



Safe Drinking Water: Public Health Laboratory Surveillance

An Update for Public Health
Workers on Laboratory
Testing for Safe Drinking
Water in British Columbia

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Laboratory Services*

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I. Background:

The Action plan for Safe Drinking Water: maps out our direction based on the amended Drinking Water Act.

At a subsequent province-wide conference for Drinking Water Regulators sponsored by the BC Ministry of Health, a framework for public health laboratory testing was presented as part of an integrated approach to monitoring, Source-to-Tap. A description of this framework is attached as Appendix A (also available at <http://www.bccdc.org>). This paper provides an update on public health laboratory-based microbial testing and surveillance within this framework.

II. Purpose:

The purpose of this paper is to provide public health workers with information on current laboratory principles and practices related to testing for safer drinking water. It is intended to help determine appropriate types of testing and to assist in the interpretation of these tests in different settings.

III. Source-To-Tap Responsibilities:



A. Watershed Protection

Providing safe drinking water begins at the water source. It focuses on generating knowledge regarding the physical, chemical, and biological characteristics of a water source, ensuring that the environmental threats that face a water supply continue to be understood, evaluated responded to. Watershed management and protection practices are fundamental to providing safe drinking water.

A program of laboratory testing of raw water quality in a watershed may be useful for two reasons:

- Firstly, understanding the microbial quality of the source water establishes a reference point on which treatment requirements can be built.
- Secondly, understanding microbial water quality in the source may help with interpretation of microbiological results from treated drinking water.

Public health and environmental managers need to work together on planning, program development and testing as well as ongoing risk assessments. Our vision is for real-time surveillance to provide timely data for public health and water purveyor intervention.



B. Monitoring Treated Drinking Water

Monitoring water quality (i.e., turbidity, water chemistry, Bacterial Indicators) or drinking water process efficiencies (i.e., chlorine residual) is another important step in the Source-to-Tap continuum. In some cases, physiochemical water quality indicators are monitored at the treatment site. Some larger water purveyors also test for total coliforms/*E. coli* on site, as part of the monitoring of water treatment procedures Quality Assurance Program.

When testing for public health purposes (rather than treatment Quality Assurance), however, water samples must be sent to a Provincial Health Officer approved laboratory, according to provincial regulations.

Monitoring for bacteriological water quality is only one of the ‘spot-checks’ for operational treatment process integrity and distribution system integrity. It provides a limited spatial and temporal picture within the whole water system.



C. Disease Surveillance

Waterborne disease surveillance is the final step in the “Source-to-Tap” continuum of public health monitoring. Surveillance for cases of disease (most often of gastrointestinal illness) is not as timely as environmental laboratory surveillance which provides the opportunity for intervention before contaminated water is consumed. Early disease detection, however, provides an alert to public health and with a rapid response, further disease transmission can be prevented.

IV. Start With A Plan:

Effective monitoring **Plans** ensure that testing of drinking water for microbiological contamination occurs under a wide variety of conditions and challenges (i.e., high precipitation, turbidity, drought, etc.).

Effective monitoring **Plans** develop a baseline trend and a detailed understanding of what conditions may compromise drinking water safety.

Effective monitoring **Plans** recognizes the need for surveillance in all 3 Source-to-Tap components noted above. Within an overall approach to each drinking water system, laboratory test results should be considered along with other indicators of treatment efficiency or water quality, to develop a comprehensive assessment of risk.

The following examples may provide some insight as to why microbiology test results should be used in conjunction with other indicators of drinking water quality:

- **EXAMPLE 1** - A water utility receives a report showing no bacterial contamination in their finished water. At the same time its staff note uncommonly high turbidity and/or particle counts in the treated water. This may indicate treatment process inefficiency or failure, and may constitute a public health concern even though there is no indication of contamination using Bacterial Indicators. In this situation the discrepancy between bacteriological results and other indicators of water quality may be explained as follows. The grab sample used for bacteriological water quality may have been taken before a surge of highly turbid, contaminated raw water entered the treatment system. Further microbiological testing would be indicated.
- **EXAMPLE 2** - The presence of *E. coli* is detected in finished drinking water yet all other indicators of process efficiency (i.e., chlorine residual, particle counts, etc.) and water quality (i.e., turbidity) show that optimal treatment efficiency was occurring before, during, and after the grab sample was taken. It was however noted that a casual employee was collecting the water samples. This person was not properly trained in sample collection. She/he may have contaminated the sample before it was sent to the lab for analysis. Further testing was carried out.

Below are general recommendations for developing effective monitoring and testing Plans:

- Implement routine testing plans that are reflective of ‘normal’ operational integrity of a water treatment system. This will help create a baseline trend.
- Include ‘event’-based’ monitoring (i.e., increased precipitation or turbidity) to assess how these challenges may affect water quality.
- Ensure that all indicators are routinely collected at the same time as bacteriological samples are collected so correlations between parameters can be made. This will help assess risk when any one parameter fails to meet standards.
- Ensure that all data is collected, retained, and available for analysis.

- Use comprehensive training programs for people who collect samples.
- Develop a public health audit plan with BCCDC Laboratory Services staff. A Laboratory Task Group of the Drinking Water Leadership Council is currently working to enhance this program.

V. General Principles of Water Microbiology:



The goal of this section is to provide a summary of:

- A. Bacterial Indicator Microbiology,
- B. Bacterial Indicator Laboratory Methods and
- C. Specific Pathogen Testing.

A. *Bacterial Indicator Microbiology*

The presence of surrogate or indicator bacteria (i.e., *E. coli*) in a drinking water sample signifies that the water may have been contaminated with feces.

A very important point to understand is that microbial indicators, such as *E. coli* or fecal coliforms, are not intended to be absolute indicators for the presence of pathogens. Rather the presence of these Bacterial Indicators in a drinking water sample is consistent with the fact that the water was likely contaminated with feces and thus water is at a higher risk for causing disease. Fecally-contaminated water may or may not

have pathogenic microorganisms in it. Consequently the consumption of bacterially-contaminated water may or may not cause disease. The concept of using bacteria as indicators of water quality and public health safety is based on risk by association.

Testing water for specific waterborne pathogens is prohibitively costly and complex. Bacteria such as *E. coli* are simple and convenient indicators to test for in the laboratory but as their name implies, they are only indicators. Their presence does not imply that human pathogens (diseases – causing organisms) are present for certain. Nor does their absence guarantee that pathogens are absent. Thus, monitoring microbial drinking water quality is based on the use of Bacterial Indicators for assessing relative health risks.

Specific pathogen-related tests are impractical for routine monitoring since each pathogen requires a unique set of laboratory-based testing algorithms and as noted above, tests are prohibitively expensive. Pathogen specific testing, however, is important in outbreak scenarios or in other very specific settings.

Bacterial Indicators that are useful for public health must fulfill two criteria:

- They should only be associated with the feces of humans or animals; and
- They should be incapable of replicating outside the host gastrointestinal system, in the environment.

Thus the presence of key Bacterial Indicators such as *E.coli* in a water supply would indicate a potential health threat as a result of fecal contamination of the water supply.

The following bacteria are the most commonly used Bacterial Indicators:

1. *E. coli* Bacterial Indicator

Escherichia coli is the most important public health indicator.

It is a coliform bacteria found predominantly in the feces of warm-blooded animals (birds and mammals). It has similar biochemical properties to the other coliforms (fecal coliforms are a sub-type of total coliforms and *E.coli* is a member of the fecal coliform group). It is distinguished by the presence of the enzyme β -glucuronidase.

Most methods of detection (Membrane Filtration or Defined Enzyme Substrate Methods) make use of the presence of this enzyme for detection of *E. coli* in water samples. Over 95% of *E. coli* isolates tested to date possess this enzyme.

Of note, most strains of *E. coli* O157 do not produce this enzyme. This type of *E.coli* is one of the very few known strains that cannot be detected by β -glucuronidase-based methods. However, the likelihood that *E. coli* O157 being the only *E. coli* strain present in a fecally contaminated water sample, is remote.

E. coli has an extremely limited ability to survive and replicate outside the host. Thus it is the most appropriate indicator of fecally-contaminated water and the key Bacterial Indicator for public health.

The presence of *E. coli* in a water sample represents an immediate public health concern. Follow-up to a positive sample should occur immediately, including resampling of the system (resampling at multiple sites throughout the distribution system may be warranted). All other water quality parameters and treatment process indicators should also be examined before, during, and after an *E. coli* positive sample was taken.

When there are other failed parameters coinciding with the timing of an *E. coli* positive result, a significant health threat must be considered and issuing of a boil water advisory warranted.

Even when there is no evidence for other failed parameters, with no other possible concrete explanations for this positive Bacterial Indicator result (i.e., poor sampling technique or leaking bottle, etc), an *E. coli* positive drinking water sample may still be grounds for issuing a boil water advisory.

2. Fecal Coliform Bacterial Indicator

Historically fecal coliforms have been extensively used as Bacterial Indicators of fecal contamination. *E. coli*, more recently, will replace this group in British Columbia, as it is a more specific indicator of contamination by human or animal feces.

Fecal coliforms are a sub-group of the total coliform group. They are distinguished from total coliforms by their ability to grow at higher temperatures (42°C - 44.5°C), a useful trait for the laboratory. *Escherichia coli* is one of the fecal coliforms. Other fecal coliforms include *Klebsiella*, *Enterobacter* and *Citrobacter*.

Culturing microbes at increased temperatures selects for microbes adapted to the gastrointestinal system of warm-blooded animals, as opposed to environmental microbes adapted to live in lower environment temperature. This is a general trait that is not true in all cases, and although incubation in the laboratory at higher temperature enriches for fecal bacteria, some environmental isolates may still grow (i.e., some species of *Enterobacter*). Moreover, some species of *Klebsiella* and *Enterobacter* have been shown to proliferate in the nutrient rich water. Thus fecal coliforms as a whole group are not as specific a Bacterial Indicator as *E.coli*.

When compared to the presence of total coliforms, the presence of fecal coliforms in a water sample adds significant weight to a possible health risk. The presence of fecal coliforms in water should be followed up immediately, including immediate resampling. All water quality parameters and treatment process indicators should be examined before, during, and after, a fecal coliform positive bacteriological sample was taken (as noted for *E.coli*).

When there are other failed parameters coinciding with the timing of the fecal coliform positive result, a significant health threat should be considered and issuing of a boil water advisory warranted.

With no other possible concrete explanations for this result (i.e., poor sampling technique or leaking bottle, etc), a fecal coliform positive drinking sample may be grounds for issuing a boil water advisory.

3. Total Coliform Bacterial Indicator

There is some debate internationally about the public health significance of this Bacterial Indicator group.

An understanding of the basic definition of this group of bacteria, however, is important to assessing possible risks as poor drinking water quality is associated with the presence of these organisms.

By definition total coliforms are bacteria that can ferment lactose and possess the enzyme β -galactosidase.

Originally, this group of bacteria included 4 groups (or genera): *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter*. These 4 groups are found in feces of warm-blooded animals, including man. However, recent scientific evidence has shown that total coliforms actually includes a much broader grouping of bacteria than these 4 original groups. Introduction of Defined Enzyme Substrate (DES) methods and more extensive research has shown that many Bacterial Indicators including *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter* plus *Yersinia*, *Serratia*, *Hafnia*, *Pantoea*, *Kluyvera*, *Cedecea*, *Ewingella*, *Moellerella*, *Leclercia*, *Rahnella* or *Yokenella* will produce a total coliform positive test. Those underlined are species of total coliforms that are considered to be primarily of environmental source. (See Appendix E). Thus the origins of this larger number of total coliform groups includes both feces and the environment.

In fact, to date there are now 19 recognized groups (comprising 80 different species), of which only 10 genera have actually been associated with feces. Several environmental species included as total coliforms, are associated with soil, vegetation, or water sediments. Thus, not all total coliforms represent bacteria coming from the feces.

Moreover, recent research has also demonstrated that some genera of total coliforms, although found in the feces of animals, are also capable of replicating in nutrient rich environments (*Klebsiella*, *Enterobacter*). This makes it difficult to assess whether the water in which total coliforms were detected was contaminated with feces or not.

Thus overall, the total coliform group has become a less specific measure of public health risk. In fact, the group violates the two basic criteria for a good indicator, these being the requirement for the microorganism to only be associated with the feces of animals and to be incapable of replicating in the environment. What then is the relevance of total coliform testing in assessing microbial water quality and how should positive results be interpreted?

Here are some scenarios where total coliform testing results may be useful:

- EXAMPLE 1 - In treated water systems the presence of total coliforms may represent treatment failure or sub-optimal treatment efficiency. Their presence however does not necessarily represent an immediate health concern. A general rule for treated water systems is that total coliforms should not be present, whether they are derived from environmental sources or not. Action protocols should reflect this. Health authorities and water purveyors should assess why total coliforms are in the system and assess other indicators of treatment process and water quality as a means of assessing risk. Repeat samples should be taken and processed again to see if the problem persists.
- EXAMPLE 2 - High quality, untreated ground water that suddenly experiences total coliforms failures is of public health concern, particularly if the ground water has no

previous history of coliform contamination. In this scenario, ground water may have suddenly been impacted by surface water, and therefore at risk of contamination (even in the absence of *E. coli*). Due to the environmental persistence of total coliforms, compared to *E. coli*, infiltration of a groundwater system with total coliforms may be seen as a precursor to more severe failures in the future (i.e., total coliforms and *E. coli*).

- **EXAMPLE 3** - Surface water receiving minimal disinfection and treatment (coagulation, flocculation, and filtration) may occasionally experience total coliform failures. These may not represent an immediate health threat in the absence of *E. coli* or fecal coliforms. However, their presence may represent poor treatment operation and should be followed up. Since these systems do not have comprehensive disinfection and treatment total coliform failures should be followed up immediately and interpreted in light of other parameters or process indicators to ensure that maximal operational integrity has been maintained in these systems.

B. Bacterial Indicator Laboratory Methods

Different testing methods use specific biological characteristics of *E.coli*, fecal coliforms or total coliforms.

Defined Enzyme Substrate (DES) broth (liquid-based MPN methods) target the production of the enzymes β -glucuronidase and β -galactosidase.

Traditional Membrane Filtration (MF) methods target lactose fermentation leading to aldehyde or acid production as a distinguishing lab criteria. New Membrane Filtration (MF) methods are based on detection of β -glucuronidase and β -galactosidase constitutive enzymes of the target bacteria by using culture media incorporated with chromogenic and/or fluorogenic substrates. MF culture media are now used world-wide and can provide simple, rapid and simultaneous detection of total coliform and *E.coli* in water.

Each method has advantages and disadvantages. All are acceptable methods.

It is most important, however, to remember that results from different methods must not be compared. For example, DES and new MF methods tend to be more sensitive and, growth permitting, result in a broader range of total coliforms detected, (isolates of fecal bacteria as well as environmental isolates).

At the same time, DES and new MF methods are quite selective for *E. coli*, more so than traditional MF methods. This is because some isolates of *E. coli* may not ferment lactose with acid and gas production. As many as 25% of *E. coli* isolates will not ferment lactose in the laboratory. Over 95% of *E. coli* isolates have the enzyme β -glucuronidase as the distinguishing criteria in the laboratory for *E. coli* in DES and new MF tests.

C. Specialized Pathogen Testing

Some waterborne microbes such as parasites and some viruses are relatively resistant to disinfection. Thus a multi-barrier approach to drinking water safety must be used to minimize the risk of disease spread of these pathogens.

As well, because of their resistance to chemical disinfection, the presence of Bacterial Indicators do not always correlate with the presence of protozoan or viral pathogens in the water, and vice versa. Many waterborne disease outbreaks have been described in which drinking water met all requirements for bacteriological water quality (as well as process efficiency indicators and other water quality parameters).

The majority of outbreaks in BC (1980-present) have been caused by protozoan parasites.

BCCDC Environmental Health Laboratory Services has a province-wide mandate to provide public health services and reference services. It plays a central role in surveillance of waterborne diseases, in outbreak detection, investigation and management, in Quality Assurance (support for the Provincial Health Officer's Enhanced Water Quality Assurance Program) and public health audits, and in drinking water related research and education. BCCDC carries out Bacterial Indicator testing (*E.coli*, fecal coliforms and total coliform tests as requested) as well as the following pathogen-specific, reference services for public health purposes.

Pathogen-specific testing for *Cryptosporidium*, *Giardia*, *Legionella*, *Pseudomonas*, *Campylobacter*, *E. coli* 0157, *Salmonella*, and other pathogens are carried out on specific request.

Specialized tests detection of and DNA genotyping or fingerprinting of *Giardia* and *Cryptosporidium* parasites, Heterotropic Plate Count (HPC) or virus detection, may also be useful in specific circumstances and are available on request from BCCDC.

Requests for these types of tests may be made by contacting BCCDC Laboratory Services (Mr. J. Fung, 604-660-1753, Ms. S. Shay, 604-660-6734 or Dr. J. Isaac-Renton, 604-660-6032). If urgent, page the Microbiologist On-Call (604-661-7033).

VI. Recommendations:

The following summary recommendations are offered to public health workers responsible for ensuring safer drinking water for British Columbians:

1. *E. coli* tests are the best indicators of fecal contamination
 - Positive (*E.coli* test results or if chosen, fecal coliforms) results should trigger immediate, extensive re-sampling of the water distribution system.

- Assessing risks should include a composite assessment of all available indicators of water quality and/or process efficiency to determine reasons for this result. A complete assessment should look at all water quality indicators that related to the timing of bacteriological water sampling. Consideration of a boil water advisory is warranted.
 - In the absence of concrete explanations that could explain a positive result (e.g., poor sampling technique), immediate corrective action should be taken including consideration of issuing a boil water advisory.
2. *E. coli* tests can currently be carried out by many Provincial Health Officer approved laboratories in British Columbia (See Appendix G and website noted).
 3. Total coliform tests are useful for determining general drinking water quality.
 - Positive total coliform test results should be followed-up with re-sampling.
 - The presence of total coliforms in drinking water should lead to the question, “Why are these bacteria present?”
 - Results of one method of total coliform testing should not be compared with another method of total coliform testing.
 4. Monitoring plans should include elements of data collection and analysis to get an overall appreciation (i.e., trends) of system water quality and potential risks to public health.
 5. Specific pathogen testing is available for specific reasons, on consultation with BCCDC senior staff.

VII. Reading and Source Materials:

- Australian Drinking Water Guidelines link, specifically Chapter 5: Microbial Quality of Drinking Water: http://www.nhmrc.gov.au/publications/_files/awgfull.pdf
- Basic Microbiology for Drinking Water Personnel. American Water Works Association, Denver, 2001. Hill, Dennis.
- BCCDC website: <http://www.bccdc.org>
At BCCDC homepage:
Scroll down menus on the left-hand side of the page to Resources and open this link.
At Resources page, scroll down menus on the left-hand side of the page to Publications.
At Publications homepage, about 1/3 of the page down, under the title Laboratory Services, Environmental Health section, are the 2 files of interest referred to in this document.
- Changing Face of Coliforms and Indicators, NHMRC, South Australia, Discussion Paper, September 2001.
- Framework for Monitoring Drinking Water Systems – Source to Tap:
http://www.healthservices.gov.bc.ca/cpa/publications/safe_drinking_printcopy.pdf
- Health Canada Drinking Water Guidelines link: http://www.hc-sc.gc.ca/ewh-semt/water-eau/drink-potab/index_e.html.
- An Operator's Guide to Bacteriological Testing. American Water Works Association, Denver, 1993. Lisle, John.
- Provincially Approved Laboratories in British Columbia - EWQA (UBC Dept of Pathology) website:
http://www.pathology.ubc.ca/education/Certificate_Programs/Enhanced_Water_Quality_Assurance/PHO_-_Approved_Laboratory_List.htm
- Total Coliform Members by Evolving Definitions: Kreig, 1984; Topley, 1997; Ewing, 1986; Ballows, 1992.

VIII. Acknowledgements:

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IX. Appendices:

Appendix A General Laboratory Principles

Appendix B Important Facts About Laboratory Testing Procedures

Appendix C BCCDC Drinking Water Collection Protocol

Appendix D Distance Education: Microbes in Your Water? What you Really Need to Know For the Public Health

Appendix E Members of the Total Coliform Group: An Evolving Definition

Appendix F Provincial Health Officer Approval: Quality Assurance for Public Health

Appendix G Summary List of Provincial Health Officer Approved Laboratories - 2006

Appendix A

General Laboratory Principles

Your need to know the answer to: When to test? Why test? Which test to use?

The following principles will hopefully assist in answering these questions, as well as helping further with the interpretation of bacteriological results.

When developing a Monitoring Plan to assess drinking water quality, consider:

- **Reason For Testing:**
The choice of test will differ depending on what question is being asked. Is the test being used as a public health audit of the entire monitoring process? Is the test being done in response to increased risk or an outbreak? Is it being done by water purveyors for treatment process quality assurance?
- **Lab Tests Are Never 100% Accurate:**
Inherent to all analytical testing is a certain degree of *uncertainty* associated with results that are generated. Uncertainty is defined by (EURACHEM/CITAC Guide CG 4: *Quantifying Uncertainty in Analytical Measurement*, 2nd Edition, pg. 4) as: *a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measure.*

In essence, the results generated by any laboratory are not absolute; a certain degree of variability occurs with any result. The entire scope of this variability is the measurement of uncertainty associated with that testing.

Uncertainty is not intended to raise suspicion about the result, but rather provide the customer with a high degree of confidence in the validity of the result.

Different types of tests are most reliably evaluated using concepts of **Positive Predictive Value** (i.e., how often the test calls a positive sample 'positive' given a specific level of disease or contamination event) and **Negative Predictive Value** (how often the test calls a negative sample, negative given a specific level of disease or contamination event).

Positive Predictive and Negative Predictive values of the test vary with the prevalence of the disease or contamination event.

In terms of monitoring drinking water quality, the more often there is a problem (defined for our purposes as bacterial contamination events), the more reliable (“predictive”) the test results will be.

- **One Size Does Not Fit All:**
The idea that one approach to testing is suitable for all monitoring plans is false. Source water quality is very diverse across the province. Treatment systems used to process drinking water, also vary. Some types of tests are more useful in some settings, e.g. In the Greater Vancouver Regional District, screening methods are useful because fecal positives are very rare. Screening types of tests are not useful in other communities where frequently contaminated surface water is observed.
- **Specific Test Methods May Be More Useful Than Others:**
Several different types of testing are currently acceptable for examination of microbial water quality (USEPA, Standard Methods for Examination of Water and Wastewater, EWQA approved). Some methods are more appropriate in some situations than in others. For example, in remote areas where shipping of samples is virtually impossible, certain types of testing may be conducive to on-site analysis with relatively simple equipment. However, it is important that all results fall under the jurisdiction of a Quality Management System that ensures the ongoing validity, acceptability, and quality of the data generated. Quality activities in the laboratory must be strong, demonstrable to clients and sustainable.
- **Acceptable Test Methods May Not Be Comparable:**
Different types of test methods have different sensitivities for Bacterial Indicator detection. One method will, for example, detect more total coliforms (increased numbers and increased frequency of positives) than another. Although these diverse laboratory tests are intended to target the same group of bacteria (i.e., total coliforms) their functionality is based on specific biochemical properties of these organisms. These biochemical characteristics can be quite diverse and thus different methods can selectively enrich for certain target populations. Although specific results may not be comparable trends that are produced may be similar. Results from one method are not directly comparable to another e.g. Most Probable Number test (MPN) vs Selective Media Membrane Filtration test (MF) vs broth or MF Defined Substrate Method (DSM) types of tests. This is particularly true for different laboratory methods for total coliform testing.
- **Unequal Distribution of Microorganisms in a Water Sample:**
One factor that can significantly affect laboratory results is the uneven distribution of microorganisms in any one water system and even within one water sample.

Variation may be temporal or spatial. Results are most variable when organisms are present in very low concentrations.

It is not uncommon for independent laboratory tests performed from a single water sample, and using the same laboratory method, to yield slightly different recoveries in a split sample analysis. This does not constitute a laboratory error even if transport conditions (time and temperatures) are identical.

- **Quality is the Touchstone**

All laboratories must be part of a Quality System-period.

- **One Must Be Careful Using Laboratory Test Results Numbers for Assessing Risk:**

Historically, drinking water guidelines have made use of numerical values believed to be important in assessing risk (i.e., maximal acceptable concentrations or MACs). Numbers can provide a false sense of risk security.

When a grab sample is taken for analysis it is intended to represent water quality. *However, grab samples provide a severely limited picture of spatial and temporal water quality.*

For example, if a grab sample is taken and results reveal a concentration of 4 total coliforms/100 ml, it may be assumed that the water has low levels of contamination and is safe based on a 10 total coliform/100 ml MAC guideline. However, it is impossible to assess whether the grab sample was taken during an *influx* or *efflux* contamination event. In this case total coliform numbers could have exceeded 500 total coliforms/100 ml five minutes prior to when the grab sample was taken (efflux).

Alternatively, the water sample may have been taken just at the beginning of a contamination event (influx) and concentrations of 500 total coliforms/100ml may have occurred 5 minutes after taking the grab sample.

The latest version of the *Guidelines for Canadian Drinking Water Quality* no longer contains maximal acceptable concentrations of bacterial indicators.

- **Importance of Correct Collection and Transport of Samples:**

Bacteria do not generally survive well in water due to a variety of factors (i.e., osmotic pressure, predation, etc). It is well known that the numbers of bacteria within a water sample rapidly decline 24 hrs after it is collected. Temperature can also affect die off within the water sample, with higher temperatures leading to greater die offs. Samples should be collected, placed on ice and shipped as soon as possible to testing laboratories. (30hr holding/transport time)

Laboratory test results are dependent on correct collection procedures (Appendix B). They are also dependent on fast, reliable transport of samples to the laboratory. Laboratories testing for public health purposes must setup test samples as soon as possible after their arrival.

Appendix B

Important Facts About Laboratory Testing Procedures

Two laboratory procedures are used to assess the presence of Bacterial Indicators:

- **Growth Amplification Procedure By Membrane Filtration (MF):**

In this procedure, a water sample is filtered through a small pore membrane (0.45 μm) and the membrane is placed on top of an agar-containing Petri dish containing a “differential” media. This media allows for growth but at the same time selects for and differentiates the target organisms (i.e., total coliforms, fecal coliforms, *E. coli*). The results after incubation (37°C) are quantitative and provide an estimate of the number of target bacteria in a water sample.

A new MF procedure for direct detection of total coliforms and *E.coli* has recently been described (Chromocult®, Merck) and has been evaluated by BCCDC.

- **Growth Amplification Procedure By Broth Media:**

For non-quantitative results (presence/absence) results, a broth media in a sterile container is used. Recent advances in the biochemical properties of total coliforms led to the development of Defined Enzyme Substrate Methods (DES) for detection of bacterial indicators in broth cultures. Some examples include Colilert® or Colisure® but there are other commercially available types as well.


In these systems, the only available carbon source for bacterial metabolic energy is in a single chemical constituent (ONPG). Degradation of this compound is dependent on the microbial production of an enzyme known as β -galactosidase. Thus, only microbes that possess the enzyme β -galactosidase are capable of growing in the broth media.

DSM procedures can simultaneously detect both total coliforms and *E. coli* in a single reaction, by targeting β -galactosidase (total coliforms) and β -glucuronidase (*E. coli*) production in a single container with broth media.


For semi-quantitative results, an MPN (Most Probable Number) broth approach is used. Many small parts of the drinking water sample are injected into many replicate, smaller separate containers with broth-media. Thus MPN can be done using either traditional broth cultures or by DES broth media.

Appendix C

BCCDC Drinking Water Collection Protocol



BC Centre for Disease Control
AN AGENCY OF THE PROVISIONAL HEALTH SERVICES AUTHORITY




A research and teaching centre affiliated with UBC

655 West 12th Avenue
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Ph: 604-660-6030 Fax: 604-660-6073
www.bccdc.org

Collection of Drinking Water Samples for Coliform Testing


A. Sampling Kit



BCCDC Sampling Kit Contains :

1. Sterile sample bottle (with sodium thiosulphate)
2. Requisition form
3. Zip lock plastic bag
4. Rubber band
5. Pen (not supplied)

B. Fill in Requisition and Bottle Label




Requisition Information Required :

1. Contact information – Health Authority and Water Supplier
2. Date and time of collection
3. Water system information
4. Precise sampling site (exact location)
5. Sender address
6. Tests requested

Sample Bottle Label Information Required :

1. Precise sampling site (exact location)
2. Submitter's information

C. Transport



Transport :

1. Ship samples in cooler with sufficient ice packs to maintain temperature at <math><10^{\circ}\text{C}</math>.
2. Ship samples early in the week by same day or overnight courier.
3. Samples exceeding 30 hours holding time (from collection to testing) will not be tested.

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D. Remove attachment



How To Collect Samples :

1. Tap without attachments - run water for 2 to 3 minutes before collecting sample.
2. Tap with mixing faucet - remove attachments such as aerators, filters, hoses, screen or splash guard, run hot water for 2 minutes and then cold water for 2 to 3 minutes before sampling.

E. Collect sample



3. Remove cap of sample container without touching the mouth of the bottle or the inside of the cap.
4. Without rinsing, fill with water sample to 200 mL fill line (marked on the container).

N.B. Collect water sample only from the cold water tap

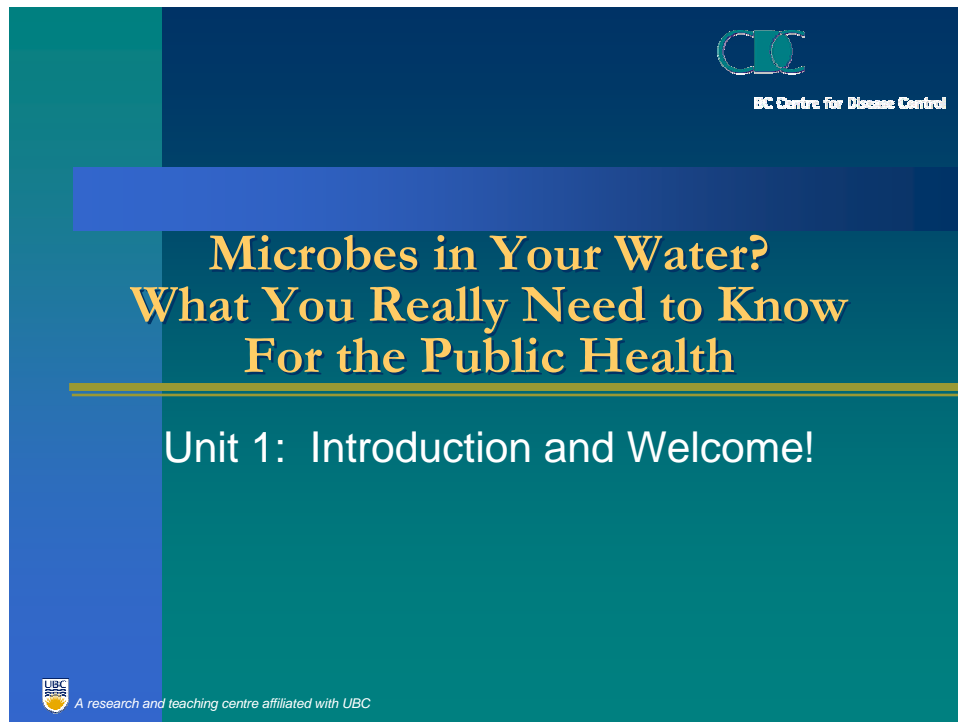
F. Replace bottle cap



5. Replace cap of sample container securely (tight).
6. Place requisition inside zip lock plastic bag and wrap it around sample container with rubber band.
7. See Transport instructions on the other side for shipping samples.

Appendix D

Distance Education: Microbes in Your Water? What You Really Need to Know For the Public Health



For further information please contact Cora Yee, BCCDC Healthy Water Program Coordinator, at 604-660-6561.

Appendix E

Members of the Total Coliform Group: An Evolving Definition

MPN Positive Test Definitions	MF Positive Test Definitions	Defined Substrate Media (DSM) Positive Test Definitions
Pre-1994 Acid and Gas From Lactose	Report 71, 1994 Acid from Lactose	(β-Galactosidase Present)
<i>Escherichia</i>	<i>Escherichia</i>	<i>Escherichia</i>
<i>Klebsiella</i>	<i>Klebsiella</i>	<i>Klebsiella</i>
<i>Enterobacter</i>	<i>Enterobacter</i>	<i>Enterobacter</i>
<i>Citrobacter</i>	<i>Citrobacter</i>	<i>Citrobacter</i>
	<i>Yersinia</i>	<i>Yersinia</i>
	<i>Serratia</i>	<i>Serratia</i>
	<i>Hafnia</i>	<i>Hafnia</i>
	<u><i>Pantoea</i></u>	<u><i>Pantoea</i></u>
	<u><i>Kluyera</i></u>	<u><i>Kluyera</i></u>
		<u><i>Cedecea</i></u>
		<u><i>Ewingella</i></u>
		<u><i>Moellerella</i></u>
		<u><i>Leclercia</i></u>
		<u><i>Rahnella</i></u>
		<u><i>Yokenella</i></u>

Bold type = coliforms which can be present in the environment as well as in human faeces.
Bold and underline = coliforms which are considered to be primarily environmental.
Source: Kreig, 1984; Topley, 1997; Ewing, 1986; Ballows, 1992.

Appendix F

Provincial Health Officer Approval: Quality Assurance for Public Health

There is an expectation by the public that environmental testing laboratories be competent in performing their tasks.

The BC Ministry of Health Provincial Health Officer (PHO) leads Laboratory Quality Assurance for testing for public health purpose. Quality Assurance is based on principles of:

- **Well-Trained and Competent Staff:**
Employing personnel with a background knowledge and understanding of environmental microbiology principles is vital to ensuring laboratory quality and competency.

There is a misconception that some relatively new testing methodologies are ‘idiot-proof’ that no real expertise in microbiology is needed to perform these tests. Although some tests are becoming more ‘user-friendly’ (eg. some DES methods), it is imperative that these tests be monitored for validity.

- **Strong Laboratory Quality Management Program in Place:**
Each PHO approved laboratory must have a Quality Management Program in place including: Each lot of media needs to be tested against reference organisms to ensure its suitability for testing. This requires the expertise and laboratory capacity to culture bacteria.

Each reagent or vessel also needs to be guaranteed sterile and fit for use.

Every sample that comes into the laboratory requires traceability in the event that a questionable result is obtained.

There are many examples of the need for a Quality Management System to ensure validity of the data generated.

- **Participation in External Quality Assessments (Proficiency Testing and On-Site Audits):**
The standard laboratory practice of proficiency testing, checks the laboratory’s abilities by requiring blind testing of samples (negative and positive). These test samples are sent to the laboratory by an external source. Laboratories testing for public health purposes must pass these regular and ongoing “examinations” of quality to maintain their Public Health Office approval.

In addition all environmental microbiology laboratories must undergo rigorous, regular (every 2 years) on-site audits to ensure that the quality of operations is maintained and all associated documentation and processes are up to date and being followed. This

PHO peer-review program is the Enhanced Water Quality Assurance (EWQA) Program.

Water purveyors using bacterial screening test for QA of treatment processes may not require such a rigorous laboratory Quality Management System. Nevertheless water purveyors carrying out testing should be aware of all the possible problems that can arise and the QA/QC issues that need to be maintained in ensuring the validity of the test data.

Appendix G

Summary List of Provincial Health Officer Approved Laboratories

LABORATORIES APPROVED BY B.C. PROVINCIAL HEALTH OFFICER FOR WATER MICROBIOLOGY TESTING AT JANUARY 12, 2006			
NAME	ADDRESS	PHONE	APPROVED FOR
ALS ENVIRONMENTAL - CALGARY, ALBERTA	#2-21 HIGHFIELD CIRCLE SE, CALGARY, AB CANADA T2G 5N6	403-214-5431	Total Coliform and <i>E.coli</i>
ALS ENVIRONMENTAL - FORT St. JOHN, BRITISH COLUMBIA	#2-8820 100TH STREET, FORT St. JOHN, BC V1J 3W9	250-785-8281	Total Coliform and <i>E.coli</i>
ALS ENVIRONMENTAL - VANCOUVER, BRITISH COLUMBIA	1988 TRIUMPH STREET, VANCOUVER BC V5L 1K5	(604)253-4188	Total Coliform, Fecal Coliform and <i>E.coli</i>
BCCDC ENVIRONMENTAL MICROBIOLOGY LABORATORY	655 WEST 12TH AVENUE, VANCOUVER BC V5Z 4R4	(604)660-1753	Total Coliform, Fecal Coliform and <i>E.coli</i>
C R D WATER LABORATORY*	479 ISLAND HIGHWAY, VICTORIA BC V9B 1H7	(250)474-9680	Total Coliform, Fecal Coliform and <i>E.coli</i>
CANTEST LTD - BURNABY, BRITISH COLUMBIA	4606 CANADA WAY, BURNABY BC V5G 1K5	(604)734-7276	Total Coliform, Fecal Coliform and <i>E.coli</i>
CANTEST LTD - VICTORIA, BRITISH COLUMBIA	1104 - 4464 MARKHAM STREET, VICTORIA BC V8Z 7X8	(250)385-6112	Total Coliform, Fecal Coliform and <i>E.coli</i>
CARO ENVIRONMENTAL SERVICES	102 - 3677 HIGHWAY 97N, KELOWNA BC V1X 5C3	(250)765-9646	Total Coliform, Fecal Coliform and <i>E.coli</i>
ECO TECH LABORATORY LTD.	10041 DALLAS DRIVE, KAMLOOPS BC V2C 6T4	(250)573-5700	Total Coliform, Fecal Coliform and <i>E.coli</i>
G V R D WATER LABORATORY	4330 KINGSWAY, VANCOUVER BC V5G 4G8	(604)451-6001	Total Coliform, Fecal Coliform and <i>E.coli</i>
IG MICROMED ENVIRONMENTAL INC	190 - 12860 CLARKE PLACE, RICHMOND BC V6V 2H1	(604)279-0666	Total Coliform, Fecal Coliform and <i>E.coli</i>
MB LABORATORIES LTD	2062 HENRY AVENUE WEST, SIDNEY BC V8L 5Y1	(250)456-1334	Total Coliform, Fecal Coliform and <i>E.coli</i>
NORTH ISLAND LABORATORIES	2755 B MORAY AVENUE, COURTENAY BC V9N 8M9	(250)338-7786	Total Coliform, Fecal Coliform and <i>E.coli</i>
NOTHERN LABORATORIES LTD	251 KAIEN ROAD, PRINCE RUPERT BC V8J 4B7	(250)627-1906	Total Coliform, Fecal Coliform and <i>E.coli</i>
NORWEST LABS	104 - 19575 - 55A AVENUE, SURREY BC V3S 8P8	(604)514-3322	Total Coliform, Fecal Coliform and <i>E.coli</i>
PSC ANALYTICAL SERVICES INC.	8577 COMMERCE COURT, BURNABY BC V5A 4N5	(604)444-4808	Total Coliform, Fecal Coliform and <i>E.coli</i>
PROVINCIAL LABORATORY FOR PUBLIC HEALTH (MICROBIOLOGY) - CALGARY	3030 HOSPITAL DRIVE NW, CALGARY AB T2N 4W4	(403)944-4563	Total Coliform, Fecal Coliform and <i>E.coli</i>
PROVINCIAL LABORATORY FOR PUBLIC HEALTH (MICROBIOLOGY) - EDMONTON	8440 - 112 STREET, EDMONTON AB T6G 2J2	(780)407-2699	Total Coliform, Fecal Coliform and <i>E.coli</i>

*CRD Water Services Laboratory does not accept any external water samples for testing.