

LABSTRACT – December 2020

Legionella: Introduction of molecular screening of environmental water samples

Audience

Public health unit professionals who submit environmental water samples for *Legionella* testing in support of *Legionella* outbreak investigations.

Overview

Effective December 9, 2020, Public Health Ontario's (PHO) laboratory will implement polymerase chain reaction (PCR) based testing as a screening method for the detection of *Legionella* in water samples. The new PCR method detects three targets including: *Legionella* species, *Legionella pneumophila*, and *Legionella pneumophila* serogroup 1.

Advantages

- The method enables rapid screening of samples with a shorter turn-around time of 2 days for the reporting of negative results. Positive screen results will be reported verbally by telephone to the submitter.
- The PCR method is more sensitive than culture and is able to identify the three targets, as outlined above, in a single test.

Background information

This new PCR screening method has been verified by PHO laboratory for the detection of *Legionella* in water samples. Swab sample submissions will continue to be analyzed using the routine culture method. Only samples for which *Legionella* is "detected" by PCR and those with indeterminate PCR test results will be further analyzed using routine culture. The isolation of *Legionella* by culture enables additional characterization and molecular typing of *Legionella* for outbreak investigation and epidemiological purposes.

For submission and testing of *Legionella* samples, consultation with the PHO's Environmental Microbiology section is recommended prior to sample collection. For additional information, the Environmental Microbiology section and the associated PHO microbiologist may be contacted through the PHO Laboratory Customer Service Centre. For information on *Legionella* clinical specimen collection, and testing details, refer to the laboratory [Test Information Index](#) webpage.

Change details

What has changed with sample collection?

- Two litres of water (potable or non-potable) is required for the analysis. This equates to 10 standard PHO water collection bottles filled to the 200 mL fill line.
- Five collection bottles (1 litre of water) will be used for PCR screening, and 5 collection bottles (1 litre of water) will be used for culture testing. The submitter must ensure that the 2 litres of water are collected aseptically, are homogeneous and collected under the same conditions.
- PCR testing will not be performed if less than 2 litres was received or on swab samples.
- Ship the samples 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.

What has changed with test ordering?

- Two separate requisitions must be completed for each set of samples submitted (5 collection bottles per requisition).
- Use the [Environmental Microbiology Investigation Requisition Form](#) (07/2019 version, or newer) and ensure all mandatory fields on the form are completed, including *Legionella* as the confirmed etiological agent.
- Refer to the [Public Health Inspector's Guide to Environmental Microbiology Laboratory Testing](#) for additional collection and submission information.

Interpretation of results

For *Legionella*, the PCR results will be reported as **Not detected**, **Detected**, and **Indeterminate** (invalid results) - see table below. Culture will be initiated for all PCR Detected or Indeterminate test results.

PCR Screening Test	Culture	Interpretation
<i>Legionella</i> species - Not detected <i>Legionella pneumophila</i> - Not detected <i>Legionella pneumophila</i> serogroup 1 - Not detected	Not performed	<i>Legionella</i> species DNA was not detected by PCR.
<i>Legionella</i> species - Detected <i>Legionella pneumophila</i> - Not detected <i>Legionella pneumophila</i> serogroup 1 - Not detected	Culture will be initiated	<i>Legionella</i> species DNA was detected in the sample.
<i>Legionella</i> species - Detected <i>Legionella pneumophila</i> - Detected <i>Legionella pneumophila</i> serogroup 1 - Not detected	Culture will be initiated	<i>Legionella pneumophila</i> DNA was detected by PCR. Note: Additional <i>Legionella</i> species may be present in the sample.
<i>Legionella</i> species - Detected <i>Legionella pneumophila</i> - Detected <i>Legionella pneumophila</i> serogroup 1 - Detected	Culture will be initiated	<i>Legionella pneumophila</i> serogroup 1 DNA was detected by PCR. Note: Additional <i>Legionella</i> serogroups and/or species may be present in the sample.
<i>Legionella</i> species - Indeterminate <i>Legionella pneumophila</i> - Indeterminate <i>Legionella pneumophila</i> serogroup 1 - Indeterminate	Culture will be initiated	<i>Legionella</i> PCR test results are not available (no internal positive control detected, and/or low or no target-specific signal detected).

References

1. Bioteccon Diagnostics. microproof® Legionella Quantification LyoKit – 5´Nuclease – , 01/2020. Available from bc-diagnostics.com/downloads/products/bpz/R_602_45_20_Legionella.pdf – conforms to ISO 12869:2012, Water quality — Detection and quantification of *Legionella* spp. and/or *Legionella pneumophila* by concentration and genic amplification by quantitative polymerase chain reaction (qPCR) standard
2. Infectious Diseases Protocol. Appendix A: Disease-Specific Chapters. Chapter: Legionellosis. Effective: February 2019. Available from: health.gov.on.ca/en/pro/programs/publichealth/oph_standards/docs/legionellosis_chapter.pdf
3. Infectious Diseases Protocol. Appendix B: Provincial Case Definitions for Diseases of Public Health Significance. Disease: Legionellosis. Effective: February 2019. Available from: health.gov.on.ca/en/pro/programs/publichealth/oph_standards/docs/legionellosis_cd.pdf

For further information

- Contact the PHO’s Laboratory Customer Service Centre at 416-235-6556 or 1-877-604-4567 (toll-free), or by email at customerservicecentre@oahpp.ca
- For PHO’s Laboratory specimen collection information and previous Labstracts, refer to publichealthontario.ca/test_directory
- The current versions of the PHO Test Requisition and other forms are available at publichealthontario.ca/Requisitions
- To subscribe to receive future Labstracts, [register on our website](#)
- To register for Autofax and receive laboratory reports by fax directly from our laboratory information system as soon as they are released, contact the PHO’s Laboratory Customer Service Centre.



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