THE WATER MANAGEMENT SOCIETY

Rapid Microbiology Industry Liaison Group

PCR Factsheet (Bio-Rad)

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.

PCR (BIO-RAD) SUMMARY TABLE

- Real-time PCR kits for detection or quantification of *Legionella* spp. or *Legionella pneumophila*.
- Kits deliver reliable results within a few days without the need of bacterial cultures.
- Protocol includes sample filtration, optional treatment to remove free DNA, DNA extraction, PCR amplification and data analysis.
- High negative predictive value i.e. negative means negative.
- See also, HSE website for further information at: ***www.hse.gov.uk/legionnaires/faqs.htm#Testing-monitoring***

The information below is based on Bio-Rad which is one of the manufacturers who have developed real-time PCR kits for the detection or quantification of *Legionella spp.* or *Legionella pneumophila*. PCR kits are available from other manufacturers.

Method	Polymerase chain reaction (detects DNA)
Bacteria detected	*Legionella* spp, *L. pneumophila*
Pre-concentration as per ISO 11731	YES
Algorithm to convert results to CFU	Possibly but may not be reliable, as results expressed in genomic units (GU).
Can differentiate between live and dead cells	Bio-Rad legionella qPCR solution will manage free DNA from cells.
Will detect Viable But Non-Culturable (VBNC) bacteria	YES
Interference from biocides and other water treatment additives	Possible - may require additional sample preparation.
Use with complex water samples e.g. from cooling towers	YES
Laboratory or field	Laboratory use only or specialist field testing areas.
Are results comparable to current plate counts?	May be difficult and should not be relied on
Would current plate technique still be required?	In some circumstances but not if sample is negative.
False positive False negative	YES - but lower if Free DNA Removal Solution (FDRS) used NO - high negative predictive value
Could rapid test give a positive result whilst culture test gives negative result?	YES perhaps due to chemical inhibition

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:	**iQ-Check® Legionella Real-Time PCR Kits**
ii.	Scientific principles / basis for test:	**Real time Polymerase chain reaction (PCR)**
iii.	Sensitivity: Specificity: Limit of detection:	**Data not provided** **Data not provided** **480 GU/L. (see Bonetta below)**
iv.	Scientific publication references:	**Touron-Bodilis A, Pougnard C, Frenkiel-Lebosse H, Hallier-Soulier S. 2011. Usefulness of real-time PCR as a complementary tool to the monitoring of *Legionella* spp. and *Legionella pneumophila* by culture in industrial cooling systems. J. Appl. Microbiol. 111:499-510.** **Bonetta S, Ferretti E, Balocco F, Carraro E. 2010. Evaluation of *Legionella pneumophila* contamination in Italian hotel water systems by quantitative real-time PCR and culture methods. J. Appl. Microbiol. 108:1576-1583.** **Collins S, Jorgensen T, Willis C, Walker J. 2017.** **Real time PCR to supplement gold-standard culture-based detection of Legionella environmental samples. J. Appl Microbiol. 122:1692 - 1703**
v.	Patents:	**NOT PROVIDED**
vi.	Countries sold into:	**Worldwide**
vii.	Manufacturer: Supplier:	**Biorad**
viii.	Commercially available:	**Yes**
ix.	Micro-organism species detected:	***Legionella spp.* and *L. pneumophila***
x.	Lab based: Field based:	**Yes** **Specialist only**
xi.	Can the test be used to determine operational control? Trend analysis?	**Yes** **Yes**
xii.	Independent end-user data:	**Yes - Contact manufacturer**
xiii.	Method validated by third party:	**Yes *Legionella* spp and *L. pneumophila*** **NF validation; NF T90-471; ISO /TS 12869**

2.	Application details	
i.	Sample quality required:	**Can be used with complex matrices such as cooling tower 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:	**As per BS 7592**

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?	**Microorganisms unlikely to interfere. Chemical, biocides used for water treatment and should be notified to lab.**
iii.	What additional equipment will be required?	**Filtration, thermocyclers and associated PCR equipment**
iv.	Is equipment specialised?	**Yes**
v.	Is the process automated? Could it be automated?	**Sample preparation is manual but PCR sections are semi-automated once loaded**
vi.	Does sample need pre-treatment prior to analysis?	**Yes – filtration or centrifugation (ISO 11731)**
vii.	Is training provided?	**Yes**
viii.	How long will test take before results are available?	**Approximately 4 hours**
ix.	How many samples can be analysed?	**Will be dependent on resources but could be up to 100 per day**
x.	What units are results expressed in?	**Genomic Units per litre (GU /L)**
xi.	Does the result correlate with standard analytical procedures such as plating?	**Not necessarily – CFU and GU are not comparable**
xii.	Is specialised training required to conduct test and interpret results?	**Yes**
xiii.	Are results reproducible:	**Yes**
xiv.	What errors (if any) could occur with analysis (weak link)?	**Experienced, qualified & competent technicians required to perform tests**
xv.	Has test been validated for environmental samples?	**Yes**
xvi.	Does the final result include VBNC?	**Yes**
xvii.	Does it detect live or dead cells, or both?	**Limited - Taylor MJ, Bentham RH, Ross KE. 2014. Limitations of Using Propidium Monoazide with qPCR to Discriminate between Live and Dead *Legionella* in Biofilm Samples. Microbiology Insights 7:15-24. Delgado-Viscogliosi P, Solignac L, Delattre JM. 2009. Viability PCR, a culture-independent method for rapid and selective quantification of viable *Legionella pneumophila* cells in environmental water samples. Appl. Environ. Microbiol. 75:3502-3512.**
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