FINAL
PROJECT REPORT

Groundwater Project Report for DNR Project #222
Meeting the Source Assessment Requirement under the RTCR: A Wisconsin Pilot Project

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EXECUTIVE SUMMARY

The goal of this project was to develop a sampling and testing algorithm to support the new site assessment requirements under the Revised Total Coliform Rule (RTCR) consistent with the "find and fix" goals of the Wisconsin Department of Natural Resources (WDNR). Because of the large number of public water supplies falling into the category of Transient Non-Community Water Systems (TNCWS) in Wisconsin, the WI DNR has elected to conduct both Level 1 and Level 2 Assessments under the RTCR for these systems. The final proposed process involves (1) sanitary surveys, (2) measurement of a suite of microbial indicator organisms, and (3) corrective action. The first two components of the protocol were developed at the Wisconsin State Laboratory of Hygiene and the last component is the responsibility of the Water Supply Section in collaboration with the property owners/well operators. The algorithm recommended for implementation as a State program involves a tiered sampling and analysis approach. In Tier 1, a site will be sampled and tested for coliforms, *E. coli,* enterococci, and adenosine triphosphate (ATP). Should any of the indicator enumerations return positive, the organisms will be speciated using the API 20E analytical profile index to determine if the species are of predominantly fecal or environmental origin or both. These results coupled with a sanitary survey and RTCR system Assessment, the decision to conduct large volume sampling and concentration followed by fecal source tracking analyses will be made. The source remediations achieved through implementation of this protocol is intended to provide a long-term solution that is sustainable, holistic, and economical for each public water supply investigated. The final analysis algorithm for transfer to a Wisconsin State Laboratory program was refined and is set for implementation in Fiscal Year 2017.

INTRODUCTION

Pathogens associated with fecal contamination are the primary cause of waterborne disease outbreaks in the United States. Water supplies are expected to become increasingly vulnerable to waterborne pathogens as a result of global climate change, and Wisconsin's groundwater is no exception. Current groundwater monitoring regulations are relatively successful at detecting potential fecal contamination, but do not provide information on its source.

Under the Revised Total Coliform Rule (RTCR) implications for unsafe sample results in transient non-community water supplies (TNCWS) could result in financially burdensome retesting and follow-up monitoring. The labor and cost required to meet these requirements is especially onerous in Wisconsin, which has over 9400 active TNCWS. To prevent a dramatic increase in the number of total coliform-positive follow-up samples as a result of the new requirements, an alternative RTCR unsafe follow-up source water assessment program was developed and piloted in coordination with the Wisconsin Department of Natural Resources (WDNR).

The goal of this project was to develop, test, and deploy a scientifically-based well assessment protocol as part of an overall well assessment program. This protocol was envisioned to consist of: large volume (100 liter) sampling capabilities among the WDNR and Wisconsin Public Health Department communities, a sanitary survey component, and development and testing of a suite of microbial indicators that can be standardized to accurately and efficiently track the sources of coliforms in public water supply groundwater wells in Wisconsin. The overall program thus contains three basic components, including: (1) sanitary surveys, (2) measurement of a suite of microbial indicator organisms, and (3) corrective action. The first two components of the protocol were developed at the Wisconsin State Laboratory of Hygiene and the last component is the responsibility of the Water Supply Section in collaboration with the property owners/well operators. The focus of the information gained from the protocol is to inform corrective actions, and is more aggressive than the well assessment protocol described in the RTCR to support the WDNR's find and fix approach.

The proposed initial suite of microbial indicators contained indicators for general fecal contamination as well as specific indicators of fecal contamination generated by the most probable sources across Wisconsin, including humans and livestock species. Genotyping and serotyping of coliphages, culturing of sorbitol-fermenting *Bifidobacteria* sp., and polymerase chain reaction (PCR) analysis of host-specific bacteria and enteric viruses (various *Bacteroides* sp., *Rhodococcus coprophilus*, and Adenovirus among others) are some of the more successful and reliable fecal source tracking (FST) methods available at the initiation of the project. However, all FST methods have limitations, suggesting that a toolbox approach utilizing multiple methods is required to consistently detect contamination sources. As the project progressed, improvements and changes in the source tracking toolbox were made based on developments in the field and discussions with science experts. These components are summarized in Tables 1 and 2 with the rationale for the changes are discussed in more detail below.

Table 1: Water Quality and Fecal Source Tracking Targets and Methods

Target Analyte	**Contamination Source**	**Methods**	**SOP**
Total coliforms and *E. coli*	Generic fecal indicators	Enzyme Substrate Test in Quanti-Tray® format	Appendix C
Enterococci	Generic fecal indicators	Enterolert® Enzyme Substrate Test in Quanti-Tray® format	Appendix D
ATP	Total microbial population	Filter, lyse cells, elute, and measure with luminometer	Appendix E
API 20E	Coliform speciation	API 20E	Appendix F
Adenovirus	Human	Polyethylene glycol precipitation, nucleic acid extraction, qPCR analysis	Appendix G & H
Bifidobacteria sp.	Human	Membrane filter HFUF concentrate, nucleic acid extraction, qPCR analysis	Appendix I , K & L
Rhodococcus coprophilus	Grazing animal	Membrane filter HFUF concentrate, nucleic acid extraction, qPCR analysis	Appendix I, K & L
Bacteroides spp.	Human Bovine		Appendix I & K
Toxigenic *E. coli*	Pathogen		Appendix J, K, &L
E. coli O157:H7	Pathogen		
Turbidity	Water quality parameter	Hach 2100N Turbidimeter	Standard Method 2130B

Table 2: Summary of Final Indicator and Molecular Testing Suite

Indicator Tests	**Test Application**
Total coliforms	Gives a general assessment of the sanitary condition of a drinking water sample. Included in the subset of total coliform is *E. coli*.
Generic *E. coli*	Good indicator of fecal pollution and possible presence of pathogens.
Enterococci	Fecal-specific subset of organisms present in the intestinal tracts of humans and warm-blooded animal species; has the ability to survive in saltwater, thus also provides detection of fecal pathogens with resistance to saline environments.
ATP	ATP analysis provides an estimate of the total microbial population of a water sample. Differences between first flush and purged well levels can be an indicator of biofilm issues.
API 20E®	Allows for classification of bacteria based on a standardized identification system. Can identify over 7800 bacteria strains, some which may be of sanitary concern.
Microbial Source Tracking Tests	
Adenovirus	Pathogenic viruses that infect and are carried by a variety of animal species. Human-specific serotypes are indicative of human fecal contamination.
Rhodococcus coprophilus	Bacteria found on vegetation that proliferates in the manure of herbivorous animals (cows, donkeys, goats, horses, and sheep). Indicates livestock or wildlife contamination.
Bacteroides spp.	Bacteria that inhabitants the human gut and most warm-blooded, non-human animal species. Enumeration of specific strains can be indicators of sewage or septic contamination as well as manure contamination.
Bifidobacteria spp.	Probiotic bacteria that inhabit the guts of humans and animals. A human-specific assay is employed to indicate for human fecal contamination.
Toxigenic *E. coli* (STEC)	A pathogenic subset of E. coli indicator bacteria. Provides definitive evidence of the presence or absence of the fecal pathogen. Typical of bovine fecal contamination, but can also include humans and other animals.
E. coli O157:H7	Specific strain of total coliform bacteria that can cause serious illness.

In addition to re-testing RTCR unsafe wells for general fecal indicators (total coliform, generic *E. coli* and enterococci by the enzyme substrate method), a flush time separated analyses of samples for adenosine triphosphate (ATP) and speciation of coliforms using the API 20E method are included in initial testing. The well assessment algorithm developed in this project is aimed at identifying sources of microbial contamination within Wisconsin's public water supplies that rely on groundwater. The primary sources of microbes being excessive biofilm growth or surface activities contaminating the aquifer. The information gathered for an individual well provides the scientific basis for developing measures to clean-up existing contamination or preventing contamination from recurring. The final testing algorithm is presented in Figure 1 and discussed in more detail below. The source remediations achieved through implementation of this protocol is intended to provide a long-term solution that is sustainable, holistic, and economical for each public water supply investigated. The final analysis algorithm for transfer to a Wisconsin State Laboratory program was refined and is set for implementation in Fiscal Year 2017.

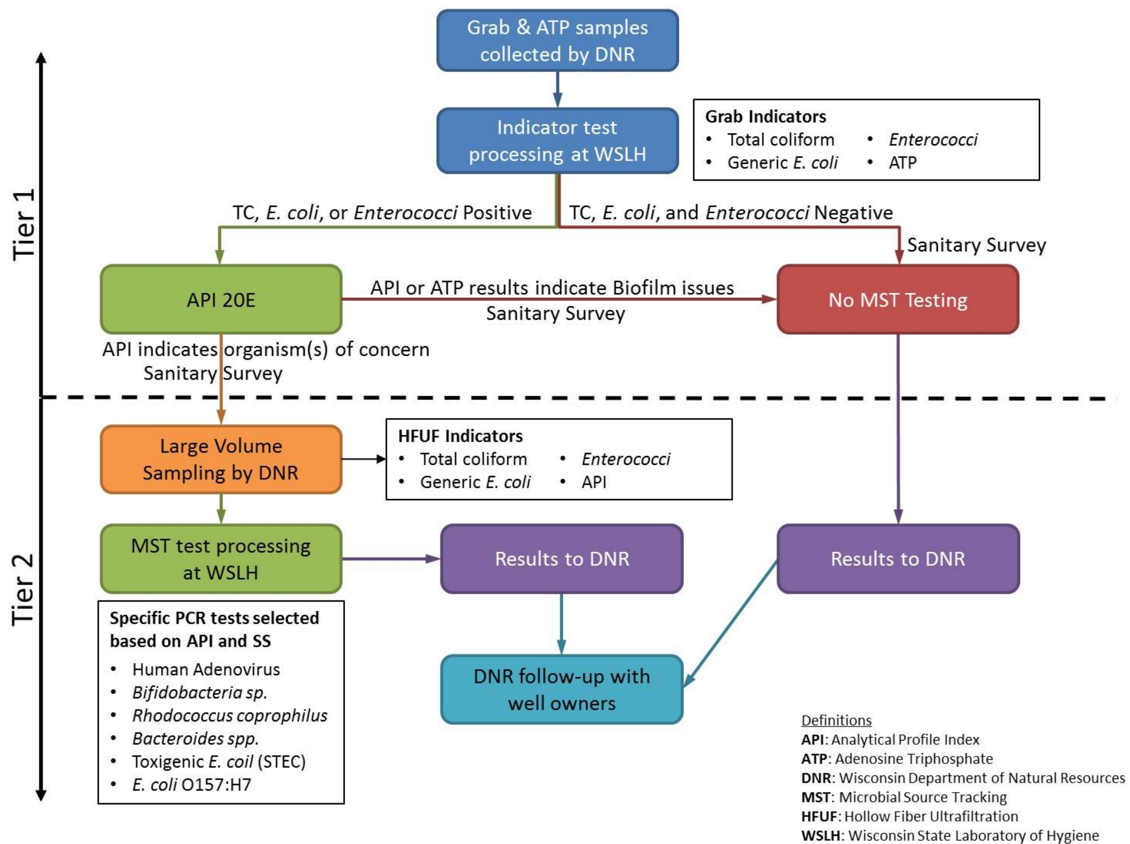


Figure 1: Final and Future Workflow

METHODS

Site Selection

Wisconsin is home to approximately 10% of all transient non-community water systems (TNCWS) in the Nation. These systems frequently return unsafe based on the 1989 Total Coliform Rule regulations (and the now current Revised Total Coliform Rule [RTCR] regulations). Thus, the Wisconsin Department of Natural Resources (WDNR) placed an emphasis on sampling TNCWS as a part of this project. In fact, all systems tested in this project are TNCWS utilizing a groundwater source.

Site selection was at the discretion of WDNR staff but was typically based on two criteria: (1) recent/repeat unsafe samples (especially after well chlorination), and/or (2) "problem" wells with a history of unsafe samples. In Wisconsin, the common treatment for wells that return a total coliform unsafe is to use shock/batch chlorination to inactivate and remove biological activity, such as planktonic microorganisms or biofilms on well infrastructure, which may be contributing to the unsafe samples. However, this common treatment method is often a temporary solution; biofilms regrow or a slug of contamination can reach the well again, triggering another unsafe sample. In the case where repeat unsafe samples occurred (e.g. 3 or more total coliform unsafes) and initial treatment methods did not remedy the problem, WDNR elected to use the large volume sampling method to better identify the cause of repeat unsafes. Wells were also selected if a recent sample returned unsafe and the testing records indicated a history of unsafe samples.

Well Water Sampling and Concentration

Sampling capacity among WDNR staff for sample collection and concentration was predominantly accomplished using prior funding, Wisconsin Department of Natural Resources Counter Terrorism Activities Project WP-00E38201. Portable "kits" were built and contain almost all supplies required for sampling and concentration. Figure presents one of the portable kits. The kits were customized to not only carry all supplies but also serve as the location of sample concentration. The kits contain supplies including a peristaltic pump, hollow fiber ultrafilter membranes, tubing sets, bottles of reagents, collection bottles, gloves, and antiseptic wipes. Items that do not fit inside of the kit itself include carboys and waste buckets.

Sample Collection and Concentration

Collection and concentration of the groundwater sample is accomplished using the Dead-end HFUF SOP for Field Filter, found in Appendix A, and the ATP and Bacteria Grab Sampling Method, found in appendix B. Fecal source tracking targets are often present at low concentrations in drinking water well samples, thus sample concentration is necessary. The dead-end HFUF SOP details all materials and steps required to concentrate the large volume well samples using hollow-fiber ultrafilter (HFUF) membrane. Particles (*i.e.* microorganisms) retained by the ultrafilter are concentrated approximately 100 times to a volume of about one liter which is then used for indicator and fecal source tracking analyses.

The ATP and Bacteria Grab Sample Method takes into account systems with pressure tanks or long distances of pipe, occurring before a sample tap, which must be purged before collection of well water, as opposed to water sitting in pipes. The method also helps control variation in ATP quantification; it was seen in past sample events that "duplicate" ATP samples (collected at the same location and within a minute apart) had varying ATP concentrations, likely a result of slugs of biofilm sheering off well infrastructure or aquifer and entering the collection bottles at varying time points. To better control for these variations, the approach collects 5L of well water in a carboy, which is then mixed and poured into the coliform/ATP collection bottles for testing.

Figure 2: Portable kit containing sampling supplies

At the start of well sampling, both a coliform and ATP sample (typically denoted "pre-grab' and "pre-ATP", respectively) are collected. After the initial bacteria and ATP samples are collected, the well is flushed for 30 or more minutes to purge water standing in the well column and pull aquifer water into the well. After the well purge, a 100L (large volume) sample is collected in the carboys. Prior to filling each carboy with well water, a sodium polyphosphate (NaPP) solution (denoted "1000X NaPP") is added to each carboy (10mL 1000X NaPP for 10L carboys or 20mL 1000X NaPP for 20L carboys). If a system being sampled has a chlorination system then a 10% sodium thiosulfate solution is also added to each carboy to neutralize residual chlorine. A second

round of coliform and ATP samples (typically denoted as "post-grab" and "post-ATP", respectively) are collected after the large volume collection.

Sample Testing Toolbox

Table 1 and Table 2, above, present an overview of all testing along with a brief descriptions of each test application and the testing methods, respectively. All laboratory tests are conducted using a standard aseptic technique to minimize and prevent contamination of the sample.

During the research project, efforts were placed on improving the level of detection/level of quantification (LOD/LOQ) of the qPCR assays by the testing of known amounts of the target DNA gene sequences to challenge the assays with low numbers of gene copies. This creates a reference level of quantification to be used in comparison with levels found in each large volume sample, allowing for conclusions to be drawn about the "amount" of contamination present in the well at the time of sampling. Standard curves have been produced for toxigenic *E. coli* (stx 1 and stx 2), *E. coli* O157:H7, *Rhodococcus coprophilus,* human *Bifidobacteria* and human adenovirus.

The microbial source tracking suite used in this research began with both a human and bovine *Bacteroides* assays; however, recent research and dialogue with project stakeholders suggested that the *Bacteroides* assays could be improved. Thus, to improve both sensitivity and specificity (i.e. reduce false-positives from cross-reactions), the primer and probe set for human B*acteroides* was switched to one that supports the use of the HF183 forward primer. The bovine-specific assay was also changed to a ruminant-specific *Bacteroides* assay. In addition, improvements were made to STEC assays; the new assay uses a 6-carboxyfluorescein (FAM) fluorescent dye as a reporter moiety for the TaqMan based assay.

Sanitary Survey

A project specific sanitary survey was developed to help identify potential pathways or inputs of contamination. The sanitary survey includes parameters such as well age, well depth, aquifer soil/rock characteristics, and numerous sources of potential fecal contamination such as animal agriculture, manure spreading, and septic systems. The survey is completed by WDNR staff in the field during sample collection. The survey information is incorporated in the overall laboratory workflow (see next section) and weight of evidence approach. The sanitary survey can be found in Appendix M.

Result Reporting

As requested by WDNR staff during the September 25, 2015 project meeting, laboratory results available after 24hr hours of sample analysis are reported via email. Once the API 20E (bacteria identification) analyses are completed, a formal "Indicator Report" is sent to WDNR which includes the 24hr results, bacterial identification, and a discussion of overall results taking into account the sanitary survey data to provide recommendations for future molecular source tracking testing. If source tracking is recommended, another formal "Molecular Report" is provided to WDNR with the testing results. This typically requires a few weeks after sample

collection so that samples may be batched for molecular analyses. The finalized "Indicator Report" and "Molecular Report" templates are presented in Appendix N.

WORK FLOW

One of the main goals of this research project is to develop an assessment protocol that can be followed to process each well sample. To help achieve this goal, a testing workflow was developed to standardize sample processing. The workflow has been improved over the course of the research as shown by the progression between Figure , Figure , to the final algorithm presented above in Figure . The first iteration of the workflow (Figure) was developed at the start of the project by Dr. Sharon Long (project principal investigator) and Mark Walter (prior graduate student working for Dr. Long) and is modeled after a general source tracking approach; the indicator and molecular tests are always completed and used together in the weight of evidence approach (along with sanitary survey data) to suggest the most likely issue contributing to unsafe samples.

The second iteration came after pilot testing of the first 18 large volume samples as well as feedback from WDNR staff. This workflow (Figure) improves on the first by adding the bacterial identification test (API 20E®). The workflow also splits into different "paths" after the indicator testing which permits samples to not undergo molecular testing if a biofilm issue is identified in the absence of fecal-specific indicators or bacteria (note the red paths in Figure). This "path" split helps achieve additional project goals only considering the testing for some or none of the qPCR assays, overall reducing testing costs. This also helps to speed up testing turnaround time by highlighting priority on only necessary testing.

The third and final workflow iteration (Figure) was developed after analysis of an additional 31 samples. This workflow is split into a two tiered approach: the "Tier 1" (screening level) and the "Tier 2" (large volume sampling level). The screening level is a preliminary assessment of the well that only requires coliform and ATP sample collection (according to the ATP and Bacteria Grab Sampling Method, Appendix B). This change help reduces sampling time and cost requirements by eliminating large volume testing and concertation on the first round of sampling. In the case that the screening level suggests contamination at a well (e.g. presence of fecal-specific indicators or identification of organisms of sanitary concern through the API 20E®), large volume sampling and molecular testing (Tier 2) is recommended as a follow-up to further elucidate the possible source(s) of contamination contributing to RTCR unsafe samples. Based on the regulatory history of the well (*e.g.* past/continued coliform or *E. coli* positives), WDNR staff may decide to proceed with both levels of assessment at once, following a workflow like that in Figure . As of June 10th, 2016, the Tier 1/Tier 2 approach is active and used by WDNR staff.

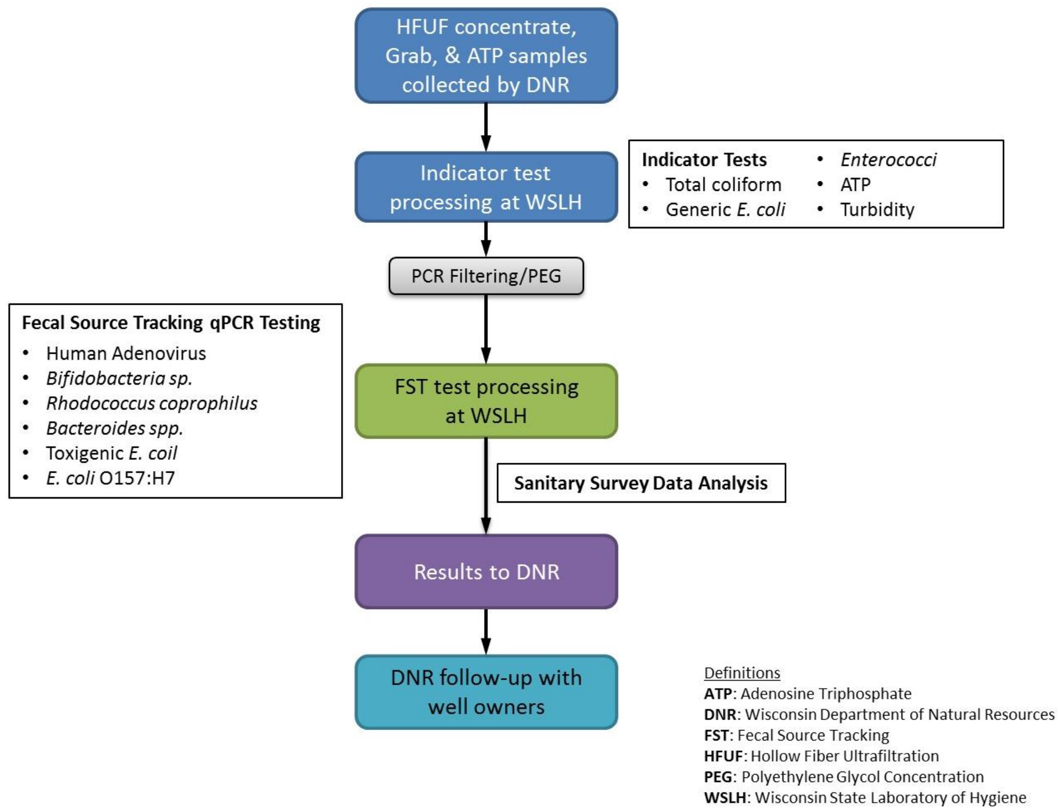


Figure 3: Original Workflow for Pilot Samples

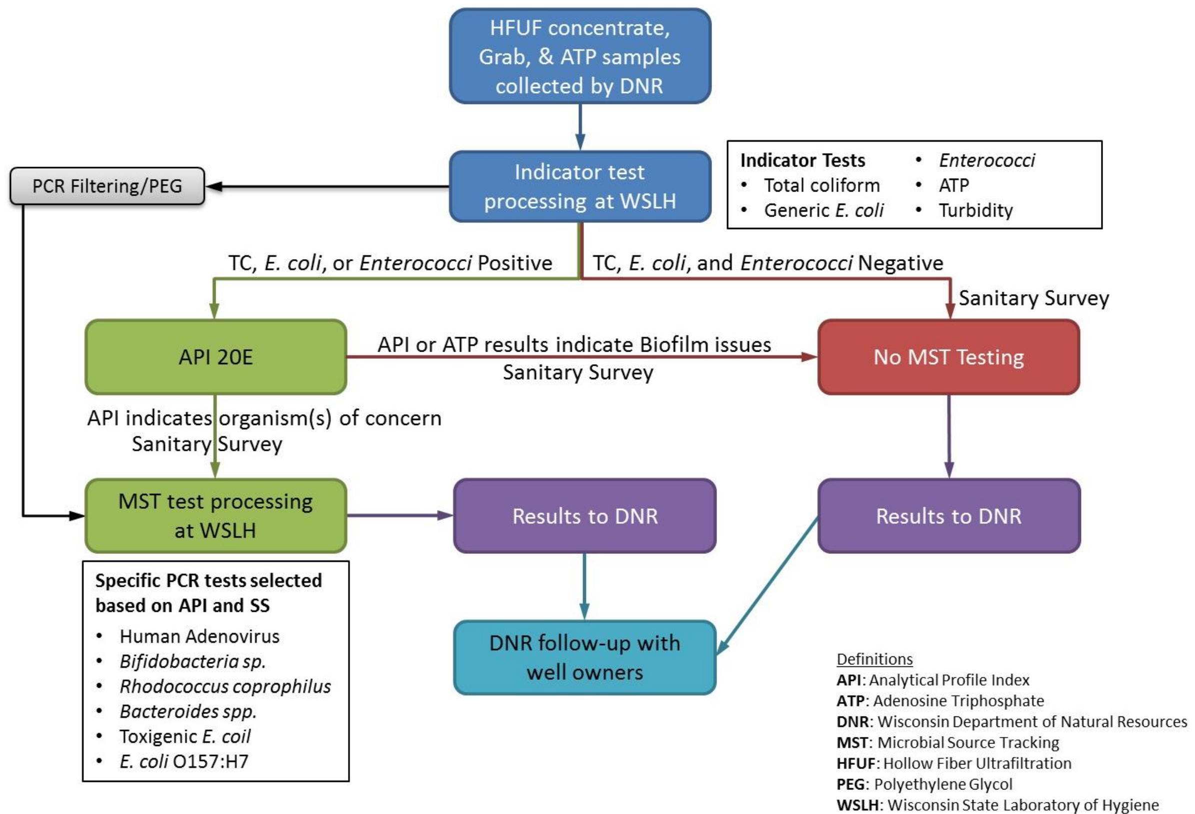


Figure 4: Updated Workflow Applied to Project

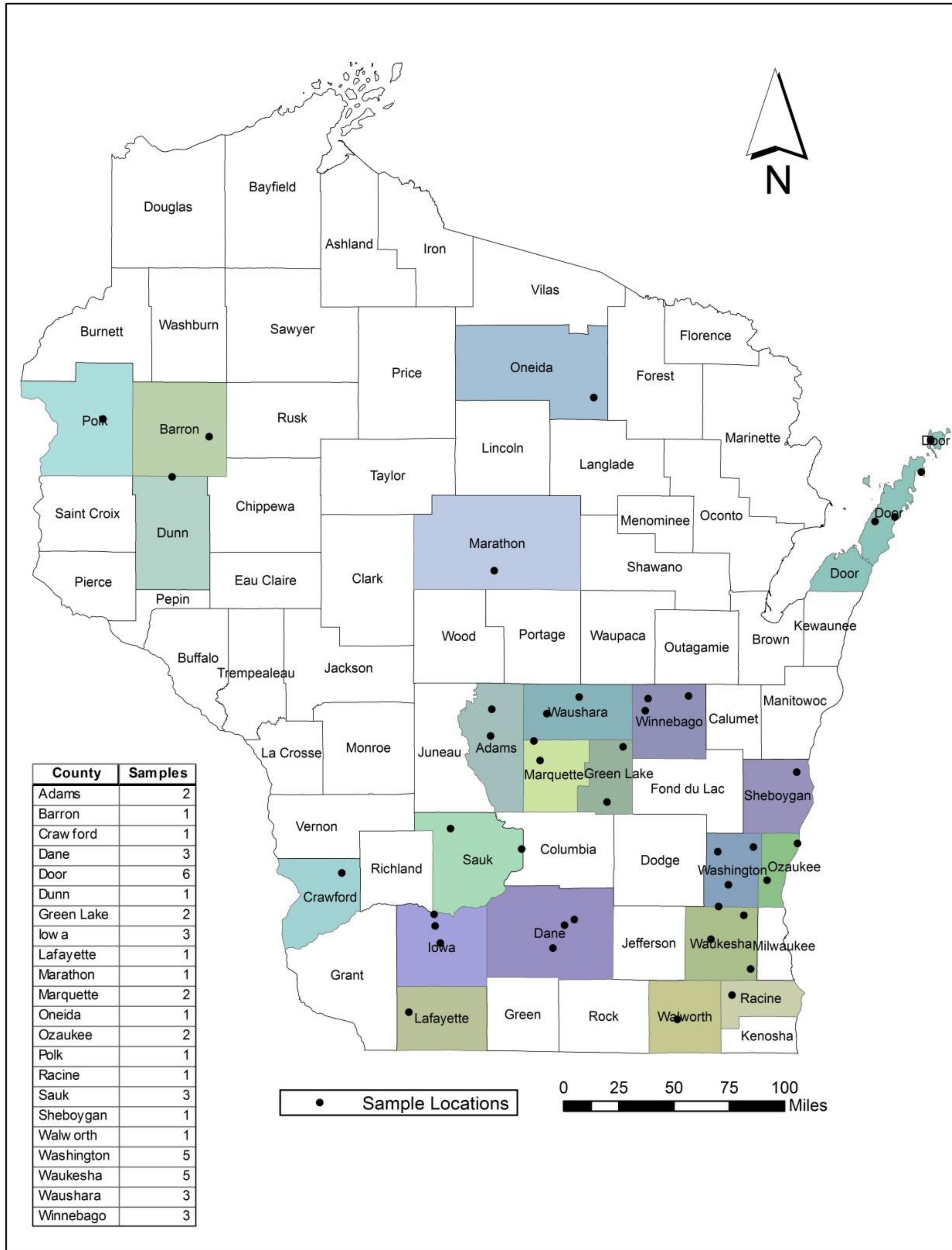
RESULTS AND DISCUSSION

The final data set for this project consists of 49 large volume sampling events, with only 48 included in the data set. It was found that one well had been sanitized prior to sampling, thus the sample was omitted from the data set. Figure presents the approximate locations all 49 sample events across Wisconsin and the count of samples from counties which had a large volume sample. Ideally, distribution of samples would be relatively consistent across all regions of Wisconsin to ensure the developed protocol applied in a variety of geographical areas; however, sampling of systems in remote regions proved difficult, owing to availability of staff and time required for sampling. As a result, the majority of samples collected for this project were concentrated in the southern areas of Wisconsin where WDNR staffing capabilities permitted easier access to sites. Roughly one-fifth of the samples were collected from more northern regions in Wisconsin.

Table 3 summarizes the results for the parameters measured with the number of samples tested for the each parameter, the percentage of samples that were positive for the parameter, and the range of numerical results. For detailed results for each sample, see Appendix O. Of the bacterial indicators (total coliforms, *E. coli*, and enterococci), total coliforms and enterococci were detected most often. Total coliforms in the grab sample (at well start-up) were detected 68.8% of the time and in the HFUF concentrated sample 91.7% of the time. The TNCWS tested as part of

this project have typically been coliform unsafe in the past, thus frequent detection of total coliforms is not unexpected and also confirms that the wells are still producing RTCR unsafe samples at the time of large volume sampling. Enterococci, a fecal-specific indicator, were detected in 56.3% of HFUF concentrated samples, indicating sites may be at risk for fecal-specific contamination. Enterococci were frequently detected in absence of *E. coli*, another fecal-specific indicator. In fact, *E. coli* was only detected 6.3% of the time in HFUF concentrated samples and never in concentrated grab samples. These findings suggest that *E. coli* may not be a suitable indicator of fecal-contamination for follow-up testing of groundwater sites in Wisconsin. The findings also suggest that fecal-specific contamination detected by enterococci is not recent, but rather historic. *E. coli* typically survive no longer than one week in the environment, thus their detection is typically more indicative of recent fecal contamination than historic contamination. Furthermore, enterococci may more readily incorporate into well biofilms and become resuspended at the time of sample collection, further emphasizing the indication of historic rather than recent contamination.

Analysis of adenosine triphosphate (ATP) to indicate for well biofilms was almost always detected. While only a few wells (n=3) indicated low levels of microbial activity not indicative of a biofilm (<500 microbial equivalents/mL), the majority of wells showed indications of elevated microbial activity. Many wells were found to have significant biofilm issues, with values of microbial equivalents/mL exceeding 10,000 times that of microbial activity in finished water provided by municipalities such as in Madison, WI.



County	Samples
Adams	2
Barron	1
Crawford	1
Dane	3
Door	6
Dunn	1
Green Lake	2
Iowa	3
Lafayette	1
Marathon	1
Marquette	2
Oneida	1
Ozaukee	2
Polk	1
Racine	1
Sauk	3
Sheboygan	1
Walworth	1
Washington	5
Waukesha	5
Waushara	3
Winnebago	3

Figure 5: Approximate locations of all 49 large volume sampling events with total count from each county tested.

Table 3: Summary of Laboratory Results for the 48 well Samples

Parameter	Number	% Positive	Range
ATP (first flush)			
Microbial equivalents/mL	n=48	100	370 – 365,430
cATP/mL	n=48	100	0.37 – 365.43
Relative light units/mL	n=48	100	61 – 61,110
ATP (after sustained pumping)			
ME/mL	n=48	97.9	BDL – 295,950
cATP/mL	n=48	97.9	BDL – 295.95
RLU/mL	n=48	100	24 – 45,242
Total coliform			
Grab sample (MPN/100 mL)	n=48	68.8	BDL - >2419.6
HFUF concentration (MPN/100 mL)	n=48	91.7	BDL – 76.78
E. coli			
Grab sample (MPN/100 mL)	n=48	0.0	N/A
HFUF concentration (MPN/100 mL)	n=48	6.3	BDL - 0.126
Enterolert (Enterococci) (MPN/100 mL)	n=48	56.3	BDL - >26.1
Bacteroides sp.			
Human (gene copies/100 mL)	n=48	14.6	BDL – $3.4x10^4$
Bovine	n=25	0.0	N/A
Ruminant	n=23	0.0	N/A
Rhodococcus coprophilus	n=48	6.3	N/A
Human Adenovirus	n=48	4.2	N/A
Toxigenic *E. coli* (STEC)	n=48	0.0 (2.1%)*	N/A
E. coli O157:H7	n=48	0.0	N/A
Human *Bifidobacteria*	n=48	0.0	N/A

*Possible presence of stx 1 gene, likely *Shigella* organism and not true STEC
BDL – below detection limits
N/A – not applicable

The human molecular marker *Bacteroides* was detected in 14.6% of samples. The other human markers for adenovirus and *Bifidobacteria* were detected 4.2% and 0.0% of the time, respectively. Bovine- and ruminant-specific *Bacteroides* were never detected, even in cases when *Rhodococcus coprophilus*, a ruminant-specific marker, was detected (6.3% of the time). Toxigenic *E. coli* (STEC) and *E. coli* O157:H7 were never detected; however, the *stx1* gene, part of toxigenic *E. coli* was detected in one sample (2.1% of the time). The presence of only the *stx1* gene is indicative of a possible *Shigella* organism, but not a true STEC organism.

One of the goals of this project was to not only develop a protocol to assess sources of total coliform RTCR violations, but to also see if a screening approach consisting of bacterial indicators (total coliforms, *E. coli*, enterococci) as well as information from the sanitary survey

and bacterial identification (API 20E®) could be used to select specific molecular tests most likely to add to the weight-of-evidence in identifying potential sources of contamination. Presented below are a couple case examples of how the screening approach performed as part of the overall project algorithm.

Example: Human Source

Following along with the workflow as discussed above, the sample at this site was evaluated for the bacterial indicators of which only total coliforms were detected. Since a positive result was provided by the coliforms, the bacterial identification was performed which identified two organisms likely of environmental origin (*Serratia liquefaciens* and *Enterobacter asburiae*) and one organism known to be part of the health human gut flora (*Kluyvera* spp.). The sanitary survey information did not indicate the presence of agriculture practices or agricultural animal presence, but did indicate hiking and hunting. Overall, the screening tests indicate the possibility of fecal contamination, especially form a human source, thus the human molecular markers were selected for testing. Of the three human markers, two were detected (*Bacteroides* and adenovirus) and one not detected (*Bifidobacteria*). As part of this research, the remaining molecular tests for animal and *E. coli* contamination sources were also conducted and ultimately not detected. In this case example, the screening tests, especially the bacterial identification of human gut bacteria, were able to both add to the scientific weight-of-evidence for fecal contamination and also help predict the molecular testing which was most likely to further elucidate the source of contamination.

Example: Biofilm

At this site, total coliforms were detected but no *E. coli* or enterococci were detected. The presence of total coliforms allowed for the bacterial identification to be conducted, which isolated only *Pantoea* spp. 3. This organism is mostly likely of environmental origin and does not suggest human or animal contamination may be present. The sanitary survey indicated nearby surface water resources, but no presence of human or animal activities. Thus, the overall body of evidence from the screen tests suggested that the molecular tests should not be conducted because RTCR violations were likely a result of environmental coliforms and not fecal pollution. As part of the research project, all molecular testing was conducted and all markers were not-detected, emphasizing the agreement between screening methods and molecular testing. Furthermore, ATP analysis suggested a strong biofilm associated with well infrastructure which was likely harboring the coliform organisms resulting in RTCR unsafes.

While these are just two examples of how the suite of indicators and molecular methods work together, analysis of the overall data set was conducted to see how often the screening tests aligned with the molecular testing. To evaluate the ability of the combination of screening tests to accurately predict appropriate scenarios to employ molecular techniques, an agree/disagree table was created and is presented by Table .

Table is divided into four quadrants according to whether or not the combination of bacterial indicators, sanitary survey, and bacterial identification indicate fecal contamination (Y = “Yes”) or do not indicate fecal contamination (N = “No”) and likewise for the molecular indicators. The four quadrants then contain agreement or disagreement between the different scenarios, with the first scenario being where both the indicators and molecular markers suggest fecal contamination

(Y and Y). This scenario occurred 18.8% (n=9) of the time. The second scenario where the indicators do not suggest the possibility of fecal contamination but the molecular marks do suggest fecal contamination (N and Y) occurred 4.2% (n=2) of the time. The third scenario where fecal indicators suggest the possibility of fecal contamination but the molecular markers do not (Y and N) occurred 45.8% (n=22) of the time. The fourth scenario where both the indicators and molecular markers do not suggest fecal contamination (N and N) occurred 31.3% (n=15) percent of the time. Ideally, the methodology would always result in scenario one or four where indicators and molecular markers are in agreement. Combing these two scenarios, the indicators and molecular markers were in agreement 50% of the time (n=24). Scenario three indicates that the fecal markers were often too conservative because the fecal molecular markers were not detected. One explanation for the frequent detection of bacterial indicators but absence of molecular markers may be that the limit of detection for molecular markers is too high; molecular methods are inherently imperfect because of the limit to which genomic information can be detected. While non-detection of fecal markers through molecular methods does not necessarily translate to "no contamination present", the weight-of-evidence may suggest that fecal contamination is unlikely or that the specific source of contamination using molecular detection methods cannot be determined. The third scenario (the "false-positive" scenario) is acceptable in terms of public health because it offers a more conservative estimate of potential pollution (*i.e.* molecular methods were employed were non-detection of molecular markers indicates that they could not have been tested). The advantage of avoiding false-positive results would be a reduction in time and cost spent on molecular methods, which are significantly more costly than the combination of indicators, sanitary survey, and bacterial identification. The final scenario includes cases where indicators suggest no fecal contamination but molecular markers do detect source(s) of fecal contamination. In terms of public health, this is the least ideal scenario as a screening approach using indicators could result in false-negative assessments of fecal contamination. However, if scenarios one, three, and four are considered as one group, then the methodology indicates approximately 95.8% certainty that the screening approach (of bacterial indicators, sanitary survey, and bacterial identification) will either agree with or is more conservative than molecular markers, which is an appropriate threshold when considering risk to public health of false-negative result.

Table 4: Agreement of Bacterial Indicators, Sanitary Survey (SS), and Bacterial Identification (API) with Fecal Molecular Markers, where Y = "Yes, some indication of fecal contamination" and N = "No indication of fecal contamination."

<table>
<tr><td colspan="2" rowspan="2">n=48</td><td colspan="2">Bacterial Indicators + SS + API</td></tr>
<tr><td>Y</td><td>N</td></tr>
<tr><td rowspan="2">Fecal Molecular Markers</td><td>Y</td><td>9
(18.8%)</td><td>2
(4.2%)</td></tr>
<tr><td>N</td><td>22
(45.8%)</td><td>15
(31.3%)</td></tr>
</table>

RECOMMENDATIONS

As the project moves forward to become a full-time program for the WDNR to meet the new requirements of the RTCR, modifications to the testing suite should be considered. For the bacterial indicators, *E. coli* were not detected in any grab samples and only in 6.3% of the concentrated samples. Overall, the *E. coli* did not contribute too often to the weight-of-evidence for fecal contamination, even in cases where molecular markers of human or animal contamination were detected. However, the *E. coli* detection method is run simultaneously (at the same time with no additional cost) with total coliform detection, thus the removal of *E. coli* is not recommended. Changes to the suite of molecular indicators are recommended. The bovine- and ruminant-specific *Bacteroides*, human *Bifidobacteria*, toxigenic *E. coli*, and *E. coli* O157:H7 molecular markers were never detected during the project. The bovine- and ruminant-specific *Bacteroides* was not detected even in cases where in the same sample *Rhodococcus coprophilus* (a ruminant animal fecal marker) was detected. It is recommended that *Bacteroides* is used strategically in scenarios where evidence (*e.g.* sanitary survey land use information, bacterial identification) suggests a strong animal presence. It is also recommended that the toxigenic *E. coli* and *E. coli* O157:H7 assays are employed strategically such as in cases where *E. coli* is detected as part of the bacterial indicators. Lastly, it is recommended that the *Bifidobacteria* assay be removed from the testing suite. The human *Bifidobacteria* molecular marker is still in its infancy; another research study running concurrently with this project has found risk of cross-reaction for the Spanish *Bifidobacteria* assay with various animals (cows, horses, etc.), suggesting the assay may not be robust enough for application in Wisconsin.

It is recommended that the total coliforms (and therefore *E. coli*), enterococci, bacterial identification, ATP, human *Bacteroides*, *Rhodococcus coprophilus*, and human adenovirus remain as part of the full-time testing suite. Information from the sanitary survey is also recommended to remain as part of the overall analysis approach. Furthermore, it is recommended that the project continue using the tiered approach (Tier 1/Tier 2) as described above (Figure 1).

While the screening tests were only agreeing with the molecular testing approximately 50% of the time, a majority of the systems tested showed indications of biofilm issues with microbial populations of environmental origins. The goal of the Tier 1 approach is to then screen out systems with biofilm issue and no proceed with large volume sampling and testing, overall reducing time and costs. For those systems where the screen approach suggests the possibility of fecal contamination, WDNR can choose to follow up with either Tier 2 large volume sampling and testing of molecular targets to further elucidate the potential for source of fecal contamination or may instead opt to proceed directly to remedial action. This tiered approach both allow for some reduction in project costs and offers greater flexibility for the WDNR to determine at what stage is appropriate to proceed with corrective action.

PROJECT DISSEMINATION

As part of the Master's degree requirements for Brandon Moss, the research assistant on the project, a presentation was given at the Civil and Environmental Engineering (CEE) seminar held for professors and graduate students in the CEE program. Work on the project was also presented at the Soil Science seminar.

Presentations at three professional conferences were delivered, the first was the American Water Works Association Illinois Chapter Annual Conference "WATERCON 2016", and where a presentation titled "An Integrated Approach to RTCR Assessments" was given. Presentations were also given as the Water Microbiology Conference at the University of North Carolina and the American Water Works Association Annual Conference and Exposition with presentations titled "An Integrated Monitoring Approach to RTCR Level 1 and 2 Assessments" and "Large Volume Sample Approach to Meet the Source Assessment Requirements under the RTCR", respectively.

CONTINUING AND FUTURE WORK

As discussed in "Recommendations", the project is both recommended and currently on course to become a full-time program used by the WDNR to meet the new requirements of the RTCR. It is anticipated that each year roughly 50-80 Tier 1 samples will be submitted, with approximately 20 followed up by large volume sampling and testing of molecular markers. The project will require one new staff hire at the WSLH to coordinate sample submission from WDNR staff, conduct laboratory testing, maintain sample capacity (*e.g.* supplies) among WDNR staff, and assist in sample analysis and write-ups.

Additionally, grant funding has been secured to allow one new graduate student to research the application of both a bovine and porcine adenovirus molecular assay for use on large volume well samples. The world of microbial source tracking has been and still is imperfect; the development of two new assays add the potential for faster, inexpensive, and more accurate detection methods of fecal sources of contamination and may ultimately bolster the scientific evidence provided to WDNR to support cases where corrective action is necessary. The research and development of these two assays will take course of a one-year period beginning in July, 2016.

ACKNOWLEDGEMENTS

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The authors would like to thank the following individuals without whose help, this project would not have been possible.

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APPENDIX A

Dead-End Hollow Fiber Ultrafiltration for Field Filtering Public Water Supply Well Samples

March 9, 2016

The purpose of this procedure is to concentrate large volumes (approximately 100 L) of well water in order to conduct in depth assessment of the source(s) of coliforms/RTCR unsafes. This method has been tested for efficacy with bacteria (*E. coli* and enterococci), viruses (coliphage, adenovirus, norovirus), and parasites (aerobic endospores as a surrogate, *Cryptosporidium*, and *Giardia*). This method is a modification of the method validated by WSLH for preparedness response incorporating modifications per Smith and Hill (2009).

Media and Reagents

<u>5% newborn calf serum (or fetal bovine serum</u>)
114mL sterile cell culture water
6mL calf serum
Prepare day of filter blocking
Filters may be blocked the evening prior to sample filtration if kept refrigerated
(this is enough for 1 filter)

<u>1000X NaPP solution</u>
*Sent pre-made from WSLH or county health department
10 g sodium polyphosphate
100 mL sterile cell culture water
In sterile container, heat in 65ºC waterbath to dissolve (may need to be warmed overnight)
Store at room temperature for up to 3 months

<u>Filter Pre-Wash Solution</u>
1 L sterile Type I laboratory water
1 mL 1000X NaPP solution
Prepare day of use
(this is enough for 1 filter)

<u>Filter Post-Wash Solution</u>
900 mL sterile Type 1 laboratory water
0.09 mL TWEEN® 80
0.9 mL 1000X NaPP solution
9.0 µL Antifoam Y-30
Prepare day of use
(this is enough for 1 filter)

<u>10% Sodium thiosulfate</u>
100 g sodium thiosulfate
1,000 mL sterile cell culture water
Autoclave, 15 min, 121ºC
Store at room temperature
(for chlorinated samples only)

Apparatus and Materials (in order of assembly)

Portable HFUF kit

Bag of 6 mL tubes of frozen calf serum (completely non-toxic and non-hazardous), remove and thaw **only** the number of tubes needed

20 L or 10 L carboys – sterilized (Fisher 02-960-20B)

Spare (empty) 1 L bottle for sample collection – sterilized

Backwash collection bottles – 1L, empty, pre-weighed, sterilized

2 filtrate (waste) buckets or carboys (or wastewater can be discharged to the sewer or onto the ground in appropriate circumstances)

Funnel – cleaned with bleach water, rinsed three times with tap or sample water, and covered with aluminum foil prior to use

ThermoSafe cooler for shipping samples

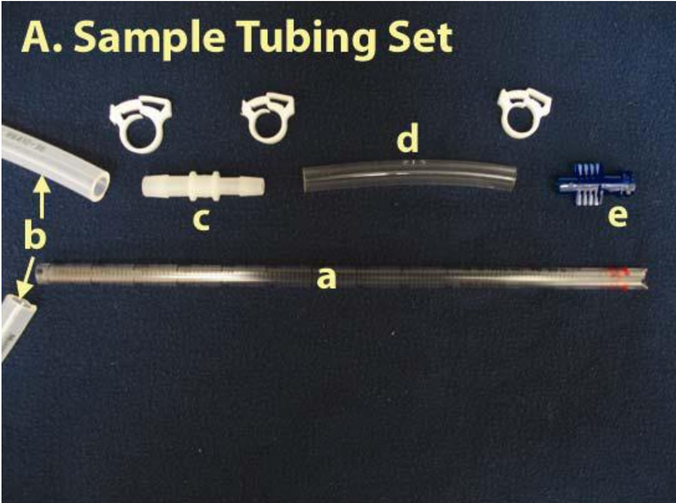
<u>Items you will find in your portable HFUF kit*</u>

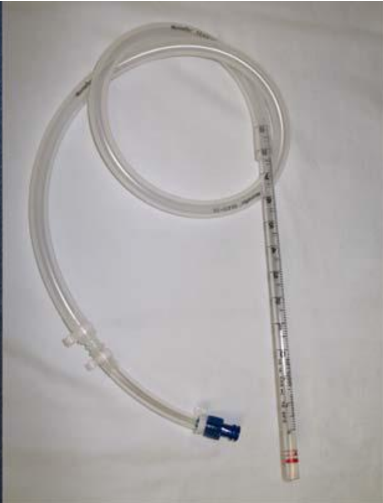
- 3 rectangular plastic bins
- Bags of various sized gloves
- Bag of antiseptic wipes
- Bag of Wypall L40 wipes (absorbent laboratory diapers)
- Bag of trash bags and zip-top bags
- Sharpie marker
- Bag of ATP sample bottles
- Bag of coliform sample bottles with Styrofoam packer
- 3 Asahi REXEED 21S filters
- 3 sterile containers of 114 mL of cell culture water
- Bag of 60 cc syringes
- Bag of 20 mL or 10 mL tubes of 1000X NaPP solution
- Bag of 10 mL or 5 mL tubes of 10% sodium thiosulfate solution
- Bottle holder containing
  - 3 bottles for preparing pre-wash
  - 3 bottles for preparing post-wash (containing 100 µL of TWEEN 80)
- Bag of 1 mL tubes of 1000X NaPP solution
- Bag of 0.9 mL tubes of 1000X NaPP solution
- Bag of Antifoam Y-30 tubes (contains only µLs, do not be alarmed if tube looks empty)
- Bag of sterile transfer pipettes

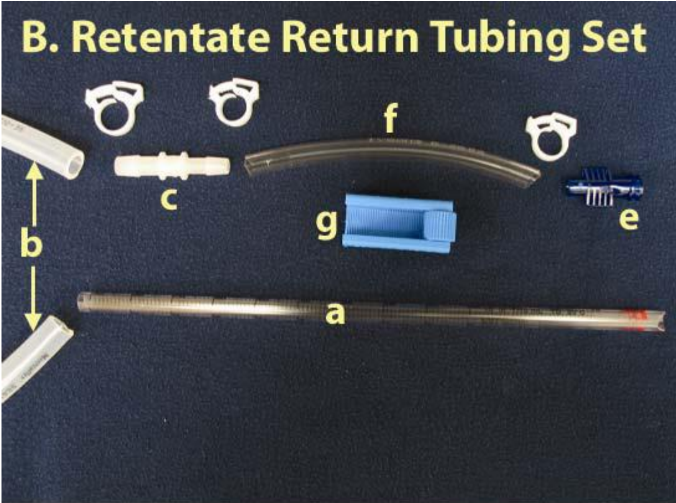
- 3 Sample tubing sets (check expiration date)
- 3 Retentate tubing sets (check expiration date)
- 3 Filtrate tubing sets (check expiration date)
- 3 Backwash tubing sets – 33 inches (check expiration date)
- 25-foot, 1” diameter tube in a closable, cylindrical, plastic bucket
- Bag of zip ties
- Metal peg board hook
- MasterFlex L/S Easy-Load II Pump Head (Fisher 77201-62) mounted on MasterFlex L/S Precision Drive (Fisher 0752810)
- MasterFlex power cord
- Extension cord
- Lighter
- Pipette bulb
- 50 mL pipettes
- Filtrate tubing clamp
- Zip tie cutters
- Zip top bag containing paperwork (sanitary survey, HFUF protocol, HFUF bench sheet, sample request form, supply re-stock sheet, shipping cooler checklist)
- Bag of large aluminum foil sheets
- Bag of small aluminum foil sheets

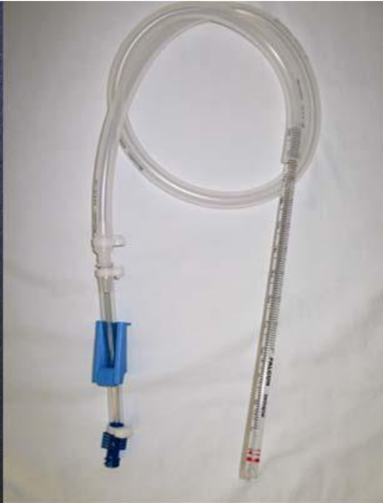
*because of space considerations, some items may be packed in your ThermoSafe box

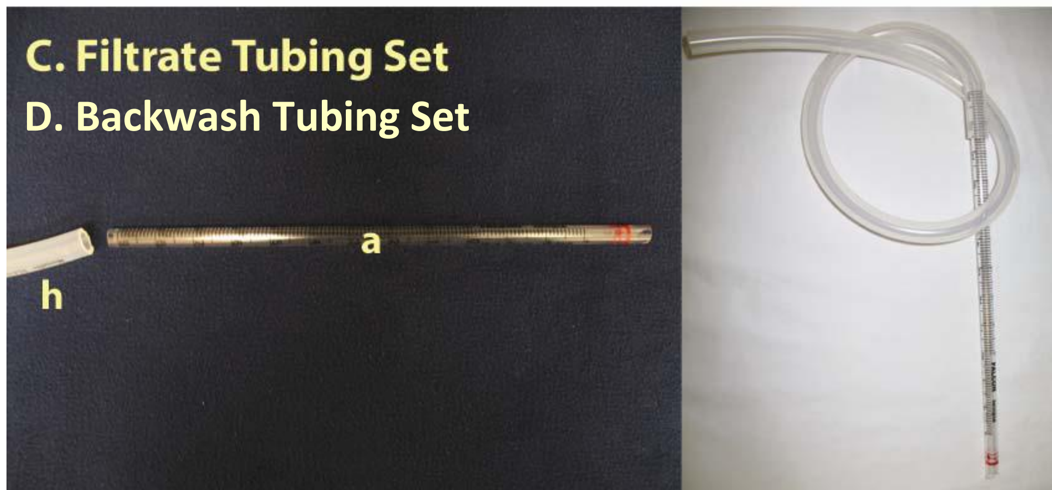
Recognizing Your Tubing Sets











Constructing Your System

I. Preparations
   1. Open field kit and remove the inverted gray bin from right-hand side.
   2. Remove pump and foam padding from field kit. Remove these 3 pieces individually to prevent damage to the pump.
   3. Lift out only one (1) of the gray bins on the right-hand side containing water bottles, box of filtration accessories, etc. Leave one bin to collect spilled water.
   4. Flip the previously removed empty gray bin upside-down so the open side is facing downwards. Place the right lip of this bin beneath the left lip of the gray bin currently in the field kit. Slide bin into place until it is flush with the bottom of the field kit.
   5. Place the pump (without foam padding) and the 6 1-L bottle holder on the left-hand bin as shown in the photo below.
   6. Record all data, or attach stickers (if applicable), on the bench sheet provided in the zip top bag labeled "Paperwork".
   7. Put on gloves. To maintain best aseptic practices, wipe gloves with antiseptic wipes.
   8. Pretreat/block one dialysis filter per sample to be filtered (up to 3) with 5% calf serum solution (can be prepared in the office/lab the evening prior to use as long as it is kept refrigerated).
      8.1. Thaw one tube of 6 mL frozen calf serum (per sample) at room temperature.
      8.2. Pour thawed calf serum from tube into bottle containing 114 mL of sterile cell culture water. Cap the bottle and swirl to mix. Discard tube.
      8.3. Lay out a clean Wypall L40 on a clean surface.

8.4. Using the zip tie cutters provided, carefully remove Asahi REXEED 21S filter from packaging. Place on clean Wypall L40 and remove end caps from filter. Do not remove the side caps. Do not discard packaging or end caps.

8.5. Position the Asahi REXEED 21S filter vertically with the top (orange end) facing up. Using a 60cc syringe (individually wrapped "BD 60 ml Syringe"), draw air into the syringe and then align with the top port (orange end). Slowly expel the contents of the 60cc syringe into the Asahi REXEED 21S filter, collecting the drained saline solution in a 5-gallon waste bucket. Repeat until all saline solution has been expelled (typically 3 or 4 times).

8.6. Position the Asahi REXEED 21S filter horizontally. Using the same 60cc syringe from Step 8.5, fill with 5% calf serum solution. Be sure to either leave the bottom port (blue end) cap off completely or attach loosely.

8.7. Align the 60cc syringe with the port on the top (orange end) of the Asahi REXEED 21S filter. Slowly expel the contents of the 60cc syringe into the Asahi REXEED 21S filter. Repeat until all solution has been used. If you are careful to keep the syringe aseptic, it may be used to block as many filters as needed (up to 3). Discard the 60cc syringe after all filters are blocked or the syringe becomes contaminated.

8.8. Seal the Asahi REXEED 21S filter end ports with end caps. Discard the bottle. Invert the filter at least 25 times to fully coat the filter with calf serum solution.

8.9. Label the Asahi REXEED 21S filter and a clean 2-gallon zip-top bag to correspond to the ID of the sample to be collected.

8.10. Place the blocked and labeled Asahi REXEED 21S filter back into its original packaging and place the packaged filter into the labeled zip-top bag.

8.11. Store filters in refrigerator or cooler with ice until use.

9. Prepare filter **pre-wash** and **post-wash** solutions (if desired, may be prepared in office/lab **same day** of filtering, prior to traveling to field site).

9.1. Obtain 1 L sterile water bottle marked "**Pre-Wash**" (1 for each sample). Pour the contents of one tube of 1 mL 1000X NaPP solution (located in Tupperware container) into 1 L bottle and label "**Pre-Wash**/*Sample Name*" on the labels provided on the bottle base and cap.

9.2. Cap tightly and mix "**Pre-Wash**/Sample" bottle by inverting 25 times. Use filter **pre-wash** solution same day of preparation.

9.3. Obtain 900 mL sterile water bottle marked "**Post-Wash**" (1 for each sample). Pour the contents of one tube of 0.90 mL 1000X NaPP solution into bottle and label "**Post-Wash**/*Sample Name*" on the labels provided on the bottle base and cap.

9.4. Using a new, sterile transfer pipette (located in Tupperware container), add a small volume of **post-wash** solution to tube containing Antifoam Y-30 (located in Tupperware container). Do not be alarmed if the tube appears empty because of the extremely small volume of Antifoam Y-30. Pipette up and down a few times to mix, the solution will appear milky-colored. Use transfer pipette to transfer

solution to **post-wash** bottle and pipette up and down to rinse transfer pipette. Discard transfer pipette.

9.5. Cap tightly and mix "**Post-Wash**/Sample" bottle by inverting 25 times. Use filter **post-wash** solution same day of preparation.

II. Sampling*

10. Collect and prepare sample. **Be sure to collect initial ATP sample, followed by coliform sample, prior to rinsing previously used 20 or 10 L carboys, 1 L sample transfer bottles, or funnel, and prior to collecting HFUF samples.**

10.1. Change gloves. Wipe gloves with antiseptic wipes.

10.2. Remove autoclave tape from carboys.

10.3. If previously used during this sampling period, rinse the inside of each carboy and 1 L sample transfer bottle 3 times with well water to get rid of residual bleach from Steps 14.4 through 14.6 and coat carboy/bottle walls with the sample to be collected. Dump rinse water into waste bucket or sewer.

10.4. Pour the contents of one tube of 1000X NaPP solution (large tubes located in Tupperware container) into each of the 20 L sample carboys or 10 mL 1000XNaPP to each 10 L carboy. Discard tubes in the trash bags provided.

10.5. If the sample is **chlorinated**, add 10 mL of 10% Na thiosulfate to each 20 L carboy or 5 mL of 10% Na thiosulfate to each 10 L carboy.

10.6. Collect sample into each carboy containing 1000X NaPP solution (and Na thiosulfate if chlorinated). Fill to the 20 or 10 L mark on the carboys, which ever you are using. If space limitations prevent direct sample collection in carboy, use spare (empty) 1 L bottle provided to fill carboys to fill mark.

10.7. Place the first sample carboy into the empty gray bin on the right side of the kit. Position the carboy so the volume markings are facing towards you (facing away from the field kit lid).

*If using 10 L transfer containers, add 10 mL tube of 1000X NaPP, and fill to the 10L mark.

III. Constructing the System

11. Construct complete filtering set-up.

    11.1. Place the blocked filter into the mounting, with the blue end down and the orange end up, and the filter side ports pointing to the right (away from the pump).

    11.2. Zip-tie the filter to the filter mount as shown in the photo above.

    11.3. Make sure cap is tight on the lower side port of the filter.
    Note: Briefly inspect all connections on tubing units as you perform steps 11.4, 11.5, and 11.7 to make sure tubing clamps are positioned properly and connections are tight to minimize chances of leaks.

    11.4. Remove cap from upper side filter port and attach filtrate tubing set (C). Remove the pipette end of the filtrate tubing set (C) from the zip-top bag and place it into the waste bucket

    11.5. Remove cap from bottom filter port (blue end) and twist luer lock connector of retentate tubing set (B) into bottom filter port (blue end). Leave the pipette end in clean zip-top bag until ready for use in step 13.

    11.6. Ensure valve is **CLOSED** on retentate tubing (B).

11.7. Remove cap from top filter port (orange end) and twist luer lock connector of sample tubing set (A) into top filter port (orange end). Leave the pipette end in clean zip-top bag until ready for use.

11.8. Load sample tubing set (A) into pump head, with pipette end remaining in clean zip-top bag. Adjust tubing to minimize slack between filter and pump head. Prevent kinks in the sample tubing set (A) by placing it over the metal peg board hook provided in your kit.

III. Prewash and sample concentration

12. The filtration process.

12.1. Loosen the cap of the 1-L bottle of filter **pre-wash** solution prepared above that corresponds with the sample to be filtered. Remove pipette end of sample tubing set (A) from zip-top bag, quickly flame sterilize using the lighter provided in your kit, and place into the **pre-wash** solution bottle that corresponds with the sample to be filtered. Take care not to melt the pipette during flame sterilization.

12.2. To wash residual calf serum out of filter, fully close the flow regulator on the retentate tubing set (B). Plug in pump. Turn the pump on using the switch on the back of the pump. Make sure the blue light indicating flow direction on the pump drive face is illuminated next to the picture with the arrow pointing towards the filter. Start pump by pushing the blue button on the far right hand side of the pump drive face. Using the up and down arrows, adjust the pump speed to 250. Be sure discharge is collected in disposal bucket or pumped to sewer. Once **pre-wash** bottle is empty, turn off pump by pressing the blue button on the far right hand side of the pump drive face.

12.3. Now you are ready to filter your sample.

12.4. Using lighter provided, quickly flame sterilize the pipette tip from the sample tubing set (A). Take care not to melt the pipette. Aseptically place the pipette end into the first carboy containing NaPP treated sample (which should already be located in your field kit from previous steps). Aseptically re-cap the **pre-wash** bottle for later use. Repeat this step for the retentate tubing set (B). Both the sample (A) and retentate tubing set (B) pipettes should now be in the sample carboy.

12.5. Place funnel into first carboy. Ensure blue flow regulator on retentate tubing set (B) is as tight as it will go.
Note: If funnel was recently cleaned, wipe funnel off with a clean Wypall-L40 to remove any excess bleach solution.

12.6. Turn on pump. Adjust pump speed to 450. If this pump speed causes cavitation or sample tubing begins to leak, reduce pump speed slowly until cavitation or leaking ceases (typically 400-425).
Note: Dead-end HFUF runs at a higher pressure, so watching for tubing leaks is crucial to not lose any sample.

12.7. Place a new sheet of aluminum foil over top of funnel while filtering.

12.8. Use funnel to transfer contents of the second carboy into the first sample carboy. **Note:** If funnel was previously used during this sampling period, rinse 3 times with current sample well water prior to this step to get rid of residual bleach from steps 14.4 and 14.6. Collect this rinse water in waste bucket or discharge directly to sewer.

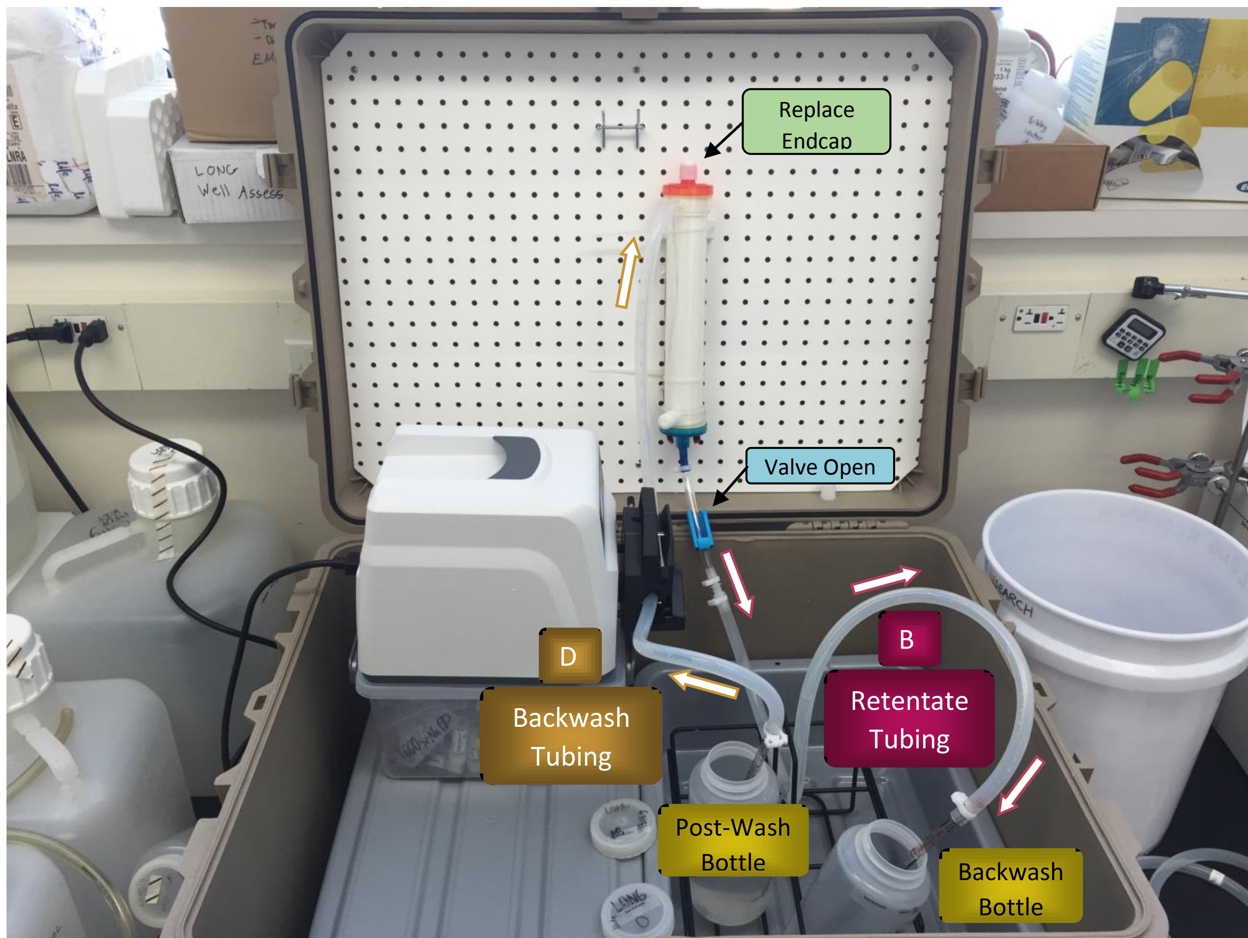
12.9. When waste bucket becomes full, switch to a second waste bucket while emptying the other.

12.10. Repeat steps 12.8 and 12.9 until all 100 L of volume has been transferred to the first carboy; continue filtering until volume in the first carboy reaches approximately 500-1000 mL. Turn off pump.

12.11. Swirl the remaining 500-1000mL around in the carboy to suspend and mix anything that may have settled during the concentration process. Transfer the remaining sample volume from the carboy into the empty **pre-wash** bottle (saved from above). **Be careful not to overfill the 1L pre-wash bottle if more than 1000mL is accidentally estimated**. Use a pipette bulb and 50 mL pipette tip to transfer remaining drops of sample from the carboy to the **pre-wash** bottle.

12.12. Turn on the pump and continue filtering all remaining sample (now in the **pre-wash** bottle). Turn off pump.

12.13. Release sample tubing (A) from the pump head. After inspecting the sample tubing (A) to make sure no spills will occur, detach the sample tubing (A) from filter and drain into **pre-wash** bottle. The volume in the pre-wash bottle should now be about 50-100 mL (a little more is not a problem as the amount remaining in the sample tubing may vary).
Note: When removing sample tubing from pump head, pressure built up from the concentration process may result in an “air burst” coming back from the filter through the tubing. Make sure to secure the tubing by holding the pipet end of the sample tubing in the **pre-wash bottle**.



13. The backwash process

13.1. To backwash remaining particles from filter: remove filtrate tubing (C) and replace with new sterile backwash tubing set (D). Thread tubing through peristaltic pump. Flame pipet without melting it and place into **post-wash** bottle.

13.2. Remove sample tubing (A), place it back in its zip top back for shipping to WSLH. Replace end cap onto top of filter.

13.3. Place retentate tubing (B) into backwash bottle and fully open flow regulator.

13.4. Ensure valve is **OPEN** on retentate tubing (B).

13.5. With pump off, adjust pump speed to **200 rpm.**

13.6. Turn on pump to pass **post-wash** through the filter and collect in backwash bottle. **Note:** Do not forget to reduce pump speed. If the pump is still set at concentration speed (400-450 rpm), the backwash tubing can be easily broken.

13.7. Turn off pump. Collect the fluid remaining in the filter and tubing sets. **Note:** When removing backwash tubing from the pump head, it is possible another "air burst" may occur. If fluid is released back from the filter into the tubing, remember to save this volume in the backwash bottle. Also, a small volume of post-wash solution may remain in the post-wash bottle. This is okay; do not save this volume (it can be dumped out in a sink/drain).

13.8. After draining the tubing sets, place them back into their original bags for shipping to WSLH for cleaning and future reuse. Cap **backwash** bottle tightly, place in cooler.

V. Post-Filtration and Clean-up or Preparing for a Second Well

14. Package samples and equipment for shipment to WSLH.

14.1. Put all used ATP sample bottles and Colilert sample bottles into sample cooler.

14.2. Put all used **pre-wash** bottles, **post-wash** bottles, **backwash** bottles and bags of tubing sets into sample cooler.

14.3. Place sanitary survey(s), HFUF bench sheet(s), test request form(s), supply re-stock sheet, and shipping cooler checklist in a zip-top-bag and place bag in sample cooler.

15. Equipment clean-up/re-use for second sample.

15.1. Using the cutters provided, cut the zip-ties you used to attach filter to mounting. Discard zip-ties and filter. Change gloves. Wipe gloves and peg board with antiseptic wipes.

15.2. Be sure all items in portable HFUF kit and sample cooler are secured for transport.

15.3. Upon returning to your office or laboratory, rinse carboys, 1 L sample transfer bottle, and funnel with tap water and place upside-down on clean Wypall L40s to dry.

15.4. Prior to re-use, use funnel to fill each carboy with 10 L of tap water solution containing approximately 5% bleach (*i.e.* 9.5 L water to 500 mL household strength bleach). Remove funnel, rinse with tap water, dry with clean Wypall L40, and cover each opening with an appropriately sized aluminum foil sheet provided in your kit. Cap carboys tightly, shake, and dump. Rinse insides of carboys 3 times with tap water. Carboys and funnel are now ready for field deployment. Rinse bottle with water flushed from the well several times before collecting sample.

15.5. Prior to re-use, fill 1 L sample transfer bottle with 500 mL of tap water solution containing approximately 5% bleach (*i.e.* 475 mL water to 25 mL household strength bleach). Cap bottle tightly, shake, and dump. Rinse insides of bottle 3 times with tap water. The bottle is now ready for field deployment. Rinse bottle bottle with water flushed from the well several times before using to collect sample.

15.6. If collecting multiple samples on the same day, Steps 15.3 through 15.5 can be done in the field using well water from the next sample location **after the initial ATP and coliform samples have been collected from that well**. Proceed to Step 10.4.

REFERENCES

Smith, C.M., and V.R. Hill. 2009. Dead-End Hollow-Fiber Ultrafiltration for Recovery of Diverse Microbes from Water. *Applied and Environmental Microbiology* 75(16): 5284-5289.

APPENDIX B

Tier 1 Assessment Only
ATP and Bacteria Grab Sampling Approach for RTCR Sites
June 1, 2016

The purpose of this procedure is to provide instructions to accurately collect ATP and bacteria grab samples at RTCR sites which have been selected for Tier 1 sampling only. The motivation for this method is to minimize past inconsistencies for ATP sampling and measurement.

Materials
One 5 L carboy (autoclaved or cleaned with bleach and tap water rinsed)
Two ATP collection bottles
Four bacteria collection bottle

Procedure
Depending on the configuration of the well that is being sampled, the sample approach will vary.

Please note the following on the sample collection/submission sheets:

1. Identify if the sample tap is immediately on or adjacent to the well head (approx. < 5 ft)
2. Determine if the well has been dormant or in continuous use. If the well has been dormant, document when the well pump last ran.
3. Identify if the system utilizes a pressure tank/storage vessel as part of the distribution system.
4. Match the system configuration with the table below and proceed to the appropriate step.

Case	Proceed to Step
Sample tap adjacent to well	5
Sample tap some distance away from well	11

Scenario 1: Sample tap is adjacent to well head:

5. If the well has a pressure tank before the sample tap (non-compliant system), purge the contents of the tank. Ensure the well pump turns on after the tank has been purged. If the well has a tap before a tank or does not have a tank, proceed to step 6.
6. Immediately upon well/pump start-up, collect the first 5L of well water into the clean 5L carboy. The well purge can now begin and should be sustained for 30 minutes or more (e.g. more time may be required to purge one entire well volume if desired, when calculated).
7. Shake/invert the 5L carboy 25 times to completely mix the contents.
8. Use the completely mixed 5L carboy to fill one ATP and two bacteria collection bottles. Ensure the bottles are labeled as “PRE”. Discard remaining sample in the 5L carboy. Save the carboy for use in step 11.

9. Allow well purge time to complete.
10. Completely rinse the inside of the 5L carboy three times with flushing/purging water from the well.
11. Fill the 5L carboy with well water. Shake/invert 25 times to completely mix the contents.
12. Use the completely mixed 5L carboy to fill another set of ATP and bacteria collection bottles (one ATP, two bacteria). Ensure the bottles are labeled with "POST".
13. Discard remaining volume in 5L carboy. The carboy should be cleaned for future uses.

Scenario 2: Sample tap is some distance away from well head:

14. If the well has a pressure tank before the sample tap (non-compliant system), purge the contents of the tank. Ensure the well pump turns on after the tank has been purged. If the well has a tap before a tank or does not have a tank, proceed to step 15.
15. Use the table below to determine which "case" your well falls into by identifying the set of information you know about the well.

Case	Proceed to Step
Known pump flow rate, pipe distance, and pipe diameter (must know all three!)	16
Unknown pump flow rate, **known** pipe distance	22

Known flow, distance, and diameter:

16. Estimate the distance of the sample tap from the well head (e.g. 25 ft, 50 ft, 100 ft, etc.)
17. Estimate the pipe diameter of the sample tap (e.g. 3/8", 1/2", etc.)
18. Using the flow rate, pipe distance, and pipe diameter, calculate the time required to purge the length of pipe before collecting the 5L sample. To calculate the time, use Equation 1 below:

Equation 1: Purge time calculation

$$Time = \frac{L * \left(\frac{d}{24}\right)^2 * 1410}{Q}$$

Where L = length of pipe from well to sample tap (feet)
d = pipe diameter (inch)
Q = flow rate of pump (gpm)
Time = purge time in seconds

19. Purge the well at the sample tap for the precise amount of time calculated in Step 18.
20. Immediately after the calculated time has passed, fill the 5L carboy.
21. Proceed to Step 7.

Unknown flow rate, known pipe distance:

22. Estimate the distance of the sample tap from the well head (e.g. 25 ft, 50 ft, 100 ft, etc.)
23. For every **foot** of distance, purge the well for time frame shown in the following table:

Pipe Dia	3/8”	1/2”	3/4”	1”
Purge Time (sec)	0.069	0.12	0.28	0.49

24. Immediately after the calculated time has passed, fill the 5L carboy.
25. Proceed to Step 7.

APPENDIX C

ESS MICRO METHOD 300

Total Coliform/*E.coli* Enzymatic Substrate

Colilert®, Colisure®, Colilert-18® in Presence Absence and Quanti-Tray® Formats SM9223B

Scope and Applicability
Summary of Method
Definitions
Interferences
Safety and Waste Management
Equipment
Reagents and Standards
Sample Handling and Preservation
Quality Control
Method Calibration
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Related Documents
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1.0 Scope and Application

1.1 The Safe Drinking Water Act and the Groundwater Rule require that all potable water be free of total coliform and *E.coli.*

1.2 The Beach Act requires recreational samples to be tested for either enterococci or *E.coli*. Wisconsin has adopted the *E.coli* standard and the Colilert® and Colilert-18® MPN methods are approved for this testing.

1.3 The method describes identifying total coliform/*E.coli* using the presence/absence and multi-well formats (MPN).

1.4 This procedure outlines the steps to simultaneously detect total coliform and *E.coli* in potable water, source water, recreational water, surface water and wastewater.

1.5 The Colisure® method can only be used for drinking water samples.

2.0 Summary of Method

2.1 The reagent is added to 100 ml of the sample.

2.2 The sample is then incubated for a specified time at 35° C ± 0.5° C.

2.3 A color change (from clear to yellow with Colilert® and Colilert-18® and from yellow to magenta for Colisure®) indicates the presence of total

coliform bacteria in the sample and is interpreted as "unsafe" for potable waters. If there is no color change, the sample is interpreted as "safe".

2.4 All unsafe samples are checked for the presence of fluorescence using a long wavelength UV light (366 nm). The presence of *E.coli* is indicated by a sky-blue fluorescence. If there is no fluorescence, the sample is absent for *E.coli.*

2.5 From a Quanti-tray ® the number of total coliform positive wells and/or the number of fluorescence wells (*E.coli)* are counted. Quanti-tray® results are reported as a most probable number (MPN) according to a statistically derived number using the manufacturer's provided chart or software.

3.0 Regulatory Deviations

3.1 The deviations are listed in Section 9.0.

4.0 Definitions

4.1 Total Coliform is defined with this method as ortho-nitrophenyl-ß-D-galactopyranoside (ONPG) or chlorophenol red-ß-D-galactopyranoside (CPRG) being hydrolyzed by the ß-D-galactosidase enzyme which is produced by total coliform and creates a color change in the sample.

4.2 *E.coli* is defined with this method as 4-methylumbelliferyl-ß-D-glucuronide (MUG) hydrolyzed by ß-glucoronidase which is produced by *E.coli* and produces a fluorescent blue that can be view with a long wavelength (UV) light.

4.3 MERI – Madison Energy Recovery, Inc

5.0 Interferences

5.1 Samples that are extremely turbid or contain high iron content could interfere with the color change for Colilert® and Colilert-18®. These samples will be tested with Colisure®.

5.2 The test should not be performed if chlorine is present in the sample. The suspect sample will be shaken 25 times and the excess poured into a clean bottle. The presence of chlorine is checked by adding a small amount (about 3 drops) of DPD to the excess sample. The development of a pink color indicates the presence of chlorine.

5.3 Samples with a heterotrophic plate count of more than 20,000/1 mL before reagent is added may cause a false-positive test.

5.4 Samples that result in colors other than method-specific color change will be rejected and a new sample will be requested from the utility or source.

6.0 Safety, Waste Management and Pollution Prevention

6.1 All samples and cultures may contain potentially harmful pathogenic organisms. Care must be taken not to contaminate work area, other staff or one self. All spills must be decontaminated with Wescodyne solution using the following procedure:

6.1.1 Place a paper towel over the spill.

6.1.2 Pour Wescodyne over the entire spill without excessive splashing.

6.1.3 Let Wescodyne sit on the spill for at least 5 minutes before wiping up and/or sweeping up the spill.

6.1.4 If broken glass is involved, sweep up with a broom and discard in the red sharps container.

6.1.5 While wearing gloves, wipe up the liquid with paper toweling and discard in the MERI barrel.

6.2 Dispose of any cultures or media containing cultures in the MERI Barrel or dish pans to be autoclaved before disposal.

6.3 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

6.4 Solutions and reagents are prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be discarded.

6.5 General safety practices for laboratory operations are outlined in the Chemical Hygiene Plan for the Agriculture Drive facility (ref. 16.5).

6.6 All laboratory waste, excess reagents and samples must be disposed of in a manner consistent with applicable rules and regulations.

6.7 Waste disposal guidelines are described in the University of Wisconsin "Laboratory Safety Guide" (ref. 16.6). Specific waste disposal guidelines are detailed in the Environmental Health Division's "Waste Management" SOP (ref. 16.7).

7.0 Equipment and Supplies

7.1 35º C incubator

7.2 150 mL clear bottles with or without sodium thiosulfate

7.3 Quanti-Tray/2000® vessels for MPNs

7.4 6 watt long wavelength (366 nm) UV light

7.5 IDEXX MPN chart or IDEXX software (IDEXX MPN 3.1)

7.6 Quanti-Tray/2000® Sealer

8.0 Reagents and Standards

8.1 Colilert®, Colilert-18®, Colisure® stored at room temperature and used before manufacturer's expiration date

8.2 Colilert® and Colilert-18® comparator

8.3 99 mL deionized water blanks

9.0 Sample Collection, Preservation, Shipping, Handling and Storage

9.1 Samples are shipped at ambient temperatures for potable drinking water samples. All samples should be tested within 30 hours of collection for public water systems. Samples for private wells are tested up to 2 days after collection. There is no regulated holding times for private samples except for new wells. The holding time for new wells is 30 hours.

9.2 "No test" situations" for drinking water samples:

9.2.1 If the sample is over 30 hours for public water systems, the sample is not tested. Samples over 2 days for private water systems are not tested.

9.2.2 Frozen samples.

9.2.3 Chlorine present in sample.

9.2.4 Sample volumes less than 98 mL.

9.3 Samples for surface or recreational waters and wastewater are shipped on ice and if the ice is melted, a temperature is taken. If the temperature is greater than 10º C, the sample is reported out as sample received warm or not tested.

9.4 The holding time for surface or recreational waters and wastewater is 6 hours from the time of collection until receipt at the lab or 8 hrs until sample is put in the incubator. Since this is not possible in most cases the data is flagged when the sample is tested over 8 hours with sample received after 6 hours.

9.5 "No test situations" for surface or recreational waters and wastewater:

9.5.1 Frozen samples may not be tested.

9.5.2 Sample volumes less than 98 mL.

9.5.3 Samples older than 1 day unless the sample is collected by the USGS. USGS requests samples to be tested no matter how old.

9.5.4 Chlorine present.

10.0 Quality Control

10.1 Please refer to the Environmental Health Division Quality Assurance Manual (ref. 16.3) for general information on quality control procedures.

10.2 Each new lot of reagent is QC'd when received (ref. 16.8)

10.3 Each new box of bottles and Quanti-Trays® are checked for sterility, volume and fluorescence when received (ref. 16.9, 16.10)

10.4 The Quanti-Tray sealer is checked for proper sealing monthly (ref. 16.11).

10.5 Reagent is stored away from light and kept at 4-30ºC.

10.6 Each lot of reagent is tested with positive and negative cultures when received and on a monthly basis. If correct reactions are not observed, the new or existing lots are not used for analysis. (ref. 16.8)

10.7 Stock cultures are checked for purity and performance. (ref. 16.13)

10.8 If dilutions are required for this method, one mL of sample is put into a 99 mL deionized dilution blank for a two log reduction and if further reduction is required the sequence is performed again with the inoculated 99 mL dilution blank, and each consecutive 99 mL dilution blank until a correct dilution is obtained.

11.0 Method Calibration and Standardization

11.1 Incubator temperatures are recorded twice daily during business days and once on weekends to insure temperature is within limits.

11.2 Thermometers are calibrated each calendar year with a NIST thermometer or NIST traceable thermometer. No mercury thermometers are used.

12.0 Procedure

12.1 Thoroughly mix sample by shaking vigorously 25 times.

12.2 Pour off sample to 100 mL ± 2 mL (top of WSLH logo on bottle).

12.3 Aseptically transfer contents of reagent into bottle.

12.4 Close cap tightly and shake to dissolve reagent.

12.5 Label cap with ID if identification of total coliform is requested.

12.6 If a client requests "numbers" or "counts" use the Quanti-Tray/2000® (QT) method:

12.6.1 Label the Quanti-Tray/2000® with the sample number and ID, if requested.

12.6.2 Pour off sample to 100 mL and add reagent, wait for reagent to dissolve.

12.6.3 Aseptically add sample to QT and run through sealer according to manufacturer instructions.

12.6.4 100 mL and 0.01 mL of sample are performed on surface water samples unless requiring more dilutions and 100 mL only are performed on beach samples.

12.7 For both presence/absence and QT samples you must create and clone a batch for sample data management into the Chemware/Horizon system and print a label with the HBN #, HBN barcode, analyst initials and date/time. Place the label on the last bottle in the batch. See ref. 16.12.

12.8 Place samples into 35ºC incubator and incubate according to the chart below (Table 1-section 17.1)

12.9 Record sample sequence numbers, time and analyst's initials in correct logbook. This process is used for analyst ease in finding samples and logging samples out since data is currently managed by the Chemware Horizon system.

12.10 Place racks on shelves corresponding to day of week and sample type.

12.11 Place racks on shelves so that first sample in sequence (usually the smallest number) is at the back of the shelf and all the way to the right and the last sample in the sequence (usually the largest number) is toward the front of the shelf and all the way to the left. If sample(s) is (are) QT(s), simply place the QTs on shelf.

12.12 Label last rack in sequence or QT with the time placed in the incubator.

12.13 Results are read after specified incubation times using the following criteria in chart below. See "Allowable Read-Out Times" chart for specific set-up read-out times. (Table 2-section 17.2)

12.14 Record in the proper logbook the time and initials when samples are read out.

12.15 All total coliform positive samples are screened for the presence of *E.coli* by turning the incubator light off and placing the sample 5 inches from a 366 nm, long wavelength UV light in the darkened room. Blue fluorescence indicates the presence of *E.coli.*

12.16 The sample results are recorded using the analytical batch in the Chemware/Horizon software as total coliform present or absent, with *E.coli* present or absent (ref. 16.12). When there is a total coliform positive or total coliform and *E. coli* positive sample, it is entered into the analytical batch and the data review must be performed by a second analyst. If all sample results are negative for total coliform and *E. coli* the data review can be performed by the same initial analyst.

12.17 For QTs, the number of chromogenic/fluorescent large and small wells is counted and recorded in the analytical batch. Chemware/Horizon will calculate the MPN according to the IDEXX™ chart provided or software. If the result is associated with a dilution of the sample either change the initial volume or the dilution factor before recording large and small wells. Results of each dilution not used for the final result for total coliform and E. coli are recorded in the "comment" section of the analytical batch. The purpose is to maintain an electronic record that the analysis had been conducted.

12.18 After the results are posted the Quality Control Report is generated and the analyst reviews the results that will be reported to the client. Corrections to results are made before the data review step is performed.

12.19 After the results are accepted the samples are thrown in the MERI barrel for disposal.

13.0 Data Analysis and Calculations

13.1 Presence/absence is reported for most drinking water samples.

13.2 For all Quanti-Tray methods if 100 mL of sample is used, the MPN (most probable number) is generated by Chemware/Horizon from the IDEXX chart or software. The MPN is check periodically to assure the correct number is generated.

13.3 If there are dilutions the MPN is determined by:

13.3.1 Total coliform or *E.coli* / 100 mL = (MPN from chart or software X 100)/volume per mL analyzed.

Example:

MPN = 24
Volume analyzed = 0.01 mL
Total coliform or *E.coli*/ 100 mL = (24 X 100)/0.01 = 240,000

Total coliform or *E.coli* MPN/ 100 mL = 240,000

13.3.2 Alternative determination is by logs per 100 mL:

- The MPN is determined by IDEXX software or chart and Table 3.
- The number of zeros is added based on the reverse of the log of ten per 100 mL.

Example:

MPN = 24
The volume used is 0.01 which is minus two logs per 1 ml or minus 4 logs per 100 mL.
So 4 zero's will be added to the result.
24 + 0000
240,000

14.0 Method Performance

14.1 The detection limit is one total coliform/*E.coli* per 100 mL.

14.2 False-positive or false-negative rates are given in the Federal Register when the methods were approved. False positive or false negative rates have not been determined for WSLH samples but the lab has passed most of the proficiency samples for these methods.

15.0 Data Assessment and Management

15.1 Samples must be incubated within the stated time parameters.

15.1.1 Corrective action: If a sample is incubated longer than stated time parameters and the sample is negative, the sample may be reported out as safe with a disclaimer on the report stating the sample was not incubated within time constraints.

15.1.2 Corrective Action: If samples are positive when incubated over the time parameters, the results are reported as lab accidents.

15.2 Other than for incubation warm up, samples were temperatures were not within limits, the results are flagged with a disclaimer and reported out (±2ºC) or the samples are reported as lab accidents.

15.3 Any other data that doesn't meet quality control standards during the testing process will be reported and flagged or the results invalidated.

16.0 Related Documents

16.1 Federal Register, National Primary and Secondary Drinking Water Regulations: Analytical Methods for Chemical & Microbiological Contaminants and Revisions to Laboratory Certification Requirements; Final Rule, 40 CFR parts 141 and 143, Vol. 64, No 230

16.2 APHA, 2005. *Standard Methods for the Examination of Water and Wastewater,* 21st Edition.

16.3 Environmental Health Division Quality Assurance Manual, Wisconsin State Laboratory of Hygiene.

16.4 2009 TNI Standard, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis, The NELAC Institute, 2009.

16.5 Wisconsin State Laboratory of Hygiene, AD Safety GENOP 102, Chemical Hygiene Plan and General Laboratory Safety Plan for the Agriculture Drive Facility, State Laboratory of Hygiene.

16.6 University of Wisconsin—Madison, Chemical & Radiation Protection Office, Safety Department (262-8769), "Laboratory Safety Guide," 2004, http://www.fpm.wisc.edu/safety

16.7 EHD GENOP 038, "Waste Management," Environmental Health Division, Wisconsin State Laboratory of Hygiene."

16.8 ESS MICRO QA 202, "Colilert®, Colilert-18 ®, Colisure ®, & Colitag ™ Quality," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.9 ESS MICRO QA 212, "Sample Bottle Sterility/Calibration/Fluorescence," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.10 ESS MICRO QA 214, "Quanti-Tray ® Sterility Check," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.11 ESS MICRO QA 218, "Quanti-Tray ® Sealer Check," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.12 ESS MICRO GENOP 411, "Cheware/Horizon Process for Analytical Testing," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.13 ESS MICRO QA 206, "Maintenance of Stock Cultures for Quality Control"

17.0 Tables and figures

17.1 Table 1—Color Change:

Reagent	**Incubation time**	**Safe**	**Unsafe**
Colilert®	24-28 hrs	Clear	Yellow*
Colilert-18®	18-22 hrs	Clear	Yellow*
Colisure®	24-48 hrs	Yellow	Magenta*

*****Color must be equal to or greater than the comparator for Colilert® and Colilert-18®. If colors are border-line, the sample may be incubated for up to 28 hours for Colilert® and 22 hours for Colilert-18®. If color is still lighter than the comparator after additional incubation, the samples are reported as safe. If the color change is indeterminate, invalidate the sample for any of the methods.

17.2 Table 2 – Readout times

Setup Time **Military Time**	**Colilert® read time -** **Next day: 24-28 hrs**	**Colilert-18® read time – Next day: 18 – 22 hrs**	**Colisure® read time – Next day to following day: 24-28 hrs**
0700	0700 - 1100	0100 - 0500	0700 – 0700 next day
0800	0800 - 1200	0200 - 0600	0800 – 0800 next day
0900	0900 - 1300	0300 - 0700	0900 – 0900 next day
1000	1000 - 1400	0400 - 0800	1000 – 1000 next day
1100	1100 – 1500	0500 - 0900	1100 – 1100 next day
1200	1200 - 1600	0600 - 1000	1200 – 1200 next day
1300	1300 – 1700	0700 - 1100	1300 – 1300 next day
1400	1400 – 1800	0800 - 1200	1400 – 1400 next day
1500	1500 - 1900	0900 - 1300	1500 – 1500 next day
1600	1600 - 2000	1000 - 1400	1600 – 1600 next day
1700	1700 - 2100	1100 - 1500	1700 – 1700 next day

17.3 Table 3 – Zero's added per 100 mL

Volume used	**Log**	**Zero added to result**
100 mL (0)	10^{2}	0
1 mL (2)	10^{0}	2
0.01 (4)	10^{-2}	4
0.0001 (6)	10^{-4}	6

18.0 Revision Tracking Table

Revision number	Revision date	Changes Made	Revision author
	12/01/2009	Add the recording of Total Coliform and *E.coli* per Groundwater Rule Changed Format to Current WSLH format for SOPs Added Table 3 to SOP	
	1/19/2009	Added action under "Interferences" regarding sample rejection due to atypical l results	
6	12/17/2012	In section 9.2.1—changed 48 hrs to 30 hrs for age of public water system samples not to be tested. In section 10.6—added testing media when received and on a monthly basis. In section 12—added info about using the Chemware/Horizon system Re-formatted	J. Olstadt

Signature Page

Written by: Jeremy Olstadt Date: 12/03/2012

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Reviewed by: Susan D. Hill Date: 12/11/12

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Approved by: Sharon Kluender Date: 1/8/2013

Title: Microbiology Supervisor

Dept: Water Microbiology

ANALYST CERTIFICATION STATEMENT

"I have read, understand and agree to perform the current revision of this method."

ESS MICRO METHOD 300, "Total Coliform/E. coli Enzymatic Substrate," Revision 6

ANALYST NAME	ANALYST SIGNATURE	DATE

APPENDIX D

ESS MICRO METHOD 356
Enterococci
(Fluorogenic Substrate)
Enterolert and Quanti-Tray/2000 Method
(Federal Register, July 21, 2003 Vol 68, No 39, pp 43271-43283)

Scope and Applicability
Summary of Method
Deviations
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1.0 Scope and Application

1.1 Enterococci is an indicator of fecal contamination.

1.2 This method can be used for drinking water, surface water and wastewater.

1.3 The method can be used for both presence/absence and quantitative by Quanti-tray™2000 (QT) for drinking water and only the Quanti-tray™2000 for surface and drinking water.

2.0 Summary of Method

2.1 The reagent Enterolert™ is added to 100 mL of sample.

2.2 The sample is incubated for 24 – 28 hrs at 41±0.5°C.

2.3 All samples are checked for the presence of enterococci using a long-wavelength UV light (366 nm). The presence of enterococci is indicated by a sky-blue fluorescence. If QT is used, a MPN is determined and reported.

2.4 If there is no fluorescence, the sample is absent for enterococci.

2.5 If there is fluorescence, the sample is reported present for presence/absence samples and a MPN is given for quantitative samples based on the number of positive well on a QT from an IDEXX chart or software.

3.0 Regulatory Deviations

3.1 On weekends the samples may be not be incubated 24 hours or incubated over 28 hours. The results will be flagged with a disclaimer.

3.2 Other deviations are listed in Section 9.0

4.0 Definitions

4.1 Enterococci are defined as 4-methylumbelliferyl-ß-D-glucoronide (MUG) being hydrolyzed by ß-glucoronidase which is produced by enterococci and produces a fluorescent blue that can be view with a long-wavelength (365 nM) UV light.

4.2 MPN – Most Probable Number

4.3 QT – Quanti-tray™2000

5.0 Interferences

5.1 The test should not be performed if chlorine is present in the sample. The suspect sample will be shaken 25 times and the excess poured into a clean bottle. The presence of chlorine is checked by adding a small amount (about 3 drops) of DPD to the excess sample. The development of a pink color indicates the presence of chlorine.

5.2 Samples with a heterotrophic plate count of more than 20,000/1 mL before reagent is added may cause a false-positive test.

6.0 Safety, Waste Management and Pollution Prevention

6.1 All samples and cultures may contain potentially harmful pathogenic organisms. Care must be taken not to contaminate work area, other staff or one self. All spills must be decontaminated with Wescodyne solution using the following procedure:

6.1.1 Place a paper towel over the spill.

6.1.2 Pour Wescodyne over the entire spill without excessive splashing.

6.1.3 Let Wescodyne sit on the spill for at least 5 minutes before wiping up and/or sweeping up the spill.

6.1.4 If broken glass is involved, sweep up with a broom and discard in the red sharps container.

6.1.5 While wearing gloves, wipe up the liquid with paper toweling and discard in the MERI barrel.

6.2 Dispose of any cultures or media containing cultures in the MERI Barrel or dish pans to be autoclaved before disposal.

6.3 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

6.4 Solutions and reagents are prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be discarded.

6.5 General safety practices for laboratory operations are outlined in the Chemical Hygiene Plan for the Agriculture Drive facility (ref. 16.5).

6.6 All laboratory waste, excess reagents and samples must be disposed of in a manner consistent with applicable rules and regulations.

6.7 Waste disposal guidelines are described in the University of Wisconsin "Laboratory Safety Guide". Specific waste disposal guidelines are detailed in the Environmental Health Division's "Waste Management" SOP (ref. 16.7).

7.0 Equipment and Supplies

7.1 41°C incubator

7.2 35°C incubator used for dark room

7.3 150 mL bottle with or without sodium thiosulfate

7.4 UV long wavelength light (365 nm)

7.5 Quanti-tray™2000

7.6 Quanti-tray™ sealer

7.7 IDEXX MPN chart or software

7.8 Pipettes if dilutions are need

8.0 Reagents and Standards

8.1 Enterolert™

8.2 99 mL Sterile deionized water if dilutions are needed.

9.0 Sample Collection, Preservation, Shipping, Handling and Storage

9.1 All samples and cultures may contain potentially harmful pathogenic organisms. Care must be taken not to contaminate work area, other staff or one self. All spills must be decontaminated with Wescodyne solution using the following procedure:

9.1.1 Place a paper towel over the spill.

9.1.2 Pour Wescodyne over the entire spill without excessive splashing.

9.1.3 Let Wescodyne sit on the spill for at least 5 minutes before wiping up and/or sweeping up the spill.

9.1.4 If broken glass is involved, sweep up with a broom and discard in the red sharps container.

9.1.5 While wearing gloves, wipe up the liquid with paper toweling and discard in the MERI barrel.

9.2 Dispose of any cultures or media containing cultures in the MERI Barrel or dish pans to be autoclaved before disposal.

9.3 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

9.4 Solutions and reagents are prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be discarded.

9.5 General safety practices for laboratory operations are outlined in the Chemical Hygiene Plan for the Agriculture Drive facility (ref. 16.5).

9.6 All laboratory waste, excess reagents and samples must be disposed of in a manner consistent with applicable rules and regulations.

9.7 Waste disposal guidelines are described in the University of Wisconsin "Laboratory Safety Guide" (ref. 16.6). Specific waste disposal guidelines are detailed in the Environmental Health Division's "Waste Management" SOP (ref. 16.7).

10.0 Quality Control

10.1 Please refer to the Environmental Health Division Quality Assurance Manual (ref. 16.3) for general information on quality control procedures.

10.2 Each new lot of reagent is QC'd when received (ref. 16.8)

10.3 Each new box of bottles and Quanti-Trays® are checked for sterility, volume and fluorescence when received (ref. 16.9, 16.10).

10.4 The Quanti-Tray sealer is checked for proper sealing monthly (ref. 16.11).

10.5 Reagent is stored away from light and kept at 4-30ºC.

10.6 Each lot of reagent is tested with positive and negative cultures when received and on a monthly basis. If correct reactions are not observed, the new or existing lots are not used for analysis (ref. 16.8).

10.7 Stock cultures are checked for purity and performance (ref. 16.13).

10.8 If dilutions are required for this method, one mL of sample is put into a 99 mL deionized dilution blank for a two log reduction and if further reduction is required the sequence is performed again with the inoculated 99 mL dilution blank, and each consecutive 99 mL dilution blank until a correct dilution is obtained.

11.0 Method Calibration and Standardization

11.1 Incubator temperatures are recorded twice daily during business days and once on weekends to insure temperature is within limits.

11.2 Thermometers are calibrated each calendar year with a NIST thermometer or NIST traceable thermometer. No mercury thermometers are used.

12.0 Procedure

12.1 Thoroughly mix sample by shaking vigorously 25 times.

12.2 Pour off sample to 100 mL ± 2 mL (top of WSLH logo on bottle).

12.3 Aseptically transfer contents of reagent into bottle.

12.4 Close cap tightly and shake to dissolve reagent.

12.5 If a client requests “numbers” or “counts” use the Quanti-Tray/2000® (QT) method:

12.5.1 Label the Quanti-Tray/2000® with the sample number.

12.5.2 Pour off sample to 100 mL and add reagent, wait for reagent to dissolve.

12.5.3 Aseptically add sample to QT and run through sealer according to manufacturer instructions.

12.5.4 100 mL and 0.01 mL of sample are performed on surface water samples unless requiring more dilutions and 100 mL is the only dilution performed on beach samples.

12.6 For both presence/absence and QT samples you must create and clone a batch for sample data management into the Chemware/Horizon system and print a label with the HBN #, HBN barcode, analyst initials and date/time. Place the label on the last bottle in the batch. See ref. 16.12.

12.7 Place samples into 41ºC incubator and incubate according to the chart below (Table 1-section 17.1)

12.8 Record sample sequence numbers, time and analyst's initials in correct logbook. This process is used for analyst ease in finding samples and logging samples out since data is currently managed by the Chemware Horizon system.

12.9 Place racks on shelves so that first sample in sequence (usually the smallest number) is at the back of the shelf and all the way to the right and the last sample in the sequence (usually the largest number) is toward the front of the shelf and all the way to the left. If sample(s) is (are) QT(s), simply place the QTs on shelf.

12.10 Label last rack in sequence or QT with the time placed in the incubator.

12.11 Results are read after specified incubation times using the following criteria in chart below. See "Allowable Read-Out Times" chart for specific set-up read-out times. (Table 2-section 0)

12.12 Record in the proper logbook the time and initials when samples are read out.

12.13 All samples are screened for the presence of enterococci by turning the 35° C incubator light off and placing the sample 5 inches from a 366 nm, long wavelength UV light in the darkened room. Blue fluorescence indicates the presence of enterococci.

12.14 The sample results are recorded using the analytical batch in the Chemware/Horizon software as enterococci present or absent (ref. 16.12).

12.15 For QTs, the number of fluorescent large and small wells is counted and recorded in the analytical batch. Chemware/Horizon will calculate the MPN according to the IDEXX™ chart provided or software. If the result is associated with a dilution of the sample either change the initial volume or the dilution factor before recording large and small wells. Results of each dilution not used for the final result for enterococci are recorded in the

"comment" section of the analytical batch. The purpose is to maintain an electronic record that the analysis had been conducted.

12.16 After the results are posted, the Quality Control Report is generated and the analyst reviews the results that will be reported to the client. Corrections to results are made before the data review step is performed.

12.17 After the results are accepted, the samples are thrown in the MERI barrel for disposal.

13.0 Data Analysis and Calculations

13.1 Presence/absence is reported for most drinking water samples.

13.2 For all Quanti-Tray methods if 100 mL of sample is used, the MPN (most probable number) is generated by Chemware/Horizon from the IDEXX chart or software. The MPN is checked periodically to assure the correct number is generated.

13.3 If there are dilutions the MPN is determined by:

13.3.1 Enterococci / 100 mL = (MPN from chart or software X 100)/volume per mL analyzed.

Example:

MPN = 24
Volume analyzed = 0.01 mL
Enterococci/ 100 mL = (24 X 100)/0.01 = 240,000
Enterococci/ 100 mL = 240,000

13.3.2 Alternative determination is by logs per 100 mL:

- The MPN is determined by IDEXX software or chart and Table 3.
- The number of zeros is added based on the reverse of the log of ten per 100 mL.

Example:

MPN = 24
The volume used is 0.01 which is minus two logs per 1 ml or minus 4 logs per 100 mL.
So 4 zero's will be added to the result.
24 + 0000
240,000

14.0 Method Performance

14.1 The detection limit is one enterococci *per* 100 mL.

14.2 False-positive or false-negative rates are given in the Federal Register when the methods were approved. False positive or false negative rates have not been determined for WSLH samples but the lab has passed most of the proficiency samples for these methods.

15.0 Data Assessment and Management

15.1 Samples must be incubated within the stated time parameters.

15.1.1 Corrective action: If a sample is incubated longer than stated time parameters and the sample is negative, the sample may be reported out as safe with a disclaimer on the report stating the sample was not incubated within time constraints.

15.1.2 Corrective Action: If samples are positive when incubated over the time parameters, the results are reported as lab accidents.

15.2 Other than for incubation warm up, samples where temperatures were not within limits, the results are flagged with a disclaimer and reported out (±2ºC) or the samples are reported as lab accidents.

15.3 Any other data that doesn't meet quality control standards during the testing process will be reported and flagged or the results invalidated.

16.0 Related Documents

16.1 Applied and Environmental Microbiology, "Evaluation of Enterolert™ in Recreational Waters", Gary E. Budnick, Robert T. Howard and Donald R. Mayo, App and Env Microm Vol 62. No. 10, Oct 1990, p3881-3884

16.2 APHA, 2005. *Standard Methods for the Examination of Water and Wastewater,* 21st Edition.

16.3 Environmental Health Division Quality Assurance Manual, Wisconsin State Laboratory of Hygiene.

16.4 2009 TNI Standard, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis, The NELAC Institute, 2009.

16.5 Wisconsin State Laboratory of Hygiene, AD Safety GENOP 102, Chemical Hygiene Plan and General Laboratory Safety Plan for the Agriculture Drive Facility, State Laboratory of Hygiene.

16.6 University of Wisconsin—Madison, Chemical & Radiation Protection Office, Safety Department (262-8769), "Laboratory Safety Guide," 2004, http://www.fpm.wisc.edu/safety

16.7 EHD GENOP 038, "Waste Management," Environmental Health Division, Wisconsin State Laboratory of Hygiene."

16.8 ESS MICRO QA 202, "Colilert®, Colilert-18 ®, Colisure ®, & Colitag ™ Quality," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.9 ESS MICRO QA 212, "Sample Bottle Sterility/Calibration/Fluorescence," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.10 ESS MICRO QA 214, "Quanti-Tray ® Sterility Check," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.11 ESS MICRO QA 218, "Quanti-Tray ® Sealer Check," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.12 ESS MICRO GENOP 411, "Cheware/Horizon Process for Analytical Testing," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

16.13 ESS MICRO QA 206, "Maintenance of Stock Cultures for Quality Control"

17.0 Tables and figures

17.1 Table 1—Fluorescent:

Reagent	Incubation time	Absent	Present
Enterolert™	24-28 hrs	Np Fluorescence	Fluorescence

17.2 Table 2 – Readout times

Setup Time **Military Time**	**Enterolert® read time -** **Next day: 24-28 hrs**
0700	0700 - 1100
0800	0800 - 1200
0900	0900 - 1300
1000	1000 - 1400
1100	1100 – 1500
1200	1200 - 1600
1300	1300 – 1700
1400	1400 – 1800
1500	1500 - 1900
1600	1600 - 2000
1700	1700 - 2100

17.3 Table 3 – Zero's added per 100 mL

Volume used	**Log**	**Zero added to result**
100 mL (0)	10^{2}	0
1 mL (2)	10^{0}	2
0.01 (4)	10^{-2}	4
0.0001 (6)	10^{-4}	6

18.0 Revision Tracking Table

Revision number	**Revision date**	**Changes Made**	**Revision author**
	12/15/2012	Added Chemware/Horizon Process for Analytical Testing	J. Olstadt
	12/15/2012	Changed Format to Current WSLH Format	J. Olstadt
	12/15/2012	Added Tables 1 through 3 to SOP	J. Olstadt
	12/15/2015	Changed hold time for public water samples	J. Olstadt

		from 48 hours to 30 hours	

Signature Page

Written By: Jeremy Olstadt__________ Date: ___12/15/12__
Title:__Advanced Microbiologist__________
Unit:__Water Micro.____________________

Reviewed by:__Susan D. Hill________ Date:__02/12/2013___
Title:__QA Coordinator___________
Unit:__EHD____________________

Approved by:__Sharon Kluender___________ Date:__02/13/2013___
Title:__Micro Supervisor________________
Unit:__Water Micro____________________

ANALYST CERTIFICATION STATEMENT

"I have read, understand and agree to perform the current revision of this method."

ESS MICRO METHOD 356, Revision 3

ANALYST NAME	ANALYST SIGNATURE	DATE

APPENDIX E

ESS MICRO METHOD 307
Microbial Equivalence by ATP Assay

Scope and Applicability
Summary of Method
Definitions
Safety and Waste Management
Sample Handling and Preservation
Interferences
Reagents and Standards
Equipment
Quality Control
Method Calibration
Procedure
Calculations
Data Management
Method Performance
Related Documents
Tables and Figures
Signatures
Certification Statement

1.0 Scope and Application

1.1 ATP (adenosine triphosphate) measures living microbiological activity. This method is used for drinking water samples where a biofilm is suspected. The method may also be used for other matrices like soil.

1.2 The ATP is measured with PhotonMaster Luminometer using a firefly luciferase assay. The limit of detection per manufacturer of the PhotonMaster Luminometer is 0.1 pg ATP/mL.

1.3 When 0.5 pg ATP/mL is assayed, this is comparable to a heterotrophic plate count of 500 cfu/mL.

1.4 A biofilm may be present when ATP is greater than 0.5 pg ATP/mL.

2.0 Summary of Method

2.1 This method measures ATP using a firefly luciferase assay measured with a PhotonMaster Luminometer.

2.2 The water sample is slowly filtered through 0.7 µm glass filter using a 60 mL syringe. Discard the filtrate. Keep track of the **total** volume of sample filtered.

2.3 The filter is washed with 4 ml LumiClean™.

2.4 Elute the ATP off the filter by using 1 ml UltraLyse 7™and place this elute/ATP solution back into the original sample container Roll to coat all surfaces of the container with the liquid. After at least five minutes the filter and original container is rinsed with 9 mL UltraLute™ (Dilution).

2.5 The dilution is mixed 3 times and 100 µL from the container is pipetted into an assay tube. Also added to the assay tube is 100 µL of the enzyme Luminase™ what causes a reaction with the ATP, oxygen and luciferin to

produce AMP, PPi, oxyluciferin and light. This allows the relative light units to be measured.

2.6 The tube is gently swirled and immediately inserted into PhotonMaster Luminometer to measure the relative light units (RLU).

2.7 The RLU_{cATP} (cellular ATP) and pg/mL are reported in LumiCalc™. The result is written on the bench sheet once the RLU is read.

2.8 The results are recorded on bench sheet to be reported in Horizon/Chemware.

2.9 The ATP concentration is automatically calculated in the LumiCalc™ software in pg/ml.

2.10 The pg/ml is converted to microbial equivalents in Horizon/Chemware.

3.0 Regulatory Deviations

3.1 This method is not used for any regulatory purposes but general requirements of NELAC accreditation are followed.

3.2 The lab uses this method to determine if a water system has a biofilm.

3.3 The method was developed by Andy Jacque and is unpublished.

4.0 Definitions

4.1 ATP - Adenosine triphosphate

4.2 AMP – Adenosine monophosphate

4.3 RLU – Relative Light Units

4.4 PPi – Pixels per inch or resolution

4.5 $_{c}$ATP – Cellular ATP

4.6 Biofilm – Large numbers of microbial cells that stick together and attached to surfaces.

4.7 pg – picogram

4.8 fg – femtogram

4.9 ME – microbial equivalents

4.10 Microbial equivalents – one *E. coli* sized bacteria contains 0.001 pg of ATP. Based on this calculation an estimate of culturable bacteria is obtained.

4.11 Other definitions are listed in the QA Manual

5.0 Interferences

5.1 No known interferences.

6.0 Safety, Waste Management and Pollution Prevention

6.1 The reagents used may cause skin irritation, so gloves and lab coats must be worn when performing the analyses.

6.2 General safety practices for laboratory operations are outlined in the Chemical Hygiene Plan for the Environmental Health Division. (ref 16.5)

6.3 All laboratory waste, excess reagents and samples must be disposed of in a manner consistent with applicable rules and regulations. Verbal communication with UW Safety – all reagents may be flushed down the drain.

6.4 Waste disposal guidelines are described in the University of Wisconsin "Laboratory Safety Guide". (ref 16.6)

6.5 Specific waste disposal guidelines are detailed in the EHD GENOP 038 "Waste Management," Environmental Health Division, Wisconsin State Laboratory of Hygiene." (16.7)

7.0 Equipment and Supplies

7.1 PhotonMaster Luminometer

7.2 Computer with LumiCalc software

7.3 Refrigerator

7.4 Ice paks

7.5 Sterile 150 mL polystyrene or 250 mL polypropylene bottles

7.6 Gloves

7.7 Lab coats

7.8 Pipet filler or pipette bulb

7.9 Micropipettor(s) – for 100 µL and 1000 µL

7.10 Wypall L40 wipes (absorbent laboratory diapers)

7.11 10% bleach/water solution

7.12 70% ethanol solution

7.13 12 x 55 mm test tubes (assay tubes)

7.14 100 µL and 1000 µL sterile pipette tips

7.15 5 mL or 10 mL sterile pipettes

7.16 60 mL sterile syringes

7.17 0.7 micron sterile glass syringe filters

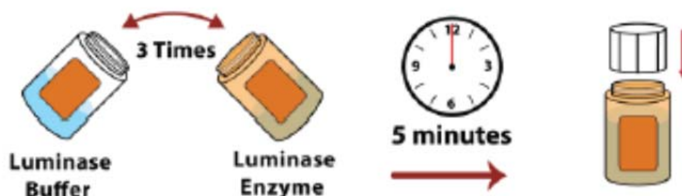
7.18 Filtrate waste receptacle

7.19 Styrofoam shipper

8.0 Reagents and Standards

8.1 Luminase™ solution (or Luminase™ Buffer and freeze-dried Luminase™ Enzyme)

8.1.1 Hydrate Luminase™, if not already prepared.



Luminase Rehydration Process

8.1.1.1 Pour 1 vial of liquid Luminase™ Buffer into 1 vial of freeze-dried Luminase™ Enzyme.

- Store buffer and enzyme for 6 months at 20°C or 12 months at 2-8°C.
- Keep buffer and enzyme in box to prevent light exposure.
- Do not attempt to re-hydrate smaller portions of Luminase™. Always mix 1 full vial of buffer with 1 full vial of enzyme.
- If possible, do not re-hydrate Luminase™ in advance. Re-hydrating only as needed extends shelf-life.

8.1.1.2 Pour the contents of the Luminase™ Enzyme vial into the Luminase™ Buffer vial to mix.

8.1.1.3 Pour the contents of the Luminase™ Buffer vial into the Luminase™ Enzyme vial to mix. Discard Luminase™ Buffer Vial.

8.1.1.4 Cap the Luminase™ Enzyme vial 5 minutes after mixing is complete.

8.1.1.5 Store re-hydrate Luminase™ at 2-8°C for up to 3 months or freeze for up to 6 months (unlimited freeze/thaw cycles).

- Keep in box to prevent light exposure.
- Always bring cold re-hydrated Luminase™ to room temperature prior to use.
- Never expose rehydrated Luminase™ to temperature ≥ 30°C for more than 1to 2 hours.

8.2 LumiClean™ solution – stored in the dark in the media cabinet at room temperature for 18 months

8.3 UltraLyse™ 7 solution – stored in the dark in the media cabinet at room temperature for 18 months

8.4 UltraLute™(Dilution) tubes – stored in the dark in the media cabinet at room temperature for 18 months

8.5 UltraCheck™ 1 (standard 1 ng ATP/mL) – stored in the dark in the media cabinet at room temperature for 18 months

9.0 Sample Collection, Preservation, Shipping, Handling and Storage

9.1 Samples are collected in sterile polystyrene or polypropylene bottles. If samples come from a chlorinated source, the samples are collected in a sodium thiosulfate bottle.

9.2 Samples are collected with the first draw and sent back to the lab in a cooler with gel paks to keep the samples cool. Wet ice is not used.

9.3 If the samples can not be processed immediately after receipt, they may be refrigerated up to 5 days at 1°C - 5°C.

10.0 Quality Control

10.1 Please refer to the Environmental Health Division Quality Assurance Manual for general information on quality control procedures.

10.2 Each new lot of reagents is checked for background RLUs.

10.2.1 The reagents are filtered through a filter (UltraClean™, UltraLyze7™ and UltraLute™) and the RLUs and pg/mL are recorded in a logbook.

10.3 UltraCheck™ 1 (standard 1 ng ATP/mL) and Luminase is tested with each batch of samples

10.3.1 If the ATP standard is under 5000 ATP/mL, a new Luminase is prepared.

10.4 A background control is tested with each batch of samples.

10.4.1 If the background control is high, new assay tubes are used.

11.0 Method Calibration and Standardization

11.1 Pipettes are calibrated on a quarterly basis by an outside vendor.

11.2 New Luminase standard is made if the reading is less than 5000.

12.0 Procedure

12.1 Most of the procedure is taken directly from test kit instructions, LumiCalc video and verbal communication with Andy Jacques.

12.2 Put on gloves and lab coat to protect the samples from contamination and the analyst from skin irritation. Wipe gloves with diapers (Wypall L40 wipes) saturated with 10% bleach/water solution, followed by 70% ethanol solution. Wipe all work areas following the same procedure.

12.3 Login into Computer SLHi0067.

12.3.1 Username: SLHi0067

12.3.2 Password: water_2601

12.3.3 From the start button go to the programs and choose LumiCalc program.

12.4 Double check the calculator icon to setup samples in Lumicalc™.

12.5 Go to Sample points to set up samples points in LumiCalc™, Press the (+) key.

12.5.1 This will prompt you for the following:

12.5.1.1 Choose the QGA for test kit application from drop-down menu.

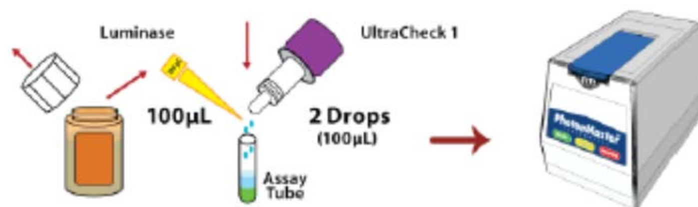
12.5.1.2 Choose QGA for test method application from drop-down menu.

12.5.1.3 Choose potable and sanitary water for application.

12.5.1.4 Name: Sample number

12.5.1.5 Click save and move on the next sample point

12.6 Calibrate ATP Standard



12.6.1 Allow all reagents to reach room temperature prior to use. (Take Luminase out of the refrigerator one hour before processing samples).

12.6.2 Waste a couple of drops of Ultracheck™ 1 before adding 2 drops (100 µL) of UltraCheck™ 1 to a new 12 x 55 mm test tube (the Assay Tube).

12.6.3 Using a sterile pipette, add 100 µL of Luminase™ to the test tube (the Assay Tube). Swirl test tube gently 5 times and immediately insert into PhotonMaster luminometer.

12.7 Measure RLU.

12.7.1 Click in the input box for standard.

12.7.2 A pop-up window will display the reading progress and output.

12.7.3 Once the test is complete, click the SAVE button.

12.7.4 Remove tube from PhotonMaster and discard in MERI barrel.

12.8 Record RLU_{ATP1} manually on the bench sheet and save in LumiCalc.

12.8.1 If RLU_{ATP1} is 5,000 or less, re-hydrate a new bottle of Luminase and use to repeat steps in 12.6.

12.8.2 It is normal for RLU_{ATP1} readings from the same batch of Luminase to decrease over time as a result of decreased luciferase enzyme activity. Step 8.1.1.5 ensures sufficient activity to meet specified detection limit.

12.9 Perform a background check. An empty assay test tube is put into the PhotonMaster and click in the input box for background value (bg).

12.9.1 A pop-up window will display the reading progress and output.

12.9.2 Once the test is complete, click the SAVE button.

12.9.3 Remove tube from PhotonMaster and discard in MERI barrel.

12.10 Filter Sample

12.10.1Mix sample to promote homogeneity.

12.10.2Attach a filter to the syringe.

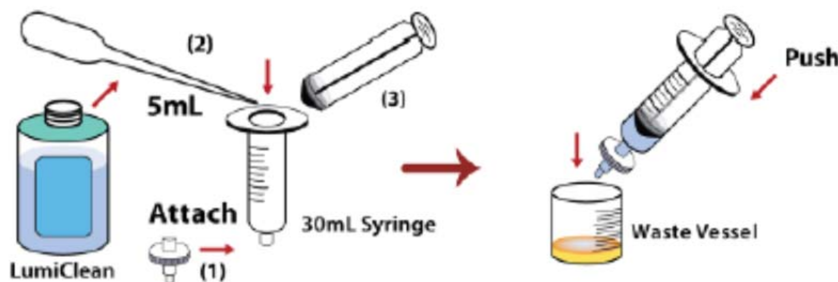
12.10.3Pour sample into syringe. Reattach filter and slowly push (3-5 mL per second) the entire sample volume in the syringe through the filter into a waste receptacle. Stop pushing once the syringe barrel is empty to ensure that the filter remains wet.

12.10.3.1 If there is more sample to filter, remove filter and remove plunger. Reattach filter and pour sample into the syringe. Reinsert plunger into syringe and continue to filter. Record volume filtered.

12.10.3.2 If the full syringe volume cannot be filtered, record the actual volume processed.

12.10.3.3 If sample will not filter, contact LuminUltra for recommendations on changing to different type of test kit.

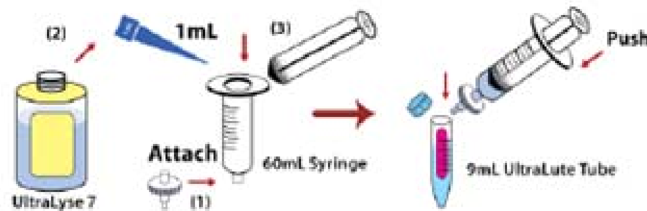
12.11 Wash filter.



12.11.1 Detach filter from syringe and remove plunger. Reattach filter to the syringe and then pipette 4 mL of LumiClean™ with a sterile pipette. (store in dark place at 20°C, approximately room temperature).

12.11.2 Re-insert the syringe plunger and slowly pass the LumiClean™ through the filter. Discard the liquid. Continue to push down on plunger until the filter is dry.

12.12 Extract ATP from filter.



UtlraLute tube should be original container 1

12.12.1 Label a 9 mL UltraLute (Dilution) Tube (store in dark place at 20°C, approximately room temperature) to correspond with the sample name.

12.12.2 Remove filter and then remove the plunger. Attach filter and pipette 1 mL of UltraLyse 7™ to the syringe barrel.

12.12.3 Re-insert the syringe plunger and slowly pass the UltraLyse 7™ through the filter and into the original collection container. Continue to push down on the plunger until the filter is dry.

12.12.4 Coat the entire inside of container with the UltraLyse 7™. Allow at least 5 minutes before rinsing with UltraLute™

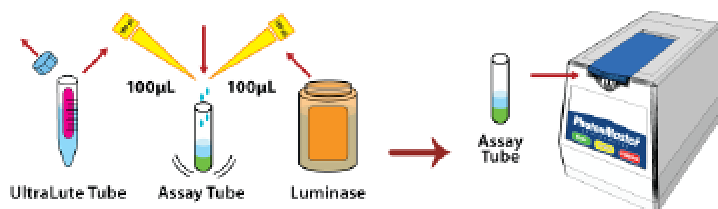
12.12.5 After at least 5 minutes remove filter from syringe then remove plunger. Reattach filter to syringe and pour the 9 mL of UltraLute into syringe. Re-

insert plunger into syringe and slowly filter the 9 mL of UltraLute into original container.

12.12.6 Invert original container 3 times to mix. At this time the sample (filter extract) is stable at room temperature for up to 4 hours.

12.12.7 Discard the syringe and filter in MERI barrel.

12.13 Perform the ATP assay on filter extract.



UtlraLute tube should be original container 2

12.13.1 Pipette 100 µL of filter extract from the original container to a new 12 x 55 mm test tube (the Assay Tube).

12.13.2 Pipette 100 µL of Luminase to the test tube (assay tube) with the filter extract. Swirl test tube gently 5 times and immediately insert into PhotonMaster luminometer.

12.13.3 Repeat step 12.7 for each sample, but click on the appropriate sample point rather than clicking the UltraCheck 1 input box.

12.13.4 Record RLU_{1ATP} (total ATP) and ATP pg on bench sheet directly from PhotonMaster luminometer. Remove tube and discard in MERI barrel.

12.13.4.1 If RLU_{cATP} (cellular ATP) is 10 or less, sample concentration is below the detection limit. Report cATP (pg ATP/mL) = 0 in calculations, or repeat analysis and use a larger sample volume in step 12.10.

12.13.4.2 If RLU_{cATP} is 50 or less, but greater than 10, measure and subtract RLU_{bg} (background RLU) from RLU_{cATP} measurement and/or repeat analysis and use a larger sample volume in step 12.10. RLU_{bg} is measured by putting an empty assay tube in PhotonMaster and taking the reading. The software will automatically subtract the RLUbg from RLU_{cATP} reading.

12.13.4.3 If "Scale Over" is returned, repeat analysis using a smaller volume in step 12.10.

12.14 Record results on the bench sheet for each sample.

12.15 Repeat steps 12.10 through 12.14 for each sample. Between each sample wipe gloves with diapers saturated with 10% bleach/water solution, followed by 70% ethanol solution. Wipe all work area following the same procedure.

12.16 When all samples have been processed, enter RLUs and cATP into Horizon. Horizon will calculate the ME/mL. (ref 16.8)

12.17 Wipe the entire bench top with 10% bleach/water solution, followed by 70% ethanol solution.

13.0 Data Analysis and Calculations

13.1 Calculate ATP concentrations

13.1.1 RLU values are converted to ATP concentrations using LumiCalc software.

13.1.2 If LumiCalc software is not available, perform the manual calculations:

13.1.2.1 Cellular ATP concentration (cATP) in pg ATP per mL. When applicable subtract RLU_{bg} from RLU_{cATP} prior to executing this calculation.

$$cATP\ (pg\ ATP/mL) = \frac{RLU_{cATP}}{RLU_{ATP1}} \times \frac{10{,}000\ (pg\ ATP)}{V_{Sample}\ (mL)}$$

13.1.2.2 Cellular ATP concentrations (cATP) in Microbial Equivalents (ME) per mL. This calculation is based on the established conversion that 1 *E. coli*-sized bacteria contains 0.001 pg (1 fg) of ATP.

$$cATP\ (ME/mL) = cATP\ (pg\ ATP/mL) \times \frac{1\,ME}{0.001\ pg\ ATP}$$

13.2 Quarterly the calculation are manually checked in LumiCalc and Horizon for accuracy and recorded in a logbook.

14.0 Method Performance

14.1 The ATP is measured with PhotonMaster Luminometer using a firefly luciferase assay. The limit of detection per manufacturer of the PhotonMaster Luminometer is 0.1 pg ATP/mL.

15.0 Data Assessment and Management

15.1 Data that doesn't meet quality control standards during the testing process may be reported and flagged or the results invalidated.

16.0 Related Documents

16.1 LuminUltra Technologies Ltd., Test kit instructions

16.2 Andrew Jacque personal correspondence, publication pending

16.3 Environmental Health Division Quality Assurance Manual, Wisconsin State Laboratory of Hygiene.

16.4 2009 TNI Standard, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis, The NELAC Institute, 2009.

16.5 Wisconsin State Laboratory of Hygiene, AD Safety GENOP 102, Chemical Hygiene Plan and General Laboratory Safety Plan for the Agriculture Drive Facility, State Laboratory of Hygiene.

16.6 University of Wisconsin—Madison, Chemical & Radiation Protection Office, Safety Department (262-8769), "Laboratory Safety Guide," 2004, http://www.fpm.wisc.edu/safety

16.7 EHD GENOP 038, "Waste Management," Environmental Health Division, Wisconsin State Laboratory of Hygiene."

16.8 ESS MICRO GENOP 411, "Chemware/Horizon Process for Analytical Testing," Water Microbiology Dept., Wisconsin State Laboratory of Hygiene.

17.0 ATP Bench sheet

ATP

Date: ________________________

Analyst: ______________________

ATP STD (RLU): _______________

BACKGROUND ATP: __________

SAMPLE #	RLU	ATP/mL (pg)	COMMENTS

18.0 Signature Page

Written by: Mark Walter — Date: 2/24/2012

Title: Research Associate

Dept: Water Microbiology

Reviewed by: Susan D. Hill — Date: 04/21/2014

Title: QA Coordinator

Dept: Environmental Health Division

Approved by: Sharon Kluender — Date: 04/21/2014

Title: Microbiology Supervisor

Dept: Water Microbiology

ANALYST CERTIFICATION STATEMENT

"I have read, understand and agree to perform the current revision of this method." ESS MICRO METHOD 307, Revision 1

ANALYST NAME	ANALYST SIGNATURE	DATE

APPENDIX F

Processing Bench Sheet
mEndo/Standard Method Plates and API 20E

Sample Location: Processing by:

Sample Date: Today's Date:

Quanti-Tray Coliform Sample / mEndo Plate ID (e.g. Grab Small, HFUF Medium)	mEndo Plate Lot # / Exp. Date: Comment/Description (e.g. sheen w/ umbinate edges)

Time/Date/Temp mEndo to Inc:

Time/Date/Temp mEndo removed from Inc:

Std Method Plates Plate ID	Plate Lot # / Exp. Date: Comment/Description

Time/Date/Temp Std Method to Inc:

Time/Date/Temp Std Method removed from Inc:

API 20E LN:
Time/Date/Temp API to Inc:
Time/Date/Temp API removed from Inc:

From ESS MICRO METHOD 328

Identification of Total Coliform Using API 20E

1. Procedure

1.1. Dip the small end of a sterile combi-loop into the unsafe sample. Streak a mENDO plate for isolated colonies; incubate at 35°C for 24 h.

1.2. Check for typical green-sheen or dark red isolated colonies on the mENDO plate.

1.3. Streak an isolated colony to a nutrient agar plate (if there is more than one distinct type of colony on the mENDO plate, streak several nutrient agar plates). Incubate at 35°C for 18-24 h.

1.4. Squirt some water on the bottom of the API tray. Place an API 20E strip on top of the water.

1.5. Pick an isolated colony and transfer to 6 ml of sterile saline then briefly vortex so the emulsion is turbid. If the emulsion is not turbid, pick more colonies and briefly vortex.

1.6. With a sterile Pasteur pipette against the side of the tube on the strip slowing distribute the emulsion so there are not bubbles.

1.6.1. For CIT, VP, GEL, fill both the tube and cupule.

1.6.2. For other tests just fill the tube.

1.6.3. For ADH, LDC, ODC, H_2S and URE overlay with mineral oil to create anaerobic conditions.

1.7. Place cover over the tray and place in the 35°C incubator for 18-24 hrs.

1.8. After incubation period the following reagents are added to the following tests:

1.8.1. TDA – add 1 drop of TDA reagent

1.8.2. IND – add 1 drop of James reagent

1.8.3. VP – add I drop of VP1 then VP2 reagents. Wait 10 minutes before recording a negative reaction

1.9. If an oxidase test needs to be performed, see SOP 332

1.10. Record reactions on API sheets, and add values for each group for profile number.

1.11. Log into the apiweb site: https://apiweb.biomerieux.com/jsp/login.jsp

1.11.1. Username:

1.11.2. Password:

1.11.3. Enter profile number into software

1.12. Record organism name on API sheet

APPENDIX G

Polyethylene Glycol Precipitation SOP
Drinking Water Samples

Version: January 27, 2011
Revised June 18, 2014; July 14, 2014; October 3, 2015

Materials

- Sterile graduated cylinders
- Sterile centrifuge tubes (choose appropriate size)
  - 50mL (make sure they are Corning rated for 15,500xg)
  - 250mL Corning
- Bacto Beef extract
- NaCl
- PEG 8000
- Alcohol burner
- Ethanol
- Absorbent diapers
- Sterile pipets
- Weigh boats
- Scoops
- Scale
- 5% Bleach solution
- Turn on Shaker Incubator set at 4°C, record that you will be using the incubator, what you are using it for, how long you will be using it, and at what temperature you have set it.

Procedure

Day 1:

1. Measure 200mL sample or HFUF concentrate
2. Aseptically pour sample into new, sterile 250mL centrifuge tubes containing 4.0 g beef extract (2% w/v final concentration), swirl to completely dissolve.
3. Add 5µL anti-foam to underside of cap. Shake to mix.
4. Add PEG ingredients (**order is important!**):
   a. Add 3.5g (0.3M final concentration) NaCl to each supernatant tube, swirl to completely dissolve.
   b. Add 20g (10% w/v final concentration) PEG 8000 to each supernatant tube, swirl to completely dissolve.
5. Calibrate pH meter
6. Clean probe with copious amounts of 70% ethanol followed by autoclaved Type I water.
7. Test pH and make sure it is between 7.2 and 7.4. Use filter sterilized 6N HCl or 1N NaOH if needed.
8. Prepare an autoclaved Type I water blank following steps 2 through 6.
9. Shake/incubate samples overnight at 4°C and 125-150rpm

Day 2

1. Gather materials:
    a. Bleach
    b. Ethanol
    c. Absorbent diapers
    d. Alcohol burner
    e. Sterilized Pasteur pipets
    f. Sterilized 1.5mL microcentrifuge tubes

2. Balance and centrifuge the PEG tubes at 4,200rpm (5,020xg) for 45min at 4°C
    a. Use the Beckman-Coulter JS rotor 4.2
3. Carefully aspirate the supernatant to the elbow of the bottle so as not to disturb the pellet (down to the elbow of the tube)
4. Balance tubes and centrifuge the remaining PEG pellet at 2,600rpm (1,500xg) for 5min at 4°C
5. Aspirate all traces of fluid without disturbing the pellet. (Tilt tube and aspirate liquid from the elbow). Compare size against Crypto oil references.
6. Let the pellets warm-up, and flick until the little remaining liquid allows the pellet to become a viscous fluid consistency.
7. Use a glass 5 mL pipet to transfer about 0.5 to 0.7 mL of pellet to MoBio Power Soil tubes. Splitting pellet between multiple tubes depending on size.
8. Use a last tube to pipet some of the lysis buffer into the centrifuge tube to wash and wash the inside of the pipet by bubbling.
9. Freeze at -80°C for at least one hour, but up to several weeks to months if needed. Proceed to nucleic acid extraction and purification

APPENDIX H

MoBio Power Soil Nucleic Acid Extraction and Clean-up SOP

Version: January 27, 2011
Revised: October 10, 2011; September 29, 2014; October 13, 2014

Always move from Level 1, to 2, to 3. Do not return to lower numbered area until showered and dressed in freshly laundered clothes.

Nucleic Acid Extraction
Gather Supplies (day before if possible) in Culture Lab:

- Sterile 1.5mL microcentrifuge tubes
- Microcentrifuge tube rack
- Finnpipette tips of various sizes
- Ice if more than one extraction is taking place (the samples can be processed on the benchtop, but it is not wise to let extracted DNA sit at room temperature)
- Clean paper spill mat
- Vortex Genie
- Microcentrifuge
- MoBio PowerBead tubes
- Absorbent diapers
- 5% Bleach solution
- 70% Ethanol
- DNA Away

MoBio PowerSoil DNA Isolation Kit – follow kit instructions – *for PEG and solid samples*

For PEG

- Quantiatively transfer all PEG pellet to PowerBead tube(s) (approximately one tube per 750 uL).

For Feces

- Add 0.20g feces to sterile 2mL tubes.

All PowerBead tubes containing sample

- Vortex 2mL tube with feces/bead/lysis buffer mixture, add 60uL of Solution C1 and invert several times
- Secure in the bead beater fitted with a 2mL tube holder assembly (e.g. Disruptor Genie) and process at "mix" speed for 10 min.

Proceed to Level 2 and continue at step 6 of the **MoBio PowerSoil kit Experienced User Protocol** (see below; steps also written in the TTV Bench sheet)

Nucleic Acid Clean-up

Gather Supplies (day before) in Level 2:

- MoBio kit solutions, tubes and spin filters
- Jars of extra 2 and 1.5 mL tubes (autoclaved)
- Boxes of PCR-ready pipet tips (1000 and 100 uL)
- Container for discarded fluid
- Microfuge racks
- Clean paper spill mat
- Level 2 lab coat
- Boxes of appropriately sized gloves
- Absorbent diapers
- 5% Bleach solution
- 70% Ethanol
- DNA Away

1. Centrifuge the PowerBead tubes at 10,000xg for 30sec at room temp, KEEP TUBES LOW TO BENCH WHEN OPENING TO MINIMIZE AEROSOL SPLATTER
2. Transfer supernatant to a clean 2mL collection tube
3. Add 250uL solution C2 and vortex for 5 sec; incubate at 4°C for 5min (could add C2 ahead of time before transfer the supernatant)
4. Centrifuge at room temp for 1min at 10,000xg
5. Transfer no more than 600uL to a clean 2mL collection tube, if more than 600 ul, prepare a second tube
6. Add 200uL of solution C3 and vortex for 5 sec; incubate at 4°C for 5min (if second tube is used in previous step, adjust the volume to maintain 3:1 ratio for the second tube), CHANGE TIPS FOR EVERY SAMPLE
7. Centrifuge at room temp for 1min at 10,000xg
8. Using oversized 2mL tubes, pipet one tube with 1200uL solution C4 for each sample tube (shake to mix solution C4 before pipeting), close caps and open only one at a time while transferring sample
9. Avoid the pellet and transfer up to 750uL supernatant to an oversized 2mL tubes containing C4 and vortex for 5 sec
10. Prepare additional oversized 2mL tubes until all supernatants are transferred
11. Load ~675uL into a clean spin filter and centrifuge at 10,000xg for 1min at room temp. **Combine tubes of the same sample at this step – record in reference table**

12. Discard the flow through and repeat the step 15 until all extract is applied to filter
13. Add 500uL solution C5 to the spin filter and centrifuge at room temp for 30sec at 10,000xg
14. Discard the flow through and centrifuge again at room temp for 1 min at 10,000xg
15. Aseptically transfer the spin filter to a new 2mL collection tube (labeled for long term storage) and add 100uL solution C6 directly to the membrane (incubate at 4°C 5 min. before centrifuging)
16. Centrifuge at room temperature for 30sec at 10,000xg
17. Aseptically discard the Spin filter
18. -The DNA is now suitable for PCR. Save extracts in Box/Ziplock in -20°C freezer (top left drawer of freezer)

Combine tubes of the same sample at step 11 and record below.

Start Tube	# C3 Tubes	# C4 Tubes	Filter Tubes

APPENDIX I

ESS ENV WATER MICRO METHOD

DNA Extraction (PowerClean Pro) for *Bacteroides, Rhodococcus coprophilus, and Bifidobacteria* Molecular Methods

Well Assessment Project

Wisconsin State Laboratory of Hygiene:

Environmental Health Division

Equipment and Supplies

- Gloves (all areas)
- Lab jacket
- Wypall L-40s (wipes)
- 10% bleach solution
- Eliminase/DNA Away wipes
- 70% ethanol solution
- 95% ethanol solution
- Sterile 99 mL phosphate buffer dilution blanks
- Sterile vessel suitable for making matrix spike
- Forceps (2)
- Bunsen burner/alcohol flame (all areas except Level 3)
- UV sterilizing box
- Vacuum manifold attached to a vacuum system
- Sterile filtration funnels/bases
- Membrane filters, GE polycarbonate, 0.4 micron, 47 mm (Cat No K04CP04700)
- Sterile pipettes, 10 mL, 25 mL, and 50 mL
- Micropippetors capable of delivering 1-10 µL, 10-100 µL and 100-1000 µL (all areas except Level 3)
- Sterile pipette tips for micropippetors - 1-10 µL, 10-100 µL and 100-1000 µL (all areas except Level 3)
- Sterile plastic petri plates 100 x 15 mm
- 2 mL conical microcentrifuge tubes with o-rings (RNA/DNA clean)
- Acid washed glass beads – 1 mm (Sigma G1277-100G)
- AE Buffer
- Salmon Sperm DNA
- BioSpec Products Mini Beadbeater
- Dead air box with UV light (Level 1)
- Microcentrifuge with adapters (Levels 1 and 2)
- 2.0 Lo-Bind Collection Tubes (three per sample)
- 2.0 PowerClean DNA Collection Tubes (one per sample)

Reagents and Standards

Preparation of the tubes containing the glass beads for bead beating is accomplished by weighing out 0.15 g (+ or – 0.01 g) acid washed glass beads (Sigma G1277-100G) in a weighing boat in the media room. Once weighed properly, dump the weighing boat of glass beads into a 2 mL conical RNA/DNA screw cap tube. When all of the tubes needed for analysis are completed, autoclave the tubes containing beads in a microcentrifuge tube rack for 15 minutes at 121°C. Tighten caps when cool.

Sample Collection, Preservation, Shipping, Handling and Storage

- Samples are collected by the customer and sent to the WSLH on ice. Samples will typically arrive in glass warden kit jars.
- Samples should be tested as soon as possible, but should be held at 4°C until analysis can begin.
- Media and reagents should be stored according to manufacturer's instructions.

Quality Control

- Please refer to the Environmental Health Division Quality Assurance Manual for general information on quality control procedures.
- Record dead air box (DAB UV) use on the log next to the DAB in room 108.
- Record use of ABI 7500 Fast PCR machine.
- Record use of the BSC in room 100C (log is taped to front of BSC).
- A matrix spike will be performed with each batch run. Negative and positive (calibrator) controls will be performed with each run.

Procedure

Put on gloves. Wipe gloves with wipes saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Wipe all work areas and equipment following the same procedure.

Prepare the membrane filtration station for filtering as stated in (ESS Micro Method SOP 310 Basic Membrane Filtration). Use fresh 95% ethanol and an unopened chem/pour-off bottle for forceps flaming. Use a 0.4 micron polycarbonate membrane with the shiny side facing up on the filter support.

Shake sample 25 times and pipet HFUF concentrate in individual 25 mL aliquots up to a maximum of 100 mL if possible.

If the filter clogs before 100 mL is filtered document the amount of sample filtered in a lab notebook or bench sheet. If you are running short of sample, record the amount filtered in a lab notebook or bench sheet and the tube if possible.

Rinse the cup and base with sterile Type I lab water with approximately 25-30 mL with each rinse. Use new cups and bases for membrane filtration of each sample.

Once a sample or samples are filtered transfer the filter to a 100x15 mm sterile plastic Petri dish.

Inside the sterile Petri dish, use two flame sterilized forceps (black handled ones from Dr. Long's area) and roll the filter into a cylinder and place into a 1.5-2 mL conical tube containing glass beads (ref 8.1). After placing the filter into the tube, pipette 300 μL of AE Buffer containing 0.2 μg/mL of salmon testes DNA (15.2) slowly down the inside of the rolled filter, if possible. If you cannot pipette it down the inside of the filter, just pipette it along the side of the filter. Salmon testes DNA is used as a control to determine if PCR inhibition is occurring during the amplification step of the assay.

Filter each sample in triplicate (one for each target) and a negative control (sterile Type I water).

To enhance cell lysis, place tubes with filters and AE/SS buffer into the -80°C freezer at least overnight (in emergency situations, leave in -80°C at least one hour or until cells are fully frozen).

With each run, also include (**may be prepared by analyst running PCR**): A calibrator (sterile Type I water spiked with known amount of *appropriate target*).

This step should be done AFTER the mastermix has been made if running PCR on the same day. Cells or potential cells in the sample are disrupted to expose their DNA by placing the microcentrifuge tube containing 1 mm glass beads and filter into a BioSpec Products Mini Beadbeater. Place the tubes into the sample ports and bead beat on **'homogenize' for 1 minute**. Use ear protection or step into the next room when bead beating because of the extreme volume of the machine. Place tubes in a rack on ice and proceed to Level 2.

Level 2 DNA Extraction. Change gloves. Wipe gloves with wipes saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Wipe all work areas following the same procedure.

Centrifuge bead-beat tubes for 1 minute @ 12,000 x *g* @ 20°C.

Carefully pipet liquid from around the membrane (avoiding beads) and place into a sterile, lo-bind microcentrifuge tube. Try to get as much liquid as you can (>200 μL). Discard the bead-beat tube.

Centrifuge all tubes for 3 minutes @ 12,000 x *g* @ 20°C.

Pipet supernatant into a new, sterile, lo-bind, 1.5 mL centrifuge tube (you want 150 µL) . Discard the old tube.

Vortex and aliquot 15 µL to master mix tubes for traditional PCR or 5 µL to capillary/plate/tubes containing master mix for qPCR if sample turbidity was **LESS THAN 5 NTU.** Otherwise, can run the extract through the MO BIO Power Clean Pro DNA Clean-Up kit as follows.

1. Add up to 100 µl of DNA sample to a **1.5 ml Lo-Bind Collection Tube**. If less than 100 µl is added, adjust the volume with distilled water.

2. Add 50 µl of **Solution DC1** to the DNA. Vortex briefly to mix.

3. Add 50 µl of **Solution DC2** to the DNA and vortex briefly to mix.

4. **Centrifuge** at the tube at 13,000 x *g* for 2 minutes at room temperature.

5. Avoiding the pellet, transfer the entire supernatant to a clean **1.5 ml Lo-Bind Collection Tube.**

   Note: *Expect 160-190 µl of supernatant at this step. The exact recovered volume depends on the nature of your starting material and is not critical for the procedure to be effective.*

6. Shake to mix Solution DC3. Add 400 µl of **Solution DC3** and vortex briefly to mix.

7. **Centrifuge** tubes briefly (30 sec) to remove any solution from the cap.

8. Load up to 600 µl onto **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard flow through.

9. Add 500 µl of **Solution DC4** to **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Discard flow through.

10. Again, Add 500 µl of **Solution DC4** to **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Discard flow through.

11. Centrifuge **Spin Filter** at maximum speed for 2 minutes at room temperature to remove any residual ethanol from the wash in steps 9 & 10.

12. Carefully place **Spin Filter** into new **2 mL MoBio Collection Tube**. Avoid splashing any **Solution DC4** onto **Spin Filter**.

    Note: *It is important to avoid any traces of the ethanol based wash solution.*

13. If starting with 50 µl of genomic DNA, add 50 µl of **Solution DC5** to center of white filter membrane.

    If starting with 100 µl of genomic DNA, add 100 µl of **Solution DC5** to center of white filter membrane.

Incubate for 1 minute at room temperature.

***Note:** For efficient elution, use a minimum of 50 µl of **Solution DC5**, irrespective of starting volume. By reducing elution volume, it is possible to obtain DNA in a more concentrated form.*

Centrifuge at 10,000 x *g* for 1 minute at room temperature.

14. Discard the **Spin Filter**. The DNA in the **2 mL Collection Tube** is now application ready. Store DNA frozen (-20° to -80°C). **Solution DC5** does not contain EDTA.

APPENDIX J

E. coli Membrane Filtration and DNA Extraction Using Zymo ZR Soil Microbe DNA Kit for Clean-up
Last Revised: June 2013

Materials and Reagents

- 0.4 µm Polycarbonate membrane filters
- Empty sterile 100 mm petri dishes
- 99 mL DI water blanks
- 2 Membrane forceps
- 70% Ethanol solution
- Eliminase or DNA Away wipes
- 10% Bleach/water solution
- Wypall L40 wipes (absorbent laboratory diapers)
- Gloves
- Flow sort aliquot(s)
- 5 mL centrifuge tube(s)
- 15 mL centrifuge tube(s)
- Plastic ice container (in drawer in Crypto microscope room)
- Ice
- Marking pens
- Tabletop vortex
- Microcentrifuge
- Bunsen burner
- 95% Ethanol solution for flaming
- 10 - 100 and 100 - 1,000 µL (or 200 - 1, 000 µL) pipette
- 100 and 1,000 µL Nuclease-free pipette tips
- Sterile, nuclease-free, 1.5 mL lo-bind microcentrifuge tubes
- Zymo ZR Soil Microbe DNA kit components
    - ZR BashingBead Lysis Tubes
    - Lysis Solution
    - Zymo-Spin IV Spin Filters
    - Collection Tubes (pre-autoclaved)
    - Soil DNA Binding Buffer
    - Zymo-Spin IIC Columns
    - DNA Pre-Wash Buffer
    - Soil DNA Wash Buffer
    - DNA Elution Buffer
- Beaker or falcon tube to collect flow-through waste
- Bead beater
- Timer
- Filter tower(s)
- 2 mL tube rack(s)
- Benchkote
- Centrifuge

Membrane Filtration Procedure (Water Microbiology Laboratory)

1. Put on gloves. Wipe gloves with diapers saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Wipe all work areas following the same procedure.
2. Label all sample containers and ZR BashingBead Lysis Tubes with either the name of the sample or blank (if not already done) and line up prior to filtering. Record all applicable dates and lot numbers of spikes or samples.
3. Aliquot the required volume of Lysis Solution (750 µL/sample) into a 5 mL and/or 15 mL centrifuge tube(s).
4. Light Bunsen burner. Flame tops of all bottles and tubes prior to opening.
5. If preparing spiked samples or standard curve samples, use an appropriate pipette to transfer flow sort to water sample or DI blank, respectively. Rinse flow sort tube with sample volume and return to sample container; pipette up and down to rinse out pipette tip to ensure all cells are transferred to sample container.
6. Place clean, autoclaved filtration tower in filtration manifold.
7. By pouring or pipetting volumetrically, filter sample volume (typically 100 mL) through 0.4 µm polycarbonate membrane (shiny side up). Record volume filtered. Rinse inside of sample container or pipette tip with 99 mL DI blank and apply this volume to the filter to rinse.
    a. Start with lowest dilution of sample.
    b. The same filtration tower may be used for the same sample if sequenced from most diluted to most concentrated.
8. Using forceps, fold filter in half and place into a clean, new empty 100 mm petri plate. Forceps should be dipped in ethanol and flamed for sterilization before each use.
9. Use both forceps to roll membrane into a cylinder. Place membrane into a ZR BashingBead Lysis Tube.
10. Pipette 750 µL of Lysis Solution down the center of the membrane cylinder and cap tube tightly. Vortex and microcentrifuge tube to mix and spin down tube contents, respectively.
11. Repeat steps 6 through 10 for all samples and filter blank control.
12. Transfer ZR BashingBead Lysis Tube(s) to -80°C freezer for at least 1 hour, preferably overnight.

Water Microbiology Laboratory Membrane Filtration Clean-up Procedure

1. Place filter towers in UV box for 2 minutes before placing them in wash bin.
2. Change gloves. Wipe gloves with diapers saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Wipe all work areas following the same procedure.

DNA Extraction Procedure (Water Microbiology Laboratory)

1. Put on gloves. Wipe gloves with diapers saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Wipe all work areas following the same procedure.
2. Remove ZR BashingBead Lysis Tubes from -80°C freezer and thaw to room temperature. This step may be skipped if sample analysis must be expedited.
3. Load ZR BashingBead Lysis Tubes into the bead beater (balanced) and bead beat on "mix" setting for 5 minutes.
4. Transfer the ZR BashingBead Lysis Tubes from the bead beater to a 2 mL tube rack and place in plastic ice container with ice.

Water Microbiology Laboratory DNA Extraction Clean-up Procedure

1. Change gloves. Wipe gloves with diapers saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Wipe all work areas following the same procedure.
2. Aseptically transfer ZR BashingBead Lysis Tubes (in 2 mL tube rack on ice) to Level 2.

DNA Extraction Procedure (Level 2)

1. Put on gloves. Wipe gloves with diapers saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Wipe all work areas following the same procedure.
2. Place all necessary materials on new Benchkote.
3. Centrifuge (balanced) ZR BashingBead Lysis Tube(s) @ 10,000 x g* @ 20°C for 1 minute.
4. Snap off base of a Zymo-Spin IV Spin Filter (orange cap for liquid samples) and place in Zymo Collection Tube. Pipette up to 400 µL of supernatant to Zymo-Spin IV Spin Filter contained in Collection Tube(s) and centrifuge (balanced) @ 7,000 x g* @ 20°C for 1 minute. Discard Zymo-Spin IV Spin Filter.
5. Pipette 1.2 mL Soil DNA Binding Buffer to filtrate in Collection Tube(s) containing sample.
6. Pipette solution up and down a few times. Transfer 800 µL of sample mixture to Zymo-Spin IIC Column in a new Collection Tubes(s) and centrifuge (balanced) @ 10,000 x g* @ 20°C for 1 minute.
7. Discard flow through from Collection Tube(s).
8. Repeat steps 6 & 7 (*i.e.* transfer 800 µL of sample mixture in initial Collection Tube and centrifuge (balanced) @ 10,000 x g* @ 20°C for 1 minute and then discard flow through). Repeat until all the liquid has been added to the Zymo-Spin IIC Column.

9. Transfer Zymo-Spin IIC Column to a new Collection Tube, pipette 200 µL of DNA Pre-Wash Buffer to top of column and centrifuge Collection Tube(s) (balanced) @ 10,000 x g* @ 20°C for 1 minute. Discard flow through.
10. Pipette 500 µL of Soil DNA Wash Buffer to each Zymo-Spin IIC Column and centrifuge (balanced) @ 10,000 x g* @ 20°C for 1 minute. Discard flow through.
11. Transfer each Zymo-Spin IIC Column to clean 1.5 mL lo-bind microcentrifuge tube and add 100 µL DNA Elution Buffer directly to the column matrix (center area of Zymo-Spin IIC Column). Centrifuge (balanced) @ 10,000 x g* @ 20°C for 30 seconds to elute the DNA. Place extract (now ready for PCR analysis) on ice. Discard Zymo-Spin IIC Column.
12. Clearly label additional extract and archive by freezing at -20°C Level 2 freezer.

Level 2 DNA Extraction Clean-up Procedure

1. Discard Benchkote.
2. Change gloves. Wipe gloves with diapers saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Wipe all work areas following the same procedure.

Notes

* $g = (1.118 \times 10^{-5}) \times R \times S^2$; where g is the relative centrifugal force, R is the radius of the rotor in centimeters, and S is the speed of the centrifuge in revolutions per minute (rpm).

APPENDIX K

Direct Extraction

This step should be done AFTER the mastermix has been made if running PCR on the same day. Cells or potential cells in the sample are disrupted to expose their DNA by placing the microcentrifuge tube containing 1 mm glass beads and filter into a BioSpec Products Mini Beadbeater. Place the tubes into the sample ports and bead beat on **'homogenize' for 1 minute**. Use ear protection or step into the next room when bead beating because of the extreme volume of the machine. Place tubes in a rack on ice and proceed to Level 2.

Level 2 DNA Extraction. Change gloves. Wipe gloves with wipes saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Wipe all work areas following the same procedure.

Centrifuge bead-beat tubes for 1 minute @ 12,000 x *g* @ 20°C.

Carefully pipet liquid from around the membrane (avoiding beads) and place into a sterile, lo-bind microcentrifuge tube. Try to get as much liquid as you can (>200 µL). Discard the bead-beat tube.

Centrifuge all tubes for 3 minutes @ 12,000 x *g* @ 20°C.

Pipet supernatant into a new, sterile, lo-bind, 1.5 mL centrifuge tube (you want 150 µL) . Discard the old tube.

APPENDIX L

Master Mix Preparation
Last Revised: June 2016 JWD

Precautions to prevent cross-contamination from previous experiments must be taken. To avoid contamination on the analyst's person, always move from Level 1, to 2, to 3. Do not return to lower numbered areas until showered and dressed in freshly laundered clothes.

Materials and Reagents

- Aluminum foil (in glassware kitchen)
- Tupperware labeled for PCR (in drawer in Crypto microscope room)
- Ice
- Mastermix calculation sheets
- 2 mL tube rack
- 2 mL centrifuge tube(s)
- 15 mL centrifuge tube(s)
- 50 mL centrifuge tube(s)
- Cold box
- 70% Ethanol solution
- Eliminase or DNA Away wipes
- 10% Bleach/water solution
- Wypall L40 wipes (absorbent laboratory diapers)
- Gloves
- 100 – 1,000, 10 – 100, and 0.2 – 10 μL pipette and corresponding tip boxes
- Alcohol burner
- 95% Ethanol solution for burner
- Nuclease-free water (NFW) (Freezer)
- Environmental Master Mix 2.0 (Freezer)
- Quantified forward and reverse primers (Freezer)
- TaqMan® Probe (Freezer)

Mastermix Preparation Procedure (Level 1)

1. Put on gloves. Wipe gloves with diapers saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Fill dedicated Tupperware container from Crypto microscope room with ice. Bring aluminum foil and ice container to Level 1.
2. Change gloves and put on a lab coat. Wipe gloves with diapers saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Wipe all work areas following the same procedure.
3. Put needed materials (including NFW, but excluding mastermix, primers, and probes) in the dead air box.
4. Open the lids of all the pipette tip boxes, close the dead air box, and turn on UV light for at least 20 minutes.

5. Turn off UV light. Close all tip boxes & organize the workspace.
6. Light the alcohol burner (flame tops of all bottles and tubes prior to opening).
7. Pipette volume of NFW indicated on mastermix calculation sheet to the 2 mL or 15 mL mastermix centrifuge tube.
8. Pipette 30 µL of NFW to a 2 mL centrifuge tube and label "NFW blank".
9. Record lot numbers for NFW on the mastermix calculation sheet. Record volume removed and the date and analyst initials on the applicable source NFW container.
10. Put away any remaining source NFW tubes.
11. Aseptically transfer appropriate volume of mastermix aliquots from -20°C freezer to dead air box to thaw.
12. Pipette volume of Environmental Master Mix 2.0 (MM) indicated on mastermix calculation sheet into 2 mL or 15 mL mastermix centrifuge tube containing NFW.
13. Record lot numbers for the MM on the mastermix calculation sheet. Mark MM aliquots that have been thawed and record volume removed and the date and analyst initials on the MM aliquots.
14. Put any remaining MM back in -20°C freezer.
15. Aseptically transfer appropriate volume of primer and probe aliquots from -20°C freezer to the top of the cold box surface in the dead air box to thaw. Be sure to wrap probe tubes in foil. If multiple master mixes are to be prepared, remove only the primer and probe for one master mix at a time to avoid cross-contamination.
16. Pipette volumes of primers and probe(s) indicated on mastermix calculation sheet into 2 mL or 15 mL mastermix centrifuge tube containing NFW/MM solution(s).
17. Record lot numbers for the primers and probe(s) on the mastermix calculation sheet. Mark primer and probe aliquots that have been thawed and record volume removed and the date and analyst initials on the primer and probe aliquots.
18. Put any remaining primer and probe aliquots back in the -20°C freezer.
19. Place 2 mL or 15 mL mastermix centrifuge tube and "NFW blank" tube in separate 50 mL centrifuge tubes and cover with foil. Place in Tupperware container filled with ice.

Level 1 Mastermix Preparation Clean-up Procedure

1. Reset pipets to largest volume.
2. Change gloves. Wipe gloves with diapers saturated with 10% bleach/water solution, followed by Eliminase/DNA Away wipes, followed by 70% ethanol solution. Wipe all work areas following the same procedure.
3. Turn on UV light in dead air box for 20 minutes.
4. Aseptically transfer mastermix tube(s) and "NFW blank" tube to clean refrigerator in Level 2 until ready for use.

Example MasterMix Calculation Sheets

Adenovirus Calculation Sheet

qPCR Environmental Master Mix		**Adenovirus A-F Assay**		
Date: 2/12/16				RUSH
Investigator: BDM				
Reagents	Conc. per rxn tube	Volume per rxn tube (µl)	Master Mix # of tubes 25	Lot # Exp dates
Add H_2O to make vol of 20 µl		9.55	239	
Amount of DNA Template		5		
Environmental MM		15	375	
lot#	exp date			
JTVXP probe	150 nM	0.15	3.75	
Probe (30.0 uM stock)				
JTVXF forward primer (100 uM stock)	500 nM	0.15	3.75	
mod-JVTXR reverse primer (100 uM stock)	500 nM	0.15	3.75	
Total Volume		30	625	
Dispense to each well			25 uL	
Primer stock prepared:	8/28/2014	exp. Aug 2017 (3 years)		
Probe stock prepared:	8/28/2014	exp. Aug 2017 (3 years)		
JTVXR concentrated stock	56.2 uM			

Step	Time	Temp °C	
UP Enzyme Activation	10 min	95	
Denaturation	10 sec	95	
Anneal and Extend	1 min	60	collect data
Cycle step 3-4	45 cycles total		

Assay modified from Jothikumar 2005

Forward	JTVXF	GGA CGC CTC GGA GTA CCT GAG
modified Reverse	mod-JTVXR	ACI GTG GGG TTT CTR AAC TTG TT
TaqMan Probe	JTVXP	6FAM-CTG GTG CAG TTC GCC CGT GCC A-TamaraSp
Threshold	0.01 RLU	

Modified gene reverse sequences
AAC AAG TTC AGA AAC CCC ACI GT
AAC AAG TTT AGA AAC CCC ACI GT

BifidoBacteria Calculation Sheet

qPCR Environmental Master Mix BIFIDOBACTERIA				**IDT primers**
For Human				**1/14/2016**
***Don't forget about Salmon sperm control!!				
Date:				
Investigator:				
Reagents	Conc. per rxn tube	Volume per rxn tube (µl)	Master Mix # of tubes 72	enter number of rxns
Add H_2O to make vol of 25 µl		7.6	547	in grey square to left
Amount of DNA Template		5		
Environmental MM		15	1080	
Probe (7.5 µM working stock)	250 nM	1	72	
Forward Primer (39 µM working stock)	900 nM	0.7	50.4	
Reverse Primer (41 µM working stock)	900 nM	0.7	50.4	
Total Volume		30	1800	
Dispense 25 uL Master Mix to each PCR tube/well, 5 uL template				

Step	Time	Temp °C
UP Enzyme Activation	10 min	95
Denaturation	15 sec	95
Anneal/Extend	1 min	60
Cycle step 2-3	40 - 60 times	choose 40 to start

Forward Primer
5' TTC GGG TTG TAA ACC GCT TTT 3'
Reverse Primer
5' TAC GTA TTA CCG CGG CTG CT 3'
B. adolescentis HUMAN probe - IDT
5' FAM-TCG GGG TGA GTG TAC CT-BHQ1 3'

qPCR Environmental Master Mix Bench Sheet
stx1 F and R primers (Sen/Ibekwe)

valid for primers prepared 08/07/2015
valid for probe prepared 11/09/2015

Date: 5/13/16

MM 1 HILL, MSS

Investigator: BDM/JWD

Master Mix # of tubes:	32
NFW # of wells:	2

Reagents	Conc. per rxn tube (µM)	Vol. per rxn tube (µL)	Total volume of reagents (µL)	Manufacturer	Aliquot vol.	Thawed/used before?	Notes
Add H_2O to get 20 (µL)		7.2	230	Roche			
Amount of DNA Template		5.0	0.0				
TaqMan Env. MM		15.0	480	ABI	775 µL		
stx1 Forward Primer (10 uM working stock)	0.3	0.9	28.8	IDT	20 µL		
stx1 Reverse Primer (10 uM working stock)	0.3	0.9	28.8	IDT	20 µL		
stx1 Probe (3.0 uM working stock)	0.1	1.0	32.0	ABI	20 µL		
Total Volume		30	800.00				
Dispense to each well			**25**				

PCR water for NFW Controls	(add extra)	10

Spin well plate in salad spinner until moisture off plastic (40-50 times)
Time in 7500 Fast Machine________
Data will be automatically saved to D:Applied Bio:7500:Expts, transfer to folder on flash drive, then put on R drive in results and/or make own folder
Export Setup and Results to same place, transfer to folder on flash drive, then R
Reference always ROX

qPCR Environmental Master Mix Bench Sheet
stx2 : 779f and 909r (Arklam)

valid for primers prepared 11/09/2015
valid for probe prepared 11/11/2015

Date: 5/13/16

HILL, MSS

Investigator: BDM/JWD

Master Mix # of tubes: 32
NFW # of wells: 2

Reagents	Conc. Per rxn tube (μM)	Vol. per rxn tube (μL)	Total volume of reagents (μL)	Manufacturer	Aliquot Vol.	Thawed/used before?	Notes
Add H_2O to get 20 (μL)		6.6	211	Roche			
Amount of DNA Template		5.0	0.0				
TaqMan Env. MM		15.0	480	ABI	775 μL		
stx2 779F Forward Primer (10 uM working stock)	0.4	1.2	38.4	IDT	30 μL		
stx2 909R Reverse Primer (10 uM working stock)	0.4	1.2	38.4	IDT	30 μL		
stx2 Probe 814p (3.0 working stock)	0.1	1.0	32.0	IDT	30 μL		
Total Volume		30	800.00				
Dispense to each well			**25**				

PCR water for NFW Controls	(add extra)	10

Spin well plate in salad spinner until moisture off plastic (40-50 times)
Time in 7500 Fast Machine________
Data will be automatically saved to D:Applied Bio:7500:Expts, transfer to folder on flash drive, then put on R drive in results and/or make own folder
Export Setup and Results to same place, transfer to folder on flash drive, then R
Reference always ROX

qPCR Environmental Master Mix Bench Sheet valid for primers and probes prepared 1/11/16 SC

Z3276 F and R primers

16S 395f and 489r

Date: 6/10/16 MSS

Investigator: Master Mix # of tubes: 16

NFW # of wells: 2

Reagents	Conc. per rxn tube (µM)	Vol. per rxn tube (µL)	Total volume of reagents (µL)	Manuf.	Aliquot vol.	Thawed/used before?	Notes
Add H_2O to get 20 (µL)		4.6	73	Roche			
Amount of DNA Template		5.0	0.0				
TaqMan Env. MM		15.0	240	ABI	775 µL		
Z3276 Forward Primer (5 uM working stock)	0.2	1.2	19.2	IDT	30 µL		
Z3276 Reverse Primer (5 uM working stock)	0.2	1.2	19.2	IDT	30 µL		
Z3276 Probe (35.2 uM working stock)	0.1	0.09	1.4	ABI	15 µL		
16S 395F Forward Primer (20 uM working stock)	0.9	1.4	22.4	IDT	35 µL		
16S 489R Reverse Primer (20 uM working stock)	0.9	1.4	22.4	IDT	35 µL		
16S 447P Probe (21.2 uM working stock)	0.1	0.14	2.2	ABI	15 µL		
Total Volume		30	400.00				
Dispense to each well			**25**				

PCR water for NFW Controls (dispense to 15mL	(add extra)	10

Spin well plate in salad spinner until moisture off plastic (40-50 times)

Time in 7500 Fast Machine________

Data will be automatically saved to D:Applied Bio:7500:Expts, transfer to folder on flash drive, then put on R drive in results and/or make own folder

Export Setup and Results to same place, transfer to folder on flash drive, then R! 1 1

Reference always ROX

APPENDIX M

Sanitary/Source Water Survey – Wisconsin Pilot Well Assessment Program

Created March 2014; Updated March 2014

Name of person completing survey: ____

DNR User ID: ____

Contact information: email ____________________**phone** __________________________

PWS ID #: **System Name:**	
Owners Name:	
Sample Location	
Sample Address	
County	**Unique Well Number:** **Entry Point ID:**
Well Construction Date:	**Well Depth** (in feet)
Is the well cased? Yes No Don't know	**Has the casing been inspected?** Yes No …N/A
Any physical well deficiencies (structural, other)? Yes No Don't know **Comment:**	**Aquifer characteristics** consolidated unconsolidated karst other soil type notes:
Is disinfection used? Yes No Don't know	
Was disinfectant residual acceptable at time of TCR sampling? Yes No Don't know	**Depth to bedrock**

Please circle all activities taking place within 1000 feet of the well and indicate approximate distance (answer to the best of your knowledge)

Animal agriculture
   (barnyard, feedlot, stable)
Animal grazing
Automobile service station
Biosolids application
   Class A
   Class B
Construction activities
Dry cleaners
Food processing
Fuel storage
   diesel
   fuel oil
   propane
   other ___________________________
Gasoline station(s) # ________________
Herbicide, pesticide, fertilizer use
Industry (other)
   Type(s) ___________________________
   ____
Landfill (within 1200 feet)
Logging operations

Manure application
Manure storage
   pile
   lagoon
Mining
   Type _____________________________
On-site wastewater treatment/septic system
   Age of system
Recreational activities
   Describe __________________________
Road salting
Slaughterhouse
Surface water resources
   lake
   pond
   stream/river
   marsh/wetland
Stormwater storage/retention pond
Wildlife habitat/sanctuary
Other(s)
   Describe __________________________

Additional Comments:

APPENDIX N

Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

Environmental Health Division
2601 Agriculture Drive, P.O. Box 7996
Madison, WI 53718
Phone: (608) 224-6202 • (800) 442-4618
Fax: (608) 224-6213 • Web: www.slh.wisc.edu

Large Volume Sampling Indicator Results

Site Name:
PWS ID:
Date Sampled:

Report Date:
Report by:

Discussion

WSLH scientist interpretation of indicator, ATP, and API 20E results.

Indicator Results

ATP

Sample Type	microbial equivalents/mL
Pre #1	
Pre #2	
Pre Average	
Post #1	
Post #2	
Post Average	

Grab (retest)

Test	MPN/100mL
Total coliforms	
E. coli	

HFUF (100L concentrate)

Test	MPN/100mL
Total coliforms	
E. coli	
Enterococci	

Miscellaneous (e.g. iron bacteria, if needed)

Bacteria Identification (API 20E)

(Include organism descriptions)

Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

Environmental Health Division
2601 Agriculture Drive, P.O. Box 7996
Madison, WI 53718
Phone: (608) 224-6202 • (800) 442-4618
Fax: (608) 224-6213 • Web: www.slh.wisc.edu

Large Volume Sampling Molecular Results

Site Name: **Report Date:**
PWS ID: **Report by:**
Date Sampled:

Discussion
WSLH scientist interpretation of molecular results for human indicators, animal indicators, and pathogens.

Molecular Results

Human

Test	Result
Adenovirus	
Bacteroides sp.	
Bifidobacteria sp.	

Animal

Test	Result
Ruminant *Bacteroides* sp.	
Rhodococcus coprophilus	

Pathogens

Test	Result
Toxigenic *E. coli* (STEC)	
E. coli O157:H7	

Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

Environmental Health Division
2601 Agriculture Drive, P.O. Box 7996
Madison, WI 53718
Phone: (608) 224-6202 • (800) 442-4618
Fax: (608) 224-6213 • Web: www.slh.wisc.edu

Tier 1 Well Assessment Results

Site Name:
PWS ID:
Date Sampled:

Report Date:
Report by:

Discussion
WSLH scientist interpretation of indicator, ATP, API 20E, and Sanitary Survey results.

Indicator Results

ATP

Sample Type	microbial equivalents/mL
Pre	
Post	

"Pre" Purge Bacteria Grab

Test	MPN/100mL
Total coliforms	
E. coli	
Enterococci	

"Post" Purge Bacteria Grab

Test	MPN/100mL
Total coliforms	
E. coli	
Enterococci	

Miscellaneous (e.g. iron bacteria, if needed)

Bacteria Identification (API 20E)
(Include organism descriptions)

APPENDIX O

Well Assessment Data

PWS ID: **42401392**
Sample date: **4/28/2014**

Start volume	100	L
Final volume	1073.7	mL
Concentration factor		93.1

Turbidity	8.09	NTU

ATP

first flush	0.68	cATP/mL	680	ME/mL	92	RLU
after pumping	0.82	cATP/mL	820	ME/mL	131	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	0/0	Yellow	0/0	Fluorescence	<1	<1 Total coliforms
HFUF conc.	21/2	Yellow	0/0	Fluorescence	29.2	0.314 Total coliforms
Enterolert HFUF conc.			2/1	Fluorescence	3.0	0.0322 Enteroocci

F+ Coliphage	0 plaques	< 0.01 PFU/100 mL

Human Bacteroides	Negative
Bovine Bacteroides	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Generic E. coli by PCR	0.025 Calibrated cell equivalents per 100 mL
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **11307318**
Sample date: **4/30/2014**

Start volume	100	L
Final volume	991.9	mL
Concentration factor		100.8

Turbidity	10.8	NTU

ATP

first flush	1.50	cATP/mL	1500	ME/mL	429	RLU
after pumping	1.18	cATP/mL	1180	ME/mL	311	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	6/2	Yellow	0/0	Fluorescence	8.4	8.4 Total coliforms
Colilert Duplicate	4/1	Yellow	0/0	Fluorescence	5.2	5.2 Total coliforms
HFUF conc.	48/16	Pink (CS)	0/0	Fluorescence	228.2	2.3 Total coliforms
Enterolert HFUF conc.			0/1	Fluorescence	1.0	0.009922 Enteroocci

F+ Coliphage	0 plaques	< 0.02 PFU/100 mL

Human Bacteroides	Positive
Bovine Bacteroides	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Generic *E. coli* by PCR	< 0.003 Calibrated cell equivalents per 100 mL
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **12501016**
Sample date: **5/12/2014**

Start volume	100	L
Final volume	964	mL
Concentration factor		103.7

Turbidity	61.6	NTU

ATP

first flush	7.26	cATP/mL	7260	ME/mL	1139	RLU
after pumping	10.25	cATP/mL	10250	ME/mL	1791	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	45/8	Yellow	0/0	Fluorescence	127.4	127.4 Total coliforms
HFUF conc.	49/48	Pink (CS)	0/0	Fluorescence	>2419.6	>23.3 Total coliforms
Enterolert HFUF conc.			1/0	Fluorescence	1.0	0.00964 Enteroocci

F+ Coliphage	0 plaques
Human Bacteroides	Negative
Bovine Bacteroides	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Generic E. coli by PCR	Negative*
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **26702775**
Sample date: **5/20/2014**

Start volume	100	L
Final volume	999.6	mL
Concentration factor		100.0

Turbidity	276	NTU

ATP

first flush	3.59	cATP/mL	3590	ME/mL	732	RLU
after pumping	2.96	cATP/mL	2960	ME/mL	659	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	2/0	Pink	0/0	Fluorescence	2	2.0 Total coliforms
HFUF conc.	33/6	Pink	0/0	Fluorescence	62	0.62 Total coliforms
Enterolert HFUF conc.			29/12	Fluorescence	61.2	0.612 Enteroocci

F+ Coliphage	0 plaques
Human Bacteroides	Negative
Bovine Bacteroides	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Generic *E. coli* by PCR	Negative*
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **26513751**

Sample date: **6/3/2014**

Start volume	100	L
Final volume	999.6	mL
Concentration factor		100.0

Turbidity	34.2–29.0	NTU

ATP

first flush	1.37	cATP/mL	1370	ME/mL	305	RLU
after pumping	0.91	cATP/mL	191	ME/mL	910	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	9/1	Yellow	0/0	Fluorescence	10.9	10.9 Total coliforms
HFUF conc.	49/48	Yellow	0/0	Fluorescence	>2419.6	>26.36 Total coliforms
Enterolert HFUF conc.			1/0	Fluorescence	1.0	0.011 Enteroocci

F+ Coliphage	0 plaques
Human Bacteroides	Negative
Bovine Bacteroides	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Generic *E. coli* by PCR	Negative*
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **13302058**
Sample date: **6/9/2014**

Start volume	100	L
Final volume	1031.9	mL
Concentration factor		96.9

Turbidity	14.4–9.7	NTU

ATP

first flush	0.41	cATP/mL	410	ME/mL	183	RLU
after pumping	0.51	cATP/mL	510	ME/mL	230	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	13/1	Yellow	0/0	Fluorescence	17.1	5.814 Total coliforms*
HFUF conc.	49/44	Pink	0/0	Fluorescence	1553.1	16.03 Total coliforms
Enterolert HFUF conc.			4/0	Fluorescence	4.1	0.042 Enteroocci

*34mL of sample given diluted to 100mL to perform test

F+ Coliphage	0 plaques	
Human Bacteroides	Negative	
Bovine Bacteroides	Negative	
Rhodococcus coprophilus	Negative	
Adenovirus	Negative	
Generic *E. coli* by PCR	< 0.03	calibrated cell equivalents per 100 mL
Toxigenic *E. coli*	Negative	
***E. coli* O157:H7**	Negative	

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **15710684**
Sample date: **6/12/2014**

Start volume	100	L
Final volume	1077.9	mL
Concentration factor		92.8

Turbidity	9.78–6.45	NTU

ATP

first flush	5.18	cATP/mL	5180	ME/mL	1566	RLU
after pumping	3.1	cATP/mL	3100	ME/mL	946	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	29/1	Yellow	0/0	Fluorescence	43.2	43.2 Total coliforms
HFUF conc.	49/45	Yellow	0/0	Fluorescence	1732.9	18.67 Total coliforms
Enterolert HFUF conc.			49/48	Fluorescence	>2419.6	> 26.1 Enteroocci

F+ Coliphage	0 plaques
Human Bacteroides	Positive
Bovine Bacteroides	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Generic *E. coli* by PCR	Negative*
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **70100195**
Sample date: **6/12/2014**

Start volume	100	L
Final volume	954.4	mL
Concentration factor		104.8

Turbidity 25.5–11.1 NTU

ATP

first flush	131.99	cATP/mL	131990	ME/mL	34082	RLU
after pumping	6.11	cATP/mL	6110	ME/mL	1594	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	49/48	Yellow	0/0	Fluorescence	>2419.6	>2419.6 Total coliforms
HFUF conc.	49/48	Yellow	0/0	Fluorescence	>2419.6	>23.09 Total coliforms
Enterolert HFUF conc.			6/1	Fluorescence	7.4	0.071 Enteroocci

F+ Coliphage 0 plaques

Human Bacteroides Negative
Bovine Bacteroides Negative

Rhodococcus coprophilus Negative

Adenovirus Negative

Generic *E. coli* by PCR < 0.03 calibrated cell equivalents per 100 mL
Toxigenic *E. coli* Negative
***E. coli* O157:H7** Negative

Bifidobacteria
Human Negative
Bovine Negative
Swine Negative

Well Assessment Data

PWS ID: **47113572**
Sample date: **6/17/2014**

Start volume	100	L
Final volume	965	mL
Concentration factor		103.6

Turbidity	38.6–13.4	NTU

ATP

first flush	4.12	cATP/mL	4120	ME/mL	1717	RLU
after pumping	3.65	cATP/mL	3650	ME/mL	1590	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	0/0	Pink	0/0	Fluorescence	< 1	< 1 Total coliforms
HFUF conc.	32/5	Pink	0/0	Fluorescence	57.3	0.553 Total coliforms
Enterolert HFUF conc.			2/0	Fluorescence	2.0	0.0193 Enteroocci

F+ Coliphage	0 plaques	
Human Bacteroides	Negative	
Bovine Bacteroides	Negative	
Rhodococcus coprophilus	Negative	
Adenovirus	Negative	
Generic *E. coli* by PCR	< 0.01	calibrated cell equivalents per 100 mL
Toxigenic *E. coli*	Negative	
***E. coli* O157:H7**	Negative	

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **12503502**
Sample date: **6/23/2014**

Start volume	100	L
Final volume	1091.2	mL
Concentration factor		91.6
Turbidity	> 100	NTU

ATP

first flush	0.37	cATP/mL	370	ME/mL	140	RLU
after pumping	1.36	cATP/mL	1360	ME/mL	489	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	10/0	Pink	0/0	Fluorescence	11	11 Total coliforms
HFUF conc.	49/30	Pink	0/0	Fluorescence	613.1	6.69 Total coliforms
Enterolert HFUF conc.			0/0	Fluorescence	< 1	< 0.0109 Enteroocci

F+ Coliphage	0 plaques	
Human Bacteroides	Negative	
Bovine Bacteroides	Negative	
Rhodococcus coprophilus	Negative	
Adenovirus	Negative	
Generic *E. coli* by PCR	< 0.04	calibrated cell equivalents per 100 mL
Toxigenic *E. coli*	Negative	
***E. coli* O157:H7**	Negative	

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **24604415**
Sample date: **7/10/2014**

Start volume	100	L
Final volume	1237.6	mL
Concentration factor		80.8

Turbidity > 100 NTU

ATP

first flush	149.06	cATP/mL	149060	ME/mL	51022	RLU
after pumping	34.65	cATP/mL	34650	ME/mL	11705	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	0/0	Pink	0/0	Fluorescence	< 1	< 1 Total coliforms
HFUF conc. 50mL	18/1	Pink	0/0	Fluorescence	23.1	0.0057 Total coliforms
HFUF conc. 1 mL	1/0	Pink	0/0	Fluorescence	1	0.0124 Total coliforms
Enterolert HFUF conc.		50 mL	7/2	Fluorescence	9.6	0.0024 Enteroocci
		1 mL	2/0	Fluorescence	2	0.0248 Enteroocci

Human Bacteroides Negative
Bovine Bacteroides Negative

Rhodococcus coprophilus Negative

Adenovirus Negative

Toxigenic *E. coli*
***E. coli* O157:H7**

Bifidobacteria
Human Negative
Bovine Negative
Swine Negative

Well Assessment Data

PWS ID: **15710211**
Sample date: **7/15/2014**

Start volume	100	L
Final volume	1031.2	mL
Concentration factor		97.0

Turbidity	3.24	NTU

ATP

first flush	3.82	cATP/mL	3820	ME/mL	914	RLU
after pumping	0	cATP/mL	0	ME/mL	24	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	0/0	Yellow	0/0	Fluorescence	< 1	< 1 Total coliforms
HFUF conc.	0/0	Pink	0/0	Fluorescence	< 1	< 0.0103 Total coliforms
Enterolert HFUF conc.			0/0	Fluorescence	< 1	< 0.0103 Enteroocci

Human Bacteroides	Negative
Bovine Bacteroides	Negative
Rhodococcus coprophilus	Negative
Adenovirus	NM
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **70101647**
Sample date: **7/28/2014**

Start volume	100	L
Final volume	977.2	mL
Concentration factor		102.3
Turbidity	> 100	NTU

ATP

first flush	31.1	cATP/mL	31100	ME/mL	10481	RLU
after pumping	28.01	cATP/mL	28010	ME/mL	8479	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	0/0	Yellow	0/0	Fluorescence	< 1	< 1 Total coliforms
HFUF conc.	42/6	Pink	7/0	Fluorescence	98.8	0.97 Total coliforms
					7.5	0.073 *E. coli*
Enterolert HFUF conc.			6/4	Fluorescence	10.6	0.10 Enteroocci

Human Bacteroides	Negative
Bovine Bacteroides	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **61702894**
Sample date: **8/11/2014**

Start volume	100	L
Final volume	1143	mL
Concentration factor		87.5
Turbidity	> 100	NTU

ATP

first flush	1.58	cATP/mL	1580	ME/mL	399	RLU
after pumping	0.87	cATP/mL	870	ME/mL	205	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	0/1	Pink	0/0	Fluorescence	1	1 Total coliforms
HFUF conc.	38/13	Pink	10/0	Fluorescence	95.8	1.09 Total coliforms
					11	0.126 *E. coli*
Enterolert HFUF conc.			3/1	Fluorescence	4.1	0.05 Enteroocci

Human Bacteroides	Negative
Bovine Bacteroides	Negative
Rhodococcus coprophilus	Positive (needs re-run)
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **43901495**
Sample date: **8/28/2014**

Start volume	100	L
Final volume	913.3	mL
Concentration factor		109.5
Turbidity	28.1	NTU

ATP

first flush	0.38	cATP/mL	380	ME/mL	61	RLU
after pumping	1.58	cATP/mL	1170	ME/mL	227	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	17/0	Yellow	0/0	Fluorescence	20.3	0.2 Total coliforms
HFUF conc.	49/47	Pink	0/0	Fluorescence	2419.6	22.1 Total coliforms
Enterolert HFUF conc.			0/0	Fluorescence	< 1	< 0.009 Enteroocci

Human Bacteroides	Negative
Bovine Bacteroides	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

Well Assessment Data

PWS ID: **11336072**
Sample date: **9/3/2014**

Start volume	100	L
Final volume	998.7	mL
Concentration factor		100.1

Turbidity 5.05 NTU (Range 4.8-5.5)

ATP

first flush	0.5	cATP/mL	500	ME/mL	129	RLU
after pumping	0.34	cATP/mL	340	ME/mL	77	RLU

					Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	12/0	Yellow	0/0	Fluorescence	13.5	13.5 Total coliforms
HFUF conc.	49/42	Yellow	0/0	Fluorescence	1299.7	13.0 Total coliforms
Enterolert HFUF conc.			14/2	Fluorescence	18.5	0.18 Enterococci

Human Bacteroides	Positive	33 gene copies/100 mL
Bovine Bacteroides	Negative	
Rhodococcus coprophilus	Positive (needs re-run)	
Adenovirus	Negative	
Toxigenic *E. coli*	Negative*	
***E. coli* O157:H7**	Negative	

Bifidobacteria

Human	Negative
Bovine	Negative
Swine	Negative

*potential presence of the stx 1 gene

Well Assessment Data

PWS ID: **42402624**
Sample date: **10/1/2014**

Start volume	100	L
Final volume	1509.5	mL
Concentration factor		66.2

Turbidity	>100	NTU

ATP

first flush	26.72	cATP/mL	26720	ME/mL	1861	RLU
after pumping	3.27	cATP/mL	3270	ME/mL	677	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Top Grab	18/1	Yellow	23.1	23.1 TC
	0/0	Fluoresence	< 1	< 1.0 *E. coli*
HFUF conc.	49/29	Yellow	579.4	8.7 TC
	0/0	Fluoresence	< 1	< 1.0 *E. coli*
Enterolert HFUF conc.	6/1	Fluorescent	7.4	0.11 Enterococci

API 20E	NM
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative
Bifidobacteria	
Human	Negative

Well Assessment Data

PWS ID: **26815580**

Sample date: **11/20/2014**

Start volume	100	L
Final volume	974	mL
Concentration factor		102.7

Turbidity >5 NTU

ATP

first flush	2.66	cATP/mL	2660	ME/mL	387	RLU
after pumping	2.55	cATP/mL	2250	ME/mL	487	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Pre Grab	1/1	Yellow	2.0	2.0 TC
	0/0	Fluorescence	< 1	< 1 *E. coli*
Post Grab	0/0	Yellow	< 1	< 1 TC
	0/0	Fluorescence	< 1	< 1 *E. coli*
HFUF conc.	34/2	Yellow	57.6	0.56 TC
	0/0	Fluorescence	3.1	0.03 *E. coli*
Enterolert	5/1	Fluorescence	6.3	0.06 enterococci

API 20E *Serratia fonticola*, *Enterobacter amnigenus* 2

Human *Bacteroides* Positive 1 gc/100mL

Bovine *Bacteroides* Negative

Rhodococcus coprophilus Negative

Human Adenovirus Negative

Toxigenic *E. coli* Negative

***E. coli* O157:H7** Negative

Bifidobacteria

Human Negative

Well Assessment Data

PWS ID: **73702794**
Sample date: **1/14/2015**

Start volume	100	L
Final volume	1129.1	mL
Concentration factor		88.6

Turbidity 3.92 NTU

ATP

first flush	1.16	cATP/mL	1160	ME/mL	350	RLU
after pumping	1.18	cATP/mL	1180	ME/mL	347	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Pre Grab	0/0	Yellow	< 1	< 1 TC
	0/0	Fluorescence	< 1	< 1 *E. coli*
Post Grab	0/0	Yellow	< 1	< 1 TC
	0/0	Fluorescence	< 1	< 1 *E. coli*
HFUF conc.	16/0	Yellow	18.9	0.2134 TC
	0/0	Fluorescence	< 1	< 0.011 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescent	< 1	< 0.011 enterococci

API 20E *Serratia Fonticola*, *Enterobacter amnigenus* 2

Human *Bacteroides* Positive 0.03 gc/100mL
Bovine *Bacteroides* Negative

Rhodococcus coprophilus Negative

Adenovirus Negative

Toxigenic *E. coli* Negative
***E. coli* O157:H7** Negative

Bifidobacteria Negative
Human Negative

Well Assessment Data

PWS ID: **12504206**
Sample date: **2/5/2015**

Start volume	100	L
Final volume	928.1	mL
Concentration factor		107.7

Turbidity 3.59-3.86 NTU

ATP

first flush	2.68	cATP/mL	2680	ME/mL	662	RLU
after pumping	2.97	cATP/mL	2970	ME/mL	760	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	46/13	Yellow	161.6	161.60 TC
	0/0	Fluoresence	< 1	< 1.0 *E. coli*
HFUF conc.	4948	Yellow	>2419.6	>22.456 TC
	0/0	Fluoresence	< 1	< 0.01 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescent	< 1	< 0.01 enterococci

API 20E *Serratia liquefaciens, Enterobacter asburiae, Kluyvera spp.*

Human *Bacteroides*	Positive	0.009 gc/100mL
Bovine *Bacteroides*	Negative	
Rhodococcus coprophilus	Negative	
Adenovirus	Positive	1’s – 10’s/ L
Toxigenic *E. coli*	Negative	
***E. coli* O157:H7**	Negative	

Bifidobacteria

Human Negative

Well Assessment Data

PWS ID: **26807660**
Sample date: **2/24/2015**

Start volume	100	L
Final volume	925.2	mL
Concentration factor		108.1

Turbidity 5.75 NTU

ATP

first flush	0.47	cATP/mL	470	ME/mL	96	RLU
after pumping	0.53	cATP/mL	530	ME/mL	111	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	11/1	Yellow	13.4	13.4 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	49/33	Yellow	727	6.73 TC
	0/0	Fluorescence	<1	< 0.01 *E. coli*
Enterolert	0/0	Fluorescence	<1	< 0.01 Enterococci
HFUF conc.				

API 20E *Yersinia pestis, Kelbsiella pneumoniae spp ozaenae, Pantoea spp* 4

Human *Bacteroides* Negative
Bovine *Bacteroides* Negative

Rhodococcus coprophilus Negative

Human Adenovirus Negative

Toxigenic *E. coli* Negative
***E. coli* O157:H7** Negative

Bifidobacteria
Human Negative

Well Assessment Data

***** Well chlorinated prior to sample collection. Omitted from data set *****

PWS ID: **64904147**

Sample date: **3/2/2015**

Start volume	100	L
Final volume	954.5	mL
Concentration factor		107.7

Turbidity 35.2 NTU

ATP

first flush	137.22	cATP/mL	137220	ME/mL	137220	RLU
after pumping	69.52	cATP/mL	69520	ME/mL	69520	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	0/0	Yellow	< 1	< 1.0 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
HFUF conc.	0/0	Yellow	< 1	< 0.01 TC
	0/0	Fluorescence	< 1	< 0.01 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	< 1	< 0.01 enterococci

API 20E N/A

Human *Bacteroides* Negative

Bovine *Bacteroides* Negative

Rhodococcus coprophilus Negative

Adenovirus Negative

Toxigenic *E. coli* Negative

***E. coli* O157:H7** Negative

Bifidobacteria

Human Negative

Well Assessment Data

PWS ID: **26807660**
Sample date: **3/10/2015**

Start volume	100	L
Final volume	956	mL
Concentration factor		104.6

Turbidity >5 NTU

ATP

first flush	7.74	cATP/mL	7740	ME/mL	2507	RLU
after pumping	1.57	cATP/mL	1570	ME/mL	475	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	40/6	Yellow	88.2	88.2 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	49/48	Yellow	>2419.6	>23.13
	0/0	Fluorescence	<1	< 0.01 *E. coli*
1 ml HFUF	32/2	Yellow	52.1	49.81 TC
	0/0	Fluorescence	<1	<0.009 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	<1	< 0.009 Enterococci

API 20E *Yersinia pestis, Kelbsiella pneumoniae spp ozaenae, Pantoea spp* 4

Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative
Bifidobacteria	
Human	Negative

Well Assessment Data

PWS ID: **47002109**

Sample date: **5/18/2015**

Start volume	100	L
Final volume	1419.7	mL
Concentration factor		70.4

Turbidity	> 5	NTU

ATP

first flush	163.52	cATP/mL	163520	ME/mL	21265	RLU
after pumping	96.44	cATP/mL	96440	ME/mL	13520	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	0/0	Yellow	< 1	< 1.0 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
HFUF conc.	0/0	Yellow	< 1	< 0.014 TC
	0/0	Fluorescence	< 1	< 0.014 *E. coli*
Enterolert HFUF conc.	0/2	Fluorescence	2.0	< 0.0284 enterococci

API 20E	No culturable orgs.
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative

Well Assessment Data

PWS ID: **41503627**
Sample date: **6/4/2015**

Start volume	100	L
Final volume	812.1	mL
Concentration factor		123.1

Turbidity >5 NTU

ATP

first flush	32.82	cATP/mL	32820	ME/mL	9228	RLU
after pumping	26.11	cATP/mL	26110	ME/mL	7783	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	0/0	Yellow	<1	<1 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	0/0	Yellow	<1	<0.008 TC
	0/0	Fluorescence	<1	< 0.01 *E. coli*
Enterolert	1/1	Fluorescence	2	0.016 Enterococci
HFUF conc.				

API 20E *Bibersteinia trehalos, Erwinia spp.*

Human *Bacteroides* Negative
Bovine *Bacteroides* Negative

Rhodococcus coprophilus Negative

Human Adenovirus Negative

Toxigenic *E. coli* Negative
***E. coli* O157:H7** Negative

Bifidobacteria
Human Negative

Well Assessment Data

PWS ID: 41503286

Sample date: 6/4/2015

Start volume	100	L
Final volume	1084.2	mL
Concentration factor		92.2

Turbidity	> 5	NTU

ATP

first flush	32.31	cATP/mL	32310	ME/mL	8965	RLU
after pumping	51.22	cATP/mL	51220	ME/mL	14497	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	0/0	Yellow	< 1	< 1.0 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
HFUF conc.	0/0	Yellow	< 1	< 0.011 TC
	0/0	Fluorescence	< 1	< 0.011 *E. coli*
Enterolert HFUF conc.	0/2	Fluorescence	2.0	0.016 enterococci

API 20E	*Cedacea* sp.
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative

Well Assessment Data

PWS ID: **20720331**
Sample date: **6/23/2015**

Start volume	100	L
Final volume	992.7	mL
Concentration factor		100.7

Turbidity >5 NTU

ATP

first flush	32.82	cATP/mL	32820	ME/mL	9228	RLU
after pumping	26.11	cATP/mL	26110	ME/mL	7783	RLU
after pumping HFUF	27.44	cATP/mL	27440	ME/mL	6931	RLU
Average	56.07	cATP/ml	56070	ME/ml	8813.5	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	11/0	Yellow	12.2	12.2 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	49/48	Yellow	>2419.6	>24.02 TC
	0/0	Fluorescence	<1	< 0.01 *E. coli*
Enterolert	1/1	Fluorescence	<1	0.099 Enterococci
HFUF conc.				

API 20E	*Serratia liquefaciens*
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative
Bifidobacteria	
Human	Negative

Well Assessment Data

PWS ID: 24608507
Sample date: 6/30/2015

Start volume	100	L
Final volume	1034.9	mL
Concentration factor		96.6

Turbidity > 5 NTU

ATP

first flush	7	cATP/mL	7000	ME/mL	1943	RLU
after pumping	3.65	cATP/mL	3650	ME/mL	1050	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	5/0	Yellow	5.2	5.2 TC
	0/0	Fluorescence	< 1	< 1 *E. coli*
HFUF conc.	49/45	Yellow	1732.9	17.93 TC
HFUF duplicate	49/44	Yellow	1553.1	16.07 TC
1mL HFUF	10/0	Yellow	11.0	11.38 TC
	0/0	Fluorescence (all HFUF)	< 1	< 0.0104 *E. coli*
Enterolert HFUF conc.	17/2	Fluorescence	22.8	0.236 Enterococci

API 20E *Vibrio Fluvialis, Pseudomonas luteola, Cedecea lapagei, enterobacter amnigenus 2, enterobacter cloacae, citrobacter youngae, escherishia vulneris, klebsiella pneumoniae ssp ozaenae*

Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human Negative

Well Assessment Data

PWS ID: **43904432**
Sample date: **7/6/2015**

Start volume	100	L
Final volume	982.5	mL
Concentration factor		101.8

Turbidity	>5	NTU

ATP

first flush	40.32	cATP/mL	40320	ME/mL	11811	RLU
after pumping	6.75	cATP/mL	6750	ME/mL	1911	RLU
after pumping HFUF	7.69	cATP/mL	7690	ME/mL	2177	RLU
Average	22.46	cATP/ml	22460	ME/ml	6141	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	0/0	Yellow	<1	<1 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	3/0	Yellow	3.1	0.0305 TC
	0/0	Fluorescence	<1	< 0.01 *E. coli*
1 ml HFUF	0/0	Yellow	<1	<0.983 TC
	0/0	Fluorescence	<1	<0.983 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	<1	0.099 Enterococci

API 20E — *Serratia liquefaciens*, *Serratia fonticola*, *Enterobacter amnigenus 2*

Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative

Well Assessment Data

PWS ID: **26713577**
Sample date: **7/16/2015**

Start volume	100	L
Final volume	970.3	mL
Concentration factor		103.1

Turbidity	> 5	NTU

ATP

first flush	36.61	cATP/mL	36610	ME/mL	7662	RLU
first flush #2	32.00	cATP/mL	32000	ME/mL	6143	RLU
after pumping	34.47	cATP/mL	34470	ME/mL	6892	RLU

			Raw MPN	Vol Adjusted MPN/100 mL
Colilert retest	47/12	Yellow	172.3	172.3 TC/100mL
	0/0	Fluorescence	< 1	< 1 *E. coli*/100mL
HFUF conc.	49/46	Yellow	1986.3	19.27 TC / 100 mL
	0/0	Fluorescence	< 1	< 0.0097 *E. coli* / 100mL
1mL HFUF	8/2	Yellow	10.8	10.48 TC / 100 mL
	0/0	Fluorescence	< 1	< 0.0097 *E. coli* / 100mL
Enterolert HFUF	0/0	Fluorescence	< 1	< 0.0097 Enterococci/100mL

API 20E	*Serratia liquefaciens*
Human Bacteroides	Negative
Ruminant Bacteroides	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative
Bifidobacteria	Negative
Human	Negative

Well Assessment Data

PWS ID: **11305151**
Sample date: **8/3/2015**

Start volume	100	L
Final volume	1127.2	mL
Concentration factor		88.7

Turbidity	>5	NTU

ATP

first flush	11.87	cATP/mL	11870	ME/mL	2709	RLU
after pumping	2.9	cATP/mL	2900	ME/mL	723	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	15/12	Yellow	32.1	32.1 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	49/48	Yellow	>2419.6	>27.27 TC
	0/0	Fluorescence	<1	< 0.01 *E. coli*
1 ml HFUF	0/0	Yellow	<1	<0.983 TC
10 ml HFUF	10/5	Yellow	11	11.38 TC
10 ml HFUF	48/15	Purple (Colisure)	218.7	24.65 TC
	0/0	Fluorescence (all)	<1	<0.011 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	<1	0.236 Enterococci

API 20E — *Pantoea spp 2, Enterobacter amnigenus 1, Enterobacter gregoviae, Klebsiella oxytoca*

Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative

Well Assessment Data

PWS ID: **60311229**

Sample date: **8/11/2015**

Start volume	100	L
Final volume	1014.7	mL
Concentration factor		98.6

Turbidity > 5 NTU

ATP

First flush 1	1.82	cATP/mL	1820	ME/mL	421	RLU
First flush 2	1.04	cATP/mL	1040	ME/mL	227	RLU
After pumping 1	1.46	cATP/mL	1460	ME/mL	288	RLU
After pumping 2	2.65	cATP/mL	2650	ME/mL	467	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	14/2	Yellow	18.5	18.5 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
HFUF conc.	0/0	Yellow	< 1	<0.01 TC
1mL HFUF	0/0	Yellow	< 1	< 1.01 TC
	0/0	Fluorescence (all HFUF)	< 1	< 0.01 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	< 1	< 0.01 Enterococci

API 20E *Kebsiella pneumoniae ssp pneumonia 2, pantoea spp 2, klebsiella oxytoca*

Human *Bacteroides*	Positive	115 gc / 100mL
Bovine *Bacteroides*	Negative	

Rhodococcus coprophilus Negative

Adenovirus Negative

Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human Negative

Well Assessment Data

PWS ID: **47002109**
Sample date: **8/31/2015**

Start volume	100	L
Final volume	996.4	mL
Concentration factor		88.7

Turbidity >5 NTU

ATP

first flush	51.6	cATP/mL	51600	ME/mL	13537	RLU
after pumping	21.6	cATP/mL	21600	ME/mL	6186	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	6/2	Yellow	8.4	8.4 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	32/7	Yellow	60.9	0.61 TC
1 ml HFUF	1/0	Yellow	1	<1 TC
	0/0	Fluorescence (all)	<1	< 1 *E. coli*
86 ml HFUF	39/1	Purple (Colisure)	72.2	0.84 TC
	0/0	Fluorescence (all)	<1	<0.011 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	<1	0.01 Enterococci

API 20E *Enterobacter cloacae, Klebsiella pneumoniae spp pneumoniae 1*

Human *Bacteroides* Negative
Bovine *Bacteroides* Negative

Rhodococcus coprophilus Negative

Human Adenovirus Negative

Toxigenic *E. coli* Negative
***E. coli* O157:H7** Negative

Bifidobacteria
Human Negative

Well Assessment Data

PWS ID: **46014914**
Sample date: **8/31/2015**

Start volume	100	L
Final volume	850.9	mL
Concentration factor		117.5

Turbidity > 5 NTU

ATP

First flush	19.73	cATP/mL	19730	ME/mL	5821	RLU
After pumping	15.02	cATP/mL	15020	ME/mL	3943	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	0/0	Yellow	< 1	< 1.0 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
HFUF conc.	11/0	Yellow	12.2	0.104 TC
1mL HFUF	0/0	Yellow	< 1	< 0.009 TC
	0/0	Fluorescence (all HFUF)	< 1	< 0.009 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	< 1	< 0.009 Enterococci

API 20E	*Serratia liquefaciens*
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative
Bifidobacteria	
Human	Negative

Well Assessment Data

PWS ID: **26704722**
Sample date: **9/8/2015**

Start volume	100	L
Final volume	955.5	mL
Concentration factor		104.7

Turbidity >5 NTU

ATP

first flush 1	51.6	cATP/mL	51600	ME/mL	13537	RLU
First Flush 2	2.74	cATP/mL	2740	ME/mL	853	RLU
after pumping	21.6	cATP/mL	21600	ME/mL	6186	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	13/0	Yellow	<114.8	14.8 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	49/48	Yellow	>2419.6	>23.12 TC
1 ml HFUF	43/9	Yellow	114.5	109.4 TC
	0/0	Fluorescence (all)	<1	< 1 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	<1	0.029 Enterococci

API 20E *Serratia Liquefaciens*

Human *Bacteroides* Negative
Bovine *Bacteroides* Negative

Rhodococcus coprophilus Negative

Human Adenovirus Negative

Toxigenic *E. coli* Negative
***E. coli* O157:H7** Negative

Bifidobacteria
Human Negative

Well Assessment Data

PWS ID: **47112384**
Sample date: **9/16/2015**

Start volume	100	L
Final volume	967.4	mL
Concentration factor		103.4

Turbidity > 5 NTU

ATP

First flush 1	48.87	cATP/mL	48.87	ME/mL	14244	RLU
First flush 2	41.22	cATP/mL	41.22	ME/mL	10887	RLU
After pumping 1	12.29	cATP/mL	12.29	ME/mL	3214	RLU
After pumping 2	22.48	cATP/mL	22.48	ME/mL	5708	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	0/1	Yellow	1.0	1.0 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
HFUF conc.	42/10	Yellow	110.6	1.07 TC
1mL HFUF	2/0	Yellow	2.0	1.93 TC
	0/0	Fluorescence (all HFUF)	< 1	< 0.01 *E. coli*
Enterolert HFUF conc.	3/0	Fluorescence	3.1	0.03 Enterococci

API 20E — *Pantoea spp* 4, *Citrobacter braakii*, *Klebsiella pneumoniae ssp pneumoniae 2*, *Serratia marcescens, Rahnella aquatilis, Aeromonas salmonicida ssp salmonicida, Pantoea spp 1*

Human *Bacteroides* — Negative
Bovine *Bacteroides* — Negative

Rhodococcus coprophilus — Negative

Adenovirus — Negative

Toxigenic *E. coli* — Negative
***E. coli* O157:H7** — Negative

Bifidobacteria
Human — Negative

Well Assessment Data

PWS ID: **15708827**
Sample date: **9/21/2015**

Start volume	100	L
Final volume	1022.9	mL
Concentration factor		103.4

Turbidity >5 NTU

ATP

first flush	5.60	cATP/mL	5600	ME/mL	855	RLU
after pumping	2.82	cATP/mL	2820	ME/mL	426	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	29/4	Yellow	48.0	48.0 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	49/44	Yellow	1553.1	15.89 TC
1 ml HFUF	15/0	Yellow	17.5	17.9 TC
	0/0	Fluorescence (all)	<1	< 1 *E. coli*
Enterolert HFUF conc.	2/0	Fluorescence	2.0	0.020 Enterococci

API 20E *Citrobacter freundii 99.9% (6 isolates)*

Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human Negative

Well Assessment Data

PWS ID: **26828670**

Sample date: **10/1/2015**

Start volume	100	L
Final volume	1091.5	mL
Concentration factor		91.6
Turbidity	5.6	NTU

ATP

First flush 1	7.70	cATP/mL	7700	ME/mL	7700	RLU
First flush 2	4.40	cATP/mL	4400	ME/mL	4400	RLU
After pumping 1	2.4	cATP/mL	2400	ME/mL	2400	RLU
After pumping 2	1.2	cATP/mL	1200	ME/mL	1200	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	1/0	Yellow	1.0	1.0 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
HFUF conc.	49/22	Yellow	387.3	4.23 TC
1mL HFUF	2/0	Yellow	2.0	2.18 TC
	0/0	Fluorescence (all HFUF)	< 1	< 0.011 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	< 1	< 0.011 Enterococci

API 20E	*Citrobacter braakii, Citrobacter freundii, Enterobacter amnigenus 2*
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative
Bifidobacteria	
Human	Negative

Well Assessment Data

PWS ID: **26701334**
Sample date: **10/5/2015**

Start volume	100	L
Final volume	1153.3	mL
Concentration factor		86.7

Turbidity	>5	NTU

ATP

First Flush 1	23.43	cATP/mL	23430	ME/mL	6967	RLU
First Flush 1	19.80	cATP/mL	19800	ME/mL	5890	RLU
First Flush 2	16.02	cATP/mL	16020	ME/mL	4681	RLU
After Pumping 1	16.74	cATP/mL	16740	ME/mL	4981	RLU
After Pumping 2	14.81	cATP/mL	14810	ME/mL	4460	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	10/0	Yellow	11.0	11 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	49/18	Yellow	307.6	3.55 TC
1 ml HFUF	0/0	Yellow	<1	<1.15 TC
	0/0	Fluorescence (all)	<1	< 1 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	<1	0.012 Enterococci

API 20E	*Serratia fonticola (x5)*
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative
Bifidobacteria	
Human	Negative

Well Assessment Data

PWS ID: **41511690**
Sample date: **10/6/2015**

Start volume	100	L
Final volume	1014.6	mL
Concentration factor		98.6

Turbidity	> 5	NTU

ATP

First flush 1	156.51	cATP/mL	156510	ME/mL	28590	RLU
First flush 2	147.03	cATP/mL	147030	ME/mL	24863	RLU
After pumping 1	36.01	cATP/mL	36010	ME/mL	6230	RLU
After pumping 2	29.73	cATP/mL	29730	ME/mL	4044	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	0/0	Magenta	1.0	1.0 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
HFUF conc.	49/29	Magenta	579.4	5.88 TC
HFUF duplicate	49/25	Magenta	461.1	4.68 TC
1mL HFUF	7/0	Magenta	7.5	7.61 TC
	0/0	Fluorescence (all HFUF)	< 1	< 0.01 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	< 1	< 0.01 Enterococci

API 20E *Pantoea* spp 3

Human *Bacteroides* Negative
Bovine *Bacteroides* Negative

Rhodococcus coprophilus Negative

Adenovirus Negative

Toxigenic *E. coli* Negative
***E. coli* O157:H7** Negative

Bifidobacteria
Human Negative

Well Assessment Data

PWS ID: **25221251**
Sample date: **10/29/2015**

Start volume	100	L
Final volume	1040.6	mL
Concentration factor		96.1

Turbidity >5 NTU

ATP

First Flush 1	23.88	cATP/mL	23880	ME/mL	4348	RLU
First Flush 2	18.17	cATP/mL	18170	ME/mL	3437	RLU
After Pumping 1	30.99	cATP/mL	30990	ME/mL	5533	RLU
After Pumping 2	28.44	cATP/mL	28443	ME/mL	4819	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	14/1	Yellow	17.3	17.3 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	46/13	Yellow	161.6	1.68 TC
1 ml HFUF	0/0	Yellow	<1	<1.15 TC
	0/0	Fluorescence (all)	<1	< 1 *E. coli*
Enterolert HFUF conc.	0/0	Fluorescence	<1	0.012 Enterococci

API 20E *Enterobacter amnigenus 2, Serratia liquefaciens, Enterobacter cloacae*

Human *Bacteroides* Negative
Bovine *Bacteroides* Negative

Rhodococcus coprophilus Negative

Human Adenovirus Negative

Toxigenic *E. coli* Negative
***E. coli* O157:H7** Negative

Bifidobacteria
Human Negative

Well Assessment Data

PWS ID: **61203769**

Sample date: **12/4/2015**

Start volume	100	L
Final volume	1073.9	mL
Concentration factor		93.1

Turbidity	7.4	NTU

ATP

First flush 1	056	cATP/mL	560	ME/mL	106	RLU
First flush 2	0.50	cATP/mL	500	ME/mL	95	RLU
After pumping 1	0.29	cATP/mL	290	ME/mL	55	RLU
After pumping 2	0.24	cATP/mL	240	ME/mL	46	RLU
After pumping 2 duplicate	0.29	cATP/mL	290	ME/mL	56	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	44/7	Yellow	115.3	115.3 TC
	0/0	Fluorescence	< 1	< 1 *E. coli*
HFUF conc.	49/48	Yellow	> 2419.6	> 25.98 TC
1mL HFUF	30/4	Yellow	50.4	54.12 TC
	0/0	Fluorescence (all HFUF)	< 1	< 0.011 *E. coli*
Enterolert HFUF conc.	35/9	Fluorescence	50.0	0.54 Enterococci

API 20E	*Pantoea spp* 1, *Citrobacter braakii*
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative

Well Assessment Data

PWS ID: **26815580**
Sample date: **12/9/2015**

Start volume	100	L
Final volume	943.2	mL
Concentration factor		106.0

Turbidity	>5	NTU

ATP

First Flush 1	105.32	cATP/mL	105320	ME/mL	35466	RLU
First Flush 2	96.01	cATP/mL	96010	ME/mL	30969	RLU
After Pumping 1	7.77	cATP/mL	7770	ME/mL	2416	RLU
After Pumping 2	8.14	cATP/mL	8140	ME/mL	2763	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest (Grab)	1/0	Yellow	1.0	1.0 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
Post Grab	0/0	Yellow	<1.0	<1.0 TC
	0/0	Fluoresence	<1	< 1 *E. coli*
HFUF conc.	9/1	Yellow	10.1	0.095 TC
1 ml HFUF	0/0	Yellow	<1	<1.15 TC
	0/0	Fluorescence (all)	<1	< 1 *E. coli*
Enterolert	3/1	Fluorescence	4.0	0.038 Enterococci

API 20E — *Klebsiella oxytoca, Klebsiella pneumoniae spp pneumonia 2, Serratia liquefaciens, Pantoea spp 1, Pantoea spp 2, Enterobacter amnigenus 2, Citrobacter braakii, and Cronobacter spp.*

Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative

Well Assessment Data

PWS ID: **41517157**

Sample date: **1/19/2016**

Start volume	100	L
Final volume	997.1	mL
Concentration factor		100.3

Turbidity	> 5	NTU

ATP

First flush 1	9.42	cATP/mL	9420	ME/mL	1097	RLU
First flush 2	10.78	cATP/mL	10780	ME/mL	1293	RLU
After pumping 1	15.82	cATP/mL	15820	ME/mL	1671	RLU
After pumping 2	16.93	cATP/mL	16930	ME/mL	1842	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	8/2	Yellow	9.2	9.2 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
HFUF conc.	49/29	Yellow	579.4	5.78 TC
1mL HFUF	1/0	Yellow	1.0	0.997 TC
	0/0	Fluorescence (all HFUF)	< 1	< 0.01 *E. coli*
Enterolert HFUF conc.	1/0	Fluorescence	1.0	0.01 Enterococci

API 20E	*Enterobacter amnigenus 2, Klebsiella oxytoca, Klebsiella pneumoniae ssp pneumonia 1, Citrobacter braakii, Pasturella pneumotropica/Mannheimia haemolytica*
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative
Bifidobacteria	
Human	Negative

Well Assessment Data

PWS ID: **47006718**
Sample date: **3/7/2016**

Start volume	100	L
Final volume	1042.7	mL
Concentration factor		95.9

Turbidity	>5	NTU

ATP

First Flush 1	8.77	cATP/mL	8740	ME/mL	2730	RLU
After Pumping 1	2.23	cATP/mL	2230	ME/mL	1671	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest (Grab)	0/0	Yellow	<1	<1.0 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	49/12	Yellow	224.7	2.34 TC
1 ml HFUF	2/1	Yellow	3.0	3.13 TC
	0/0	Fluorescence (all)	<1	< 1 *E. coli*
Enterolert	0/0	Fluorescence	<1	<0.01 Enterococci

API 20E	*Enterobacter amnigenus 2*
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative
Bifidobacteria	
Human	Negative

Well Assessment Data

PWS ID: **41505728**
Sample date: **4/13/2016**

Start volume	100	L
Final volume	924.8	mL
Concentration factor		108.1

Turbidity	> 5	NTU

ATP

First flush 1	15.16	cATP/mL	15160	ME/mL	2289	RLU
After pumping 1	6.81	cATP/mL	6810	ME/mL	1037	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Pre	6/1	Yellow	7.4	7.1 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
Coliform Post	14/0	Yellow	16.1	16.1 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
HFUF conc.	49/36	Yellow	866.4	8.01 TC
	8/1	Fluorescence	9.7	0.09 *E. coli*
1mL HFUF	3/0	Yellow	3.1	2.87 TC
	0/0	Fluorescence	< 1	< 0.92 *E. coli*
Enterolert HFUF conc.	14/1	Fluorescence	16.1	0.15 Enterococci

API 20E — *Eschericia coli, Yersinia pestis, Escherichia vulneris, Serratia liquefaciens, Citrobacter Braakii, Serratia fonticola, Serratia rubidaea, Pantoea spp 1*

Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Positive
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative

Well Assessment Data

PWS ID: **41517861**
Sample date: **4/13/2016**

Start volume	100	L
Final volume	874.5	mL
Concentration factor		114.4
Turbidity	>5	NTU

ATP

First Flush 1	365.43	cATP/mL	365430	ME/mL	61110	RLU
After Pumping 1	295.95	cATP/mL	295950	ME/mL	45242	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest (Grab)	2/0	Yellow	2.0	2.0 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
Coliform Post (Grab)	49/13	Yellow	235.9	235.9 TC
	0/0	Fluorescence	<1	<1 *E. coli*
HFUF conc.	49/48	Yellow	>2419.6	>21.16 TC
1 ml HFUF	42/2	Yellow	87.8	2.87 TC
	0/0	Fluorescence (all)	<1	< 1 *E. coli*
Enterolert	49/48	Fluorescence	>2419.6	>21.16 Enterococci

API 20E	*Enterobacter amnigenus 2, Citrobacter youngae, Serratia fonticola, Serratia liquefaciens, Enterobacter amnigenus 1 (other possible(?), Klyuvera spp, Citrobacter braakii)*
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative
Bifidobacteria	
Human	Negative

Well Assessment Data

PWS ID: **47105949**

Sample date: **4/13/2016**

Start volume	100	L
Final volume	851.5	mL
Concentration factor		114.4

Turbidity	1.8 – 1.9	NTU

ATP

First flush 1	1.13	cATP/mL	1310	ME/mL	208	RLU
After pumping 1	4.01	cATP/mL	4010	ME/mL	629	RLU

			Raw MPN	Vol Adjusted MPN/100mL
Coliform Retest	9/1	Yellow	10.9	10.9 TC
	0/0	Fluorescence	< 1	< 1.0 *E. coli*
HFUF conc.	49/40	Yellow	1119.9	9.54 TC
	0/0	Fluorescence	< 1.0	< 0.01 *E. coli*
1mL HFUF	2/0	Yellow	2.0	1.70 TC
	0/0	Fluorescence	< 1	< 0.85 *E. coli*
Enterolert HFUF conc.	1/0	Fluorescence	1.0	0.01 Enterococci

API 20E	*Pantoea spp 1, Rahnella Aquatilis, Ewingella americana, Serratia fonticola, Serratia liquefaciens*
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative

Bifidobacteria

Human	Negative

Well Assessment Data

PWS ID: **74402163**
Sample date: **4/27/2016**

Start volume	100	L
Final volume	1038.7	mL
Concentration factor		96.3

Turbidity >5 NTU

ATP

First Flush 1	36.64	cATP/mL	34640	ME/mL	7088	RLU
After Pumping 1	16.72	cATP/mL	16720	ME/mL	3444	RLU

			raw MPN	Vol Adjusted MPN/100mL
Coliform Retest (Grab)	0/0	Yellow	<1	<1.0 TC
	0/0	Fluorescence	<1	< 1 *E. coli*
HFUF conc.	3/0	Magenta	3.1	0.032 TC
1 ml HFUF	0/0	Yellow	<1	<1 TC
	0/0	Fluorescence (all)	<1	< 1 *E. coli*
Enterolert	0/0	Fluorescence	<1	<0.010 Enterococci

API 20E	*Citrobacter koseri/amalonaticus*
Human *Bacteroides*	Negative
Bovine *Bacteroides*	Negative
Rhodococcus coprophilus	Negative
Human Adenovirus	Negative
Toxigenic *E. coli*	Negative
***E. coli* O157:H7**	Negative
Bifidobacteria	
Human	Negative