Review on Effects of Holding Time Exceedances on Ambient Water Quality

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Abstract: Globally, contamination of water bodies by microbial pathogens is a significant public health concern. Fecal indicator bacteria such as *Escherichia coli (E. coli)* typically identify the deterioration of water bodies. In order to determine water quality, monitoring *E. coli* concentrations is important. During collection, storage, and transportation of water samples, holding time can have a significant impact on the density of indicator pathogens. Although many studies have reported on the effects of holding time exceedances on water quality, there is a lack of comprehensive review of these studies. The objective of this work is to provide a complete review on the effects of holding time exceedances on water quality. The results of this study suggest that most *E. coli* samples can be analyzed beyond 8 hr and up to 48 hr after sample collection while still generating comparable data if the samples are stored below 10°C.

Introduction

Water used for domestic or recreational purposes has an important impact on human health. As many as 3.4 million people die from water-related diseases due to poor water quality (World Health Organization [WHO], 2014). Each day, 4,000 children die due to contaminated water according to the United Nations Children's Fund (2014). The presence of pathogens in the surface water is increasingly turning into a concern throughout the world. According to the Clean Water Act Sections §305(b) and §303(d), more streams and rivers remain impaired due to pathogens than any other pollutants (Figure 1). Pathogen impairment has negatively affected 480,000 km of rivers and 2 million hectares (ha) of lakes in the United States (US) (United States Environmental Protection Agency [USEPA], 2014).

Sources of Pathogens and Control Measures

Contamination of water bodies by pathogens may result from a point and non-point sources. Non-point sources include agricultural runoff, urban stormwater, and streams. Point sources consist of overflows from wastewater treatment plants, spills, or runoff from livestock housing or manure storage facilities. All these sources are linked to increase microbial loads to natural bodies of water (McLellan, 2004). Some microbial contaminants can be removed by water treatment coagulation and filtration processes.

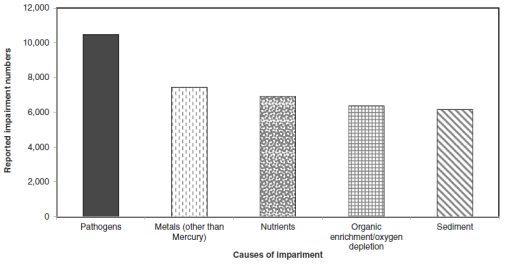

Figure 1: Causes of impairment in the U.S. (source: U.S. EPA, 2014)

The most important and cost-effective protection for water bodies is to prevent pathogen entry into source water. Point sources can often be controlled by treatment at the source. Control of non-point pollution requires a diminished release of pathogens to the atmosphere and run-off.

Indicator Organism

Indicator organisms are monitored to assess the level of microorganisms in water bodies. Water quality standards related to fecal contamination are measured in reference to *Escherichia coli* (*E. coli*) and *Enterococci* count. The presence of microorganisms such as *E. coli* in a water sample from an environmental source provides direct evidence of fecal contamination. The presence of microorganisms such as this is a great concern for human health.

Among the various microorganism, *E. coli* is a specific indicator of fecal pollution. According to Odonkor and Ampofo (2013), two key factors led to the use of *E. coli* as the preferred indicator for the detection of fecal contamination. First, some fecal coliforms could be of non-fecal origin. Second, the development of improved testing methods for *E. coli* makes testing more accurate. They are absent in uncontaminated water, survive at least as long as other waterborne microorganisms, and are thus considered by scientists as a good indicator organism (WHO, 2016).

The result of various studies demonstrates that *E.coli* is present in fecally contaminated water. The US Environmental Protection Agency (EPA) published a report recommending *E. coli* or *Enterococci* as the preferred fecal indicator bacteria (FIB) for freshwater (USEPA, 1986). This study is focused on *E. coli* in freshwater, where reports on its quantification are more prevalent than other water microorganisms.

Overview of Methods used to Enumerate *E. coli*

Over the years, differentiation of coliforms had come to a series of correlations that suggested indole production, gelatin liquefaction, sucrose fermentation, and Voges-Proskauer reaction were among the more important tests for determining fecal contamination. These developments culminated in the IMViC (Indole, Methyl red, Voges-Proskauer and Citrate) tests to differentiate fecal coliforms. One of the first generally accepted simpler methods for coliforms was called the Multiple-Tube Fermentation Test. The test method has evolved continually to become more specific. Some of the more significant developments were the so-called fecal coliform test, which selects for coliforms of fecal origin by using a higher incubation temperature (Odonkor and Ampofo, 2013).

Table 1. Approved CWA *E. coli* Test Methods

Number	Method Title
1103.1	*Escherichia coli (E. coli)* in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC)
1603	*Escherichia coli (E. coli)* in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC)
1604	Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)

(Source: USEPA, https://www.epa.gov/cwa-methods/approved-cwa-microbiological-test-methods)

Culture-based methods, multiple-tube fermentation (MTF) or membrane filtration (MF) followed by incubation on selective media are traditionally used for the enumeration of *E. coli* in waters. MTF and MF methods are now widely used for the routine analysis of microbiological water quality in Europe and North America(Prats et al., 2007). The US Environmental Protection Agency (USEPA) Method 1103.1 describes a membrane filter (MF) procedure for the detection and enumeration of *E. Coli* bacteria in ambient water (USEAP, 2010). EPA approved methods for *E. coli* Test in ambient water is listed in Table 1.

Eccles et al. (2004, examined the suitability of membrane filtration techniques and most probable number methods for isolating and enumerating *Escherichia coli*. The result showed that the tested methods gave comparable recoveries, and did not vary by greater than one order of magnitude (1 log). Likewise, Hamilton et al. (2006), examined enzyme-specific media and compared to levels determined with conventional culture media (m-FC and m-TEC) and concluded that levels observed with all tests were highly correlated, and significantly fewer *E. coli* were enumerated with m-TEC than with enzyme-specific media.

Water Quality and Holding Time

The Clean Water Act requires the US Environmental Protection Agency (USEPA) to update the environmental water quality status of our nation's waters on a regular basis. Water quality samples from various water bodies are collected and analyzed regularly by individual state agencies and their partners in order to accomplish this goal. Water samples collected for microbiological analysis should be examined as soon as possible. This is because changes could occur in the bacterial densities due to external factors such as storage temperature, time, and exposure to the atmosphere (United States Geological Survey [USGS], 2006). It is not possible to analyze the water sample immediately due to technical and procedural difficulties.

It is important to apply relevant standard operating procedures in the collection, handling, and preservation of water samples in order to ensure they are accurate representations of the water body being sampled (Ferguson, 1994). Holding time is critical during water quality sampling. The USEPA recommends analyzing samples immediately after the collection due to density differences over time. There is concern that the reliability of data is compromised because of holding time exceedances. It is important universally to determine proper holding time, which is defined as the length of time a sample can be stored after collection and prior to analysis without affecting the results.

Many investigations have shown that the increase in holding time results in a decrease in pathogen count (Aulenbach, 2010; Lonsane, Parhod, & Rao, 1967; Mcdaniels & Bordner, 1983). Pope et al. (2003) assessed the effects of holding time on *E. coli* in surface water samples that included 11 laboratories and 24 sites across the United States (US). Many previous studies measured *E. coli* concentrations between different holding times. Standridge and Lesar (1977) measured these differences between 4 hours (hr) and 24 hr. Pope et al. (2003) conducted similar studies between 0 to 48 hr. The Texas Commission on Environmental Quality's study looked at the differences between 6 and 30 hr and Harmel et al. (2016a) looked at times of ≤24 hr and >24 hr. They all concluded that increases in holding times resulted in decreases in pathogen counts.

Oklahoma Conservation Commission and Water Quality

In the state of Oklahoma, the Oklahoma Conservation Commission's (OCC) Water Quality Division is responsible for identifying waters impaired by non-point source pollution (NPS). NPS is pollution that comes from multiple diffuse sources such as pesticides, fertilizers, sediment, and animal waste run-off. OCC works to prioritize and implement projects to reduce pollutants and improve water quality. The bacteria analysis under OCC operating procedure is conducted only during the period from May 1 through September 30. Their analysis commonly includes *E.coli* and

Table 2. Required Containers, Preservation Techniques, and Holding Times Applicable to all Non-Potable Water Samples (includes wastewater, surface water, and groundwater)

	EPA Procedure[1]	OCC Procedure[2]
Bacterial Tests	*E. coli*	*E.coli*
Container	Plastic, Glass	Plastic, Glass
Preservation	Cool, <10 °C, 0.0008% $Na_2S_2O_3$	Ice, 4 °C
Maximum holding time	8 hours	6 hr/24 hr/48 hr[3]

(Sources: [1]USEPA, 1986; [2] OCC, 2014)
[3]standard violation 6 hr holding time is required; for OCC purposes, 24 hr is preferred but 48 hr is acceptable

Enterococcus. Table 2 lists OCC-recommended sampling and preservation procedures for bacteria samples.

Objective

The Oklahoma Conservation Commission is the state agency responsible for the state of Oklahoma's Non-Point Source Pollution Program. They are responsible for monitoring streams around the state for a wide variety of parameters, including pathogens. It would benefit the agency to better understand the effect of holding time on its pathogen counts.

There is currently inconsistency in the maximum acceptable holding times for water sampling of pathogens nationally. Although many studies reported on the effects of holding time exceedances on water quality, there is a lack of comprehensive review of these studies. The objective of this work is to provide an inclusive review on the effects of holding time exceedances on water quality.

Materials and Methods

A literature review was performed to collect and compile data pertaining to the effect of holding time exceedances on water quality. We searched handbooks, official guidelines, and scientific papers. They were qualitatively analyzed with respect to holding time and *E. coli* count in order to determine the relativity to this particular study. If the analyzed document discussed changes in bacteria count due to holding time, it was considered relevant.

Sources for the literature review included official websites of government agencies such as the USEPA, state agencies, and scientific peer review journals. Scientific papers were searched in databases such as Google Scholar and Web of Science using keywords: holding time, fecal indicator bacteria, and water quality. Literature sources were selected based on whether the results discussed the effects of holding time on pathogen counts, such as the number of *E. coli* or *Enterococci*. Nine reports, fourteen journal articles and one handbook were reviewed. Department of Environmental Quality or similar state agency websites of all 50 states and Washington DC were also reviewed. The literature source, holding time, sample storage hour, and effect of holding time on microbial density were tabulated for further discussion.

Results and Discussion

Holding Time and Bacteria Count

The USEPA-recommended water quality criteria for *E. coli* in freshwater systems is presented in Table 3 (USEPA, 2018). According to their recommendation, the maximum allowable value is 126 (MPN/100 ml) and it is expressed as the geometric mean value. The states in USEPA Region 6 all have a 126 maximum allowable value (MPN/100 ml) for freshwater that is used for primary contact recreation. For most of the other states, the standard required (a geometric mean during any consecutive 30-day period during the recreational season) is to be less than 126 CFU/100 mL (Table 4). Alaska, Illinois, North Carolina, and West Virginia apply a standard of 200 CFU/100 mL.

Collected water samples are preserved by placing the sample on ice and transporting

Table 3: Water quality criteria for *E.coli* in freshwater systems

Single Sample Maximum Allowable Values (MPN/100 ml) for *E. coli*		
	EPA Standard	Oklahoma standard
Geometric Mean Value	126[a]	126[a]
Designated Bathing Area	235	235[b]
Moderate Full Contact Recreation	298	-
Lightly used Full Body Contact Recreation	409	406[c]
Infrequent used Full Body Contact Recreation	576	-

(Source: USEPA, 2018)

[a] *E. coli shall not exceed a monthly geometric mean of 126/100 ml based upon a minimum of not less than five (5) samples collected over a period of not more than thirty (30) days*
[b] *in lakes and high use waterbodies*
[c] *all other primary body contact recreation beneficial use areas*

it to a refrigerator. It is best to minimize the time between the collection, storage, and analysis. As per the American Public Health Association (APHA) guidelines (1998) for non-potable water samples, the standard storage time between collection and processing is 6 hr, and the samples should be kept below 10ºC (Selvakumar, Borst, Boner, & Mallon, 2004). At present, the maximum acceptable holding time across the globe is inconsistent. The Clean Water Act Alternate Test Procedure, as described in Section 40 CFR Part 136 TABLE II, requires 8 hr as maximum holding time for all non-potable water samples (Table 2) (USEAP, 1986).

Comparison on maximum holding time varies by state (Table 4). The maximum allowable holding time found is 30 hr for Idaho and Iowa. Kansas, Montana, and Oklahoma allow up to 24 hr. In most of the other states, the water samples are required to be delivered to the lab within 6 to 8 hr of collection.

Previous studies (Table 5) have examined municipal and industrial effluent (Dutka & El-Shaarawi, 1980; Selvakumar et al., 2004; Standridge & Lesar, 1977), stormwater (Characklis et al., 2005; Selvakumar et al., 2004), water from lakes and rivers (Aulenbach, 2010; Dutka & El-Shaarawi, 1980; Pope et al., 2003; Standridge & Lesar, 1977), and water from municipal distribution systems (McDaniels & Bordner, 1983) for fecal indicator bacteria (FIB). According to these studies, survival rates for different FIB vary. Total coliform often decreases shortly after collection, whereas fecal coliform generally survives longer. It was noted that they could possibly live up to 62 hr (Aulenbach, 2010).

Lonsane et al. (1967) observed that the concentration decreased with an increase in storage time while the differences were not significant for marginally polluted water. Standridge and Lesar (1977) examined 28 water samples with initial coliform counts between 102/mL and 106/mL and found little change after storage at 2ºC to 4ºC for 24 hr. McDaniels and Bordner (1983) observed a significant decrease in coliform populations after 24 hr at temperatures 5°C and 22°C. The rate of decline was 2.5 magnitudes greater at 22°C than at 5°C. Average losses in 24 hr were 34% at 5°C and 87% at 22ºC. Some studies did not observe a significant decrease in *E. coli* density between 18 to 27r (Aulenbach, 2010; Selvakumar et al., 2004), while other studies suggested that up to 48 hr was acceptable (Pope et al., 2003).

In a study by the Texas Commission on Environmental Quality, decreases in *E. coli* concentrations were observed with an increase in holding time from 8 hr, 24 hr and 48 hr (Texas Commission on Environmental Quality [TCEQ], 2008). In a similar study where the number of *E. coli* was examined after a holding

Table 4: State by State comparison on holding time and maximum allowable value of *E.coli*

States	Holding time (hrs)	Geometric Mean*, Maximum Allowable Values (MPN/100 ml)**	Source
Alabama	8	126	www.adem.state.al.us
Alaska	6	200	www.dec.alaska.gov
Arizona	6	245 CFU[1]	www.legacy.azdeq.gov
Arkansas	-	126	-
California	6	126	www.waterboards.ca.gov
Colorado	8	126	www.colorado.gov www.colorado.gov/pacific/sites/default/files
Connecticut	8	126	www.portal.ct.gov
Delaware	6	100	www.dnrec.delaware.gov
Florida	6	126	www.floridadep.gov
Georgia	24[2]	126	www.epd.georgia.gov
Hawaii	-	-	-
Idaho	30	126	www.ci.moscow.id.us
Illinois	8	200	www.idph.state.il.us
Indiana	6	125	www.in.gov/idem
Iowa	30	126	www.iowadnr.gov
Kansas	24	160	www.kdheks.gov
Kentucky	8	130	www.water.ky.gov
Louisiana	6	-	www.deq.state.la.us
Maine	-	-	-
Maryland	6	126	www.health.maryland.gov
Massachusetts	8	126	www.mass.gov/doc/hudson-river-basin-water-quality-assessment-report-2002-appendices-0
Michigan	6	130	https://www.michigan.gov/documents/deq
Minnesota	6	126	www.pca.state.mn.us
Mississippi	6	126	www.mdeq.ms.gov
Missouri	6	126	dnr.mo.gov/env
Montana	24	126	waterquality.montana.edu
Nebraska	-	126	-
Nevada	6	126	www.ndep.nv.gov
New Hampshire	-	-	-
New Jersey	8	126	www.nj.gov /dep/srp/guidance
New Mexico	6	206	www.nmhealth.org
New York	8	200	www.wadsworth.org
North Carolina	6	200	www.files.nc.gov
North Dakota	-	126	
Ohio	6	126	www.epa.ohio.gov
Oklahoma	24	126	www.owrb.ok.gov
Oregon	6	126	www.deq.state.or.us
Pennsylvania	8	200	www.sfiles.dep.state.pa.us
Rhode Island	6	200	www.dem.ri.gov
South Carolina	-	200	-
South Dakota	6	126	www.denr.sd.gov
Tennessee	8	126	www. publications.tnsosfiles.com
Texas	8	126	www.tceq.texas.gov
Utah	8	126	www.deq.utah.gov
Vermont	8	126	www.dec.vermont.gov
Virginia	-	126	-
Washington	6	126	www.fortress.wa.gov
West Virginia	6	200	www.dep.wv.gov
Wisconsin	-	126	-
Wyoming	8	126	http://deq.wyoming.gov

** calculated using data from at least five different samples collected in separate 24-hr periods*

*** (Source: www.epa.gov)*

[1] Applicable Standard or Other Criteria

[2] All samples must be plated preferably as soon as possible, but no more than 24 hours after collection

Table 5. Summary of previous studies on effects of holding time on *E. coli* in water samples

Holding time (hours)	Temperature (°C)	Type of Bacteria	Conclusion/Remarks	Reference
24, 48, and 72	20 and 4	*E.coli*	– Concentration decreased with storage time	Lonsane et al., (1967)
4 and 24	4	*E.coli*	– Many samples can successfully be stored at 4°C for 24 hr	Standridge & Lesar, (1977)
2, 24, 30, and 48	1.5	*E.coli*	– More than 75% of the samples were microbiologically stable for at least 24 hr	Dukta & El-Shaarawi, (1980)
24, 30, and 48	22 and 5	*E.coli*	– Coliform populations declined significantly at both temperatures after 24 hrs. The rate of decline was 2.5 orders of magnitude greater at 22°C than at 5°C. Average losses in 24 hr were 34% at 5°C and 87% at 22 hr	McDaniels & Bordner, (1983)
9 and 18	20	*E.coli*	– Total coliform counts varied significantly when water samples were stored for either 9 or 18 hr – Results signify an inherent difference between samples collected manually and those collected automatically	Ferguson, (1994)
0, 8, 24, 30, and 48	10	*E.coli*	– If samples are held below 10°C and are not allowed to freeze, most surface water E. coli samples analyzed by commonly used methods beyond 8 hr – No significant difference in bacterial densities throughout the 48 hr	Pope et al., (2003)
24	4	*E.coli*	– The concentration of fecal coliform measured during the first 7 hr holding time was slightly greater than concentrations measured beyond 24 hr holding time	Selvakumar, et al., (2004)
6 and 24	<10°C	*E. coli*	– Samples can be analyzed 24 hr after sample collection and still generate data comparable to those generated at 6 hr after sample collection	USEPA, (2006)
Up to 62	-	*E. coli*	-Fecal and total coliform densities did not change significantly with holding times up to about 27 hr	Aulenbach, (2010)

time of 6 hr, decreases were observed by 20% (USEPA, 2006). A study (Karthikeyan's unpublished data, as cited in Harmel et al., 2016b) showed an increase in the concentration of *E. coli* in the first 2 hr by an average of 15%. It decreased from 3% to 17% for the next 3 to 48 hr. Karthikeyan et al. (unpublished data, as cited in Harmel et al., 2016b) observed higher uncertainty in counts when samples were stored at 25°C compared to 15°C. McCarthy et al. (2008) also examined the effects of temperature on pathogen count for samples stored for up to 24 hr, and the result showed that the number of hours a sample is stored in the field was not statistically significant.

A comprehensive study was performed by Pope et al. (2003) to examine the effects of holding time exceedances on pathogen counts. It included multiple laboratories and many sites across the US. It also included more than one monitoring method for *E. coli* (Table 6). There was no significant decrease in *E. coli* densities from samples that were analyzed with the Colilert method and stored at 4ºC and 10°C. Significant differences occurred when the samples had been held for at least 48 hr. *E. coli* densities for samples stored at 20°C and 35°C were significantly reduced within 8 to 48 hr.

Samples from the Southern Nevada Pumping Plant 1 showed a significant increase with time. The increase in *E. coli* density at this site was related to holding temperature and was attributed to samples not being maintained below 10°C after 12 hr (Pope et al., 2003). The study (Pope et al., 2003) demonstrates that 8 of 13 sites showed no significant difference in *E. coli* densities between time 0 and the 48-hr holding time, regardless of the evaluation

Table 6. Summary of test results for time 0 comparison (Source: Pope et al., 2003)

Laboratory	Site	Method	Coolant	Mean no. of *E. coli*/100 ml at time 0	No. of E. coli/100 ml (significant change in density) at indicated time (h) after sample collection[a]			
					8	24	30	48
Fairfax County Water	Potomac River	Colilert	Wet ice	73	NS	51 (D)	NS	NS
Fort Worth Water	Rolling Hills WTP[b]	Colilert	Utek	63	NS	NS	NS	NS
Indianapolis Water	Fall Creek	Colilert	Utek	337	NS	NS	NS	NS
	White River	Colilert	Utek	534	NS	NS	NS	NS
City of Phoenix	Squaw Peak WTP	Colilert	Wet ice	11	NS	NS	NS	NS
	Union Hills WTP	Colilert	Wet ice	69	NS	NS	NS	NS
Jefferson Parish	Mississippi River	mTEC	Wet ice	310	NS	NS	NS	NS
Southern Nevada	SNWS Pumping Plant	mTEC	Utek	17	30 (I)	32 (I)	34 (I)	44 (I)
Passaic Valley	Passaic & Ramapo Rivers	mFC/NA-MUG	Blue ice	193	NS	90 (D)	108 (D)	85 (D)
Portland Water Bureau	Station 2	mFC/NA-MUG	Blue ice	44	NS	55 (I)	NS	NS
Mohawk Valley	Hinckley Reservoir	mFC/NA-MUG	Utek	42	97 (I)	NS	NS	NS
Wisconsin State Laboratory of Hygiene	Willow Creek	mFC/NA-MUG	Wet ice	560	NS	NS	NS	NS
	Wingra Springs	mFC/NA-MUG	Wet ice	367	NS	NS	NS	NS

D, significant decrease in E. coli density compared to the time 0 results; I, significant increase in E. coli density compared to the time 0 results; NS, no significant difference in E. coli density compared to the time 0 results; WTP, water treatment plant

method and the coolant used. The results of the Pope et al. (2003) investigation suggested that *E. coli* samples can be analyzed beyond 8 hr and up to 48 hr after sample collection while still generating comparable *E. coli* data, provided that the samples are stored below 10°C.

To ensure that the most accurate data are generated, *E. coli* samples collected from surface waters should be analyzed immediately and within 6 to 8 hr when on-site facilities are available (Pope et al., 2003). Many of the studies (Aulenbach, 2010; Pope et al., 2003; Selvakumar et al., 2004) have reported that water samples can be analyzed beyond the 8 hr holding time and generate reliable data. However, an overall decrease in bacterial density with an extended holding time of > 8 hr was observed in some cases (Ferguson, 1994). The magnitude of the decrease is attributed to a decrease in nutrient concentrations and other parameters, including temperature, storage condition, and initial bacterial density (Volk & LeChevallier, 1999). As unpredictability exists within survival rates of *E. coli* and other pathogens, a comparison between the results of different studies is also challenging. The ability to compare water-quality of different sources largely depends on the uniformity in the sampling, preservation, storage, and transportation.

Collection/Storage Method and Bacteria Count

Sample preservation and storage protocols maybe even more critical for microbial samples due to their transient nature and susceptibility to environmental conditions. Typical preservation procedures involve placing the sample on ice after collection and transporting it to a refrigerator. The standard storage time between collection and processing is ≤ 8 hr, with the sample held below 10°C during this period (American Public Health Association [APHA], 1998). However, utilizing hold times longer than 8 hr for fecal indicator bacteria is supported by studies such as Pope et al. (2003) and Selvakumar

et al. (2004). Thus, numerous research studies have utilized 24 hr as a hold time threshold. Pope et al. (2003) reported that when samples were stored in coolers with wet ice or Utek ice packs, five of seven sites showed no significant difference between 0 and 24 hr of holding time, four sites showed no significant difference at 30 hr of holding time, and only two of seven sites showed no significant difference between 0 and 48 hr of holding time. Likewise, a study where samples were put on ice and brought back to the laboratory and refrigerated until processing indicated that bacteria densities do not change significantly with holding times up to about 27 hr for total coliform and possibly as long as 62 hr (Aulenbach, 2010). In another similar study (Harmel et al., 2016a), samples were stored in a cooler on the ice during transport to the laboratory and tested for short events (≤24 hr holding time) and long events (>24 hr). The results showed that there were no statistically significant *E. coli* concentration differences in samples stored for long runoff events (>24 hr). Storage uncertainty relates to the fact that collected samples can often be left in the field, without preservation or refrigeration, for a number of hours before analysis (McCarthy et al., 2008). According to, McCarthy et al. (2008) *Hours in Field* is not a significant factor for the *E. coli* level of stored samples up to 24 hr. However, when comparing holding times for samples stored in environmental conditions, McCarthy et al. (2008) reported an initial increase in *E. coli* concentrations (4 and 8 hr) but decreased after 24 hr.

Conclusion

It is not always possible to transport water samples to the testing facilities immediately, thus increasing holding time. Holding time and temperature can have a significant effect on the density of indicator species (*E. coli*). To ensure that the most accurate data are generated, *E. coli* samples collected from surface waters should always be analyzed as soon as possible. The results of this review suggest that pathogens (*E. coli*) present in water samples can be analyzed beyond 8 hr after sample collection while still generating comparable *E. coli* data. However, water samples need to be stored below 10°C.

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