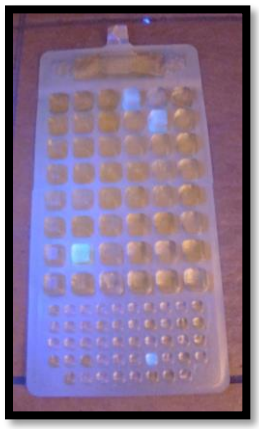
Montana Department of Environmental Quality

Standard Operating Procedure

Sample Collection, Handling, and Analysis of *Escherichia coli*



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The Montana Department of Environmental Quality (DEQ) Water Quality Planning Bureau (WQPB) Standard Operating Procedures (SOPs) are adapted from published methods or developed by in-house technical and administrative experts. Their primary purpose is for WQPB internal use, although they may also have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method. This document does not contain regulatory or statutory requirements unless specified.

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Although the WQPB follows this SOP in most cases, there may be situations where an alternative methodology, procedure, or process is used to meet specific project objectives. In such cases, the project manager is responsible for documenting deviations from these procedures in Quality Assurance Project Plans (QAPPs), Sampling and Analysis Plans (SAPs), and end of project summary reports.

Document Revision History

Revision Date	Version number	Summary of change(s)	Revised sections(s)	Revised by
December 2006	1.0	Initial version	All	David Feldman
August 2019	2.0	Wrote all sections to align with 2019 SOP template. Added sections on cautions, interferences, data and records management, and quality control sections. Expanded procedural steps for clarity.	All	Katie Makarowski

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ACRONYMS

ARM	Administrative Rules of Montana
CFR	Code of Federal Regulations
DEQ	Department of Environmental Quality
EPA	Environmental Protection Agency
FIB	Fecal Indicator Bacteria
GI	Gastrointestinal
GPS	Global positioning system
HAZWOPER	Hazardous Waste Operations and Emergency Response
LED	Light-Emitting Diode
MPN	Most probable number
QA	Quality Assurance
QC	Quality Control
SAP	Sampling and analysis plan
SDS	Safety Data Sheets
SOP	Standard Operating Procedure
SVC	Site Visit Code
SVF	Site Visit Form
UV	Ultraviolet

1.0 PURPOSE

This document presents the Montana Department of Environmental Quality (DEQ) Water Quality Planning Bureau (WQPB) Standard Operating Procedure (SOP) for enumeration of *Escherichia coli (E. coli)* in water samples. *E. coli* is a subgroup of total coliform bacteria which comes primarily from the feces of warm blooded animals. *E. coli* are a large, diverse group of bacteria (CDCP, 2015). Many strains of *E. coli* are not pathogenic and will not cause illness but, because 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 a fecal indicator bacteria (FIB) or pathogen indicator, a term defined by the Clean Water Act as "a substance that indicates the potential for human infectious diseases" (CWA §502(23); EPA, 2012).

2.0 APPLICABILITY

This SOP focuses on analysis of surface water samples collected from Montana's lakes, reservoirs, rivers, and streams, including streams and rivers of all Strahler order (Strahler, 1952), Montana's large rivers (Flynn and Suplee, 2010), lakes and reservoirs.

E. coli data are used to determine attainment of water quality standards. Swimming and other recreational activities in water contaminated with pathogens can make people ill (EPA, 2016). In Montana, *E. coli* is a pollutant evaluated by DEQ when assessing suitability of surface waters for recreational beneficial use and, in some cases, drinking water beneficial use. Refer to DEQ's *Escherichia coli* (*E. coli*) Assessment Method for State Surface Waters (Makarowski, 2019) for more information about monitoring design, data analysis, and assessment decisions. For additional information or guidance regarding this SOP, contact DEQ's Water Quality Monitoring and Assessment Program.

3.0 METHOD SUMMARY

This SOP details sample collection, handling and analysis procedures for enumeration of *E. coli* in surface waters. Three analytical methods are acceptable for ambient water: A9223B (also known as the Colilert method), EPA Method 1604, and EPA Method 1603 (**Section 6.8**). Sample collection and handling procedures are the same regardless of which of the three analytical methods is selected (**Section 10.4**). Samples of a specified volume (typically 100ml) are collected in sterile containers in the field using grab sample techniques. Sodium thiosulfate ($Na_2S_2O_3$) preservative is added to neutralize chlorine if residual chlorine is likely in the water sample. Samples are stored at < 10°C, have a 6-hour holding time, and must be processed in the laboratory within 8 hours of collection (EPA, 2007).

Short holding time requirements (i.e., 6 hours) often limit the feasibility of transporting *E. coli* samples to a professional laboratory in a timely manner. Therefore, sample processing and analysis is often performed by field personnel using the Colilert method in a portable laboratory with the Colilert method (**Section 10.5**). One packet of Colilert (24-hour) reagent is poured into each sample bottle and agitated gently to dissolve. Samples are transferred to 97-well Quanti-Tray/2000s and sealed using a Quanti-Tray sealer. The samples are incubated at 35 ± 0.5°C for 24 to 28 hours. A color change in tray wells from clear to yellow observed under ambient lighting indicates the presence of total coliform bacteria. When

the yellow cells are viewed under ultraviolet (UV) light, blue fluorescence indicates the presence of *E. coli*. Counts of wells that are yellow and yellow with blue fluorescence are used in conjunction with the IDEXX most probable number (MPN) table to enumerate total coliform and *E. coli, respectively*.

E. coli results are reported as colony forming units (cfu) per 100 ml (direct count methods) or most probable number per 100ml (probability-based methods).

4.0 DEFINITIONS

Colilert: A bacterial testing procedure which simultaneously detects or quantifies both total coliforms and *Escherichia coli* with results in 24 hours (IDEXX Laboratories, Inc., 2019); approved by EPA in 2007 in Standard Methods for Examination of Water and Wastewater (EPA, 2007); officially referred to as the Enzyme Substrate Coliform Test (9223B) (NWQMC, 2017).

Escherichia coli (E. coli): A species of bacteria that inhabit the intestinal tract of warm-blooded animals and remain viable (alive and capable of infecting another organism) in water for a variable period of time. While *E. coli* are normally harmless and live in the intestines of healthy people and animals, a few strains may cause illness. The presence of *E. coli* bacteria in water indicates fecal contamination by a warm-blooded animal; harmful bacteria, viruses, or protozoa associated with fecal contamination may also be present (Washington DOE, 2014).

Fecal coliforms: Fecal coliforms are traditionally defined as coliforms that ferment lactose at 44.5 °C in a medium with bile salts (Cabral, 2010); generally, originate in the intestines of warm-blooded animals.

Fecal indicator bacteria (FIB): A group of organisms that indicate the possible presence of pathogenic (disease-causing) bacteria. Although FIB are not generally harmful themselves, they indicate the possible presence of pathogenic (disease-causing) bacteria, viruses, and protozoa that also live in the human and animal digestive systems. Therefore, their presence in streams suggest that pathogenic microorganism might also be present (Washington DOE, 2014).

Most probable number (MPN): A statistical representation of the number of organisms in a sample (40 CFR 136.3(b)); water quality criteria for *Escherichia coli* are expressed in colony forming units per 100 milliliters of water or as most probable number (ARM 17.30.621-629).

Pathogen indicator: A substance that indicates the potential for human infectious disease (33 USCS § 1362(23)).

Total coliform bacteria: Gram-negative, oxidase-negative, non-spore forming rods, that ferment lactose with gas production at 35 to 37 °C, after 48 hours, in a medium with bile salts and detergents (Cabral, 2010); contain the subgroup fecal coliforms which includes *E. coli* bacteria.

5.0 HEALTH AND SAFETY WARNINGS

Field personnel should be aware of job hazards associated with collecting field samples that could result in personal injury or loss of life. Consult the Water Quality Planning Bureau Job Hazard Analysis form.

Personnel must be familiar with any health hazards associated with fixing or preserving agents to be used. Safety Data Sheets (SDS) for all chemicals used in field operations must be available to sampling team members. Personal protective equipment should be worn or used as needed, including gloves, eye protection, respirators, and personal floatation devices. If sampling will be performed in an exclusion or contaminant reduction zone of a hazardous waste site, sampling personnel are required to have Hazardous Waste Operations and Emergency Response (HAZWOPER) training. Always use caution when driving or wading during field activities.

Adhere to the following health and safety warnings associated with sample collection and processing:

- Samples may contain pathogenic microorganisms and personnel who collect and process the samples should protect themselves from waterborne illnesses by wearing clean disposable gloves, washing hands frequently, and consider using hand sanitizer between collections in the field if hand-washing is not possible.
- When opening the Colilert reagent snap pack, open the pack so that the pack is facing away from you. Note: The Colilert reagent is not hazardous according to the manufacturer's Material Safety Data Sheet, but inhaled powder may cause lung irritation.
- Use caution when using the Quanti-Tray Sealer as it might be hot.
- Wear safety glasses and do not look directly into the UV light when reading sample results.

6.0 CAUTIONS

This section indicates activities that could result in equipment damage or loss, degradation of sample, or possible invalidation of results.

6.1 PREVENTING CONTAMINATION

Samples that are damaged or are known or suspected to be contaminated by fecal sources should not be analyzed and should be discarded. When collecting and handling samples, the following actions will help prevent contamination:

- Wash hands before and after sample collection and handling if possible. Use sanitizing wipes or hand sanitizer as an alternative if facilities are not available.
- Wear gloves (latex or nitrile, powder-free) when collecting and handling *E. coli* samples
- Collect samples from downstream to upstream to minimize disturbance
- Do not touch the inside, lip, or cap of the sampling container or Quanti-Tray
- Secure the lid of the sample bottle immediately following sample collection and open the lid only briefly to add Colilert reagent to the bottle just prior to filling the Quanti-Tray
- Store samples upright in the cooler
- Replenish ice and drain the sample cooler regularly so sample bottles remain at the desired temperature and are not submerged in standing water
- Once a sealed package of Quanti-Trays has been opened, store unused Quanti-Trays by folding over the opened end of the bag and seal with tape or store unused trays in a clean container (e.g., zipper bag) to limit exposure to the open environment.
- Clean and disinfect surfaces at the location where sample preparation and incubation will take place (if using a portable laboratory)

6.2 EQUIPMENT USE AND MAINTENANCE

Field personnel are responsible for proper use, maintenance, and storage of all equipment associated with procedures described in this SOP. Common equipment used during *E. coli* monitoring includes a Quanti-Tray sealer, incubator, and a portable UV light. Field personnel should adhere to user manuals, clean and calibrate as needed, inspect prior to use, and store equipment in a secure location when not in use. Field personnel should report any problems encountered with equipment or supplies to WQPB staff responsible for inventorying and maintaining equipment and supplies.

6.3 SAMPLE CONTAINERS

The volume of ambient water typically needed for *E. coli* analysis is 100 ml. *E. coli* samples must be collected in sterile containers made of either glass or any plastic that is made of a sterlizable material (polypropylene or other autoclavable plastic) (EPA, 2007; 40 CFR Parts 136 and 503). Generally, DEQ's contracted laboratories provide new, sealed 100 ml high density polyethylene (HDPE) bottles that are pre-preserved with sodium thiosulfate ($Na_2S_2O_3$) (**Section 6.5**).

6.4 SAMPLE COLLECTION

When using the Colilert method, sample bottles must contain 100 ml of sample or the Quanti-Tray may not fill completely, resulting in invalid results.

6.5 SAMPLE PRESERVATION

Sample containers are often received from the laboratory pre-preserved with sodium thiosulfate ($Na_2S_2O_3$), a reducing agent which neutralizes residual chlorine in a water sample. A reducing agent is generally added to samples only if an oxidant (e.g., chlorine) is present (EPA, 2007). Therefore, sodium thiosulfate is mandatory for *E. coli* samples that are likely to contain chlorine, such as samples of tap water or wastewater effluents. For ambient water where chlorine is not usually found in significant amounts, sodium thiosulfate preservative is not necessary. However, it is acceptable to use sample containers pre-preserved with sodium thiosulfate even if the water being sampled is not expected to be chlorinated as the behavior of coliform counts of samples after 6-hours of refrigeration appears to be unaffected by the presence of thiosulphate (PHLSWS, 1953).

Samples must be held on regular (wet) ice at < 10°C until samples are analyzed (EPA, 2007). If this temperature is exceeded, the samples may be analyzed but results should be flagged. Do not allow samples to freeze as freezing can damage bacteria cells (Utah DEQ, 2013).

6.6 HOLDING TIME

The holding time specifies the maximum time that samples may be held before the start of analysis and still be considered valid (EPA, 2007). For *E. coli* samples, sample processing in preparation for incubation should begin as soon as possible after sample collection. The maximum transport time allowed to the laboratory is 6 hours, and samples should be processed within 2 hours of receipt at the laboratory (EPA, 2007; Pope, *et al*., 2003; Myers and Sylvester, 1997; NWQMC, 2017; APHA, *et al.,* 1998).

To ensure that samples can be processed within this 6-hour holding time, field personnel must carefully note collection and travel times either to the portable laboratory or to the professional laboratory. If

giving samples to a professional lab for analysis, field personnel must provide the lab personnel with advance notice of when to expect samples so the lab can be prepared to meet time-sensitive analytical requirements.

For a grab sample, the holding time begins at the time of collection. If samples are analyzed out of holding time, data is qualified with an "H" flag in the database. If samples exceed the holding time prior to analysis, laboratory staff may contact project managers to confirm whether the lab should proceed with analysis.

6.7 SAMPLE DELIVERY AND SHIPPING

Incubation and enumeration of samples can be performed by field personnel in a portable laboratory or by a professional laboratory. Due to the short 6-hour holding time for *E. coli* samples, unless the sampling occurs near an approved professional laboratory, field personnel that collect the samples often also perform sample processing and analysis at a portable laboratory location in the field or at a nearby hotel. This short holding time also makes shipping samples impractical, so samples analyzed by a professional laboratory must be hand-delivered:

- Indicate "hand" as delivery method on the chain-of-custody form
- Confirm with lab staff receiving samples that proper temperatures were achieved
- Confirm all samples are relinquished and received

6.8 ANALYTICAL METHODS

E. coli samples must be "analyzed by the most probable number or equivalent membrane filter methods" (ARM 17.30.620(2)). Three analytical methods are acceptable for enumeration of *E. coli* in ambient surface waters: Colilert Method (9223B), EPA Method 1604, and EPA Method 1603. All are acceptable for use when making water quality standards attainment decisions. DEQ's Water Quality Planning Bureau Monitoring Suite table (DEQ, 2019) indicates the Colilert method as the preferred method; this method is commonly completed by field personnel using a portable laboratory and the procedures are detailed throughout this SOP. The other two alternate methods are more typically completed by professional laboratories.

1. Colilert Method (9223B) (preferred method)

The Colilert method (IDEXX Laboratories, Inc., 2017) is officially referred to as the Enzyme Substrate Coliform Test (9223B) (NWQMC, 2017). In 2007, EPA approved the Colilert bacterial testing procedures (EPA 2007). For this method, a specified volume of sample (typically 100ml) is mixed with commercially prepared enzyme substrate. Samples are transferred to 97-well Quanti-Tray/2000s and sealed using a Quanti-Tray sealer and incubated at 35 +/- 0.5 ^{o}C for 24 to 28 hours. An enzyme produced by *E. coli*, β-glucuronidase, is detected by hydrolysis of the fluorescent substrate MUG (4-methylumbelliferyl-beta-D-glucuronide). Quanti-Tray wells that are yellow when viewed under ambient light indicate presence of total coliform bacteria. Yellow wells that exhibit blue fluorescence when viewed under long-wavelength (366-nm) ultraviolet light indicate presence of *E. coli*. Counts of yellow wells, and counts of yellow wells that show blue fluorescence, are used in conjunction with the IDEXX most probable number (MPN) table to determine the number of total coliform bacteria and *E. coli* bacteria, respectively. Results are reported as MPN/100 ml, a surrogate for colony forming units.

2. EPA Method 1604 (alternate method)

EPA Method 1604 is "Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)" (EPA, 2002). A volume of sample is filtered through a 0.45 µm cellulose ester membrane filter that retains the bacteria present in the sample. This filter is placed on MI medium (a plate of MI agar or absorbent pad saturated with MI broth) and incubated at 35°C for up to 24 hours. The medium includes two enzyme substrates, the fluorogen 4-Methylumbelliferyl-β-D-galactopyranoside (MUGal) and a chromagen Indoxyl- β-D-glucuronide (IBDG). The bacterial colonies that grow on the plate are inspected for 1) the presence of blue color from the breakdown of IBDG by the *E. coli* enzyme β-glucuronidase, and 2) fluorescence under longwave ultraviolet light (366nm) from the breakdown of MUGal by the total coliform enzyme β-galactosidease (EPA, 2002).

3. EPA Method 1603 (alternate method)

In 2007, EPA approved the EPA Method 1603 bacterial testing procedures (EPA, 2007). EPA Method 1603 is "*Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC)" (EPA, 2014). A sample is filtered through the membrane which retains the bacteria, and the membrane is placed on a selective and differential medium (modified mTEC agar), incubated at 35°C ± 0.5°C for 2 ± 0.5 hours to resuscitate injured or stressed bacteria, and then incubated at 44.5°C ± 0.2°C for 22 ± 2 hours. The modified medium contains a chromogen (5-bromo-6-chloro-3-indolyl-β-D-glucuronide), which is catabolized to glucuronic acid and a red- or magenta-colored compound by *E. coli* that produces the enzyme β-D-glucuronidase. Method 1603 provides a direct count of *E. coli* based on the development of red or magenta colonies that grow on the membrane filter and agar (EPA, 2014).

6.9 COLILERT REAGENT

Verify expiration dates of all materials. The shelf life of the Colilert reagent is up to 12 months from the date of manufacture for the snap pack formats (100 mL). Colilert reagent should be stored at 2 to 30°C and away from light. Colilert reagent powder should be white to off-white in color, and it should be dry and free-flowing. The shelf life of Quanti-Tray/2000 is up to 3 years from date of manufacture. Discard and replace expired supplies and contact IDEXX Technical Services with concerns or questions.

Once Colilert reagent is added to the sample, samples are agitated to dissolve the reagent prior to pouring the solution into the Quanti-Tray. If samples are shaken too vigorously, excessive foam may occur and result in trays that are difficult to read or interpret. Avoid excessive foaming of the sample and allow foam to settle before pouring it into the tray.

6.10 INCUBATION TIME

When using the Colilert method, samples must be incubated for 24 to 28 hours. Samples must remain in the incubator for the full term as test sensitivity may be affected by taking the samples out of the incubator too soon or after too long, resulting in false negatives or false positives. The Colilert reagent system was designed to suppress non-coliform positive bacteria for at least 28 hours (IDEXX Laboratories, Inc., 2017).

If incubation extends past 28 hours, it is possible that such bacteria will overcome the Colilert suppression system and react with the nutrient indicators in Colilert, producing invalid results. Colilert samples can be read after 28 hours of incubation and produce valid results only if they are negative.

That is, if an inoculated Colilert sample incubated over 28 hours lacks yellow color, it is a valid negative test, whereas a yellow color after 28 hours is not valid (IDEXX Laboratories, Inc., 2017).
Alternately, Colilert samples can be read before 24 hours of incubation and produce valid results only if they are positive. That is, an inoculated Colilert sample that is positive for coliforms and *E. coli* before 24 hours is a valid positive test for both coliforms and *E. coli*. If the sample is positive before 24 hours for coliforms only, the sample is a valid positive test for coliforms. However, the sample should be incubated for a longer period (up to 24 hours, but not greater than 28 hours total time) to determine whether *E. coli* are present (IDEXX Laboratories, Inc., 2017).

7.0 INTERFERENCES

This section describes components of the procedure or environmental factors that may interfere with the accuracy of the final product.

7.1 MONITORING SITE LOCATIONS

Sampling site locations are selected based on a project's monitoring objectives and latitude/longitude coordinates are proposed in the project's sampling and analysis plan (SAP). Proposed sites are often subject to change pending landowner access permission, safety or other site-specific considerations. Refer to DEQ's *Escherichia coli* (*E. coli*) Assessment Method for State Surface Waters (Makarowski, 2019) for guidance on spatial and temporal independence of *E. coli* monitoring sites if data is to be used for assessment.

7.2 GENERAL GUIDANCE FOR SELECTING SAMPLE COLLECTION LOCATIONS WITHIN SITES

Upon arrival at a site, field personnel must determine the specific location where to collect *E. coli* samples. The following characteristics of preferred sampling locations help guide this determination:

- Near the proposed latitude/longitude supplied in the project SAP
- Field personnel can safely wade into, stand, and collect samples
- Upstream from recent disturbances to the substrate or water column
- In the main channel (i.e., the channel with most of the flow) if channel is braided or split
- In a well-mixed area of the waterbody (i.e., avoid side channels, backwater areas, eddies, stagnant shorelines)
- In areas where bathers tend to congregate; swimmer density can influence the quality of beach water (Wymer *et al*. 2005; Smith and Dufour 1993)
- Avoid surface scum; water should be sufficiently deep so water sample bottles can be fully submerged below the water surface and so the mouth of the bottle is elevated away from the bottom substrate
- Avoid sampling near areas of recent disturbances of the substrate or water column

7.3 TIME OF YEAR

E. coli sampling can occur year-round, though seasonal differences in water quality standards may apply (e.g., standards that apply from April 1 to October 31 differ from those that apply from November 1 to March 31 for B and C stream classifications).

7.4 WET WEATHER

Rainfall is a factor that significantly influences the measurement of indicator microbes (Olivieri, *et al*., 1977; Wymer, *et al.,* 2005). *E. coli* data used to develop criteria based on accepted illness rates were collected during steady state dry weather conditions (EPA, 1986). Therefore, it is preferable to collect *E. coli* samples during steady state dry weather conditions. Avoid sampling during or shortly after storm events or floods, unless these periods are being targeted per the project SAP.

7.5 TEMPORAL INDEPENDENCE

Montana's general *E. coli* water quality standard states, "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)). Therefore, samples collected at an individual sampling location within a 30-day period should generally be collected at least 24-hours apart.

7.6 TIME OF DAY AND SUNLIGHT

Samples may be collected during any time of day. However, incoming solar radiation is arguably the most potent physicochemical factor in the inactivation or killing of *E. coli* in water and cloud cover has been shown to exert a strong effect on *E. coli* concentrations (Whitman, *et al*., 2004). *E. coli* concentrations are affected by water temperature and UV radiation. Levels at beaches tend to be higher in the morning than afternoon (Wymer, *et al*., 2005) and higher in the shaded areas than in areas exposed to full sun (Whitman, *et al*., 2004). If possible, sample in the morning in shaded locations, and be consistent regarding the sampling time and sun exposure during sample collection at a site.

7.7 DEPTH

Depth has been shown to have a significant impact on microbial indicator densities; bacterial densities become progressively lower as one moves from ankle-deep to knee-deep to chest-deep water (Wymer, *et al.,* 2005). The usual route of exposure for gastrointestinal effects is through ingestion so consideration should be given to the likely depths where bathers are found, especially at public swimming beaches. Targeted depths may vary depending on the bather population that may be impacted; for example, small children may be more likely exposed to contamination in shallow water, whereas adults and older children are more likely exposed in deeper waters (Wymer, *et al.,* 2005).

Project QAPPs or SAPs may indicate a preferred sampling depth or depth-integrated sampling approach depending on project objectives. Unless specified otherwise in the project SAP, samples for *E. coli* analysis should be collected using a hand-dipped grab sample collection technique following this guidance:

- In the area used for swimming, wade into an area at 0.7- to 1-m water depth.
- Maintain consistency in water depth throughout the sampling period.

- Collect samples at a depth 15 to 30 cm below the water surface (Myers, *et al*., 2014).
- Fully submerge the bottle so the mouth is below the water surface to avoid surface scum.
- Elevate the bottle above the stream or lake bed surface enough that bottom sediments are not disturbed or drawn into the sample bottle.

7.8 WIND

Wind direction may affect the quality of beach waters either by driving contamination toward the beach or away from the beach (Wymer, *et al.,* 2005). Wave action along shorelines may agitate substrates and subsequently suspend particles which may contain associated bacteria.

7.9 REPLICATE SAMPLES

Project-specific objectives and sampling designs may require replicate samples to more accurately represent variability at a monitoring location (e.g., from multiple depths, widths, or longitudinal distances along the shoreline) and replicate samples may be specified in a project's SAP.

If replicates are not specified in a SAP, a single sample will be collected per sample collection event per sampling location. Replicate sampled are generally analyzed separately, and project managers should plan accordingly to ensure adequate space in the incubator. Alternately, project SAPs should specify whether replicate samples are to be composited and subsampled prior to incubation and enumeration. Depending on project objectives, result values from replicate samples may be individually incorporated into a dataset or, for example, a geometric mean of replicates may be calculated and that mean incorporated into the dataset.

8.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

8.1 PERSONNEL QUALIFICATIONS AND TRAINING

Field personnel must be familiar with proper sampling and analysis techniques, sample handling, safety procedures, and record keeping. All field personnel will be provided written SOPs and in-person training prior to applying these procedures and must be accompanied in the field by experienced staff until competence is demonstrated. Field audits may be conducted by project managers or the quality assurance officer to verify protocols are followed correctly.

8.2 RESPONSIBILITIES AND FIELD PREPARATIONS

Project managers are responsible for:

- Developing SAPs and ensuring they are approved prior to the first sampling event
- Distributing SAPs and SOPs to field personnel
- Ensuring site access permission is granted
- Ensuring field personnel have received training and demonstrated proficiency in all sampling and analysis protocols described in this SOP

- Providing detailed instructions for individual sampling trips and reviewing them with field personnel, including sufficiently detailed site access instructions, landowner contact information, sites to be visited, allowable deviations from sampling plans, etc.
- Communicating to ensure samples are analyzed within holding times
- Updating site lists, landowner contact records, and/or sampling plans based on feedback received from field personnel prior to subsequent sampling events

Field personnel are responsible for:

- Their safety
- The quality of the work performed
- Familiarity with study objectives and the purpose for each type of data being collected
- Understanding the system that each sample is striving to represent
- Packing necessary equipment and supplies
- Following sampling and decontamination protocols
- Completing all required documentation and field forms
- Following site access instructions from landowners and not trespassing
- Equipment and supplies, including pre-field checks, use and maintenance
- Sample handling and storage
- Communicating to project manager (or designated appointee) regarding sample delivery
- Communicating with project manager and supervisor regarding evening safety check-ins and completion of sampling event
- Reporting vehicle maintenance issues
- Collecting the required number of quality control samples (blanks and duplicates)
- Safety requirements and recommendations

9.0 EQUIPMENT AND SUPPLIES

- Site Visit Form(s)
- Site Visit Code(s)
- Bacteria Sample Collection & Analysis Form(s)
- GPS unit
- Cooler
- Ice
- Paper towels
- Hand Sanitizer
- Waders/boots
- Gloves
- Pencils
- Permanent fine point markers
- Sample labels (if delivering to external lab)
- Clear tape

- Sterile 120mL sample bottle(s) pre-preserved with sodium thiosulfate ($Na_2S_2O_3$) (one bottle per sample)
- Colilert™ packets (one packet per sample)
- Quanti-Tray/2000 (97-well) sample trays (one tray per sample)
- Quanti-Tray® Sealer with power cord
- Quanti-Tray® /2000 rubber insert for the sealer
- Scissors
- Portable incubator (Millipore™ Dual Chamber Portable Incubator) (Millipore Corporation 2000)
- Incubator power supplies (**Appendix B**; for example, AC power supply cord, vehicle DC power supply cord, rechargeable batteries and charger(s), external battery cable and external battery)
- Safety glasses to protect eyes when using UV light
- Portable 6-watt, 365-nm UV light with spare batteries
- Heavy blanket (for blocking light when using UV light to check fluorescence)

10.0 PROCEDURAL STEPS

This section describes in order the procedural steps taken to collect, handle and analyze *E. coli* samples.

10.1 ORDER OF OPERATIONS OVERVIEW

This section provides a quick-glance overview of order of operations when collecting and analyzing *E. coli* samples; detailed procedural steps follow:

1. Conduct pre-field checks.
2. Collect and label the sample, and record the sampling event on the Site Visit Form and the Bacteria Sample Collection & Analysis Form (**Appendix A**).
3. Store and transport the samples to either the professional laboratory or the portable laboratory location within the 6-hour holding time.
4. If using the Colilert method, turn on the incubator and set it to 35°C.
5. Add Colilert reagent to each sample and shake/swirl gently to dissolve.
6. Pour each sample into a Quanti-Tray and label both the top and bottom of each tray with the Site Visit Code.
7. Once the Quanti-Tray sealer ready light is illuminated, place the Quanti-Tray into rubber insert and pass it through sealer.
8. Once the incubator ready light is illuminated, confirm the temperature is 35°C ± 0.5°C with the internal thermometer.
9. Place the Quanti-Trays into the incubator (cut in half first to fit if needed), latch the doors, and record the start incubation time on the Bacteria Sample Collection & Analysis Form.
10. After 24 to 28 hours, remove the trays from the incubator and match the top and bottom trays; record the stop incubation time on the Bacteria Sample Collection & Analysis Form.
11. Mark any cells that have been turned yellow with a fine-line permanent marker; count and record the number of large and small cells that are yellow on the Bacteria Sample Collection & Analysis Form (use a Colilert Comparator if needed).
12. Turn off the lights and view the trays using the UV light; mark any of the yellow cells that are also fluorescing; count and record the number of large and small cells that are both yellow and

fluorescing on the Bacteria Sample Collection & Analysis Form (use a Colilert Comparator if needed).

13. Use the most probable number (MPN) table to determine the MPN count of total coliform (yellow) and *E. coli* (yellow *and* fluorescent) and record the MPN/100mL on the Bacteria Sample Collection & Analysis Form.
14. Discard used supplies.

10.2 PRE-FIELD CHECKS

Complete the following pre-field checks well in advance of departure to allow sufficient time in case repair or replacement is necessary:

- Inspect all equipment and ensure it is in working order. For example, the incubator turns on and reaches the required 35°C temperature, the sealer turns on and the green ready indicator light turns on, the blacklight bulb is intact and battery charge is sufficient.
- Charge and test the back-up (rechargeable) batteries for the incubator
- Inspect and test the vehicle cigarette lighter power cord and/or other power or battery supplies (**Section 9**, **Appendix B**).
- Confirm that all supplies are not expired and were stored properly (e.g., Colilert reagent, Quanti-Trays)

10.3 INITIATE FIELD FORMS

1. Place a unique Site Visit Code on a Site Visit Form (**Appendix A**) and fill out all required header information; check the Bacteria Sample Collection box.
2. Place a Site Visit Code on a Bacteria Sample Collection & Analysis Form and fill out all required information regarding sample collection.

10.4 SAMPLE COLLECTION, HANDLING AND STORAGE

Prior to collecting samples, refer to the Cautions (**Section 6**) and Interferences (**Section 7**) sections of this document, and refer to the project SAP for sampling locations and additional guidance.

Collect an unfiltered grab sample in a 120 mL sterile sample container:

1. Label the sample bottle with the Site Visit Code. If samples are to be delivered to an external laboratory, adhere a label with Site Visit Code, waterbody name, personnel name, and date; cover the sample label with clear tape.
2. Put on gloves.
3. Carry the sample bottle to a suitable sampling location (**Section 7**).
4. Hold the bottle from the bottom and remove the lid.
5. Face upstream into the direction of the flow (if applicable).
6. Use a hand-dipped grab sample collection technique to collect the sample: use a swift sweeping motion to submerge the sample bottle to the appropriate depth, generally 15-30 cm (**Section 7)** until it fills to the 100 ml line, and draw the bottle up out of the water column. While filling the bottle, ensure the mouth of the bottle is below the water surface to prevent surface scum floating on the water surface from entering the bottle, elevated above the bottom to prevent

substrate from entering the sample bottle, and take care not to dump out the sodium thiosulfate ($Na_2S_2O_3$) preservative if the bottle is pre-preserved.

NOTE: Typically, exactly 100 ml of water is needed. If there is less than 100 ml, use the lid of the bottle to scoop and add small amounts of ambient water to the bottle to reach the 100 ml mark. If there is more than 100 ml, carefully pour out the excess to achieve the correct volume.

7. Gently invert the sample bottle several times to dissolve the sodium thiosulfate preservative.

NOTE: Minimize contamination risk by keeping sample bottles closed whenever possible and avoid touching the inside of the bottle lid or lip.

8. Securely tighten the lid.
9. Store samples upright in a cooler on ice at < 10°C and transport them to the professional or portable laboratory.

NOTE: *E. coli* samples have a very short holding time (**Section 6**) and sample analysis should begin as soon as possible following sample collection; the maximum allowable transport time to the laboratory is 6 hours, and samples should be processed within 2 hours of receipt at the laboratory (EPA, 2007).

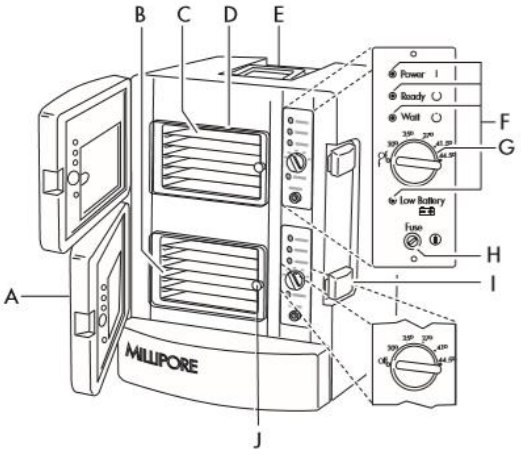
10.5 SAMPLE ANALYSIS USING THE IDEXX COLILERT METHOD

The Colilert method is approved by EPA (EPA, 2007) and is officially referred to as the Enzyme Substrate Coliform Test (9223B) (NWQMC, 2017) (**Section 6.8**).

Prepare the equipment and samples:

1. Place the dual-chamber incubator (**Figure 1**) on a level surface. Position the thermometer with clips onto one of the inner chamber shelves so it is visible and can be read through the heated interior doors. Close and latch both inner and outer chamber doors. Plug in the incubator. Both chambers of the dual chamber incubator have their own temperature control; turn both temperature knobs to 35°C. The green Power and yellow Wait LEDs light. When the inside chamber reaches the set temperature (may take 30 minutes or more), the Wait LED goes out and the green Ready LED lights. Verify the temperature in the chamber by looking through the inner chamber doors at the thermometer.

NOTE: Instructions for connecting the incubator to an external battery is contained in **Appendix B**.



Letter	Part
A	Exterior door
B	Bottom chamber with shelves
C	Top chamber with shelves
D	Heated interior door
E	Carrying handle
F	Indicator LEDs
G	Temperature selectors
H	Fuse
I	Exterior door latch
J	Interior door knob

Figure 1 – Millipore portable dual-chamber incubator (Millipore Corporation, 2014)

NOTE: Nine Quanti-Trays can fit in the incubator at one time.

2. Place the Quanti-Tray sealer on a level surface with adequate space for Quanti-Trays to be inserted and ejected and plug it in. Attach the input shelf to the sealer by inserting the shelf tabs into the two slots on the front of the sealer. Turn on the Quanti-Tray sealer. The amber Power light should illuminate. Allow the sealer to warm up and the green Ready Light to come on (up to 10 minutes), indicating that the unit has reached operating temperature.

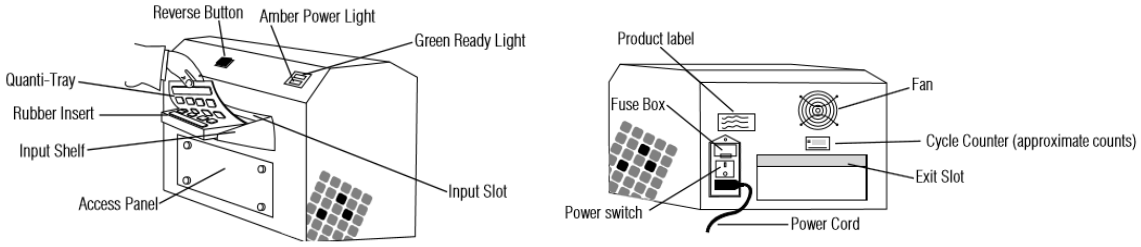


Figure 2 – IDEXX Quanti-Tray Sealer (IDEXX Laboratories, Inc. 2019)

3. Wash hands and put on gloves.
4. Remove samples from the cooler. Tap the Colilert snap packet to concentrate the reagent medium at the bottom. Aim the Colilert packet away from the face and snap open the pack. Add the contents of one packet of Colilert reagent to each 100 ml sample (**Figure 3**). Gently shake/swirl/invert the bottle to completely dissolve the reagent into the sample.

NOTE: Excessive shaking can cause the Colilert to foam, potentially producing inaccurate results. Avoid excessive foaming or allow foam to subside prior to pouring the solution into the Quanti-Tray.

Figure 3 – Adding Colilert reagent packets to *E. coli* samples (IDEXX Laboratories, Inc., 2019)

5. Once the Colilert is fully dissolved, pour each sample into a Quanti-Tray. Use one hand to hold a Quanti-Tray upright with the well side facing the palm. Squeeze the upper part of the Quanti-Tray so that the Quanti-Tray bends toward the palm. Gently pull the foil tab to separate the foil from the tray. Avoid touching the inside of the foil or the tray (**Figure 4**).

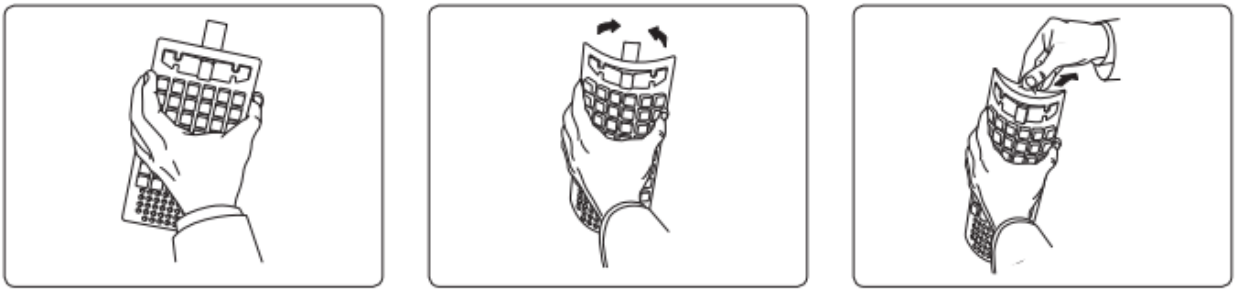


Figure 4 – Opening Quanti-Tray/2000 sample trays (IDEXX Laboratories, Inc., 2019)

6. Pour the reagent/sample mixture directly into the Quanti-Tray, avoiding contact with the foil tab (**Figure 5**). Tap the small wells 2-3 times to release any air bubbles. Allow foam to settle.

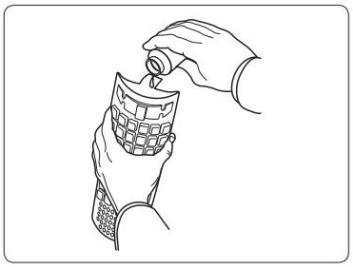


Figure 5 - Adding a sample to a Quanti-Tray/2000 sample tray (IDEXX Laboratories, Inc., 2019)

7. Once the Quanti-Tray sealer has warmed up and the green Ready Light is illuminated (up to 10 minutes after its been plugged in) indicating that the unit has reached operating temperature, place a rubber sealer on the input shelf with the large cutout facing away from the sealer. Place the sample-filled Quanti-Tray onto the rubber insert with the plastic well side of the Quanti-Tray facing down. Gently guide the tray into the sealer until the motor grabs the rubber insert and begins to draw it into the sealer. Seal the tray from the bottom up so it does not spill. In about 15 seconds, the tray will be sealed and will be ejected from the rear of the sealer.

NOTE: If at any time you wish to reverse the motor drawing the rubber insert into the sealer (for example, if a misaligned tray is accidentally fed into the sealer), press and hold the Reverse button. However, do not reverse the motor once the rubber insert has been drawn fully into the sealer.

NOTE: Having up to two empty wells will not result in a statistically significant difference in measured total coliform/*E. coli* concentrations; if more than two wells are empty, inadequate sample volume was collected and the sample should be considered invalid (Utah DEQ, 2014).

Seal and incubate the samples:

8. Once the tray is sealed, label both the top and bottom of each tray with the Site Visit Code using a permanent marker. Each Quanti-Tray must be cut in half to fit inside the chambers of the incubator. Cut the sealed Quanti-Tray in half with scissors; be sure to cut in between the row of the cells and do not damage or cut any of the cells. If cells are damaged the sample must be rejected.
9. Once the incubator ready light is illuminated, confirm the temperature is 35°C ± 0.5°C with the internal thermometer.
10. Place the Quanti-Trays into the incubator, latch all doors securely, and record the start incubation time on the Bacteria Sample Collection & Analysis Form.
11. After 24 to 28 hours, remove the trays from the incubator and match the top and bottom portions of cut trays; record the stop incubation time on the Bacteria Sample Collection & Analysis Form

10.6 Enumerate E. coli using Most Probable Number Table

NOTE: Each Quanti-Tray has 49 large cells (including the large well on top) and 48 small cells (97 wells total).

12. Mark any cells that have turned yellow (when viewed under normal lighting) with a fine-line permanent marker; count and record the number of large and small cells that are yellow on the Bacteria Sample Collection & Analysis Form (use a Colilert Comparator to distinguish between minimal positive and negative test results).

13. Look for fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample. Mark the yellow cells that are also fluorescing; count and record the number of large and small cells that are both yellow and fluorescing on the **Bacteria Sample Collection & Analysis Form** (use a Colilert Comparator to distinguish between minimal positive and negative test results).

CAUTION: You should never look directly into ultraviolet lamps. Eye damage can occur if they are exposed to direct UV radiation.

14. Use the most probable number (MPN) table to determine the MPN count of total coliform (yellow) and *E. coli* (yellow *and* fluorescent) and record the values (MPN/100ml) on the **Bacteria Sample Collection & Analysis Form**.

Complete the procedure:

1. Discard used materials (trays, sample bottles, empty Colilert packets)
2. Turn off and unplug all equipment, allow to cool, and repack.
3. Remove gloves and wash hands.

10.7 COLILERT COMPARATOR

Use of a Colilert Comparator (**Figure 6**) to minimize false positive or false negative readings is strongly recommended. The Colilert comparator is a liquid color and fluorescent reference. Its purpose is to assist in distinguishing a minimal positive from a negative test result. At 24 to 28 hours, any yellow color or fluorescence equal to or greater than the comparator is considered a positive test result (IDEXX Laboratories, Inc., 2017).

Figure 6 – Colilert Comparator (IDEXX Laboratories, Inc., 2017)

Table 1 – Interpretation of results compared to the comparator (IDEXX Laboratories, Inc. 2017)

Appearance	Result
Less yellow than the comparator	Negative for total coliforms and *E. coli*
Yellow equal to or greater than the comparator	Positive for total coliforms
Yellow and fluorescence equal to or greater than the comparator	Positive for *E. coli*

10.8 DETECTION LIMIT

Colilert detects total coliforms and *E. coli* at 1 organism/100 ml.

10.9 UNITS

Bacteria criteria are expressed as colony forming units (cfu) per 100 ml, or as most probable number (mpn) per 100 ml when the Colilert mpn table is used. Montana rules state, “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 17.30.621-629). For purposes of reporting, EPA has used these units (cfu/100 ml or mpn/100 ml) interchangeably and approved both in federal rule for ambient water, wastewater and sludge (40 CFR 136.3, 2003).

11.0 DATA AND RECORDS MANAGEMENT

All sample collection should be described in a DEQ-approved Sampling and Analysis Plan (SAP). Sampling and analysis plans (SAPs) will describe sampling activities for which this SOP is applied and will specify, for example, the location of monitoring sites, the number of samples, the type and amount of quality control samples required, sampling timing, etc. Field forms (**Appendix A**) must be filled out and reviewed in the field by field personnel and scanned and archived after sampling is complete.

DEQ provides detailed guidance to organizations, individuals, and laboratories on how to submit ambient water quality data to DEQ (http://deq.mt.gov/Water/SurfaceWater/SubmitData). Montana uses a system called the Montana EQuIS Water Quality Exchange (MT-eWQX) to store water quality monitoring data, including physical, chemical, biological and habitat data, from locations across the state. Once verified, this data is submitted to EPA's Water Quality Portal. All sample result values must be accompanied by metadata required for DEQ's data management process, including site identifiers including latitude and longitude, date of collection, name of collector, analytical method, and units (cfu/100 ml or mpn/100 ml), time of sample collection, incubator temperature, and incubation time. Any deviations from this SOP should be documented and, as appropriate, submitted as comments that accompany database submissions.

12.0 QUALITY ASSURANCE AND QUALITY CONTROL

Refer to relevant program quality assurance program plans (QAPPs) and sampling and analysis plans (SAPs) for project-specific quality assurance requirements, documentation, and quality control sample guidance (i.e., duplicates, blanks).

12.1 FIELD DUPLICATES

Field duplicate samples are multiple samples collected by the same person, at the same time and place, following the same method, and using the same equipment as was used to collect routine samples. Duplicate *E. coli* samples are used to quantify the variability in sample collection as well as sample handling and analysis.

- Refer to the SAP for specific guidance regarding total number of duplicate samples required for a project (often 10% of the total number of samples collected throughout a sampling season).
- Duplicate samples are generally collected at randomly-selected sites that allow for the side-by-side collection of two sets of samples upstream from previous disturbances.
- Use a separate Site Visit Form and a unique Site Visit Code to distinguish duplicate samples from routine samples. Label duplicate samples in the same way as routine samples (**Section 10.4**). On the Site Visit Form, indicate "field duplicate" by checking the box and recording the Site Visit Code of the routine samples they accompany in the space provided.

- Follow the exact same procedures used in rinsing, collecting, preserving, handling, and storing routine samples for the duplicate samples so two identical samples are produced (**Section 10**).
- Submit duplicate samples to the laboratory at the same time routine samples are submitted or, if processing samples in a portable laboratory, process duplicates alongside routine samples.

12.2 FIELD BLANKS

Field blanks are samples of analyte free water poured into a container in the field, preserved and delivered to the laboratory with field samples (EPA, 2009). The primary purpose of field blanks is to measure the magnitude of contaminant concentration that might have been introduced into the samples a result of sampling-related activities (Myers, *et al*., 2014) and during sample analysis.

- Prepare one set of field blank samples during each sampling event (i.e., to accompany each "batch" of *E. coli* samples).
- Prepare field blanks using laboratory-grade distilled or deionized water only.
- Prepare field blank samples while in the field.
- Use a separate Site Visit Form and a unique Site Visit Code to distinguish field blank samples from routine samples. Label field blank samples in the same way as routine samples (**Section 10.4**). On the Site Visit Form, indicate "field blank" by checking the box provided.
- Follow exactly the same procedures used in collecting, preserving, handling, storing and analyzing routine samples for the field blank samples except use laboratory-grade distilled or deionized water rather than ambient stream water (**Section 10**).
- Analyze field blanks for fecal indicator bacteria. If no growth is observed, the sample was collected by use of sufficiently sterile procedures (Myers, *et al*., 2014).

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APPENDIX A – FIELD FORMS

Forms associated with this Standard Operating Procedure (SOP):

1. **Site Visit Form**

Site Visit Forms and accompanying Site Visit Codes are required for DEQ personnel only; contact the Water Quality Planning Bureau data management team or the Water Quality Monitoring and Assessment Section for more information. Site Visit Forms are updated regularly to meet project-specific needs. Generally, when conducting *E. coli* sampling, check the "Bacteria" box.

Field Assessments

Photos ☐ Aquatic Plant Visual Assessment ☐ SAM ☐
Aquatic Plant Tracking ☐ Rosgen ☐ NRCS ☐
EMAP ☐ Total Discharge ☐ Channel X-Section ☐
Wetland ☐ Bacteria ☒ Other:_____

2. **Bacteria Sample Collection and Analysis Form** (see next page)

Bacteria Sample Collection and Analysis Forms are required for all projects that involve field personnel using the Colilert method, including DEQ personnel, Contractors, or other external users submitting data to DEQ for use in decision-making.

Bacteria Sample Collection and Analysis Form

Waterbody: _____

Date Collected: _____

Site Visit Code: _____

Personnel: _____

Visit No: _____

Colilert Quanti-Tray/2000 *E. coli* Analysis Results

					Total Coliform (yellow)			***E. coli* (yellow & fluorescent)**		
Time Collected	**Incubation Start Time**	**Date Analyzed**	**Incubation Finish Time**	**Incubator Temp (°C)**	**# Large Positive**	**# Small Positive**	**MPN Count (CFU/ 100ml)**	**# Large Positive**	**# Small Positive**	**MPN Count (CFU/ 100ml)**

NOTE: Each Quanti-Tray/2000 has 49 large cells (including the large well on top) and 48 small cells (97 wells total).

Circle:

Recent Rain:	None	24 hours	48 hours	72 hours	4-7 days	> 1 week
Weather:	Clear/Sun	Hazy	Partly Cloudy	Cloudy	Light Showers	Rain
Turbidity:	Clear	Slight	Turbid	Opaque		

Comments:

APPENDIX B –ALTERNATE METHODS TO POWER THE INCUBATOR OR SEALER

The Portable Millipore incubator can be powered several ways, including plugging the unit into a wall outlet, connecting to a vehicle battery, connecting to a rechargeable battery, or connecting to an external battery (Millipore Corporation 2000). The appropriate power supply depends on where you use the incubator. For example, if you use the incubator in the field, you can use the rechargeable nickel cadmium battery as a power supply.

Connecting the Incubator Using an Alternate Current (AC) Power Supply
Connect the incubator to an AC 115 V AC or 220/240 V AC power supply that comes with the kit. It is also available as an accessory.

NOTE: Prolong the battery life by using AC power to warm-up the incubator.

1. Plug the AC power supply cord into the incubator; the plug is located at the rear of the incubator.
2. Plug the prong-end of the cord into a grounded, AC outlet.
3. Turn the temperature selector knob to the temperature setting you want.

Connecting the Portable Incubator to a Vehicle Battery
The incubator can be operated in a laboratory or other locations using the 12 V DC cigarette lighter power supply that comes with the kit.

1. Loosen the thumbscrews on the back plate of the incubator by turning them to the left. Then remove the screws and plate and set them aside.
2. Connect the power jack end of the cigarette lighter power supply to the incubator power plug. Then plug the other end of the cable to the vehicle's cigarette lighter.
3. Turn the temperature selector knob to the temperature setting you want.

Connecting the Portable Incubator to an External Battery
An external 12 V DC battery may be used to supply power to the incubator. To connect the incubator to the external battery:

1. Loosen and remove the captive thumbscrews on the back plate by turning them to the left. Then remove the screws and plate.
2. Insert the power jack end of the battery cable to the incubator power plug.
3. Connect the clamp on the black cable to the negative (-) battery terminal of the battery. Then connect the clamp on the red cable to the positive (+) battery terminal of the battery.
4. Turn the temperature selector knob to the temperature setting you want.

Connecting the Quanti-Tray sealer to a Generator
A generator maybe used to provide a short-term 110 V power supply to run the Quanti-Tray sealer. Do not run a generator in a vehicle. Using a generator to run the incubator is not recommended as continuous power supply is required.

APPENDIX C – MOST PROBABLE NUMBER (MPN) TABLE

IDEXX Quanti-Tray®/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

IDEXX Quanti-Tray®/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6