

Latest *Legionella* research

Associate Professor Harriet Whiley
Harriet.Whiley@flinders.edu.au



Overview

- *Legionella* testing and VBNC state
- Thermotolerance
- Amoeba and *Legionella* in Australian drinking water
- *Legionella* risk and water stagnation through intermitted usage

Detection of *Legionella* from water sources

Culture – the ‘gold standard’ for *Legionella* testing

7-10 days

AS 5132:2017 ‘Waters-examination for *Legionella* spp. including *Legionella pneumophila* – using concentration’.

Limit of detection = 100 CFU/L

AS/NZS 3896:2017 Waters-examination for *Legionella* spp. including *Legionella pneumophila*

Limit of detection = 10 CFU/mL = 10,000 CFU/L

Culture – doesn’t detect VBNC *Legionella*

qPCR – detects DNA (live and dead *Legionella*)

(ISO/TS12869:2019)

Viable but non culturable *Legionella*

- VBNC *Legionella* still pathogenic – can infect amoeba and human cell lines.
- Induced by stress – heat, low nutrients, disinfectants etc



International Journal of
*Environmental Research
and Public Health*



Commentary

***Legionella* Risk Management and Control in Potable Water Systems: Argument for the Abolishment of Routine Testing**

Harriet Whiley

Health and the Environment, School of the Environment, Flinders University, GPO Box 2100, Adelaide 5001, Australia; Harriet.Whiley@flinders.edu.au; Tel.: +61-8-7221-8580

**Critical Reviews
in Microbiology**

<http://informahealthcare.com/mby>
ISSN: 1040-841X (print), 1549-7828 (electronic)
Crit Rev Microbiol, 2016; 42(1): 65–74
© 2014 The Author(s). Published by Informa Healthcare USA, Inc.
DOI: 10.3109/1040841X.2014.885930

**informa
healthcare**

REVIEW ARTICLE

***Legionella* detection by culture and qPCR: Comparing apples and oranges**

Harriet Whiley and Michael Taylor

frontiers in
MICROBIOLOGY

REVIEW ARTICLE
published: 24 September 2014
doi: 10.3389/fmicb.2014.00501



Uncertainties associated with assessing the public health risk from *Legionella*

Harriet Whiley^{1*}, Alexandra Keegan², Howard Fallowfield¹ and Kirstin Ross¹

¹ Health and the Environment, Flinders University, Adelaide, SA, Australia
² South Australian Water Corporation, Adelaide, SA, Australia



Standard culture method AS5132:2017

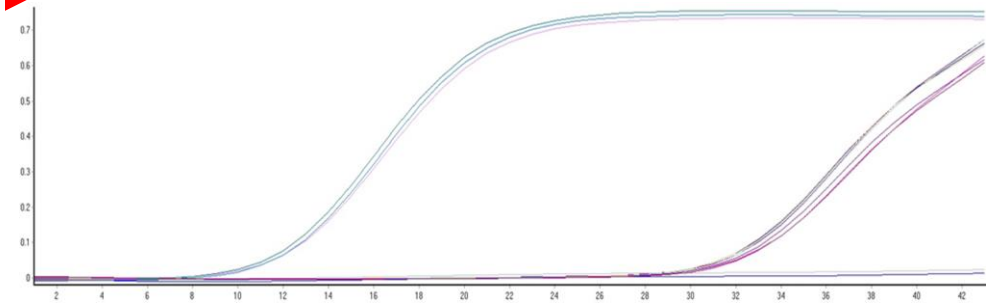


Dead cells

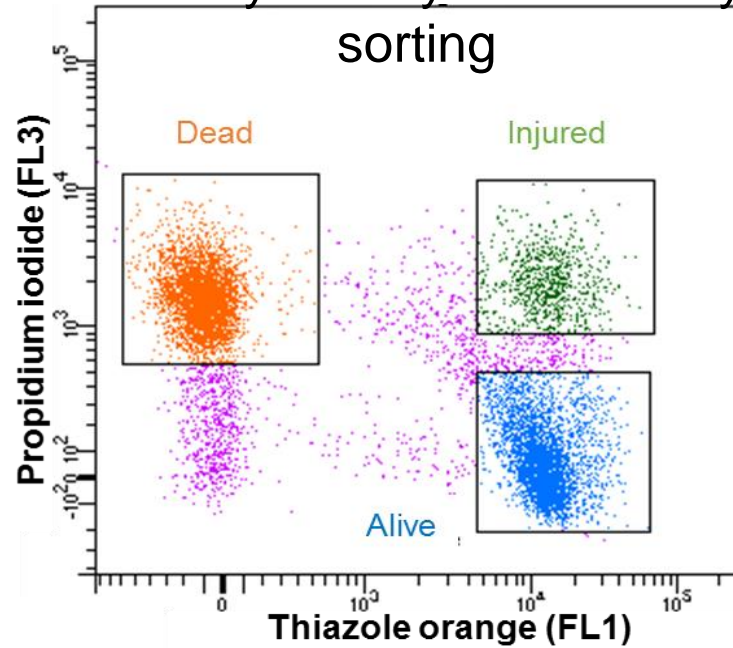
Injured cells

Alive cells

qPCR enumeration of injured (VBNC) *Legionella* (ISO/TS12869:2019)



Flow cytometry with viability sorting



frontiers | Frontiers in Microbiology

TYPE Methods
PUBLISHED 30 January 2023
DOI 10.3389/fmicb.2023.1094877

Check for updates

OPEN ACCESS

EDITED BY
Hector Mora Montes,
University of Guanajuato,
Mexico

REVIEWED BY
Maria Luisa Ricci,
National Institute of Health (ISS), Italy
Sebastian P. Faucher,
McGill University,
Canada

*CORRESPONDENCE
Harriet Whitley
harriet.whitley@flinders.edu.au

SPECIALTY SECTION
This article was submitted to
Infectious Agents and Disease

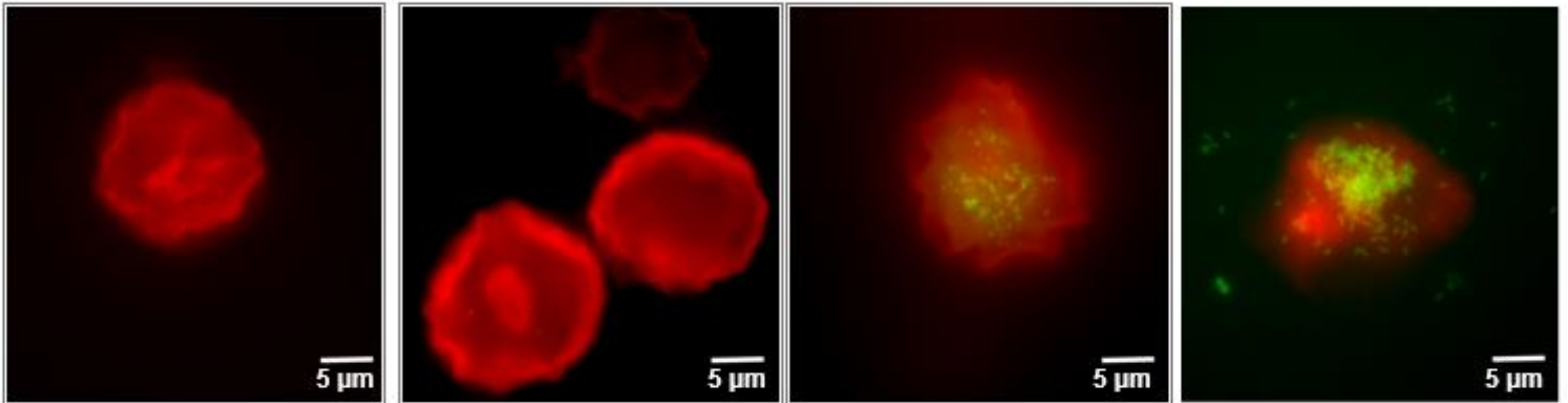
Detection and quantification of viable but non-culturable *Legionella pneumophila* from water samples using flow cytometry-cell sorting and quantitative PCR

Muhammad Atif Nisar¹, Kirstin E. Ross¹, Melissa H. Brown¹, Richard Bentham¹, Giles Best^{2,3} and Harriet Whitley^{1*}

¹College of Science and Engineering, Flinders University, Bedford Park, SA, Australia, ²College of Medicine and Public Health, Flinders University, Bedford Park, SA, Australia, ³Flow Cytometry Facility, Flinders University, Bedford Park, SA, Australia



VBNC *Legionella* still infects amoeba hosts



Red = *Acanthamoeba polyphaga*; Green = VBNC *Legionella*

Hospital case study

- From March 2021 to June 2022, water (n = 120) and biofilm (n = 46) samples were collected from the showers and handbasins of patient ensuites in a private NSW hospital
- 21% (36 /166) samples were positive for VBNC *Legionella* spp.
- 21% (36 /166) samples were qPCR positive (ISO/TS12869:2019)
- only 2.5% (4/166) samples were positive using the standard culture method (AS 5132:2017)

Standard culturing returned a false negative result in 89% (32/36) of samples positive for VBNC *Legionella*

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 07 June 2023
DOI 10.3389/fcimb.2023.1190631



OPEN ACCESS

EDITED BY
Hiroyuki Yamaguchi,
Health Sciences University of Hokkaido,
Japan

REVIEWED BY
Lucianna Vaccaro,
CEU San Pablo University, Spain
Carolina Hurtado Marcos,
CEU San Pablo University, Spain

*CORRESPONDENCE
Harriet Whitley
✉ Harriet.Whitley@flinders.edu.au

RECEIVED 21 March 2023
ACCEPTED 18 May 2023
PUBLISHED 07 June 2023

CITATION
Nisar MA, Ros KE, Brown MH, Bentham R,
Best G, Xi J, Hinds J and Whitley H (2023)
Stagnation arising through intermittent
usage is associated with increased viable
but non culturable *Legionella* and amoeba

Stagnation arising through intermittent usage is associated with increased viable but non culturable *Legionella* and amoeba hosts in a hospital water system

Muhammad Atif Nisar¹, Kirstin E. Ros¹, Melissa H. Brown^{1,2}, Richard Bentham¹, Giles Best^{3,4}, James Xi⁵, Jason Hinds⁵ and Harriet Whitley^{1,2*}

¹College of Science and Engineering, Flinders University, Bedford Park, SA, Australia, ²Australian Research Council Training Centre for Biofilm Research and Innovation, Flinders University, Bedford Park, SA, Australia, ³College of Medicine and Public Health, Flinders University, Bedford Park, SA, Australia, ⁴Flow Cytometry Facility, Flinders University, Bedford Park, SA, Australia, ⁵Enware Australia Pty Ltd., Caringbah, NSW, Australia

Thermotolerance

- Water risk management plan
- Multiple barriers of control

Technology	Description	Strengths	Weaknesses
Heat disinfection (pasteurisation or thermal shock disinfection)	Periodic heating of the calorifier or water heater to a temperature sufficient to achieve 70°C at all outlets and then flushing heated water through all heated ring mains, heated water pipework and heated water outlets to control <i>Legionella</i>	<ul style="list-style-type: none"> • Relatively simple (theoretically but generally not in practice) • Does not require addition of chemicals 	<ul style="list-style-type: none"> • Scalding hazards from the super-heated water • Requires considerable hours of labour • Results in a high volume of wastewater • Uses a large amount of energy to heat water • Many facilities do not have sufficient hot water capacity to offer this method • Has poor long-term control • May unintentionally lead to significant heat transfer to cold water • Cannot be used to disinfect cold water pipework



Legionella Persistence in Manufactured Water Systems: Pasteurization Potentially Selecting for Thermal Tolerance

Harriet Whaley*, Richard Bentham and Melissa H. Brown

College of Science and Engineering, Flinders University, Bedford Park, SA, Australia

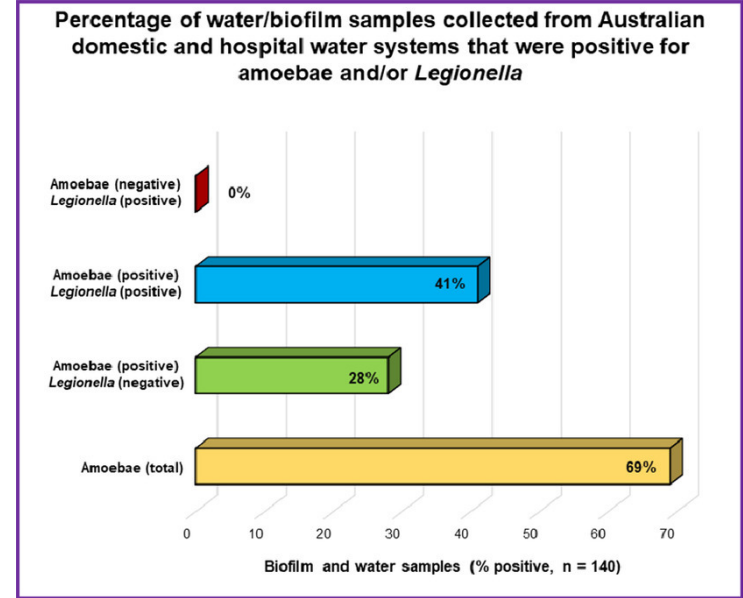
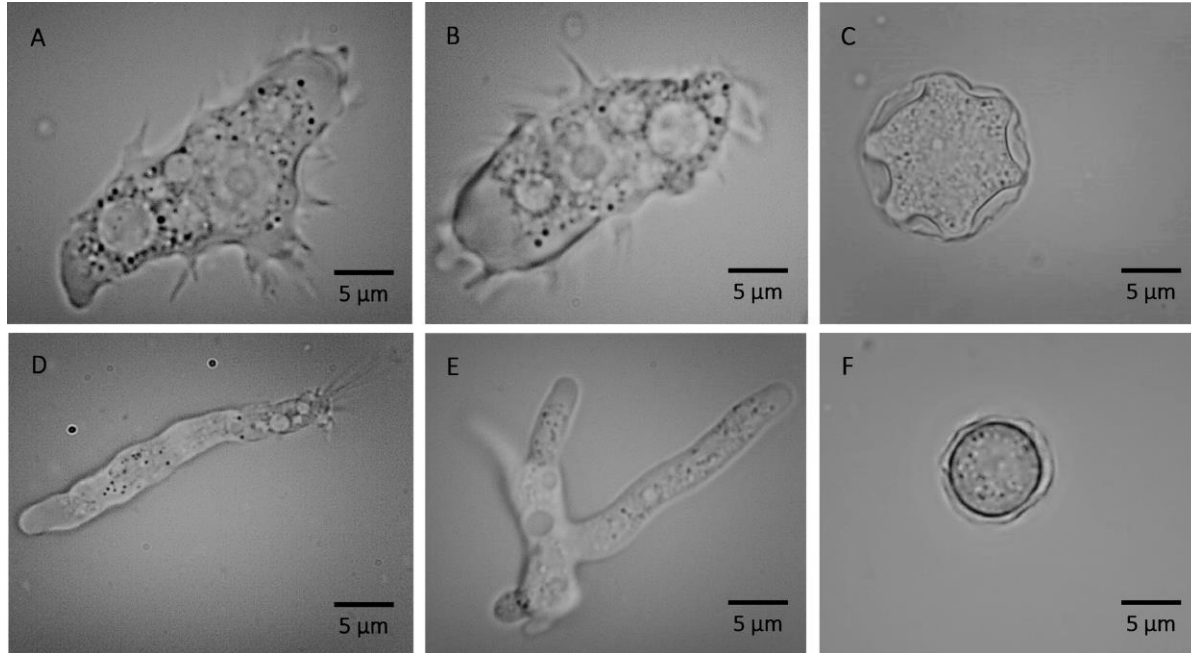
Thermotolerance of strains

Sample ID	Origin	Serogroup	Thermal Treatment	
			65°C	70°C
LpINPI	Clinical	sg1	VBNC	20 minutes
LpIp2	Clinical	sg1	VBNC	20 minutes
LpIp3	Clinical	sg1	VBNC	20 minutes
LpIp4	Clinical	sg1	VBNC	20 minutes
LpI-5	Clinical	sg1	VBNC	20 minutes
LpI-6	Clinical	sg1	30 minutes	20 minutes
Lp2.14.7	Clinical	sg2-14	20 minutes	20 minutes
Lp2.14.8	Clinical	sg2-14	VBNC	20 minutes
71S1	Environmental	sg1	VBNC	20 minutes
CH02	Environmental	sg1	30 minutes	20 minutes
FL3.1	Environmental	sg1	VBNC	20 minutes
FL3.2	Environmental	sg1	VBNC	20 minutes
FL3.3	Environmental	sg1	VBNC	20 minutes
FL3.4	Environmental	sg1	VBNC	20 minutes
EK03	Environmental	sg2-14	VBNC	20 minutes
EK04	Environmental	sg2-14	VBNC	20 minutes

Unpublished data

- These results are from pure cultures
- In the environment, *Legionella* would also be protected by other cells, biofilm and amoeba hosts

Amoeba hosts



Water Research 226 (2022) 119238



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Water Research

journal homepage: www.elsevier.com/locate/watres



Molecular screening and characterization of *Legionella pneumophila* associated free-living amoebae in domestic and hospital water systems

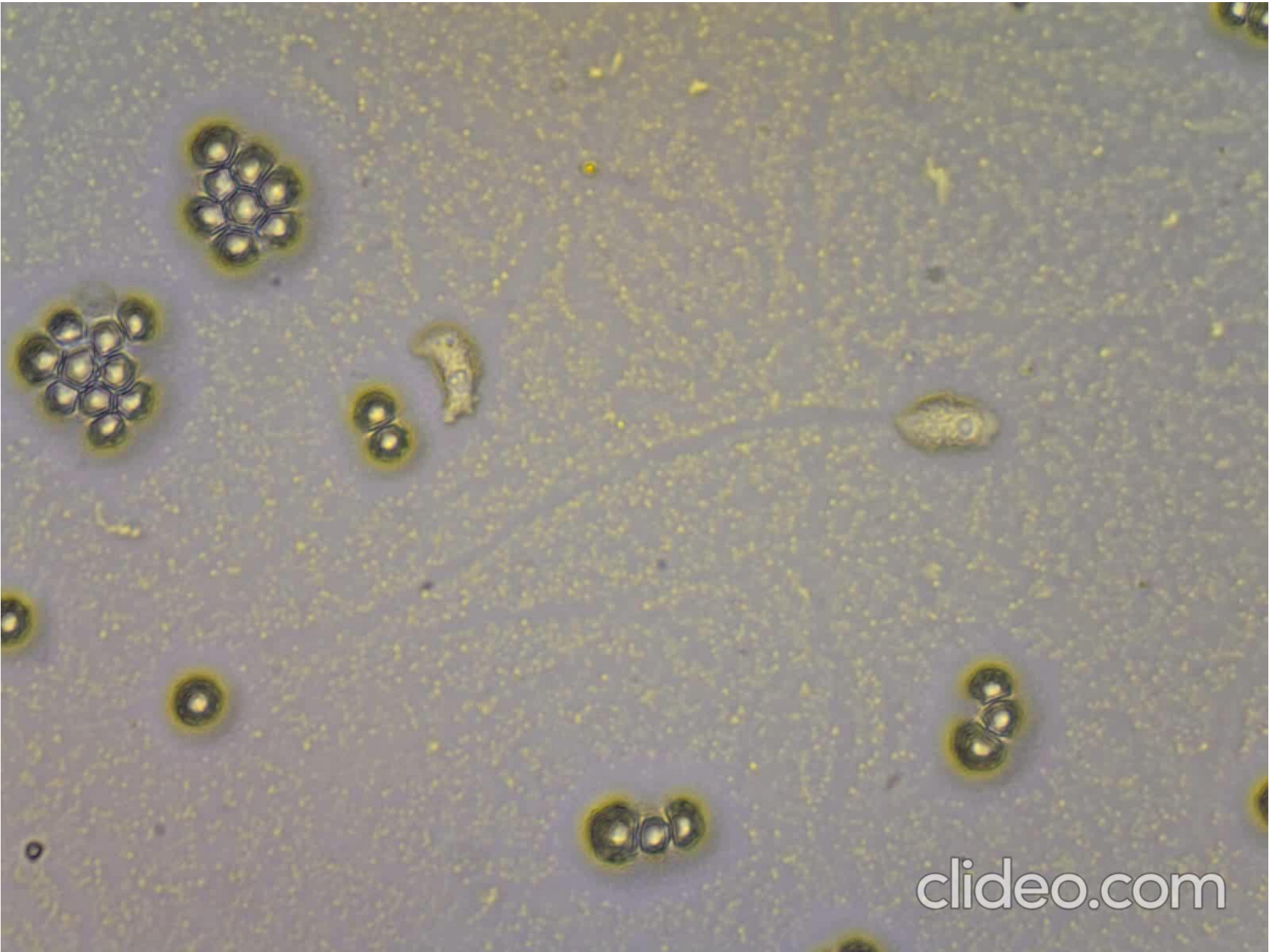
Muhammad Atif Nisar^a, Kirstin E Ross^a, Melissa H Brown^a, Richard Bentham^a, Jason Hinds^b, Harriet Whiley^{a,*}



Review
***Legionella pneumophila* and Protozoan Hosts: Implications for the Control of Hospital and Potable Water Systems**

Muhammad Atif Nisar, Kirstin E. Ross^{ORCID}, Melissa H. Brown^{ORCID}, Richard Bentham and Harriet Whiley^{*ORCID}

Acanthamoeba
10X objective



*Vermamoeba
vermiformis*
20X objective



clideo.com

Water stagnation



Permanent stagnation



Temporal stagnation

 **frontiers**
in Environmental Science

SYSTEMATIC REVIEW
published: 14 December 2020
doi: 10.3389/fenv.2020.611611



**Water Stagnation and Flow
Obstruction Reduces the Quality of
Potable Water and Increases the Risk
of Legionellosis**

Muhammad Atif Nisar, Kirstin E. Ross, Melissa H. Brown, Richard Bentham and
Harriet Whitley*

College of Science and Engineering, Flinders University, Bedford Park, SA, Australia



Enware operational monitoring hospital case study

Enware Smart[®] Flow monitoring system

- Water flow was measured using flow switches located at the hot water inlet and cold water inlet of both the TMVs and hand basin faucets
- Temperature at the hot water inlet, cold water inlet, and outlet of the thermostatic mixing valves (TMV) and the hot water inlet and cold water inlet of hand basin faucets

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 07 June 2023
DOI 10.3389/fcimb.2023.1190631

Check for updates

OPEN ACCESS

EDITED BY
Hiroyuki Yamaguchi,
Health Sciences University of Hokkaido,
Japan

REVIEWED BY
Lucianna Vaccaro,
CEU San Pablo University, Spain
Carolina Hurtado Marcos,
CEU San Pablo University, Spain

*CORRESPONDENCE
Harriet Whitley
✉ Harriet.Whitley@flinders.edu.au

RECEIVED 21 March 2023
ACCEPTED 18 May 2023
PUBLISHED 07 June 2023

CITATION
Nisar MA, Ros KE, Brown MH, Bentham R,
Best G, Xi J, Hinds J and Whitley H (2023)
Stagnation arising through intermittent
usage is associated with increased viable
but non culturable *Legionella* and amoeba

Stagnation arising through intermittent usage is associated with increased viable but non culturable *Legionella* and amoeba hosts in a hospital water system

Muhammad Atif Nisar¹, Kirstin E. Ros¹, Melissa H. Brown^{1,2}, Richard Bentham¹, Giles Best^{3,4}, James Xi⁵, Jason Hinds⁵ and Harriet Whitley^{1,2*}

¹College of Science and Engineering, Flinders University, Bedford Park, SA, Australia, ²Australian Research Council Training Centre for Bioluminescence Research and Innovation, Flinders University, Bedford Park, SA, Australia, ³College of Medicine and Public Health, Flinders University, Bedford Park, SA, Australia, ⁴Flow Cytometry Facility, Flinders University, Bedford Park, SA, Australia, ⁵Enware Australia Pty Ltd., Caringbah, NSW, Australia

Smart Flow[®]
Water Risk Management System
Simplified for Healthcare

- ✓ REDUCE RISK OF WATERBORNE INFECTIOUS DISEASES
- ✓ IMPROVE COMPLIANCE THROUGH AUTOMATED MONITORING & REPORTING
- ✓ SAVE UP TO 65% OPERATING COSTS
*Compared to manual best practice

85% 100% 75%

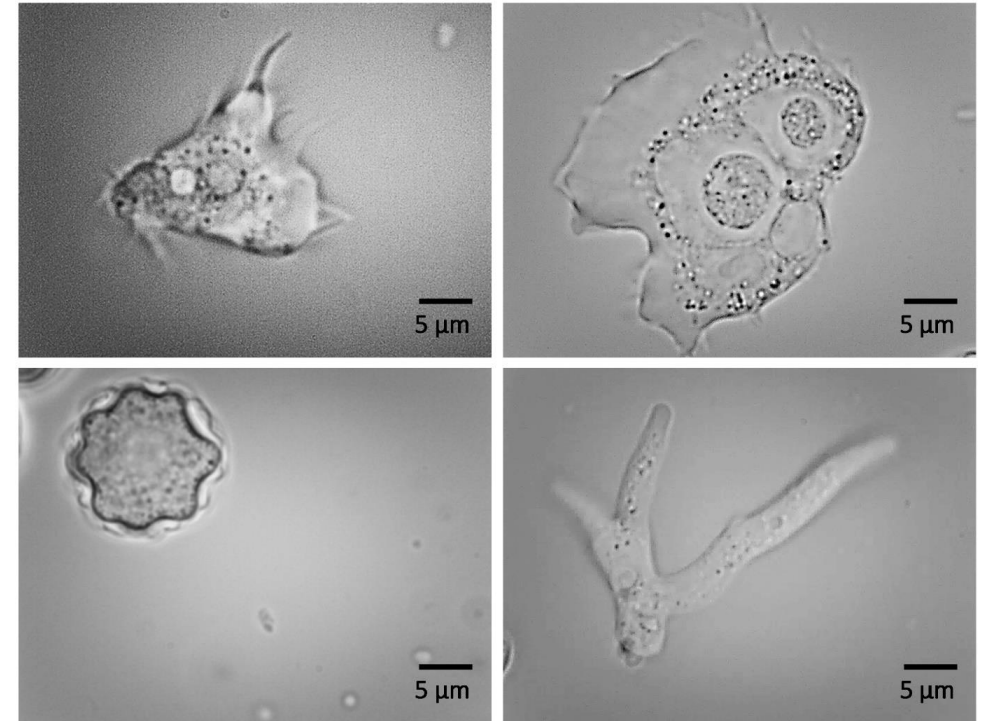
GOOD DESIGN AWARD WINNER 2022

The advertisement features a 3D architectural rendering of a hospital building. Three circular progress indicators are overlaid on the building: a purple one at 85%, a green one at 100%, and a yellow one at 75%. A 'GOOD DESIGN AWARD WINNER 2022' badge is positioned in the upper right corner of the image.

Enware operational monitoring case study

Relationships between *Legionella*, amoeba and HPC

- All samples positive for *Legionella* were also positive for amoeba host
- Samples with a very high heterotrophic plate count (HPC $\geq 5 \times 10^3$ CFU/L) were also statistically significantly associated with high concentrations of *Legionella* DNA (alive and dead cells), VBNC *Legionella* and *V. vermiformis*.

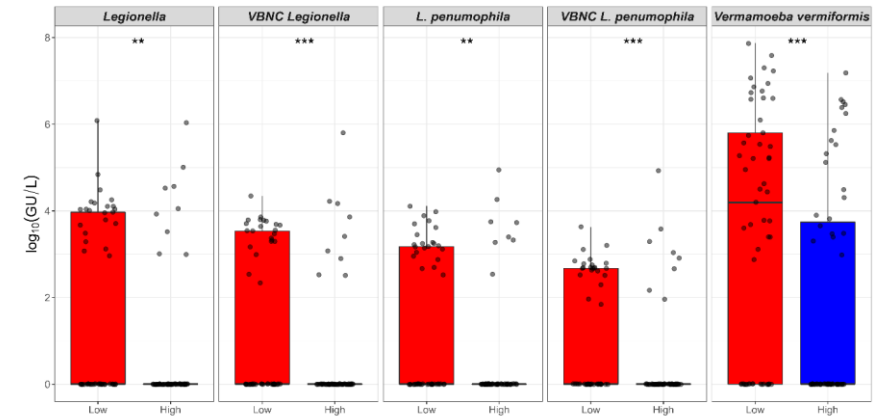


Detected amoeba

Enware operational monitoring case study

Relationships between microbes and water flow / stagnation

- temporary water stagnation arising through intermittent usage (< 2 hours of usage per month) significantly ($p < 0.01$) increased the amount of *Legionella* DNA, VBNC *Legionella*/*L. pneumophila*, and *V. vermiformis*



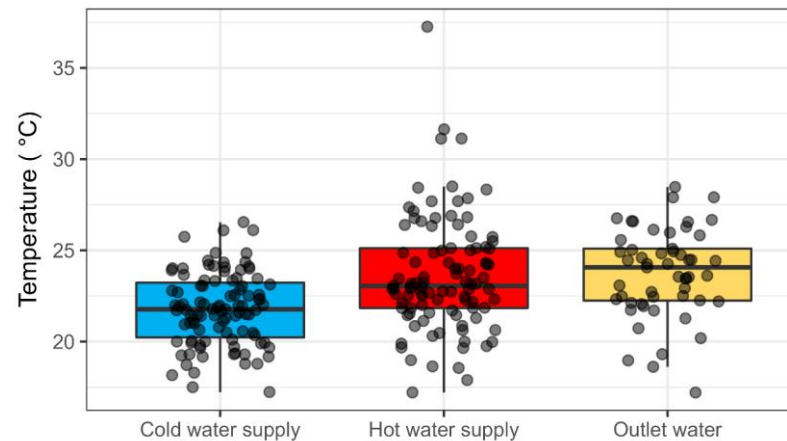
Relationship between intermittent water usage and the presence of *Legionella*/*Vermamoeba vermiformis*

Temporary stagnation arising through intermittent usage increases *Legionella* and amoeba hosts

Enware operational monitoring case study

Relationships between microbes and water temperature

- No associations found (likely due to our data handling approach – more research needed)
- study averaged water temperatures across one week or one month prior to sampling for both the hot and cold water pipelines/outlets the water temperatures were more similar to each other than anticipated



Average temperature (one week prior to sampling) of hot water supply, cold water supply and outlet water

Take home messages

- *Legionella* testing results in false negatives
- Operational monitoring: understanding your system is more important than testing
- Thermotolerance: 70°C for 20 min needed to kill all tested strains (may be higher in a complex system)
- *Legionella* always found with amoeba hosts – future disinfection strategies should target the hosts
- Temporary stagnation arising through intermitted usage associated with increased *Legionella* and amoeba

Acknowledgements

- Muhammad Atif Nisar, Flinders University
- Dr. Giles Best Flinders Flow Cytometry
- Mrs. Pat Vilimas Flinders Microscopy
- Professor Melissa Brown, Flinders University
- Professor Kirstin Ross, Flinders University
- Assoc. Prof Richard Bentham, Flinders University and Built Water Solutions
- Jason Hinds, Enware
- James Xi, Enware

Contact me

Harriet.Whiteley@flinders.edu.au



<https://www.microbialtestingandresearchservices.net/>

E.g.

- Water quality / *Legionella*
- Air quality monitoring
- Determining disinfection efficacy
- Evaluating antimicrobial surfaces/materials



<https://www.flinders.edu.au/arc-biofilm>