Rapid Microbiology Industry Liaison Group

FACTSHEETS

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Agenda

- Background
- Current Position
- Factsheets
  - PCR, IMS, MALDI-ToF, MPN
- Buyer Beware
- Next Steps



Background

- HSE requirement for “evidence-base”
- Consumer demand
- Independent liaison group
  - HSE, PHE, WMSoc, LCA
- Strengths and weaknesses of techniques

Aims & Objectives

- Increase awareness of techniques
  - Review verification & validation data
  - Provide publications in “Waterline”
  - Events to communicate findings
  - Factsheets to guide membership

Current Position



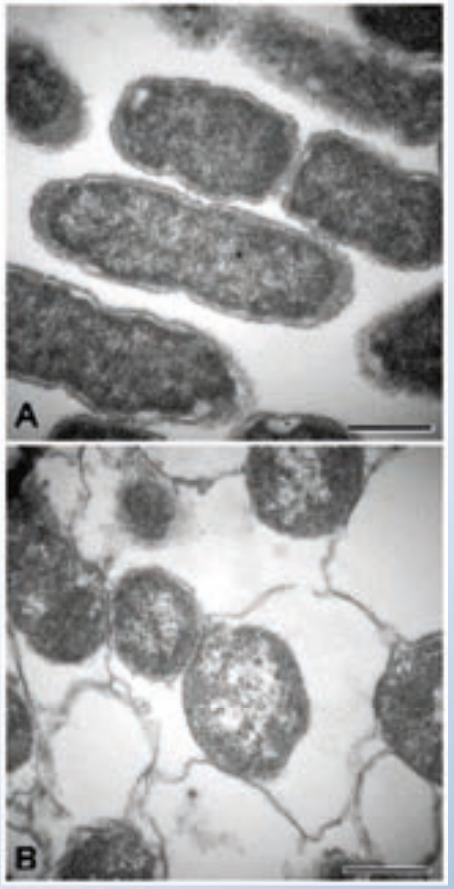
ISO 11731:2017

- Water quality – Enumeration of *Legionella* spp
  - Selection on water type and bacterial count
  - Matrix of testing procedures
    - Concentration step
  - Variable sensitivity



VBNC

- Viable But Non-Culturable
  - Very low metabolic activity
  - Do not divide, **but** are alive
  - Are able to become culturable once resuscitated



Pawlowski DR, *et al*. (2011) PLoS ONE 6(3): e17585. doi:10.1371/journal.pone.0017585

HSG 274 – Part 2

Legionella testing should be performed:

- In UKAS accredited labs
- With current ISO *Legionella* standard methods within scope of accreditation
- Should take part in proficiency testing scheme (accredited to ISO 17043)

www.hse.gov.uk/pubns/priced/hsg274part2.pdf

ISO/IEC 17043:2010

- Competence of providers
- Development & operation

of proficiency testing schemes

Table of contents

https://www.iso.org/standard/29366.html

HSG 274 – Part 2

Alternative quantitative testing methods may be used as long as they have been validated using ISO 17994 and meet the required sensitivity & specificity

Legionella bacteria (cfu/l)
>100 cfu/l and up to 1000
>1000 cfu/l

www.hse.gov.uk/pubns/priced/hsg274part2.pdf

ISO 17994:2014

- Water quality - Requirements for comparison of relative recovery of microorganisms by two quantitative methods

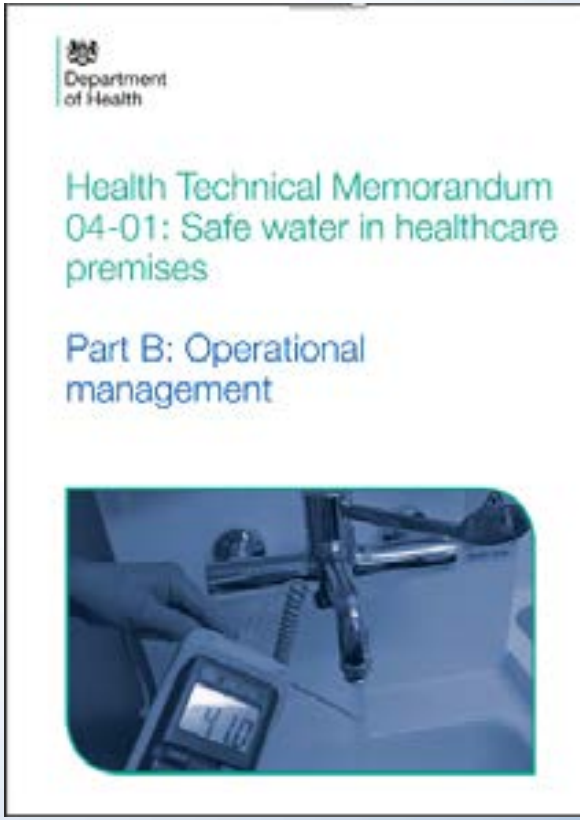
https://www.iso.org/obp/ui/#iso:std:iso:17994:ed-2:v1:en

HTM 04-01 – Part B

All microbiological measurements should be:

- By approved methods and/or
- Performed by UKAS accredited labs for the method being used

Dip slides not acceptable on hot & cold water systems

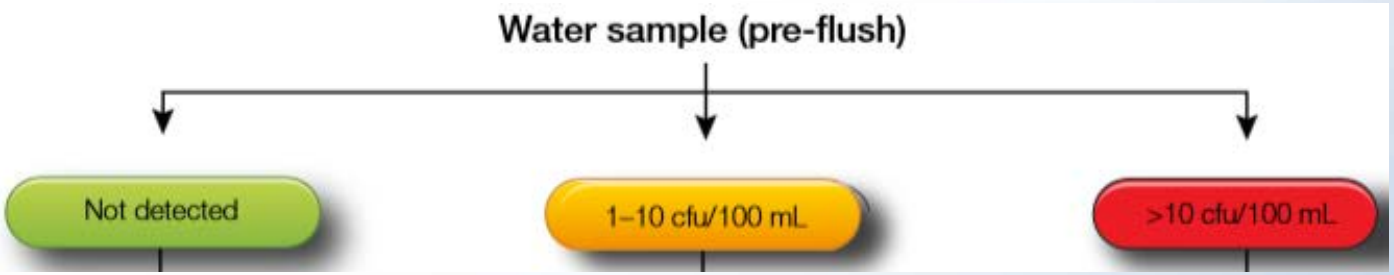


https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/524882/DH_HTM_0401_PART_B_acc.pdf

HTM 04-01 – Part B

Legionella bacteria (cfu/L)
Not detected or up to 100
>100 and up to 1000
>1000

Appendix D Testing for *P. aeruginosa*



https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/524882/DH_HTM_0401_PART_B_acc.pdf

Legionella Testing Market

- 2016
  - US$ 180 m (£129 m)
- 2025
  - US$ 398.7 m (£286 m)

FACTSHEETS



WMSoc Fact Sheets

- PCR
- MALDI ToF
- IMS
- MPN

WMSoc Disclaimers

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.

WMSoc Disclaimers

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.



PCR (BIORAD) SUMMARY TABLE

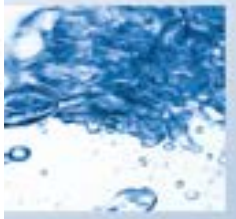
- Bio-Rad has developed real-time PCR kits for detection or quantification of *Legionella* spp. or *Legionella* pneumophila.
- Kits deliver reliable results within a few days without the need of bacterial cultures.
- Protocol includes sample filtration, DNA extraction, PCR amplification and data analysis.
- 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, mycobacteria , Pseudomonas etc. Different kits will be required for different bacteria.*
Pre-concentration as per ISO 11731	YES
Algorithm to convert results to CFU	Possibly but may not be reliable. Results expressed in genomic units (GU).
Can differentiate between live and dead cells	Not reliably - will depend on manufacturer.
Will detect 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 NO
Could rapid test give a positive result whilst culture test gives negative result?	YES

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:	Sensitive enough to detect lower levels of contamination than the culture method. Specific – yes lOD - 480 GU/L. (see Bonetta)
iv.	Scientific publication references:	Touron-Bodilis A, Pougnard C, Frankiel-Labosse 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, Jorgenson 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:	
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
xi.	Can the test be used to determine operational control? Trend analysis?	Yes Yes
xii.	Independent end-users:	Yes
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
ii.	What sample preparation on-site is required:	Filtration and preparation as per instructions for PCR analysis
iii.	Does the sample need to be tested within a prescribed time scale (courier)?	Once prepared, samples can be stored until analysis
iv.	Sample bottle type: Sample volume required:	(refer to relevant ISO/BSI for specific organism of interest)



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?	**Chemical, biocides**
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?	**Approx. 4 hour**
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?	**GU/L**
xi.	Does the result correlate with standard analytical procedures such as plating?	**Not necessarily – CFU and GU are not always 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**
xviv.	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

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

PCR – polymerase chain reaction

VBNC – viable but non-culturable

THE WATER MANAGEMENT SOCIETY

BUYER BEWARE



Laboratory v Point of Care

- Why test?
  - WSP/Compliance/ Trend analysis/monitoring
  - Outbreaks
- Where to test?
  - Compliance
  - Equipment outlay



Quality Control

- Positive/negative controls
- Records
  - Batch no
  - Use by date
  - Manufacturer QC

Image courtesy of: Chris 73 / Wikimedia Commons

Training

- Sampling standards
  - ISO 5667/19458
  - BS 6068/7592/8554
  - Aseptic technique
- Test standards
  - ISO 17025, 11731, 16266, 17994, 17043

Training

- Manufacturer
  - Instructions for use
  - You-Tube
  - Bespoke
- Independent

Next Steps



Next Steps

- Peer Review
  - Load onto WMSoc members area
- New Group Members
  - Comparative matrix
  - Factsheets for new technologies
  - Liaise with regulators