THE WATER MANAGEMENT SOCIETY

Rapid Microbiology Industry Liaison Group

MPN (Legiolert™) Factsheet

These guides are intended to give unbiased views regarding a number of technologies and test kits and their potential capabilities.

The opinions expressed are the views of individual members of the Rapid Microbiology Industry Liaison Group and are supplied in good faith. Additional third party opinions are provided in the attached literature references and on-line links. The WMSoc cannot be held responsible for any misunderstanding or subsequent misapplication of this information.

The manufacturer should be contacted regarding details about availability, pricing, repairs, calibration, QA/QC recommended protocols, validation data, and performance data, etc.

The factsheets cannot give advice regarding specific water testing alert levels – these need to be advised by the regulators.

LEGIOLERT SUMMARY TABLE

- This test detects all serogroups of *L. pneumophila*. It does not detect *Legionella* spp.
- A confirmed *L . pneumophila* result is available in seven days without any additional steps.
- Very simple and robust method that can be carried out in a basic test room laboratory with minimum facilities. There are three protocols: 100 mL (drinking water) 10 mL (drinking water) and 1mL (cooling tower and other non-potable samples). Potable water samples are incubated at 39 ± 0.5 °C; Non-potable water samples are incubated at 37 ± 0.5 °C.
- No sample filtration step is required.

*Sample concentration may be performed if required to reflect the practice in some labs.

Method	Most probable number technique to detect *Legionella pneumophila*
Bacteria detected	*L. pneumophila*, all serogroups, including Serogroup 1 and Serogroups 2-14. Serotyping and isolation can be done from a positive well.
Pre-concentration as per ISO 11731	No concentration required*
Algorithm to convert results to CFU	None required. Calculated/determined MPN is scientifically equivalent to CFU
Can differentiate between live and dead cells	Live only
Will detect VBNC bacteria	May encourage growth due to liquid medium
Interference from biocides and other water treatment additives	None detected in studies to date
Use with complex water samples e.g. from cooling towers	Yes (1 mL protocol)
Laboratory or field	Laboratory or clean area with required equipment
Are results comparable to current plate counts?	Yes – Comparison done according to ISO 17994 against ISO 11731-2:2004 and ISO 11731 (see scientific publication references)
Would current plate technique still be required?	No
False positive False negative	< 1% (measured according to ISO 13843) 4.2% (measured according to ISO 13843)
Could rapid test give a positive result whilst culture test gives negative result?	Possibly. Liquid culture growth medium may enhance/encourage growth of VBNC

Suitable verification data should be supplied by any laboratories undertaking the testing (or UKAS accreditation).
All tests should have positive and negative control data available - irrespective of whether laboratory or field-based.
N.B. Not all methods are suitable for field-based testing.

1.	General	
i.	Name of Test:	**Legiolert® (for detection of *Legionella pneumophila*)**
ii.	Scientific principles / basis for test:	**This liquid culture test is based on a bacterial enzyme-detection technology that signals the presence of *L. pneumophila* through utilization of a substrate present in the Legiolert reagent. *L. pneumophila* cells grow rapidly and reproduce using the rich supply of amino acids, vitamins, and other nutrients present in the Legiolert reagent. Actively growing strains of *L. pneumophila* use the added substrate to produce a brown colour/turbidity indicator.**
iii.	Sensitivity: Specificity: Limit of detection:	**Sensitivity: 98% (measured according to ISO 13843)** **Specificity: >99% (measured according to ISO 13843)** **Limit of Detection: When using the 100 mL protocol for potable water, the Legiolert test detects *L. pneumophila* at ≥1 organisms/100 mL. When using the 1.0 mL protocol for non-potable water, the Legiolert test detects *L. pneumophila* at ≥1 organisms/mL.**
iv.	Scientific publication references:	***Potable Water*** **K Spies, S Pleischl, B Lange, B Langer, I Hubner, L Jurzik, K Luden, M Exner "Comparison of the Legiolert/Quanti-Tray* MPN test for the enumeration of *Legionella pneumophila* from potable water samples with the German regulatory requirements methods ISO 11731-2 and ISO 11731." International Journal of Hygiene and Environmental Health 221(7): 1047-1053; August 2018; https://www.sciencedirect.com/science/article/pii/S1438463917306818?via%3Dihub** **DP Sartory, K Spies, B Lange, S Schneider, B Langer "Evaluation of a most probable number method for the enumeration of *Legionella pneumophila* from potable and related water samples." Letters of Applied Microbiology 64(4); 271-275; April 2017; https://www.ncbi.nlm.nih.gov/pubmed/28117485** ***Potable and Non-potable Water*** **R Petrisek, J Hall "Evaluation of a most probable number method for the enumeration of *Legionella pneumophila* from North America potable and nonpotable water samples." Journal of Water and Health 16 (1):25-33; February 2018; https://www.ncbi.nlm.nih.gov/pubmed/29424715** ***Non-potable Water*** **Rech, M.M., Swalla, B.M. & Dobranic, J.K."Evaluation of Legiolert for Quantification of *Legionella pneumophila* from Non-potable Water." Curr. Microbiol. Pages 1-8; July 2018 https://doi.org/10.1007/s00284-018-1522-0** **Swalla, Brian "Strategies for Reducing Uncertainty in Legionella Analysis", Technical Paper 18- 22, Cooling Tower Institute; 2018; http://www.cti.org/tech_papers/legionella.php**
v.	Patents:	**Patent pending**
vi.	Countries sold into:	**Worldwide**
vii.	Manufacturer: Supplier:	**IDEXX Laboratories, Inc.**
viii.	Commercially available:	**Worldwide**
ix.	Micro-organism species detected:	***L. pneumophila*, all serogroups, including Serogroup 1 and Serogroups 2-14. Serotyping and isolation can be done from a positive well.**
x.	Lab based: Field based:	**Yes** **Basic test area and equipment required**
xi.	Can the test be used to determine operational control? Trend analysis?	**Yes** **Yes**
xii.	Independent end-user data:	**Yes. See scientific publication references above.**
xiii.	Method validated by third party:	**Yes. See scientific publication references above.**

2.	Application details	
i.	Sample quality required:	**Can be used with complex matrices other than potable water**
ii.	What sample preparation on-site is required:	**None**
iii.	Does the sample need to be tested within a prescribed time scale (courier)?	**As per BS 7592**
iv.	Sample bottle type: Sample volume required:	**Standard** **Up to 100 mL, depending on protocol to be used**

3.	Analytical procedures	
i.	Does procedure require initial isolation of test organism by culture?	**No**
ii.	Which other substances and/or microorganisms are potential interferences or inhibitors?	**Appears to be unaffected by typical levels of other waterborne bacteria. Contact supplier for latest information on potential chemical interferences.**
iii.	What additional equipment will be required?	**Quanti-Tray® Sealer PLUS Incubator: Potable water samples 39 ± 0.5 °C; Non-potable water samples 37 ± 0.5 °C**
iv.	Is equipment specialised?	**Yes, but inherently very simple to use**
v.	Is the process automated? Could it be automated?	**Sample preparation is manual and very simple Not currently required**
vi.	Does sample need pre-treatment prior to analysis?	**100 mL potable protocol requires a total hardness (TH) determination using a supplied paper test strip to decide if the TH is high or low. Depending upon the result, supplied supplement neutraliser is added to 100 mL of the potable water sample. 1 mL non-potable protocol requires a 1 minute pretreatment to eliminate heterotroph interference.**
vii.	Is training provided?	**Video instruction provided online.**
viii.	How long will test take before results are available?	**Up to 7 days to for confirmed positive result. Requires full 7 days for a negative result**
ix.	How many samples can be analysed?	**Will be dependent on resources but could be up to 100 per technician. Typically up to 2 min "hands on" time per sample**
x.	What units are results expressed in?	**MPN per unit volume tested, scientifically equivalent to CFU per unit volume tested**
xi.	Does the result correlate with standard analytical procedures such as plating?	**Yes**
xii.	Is specialised training required to conduct test and interpret results?	**No, but advised to demonstrate evidence of competency. WMSoc training course available: HTM04-01- Monitoring the Risk of Waterborne Pathogens - Best Practices - W038**
xiii.	Are results reproducible:	**Yes. Measured according to ISO 13843: Reproducibility: <.01 (99%) Repeatability: <.01 (99%)**
xiv.	What errors (if any) could occur with analysis (weak link)?	**Method appears to be simple and robust**
xv.	Has test been validated for environmental samples?	**Yes, see scientific publication references**
xvi.	Does the final result include VBNC?	**Possibly – it will encourage growth of VBNC**
xvii.	Does it detect live or dead cells, or both?	**Live cells only. Does not detect dead cells**
xviii.	Has test been used in an EQA process or could it be?	**Yes**
xix.	Will it be possible for a user organisation to gain UKAS ISO 17025 accreditation?	**Yes**

Glossary:

Algorithms - can enable calculation between different measures (e.g. MPN to CFU)

Colony forming units - used to estimate the number of viable bacteria or fungal cells in a sample

Genus - a way of classifying bacteria. Genus comes above species & below family

Sensitivity - (also called the true positive rate or probability of detection) measures proportion of positives that are correctly identified as such

Species - a group of living things that all share common characteristics and that are all classified as alike in some manner

Specificity - (also called the true negative rate) measures proportion of negatives that are correctly identified as such

Strain - a particular variety of bacteria

Viable - the ability (of bacteria) to multiply

List of abbreviations:

ATP – Adenosine tri-phosphate

CFU – Colony Forming Units

EQA – External quality assurance

GU – Genomic unit

IMS – immunomagnetic separation

LOD - Limit of detection - the lowest quantity of bacteria that can be distinguished from the absence of that bacteria (a blank value) with a stated confidence level (generally 99%).

MPN - Most Probable Number

MALDI ToF – Matrix Assisted Laser Desorption/Ionization Time of Flight

NF Validation - Third party certification

PCR – Polymerase Chain Reaction

qPCR – Quantitive Polymerase Chain Reaction

RTPCR - Real Time Polymerase Chain Reaction

VBNC – Viable but Non-Culturable