastewater (20th and 21st editions) addresses dechlorination procedures.
- Add a reducing agent only if an oxidant (e.g., chlorine) is present. Reducing agents shown to be effective are sodium thiosulfate (Na₂S₂O₃), ascorbic acid, sodium arsenite (NaAsO₂), or sodium borohydride (NaBH₄). However, some of these agents have been shown to produce a positive or negative cyanide bias, depending on other substances in the sample and the analytical method used. Therefore, do not add an excess of reducing agent. Methods recommending ascorbic acid (e.g., EPA Method 335.4) specify adding ascorbic acid crystals, 0.1–0.6 g, until a drop of sample produces no color on potassium iodide (KI) starch paper, then adding 0.06 g (60 mg) for each liter of sample volume. If NaBH₄ or NaAsO₂ is used, 25 mg/L NaBH₄ or 100 mg/L NaAsO₂ will reduce more than 50 mg/L of chlorine (see method “Kelada-01” and/or Standard Method 4500-CN—for more information). After adding reducing agent, test the sample using KI paper, a test strip (e.g. for chlorine, SenSafeTMTotal Chlorine Water Check 480010) moistened with acetate buffer solution (see Standard Method 4500-Cl.C.3e), or a chlorine/oxidant test method (e.g., EPA Method 330.4 or 330.5), to make sure all oxidant is removed. If oxidant remains, add more reducing agent. Whatever agent is used, it should be tested to assure that cyanide results are not affected adversely.
- ⁶ Samples that cannot be assayed within 2 hours may be refrigerated, but shall be plated within 24 hours of collection.
- ⁷ Per Annex B of ISO 19458:2006(E), the maximum storage time including transport is 24 hours (recommended) and 48 hours (acceptable) for *Legionella spp.* If the acceptable time is exceeded, the lab is to qualify results for not meeting the holding time on client reports. The recommended storage water temperature is 5 +/- 3 °C; the acceptable storage temperature is ambient.
- ⁸ Please refer to ISO 19458:2006(E), Section 4.2.3 Inactivation of disinfectants. Typically, the reducing agent, sodium thiosulfate, is added to stop the action of the oxidant (i.e. chlorine). For other disinfectants, corresponding inactivation measures need to be taken. The lab’s client needs to disclose what biocide they are using as part of the lab’s sample acceptance, and the lab needs to check for and document the presence or absence of the biocide at the time of sample receipt. If inactivation is not possible or feasible, it has to be reported.

B. Reference

40 CFR 136, Table II: “Cool, < 10 C”. Footnote 2 states: “Except where noted in this Table II and the method for the parameter, preserve each grab sample within 15 minutes of collection. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR part 403, Appendix E), refrigerate the sample at ≤ 6 C during collection unless specified otherwise in this Table II or in the method(s). For a composite sample to be split into separate aliquots for preservation and/or analysis, maintain the sample at ≤ 6 C, unless specified otherwise in this Table II or in the method(s), until collection, splitting, and preservation is completed...”

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C. Holding Time

The maximum **holding time** includes the time elapsed from collection of the sample to placement into the incubator, or stated another way: it is from the time of collection to the time of incubation. Refer to table in Section A for specific holding times.

When holding time requirements are not met, a statement of compliance / non-compliance with these requirements must be included on the report to the client. As an example, a laboratory may qualify the result with an "H"-flag.

D. Sampling

For drinking waters, at least a 100-mL sample must be collected.

Samples collected for certain analytes, such as *Cryptosporidium*, require a larger sample size. (**Note:** ELAP does not certify laboratories for *Cryptosporidium*. The US EPA through its Laboratory Quality Assurance (QA) Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act grants "Approved" status. The purpose of the Lab QA Program is to identify laboratories that can reliably measure the occurrence of *Cryptosporidium* in surface water using EPA Method 1622 and/or EPA Method 1623. Approved *Cryptosporidium* laboratories can be found at this link: http://water.epa.gov/lawsregs/rulesregs/sdwa/lt2/lab_home.cfm#listapprovedlabs.)

For bacteriological tests, when the sample is collected, leave ample air space in the bottle (at least 2.5 cm or 1 in) to facilitate mixing by shaking.

For sewage sludge samples, collect the samples in sterile, non-toxic glass or plastic containers with leak-proof lids. The most appropriate location for biosolid sample collection is the point prior to leaving the wastewater treatment plant. Samples may be taken from pipes, conveyor belts, bins, compost heaps, drying beds, and stockpiles.

E. Legionella Quality System and Control Requirements

The approved method for the culture analysis of Legionella is **ISO 11731:2017(E)**. The lab must follow the quality system and control requirements for microbiological testing as required by ELAP and set forth in the NELAC Institute (TNI) Standards.

F. Total Microcystins

The approved method for analysis of Total Microcystins in both ambient and drinking waters is EPA Method 546. Samples are collected into amber glass bottles fitted with PTFE-lined screw caps. Residual chlorine in drinking water samples must be neutralized at the time of collection. Samples are neutralized with a solid form of sodium thiosulfate (10mg/100ml). Also, samples must be confirmed to be at, or below 10 C when they are received by the laboratory.