SOURCE WATER MONITORING GUIDANCE MANUAL FOR PUBLIC WATER SYSTEMS

FOR THE FINAL LONG TERM 2 ENHANCED SURFACE WATER TREATMENT RULE

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ACRONYMS

CFU	Colony-forming unit
CNFG	Confluent growth
DAPI	4, 6-diamidino-2-phenylindole
DCTS	LT2/Stage 2 Data Collection and Tracking System
DIC	Differential interference contrast
EPA	United States Environmental Protection Agency
FA	Fluorescence assay
FITC	Fluorescein isothiocyanate
GWUDI	Ground water under the direct influence [of surface water]
ICR	Information Collection Rule
IDC	Initial demonstration of capability
IESWTR	Interim Enhanced Surface Water Treatment Rule
IFA	Immunofluorescence assay
IMS	Immunomagnetic separation
IPMC	Information Processing and Management Center
IPR	Initial precision and recovery
IPT	Initial proficiency testing
L	Liter
LT2 Rule	Long Term 2 Enhanced Surface Water Treatment Rule
LT2ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule
mL	Milliliter
MPC	Magnetic particle concentrator
MPN	Most probable number
MS	Matrix spike
MS/MSD	Matrix spike/matrix spike duplicate
μm	Micrometer
NA-MUG	Nutrient agar (NA) with 4-methylumbelliferyl-beta-D-glucuronide (MUG)
NELAC	National Environmental Laboratory Accreditation Conference
nm	Nanometer
NPDWR	National Primary Drinking Water Regulations
NTU	Nephelometric turbidity unit
OPR	Ongoing precision and recovery
OPT	Ongoing proficiency testing
PBMS	Performance-based measurement system
PT	Proficiency testing
PWS	Public water system
QA	Quality assurance
QAP	Quality assurance plan
QC	Quality control
RSD	Relative standard deviation
SDWA	Safe Drinking Water Act
SOP	Standard operating procedure
TNTC	Too numerous to count
UV	Ultraviolet

SECTION 1: GENERAL REQUIREMENTS

1.1 Introduction

The Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule) (Reference 8.1) requires public water systems (PWSs) that use surface water or ground water under the direct influence (GWUDI) of surface water to monitor their source water (influent water prior to treatment plant) for *Cryptosporidium* and/or *E. coli*, and turbidity for a limited period [40 CFR § 141.701]. In support of the monitoring requirements specified by the rule, three documents have been developed to provide guidance on monitoring and reporting data under the LT2 Rule to the affected PWSs and the laboratories that support them:

- *Source Water Monitoring Guidance Manual for Public Water Systems for the Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule)* (this document). This guidance manual for PWSs affected by the rule provides information on laboratory contracting, sample collection procedures, and data evaluation and interpretation. This guidance manual also provides information on grandfathering requirements for *Cryptosporidium* and *E. coli* data.

- *Microbial Laboratory Guidance Manual for the Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule)*. This manual provides *Cryptosporidium* and *E. coli* laboratories analyzing samples in support of the LT2 Rule with guidance and detailed procedures for all aspects of microbial analyses under the rule to maximize data quality and consistency.

- *Users' Manual for the LT2/Stage 2 Data Collection and Tracking System (DCTS)*. This manual provides PWSs and laboratories with instructions on using the DCTS for entry, review, and approval of electronic data and provides instructions for States and EPA for generating tracking reports.

These manuals, an Online Microscopy Training Module, and other guidance and information are available at http://www.epa.gov/safewater/disinfection/lt2/compliance.html

This guidance document is provided to help implement the LT2 Rule. This guidance document does not, however, substitute for the LT2 Rule or the analytical methods approved for use under the rule. The material presented here is intended solely for guidance and does not alter any regulatory or analytical method requirements.

This manual provides guidance on the following aspects of the LT2 Rule:

- **Section 1:** Overview of the rule's monitoring requirements
- **Section 2:** Establishing a *Cryptosporidium* laboratory contract
- **Section 3:** Sampling location and schedule guidance
- **Section 4:** Guidance on collecting and shipping LT2 monitoring samples
- **Section 5:** Guidance on submitting historical data ("grandfathering")
- **Section 6:** Understanding *Cryptosporidium* data and analyses
- **Section 7:** Understanding *E. coli* data and analyses

The LT2 Rule is a National Primary Drinking Water Regulation that requires monitoring, reporting, and public notification for all PWSs that use surface water or GWUDI sources. The LT2 Rule was developed to improve the control of microbial pathogens, including specifically the protozoan *Cryptosporidium*, in drinking water and to address risk trade-offs with disinfection byproducts.

The LT2 Rule requires PWSs that use surface water or GWUDI to monitor their source water (influent water prior to treatment plant) for *Cryptosporidium*, and/or *E. coli*, and turbidity [40 CFR § 141.701(a) and (c-h)]. Monitoring requirements vary by PWS size (large or small), treatment type (filtered or unfiltered) and selling relationship (wholesaler or non-wholesaler). Monitoring results will be used to determine whether additional treatment is required at PWSs and to refine the relationship established between *Cryptosporidium* and *E. coli* levels in source water. All PWSs that must comply with the requirements of the LT2 Rule must initiate monitoring according to the schedule in **Table 1-1** [40 CFR § 141.701(c)]. A second round of monitoring is also required for all PWSs after the initial round of monitoring described in this guidance [40 CFR § 141.701(c)].

Every PWS that is subject to the LT2 Rule should receive a letter from EPA or the state with information on the LT2 Rule and a determination of source water monitoring requirements and schedule (schedule 1, 2, 3, or 4). Systems that EPA or the state anticipates are on schedules 1 and 2 should receive a letter in February 2006. Systems that EPA or the state anticipates are on schedules 3 and 4 should receive a letter in July 2006. States determined your requirements and schedule based on their records on your population served and connections to other systems. You should make sure the schedule determination in the letter is consistent with your system size, source water type, and buying/selling relationships with other PWSs before proceeding. If you have questions about your schedule and requirements, please discuss them with your point of contact for the LT2 listed at http://www.epa.gov/safewater/disinfection/lt2/compliance.html.

1.2 Schedules 1-3: Large Systems and Wholesalers (as applicable)

Large systems (those serving at least 10,000 people) affected by the LT2 Rule include both filtered and unfiltered PWSs.

- A large, filtered system in the LT2 Rule is a system that
  - Uses surface water or GWUDI
  - Serves at least 10,000 people
  - Provides filtration or is unfiltered, but required to install filtration because the system no longer meets all filtration avoidance criteria

  Large filtered PWSs and wholesalers (as applicable – see section 1.2.1) are required to conduct initial source water monitoring that includes sampling for *Cryptosporidium*, *E. coli*, and turbidity at least once per month for two years [40 CFR § 141.701(a)(1)].

- A large, unfiltered system in the LT2 Rule is a system that:
  - Uses surface water or ground GWUDI
  - Serves at least 10,000 people
  - Does not currently provide filtration and meets all filtration avoidance criteria

Large unfiltered PWSs and wholesalers (as applicable – see section 1.2.1) are required to conduct initial source water monitoring that includes sampling for only *Cryptosporidium* at least once per month for two years [40 CFR § 141.701(a)(2)].

All of the *Cryptosporidium* sampling requirements and guidance discussed in this document apply equally to both filtered and unfiltered PWSs, unless otherwise stated. However, the *E. coli* and turbidity guidance in this document does not apply to unfiltered PWSs.

The steps suggested and required for LT2 Rule compliance for large PWSs on the first three schedules and the timelines for these steps are summarized in **Tables 1-2** through **1-4**. PWSs that will be submitting previously collected data ("grandfathering") should also consult Section 5 of this guidance. Details on the use of the *Cryptosporidium* and *E. coli* data collected under the LT2 Rule are provided in Sections 6 and 7.

1.2.1 Wholesale Systems and Combined Distribution Systems

A wholesale system is a public water system that treats source water as necessary to produce finished water and then delivers some or all of that finished water to another public water system [40 CFR § 141.2]. Wholesale systems must comply with the requirements based on the population of the largest system in the combined distribution system [40 CFR § 141.700(b)(1)].

A combined distribution system is the interconnected distribution system consisting of the distribution systems of wholesale systems and the consecutive systems that receive finished water [40 CFR § 141.2].

As described in Section 1.1, States have made determinations of wholesalers, combined distribution systems and monitoring requirements and schedules based on information available regarding buying/selling relationships and interconnection types (permanent, seasonal or emergency) and their usage. If you have questions about your requirements or combined distribution system status, contact your point of contact listed at http://www.epa.gov/safewater/disinfection/lt2/compliance.html or send an email to stage2mdbp@epa.gov.

Table 1-1. Summary of LT2 Rule Monitoring Requirements (Initial Round)

SCHEDULE	Monitoring begins	Monitoring duration	Monitoring parameters and sample frequency requirements		
			Cryptosporidium	***E. coli***	***Turbidity***
SCHEDULE 1*: *Large systems serving ≥100,000*	*October 1, 2006*	*2 years* [a]	*Minimum 1 sample/month* [b]	*Minimum 1 sample/month* [c]	*Minimum 1 sample/month* [c]
SCHEDULE 2*: *Large systems serving ≥50,000 and <100,000*	*April 1, 2007*	*2 years* [a]	*Minimum 1 sample/month* [b]	*Minimum 1 sample/month* [c]	*Minimum 1 sample/month* [c]
SCHEDULE 3*: *Large systems serving ≥10,000 and <50,000*	*April 1, 2008*	*2 years* [a]	*Minimum 1 sample/month* [b]	*Minimum 1 sample/month* [c]	*Minimum 1 sample/month* [c]
***SCHEDULE 4*:** *Small filtered systems (serving fewer than 10,000)*	*October 1, 2008*	*1 year* [a,d]	*See next row* **§**	*Every two weeks*	*N/A*
Small unfiltered systems (serving fewer than 10,000) *and* **§** *Small filtered systems exceeding E. coli* trigger levels [d] *or that elect to proceed directly to Cryptosporidium monitoring or that fail to conduct E. coli monitoring*	April 1, 2010	1 year [b], 2 *Cryptosporidium* samples per month, or 2 years [e], 1 *Cryptosporidium* sample per month		N/A	N/A

*** Wholesale systems must comply with the requirements based on the population of the largest system in the combined distribution system [40 CFR § 141.700(b)(1)]**

[a] PWSs may be eligible to use (grandfather) data collected prior to the applicable monitoring start date if certain requirements are met (see Section 5) [40 CFR § 141.707(a)(1)]

[b] PWSs monitoring for *Cryptosporidium* may collect more than one sample per month if sampling is evenly spaced over the monitoring period [40 CFR § 141.701(a)(7)]

[c] Unfiltered systems serving ≥10,000 are not required to perform *E. coli* or turbidity monitoring but to conduct source water monitoring that includes *only Cryptosporidium* sampling [40 CFR § 141.701(a)(2)]

[d] Filtered systems serving fewer than 10,000 people must monitor for *Cryptosporidium* only if their *E. coli* annual mean concentrations is greater than 10 *E. coli*/100 mL for systems using lakes/reservoirs or is greater than 50 *E. coli*/100 mL for systems using flowing streams [40 CFR § 141.701(a)(4)]

[e] Small systems collecting one sample per month for 2 years are still required, where applicable, to meet the treatment technique implementation deadlines in 40 CFR § 141.713 (c). The same treatment compliance dates apply to the PWS regardless of which *Cryptosporidium* sampling frequency is used (i.e., selecting the 2 year *Cryptosporidium* sampling frequency does not extend *Cryptosporidium* treatment compliance deadlines).

N/A = Not applicable. No monitoring required.

Table 1-2. SCHEDULE 1: Timeline for required monitoring (Initial Round)

- Large Systems serving at least 100,000 and
- Systems that sell water and are part of a network of systems (a combined distribution system) with its largest system serving 100,000 or more persons

Event	Timeline	Duration
Establish contract with a *Cryptosporidium* laboratory approved under EPA's Lab QA Program (see Section 2.3.1) (Reference 8.2)	As soon as possible, recommended by June 2006	N/A - single event
Verify that the laboratory you plan to use to perform *E. coli* analyses under LT2 is certified under the drinking water laboratory certification program to perform a similar technique to the *E. coli* method that has been selected for use [a] (See Section 2.3.2)		
Verify that the party who will measure turbidity has been approved by the State [a]		
Work with your *Cryptosporidium* laboratory to establish a mutually acceptable sampling schedule (see Section 2.1.3 and Section 3.2)		
Submit sampling schedule (see Section 3.2) or submit notice of intent to provide full treatment as defined in 40 CFR § 141.701(d) (see Section 1.5)	**Required–** No later than July 1, 2006	
Submit sampling location and source water monitoring description (see Section 3.1)		
Submit notice of intent to grandfather (if applicable) (see Section 5) [b,c]	**Required** (if grandfathering) - No later than July 1, 2006	
Submit grandfathered *Cryptosporidium* data package (if applicable) (see Section 5) [b,c]	**Required** (if grandfathering) - No later than December 1, 2006	
Collect monitoring samples [c] (see Section 4)	**Required–** No later than the month beginning October 1, 2006	At least once per month for 2 years[d]
Submit monitoring results [c] (see Sections 6.3 and 7.3)	**Required–** No later than 10 days after the end of the first month following the month that the sample was collected (approximately 40 to 70 days after sample collection, depending on when during the month the sample is collected)	

[a] Not applicable to large, *unfiltered* systems because these systems are not required to monitor for *E. coli* or turbidity [40 CFR § 141.701(a)(2)]

[b] PWSs with fewer than two years of grandfathered data at the time of LT2 Rule promulgation, or that have at least two years of grandfathered data but intend to conduct monitoring under the LT2 Rule, must also submit a sampling schedule [40 CFR § 141.702(a) and 40 CFR § 141.707(f)(1)]

[c] PWSs may be eligible to use historical (grandfathered) data in lieu of monitoring requirements if certain quality assurance and quality control criteria are met (see Section 5) [40 CFR § 141.707]

[d] PWSs monitoring for *Cryptosporidium* may collect more than one sample per month if sampling is evenly spaced over the monitoring period [40 CFR § 141.701(a)(7)]

N/A = Not applicable

Table 1-3. SCHEDULE 2: Timeline for required monitoring (Initial Round)

- Large Systems serving ≥ 50,000 and <100,000, and
- Systems that sell water and are part of a network of systems (a combined distribution system) with its largest system serving 50,000–99,999 persons

Event	Timeline	Duration
Establish contract with a *Cryptosporidium* laboratory Approved under EPA's Lab QA Program (see Section 2.3.1)	As soon as possible, recommended by December 2006	N/A - single event
Verify that the laboratory you plan to use to perform *E. coli* analyses under LT2 is certified under the drinking water laboratory certification program to perform a similar technique to the *E. coli* method that has been selected for use [a] (see Section 2.3.2)		
Verify that the party who will measure turbidity has been approved by the State [a]		
Work with your *Cryptosporidium* laboratory to establish a mutually acceptable sampling schedule (see Section 2.1.3 and Section 3.2)		
Submit sampling schedule (see Section 3.2) or submit notice of intent to provide full treatment as defined in §141.701 (d) (see Section 1.5)	**Required–** No later than January 1, 2007	
Submit sampling location and source water monitoring description (see Section 3.1)		
Submit notice of intent to grandfather (if applicable) (see Section 5) [b,c]	**Required** (if grandfathering) - No later than January 1, 2007	
Submit grandfathered *Cryptosporidium* data package (if applicable) (see Section 5) [b,c]	**Required** (if grandfathering) - No later than June 1, 2007	
Collect monitoring samples [c] (see Section 4)	**Required–** No later than the month beginning April 1, 2007	At least once per month for 2 years[d]
Submit monitoring results [c] (see Sections 6.3 and 7.3)	**Required–** No later than 10 days after the end of the first month following the month that the sample was collected (approximately 40 to 70 days after sample collection, depending on when during the month the sample is collected)	

[a] Not applicable to large, *unfiltered* systems because these systems are not required to monitor for *E. coli* or turbidity [40 CFR § 141.701(a)(2)]

[b] PWSs with fewer than two years of grandfathered data at the time of LT2 Rule promulgation, or that have at least two years of grandfathered data but intend to conduct monitoring under the LT2 Rule, must also submit a sampling schedule [40 CFR § 141.702(a) and 40 CFR § 141.707(f)(1)]

[c] PWSs may be eligible to use historical (grandfathered) data in lieu of monitoring requirements if certain quality assurance and quality control criteria are met (see Section 5) [40 CFR § 141.707]

[d] PWSs monitoring for *Cryptosporidium* may collect more than one sample per month if sampling is evenly spaced over the monitoring period [40 CFR § 141.701(a)(7)]

N/A = Not applicable

Table 1-4. SCHEDULE 3: Timeline for required monitoring (Initial Round)

- Large Systems serving ≥ 10,000 and <50,000, and
- Systems that sell water and are part of a network of systems (a combined distribution system) with its largest system serving 10,000–49,999 persons

Event	Timeline	Duration
Establish contract with a *Cryptosporidium* laboratory Approved under EPA's Lab QA Program (see Section 2.3.1)	Recommended by December 2007	N/A - single event
Verify that the laboratory you plan to use to perform *E. coli* analyses under LT2 is certified under the drinking water laboratory certification program to perform a similar technique to the *E. coli* method that has been selected for use [a] (see Section 2.3.2)		
Verify that the party who will measure turbidity has been approved by the State [a]		
Work with your *Cryptosporidium* laboratory to establish a mutually acceptable sampling schedule (see Section 2.1.3 and Section 3.2)		
Submit sampling schedule (see Section 3.2) or submit notice of intent to provide full treatment as defined in § 141.701 (d) (see Section 1.5)	**Required–** No later than January 1, 2008	
Submit sampling location and source water monitoring description (see Section 3.1)		
Submit notice of intent to grandfather (if applicable) (see Section 5) [b,c]	**Required** (if grandfathering) - No later than January 1, 2008	
Submit grandfathered *Cryptosporidium* data package (if applicable) (see Section 5) [b,c]	**Required** (if grandfathering) - No later than June 1, 2008	
Collect monitoring samples [c] (see Section 4)	**Required–** No later than the month beginning April 1, 2008	At least once per month for 2 years[d]
Submit monitoring results [c] (see Sections 6.3 and 7.3)	**Required–** No later than 10 days after the end of the first month following the month that the sample was collected (approximately 40 to 70 days after sample collection, depending on when during the month the sample is collected)	

[a] Not applicable to large, *unfiltered* systems because these systems are not required to monitor for *E. coli* or turbidity [40 CFR § 141.701(a)(2)]

[b] PWSs with fewer than two years of grandfathered data at the time of LT2 Rule promulgation, or that have at least two years of grandfathered data but intend to conduct monitoring under the LT2 Rule, must also submit a sampling schedule [40 CFR § 141.702(a) and 40 CFR § 141.707(f)(1)]

[c] PWSs may be eligible to use historical (grandfathered) data in lieu of monitoring requirements if certain quality assurance and quality control criteria are met (see Section 5) [40 CFR § 141.707]

[d] PWSs monitoring for *Cryptosporidium* may collect more than one sample per month if sampling is evenly spaced over the monitoring period [40 CFR § 141.701(a)(7)]

N/A = Not applicable

1.3 Schedule 4 - Small Systems

Small systems (those serving fewer than 10,000 people) affected by the LT2 Rule include both filtered and unfiltered systems.

A small filtered system under the LT2 Rule is a system that

- Uses surface water or ground water under the direct influence of surface water
- Serves fewer than 10,000 people
- Provides filtration, or is unfiltered but required to install filtration because the system no longer meets all filtration avoidance criteria.

Small, filtered systems are required to conduct initial source water monitoring for *E. coli* biweekly as an indicator of *Cryptosporidium.* Those small filtered systems that exceed *E. coli* trigger levels, those that opt to proceed directly to *Cryptosporidium* monitoring without collecting *E. coli* data, and those that fail to conduct *E. coli* monitoring must conduct monitoring for *Cryptosporidium*. [40 CFR § 141.701(a)(3)].

A small unfiltered system under the LT2 Rule is a system that

- Uses surface water or ground water under the direct influence of surface water
- Serves fewer than 10,000 people
- Does not currently provide filtration and meets all filtration avoidance criteria

Small, unfiltered systems are required to conduct initial source water monitoring that includes *Cryptosporidium* sampling only (small unfiltered systems do not conduct *E. coli* indicator monitoring). [40 CFR § 141.701(a)(6)].

The steps required for LT2 Rule compliance for small PWSs and the schedule for these steps are summarized in **Table 1-5**.

Table 1-5. SCHEDULE 4: Timeline for required monitoring (Initial Round)

- Small systems serving <10,000 persons, and
- Systems that sell water and are part of a network of systems (a combined distribution system) with its largest system serving <10,000 persons

Filtered Systems – *E. coli* monitoring		
Event	**Timeline**	**Duration**
Verify that the laboratory that will perform your *E. coli* analyses under the LT2 Rule is certified under the drinking water laboratory certification program to perform the analytical method you plan to use (See Section 2.3.2)	Recommended by June, 2008	N/A - single event
Submit sampling schedule (see Section 3.2) or submit notice of intent to provide full treatment as defined in § 141.701 (d) (see Section 1.5) or submit notice of intent to avoid *E. coli* monitoring by monitoring for *Cryptosporidium*	**Required–** No later than July 1, 2008	
Submit sampling location and source water monitoring description		
Submit notice of intent to grandfather (if applicable) (see Section 5) [d,e]	**Required** (if grandfathering) - No later than July 1, 2008 [d,e]	
Submit grandfathered *E. coli* data package (if applicable) (see Section 5) [d,e]	**Required** (if grandfathering) - No later than December 1, 2008 [d,e]	
Collect *E. coli* samples [e] (See Section 4)	**Required–** No later than the month beginning October 1, 2008	One year (one sample every two weeks) [b]
Submit *E. coli* monitoring results (See Section 7.3)	**Required–** No later than 10 days after the end of the first month following the month that the sample was collected (approximately 40 to 70 days after sample collection)	At least once per month for one year

Table 1-5 continued on the following page

Table 1-5. SCHEDULE 4: Timeline for required monitoring (Initial Round) (continued)

Unfiltered Systems and Filtered Systems that Exceed *E. coli* Trigger Levels[a] or that Elect to Bypass *E. coli* Monitoring and Proceed Directly to *Cryptosporidium* Monitoring or that fail to conduct *E. coli* monitoring		
Establish contract with a *Cryptosporidium* laboratory approved under EPA's Lab QA Program	Recommended by December, 2009	N/A - single event
Work with your *Cryptosporidium* laboratory to establish a mutually acceptable sampling schedule		
Submit sampling schedule (see Section 3.2) or submit notice of intent to provide full treatment as defined in § 141.701 (d) (see Section 1.5)	**Required–** No later than January 1, 2010	
Submit sampling location and source water monitoring description		
Submit notice of intent to grandfather (if applicable) (see Section 5) [d,e]	No later than January 1, 2010 [d,e]	
Submit grandfathered *Cryptosporidium* data package (if applicable) (see Section 5) [d,e]	No later than June 1, 2010 [d,e]	
Collect *Cryptosporidium* samples	**Required–** No later than the month beginning April 1, 2010	One year (two samples per month)[b] or two years (one sample per month) [c]
Submit *Cryptosporidium* monitoring results	**Required–** No later than 10 days after the end of the first month following the month that the sample was collected (approximately 40 to 70 days after sample collection)	At least once per month for one year

[a] Small filtered systems are required to monitor for *Cryptosporidium*, beginning six months after completion of *E. coli* monitoring if the *E. coli* annual mean concentration exceeds 10 *E. coli*/100 mL for systems using lakes/reservoirs or exceeds 50 *E. coli*/100 mL for systems using flowing streams

[b] PWSs may sample more frequently if the sampling frequency is evenly spaced over the monitoring period [40 CFR § 141.701(a)(7)]

[c] Small systems collecting one sample per month for 2 years are still required, where applicable, to meet the treatment technique implementation deadlines in 40 CFR § 141.713 (c). The same treatment compliance dates apply to the PWS regardless of which *Cryptosporidium* sampling frequency is used (i.e., selecting the 2 year *Cryptosporidium* sampling frequency does not extend *Cryptosporidium* treatment compliance deadlines).[d] PWSs may be eligible to use historical (grandfathered) data in lieu of monitoring requirements if certain quality assurance and quality control criteria are met (see Section 5) [40 CFR § 141.707]

[e] Small systems with less than a complete set of grandfathered data, or that intend to conduct additional monitoring beyond the required minimum under the LT2 Rule, must also submit a sampling schedule [40 CFR § 141.702(a) and 40 CFR § 141.707(f)(1)]

N/A = Not applicable

1.4 Consecutive Systems

A consecutive system is a public water system that receives some or all of its finished water from one or more wholesale systems. Delivery may be through a direct connection or through the distribution system of one or more consecutive systems [40 CFR § 141.2].

Consecutive systems are not exempt from the requirements of the LT2 Rule. However, consecutive systems may receive water that a wholesale system has monitored, and treated if required, to comply with the LT2 Rule. In this case, the consecutive system is not required to conduct additional monitoring or install additional treatment on that water under the requirements of the LT2 Rule.

1.5 Monitoring Avoidance

Filtered systems are not required to conduct source water monitoring under the LT2 Rule if the system will provide a total of at least 5.5-log of treatment for *Cryptosporidium*, equivalent to meeting the treatment requirements of Bin 4 in the LT2 Rule [40 CFR § 141.701(d)(1) and 40 CFR § 141.711].

Unfiltered systems are not required to conduct source water monitoring under the LT2 Rule if the system will provide a total of at least 3-log *Cryptosporidium* inactivation, equivalent to meeting the treatment requirements for unfiltered systems with a mean *Cryptosporidium* concentration of greater than 0.01 oocysts/L in 40 CFR § 141.712 [40 CFR § 141.701(d)(2)].

If a system chooses to provide the level of treatment as described above, rather than start source water monitoring, the system must submit written notification (**Appendix A**) no later than the date the system is otherwise required to submit a sampling schedule for monitoring. Alternatively, a system may choose to stop sampling at any point after it has initiated monitoring if it provides written notification that it will provide this level of treatment.

Systems that are considering this option to avoid monitoring, or that believe that they already meet the requirements to avoid monitoring, should consult with their point of contact as listed on the contact list on the LT2 web site at http://www.epa.gov/safewater/disinfection/lt2/compliance.html. Systems should review with the point of contact the microbial toolbox credits provided in the LT2 Rule, and the PWS' existing treatment capabilities and any credits already determined by the State, to be sure of the treatment capabilities needed to fulfill the LT2 Rule requirements. PWSs must install and operate technologies to provide this level of treatment by the applicable treatment compliance date in 40 CFR § 141.713 [40 CFR § 141.701(d)(3)].

1.6 Reports and Notices

For the Initial Round of monitoring, the required reports and notifications for PWSs on **Schedules 1-3** should be submitted through the options listed below in Table 1-6. Reporting procedures for **PWSs on Schedule 4** will be provided at a later date.

Table 1-6. Submitting Reports and Notices

	Options for submitting reports and notices		
Report/Notice	**E mail**	**Hardcopy**	**DCTS**
Sampling Schedule *			✓
Sample Location Description	✓	✓	
Intent to Provide Maximum Treatment		✓	
Intent to Grandfather Data	✓	✓	
Grandfathered Data	✓	✓	✓
Grandfathered Data Supporting Documentation	✓	✓	
Initial Round Monitoring Data *			✓

***** Sampling schedules and Initial Round monitoring data must be submitted through the DCTS unless EPA or State approve an alternative means [40 CFR §141.702(a)(2) and 40 CFR § 141.706(b)]. If the schedule is not submitted through the DCTS by the required date, you may not be able to use the DCTS to input your schedule and should call your point-of-contact as indicated on the contact list at http://www.epa.gov/safewater/disinfection/lt2/compliance.html for assistance).

Depending on the option chosen, reports and notices should be submitted as follows:

Via email to: stage2mdbp@epa.gov

Hardcopies mailed or faxed to: LT2ESWTR and Stage 2 DBPR
P.O. Box 98
Dayton, OH 45401

Fax: (937)586-6557

Submissions through the LT2/Stage 2 Data Collection and Tracking System (DCTS):

Access the DCTS through the LT2 web site at http://www.epa.gov/safewater/disinfection/lt2

1.7 Questions?

In some cases, EPA will be your main point of contact during the first phases of the source water monitoring. In other cases, your state will be your main point of contact. To identify your point of contact for LT2 source water monitoring, visit EPA's website at http://www.epa.gov/safewater/disinfection/lt2/compliance.html.

If after reading this guidance you still have questions, please submit them as follows:

- Submit them to your point-of-contact as indicated on the contact list at http://www.epa.gov/safewater/disinfection/lt2/compliance.html.

  Or

- Send them via email to stage2mdbp@epa.gov.

  Or

- Call the Safe Drinking Water Hotline at 1-800-426-4791

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SECTION 2: CONTRACTING FOR LABORATORY SERVICES

Although many public water systems (PWSs) have established procedures and policies governing the purchase of services and supplies, these procedures may not lend themselves to the purchase of analytical services. This section provides a basic framework for addressing the technical and contractual issues associated with purchasing laboratory services for the LT2 Rule, awarding contracts, and working with contract laboratories. In several instances a separate laboratory will be necessary for *Cryptosporidium* analysis from the laboratory performing *E. coli* analysis. Many *Cryptosporidium* laboratories do not perform *E. coli* analysis, and PWSs will need to have their coliform laboratory perform the *E. coli* analysis, or contract with a different laboratory.

Successfully contracting for laboratory services for LT2 Rule monitoring relies on the following steps:

Step 1: Define the scope of your analytical requirements to develop a detailed contract

Step 2: Develop a standardized bid sheet/cost estimate (**Appendices B** and **C**)

Step 3: Solicit approved/certified laboratories

Step 4: Award contracts to a primary laboratory(ies) and a backup laboratory(ies)

Step 5: Work closely with your laboratory(ies) before monitoring begins and maintain communications throughout monitoring

These general steps, and details on the activities associated with each, are discussed in Sections 2.1 through 2.5. Whether you contract with one laboratory for both *Cryptosporidium* and *E. coli* analyses or separate laboratories, the same general procedures apply.

Remember: you must use an approved laboratory for *Cryptosporidium* analysis and a certified laboratory for *E. coli* analysis, as described in Section 2.3 below [40 CFR § 141.705].

2.1 Defining Your Needs and Developing a Contract

The first step in developing an analytical services contract for analyses for LT2 Rule monitoring is identifying the *"who," "what," "when,"* and *"how"* of the project for your system (the *"why"* is the LT2 Rule itself). A well-written contract will address each of these issues, as well as the administrative issues, such as laboratory payments and adjustments.

The best way to ensure that you get the data you need for LT2 Rule monitoring within the required time period is to specify your requirements *in detail* in the contract. A well-written contract can minimize or eliminate many common problems in procuring analytical services, and enable you to collect reliable and timely results.

Recommendations on the factors to consider in defining the scope of the services you need, and the information you should be sure to include in your contract, are provided below.

2.1.1 Client Information

"Who" defines your PWS to the laboratories that you would like to submit bids for the project. Will you be contracting for laboratory services for a single plant or will this contract require analyses to fulfill monitoring requirements for multiple plants in a system?

> ☞ *Clearly identify in your contract the name and public water system identification number (PWSID) of your PWS, as well as the name(s) and identification number of the facility(ies) for which samples need to be analyzed. This information ultimately will be used to identify your samples in the LT2/Stage 2 Data Collection and Tracking System (DCTS); the laboratory(ies) you use for sample analyses will need to know this information. (Alternately, you can provide this information after award to the awarded laboratory(ies) only.)*

2.1.2 Sample Information

"What" describes the samples to be analyzed. As noted in Sections 2.1.2.1 through 2.1.2.5, this encompasses a variety of factors, each of which should be evaluated and defined before you develop your contract.

2.1.2.1 Number of Samples

What is the total number of samples the laboratory(ies) will need to analyze? Will your PWS monitor once per month or twice or more per month? This total includes not only routine monitoring samples (field (monitoring) samples), but also the *Cryptosporidium* matrix spike (MS) samples that are required at a frequency of 1 per 20 field (monitoring) samples. Field (monitoring) samples and MS samples are considered "billable" samples (sample analyses for which the laboratory will be paid their per-sample cost).

Internal laboratory quality control (QC) samples, such as method blanks and ongoing precision and recovery (OPR) samples should be considered "unbillable" samples—sample analyses that are required, but apply to multiple PWS clients. Rather than charging clients for these samples directly, laboratories typically will distribute the costs of these samples across billable samples.

If your PWS has not sampled for *Cryptosporidium* or worked with the lab previously, you should consider adding a couple of practice samples to make sure all sampling systems are functioning properly before starting required monitoring.

If a sample is collected and sent to the laboratory, but cannot be submitted under the LT2 Rule because of a problem unrelated to laboratory performance (such as shipping delays that violate the sample holding time), your PWS will be required to collect a replacement sample for *Cryptosporidium* analysis (see Section 3.2.2 for details). You should add to the contract, as an option to be exercised at your direction in such an event, additional sample analyses as replacement samples. Appendices B and C provide a worksheet to estimate the number of samples including matrix spike, practice and replacement samples.

☞ *Clearly indicate in your Cryptosporidium contract the total number of: (1) field (monitoring) samples and (2) MS samples that the laboratory will be required to analyze. Be sure to include additional optional sample analyses that can be exercised if replacement samples are required due to problems unrelated to laboratory performance. Adding practice samples is also a good idea.*

2.1.2.2 Type of Samples

Will your PWS collect and ship bulk water samples to the laboratory for filtration and processing or will your PWS filter samples onsite and ship the filter to the laboratory? Shipping and analytical costs are likely to be lower if you filter your samples onsite, but you will need to purchase or rent sample filtration equipment (see Section 4.1 for details) and have staff trained to use the required procedures or pay for the laboratory or another firm to perform these tasks.

☞ *Clearly specify in the contract whether the laboratory will receive bulk water samples or filtered samples. If filtered samples will be sent, indicate which filter you will use (see Section 2.1.4.2).*

If you will be filtering onsite, and will be using your own equipment to filter the samples, you can purchase filters directly from the vendor or through your *Cryptosporidium* contract laboratory. Be sure to indicate clearly in the contract whether filters should be included in the laboratory's' bid price. (Additional information on filtering samples onsite and purchasing filters is provided in Section 4.1.2). Matrix spike samples must be shipped to the lab as 10 L bulk samples [40 CFR § 141.704(a)(2)].

☞ *If your PWS will be purchasing filters directly, specify this in the contract, so the laboratory knows not to include this in their per-sample price. Be sure to purchase extra filters if the source water to be sampled is highly turbid. PWSs with highly turbid source water must filter as much volume as two filters can accommodate before clogging.*

For *E. coli*, all samples should be collected in 125 mL or 250 mL bottles and shipped to the laboratory for analysis.

2.1.2.3 Anticipated Sample Volume

The LT2 Rule [40 CFR § 141.704(a)(1)] requires that at least 10 L be analyzed for each sample for *Cryptosporidium* (with some exceptions - see Section 4.2). Will your PWS collect 10 L samples or collect higher-volume samples, such as 50 L samples? If your PWS will be shipping bulk water samples to the laboratory, greater sample volumes will result in higher shipping costs and will likely result in higher analytical costs. If your PWS will be filtering samples on-site and shipping filters to the laboratory, the sample volume should not affect shipping or analytical costs, but the greater sample volumes filtered may result in higher packed pellet volume and multiple subsamples (Section 2.1.2.4, below).

Clearly indicate in the contract the volume you anticipate collecting for each *Cryptosporidium* sample.

For *E. coli* samples, the sample volume collected should be at least 100 mL.

> ☞ *Clearly indicate in your contract for Cryptosporidium analyses that different sample prices are needed for: (1) full sample analyses, (2) subsample analyses, and (3) extra filters and the cost of analysis of the extra filters.*

2.1.2.4 Subsamples and Filter Clogs

Additional steps are required at the laboratory for samples that generate more than 0.5 mL of packed pellet volume or samples that clog before 10 L have been filtered. Specifically, the laboratory will need to process two or more "subsamples" through the method or process two filters to meet LT2 Rule sample volume analysis requirements [40 CFR § 141.704(a)(1)]. If you know that the source water(s) to be monitored by your PWS are sometimes characterized by turbidity levels containing excessive particulates that interfere with filtration or sample purification, you will need to consider that some of your samples may need to be processed as multiple subsamples or may require two filters. One option for estimating the number of subsamples is to send the laboratory a 10L sample (either bulk or filtered) during a peak turbidity event for concentration by the lab. Alternatively, you may want to allow for the possibility that some samples may require multiple analyses. By including subsample costs in the original contract (which would apply only if you encountered this issue), you will avoid changes to the contract on short notice if subsamples are required during monitoring.

For *E. coli*, if the laboratory uses a membrane filter method and experiences clogging of the membrane filter, smaller volumes should be filtered or another method should be chosen.

2.1.2.5 Extra Services

Will any additional services be required of the laboratory outside of actual sample analyses? Possible services include:

- Sampling kit rental or purchase for on-site filtration
- Sample shipping containers
- Sample archiving for *Cryptosporidium*
- Training for sample collection personnel
- Extra analytical time for challenging water matrices

Some of these services may be included in the sample analysis cost by some laboratories. Defining the specific services your PWS will need, and specifying these services clearly in the contract will enable the laboratories to better assess whether the requested services are included in their routine costs or are extra, and respond accordingly.

> ☞ *Clearly specify in your contract any services required in addition to routine sample analysis.*

2.1.3 Sampling Schedules

Table 1-1 and Section 1 of this guidance provide information regarding the minimum required sampling frequency for PWSs. As described in Section 2.1.2.1 earlier, you should consider the minimum requirements for your PWS and determine if you will sample more often.

If at all possible, *do not establish a firm sampling schedule with specific dates at this point*. Most of the laboratories available to perform *Cryptosporidium* analyses have multiple PWS clients and need to evenly distribute their sample load within each week and across weeks in a month to meet holding time requirements. Rather than dictating a sample collection schedule to the laboratory–and potentially discouraging laboratories from bidding on the work or risk violating holding times during monitoring–work with the awarded laboratory to establish a schedule that will comply with LT2 Rule requirements and is mutually acceptable to your PWS and the laboratory. Section 3 provides information on the sampling schedule that must be submitted prior to monitoring.

> ☞ *Indicate in your contract the month that you plan to begin monitoring and how frequently you will monitor. . If possible, do not specify actual sample collection dates and days during the week; work with the awarded laboratory to establish a schedule that meets your needs and does not cause problems for the laboratory.*

2.1.4 Analytical Methodology for *Cryptosporidium*

"How" describes the analytical method that the laboratory will use. This involves two sets of options for *Cryptosporidium*: which method to use (EPA Method 1622 or EPA Method 1623) and which filter to use, regardless of method. It also refers to the QC requirements that must be met during sample processing and analysis (Reference 8.3 and 8.4).

2.1.4.1 EPA Method 1622 Versus EPA Method 1623

Will your PWS monitor for *Cryptosporidium* only or *Cryptosporidium* and *Giardia*? Method 1623 targets both *Cryptosporidium* and *Giardia*. Method 1622 is identical but reagents are specific to only *Cryptosporidium*. The LT2 rule does not require monitoring for *Giardia*, and only *Cryptosporidium* data need to be submitted. However, most laboratories analyze samples for both *Cryptosporidium* and *Giardia* using EPA Method 1623 because *Giardia* serves as a good internal control, provides extra information to the PWS and may be offered at a less expensive price than *Cryptosporidium*-only due to QA/QC considerations. The method should be agreed upon by the laboratory and the PWS, and specified in the contract. Some labs may not offer 1622 analysis if they don't have sufficient clients requesting it.

2.1.4.2 Filter Options

Unless you have a reason for specifying a particular filter, leave this up to the laboratory in your contract. If your PWS has experience monitoring for *Cryptosporidium* and has a filter preference, you will need to indicate this to the laboratories interested in bidding on the project, as not all laboratories are approved by EPA through the Lab QA Program to perform all versions of the methods.

☞ *If your PWS has experience with* Cryptosporidium *sampling and would like analyses performed using a specific filter, clearly indicate this in the contract. Otherwise, do not specify a filter type.*

2.1.4.3 Quality Control Requirements

Although EPA Methods 1622 and 1623 (Reference 8.3 and 8.4) specify the QC requirements that must be met during performance of the method, your contract should reiterate that all of the QC requirements for the method must be met at the required frequency during processing and analysis of your samples. As noted earlier in Section 2.1.2.1, the costs for the method blank, ongoing precision and recovery, and staining control tests should be distributed by the laboratory across the cost of sample analysis.

☞ *Reiterate in the contract that method blanks, ongoing precision and recovery tests, and staining controls must be performed at the frequency required in the method, and that all holding times must be met.*

2.1.5 Analytical Methodology for *E. coli*

The choice of method may depend on what methods your laboratory is certified to perform and the quality of your source water. Unless you have a reason to select a particular method, it is best to allow the laboratory to determine the *E. coli* method.

None of the QC requirements should be billable, but rather should be distributed by the contract laboratory across the cost of monitoring samples for all of their clients.

2.1.6 Data Deliverables and Other Contract Issues

In addition to the *"who," "what," "when,"* and *"how"* questions that should be addressed by the contract, you also should provide details on data delivery, adjustments for lateness, and sample reanalysis cost issues. These issues are discussed in Sections 2.1.6.1 through 2.1.6.5.

2.1.6.1 Data Submission

EPA has developed the web-based LT2/Stage 2 Data Collection and Tracking System (DCTS) to allow laboratories to report data to PWSs electronically and allow PWSs to verify the data electronically before submitting the monitoring results to EPA [40 CFR § 141.706]. This reporting process is summarized in Section 6.3 for *Cryptosporidium* data and Section 7.3 for *E. coli* and turbidity data, and discussed in detail in the *Users' Manual for the LT2/Stage 2 Data Collection and Tracking System (DCTS)*. The laboratory, at a minimum, should submit the results for each monitoring sample to you electronically. (Although your PWS also could enter these data into the DCTS, based on hardcopy results from the laboratory, this is strongly discouraged, as the potential for error increases when personnel unfamiliar with the generation of the data for a sample enter these data into the DCTS.)

☞ *Clearly indicate in your contract that the laboratory is required to enter monitoring results for your samples into the LT2/Stage 2 Data Collection and Tracking System. Specify that all laboratory data must be recorded on appropriate laboratory bench sheets.*

2.1.6.2 Hardcopy Data Deliverables

Note: If you do not intend to review all of the raw data generated by the laboratory, this section is not relevant and can be ignored.

If your PWS intends to review all of the raw data associated with your LT2 samples (discussed in Section 6), you should request copies of the forms used by the laboratory to record sample measurements, sample processing times, and sample examination results, as well as information on the QC samples associated with your monitoring sample. (Original data forms should stay at the laboratory; copies can be sent to the PWS. If bench sheets, etc., are requested on a monthly basis, the PWS may expect additional charges from the laboratory)

PWSs that want to review raw data for each *Cryptosporidium* analysis should request the following:

- **Monitoring sample identification information**
- **Monitoring sample result**, in oocysts/L
- **Laboratory quality control batch** associated with the sample
- **ID number and result for the ongoing precision and recovery (OPR) sample** analyzed for this QC batch
- **ID number and result for the method blank sample** analyzed for this QC batch
- **LT2 sample collection form** initiated by your utility and completed with sample receipt information by the laboratory
- **Method 1622/1623 Bench Sheet** with raw data associated with the monitoring sample (and MS sample, if applicable)
- **Method 1622/1623 *Cryptosporidium* Slide Examination Form** with raw data for the monitoring sample (and MS sample, if applicable)
- **Laboratory comments.** If the laboratory provided comments on the sample analyses or results that require follow-up, contact the laboratory to discuss, if necessary. Comments may include any applicable data qualifiers. The following is a list of potential data qualifiers:
  - The recovery for the associated ongoing precision and recovery (OPR) sample did not meet method requirements
  - Oocysts were detected in the method blank
  - Positive and negative staining controls were not acceptable or not examined
  - Method holding times were not met
  - Sample arrived at the laboratory in unacceptable condition

PWSs that want to review the raw data for each *E. coli* analysis should request the following:

- **Monitoring sample identification information**
- **Monitoring sample result**, in *E. coli*/100 mL
- ***E. coli* Method Bench Sheet** with raw data for the monitoring sample
- **Laboratory comments.** If the laboratory provided comments on the samples analyses or results that require follow-up, contact the laboratory to discuss, if necessary. Comment may include any applicable data qualifiers. See section 7.4.1 of this manual for a list of possible data qualifiers for *E. coli* analysis.

> ☞ *If you want the laboratory to submit hardcopy results (this should not be requested unless you intend to review all of the raw data), clearly indicate in your contract the materials that are required. You may also choose to request a hard copy of only the summary results.*

2.1.6.3 Data Turnaround Requirements

Under the LT2 Rule, PWSs are required to submit data no later than 10 days after the end of the first month following the month when the sample is collected (this is approximately 40 to 70 days after sample collection, depending on when during the month the sample is collected) [40 CFR § 141.706(a)]. For example, if a sample is collected on March 17, data must be submitted no later than May 10.

The turnaround requirement for the laboratory should be shorter to provide your PWS time to review the data before the submission deadline to EPA. The required data turnaround should be stated clearly in the contract. This turnaround time should be expressed in calendar days (not working days), and should start from the sample collection date. The data turnaround time calculations should consider the day that the sample is collected "day zero," and the following day as "day one." (Data turnaround times in analytical contracts typically start from the receipt of the sample at the laboratory, but calculating it from the sample collection date is more logical in this case because the LT2 Rule's data submission requirements are based on sample collection date.)

If the data turnaround time starts from sample collection, rather than sample receipt by the laboratory, this turnaround should accommodate the potential for shipping delays that will be outside of the laboratory's control. The maximum shipping time is 4 days, including delays. This includes up to 4 days between sample collection and initiation of the elution step, which effectively is the maximum time for any shipping delay, as samples received more than 4 days after collection will not be valid and cannot be submitted through the DCTS. As a general rule, the data turnaround time should be less than 15 days.

Using the 15 days allowed for sample analysis by the methods (plus additional time to compile the data package and mail the results, if hardcopies are required) as the shortest realistic turnaround time, determine when you will actually need the results. The same turnaround time can be specified for both submission of electronic data and receipt of hardcopy materials.

2.1.6.4 Liquidated Damages and Penalties

You should consider including penalty or damage clauses in your contract as incentives to preclude laboratories from submitting data late or performing analyses improperly. Due to the nature of the services provided, assessing actual damages caused by improperly performed analyses is often difficult. Liquidated damages often are used in analytical services contracts in lieu of actual damages. Liquidated damages typically specify that, if the laboratory fails to deliver the data specified in the deliverables section of the contract, or fails to perform the services within the specified data turnaround time, the laboratory will pay a fixed, agreed, price to compensate the organization to whom the services should have been delivered. For example, some EPA contracts for analytical services specify that the laboratory will pay, as fixed, agreed, and liquidated damages, 2 percent of the analysis price per calendar day of delay, to a maximum reduction of 50 percent of the analysis price.

If liquidated damages or penalties are involved, they should (1) be based in terms of cost by each late day, (2) be strong enough to discourage late delivery, and (3) be reasonable enough that they will not discourage laboratories from bidding. The contract should specify that the laboratory will not be charged with liquidated damages when the delay in delivery or performance arises out of causes beyond the control and without the fault or negligence of the laboratory. It also may be necessary to limit damages to a certain dollar value or scope.

Other types of damages that should be considered and may be included in the contract include costs for resampling and administrative costs associated with the evaluation and processing of unacceptable data (data that do not meet the requirements specified in the contract or the QC requirements specified in the analytical method).

☞ *Clearly indicate in your contract whether liquidated damages will be applied to late data or other problems, how these liquidated damages are calculated, and the limits and conditions associated with the damages.*

2.1.6.5 Re-Analysis Costs

Every laboratory periodically produces data that are associated with unacceptable QC data or are invalid for other reasons. The contract should stipulate that the laboratory will reanalyze samples at no cost to your PWS if the problems are due to laboratory error. If the problems are due to an error outside of the laboratory's control (such as the laboratory's rejection of a *Cryptosporidium* sample received at > 20°C that results in resampling by your PWS), the laboratory should not be responsible for the additional costs that may result.

☞ *Clearly indicate in your contract when the laboratory would be required to bear the costs of sample re-analysis and when these costs will be borne by your PWS.*

The contract also should state that you have the right to inspect the results, and if they do not meet the requirements in the contract, you have the right to reject the data, returning them to the laboratory without

payment. Rejection of data should be based on sound technical review of the results. It also obligates you to make no use of those results without making some payment to the laboratory.

> ☞ *Clearly indicate in your contract that your PWS has the right to inspect results and reject the results if they do not meet contract requirements.*

2.2 Developing a Bid Sheet

After all project requirements have been established, you should develop a bid sheet to accompany the analytical requirements summary during the solicitation. The bid sheet allows laboratories to submit bids in the same format, making bid evaluations easier, and also helps to clarify the project. Development and use of a bid sheet is recommended regardless of whether your PWS solicits the project competitively to multiple laboratories, or is simply requesting a quote from a laboratory you already know you will be using, as it provides a very clear vehicle for submitting and evaluating costs.

Bid sheets for analytical services typically are formatted as a table, with costs in the columns and descriptions of services and supplies heading the rows (**Appendices B** and **C**).

The bid sheet should include the following information:

- Project identifier (e.g. "LT2 Monitoring Sample Analyses for [PWS name and/or facility name]")
- Space for laboratory identification information (for when they submit their bid)
- Day, date, and time (including time zone) of the bid deadline
- PWS information (contact and mailing address, fax number, phone number, and/or email address)
- Estimated award date
- Laboratory period of performance (includes the period of time during which the laboratory is obliged to resolve issues associated with analysis of the samples—generally 6 months after shipment of last sample)
- Data turnaround time (time from sample collection to reporting results)
- Bid validity period (period of time during which bid prices are considered valid—generally 45 days after the bid deadline; if the project is awarded after the period you specify, you must contact bidding laboratories to determine whether their bid is still valid, or needs to be revised)
- A summary of the analytical requirements:
  - Method (e.g., *Cryptosporidium* and *Giardia* by EPA Method 1623)
  - *E. coli* method of choice (if needed)
  - Filter preference, if any. (This should *not* be specified unless your PWS has experience with *Cryptosporidium* and a basis for requesting the use of a specific filter; if you know that you will be field filtering using a specific filter and shipping this to the laboratory, you should specify this)
  - Whether samples will be shipped as filtered samples or bulk water samples for *Cryptosporidium*
  - Sample volume for *Cryptosporidium* (e.g., 10 L, 50 L)
- Total number of field (monitoring) samples to be analyzed, plus extra, in case of replacement samples

- Two optional "practice" samples
- Total number of MS samples to be analyzed for *Cryptosporidium*, at least 1 for every 20 samples.
- Total number of potential subsamples to be analyzed for *Cryptosporidium* (expressed as "Up to [no.] subsamples" so you are not committing to this – just leaving it as an option)
  - The number generally should not exceed four per sample
  - If you have high-turbidity water, you may need to specify up to four subsamples for all of your field (monitoring) and MS samples
  - If you have a low-turbidity water, you should specify a minimal number, just in case the need arises

  (These costs would not be incurred unless subsamples actually have to be analyzed)
- Total number of potential extra filters for *Cryptosporidium* (in case one or more samples clog during LT2 Rule monitoring):
  - If you will be shipping bulk samples to the laboratory, express this as "Up to [no.] extra filters/elutions"
  - If you will be filtering samples in the field, but receiving filters from the laboratory, express this as "Up to [no.] extra filters"

  (These costs would not be incurred unless more than one filter actually has to be used)
- Columns for laboratories to enter per-analysis and total costs
- Costs for cubitainers or carboys, if you would like the laboratory to provide this
- Cost of sampling apparatus, if you would like the laboratory to provide this
- Cost of shipping supplies to PWS, if applicable

2.3 Soliciting the Contract

Procedures for soliciting and awarding contracts to perform analytical services can vary, depending upon the scope of the project and purchasing requirements within the organization that is issuing the contract. At one end of the spectrum are contracts that are awarded after placing a single phone call and obtaining a quote from a single laboratory. The opposite end of the spectrum are contracts awarded after a competitive solicitation and bidding process involving the distribution of a detailed project description and a formal bid sheet via fax or mail.

2.3.1 Approved Laboratories for *Cryptosporidium*

Regardless of whether you will be soliciting the project to multiple laboratories or working with a single laboratory (although a backup laboratory is strongly recommended—see below), you must limit your laboratories to only those approved by EPA through the Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* Under the Safe Drinking Water Act (Laboratory QA Program) or approved for *Cryptosporidium* by an equivalent State approval program [40 CFR § 141.705(a)]. However, at the time of publication of this guidance document there were no equivalent State programs for approval of *Cryptosporidium* laboratories. Information on the Laboratory QA program and a list of

approved laboratories are posted at http://www.epa.gov/safewater/disinfection/lt2/lab_home.html. The Laboratory QA Program is also described in detail in the *Microbial Laboratory Guidance Manual for the*

Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule) that is also available at http://www.epa.gov/safewater/disinfection/lt2/lab_home.html.

Briefly, the objectives of the program are to evaluate laboratories' competency to measure reliably for the occurrence of *Cryptosporidium* in surface water using EPA Method 1622/1623. Each laboratory participating in the program is required to complete the following steps to be qualified through this program:

- Participate in an on-site evaluation of their technical, data management, and quality assurance procedures
- Acceptably perform proficiency testing on blind samples every 4 months

2.3.2 Certified Laboratories for *E. coli*

The PWS must choose a laboratory for *E. coli* analysis that is certified by EPA, the National Environmental Laboratory Accreditation Conference (NELAC) or the State for total coliform or fecal coliform analysis under 40 CFR § 141.74. **The laboratory must use the same technique for *E. coli* analysis as the technique for which they are certified to perform total or fecal coliform analyses under 40 CFR § 141.74**. For a list of certified laboratories, the PWS may refer to their State's drinking water laboratory certification web page (if available) or contact their State drinking water laboratory certification officer. A list of *E. coli* methods that certified laboratories can perform under the LT2 Rule is provided in **Table 7-1.** EPA notes that this approach differs from the approach typically used in its Laboratory Certification program in that the latter program is based on certification for the specific method (not simply the same technique) being used in compliance monitoring.

2.3.3 Primary and Backup Laboratory Contracts

Because a laboratory's approval status could change during the LT2 Rule monitoring period, you should plan to award a primary contract and a backup contract. If no performance problems or other problems are encountered during the LT2 Rule monitoring period by the laboratory awarded the primary contract, then this laboratory would provide uninterrupted sample analysis support for the entire monitoring period. However, if the laboratory encountered performance problems and was disapproved, or was otherwise unable to meet contract requirements, your PWS could switch sample analyses to the backup laboratory under the contract you established with this laboratory before monitoring began. You may discuss the award of primary and backup contracts with the laboratories in the contract solicitation.

2.4 Evaluating Bids

After the laboratories have received the solicitation and submitted their bids, you may evaluate the bids to identify the laboratory that will be awarded the analytical services contract. Specific procedures for evaluating bids may vary, depending upon the requirements of your organization, but the bid evaluation process generally entails evaluation and comparison of each laboratory's proposed cost and capability to meet the analysis requirements.

2.4.1 Identifying Responsive Bidders

You may consult your legal department or purchasing department to identify any applicable requirements for evaluating competitive bids. Review all bids and recalculate subtotals and totals to ensure that the bidding laboratories did not make any mathematical errors. In addition, you may want to verify that there

are no unacceptable contingencies associated with any of the bids. Either eliminate from consideration bids from laboratories that bid with contingencies, or contact the laboratory(ies) to discuss the bid and verify that the laboratory cannot perform the specified services.

Of the remaining (responsive) bids, identify the lowest bidder (or the laboratory that best meets your requirements) to award the primary contract and a second bidder to award the backup contract. If additional assessments of a laboratory's performance or responsibility are needed, you may want to contact references.

2.4.2 References

If you have not worked with a particular laboratory before and would like to verify that the laboratory will meet your needs throughout the monitoring period, you can ask the laboratory to provide contacts and phone numbers of utility or government clients for whom the laboratory has performed *Cryptosporidium* or *E. coli* sample analyses or other comparable services.

Questions to ask the references include:

- Did the laboratory provide data by the required due date?
- Were the data provided by the laboratory of acceptable quality and compliant with contract requirements and in an easy to understand format?
- Were laboratory personnel easy to work with when problems arose during all phases of the project, including sample scheduling, sample analysis, and data review? If problems were noted during data review, was the laboratory prompt and responsive in addressing your concerns?
- Do you have any reservations in recommending this laboratory?

2.5 Communicating with the Laboratory

After the analytical services contract is awarded, request laboratory contact information for the following roles, and provide the laboratory with PWS contacts for the same roles:

- A technical contact for analytical questions or problems
- A sample control contact for shipping delays on the PWS end and sample receipt problems on the laboratory end
- An administrative contact for invoicing and payment

Maintaining communications with the laboratory is critical to identifying and resolving problems quickly and minimizing the need for resampling and reshipments. At a minimum, notify the laboratory of sample shipments the day you ship and confirm that the laboratory received the sample on time and in acceptable condition. You also can consider contacting the laboratory each week before you sample to verify that they know to expect samples.

Although most communications are typically conducted over the phone, these communications also can be conducted via email, which has the added benefit of providing your PWS and the laboratory with a written record of sample receipt confirmations, problem notifications, and problem resolutions.

SECTION 3: SAMPLING LOCATION AND SCHEDULE

Public Water Systems (PWSs) required to monitor under the LT2 Rule must meet specific requirements regarding where and when to sample, and must submit a sample location description and sample schedule. As noted previously, monitoring requirements for each system size and the schedule for each stage of monitoring are described in **Table 1-1**. This section provides guidance for meeting the requirements regarding submission of sampling location descriptions and sampling schedules as required by the rule.

3.1 Sample Collection Location and Description

LT2 Rule monitoring is intended to assess the mean *Cryptosporidium* level in the influent to drinking water plants that treat surface water or ground water under the direct influence (GWUDI) of surface water.

PWSs are required to collect source water samples for the LT2 Rule from each plant intake prior to chemical treatment, unless the State approves the system to collect a source water sample after chemical treatment [40 CFR § 141.703(b)(2)]. To grant this approval, the State must determine that collecting a sample prior to chemical treatment is not feasible for the system and that the chemical treatment is unlikely to have a significant adverse effect on the analysis of the sample [40 CFR § 141.703(b)]. PWSs that recycle filter backwash water must collect source water samples prior to the point of filter backwash water addition [40 CFR § 141.703(c)]. All *Cryptosporidium*, *E. coli*, and turbidity source water samples collected under LT2 Rule requirements must be collected prior to chemical treatment [40 CFR § 141.703(b)] and should be collected from the same sampling location.

Generally, monitoring is required for each plant that treats a surface water or GWUDI source. However, where multiple plants receive all of their water from the same influent (e.g., multiple plants draw water from the same pipe or intake) the State may approve one set of monitoring results to be used to satisfy the requirements for all plants [40 CFR § 141.703(a)].

PWSs must submit a description of their sampling location(s) at the same time as the sampling schedule required under this rule [40 CFR § 141.703(f)]. This description must address the position of the sampling location in relation to the system's water source(s) and treatment processes, including pretreatment, points of chemical treatment, and filter backwash water recycle.

Appendix D is provided as a worksheet to assist PWSs in describing proper sampling locations. Some of the information from **Appendix D** should be submitted with each sample and will be useful to know when determining sample location, such as

- **Public Water System Identification (PWSID)**
- **Water system facility identification.** This is the ID, usually assigned by the State, which uniquely identifies a water system facility. In the case of the LT2 Rule, this is an individual water treatment plant. If there is no State-generated or other number provided by the PWS, the DCTS will autogenerate one. The PWS can change this number in the DCTS if desired.

- **Source water sample collection point number.** This is a number, usually assigned by the State, which uniquely identifies a point within a water system facility from which the sample is collected. The DCTS uses this number to track sample results. If there is no State-generated or other number provided by the PWS, the DCTS will autogenerate one. The PWS can change this number in the DCTS if desired.

Appendix E provides 9 example sample location schematics to illustrate the correct sample collection location. A blank sample location schematic is also provided. A completed worksheet (**Appendix D**) accompanied by a sampling location schematic from **Appendix E** (a marked up version of an example schematic or a blank schematic (figure 10)) may be submitted to meet the sampling location description requirement.

Additional guidance on sampling at plants is provided below, in Sections 3.1.1 through 3.1.6.

3.1.1 Plants That Do Not Have a Sampling Tap Located Prior to Any Treatment

Plants in this situation may pursue one of the following options:

- Manually collect source water samples as close to the intake as is feasible, at a similar depth and distance from shore.
- Install a sampling tap prior to treatment.
- Collect a source water sample after chemical treatment only if it is determined by the State that collecting a sample prior to chemical treatment is not feasible and that the chemical treatment is unlikely to have a significant adverse effect on the analysis of the sample [40 CFR § 141.703(b)(2)].

3.1.2 Plants That Use Different Water Sources at the Same Time

This includes multiple surface water sources and blended surface water and ground water sources. The use of multiple sources during monitoring must be consistent with routine operational practice.

Plants in this situation should pursue one of the following options:

- If there is a sampling tap after the sources are combined but prior to treatment, the sample must be collected from the tap [40 CFR § 141.703(e)(1)].
- If a sampling tap located after the sources are combined but prior to treatment is not available, PWSs must collect samples at each source near the intake on the same day and must use one of the following options for sample analysis [40 CFR § 141.703(e)(2)]:
    - **Compositing**: Samples may be collected manually at each source near the intake on the same day and composited into one sample. The volume of sample from each source should be weighted according to the proportion of that source used by the plant. For example, if a plant has two sources and 75 percent of the drinking water is from Source A and 25 percent is from Source B, then for a 10 L sample, 7.5 L would be collected from Source A and combined with 2.5 L collected from Source B. Compositing of samples should reflect plant operation at the time the sample is collected and may change during the monitoring period.
    - **Weighted Average**: Separate samples may be collected manually at each source near the intake on the same day and analyzed independently. The individual results must then be used to calculate a weighted average of the analysis results. The weighted average must be calculated by multiplying the analysis result for each source by the fraction of the source contribution to total plant flow at the time the samples were collected, and then summing these values. For example, if a plant has two sources and 75 percent of the drinking water is from Source A and 25 percent is from Source B, then one sample would be collected from each source and analyzed independently. If the concentration of oocysts for the sample from Source A was 5 oocysts/L and

the concentration of the sample from Source B was 1 oocyst/L, the final result for the plant for this sampling event would be 4 oocysts/L ([5 oocysts/L x 0.75] + [1 oocyst/L x 0.25]).

3.1.3 Plants That Use Presedimentation

PWSs may collect samples after presedimentation if no chemical treatment (for example, coagulation) is used in or prior to the presedimentation basin. If chemical pretreatment is used, PWSs must collect samples prior to presedimentation or get permission from the State to sample after chemical treatment as described in Section 3.1. Use of presedimentation basins during monitoring should be consistent with routine operational practice and should be documented. However, if samples are collected after (i.e., downstream of) the presedimentation process, the PWS will not be eligible for *Cryptosporidium* treatment credit for the presedimentation process. In this case, the removal achieved by the presedimentation process will be reflected in the monitoring results and bin classification. PWSs that collect samples prior to the presedimentation basins may be eligible for additional treatment credit as described in 40 CFR § 141.717.

3.1.4 Plants That Use Raw Water Off-Stream Storage

For these plants, source water samples should be collected after the off-stream storage reservoir. Use of off-stream storage during monitoring should be consistent with routine operational practice and should be documented.

3.1.5 Plants That Use Bank Filtration

The correct sampling location for PWSs with plants using bank filtration differs depending on whether the bank filtered water is treated by subsequent filtration for compliance with the Interim Enhanced Surface Water Treatment Rule (IESWTR) [40 CFR § 141.703(d)].

- PWSs that receive *Cryptosporidium* treatment credit for bank filtration towards compliance with the IESWTR or the Long Term1Enhanced Surface Water Treatment Rule, as applicable, must take source water samples in the surface water prior to bank filtration [40 CFR § 141.703(d)(1)].
- PWSs that use bank filtration as pretreatment to a filtration plant must collect source water samples from the well (i.e., after bank filtration). Use of bank filtration during monitoring must be consistent with routine operational practice and should be documented. PWSs collecting samples after a bank filtration process may not receive treatment credit for the bank filtration under 40 CFR § 141.717(c)[40 CFR § 141.703(d)(2)].

3.1.6 Plants That Use Ground Water Under the Direct Influence of Surface Water

PWSs using a source of ground water under the direct influence of surface water must collect source water samples from the ground water (e.g., the well) unless they received credit for bank filtration as described above.

3.1.7 Submitting Sample Location Descriptions

Sample location descriptions will not be considered confidential business information and are subject to the Freedom of Information Act. Therefore, the submission should not contain information that poses a security risk to the system. The worksheet provided in **Appendix D** and the schematics provided in **Appendix E** are generic enough that they should not pose a security risk.

PWSs must submit the sampling location descriptions by the deadlines indicated in **Tables 1-2 through 1-5**. The sampling location descriptions should be submitted as directed in **Section 1.6** of this manual.

3.2 Source Water Sampling Schedule

PWSs required to conduct source water monitoring must submit a sampling schedule that specifies the calendar dates when the system will collect each required sample. PWSs are required to collect samples in accordance with the schedule, which should be established and agreed to by the PWS and the laboratory prior to submission. PWSs should contact the laboratory as early as possible to establish a mutually acceptable sampling schedule if they want to be sure that the laboratory can analyze samples on the desired dates. PWSs may collect samples more frequently than required (e.g., twice-per-month, weekly), provided the samples are evenly spaced throughout the monitoring period [40 CFR § 141.701 (a)(7)]. PWSs are required to collect samples within two days before or after the dates indicated in their sampling schedules [40 CFR § 141.702(b)].

The schedule should be entered using the scheduler function within the LT2/Stage 2 Data Collection and Tracking System (DCTS). Details on the use of the scheduler are provided in the *Users' Manual for the LT2/Stage 2 Data Collection and Tracking System (DCTS)*. The DCTS can be accessed at http://www.epa.gov/safewater/disinfection/lt2/. PWSs that are unable to submit their schedules electronically should contact their point of contact as indicated on the contact list at http://www.epa.gov/safewater/disinfection/lt2/compliance.html, at least one month prior to the deadline for submission.

3.2.1 Part-Year Plants

Water treatment plants that use surface water or ground water under the direct influence (GWUDI), but are operated only part of the year should follow these requirements [40 CFR § 141.701(e)]:

- PWSs must sample their source water only during the months that the plant operates, unless the State specifies another monitoring period based on plant operating practices.
- PWSs with plants that operate less than six months per year and that monitor for *Cryptosporidium* must collect at least six *Cryptosporidium* (plus *E. coli* and turbidity, if applicable) samples per year during each of the 2 years of monitoring. Samples must be evenly spaced throughout the monitoring period when the plant is operating.

3.2.2 Sample Collection Problems

Permissible exceptions to collecting samples on the dates specified in the sampling schedule are noted as follows:

- If extreme conditions or situations exist that may pose danger to the sampler, or which are unforeseen or cannot be avoided and which cause the system to be unable to sample in the required time frame, the PWS must sample as close to the scheduled date as feasible, unless the State approves an alternative sampling date. The PWS must submit an explanation for the delayed sampling date to EPA/the State concurrent with the shipment of the samples to the laboratory [40 CFR § 141.702(b)(1)]. Sample results and explanations should be submitted through the LT2/Stage 2 Data Collection and Tracking System.

- If a PWS is unable to report a valid analytical result for a scheduled sampling date due to equipment failure, loss of our damage to the sample, failure to comply with the analytical method requirements, including the quality control requirements in 40 CFR § 141.704 or the failure of an approved laboratory to analyze the sample, the PWS must collect a replacement sample. The PWS must collect the replacement sample not later than 21 days after receiving information that an analytical result cannot be reported for the scheduled date, unless the PWS demonstrates that collecting a replacement sample within this time frame is not feasible or EPA/the State approves an alternative resampling date. The PWS must submit an explanation for the delayed sampling date to EPA/the State concurrent with the shipment of the sample to the laboratory [40 CFR § 141.702(b)(2)].

Alternative sample collection dates should be timed so as not to coincide with another scheduled *Cryptosporidium* sample collection date.

Monitoring results with sample collection dates that do not comply with the schedule entered into the DCTS by the PWS before monitoring began will be flagged. If EPA approves the explanations for the missed sampling dates, the DCTS will automatically update the schedule to permit the analytical results to be submitted through the DCTS without qualification. The *Users' Guide for the LT2/Stage 2 Data Collection and Tracking System* provides more information on the procedures for reporting results in these circumstances.

3.2.3 Changing the Sampling Schedule

Before the sampling schedule deadline, if EPA or the State has not already approved the sampling schedule, the PWS can access the scheduler module of the DCTS any time to make changes to the schedule submitted. After the schedule has been approved, or after the deadline to submit the schedule, changes to the schedule can only be made through contacting your point of contact as indicated on the contact list at http://www.epa.gov/safewater/disinfection/lt2/compliance.html

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SECTION 4: COLLECTING AND SHIPPING SOURCE WATER SAMPLES

4.1 Sample Collection Guidance

During each of the scheduled sampling events, several actions should be performed in addition to collecting the sample. Guidance and procedures for each of these sample collection activities is provided in Sections 4.1.1 - 4.1.5, below.

4.1.1 Sample Collection Documentation

PWSs should be prepared to record the information in **Table 4-1** during sample collection to link the monitoring result to the plant, meet the data reporting requirements of the LT2 Rule, provide the laboratory with the information needed to meet holding times, and to provide information that will be used to refine the relationship between *Cryptosporidium* and *E. coli* levels.

For samples that are shipped offsite, this information should be documented on an LT2 sample collection form (**Appendix F**), or similar form provided by your contract laboratory. For samples analyzed onsite by your utility's laboratory, this information can be documented in a sampling log book or other standard form used by your utility; the LT2 sample collection form can also be used.

Sample collection personnel must select from four source water types on the LT2 sample collection form:

- Flowing stream (defined under the LT2 Rule as "a course of running water flowing in a definite channel")
- Reservoir/lake (defined under the LT2 Rule as "a natural or man made basin or hollow on the Earth's surface in which water collects or is stored that may or may not have a current or single direction of flow")
- Ground water under the direct influence (GWUDI) of surface water, with flowing stream as the nearest surface water body
- GWUDI with reservoir/lake as the nearest surface water body

The source water type should be selected based on the type of source water that accounts for *the majority of the surface water used as source water at the time of sample collection*. For example, if the plant uses a mix of approximately 55 percent reservoir/lake water and 45 percent flowing/stream water, the "reservoir/lake" option should be circled on the LT2 sample collection form.

For GWUDI systems, the selection of source water type is based on the type of surface water that is the nearest surface water body; if no surface water body is nearby, the PWS must select GWUDI with lake/reservoir [40 CFR § 141.701(a)(4)(iv)].

Cryptosporidium sample collection procedures are discussed in Section 4.1.2; *E. coli* sample collection procedures and turbidity measurement procedures are discussed in Section 4.1.4 and 4.4, respectively.

Table 4-1. Minimum Data Elements to Record During Sample Collection

Sampling Information	Required	Recommended
PWS name		✓
Public Water System Identification (PWSID) number [a]	✓	
Facility name		✓
Facility ID [a]	✓	
Sample collection point name		✓
Sample collection point ID		✓
Source water type [b]	✓	
Requested analysis		✓
Sample collection date [a]	✓	
Sample collection time (start and stop time for field-filtered samples) [c]		✓
Meter readings *(for field-filtered samples only)*		✓
Source water temperature		✓
Turbidity [d]	✓	

[a] The combination of these elements constitute the unique sample identifier for LT2 monitoring samples
[b] This information must be recorded with the *E. coli* sample collection information.
[c] Not required, but important for calculating holding time.
[d] This information must be recorded with the *E. coli* sample collection information for filtered PWSs serving ≥10,000 people.

4.1.2 *Cryptosporidium* Sample Collection

Several options are available to the PWS in collecting untreated surface water samples for *Cryptosporidium* analysis, including the following:

- On-site filtration of water samples using the Pall Gelman Envirochek™ or Envirochek™ HV capsule filter. A detailed protocol for filtering samples onsite from pressurized and unpressurized sources is provided as **Appendix G**.
- On-site filtration of water samples using the IDEXX Filta-Max® foam filter. A detailed protocol for filtering samples onsite from pressurized and unpressurized sources is provided as **Appendix H**.
- Collection of bulk water samples for shipment to the laboratory for filtration and analysis. A detailed protocol for collecting, packing, and shipping bulk samples is provided as **Appendix I**.

The sample must be eluted from the filter within 96 hours of sample collection, regardless of the procedure used to collect *Cryptosporidium* samples, per EPA Method 1622/1623 [40 CFR § 141.704]; additional information on the methods is provided in Section 8.2 of this manual. If this holding time is violated, the sample will be considered non-compliant and the laboratory will reject the sample. Your PWS will be required to recollect and reship the sample [40 CFR § 141.702(b)(2)].

LT2 Rule requirement: *Each sample must meet EPA Method 1622/1623 requirements, which include holding time requirements [40 CFR § 141.704(a)].*

4.1.2.1 Purchasing Filters

If one of the field filtration options is used, you may want to consider purchasing filters in bulk from the manufacturer (or the manufacturer's local distributor), as opposed to purchasing the filters from your *Cryptosporidium* contract laboratory as part of the sampling kit. The sampling kit contains tubing, flow meter and flow control valve etc. see appendices G and H for list of materials and equipment needed for sampling. This approach provides your PWS with a ready supply of extra filters onsite in case a filter clogs during a sampling event. Plants wishing to explore this option should call one of the contacts in **Table 4-2**.

Table 4-2. Contacts for Filters Approved for Using EPA Method 1622/1623

Pall Life Sciences (Envirochek™ and Envirochek™ HV capsule filters)	IDEXX (Filta-Max® foam filters)
www.pall.com/gelman 600 South Wagner Road Ann Arbor, MI 48103 Sales: Phone: (800) 521-1520 ext.2 Fax: (734) 913-6114 Technical Support: Phone: (800) 521-1520 ext.3 Fax: (734) 913-6114	www.idexx.com Sales: Phone: (800) 321-0207 ext.1 Fax: (207) 856-0630 Technical Support: Phone: (800) 321-0207 ext.2 Fax: (207) 856-0630 E-mail: water@idexx.com

Your PWS can also purchase and assemble the entire sampling kit and maintain this kit on site, rather than shipping it back and forth between the *Cryptosporidium* laboratory and the plant. If the filters you use have associated shelf lives and storage conditions, ensure that the filters are stored according to the manufacturers' directions and are not used past the specified shelf life.

The components and part numbers for the sampling kit are specified in the individual protocols for each filter. If the sampling kit is maintained onsite by the utility, the utility should use disposable materials wherever possible to mitigate the risk of cross-contamination between samples or sampling events, and should disinfect the non-disposable sampling equipment between uses.

Sampling kit cleaning may consist of the following:

- Cleaning equipment with warm detergent solution and exposing to hypochlorite solution (5 percent solution of fresh bleach and water) for at least 30 minutes at room temperature
- Rinsing the equipment with reagent water and placing the equipment in an area free of potential *Cryptosporidium* contamination until dry

4.1.2.2 *Cryptosporidium* Sample Volumes

Under the LT2 Rule *Cryptosporidium* sample volume requirements [40 CFR § 141.704(a)(1)], PWSs are required to analyze, at a minimum, either:

- 10 L of sample, *or*
- 2 mL of packed pellet volume, *or*

- As much volume as two filters can accommodate before clogging (this condition applies only to filters that have been approved by EPA for nationwide use with EPA Method 1622/1623—the Pall Gelman Envirochek™ and Envirochek™ HV filters, or the IDEXX FiltaMax® foam filter).

The LT2 Rule sample volume analysis requirement of 10 L (rather than 10.0 or 10.00 L) accommodates the potential for imprecisely filled sample containers or filters. Therefore, sample volumes of 9.5 L and higher would meet the rule requirements, and PWSs may analyze volumes larger than 10 L. Larger volumes analyzed should increase analytical sensitivity (detection limit) and representativeness, provided method performance is acceptable. EPA encourages PWSs to analyze similar sample volumes throughout the monitoring period. However, data sets including different samples volumes will be accepted provided the system meets the minimum sample volume requirements noted above.

PWSs with turbid source waters containing excessive particulates that interfere with filtration or sample purification are more likely to clog filters than PWSs with lower turbidity waters. If your PWS encounters highly turbid water regularly, or variable water quality that clogs the filter unpredictably, you should routinely bring two filters to the sampling point for each sampling event:

- If the water quality allows a full 10 L to be filtered without clogging, your PWS can simply ship the filter to the laboratory and save the remaining materials for subsequent events.
- If the first filter clogs after 5 L or more have been filtered, your PWS should be able to filter the remaining volume through the second filter and ship both filters to the laboratory for processing.

If two filters clog, be sure to document the volume passed through each filter. Although more expensive to ship, utilities likely to clog filters may want to consider sending the laboratory a 10 L bulk water sample for processing with centrifugation instead of filtration.

4.1.3 Matrix Spike Sample Collection

In addition to routine monitoring samples, matrix spike (MS) samples are also required by the LT2 Rule [40 CFR § 141.704(a)(2)]. An extra bulk water sample must be collected so that the laboratory can spike it with *Cryptosporidium* oocysts and filter it in the laboratory [40 CFR § 141.704(a)(2)(i)] to assess recovery in your PWS' source water matrix. Section 9.1.8 of EPA Method 1622/1623 specifies that MS samples be analyzed at a frequency of 1 MS per 20 routine monitoring samples.

For all PWSs, the first MS sample should be collected and analyzed during the first sampling event under the monitoring program, per Section 9.1.8 of EPA Method 1622/1623. If it is not possible to analyze an MS sample for the first sampling event (due to laboratory capacity or schedule, for example), the first MS sample should be analyzed as soon as possible to identify potential method performance issues with the matrix. The laboratory and the PWS may evaluate the MS recoveries, as well as other attributes of sample processing and examination with that particular site, and work together to determine whether sample filtration and processing procedures are working acceptably, or need to be re-evaluated. Matrix spike samples may be analyzed more frequently than one every 20 field (monitoring) samples to better characterize method performance in the matrix.

The MS frequency specified in EPA Method 1622/1623 (one at the first sampling event and one for every 20 field (monitoring) sample thereafter) translates to the following, for each plant category:

- For large PWSs that perform monthly monitoring for 2 years (resulting in 24 monitoring samples), two MS samples will need to be collected and analyzed
- For large PWSs that perform semi-monthly monitoring for 2 years (resulting in 48 samples), a minimum of three MS samples will need to be collected and analyzed
- For large PWSs that perform weekly monitoring for 2 years (resulting in 104 samples), a minimum of six MS samples will need to be collected and analyzed

- For small PWSs that are triggered into *Cryptosporidium* monitoring and collect semi-monthly samples for 1 year or monthly samples for 2 years (resulting in 24 samples), two MS samples must be collected and analyzed

As specified in Section 9.5 of EPA Method 1622/1623, the MS sample volume analyzed must be within 10 percent of the volume analyzed for the associated monitoring sample. If the volume of the MS sample is greater than 10 L, the system is permitted to filter all but 10 L of the MS sample in the field, and ship the filtered sample and the remaining 10 L of source water to the laboratory to have the laboratory spike the remaining 10 L of water and filter it through the filter used to collect the balance of the sample in the field [40 CFR § 141.704(a)(2)(ii)].

Utilities should split their sample stream and collect the monitoring sample volume and MS sample volume simultaneously:

- The sample stream should be split using flow controllers on both sides of the split to regulate the pressure difference between the side being subjected to filtration (resulting in higher pressure) and the side flowing into a bulk sample container
- If splitting the sample stream is not practical, the utility should collect the MS sample immediately before or after the monitoring sample

MS sample results will not be used to adjust *Cryptosporidium* recoveries at any individual source water; the MS results will be used collectively to assess overall recovery and variability for EPA Method 1622/1623 in source water. No resampling will be necessary for MS samples that do not meet Method 1622/1623 recovery guidelines.

LT2 Rule requirements: *Each sample must meet EPA Method 1622/1623 requirements [40 CFR § 141.704(a)], which include the following: (1) The MS and field (monitoring) sample must be collected from the same sampling location by splitting the sample stream or collecting the samples sequentially (method Section 9.5.1). (2) The volume of the MS sample analyzed must be within 10% of the volume of the field (monitoring) sample analyzed (method Section 9.5.1). (3) The MS and field (monitoring) sample must be analyzed by the same procedure (method Section 9.5.1).*

4.1.4 *E. coli* Sample Collection

For most large PWSs, *E. coli* analyses will be conducted onsite and samples will not be shipped. However, many small PWSs will collect *E. coli* samples and ship them offsite for analysis. Regardless of whether the samples are analyzed by the utility's own laboratory or by a commercial laboratory, laboratories analyzing *E. coli* samples for the LT2 Rule must use an *E. coli* method approved for use under the rule and must be certified for total coliform or fecal coliform analysis that uses the same technique as the *E. coli* method the laboratory plans to use for LT2 Rule monitoring [40 CFR § 141.704(b) and 141.705(b)]. Approved *E. coli* methods and the drinking water certification program are provided in the *Microbial Laboratory Guidance Manual for the Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule)*. Information on these methods is also provided in **Table 7.1** of this document.

PWSs monitoring for *E. coli* under the LT2 Rule should collect and analyze at least 100 mL of sample to ensure sufficient volume for sample analysis. If spillage or leakage occurs during shipment, the sample may have become contaminated, and the sample should not be analyzed (see Section 7.4.1). Collect *E. coli* samples in sterile, non-toxic, plastic, or glass containers with a leak-proof lid. The capacity of sample containers should be at least 120 mL (4 oz.) or 250 mL (8 oz.) to allow for sufficient sample volume and at least a 1-inch head space to facilitate mixing of the sample by shaking prior to analysis. A detailed protocol for collecting source water samples for *E. coli* analysis, as well as packing and shipping guidance for utilities that transport samples offsite for analysis, is provided as **Appendix J**.

EPA encourages laboratories to analyze samples as soon as possible after collection. *E. coli* samples must be analyzed within 30 hours of sample collection [40 CFR § 141.704(b)(1)]. If the State determines that it is not feasible for the sample to analyze the sample within the 30-hour holding time, the State may authorize the holding time to be extended to 48 hours but only for the Colilert method. The holding time can be extended to 48 hours only when authorized by the State, and is done on a case-by-case basis [40 CFR § 141.704(b)(2)]. ***Note:*** This is a longer time period than currently permitted for analysis of samples under the Surface Water Treatment Rule.

E. coli samples should be maintained between 0°C and 10°C by storing in a refrigerator or in a cooler with wet ice, blue ice, or gel packs, etc. [40 CFR § 141.704(b)(3)]. Additional guidance on monitoring sample temperature is available in Section 4.2 of this manual.

4.2 Sample Temperatures

Source water samples are dynamic environments and, depending on sample constituents and environmental conditions, *Cryptosporidium* oocysts present in a sample can degrade and *E. coli* present in a sample can grow or die off, biasing analytical results. To reduce biological activity and preserve the state of source water between collection and analysis, samples should be chilled. However, freezing is also a concern for *Cryptosporidium* filters and 120 mL or 250 mL *E. coli* samples that are shipped offsite with coolant materials, such as wet ice, blue ice, or gel packs. Samples can freeze under these conditions if not packed properly.

Upon receipt, the laboratory must record the sample temperature and reject *Cryptosporidium* samples that are received at >20°C or frozen, or *Cryptosporidium* samples that the laboratory has determined exceeded 20°C or froze during shipment [Section 8.1.3 of EPA Method 1622/1623]. *E. coli* samples that are received at >10°C or frozen, or *E. coli* samples that the laboratory has determined exceeded 10°C or froze during shipment, must also be rejected.

After receipt, *Cryptosporidium* samples must be stored at the laboratory between 1°C and 10°C, and not frozen, until processed (Section 8.1.3 of EPA Method 1622/1623) [40 CFR § 141.704(a)]. *E. coli* samples must be stored at the laboratory between 0°C and 10°C, and not frozen, until processed [40 CFR § 141.704(b)(3)].

The following steps should help maintain acceptable temperatures:

- If *Cryptosporidium* and *E. coli* samples are collected early in the day, chill samples by storing in a refrigerator between 1°C and 10°C or pre-icing the sample in a cooler. If the sample is pre-iced before shipping, replace with fresh ice immediately before shipment.
- If *Cryptosporidium* samples are collected later in the day, these samples may be chilled overnight in a refrigerator between 1°C and 10°C. Overnight refrigeration is recommended for bulk water *Cryptosporidium* samples that will be shipped offsite, as this minimizes the potential for water samples collected during the summer to melt the ice in which they are packed and arrive at the laboratory at >20°C.

The sample collection procedures in Sections 4.1.2 through 4.1.4 and **Appendices G, H, I, and J** provide sample packing procedures for the various ways of collecting *E. coli* and *Cryptosporidium* samples, as well as guidance on packing samples to maintain appropriate temperatures. Utility personnel should follow these procedures to ensure that samples remain at acceptable temperatures during shipment. Several options are available to measure sample temperature upon receipt at the laboratory and, in some cases, during shipment:

- **Temperature sample.** One option, for *Cryptosporidium* filtered samples (not for 10 L bulk samples) and *E. coli* 120 and 250 mL samples, is for the PWS to fill a small, inexpensive sample bottle with water and pack this "temperature sample" next to the field (monitoring) sample. The temperature of this extra sample volume is measured upon receipt to estimate the temperature of the field (monitoring) sample. Temperature sample bottles are not appropriate for use with 10 L bulk samples because of the potential effect that the difference in sample volume may have in temperature equilibration in the sample cooler. Example product: Cole Parmer cat. no. C-06252-20 or equivalent.

- **Temperature vial.** A similar option is to use a thermometer that is securely housed in a liquid-filled vial. Temperature vials are not appropriate for use with 10 L bulk samples for the reasons stated above. Unlike temperature samples, the laboratory does not need to perform an additional step to monitor the temperature of the vial upon receipt, but instead just reads the thermometer. Example product: Eagle-Picher Sentry Temperature Vial 3TR-40CS-F or 3TR-40CS or equivalent.

- **iButton.** Another option for measuring the sample temperature during shipment and upon receipt is a Thermocron® iButton. An iButton is a small, waterproof device that contains a computer chip to record temperature at different time intervals. The information is then downloaded from the iButton onto a computer. The iButton should be placed in a temperature sample in the cooler rather than placed directly in the cooler, where it may be affected by close contact with the coolant. Again, this option is not appropriate for use with 10 L bulk samples. Example product: Thermocron® iButtons or equivalent.

All temperature measurement devices should be calibrated routinely, where possible, to ensure accurate measurements. See the U.S. EPA *Manual for the Certification of Laboratories Analyzing Drinking Water* (Reference 8.5) for more information.

4.3 Measuring Turbidity

Filtered PWSs collecting samples for *Cryptosporidium* analysis must measure the turbidity of the source water at the time of *Cryptosporidium* and *E. coli* sample collection during LT2 Rule monitoring [40 CFR § 141.701(a)]. Turbidity must be measured by a party approved by the State [40 CFR § 141.705(c)] using approved methods for turbidity measurement [40 CFR § 141.74(a)(1) and 40 CFR § 141.704(c)]. These methods include:

- Method 2130B, published in *Standard Methods for the Examination of Water and Wastewater* (19th or 20th Edition) (Reference 8.6)
- GLI Method 2, "Turbidity," November 2, 1992, Great Lakes Instruments, Inc., 8855 North 55th Street, Milwaukee, Wisconsin 53223
- EPA Method 180.1, "Methods for the Determination of Inorganic Substances in Environmental Samples," EPA/600/R-93/100, August 1993; available at NTIS, PB94-121811
- Hach FilterTrak Method 10133; a description of the Hach FilterTrak Method 10133, "Determination of Turbidity by Laser Nephelometry," January 2000, Revision 2.0, can be obtained from: Hach Co., P.O. Box 389, Loveland, Colorado 80539-0389. Phone: 800-227-4224

Many States have designated certified operators and professional engineers as approved parties for turbidity measurement. If you are not sure about your State's requirements, please contact your State drinking water program representative.

4.3.1 Measuring Sample Turbidity During LT2 Monitoring

When measuring turbidity, cuvettes should be clear, colorless glass or plastic. The tube should be kept clean, both inside and out, to provide accurate readings. If a sample tube is scratched, it must be discarded.

- Measuring Sample Turbidity Using SM 2130B. Measure turbidity immediately after sample collection to prevent temperature changes, particle flocculation, and sedimentation from changing sample characteristics. Shake sample well before pouring into cuvette. Gently agitate to remove air bubbles from the inside of the sample before pouring the sample into cell. Wait until all the air bubbles disappear and remove all moisture from the outside of the sample cell before placing it into the instrument. If fogging occurs, warm the sample cell in a warm water bath for a short time, then re-agitate the sample before placing it in the turbidimeter. Read turbidity directly from instrument display. *Note: Measurements should be within the calibration range.*
- Measuring Sample Turbidity Using GLI Method 2 or Revised EPA Method 180.1. Different procedures should be followed, depending on the turbidity of the sample:
  - For turbidities estimated to be less than 40 NTU. Shake the sample thoroughly to disperse the solids. After waiting for the air bubbles to disappear pour the sample into the turbidimeter tube and read directly from the instrument scale.
  - For turbidities estimated to be greater than 40 NTU. Dilute the sample with turbidity-free water and compute the turbidity with the dilution factor included.

4.3.2 General Quality Control for Turbidity Measurements

Utilities performing environmental sample measurements must be approved by the State (or EPA Region, for States that do not have primacy) under the drinking water laboratory certification program [40 CFR § 141.705(c)]. Each utility laboratory should operate a formal quality control (QC) program and maintain performance records that define the quality of the data generated. Two types of calibration are recommended for turbidity measurements:

- A primary suspension standard. The primary suspension standard should be used to calibrate the turbidimeter initially and at least every four months in order to prevent instrument drift. The calibration should be documented. The standards should be replaced when they exceed the expiration date. Acceptable primary suspensions include Formazin (recipes for preparation can be found in EPA Method 180.1 and Standard Method 2130B), AMCO CLEAR (available from GFS Chemicals), and Hach StablCal Stabilized Formazin Standards (available from Hach Company). Please note that Formazin standards are relatively unstable, particularly at low concentrations. Therefore, dilutions used for calibration need to be prepared on the day they will be used. Stock solutions may be stable for a month (at 400 NTU) to one year (at 4000 NTU). Consult an approved method for more information.
- A secondary suspension standard. The secondary suspension standard is used daily to check the calibration of the instrument. The calibration should be documented, and should not vary by more than 10% from the initial calibration values (if they do vary by more than 10%, the system should be recalibrated so that performance is acceptable). The standards should be replaced when they exceed the expiration date. Acceptable secondary standards include all primary standards, or other materials that are suggested by instrument manufacturers – such as sealed sample cells filled with a labeled suspension or metal oxide particulates in a polymer gel, or a turbid glass cube. The purpose of the secondary standard is to provide a quick check of calibration. The secondary standards should have a fixed turbidity that does not vary from use to use.

SECTION 5: GRANDFATHERING *CRYPTOSPORIDIUM* DATA

"Grandfathered" *Cryptosporidium* data are results generated before monitoring begins as required under the LT2 Rule and that a PWS intends to use in determining its bin classification (Section 6.1.1) or mean *Cryptosporidium* level under the rule. Grandfathered data may be used in lieu of, or in addition to, results generated for the LT2 Rule initial round of monitoring [40 CFR § 141.707]. This section of the manual is designed to assist PWSs in producing and reporting grandfathered data that should be equivalent to the data collected during LT2 Rule initial round of monitoring and therefore eligible for use in bin classification or establishing a mean *Cryptosporidium* level.

5.1 Intent to Grandfather

If your PWS will submit grandfathered data, you must first report that you intend to submit previously collected monitoring results (**Appendix K**). This report must specify the number of previously collected results that you will submit, the dates of the first and last sample, and whether your PWS will collect additional source water monitoring samples to meet the requirements of [40 CFR 141.701(a)]. PWSs must report this information no later than the date the PWS is required to submit a schedule for sampling under the LT2 Rule, as described in **Tables 1-2** through **1-5** [40 CFR § 141.707(f)(1)]. The information must be submitted as indicated in Section 1.6 of this manual.

If your PWS plans to collect samples in addition to the grandfathered data, you must also submit a monitoring schedule as specified in **Tables 1-2** through **1-5** and Section 3.2 of this guidance manual.

5.2 Requirements for Grandfathered *Cryptosporidium* Data

To be eligible for grandfathering, previously collected *Cryptosporidium* monitoring must meet the requirements in section 141.707 of the LT2 rule and the State must approve of the grandfathering. If the previously collected data does not meet requirements, it may not be approved. **Appendix L** provides a checklist for PWSs that plan to begin collecting data for grandfathering. The remainder of this section provides information on the grandfathering requirements.

5.2.1 Sample Collection Location and Volume

The sampling location for grandfathered samples must meet the same location requirements as those that apply to new sampling conducted under the LT2 Rule [40 CFR § 141.707(d)]. These sample location criteria are described in Section 3.1 of this guidance. The samples must be representative of a plant's source water(s) and the source water(s) must not have changed since the samples were collected [40 CFR § 141.707(f)(2)(iii)].

PWSs must provide a description of the sampling location that addresses the position of the sampling location in relation to the water source(s) and treatment processes, including points of chemical addition and filter backwash recycle [40 CFR § 141.707(f)(2)(iii)]. PWSs may submit **Appendix D** (Sample Location Worksheet) and one of the schematics in **Appendix E** with the grandfathered date package to meet this requirement.

Samples collected for grandfathering must meet the same volume analysis requirements as for LT2 Rule monitoring [40 CFR § 141.707(c)(2)]. These requirements are described in Section 4.2 of this manual.

5.2.2 Sample Collection Frequency and Schedule

Cryptosporidium samples submitted for grandfathering must have been collected no less frequently than each calendar month on a regular schedule, beginning no earlier than January 1999 (when EPA Method 1622 was first released as an interlaboratory-validated method) [40 CFR § 141.707(e)]. Sample collection intervals may vary for the conditions described in Section 3.2.2 of this manual. The PWS must provide documentation of the conditions that caused the variability in sampling when reporting the grandfathered results.

Before beginning to monitor PWSs should develop a schedule listing the calendar date on which each *Cryptosporidium* sample will be collected, and include this schedule when submitting the grandfathered data package. If your PWS has been monitoring without an established sampling schedule, you should develop a schedule that summarizes previously collected sampling and specifies the dates for the collection of remaining samples. PWSs should collect remaining samples within 2 days before or after the dates indicated in their sampling schedules unless they can document the conditions in Section 3.2.2 of this manual as indicated above. PWSs that will collect additional samples on or after the applicable beginning date for required monitoring [40 CFR § 141.701(c) and Section 1 of this manual] should submit a schedule for the additional samples so that the laboratory can submit sample results through the DCTS.

5.2.3 *Cryptosporidium* Analytical Methods for Grandfathered Data

The LT2 rules requires methods 1622 or 1623 for analysis of grandfathered *Cryptosporidium* monitoring [40 CFR § 141.707(c)(1)]. The procedures in EPA Methods 1622/1623 are performance-based and allow for modifications, if the laboratory was approved to use the modified procedures under the Lab QA Program discussed in Section 5.2.3. The following are acceptable versions of Methods 1622 and 1623 for grandfathered monitoring data collected before the LT2 Rule effective date of March 6, 2006:

- *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA.* U.S. Environmental Protection Agency, EPA-815-R-05-002. 2005.
- *Method 1622: Cryptosporidium in Water by Filtration/IMS/FA.* U.S. Environmental Protection Agency, EPA-815-R-05-001. 2005.
- *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA.* U.S. Environmental Protection Agency, Office of Water. 2003. *(**Note:** This was the proposed version of the 2005 method, which was available via the EPA website)*
- *Method 1622: Cryptosporidium in Water by Filtration/IMS/FA.* U.S. Environmental Protection Agency, Office of Water. 2003. *(**Note:** This was the proposed version of the 2005 method, which was available via the EPA website)*
- *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA.* U.S. Environmental Protection Agency, Office of Water. EPA-821-R-01-025. 2001.
- *Method 1622: Cryptosporidium in Water by Filtration/IMS/FA.* U.S. Environmental Protection Agency, Office of Water. EPA-821-R-01-026. 2001.

- *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA.* U.S. Environmental Protection Agency, Office of Water. EPA-821-R-99-006. 1999.
- *Method 1622: Cryptosporidium in Water by Filtration/IMS/FA.* U.S. Environmental Protection Agency, Office of Water. EPA-821-R-99-001. 1999.

On and after March 6, 2006 (the effective date of the LT2 Rule), laboratories should use the 2005 version of the methods or the PWS may run the risk of the State rejecting the data.

5.2.4 *Cryptosporidium* Laboratories for Grandfathered Data

EPA has established the Laboratory Quality Assurance Evaluation Program for the Analysis of *Cryptosporidium* in Water (Lab QA Program) to approve laboratories to perform *Cryptosporidium* analyses under the LT2 Rule. EPA recognizes that some PWSs could begin generating grandfathered *Cryptosporidium* data prior to when the Lab QA Program is fully implemented (e.g., before EPA is able to evaluate all laboratories that will participate in the program). Consequently, PWSs should ensure that their grandfathered *Cryptosporidium* samples are analyzed by laboratories that are approved or will be seeking approval under the Lab QA Program. Data from samples analyzed by laboratories that do not meet the criteria for approval under the LT2 Rule may not be accepted for grandfathering. Information about the Lab QA program and a list of currently approved laboratories can be found at http://www.epa.gov/safewater/disinfection/lt2/lab_home.html.

5.2.5 *E. coli* and Turbidity Measurements

Filtered PWSs may grandfather *Cryptosporidium* samples to meet the requirements of § 141.701(a) when the system does not have corresponding *E. coli* and turbidity samples [40 CFR § 141.707(a)(2)]. However, EPA continues to recommend that filtered PWSs conducting early (i.e., grandfathered) *Cryptosporidium* monitoring also collect and analyze *E. coli* samples with each *Cryptosporidium* sample and measure turbidity during each sampling event.

E. coli samples analyzed for grandfathering must meet the analytical method and approved laboratory requirements in §§ 141.704 and 141.705 of the LT2 rule, which are described in Section 7 of this guidance.

5.3 Checklists for Grandfathering *Cryptosporidium* Data

To help PWSs interested in using grandfathered *Cryptosporidium* data (or in monitoring before your scheduled start date), two checklists have been developed. The "Checklist for Beginning Grandfathered *Cryptosporidium* Monitoring" (**Appendix L**) is designed to be used by PWSs to check their intended monitoring approach before proceeding with monitoring. The "Grandfathered *Cryptosporidium* Data Submittal Report" (**Appendix M**) is designed to be used by PWSs to check their data package when submitting grandfathered monitoring data for review.

5.4 Reporting Grandfathered Data

PWSs must submit previously collected monitoring results for grandfathering along with all required documentation described below no later than two months after the date the system is required to start monitoring. [40 CFR § 141.707(f)(2)]. The documentation may be submitted with the notice of intent to

Grandfather (Section 5.1) which is required no later than 3 months prior to the date the system is required to start monitoring. Section 1.6 of this guidance provides information regarding where to send

grandfathered data packages. **Appendix M** contains a checklist that PWSs can use to assure that their grandfathered data package is complete. The information to be submitted is described below.

5.4.1 Grandfathered Data Package Contents

The grandfathered data package should include the following [40 CFR § 114.707(f)(2)]:

- A signed cover letter from the PWS certifying that the data represent the plant's current source water and that all source water *Cryptosporidium* monitoring results generated during the time period beginning with the first reported result and ending with the final reported result are included in the package (See Section 5.3.1.1 for details)
- Sample collection schedule (recommended)
- Sampling location description
- Where applicable, documentation addressing the dates and reason(s) for re-sampling, as well as the use of presedimentation, off-stream storage, or bank filtration during monitoring
- A list of *Cryptosporidium* field (monitoring) and MS samples submitted in the data package (see Section 5.3.1.1, below, for details), identified by sample ID and collection date
- *Cryptosporidium* sample results for all field (monitoring) and MS samples (see Section 5.3.1.2, below, for details) and
- Documentation that all method-required quality control requirements were acceptable for every *Cryptosporidium* field (monitoring) and MS sample submitted with the package (see Section 5.3.1.3, below, for details).

5.4.1.1 Sample Results to be Reported

PWSs that conduct monitoring for grandfathering must submit results for all source water *Cryptosporidium* samples generated during the time period beginning with the first reported result and ending with the final reported result [40 CFR 14.707(f)(2)(ii)]. This will include all samples that were:

- Collected from the sampling location designated for LT2 Rule monitoring
- Not spiked
- Analyzed using the laboratory's routine process for Method 1622/1623 analyses, including analytical technique and QA/QC.

For example, if your PWS monitored monthly, but collected additional samples periodically from the same monitoring location and analyzed them using the same procedure as for the routine monitoring samples, you must include these results with your grandfathered data.

Sample results generated after the last sample result in the PWS's data package are considered outside the PWS's LT2 Rule monitoring period and need not be submitted to EPA for LT2 Rule binning purposes. However, these results may be subject to reporting requirements under other federal or State regulations.

The grandfathered data set must also include matrix spike (MS) samples at the required frequency, one MS sample for every 20 field (monitoring) samples. The requirements for analysis of MS samples are the same for LT2 Rule monitoring and for grandfathered data [40 CFR § 141.707(f)(2)(i)]. These requirements, and guidance on MS sample collection, are discussed in Section 4.1.3 of this manual.

5.4.1.2 Data Elements to be Reported for Each Sample Result

The following data elements, at a minimum, must be submitted for each *Cryptosporidium* monitoring sample and MS sample [40 CFR § 141.707(f)(2)(i)]:

- PWS ID
- Facility ID
- Sample collection date
- Sample type (field (monitoring) or MS)
- Sample volume filtered (L), to nearest ¼ L
- Was 100% of filtered volume examined?
- Number of oocysts counted
- For matrix spike samples, PWSs must also report the sample volume spiked and estimated number of oocysts spiked. These data are not required for field (monitoring) samples.
- For samples in which less than 10 L is filtered or less than 100% of the sample volume is examined, PWSs must also report the number of filters used and the packed pellet volume.
- For samples in which less than 100% of sample volume is examined, PWSs must also report the volume of resuspended concentrate and volume of this resuspension processed through immunomagnetic separation.

EPA recommends that these data elements be reported by submitting a completed sample collection form, laboratory bench sheet, and *Cryptosporidium* slide examination form for each sample. Example bench sheets and Crypto Slide Examination Forms are provided as attachments in the *Microbial Laboratory Guidance Manual for the Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule)*, available for download from http://www.epa.gov/safewater/disinfection/lt/compliance.html. Sample documentation forms that are different from these examples, but that contain the minimum required data elements listed above, may be acceptable.

5.4.1.3 Supporting Quality Control Information

The grandfathered data package must include a signed letter from the laboratory certifying that all method-required quality control elements (including sample temperature upon receipt, ongoing precision and recovery (OPR) and method blank results, holding times, and positive and negative staining controls) were performed at the required frequency, and were acceptable for every monitoring and MS sample submitted with the package. The letter may include a list of the applicable monitoring and MS samples, and the corresponding OPR and method blank sample ID for each. [40 CFR § 141.707(f)(2)(iv)].

Alternately, the PWS may include the bench sheet and *Cryptosporidium* slide examination form (or comparable detailed data reporting forms) for each OPR and method blank sample associated with the field (monitoring) and MS samples in the grandfathered data package. If this option is selected, the letter

from the laboratory should certify that sample temperature upon receipt, holding times, and positive and negative staining controls were acceptable for all samples. (The letter is not necessary if detailed data reporting forms containing this information are submitted for the field (monitoring) and MS sample results.)

5.5 Rejected or Missing *Cryptosporidium* Data

The State may approve grandfathering of previously collected data where there are time gaps in the sampling frequency if the system conducts additional monitoring as specified by the State to ensure that the data used to comply with the initial source water monitoring requirements of 40 CFR § 141.701(a) are seasonally representative and unbiased [40 CFR § 141.707(e)(1)].

If some of the data that a PWS submits are rejected because they do not meet the requirements for grandfathering, the PWS must conduct additional monitoring to replace rejected data on a schedule that the State approves. The PWS is not required to begin this additional monitoring until two months after notification that data has been rejected and additional sampling is necessary [40 CFR § 141.707(h)].

If the State determines that a previously collected data set was generated during source water conditions that were not normal for the PWS, such as a drought, the State may disapprove the data. Alternatively, the State may approve the previously collected data if the system reports additional source water monitoring, as determined by the State, to ensure that the data set represents average source water conditions for the PWS [40 CFR § 141.707(g)(1)].

If the *Cryptosporidium* sampling frequency varies from month to month, and the monitoring results are approved, the monthly averaging procedure in 40 CFR § 141.710(b)(5) or 40 CFR § 141.712(a)(3), as applicable, must be used to calculate the bin classification for filtered PWSs or the mean *Cryptosporidium* concentration for unfiltered PWSs [40 CFR § 141.707(e)(2)]. This procedure is described in Section 6.1.1.1 of this manual.

SECTION 6: *CRYPTOSPORIDIUM* DATA: USE, RECORDING, SUBMITTING AND REVIEW

When *Cryptosporidium* samples are processed and analyzed by the laboratory, data on sample measurements, sample processing times, and slide examination results are recorded at the laboratory on the bench sheet and reported to the PWS through the LT2/Stage 2 Data Collection and Tracking System (section 6.3). This data may also be reported to the PWS via hardcopy forms as indicated in the contract agreement. This section provides an overview of the data recording and reporting processes and describes the significance of the examination results reported by the laboratory. This information is discussed in more detail in the *Microbial Laboratory Guidance Manual for the Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule)*.

6.1 Use of *Cryptosporidium* Data

Two types of *Cryptosporidium* data are collected under the LT2 Rule: *Cryptosporidium* occurrence data from the analysis of monitoring (field) samples, and method performance data from the analysis of matrix spike (MS) samples. The use of occurrence data from monitoring samples is discussed in Sections 6.1.1 and 6.1.2; the use of method performance data from MS samples is discussed in Section 6.1.3.

6.1.1 Determining Bin Classification – Filtered Systems

Filtered PWSs monitoring for *Cryptosporidium* will use the concentration of *Cryptosporidium* oocysts in source water samples analyzed under the LT2 Rule to calculate a mean *Cryptosporidium* bin concentration for each treatment plant for which monitoring was required. The mean *Cryptosporidium* bin concentration is then used to classify each treatment plant into a treatment requirements "bin" [40 CFR § 141.710]. These bin classifications are provided in **Table 6-1** for filtered PWSs. The treatment bin classifications established for filtered PWSs are used to determine whether additional treatment is needed. PWSs in Bin 1 are not required to implement additional treatment; PWSs in Bins 2 - 4 will be required to implement increasing levels of treatment [40 CFR § 141.711].

Table 6-1. Bin Classifications for Filtered PWSs

***Cryptosporidium* Bin Concentration**	**Bin Classification**
Cryptosporidium < 0.075 oocysts/L	Bin 1
0.075 oocysts/L ≤ *Cryptosporidium* < 1.0 oocyst/L	Bin 2
1.0 oocyst/L ≤ *Cryptosporidium* < 3.0 oocysts/L	Bin 3
Cryptosporidium ≥ 3.0 oocysts/L	Bin 4
PWSs that serve fewer than 10,000 people and NOT required to monitor for *Cryptosporidium* [a]	Bin 1

[a] Filtered PWSs serving fewer than 10,000 people are not required to monitor for *Cryptosporidium* if they monitor for *E. coli* and demonstrate a mean concentration of *E. coli* less than or equal to 10 *E. coli*/100 mL for lake/reservoir sources or 50 *E. coli*/100 mL for flowing stream sources or do not exceed an alternative State-approved indicator trigger [40 CFR § 141.701(a)(4) and § 141.710(c)].

6.1.1.1 Calculating Bin Concentrations

The method used to average individual sample concentrations to determine a PWS's bin classification depends on the number of samples collected and the length of the sampling period. For filtered PWSs monitoring for *Cryptosporidium* under the LT2 Rule, bin concentrations must be calculated as follows:

- For PWSs that collect at least 48 samples during the required monitoring period, the *Cryptosporidium* bin concentration is equal to the arithmetic mean of all sample concentrations [40 CFR § 141.710(b)(1)]
- For PWSs that collect at least 24 samples, but not more than 47 samples, during the required monitoring period, the *Cryptosporidium* bin concentration is equal to the highest arithmetic mean of all sample concentrations in any 12 consecutive months in the monitoring period [40 CFR § 141.710(b)(2)]
- For PWSs serving fewer than 10,000 people and monitoring *Cryptosporidium* for only one year (i.e., collect 24 samples in 12 months), the bin concentration is equal to the arithmetic mean of all sample concentrations [40 CFR § 141.710(b)(3)]
- For PWSs with plants operating only part of the year that monitor fewer than 12 months per year, the bin concentration is equal to the highest arithmetic mean of all sample concentrations during any year of *Cryptosporidium* monitoring [40 CFR § 141.710(b)(4)]
- For filtered PWSs in which sampling frequency varies, PWSs must first calculate a monthly average for each month of monitoring. The PWS must then use these monthly average concentrations, rather than individual sample concentrations, in the applicable calculation for bin classification [40 CFR § 141.710(b)(5)].

The *Cryptosporidium* bin concentration is then used with the table above to determine the bin classification.

6.1.2 Determining Mean *Cryptosporidium* Levels – Unfiltered Systems

The level of *Cryptosporidium* inactivation unfiltered PWSs must provide is based on mean *Cryptosporidium* levels. Unfiltered PWSs with a mean *Cryptosporidium* level of ≤0.01 oocysts/L must provide at least 2-log *Cryptosporidium* inactivation. If the mean *Cryptosporidium* level is >0.01oocysts/L, the unfiltered system must provide at least 3-log *Cryptosporidium* inactivation [40 CFR § 141.712(b)].

Unfiltered PWSs must calculate the arithmetic mean of all *Cryptosporidium* samples concentrations [40 CFR § 141.712(a)(1)]. For unfiltered PWSs in which sampling frequency varies, PWSs must first calculate a monthly average for each month of monitoring. PWSs must then use these monthly average concentrations, rather than individual sample concentrations, in the calculation of the mean *Cryptosporidium* level [40 CFR § 141.712(a)(3)].

6.1.3 *Cryptosporidium* Matrix Spike Data

During LT2 Rule *Cryptosporidium* monitoring, PWSs are required to collect one matrix spike (MS) sample for every 20 monitoring samples from their source water starting with the first sampling event or as soon as possible, per the requirements in EPA Methods 1622/1623 (Section 9.1.8, per EPA Method 1622/1623). For PWSs that perform monthly *Cryptosporidium* monitoring for two years and collect 24 monitoring samples and for small PWSs that are triggered into *Cryptosporidium* monitoring and collect 24 monitoring samples, two MS samples will be analyzed. For large PWSs that perform semimonthly or more frequent monitoring for two years and collect 48 or more samples, a minimum of three MS samples will be analyzed.

Although MS sample results will not be used to adjust *Cryptosporidium* recoveries at any individual source water, the results will be used collectively to assess overall recovery and variability for EPA Method 1622/1623 in source water.

6.2 *Cryptosporidium* Data Recording at the Laboratory

The *Cryptosporidium* laboratory may record LT2 Rule monitoring data using the following standardized forms:

6.2.1 LT2 Sample Collection Form

This form (an example of which is provided as **Appendix F**) is initiated at the plant upon sample collection and is completed at the laboratory. The following information is recorded on this form by the *Cryptosporidium* laboratory:

- Date and time of sample receipt
- Laboratory personnel receiving the sample
- Sample temperature upon receipt
- Sample condition upon receipt

Although none of this information is entered into the LT2/Stage 2 Data Collection and Tracking System, it provides documentation for the utility, the laboratory, and EPA or State officials on sample receipt information relevant to LT2 Rule requirements regarding sample temperatures and sample holding times.

6.2.2 Method 1622/1623 Bench Sheet

The laboratory uses the bench sheet to record all information associated with sample processing, up to, but not including, sample examination. Information on filtration (if performed in the laboratory), elution, concentration, immunomagnetic separation, and sample staining are documented on this form. These data include:

- Dates and times for all steps associated with method-required holding times
- All primary measurements used to calculate sample volume analyzed, if less than 100% of the volume filtered was analyzed. This information includes the following:
  - The volume of the sample after the concentrate (packed pellet) has been resuspended
  - The volume of this resuspended concentrate that was actually analyzed
  - (These two values are used to calculate the percent of the sample volume analyzed, if less than 100% of the volume filtered was analyzed.)
- Filter clog and packed pellet information, which should be provided to demonstrate compliance with LT2 Rule sample analysis requirements if less than 10 L was analyzed
- *Cryptosporidium* spiking information for OPR and MS samples
- Analyst names or initials for each step
- Reagent and filter lot information

6.2.3 Method 1622/1623 *Cryptosporidium* Slide Examination Form

The laboratory uses the slide examination form to document detailed information on slide examination. This information includes the following:

- Date and time the examination was completed

- Positive and negative staining control results
- Detailed information on the characteristics of each object on the slide that the analyst determined was a *Cryptosporidium* oocyst, including the following:
  - Size of the oocyst
  - Shape of the oocyst
  - Whether the DAPI stain applied to the sample was negative or whether it was positive with intense blue staining or revealed the presence of nuclei, and, if so, how many were observed by the analyst
  - Whether during DIC examination the analyst observed empty or amorphous oocysts or oocysts with internal structures

6.3 Submitting *Cryptosporidium* Data through the LT2/Stage 2 Data Collection and Tracking System (DCTS)

During the LT2 Rule, laboratories report *Cryptosporidium* data through the LT2/Stage 2 Data Collection and Tracking System (DCTS), where it is also available to their PWS clients. The DCTS is a web-based application that allows laboratory users to enter or upload data, then electronically "release" the data to the PWS for review, approval, and submission to EPA and the State. Although ownership of the data resides with the PWS throughout this process, the DCTS increases the ease and efficiency of the data entry and transfer process from one party to another by transferring the ability to access the data from the laboratory to the PWS to EPA and the State, and ensuring that data cannot be viewed or changed by unauthorized parties.

A summary of the data entry, review, and transfer process through the DCTS is provided below in **Table 6-2** and in Sections 6.3.1 through 6.3.3. More detailed information about the DCTS is provided in the *Users' Manual for the LT2/Stage 2 Data Collection and Tracking System*, including detailed information on the PWS and Laboratory user registration process. Information on the DCTS and a downloadable Users' Manual are available at http://www.epa.gov/safewater/disinfection/lt2.

Table 6-2. LT2/Stage 2 Data Collection and Tracking System (DCTS) Data Entry, Review, and Transfer Process

Laboratory actions • Laboratory posts analytical results to the DCTS • DCTS reduces data and checks for completeness and compliance with LT2 Rule requirements • Laboratory Principal Analyst confirms that data meets quality control requirements • Laboratory "releases" results electronically to the PWS for review • Laboratory user cannot edit data after it is released to the PWS	EPA does not have access to data
⬇	
PWS actions • PWS reviews electronic data through the DCTS • PWS cannot edit data - only review data and either return to laboratory to resolve errors or submit to EPA • PWS "releases" data back to the laboratory if it has questions • If no questions, PWS submits data to EPA as "approved" or "contested" (indicating that samples have been correctly analyzed, but that the PWS contends that they are not valid for use in LT2 binning) • If the PWS does not review the sample result by the deadline for submitting it to EPA (no later than 10 days after the end of the first month following the month when the sample was collected) the sample result status in the DCTS is automatically changed to "approved – not reviewed" to prevent a monitoring violation report from generating.	
⬇	
EPA and State actions • EPA and State users cannot edit data - only review data • EPA and State review data through the DCTS and approve results where appropriate • Contested results - If EPA/the State rejects the PWS explanation for the contested sample, the sample is marked "EPA approved" in the DCTS - If EPA/the State accepts the PWS explanation for the contested sample, the sample is invalidated and the PWS must resample	

6.3.1 Data Entry/Upload

The analyst or another laboratory staff member enters a subset of the data recorded at the bench (Section 6.2) into the DCTS, either by entering the data using web forms or by uploading data in XML format. This information includes the following:

- PWS ID
- Facility ID
- Sample collection date
- Sample type (field (monitoring) or MS)
- Sample volume filtered (L), to nearest ¼ L
- Was 100% of filtered volume examined?
- Number of oocysts counted
- For samples in which less than 10 L is filtered or less than 100% of the sample volume is examined, the laboratory also must enter or upload the number of filters used and the packed pellet volume.
- For samples in which less than 100% of sample volume is examined, the laboratory also must report the volume of resuspended concentrate and volume of this resuspension processed through immunomagnetic separation.
- For matrix spike samples, the laboratory also must report the sample volume spiked and estimated number of oocysts. These data are not required for field (monitoring) samples.

By entering *Cryptosporidium* data into the DCTS, the laboratory acknowledges that the following QC requirements were met: all holding times, sample condition on receipt, results of associated method blank, OPR, and positive and negative staining controls.

After the information has been entered or uploaded into the DCTS, it will reduce the data to yield final sample results, in oocysts/L, verify that LT2 Rule *Cryptosporidium* sample volume analysis requirements were met for samples in which less than 10 L were analyzed (see Section 4.2), and calculate MS recoveries.

The laboratory's Principal Analyst under the Lab QA Program is responsible for verifying the quality and accuracy of all sample results in the laboratory. The principal analyst or other senior laboratory representative (e.g., laboratory manager or QA officer) should review and approve the results before they are submitted to the PWS for review. If inaccuracies or other problems are identified, the Principal Analyst discusses the sample information with the analyst or data entry staff and resolves the issues before the data are approved for PWS review.

If no inaccuracies or other issues are identified, the laboratory approves the reported data for "release" to the PWS for review (EPA does not receive the data at this point). When the data are approved, the rights to the data are transferred electronically by the DCTS to the PWS, and the data can no longer be changed by the laboratory.

6.3.2 PWS Data Review

After the laboratory has released *Cryptosporidium* data electronically to the PWS using the DCTS, the PWS will review the results. The PWS user cannot edit the data, but if the PWS has an issue with the sample result, such as if the PWS believes that the collection date is incorrect, the PWS can release the results back to the laboratory for issue resolution. In addition to noting in the DCTS the reason for the return of the data to the laboratory, the PWS should contact the laboratory to discuss the issue.

If the PWS determines that they have no issues with the data, the PWS releases the results to EPA (and the State, if applicable) as "approved" results. If the PWS believes that the data are not valid for LT2 binning purposes, the PWS can release the results to EPA and the State as "contested." Contested samples are those that have been correctly analyzed, but that a PWS contends are not valid for use in LT2 binning and has submitted to EPA for evaluation. To contest the data, the PWS must indicate in the DCTS its reason for contesting the results. If EPA or the State concurs with the contesting, the data will be invalidated and the PWS will be able to work with EPA or the State to reschedule a sample. If EPA or the State believe the sample result is valid, the result will be approved and must be included in the binning calculation.

6.3.3 EPA/State Review

After the PWS has released the results as approved or contested, they are available to EPA and State users to review through the DCTS. EPA and State users cannot edit the data.

6.3.4 Archiving Examination Results

Although not required, laboratories also may archive slides and/or take photographs of slides to maintain for clients. Slides should be stored in a humid chamber in the dark at 1°C to 10°C. An alternative mounting medium may be used, which might preserve slides longer. Details are provided in the *Microbial Laboratory Guidance Manual for the Long Term 2 Enhanced Surface Water Treatment Rule.*

6.4 (*Optional*) Review and Validation of Raw *Cryptosporidium* Data

If your PWS plans to review the raw data generated by the laboratory, you should request from the laboratory the hardcopy data needed to verify the electronic results (see Section 2.1.6). This step is *not* required. However, for those PWSs interested in taking this extra step, Sections 6.4.1 through 6.4.3 provide guidance on how to review and validate hardcopy data and verify accuracy.

6.4.1 Data Completeness Check

Upon receipt of the hardcopy sample results for a monitoring sample, verify that the laboratory has submitted the following information:

- Monitoring sample identification information
- Monitoring sample result, in oocysts/L
- Laboratory quality control batch associated with the sample
- Result for the ongoing precision and recovery (OPR) sample analyzed for this QC batch
- Result for the method blank sample analyzed for this QC batch
- **LT2 sample collection form** initiated by your utility and completed with sample receipt information by the laboratory
- **Method 1622/1623 Bench Sheet** with raw data associated with the monitoring sample (and MS sample, if applicable)
- **Method 1622/1623 *Cryptosporidium* Slide Examination Form** with raw data for the monitoring sample (and MS sample, if applicable)
- **Laboratory comments.** If the laboratory provided comments on the sample analyses or results that require follow-up, contact the laboratory to discuss, if necessary. Comments may include any applicable data qualifiers. The following is a list of potential data qualifiers:
  - The recovery for the associated ongoing precision and recovery (OPR) sample did not meet method requirements

- Oocysts were detected in the method blank
- Positive and negative staining controls were not acceptable or not examined
- Method holding times were not met
- Sample arrived at the laboratory in unacceptable condition

Any of the above data qualifiers would result in the sample being considered invalid for LT2 use and the laboratory should not report the results for the sample to EPA. The PWS may be required to resample.

If forms are missing, incomplete, or incorrect, contact the laboratory immediately to discuss and request resubmission of the missing forms and/or spreadsheets.

6.4.2 Evaluation of Data Against Method Quality Control Requirements

To verify that the laboratory analyzed your monitoring sample within the analytical controls specified by the method, check the following information:

- **Sample condition upon receipt.** Verify on the completed LT2 sample collection form that your *Cryptosporidium* sample was received in acceptable condition (not leaking, etc.), and at a temperature $\leq 20^{\circ}C$, but not frozen.
- **Method blank.** Verify that the laboratory analyzed a method blank with the monitoring sample's QC batch and confirm that the method blank did not contain any oocysts.
- **Ongoing precision and recovery sample.** Verify that the laboratory analyzed an OPR sample with the monitoring sample's QC batch and that the OPR sample recovery was between 11% and 100%, as required by EPA Methods 1622 and 1623.
- **Holding times.** Using the sample collection date and time on the LT2 data collection form and the dates and times of the method steps recorded by the laboratory on the Method 1622/1623 bench sheet and slide examination form for the monitoring sample, verify the following:
  - The laboratory began elution no more than 96 hours from sample collection
  - The laboratory performed the elution, concentration, purification, and slide preparation (application of the purified sample to the slide) within one working day (the date of the elution step should be the same as the date of the slide preparation step)
  - The laboratory stained the slide within 72 hours of application of the purified sample to the slide (the date and time next to the slide staining step should be no more than 72 hours later than the date and time next to the slide preparation step)
  - The laboratory examined the slide within 168 hours (seven days) of staining (the examination date should be no more than seven days from the slide staining date)
- **Positive and negative staining controls.** Based on the information at the top of the Method 1622/1623 *Cryptosporidium* Slide Examination Form, verify that the laboratory performed positive and negative staining controls, and that the results of these controls were acceptable.

6.4.3 Calculation Verification

The laboratory does not directly report the final concentration of oocysts/L in the sample to EPA. Instead, they report a series of primary measurements that are used by the LT2 Data Collection System to automatically calculate the final concentration. The volume filtered, the total volume of resuspended concentrate, and the volume transferred to IMS are used to determine the volume analyzed. The laboratory also records the total count of oocysts detected, which is divided by the volume analyzed to determine the final concentration of oocysts/L. Although the final results are automatically calculated by the LT2 Data Collection System using the primary measurements supplied by the laboratory, the plant

still may wish to verify them. Guidance on recalculating and verifying final results using primary measurements is provided below.

6.4.3.1 Field (monitoring) Sample Calculations

To calculate the concentration of *Cryptosporidium* in your field (monitoring) sample, reported as oocysts/L, the following information is needed:

- Number of oocysts detected in the sample (recorded as a primary measurement from the examination results form)
- Volume analyzed

Using these two data elements, the final concentration is calculated as:

$$\text{final concentration} = \frac{\text{oocysts detected in the sample}}{\text{volume analyzed (L)}}$$

If 100% of the sample volume filtered is examined, then the volume analyzed equals the volume filtered. This applies whether one filter or more than one filter was used; if more than one filter was used, and all of the volume filtered through the multiple filters is processed through the remainder of the method, then the volume examined is simply the sum of the volumes filtered through each of the filters used.

If less than 100% of the volume filtered was processed through the remainder of the method, then additional calculations are needed to determine the volume analyzed. This is discussed below.

Determining Volume Analyzed when Less than 100% of Sample Was Examined

When less than 100% of the sample filtered is processed through the remainder of the method and examined (such as when the volume filtered yields greater than 2 mL of packed pellet volume after centrifugation), then the volume analyzed must be determined using the following equations to determine the percentage of the sample that was examined.

$$\text{percent examined} = \frac{\text{total volume of resuspended concentrate transferred to IMS (see Section 6.2.2)}}{\text{total volume of resuspended concentrate produced}}$$

$$\text{volume analyzed (L)} = \text{percent examined} \times \text{sample volume filtered}$$

Determining the Volume of Resuspended Concentrate to Use for Packed Pellets > 0.5 mL

Packed pellets with a volume greater than 0.5 mL must be divided into subsamples. Use the formula below to determine the total volume of resuspension required in the centrifuge tube before separating the concentrate into two or more subsamples and transferring to IMS.

$$\text{total volume of resuspended concentrate (ml) required} = \frac{\text{pellet volume (ml) after centrifugation}}{\text{0.5 mL}} \times 5 \text{ mL}$$

Example. A 10 L field (monitoring) sample was filtered and processed, producing a packed pellet volume of 2.7 mL. The laboratory transferred 20 mL of the 27 mL total resuspended concentrate to IMS and examination. The laboratory detected 20 oocysts during examination. The following calculations were performed to determine the volume analyzed and final concentration.

$$\text{total volume of resuspended concentrate (ml) required} = \frac{2.7\ \text{mL}}{0.5\ \text{mL}} \times 5\ \text{mL} = 27\ \text{mL}$$

$$\text{percent examined} = \frac{20\ \text{mL}}{27\ \text{mL}} = 0.74\ (74\%)$$

$$\text{volume analyzed (L)} = 0.74 \times 10\ \text{L} = 7.4\ \text{L}$$

$$\text{final concentration (oocysts/L)} = \frac{20\ \text{oocysts}}{7.4\ \text{L}} = 2.7\ \text{oocysts/L}$$

6.4.3.2 Matrix Spike Sample Calculations

For matrix spike (MS) samples, the laboratory records all of the same information that is recorded for field (monitoring) samples, in addition to information specific to matrix spike samples. The sample volume spiked and the estimated number of oocysts spiked into the sample are used to calculate the concentration of spiked organisms in the sample. To correct for background concentration, the number of oocysts detected in the unspiked field (monitoring) sample is subtracted from the number of oocysts detected in the MS sample.

To determine the percent recovery for a matrix spike (MS) sample, the following information is needed:

- The number of oocysts detected in the MS sample
- The true value of the oocysts spiked into the MS sample
- The number of oocysts detected in the unspiked field (monitoring) sample (to correct for background concentration)

$$\text{percent recovery} = \frac{\text{oocysts counted in MS sample - oocysts counted in unspiked field (monitoring) sample}}{\text{oocysts spiked into MS sample}} \times 100\%$$

This calculation assumes that the same sample volume was examined for both the field (monitoring) and MS samples. If the sample volumes examined are different, calculate the number of oocysts per L for both the field (monitoring) and MS samples before calculating percent recovery.

6.4.4 Data Archiving Requirements

The PWS is required to keep all original, hardcopies of monitoring results including quality control data associated with LT2 sample analyses (both initial and second round of monitoring) for 3 years after bin classification for filtered PWSs or determination of mean *Cryptosporidium* level for unfiltered PWSs [40 CFR § 141.722(a)]. Although it is the PWS's responsibility to meet LT2 Rule data storage requirements for compliance monitoring samples, the PWS may contract this work to the laboratory.

SECTION 7: *E. COLI* DATA: USE, RECORDING, SUBMITTING AND REVIEW

When *E. coli* samples are processed and analyzed by the laboratory, data on sample results and sample processing times are recorded at the laboratory and reported to the PWS through the LT2/Stage 2 Data Collection and Tracking System (DCTS). This section provides an overview of the data recording and reporting processes and provides guidance on how to review the data you receive from the laboratory. Sample analysis and data review are also described in more detail in the *Microbial Laboratory Guidance Manual for the Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule)*.

7.1 Use of *E. coli* Data

E. coli data are being collected by large filtered PWSs and applicable wholesalers during LT2 Rule monitoring to confirm or refine the *E. coli* levels that trigger *Cryptosporidium* monitoring by small, filtered PWSs. *E. coli* data are being collected by small filtered PWSs because only those small filtered PWSs with mean *E. coli* levels that exceed the trigger level will be required to monitor for *Cryptosporidium* to determine bin placement [40 CFR § 141.701 (a)(4)]. Based on the data from the Information Collection Rule (ICR) and Information Collection Rule Supplemental Survey (ICRSS), the *E. coli* trigger levels were set at a mean of 50 *E. coli*/100 mL for flowing stream–type source waters and 10 *E. coli*/100 mL for reservoir/lake source waters. The *E. coli* and turbidity data from large PWS monitoring will be reviewed and, if appropriate, guidance on alternate indicator trigger values will be issued to States prior to when small PWSs begin monitoring. States are allowed to approve alternative approaches to indicator monitoring for small PWSs [40 CFR § 141.701(a)(5)].

Approved methods for *E. coli* analysis under the LT2 Rule are listed below in **Table 7-1**.

7.2 *E. coli* Data Recording at the Laboratory

The laboratories performing *E. coli* analyses during the LT2 Rule should record the following general types of information (For further detail see section 4.6 of *Microbial Laboratory Guidance Manual for the Long Term 2 Enhanced Surface Water Treatment Rule*):

- Sample identification information
- All primary measurements used to calculate the final *E. coli* concentration for each sample
- The incubation start and read times for each method to verify that method requirements were met
- The name of the analyst performing the sample analysis
- Quality control (QC) analysis results (e.g., positive/negative controls, blanks, etc.)

Table 7-1. Approved *E. coli* Methods for LT2 Rule

***E. coli* Methods Approved for LT2 Rule**	***Method Format***	***Method Citation***
Standard Methods 9221B.1/9221F (LTB-EC-MUG)	multiple tube fermentation/ most probable number	Standard Methods for the Examination of Water and Wastewater (Reference 8.6)[2]
Standard Methods 9223B (Colilert®/Colilert-18®)	multiple tube/multiple well	Standard Methods for the Examination of Water and Wastewater (Reference 8.6)[2]; IDEXX Laboratories, Inc. (Reference 8.8)
Standard Methods 9222B/9222G[1] (mEndo/LES-Endo-NA-MUG)	membrane filtration, two step	Standard Methods for the Examination of Water and Wastewater (Reference 8.6)[2]
Standard Methods 9222D/9222G (mFC-NA-MUG)	membrane filtration, two step	Standard Methods for the Examination of Water and Wastewater (Reference 8.6)[2]
Standard Methods 9213D/ EPA Method 1103.1 (mTEC)	membrane filtration, one step	EPA Method 1103.1 (Reference 8.9); Standard Methods for the Examination of Water and Wastewater (Reference 8.6)[2]
EPA Method 1603 Modified mTEC	membrane filtration, one step	EPA Method 1603 (Reference 8.10)
EPA Method 1604 MI medium [1]	membrane filtration, one step	EPA Method 1604 (Reference 8.11)
m-ColiBlue24® Broth [1]	membrane filtration, one step	Hach Company (Reference 8.12)

[1] If high levels of non-*E. coli* total coliforms interfere with the ability to accurately enumerate *E. coli* despite additional dilutions, an alternate method should be used (i.e., SM 9222D/9222G, SM 9213D/EPA Method 1103.1, EPA Method 1603, SM 9221B.1/9221F, and SM 9223B)
[2] 18th, 19th, or 20th Editions of Standard Methods for the Examination of Water and Wastewater may be used.

7.2.1 Sample Identification Information

Sample identification information is used to track the sample through sample collection, analysis, and data reporting. At a minimum, the laboratory records the sample ID, the target parameter (*E. coli*), and the method being used (e.g., Membrane Filtration: SM 9222D/SM 9222G).

7.2.2 Primary Data

The laboratory should record all primary measurements needed to calculate the final concentration of *E. coli* per 100 mL. Primary measurements for membrane filtration methods will include the volumes filtered and the plate counts for each volume filtered. The multiple-well and multiple-tube formats will include the volumes or dilutions of samples analyzed and the number of positive wells or tubes per each volume analyzed.

7.2.3 Sample Processing and Quality Control Information

The laboratory should record information on the bench sheet on how they processed and analyzed the sample, including incubation start/end date and times, and the analyst performing each step of the method. The lot numbers of reagents, media, and materials used to process the sample and the results of QC analyses should be recorded in a media log book or QC checklist. In addition to being used to resolve

questions regarding validity of results, this information may be used by the laboratory to determine the source of any problems the laboratory is having with method performance.

7.2.4 Sample Results

The final *E. coli* concentration for field (monitoring) samples will be reported as CFU/100 mL or MPN/100 mL depending on the method used for analysis. If no *E. coli* are detected in the sample, the detection limit based on the volume of sample analyzed may be reported (e.g., <1 CFU /100 mL or <1.8 MPN/100 mL) or a zero for purposes of the DCTS.

7.3 Submitting *E. coli* Data through the LT2/Stage 2 Data Collection and Tracking System (DCTS)

During the LT2 Rule, laboratories report *E. coli* data electronically through EPA's LT2/Stage 2 Data Collection and Tracking System (DCTS) to the PWS staff responsible for approving and submitting monitoring results to EPA. The DCTS is a web-based application that allows laboratory users to enter or upload data, then electronically "release" the data to the appropriate PWS staff for review, approval, and submission to EPA and the State. Although ownership of the data resides with the PWS throughout this process, the DCTS increases the ease and efficiency of the data entry and transfer process from one party to another by transferring the ability to access the data from the laboratory to the PWS to EPA and the State, and ensuring that data cannot be viewed or changed by unauthorized parties. A summary of the data entry, review, and transfer process through the Data Collection System for both *Cryptosporidium* and *E. coli* samples is provided in **Table 6-2**, in Section 6.3 of this manual.

The data reporting process is summarized below, in Sections 7.3.1 through 7.3.3, and discussed in detail in the *Users' Manual for the LT 2/Stage 2 Data Collection and Tracking System*. The Users' Manual also provides detailed information on the laboratory registration process. Information on the DCTS and a downloadable Users' Manual are available at http://www.epa.gov/safewater/disinfection/lt2.

7.3.1 Data Entry/Upload

The analyst or another laboratory staff member enters a subset of the data recorded at the bench (Section 7.2) into the DCTS either by entering the data using web forms or by uploading data in XML format. This required information includes the following [40 CFR § 141.706 (e)(2)]:

- PWS ID
- Facility ID
- Sample collection date
- Analytical method number
- Method type
- Source water type (provided by PWS on sample collection form)
- Turbidity result (provided by PWS on sample collection form) (if applicable)
- *E. coli*/100 mL (see note below)

Note: The laboratory may then enter the final result for the sample that was calculated at the laboratory, or may enter the primary measurements recorded at the bench and have the DCTS automatically calculate the final sample concentration. Because this information is specific to method type (membrane filtration, multiple tube fermentation, 51-well, and 97-well), the system provides different entry screens for each method type. The laboratory staff entering the data should verify that all holding times and other QC specifications were met.

The laboratory's official contact is responsible for verifying the quality and accuracy of all sample results in the laboratory, and should review and approve the results before they are submitted to the PWS for review. If inaccuracies or other problems are identified, the official contact discusses the sample information with the analyst or data entry staff and resolves the issues before the data are approved for PWS review.

If no inaccuracies or other issues are identified, the laboratory's official contact approves the data for "release" to the PWS for review (EPA does not receive the data at this point). When the data are approved, the rights to the data are transferred electronically by the DCTS to the PWS, and the data can no longer be changed by the laboratory.

7.3.2 PWS Data Review

After the laboratory has released *E. coli* data electronically to the PWS using the DCTS, the PWS will review the results. The PWS user cannot edit the data, but if the PWS has an issue with the sample result, such as if the PWS believes that the collection date is incorrect, the PWS can release the results back to the laboratory for issue resolution. In addition to noting in the DCTS the reason for the return of the data to the laboratory, the PWS should contact the laboratory to discuss the issue.

If the PWS determines that they have no issues with the data, the PWS releases the results to EPA (and the State, if applicable) as "approved" results. If the PWS believes that the data are not valid for calculating mean *E. coli* level, the PWS can release the results to EPA and the State as "contested." Contested samples are those that have been correctly analyzed, but that a PWS contends are not valid for use in calculating mean *E. coli* level and has submitted to EPA for evaluation. To contest the data, the PWS must indicate in the DCTS its reason for contesting the results. If EPA or the State concurs with the contesting, the data will be invalidated and the PWS will be able to work with EPA or the State to reschedule a sample. If EPA or the State believe the sample result is valid, the result will be approved and must be included in calculating the mean *E. coli* level.

7.3.3 EPA/State Review

After the PWS has released the results as approved or contested, they are available to EPA and State users to review through the DCTS. EPA and State users cannot edit the data.

7.4 (*Optional*) Reviewing and Validating Raw *E. coli* Data

If the PWS staff responsible for submitting data to EPA plans to review the raw data generated by the laboratory, the original, hardcopy records (whether generated by an in-house laboratory or a contract laboratory) should be compared to the electronic results. However, this step is *not* required. Sections 7.4.2 through 7.4.3 provide guidance on how to review and validate the hardcopy data and verify accuracy.

7.4.1 Data Completeness Check

Upon receipt of hardcopy sample results for a monitoring sample, verify that the following information is included on the applicable forms:

- Monitoring sample identification information
- Monitoring sample result, in *E. coli*/100 mL
- Laboratory quality control checklist (or other verification from the laboratory that all QC specifications were met)
- **LT2 sample collection form** initiated at the time of sample collection and completed with sample receipt information by the laboratory

- ***E. coli* Method Bench Sheet** completed by the laboratory with primary sample processing and analysis data associated with the monitoring sample

Laboratory comments. If the laboratory provided comments on the sample analyses or results that require follow-up, contact the laboratory to discuss, if necessary. Comments may include any applicable data qualifiers. The following is a list of potential data qualifiers:

- Sample arrived at the laboratory in unacceptable condition (e.g., leaking)
- Sample holding time exceeded
- Sample holding temperature not within acceptable range
- Unacceptable blank sample result
- Unacceptable positive or negative culture control result
- Media sterility checks were not acceptable
- Method incubation times or temperatures were not within acceptable ranges
- Membrane filtration: Too much sediment on the filter
- Membrane filtration: Confluent growth of non-target organism (CNFG)
- Membrane filtration: Colonies too numerous to count (TNTC)
- Membrane filtration: Pre- or post- filtration series sterility check not acceptable (e.g., contamination with *E. coli* or other organisms)
- Quanti-Tray® was damaged or leaked
- Sample was not distributed to all wells in Quanti-Tray®
- All rows of tubes were not inoculated
- Presumptive positive tubes were not transferred into the appropriate confirmatory medium

Any of the above data qualifiers would result in the sample being considered invalid for LT2 use, and the results for the sample should not be entered into the LT2/Stage 2 Data Collection and Tracking System (DCTS). If the laboratory enters the results into the DCTS, the PWS should not submit the results to EPA. If forms are missing, incomplete, or incorrect, contact the laboratory immediately to discuss and request resubmission of the missing forms and/or spreadsheets.

7.4.2 Evaluation of Data Against Method Quality Control Requirements

To verify that the laboratory analyzed your monitoring sample within the analytical controls specified by the method, check the following information:

- **Sample condition upon receipt.** If the sample was shipped to the laboratory, verify on the completed LT2 sample collection form that your sample was received in acceptable condition (e.g., not leaking, etc.), and at a temperature between 0°C and 10°C, but should not be frozen.
- **QC samples associated with field (monitoring) samples.** The frequency of analysis of quality control samples including method blanks, positive and negative controls, etc. varies according to

method requirements and specifications of the certifying authority. Verify that the required QC samples were analyzed with the field (monitoring) sample.

- **Holding time.** Using the sample collection date and time on the LT2 data collection form and the date and time of the first method step recorded by the laboratory on the *E. coli* method bench sheet, verify that the laboratory began sample analysis within 30 hours of sample collection (or 48 hours with State approval if using Colilert).

7.4.3 Calculation Verification

Method-specific data which should be recorded for each of the individual method types as well as standardized calculations for each method type are discussed in Sections 7.4.3.1 through 7.4.3.4.

7.4.3.1 Calculations for Determining the *E. coli* Concentration Using the Colilert® Quanti-Tray 2000® (97-well)

A. **Select appropriate dilution to yield countable results.** If multiple dilutions are used, the tray exhibiting positive wells in the 40% to 80% range (39 to 78 total positive large and small wells) should be used to determine MPN value.

Note: The analytical result can be automatically calculated using the LT2/Stage 2 Data Collection and Tracking System. See Section 7.3.1 for additional information.

B. **Determine MPN.** Using the number of positive wells from the appropriate dilution, identify the corresponding MPN/100 mL in the table provided by the vendor. Using the number at the intersection of large positive wells and small positive wells, identify the corresponding MPN/100 mL in the table provided by the vendor. Large well values are located in the left column; small well values are located in the top row. For example, if a 100 mL sample was analyzed, and there were 29 large positive wells and five small positive wells, the corresponding MPN would be 49.6 MPN/100 mL.

C. **Adjust for dilution factor.** Because the MPN/100 mL values in the table are based on 100 mL samples, the MPN value should be adjusted if less than 100 mL of sample volume was analyzed. Use the following calculation to adjust the MPN to account for the dilution:

$$\text{Analytical Results} = \text{MPN Value} \times \frac{100}{\text{mL of sample analyzed}}$$

Example:

Volume analyzed = 10 mL of sample (in 90 mL of dilution water)
Large wells positive = 39
Small wells positive = 5
The MPN value calculated based on the intersection of 39 and 5 in the table.
MPN = 81.3

$$\text{Analytical result} = 81.3 \times \frac{100}{10} = 813 \textit{ E. coli} \text{ MPN/100 mL}$$

7.4.3.2 Calculations for Determining the *E. coli* Concentration Using the Colilert® Quanti-Tray 51® (51-well)

A. **Select appropriate dilution.** If multiple dilutions are used, the tray exhibiting 80% positive wells (41 positive wells) should be used to determine MPN value.

Note: The analytical result can be automatically calculated using the LT2 Data Collection System. See Section 7.3.1 for additional information.

B. **Determine MPN.** Using the number of positive wells from the appropriate dilution, identify the corresponding MPN/100 mL in the table provided by the vendor. For example, if a 100 mL sample was analyzed, and there were 41 positive wells, the corresponding MPN would be 83.1 MPN/100 mL

C. **Adjust for dilution factor.** Because the MPN/100 mL values in the table are based on 100 mL samples, the MPN value should be adjusted if less than 100 mL of sample volume was analyzed. Use the following calculation to adjust the MPN to account for the dilution:

$$\text{MPN value} \times \frac{100}{\text{mL sample analyzed}} = \textit{E. coli}\ \text{MPN/100 mL}$$

Example:

Volume analyzed (mL) = 10 mL (in 90 mL of dilution water)
Number of positive wells = 41
MPN = 83.1

The analytical result is calculated as follows:

$$83.1 \times \frac{100}{10} = 831\ \textit{E. coli}\ \text{MPN/100 mL}$$

7.4.3.3 Calculations for Determining the *E. coli* Concentration Using Membrane Filter Data (adapted from Reference 8.6)

A. *E. coli* counts should be determined from the volume(s) filtered that yielded 20 to 80 *E. coli* colonies (20-60 for mFC-NA-MUG), and not more than 200 total colonies per plate. (Guidance for samples that do not yield countable plates is provided in Sections E and F)

Note: The analytical result can be automatically calculated using the LT2/Stage 2 Data Collection and Tracking System. See Section 7.3.1 for additional information.

B. If there are greater than 200 colonies per membrane, even for the lowest dilution, the result is recorded as "too numerous to count" (TNTC). These results cannot be reported for LT2 monitoring, as they cannot be used for the required data analyses. During the next sampling event, analyze an additional, lower dilution volume (the highest dilution volume may be omitted) unless conditions were unusual (e.g., heavy rains, flooding, etc.) during the sampling event yielding TNTC for all dilutions.

C. If colonies are not sufficiently distinct for accurate counting, the result is recorded as "confluent growth" (CNFG). To prevent CNFG from occurring, smaller sample aliquots should be filtered. For example, if sample volumes of 100, 10, 1, and 0.1 mL are analyzed and even the 0.1 mL plate results in CNFG, then potentially 0.01 mL should be analyzed during the next sampling event. For sample volumes less than 1 mL, serial dilutions should be used, and 1 mL volumes of the dilutions should be filtered. The 100 mL volume can be eliminated.

Note: If growth is due to high levels of total coliforms but low E. coli then another method should be chosen for analyses that does not rely on total coliform determination prior to or simultaneous with E. coli determination.

Note: Results that are TNTC or CNFG are not appropriate for LT2 microbial data analysis, and cannot be entered into the LT2/Stage 2 Data Collection and Tracking System.

D. Using the *E. coli* counts from the appropriate dilution, *E. coli* CFU/100 mL is calculated based on the following equation:

$$\textit{E. coli}\ \text{CFU} \times \frac{100}{\text{mL sample filtered}} = \textit{E. coli}\ \text{CFU/100 mL}$$

Example 1:

Filter 1 volume = 100 mL	CFU = TNTC
Filter 2 volume = 10 mL	CFU = 40
Filter 3 volume = 1.0 mL	CFU = 9
Filter 4 volume = 0.1 mL	CFU = 0

Using the guidance on countable colonies in Step A, the counts from the 10 mL plate will be used to calculate the *E. coli* concentration for the sample:

$$40\ \textit{E. coli}\ \text{CFU} \times \frac{100}{10\ \text{mL}} = 400\ \textit{E. coli}\ \text{CFU/100 mL}$$

E. If no *E. coli* colonies are present, the detection limit (i.e., 1 CFU per volume filtered) is calculated and reported per 100 mL (see example below).

Example 2:

Filter 1 volume (mL) = 100 mL	CFU = 0
Filter 2 volume (mL) = 10 mL	CFU = 0
Filter 3 volume (mL) = 1.0 mL	CFU = 0

$$\text{Detection limit} = \frac{100\ \text{mL}}{\text{Largest volume filtered}} = \textit{E. coli}\ \text{CFU/100 mL}$$

$$\frac{100\ \text{mL}}{100\ \text{mL}} = <1\ \textit{E. coli}\ /100\ \text{mL}$$

Example 3:

Filter 1 volume (mL) = 100 mL	CFU = Lab accident, no data available
Filter 2 volume (mL) = 10 mL	CFU = 0
Filter 3 volume (mL) = 1.0 mL	CFU = 0

Calculation of *E. coli*/100 mL:

$$\frac{100\ \text{mL}}{10\ \text{mL}} = <10\ \textit{E. coli}\ \text{CFU}\ /100\ \text{mL}$$

F. If there are no filters with *E. coli* counts in the 20-80 colony range (20-60 for mFC-NA-MUG), sum the *E. coli* counts on all filters, divide by the volume filtered and report as number per 100 mL.

Example 4:

Filter 1 volume (mL) = 50 mL	CFU = 15
Filter 2 volume (mL) = 25 mL	CFU = 6
Filter 3 volume (mL) = 10 mL	CFU = 0

The analytical result is calculated as:

$$(15 + 6 + 0) \times \frac{100}{(50+25+10)} = 25 \textit{ E. coli} \text{ CFU/100 mL}$$

Example 5:

Filter 1 volume (mL) = 50 mL	CFU = 105
Filter 2 volume (mL) = 25 mL	CFU = 92
Filter 3 volume (mL) = 10 mL	CFU = 85

The analytical result is calculated as:

$$(105 + 92 + 85) \times \frac{100}{(50 + 25 + 10)} = 332 \textit{ E. coli} \text{ CFU/100 mL}$$

Example 6:

Filter 1 volume (mL) = 100 mL	CFU = 82
Filter 2 volume (mL) = 10 mL	CFU = 18
Filter 3 volume (mL) = 1.0 mL	CFU = 0

The analytical result is calculated as:

$$(82 + 18 + 0) \times \frac{100}{(100 + 10 + 1)} = 90 \textit{ E. coli} \text{ CFU/100 mL}$$

Example 7:

Filter 1 volume (mL) = 50 mL	CFU = TNTC
Filter 2 volume (mL) = 25 mL	CFU = TNTC
Filter 3 volume (mL) = 10 mL	CFU = 83

The analytical result is calculated as:

$$83 \times \frac{100}{10} = 830 \textit{ E. coli} \text{ CFU/100 mL}$$

7.4.3.4 Calculation of *E. coli* Concentrations Using Multiple-Tube Methods

The guidance and examples for determining *E. coli* concentrations using multiple-tube methods are based on the revision of Standard Methods 9221C included in the *2001 Supplement to the 20th Edition of Standard Methods* (Reference 8.13), approved by the Standard Methods Committee in 1999.

Note: The analytical result can be automatically calculated using the LT2 Data Collection System. See Section 7.3.1 for additional information.

A. For each sample volume (e.g., 10, 1, 0.1, and 0.01 mL or additional sample volumes as necessary), determine the number of positive tubes out of five.

B. A dilution refers to the volume of original sample that was inoculated into each series of tubes. Only three of the dilution series will be used to estimate the MPN. The three selected dilutions are called significant dilutions and are selected according to the following criteria. Examples of significant dilution selections are provided in **Table 7-2**, below.

- Choose the highest dilution (the most dilute, with the least amount of sample) giving positive results in all five tubes inoculated and the two succeeding higher (more dilute) dilutions. (**Table 7-2**, Example A).
- If the lowest dilution (least dilute) tested has fewer than five tubes with positive results, select it and the two next succeeding higher dilutions (**Table 7-2**, Examples B and C).
- When a positive result occurs in a dilution higher (more dilute) than the three significant dilutions selected according to the rules above, change the selection to the lowest dilution (least dilute) that has less than five positive results and the next two higher dilutions (more dilute) (**Table 7-2**, Example D).
- When the selection rules above have left unselected any higher dilutions (more dilute) with positive results, add those higher-dilution positive results to the results for the highest selected dilution (**Table 7-2**, Example E).
- If there were not enough higher dilutions tested to select three dilutions, then select the next lower dilution (**Table 7-2**, Example F).

C. MPN values must be adjusted based on the significant dilutions series selected above. Because the MPN/100 mL values in the table are based on the 10 mL, 1 mL, and 0.1 mL dilutions, per method requirements, the MPN value must be adjusted if these are not the significant dilutions selected. Use the following calculation to adjust the MPN when the 10 mL, 1 mL, and 0.1 mL dilutions are not the significant dilutions selected:

$$\text{Analytical result} = \frac{\text{MPN value}}{\text{\# of mL in middle dilution}} = \textit{E. coli}\ \text{MPN/100 mL}$$

Table 7-2. Examples of Different Combinations of Positive Tubes (Significant Dilution Results Are in Bold and Underlined)

Example	Least dilute (Lowest)				Most dilute (Highest)	Combination of positives	MPN Index from Standard Methods	*E. coli*/100 mL (after adjustment)
	10 mL	1 mL	0.1 mL	0.01 mL	0.001 mL			
A	5	***5***	***1***	***0***	0	5-1-0	33	330
B	***4***	***5***	***1***	0	0	4-5-1	48	48
C	***0***	***0***	***1***	0	0	0-0-1	1.8	1.8
D	5	***4***	***4***	***1***	0	4-4-1	40	400
E	5	***4***	***4***	***0***	***1***	4-4-1	40	400
F	5	5	***5***	***5***	***2***	5-5-2	540	54,000

Example A: *The significant dilution series for the 5-1-0 combination of positives includes the 1 mL, 0.1 mL, and 0.01 mL dilutions. Because the 10 mL, 1 mL, and 0.1 mL dilutions were not selected, an adjustment is necessary to account for the dilutions selected:*

$$\textit{Analytical result} = \frac{33}{0.1} = 330\ \textit{E. coli}\ /\ 100\ \text{mL}$$

Example B: Because the 10 mL, 1 mL, and 0.1 mL dilutions are the significant dilutions, no adjustment is necessary and the result is 48 *E. coli*/100 mL.

Example C: Because the 10 mL, 1 mL, and 0.1 mL dilutions are the significant dilutions, no adjustment is necessary and the result is 1.8 *E. coli*/100 mL.

Examples D and E: The significant dilution series for the 4-4-1 combination of positives includes the 1 mL, 0.1 mL, and 0.01 mL dilutions. Because the 10 mL, 1 mL, and 0.1 mL dilutions were not selected, an adjustment is necessary to account for the dilutions selected:

$$\text{Analytical result} = \frac{40}{0.1} = 400\ \textit{E. coli}\ /\ 100\ \text{mL}$$

Example F: The significant dilution series for the 5-5-2 combination of positives includes the 0.1 mL, 0.01 mL and 0.001 mL dilutions. Because the 10 mL, 1 mL, and 0.1 mL dilutions were not selected, an adjustment is necessary to account for the dilutions selected:

$$\text{Analytical result} = \frac{540}{0.01} = 54{,}000\ \textit{E. coli}\ /\ 100\ \text{mL}$$

7.4.4 Data Archiving Requirements

The PWS is required to keep all original, hardcopy quality control data associated with LT2 sample analyses (both initial and second round of monitoring) for 3 years after bin classification or determination of the mean *Cryptosporidium* level [40 CFR § 141.722(a)]. Although it is the PWS's responsibility to meet LT2 Rule data storage requirements for compliance monitoring samples, the PWS may contract this work to the laboratory.

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SECTION 8: REFERENCES

8.1 USEPA. 2006. National Primary Drinking Water Regulations: Long Term 2 Enhanced Surface Water Treatment Rule. 40 CFR parts 9, 141, and 142. Supporting guidance documents are available at: http://www.epa.gov/safewater/disinfection/lt2/.

8.2 USEPA. 2005. Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act; Agency Information Collection: Proposed Collection; Comment Request. Federal Register: June 3, 2005. 70 FR 32607.

8.3 USEPA. 2005. Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA-815-R-05-001. Document is available for download at: http://www.epa.gov/safewater/disinfection/lt2/.

8.4 USEPA. 2005. Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA-815-R-05-002. Document is available for download at: http://www.epa.gov/safewater/disinfection/lt2/.

8.5 USEPA 2005. *Manual for the Certification of Laboratories Analyzing Drinking Water; Criteria and Procedures; Quality Assurance*. Fifth Edition. EPA 815-R-05-004. Office of Ground Water and Drinking Water, U.S. Environmental Protection Agency, 26 Martin Luther King Drive, Cincinnati, OH 45268.

8.6 American Public Health Associated. 1998. Standard Methods for the Examination of Water and Wastewater; 20th Edition. American Public Health Association, Washington D.C. Standard Methods may be ordered from: American Water Works Association Bookstore, 6666 West Quincy Avenue, Denver, CO 80235.

8.7 Connell, Kevin, et al. 2000. ICRSS - Building a Better Protozoa Data Set, J. AWWA. 92(10): 30 - 43.

8.8 IDEXX Laboratories, Inc., Description of Colilert®, Colilert-18®, Quanti-Tray®, Quanti-Tray®/2000, and Colisure™ methods may be obtained from: IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092.

8.9 USEPA. 2002. Method 1103.1: *Escherichia coli* in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC). U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA-821-R-02-020.

8.10 USEPA. 2002. Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* agar (Modified mTEC). U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA-821-R-02-023.

8.11 USEPA. 2002. Method 1604: Total coliforms and *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium). U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA-821-R-02-024.

8.12 Hach Company, Inc. m-ColiBlue24 Method is available from: Hach Company, P.O. Box 389, Loveland, CO 80539

8.13 2001 Supplement to the 20th Edition of Standard Methods 9221 C: Explanation of Bacterial Density. This supplement is available for download at http://www.techstreet.com/cgi-bin/detail?product_id=923645.

8.14 USEPA. 2005. *Microbial Laboratory Guidance Manual for the Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule)*. This manual is available for download from http://www.epa.gov/safewater/disinfection/lt2/compliance.html

Appendix A

Intent to Provide Maximum Treatment
Example Notice

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Long Term 2 Enhanced Surface Water Treatment Rule
Intent to Provide Maximum Treatment – Example Notice

Public Water Systems (PWSs) who choose to provide the maximum level of treatment for *Cryptosporidium* applicable to their plant type rather than start source water monitoring must submit written notification no later than the date the PWS is otherwise required to submit a sampling schedule for monitoring. Alternatively, a PWS may choose to stop sampling at any point after it has initiated monitoring if it provides written notification that it will provide this level of treatment. This form is an example of the necessary written notification. PWSs must install and operate technologies to provide this level of treatment by their applicable treatment compliance date.

PWS Information	
PWS Name:	PWS ID:
PWS Address:	
Email Address:	
Water Treatment Plant Name:	
Water System Facility ID:	
Type of system that will be providing maximum treatment instead of conducting monitoring: ☐ **Filtered system** providing a total of at least 5.5-log of treatment for *Cryptosporidium* [40 CFR part 141.701 (d)(1)]. ☐ **Unfiltered system** providing a total of at least 3-log *Cryptosporidium* inactivation [40 CFR part 141.701 (d)(2)].	
Planned date of treatment compliance:	
Planned treatment to achieve compliance:	
☐ **Yes** ☐ **No**	I understand the treatment requirements that my PWS is required to meet and am aware of the deadline for providing the treatment.
☐ **Yes** ☐ **No**	I have discussed these requirements with a State or U.S. EPA representative
Signature:	Date:
Name (print)	Phone:

This notice may be submitted using one of the following options:

- **As an email attachment sent to stage2mdbp@epa.gov**
- **By mail or fax to the following:** **LT2ESWTR and Stage 2 DBPR**
  P.O. Box 98
  Dayton, OH 45401

  FAX: (937)-586-6557

Appendix B

Cost Estimate for Bulk Water Sample Analysis

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LT2 Monitoring Bulk Water Sample Analysis for [PWS name and/or facility name]

PWS required field:

For further information on this bid sheet, refer to Section 2.2 of the Source Water Monitoring Guidance Manual for Public Water Systems

		Submit bid to:	
Laboratory name:		**PWS name:**	
Laboratory address:		**PWS address:**	
Laboratory contact:		**PWS contact:**	
Phone/fax/email:		**Phone/fax/email:**	

Bid deadline (day, date, time (including time zone)):	**Laboratory period of performance:**
Estimated award date:	**Results turnaround time:**
Bid validity period:	**Extra Services (if applicable):**

Costs for *Cryptosporidium*-Only or *Cryptosporidium*/*Giardia* Analysis

			(A)	(B)	(A x B)
Sample PWS requests bid for *Cryptosporidium* analysis using Method 1623* [Specify sample volume if other than 10 L]	**Number of plants**	**Samples required per plant**	**Total samples**	**Cost per sample**	**Total cost**
Bulk water samples - full analysis					
Matrix spike samples					
Practice samples					
Potential replacement samples		Up to [no.]			
Estimated subsamples**		Up to [no.]			
Equipment	**Number of plants**	**Equipment required per plant**	**Total equipment**	**Cost per unit**	**Total cost**
Cubitainers/Carboys***					
Sampling if conducted by the laboratory or subcontractor (including turbidity)					
Shipping**	**Number of plants**	**Shipments per plant**	**Total shipments**	**Cost per shipment**	**Total cost**
Shipment of cubitainer/carboy to PWS					
Shipment of collected samples to laboratory					

Total

Costs for *E. coli* analysis

			(A)	(B)	(A x B)
Sample PWS requests bid for *E.coli* analysis using [Specify method]	**Number of plants**	**Units required per plant****	**Total units**	**Cost per unit**	**Total cost**
Field sample analysis					
Equipment					
Sample collection bottles					
Shipping**					
Shipment of sample collection bottles to PWS					
Shipment of collected samples to laboratory					

Total

*Laboratories may require special schedule and increased QA costs if 1622 is requested by the PWS.

**IMS, staining, and examination of each 0.5-mL portion of a sample concentrate that exceeds 0.5 mL packed pellet volume; include number for field, matrix spike, practice, and replacement samples

***All cubitainers/carboys required for field samples, matrix spike samples, practice samples, and potential replacement samples (may be purchased directly from supplier)

****Shipment cost of replacement equipment and samples should also be discussed and decided with laboratory

Appendix C

Cost Estimate for Field-Filtered Sample Analysis

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LT2 Monitoring Field-Filtered Sample Analysis for [PWS name and/or facility name]

PWS required field:

For further information on this bid sheet, refer to Section 2.2 of the Source Water Monitoring Guidance Manual for Public Water Systems

		Submit bid to:	
Laboratory name:		**PWS name:**	
Laboratory address:		**PWS address:**	
Laboratory contact:		**PWS contact:**	
Phone/fax/email:		**Phone/fax/email:**	

Bid deadline (day, date, time (including time zone)):	**Laboratory period of performance:**
Estimated award date:	**Results turnaround time:**
Bid validity period:	**Extra Services (if applicable):**

Costs for *Cryptosporidium*-Only or *Cryptosporidium*/*Giardia* Analysis

			(A)	(B)	(A x B)
Sample PWS requests bid for *Cryptosporidium* analysis using Method 1623* [Specify sample volume if other than 10 L]	**Number of plants**	**Samples required per plant**	**Total samples**	**Cost per sample**	**Total cost**
Field-filtered samples - full analysis					
Matrix spike samples (bulk sample)					
Practice samples					
Potential replacement samples		Up to [no.]			
Estimated subsamples**		Up to [no.]			
Equipment	**Number of plants**	**Equipment required per plant**	**Total equipment**	**Cost per unit**	**Total cost**
Filters [Envirochek™, Envirochek™ HV or Filta-Max®]***(extra if clogging expected)					
Sampling apparatus for rental or purchase (used during monitoring period)					
Cubitainer/Carboy (for use with each matrix spike)					
Sampling if conducted by the laboratory or subcontractor (including turbidity)					
Shipping	**Number of plants**	**Shipments per plant**	**Total shipments**	**Cost per shipment**	**Total cost**
Shipment of filters to PWS***					
Shipment of cubitainer/carboy (for matrix spike) to PWS					
Shipment of filter apparatus to PWS					
Shipment of cubitainer/carboy (for matrix spike) to laboratory					
Shipment of collected samples to laboratory***					
			Total		

Costs for *E. coli* analysis

			(A)	(B)	(A x B)
Sample PWS requests bid for *E.coli* analysis using [Specify method]	**Number of plants**	**Units required per plant****	**Total units**	**Cost per unit**	**Total cost**
Field sample analysis					
Equipment					
Sample collection bottles					
Shipping**					
Shipment of sample collection bottles to PWS					
Shipment of collected samples to laboratory					
			Total		

*Laboratories may require special schedule and increased QA costs if 1622 is requested by the PWS.

**IMS, staining, and examination of each 0.5-mL portion of a sample concentrate that exceeds 0.5 mL packed pellet volume; include number for field, matrix spike, practice, and replacement samples

***All filters required for field samples, matrix spike samples, practice samples, and potential replacement samples (may be purchased directly from supplier)

****Shipment cost of replacement equipment should be discussed and decided with laboratory

Appendix D

Sampling Location Worksheet

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Long Term 2 Enhanced Surface Water Treatment Rule
Sampling Location Worksheet

Public Water System (PWS) Name: _____ PWS ID: _____

Water Treatment Plant Name: _____ Water System Facility ID: _____

1. Source Name		
2. Source Type Flowing stream, Lake/Reservoir, or GWUDI		
3. Source Water Sampling Location Provide State assigned number		
4. Usage All year, Part-year, or Emergency (Describe conditions, constraints, months in operation)		
5. Proportion of typical average daily flow	_____ %	_____ %
6. Pretreatment Practices Presedimentation, Bank filtration, or Off-stream storage		
7. Recycling Practices (if applicable) Description and return flow location		
8. Chemical Pretreatment (Indicate location on plant schematic)		
9. Sample Compositing Procedure (if applicable) Blended sample tap, Composite sample, or Weighted		

Use additional sheets or reverse side to provide more information

Additional comments (Indicate item number from table on reverse side)

Appendix E

LT2 Sample Collection Location Schematics

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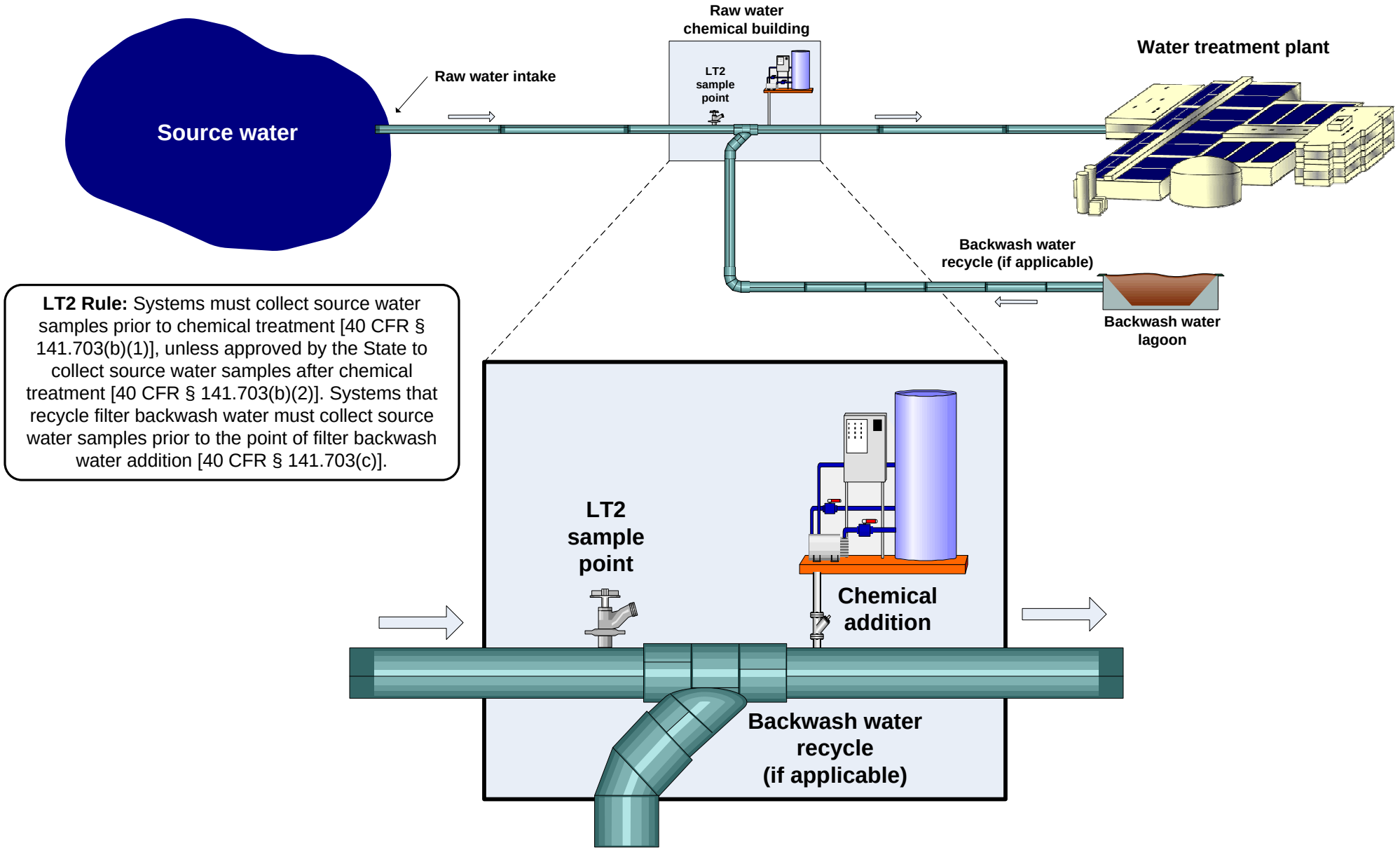
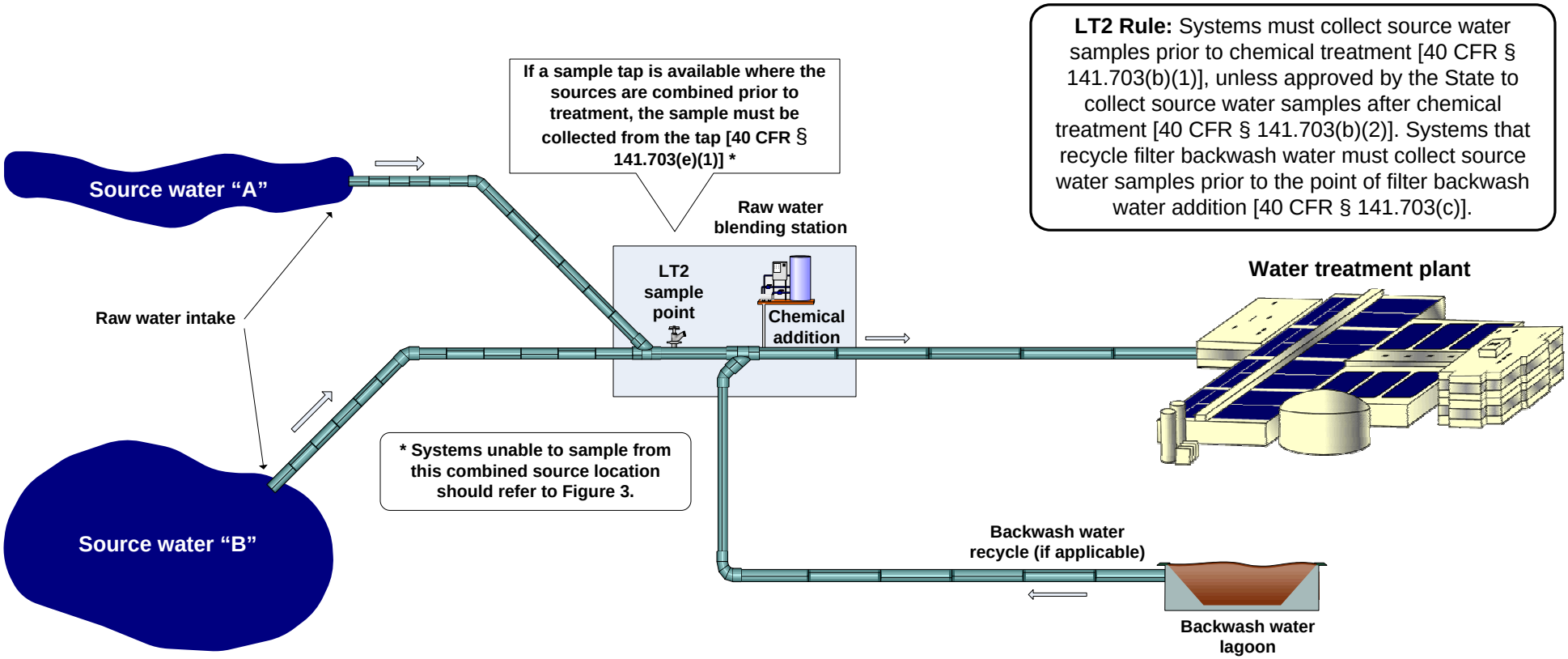
Figure	Description
1	Sample tap before chemical treatment and backwash water recycle (if applicable)
2	Multiple sources: sample tap after two combined sources
3	Multiple sources: two (or more) sources to be composited
4	Multiple plants with the same influent
5	Bank filtration
6	Ground water under the direct influence of surface water
7	Presedimentation basin
8	Raw water off-stream storage
9	Mixed source water: ground water and surface water sources
10	Blank schematic for submission to EPA

Figure 1. Sample Tap before Chemical Treatment and Backwash Water Recycle (if applicable)



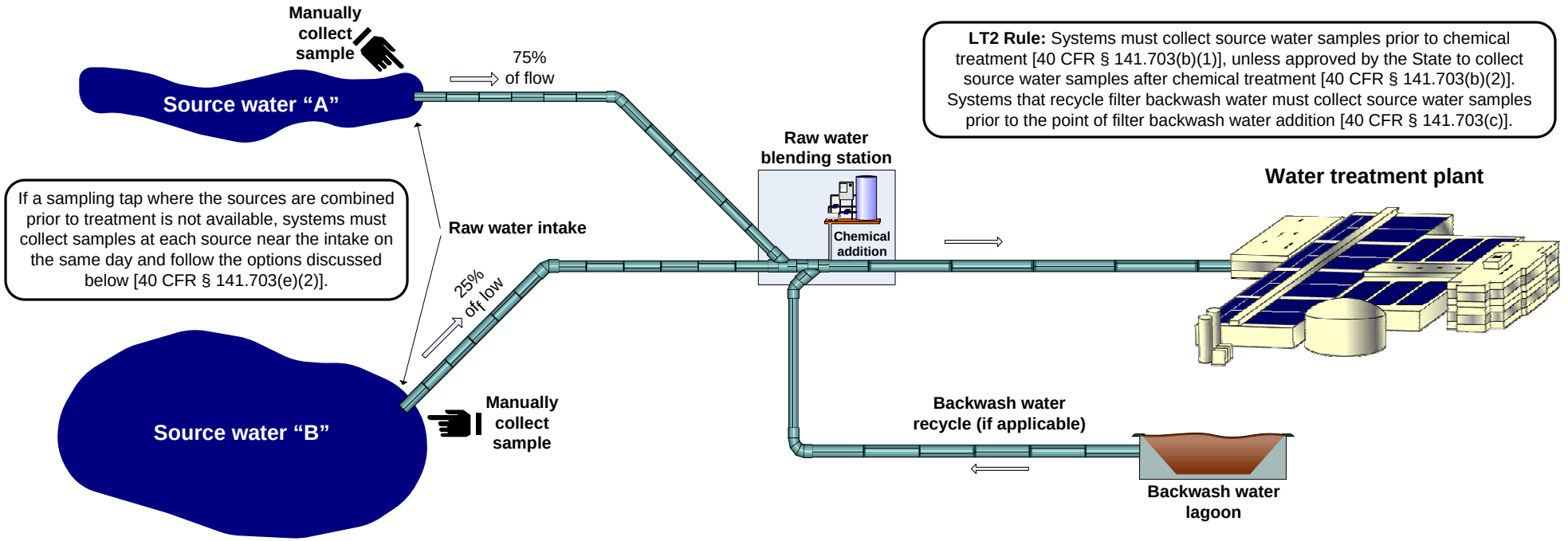
LT2 Rule: Systems must collect source water samples prior to chemical treatment [40 CFR § 141.703(b)(1)], unless approved by the State to collect source water samples after chemical treatment [40 CFR § 141.703(b)(2)]. Systems that recycle filter backwash water must collect source water samples prior to the point of filter backwash water addition [40 CFR § 141.703(c)].

Figure 2. Multiple Sources: Sample Tap after Two Combined Sources



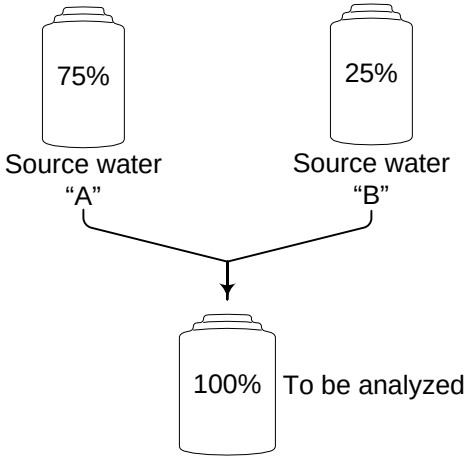
Multiple Alternating Sources:
Systems that use multiple alternating sources (seasonal or due to water quality changes) must sample from the source water in use on the designated sampling date following normal operating practices [40 CFR § 141.703(e)].

Figure 3. Multiple Sources: Two (or More) Sources to be Composited



OPTION 1 (Recommended Option):

Collect samples manually at each source near the intake on the same day and composite them into one sample to be analyzed. The volume of sample from each source must reflect its proportion of the total plant flow at the time the samples were collected [40 CFR § 141.703(e)(2)(i)].



OPTION 2:

Collect samples manually at each source near the intake on the same day and analyze each independently, then calculate a weighted average of the analysis results. This is done by multiplying the result for each source by the percentage of its contribution to the total plant flow at the time the samples were collected, and then summing these values [40 CFR § 141.703(e)(2)(ii)].

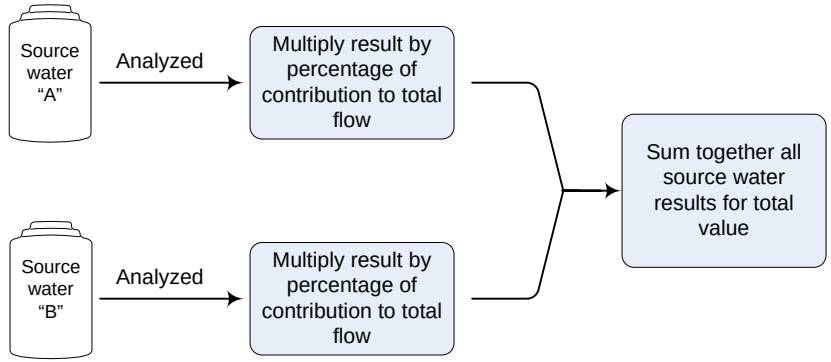
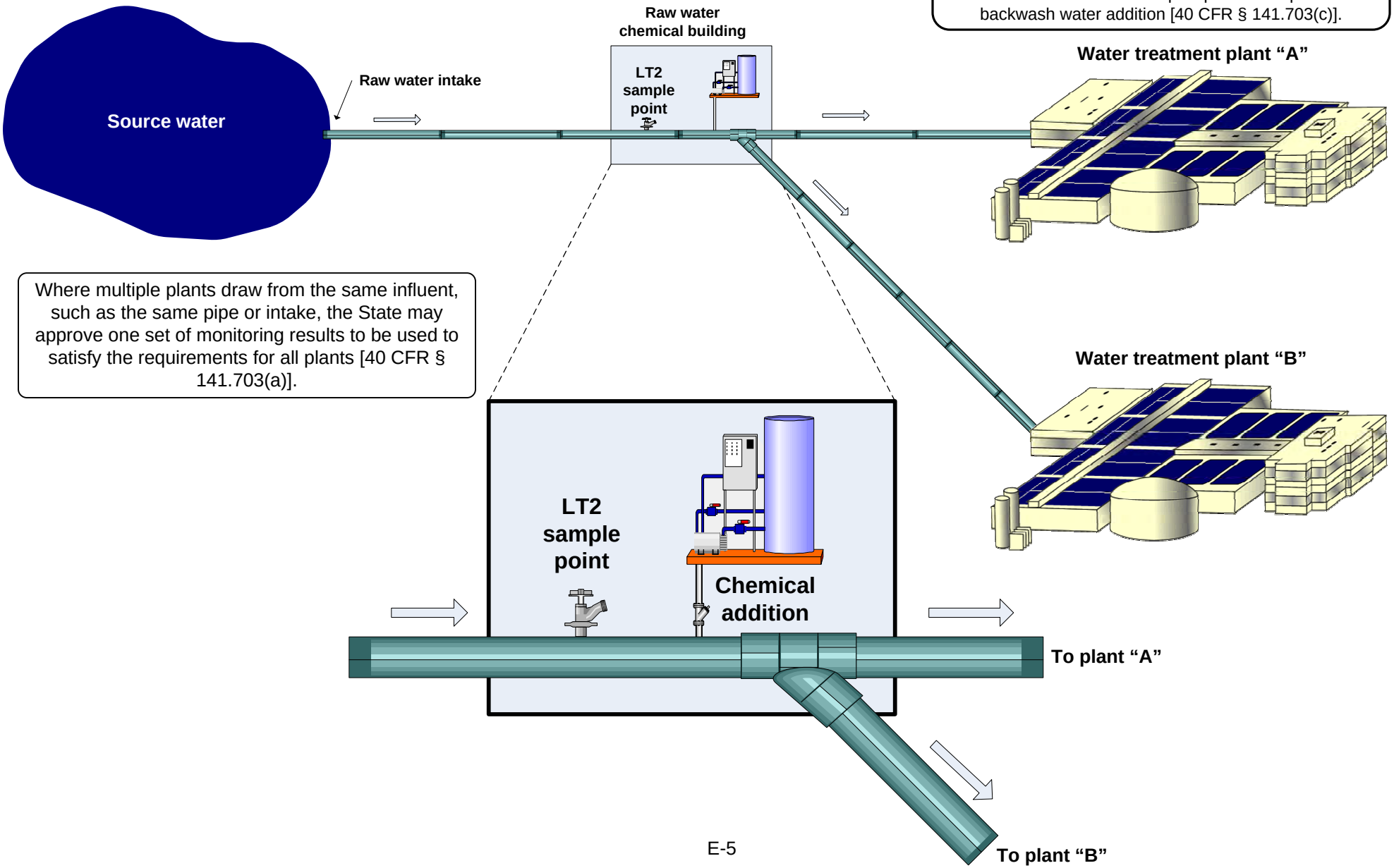


Figure 4. Multiple Plants with the Same Influent

LT2 Rule: Systems must collect source water samples prior to chemical treatment [40 CFR § 141.703(b)(1)], unless approved by the State to collect source water samples after chemical treatment [40 CFR § 141.703(b)(2)]. Systems that recycle filter backwash water must collect source water samples prior to the point of filter backwash water addition [40 CFR § 141.703(c)].

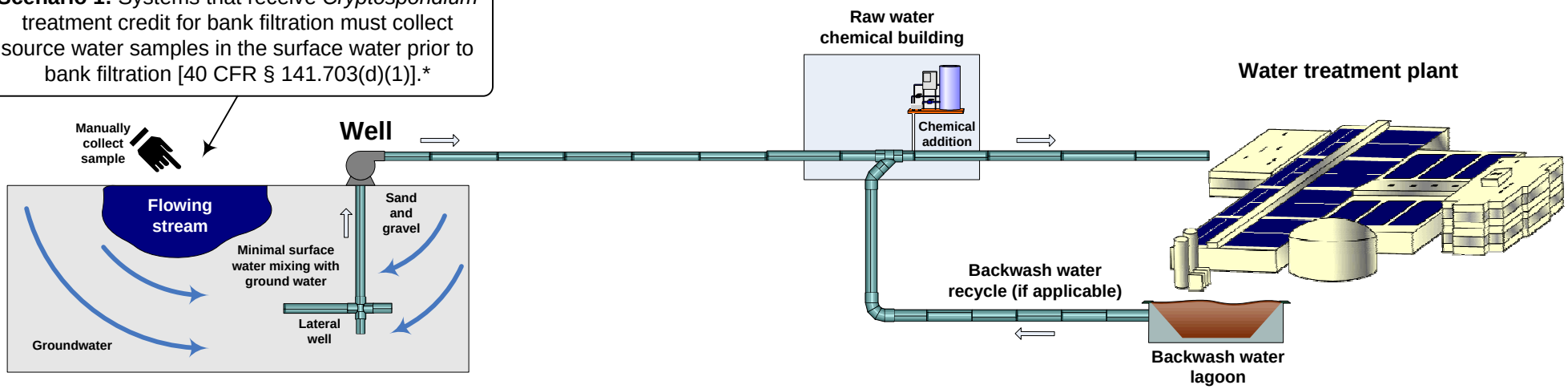


Where multiple plants draw from the same influent, such as the same pipe or intake, the State may approve one set of monitoring results to be used to satisfy the requirements for all plants [40 CFR § 141.703(a)].

Figure 5. Bank Filtration

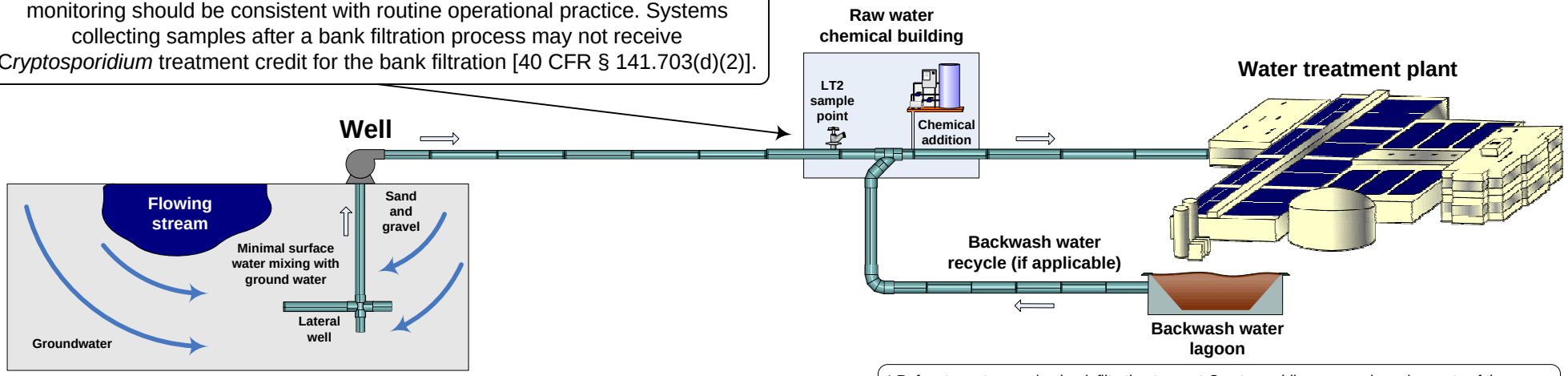
The correct sampling location for systems using bank filtration differs depending on whether the bank filtered water is treated by subsequent filtration:

Scenario 1: Systems that receive *Cryptosporidium* treatment credit for bank filtration must collect source water samples in the surface water prior to bank filtration [40 CFR § 141.703(d)(1)].*



Bank filtration cross section

Scenario 2: Systems using bank filtered water that is treated by subsequent filtration must collect source water samples from the well source (i.e., after bank filtration) but before any other treatment.** Use of bank filtration during monitoring should be consistent with routine operational practice. Systems collecting samples after a bank filtration process may not receive *Cryptosporidium* treatment credit for the bank filtration [40 CFR § 141.703(d)(2)].



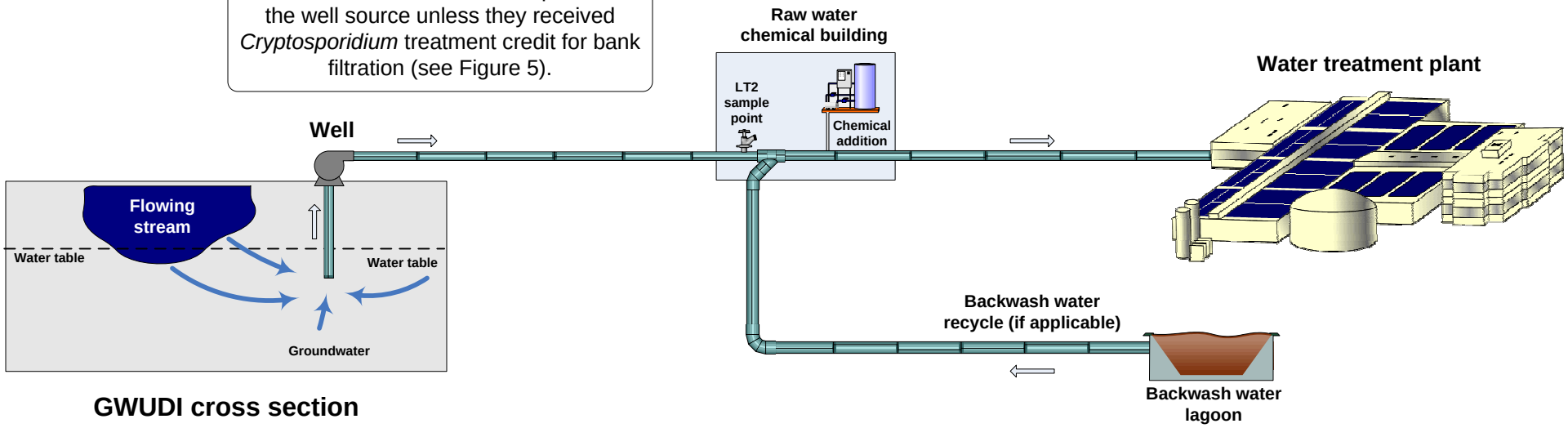
Bank filtration cross section

* Refers to systems using bank filtration to meet *Cryptosporidium* removal requirements of the Interim Enhanced Surface Water Treatment Rule (IESWTR) or Long Term 1 ESWTR under 40 CFR § 141.173(b) or 40 CFR § 141.522(a).
** Refers to systems where bank filtration serves as pretreatment to a filtration plant.

Figure 6. Ground Water Under the Direct Influence of Surface Water (GWUDI)

LT2 Rule: Systems must collect source water samples prior to chemical treatment [40 CFR § 141.703(b)(1)], unless approved by the State to collect source water samples after chemical treatment [40 CFR § 141.703(b)(2)]. Systems that recycle filter backwash water must collect source water samples prior to the point of filter backwash water addition [40 CFR § 141.703(c)].

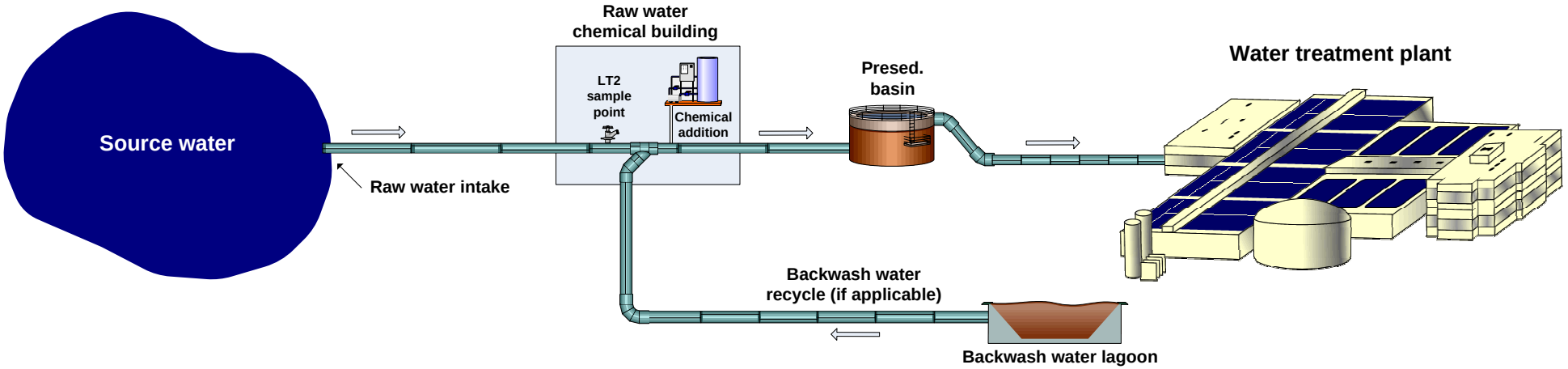
Systems that use ground water under the direct influence of surface water (GWUDI) must collect source water samples from the well source unless they received *Cryptosporidium* treatment credit for bank filtration (see Figure 5).



GWUDI cross section

Figure 7. Presedimentation Basin

Scenario 1:
Systems using a presedimentation basin with chemical addition should collect source water samples prior to chemical treatment, unless approved by the State to collect source water samples after chemical treatment. Systems that recycle filter backwash water must collect source water samples prior to the point of filter backwash water addition [40 CFR § 141.703(c)].



Scenario 2:
Systems without chemical addition prior to or in a presedimentation basin, or that have been approved by the State to collect source water samples after chemical treatment, may sample after the presedimentation basin but will not receive any treatment credit for presedimentation.

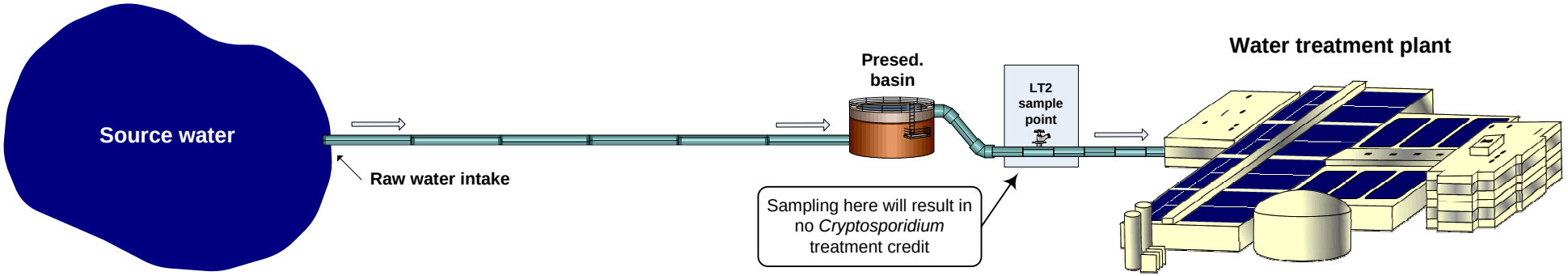


Figure 8. Raw Water Off-Stream Storage

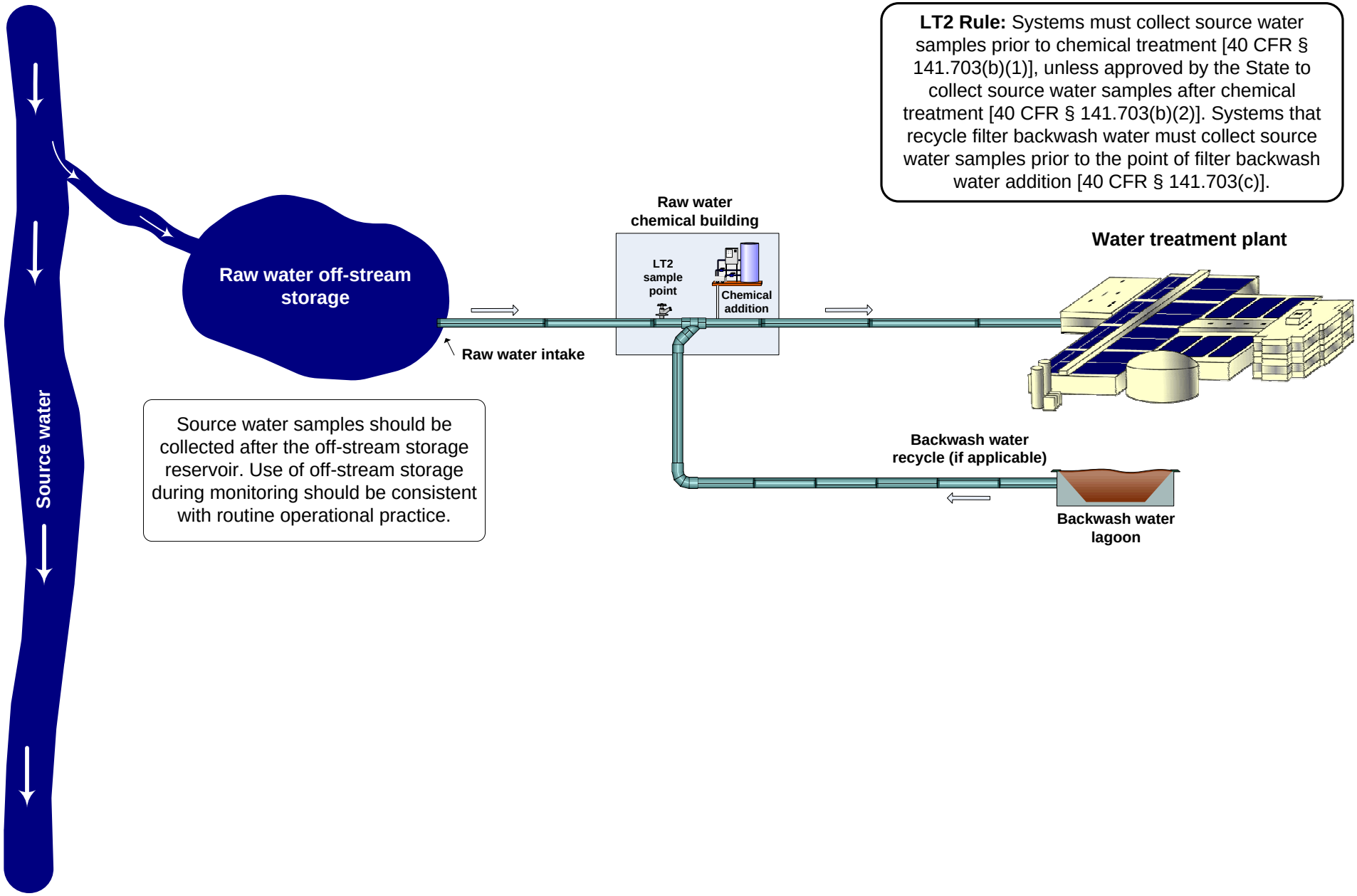


Figure 9. Mixed Source Water: Ground Water and Surface Water Sources

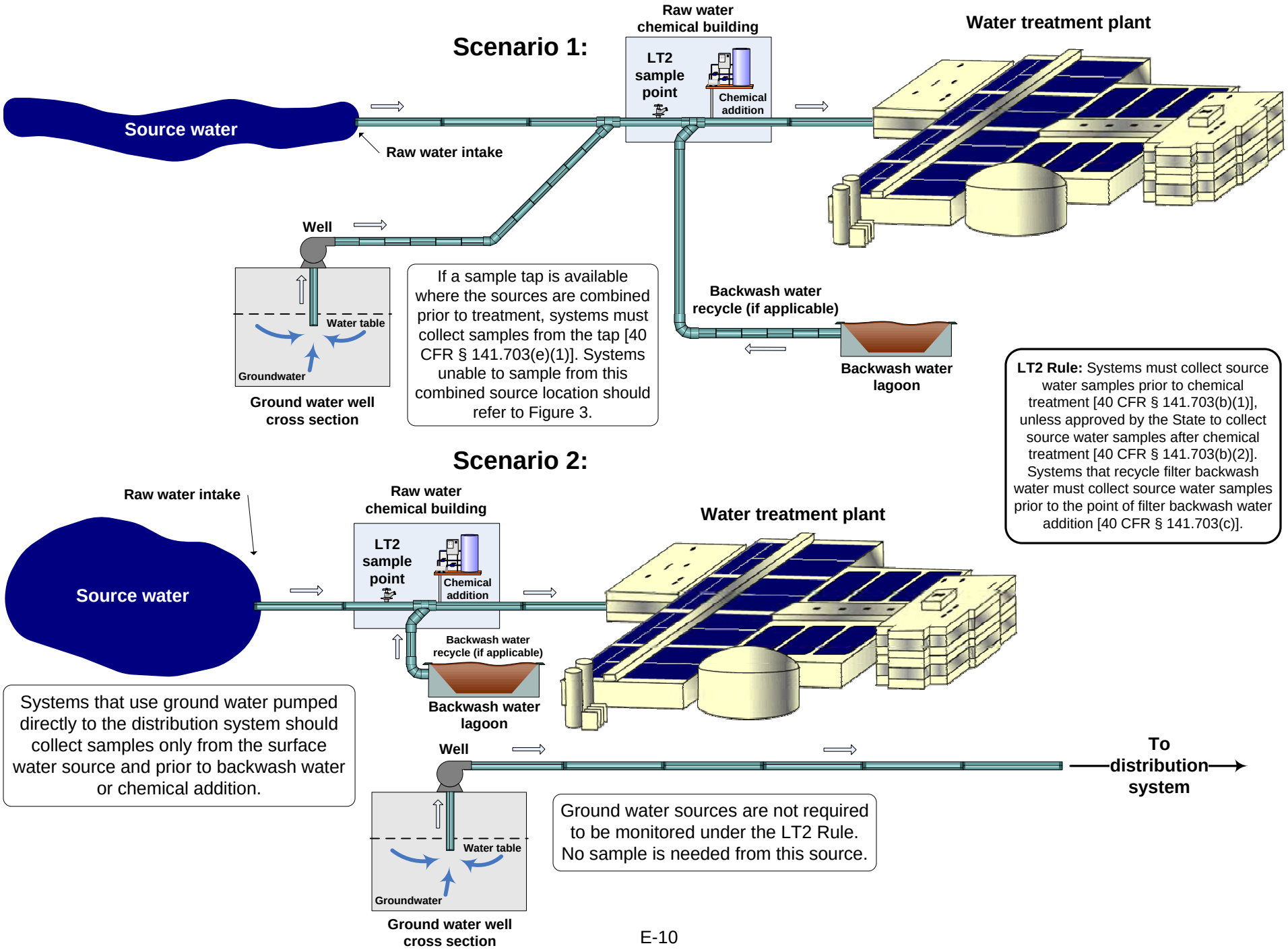


Figure 10. Blank Schematic for Submission to EPA

Public Water System (PWS) name: _____

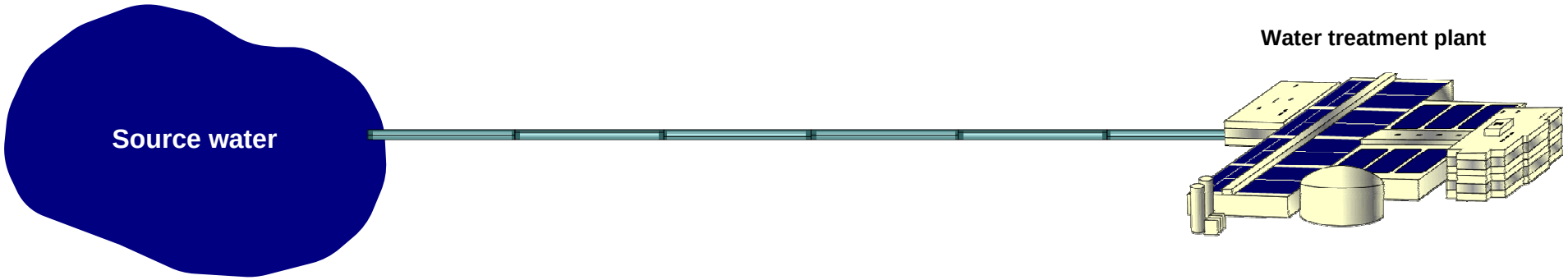
PWS ID: _____

Water treatment plant name: _____

Water system facility ID: _____

Indicate the following on the diagram that best represents your facility type (if applicable):

1. LT2 sampling location
2. Points of chemical treatment prior to the treatment plant
3. Filter backwash water addition
4. Pretreatment processes (e.g., presedimentation basins, bank filtration)
5. Multiple source waters (show by adding additional sources)



Appendix F

LT2 Sample Collection Form

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Long Term 2 Enhanced Surface Water Treatment Rule
Sample Collection Form

Utility Information	Shipping Information	For Lab Use Only
PWS name: PWS address:	Lab name: Lab address:	Date received: Time received: Received by: Sample temperature on receipt: Sample condition on receipt:
Sampler name:	Date shipped: Tracking number:	

Sample Identification Information (*the combination of bolded items are used to identify the sample under LT2*)	
Sample ID (optional):	
PWS ID:	
Facility ID:	
Facility name:	
Sample collection point ID:	
Sample collection point name:	
Sample collection date:	

Source water type[a] (circle one):	Flowing stream (FS) GWUDI[b] - FS	Reservoir/lake (RL) GWUDI[b] - RL
Requested analysis (circle one):	*Cryptosporidium* field sample *E. coli*	*Cryptosporidium* matrix spike

Sample Collection Information

Cryptosporidium	***E. coli***
Initial meter reading (*field-filtered samples only*):	Sample collection time:
Final meter reading (*field-filtered samples only*):	Turbidity (NTU):
Sample collection time (*or start time, if field filtering*):	
Sample collection stop time *(field-filtered samples only)*:	
Source water temperature:	

Additional comments:

Sampler signature: Date:

[a] The source water type should be selected based on the type of source water that accounts for the majority of the surface water used as source water at the time of sample collection

[b] Ground water under the direct influence of surface water

Appendix G

Envirochek™ Field Filtration for *Cryptosporidium*

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Procedure for Field-Filtering Samples for *Cryptosporidium* Analysis Using the Pall Life Sciences Envirochek™ or Envirochek™ HV Capsules

1.0 Materials

The following materials should be available before collecting sample:

- □ Several pairs of new, powder-free latex gloves
- □ Sample collection form (**Appendix F**)
- □ Pall Life Sciences Envirochek™ or Envirochek™ HV capsule (recommend that two capsules be kept on hand in case the first one clogs prior to filtering 10 L)
- □ Sanitary ≥30 L carboy (if sample is not from a pressurized source)
- □ Stopwatch
- □ Sample label
- □ Cooler, approximately 16-quart
- □ Temperature monitoring device (e.g., thermometer vial, Thermochron™ iButton, or equivalent) (if measuring temperature during shipment)
- □ Two large plastic trash bags
- □ One 8-lb bag of ice or gel ice packs
- □ Three gallon size ziplock bags
- □ Strapping tape
- □ Two self-adhesive plastic airbill sleeves
- □ Airbill for shipment

The following items may be purchased as a unit for sampling from a vendor or laboratory supplying sampling apparatus for field filtering *Cryptosporidium* samples:

- □ Pump (if sample is not from a pressurized source) - Electric centrifugal or Electric peristaltic pump, or any equivalent pump that can create a flow of approximately 2 L/min
- □ Two 0.5-in. X 0.375-in. barbed reducing connectors
- □ Five lengths of clean 12.7-mm (0.5-in.) internal-diameter clear, vinyl, laboratory tubing
- □ Five pairs of hose clamps fit to tubing
- □ One 0.5-in. x 3-in. nipple
- □ One coupling to fit 0.5-in. internal diameter tubing
- □ One roll teflon tape
- □ One 0.5-in. x 0.5-in. x 0.25-in. tee
- □ Six 0.5-in. barbed male adapters
- □ One garden hose barbed adapter
- □ Pressure regulator

- □ Pressure gauge (capsule's maximum operating pressure, Envirochek™ - 30 PSID, Envirochek™ HV - 60 PSID)
- □ Water meter (flow totalizer)
- □ Flow rate meter with valve or flow control valve (capsule's maximum flow rate, Envirochek™ - 2 L/min, Envirochek™ HV - 4 L/min)

2.0 Collecting the Sample

If the sample will be collected from a pressurized source, use the sample collection procedures in Section 2.1. If the sample will be collected from an unpressurized source, use the sample collection procedures in Section 2.2.

2.1 Sample Filtration from a Pressurized Source

2.1.1 Before connecting the sampling system to the tap or source, turn on the tap and allow the water to flow for 2 to 3 minutes until the temperature has stabilized or until any debris that has accumulated in the source water lines has cleared and the turbidity in the water becomes visibly uniform. Turn off the tap.

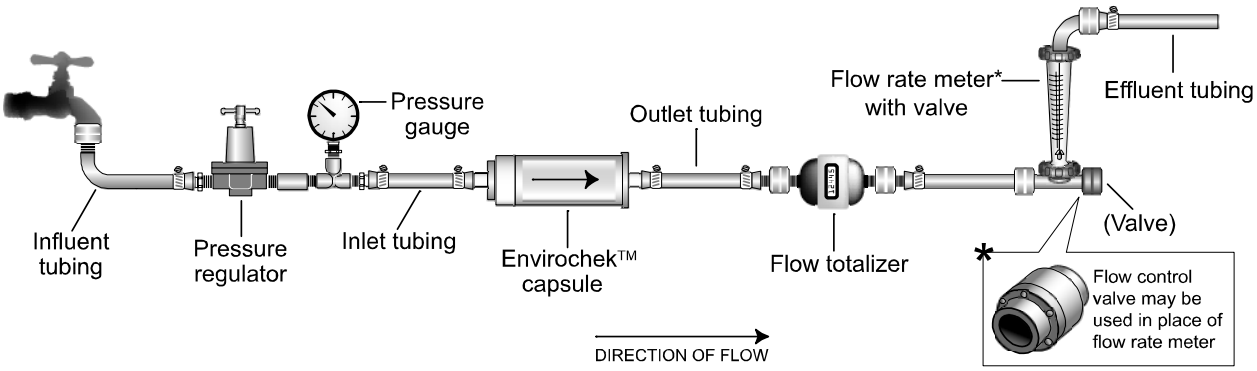
2.1.2 Put on a pair of powder-free latex gloves to prevent contamination from outside sources. Any contamination inside the sampling apparatus may bias the final results.

2.1.3 Determine the pressure of the water source with the pressure gauge.

2.1.4 Assemble the sampling system, minus the Envirochek™ /Envirochek™ HV capsule. In place of the Envirochek™ /Envirochek™ HV capsule, insert a 0.5-in. barbed connector between the outlet tubing from the sample valve or the pressure regulator or gauge and the inlet tubing of the flow totalizer, flow meter or control valve. For high pressure (>30 PSIG for Envirochek™ capsule or >60 PSIG for Envirochek™ HV capsule) sites, the sampling system should be assembled in the following order, as shown in **Figure 1** below:

- □ Reinforced influent tubing
- □ Pressure regulator
- □ Pressure gauge
- □ Reinforced inlet tubing
- □ Envirochek™ /Envirochek™ HV capsule
- □ Reinforced outlet tubing
- □ Flow totalizer (mechanical or graduated collection device)
- □ Flow rate meter with valve or flow control valve
- □ Effluent tubing to drain

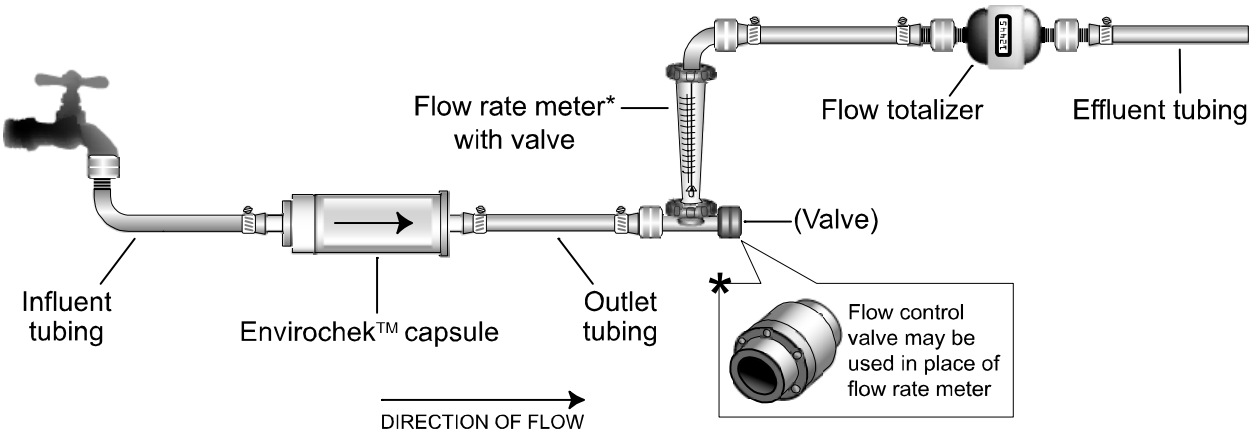
Figure 1.Sample System Setup for Collecting *Cryptosporidium* Samples from a Pressurized Source (>30 PSIG for Envirochek™ capsule or >60 PSIG for Envirochek™ HV capsule)



For low pressure (1 to 30 PSIG for Envirochek™ capsule or 1 to 60 PSIG for Envirochek™ HV capsule) sites, the sampling system should be assembled at the sample port valve in the following order, as shown in **Figure 2** below :

- ☐ Influent tubing
- ☐ Envirochek™ /Envirochek™ HV capsule
- ☐ Outlet tubing
- ☐ Flow rate meter with valve or flow control valve
- ☐ Flow totalizer (mechanical or graduated collection device)
- ☐ Effluent tubing to drain

Figure 2.Sample System Setup for Collecting *Cryptosporidium* Samples from a Pressurized Source (1 - 30 PSIG for Envirochek™ capsule or 1 - 60 PSIG for Envirochek™ HV capsule)



2.1.5 Connect the sampling system, with the connector in place of the Envirochek™ /Envirochek™ HV capsule, to the pressurized water system, using appropriate fittings and clamps.

2.1.6 Slowly turn the tap to fully open. Allow a minimum of 20 L to flush the system. During this period, perform the following steps:

2.1.6.1 Measuring the flow rate with the flow rate meter or the flow totalizer and a stopwatch, adjust the flow rate to approximately 2 L/min (approximately 0.5 gpm) for the Envirochek™ capsule or 4 L/min (approximately 1.0 gpm) for the Envirochek™ HV capsule. If a flow control valve at the appropriate flow rate is used, no adjustment of the flow rate is necessary. Using the pressure regulator, adjust the pressure to a maximum of 30 PSIG if using the Envirochek™ or 60 PSIG if using the Envirochek™ HV. Observe the system for leaks and take the necessary corrective action if any are present.

2.1.6.2 Record the following information on the sample collection form (**Appendix F**):

- ✎ Public water system (PWS) name
- ✎ PWS address
- ✎ Sampler name
- ✎ Sample ID (optional)
- ✎ Public Water System Identification (PWS ID) number
- ✎ Public Water System facility ID number
- ✎ Facility name
- ✎ Sample collection point ID
- ✎ Sample collection point name
- ✎ Sample collection date
- ✎ Source water type (optional [but required for *E. coli* sample forms])
- ✎ Requested analysis (circle *Cryptosporidium* field sample for routine monitoring sample; circle both "*Cryptosporidium* field sample" and "*Cryptosporidium* matrix spike" sample if you are sending an additional sample with the monitoring sample for matrix spike analysis)

2.1.6.3 After the assembly has been flushed, measure the turbidity of the source water and any optional water quality parameters such as temperature, and/or pH.

2.1.7 Turn off the water at the sample port valve when the flow rate has been adjusted and the system has been flushed.

2.1.8 Record the following information on the capsule label with a waterproof pen:

- ✎ PWS ID
- ✎ Facility name
- ✎ Date of sample collection

2.1.9 Record the following information on the sample collection form:

- ✎ Initial meter reading

2.1.10 Remove the connector and in its place, install the Envirochek™ /Envirochek™ HV capsule in line, securing the inlet and outlet ends with the appropriate fittings/clamps.

Note! Retain the vinyl caps provided with the Envirochek™ /Envirochek™ HV capsule. These caps will be needed to seal the capsule for shipment.

2.1.11 Slowly turn on the pressurized water source. Adjust the flow rate to approximately 2 L/min for Envirochek™ capsule or 4 L/min for Envirochek™ HV capsule, if necessary. Record the following information on the capsule label or sample collection form:

- ✎ Start time

2.1.12 Vent the residual air in the capsule using the bleed valve by turning it counter-clockwise. When the capsule is full of water, close the bleed valve.

2.1.13 Monitor the water meter. When the targeted volume (actual sample volumes will be selected by the utility, but volumes are typically 10 L [2.64 gal] to 50 L [13.2 gal] and preferred to be consistent between sampling events for each source) has passed through the Envirochek™ /Envirochek™ HV capsule, shut off the water source. Allow the pressure to decrease until the water stops.

2.1.14 Record the following information on the capsule label and/or sample collection form:

- ✎ Stop time (when the water was shut off)
- ✎ Final meter reading
- ✎ Comments to laboratory, if needed

2.1.15 With the capsule inlet pointed up, loosen the outlet end of the Envirochek™ /Envirochek™ HV capsule and allow water to drain as much as possible. Water drainage from the capsule through the outlet is acceptable, as the sample has passed through the membrane. Opening the bleed valve during the draining will speed the process. Be sure to close the valve when finished.

2.1.16 Disconnect the inlet end of the Envirochek™ /Envirochek™ HV capsule, making sure not to spill any of the water remaining in the capsule through the inlet port. This water is part of your sample. Capsule may be shipped with or without residual water.

2.1.17 Seal the inlet of the capsule with the vinyl end cap that was previously saved.

2.1.18 Seal the outlet of the capsule with the vinyl end cap that was previously saved. Place the Envirochek™ /Envirochek™ HV capsule in a plastic ziplock bag for shipment.

2.1.19 Immediately following sample collection, place the bag containing the capsule in a refrigerator to chill prior to packing the shipping cooler for shipment. If no refrigerator is available, and the sample will not be shipped for several hours, place the bag in the shipping cooler with ice to chill. Replace the ice with fresh ice before shipping.

Note! Method 1622/1623 requires that the temperature of the sample upon arrival at the laboratory must be $\leq$20°C (but not frozen), and the laboratory must begin sample processing within 96 hours of sample collection. If the sample temperature and holding time requirements are not met, then the sample is invalid and must be recollected.

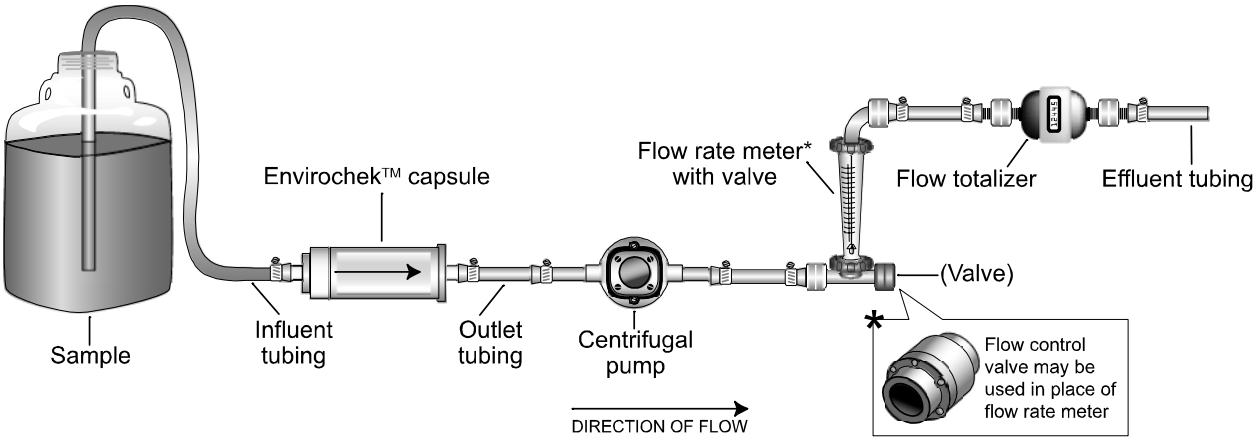
2.2 Sample Filtration Using an Unpressurized Source

2.2.1 Put on a pair of powder-free latex gloves to prevent contamination from outside sources. Any contamination inside the sampling apparatus may bias the final results.

2.2.2 If sampling from a source of unlimited volume, it may be desirable to pre-flush the sampling system. Assemble the sampling system, minus the Envirochek™ /Envirochek™ HV capsule. In place of the Envirochek™ /Envirochek™ HV capsule, insert a 0.5-in. coupling to connect the influent tubing to the inlet tubing of the pump. The sampling system should be assembled in the following order, as shown in **Figure 3** below:

- ☐ Influent tubing
- ☐ Envirochek™ /Envirochek™ HV capsule
- ☐ Outlet tubing
- ☐ Centrifugal or peristaltic pump
- ☐ Tubing
- ☐ Flow rate meter with valve or flow control valve
- ☐ Flow totalizer (mechanical or graduated collection device)
- ☐ Effluent tubing to drain

Figure 3. Sample System Setup for Collecting *Cryptosporidium* Samples from an Unpressurized Source



When assembling sample chain, make sure that it is as airtight as possible in order to create a vacuum. To accomplish this, make sure that clamps are used at each connection and that rubber washers are inserted into the hose connections on the inlet and outlet ends of the centrifugal pump.

2.2.3 Place the inlet end of the inlet tubing into the sample source, away from any walls, bottom, or other environmental surfaces.

2.2.4 Turn on the pump and allow a minimum of 20 L to flush the system. If sampling source water from a carboy, continuously refill the carboy as necessary to flush the system. As a recommendation, the carboy should not be removed from the sampling chain and should be refilled using a separate container. Observe the system for leaks and take the necessary corrective action if any are present. During this period, perform the following steps:

2.2.4.1 Measuring flow rate with the flow rate meter or the flow totalizer and a stopwatch, adjust the flow rate to approximately 2 L/min (approximately 0.5 gpm) for the Envirochek™ capsule or 4 L/min (approximately 1.0 gpm) for the Envirochek™ HV capsule by varying the pump speed or adjusting the valve (if pump is not variable speed). If a flow control valve at the appropriate flow rate is used, no adjustment of the flow rate is necessary.

2.2.4.2 Record the following information on the sample collection form:

- ✎ Public water system (PWS) name
- ✎ PWS address
- ✎ Sampler name
- ✎ Sample ID (optional)
- ✎ Public Water System Identification (PWS ID) number
- ✎ Public Water System facility ID number
- ✎ Facility name
- ✎ Sample collection point ID
- ✎ Sample collection point name
- ✎ Sample collection date
- ✎ Source water type (optional [but required for *E. coli* sample forms])
- ✎ Requested analysis (circle *Cryptosporidium* field sample for routine monitoring sample; circle both "*Cryptosporidium* field sample" and "*Cryptosporidium* matrix spike" sample if you are sending an additional sample with the monitoring sample for matrix spike analysis)

2.2.5 Turn off the pump when the flow rate has been adjusted and the assembly has been flushed.

2.2.6 Record the following information on the capsule label:

- ✎ PWS ID
- ✎ Facility name
- ✎ Date of sample collection

2.2.7 Record the following information on the sample collection form:

- ✎ Initial meter reading

2.2.8 Install the Envirochek™ /Envirochek™ HV capsule in line, securing the inlet and outlet ends with the appropriate fittings/clamps.

Note! Retain the vinyl caps provided with the Envirochek™ /Envirochek™ HV capsule. These caps will be needed to seal the capsule for shipment.

2.2.9 Refill the carboy with the sample to be filtered. As a recommendation, the carboy should not be removed from the sampling assembly and should be refilled using a separate container.

2.2.10 Turn on the pump. Adjust the flow rate to approximately 2 L/min for the Envirochek™ capsule or 4 L/min for the Envirochek™ HV capsule, if necessary. Record the following information on the capsule label and/or sample collection form:

- ✎ Start time

2.2.11 Monitor the water meter continuously refilling the carboy as needed. When the targeted volume (actual sample volumes are selected by the utility, but volumes are typically 10 L [2.64 gal] to 50 L [13.2 gal] and preferred to be consistent between sampling events for each source) is reached, remove tubing from the carboy and then shut off the pump.

2.2.12 Record the following information on the capsule label and/or sample collection form:

- ✎ Stop time (when the pump was shut off)
- ✎ Final meter reading
- ✎ Comments to laboratory, if needed

2.2.13 With the capsule inlet pointed up, loosen the outlet end of the Envirochek™ /Envirochek™ HV capsule and allow water to drain as much as possible. Water drainage from the capsule through the outlet is acceptable, as the sample has passed through the membrane. Opening the bleed valve during the draining will speed the process. Be sure to close the valve when finished.

2.2.14 Disconnect the inlet end of the Envirochek™ /Envirochek™ HV capsule, making sure not to spill any of the water remaining in the capsule through the inlet port. This water is part of your sample. Capsule may be shipped with or without residual water.

2.2.15 Seal the inlet of the capsule with the vinyl end cap that was saved previously.

2.2.16 Seal the outlet of the capsule with the vinyl end cap that was saved previously. Place the Envirochek™ /Envirochek™ HV capsule in a plastic ziplock bag for shipment.

2.2.17 Immediately following sample collection, place the bag containing the capsule in a refrigerator to chill prior to packing the shipping cooler for shipment. If no refrigerator is available, and the sample will not be shipped for several hours, place the bag in the shipping cooler with ice to chill. Replace the ice before shipping.

Note! Method 1622/1623 requires that the temperature of the sample upon arrival at the laboratory must be ≤20°C (but not frozen), and the laboratory must begin sample processing within 96 hours of sample collection. If the sample temperature and holding time requirements are not met, then the sample is invalid and must be recollected.

3.0 Packing the Sample

3.1 Insert two large plastic trash bags into the shipping cooler to create a double liner. Immediately before packing the cooler, create two 4-pound bags of ice in two separate ziplock bags. To prevent leaks place each ice pack into an additional ziplock bag. Gel packs or blue ice may be used instead of wet ice, as long as the sample is maintained in the appropriate temperature range. Seal the ziplock bags, expelling as much air as possible, and secure top with tape.

Note! Shipping companies may delay sample shipments if leakage occurs. Double liners and ziplock bags around ice will prevent leakage and delays.

3.2 Place the bag containing the capsule into the shipping container. Wrap the capsule in bubble wrap to prevent freezing. Inflated, empty sample bags can be placed between the capsule and the ice packs to prevent the sample from freezing.

3.3 If you will be monitoring sample temperature during shipment, place in the cooler the temperature monitoring device (e.g., extra sample bottle for measuring sample temperature upon receipt at the laboratory, thermometer vial, or Thermochron™ iButton). Seal each liner bag by twisting top of bag and tying in a knot.

3.4 Peel the backing off one of the plastic airbill sleeves and attach the sleeve to the inside of the cooler lid.

✎ Sign and date the sample collection form.

Fold the completed sample collection form, and place it inside the plastic sleeve.

3.5 Close the cooler lid, seal the horizontal joints with strapping tape, and secure the lid with tape by taping the cooler at each end, perpendicular to the seal.

Note! Shipping companies may delay sample shipments if leakage occurs. Be sure to seal the cooler joints.

3.6 Peel the backing off of the second airbill sleeve and attach the sleeve to the outside of the cooler lid. Complete the shipping airbill with the laboratory address, billing information, sample weight, and shipping service. Remove the shipper's copy of the airbill, and place the remainder of the airbill inside the plastic sleeve.

4.0 Shipping and Tracking

4.1 Ship samples on the day of collection and use a reliable shipping service for overnight delivery. If samples are not shipped the day of collection, the sample must be maintained between 1°C and 10°C (but not frozen) by chilling in a refrigerator or cooler filled with ice.

4.2 Contact the laboratory to notify them of the sample shipment. Request that the laboratory contact you the next day if the sample is not received.

4.3 Using the airbill number on the shipper's copy of the airbill, track the sample shipment using the shipping company's web page or by contacting the shipping company over the phone.

4.4 If problems are encountered with the shipment, communicate with the shipping company to resolve, and update the laboratory regarding the status of the shipment.

Appendix H

Filta-Max® Field Filtration for *Cryptosporidium*

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Procedure for Field-Filtering Samples for *Cryptosporidium* Analysis Using IDEXX Filta-Max® Filters

1.0 Materials

The following materials should be available before collecting your sample:

- □ Several pairs of new, powder-free latex gloves
- □ Sample collection form (**Appendix F**)
- □ Filta-Max® foam filter module (IDEXX, cat. number, FMC 10603) with housing (IDEXX, cat. number, FMC 10504)
- □ Sanitary ≥30 L carboy (if sample is not from a pressurized source)
- □ Stopwatch
- □ Sample number label
- □ Cooler, approximately 16-quart
- □ Temperature monitoring device (e.g., thermometer vial, Thermochron™ iButton, or equivalent) (if measuring temperature during shipment)
- □ Two large plastic trash bags
- □ One 8-lb bag of ice or gel ice packs
- □ Three gallon size ziplock bags
- □ Strapping tape
- □ Two self-adhesive plastic airbill sleeves
- □ Airbill for shipment

The following items may be purchased as a unit for sampling from a vendor or laboratory supplying sampling apparatus for field filtering *Cryptosporidium* samples:

- □ Electric peristaltic pump (if sample is not from a pressurized source)
- □ Two 0.5-in. x 0.375-in. barbed reducing connectors
- □ Five lengths of clean 12.7-mm (0.5-in.) internal-diameter clear, vinyl, laboratory tubing
- □ Five pairs of hose clamps fit to tubing
- □ One 0.5-in. x 3-in. nipple (if sample line pressure is >120 PSIG)
- □ One coupling to fit 0.5-in. internal diameter tubing
- □ One roll teflon tape
- □ One 0.5-in. x 0.5-in. x 0.25-in. tee (if sample line pressure is >120 PSIG)
- □ Six 0.5-in. barbed male adapters
- □ One garden hose barbed adapter
- □ Pressure regulator (if sample line pressure is >120 PSIG)
- □ Pressure gauge (filter's maximum operating pressure 120 PSIG)

- □ Water meter (flow totalizer)
- □ Flow rate meter or flow control valve (filter's maximum flow rate is 3-4 L/min)

2.0 Collecting the Sample

If the sample will be collected from a pressurized source, use the sample collection procedures in Section 2.1. If the sample will be collected from an unpressurized source, use the sample collection procedures in Section 2.2.

2.1 Sample Filtration from a Pressurized Source

2.1.1 Before connecting the sampling system to the tap or source, turn on the tap and allow the water to flow for 2 to 3 minutes until the temperature has stabilized or until any debris that has accumulated in the source water lines has cleared and the turbidity in the water becomes visibly uniform. Turn off the tap.

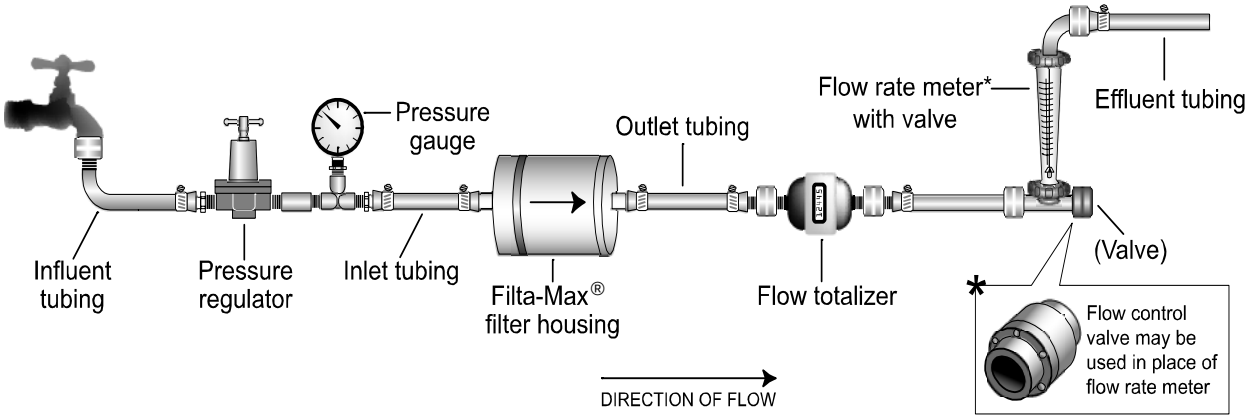
2.1.2 Put on a pair of powder-free latex gloves to prevent contamination from outside sources. Any contamination inside the sampling apparatus may bias the final results.

2.1.3 Determine the pressure of the water supply using a pressure gauge.

2.1.4 Assemble the sampling system, minus the Filta-Max® filter. For high pressure (>120 PSIG) sites, the sampling system should be assembled at the sample port valve in the following order, as shown in **Figure 1** below:

- □ Reinforced influent tubing
- □ Pressure regulator
- □ Pressure gauge
- □ Reinforced inlet tubing
- □ Filta-Max® filter housing, with the direction of flow depicted with an arrow
- □ Reinforce outlet tubing
- □ Flow totalizer (mechanical or graduated collection device)
- □ Flow rate meter with valve or flow control valve
- □ Effluent tubing to drain

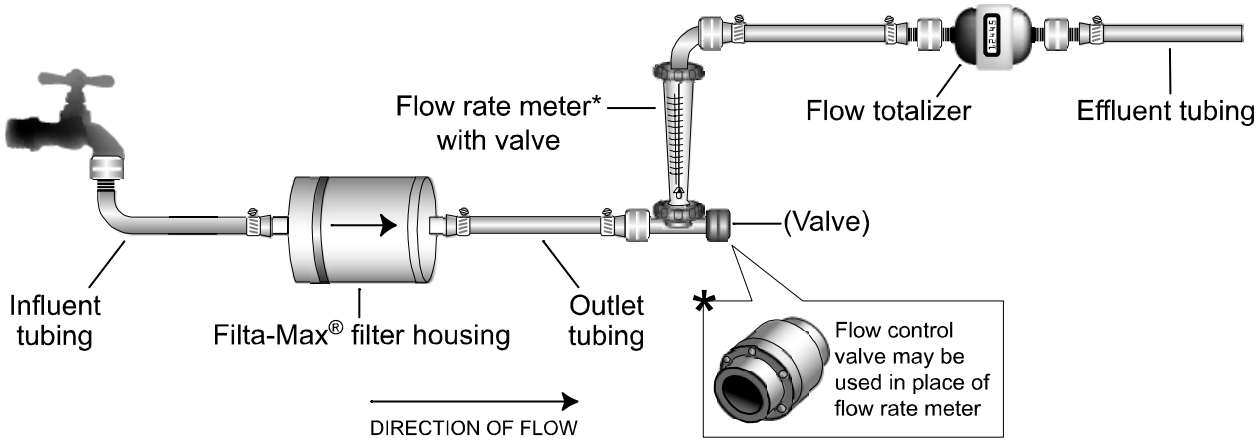
Figure 1. Sample System Setup for Collecting *Cryptosporidium* Samples from a Pressurized Source (>120 PSIG)



For a low-pressure (7.5 to 120 PSIG) site, the sampling system should be assembled at the sample port valve in the following order, as shown in **Figure 2** below:

- ☐ Influent tubing
- ☐ Filta-Max® filter housing, with the direction of flow depicted with an arrow
- ☐ Outlet tubing
- ☐ Flow rate meter with valve or flow control valve
- ☐ Flow totalizer (mechanical or graduated collection device)
- ☐ Effluent tubing to drain

Figure 2. Sample System Setup for Collecting *Cryptosporidium* Samples from a Pressurized Source (7.5 - 120 PSIG)



2.1.5 Connect the sampling system, with an empty Filta-Max® filter housing, to the pressurized water system. Verify that the filter housing is installed so that the end closest to the screw top cap is the inlet and the opposite end is the outlet.

2.1.6 Slowly turn the tap to fully open. Allow a minimum of 20 L to flush the system. During this period, perform the following steps:

2.1.6.1 Measuring the flow rate with the flow rate meter or the flow totalizer and a stopwatch, adjust the flow rate to approximately 3 - 4 L/min (approximately 0.8 - 1 gpm). If a flow control valve at the appropriate flow rate is used, no adjustment of the flow rate is necessary. At high pressure sites, using the pressure regulator, adjust the pressure to a maximum of 120 PSIG. Observe system for leaks and take the necessary corrective action if any are present. A differential pressure of 7.5 PSI is required to create flow through the filter. The recommended differential pressure to produce the flow rate of 3 to 4 L/min is 75 PSI. Do not exceed the maximum operating pressure of 120 PSIG.

2.1.6.2 Record the following information on the sample collection form (**Appendix F**):

- ✎ Public water system (PWS) name
- ✎ PWS address
- ✎ Sampler name
- ✎ Sample ID (optional)
- ✎ Public Water System Identification (PWS ID) number
- ✎ Public Water System facility ID number
- ✎ Facility name
- ✎ Sample collection point ID
- ✎ Sample collection point name
- ✎ Sample collection date
- ✎ Source water type (optional [but required for *E. coli* sample forms])
- ✎ Requested analysis (circle *Cryptosporidium* field sample for routine monitoring sample; circle both "*Cryptosporidium* field sample" and "*Cryptosporidium* matrix spike" sample if you are sending an additional sample with the monitoring sample for matrix spike analysis)

2.1.6.3 After the system has been flushed, measure the turbidity and any optional water quality parameters such as temperature and/or pH.

2.1.7 Turn off the water at the sample port valve when the flow rate has been adjusted and the system has been flushed.

2.1.8 Record the following information on the sample collection form (**Appendix F**):

- ✎ Initial meter reading

2.1.9 Install the Filta-Max® filter into the housing and secure the housing cap by hand tightening. Apply gentle pressure to create a seal between the module and the "O" rings in the base and lid of the housing. Excessive tightening is not necessary, and may shorten the life of the "O" rings. A

light application of vacuum grease may be used to lubricate the "O" rings, but too much grease will produce a negative effect.

Note! Retain the rubber stoppers provided with the filter housing. These stoppers will be needed to seal the housing for shipment.

2.1.10 Slowly turn on the pressurized water source. Adjust flow to within 3 to 4 L/min, if necessary.

- ✎ Record start time on the sample collection form.

2.1.11 Monitor the water meter. When the targeted volume (actual sample volumes will be selected by the utility but volumes are typically 10 L [2.64 gal] or 50 L [13.2 gal] and preferred to be consistent between sampling events for each source) has passed through the Filta-Max® filter, shut off the water source. Allow the pressure to decrease until the water stops.

2.1.12 Record the following information on the sample collection form (**Appendix F**):

- ✎ Stop time (when the water was shut off)
- ✎ Final meter reading
- ✎ Comments to laboratory, if needed

2.1.13 Disconnect the inlet end of the filter housing, making sure not to spill any of the water remaining in the housing. This water is part of your sample. Disconnect the outlet end of the filter housing.

2.1.14 The filter can either be shipped in the filter housing or removed from the filter housing and shipped alone.

2.1.15 If the filter will be shipped without the housing, open the housing while wearing a fresh pair of gloves and dump the filter and the water remaining in the housing into a ziplock bag. Place this bag inside a second ziplock bag and seal.

2.1.16 If the filter will be shipped in the housing, seal the inlet and outlet of the housing with the rubber stoppers that were previously saved. Place the filter housing containing the filter in a plastic ziplock bag for shipment. Ensure that there is residual water in the housing before shipping.

2.1.17 Place a label on the outer ziplock bag containing the filter and using a waterproof pen record the following information:

- ✎ PWS ID
- ✎ Facility name
- ✎ Date of sample collection

2.1.18 Immediately following sample collection, place the bag containing the filter (with or without filter housing) in a refrigerator to chill prior to packing the shipping cooler for shipment. If no refrigerator is available, and the sample will not be shipped for several hours, place the bag in the shipping cooler with ice to chill, and replace the ice before shipping.

Note! Method 1622/1623 requires that the temperature of the sample upon arrival at the laboratory must be ≤20°C (but not frozen), and the laboratory must begin sample

processing within 96 hours of sample collection. If the sample temperature and holding time requirements are not met, then the sample is invalid and must be recollected.

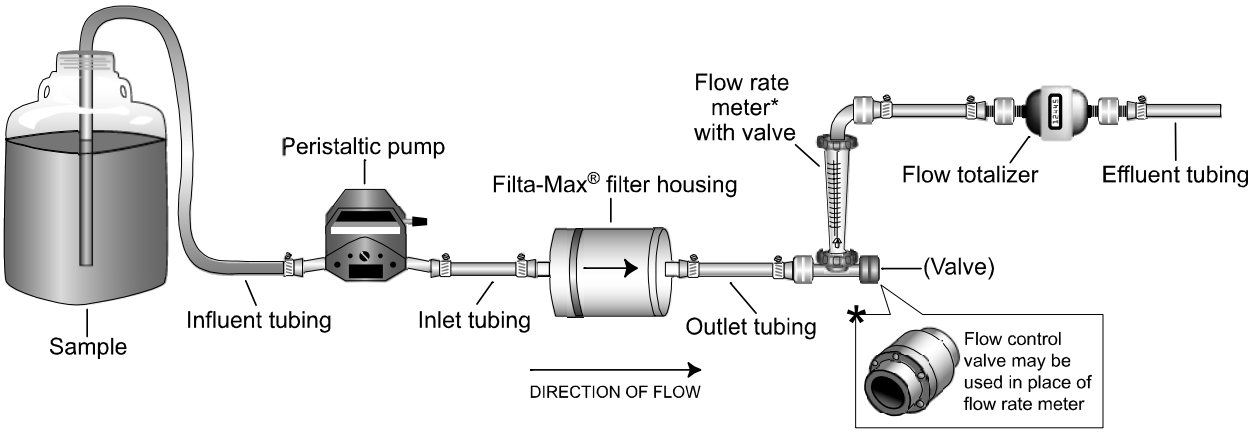
2.2 Sample Filtration from an Unpressurized Source

2.2.1 Put on a pair of powder-free latex gloves to prevent contamination from outside sources. Any contamination inside of the sampling apparatus may bias the final results.

2.2.2 If sampling from a source of unlimited volume, it may be desirable to pre-flush the sampling system. Assemble the sampling system, minus the Filta-Max® filter. Verify that the filter housing is installed so that the end closest to the screw top cap is the inlet and the opposite end is the outlet. The sampling system should be assembled in the following order, as shown in **Figure 3** below:

- ☐ Influent tubing
- ☐ Peristaltic pump
- ☐ Inlet tubing
- ☐ Filta-Max® filter housing, with the direction of flow depicted with an arrow
- ☐ Outlet tubing
- ☐ Flow rate meter with valve or flow control valve
- ☐ Flow totalizer (mechanical or graduated container)
- ☐ Effluent tubing to drain

Figure 3. Sample System Setup for Collecting *Cryptosporidium* Samples from an Unpressurized Source



2.2.3 Place the inlet end of the inlet tubing in sample source, away from any walls, bottom, or other environmental surfaces.

2.2.4 Turn on the pump and allow a minimum of 20 L to flush the system. If sampling source water from a carboy, continuously refill the carboy as necessary to flush the system. As a

recommendation, the carboy should not be removed from the sampling assembly and should be refilled using a separate container. Observe the system for leaks and take the necessary corrective action if any are present. During this period, perform the following steps:

2.2.4.1 Measuring the flow rate with the flow rate meter or the flow totalizer and a stopwatch, adjust the flow rate to approximately 3 - 4 L/min (approximately 0.8 - 1 gpm) by varying the pump speed or adjusting the valve (if pump is not variable speed). If a flow control valve at the appropriate flow rate is used, no adjustment of the flow rate is necessary.

2.2.4.2 Record the following information on the sample collection form:

- ✎ Public water system (PWS) name
- ✎ PWS address
- ✎ Sampler name
- ✎ Sample ID (optional)
- ✎ Public Water System Identification (PWS ID) number
- ✎ Public Water System facility ID number
- ✎ Facility name
- ✎ Sample collection point ID
- ✎ Sample collection point name
- ✎ Sample collection date
- ✎ Source water type (optional [but required for *E. coli* sample forms])
- ✎ Requested analysis (circle *Cryptosporidium* field sample for routine monitoring sample; circle both "*Cryptosporidium* field sample" and "*Cryptosporidium* matrix spike" sample if you are sending an additional sample with the monitoring sample for matrix spike analysis)

2.2.5 Turn off the pump when the flow rate has been adjusted and the assembly has been flushed. Following flushing, or if flushing is not performed, proceed with the following steps:

2.2.6 Record the following information on the sample collection form:

- ✎ Initial meter reading

2.2.7 Install the Filta-Max® filter into the housing and secure the housing cap by hand tightening. Apply gentle pressure to create a seal between the module and the "O" rings in the base and lid of the housing. Excessive tightening is not necessary, and may shorten the life of the "O" rings. A light application of vacuum grease may be used to lubricate the "O" rings, but too much grease will produce a negative effect.

Note! Retain the rubber stoppers provided with the filter housing. These rubber stoppers will be needed to seal the housing for shipment.

2.2.8 Turn on the peristaltic pump. Adjust the flow rate to approximately 3 - 4 L/min, if necessary. Record the following on the sample collection form:

- ✎ Start time

2.2.9 Monitor the water meter continuously refilling the carboy as needed. When the targeted volume (actual sample volumes will be selected by the utility, but volumes are typically 10 L [2.64 gal] to 50 L [13.2 gal] and preferred to be consistent between sampling events for each source) is reached, remove tubing from the carboy. Turn off the pump and allow the pressure to decrease until the water stops.

2.2.10 Record the following information on the sample collection form:

- ✎ Stop time (when the pump was shut off)
- ✎ Final meter reading or measured total volume sample
- ✎ Comments to laboratory, if needed

2.2.11 Disconnect the inlet end of the filter housing, making sure not to spill any of the water remaining in the capsule. This water is part of your sample. Disconnect the outlet end of the filter housing.

2.2.12 The filter can either be shipped in the filter housing or removed from the filter housing and shipped alone.

2.2.13 If the filter will be shipped without the housing, open the housing while wearing a fresh pair of gloves and dump the filter and the water remaining in the housing into a ziplock bag. Place this bag inside a second ziplock bag and seal.

2.2.14 If the filter will be shipped in the housing, seal the inlet and outlet of the housing with the rubber stoppers that were saved previously. Place the filter housing containing the filter in a plastic ziplock bag for shipment. Ensure that there is residual water in the housing before shipping.

2.2.15 Place a label on the outer ziplock bag containing the filter and using a waterproof pen record the following information:

- ✎ PWS ID
- ✎ Facility name
- ✎ Date of sample collection

2.2.16 Immediately following sample collection, place the bag containing the filter housing in a refrigerator to chill prior to packing the shipping cooler for shipment. If no refrigerator is available, and the sample will not be shipped for several hours, place the bag in the shipping cooler with ice to chill. Replace the ice before shipping.

Note! Method 1622/1623 requires that the temperature of the sample upon arrival at the laboratory must be ≤20°C (but not frozen), and the laboratory must begin sample processing within 96 hours of sample collection. If the sample temperature and holding time requirements are not met, then the sample is invalid and must be recollected.

3.0 Packing the Sample

3.1 Insert two large plastic trash bags into the shipping cooler to create a double liner. Immediately before packing the cooler, create two 4-lb packs of ice in two separate ziplock bags. To prevent leaks place each ice pack into an additional ziplock bag. Gel packs or blue ice may be used

instead of wet ice, as long as the sample is maintained in the appropriate temperature range. Seal the ziplock bag, expelling as much air as possible, and secure top with tape.

Note! Shipping companies may delay sample shipments if leakage occurs. Double liners and ziplock bags around ice will prevent leakage and delays.

3.2 Place the bag containing the filter, or filter within housing, into the shipping container. Wrap the filter in bubble wrap to prevent freezing. Inflated, empty sample bags can be placed between the filter housing and the ice packs to prevent the sample from freezing.

3.3 If you will be monitoring sample temperature during shipment, place in the cooler the temperature monitoring device (e.g., extra sample bottle for measuring sample temperature upon receipt at the laboratory, thermometer vial, or Thermochron™ iButton). Seal each liner bag by twisting top of bag and tying in a knot.

3.4 Peel the backing off one of the plastic airbill sleeves and attach the sleeve to the inside of the cooler lid.

✎ Sign and date the sample collection form

Fold the completed sample collection form, and place it inside the plastic sleeve.

3.5 Close the cooler lid, seal the horizontal joints with strapping tape, and secure the lid with tape by taping the cooler at each end, perpendicular to the seal.

Note! Shipping companies may delay sample shipments if leakage occurs. Be sure to seal the cooler joints.

3.6 Peel the backing off of the second airbill sleeve and attach the sleeve to the outside of the cooler lid. Complete the shipping airbill with the laboratory address, billing information, sample weight, and shipping service. Remove the shipper's copy of the airbill, and place the remainder of the airbill inside the plastic sleeve.

4.0 Shipping and Tracking

4.1 Ship samples on the day of collection and use a reliable shipping service for overnight delivery. If samples are not shipped the day of collection, the sample must be maintained between 1°C and 10°C (but not frozen) by chilling in a refrigerator or cooler filled with ice.

4.2 Contact the laboratory to notify them of the sample shipment. Request that the laboratory contact you the next day if the sample is not received.

4.3 Using the airbill number on the shipper's copy of the airbill, track the sample shipment using the shipping company's web page or by contacting the shipping company over the phone.

4.4 If problems are encountered with the shipment, communicate with the shipping company to resolve, and update the laboratory regarding the status of the shipment.

Appendix I

Collecting Bulk Water Samples for Laboratory Filtration and *Cryptosporidium* Analysis - Recommended Procedure

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Recommended Procedure for Collecting Bulk Water Samples for Laboratory Filtration and *Cryptospordium* Analysis

1.0 Materials

The following materials should be available before collecting samples:

- □ Several pairs of new, powder-free latex gloves
- □ Sample collection form (**Appendix F**)
- □ Sanitary 10 L cubitainer
- □ Sample number label
- □ Waterproof pen
- □ Cooler, approximately 34-quart
- □ Two large plastic trash bags
- □ Two 8-lb. bags of ice, or the equivalent number of ice packs sufficient to maintain the sample between 1°C and 10°C
- □ Four gallon size ziplock bags
- □ Strapping tape
- □ Two self-adhesive plastic airbill sleeves
- □ Airbill for shipment

2.0 Collecting the Sample

2.1 Put on a pair of powder-free latex gloves.

2.2 If sampling from a pressurized sample tap, turn on the influent tap and flush the system by allowing source water to flow for 2 to 3 minutes or until the temperature has stabilized and any debris that has accumulated has cleared or the turbidity in the water becomes visibly uniform.

2.3 While the system is flushing or if you are collecting a sample directly from the source water, record the following information on the sample collection form (**Appendix F**):

- ✎ Public water system (PWS) name
- ✎ PWS address
- ✎ Sampler name
- ✎ Sample ID (optional)
- ✎ Public Water System Identification (PWS ID) number
- ✎ Public Water System facility ID number
- ✎ Facility name

- ✎ Sample collection point ID
- ✎ Sample collection point name
- ✎ Sample collection date
- ✎ Source water type (optional [but required for *E. coli* sample forms])
- ✎ Requested analysis (circle *Cryptosporidium* field sample for routine monitoring sample; circle both "*Cryptosporidium* field sample" and "*Cryptosporidium* matrix spike" sample if you are sending an additional sample with the monitoring sample for matrix spike analysis)

2.4 After the system has been flushed, measure the turbidity of the source water and any optional water quality parameters such as temperature, and/or pH.

2.5 Fill the 10 L (or multiple) cubitainer(s). If both a field sample and a matrix spike sample are being collected, fill one immediately after the other. Record the following information on the sample collection form:

- ✎ Sample collection time
- ✎ Comments to laboratory, if needed

2.6 Immediately following sample collection, tighten the cubitainer cap(s) and place the cubitainer(s) in a refrigerator to chill to <10°C prior to packing the shipping cooler for overnight shipment. If no refrigerator is available, place the cubitainer(s) in the shipping cooler with ice to chill, and replace the ice with fresh ice before shipping.

Note! Method 1622/1623 requires that the temperature of the sample upon arrival at the laboratory must be ≤20°C (but not frozen), and the laboratory must have time to process the sample before 96 hours elapses after sample collection. Source water samples that are collected above 10°C, should be chilled before shipment. If the sample temperature and holding time requirements are not met, then the sample is invalid and must be recollected.

3.0 Packing the Sample

3.1 Insert two large plastic trash bags into the shipping cooler to create a double liner. Immediately before packing the cooler, place a fresh 8-lb bag of ice into each of the two plastic, ziplock bags. To prevent leaks place each ice pack into an additional ziplock bag. Seal each ziplock bag, expelling as much air as possible, and secure top with tape.

Note! Shipping companies may delay sample shipments if leakage occurs. Double liners and ziplock bags around ice will prevent leakage and delays.

3.2 Place the chilled cubitainer upright into the center of the lined cooler. Place the two ice packs into the cooler, one on each side of the cubitainer.

3.3 If you will be monitoring sample temperature during shipment, place in the cooler the temperature monitoring device (e.g., extra sample bottle for measuring sample temperature upon receipt at the laboratory, thermometer vial, or Thermochron™ iButton). Seal each liner bag by twisting top of

bag and tying in a knot.

3.4 Peel the backing off one of the plastic airbill sleeves and attach the sleeve to the inside of the cooler lid.

✎ Sign and date the sample collection form.

Fold the completed sample collection form, and place it inside the plastic sleeve.

3.5 Close the cooler lid, seal the horizontal joints with strapping tape, and secure the lid with tape by taping the cooler at each end, perpendicular to the seal.

Note! Shipping companies may delay sample shipments if leakage occurs. Be sure to seal the cooler joints.

3.6 Peel the backing off of the second airbill sleeve and attach the sleeve to the outside of the cooler lid. Complete the shipping airbill with the laboratory address, billing information, sample weight, and shipping service. Remove the shipper's copy of the airbill, and place the remainder of the airbill inside the plastic sleeve.

4.0 Shipping and Tracking

4.1 Ship sample after it has chilled to between 1°C and 10°C and use a reliable shipping service for overnight delivery. If samples are not shipped the day of collection, the sample must be maintained between 1°C and 10°C by chilling in a refrigerator or cooler filled with ice.

4.2 Contact the laboratory to notify them of the sample shipment. Request that the laboratory contact you the next day if the sample is not received.

4.3 Using the airbill number on the shipper's copy of the airbill, track the sample shipment using the shipping company's web page or by contacting the shipping company over the phone.

4.4 If problems are encountered with the shipment, communicate with the shipping company to resolve, and update the laboratory regarding the status of the shipment.

Appendix J

Collecting Source Water Samples for *E. coli* Analyses - Recommended Procedure

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Recommended Procedure for Collecting Source Water Samples for *E. coli* Analyses

1.0 Materials

The following materials should be available before collecting samples:

- □ Several pairs of new, powder-free latex gloves (optional)
- □ Sterile, non-toxic, glass or plastic container with a leak-proof lid. Container should be capable of holding 120-mL or 250-mL with ample headspace to facilitate mixing of sample by shaking prior to analysis
- □ Sample number label

The following additional materials may be needed if the sample will be shipped off-site for analysis:

- □ Sample collection form
- □ Gallon size zippered plastic bag
- □ Cooler, approximately 9-quart
- □ Two large plastic trash bags
- □ One 8-lb. bag of ice or gel ice packs
- □ Strapping tape
- □ Bubble wrap
- □ Two self-adhesive plastic airbill sleeves
- □ Airbill for shipment
- □ Temperature monitoring device (thermometer vial, or Thermochron™ iButton) (if measuring temperature during shipment)

2.0 Collecting the Sample

2.1 Record the sample number, sample location, samplers name, observations, and sampling date and time in a sampling log book if the sample will be analyzed on-site.

If the sample will be shipped off-site, record the following information on the sample collection form (**Appendix F**):

- ✎ Public water system (PWS) name
- ✎ PWS address
- ✎ Sampler name
- ✎ Sample ID (optional)
- ✎ Public Water System Identification (PWSID) number
- ✎ Public Water System facility ID number

- ✎ Facility name
- ✎ Sample collection point ID
- ✎ Sample collection point name
- ✎ Sample collection date
- ✎ Source water type
- ✎ Requested analysis (circle "*E. coli*")

2.2 Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. The service line should be cleared before sampling by maintaining a steady water flow for at least two minutes (until the water changes temperature). Please note: Pre-rinsing the sample containers with sample is prohibited when collecting *E. coli* samples.

2.3 Adjust the flow of water out of the tap or hose so the water will not splash out when it is collected into the sample container.

2.4 If there is not an inline tap that allows for the sampling of source water prior to treatment, samples should be collected as close to the intake as possible from either land or boat. Source water samples should be collected close to the surface using a grab sampling technique. Samples may be collected manually by direct submersion of the bottle into the water or by using a grab sampling device, as simple as a metal pole with an adjustable clamp at one end that holds the sampling bottle in place.

2.5 Using aseptic technique (i.e., sanitize tap, do not touch the inside of the sample container, etc.), fill the *E. coli* sample container, leaving at least 1 inch of head space, if possible. Volume collected will depend on anticipated organism density. It is recommended that utilities collect at least 100 mLs. Do not expose an opened container any longer than necessary. Record the system name, sampler's name, sample number, date and time of sample collection, sample location, and analysis requested on the sample container.

2.6 Immediately following sample collection, tighten the sample container lid. If the sample will be shipped off-site for analysis, and will not be shipped for several hours, place the sample container upright in a refrigerator to maintain the sample at a temperature between 1°C and 10°C, but not freezing, prior to shipment. If a refrigerator is not available, wrap the sample with insulation such as bubble wrap or paper towels (to prevent freezing), place the sample in a ziplock bag, and place the bag containing the sample in the shipping cooler with wet ice or ice packs. Replace with fresh ice or ice packs immediately prior to shipment.

3.0 Suggested Packaging of the Sample (Applicable to Samples Shipped Off-Site for Analysis)

3.1 Insert two large plastic trash bags into the shipping cooler to create a double liner. Immediately before packing the cooler, disperse 6 pounds of ice into 3 to 4 plastic, zippered plastic bags. Gel packs or blue ice may be used in lieu of wet ice, as long as the sample is maintained in the appropriate temperature range. Seal the zippered plastic bags, expelling as much air as possible, and secure top with tape.

Note! Shipping companies may delay sample shipments if leakage occurs. Double liners and ziplock bags around ice will prevent leakage and delays.

3.2 Place the bag containing the samples into the shipping container. Cover sample bottles with bubble wrap, to prevent freezing, and place ice or ice packs around the sample bag.

3.3 If you will be monitoring sample temperature during shipment, place in the cooler the temperature monitoring device (e.g., extra sample bottle for measuring sample temperature upon receipt at the laboratory, thermometer vial, or Thermochron™ iButton). Seal each liner bag by twisting top of bag and tying in a knot.

3.4 Peel the backing off one of the plastic airbill sleeves and attach the sleeve to the inside of the cooler lid.

✎ Sign and date the sample collection form.

Fold the completed sample collection form, and place it inside the plastic sleeve.

3.5 Close the cooler lid, seal the horizontal joints with strapping tape, and secure the lid with tape by taping the cooler at each end, perpendicular to the seal.

Note! Shipping companies may delay sample shipments if leakage occurs. Be sure to seal the cooler joints.

3.6 Peel the backing off of the second airbill sleeve and attach the sleeve to the outside of the cooler lid. Complete the shipping airbill with the laboratory address, billing information, sample weight, and shipping service. Remove the shipper's copy of the airbill, and place the remainder of the airbill inside the plastic sleeve.

4.0 Shipping and Tracking

4.1 Ship samples on the day of collection and use a reliable shipping service for next-day delivery.

Note! Under LT2, *E. coli* samples must be analyzed within 30 hours of sample collection. Samples that will be shipped off-site for analysis, need to be shipped the same day of collection. If the sample holding time or temperature requirements are exceeded upon receipt at the laboratory, the sample will be rejected and will need to be re-collected.

4.2 Contact the laboratory to notify them of the sample shipment. Request that the laboratory contact you the next day if the sample is not received.

4.3 Using the airbill number on the shipper's copy of the airbill, track the sample shipment using the shipping company's web page or by contacting the shipping company over the phone.

4.4 If problems are encountered with the shipment, communicate with the shipping company to resolve, and update the laboratory regarding the status of the shipment.

Appendix K

Intent to Grandfather –
Example Notice

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Long Term 2 Enhanced Surface Water Treatment Rule
Intent to Grandfather - Example Notice

Public Water Systems (PWSs) that will submit grandfathered data for the LT2 Rule must first submit a notice that they intend to submit previously collected monitoring results. This notice must specify the number of previously collected results that the system will submit, the dates of the first and last sample, and whether they will collect additional source water monitoring samples to meet the requirements of the rule. If a PWS plans to collect additional samples, it must also submit a monitoring schedule. Submitting this form satisfies the requirement to submit a notice of intent to grandfather.

Submit this information no later than 3 months prior to the month that the PWS is required to start monitoring.

PWS Information	
PWS Name:	PWS ID:
PWS Address:	
Email Address:	
Water Treatment Plant Name:	
Water System Facility ID:	
Grandfathered Data Information	
Number of previously collected sample results that will be submitted:	
Date of first sample: / /	
Date of last sample: / /	
Will you be collecting additional source water monitoring samples to meet the requirements of your plant? ☐ YES ☐ NO If yes, you must also submit a monitoring schedule that is required for your plant.	
Signature:	Date:
Name (print)	Phone:

This notice may be submitted using one of the following options:

- **Through the LT2/Stage 2 Data Collection and Tracking system available at http://www.epa.gov/safewater/disinfection/lt2**
- **As an email attachment and sent to stage2mdbp@epa.gov**
- **By mail or fax to the following:** **LT2ESWTR and Stage 2 DBPR**
  P.O. Box 98
  Dayton, OH 45401

  FAX: (937)-586-6557

Appendix L

Recommended Checklist for Beginning Grandfathered *Cryptosporidium* Monitoring

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Recommended Checklist for Beginning Grandfathered *Cryptosporidium* Monitoring

- □ ***Cryptosporidium* laboratory qualifications.** A list of laboratories approved for LT2 *Cryptosporidium* analysis is posted at http://www.epa.gov/safewater/disinfection/lt2/lab_home.html.
- □ **Sampling location.** Is sample collection from the plant intake prior to chemical treatment (Section 3.1)? Have you prepared a description of the sampling location that addresses the position of the sampling location in relation to the system's water source(s) and treatment processes, including points of chemical addition and filter backwash water recycle? **(Appendices D and E)**
- □ **Sampling schedule.** Is the sampling schedule designed to monitor for *Cryptosporidium* at least monthly (Section 3.2) on a regular schedule?
- □ **Laboratory coordination.** Have you verified that your intended sampling schedule can be accommodated by an approved *Cryptosporidium* laboratory (to avoid sample rejection) (Section 2.3.1)?
- □ **Matrix spikes.** Does the sampling schedule include collection of an extra bulk 10 L sample, in addition to the required monitoring samples, along with the 1st and 20th samples (Section 4.1.3)?
- □ **Method version.** Is EPA Method 1622 or EPA Method 1623 used to analyze *Cryptosporidium* samples (Section 5.2.2)?
- □ **Sample volume issues.** Is a minimum of 10 L collected for each monitoring and matrix spike sample (Section 4.2.1)?
- □ ***E. coli* laboratory qualifications.** Is the laboratory certified under the drinking water laboratory certification program for total or fecal coliform analysis using the same technique as one of the LT2 *E. coli* methods (Table 7.1)?
- □ **Turbidity measurements.** Will turbidity measurements be made for each sample

Appendix M

Grandfathered *Cryptosporidium* Data Package Report Checklist

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Long Term 2 Enhanced Surface Water Treatment Rule
Grandfathered *Cryptosporidium* Data Package – Report Checklist

Public Water Systems (PWSs) that must monitor for *Cryptosporidium* can use this checklist to prepare to report required information to EPA, if they wish to grandfather data. PWSs must submit previously collected monitoring results for grandfathering along with all required documentation no later than 2 months after the month the PWS is required to start monitoring.

PWS Information	
PWS Name:	PWS ID:
PWS Address:	
Email Address:	
Water Treatment Plant Name:	
Water System Facility ID:	
Grandfathered Data Information	
Have you submitted the required Intent to Grandfather Report, at least 3 months before you would be required to conduct monitoring?	☐ YES ☐ NO
Does the data package include a signed cover letter certifying that the data represent the plant's current source water and that all source water *Cryptosporidium* monitoring results collected during LT2 monitoring are included in this package?	☐ YES ☐ NO
Does the data package include a sample collection schedule specifying calendar dates, including first and last samples? Have you submitted a sampling schedule for additional new monitoring you will conduct, if any?	☐ YES ☐ NO ☐ YES ☐ NO
Have you included additional documentation regarding dates and reasons for resampling, the use of presedimentation, and/or off-stream storage during routine plant operation?	☐ YES ☐ NO
Does the data package include a list of the field and matrix spike (MS) samples submitted in the data package, identified by sample ID and collection date?	☐ YES ☐ NO
Are all data elements from field sample results from the monitoring period included?	☐ YES ☐ NO
Are all data elements from MS sample results from the monitoring period included?	☐ YES ☐ NO
Are the volumes analyzed for all field samples at least 10 L? For samples in which less that 10 L was examined, were at least 2 mL of packed pellet volume analyzed or did two filters clog?	☐ YES ☐ NO ☐ YES ☐ NO
Does the data package include a letter from the laboratory certifying that all method QC requirements were acceptable for every field and MS sample submitted with the package, including OPR, Method blank, holding times, staining controls, etc.? Alternatively, bench sheets and report forms documenting this QC information may be included, rather than a laboratory letter.	☐ YES ☐ NO
Was the temperature of all *Cryptosporidium* samples ≤20°C (and not frozen) upon receipt?	☐ YES ☐ NO

PWSs that must monitor for *Cryptosporidium* may submit this checklist with the grandfathered data package using one of the following options:

- **As an email attachment sent to stage2mdbp@epa.gov**
- **By mail or fax to the following:** **LT2ESWTR and Stage 2 DBPR**
  P.O. Box 98
  Dayton, OH 45401

  FAX: (937)-586-6557

Grandfathered data can be submitted separately from supporting grandfathered data package information through the LT2/Stage 2 Data Collection and Tracking system available at http://www.epa.gov/safewater/disinfection/lt2.