



Methods for the Determination of *Legionella* in Warm Water Systems

Overview

On the 1 February 2017, it became mandatory for all Queensland Health Hospitals with inpatients, Queensland Health operated residential aged care facilities and licensed private health facilities to have a Water Risk Management Plan. These plans are to include a schedule for testing water for *Legionella* and other identified hazards, and require that confirmed *Legionella* detections be reported to the Department of Health within one business day of detection.

As per the amended Public Health Regulation 2005, a prescribed test for *Legionella*

- quantifies the number of *Legionella* colony forming units in the sample tested: and
- is carried out by an accredited laboratory and is identified in the scope of the laboratory's accreditation

ALS is accredited by the National Association of Testing Authorities Australia (NATA) for the following:

- Sample collection
- *Legionella* spp. <1CFU/100mL
- *Legionella* spp. <10CFU/mL
- Rapid confirmation of *Legionella* by MALDI-TOF MS

METHOD AND LOR INFORMATION

ALS METHOD CODE

MM527/MW021 – AS 3896:2008 *Legionella* spp. (incl *L. pneumophila*) (AS/NZS 3896:2008) – Standard Level

MM570 – *Legionella* spp. (incl *L. pneumophila*) (ISO 11731-2:2004) – Low Level

LIMIT OF DETECTION

MM527/MW021 – <10cfu/mL

MM570 - <1cfu/100mL

MALDI-TOF MS *Legionella* Bio typing

ALS can provide rapid MALDI-TOF MS confirmation on both standard level and low level *Legionella* culture methods.

Traditional methods of detecting *Legionella* in a water sample take up to 10 days for the standard method and 12 days for the low-level method. This involves using a cultural method incubated for 7 (standard) to 10 (low level) days, followed by biochemical confirmation, which typically takes a further 2 to 3 days to produce a confirmed detected result.

MALDI-TOF MS (Matrix assisted laser desorption ionization-time of flight mass spectrometry) eliminates the 2-3 day confirmation time, giving a confirmed *Legionella* result within minutes. This means that confirmed *Legionella* results are available in 7 days for the standard method and 10 days for the low-level method.

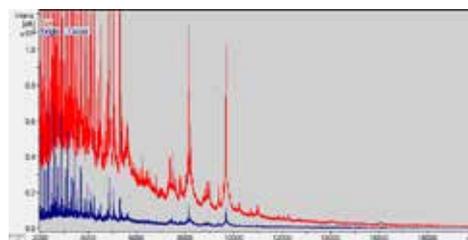


Figure 1 – Example of a confirmed *Legionella* spp. Mass Spectrum from MALDI-TOF MS

MALDI-TOF MS uses Mass Spectrometry to measure a unique molecular fingerprint of an organism. Specifically, it identifies highly abundant biomolecules that are found in all microorganisms. The characteristic patterns of these molecules are matched against an extensive open database comprising thousands of individual strains of micro-organisms to identify the organism to the species level.

ALS in the United Kingdom was one of the first Environmental laboratories in the world to validate MALDI-TOF technology for *Legionella* and has characterised one of the world's largest culture collections for *Legionella* Species. This innovation led to ALS Environmental being awarded the UK [Combating Legionella Supplier of the Year award](#) in 2016.

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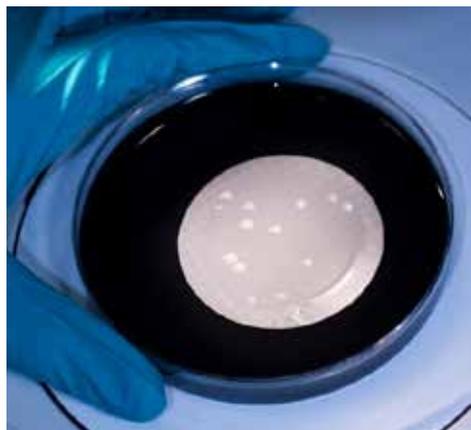
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Cultural Methods for Legionella analysis available at ALS

AS/NZS 3896:2008 – STANDARD LEVEL LEGIONELLA METHOD (MM527/MW021)

A water sample is treated by several independent steps to optimize the recovery of *Legionella* spp. This involves direct plating, heating and acid treatments with the spread plate technique onto selective culture media. Culture plates are then incubated at 36 degrees for 7 days. Plates are examined at 4 and 7 days and colonies that are present are counted and then confirmed to a species level. Confirmed results are reported as colony forming units (CFU)/mL, with a lower detection limit of 10CFU/mL. This method is the most commonly used method for analysing *Legionella* in Australia.

Note: ALS is currently preparing a submission to NATA for the recently released **AS/NZS 3896:2017**. Method references and procedures will be updated accordingly.



ISO 11731-2:2004 – LOW LEVEL LEGIONELLA METHOD (MM570)

A water sample is acid treated to remove interfering bacteria of other species and is filtered through a membrane, which is placed onto a culture media and incubated for 10 days. The volume of water filtered depends on the particulate content of the water and the degree of sensitivity required. This can range from 1mL to 100mL of water. Bacterial colonies are counted and confirmed *Legionella* reported to a limit of 1CFU/100mL.

This lower limit of reporting can provide Hospitals and Health facilities with further assurance that any detected *Legionella* in a system has been eliminated by the treatment processes outlined in their Water Risk Management Plans. This method of testing is only recommended when there is a low level of *Legionella* expected to be detected in a sample, and as the growth of *Legionella* may be inhibited by overgrowth of other bacterial colonies on the membrane, the method is only suitable for waters containing low bacterial counts.

Sampling

ALS has provided sampling services to the water industry for over 40 years and can provide independent NATA Accredited onsite sampling services to collect samples according to the individual facility's monitoring program or Water Risk Management Plan. Our full-time Field Technicians have extensive experience sampling from multiple hospital and healthcare facility types, including small dental clinics and day hospitals to large multi-facility public and private hospitals.

Samples are collected from multiple distal points throughout the facility, such as basin taps, showers, toilets, cold/hot water units, and mains supply. Typically, the monitoring program incorporates both an initial sample without flushing, then another after flushing the distal point for a period of time. Capturing both provides an important indication of the water quality supplying the distal point, as well as that within the tap/faucet/hardware upon first use by the consumer.

If the client would prefer to sample themselves, ALS provides appropriate, QC checked sample containers and eskies free of charge to ensure recommended preservation is maintained.



REFERENCES

AS/NZS 3896:2008 Waters - Examination for *Legionella* spp. including *Legionella pneumophila*

ISO 11731-2:2004 Water quality - Detection and enumeration of *Legionella* - Part 2: Direct membrane filtration method for waters with low bacterial counts

Public Health Act 2005, Chapter 2A (Water Risk Management Plans)

<https://www.legislation.qld.gov.au/LEGISLTN/CURRENT/P/PubHealA05.pdf>

Public Health Regulation 2005, part 1B (Water Risk Management Plans)

<https://www.legislation.qld.gov.au/LEGISLTN/CURRENT/P/PubHealR05.pdf>

<https://www.alsenvironmental.co.uk/our-services/water-treatment/maldi-tof-analysis>

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