THE WATER MANAGEMENT SOCIETY

Rapid Microbiology Industry Liaison Group

PCR Factsheet (Biotecon Diagnostics)

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 (BIOTECON DIAGNOSTICS) SUMMARY TABLE

- Real-time PCR kit for the detection, differentiation and quantification of *Legionella spp.*, *Legionella pneumophila* and *L. pneumophila* serogroup 1 all within a single PCR reaction.
- Kits deliver reliable results within 4 hours of beginning processing the sample.
- No need for enrichment using bacterial culture media.
- Protocol includes sample filtration, optional treatment to remove dead and free cell DNA, DNA extraction, PCR amplification and data analysis.
- Following filtration, cells are rinsed off the filter and left intact. Samples may then be split for PCR processing and separate culturing protocols if desired.
- High negative predictive value, i.e. negative means negative.
- Proven Live/Dead cell differentiation (not just free DNA removal).
- See also, HSE website for further information at: ***www.hse.gov.uk/legionnaires/faqs.htm#Testing-monitoring***

The information below is from BIOTECON Diagnostics, the manufacturer and developer of the **micro**proof Legionella Quantification LyoKit, which detects, differentiates and quantifies *Legionella spp.*, *Legionella pneumophila* and *L. pneumophila* serogroup 1 all within a single PCR reaction.

Method	Polymerase Chain Reaction (PCR) detects DNA
Bacteria detected	Detection, differentiation and quantification of *Legionella spp.*, *Legionella pneumophila* and *L. pneumophila* serogroup 1 all within a single PCR reaction.
Bacteria Quantified	Detection, differentiation and quantification of *Legionella spp.*, *Legionella pneumophila* and *L. pneumophila* serogroup 1 all within a single PCR reaction.
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	YES, can remove both free and dead cell DNA to reliably provide quantification of live / viable cells
Will detect Viable But Non-Culturable (VBNC) bacteria	YES
Interference from biocides and other water treatment additives	NO, as washing steps have been incorporated
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?	Depends on amount of VBNC present in samples.
Would current plate technique still be required?	In some circumstances, but not if sample is negative.
False positive False negative	NO false-positives if performing Live / Dead cell differentiation; VBNC's will be positive NO false-negatives (high negative predictive value)
Could rapid test give a positive result whilst culture test gives negative result?	YES, due to VBNC, but not due to free DNA or dead cells

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:	**BIOTECON microproof Legionella Quantification LyoKit**
ii.	Scientific principles / basis for test:	**Quantitative real-time Polymerase Chain Reaction (PCR)**
iii.	Sensitivity: Specificity: Limit of detection: Limit of quantification:	**Sensitivity is better than culture methods** **100%** **3 genomic units/reaction** **10 genomic units/reaction**
iv.	Scientific publication references:	**Fast and reliable quantification of *Legionella spp* and *L pneumophila* with simultaneous detection of *L pneumophila* serogroup 1 in water by real-timePCR including live/dead discrimination.** **Priller F *et al*, ESGLI 2018** **Comparison of the culture method with multiplex PCR for the confirmation of Legionella spp. and Legionella pneumophila. Eble *et al*, 2021**
v.	Patents:	**No**
vi.	Countries sold into:	**Worldwide**
vii.	Contact details:	**Manufacturer:** Biotecon Diagnostics GmbH Hermannswerder 17, 14473 Potsdam, Germany Tel +49 (0)331 2300-200 www.bc-diagnostics.com **Supplier:** Oxford Biosystems Ltd 184B Park House, Park Drive, Milton Park, Abingdon, Oxfordshire OX14 4SR Tel: +44(0)1235 431390 www.oxfordbiosystems.com
viii.	Commercially available:	**Yes**
ix.	Micro-organism species detected:	***Legionella spp*, *Legionella pneumophila*, *L pneumophila* serogroup 1 are all individually detected and quantified within a single reaction.**
x.	Lab based: Field based:	**Yes** **Specialist only**
xi.	Can the test be used to determine operational control? Trend analysis?	**Yes** **Yes**
xii.	Contact details of end-users:	**Available on request**
xiii.	Is method validated by a third party:	**In planning**

2.	Application details	
i.	Sample quality required:	**Can be used with clear water and complex matrices**
ii.	What sample preparation on-site is required:	**None. Filtration and preparation as per instructions for PCR is done in the lab**
iii.	Does the sample need to be tested within a prescribed time scale?	**The sooner the better to enable differentiation between live and dead.** **Once prepared samples can be stored until analysis.**
iv.	Sample bottle type and sample volume:	**As per BS7592**

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?	**Very high salinity or freshly added biocides in high concentrations.**
iii.	What lab equipment will be required?	**Filtration equipment, real-time PCR cycler and associated PCR equipment. D-Light box required for live/dead differentiation.**
iv.	Is equipment specialised?	**Specialised but all readily available from supplier**
v.	Is the process automated? Could it be automated?	**Sample preparation is manual.** **PCR is semi-automated once loaded**
vi.	Does sample need pre-treatment prior to analysis?	**No heat- or acid-pre treatment. Water sample is filtered.**
vii.	Is training provided?	**Yes**
viii.	How long will test take before results are available?	**Approximately 4 hours**
ix.	How many samples per day can be analysed?	**Depends on resources available. 180 per PCR cycler**
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?	**Potentially. GU and CFU are not always comparable.** **Better correlation seen if live/dead differentiation is included.**
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)?	**Qualified and competent technicians are required to perform the tests and interpret the results.**
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?	**Detects both and differentiates between live and dead**
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