

Public Health Inspector's Guide to Environmental Microbiology Laboratory Testing



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Best Practice

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Public Health Ontario

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Authors

Sandra Edelsward, B.Sc., MLT
Program Coordinator, Drinking Water Testing
Quality, Regulatory Affairs and Customer Service Department
Public Health Ontario

Allana Murphy, B.Sc.
Senior Laboratory Lead - Environmental Microbiology
Clinical and Environmental Bacteriology Department
Public Health Ontario

Anna Majury, DVM, PhD
Clinical Microbiologist
Microbiology and Laboratory Science Department
Public Health Ontario

Anne Maki, MLT
Senior Manager
Clinical and Environmental Bacteriology Department
Public Health Ontario

Analyn Peralta, MLT
Manager
Clinical and Environmental Bacteriology Department
Public Health Ontario

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- Canadian Food Inspection Agency (CFIA)
- Canadian Institute of Public Health Inspectors (CIPHI)
- Ministry of the Environment, Conservation and Parks (MECP)
- Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)
- Ministry of Health Environmental Health Protection Policy and Partnerships Branch (MOH-EH)

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Disclaimer

This document was developed by Public Health Ontario (PHO). PHO provides scientific and technical advice to Ontario's government, public health organizations and health care providers. PHO's work is guided by the current best available evidence at the time of publication.

This document is intended to assist public health inspectors in decision-making by describing PHO's laboratory testing services for environmental microbiology. This document should not be considered inclusive of all proper methods of environmental testing or exclusive of other methods of environmental testing reasonably directed at obtaining the same results. The application and use of this document is the responsibility of the user. PHO disclaims any responsibility for its application or use in any way.

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Introduction

The Public Health Inspector's Guide to Environmental Microbiology Laboratory Testing (the Guide) is designed to support effective public health practice through the provision of timely information on PHO's Laboratory services and expertise.

As an 'evergreen' document the Guide will be continually edited and updated. Any current changes will be noted in revision histories located at the end of each section of the document. For a full list of changes please see [Appendix F](#). If you print a copy of the Guide please check the [Public Health Ontario website](#) to ensure you are using the most recent version.¹

Public Health Ontario appreciates the ongoing engagement and generosity of all who are involved in the delivery of environmental health programs and services in Ontario.

Environmental Matrices Analysed by the PHO Laboratory

- Microbiological analyses of foods
- Environmental swab analyses for pathogen specific requests (outbreaks)
- Drinking water analyses for private citizens who rely on private drinking water systems (Total Coliform and *Escherichia coli* only)
- Drinking water analyses requested by boards of health (BOH) (specific microbiological analysis only)
- Ice made from treated water
- Recreational water facilities and public pools (R.R.O. 1990, Reg. 565: Public Pools) /public spas (O. Reg. 428/05) (specific microbiological analysis only)
- Public beach water testing
- Water samples suspected of being contaminated with sewage
- Environmental sampling for *Legionella* species (outbreak/cluster investigations)

Note: Consultation with the microbiologist is required for requests that fall outside of the scope of the PHO's laboratory test menu. Board of health staff are encouraged to contact [PHO's Laboratory Customer Service Centre](#) with inquiries.

Each PHO laboratory site holds a drinking water testing license ([Ministry of the Environment, Conservation and Parks](#)) for drinking water tests, which are limited to the [scope of testing](#) and accredited by the [Canadian Association for Laboratory Accreditation Inc. \(CALA\)](#) to [ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories](#).²⁻⁴

PHO's laboratory uses references and methods as guidance documents for testing from agencies such as [Health Canada \(HC\)](#), [American Public Health Association \(APHA\)](#), the [AOAC](#) (Association of Official Analytical Chemists) and the US [Food and Drug Administration Bacteriological Analytical Manual \(FDA-BAM\)](#).⁵

Investigations and Analyses Not Available at the PHO Laboratory

PHO's laboratory does not test environmental samples for the following parameters; please see other resources below for testing options:

- Algal toxin testing (Blue-green algae)
- Chemical/radiological analysis
- Foreign objects
- Fungi
- Infestations (e.g., products containing parasites, insects)
- Flootation tank/isopod or foot throne samples
- Virus detection in food or water
- Botulism investigations
- *Giardia* and *Cryptosporidium* in water

Other Resources

- [Botulism Reference Service](#), Health Canada⁶ and [Botulism Guide for Health Care Professionals \(ontario.ca\)](#), Ontario⁷.
- [Canadian Association for Laboratory Accreditation](#) (CALA) for a list of laboratories accredited with CALA in Ontario
- [Canadian Food Inspection Agency](#) (CFIA)
- Ministry of Health Environmental Health Protection Policy and Partnerships Branch (MOH-EH) environmental.health@ontario.ca
- [Ministry of the Environment, Conservation and Parks](#) (MECP)
- [Ontario Ministry of Agriculture, Food, and Rural Affairs](#) (OMAFRA)
- [Standards Council of Canada](#) (SCC) for a list of laboratories accredited with SCC in Ontario
- [University of Guelph Laboratory Services](#)
- Public Health Ontario laboratory [Criteria for Acceptance of Patient Specimens](#) webpage and [Test Information Sheets](#) for clinical specimens

Legal Considerations

The microbial analyses and acceptable limits outlined for the various parameters and sample type/source are based on current standards, where available, and/or guidelines based on current scientific evidence. References have been included to assist the user in obtaining further information.

These guidelines are intended to serve as a general statement regarding the environmental microbiological testing services available at PHO's laboratory and are of no legal force or effect. If a local board of health considers submitting environmental samples as part of an investigation with overlapping jurisdictions (e.g., federally or provincially inspected food plant combined with retail food outlet), and there is a discrepancy between federal and/or provincial microbial standards/acceptable limits, the local board of health should consult with its legal counsel.

By submitting a sample for testing, the submitter accepts Public Health Ontario's methodology, and represents and warrants that the sample was taken from the location indicated on the test requisition, and that the information provided is true in all material respects at the time of submission. Public Health Ontario assumes no responsibility for the accuracy of the information provided on the requisition, the manner in which the sample was collected or the mode by which it was transported to the laboratory.

Legal Samples – Submission Instructions

PHO's laboratory must receive prior notification that a legal sample has been collected and will be submitted.

Legal sample is defined as:

Any laboratory sample that has been submitted for testing to PHO's laboratory with an intact legal seal and for which the chain of custody has been maintained.

It is essential to ensure that legal samples are handled according to proper guidelines and adhere to the following instructions:

- Notify [PHO's laboratory Customer Service Centre](#) when legal samples are to be submitted.
- Affix and secure an official PHO laboratory (see [Appendix B](#)) and/or a law enforcement agency legal seal to the legal sample such that the sample cannot be opened without breaking the seal. The official legal seal must be initialed and dated by the individual who has affixed the legal seal. The sealed sample must be placed in an appropriate tamper evident container. Contact the [PHO's laboratory Customer Service Centre](#) to obtain official legal seals.

- Once the legal sample is received at the laboratory, a Chain of Custody form (e.g., the “Relinquished By” section of the water sample requisition) must be signed by the individual delivering the samples, and the legal seal verified as intact by both the individual delivering and receiving the package. Refer to [Instructions for Official Agencies Submitting Water Samples to the Public Health Ontario laboratory](#) for additional details on legal submissions.⁸

Chain of custody is defined as:

An unbroken, documented, chain of events from the time of sample collection, through sample transport, testing and reporting.

Maintaining a proper chain of custody from the time of collection of the sample through transport, testing and reporting ensures the integrity of the legal sample remains intact and supports the validity of the laboratory results in a legal case. Test results from legal samples received at the laboratory without a intact chain of custody may not be accepted in legal proceedings. Consult with legal counsel and the applicable legislative and regulatory requirements as to when legal seals are required.

Revision History

As an ‘evergreen’ document the revisions noted below reflect current changes to this Guide. Printed versions of this document should be checked against the [Public Health Ontario website](#) to ensure the most recent version is being referenced.¹

Date of Implementation	Description and Change
September 13, 2024	<ul style="list-style-type: none"> • Updated Ministry of Health branch name throughout • Legal Considerations – Outlined submitter’s responsibilities • Added updated Botulism Guide for Health Care Professional, Ministry of Health, Ontario

Food Sample Analysis

Microbiological Analyses Test Menu

PHO's laboratory performs testing of food samples submitted by Ontario's boards of health (BOH) for microbiological analyses including indicator and pathogenic organisms as well as physicochemical parameters. Food samples may be collected to assist with an outbreak investigation (part of meal, control or follow-up (after remedial action)) or for microbial risk assessment in support of a Hazard Analysis Critical Control Point (HACCP) assessment or routine surveillance. Food samples may also be submitted as a 'complaint' submission (food with off-odour, off-colour, texture, etc.) for laboratory evaluations that are not associated with an investigation of foodborne illness.

Analyses available at PHO's laboratory for food testing are listed below. Test information including testing frequency, turnaround times and reporting limits, is dependent on the specific analysis. [Click on individual testing links for [test directory](#) details – see below.]⁹

Indicator Bacteria¹⁰

- [Aerobic Colony Count](#)
- [Total Coliform](#)
- [Escherichia coli](#)
- [Total Gram Negative](#)
- [Yeast and Mould](#)

Foodborne Pathogens¹⁰

- [Bacillus cereus group](#)
- [Campylobacter species](#)
- [Clostridium perfringens](#)
- [Escherichia coli O157:H7](#)
- [Listeria monocytogenes](#)
- [Salmonella species](#)
- [Shigella species](#)
- [Staphylococcus aureus](#)
- [Staphylococcus aureus toxin](#)
- [Vibrio species](#)
- [Yersinia enterocolitica](#)

Physiochemical¹⁰

- [pH](#)
- [Phosphatase](#)
- [Water activity \(a_w\)](#)

Routine testing of cooked, ready-to-eat foods includes: Aerobic Colony Count, *Bacillus cereus*, Total Coliform, *Escherichia coli*, total gram negative, *Salmonella* species and *Staphylococcus aureus*. Other indicator bacteria and foodborne pathogens will be tested depending on the food type, clinical laboratory information and epidemiological details provided on the [Food Bacteriology Requisition](#).

Physicochemical testing for pH, phosphatase and/or water activity (a_w) will be performed upon request. For a list of etiological agents associated with foodborne illness, including incubation periods, symptoms, modes of transmission and associated foods, refer to [Appendix C: Major Foodborne Diseases: General Features](#).

Analyses NOT available at PHO's laboratory as part of the for food testing program:

- *Cryptosporidium*
- *Cyclospora*
- *Giardia*
- Hepatitis A
- Norovirus
- Prion disease
- *Toxoplasma*
- *Trichinella*
- Ciguatera poisoning
- Paralytic shellfish poisoning

The above listed test requests will be referred to [CFIA](#), Ontario-Area Recall Coordinator (ON-ARC), as per [Ontario Foodborne Illness Outbreak Response Protocol \(ON-FIORP\) 2023](#).^{11,12} Additional information such as commercial product details, reason for test request, clinical implications and priority level will be required by CFIA for the test referral. Contact the environmental laboratory through [PHO's laboratory Customer Service Centre](#) at 416-235-6556 or toll free 1-877-604-4567 to discuss any other test request not listed above.

Botulism test requests – contact [Health Canada’s Botulism Reference Service](#) directly at (613) 957-0902 or contact the Ministry on-call after hours at 1 (866) 212-2272.⁶ Also refer to [Botulism Guide for Health Care Professionals \(ontario.ca\)](#)⁷.

Food Surveys or Studies

If a board of health would like to initiate a special food survey or study that involves laboratory testing, contact the environmental laboratory program microbiologist through [PHO’s laboratory Customer Service Centre](#) at 416-235-6556 or toll free 1-877-604-4567 to discuss the study outline and impact on the laboratory.

Sample Selection

Instructions

- Selection of food items for collection and submission related to a foodborne outbreak should be determined based on all available environmental, epidemiological and clinical laboratory information related to the outbreak. Information about the clinical pathogen, if known or suspected, should be included to facilitate prioritization of food items both collected and tested.
- If unsafe food-handling practices are identified during the inspection of a food premise, selection of food items for a microbial risk assessment is based on the health inspector’s discretion. The submission of foods is not a requirement in these circumstances but may provide an additional tool available to support the assessment.

Considerations

- During a foodborne outbreak investigation, the results of an environmental investigation, such as a Hazard Analysis Critical Control Point (HACCP) review, and epidemiological analyses, such as the calculation of attack rate, will facilitate prioritization of food items that are most likely to be involved. If attack rates are unavailable, incubation period, clinical symptoms and epidemiologic information about related cases should be used.
- If risk stratification of food sources is not performed by the submitting public health inspector, and there is an abundance of samples submitted for testing, prioritization will be determined by PHO’s laboratory Environmental Microbiology section. The communication of all available clinical, epidemiological and environmental information to PHO’s laboratory is of great importance in guiding these decisions.
- Avoid submitting raw foods which require processing or cooking before consumption, unless they are linked to a specific outbreak or a confirmed etiological agent. If testing of raw foods is required, please contact the [PHO’s laboratory Customer Service Centre](#) at 416-235-6556 or toll free 1-877- 604-4567.

Containers for Sampling

Instructions

- Sterile plastic bags with wire closure are the preferred sample container for all food types.
- Food samples found in the original container can be submitted in the original packaging providing that the container is air-tight and will not leak during transit.
- If the original container has been opened or if the packaging is fragile and may be damaged during transit, samples should be transferred from the original container to a sterile plastic sampling bag with wire closure.

Considerations

- If a commercial packaged product has been submitted, record the following product information on the [Food Bacteriology Requisition](#): brand name, lot number, best before date, expiry date, use by date, Universal Product Code (UPC), name of manufacturer/importer/legal agent, unit size, non-intact (open) and intact (closed). Photographs may also be taken and maintained by the public health inspector to capture this information at the time of sample collection which may facilitate a food recall if required. Photographs should not be submitted to the laboratory.
- For foodborne outbreak investigations: when collecting an opened commercial packaged product, collection of a closed sample with the same lot may be useful to determine if the product was contaminated prior to consumer handling. PHO's laboratory will only analyze closed samples in conjunction with a foodborne outbreak investigation and only following consultation and approval by the laboratory. Please consult with the CFIA prior to submission as appropriate.¹¹
- Styrofoam and box-board packaging used for fast-food products are examples of containers that are not suitable for microbiological testing. They are not air tight and may leak, potentially resulting in cross contamination of sample(s). These containers should not be put inside a sterile sample bag since the outside surfaces of the containers are a potential source of contamination and the sample will be compromised.

Sample Preparation

Instructions

The following steps are recommended to organize and minimize food sample collection time:

- Investigate before sampling to determine a sampling plan.
- Ensure all materials required are available prior to collection.
- Label sample bags using a permanent marker.

- Complete requisition(s) before or after, never during, sample collection.
- After collection, double check to ensure the food sample unique identifier on the sample bag and requisition are legible and match.

Sample Collection

Instructions

- Use aseptic technique at all stages of sample collection. If sterile gloves or tongs are not available, an inverted sampling bag may be used as a glove to collect the sample.
- Collect samples that are representative of each item of food that is being investigated.
- Meals that contain multiple discreet items (e.g. rice and chicken) may be collected individually or combined as a single sample. If collected as a single sample, components should be collected in approximately equal amounts and will be tested as one sample.
- Foods that contain multiple items (e.g., sandwiches) may be collected as a single sample.
- If an individual component is of greater interest than other components of a meal or mixed food, sample the items individually so they can be tested as individual samples.
- Once collection is completed, close the mouth of the sterile plastic sampling bag with the wire closure, and roll down several times twisting the ends together tightly to prevent leaking during transport.
- Submit a minimum of 200 grams of each sample if possible, as multiple tests may be required. Samples that are less than 10 grams will not be tested.

Considerations

- Failure to follow these instructions will compromise the sample integrity and the laboratory results.
- Separation of meals or mixed foods must be performed at the sampling stage. The laboratory will not separate components of a sample once received in the laboratory.
- Leaking samples will not be accepted by the laboratory. Refer to the Food – Laboratory Acceptance Criteria section for a complete list of acceptance criteria.

Sample Collection for Large Lots of Food

Most pre-packaged foods are produced in very large lots (a batch or production unit which may be identified by the same code; i.e., lot number, production code or other unique identifiers).

Instructions

- Where possible, mix the same lot of food before sampling.

Considerations

- If a lot of food is larger than 2 kilograms, take five sub-samples of 200 grams each and submit as individual samples for analysis.
- Ensure sub-samples are clearly and distinctly labeled with a unique identifier for tracking purposes on both the sampling bag and requisition.

Sample Collection for Small Quantities of Food (<200 grams)

During some investigations, it may be determined that there is very little sample available for analysis.

Instructions

- In foodborne outbreak investigations, small quantity samples can be processed by the laboratory, but complete analysis may not be possible. Tests performed will be prioritized based on the type of food/suspected etiological agent.
- Samples that are less than 10 grams will not be tested.

Considerations

- If a particular etiological agent(s) is suspected or confirmed, indicate the information on the requisition form and the analysis for this agent(s) will be given priority. In the absence of such information, the laboratory will aim to perform those analyses most likely to provide information useful to the investigation.

Completion of the Food Bacteriology Requisition

The information below outlines the instructions for completing the [Food Bacteriology Requisition](#). Complete instructions are also located on the reverse or page 2 of the requisition and include, submitter information, sample collection, reason for test request, sample details and shipping instructions.

Instructions

- Complete all areas of the [Food Bacteriology Requisition](#). The information included on the requisition will also be provided on the final laboratory report.

Shipping Instructions

- Ship dry foods and other shelf stable foods in a closed container at ambient temperature.
- Ship frozen foods in an insulated container with sufficient ice packs to maintain the frozen state.
- Ship perishable foods in an insulated container with sufficient cold packs to maintain a temperature as close to 4°C as possible. If ice is used, contain the ice in a manner that does not allow water contact with the samples.
- Submit all food samples to the [closest PHO’s laboratory](#) as soon as possible after collection.

Considerations

- Foods that exhibit temperature abuse during shipping may be rejected and not processed. If the product appears to have undergone a change of state such as from a frozen state to a liquid state, the product will be rejected.

Laboratory Acceptance Criteria

The accuracy of the test results may be affected by improper collection, handling and/or shipping. Food samples that do not meet the acceptance criteria may be rejected by the laboratory and a new sample would be required to be re-submitted with a newly completed requisition. The acceptance criteria are outlined below.

Table 1: Food Acceptance Criteria

Description	Acceptance Reason
Submitter	<ul style="list-style-type: none"> • Sample must be received from an authorized board of health.
Sample Type	<ul style="list-style-type: none"> • Food samples are collected to assist with an outbreak/ investigation (part of meal, control or follow-up (after remedial action)), complaint or for a routine monitoring microbial risk assessment (HACCP or routine surveillance). • PHO’s laboratory may analyze raw food samples (e.g., raw chicken), which require processing or cooking before consumption, to support a foodborne outbreak investigation with a confirmed etiological agent.
Sample Requisition	<ul style="list-style-type: none"> • The requisition must be completed when it is received at the laboratory including date, time and location of collection. The requisition must have a unique identifier that matches the identifier on the sample collection bag. • If a sample is received without a requisition it will not be processed; however, the sample will be held for two weeks.

Description	Acceptance Reason
Sample Collection	<ul style="list-style-type: none"> The sampling container must not compromise the integrity of the sample. Refer to the Containers for sampling section. The full panel of tests may not be performed if a small amount of food (<200 grams) is received. At least 25 grams of sample is required for each analysis. Please contact the PHO's laboratory Customer Service Centre at 416-235-6556 or toll free 1-877-604-4567, if collecting a small amount of food.
Sample Transport	<ul style="list-style-type: none"> The sample container must be secure to avoid leaks during transport. The sample temperature must meet the requirements listed below when received at the laboratory: <ul style="list-style-type: none"> Frozen foods should be received frozen ($\leq 0^{\circ}\text{C}$); if frozen foods have a temperature $\geq 15^{\circ}\text{C}$ when received at the laboratory, they will not be tested. Foods normally held at refrigeration temperatures should be between 2.0 to 8.0°C when received at the laboratory and must not be at or $< 0^{\circ}\text{C}$ or $\geq 25^{\circ}\text{C}$. Shelf stable foods should be shipped at ambient temperatures and must be between 0°C and 30°C when received at the laboratory.

Reporting and Interpretation of Environmental Microbiological Test Results

Satisfactory microbial limits for a food sample will be dependent on the food preparation, conditions (raw, ready-to-eat, and frozen, refrigerated, shelf stable) and commodity type (e.g., dairy, meat, etc.) in relation to a specific analysis. Refer to Health Canada's Health Products and Food Branch (HPFB).

Standards and Guidelines for Microbiological Safety of Food - [An Interpretive Summary](#) for food commodity type details.¹³

The tables below describe the reporting limit and unsatisfactory level for ready-to-eat foods for indicator bacteria and foodborne pathogens according to [Health Canada](#), and physicochemical testing available at PHO laboratory.¹⁴ If a board of health would like further interpretation on food results, contact [PHO's laboratory Customer Service Centre](#) at 416-235-6556 or toll free 1-877-604-4567, and provide the sample identification number.

Indicator Bacteria

Table 2: Indicator Bacteria Reporting Limits

Testing Type	Reporting Limit (CFU/g) ^a	Unsatisfactory Level (CFU/g) ^{a, c}
<u>Aerobic Colony Count</u>	< 1,000 to > 200,000	Category 1: $\geq 10^5$, Category 2: $\geq 10^7$, Category 3: N/A
<u>Total Coliform</u>	< 3 to > 1,100 ^b	$\geq 10^3$
<u>Escherichia coli</u>	< 3 to > 1,100 ^b	≥ 100
<u>Total gram negative</u>	< 1,000 to > 200,000	$\geq 10^4$ ^d
<u>Yeast and mould</u>	< 10 to > 150,000	No reportable limit available

a: CFU (colony forming units)

b: MPN (most probable number) is used for this test method

c: Microbial guidelines as per Health Canada¹⁴

d: Microbial guidelines as per Alberta Health Services¹⁵

Unsatisfactory levels of indicator bacteria indicate improper food preparation, handling, storage or holding conditions, temperature abuse, and/or sanitation. Refer to specific test details below for more information.

Aerobic Colony Count (ACC): It is reasonable to expect properly prepared and handled ready-to-eat processed or cooked foods, where no additional food handling was required after food preparation, to have an ACC of $< 10^4$ CFU per gram. An ACC of $> 10^5$ CFU per gram is usually evidence of post processing temperature abuse, inadequate cooking or improper storage conditions. ACC cannot be applied to raw, uncooked, unprocessed foods (e.g., fruits or vegetables), cultured or fermented products (e.g., cheese, yogurt or salami, etc.) since these foods are known and expected to have naturally occurring microbial flora. There are three categories of ACC listed in the table above, which are based on food type and the processing/handling the food has undergone.

Category 1 – cooked foods that do not require handling or processing; i.e., soups, bread, quiche, cooked meat, fish & seafood, vegetables, etc.

Category 2 – cooked foods that require further handling prior to or during the preparation of the final product; i.e., hot dogs, sandwiches, burgers, etc.

Category 3 – foods that have a high ACC due to the normal microbial flora associated; i.e., pitas, potato or pasta salad, salad rolls, fresh fruits, raw vegetables, cheese, yogurt, deli meats, etc.

Total Coliform: Presence of coliforms in a processed, cooked, ready-to-eat food is indicative of inadequate processing or post processing contamination resulting from poor handling of the product. A satisfactory limit cannot be applied to uncooked or raw foods such as salads and salad ingredients, raw fruits and vegetables, raw meats as well as some fermented foods, etc., since coliforms are regularly found in and/or on these foods.¹⁶

Escherichia coli: The presence of Escherichia coli in food is indicative of direct or indirect contamination of the food product with fecal material, thus, enteric pathogens may also be present. The presence may be indicative of inadequate processing, post processing contamination and poor sanitation.

Total gram negative: Total gram negative (TGN) count has the same significance as presence of coliforms. Elevated TGN count should not be detected in cooked or processed ready-to-eat foods. Absence of gram negative organisms in a food sample is another indicator of food safety.

Yeast and mould: The presence of yeast and moulds can cause various degrees of deterioration and decomposition of food, resulting in abnormal odours, flavours and colours. Both yeast and mould can also be added intentionally to a product for a desirable flavour such as mould for blue and brie cheeses and yeast for beer, ciders and wines.

Foodborne Pathogens

Table 3: Foodborne Pathogen Reporting Limits

Testing Type	Reporting Limit (CFU/g) ^a	Unsatisfactory Level (CFU/g) ^{a, b}
<u>Bacillus cereus group</u>	< 100 to > 200,000	≥ 10 ⁴ potentially hazardous
<u>Campylobacter species</u>	Not Detected / Detected	Detected
<u>Clostridium perfringens</u>	< 100 to > 200,000	≥ 10 ⁴ potentially hazardous
<u>Escherichia coli O157:H7</u>	Not Detected / Detected	Detected
<u>Listeria monocytogenes</u>	Not Detected / Detected	Detected
<u>Salmonella species</u>	Not Detected / Detected	Detected
<u>Shigella species</u>	Not Detected / Detected	Detected
<u>Staphylococcus aureus</u>	< 100 to > 200,000	≥ 10 ⁴ potentially hazardous
<u>Staphylococcus aureus toxin</u>	Not Detected / Detected	Detected
<u>Vibrio species</u>	Not Detected / Detected	Detected
<u>Yersinia enterocolitica</u>	Not Detected / Detected	Detected

a: CFU (colony forming units)

b: Microbial guidelines as per Health Canada¹³

Foodborne pathogens should not be detected from a ready-to-eat food and would indicate improper food preparation, poor handling, inadequate sanitation practices or possible contamination from a positive food source or an ill food handler. [Appendix C: Major Foodborne Diseases: General Features](#) provide additional information regarding the organisms incubation period, clinical symptoms, mode of transmission and associated foods.

Physicochemical

Table 4: Physicochemical Reporting Limits

Testing Type	Reporting Limit
pH	1.00 to 14.00
Phosphatase	Not Detected / Detected
Water activity (a_w)	0.000 to 1.000 IU/L

pH: The pH is a function of the hydrogen ion concentration in the food and is a measure of food acidity and alkalinity, which varies with food type. pH is measured on a scale of 1 to 14, with 7 being neutral, lower numbers indicating greater acidity and higher numbers indicating greater alkalinity. pH is just one factor that influences bacterial growth, and can interact with the following to inhibit growth of pathogens and other organisms: water activity (a_w), salt, redox potential, preservatives and temperature. As the pH decreases, a lower temperature is needed to inactivate microbes.¹⁷ Foods with an equilibrium pH ≤ 3.7 will not support the growth of bacterial foodborne pathogens, regardless of the a_w . However, if the pH of the food (jarred/canned products) is ≤ 4.6 , microorganisms are inhibited providing the a_w is ≤ 0.85 . According to Public Health Ontario's [Home Canning Literature Review](#), June 2014, a hermetically sealed acidic canned or jarred food with an equilibrium pH ≤ 4.6 that has gone through sufficient heat treatment to eliminate vegetative microorganisms (bacteria, yeasts and moulds), will not support the germination and growth of botulinum spores and production of the toxin.¹⁸

Phosphatase: The enzyme alkaline phosphatase (ALP) is associated with the cream or fat globules of raw milk. It is inactivated below the detection limit of conventional methods following complete pasteurization. The detection of residual alkaline phosphatase indicates a reduction in pasteurization temperature of at least 1.5°C, a 5 minute reduction in holding time, or the presence of $\geq 0.3\%$ raw milk. Since ALP is more heat stable than most pathogens (which may be present in milk), it is used as an indicator of pasteurization; however, a negative ALP test does not guarantee that the product is pathogen free.¹⁹

Water activity (a_w): The availability of water for microbial, enzymatic or chemical activity determines the shelf life of foods. This water availability is measured as water activity (a_w). Water activity is the ratio of water vapor pressure of the food substrate to the vapor pressure of pure water at the same temperature. Water activity is measured on a scale of 0 to 1; pure water has an a_w of 1.00 and the a_w of a completely dehydrated food is 0.00. For most foods to be considered safe to store at room temperature, they should have an $a_w \leq 0.85$. Bacterial foodborne pathogens are inhibited at this a_w ; however, for those microorganisms that can cause spoilage (e.g., yeasts and moulds), the a_w should be < 0.60 to prevent food spoilage by inhibiting growth of these organisms.²⁰

Revision History

As an 'evergreen' document the revisions noted below reflect current changes to this Guide. Printed versions of this document should be checked against the [Public Health Ontario website](#) to ensure the most recent version is being referenced.¹

Date of Implementation	Description and Change
September 13, 2024	<ul style="list-style-type: none">Updated the Botulism after hours contact information and added the Botulism Guide for Health Care Professionals document from Ministry of Health, Ontario

Environmental Swabs

Microbiological Analysis Test Menu

PHO's laboratory performs microbiological pathogen specific testing of environmental swabs to support foodborne outbreak investigations. Environmental swabs processed during foodborne outbreak investigations will be tested for the presence or absence of a specified clinically confirmed bacterial pathogen from a physical environment (e.g., cutting board, meat slicer, etc.).

Sampling instructions for environmental swabs are provided below. Test information including testing frequency, turnaround times and reporting limits, is dependent on the specific analysis. [Click on individual testing links for [test directory](#) details - [Salmonella spp.](#), [Listeria monocytogenes](#) and [E. coli O157:H7.](#)]^{9,10}

Sample Selection

Instruction for sample selection of swabs for an outbreak investigation:

To identify the source of the pathogen, selection of sampling sites for the investigation should be informed by environmental and epidemiological factors. Random sampling may delay the identification of the source of the outbreak. Samples deemed as most likely to be positive should be sampled and submitted first.

PHO's laboratory should be notified prior to sample submission to allow for preparation of materials required for testing. Please contact the laboratory by calling [PHO's laboratory Customer Service Centre](#) at 416-235-6556 or toll free 1-877-604-4567.

Containers for Sampling

Instructions

- Environmental swabs are available from PHO's laboratory warehouse and can be ordered by calling the [PHO's laboratory Customer Service Centre](#) at 416-235-6556 or toll free 1-877-604-4567. The swab vial contains neutralizing solution as transport medium. This medium is able to neutralize quaternary ammonium compounds and phenolic disinfectants.

- Refer to the specific foodborne pathogen test directories, i.e., [Salmonella spp.](#), [Listeria monocytogenes](#) or [E. coli O157:H7](#) for additional details.^{9,10}
- Each sample and its accompanying requisition must be labeled with a unique identifier.

Sample Preparation

The following steps are recommended to organize and minimize sample collection time:

- Investigate before sampling to determine a sampling plan.
- Ensure all materials required are available prior to collection.
- Label sample container using a permanent marker.
- Complete requisition(s) before or after, never during, sample collection.
- After collection, double check to ensure the sample unique identifier on the sample container and requisition are legible and match.

Sample Collection

Instructions

- Using aseptic technique, open the sterile swab container; grasp the handle end of the swab.
- Remove the swab aseptically being careful not to touch any portion that might be inserted into the vial.
- Moisten the swab head and press out the excess solution against the interior wall of the vial with a rotating motion.
- Hold the swab handle to make a 30 ° angle contact with the surface. Rub the surface slowly and thoroughly over approximately 50 cm² of the surface three times, reversing direction between strokes.
- After the area has been swabbed, using aseptic technique, return the swab to the vial.
- Complete all sections of the [Environmental Microbiology Investigation Requisition](#) form with appropriate information to ensure accurate tracking of the sampling and complete all fields as outlined on page 2 of the form.
- Ensure samples are submitted to the laboratory under refrigeration conditions (2 – 8 °C) in a manner that will not leak during transport.

Considerations

- Leaking swab vials will be rejected by the laboratory. Ensure the vial is closed tightly to prevent leakage resulting in sample rejection.
- Absence of a unique identifier linking sample(s) to a sampling location on a requisition will result in rejection of sample(s) by the laboratory.
- For an investigation where sampling areas are smaller and physically different, a new swab should be used for each different area.
- For an investigation where the sample area is large, multiple sections may be swabbed with one swab. Sampling details should be accurately recorded to ensure traceability when laboratory results are received to ensure appropriate interpretation of results.

Laboratory Acceptance Criteria

The accuracy of the test results may be affected by improper collection, handling and/or shipping. Swab samples that do not meet the acceptance criteria may be rejected by the laboratory and a new sample may be re-submitted with a newly completed requisition. The acceptance criteria are outlined below.

Table 5: Environmental Swab Acceptance Criteria

Description	Acceptance Reason
Submitter	<ul style="list-style-type: none"> • Swab samples must be received from an authorized board of health submitter.
Sample Type	<ul style="list-style-type: none"> • Swabs will only be accepted for testing to support a foodborne outbreak investigation.
Sample Requisition	<ul style="list-style-type: none"> • The requisition must be completed upon receipt at the laboratory, including date and location of collection. If a sample is received without a requisition it will not be processed; however, the sample will be held for 24 hours. • The requisition must have a unique identifier that matches the unique identifier on the swab collection vial.
Sample Collection	<ul style="list-style-type: none"> • Environmental swabs are available from the PHO's laboratory warehouse and can be ordered by calling PHO's laboratory Customer Service Centre at 416-235-6556 or toll free 1-877-604-4567. Unapproved swabs will not be accepted.
Sample Transport	<ul style="list-style-type: none"> • The sample container must be secure to avoid leaks during transport. • The sample temperature must meet the requirements listed below upon receipt at the laboratory: <ul style="list-style-type: none"> • Swabs should be shipped with cold packs to maintain a temperature between 2.0 to 8.0 °C during transport to the laboratory. • Swabs received frozen or greater than room temperature may be rejected and not processed.

Reporting and Interpretation of Environmental Microbiological Test Results

The following tables outline the reporting limits and interpretation of the environmental swab analyses. Information such as collection conditions, time and date, and sanitation details should be considered when interpreting swab results.

Foodborne Pathogens

Table 6: Foodborne Pathogens Reporting Limits

Testing Type	Reporting Limit	Unsatisfactory Level
<u><i>Escherichia coli</i> O157:H7</u>	Not Detected / Detected	Detected
<u><i>Listeria monocytogenes</i></u>	Not Detected / Detected	Detected
<u><i>Salmonella</i> species</u>	Not Detected / Detected	Detected

Foodborne pathogens should not be detected from a ready-to-eat food preparation surface swab and would indicate poor food handling, inadequate work surface sanitation practices or possible contamination from an ill food handler. [Appendix C: Major Foodborne Diseases: General Features](#) provide additional information regarding the organisms incubation period, clinical symptoms, mode of transmission and associated foods.

Revision History

As an ‘evergreen’ document the revisions noted below reflect current changes to this Guide. Printed versions of this document should be checked against the Public Health Ontario website to ensure the most recent version is being referenced.²

Date of Implementation	Description and Change
September 13, 2024	<ul style="list-style-type: none"> No changes noted

Water Sample Analysis

Microbiological Analysis Test Menu

PHO's laboratory performs microbiological testing of water samples for the detection of bacterial indicators of contamination and specific pathogens in water. Each water sample is tested for specific indicators according to the water source.

If testing is required on a water source that is not listed in PHO's laboratory water testing menu below, or testing is required to identify specific etiological agents in water related to a laboratory confirmed clinical case or outbreak investigation, boards of health are asked to [consult](#) with the microbiologist, or designate, overseeing the water testing program prior to sample submission.

Sample collection requirements, sample handling, shipping conditions, test information including testing frequency, turnaround times and reporting limits are dependent on the specific water source. For disinfected water sources, ensure the sodium thiosulfate preservative in the collection bottle has not expired prior to sampling. Samples will not be processed if the requisition is not completely and accurately filled in when received at the laboratory; and a new sample and completed form will be required to be submitted. [Click on individual [testing links](#) for test directory details – see below.]⁹

- [Drinking water \(including bottled water\)](#)²¹
- [Drinking water – Private citizen](#)²²
- [Ice – Treated](#)²³
- [Public Beach Water](#)²⁴
- [Recreational Water Facilities, Public pools/Spas](#)²⁵
- [Suspected Sewage Contamination – Water](#)²⁶

For specific etiological agent requests, please contact the [PHO's laboratory Customer Service Centre](#) at 416-235- 6556 or toll free 1-877-604-4567.

For general information related to sample collection, labelling, storage, transportation and chain-of-custody of drinking water samples that fall under the Drinking Water Systems Regulation (Ontario Regulation 170/03) and Small Drinking Water Systems (Ontario Regulation 319/08), refer to the Ministry of Environment, Conservation and Park's [Practices for the Collection and Handling of Drinking-Water Samples](#).²⁷

Important Information Related to Drinking Water Analysis

Type of Drinking Water System

As part of an investigation under the [Health Protection and Promotion Act](#) (HPPA), public health inspectors may collect drinking water samples in accordance with the following sections of the Health Protection and Promotion Act ([HPPA](#)): Section 13, Section 41, Subsections (1), (2), (4), and (5)²⁸. This may include collecting samples at drinking water systems regulated under O. Reg. 170/03 and O. Reg. 319/08 as well as unregulated systems where the public has access to the water (e.g., campground with fewer than 6 connections). Consult with Ministry of Health Environmental Health Protection Policy and Partnerships Branch (MOH-EH) or legal counsel for additional information on the classification of the drinking water systems.

All drinking water testing must be conducted by a licensed laboratory using licensed drinking water testing methods.

The type of drinking water system identified on the requisition triggers specific notification requirements. Laboratories have an obligation to report test results as outlined in section 18 and 18.1 of the [Safe Drinking Water Act, 2002](#) for drinking water samples submitted under [O. Reg. 170/03 Drinking Water Systems](#) or [O. Reg. 319/08 Small Drinking Water Systems](#).²⁹⁻³¹

The details are outlined in the [Drinking Water – Official Agencies](#) Test Information Sheet.²¹

Completing the requisition and submitting the sample according to [Instructions For Official Agencies Submitting Water Samples to the Public Health Ontario Laboratory](#)⁸ will help to ensure samples are accepted.

Chain of Custody

The chain of custody must be maintained for drinking water samples collected under [O. Reg. 170/03 Drinking Water Systems](#) or [O. Reg. 319/08 Small Drinking Water Systems](#).^{30,31} Drinking water samples submitted under these regulations will not be accepted if the chain of custody is not maintained.

For drinking water samples indicated as “HPPA O. Reg 319/08” or “SDWA O. Reg 170/03”, there are two ways to maintain the chain of custody:

Table 7: Options for Maintaining a Drinking Water Sample Chain of Custody

Situation	Action
Courier - Indirect delivery of the sample to the laboratory (cannot deliver the sample directly to the laboratory)	<p>For samples delivered by courier:</p> <ul style="list-style-type: none">Place a signed and dated "Regulated Water Seal" (refer to Appendix B: Laboratory Submission Forms) up and over the cap of the sample in such a way that the two ends of the seal are attached to the shoulder of the bottle, and the midpoint of the seal is stuck to the top of the cap.The sample is sent to the laboratory with a completed requisition according to the sample transport instructions in the test directory – test information sheet.⁹

Situation	Action
In person - Direct delivery of the sample to the laboratory	<p>Complete the following fields in the “For Regulated Drinking Water or Legal Samples” section of the requisition:</p> <ul style="list-style-type: none"> • The printed name of the person relinquishing the sample to laboratory personnel • The signature of the person relinquishing the sample • Date and time the sample(s) is relinquished <p>The sample is sent to the laboratory with a completed requisition according to the sample transport instructions in the test directory – test information sheet.⁹</p>

Compliance versus Audit Samples

Compliance Samples

Owner/operators of drinking water systems under [O. Reg. 170/03](#) or [O. Reg. 319/08](#) are required to follow the requirements under those regulations. If sampling is performed to demonstrate compliance with the required monitoring frequency, they are to use a licensed commercial laboratory for their testing.^{30,31}

The exception is [O. Reg. 319/08](#) small drinking water systems that supply Ontario Parks; routine compliance samples are analyzed by the PHO’s laboratory.³¹

Audit Samples

Public health inspectors may sample regulated drinking water systems as part of an audit and these are considered audit samples. Routine monitoring of these drinking water systems by Board of Health staff is not recommended.

Drinking Water Sample Hold Time

Drinking water must be analyzed in accordance with a licensed test method. PHO’s laboratory uses a modified versions of the MECP methods; namely, E3407 Membrane Filtration Method Using DC Agar for the Simultaneous Detection and Enumeration of Total Coliforms and *Escherichia coli* in Drinking Water, and E3408: The Spread Plate Method for the Enumeration of Aerobic, Heterotrophic Bacteria in Drinking Water. These methods include a sample hold time of 48 hours.^{32,33} For results to be valid for the purposes of the [Safe Drinking Water Act, 2002](#), samples must be tested within the hold time.²⁹

Etiological Agent Testing

A Public Health Inspector or designate under the authority of the Medical Officer of Health may collect water samples for identification of an etiological agent (e.g., *Legionella* spp., *Salmonella* spp., etc.) from drinking water systems if required for investigational purposes or outbreak situations. On the [Bacteriological Analysis Water - Multiple Sample Requisition for Official Agencies](#), these samples must be identified as “Non-Regulated”, “HPPA Regulated Premises”, or “Private Residence”, and marked as an “Outbreak Investigation” with the outbreak/investigation number, etiological agent and clinical case identification (e.g., iPHIS, PHO’s laboratory barcode identification). Alternatively, complete all sections of the [Environmental Microbiology Investigation Requisition](#) with appropriate information to ensure accurate tracking of the sampling and complete all fields as outlined on page 2 of the form.

Drinking Water Submissions under a Provincial Officer’s Order or Approval (Directive)

Environmental officers and public health inspectors have the legal authority to enter a facility at any time to ensure compliance (i.e., MECP Provincial Officers for [Ontario’s environmental laws](#) and public health inspectors under the [HPPA](#)²⁸). If it is determined that a serious non-compliance has occurred, an Order can be issued that sets out obligations for a specific person or persons in relation to a specific operation.

If drinking water testing is one of the obligations, and PHO’s laboratory is to perform analysis for a bacteriological health related parameter not included in [Ontario Drinking Water Quality Standards Ontario Regulation 169/03](#),³⁴ e.g., Heterotrophic Plate Count (HPC) or Background, contact [PHO’s laboratory Customer Service Centre](#) and ask to speak to the Program Coordinator Drinking Water Testing or designate.

Drinking Water Research

If board of health personnel wish to engage in a study involving drinking water (e.g., to identify trends associated with ground water contamination in various geographic areas, etc.) contact [PHO’s laboratory Customer Service Centre](#) and ask to speak to the Program Coordinator, Drinking Water Testing.

Research projects must be reviewed and approved by the microbiologist overseeing PHO’s laboratory Environmental Microbiology program before research begins.

The [Drinking Water Testing Services regulation O. Reg. 248/03](#) outlines the requirements related to drinking water quality research (e.g., scientific studies, including special audits or surveys to assess drinking water quality).³³

A [Notification Form for Drinking Water Testing Research and Method Development](#) must be submitted to the Ministry of the Environment, Conservation and Parks.

Laboratory Acceptance Criteria

The accuracy of the test results may be affected by collection, handling and shipping. Submit water samples in a timely manner to PHO’s laboratory. Water samples that do not meet the acceptance criteria may not be accepted by the laboratory. A new sample may be submitted with a new requisition. The acceptance criteria are outlined below.

Table 8: Acceptance Criteria for Water Sample Submissions

Description	Acceptance Reason
Submitter	<p>Water samples must be submitted by an authorized submitter:</p> <ul style="list-style-type: none"> • Official Agencies: Local board of health or an Ontario Park under the Ministry of Natural Resources and Forestry (MNRF). Requests for testing by any other organization (e.g., other public health partners or Ontario Ministries) will require approval from the Director Quality, Regulatory Affairs and Customer Service and microbiologist overseeing the water testing program, or designate, prior to submission. • Private drinking water systems (e.g., well water): Private citizen submissions of samples collected from a “single household” and serves fewer than 6 private residences but not the public.
Sample Type	<ul style="list-style-type: none"> • The water sample must be collected from within the province of Ontario. • The following types of water sources are analyzed at PHO’s laboratory. For specific analyses, refer to PHO’s laboratory test directory – test information sheet:⁹ <ul style="list-style-type: none"> • Local board(s) of health: <ul style="list-style-type: none"> • Drinking water (including bottled water) • Ice - Treated • Public Beach Water • Recreational Water Facilities, Public pools/Spas • Suspected Sewage Contamination – Water • Specific etiological agent • MNRF Ontario Parks: <ul style="list-style-type: none"> • Drinking Water – Official Agency • Public Beach Water • Private Citizen <ul style="list-style-type: none"> • Drinking water – Private citizen • Refer to the acceptance criteria

Description	Acceptance Reason
Sample Requisition	<ul style="list-style-type: none"> The sample must be received with a requisition that is complete and accurate. Submitters will be required to submit clarification in writing when there is conflicting information on the form (e.g., a drinking water system is listed as a “HPPA regulated premise”, but the requisition includes a regulated drinking water system number). A unique identifier is used to link the sample with the requisition: <ul style="list-style-type: none"> For Official Agency water samples, the sample barcode or barcode number must be present on both the requisition and the sample when it is received at the laboratory. For treated ice samples, a unique identifier is written on the sample bag and the requisition; they must match. For Private Citizen drinking water samples, a unique identifier, e.g., the submitter’s name can be used as the unique identifier to link the requisition and the sample; however, samples are accepted without a unique identifier when the sample is received at the laboratory with the requisition secured around the bottle. Water samples for identification of an etiological agent (e.g., <i>Legionella</i> spp., <i>Salmonella</i> spp., etc.): <ul style="list-style-type: none"> These samples must be identified as “Non-Regulated”, “HPPA Regulated Premises”, or “Private Residence”, and marked as an “Outbreak Investigation” with the outbreak/investigation number, etiological agent and clinical case identification (e.g., iPHIS, PHO’s laboratory barcode identification).
Sample Collection	<ul style="list-style-type: none"> Water samples must be collected in water collection bottles supplied by PHO’s laboratory. Drinking water samples must be collected from the cold water tap and not the hot water tap. Treated ice samples must be collected in a sterile plastic bag with wire closure. Sufficient amount of sample for the analyses is required (i.e., 200 mL unless otherwise specified in the test directory – test information sheet).⁹ Drinking water samples must be aesthetically acceptable; i.e., clear, colourless and odourless, and does not contain debris or substances that interfere with the test. For Private Citizen drinking water submissions, only requisitions with a version number of 3743-44 (01/01) or newer are accepted.
Sample Transport	<ul style="list-style-type: none"> The sample container must be secure and not broken or damaged when received at the laboratory. Samples should be stored and transported at temperatures between 2 and 8 °C following collection. Drinking water samples must be less than 25 °C (preferably between 2 – 10 °C) but not frozen when received at the laboratory. For details on sample transport for public beach, recreational water facility or public pool/spa refer to the test directory – test information sheet.⁹

Description	Acceptance Reason
Sample Hold Time	<ul style="list-style-type: none"> Drinking water samples must be sent to the laboratory as soon after collection as possible since they must be tested within the hold time for the test as specified in the test directory – test information sheet for the analysis required.⁹ Non potable water (e.g., public beach, public pool/spa, recreational water facility, sewage, etc.) must be tested within one calendar day of collection.

Reporting and Acceptable Limits of Environmental Microbiological Test Results

The following tables outline the reporting and acceptable limits for potable and non-potable water testing. Refer to [Appendix D: Water Testing Fact Sheets](#) for interpretation and additional details on Background or Non-target bacteria, *Campylobacter* spp., Coliforms, *Escherichia coli*, Heterotrophic Plate count (HPC), *Legionella* spp., *Pseudomonas aeruginosa*, *Salmonella* spp. and *Staphylococcus aureus*.

Potable Water

OFFICIAL AGENCY – POTABLE / ICE-TREATED

Table 9: Reporting and Acceptable Limits for Potable Treated Ice Samples

Testing Type	Reporting Limit	Acceptable Limit
Background or Non-Target Bacteria (CFU / 100 mL)	0 to *NDOGN/ NDOGT	At the discretion of the MOH. Only reported on regulated drinking water samples submitted under a “Provincial Officer’s Order” or “Approval (Directive)”.
Total Coliform ^a (CFU / 100 mL)	0 to > 80	0
<i>Escherichia coli</i> ^a (CFU / 100 mL)	0 to > 80	0
Heterotrophic Plate Count (HPC) (CFU / 1 mL)	< 10 to > 3.0 x 10 ³ , **NDOGHPC	< 500 ***

*NDOGN - No Data Overgrown with non-target/NDOGT - No Data Overgrown with target

**NDOGHPC - No Data Overgrown heterotrophic plate count

*** The standard for HPC under O. Reg. 169/03 was revoked June 6, 2006 (O. Reg. 248/06) and therefore, HPC is not reportable under the Safe Drinking Water Act. The acceptable limit listed is based on United States Environmental Protection Agency [standards](#)³⁵.

OFFICIAL AGENCY – BOTTLED WATER

Table 10: Reporting and Acceptable Limits for Bottled Water Samples

Testing Type	Reporting Limit	Acceptable Limit
Total Coliform ^a (CFU / 100 mL)	0 to > 80	0
<i>Escherichia coli</i> ^a (CFU / 100 mL)	0 to > 80	0
Heterotrophic Plate Count (HPC) (CFU / 1 mL)	< 10 to > 3.0 x 10 ³ , *NDOGHPC	< 500
<i>Pseudomonas aeruginosa</i> (CFU / 100 mL) (CFU / 50 mL) ^b	0 to > 100 < 2 to > 200 ^b	0
<i>Staphylococcus aureus</i> (CFU / 100 mL) (CFU / 50 mL) ^b	0 to > 100 < 2 to > 200 ^b	0

*NDOGHPC - No Data Overgrown heterotrophic plate count

PRIVATE CITIZEN – PRIVATE DRINKING WATER SYSTEMS

Table 11: Reporting and Acceptable Limits for Private Drinking Water Samples

Testing Type	Reporting Limit	Acceptable Limit
Total Coliform ^a (CFU / 100 mL)	0 to > 80	≤ 5
<i>Escherichia coli</i> ^a (CFU / 100 mL)	0 to > 80	0

^a An overgrown sample; that is, crowding and/or confluent and/or non-identifiable microbial growth on a plate, is considered an adverse result. When there is an overgrown condition and there is no evidence of Total Coliform or *E. coli*, NDOGN - No Data Overgrown with non-target is reported. The water may be unsafe to drink. When there is an overgrown condition and there is evidence of Total Coliform and/or *E. coli*, NDOGT - No Data Overgrown with target is reported. The water is unsafe to drink.

^b When 50 mL is analyzed.

NON POTABLE WATER

OFFICIAL AGENCY – PUBLIC BEACH WATER

Table 12: Reporting and Acceptable Limits for Public Beach Water Samples

Testing Type	Reporting Limit	Acceptable Limit
<i>Escherichia coli</i> (CFU / 100 mL)	< 10 to > 1.0 x 10 ³	≤ 200

OFFICIAL AGENCY – SUSPECTED SEWAGE CONTAMINATION

Table 13: Reporting and Acceptable Limits for Suspected Sewage Contamination Water Samples

Testing Type	Reporting Limit	Acceptable Limit
<i>Escherichia coli</i> (CFU / 100 mL)	< 1.0 x 10 ³ to > 1.0 x 10 ⁶	Not Applicable

OFFICIAL AGENCY – RECREATIONAL WATER FACILITIES, PUBLIC POOLS/SPAS

Table 14: Reporting and Acceptable Limits for Recreational Water Facilities and Public Pools/Spas Samples

Testing Type	Reporting Limit	Acceptable Limit
Background or Non-target bacteria (CFU / 100 mL)	Only reported if NDOGN/NDOGT	Not Applicable
Total Coliform ^a (CFU / 100 mL)	< 2 to > 160	< 2
<i>Escherichia coli</i> ^a (CFU / 100 mL)	< 2 to > 160	< 2
Heterotrophic Plate Count (HPC) (CFU / 1 mL)	< 10 to > 3.0 x 10 ³ , *NDOGHPC	Refer to Appendix D : Water Testing Fact Sheets
<i>Pseudomonas aeruginosa</i> (CFU / 100 mL) (CFU / 50 mL) ^b	0 to > 100 < 2 to > 200 ^b	< 10
<i>Staphylococcus aureus</i> (CFU / 100 mL) (CFU / 50 mL) ^b	0 to > 100 < 2 to > 200 ^b	< 50

^a An overgrown sample; that is, crowding and/or confluent and/or non-identifiable microbial growth on a plate, is considered an adverse result. When there is an overgrown condition and there is no evidence of Total Coliform or *E. coli*, NDOGN - No Data Overgrown with non-target is reported. When there is an overgrown condition and there is evidence of Total Coliform and/or *E. coli*, NDOGT - No Data Overgrown with target is reported.

^b When 50 mL is analyzed.

*NDOGHPC - No Data Overgrown heterotrophic plate count

Water Status Codes and Associated Interpretation Statements for Drinking Water Reports

Table 15: Drinking Water Report Status Codes and Associated Interpretation

Status code	Interpretation
0	Not yet tested
1	No significant evidence of bacterial contamination
2	Significant evidence of bacterial contamination
3	UNSAFE TO DRINK, evidence of fecal contamination
4	No Data: Overgrown with Non-Target
5	The sample was taken from an unprotected source, such as a lake or river
7	The sample was too old
8	The bottle was received broken or damaged
10	Aesthetically unacceptable - The appearance or odour makes the sample unacceptable as drinking water
11	The requisition was received separated from the sample bottle
12	The sample was not collected in the proper bottle
13	The sample was received frozen
14	The sample was collected from a hot water tap
15	Insufficient sample was submitted
17	The sample leaked in transit
18	The sample was collected from a source located outside of Ontario
19	The sample was collected from a municipal water supply
20	Insufficient information was supplied on the sample
21	Not tested for some other reason
22	No Data: Overgrown with Target

Status code	Interpretation
24	Sample received was very warm upon receipt
25	Bottled water submitted
26	Interfering substances in the sample
27	Unique identifier missing
28	Outdated collection kit received
29	Unauthorized submitter
30	Laboratory error
32	Broken chain of custody
33	Sample received outside of operating hours
50	Technical difficulties with the barcode

Revision History

As an 'evergreen' document the revisions noted below reflect current changes to this Guide. Printed versions of this document should be checked against the Public Health Ontario website to ensure the most recent version is being referenced.¹

Date of Implementation	Description and Change
September 13, 2024	<ul style="list-style-type: none">• Important Information Related to Drinking Water Analysis section – Removed the table, “Examples of Drinking Water that Fall Outside Ontario Drinking Water Regulations”• Compliance versus Audit Samples section – Clarified drinking water system owner/operator responsibilities• Drinking Water Submissions under a Provincial Officer’s Order or Approval (Directive) section - Added context for Orders or Directives• Drinking Water Research section – Included a link to the “Notification Form for Drinking Water Testing Research and Method Development” form• Water – Laboratory Acceptance Criteria section – Added a requirement for submitters to provide written clarification when there is conflicting information on the requisition• Water – Reporting and Acceptable Limits of Environmental Microbiological Test Results section – Added a qualifier for the HPC acceptable limit for drinking water• Water Status Codes and Associated Interpretation Statements for Drinking Water Reports section – Added codes 32 and 33

Legionella Investigations

Microbiological Analysis Test Menu

PHO’s laboratory performs environmental testing for Legionella for investigations of confirmed culture clinical cases or outbreaks of legionellosis. Clinical case information must be provided to the laboratory when environmental testing is requested and prior to sample collection. Sample collection requirements, sample handling, shipping conditions, test information including testing frequency, turnaround times and reporting limits are dependent on the specific matrix. [Click on individual testing links for [test directory](#) details - [Legionella \(water\)](#) and [Legionella \(swab\)](#).]^{9,36,37}

The [WHO](#) outlines Legionella infection by category; community acquired, travel associated and nosocomial infection in relation to environmental sampling sites.³⁸

Table 16: Environmental Sampling Locations

Category	Community Acquired	Travel Associated	Nosocomial
Modes of transmission	Inhalation of contaminated aerosol	Inhalation of contaminated aerosol	Inhalation of contaminated aerosol, aspiration, wound infection
Sources of Legionella	Cooling towers; hot and cold-water systems; spa pools, thermal pools, springs; humidifiers; domestic plumbing; potting mixes and compost	Cooling towers; hot and cold-water systems; spa pools, thermal springs and pools; humidifiers	Cooling towers; hot and cold-water systems; spa pools, natural pools, thermal springs; respiratory therapy equipment; medical treatment
Reservoir of Legionella	Industrial sites, shopping centres, restaurants, clubs, leisure centres, sports clubs, private residences	Hotels, cruise ships, camp sites, shopping centres, restaurants, clubs, leisure centres, sports clubs	Hospitals, medical equipment

Sample Selection

Instructions

- To identify the source of the *Legionella*, selection of sampling sites for the investigation should consider both environmental and epidemiological factors. The CDC provides an example of the Legionella Environmental Assessment Form which can be used to document details on the water systems, and information on developing a sampling plan.³⁹
- *Legionella* outbreaks occur because of aerosolization and inhalation of high numbers of *Legionella*. Investigators should try to determine where in a water system there is stagnation of water and subsequent aerosolization of stagnant water; for example any dead ends in a water system may allow Legionella to grow to high numbers. *L. pneumophila* survived and multiplied in water at temperatures between 20 °C and 50 °C, with an optimal temperature range of 32–42 °C
- Samples from locations based on the environmental assessment and most likely to be positive and associated with the clinical case exposure history should be collected and submitted.

The [WHO](#) also provided a list of environmental sampling sites which was adapted from [Barbaree JM, et al. 1987](#).^{38,40} This information can be found on the table below and it can be used as a guide for selection of sampling sites, but it is not a list of mandatory sampling sites.

Table 17: Environmental Sampling Sites

Environmental Sites	Examples
Potable water outside or on the boundary of the health-care facility property	Treatment plant (raw and refined water), guardhouse or other facility if water is not fed from health-care facility, fire hydrants
General potable water system for healthcare facility	Incoming water pipe(s), water softener (pre and post), preheater (discharge side), primary heater (discharge side), circulating pumps, holding tanks (cold water, discharge side), expansion tank for hot water, back drain on sprinkler system(s), fire line where it branches off main system, water used for respiratory therapy equipment, outlets in patients’ rooms
Air compressor system	Vacuum water source Positive pressure equipment side: Condensate from tank(s), water separator(s) directly off compressors, water source(s) near air intake(s), air samples where patients were ill with legionellosis
Potable water final distribution outlets	Haemodialysis water source, before or after demineralizer

Environmental Sites	Examples
Intensive care units	Respiratory therapy (patient’s room), cardiac, services with different geographical locations, ice-maker (entry water) and ice
Air-conditioning system	Air handling unit serving area where disease occurred Cooling towers: return from heat exchanger to water (spray/trough and gutter) distribution or pond (sump), water supply
Hot tubs	Pool and balance tank (if fitted), jets and pipes (swab)
Other	Decorative fountain, creeks, ponds, sites of stagnant water

Considerations

- Ideally, sampling should be performed before disinfection of the water system.³⁹
- Dead-ends in the water system may be disconnected where pipes are capped instead of completely removed. Any fixtures and water lines that are not used on a regular basis may allow Legionella to grow to high numbers.
- Random sampling may delay the identification of the source of the outbreak. The most obvious sampling sites would be water sources in close proximity to the suspected clinical case exposure.

The table below describes types of environmental sampling sites with typical outbreak incubation period pattern and implicated *Legionella* species.³⁸

Table 18: Environmental Sampling Sites and Commonly Implicated *Legionella* species

Sampling Site	Disease Outbreaks	Commonly Implicated <i>Legionella</i> species
Cooling water systems	Rapid onset over wide area, resolve within incubation period	Predominantly <i>L. pneumophila</i> sg 1
Hot and cold-water systems	Low numbers of cases over prolonged periods	<i>L. pneumophila</i> sg 1, 2, 4, 6, 12, <i>L. micdadei</i> , <i>L. bozemanii</i> , <i>L. feeleii</i> and others
Hot tubs, natural spa pools, thermal springs	Rapid onset confined to users and those in close proximity	<i>L. pneumophila</i> sg 1, <i>L. micdadei</i> , <i>L. gormanii</i> , <i>L. anisa</i>
Humidifiers, respiratory equipment	Low numbers over prolonged periods. Rapid onset confined to users and those in close proximity	<i>L. pneumophila</i> sg 1, 3, and others
Potting mixes, compost	Low numbers of cases over prolonged periods	Exclusively <i>L. longbeachae</i>

Containers for Sampling

Instructions

- PHO's laboratory water collection bottles and environmental swabs are available from the PHO's laboratory warehouse and can be ordered by calling the [PHO's laboratory Customer Service Centre](#) at 416-235-6556 or toll free 1- 877-604-4567. PHO's laboratory water collection bottles contain sodium thiosulfate which is a dechlorination agent used to remove any residual halogen such as chlorine and prevents continuation of bacterial action during sample transit. The swab vial contains neutralizing solution as transport medium. This medium can neutralize quaternary ammonium compounds and phenolic disinfectants. [Click on individual testing links for [test directory](#) details for information on obtaining supplies from PHO's laboratory for *Legionella* outbreak investigations - [Legionella \(water\)](#) and [Legionella \(swab\)](#)].^{9,36,37}
- Prior to collecting environmental samples from sources other than water or environmental swabs, contact the environmental laboratory through [PHO's laboratory Customer Service Centre](#) at 416-235-6556 or toll free 1-877-604-4567.
- Detailed instructions are also available in the reference document [CDC Sampling Procedure and Potential Sampling Sites](#).⁴¹

Sample Preparation

The following steps are recommended to organize and minimize sample collection time:

- Investigate before sampling to determine a sampling plan. Swab samples should be collected first to capture any biofilm from the shower head or aerator. Then collect a water sample slowly into PHO's laboratory water bottle to collect any dislodged material.
- Ensure all materials, including appropriate personal protective equipment, are available prior to collection.
- Label sample containers using a permanent marker.
- Complete requisition(s) before or after, never during, sample collection.
- After collection, double check to ensure the sample unique identifier on the sample container and requisition are legible and match.

Sample Collection – Swabs

Instructions

- Use one swab per site, e.g., jets or shower head.
- Refer to the test directory link for sample collection details - [Legionella \(swab\)](#).³⁷
- Complete all sections of the [Environmental Microbiology Investigation Requisition](#) form with appropriate information to ensure accurate tracking of sampling and complete all fields as outlined on page 2 of the form.

Considerations

- Leaking swab vials will be rejected by the laboratory. Ensure the vial is closed tight to prevent leakage.
- Absence of a unique identifier linking sample(s) to a sampling location on a requisition will result in rejection of sample(s) by the laboratory.

Sample Collection – Water

Instructions

- Using aseptic technique, collect the water sample in PHO's laboratory water collection bottles. For both potable and non-potable water, collect two litres of water. Ten standard PHO laboratory water bottles filled to the 200 mL fill line will satisfy this requirement.
- Complete all sections of the [Environmental Microbiology Investigation Requisition](#) form with appropriate information to ensure accurate tracking of sampling and complete all fields as outlined on page 2 of the form.
- Samples should be stored at refrigeration temperature (2 to 8 °C) and shipped to [PHO's laboratory](#) as soon as possible as outline in the test directory for - [Legionella \(water\)](#).³⁶

Considerations

- For potable water systems, e.g., taps or showers, pre-flush samples should be collected to represent the water held in the tap or fitting. Do not allow the faucets to run before sampling. Turn water on and allow the water to run slowly into the sample bottle to minimize aerosol production. Removing the aerator from the faucet can also help to minimize aerosol production.
- Use of Personal Protective Equipment (PPE) should be guided by local board of health internal health and safety guidelines.

Laboratory Acceptance Criteria

The accuracy of the test results may be affected by improper collection, handling and/or shipping. *Legionella* samples that do not meet the acceptance criteria may be rejected by the laboratory and a new sample may be re-submitted with a newly completed requisition. The acceptance criteria are outlined in the table below.

Table 19: *Legionella* Acceptance Criteria

Description	Acceptance Reason
Submitter	<ul style="list-style-type: none"> Sample must be received from an authorized board of health submitter.
Sample Type	<ul style="list-style-type: none"> Swabs and water will be accepted for testing to support investigations of confirmed clinical cases or an outbreak investigation of legionellosis.
Sample Requisition	<ul style="list-style-type: none"> The requisition must be completed when it is received at the laboratory including date and location of collection. If a sample is received without a requisition it will not be processed; however, the sample will be held for 24 hours. The requisition must have a unique identifier that matches the identifier on the water collection bottle or swab collection vial.
Sample Collection	<ul style="list-style-type: none"> PHO's laboratory water collection bottles and environmental swabs are available from PHO's laboratory warehouse and can be ordered by calling PHO's Laboratory Customer Service Centre at 416-235-6556 or toll free 1-877-604-4567. Unapproved containers will not be accepted.
Sample Transport	<ul style="list-style-type: none"> The sample container must be secure to avoid leaks during transport. The sample temperature must meet the requirements listed below when received at the laboratory: <ul style="list-style-type: none"> Samples should be shipped with cold packs to maintain a temperature between 2.0 to 8.0 °C when received at the laboratory. Swabs received frozen or greater than room temperature may be subjected to cancellation.

Reporting and Interpretation of Environmental Microbiological Test Results

Several factors must be considered when interpreting *Legionella* results, such as sampling points, sampling in relation to water system treatment (use of biocide or thermal shock), conditions to support microbial growth such as temperature and stagnation of water; and any delay or temperature changes in shipping conditions. Collection of water and swabs can be used to indicate the colonization of the organism within the system at the time of collection only, recognizing that conditions can change over time. The following table describes the reporting and acceptable limits for *Legionella*.

Table 20: *Legionella* Reporting Limits

Testing Type	Reporting Limit	Acceptable Limit
<u><i>Legionella</i> (water)</u>	Detected / Not detected	Not detected
<u><i>Legionella</i> (swab)</u>	Detected / Not detected	Not detected

Legionella is ubiquitous to the environment – water, soil and sediment; and can grow in a wide range of temperatures. If *Legionella* was isolated from an environmental sampling site in connection to an outbreak investigation and is a match to the clinical case, remediation is required and consultation with PHO’s laboratory microbiologist is recommended. If *Legionella* was isolated and is not a match to the clinical case(s), consultation with the microbiologist may be warranted.

Remedial action may be performed immediately if there is strong epidemiological information to suggest potential *Legionella* exposure to a susceptible population. Post remediation samples may be collected and submitted to verify if a previous laboratory confirmed positive source has been effectively treated after remedial action.³⁹

Revision History

As an ‘evergreen’ document the revisions noted below reflect current changes to this Guide. Printed versions of this document should be checked against the [Public Health Ontario website](#) to ensure the most recent version is being referenced.¹

Date of Implementation	Description and Change
September 13, 2024	No changes noted

Glossary

Specific organism information can be found on the [Public Health Agency of Canada website, Pathogen Safety Data Sheet](#).⁴² Refer to [Appendix D: Water Testing Fact Sheets](#) for interpretation and additional details on Background or Non-target bacteria, Total Coliform, *Escherichia coli*, Heterotrophic Plate count (HPC), *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Aerobic colony count (ACC) – An enumeration of viable aerobic bacteria (bacteria is able grow in an oxygenated or aerobic environment) on microbiological media. This may also be referred to as Standard Plate Count (SPC).

Aerobe – An organism that requires the presence of air or free oxygen to live.

Aerosolization – Dispersion in air of a liquid material or a solution in the form of a fine mist.

Aesthetically unacceptable drinking water – Drinking water that is not clear, colourless or odourless.

Anaerobe – An anaerobic organism lives in the absence of air or free oxygen.

Approval (Directive) – A directive is issued by a Medical Officer of Health that requires a drinking-water system owner/operator to test for one or more health-related parameter. The parameter and maximum concentration are indicated in the directive.

Aseptic technique – A technique where sterile supplies or equipment and clean practices (e.g., hand washing/ sanitation using alcohol based hand rub), are used to prevent bacterial contamination.

Audit sample – A sample taken outside of the routine testing schedule as part of an audit.

Biofilm – A biofilm is a collection of organic and inorganic, living and dead material collected on a surface. In drinking water pipe networks, biofilms can result in reduced disinfectant residuals, increased bacterial levels and other operational issues.

Blowdown – The water that is drained from cooling equipment to remove mineral build-up.

Bottled water – Potable water in commercially sealed containers. In Canada, bottled water is regulated as a food and therefore it must comply with the [Food and Drugs Act](#).⁴³ Section 4 of the Act prohibits the sale of foods which contain poisonous or harmful substances and must be for human consumption. A bottled water manufactured from an inadequately treated source would be in contravention of the Food and Drugs Act.

Chain of custody – An unbroken documented chain of events from the time of sample collection until it is processed and completed.

Chromogenic media – Chromogenic media is microbiological media suitable for incubation, differentiation, or selection of different microorganisms as a result of colour production.

Cistern – A reservoir, tank, or container for storing or holding water.

Colony forming units (CFU) – A unit of measurement used to indicate the number of viable microorganisms identified on a specific bacteriological medium for a specific quantity of sample.

Compliance sample – A sample taken to fulfil the requirements of an Ontario drinking water regulation.

Complaint sample (food – no illness) – A food sample submitted for laboratory evaluations that are not associated with an investigation of foodborne illness. Complaints (food with off-odour, off-colour, texture, etc.) usually come from the general public to the local board of health office. The sample could be part of the actual meal, a control sample or a follow-up sample after remedial action.

Control sample (food - illness) – A food sample submitted in conjunction with a suspected foodborne illness incident which were not part of the suspect meal, but which are of the same type and/or prepared and stored under similar conditions as those actually consumed.

Control sample (food – no illness) – A food sample submitted due to a complaint, part of a HACCP investigation or routine surveillance investigation, which are prepared and stored under typical conditions for consumption.

Control sample (water) – A water sample taken prior to a change in a routine or prior to the application of a corrective action.

Cooling tower – A cooling tower is a specialized heat exchanger that allow air and water to meet resulting in the reduction of the water temperature. As a result, evaporation of a small volume of water reduces the temperature of the water being circulated through the tower.

Demineralizer – An instrument, apparatus or chemical used to remove minerals from water.

Designated facility – A facility as described in [Ontario Regulation 170/03 Drinking Water Systems](#) i.e., the following facilities: children and youth care, delivery agent care, health care, social care, a children’s camp or a school, private school, university, a college of applied arts and technology, or an institution with authority to grant degrees.³⁰

Distribution system – The section of a drinking water system that transports drinking water to the end user.

Etiological agent – The organism identified or suspected as causing foodborne or waterborne illness as evidenced through epidemiological information and/or laboratory investigation.

Facultative anaerobe – An organism that can live in the absence as well as in the presence of atmospheric oxygen.

Follow-up samples – Samples taken after remedial action has been taken to determine whether or not the problem has been resolved.

Frozen – Water at or below 0°C or containing obvious ice fragments.

Geometric mean – The geometric mean is a calculation used to average the bacterial levels of E. coli in samples collected from recreational water.

$$\text{Geometric Mean} = ((X1)(X2)(X3)\dots\dots(Xn))^{1/n}$$

where X1, X2, etc. represents the individual data points and n is the total number of data points used in the calculation.⁴⁴

Hazard Analysis Critical Control Point (HACCP) – A systematic preventive approach to food safety from biological, chemical, and physical hazards in production processes that can cause the finished product to be unsafe, and designs measurements to reduce these risks to a safe level.⁴⁵ For food testing, food samples identified as HACCP on the [Food Bacteriology Requisition](#) means, food collected in association with evaluating or monitoring critical control points in food preparation, production and processing.

Heterotrophic Plate Count (HPC) – A microbiological test method used to estimate the number of viable, heterotrophic bacteria in a matrix. It can also be used to measure the changes that occur to bacterial populations during water treatment and distribution. This may also be referred to as Standard Plate Count (SPC).

Hold time – The time from sample collection to time of analysis.

HPPA O. Reg 319/08 Drinking Water – Drinking water that is subject to the requirements as described in [Ontario Regulation 319/08](#) Small Drinking Water Systems under the *Health Protection and Promotion Act*.³¹

HPPA Regulated Premises – Premises/facilities regulated under HPPA, (i.e., Food premises, recreational water places, etc.) that fall outside the scope of [Ontario Regulation 319/08](#) Small Drinking Water Systems.³¹

Ice – Potable water which has been cooled to a temperature less than 0 °C.

Illness (as it relates to completion of the [Food Bacteriology Requisition](#)) – Foods associated to suspect foodborne illness incidents, are to be submitted using the [Food Bacteriology Requisition](#) under the category 'Illness' for reason for test request. These foods can be part of an investigation and/or outbreak; and the samples can be part of the actual meal, control samples or follow-up samples taken after remedial action. All available epidemiological information in conjunction with the foodborne illness incident must also be documented. Epidemiological information includes symptoms, time and date of onset of symptoms, incubation period, and the number of presumed ill and at risk. Documenting the iPHIS case ID number will allow the laboratory to link the food samples to the associated clinical case.

Indicator bacteria (water) – Types of bacteria used to detect and estimate the level of contamination. Some examples include Total Coliforms and *E. coli* (*Escherichia coli*). Indicator bacteria can also be used as process microbial indicators to evaluate the efficacy of a process (e.g., Heterotrophic Plate Count or Total Coliforms for chlorine disinfection), as fecal indicators (*E. coli*) or index and model organisms which is a group/or species indicative of pathogen presence and behaviour respectively (e.g., *E. coli* as an index for *Salmonella*).⁴⁶ Non-enteric pathogens such as Legionella, non-tuberculous mycobacteria (NTM), *Pseudomonas aeruginosa*, *Aeromonas* sp., *Leptospira*, *Staphylococcus aureus*, enteric viral or protozoan pathogens and naturally occurring or free-living organisms, are not related to fecal contamination and therefore the presence of fecal indicator organisms is not associated with the presence of non-enteric pathogens. Fecal pathogens are a more common cause of human illness than non-enteric pathogens in recreational water settings.⁴⁷

Interactive Voice Response (IVR) – A technology that allows a computer to interact with humans through the use of voice and dual-tone multi-frequency signaling keypad inputs. Private Citizen drinking water test results and the interpretation can be accessed by calling a toll free telephone number and keying in the sample barcode when prompted.

Interfering substances – A substance in a water sample that either prevents the water from passing through the membrane filter during the testing process or post incubation, interferes with the determination of the colony count due to interfering or inhibiting substances on the membrane filter (e.g., a layer of sediment on the membrane filter, etc.).

Legal sample – Any laboratory specimen that has been submitted for testing to PHO's laboratory with an intact legal seal and for which chain of custody has been maintained.

Legal seal – A tamper evident label attached to a sample or transport container that must be broken before access can be obtained.

Membrane filter – A 47 millimetre diameter cellulose ester membrane with a 0.45 µm pore size used in the membrane filtration water testing method. A vacuum draws a measured volume of water through the membrane filter enabling the capture of bacteria if present in a sample.

Most Probable Number (MPN) – A method used in microbiology to determine a statistical value that represents the viable microorganisms in a sample through the use of dilution and multiple tube inoculations.

Municipal Drinking Water System – Means a drinking water system or part of a drinking water system, (a) that is owned by a municipality or by a municipal service board established under the [Municipal Act, 2001](#) or a city board established under the [City of Toronto Act, 2006](#), (b) that is owned by a corporation established under sections 9, 10 and 11 of the Municipal Act, 2001 in accordance with section 203 of that Act or under sections 7 and 8 of the City of Toronto Act, 2006 in accordance with sections 148 and 154 of that Act, (c) from which a municipality obtains or will obtain water under the terms of a contract between the municipality and the owner of the system, or (d) that is in a prescribed class.^{48,49}

Non-Municipal Drinking Water System – Means a drinking water system that is not a municipal drinking water system.²⁹

Non-Regulated Drinking Water Systems – Means systems that are not captured under a specific regulation.

Official Agency – The following agencies that submit samples for testing to PHO’s laboratory are considered Official Agencies:

- Board of health (public health unit): An official health agency established by a group of urban and rural municipalities to provide a more efficient community health program, carried out by full-time, specially qualified staff, or
- Ministry of Natural Resources and Forestry Ontario Park: Ontario land dedicated to the people of Ontario and visitors for their inspiration, education, health, recreational enjoyment and other benefits with the intention that these areas shall be managed to maintain their ecological integrity and to leave them unimpaired for future generations.⁵⁰

Order (Provincial Officer’s Order) – An Order is a legal document that sets out obligations for a specific person or persons in relation to a specific operation. A provincial officer may issue an order under the [Safe Drinking Water Act, 2002](#) (MECP inspectors only) or the [Health Protection and Promotion Act R.S.O 1990, c.H.7](#) (e.g., public health inspectors) where, in the opinion of the Director or Medical Officer of Health or designate, an imminent drinking water health hazard exists in respect of a regulated drinking water system, the Director/Medical Officer of Health or designate may issue an order to the owner or the operating authority for the system, requiring the owner or operating authority to comply, with the directions specified in the order.^{28,29} Provincial officer means a person who is designated by the Minister as a provincial officer for the purposes of the Act and the regulations.

Outbreak – The occurrence of two or more cases of illness (except botulism, where one case constitutes an outbreak) among unrelated persons that are epidemiologically linked in time and experience similar illness after a common source of exposure (which may or may not be identified). An outbreak may be identified through laboratory surveillance or detection of an increase in illness that is unusual in terms of time and/or geography. Presence of an outbreak is confirmed through supporting laboratory and/or epidemiological evidence.¹²

Outbreak number – The number assigned to an outbreak by board of health staff (e.g., public health unit) for tracking purposes.

Overgrown: NDOGHPC - No Data: Overgrown Heterotrophic Plate Count – Crowding and/or confluent microbial growth on standard plate count agar.

Overgrown: NDOGN - No Data Overgrown with non-target – Crowding and/or confluent and/or non-identifiable microbial growth on an agar plate with no evidence of Total Coliform or *E. coli*. The test is considered overgrown with background bacteria, i.e., organisms often found in the environment. This heavy load of background bacteria can interfere with the detection of coliforms and/or *E. coli*. The water may be unsafe to drink.

Overgrown: NDOGT - No Data Overgrown with target – Crowding and /or confluent and/or non-identifiable microbial growth on an agar plate, with evidence of Total Coliforms or Total Coliforms and *E. coli* present. The water is unsafe to drink.

Oxidase – A test performed to differentiate bacteria that produce cytochrome oxidase, an enzyme of the bacterial electron transport chain. When present, the cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine) to (indophenols) and a purple colour end product results. The majority of coliforms are oxidase negative whereas the majority of *Pseudomonas aeruginosa* and *Aeromonas hydrophila* are oxidase positive.

Part of meal – Foods submitted in conjunction with a suspect foodborne illness incident or complaint which is part of the actual meal implicated.

Potable – Fit or suitable for drinking.

Perishable – A type of food with a limited shelf life if it is not refrigerated.

Personal Protective Equipment (PPE) – Protective clothing, helmets, goggles, gloves or other garments or equipment designed to protect the wearer's body from injury or infection.

Prion – A small proteinaceous infectious disease-causing agent that is believed to be the smallest infectious particle; it is neither bacterial nor fungal nor viral and contains no genetic material.

Private Citizen – A person residing in Ontario who:

- does not have access to a treated municipal drinking water supply and
- draws their drinking water from a protected source within the Province of Ontario for personal use or applies appropriate treatment to water drawn from an unprotected source.

Private Citizen drinking water – Drinking water from a “single household”.

Private residence – A dwelling place occupied for an extended period of time by the same persons, if,

- the residents have a reasonable expectation of privacy;
- food preparation, personal hygiene, and sleeping accommodations are not communal in nature; and
- any use of the dwelling place by a resident for a home occupation, trade, business, profession or craft is secondary to the use of the dwelling place as a residence and does not use more than 25 per cent of the indoor floor area.⁵¹ (e.g., water source can include a private well, cisterns, treated surface water, etc.)

Public beach – Any public bathing area owned/operated by a municipality to which the general public has access, and where there is reason to believe that there is recreational use of the water (e.g., beach signage, sectioned off swimming area, water safety/rescue equipment, lifeguard chairs, etc.), which may result in waterborne illness or injury as determined by the local medical officer of health.⁵²

Public pool – A structure, basin, chamber or tank containing or intended to contain an artificial body of water for swimming, water sport, water recreation or entertainment, but does not include,

- one that is located on a private residential property under the control of the owner or occupant and that is limited to use for swimming or bathing by the owner or occupant, members of their family and their visitors, or
- one that is used solely for commercial display and demonstration purposes.⁵³

Public spa – A hydro-massage pool containing an artificial body of water that is intended primarily for therapeutic or recreational use, that is not drained, cleaned or refilled before use by each individual and that utilizes hydrojet circulation, air induction bubbles, current flow or a combination of them over the majority of the pool area.⁵⁴

Raw foods – Foods which have NOT been subjected to some form of processing in order to render them RTE (most often cooking) and/or which have been subjected to another process to extend their shelf-life, including but not restricted to the use of heat, chemicals, reduction of pH, reduction of water activity, or special packaging.⁵⁵

Raw Water – Water that is in a drinking water system or in plumbing that has not been treated. For regulated drinking water systems, this is in accordance with,

- the prescribed standards and requirements that apply to the system, or
- such additional treatment requirements that are imposed by the licence or approval for the system, if the system is licensed or approved under the Safe Drinking Water Act.

Raw water collected from a regulated drinking water system has no adverse reporting requirements, unless it is intended to be consumed without treatment (see Raw Water for Consumption).²⁹

Raw Water for Consumption – Raw water that will be consumed without being treated first. For regulated drinking water systems, adverse results from samples identified as raw water intended for human consumption are reportable under section 18 and 18.1 of the Safe Drinking Water Act.

Ready-to-eat foods – The status of the food being ready for immediate consumption at the point of sale. It could be raw or cooked, hot or chilled, and can be consumed without further heat-treatment including re-heating.⁵⁶

Recreational water facility – A recreational water facility can include:

- regulated recreational water facilities including public pools and public spas and
- non-regulated recreational water facilities including public wading pools, splash pads/spray pads and water slide receiving basins.⁵²

Regulated water seal – A tamper evident label attached to the cap of a drinking water sample submitted under a drinking water regulation that must be broken before access can be obtained.

Relinquish – To release a sample ensuring the chain of custody is not compromised.

Resample – A sample taken from the same location, and in some cases upstream and downstream, usually in response to an adverse test result or incident.

Routine surveillance (food) – Regular on-going sanitary monitoring programs of food prepared/served in retail food establishments. Samples collected in the absence of foodborne disease.

Sanitizer – A substance or preparation for killing germs, designed for use especially on food-processing equipment.

SDWA O. Reg 170/03 Drinking Water – Drinking water that is subject to the requirements as described in [Ontario Regulation 170/03 Drinking Water Systems](#).³⁰ (e.g., some Municipal, Non-municipal and designated facilities).

Single Household – In the context of a private citizen drinking water, a single household is defined as water intended to be potable and for human consumption collected from a drinking water system (e.g., a well) that serves one or fewer than six (6) private residences. It is not from a regulated drinking water system (i.e., water for public consumption, e.g., city water) or from a commercial bottled water product.

Spread plate – The technique used to determine the load of heterotrophic bacteria in a water sample. A small volume of sample (0.1 mL) is inoculated on to the surface of an agar plate, spread using aseptic technique and the plate is then incubated according to the test method.

Surface water – Water collecting on the ground or in a stream, river, lake, etc.

Suspected sewage contamination – Water suspected of being contaminated by fecal material.

Teletypewriter (TTY) – A telegraphic apparatus by which signals are sent by striking the letters and symbols of the keyboard of an instrument resembling a typewriter and are received by a similar instrument that automatically prints them in type corresponding to the keys struck.

Test for Potability – Total Coliform and *E. coli* analysis.

Treated water – Water that is subjected to disinfection or other means of removing contaminants.

Turnaround time (TAT) – The number of business days to complete testing based on date of receipt in the laboratory. This turnaround time definition does not apply to tests that are on hold awaiting information to continue testing. Turnaround times posted in this Guide are estimates for routine tests. For some tests or at times of public health emergencies, outbreaks or exigent circumstances, turnaround times may be longer than what is listed.

Unauthorized Submitter: Official Agency – A submitter representing an agency other than a board of health or Ontario Parks under the Ministry of Natural Resources and Forestry.

Unauthorized Submitter: Private Citizen – A person who submits a drinking water sample that does not meet the definition of “Private Citizen” or “Single Household”.

Unique identifier – A sample identifier that uniquely distinguishes it from any other sample submitted. For water samples, the sample barcode is used for this purpose.

Untreated – Water that is not subjected to disinfection or other means of removing contaminants.

Water well – A hole drilled, bored or dug into the earth to obtain water.

Revision History

As an ‘evergreen’ document the revisions noted below reflect current changes to this Guide. Printed versions of this document should be checked against the [Public Health Ontario website](#) to ensure the most recent version is being referenced.¹

Date of Implementation	Description and Change
September 13, 2024	<ul style="list-style-type: none">• Removed HPPA O. Reg. 318/08 Drinking Water• Updated definitions for: Indicator bacteria (water), Non-Regulated Drinking Water Systems, Order (Provincial Officer’s Order), Outbreak and Single Household• Added definitions for Raw Water and Raw Water for Consumption

Acronyms

AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
ACC	Aerobic Colony Count
ASPHIO	Association of Supervisors of Public Health Inspectors of Ontario
Aw	Water activity
BOH	Board of health
CALA	Canadian Association for Laboratory Accreditation
CIPHI	Canadian Institute of Public Health inspectors
COC	Chain of custody
CFIA	Canadian Food Inspection Agency
CFU	Colony forming unit
FDA-BAM	US Food and Drug Administration Bacteriological Analytical Manual
HACCP	Hazard Analysis Critical Control Point
HC	Health Canada
HPC	Heterotrophic Plate Count
HPPA	Health Protection and Promotion Act
iPHIS	Integrated Public Health Information System
IU	International units
MNRF	Ministry of Natural Resources and Forestry
MECP	Ministry of the Environment, Conservation and Parks
MOH	Medical Officer of Health
MOH-EH	Ministry of Health (Ministry of Health Environmental Health Protection Policy and Partnerships Branch)
MPN	Most Probably Number

NDOGHPC	No Data: Overgrown Heterotrophic Plate Count
NDOGN	No Data: Overgrown with Non Target
NDOGT	No Data: Overgrown with Target
OAHP	Ontario Agency for Health Protection and Promotion
OMAFRA	Ontario Ministry of Agriculture, Food, and Rural Affairs
ON-ARC	Ontario-Area Recall Coordinator
O. Reg	Ontario Regulation
PHI	Public health inspector
PHO	Public Health Ontario
PHU	Public health unit
PPE	Personal protective equipment
SCC	Standards Council of Canada
SDWA	Safe Drinking Water Act
SDWS	Small Drinking Water Systems
TAT	Turnaround time
UPC	Universal Product Code

Revision History

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Date of Implementation	Description and Change
September 13, 2024	<ul style="list-style-type: none"> Added MPN

Appendix A: Supplies

Board of health staff should ensure that environmental sample collection and submission supplies are readily available. Collection kits or other PHO laboratory supplies can be ordered by completing the [Requisition for Specimen Containers and Supplies](#) form and faxing it to the PHO laboratory Toronto fax number listed on the form or to your local [PHO laboratory](#). Supplies will be shipped within 1 – 5 days of request.

Ordering instructions for supplies that are not listed on the [Requisition for Specimen Containers and Supplies](#) form:

Table A1: Supplies List

Item	Catalogue #	Ordering Instructions
Regulated water seal	L-8001	Order directly from local PHO laboratory
Legal seal	L-8002	Order directly from local PHO laboratory
Sterile sampling bags with wire closure	300240	Record “Food Bag PT# 300240 B /250” on the comment line of the Requisition for Specimen Containers and Supplies form.
Sewage Collection Kits	TC 125-B	Order directly from local PHO laboratory
Swab	—	Record “Swab” on the comment line of the Requisition for Specimen Containers and Supplies form
Water – Private Citizen collection kit	390040	Order directly from local PHO laboratory
Water – Official Agency collection bottles	300013	Order directly from local PHO laboratory

Revision History

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Date of Implementation	Description and Change
September 13, 2024	<ul style="list-style-type: none"> Updated supply list to include: Water – Private Citizen collection kit and Water – Official Agency collection bottles

Appendix B: Laboratory Submission Forms

The most current version of the forms can be accessed from the links below:

- [Bacteriological Analysis of Drinking Water for Private Citizen, Single Household Only](#)
- [Bacteriological Analysis of Water - Multiple Sample Requisition for Official Agencies](#)
- [Environmental Microbiology Investigation Form](#)
- [Food Bacteriology Requisition Form](#)
- [Potability of Water Release Form](#)

Figure B1: Regulated water seal (L-8001)




 	Public Health Ontario Laboratories Laboratoires de Santé publique Ontario	Seal no. Numero du Sceau	Note - Place centre of seal over cap so that the bottle cannot be opened without breaking the seal. Ensure that seal is securely fastened. Veuillez vous assurez que le centre du sceau soit placé au dessus du capuchon, et le tout scellé fermement.
REGULATED WATER SEAL SCEAU POUR USAGE OFFICIEL			
Initial of person attaching official seal L'initial d'individu apposant le sceau	<input style="width: 50px; height: 30px;" type="text"/> <input style="width: 50px; height: 30px;" type="text"/>	Date	
Initial of person receiving specimen Initiales de la personne recevant l'échantillon		<input style="width: 30px; height: 20px;" type="text"/> <input style="width: 30px; height: 20px;" type="text"/> <input style="width: 30px; height: 20px;" type="text"/>	10/15 

Figure B2: Legal seal (L-8002)

 	Public Health Ontario Laboratories Laboratoires de Santé publique Ontario	Seal no. Numero du Sceau	Note - Place centre of seal over cap so that the bottle cannot be opened without breaking the seal. Ensure that seal is securely fastened. Veuillez vous assurez que le centre du sceau soit placé au dessus du capuchon, et le tout scellé fermement.
LEGAL SEAL SCEAU LÉGAL			
Initial of person attaching official seal L'initial d'individu apposant le sceau	<input style="width: 50px; height: 30px;" type="text"/> <input style="width: 50px; height: 30px;" type="text"/>	Date	
Initial of person receiving specimen Initiales de la personne recevant l'échantillon		<input style="width: 30px; height: 20px;" type="text"/> <input style="width: 30px; height: 20px;" type="text"/> <input style="width: 30px; height: 20px;" type="text"/>	10/15 

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Date of Implementation	Description and Change
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Appendix C: Major Foodborne Diseases:

General Features

Information extracted from World Health Organization (2008). Foodborne Disease Outbreaks: Guidelines for Investigation and Control. Geneva, Switzerland, 2008.⁵⁷

The following tables includes the general features of major foodborne diseases including: etiological agent, incubation period, symptoms, and mode of transmission and associated foods.

Table C1: *Bacillus cereus* General Features

Features	Description
Etiological Agent	<i>Bacillus cereus</i> (Bacterial toxin)
Incubation Period	a) Diarrheal syndrome: 8 – 16 hours b) Emetic syndrome: 1 – 5 hours
Symptoms	a) Diarrheal syndrome: acute diarrhea, nausea and abdominal pain. b) Emetic syndrome: acute nausea, vomiting and abdominal pain and sometimes diarrhea.
Mode of Transmission and Associated Foods	Ingestion of food that has been stored at ambient temperatures after cooking, permitting the growth of bacterial spores and toxin production. Many outbreaks (particularly those of the emetic syndrome) are associated with cooked or fried rice that has been kept at ambient temperature. Foods involved include starchy products such as boiled or fried rice, spices, dried foods, milk, dairy products, vegetable dishes, and sauces.

Table C2: Campylobacteriosis General Features

Features	Description
Etiological Agent	<i>Campylobacter jejuni</i> and <i>Campylobacter coli</i>
Incubation Period	Typically 2 – 5 days (range 1 – 11 days)
Symptoms	Fever, severe abdominal pain, nausea and diarrhea which can vary from slight to profuse and watery sometimes containing blood or mucus.
Mode of Transmission and Associated Foods	<p>Principally through ingestion of contaminated food. Main food sources are raw milk and raw or undercooked poultry. Spread to other foods by cross-contamination or contamination with untreated water; contact with animals or birds. Other sources of transmission are contact with live animals (pets and farm animals). Person-to-person transmission occurs during the infectious period that ranges from several days to several weeks.</p> <p>Foods involved include raw milk, poultry, beef, pork and drinking- water</p>

Table C3: *Clostridium botulinum* (Botulism) General Features

Features	Description
Etiological Agent	<i>Clostridium botulinum</i>
Incubation Period	12 – 36 hours (range several hours to 8 days)
Symptoms	Vomiting, abdominal pain, fatigue, muscle weakness, headache, dizziness, ocular disturbance (blurred or double vision, dilated pupils, unreactive to light), constipation, dry mouth and difficulty in swallowing and speaking, and ultimately paralysis and respiratory or heart failure.
Mode of Transmission and Associated Foods	<p>Ingestion of toxin pre-formed in food. This may occur when raw or under-processed foods are stored in anaerobic conditions that allow growth of the organism. Most outbreaks are due to faulty preservation of food (particularly in homes or cottage industries), e.g. canning, fermentation, curing, smoking, or acid or oil preservation.</p> <p>Foods involved include vegetables, condiments (e.g. pepper), fish and fish products, meat and meat products, honey, fruit and vegetable juices. Several outbreaks have occurred as a result of consumption of uneviscerated fish, garlic in oil, and baked potatoes.</p>

Table C4: *Clostridium perfringens* Enteritis General Features

Features	Description
Etiological Agent	<i>Clostridium perfringens</i>
Incubation Period	8 – 24 hours
Symptoms	Abdominal pain, diarrhea, rarely vomiting and fever.
Mode of Transmission and Associated Foods	Illness usually caused by cooked meat and poultry dishes subject to time/temperature abuse. Dishes are often left for too long at ambient temperature to cool down before storage, or cooled inadequately. This allows spores that survive the cooking process to germinate and grow, producing large numbers of vegetative cells. If a dish is not reheated sufficiently before consumption, the vegetative cells can cause illness. Foods involved include meat and poultry (boiled, stewed or casseroled).

Table C5: Cryptosporidiosis General Features

Features	Description
Etiological Agent	<i>Cryptosporidium parvum</i>
Incubation Period	2 – 4 days
Symptoms	Persistent diarrhea, nausea, vomiting and abdominal pain, sometimes accompanied by an influenza-like illness with fever.
Mode of Transmission and Associated Foods	Spread through the fecal-oral route, person-to-person contact or consumption of fecally contaminated food and water, bathing in contaminated pools. Foods involved include raw milk, drinking-water and apple cider.

Table C6: Cyclosporiasis* General Features

Features	Description
Etiological Agent	<i>Cyclospora cayetanensis</i>
Incubation Period	2 – 11 days
Symptoms	Watery diarrhea (this is the most common symptom), abdominal bloating and gas, fatigue, (tiredness), stomach cramps, loss of appetite, weight loss, mild fever and nausea.
Mode of Transmission and Associated Foods	<p>Cyclosporiasis is unlikely to spread directly between people. This is because the parasite can only infect others once it leaves your body through feces. To be able to spread, the parasite needs to be outside the body for about 7 to 15 days.</p> <p>Food can be a source of cyclosporiasis for Canadians when imported from countries where <i>Cyclospora</i> is common. Foods imported to Canada that have been linked to the <i>Cyclospora</i> parasite include: basil, cilantro, raspberries, blackberries, mesclun lettuce, snow and snap peas and pre-packaged salad mix.</p>

*Organism specific reference taken from Government of Canada website⁵⁸

Table C7: *Escherichia coli* Infection General Features

Features	Description
Etiological Agent	<p><i>Escherichia coli</i></p> <p>a) Enteropathogenic <i>E. coli</i> (EPEC)</p> <p>b) Enterotoxigenic <i>E. coli</i> (ETEC) producing a heat-labile (LT) and a heat-stable (ST) enterotoxin</p> <p>c) Enteroinvasive <i>E. coli</i> (EIEC)</p> <p>d) Enterohaemorrhagic <i>E. coli</i> (EHEC) or verocytotoxin producing <i>E. coli</i> (VTEC), also referred to as Shiga-toxin producing <i>E. coli</i> (STEC), of which the most commonly recognized is <i>E. coli</i> O157.</p>
Incubation Period	<p>a) EPEC: 1 – 6 days; as short as 12 – 36 hours</p> <p>b) ETEC: 1 – 3 days; as short as 10–12 hours</p> <p>c) EIEC: 1 – 3 days; as short as 10–18 hours</p> <p>d) EHEC: 3 – 8 days, median of 4 days</p>
Symptoms	<p>a) EPEC adheres to the mucosa and changes its absorption capacity, causing vomiting, diarrhea, abdominal pain and fever.</p> <p>b) ETEC mediates its effects by enterotoxins. Symptoms include diarrhea (ranging from mild to a severe, cholera-like syndrome), abdominal cramps and vomiting, sometimes leading to dehydration and shock.</p> <p>c) EIEC causes inflammatory disease of the mucosa and submucosa by invading and multiplying in the epithelial cells of the colon. Symptoms include abdominal pain, vomiting and watery diarrhoea (in <10% of cases stools may become bloody and contain mucus).</p> <p>d) EHEC causes abdominal cramps and watery diarrhea that may also develop into bloody diarrhea (hemorrhagic colitis). Fever and vomiting may also occur.</p>
Mode of Transmission and Associated Foods	<p>(a – c) EPEC, ETEC, EIEC: consumption of food and water contaminated with fecal matter. Time/temperature abuse of such foods increases risk of illness. Up to 25% of infections in infants and young children in developing countries are due to <i>E. coli</i>, in particular ETEC and EPEC (10 – 20% and 1 – 5% of cases at treatment centres, respectively). ETEC is a major cause of traveller’s diarrhea in developing countries.</p> <p>d) EHEC is transmitted mainly through consumption of foods such as raw or undercooked ground-meat products and raw milk from infected animals. Fecal contamination of water and other foods, as well as cross-contamination during food preparation, will also lead to infection.</p> <p>Foods involved include ground (minced) meat, raw milk, and vegetables. Secondary transmission (person-to-person) may also occur during the period of excretion of the pathogen which is less than a week for adults but up to 3 weeks in one-third of affected children</p>

Table C8: Giardiasis General Features

Features	Description
Etiological Agent	<i>Giardia lamblia</i>
Incubation Period	7 – 10 days (range 4 – 25 days)
Symptoms	Diarrhea (which may be chronic and relapsing), abdominal cramps, fatigue, weight loss, anorexia and nausea. Symptoms may be caused by a protein toxin.
Mode of Transmission and Associated Foods	<p>Infected individuals excrete <i>Giardia</i> cysts in large numbers. Illness is spread by fecal-oral route, person-to-person contact or fecally contaminated food and water. Cysts have been isolated from lettuces and fruits such as strawberries. Infection also associated with drinking-water from surface waters and shallow wells.</p> <p>Foods involved include water, home-canned salmon, fruit and vegetables and noodle salad.</p>

Table C9: Hepatitis A General Features

Features	Description
Etiological Agent	Hepatitis A
Incubation Period	25 – 28 days (range 2 – 6 weeks)
Symptoms	Loss of appetite, fever, malaise, abdominal discomfort, nausea and vomiting, followed by symptoms of liver damage (passage of dark urine, pale stools, jaundice).
Mode of Transmission and Associated Foods	<p>Spread by fecal-oral route, primarily person-to-person. Can also be transmitted through food and water as a result of sewage contamination or infected food handlers. Risk of transmission is greatest during the second half of the incubation period until a few days after the appearance of jaundice.</p> <p>Foods involved include shellfish, raw fruit and vegetables, bakery products.</p>

Table C10: Listeriosis General Features

Features	Description
Etiological Agent	<i>Listeria monocytogenes</i>
Incubation Period	3 – 70 days
Symptoms	Influenza-like symptoms such as fever, headache and occasionally gastrointestinal symptoms.
Mode of Transmission and Associated Foods	A substantial proportion of cases of listeriosis are foodborne. Foods involved include raw milk, soft cheese, meat-based paste, jellied pork tongue, raw vegetables and coleslaw.

Table C11: Salmonellosis General Features

Features	Description
Etiological Agent	Non-typhoid <i>Salmonella</i> serotypes
Incubation Period	6 – 48 hours, occasionally up to 4 days
Symptoms	The principal symptoms are fever, headache, nausea, vomiting, abdominal pain and diarrhea.
Mode of Transmission and Associated Foods	Main route of transmission is by ingestion of the organisms in food (milk, meat, poultry, eggs) derived from infected food animals. Food can also be contaminated by infected food-handlers, pets and pests, or by cross-contamination as a result of poor hygiene. Contamination of food and water from the feces of an infected animal or person may also occur. Problems caused by initial contamination may be exacerbated by prolonged storage at temperatures at which the organism may grow. Direct person to- person transmission may also occur during the course of the infection. Foods involved include unpasteurized milk, raw eggs, poultry, meat, spices, salads and chocolate.

Table C12: *Salmonella* Typhi and *Salmonella* Paratyphi types a–c (Typhoid Fever, Paratyphoid Fever) General Features

Features	Description
Etiological Agent	<i>Salmonella</i> Typhi and <i>Salmonella</i> Paratyphi types a–c
Incubation Period	10 – 20 days (range 3 days to 8 weeks)
Symptoms	Systemic infections characterized by high fever, abdominal pains, headache, vomiting, diarrhea followed by constipation, rashes and other symptoms of generalized infection.
Mode of Transmission and Associated Foods	Ingestion of food and water contaminated with fecal matter. Food-handlers may carry the pathogen and be a source of food contamination. Secondary transmission may also occur. Foods involved include prepared foods, dairy products (e.g. raw milk), meat products, shellfish, vegetables, and salads.

Table C13: Shigellosis (Bacillary Dysentery) General Features

Features	Description
Etiological Agent	<i>Shigella dysenteriae</i> , <i>S. flexneri</i> , <i>S. boydii</i> , <i>S. sonnei</i>
Incubation Period	1 – 3 days, up to 1 week for <i>S. dysenteriae</i>
Symptoms	Abdominal pain, vomiting, fever, diarrhea ranging from watery (<i>S. sonnei</i>) to dysenteric with bloody stools, mucus and pus (<i>S. dysenteriae</i> and, to a lesser extent <i>S. flexneri</i> and <i>S. boydii</i>).
Mode of Transmission and Associated Foods	Food and water contaminated with fecal matter. Person-to-person transmission through the fecal–oral route is an important mode of transmission. Food can be contaminated by food-handlers with poor personal hygiene or by use of sewage/wastewater for fertilization. Foods involved include uncooked foods that have received extensive handling, such as mixed salads and vegetables, water and raw milk.

Table C14: *Staphylococcus aureus* Intoxication General Features

Features	Description
Etiological Agent	<i>Staphylococcus aureus</i>
Incubation Period	2 – 6 hours
Symptoms	Intoxication, sometimes of abrupt and violent onset. Severe nausea, cramps, vomiting and prostration, sometimes accompanied by diarrhea.
Mode of Transmission and Associated Foods	Consumption of foods containing the toxin. Foods are contaminated by food-handlers. If storage conditions are inadequate, the bacteria may multiply to produce toxin. Intoxication is often associated with cooked food e.g. meat, in which competitive bacteria have been destroyed. Foods involved include prepared foods subject to handling in their preparation (ham, chicken and egg salads, cream-filled products, ice cream, cheese).

Table C15: *Vibrio cholerae* O1 and O139 (Cholera) General Features

Features	Description
Etiological Agent	<i>Vibrio cholerae</i> O1 and O139
Incubation Period	1 – 3 days
Symptoms	Profuse watery diarrhea, which can lead to severe dehydration, collapse and death within a few hours unless lost fluid and salt are replaced; abdominal pain and vomiting.
Mode of Transmission and Associated Foods	Food and water contaminated through contact with fecal matter or infected food handlers. Contamination of vegetables may occur through sewage or wastewater used for irrigation. Person-to- person transmission through the fecal-oral route is also an important mode of transmission. Foods involved include seafood, vegetables, cooked rice and ice.

Table C16: *Vibrio parahaemolyticus* Gastroenteritis General Features

Features	Description
Etiological Agent	<i>Vibrio parahaemolyticus</i>
Incubation Period	9–25 hours, up to 3 days
Symptoms	Profuse watery diarrhea, abdominal pain, vomiting, and fever. A dysenteric syndrome has been reported from some countries, particularly Japan.
Mode of Transmission and Associated Foods	Associated with consumption of raw or undercooked fish and fishery products or cooked foods subject to cross contamination from raw fish.

Table C17: *Vibrio vulnificus* Infection General Features

Features	Description
Etiological Agent	<i>Vibrio vulnificus</i>
Incubation Period	12 hours – 3 days
Symptoms	Profuse diarrhea with blood in stools. Organism is associated with wound infections and septicaemia may originate from the gastrointestinal tract or traumatized epithelial surfaces.
Mode of Transmission and Associated Foods	All known cases are associated with seafood, particularly raw oysters.

Table C18: Yersiniosis General Features

Features	Description
Etiological Agent	<i>Yersinia enterocolitica</i>
Incubation Period	24 – 36 hours (range 1 – 11 days)
Symptoms	Abdominal pain, diarrhea, mild fever, sometimes vomiting.
Mode of Transmission and Associated Foods	Illness is transmitted through consumption of pork products (tongue, tonsils, gut), cured or uncured, as well as milk and milk products.

Table C19: Viral Gastroenteritis General Features

Features	Description
Etiological Agent	<p>Viral gastroenteritis</p> <p>Many different viruses can cause viral gastroenteritis, including adenoviruses, coronaviruses, rotaviruses, parvoviruses, caliciviruses and astroviruses. Those viruses most commonly associated with foodborne outbreaks are norovirus and hepatitis A.</p>
Incubation Period	15 – 50 hours
Symptoms	Diarrhea and vomiting, which is often severe and projectile with sudden onset.
Mode of Transmission and Associated Foods	Gastroenteritis viruses usually spread by fecal–oral route. Food and drinking-water may be contaminated either at source when exposed to sewage/wastewater in the environment or used for irrigation, or by an infected food-handler. Filter-feeding shellfish most common food contaminated at source, but a wide range of different cooked and uncooked foods have been implicated in secondary contamination by food-handlers.

Revision History

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Date of Implementation	Description and Change
September 13, 2024	<ul style="list-style-type: none"> No changes noted

Appendix D: Water Testing Fact Sheets

The following tables includes the general information for water testing including information about the organism, distinguishing features, where the organism came from, potential health impacts and impact/importance. Reporting and acceptable limits are also presented for each organism in the tables below. Additional information about waterborne pathogens can be found in Health Canada’s Guidance on Waterborne Pathogens in Drinking Water⁵⁹ and Guidelines for Canadian Recreational Water Quality Guideline Technical Document – Microbiological Pathogens and Biological Hazards⁴⁷.

Background Bacteria / Non Target Bacteria / Overgrown

Table D1: Background Bacteria / Non Target Bacteria / Overgrown Features

Features	Description
What is it?	Background bacteria are bacteria known as non-target organisms in drinking water testing.
Distinguishing features	These bacteria do not ferment lactose when grown on culture media designed for coliform detection unlike the typical the indicator organisms, coliforms and <i>E. coli</i> .
Where do they come from?	Background bacteria are found in the environment (e.g., soil) and may include potentially “opportunistic” bacterial pathogens most of which are non-coliforms (e.g., <i>Pseudomonas</i> , <i>Aeromonas</i> , etc.) and are not often found in drinking water which has been properly disinfected. ⁶⁰
Potential health impacts	There is no evidence of an association of any of these organisms with gastrointestinal infection through ingestion of drinking-water in the general population. ⁶¹
What does it mean?	<p>When water samples are heavily contaminated (i.e., overgrown where the plate is crowded and/or confluent and/or contains non-identifiable microbial growth) with background bacteria these organisms can interfere with the detection of coliforms or <i>Escherichia coli</i> that may be present in the sample and in some cases can release inhibitory substances (bacteriocins).^{29,60,62}</p> <p>Drinking Water</p> <p>There are two overgrown conditions:</p> <ul style="list-style-type: none"> • NDOGN - No Data Overgrown with non-target: <ul style="list-style-type: none"> • The crowding and /or confluent and/or non-identifiable microbial growth (i.e. non-target organisms) on a plate, and there is no evidence of coliforms and / or <i>E. coli</i>. The water may be unsafe to drink.

Features	Description
	<ul style="list-style-type: none"> • NDOGT - No Data Overgrown with target: <ul style="list-style-type: none"> • The crowding and /or confluent and/or non-identifiable microbial growth (i.e. non-target organisms) on a plate, and there is evidence of coliforms and / or <i>E. coli</i> present. The water is unsafe to drink. <p>If <i>Aeromonas</i> spp. or <i>Pseudomonas aeruginosa</i> is detected in a regulated drinking water sample, it is considered an adverse result for the purpose of section 18 of the Safe Drinking Water Act.²⁹</p> <p>Recreational Water Facilities</p> <p>An overgrown result is indicative of poor water quality.</p>

Table D2: Reporting and Acceptable Limits for Official Agency Potable Water / Ice – Treated / Recreational Water Facilities

Testing Type	Reporting Limit	Acceptable Limit
Background or Non-target bacteria (CFU / 100 mL)	0 to *NDOGN /NDOGT	<p>At the discretion of the MOH. Only reported on Regulated drinking water samples submitted under a “Provincial Officer’s Order” or “Approval (Directive)”.</p> <p>An overgrown result is considered an adverse condition under Ontario drinking water regulations.</p>

*NDOGN - No Data Overgrown with non-target / NDOGT- No Data Overgrown with target

Campylobacter Species

Table D3: Campylobacter species Features

Features	Description
Organism	<i>Campylobacter</i> species
What is it?	<i>Campylobacter</i> is a genus of Gram-negative bacteria.
Distinguishing features	<i>Campylobacter</i> are Gram-negative, oxidase and catalase positive and motile. They are microaerophilic (prefer environments with low oxygen) and are best cultured at 42 °C. They generally appear comma or s-shaped, but when exposed to atmospheric oxygen, the pathogenic strain, <i>C. jejuni</i> , is able to change into a coccal form. ⁶³

Features	Description
Where do they come from?	<i>Campylobacter jejuni</i> can be isolated from animals with poultry, wild birds and cattle being significant reservoirs. ⁶⁰
Potential health impacts	Refer to Appendix C .
What does it mean?	<p>Water</p> <p>Analysis of water samples for <i>Campylobacter</i> should only be undertaken when there has been clinical confirmation of an outbreak and a water source has been incriminated epidemiologically.</p> <p>Potable water</p> <p>Drinking-water supplies contaminated with <i>Campylobacter</i> spp. are usually associated with unchlorinated or inadequately chlorinated surface water supplies. Since <i>Campylobacter</i> spp. are fecally borne pathogens and are not particularly resistant to disinfection, <i>E. coli</i> is an appropriate indicator for the presence/absence of <i>Campylobacter</i> spp. in drinking-water supplies.⁶⁰</p> <p>Recreational water</p> <p>The occurrence of <i>Campylobacter</i> in surface waters has proved to be strongly dependent on rainfall, water temperature and the presence of waterfowl.⁶⁴</p>

Table D4: Reporting And Acceptable Limits for *Campylobacter* species

Sample Type	Reporting Limit (per volume of water tested)	Acceptable Limit (per volume of water tested)
Water	Not Detected / Detected	Not detected

Coliform

Table D5: Coliform Features

Features	Description
What is it?	Coliforms are non-specific indicators of bacterial contamination.
Distinguishing features	<p>They are a group of Gram-negative, non spore-forming, rod shaped bacteria capable of fermenting lactose with the production of acid and gas, within 48 hours, when grown in a lactose-containing culture medium incubated at 36 +/- 1.0 °C. Some coliforms are slow lactose fermenters that can produce the enzyme β-D galactopyranosidase that hydrolyzes chromogenic substrates, such as ortho-nitrophenyl- β -D-galactopyranoside (ONPG) or chlorophenol red- β -D-galactopyranoside (CPRG). This reaction generally occurs within 24 +/- 2 h or less at 35 °C. <i>Escherichia coli</i> and thermotolerant coliforms are a subset of the Total Coliform group.</p> <p>There are some lactose-fermenting strains of species within the genus <i>Aeromonas</i> which produce a coliform-like reaction on media used for the detection of bacterial indicators of contamination. <i>Aeromonas</i> species are oxidase-positive and generally fail to produce gas during lactose fermentation in liquid media. They do not, therefore, conform to the classical definition of a coliform. Regardless, they are often included in the Total Coliform count because the growth medium fails to differentiate this organism from the true coliforms.</p>
Where do they come from?	Coliforms occur naturally in soil and decaying vegetation, but may also be associated with human or animal fecal contamination, so the presence of coliforms does not necessarily provide specific evidence of fecal contamination. ⁶⁵
Potential health impacts	The presence of coliforms indicates the water source is not secure increasing an individual’s health risk if the water is consumed.
What does it mean?	<p>Potable water</p> <p>Coliforms may be indicative of a contaminated water supply and/or inadequate treatment. The presence of Total Coliforms in drinking water distribution systems and stored water supplies may reveal regrowth and possible biofilm formation or contamination from foreign material, including soil or plants.</p> <p>In private drinking water systems, follow-up actions should be taken to investigate the cause of the positive results.⁶⁶ Given the vulnerability of well water to external influences, such as human, environmental and agricultural impacts, it is important to test water frequently.</p>

Features	Description
	<p>Bottled water</p> <p>Although rarely found in bottled water, coliforms are used as an indicator of insanitation or possible contamination. ⁶⁵</p> <p>The Food and Drug Regulations specify that prepackaged water represented as mineral water or spring water and prepackaged ice, shall not contain any coliform bacteria. ⁶⁷</p> <p>Recreational water facilities</p> <p>Total Coliform testing on its own may be insufficient to judge the microbiological quality of whirlpool water; it is advisable to consider the outcome of all the test results for a sampling session. ⁶¹</p>

Table D6: Reporting And Acceptable Limits for Coliforms

Sample Type	Reporting Limit (CFU/100 mL)	Acceptable Limit (CFU / 100 mL)
Official Agency Potable Water including bottled water / Ice – Treated	0 to > 80, *NDOGT	0
Private Citizen – Private Drinking Water Systems	0 to > 80, *NDOGT	≤ 5
Official Agency - Recreational Water Facilities, Public Pools/Spas	< 2 to > 160, *NDOGT	< 2

*NDOGT- No Data Overgrown with target

Escherichia coli (E. coli)

Table D7: Escherichia coli (E. coli) Features

Features	Description
What is it?	<i>E. coli</i> is an indicator of fecal contamination in water.
Distinguishing features	<i>E. coli</i> are Gram-negative, facultative anaerobic, non spore-forming, lactose-fermenting, rod-shaped bacteria which are oxidase negative. When using chromogenic media, <i>E. coli</i> are defined as coliform bacteria with the enzyme β -glucuronidase. This enzyme splits the substrate 5-bromo-4-chloro-3-indolyl- β -D-glucuronide (i.e., BCIG) within 24-72 h at 36 °C and hydrolyzes 4-methylumbelliferyl- β -D-glucuronide (MUG) to yield a fluorogenic product that is detectable under long-wave (366 nm) UV light within 4 to 24 hours. Approximately 97% of <i>E. coli</i> strains can produce the enzyme β -D-glucuronidase. ⁶⁸
Where do they come from?	The majority of <i>E. coli</i> strains are not pathogenic and live in the intestinal tract of animals, including humans, at high densities (10^7 to 10^8 cells per gram of stool). ⁶⁹
Potential health impacts	Some <i>E. coli</i> strains can cause diarrhea when contaminated water are consumed, or through contact with contaminated animals or human feces. Refer to Appendix C- Major Foodborne Diseases: General Features .
What does it mean?	<p>The presence of <i>Escherichia coli</i> in a water source may indicate fecal contamination from a human or animal source and thus the possible presence of fecal pathogenic bacteria, viruses and protozoa which can cause gastrointestinal illness and in some instances potential health complications (e.g., hemolytic uremic syndrome from <i>E. coli</i> O157:H7).</p> <p>Although <i>E. coli</i> is a good indicator of the presence of enteric bacterial pathogens, it is important to realize enteric viruses and protozoa are more resistant to disinfection than bacteria, the absence of <i>E. coli</i> does not necessarily mean these organisms are not present. Even with this limitation, <i>E. coli</i> continues to be one of the targets of choice to monitor water quality by the World Health Organization and regulatory bodies.^{57,66}</p> <p>Potable / bottled water and treated ice</p> <p><i>E. coli</i> should not be present in drinking water, including bottled/prepackaged water and ice.^{33,65,67,70}</p>

Features	Description
	<p>Recreational water</p> <p>Studies have shown a strong correlation between the concentration of <i>E. coli</i> in fresh waters and the risk of gastrointestinal illness among swimmers, but enteric pathogens, especially enteric pathogenic viruses and protozoa, may be present in water even when <i>E. coli</i> is absent. ^{66,71,72} A guideline value of ≤ 200 <i>E. coli</i> CFU/100 mL is used in Ontario and is based on a geometric mean concentration of a minimum of five public beach samples. ⁷³ Refer to the Ministry of Health’s Operational Approaches for Recreational Water Guideline document for direction when test results exceed the recreational water level for <i>E. coli</i>.</p> <p>Sewage</p> <p><i>E. coli</i> levels in water $>10^5$ CFU per 100 mL are indicative of fecal contamination. ⁷⁴</p>

Table D8: Reporting And Acceptable Limits for *Escherichia coli* (*E. coli*)

Sample Type	Reporting Limit (CFU/100 mL)	Acceptable Limit (CFU / 100 mL)
Official Agency Potable Water including bottled water / Ice – Treated	0 to > 80, *NDOGT	0
Private Citizen – Private Drinking Water Systems	0 to > 80, *NDOGT	0
Official Agency - Public Beach Water	< 10 to > 1.0×10^3	≤ 200
Official Agency – Suspected Sewage Contamination	< 1.0×10^3 to > 1.0×10^6	Not Applicable
Official Agency - Recreational Water Facilities, Public Pools/Spas	< 2 to > 160, *NDOGT	< 2

*NDOGT- No Data Overgrown with target

HETEROTROPHIC PLATE COUNT (HPC)

Table D9: Heterotrophic Plate Count (HPC) Features

Features	Description
What is it?	The Heterotrophic Plate Count (HPC) is a process of estimating the number of viable, heterotrophic bacteria in a sample, i.e., bacteria that use organic (carbon-containing) compounds as a source of energy and carbon, and measure the changes that occur to bacterial populations during water treatment and distribution.
Distinguishing features	The non-selective medium used for the HPC test includes carbohydrate, carbon and nitrogen sources that can sustain the growth of a wide variety of organisms in the presence of oxygen. ⁷⁵
Where do they come from?	These organisms are ubiquitous and can be found in the environment.
Potential health impacts	For healthy individuals, drinking water that contains heterotrophic bacteria does not necessarily pose a public health risk. ⁷⁶
What does it mean?	<p>Potable water / ice</p> <p>Water with an HPC result ≤ 500/mL is deemed to have detectable residual disinfectant so an HPC > 500 per 1 mL in a distribution system or other treated water is indicative of poor water quality and possible problems with the disinfection procedure.³⁵ The test has little value as an indicator of pathogen presence but can be useful in operational monitoring as a treatment and disinfectant indicator. In addition, HPC measurement can be used in assessing the cleanliness and integrity of distribution systems and the presence of biofilms. ³⁵</p> <p>Bottled water</p> <p>Water in sealed containers, other than water represented as mineral water or spring water, shall not contain more than 100 total aerobic bacteria (i.e., heterotrophic bacteria) per millilitre as determined by an HPC test. ⁷⁷ When an HPC test shows unacceptable results, an investigation should be carried out to determine the cause, and that corrective action taken. ⁷⁸</p> <p>Recreational water facility</p> <p>A heterotrophic plate count is the primary indicator of disinfection efficacy. ⁷⁹</p> <p>For public swimming pools, not more than 15 % of samples collected during any 30 day period should have an HPC count of 200 CFU/mL. ⁶¹</p>

Table D10: Reporting And Acceptable Limits for Heterotrophic Plate Count (HPC)

Sample Type	Reporting Limit (CFU per 1 mL)	Acceptable Limit (CFU per 1 mL)
Official Agency Potable Water including bottled water / Ice - Treated	< 10 to > 3.0 x10 ³ , * NDOGHPC	< 500**
Official Agency - Recreational Water Facilities, Public Pools/Spas	< 10 to > 3.0 x10 ³ , * NDOGHPC	Refer to Appendix D: Water Testing Fact Sheets

*NDOGHPC: No Data: Overgrown Heterotrophic Plate Count

** The standard for HPC under O. Reg. 169/03 was revoked June 6, 2006 (O. Reg. 248/06) and therefore, HPC is not reportable under the Safe Drinking Water Act. The acceptable limit listed is based on United States Environmental Protection Agency [standards](#)³⁵.

Legionella species

Table D11: Legionella species Features

Features	Description
What is it?	<i>Legionella</i> is a bacterium. <i>L. pneumophila</i> is the most common cause of legionellosis which can present as two forms of respiratory illness in humans: Legionnaires’ disease causing pneumonia and the less severe Pontiac fever that causes a self-limited flu-like illness. ⁸⁰
Distinguishing features	<i>Legionella</i> are gram-negative, aerobic, rod-shaped bacteria that are fastidious and require specific nutrients in order to grow on bacteriologic medium. ⁸⁰
Where do they come from?	Water is the major reservoir for legionellae. Stagnant water, warm water (between 20°C and 50°C) and biofilm, scale and sediment are all favourable environments for this organism. ⁸¹
Potential health impacts	They are commonly found in natural water sources (e.g., freshwater lakes, rivers, creeks and hot springs) and can be found in poorly maintained artificial water systems (i.e., cooling towers, air conditioning evaporative condensers, hot and cold water systems and whirlpool spas). Legionellae survive in these aquatic environments as intracellular parasites of free-living protozoa and within biofilms. ⁸²

Features	Description
What does it mean?	If <i>Legionella</i> was isolated from an environmental sampling site in connection to an outbreak investigation and matches the serotype of the clinical case, remediation is required and consultation with PHO's laboratory microbiologist is recommended. If <i>Legionella</i> was isolated and is not a match to the clinical case, remediation may be required and PHO's laboratory microbiologist is available for consultation. Refer to PHO's Legionellosis webpage for supporting documentation. ⁸³

Table D12: Reporting And Acceptable Limits for *Legionella* species

Sample Type	Reporting Limit	Acceptable Limit
Water	Detected / Not detected	Not detected

Pseudomonas aeruginosa

Table D13: *Pseudomonas aeruginosa* Features

Features	Description
What is it?	<i>Pseudomonas aeruginosa</i> is an opportunistic bacterial pathogen that can cause skin infections when water is contaminated with significant levels of the bacteria.
Distinguishing features	<i>Pseudomonas aeruginosa</i> is a gram negative, non-fermentative, oxidase positive, rod shaped bacteria, which grows well at 42 °C, is actively motile, and under appropriate incubation conditions produces a fluorescent pigment and often a visible pigment.
Where do they come from?	<i>Pseudomonas aeruginosa</i> can be found in feces, soil, natural waters such as lakes and rivers in concentrations of 10/100 mL to > 1,000/100 mL and sewage. They can multiply in water environments and on the surface of suitable organic materials in contact with water (e.g., in drinking water distribution systems and/or biofilms). ^{60,84,85} <i>Pseudomonas aeruginosa</i> can be found in swimming pools, waterslides and is frequently isolated from whirlpools and hot tubs when the organism is shed by infected human or when the organisms is transferred from a bather's feet and hands to the water. It thrives in warm temperatures, and aeration of the water can cause sloughing off of skin cells which also enhances its growth; these cells can act as a nutrient source and can reduce the overall residual disinfectant level as the organic load increases. ^{86,87}

Features	Description
Potential health impacts	<p>The risk of colonization from ingesting <i>Pseudomonas aeruginosa</i> in drinking water is low. ⁸⁴</p> <p>There are two routes of transmission of <i>Pseudomonas aeruginosa</i> in recreational water facilities:</p> <ul style="list-style-type: none"> • Skin exposure, a primary cause of ear, eye and skin infections (e.g., folliculitis) among swimmers⁶⁰ and • Lung exposure from inhaling aerosols⁸⁴ <p>One study reported the infective dose for healthy individuals is greater than 1,000 organisms per ml. ⁸⁸</p>

Potable / bottled water

High numbers of *Pseudomonas aeruginosa* in potable water, particularly packaged water, can result in off- taste, odour and turbidity. Ensuring adequate disinfection and taking steps to control biofilm growth can reduce the growth of *Pseudomonas aeruginosa*. At source and during marketing, a natural mineral water, spring water or other bottled waters offered for sale is are to be free from *Pseudomonas aeruginosa* in any 250 ml sample.^{60,89} If *Pseudomonas aeruginosa* is detected in a regulated drinking water sample, it is considered an adverse result for the purpose of section 18 of the [Safe Drinking Water Act](#) since it is a strong indicator the drinking water system lacks appropriate disinfection or a system malfunction has occurred (i.e., backflow or pipe breakage). ^{29,90}

What does it mean?

Recreational water facilities

Pseudomonas and *Staphylococcus* account for a large percentage of bacterial swimming-pool-associated illnesses. ⁷⁹

Pseudomonas aeruginosa is the primary indicator of disinfection efficacy, with Total Coliforms, heterotrophic plate count, and staphylococci as supporting indicators of water quality for whirlpools, and is frequently isolated from whirlpool water that is coliform-negative. ⁷⁹

Well operated pools should not normally contain *Pseudomonas aeruginosa*. If the count is over 10 CFU of *Pseudomonas aeruginosa* per 100 mL, repeat testing should be undertaken. Where repeated samples contain unacceptable amounts of *Pseudomonas aeruginosa* filtration and disinfection processes should be examined to determine whether there are areas within the pool circulation where the organism is able to multiply. ⁹¹

Table D14: Reporting And Acceptable Limits for *Pseudomonas aeruginosa*

Sample Type	Reporting Limit (CFU per100 mL)	Acceptable Limit (CFU per 100 mL)
Official Agency Potable Water including bottled water	0 to > 100 < 2 to > 200 *	0
Official Agency - Recreational Water Facilities, Public Pools/Spas	0 to > 100 < 2 to > 200 *	< 10

Salmonella species

Table D15: *Salmonella* species Features

Features	Description
What is it?	<i>Salmonella</i> is a bacterium.
Distinguishing features	<i>Salmonella</i> are rod-shaped gram-negative bacteria of the family Enterobacteriaceae.
Where do they come from?	<i>Salmonella</i> are carried in the intestinal tract of domestic and wild animals such as cattle, swine, poultry, wild birds, and pets (particularly reptiles) as well as flies. For <i>Salmonella</i> Typhi, humans are the only known host. ⁹² Since <i>Salmonella</i> cells can be injured or present in low numbers in aqueous environments, large sample volumes are necessary to allow for a concentration procedure as part of the analysis. ⁹³
Potential health impacts	Refer to Appendix C .
What does it mean?	Water <i>Salmonella</i> sp. is a pathogen and should not be present in potable water. It is relatively sensitive to disinfection so <i>Escherichia coli</i> is a generally reliable index for <i>Salmonella</i> spp. in drinking-water supplies. ⁶⁰

Table D16: Reporting And Acceptable Limits for *Salmonella* species

Sample Type	Reporting Limit	Acceptable Limit
Water	Not Detected / Detected	Not detected

Staphylococcus aureus

Table D17: Staphylococcus aureus Features

Features	Description
What is it?	<i>Staphylococcus aureus</i> is an opportunistic bacterial pathogen.
Distinguishing features	Gram-positive, non-motile, non-spore-forming coccus, catalase negative facultative anaerobe of the family Micrococcaceae that produces a wide array of toxins and can coagulate rabbit plasma.
Where do they come from?	<i>Staphylococcus aureus</i> are typically coagulase-positive which differentiates them from non-hazardous coagulase-negative strains that are found on skin. Healthy individuals can carry <i>Staphylococcus aureus</i> in their nose, on their skin and in their feces and shed them when they immerse themselves in water. ⁶⁰
Potential health impacts	Refer to Appendix C . <i>S. aureus</i> is a major pathogen responsible for purulent infections of the skin (e.g., folliculitis), eyes and ears and may not become apparent until 48 hours after contact with the contaminated water. ⁶⁰
	Potable / bottled water There is no evidence of disease transmission of <i>S. aureus</i> through secondary to the consumption of drinking water. Since fecal material is not usually the source of <i>S. aureus</i> , <i>E. coli</i> is not a suitable indicator for the presence of <i>S. aureus</i> in drinking-water. ⁶⁰ If <i>S. aureus</i> is detected in a regulated drinking water sample, it is considered an adverse result for the purpose of section 18 of the Safe Drinking Water Act since it is a strong indicator the drinking water system lacks appropriate disinfection or a system malfunction has occurred (e.g., backflow or pipe breakage). ^{29,90}
What does it mean?	Recreational water facilities <i>Pseudomonas</i> and <i>Staphylococcus</i> account for a large percentage of bacterial swimming-pool-associated illnesses. ⁷⁹ The presence of <i>S. aureus</i> in swimming pools and spas is a good index of the health hazard associated with a high bather density, and accounts for a large percentage of swimming-pool-associated illnesses. Whenever swimming pool samples are examined for total staphylococci or <i>Staphylococcus aureus</i> , not more than 50 organisms/100 mL should be present. Maintaining free chlorine levels greater than 1 mg/L or equivalent disinfection efficiency should provide adequate inactivation of <i>S. aureus</i> in swimming pools. ^{60,61,94}

Table D18: Reporting And Acceptable Limits for *Staphylococcus aureus*

Sample Type	Reporting Limit (CFU per100 mL)	Acceptable Limit (CFU per 100 mL)
Official Agency Potable Water including bottled water	0 to >100 <2 to >200 *	0
Official Agency - Recreational Water Facilities, Public Pools/Spas: Staphylococci (Presumptive or <i>S. aureus</i>)	0 to >100 <2 to >200 *	<50 **

* When 50 mL is analyzed

** For total staphylococci or *Staphylococcus aureus*

Revision History

As an ‘evergreen’ document the revisions noted below reflect current changes to this Guide. Printed versions of this document should be checked against the Public Health Ontario website to ensure the most recent version is being referenced.¹

Date of Implementation	Description and Change
September13, 2024	<ul style="list-style-type: none"> HETEROTROPHIC PLATE COUNT (HPC) section – Added a qualifier for the HPC acceptable limit for drinking water

Appendix E: Ontario Public Health Standards and Supporting Legislation

The Ontario Public Health Standards (OPHS): Requirements for Programs, Services, and Accountability (Standards) (OPHS) are currently being updated. Associated protocols, guidelines and indicators are also undergoing review. Further to release of the updated Requirements for Programs, Services, and Accountability (Standards) (OPHS) by the Ministry of Health, these references will be updated and the implications of any changes will be considered throughout the Guide.

Ontario Public Health Standards

- Ontario. Ministry of Health. Ontario public health standards: requirements for programs, services, and accountability (standards) [Internet]. Toronto, ON: Queen's Printer for Ontario; 2021 [cited 2023 Mar 17]. Available from: <https://www.ontario.ca/page/ontario-public-health-standards-requirements-programs-services-and-accountability>

Ontario Public Health Protocols

- Ontario. Ministry of Health and Long-Term Care. Food safety protocol, 2019. Toronto, ON: Queen's Printer for Ontario; 2019. Available from: <https://files.ontario.ca/moh-food-safety-protocol-en-2019.pdf>
- Ontario. Ministry of Health and Long-Term Care. Safe drinking water and fluoride monitoring protocol, 2019. Toronto, ON: Queen's Printer for Ontario; 2019. Available from: <https://files.ontario.ca/moh-safe-drinking-water-fluoride-monitoring-protocol-en-2019.pdf>
- Ontario. Ministry of Health and Long-Term Care. Recreational water protocol, 2019. Toronto, ON: Queen's Printer for Ontario; 2019. Available from: <https://files.ontario.ca/moh-recreational-water-protocol-en-2019.pdf>

Ontario Public Health Standards Guideline Documents

- Ontario. Ministry of Health and Long-Term Care. Operational approaches for food safety guideline, 2019. Toronto, ON: Queen's Printer for Ontario; 2019. Available from: <https://files.ontario.ca/moh-guidelines-operational-approaches-for-food-safety-guideline-en-2019.pdf>
- Ontario. Ministry of Health and Long-Term Care. Operational approaches for recreational water guideline, 2018. Toronto, ON: Queen's Printer for Ontario; 2018. Available from: <https://files.ontario.ca/moh-guidelines-operational-approaches-recreational-water-guideline-en-2018.pdf>

- Ontario. Ministry of Health and Long-Term Care. Food safety: a guide for Ontario's food handlers. Toronto, ON: Queen's Printer for Ontario; 2018. Available from: <https://www.ontario.ca/files/2024-03/moh-food-safety-manual-en-2024-03-07.pdf>
- Ontario. Ministry of Health and Long-Term Care. Response to adverse drinking water quality incidents [Internet]. Toronto, ON: Queen's Printer for Ontario; 2009 [cited 2023 Mar 17]. Available from: <https://collections.ola.org/mon/23007/293938.pdf>
- Ontario. Ministry of Health and Long-Term Care. Small drinking water systems risk assessment guideline, 2018. Toronto, ON: Queen's Printer for Ontario; 2018. Available from: <https://files.ontario.ca/moh-guidelines-small-drinking-water-systems-risk-assessment-guideline-en-2018.pdf>

Health Protection and Promotion Act And Regulations

Please note: These links are provided for your convenience. To access official copies of Ontario's statutes and regulations, please visit: <https://ontario.ca/laws>

- *Health Protection and Promotion Act*, RSO 1990, c H.7. Available from: <https://ontario.ca/laws/statute/90h07>
- *Small Drinking Water Systems*, O Reg 319/08. Available from: <https://www.ontario.ca/laws/regulation/080319>
- *Public Spas*, O Reg 428/05. Available from: https://e-laws.gov.on.ca/html/regs/english/elaws_regs_050428_e.htm
- *Food Premises*, O Reg. 493/17. Available from: <https://ontario.ca/laws/regulation/170493>
- *Public Pools*, RRO 1990, Reg 565. Available from: https://e-laws.gov.on.ca/html/regs/english/elaws_regs_900565_e.htm

Safe Drinking Water Act and Regulations

Please note: These links are provided for your convenience. To access official copies of Ontario's statutes and regulations, please visit: <https://ontario.ca/laws>

- *Safe Drinking Water Act*, SO 2002, c 32. Available from: https://e-laws.gov.on.ca/html/statutes/english/elaws_statutes_02s32_e.htm
- *Ontario Drinking Water Quality Standards*, O Reg 169/03. Available from: <https://www.ontario.ca/laws/regulation/030169>
- *Drinking Water Systems*, O Reg 170/03. Available from: https://e-laws.gov.on.ca/html/regs/english/elaws_regs_030170_e.htm

- *Definitions of Words and Expressions Used in the Act*, O Reg 171/03. Available from: https://e-laws.gov.on.ca/html/regs/english/elaws_regs_030171_e.htm
- *Schools, Private Schools and Child Care Centres*, O Reg 243/07. Available from: <https://www.ontario.ca/laws/regulation/070243>
- *Drinking Water Testing Services*, O Reg 248/03. Available from: <https://www.ontario.ca/laws/regulation/030248>

Other Relevant Legislation

Please note: These links are provided for your convenience. To access official copies of Ontario's statutes and regulations: <https://ontario.ca/laws>

- *Environmental Protection Act*, RSO 1990, c E.19. Available from: https://e-laws.gov.on.ca/html/statutes/english/elaws_statutes_90e19_e.htm#BK7
- *Food Safety and Quality Act*, 2001, S.O. 2001, c. 20. Available from: <https://ontario.ca/laws/statute/01f20>
- *Ontario Water Resources Act*, 1990. RSO 1990, c 1990. c O.40. Available from: <https://ontario.ca/laws/statute/90o40>
 - *Wells*, RRO 1990, Reg 903. Available from: https://e-laws.gov.on.ca/html/regs/english/elaws_regs_900903_e.htm
- *Provincial Parks and Conservation Reserves Act*, SO 2006, c 12. Available from: https://e-laws.gov.on.ca/html/statutes/english/elaws_statutes_06p12_e.htm

Foodborne Illness Outbreak Guidance Documents

- Health Canada; Public Health Agency of Canada; Canadian Food Inspection Agency. Weight of evidence: factors to consider for appropriate and timely action in a foodborne illness outbreak investigation. Ottawa, ON: Her Majesty the Queen in Right of Canada, represented by the Minister of Health; 2011. Available from: https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/fn-an/alt_formats/pdf/pubs/securit/2011-food-illness-outbreak-eclosion-malad-ailments-eng.pdf
- Ontario. Ministry of Health. Ontario's Foodborne Illness Outbreak Response Protocol (ON-FIORP) [Internet]. Toronto, ON: King's Printer for Ontario; 2023 [cited 2024 Jan 9]. Available from: <https://www.ontario.ca/files/2023-12/moh-ohs-ref-foodborne-illness-outbreak-response-protocol-en-2023-12-15.pdf>

- Public Health Agency of Canada. Canada’s Foodborne Illness Outbreak Response Protocol (FIORP): a guide to multi-jurisdictional enteric outbreak response. Ottawa, ON: Her Majesty the Queen in Right of Canada, as represented by the Minister of Health; 2017. Available from: <https://www.canada.ca/content/dam/phac-aspc/documents/services/publications/health-risks-safety/64-02-17-1879-FIORP-2015-EN-04.pdf>

Supporting Documentation

- American Public Health Association. Compendium of methods for the microbiological examination of foods. 5th ed. Washington, DC: American Public Health Association; 2015.
- Centers for Disease Control and Prevention. Sampling procedure and potential sampling sites [Internet]. Atlanta, GA: Centers for Disease Control and Prevention; 2015 [cited 2023 Mar 17]. Available from: <https://cdc.gov/legionella/downloads/cdc-sampling-procedure.pdf>
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- Heymann DL, ed. Control of communicable diseases manual. 20th ed. Washington, DC: American Public Health Association; 2015.
- Ontario Agency for Health Protection and Promotion (Public Health Ontario). Home canning: literature review. Toronto, ON: Queen’s Printer for Ontario; 2014. Available from: <https://www.publichealthontario.ca/-/media/documents/H/2014/home-canning.pdf>
- World Health Organization. Foodborne disease outbreaks: guidelines for investigation and control. Geneva: World Health Organization; 2008. Available from: <https://www.who.int/publications/i/item/9789241547222>

Revision History

As an ‘evergreen’ document the revisions noted below reflect current changes to this Guide. Printed versions of this document should be checked against the [Public Health Ontario website](#) to ensure the most recent version is being referenced.¹

Date of implementation	Description and Change
September 13, 2024	<ul style="list-style-type: none"> • Updated references

Appendix F: Full Revision History

Introduction

Date of Implementation	Description and Change
October 2, 2017	<p>Changes from 2013:</p> <ul style="list-style-type: none">• The title of the Guide has been changed from Public Health Inspector's Guide to the Principles and Practices of Environmental Microbiology to Public Health Inspector's Guide to Environmental Microbiology Laboratory Testing• Removed Contact Directory. Refer clients to PHO's laboratory Customer Service Centre• Added a note stating PHO's laboratory does not analyze floatation tank/isopod and foot throne samples (no provincial guidelines for microbiological testing or standards available)
May 6, 2019	<ul style="list-style-type: none">• Updated the number of boards of health within the province• Environmental swabs submitted for RRO 1990, Reg. 562: <i>Food Premises</i>, Section 80 will no longer be accepted for testing.⁹⁵ All applicable areas have been updated to reflect this change.• Updated the Ministry of the Environment, Conservation and Parks (MECP) name and link
September 13, 2024	<ul style="list-style-type: none">• Updated Ministry of Health branch name throughout• Legal Considerations – Outlined submitter's responsibilities• Added updated Botulism Guide for Health Care Professional, Ministry of Health, Ontario

Food Sample Analysis

Date of Implementation	Description and Change
	Changes from 2013:
October 2, 2017	<ul style="list-style-type: none"> Added links to test directory - test information sheets for sample details (e.g., sample requirements (container, requisition, volume), sample handling and transport, test information, testing frequency, turnaround time and reporting) Updated food bacteriology requisition section to reflect updated requisition (1722- 44 (03/2014)) Updated food-laboratory rejection criteria to reflect acceptance protocol
May 6, 2019	<ul style="list-style-type: none"> Updated the number of boards of health within the province
September 13, 2024	<ul style="list-style-type: none"> Updated the Botulism after hours contact information and added the Botulism Guide for Health Care Professionals document from Ministry of Health, Ontario

Environmental Swabs

Date of Implementation	Description and Change
	Changes from 2013:
October 2, 2017	<ul style="list-style-type: none"> Added links to test directory - test information sheets for sample details (e.g., sample requirements (container, requisition, volume), sample handling and transport, test information, testing frequency, turnaround time and reporting) Updated swab-laboratory rejection criteria to reflect acceptance protocols and added interpretation for swabs
May 6, 2019	<ul style="list-style-type: none"> Environmental swabs submitted for R.R.O. 1990, Reg. 562: Food Premises, Section 80 will no longer be accepted for testing, as Section 80 was revoked on July 1, 2018.⁹⁵ All applicable areas have been updated to reflect this change.
August 8, 2021	<ul style="list-style-type: none"> Updated the swab requisition details from Environmental Bacteriology Swab Test requisition to Environmental Microbiology Investigation Requisition
September 13, 2024	<ul style="list-style-type: none"> No changes noted

Water Sample Analysis

Date of Implementation	Description and Change
October 2, 2017	<p>Changes from 2013:</p> <ul style="list-style-type: none"> • Added links to test directory (test information sheets) for sample details (e.g., sample requirements (container, requisition, volume), sample handling and transport, test information, testing frequency, turnaround time and reporting) • Removed “Plumbing in the following circumstances” passage from the <i>Drinking Water that fall outside Ontario Drinking Water Regulations</i> chart • Updated water-laboratory rejection criteria to reflect acceptance protocols
May 6, 2019	<ul style="list-style-type: none"> • Changed acceptable level of <i>E. coli</i> for public beaches from 100 CFU/100 mL to 200 CFU/100 mL. • Updated the Ministry of the Environment, Conservation and Parks (MECP) name and link. • Added table: Water Status Codes and Associated Interpretation Statements.
August 8, 2021	<ul style="list-style-type: none"> • Added the option to submit water samples using Environmental Microbiology Investigation Requisition for etiological agent testing.
September 13, 2024	<ul style="list-style-type: none"> • Important Information Related to Drinking Water Analysis section – Removed the table, “Examples of Drinking Water that Fall Outside Ontario Drinking Water Regulations” • Compliance versus Audit Samples section – Clarified drinking water system owner/operator responsibilities • Drinking Water Submissions under a Provincial Officer’s Order or Approval (Directive) section - Added context for Orders or Directives • Drinking Water Research section – Included a link to the “Notification Form for Drinking Water Testing Research and Method Development” form • Water – Laboratory Acceptance Criteria section – Added a requirement for submitters to provide written clarification when there is conflicting information on the requisition • Water – Reporting and Acceptable Limits of Environmental Microbiological Test Results section – Added a qualifier for the HPC acceptable limit for drinking water • Water Status Codes and Associated Interpretation Statements for Drinking Water Reports section – Added codes 32 and 33

Legionella Investigations

Date of Implementation	Description and Change
October 2, 2017	<p>Changes from 2013:</p> <ul style="list-style-type: none"> Added links to test directory (test information sheets) for sample details (e.g., sample requirements (container, requisition, volume), sample handling and transport, test information, testing frequency, turnaround time and reporting) Added details on environmental sample site recommendations from WHO Added laboratory rejection criteria to reflect acceptance protocols
May 6, 2019	<ul style="list-style-type: none"> Removed Bacteriological Analysis of Water - Single Sample Requisition for Official Agencies
August 8, 2021	<ul style="list-style-type: none"> Updated the details of the swab requisition from Environmental Bacteriology Swab Test requisition to Environmental Microbiology Investigation Requisition Updated the volume requirement for water testing
September 13, 2024	<ul style="list-style-type: none"> No changes noted

Glossary

Date of Implementation	Description and Change
October 2, 2017	<p>Changes from 2013:</p> <ul style="list-style-type: none"> Glossary expanded and list of acronyms added.
May 6, 2019	<ul style="list-style-type: none"> Updated the Ministry of the Environment, Conservation and Parks (MECP) name and link.
August 8, 2021	<ul style="list-style-type: none"> Added hold time definition.
September 13, 2024	<ul style="list-style-type: none"> Removed HPPA O. Reg. 318/08 Drinking Water Updated definitions for: Indicator bacteria (water), Non-Regulated Drinking Water Systems, Order (Provincial Officer's Order), Outbreak and Single Household Added definitions for Raw Water and Raw Water for Consumption

Acronyms

Date of Implementation	Description and Change
August 8, 2021	<ul style="list-style-type: none"> Updated MOHLTC to MOH-EH
September 13, 2024	<ul style="list-style-type: none"> Added MPN

Appendix A

Date of Implementation	Description and Change
May 6, 2019	<ul style="list-style-type: none"> Removed Bacteriological Analysis of Water - Single Sample Requisition for Official Agencies
September 13, 2024	<ul style="list-style-type: none"> Updated supply list to include: Water – Private Citizen collection kit and Water – Official Agency collection bottles

Appendix B

Date of Implementation	Description and Change
August 8, 2021	<ul style="list-style-type: none"> Updated the details of the swab requisition from Environmental Bacteriology Swab Test requisition to Environmental Microbiology Investigation Requisition

Appendix C

Date of Implementation	Description and Change
	Changes from 2013:
October 2, 2017	<ul style="list-style-type: none"> Reformatted the chart (there was no change to the content) Added Cyclosporiasis section
May 6, 2019	<ul style="list-style-type: none"> No changes noted
September 13, 2024	<ul style="list-style-type: none"> No changes noted

Appendix D

Date of Implementation	Description and Change
October 2, 2017	Changes from 2013 <ul style="list-style-type: none">• New appendix• Expanded the interpretation and added references
May 6, 2019	<ul style="list-style-type: none">• Changed acceptable level of <i>E. coli</i> for public beaches from 100 CFU/100 mL to 200 CFU/100 mL, as per Operational Approaches for Recreational Water Guideline, 2018.
September 13, 2024	<ul style="list-style-type: none">• HETEROTROPHIC PLATE COUNT (HPC) section – Added a qualifier for the HPC acceptable limit for drinking water

Appendix E

Date of implementation	Description and Change
October 2, 2017	Changes from 2013: <ul style="list-style-type: none">• Updated references
May 6, 2019	<ul style="list-style-type: none">• Updated references
August 8, 2021	<ul style="list-style-type: none">• Updated references
September 13, 2024	<ul style="list-style-type: none">• Updated references

References

Date of Implementation	Description and Change
October 2, 2017	Changes from 2013: <ul style="list-style-type: none">• Updated references
August 8, 2021	<ul style="list-style-type: none">• Updated references
September 13, 2024	<ul style="list-style-type: none">• Updated references

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Revision History

As an 'evergreen' document the revisions noted below reflect current changes to this Guide. Printed versions of this document should be checked against the Public Health Ontario website to ensure the most recent version is being referenced.

Date of Implementation	Description and Change
September 13, 2024	<ul style="list-style-type: none"><li data-bbox="646 506 932 537">• Updated references

Public Health Ontario

661 University Avenue, Suite 1701

Toronto, Ontario

M5G 1M1

416.235.6556

communications@oahpp.ca

publichealthontario.ca

