Escherichia coli (E. coli) Assessment Method for State Surface Waters

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1.0	July 2020	Kathryn Makarowski	All	Initial document

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ACRONYMS

ARM	Administrative Rules of Montana
AU	assessment unit
BST	bacterial source tracking
CFR	Code of Federal Regulations
CFU	colony forming units
CWA	Clean Water Act
CWAIC	Clean Water Act Information Center
DEQ	Department of Environmental Quality
DQA	data quality assessment
EPA	Environmental Protection Agency
FIB	fecal indicator bacteria
GI	gastrointestinal
GM	geometric mean
MCA	Montana Code Annotated
MPN	most probable number
MST	microbial source tracking
QA	quality assurance
QAPP	quality assurance project plan
QC	quality control
RPD	relative percent difference
SAP	sampling and analysis plan
SOP	standard operating procedure
SSM	single sample maximum
STV	statistical threshold value
TMDL	total maximum daily load
WARD	Water Quality Assessment and Reporting Documentation
WQPB	Water Quality Planning Bureau

1.0 INTRODUCTION

This document details the Montana Department of Environmental Quality (DEQ) assessment method for determining attainment of *Escherichia coli* (*E. coli*) water quality standards in state surface waters. *E. coli* is a pollutant assessed when evaluating whether surface waters support the contact recreation beneficial use for all waters, and the drinking water beneficial use for A-1 and A-closed waters only.

1.1 APPLICABILITY

This assessment method is applicable to all surface waters under state jurisdiction, including streams, large rivers (as defined by Flynn and Suplee, 2010), lakes, and reservoirs.

1.2 BACKGROUND INFORMATION

State waters are classified in accordance with their present and future beneficial uses per the Montana Water Quality Act (75-5-301(1), MCA). Water quality in waters classified A, B, C and I is to be maintained suitable for bathing, swimming and recreation (ARM 17.30.621 through 629). A comparable primary goal of the federal Clean Water Act is to prevent, reduce or eliminate pollution to conserve navigable waters for recreation (CWA, 2002; 33 U.S.C. 102(a)).

Swimming and other recreational activities in water contaminated with pathogens can make people ill (EPA, 2016), as can ingesting water before adequate treatment. Fecal coliform and *E. coli* are subgroups in the total coliform group of bacteria which come primarily from the feces of warm blooded animals (**Figure 1**). *E. coli* are a large, diverse group of bacteria (CDC, 2015). Many strains of *E. coli* are not pathogenic and will not cause illness but, since they are fecal in origin and have simple methods of detection, they perform consistently well as an indicator of the potential presence of fecal pathogens in fresh water that could cause gastrointestinal (GI) illness (EPA, 2012). Therefore, *E. coli* is referred to as fecal indicator bacteria (FIB) or a pathogen indicator, "a substance that indicates the potential for human infectious diseases" (CWA §502(23); EPA, 2012).

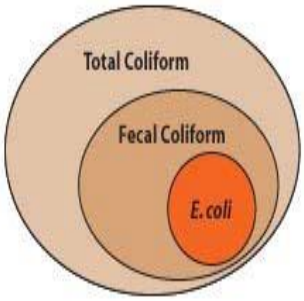

Figure 1 – Total coliform, fecal coliform and *Escherichia coli* (*E. coli*) (from Sigler and Bauder, 2017)

1.3 COMMON SOURCES, PATHWAYS AND FACTORS INFLUENCING *E. COLI* OCCURRENCE

E. coli originate in the gut of humans and warm-blooded animals. Human sources of *E. coli* in the environment include leaking septic tanks and sewer lines, municipal wastewater treatment plants, land application of biosolids, and urban storm water runoff (GA DNR, 2017). Concentrations of FIB including *E. coli* have been positively correlated with human population density (Frenzel and Couvillion, 2002). Animal sources of *E. coli* include livestock and recreational stock, land application of animal waste, domestic pet waste, and wildlife.

Fecal contaminants are often transported to waterways through runoff from residential or agricultural lands especially during storm events, direct deposition where livestock or wildlife access the water, point source discharges (e.g., wastewater facilities or stormwater outfalls), leaking septic systems via groundwater inflows to surface water, and resuspension of bacteria contained in sediments (GA DNR, 2017; EPA, 2012).

The persistence of *E. coli* inside human or animal hosts and in open environments such as soil, manure and water differs depending on the strain. Although conditions for survival of *E. coli* in open environments are less favorable than in the intestinal system, *E. coli* can survive and grow in open environments when resources are available and abiotic conditions are favorable. The organisms have survived for days to almost a year in open environments, and they can migrate between habitats; for example, *E. coli* can reach groundwater from top soil layers (vanElsas *et al.*, 2011).

E. coli growth and death rates are determined by local environmental conditions and by how the microorganism copes with local conditions by regulating gene expression patterns (vanElsas *et al.*, 2011). Factors that influence bacteria density, occurrence and distribution, and thus relative risk of human illness, include source and age of fecal contamination, availability of nutrients and carbon substrates, solar radiation, water salinity, acidity, turbidity, dissolved organic matter, and water temperature (EPA, 2012; vanElsas *et al.*, 2011). Predation, competition, and the release and resuspension of bacteria from sediments in the water column are also factors (EPA, 2012; Whitman *et al*., 2004; Park *et al*., 2017).

2.0 E. COLI WATER QUALITY STANDARDS

Guided by state law and federal recommendations, Montana developed water quality standards for *E. coli* to protect people from pathogens in waters.

2.1 EPA'S RECOMMENDED CRITERIA FOR BACTERIA FOR FRESH WATERS (RECREATIONAL USE)

In 1968, fecal coliforms were recommended as the indicator organism for evaluating the microbiological suitability of recreation waters, and federal water quality criteria recommendations for bacteria were first proposed by the National Technical Advisory Committee of the US Department of the Interior (FWPCA, 1968):

> *"As determined by multiple-tube fermentation or membrane filter procedures and based on a minimum of not less than five samples taken over not more than a 30-day period, the fecal coliform content of primary contact recreation waters shall not exceed a log mean of 200/100 ml, nor shall more than 10 percent of total samples during any 30-day period exceed 400/100 ml" (EPA, 1986).*

In 1976, this criterion was recommended again by the Environmental Protection Agency (EPA) (EPA, 1976). In the late 1970s and early 1980s, EPA conducted epidemiological studies to evaluate several additional organisms as possible indicators of fecal contamination; these studies showed that *E. coli* is a better predictor of gastrointestinal (GI) illness in fresh waters than fecal coliform (EPA, 2012). Therefore, in 1986, EPA released ambient water quality criteria for *E. coli* which represented the desired ambient condition necessary to protect the primary contact recreation designated use in fresh recreational waters (EPA, 1986; EPA, 2012). These criteria values are based on levels of risk correlating to no more than eight cases of acute GI illness per 1,000 swimmers (EPA, 1986), above which the health risk from

waterborne disease is unacceptably high (EPA, 2002a). The 1986 criteria recommendations included a steady state geometric mean[1] of 126 cfu/100ml and four single sample maximum (SSM) values which varied depending on recreational use intensity of the water in question:

- Designated beach area
- Moderately used for full body contact recreation
- Lightly used for full body contact recreation
- Infrequently used for full body contact recreation.

These four SSMs corresponded to the 75th percentile (235 cfu/100ml), 82nd percentile (298 cfu/100ml), 90th percentile (409 cfu/100ml), and 95th percentile (575 cfu/100ml) of the expected water quality sampling distribution of geometric mean values calculated during the epidemiological studies from the late 1970s and 1980s (EPA, 1986). Noncompliance was signaled if either the geometric mean exceeded the criteria value or if there was an unacceptably high value for any single bacterial sample.

In 2012, EPA released updated recreational water quality criteria recommendations which consist of a magnitude, duration, and frequency of exceedance. EPA recommended the criteria magnitude be expressed 1) as a geometric mean (GM) corresponding to the 50th percentile (126 *E. coli* cfu/100ml), and 2) as a statistical threshold value (STV) corresponding to the 90th percentile (410 *E. coli* cfu/100ml) of the same water quality distribution, and thus associated with the same level of public health protection (EPA, 2012). EPA's 2012 single statistical threshold value replaced the 1986 mutliple single sample maximum values that varied depending on recreational use intensity.

The duration and frequency components are that the waterbody should not have *E. coli* measurements greater than the selected GM magnitude during any 30-day interval, and there should not be greater than ten percent excursion of the selected STV magnitude in the same 30-day interval (EPA, 2012). To be adequately protective of the primary contact recreation use, EPA recommends using both GM and STV criteria because, together, they account for both average conditions and spikes in *E. coli* concentrations (EPA, 2012). In essence, limiting the number of samples allowed to exceed the STV before deciding water quality is impaired encourages monitoring because once an exceedance is observed, at least ten more samples need to be below the STV before water quality is considered unimpaired (EPA, 2012).

2.2 MONTANA *E. COLI* WATER QUALITY STANDARDS

Montana's *E. coli* water quality standards are contained in the Administrative Rules of Montana (ARM). General surface water quality standards state, "standards for *Escherichia coli* bacteria are based on a minimum of five samples obtained during separate 24-hour periods during any consecutive 30-day period analyzed by the most probable number or equivalent membrane filter methods" (ARM 17.30.620(2)). Montana's *E. coli* criteria vary depending on use classification, recreation season, and type of criteria (**Table 1**) (ARM 17.30.621 through 629, and 17.30.650 through 657). For example, for B-1 waters (ARM 17.30.623), the standard reads:

"The water quality standard for *Escherichia coli* bacteria (*E-coli*) varies according to season, as follows: from April 1 through October 31, the geometric mean number of *E-coli* may not exceed 126 colony forming units per 100 milliliters and 10% of the samples may not exceed 252 colony forming units per 100 milliliters during any 30-day period; and from November 1 through March 31, the geometric mean

[1] Calculation of a geometric mean is described in detail in **Section 5.2.1**.

number of *E-coli* may not exceed 630 colony forming units per 100 milliliters and 10% of the samples may not exceed 1,260 colony forming units per 100 milliliters during any 30-day period."

Table 1. Montana's *E. coli* Criteria

Use Classification	Beneficial Use	Applicable Time	Criteria (cfu/100ml or mpn/100ml)	
			Geometric Mean (may not exceed)	**Statistical Threshold Value** (10% may not exceed)
A-1 and A-closed	Drinking water	year-round	32	64
	Primary contact recreation	April 1 - October 31	126	252
	Secondary contact recreation	November 1 - March 31	630	1260
B, C, and I	Primary contact recreation	April 1 - October 31	126	252
	Secondary contact recreation	November 1 - March 31	630	1260
D, E, F, G	Secondary contact recreation	Year-round	630	1260

Montana's primary contact recreation *E. coli* criteria are based on the conversion of EPA's recommended fecal coliform criteria to *E. coli* criteria while maintaining the same acceptable illness rate (8 illnesses per 1,000 swimmers): fecal coliform geometric mean of 200 cfu/100ml converts to *E. coli* geometric mean of 126 cfu/100ml with a ratio of 0.63 (126/200 = 0.63). Using this same ratio, the recommended criteria for fecal coliform of 400 cfu/100ml converts to a statistical threshold value recommended criteria for *E. coli* of 252 cfu/100ml (252/400 = 0.63). These standards apply during the season when primary contact recreation uses are supported by Montana's climate (April 1 – October 31) (**Table 1**).

Where primary contact recreation is limited during months when ambient air and water temperatures are too cold for most people to recreate, states may adopt seasonal water quality criteria that are less stringent. These seasonal criteria must be based on EPA's recommendation for secondary contact in order to protect for incidental ingestion, and not exceed a geometric mean five times EPA's recommended water quality criteria for bacteria (EPA, 2000a). Hence, Montana's secondary contact recreation *E. coli* criteria for November 1 to March 31 (630 and 1260 cfu/100ml) are five times greater than those applicable from April 1 to October 31 (**Table 1**).

Montana's *E. coli* criteria for A use class waters are associated with drinking water and are more stringent because, for example, A-closed waters are to be maintained suitable for drinking water use after simple disinfection rather than conventional treatment (ARM 17.30.621) and thus drinking water is the most sensitive beneficial use. The same ratio (0.63) used to convert fecal coliform to *E. coli* for recreational criteria was used to convert 50 cfu/100 ml fecal coliform (the limit identified in Montana since the 1960s as protective of the drinkin water use) to 32 cfu/100ml *E. coli* for A waters' criteria.

The contact recreation criteria that are specified in rule for B through F use classes are applied to A class waters when assessing primary or secondary contact recreation use support in those waters.

3.0 SAMPLING AND DATA QUALITY CONSIDERATIONS FOR *E. COLI* ASSESSMENT

Waterbody condition must be evaluated based on all existing and readily available data and information (§75-5-702, MCA; 40 CFR 130.7(b)(5)). This section describes several considerations for developing monitoring designs and assessing data quality when performing *E. coli* assessments. Additional guidance is included in DEQ's standard operating procedure for sample collection, handling and analysis of *Escherichia coli* (Makarowski, 2019).

3.1 *E. COLI* SAMPLE COLLECTION, ANALYSIS, AND UNITS

E. coli data used to evaluate standards attainment must adhere to DEQ's standard operating procedure (SOP) for *E. coli* sample collection, handling and analysis (Makarowski, 2019).

E. coli samples must be "analyzed by the most probable number or equivalent membrane filter methods" (ARM 17.30.620(2)). Three methods are approved for *E. coli* analysis in ambient water (40 CFR Parts 136 and 503) and are acceptable for use when making water quality standards attainment decisions: the Colilert method, EPA Method 1603, and EPA Method 1604. The Colilert method is commonly used in Montana because it can be performed either by field personnel in a portable laboratory or by a professional lab and is indicated as the preferred method on Montana DEQ's WQPB Monitoring Suite Table (DEQ, 2019); the Colilert method (A9223B) is officially referred to as the Enzyme Substrate Coliform Test (NWQMC, 2017). EPA methods 1603 and 1604 are typically performed by professional labs and are alternate methods on DEQ's WQPB Monitoring Suite Table (DEQ, 2019). Analytical method selection generally depends on the feasibility of transporting samples to an approved professional lab within the short (6-8 hour) holding time and availability of portable laboratory equipment.

Traditional plate tests for *E. coli* (including membrane filtration) provide a direct count of *E. coli* bacteria colonies in water based on the development of colonies in/on media and are reported as colony forming units (cfu). While these microscopic counts may be more accurate, they can be costly and time consuming (DEQ, 2017). When the Colilert method is used, a most probable number (MPN) statistical probability table is used to estimate the number of organisms in the sample (IDEXX Laboratories, Inc., 2017) and results are reported as MPN/100ml. MPN analyses do not result in a direct count or concentration density of the bacteria being enumerated but rather rely on probabilities (EPA, 2012). These units (colony forming units and most probable number) are used interchangeably by EPA (40 CFR 136.3, 2003) and Montana rules state that "Water quality criteria for *Escherichia coli* are expressed in colony forming units per 100 milliliters of water or as most probable number, which is a statistical representation of the number of organisms in a sample, as incorporated by reference in 40 CFR 136.3(b)" (ARM 17.30.621 through 629).

3.2 SAMPLING TIMEFRAME AND TEMPORAL INDEPENDENCE

E. coli growth rate is influenced by a variety of factors (**Section 1.3**) and bacteria densities can vary substantially in relatively short periods of time, increasing exponentially under optimal growing conditions or experiencing rapid die-off. This improves the likelihood that samples collected over relatively short timeframes are temporally independent. However, in general, collecting more samples

during additional consecutive 30-day periods and over multiple years will provide a more accurate representation of *E. coli* conditions.

3.2.1 Time of Year

E. coli samples may be collected year-round. For recreation use, Montana's *E. coli* criteria differ between two recreation seasons (**Table 1**). Generally, when assessing suitability of waters for contact recreation use, DEQ will prioritize *E. coli* monitoring and assessment during the primary contact recreation season. Listing decisions for *E. coli* impairment may be made during either season, though determinations of primary contact recreation use support must be based on data collected from April 1 to October 31. If minimum data requirements are met during the secondary contact recreation season, DEQ will apply the less stringent criteria but the same decision framework used to assess primary contact recreation use support will be used to assess secondary contact recreation use support.

3.2.2 Wet Weather

Wet weather and runoff can significantly affect bacteria densities (Dorner *et al*., 2007; Baxter-Potter and Gilliland, 1988). EPA's *E. coli* geometric mean indicator densities, which correspond to accepted GI illness rates, are for steady state dry weather conditions (EPA, 1986). In general, samples should be collected during dry weather periods to establish "steady state" conditions; special studies may be necessary to evaluate the effects of wet weather conditions on waters of interest, especially if sanitary surveys indicate the area may be subject to stormwater effects (EPA, 1986). Best professional judgement may be used to determine when it is appropriate to incorporate wet weather data or spring runoff data during standards attainment and beneficial use assessment.

3.2.3 Sampling Frequency, Consecutive 30-day Sampling Period, Separate 24-hour Periods

When interpreting bacteria criteria, a short period of record can indicate impairment in cases of gross exceedances of criteria (EPA, 2002a). Montana's general *E. coli* standard provides guidance for sample collection timeframe, stating, "standards for *Escherichia coli* bacteria are based on a minimum of five samples obtained during separate 24-hour periods during any consecutive 30-day period…" (ARM 17.30.620(2)). This 30-day period is based on EPA's belief that, while a longer duration would typically allow for more samples to be collected and thus improving accuracy of water quality characterization, a shorter duration (i.e., 30 days) coupled with limited excursions above the statistical threshold value allows for the detection of transient fluctuations in water quality in a timely manner and is thus more protective of primary contact recreation (EPA, 2012). EPA recommends that states conduct at least weekly sampling when evaluating 30-day periods and encourages more frequent sampling at more densely populated beaches (EPA, 2012). Consecutive 30-day periods should be considered on a rolling basis, not calendar month; that is, data should be grouped by each 30-day period that occurs as the date progresses over the course of the recreation season or year.

Montana's *E. coli* assessment decisions (**Section 5**) are made using data that is first grouped by year, then (for recreation use) grouped according to the recreation season (either primary, April 1 to October 31, or secondary, November 1 to March 31) during which data was collected. That is, data is not combined during analysis if it was collected during different years or recreation seasons. When the preferred approach is applied, *E. coli* data is aggregated according to recreation season first, then over 30-day periods, whereas when the alternate approach is applied, data is aggregated according to recreation season only.

E. coli monitoring in Montana is logistically challenging because travel distances are often great and *E. coli* samples have a very short (6-8 hour) holding time. Effort should be made to balance spatial and temporal sample independence to obtain a representative dataset, especially when multiple samples are collected from a waterbody assessment unit within a narrower time frame than 30 days. For example, multiple sampling events may occur on a waterbody within a shorter (one- or two-week) period during which intensive sampling is completed rather than being evenly spaced (e.g., weekly) over a 30-day period. This is acceptable as long as samples collected at a single monitoring site, or at multiple sites that are spatially dependent, are collected during separate 24-hour periods. If multiple samples were collected at a site within a 24-hour period, or from spatially dependent sites, treat these temporally and/or spatially dependent samples as replicates and calculate their geometric mean, then incorporate this calculated value into the 30-day or recreation season geometric mean calculation. Consider the geometric mean calculated from replicates as a single result value when evaluating minimum sample size (i.e., n ≥ 5) for the assessment unit (MPCA, 2014; Flemer *et al*., 2014).

It is acceptable to base water quality standards attainment decisions on data collected during a single 30-day period (**Section 5.3.1, preferred approach**). Alternately, data may be collected over a longer duration (i.e., over a recreation season) without achieving the minimum data requirement of ≥ 5 samples from separate 24-hour periods within a 30-day period. In this case, it is acceptable to make water quality standards attainment decisions for contact recreation based on data collected over the course of a single recreation season if the minimum sample size required for the alternate approach is met (i.e., at least 11 samples) (**Section 5.3.1, alternate approach**).

3.2.4 Time of Day

Samples collected during any time of day (i.e., morning, afternoon, night) may be included when making water quality standards attainment and beneficial use support determinations for *E. coli*. Refer to DEQ's *E. coli* standard operating procedure (Makarowski, 2019) for additional guidance on best practices regarding sample collection time of day.

3.3 Sampling Locations and Spatial Independence

Guidance for selecting sampling locations is intended to help ensure spatial independence of data.

3.3.1 Assessment unit selection

E. coli assessment decisions are made for assessment units (i.e., waterbodies or waterbody segments). DEQ is more likely to prioritize *E. coli* assessment for assessment units that are commonly used for primary contact recreation, particularly those with public access such as designated swimming areas. DEQ may also prioritize waters that have been previously identified as impaired, waters at higher risk of *E. coli* impairment due to human activities, or other factors.

3.3.2 Assessment reaches

If an assessment unit exhibits one or more significant shifts in type and intensity of potential *E. coli* sources such that clear breaks could be made to designate homogenous sub-reaches, sub-segmenting may be justified (Suplee and Sada, 2016). For example, if a relatively un-impacted upstream reach can be isolated and its condition is likely substantially different from other downstream parts of the assessment unit, the assessment unit may be split into two sub-reaches for assessment purposes:

- If one reach indicates impairment, the entire assessment unit receives the impairment determination.

- Each reach has the same general data requirements (e.g., dataset minimums) as the parent assessment unit would have had if it hadn't been divided.
- It is better to lump than split reaches to avoid excessive sub-segmentation and the consequential administrative and sampling requirements that result.
- An assessor must decide whether to split an assessment unit into multiple assessment reaches before data collection; this will help ensure that reach breaks are based on considerations of land use and sources rather than on differences in *E. coli* concentrations among sites discovered after monitoring has occurred.

3.3.3 Total number of sites

Assessment determinations are made using data pooled for the entire assessment unit (or assessment reach per **Section 3.3.2**), not individual sites. Best professional judgement may be applied to determine how many sites are needed to adequately represent the range of potential human sources influencing the assessment unit. It is preferable to incorporate data collected at multiple sites to better capture variability in bacteria density throughout the assessment unit.

Also, as resources allow, it is preferable to collect multiple samples from each monitoring site selected (e.g., ≥ 5 samples 24 hours apart and within a 30-day period per site). This enables a multifaceted approach to data analysis; for example, in addition to pooling data from the entire assessment unit to make impairment determinations, an assessor may strive for enough data to analyze individual sites to perform a thorough source assessment. However, assessment decisions can be based on data collected at a single sampling location only if that single sampling location can be reasonably considered representative of portions of the assessment unit that experience relatively higher potential risk from *E. coli* sources.

3.3.4 Site selection

Generally, sampling designs for *E. coli* assessment should target sites that are most likely influenced by pathogen sources rather than random sampling designs intended to represent all potential impairment and non-impairment conditions throughout the assessment unit or reach. Data used to assess *E. coli* must be collected from the assessment unit itself and sampling locations that may be prioritized for monitoring include:

1. Sites where contact recreation use is the most likely (e.g., public swimming beaches, parks, campgrounds, fishing access sites, and other public access points)
2. Sites with higher risk of fecal contamination because they are near or downstream from *E. coli* source areas. Note: Samples incorporated into beneficial use assessments cannot be within the mixing zone of permitted point source discharges.

Other site locations that may be useful for source assessment purposes include sites that represent natural background conditions (e.g., upstream from human sources), sites that bracket tributary confluences or source areas, and sites on waters that are hydrologically connected to the assessment unit (e.g., tributaries, ditches, point source discharges, wetlands, reservoirs).

For site-specific sampling guidance such as depth and distance from shore, refer to DEQ's *E. coli* SOP (Makarowski, 2019).

3.3.5 Spatial independence

E. coli growth rate is influenced by a variety of factors (**Section 1.3**) and the likelihood of achieving spatial independence among sites is improved because of these relatively rapid responses of bacteria growth to site-specific conditions such as temperature, sunlight, nutrient availability, etc. Determining spatial independence relies on best professional judgement, particularly when working in lotic systems where water flowing downstream can influence spatial independence of downstream sites. The following guidance for achieving spatial independence for bacterial testing aligns with similar guidance in several of DEQ's other assessment methods (Suplee and Sada, 2016; Drygas, 2012):

- Select sites that are at least one stream mile apart unless there is a flowing tributary that confluences with the segment or a discrete source is located between the two sites.
- Consider land use and land form changes to help identify potential sources of fecal contamination and sites representative of natural background conditions

3.4 SAMPLE SIZE

Data quality requirements including minimum sample size should be reviewed prior to designing a monitoring strategy for *E. coli* assessment. Although minimum sample sizes are specified in **Section 3.4.1**, it is desirable to surpass these minimum requirements as resources allow to obtain better spatial and temporal representation and a more robust data set. For example, although minimum sample size can technically be met by sampling at a single site location, it is generally preferable to strategically collect samples at multiple independent sites that represent a range of conditions along a waterbody (**Section 3.3**). It is also preferable to strive to meet minimum sample size requirements during multiple groupings of data (e.g., multiple 30-day periods or multiple recreation seasons) to better capture temporal variability.

3.4.1 Minimum Sample Size

The basis for minimum data requirements for *E. coli* assessment is stated in ARM: "standards for *Escherichia coli* bacteria are based on a minimum of five samples obtained during separate 24-hour periods during any consecutive 30-day period…" (ARM 17.30.620(2)). This aligns with EPA guidance (**Section 3.2.3**). Samples must be independent to count toward minimum sample size (**Sections 3.2 and 3.3**).

Minimum sample size requirements vary depending on the assessment approach taken (**Section 5.3**):

1. To apply the preferred assessment approach (recreation and drinking water use), at least one 30-day period with a minimum of five samples collected during separate 24-hour periods is required to apply the geometric mean and statistical threshold value (≥ 10% exceedance) criteria.
2. To apply the alternate assessment approach (recreation only), at least eleven samples must be collected over the span of a single recreation season within a single year. Eleven was selected as the minimum to prevent a single exceedance from resulting in an impairment determination with respect to the ≥ 10% exceedance criteria.
3. Applying overwhelming evidence of an impairment (recreation and drinking water use) requires a minimum of five samples collected over the span of a single recreation season (recreation use) or a single year (drinking water use).

3.4.2 Number of Replicate Samples Used to Represent Each Site

Typically, one sample will be collected per site visit, regardless of whether the assessment unit being monitored is a stream, river, lake, reservoir, or other waterbody. Additional replicates (i.e., two or more samples collected during a single site visit) may be collected in places where there is a known or suspected source or where site-specific variability in *E. coli* levels are suspected. See **Section 3.2.3** for guidance on analyzing replicate data from samples collected at same site within one 24-hour period.

3.5 DATA CURRENCY

Because *E. coli* concentrations can vary greatly over short periods of time, it is preferable to base *E. coli* standards attainment assessment decisions on recent data. When making *E. coli* assessment decisions, only data collected since the previous water quality standards assessment decision was made should be included. Also, typically only data collected within ten years of the assessment should be considered. Assessors should evaluate the data to determine how well it represents current conditions; if conditions or land management have changed substantially within a site or reach that could affect *E. coli* loading, it may be appropriate to exclude data collected prior to the change even if it was collected less than ten years ago.

3.6 PARAMETERS REQUIRED FOR *E. COLI* ASSESSMENT

E. coli densities (cfu/100ml or mpn/100ml) are the only parameter required for making E. coli water quality standards attainment. Data must be *E. coli* result values, not fecal coliform or total coliform. See **Section 6.0** for supplemental parameters.

3.7 QUALITY CONTROL SAMPLES: FIELD DUPLICATES AND FIELD BLANKS

Field duplicates are samples collected as close as possible to the same point in space and time; duplicates should be collected by the same person and using the same collection method, though they are stored in separate containers and analyzed independently. Any *E. coli* sampling design intended for assessing water quality standards attainment should incorporate field duplicates and the frequency of duplicate sampling should be documented in a quality assurance project plan (QAPP) or sampling and analysis plan (SAP); often field duplicates are collected at a minimum frequency of 10% of total samples. Field duplicates collected for data quality control differ from replicates intentionally collected from the same site to better represent variability within a site.

Field blanks are samples collected and handled following the same methods as routine samples except laboratory-grade deionized or distilled water is used rather than ambient water. Field blanks represent total ambient conditions during sampling and laboratory sources of contamination (EPA, 2009). Any *E. coli* sampling design intended for assessing water quality standards attainment should incorporate field blanks and the frequency should be documented in a quality assurance project plan (QAPP) or sampling and analysis plan (SAP). Typically, field blanks are prepared at the end of the sampling event and at least one field blank is analyzed along with each batch of routine samples.

4.0 DATA QUALITY

Established policies and procedures of DEQ's Water Quality Division for quality assurance and quality control, beneficial use assessment, and data management apply to this assessment method. Data

quality requirements apply to all data incorporated while making assessment decisions, whether collected internally or externally.

4.1 DATA QUALITY ASSESSMENT OVERVIEW

Data quality assessment (DQA) is the scientific and statistical evaluation of data to determine whether data obtained from monitoring operations are of the right type, quality, and quantity to support water quality assessments (EPA, 2002a). Assessors use DEQ's Water Quality Assessment and Reporting Documentation (WARD) System to document the DQA outcome (pass or fail) for each parameter group being assessed per beneficial use. All data quality indicators must be met to pass the DQA; if a single indicator is not met, the DQA fails for that parameter group. An assessor may override pass or override fail a DQA but they must accompany this override with adequate justification.

Additional data quality screening may be necessary before the data set is ready to support attainment decisions (CALM, 2002), for example:

- handling non-detects
- evaluating database flags
- evaluating QC samples (i.e., field duplicates and field blanks)
- verifying holding time and incubation times were adhered to
- reviewing QA/QC reports
- investigating errors in collection or analysis
- addressing missing data, and
- reviewing deviations from SOPs and SAPs.

Once DEQ determines the data meets basic documentation requirements, the data is ready to be analyzed to support water quality standards attainment decisions (CALM, 2002).

4.2 EVALUATING FIELD DUPLICATES

Relative percent difference (RPD) is used to evaluate results between two duplicate samples:

$$RPD = \frac{|(result\ 1 - result\ 2)|}{(result\ 1 + result\ 2)/2} \text{ X } 100$$

Field duplicates (**Section 3.7**) should generally be within 25% RPD of one another. However, bacteria replicates are typically variable in nature (Wymer, *et al*., 2005) so RPD should be used primarily as a descriptive statistic. If greater than 25% RPD is found among field duplicates, the assessor should verify data quality to confirm that the routine result values are valid for inclusion in assessment.

4.3 EVALUATING FIELD BLANKS

Assessors may decide to reject samples collected during a sampling event in which a field blank returns detectable levels of *E. coli*. If field blank detections are found, assessors should attempt to identify the probable source of contamination, provide additional training or adjust collection, handling, storage, and analysis processes, as necessary.

5.0 DATA ANALYSIS TO SUPPORT WATER QUALITY STANDARDS ATTAINMENT DECISIONS

E. coli density (cfu/100ml or mpn/100ml) is the only type of data applied directly during water quality standards attainment assessment to identify *E. coli* impairments. EPA's independent applicability policy[2] is intended to protect against dismissing valuable information when evaluating aquatic life use attainment, particularly in detecting impairment (EPA, 2002a). Since *E. coli* assessment is associated with contact recreation and drinking water use attainment rather than aquatic life and involves a single data type rather than multiple data types, independent applicability does not apply.

5.1 OVERVIEW OF THE ASSESSMENT DECISION FRAMEWORK

The same process and decision framework are applied whether a waterbody was previously listed as impaired by *E. coli* or not and whether data was collected during the primary contact recreation season (April 1 – October 31) or the secondary contact recreation season (November 1 – March 31). For each assessment unit (or assessment reach), data is compiled, data quality is evaluated, and the data is prepared for assessment (**Section 5.2**). Sample result values are then compared against the applicable geometric mean (**Section 5.3**) criteria and the statistical threshold value (**Section 5.4**) criteria, and impairment listing and beneficial use support decisions are made. Waterbodies that are determined to be impaired due to *E. coli* are not fully supporting their contact recreation beneficial use or drinking water beneficial use (for A waters only) and the impairment cause name is "*Escherichia coli* (*E. coli*)."

Several scenarios are accounted for in the decision framework (**Section 5.5, Figure 2**):

- The preferred approach in which there are at least five sample result values collected within a 30-day period during separate 24-hour periods,
- An alternative approach in which there are many sample result values, but sample collection times were spread over a longer period (i.e., a recreation season),
- Overwhelming evidence in which there are few samples but many exceedances, and
- Instances where minimum data requirements are not met so assessment decisions cannot be made, but where there is higher potential risk to human health (i.e., exceedances are identified in waters routinely used for contact recreation).

5.2 PREPARING THE DATA FOR ASSESSMENT

1. Compile all *E. coli* data for an assessment unit or assessment reach (**Section 3.3**).

[2] For the purposes of water quality standards attainment decisions, the policy of independent applicability says 1) when evaluating multiple types of data (e.g., biological, chemical) and any one type of data indicates an element of a water quality standard is not attained, the water should most likely be identified as impaired, and 2) if there is reason to doubt the nonattainment finding, re-evaluate all of the data sets to resolve discrepancies; the intent of the independent applicability policy is to protect against dismissing valuable information when evaluating aquatic life use attainment, particularly in detecting impairment (EPA, 2002a).

2. Perform data quality assessment to identify the usable dataset (**Section 4**).
3. Organize data by year.
4. Organize data by season according to Montana's *E. coli* standards (**Table 1**):
    - Data collected from April 1st to October 31st is included in decision-making regarding primary contact use support and impairment status.
    - Data collected from November 1st to March 31st is included in decision-making regarding secondary contact recreation use support and impairment status. Determine the appropriate geometric mean and statistical threshold criteria depending on the use classification and recreation season (**Section 2.2**).
    - Data collected year-round is used for decision-making regarding drinking water use support.
5. Group result values by the consecutive 30-day period during which they were collected. Within each 30-day period, identify sample result values that are spatially independent. Organize samples by site and evaluate spatial independence of sites relative to one another (**Section 3.3**). If sites are not spatially independent, aggregate data from spatially dependent sites prior to evaluating temporal independence.

Note: Consecutive 30-day periods should be considered on a rolling basis, not calendar month; that is, data should be grouped by each 30-day period that occurs as the date progresses over the course of the recreation season or year.

6. Within each 30-day period, identify sample result values that are temporally independent (i.e., collected at spatially independent sites in separate 24-hour periods) (**Section 3.2**). Organize samples by date and time. Due to sampling design, datasets at a single sampling location may contain replicate samples or multiple samples collected in the same day. However*, E. coli* assessments require values for a single collection event. The following may be considered a single, temporally-independent collection event (Flemer *et al*., 2014):
    - The daily result value collected in an individual 24-hour period at a site.
    - A geometric mean of replicates where multiple samples are collected at the same site within the same 24-hour period (**Section 3.2.3**).

Note: To assess there must be at least five samples collected during separate 24-hour periods in a consecutive 30-day period (recreation and drinking water uses) or at least eleven samples collected during ≥ 5 separate 24-hour periods throughout a single recreation season (recreation use only). There is one overwhelming evidence provision in the decision frameworks that is an exception to this rule.

5.2.1 Geometric Means

"Geometric mean" is the value obtained by taking the Nth root of the product of the measured values where zero values for measured values are taken to be the detection limit (ARM 17.30.602(11)). Other definitions include "the n-th root of the product of n numbers," or, "the average of the logarithmic values of a data set, converted back to a base 10 number" (Costa, 1997). A geometric mean, unlike an arithmetic mean, tends to dampen the effect of very high or low values; this is helpful when analyzing bacteria concentrations because levels may vary anywhere from 10 to 10,000-fold over a given period (Costa, 1997).

Calculate the geometric means using the function "=GEOMEAN(x:x)" in Microsoft Excel, or the equation:

$$\sqrt[n]{\prod_{i=1}^{n} Xi}$$

There are two options for calculating geometric mean in the decision framework; these geometric means are compared to the relevant geometric mean criteria in **Table 1**.

1. A 30-day geometric mean is calculated for any 30-day period with at least five independent samples.
2. A recreation season geometric mean may be calculated when there are at least eleven samples collected in ≥ five separate 24-hour periods throughout a single recreation season (i.e., primary contact April 1 to October 31, or secondary contact November 1 to March 31) within a single year.

Note: Site-by-site geometric means may also be calculated using result values collected from individual monitoring sites which have at least five samples collected 24-hours apart within a 30-day period. However, site-by-site analyses are only used to identify potential pollutant sources or hot spots and are not applied directly into the assessment as impairment determinations are based on an evaluation of data pooled from the entire assessment unit, not individual sites.

If replicate sample result values exist for a site visit, the geometric mean of those replicates are included in the calculation of geometric mean for the assessment unit (as opposed to each individual replicate's sample result value) (**Section 3.2.3**).

5.2.2 Statistical Threshold Value Criteria

When comparing sample results values against the relevant statistical threshold value criteria in **Table 1**, result values are grouped by the 30-day period (for the preferred approach) or the recreation season (for the alternate approach) within which they were collected, and each individual result value should be considered regardless of spatial or temporal independence, including individual result values for replicate samples. Whereas geometric mean criteria is used to evaluate the average conditions and relates especially to the magnitude of exceedances, the statistical threshold value pertains to the frequency of exceedances; including each individual sample result value when calculating percent exceedance regardless of spatial or temporal independence helps to ensure that all exceedances of the criteria are considered.

5.2.3 Handling Non-Detect and Greater-Than-Detect Values

The required reporting limit for *E. coli* using EPA Method 1603 (and 1604) is 1 cfu/100ml, and the maximum achievable detection limit when using most probable number approach is 2,419.6 cfu/100ml. For data points reported below detectable limits, the geometric mean calculation should be based on the assumption that those observations were present at the detection limit (EPA, 2012). Therefore, any *E. coli* sample result value that is reported as below detection (< 1 cfu/100ml) will be treated as 1 cfu/100ml. Likewise, any *E. coli* sample result value reported as above the maximum detection (> 2,419.6 cfu/100ml) will be treated as 2419.6 cfu/100ml for the calculation of the geometric mean (Flemer *et al*., 2014).

5.2.4 Large Datasets

While uncommon due to logistical challenges, there may be instances where a large dataset of *E. coli* result values (e.g., weekly sampling over a multi-year period) is available for a waterbody assessment unit. Aggregating data by individual month across years (e.g., all April values, all May values, etc.) for very large datasets diminishes the value of the data and assessment, making it less likely that periodic *E. coli* exceedances will be identified that indicate impairment (MPCA, 2014). Therefore, large datasets should be treated the same as small datasets with respect to data analysis (i.e., pooling data collected per 30-day period and per recreation season and evaluating each period against both the geometric mean and statistical threshold value criteria). Refer to **Section 3.5** for information about evaluating data currency when deciding which data to include when making assessment decisions.

5.3 ASSESSMENT DECISION FRAMEWORK

Two decision frameworks are used to determine whether assessment units are impaired due to *E. coli.* The first (**Figure 2**) pertains to contact recreation use support for all waters. The second (**Figure 3**) pertains to decisions about drinking water use support for waters with A-1 and A-closed use classifications since more stringent criteria apply only to these use classes (**Section 2.2**). Multiple scenarios are built into each decision-making framework to consider multiple approaches for grouping data and to help ensure that all existing and readily available data is considered when making attainment and non-attainment decisions.

5.3.1 Impairment Listing Decisions for Contact Recreation Use Support

This decision framework (**Figure 2**) applies when making *E. coli* impairment listing decisions for contact recreation use support for all waters (i.e., A through F use classes), though it is important to note the following specifications that determine which criteria apply (**Section 2.2**):

- The recreation-based criteria for primary and secondary contact recreation apply seasonally to waters with B, C and I use classifications
- The recreation-based criteria for primary and secondary contact recreation specified in rule for B, C and I waters is applied to A-1 and A-closed waters.
- The recreation-based criteria for secondary contact recreation specified in rule for waters with D, E and F use classifications is applied year-round (i.e., primary contact recreation criteria do not apply).

The contact recreation decision framework incorporates four scenarios: preferred approach, alternate approach, overwhelming evidence, and final risk screening. Data is compiled for the assessment unit, evaluated for spatial and temporal independence and aggregated as needed (**Section 3.3**), reviewed for data quality (**Section 4.0**), and grouped by contact recreation season and by 30-day period. Then, the data is run through the four scenarios. The assessment record should state whether any listing was prompted by primary contact recreation or secondary contact recreation criteria exceedances, or both.

Even if a decision of impairment is made before all data is reviewed (e.g., from a single 30-day period or recreation season grouping of data), proceed to pass every existing group of data through the decision framework so all available data can be used to describe current conditions and variability.

SCENARIO 1 – preferred approach (30-day analysis)

The preferred assessment approach is taken for all consecutive 30-day periods for which there are at least five independent result values collected during separate 24-hour periods. For each 30-day period that achieves this minimum data requirement, the 30-day geometric mean is calculated and compared to the geometric mean criteria, and individual result values are compared to the statistical threshold value criteria. If either the geometric mean exceeds the criteria, or if ten percent or more of samples exceeds the statistical threshold value criteria for any single consecutive 30-day period, the assessment unit is impaired by *E. coli* and is not fully supporting its contact recreation beneficial use.

To ensure that all existing and readily available data is considered when making attainment or non-attainment decisions, proceed to the alternate approach: 1) if no 30-day period meets the minimum data requirements for the preferred approach, or 2) if all 30-day periods which meet the minimum data requirement for the preferred approach do not indicate impairment and there is additional existing and readily available data that has not yet been used to evaluate water quality standards attainment.

SCENARIO 2 – alternate approach (recreation season analysis)
The alternate approach may be taken in instances where the minimum data requirement for the preferred approach (i.e., at least five independent result values during separate 24-hour periods within a consecutive 30-day period) is not met or if the preferred approach does not already indicate impairment. The alternative approach is possible when there are a substantial number of result values obtained throughout a single recreation season.

For each contact recreation season in a single year with at least eleven independent result values collected during at least five separate 24-hour periods, the seasonal geometric mean is calculated and compared to the geometric mean criteria, and individual result values are compared to the statistical threshold value criteria. If either the seasonal geometric mean exceeds the criteria, or if ten percent or more of samples exceeds the statistical threshold value criteria for any single recreation season, the assessment unit is impaired by *E. coli* and is not supporting its contact recreation beneficial use.

If contact recreation seasons that meet the minimum data requirement for the alternate approach do not indicate impairment, the assessment unit is not impaired due to *E. coli* unless remaining data that has not yet been used to evaluate water quality standards attainment indicates that there is overwhelming evidence which suggests otherwise.

SCENARIO 3 – overwhelming evidence
If there are at least five result values obtained during separate 24-hour periods within an individual contact recreation season and greater than or equal to four of those values exceed the statistical threshold value criteria, there is overwhelming evidence to determine the assessment unit is impaired due to *E. coli* and is not fully supporting its contact recreation beneficial use.

SCENARIO 4 – final risk screening
This final risk screening scenario is intended to prompt consideration of exceedances that may be found in a data set and to consider future action if there is insufficient information to assess because the minimum data requirements are not met for the preferred, alternate, and overwhelming evidence approaches. For each contact recreation season for which data exists, compare individual result values against the statistical threshold value to screen for exceedances.

If zero or one result value exceeds the statistical threshold value, there is insufficient information to assess and no further action is anticipated. If there are two or more result values that exceed the

statistical threshold value criteria, the assessor should discern whether there is high likelihood that the waterbody is routinely used for primary contact recreation; for example, if there are publicly accessible designated swimming areas or known sites that are frequented for primary contact recreation use. For remote waters that are not designated as public beaches and that are unlikely to be used for primary contact recreation, there is insufficient information to assess and no further action is anticipated. Alternately, if the assessment unit is routinely used for primary contact recreation and there are two or more exceedances, the state will consider additional monitoring (as resources allow) to meet the data requirements of the preferred or alternate assessment approaches.

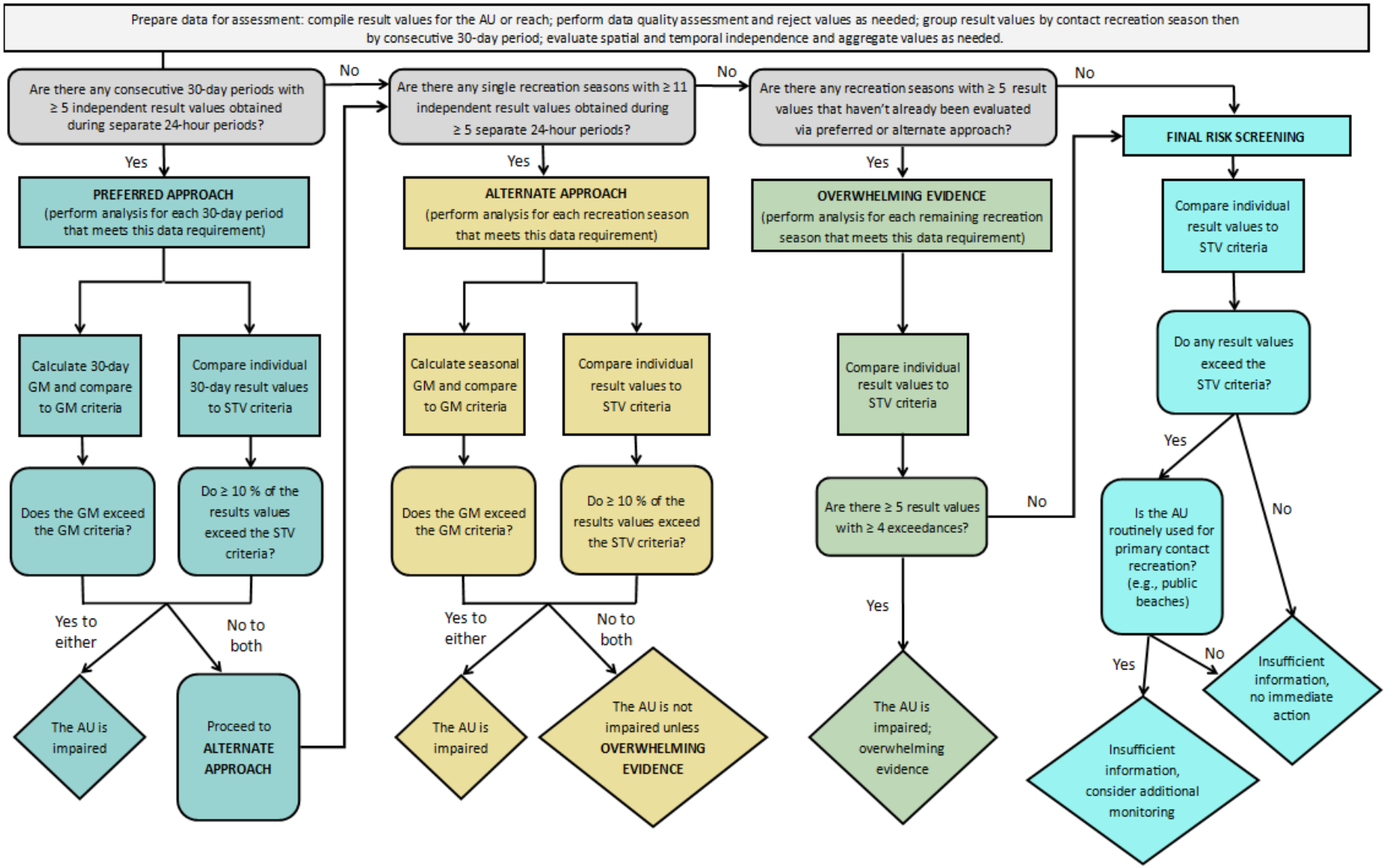

Figure 2 – *E. coli* Assessment Decision Framework for Contact Recreation Beneficial Use Support

5.3.2 Impairment Listing Decisions for Drinking Water Use Support

This decision framework (**Figure 3**) applies when making *E. coli* impairment listing decisions for drinking water use support for waters with A-closed and A-1 use classifications. The criteria for waters with A-closed and A-1 use classifications pertains to drinking water use after simple disinfection (**Section 2.2**).

Even if a decision of impairment is made before all data is reviewed (e.g., from a single 30-day period), proceed to pass every existing group of data through the decision framework so all available data can be used to describe current conditions and variability.

The drinking water beneficial use support decision framework incorporates two scenarios:

SCENARIO 1 – preferred approach (30-day analysis)
The preferred assessment approach is taken for all consecutive 30-day periods for which there are at least five independent result values collected during separate 24-hour periods. For each 30-day period that achieves this minimum data requirement, the 30-day geometric mean is calculated and compared to the geometric mean criteria, and individual result values are compared to the statistical threshold value criteria. If either the geometric mean exceeds the criteria, or if ten percent or more of samples exceeds the statistical threshold value criteria, for any single consecutive 30-day period, the assessment unit is impaired by *E. coli* and is not fully supporting its drinking water beneficial use.

If the minimum data requirements for this approach are not met for any 30-day period, or if no 30-day period results in an impairment determination, proceed to the overwhelming evidence scenario.

SCENARIO 2 – overwhelming evidence
If there are at least five result values within an individual year and at least four of those values exceed the statistical threshold value criteria, there is overwhelming evidence to determine the assessment unit is impaired due to *E. coli* and is not fully supporting its drinking water beneficial use.

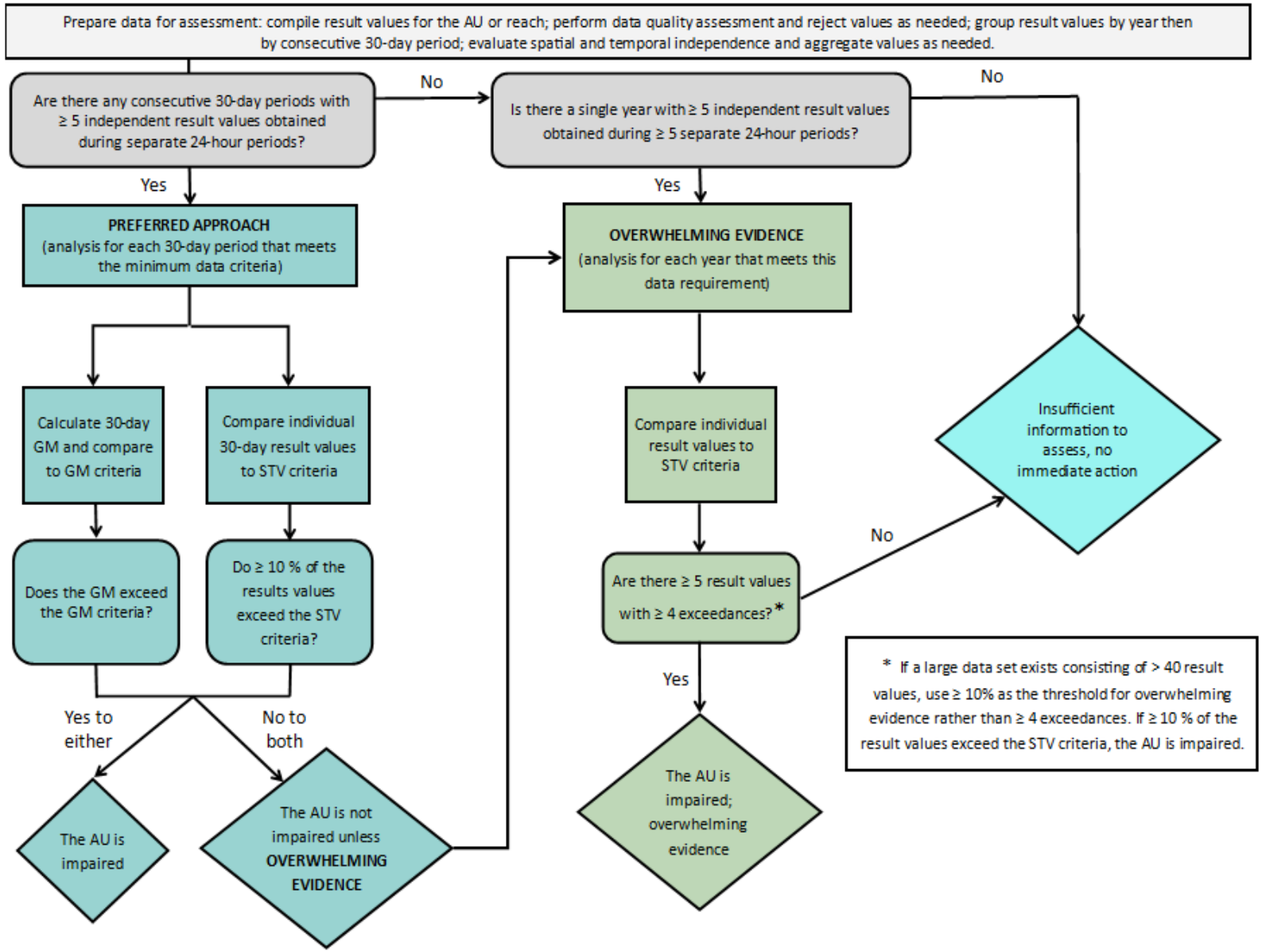

Figure 3 – *E. coli* Assessment Decision Framework for Drinking Water Beneficial Use Support

5.4 Document Assessment Decisions and Review with Management

The assessor must document all data and decisions made pertaining to *E. coli* impairment and beneficial use support determinations for assessment units. Assessment outcomes for individual assessment units, including data summaries, impairment determinations and beneficial use support determinations are documented via Montana DEQ's Clean Water Act Information Center (CWAIC) (available at www.cwaic.mt.gov). Waterbodies identified as impaired due to *E. coli* are included in Montana's biennial Water Quality Integrated Report and list of impaired waters; *E. coli* is a pollutant for which total maximum daily loads (TMDLs) are developed. Assessment decisions are reviewed by the Monitoring and Assessment Section Supervisor and may be reviewed by the QA Officer, managers or staff from other DEQ programs.

5.5 Decision Error

False rejection error (Type I)
Type I error is incorrectly rejecting a true null hypothesis (EPA, 2000b). In the context of *E. coli* assessment, Type I error would be mistakenly identifying an assessment unit as not impaired for *E. coli* when, in fact, the waterbody is impaired. A potential consequence of a false rejection error includes risk to human health if the public is not aware of an impairment that may lead to higher illness following exposure during primary contact recreation. To reduce this error, multiple scenarios (e.g., 30-day analysis, recreation season analysis, overwhelming evidence, and final risk screening) are built into each decision-making framework to identify potential impairments via multiple analyses and groupings of data.

False acceptance error (Type II)
Type II error is incorrectly retaining a false null hypothesis (EPA, 2000b). For example, if the null hypothesis states that the waterbody is impaired, Type II error would be identifying an assessment unit as impaired for *E. coli* when, in fact, the waterbody is not impaired. A potential consequence of false acceptance decision error is unnecessary resource expenditure to address a problem that does not exist. This method minimizes the likelihood of Type II error by setting minimum data requirements, by incorporating both geometric mean and allowable exceedance rate analyses, and by ensuring that scenarios only result in an impairment listing when there are numerous exceedances of criteria. While incorporating multiple approaches to grouping data (e.g., by 30-day period as well as by recreation season) may increase the likelihood of concluding an assessment unit is impaired when it is not, this decision-making framework errs toward being protective of human health.

6.0 Source Assessment and Supplemental Information

6.1 Probable Sources

Probable sources of impairment are the activities, facilities, or conditions that generate the pollutants that prevent waters from meeting water quality standards. The following sources are the most commonly associated with *E. coli* impairment listings in Montana; additional selections are available in the Water Quality Assessment and Reporting Documentation (WARD) system if needed:

- Livestock (Grazing or Feeding Operations)
- On-site Treatment Systems (Septic Systems and Similar Decentralized Systems)
- Municipal Point Source Discharges

- Combined Sewer Overflows
- Septage Disposal
- Impacts from Land Application of Wastes
- Wastes from Pets
- Accidental release/Spill
- Natural Sources

If water quality data is available that proves a probable source is contributing loads or increasing concentrations, the assessor should check the Source Confirmed box in WARD, whereas if probable sources are present in the watershed but are not confirmed, the assessor should check the Source Not Confirmed box. The assessor may also include a brief description of sources in the overall condition of the waterbody summary in WARD.

Since warm-blooded animals are a source of *E. coli* and wildlife are abundant, natural sources of *E. coli* are present in most environments. However, DEQ prioritizes *E. coli* monitoring and assessment activities in waters where there are probable sources related to human activities; this is because human sources of fecal contamination were the primary basis for the development of *E. coli* water quality standards and because human sources tend to be controlled though implementation of best management practices or other pollution control measures.

6.2 SUPPLEMENTAL INFORMATION

E. coli bacteria density data is the only data type required for assessment (**Section 3.6**), though additional data types may be useful to consider during risk assessment, monitoring design or source assessment:

Fecal coliform data
Fecal coliform data is not used for assessment using *E. coli* standards. If fecal coliform data exists and it is used to screen for risk of pathogen contamination, fecal coliform densities (cfu/100ml) may be converted to *E. coli* densities using the ratio of 200 fecal coliform to 126 *E. coli* (0.63).

Flow
Discharge data collected concurrently with E. coli samples can be used to calculate loads:
Load = Concentration x Flow x Unit conversion factor

Land Use Information
Land use information related to pathogens (e.g., septic density, domestic pet parks, recreational stock (e.g., hobby horses) access, grazing allotments, wildlife use, land application, animal feeding operations, stormwater outfalls and wastewater treatment plants) supports monitoring designs and source assessment. Information about public access and recreation sites such as public beaches, fishing access points, campgrounds, parks, etc., may also inform monitoring location selection.

Reconnaissance, Photos, and Visual Observations
Visual observations and photos can help to substantiate assessment decisions and source assessments, such as observations of septic leakage, dumping, or fecal excrement in or near a waterbody.

Natural background information
Information that helps distinguish between natural and human pathogen sources supports source assessment and load allocations (e.g., observations of heavy wildlife use, data collected upstream from human sources).

Data from connected waters
E. coli data from tributaries, ditches, point source discharges, wetlands, reservoirs, etc., that are hydrologically connected to the assessment unit may be useful when evaluating location and magnitude of sources and seasonal variability.

Precipitation data
Information about timing of precipitation relative to sampling events may help explain peaks in *E. coli* concentrations, evaluate overland runoff sources, or make best professional judgements regarding inclusion of wet-weather samples during assessment.

Bacterial source tracking (BST) or microbial source tracking (MST)
Bacterial source tracking (BST), also known as microbial source tracking (MST), is a new set of methods used to identify the source of fecal bacteria in environmental samples, sometimes simply by identifying whether the source is human versus animal and sometimes identifying the source down to the species (e.g., cow, dog, deer) (EPA 2002b; Source Molecular Corporation, 2018). MST analytical methods commonly include molecular analysis of genetic material (e.g., DNA or RNA) to determine which human or animal source contributed the bacteria or viruses observed in the water sample (Tetra Tech, Inc. and Herrera Environmental Consultants, 2011).

Identifying sources of fecal pollution is a key component in effectively implementing a pollution management program and is needed to target best management practices and develop bacterial total maximum daily loads (TMDLs); this information may also be useful to properly assess risk in contact recreation. Bacterial source tracking can identify primary sources of *E. coli*, illustrate the relative abundance of *E. coli* from identified sources, determine the presence or absence of major watershed sources, inform watershed management decisions and allow resources to be focused where pollutant reductions are needed most (TWRI, 2017).

Because MST studies can be expensive and resource-intensive it is important to carefully weigh the benefits, needs and goals against the expected expense. MST methods should be used to supplement rather than replace current methods and tools for evaluating and identifying fecal bacteria sources - tools such as traditional monitoring of fecal bacteria indicators, sanitary surveys, watershed tours, and local knowledge (Tetra Tech, Inc. and Herrera Environmental Consultants, 2011).

7.0 PUBLIC INFORMATION

E. coli data collected by DEQ is stored in DEQ's MT-eWQX Enterprise (EQuIS) database and is uploaded weekly to the Water Quality Portal (EPA, USGS and NWQMC, 2018). Assessment outcomes for individual assessment units, including data summaries, impairment determinations, and beneficial use support determinations, are documented via Montana DEQ's Clean Water Act Information Center (CWAIC) (available at www.cwaic.mt.gov).

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