United States Environmental Protection Agency

Disinfection of Biofilm-Associated *Legionella pneumophila*

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November 20, 2019

2019 U.S. EPA International Decontamination Research and Development Conference

Office of Research and Development
National Homeland Security Research Center
Water Infrastructure Protection Division

Legionella spp.

66 species (serogroups)

L. adelaidensis
L. anisa
L. beliardensis
L. birminghamensis
L. bozemanii (2)
L. brunensis
L. busanensis
L. cardiaca
L. cherrii
L. cincinnatiensis
L. clemsonensis
L. donaldsonii
L. drancourtii
L. dresdenensis
L. drozanskii
L. dumoffii
L. erythra (2)
L. fairfieldensis
L. fallonii
L. feeleii (2)
L. geestiana
L. genomospecies
L. gormanii
L. gratiana
L. gresilensis
L. hackeliae (2)
L. impletisoli
L. israelensis
L. jamestowniensis
L. jeonii
L. jordanis
L. lansingensis
L. londiniensis
L. longbeachae (2)
L. lytica
L. maceachernii
L. massiliensis
L. micdadei
L. monrovica
L. moravica
L. nagasakiensis
L. nautarum
L. norrlandica
L. oakridgensis
L. parisiensis
L. pittsburghensis
L. pneumophila (15)
L. quateirensis
L. quinlivanii (2)
L. rowbothamii
L. rubrilucens
L. sainthelensi (2)
L. santicrucis
L. shakespearei
L. spiritensis
L. steelei
L. steigerwaltii
L. saoudiensis
L. taurinensis
L. thermalis
L. tucsonensis
L. tunisiensis
L. wadsworthii
L. waltersii
L. worsleiensis
L. yabuuchiae

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L. worsleiensis
L. yabuuchiae

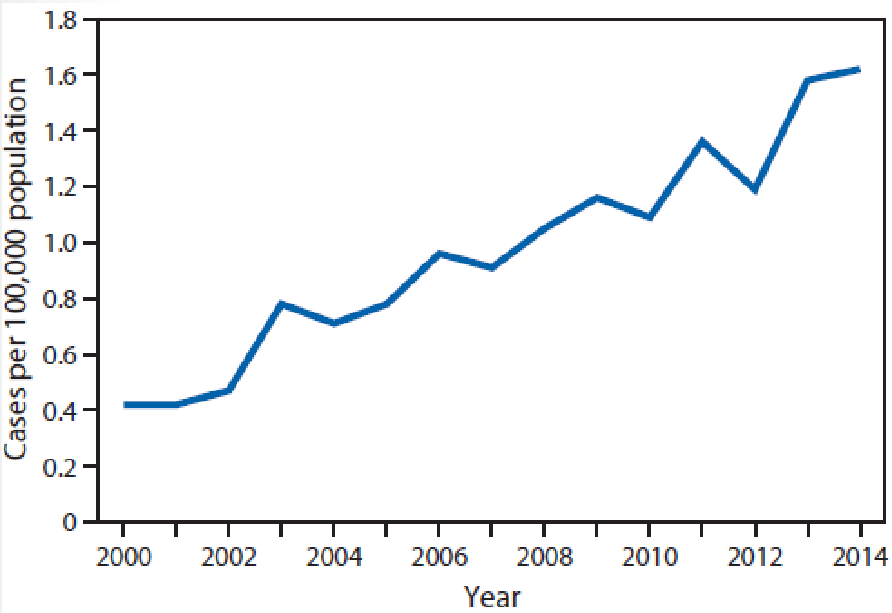
19 species associated with human disease

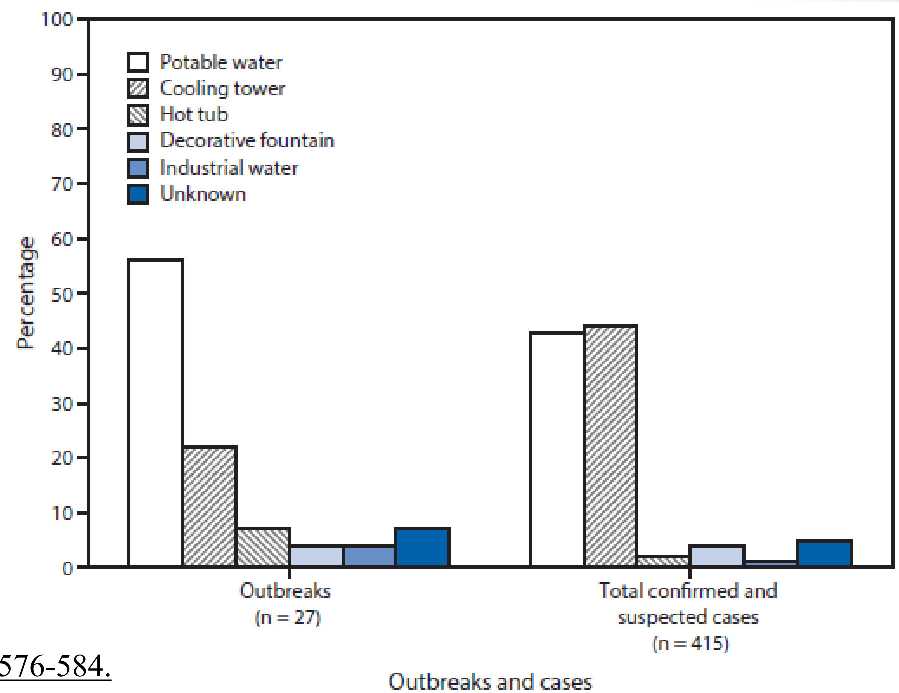
Dresden panel of mAbs (17), groups Lp into sg 1 to 15

Lück, C. et al. 2013. Typing Methods for *Legionella*, p. 119-148. *In* C. Buchrieser and H. Hilbi (ed.), *Legionella*.

sg1, >80% clinical isolates

Legionnaires Disease – A Public Health and Economic Burden



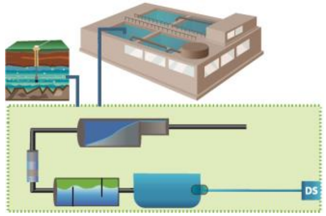


Garrison, L. E., et al. (2016). MMWR Morb Mortal Wkly Rep **65**(22): 576-584.
Collier, S. A., et al. (2012). Epidemiol Infect **140**(11): 2003-2013.

- Incidence rate up from 0.4 to 1.6 cases per 100,000 people; exposure to potable water responsible for the majority of outbreak cases
- Between 2004-2007, LD cases had associated healthcare costs of $9.4 million (Medicaid and Medicare patients)

Legionella Environmental Distribution

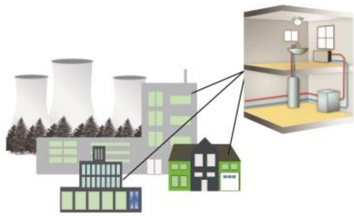
Treated water - reclaimed (i.e. sewage treatment plant), ground, and surface



- 3 x 10^3 – 8 x 10^4 CFU L^{-1}
- 290 – 2.5 x 10^3 CE L^{-1}

L. anisa
L. adelaidensis
L. bozemanii
L. donaldsonii
L. dumoffi
L. fairfieldensis
L. fallonii
LLAP 2, 3, 4, 7, 8
L. londiniensis
L. micdadei
L. pneumophila
L. quateirensis
L. sterigerwaltii
L. wadsworthii
L. waltersii
L. worsleiensis

Distributed water - premise plumbing, e.g. residences, apartments, recreational facilities, hospitals, and cooling towers



- 43 – 6 x 10^5 CFU L^{-1}
- < 2 x 10^3 – 8 x 10^5 GU L^{-1}
- 1.6 x 10^5 – 2 x 10^6 CE L^{-1}

L. anisa
L. bozemanii
L. cherrii
L. dumoffi
L. erythra
L. feelii
L. gormanii
L. gresilensis
L. hackeliae
L. longbeacheae
L. lytica
L. maceachernii
L. micdadei
L. pneumophila
L. rubrilucens
L. saintcrucis
L. sterigerwaltii

Soil – garden, compost, potting

- 10^2 - 10^8 CFU g^{-1}
- 10^4 - 10^6 GU g^{-1}

L. anisa
L. birminghamensis
L. bozemanii
L. cincinnatiensis
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L. saintcrucis
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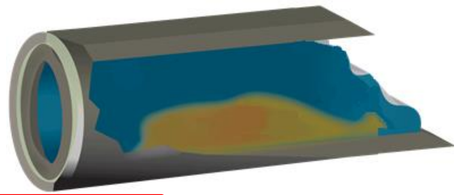
L. wadsworthii
L. yabuuchiae

Papadakis et al. 2018. Int J Environ Res Public Health
Schalk et al. 2014. Int J Infect Dis.
van Heijnsbergen et al. 2016. Appl Environ Microbiol.
van Heijnsbergen et al. 2014. J Appl Microbiol.

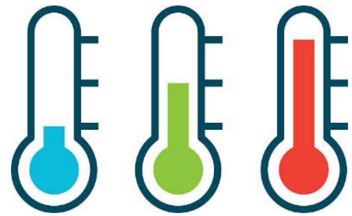
Adapted from Buse et al. 2012. Water Res 46: 921-933.

Conditions within Premise Plumbing

generation of respirable, DW-derived aerosols



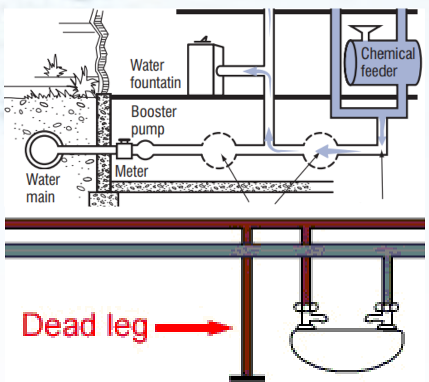
biofilms and sediments



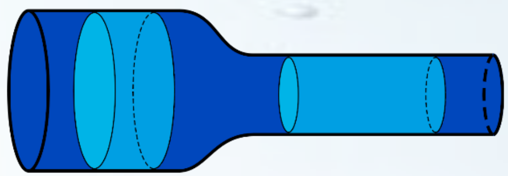
amenable growth temperatures

low to absent residual

nutrients (metals, minerals, microbes, etc.)



cross-connections



high surface area to volume ratios

eukaryotic hosts

water stagnation

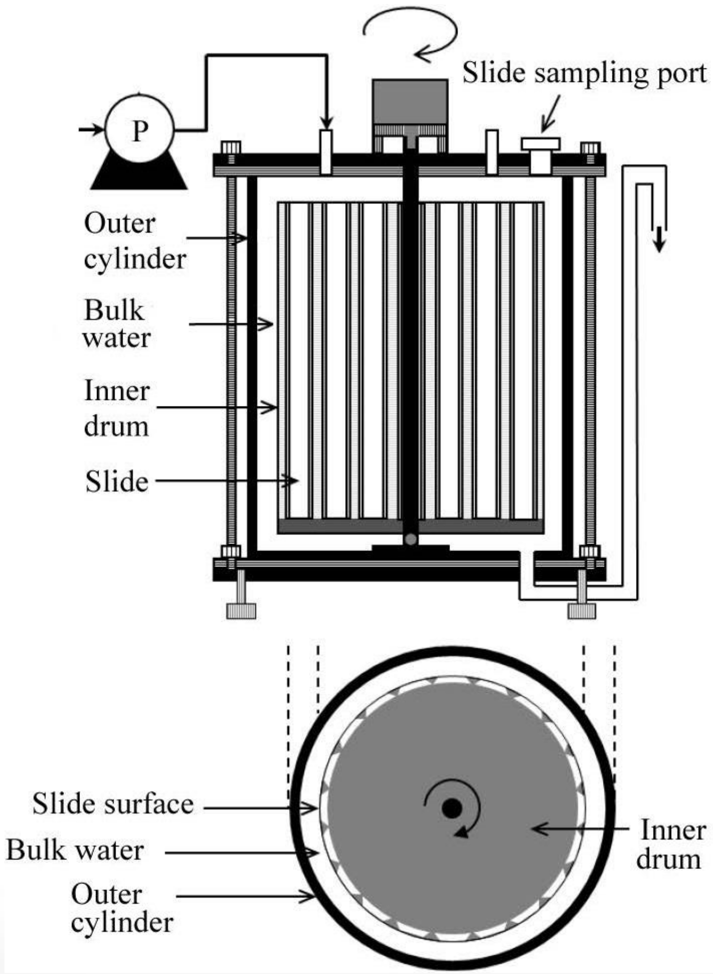
Drinking Water Biofilm Development

Project goal: determine the disinfection efficacy of chlorine and monochloramine, on DW biofilm-associated *L. pneumophila* established on copper (Cu) and polyvinylchloride (PVC) surfaces

BAR – biofilm annular reactor (1, 2, and 3)
M – motor
CW – cloth wrap
AW – aluminum wrap
Mf – manifold
V – value
C – controller
T – timer

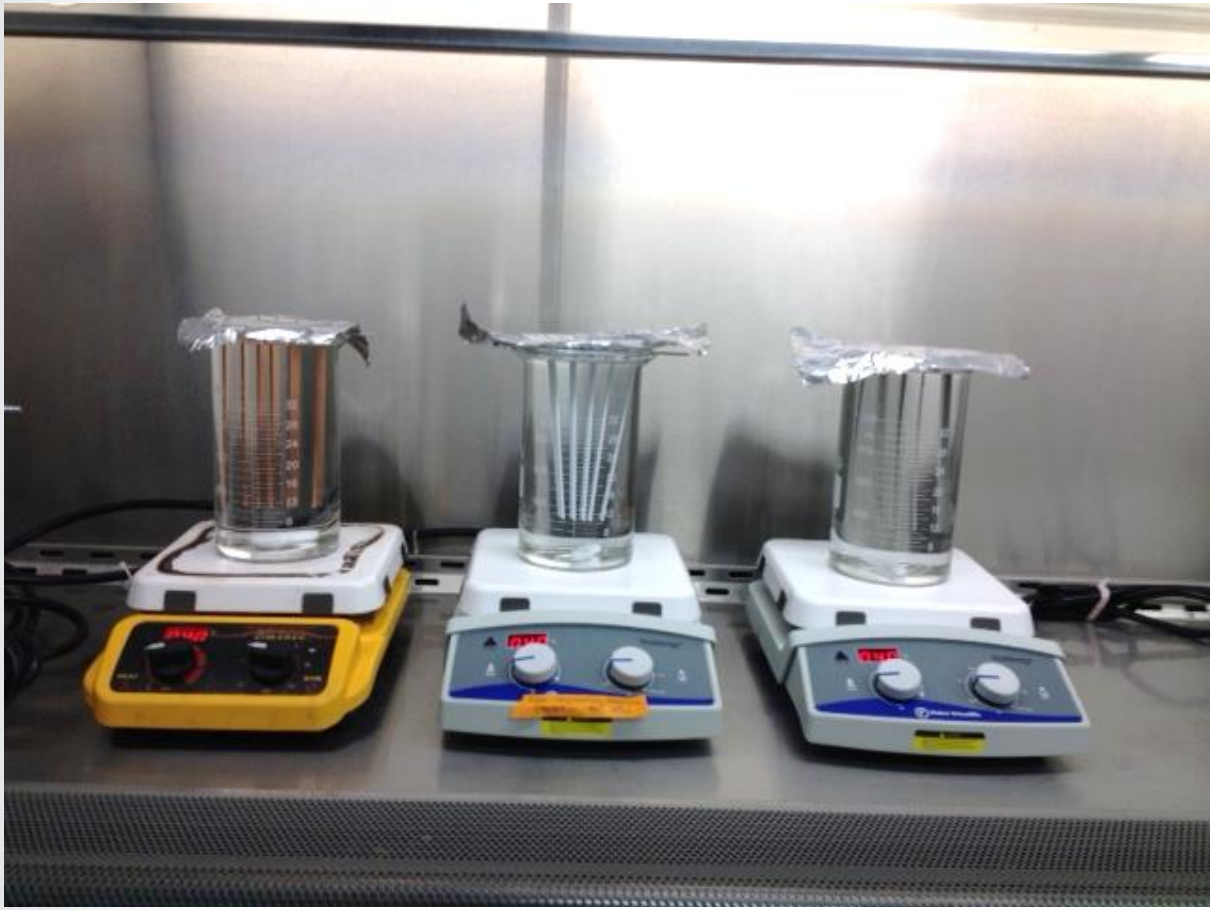
- Developed mature (1.5-2 yo) drinking water biofilms on Cu and PVC surfaces
- Conditions set to mimic private residential water flow conditions
- Monitor water quality: TOC, HPC, chlorine decay, pH, temperature, hardness, turbidity, organics, inorganics, metals, etc.

DW Biofilms on Cu and PVC surfaces



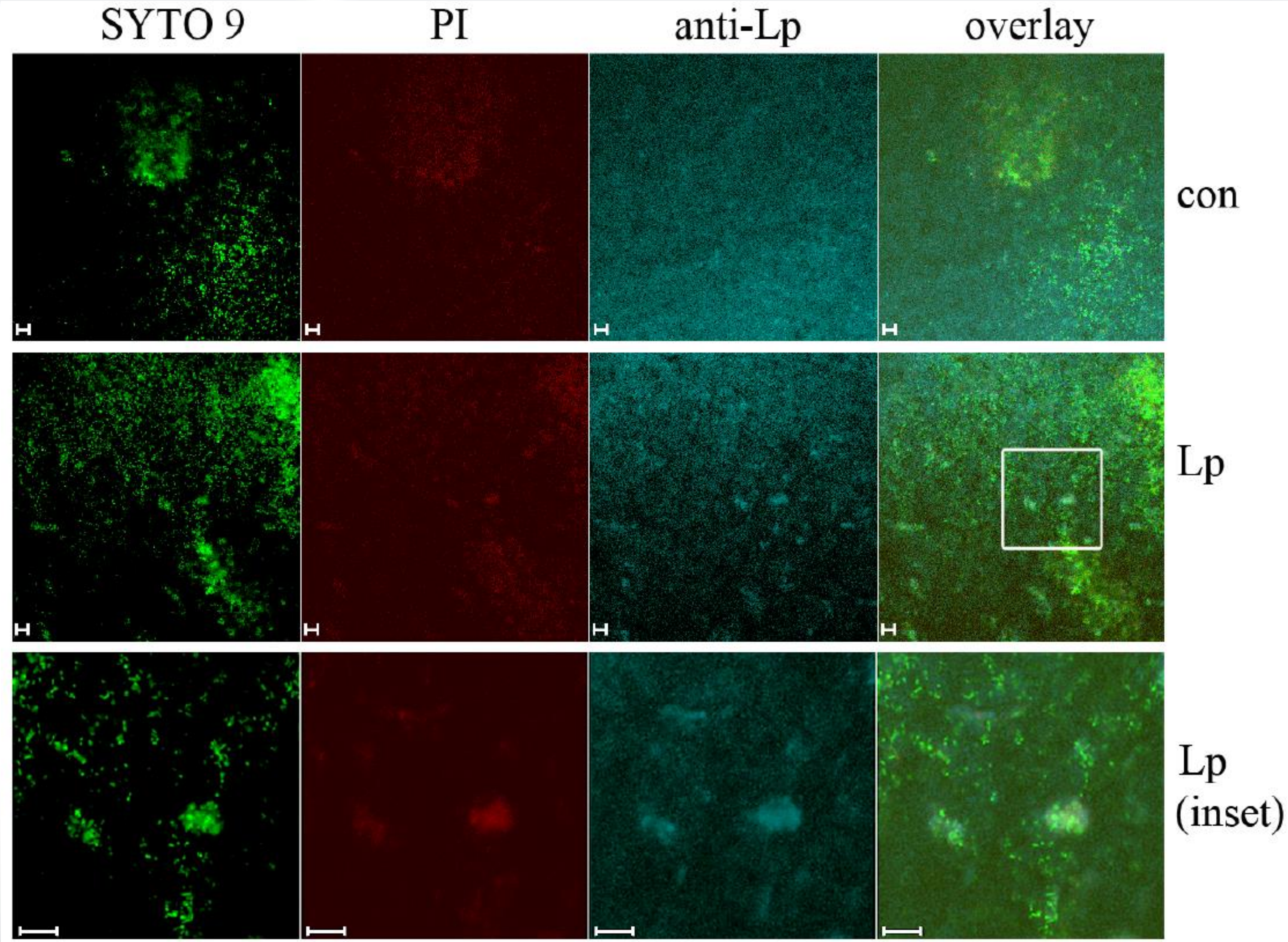
Adapted from Park et al. 2015. K J Microbiol 51:355.

L. pneumophila Inoculation Method

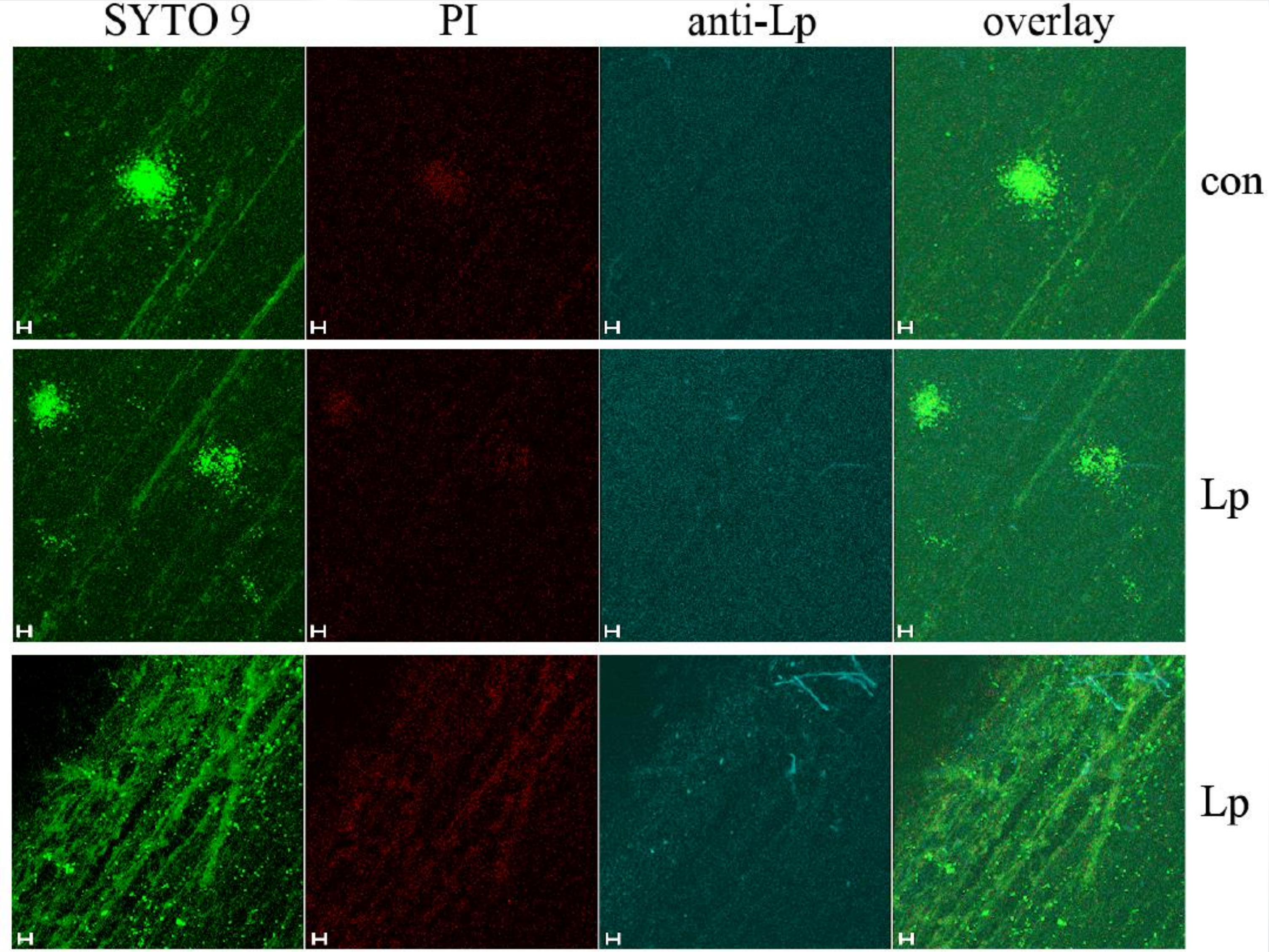


- slides removed from reactors
- suspended in beakers with 10^6 CFU mL^{-1} Lp sg 1 in 0.22 µm filtered, UV dechlorinated (5 d) drinking water (dfH_2O)
- batch mode for 5 d
- slides rinsed twice in 250mL dfH_2O
- incubated for additional 24 h in dfH_2O

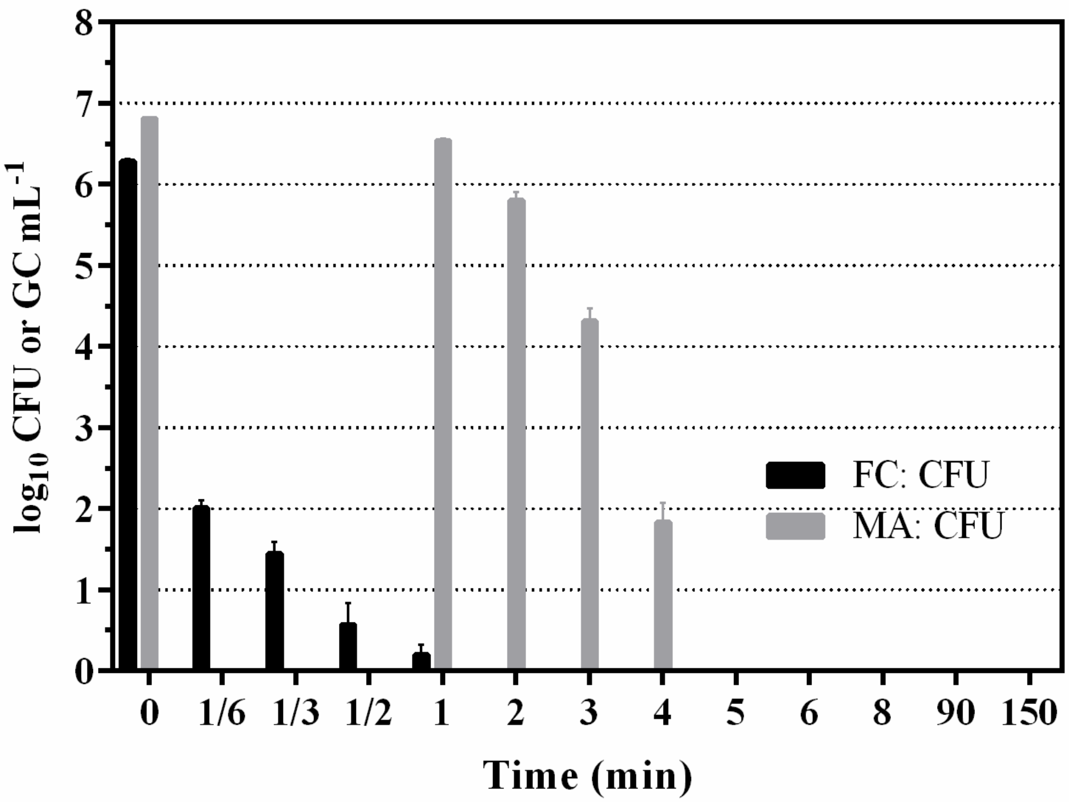
Confocal Microscopy – Cu Biofilms

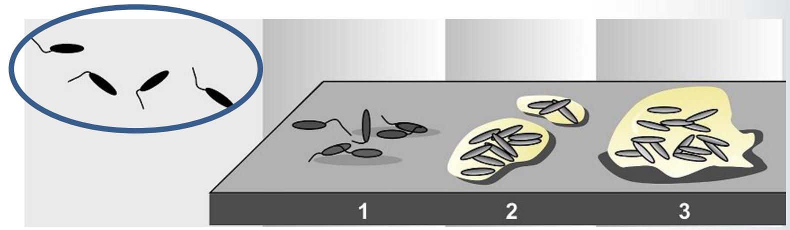


Confocal Microscopy – PVC Biofilms



Inactivation of Planktonic *L. pneumophila*

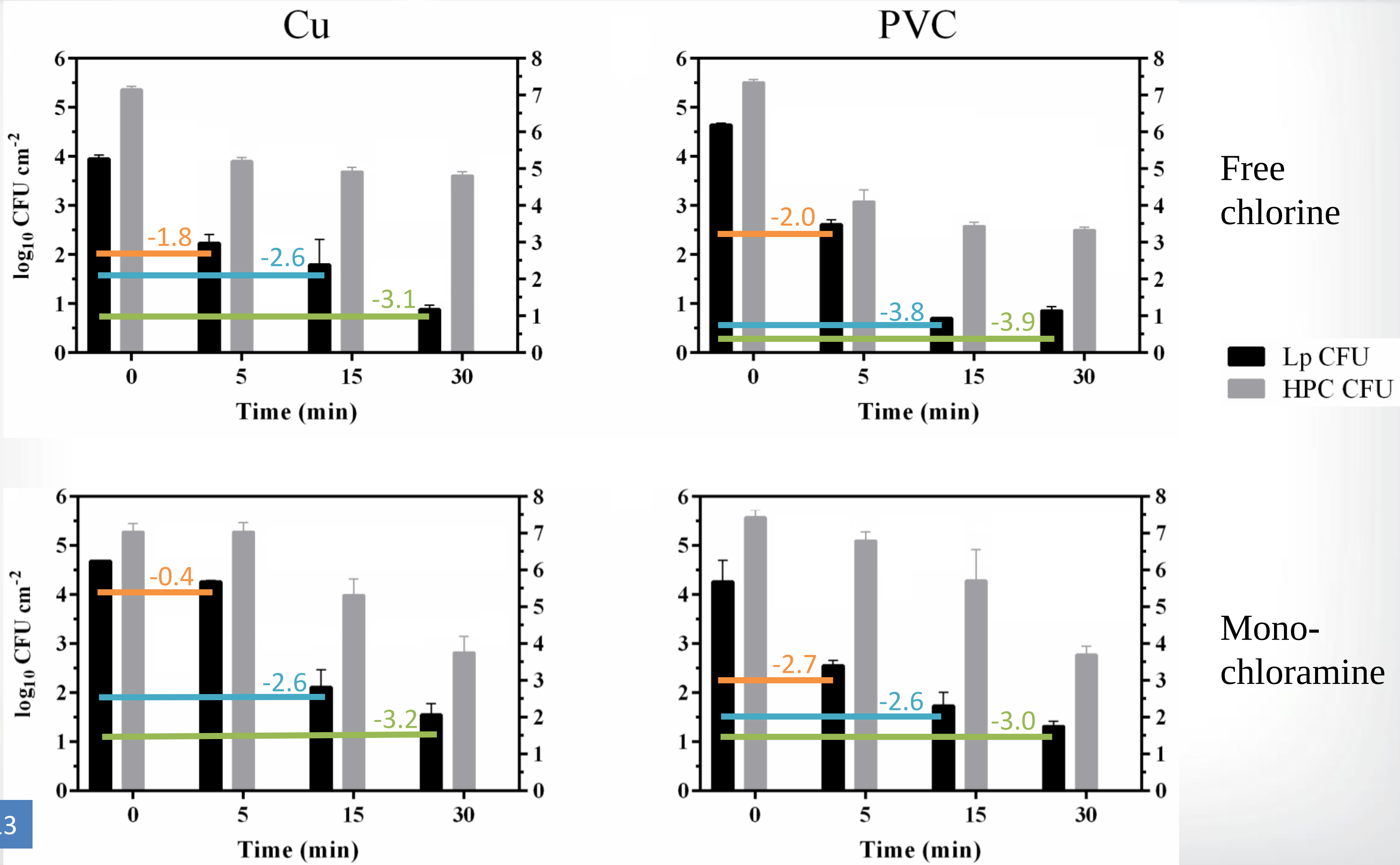




21°C, pH 8 conditions
Free chlorine (FC): 2.1±0.1 mg L^{-1}
Monochloramine (MA): 2.2±0.1 mg L^{-1}

Inactivation Ct value for:	FC	MA
2-$\log_{10}$	-	5.35
3-$\log_{10}$	0.11	6.58
4-$\log_{10}$	0.30	7.81

Inactivation of Lp-Colonized Cu and PVC DW Biofilms



Ct values for Planktonic- and Biofilm-Lp inactivation

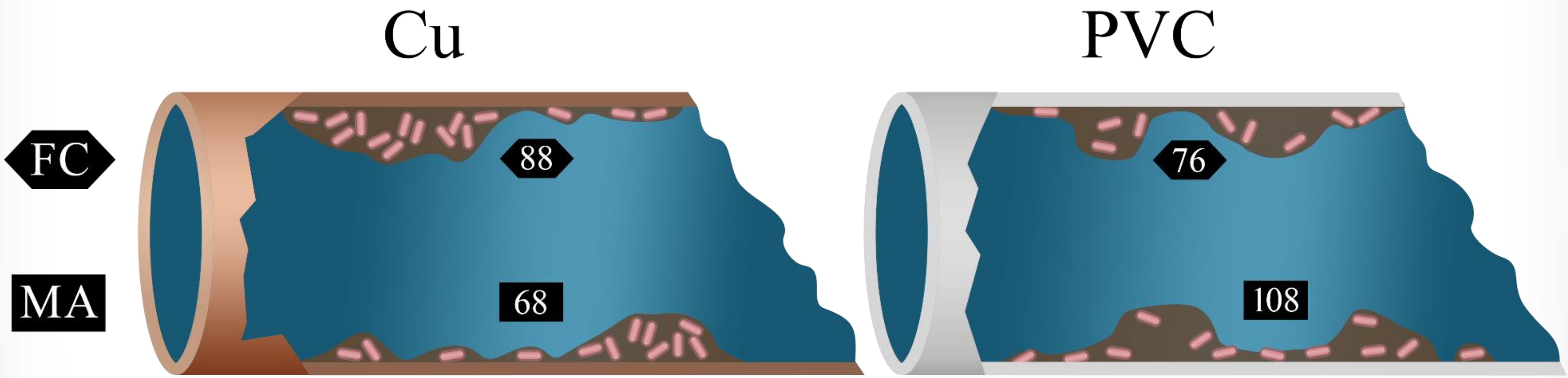
Inactivation Ct value for:	Planktonic Lp		Drinking Water Biofilm-associated Lp			
	Free chlorine	Monochloramine	←Lower *Ct*			Higher *Ct*→
$2\text{-}log_{10}$	-	5.35	Free chlorine PVC 8.86	Free chlorine Cu 13.18	Monochloramine PVC 17.16	Monochloramine Cu 34.86
$3\text{-}log_{10}$	0.11	6.58	Free chlorine PVC 36.11	Free chlorine Cu 50.83	Monochloramine Cu 55.38	Monochloramine PVC 62.8
$4\text{-}log_{10}$	0.30	7.81	Free chlorine PVC 63.67	Monochloramine Cu 75.90	Free chlorine Cu 88.48	Monochloramine PVC 108.44

- 2- and 3-log reduction biofilm-Lp: free chlorine more effective at inactivation
- For 4-log reduction: Effectiveness seemed to be substratum and disinfectant dependent
  - Biofilm penetration? (Cu vs. PVC biofilm structures)
  - Chemical interactions between disinfectant and substratum?

Summary Points

- For planktonic *L. pneumophila*, free chlorine (FC) was more effective at inactivation compared to monochloramine (MA) treatment

- For biofilm-associated *L. pneumophila* and 4-log inactivation levels, MA was more effective on Cu biofilms, while FC was more effective on PVC biofilms

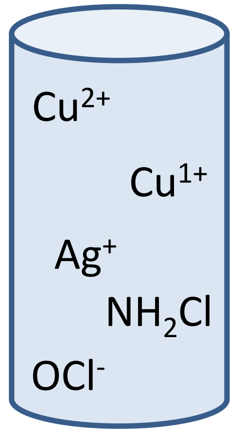


- Free chlorine treatment negatively impacted 16S rRNA gene transcript levels and may act synergistically with Cu surfaces to further reduce their levels (data not presented here)

Ongoing Decontamination Work

Current study: determining the effectiveness of metal ions and various water quality parameters on *L. pneumophila* inactivation (bench scale and pilot scale studies)

- Cl_2 + Cu/Ag metal biocides effectiveness
- monitor water quality
- pathogen quantification (culture and molecular)
- microbial community analyses (bulk and biofilm phase)



Bench scale: evaluate combinations of microbes and inactivating agents

Evaluating decontamination technologies for drinking water distribution systems

Ongoing & Planned Work

➢ **Characterization of contaminant spread via aerosolization and inhalation**

- Assess efficiency of drinking water to air partitioning for *L. pneumophila*
- Determine their viability and concentrations within the aerosols (and infectivity in murine models)

➢ **Mechanisms of Pathogen Mobility and Adherence to Infrastructure Surfaces**

NRMRL
Darren Lytle, PhD
Jill Holle
Christy Muhlen

NRMRL ORISE
Min Tang

CDC — Centers for Disease Control and Prevention
Claressa Lucas, PhD
Brian Raphael, PhD

Buse, Hoelle, Muhlen, Lytle 2018 FEMS ML

Understanding contaminant mobility (surface charge, hydrophobicity) and adhesion to pipe walls (microbalance) can help design effective decontamination strategies and/or engineering operations to minimize pathogen occurrence

Ongoing & Planned Work

- **Evaluating the effectiveness of flushing for controlling *Legionella pneumophila* growth in building water systems and reducing their levels in premise plumbing**
  - Conduct longitudinal study on the impacts of flushing and thermal inactivation on *L. pneumophila, Pseudomonas aeruginosa*, and NTMs in hot water heaters, plumbing, and fixtures
  - Study design: four hot water heaters, two of conventional design and two prototypes with passive control of microbial growth

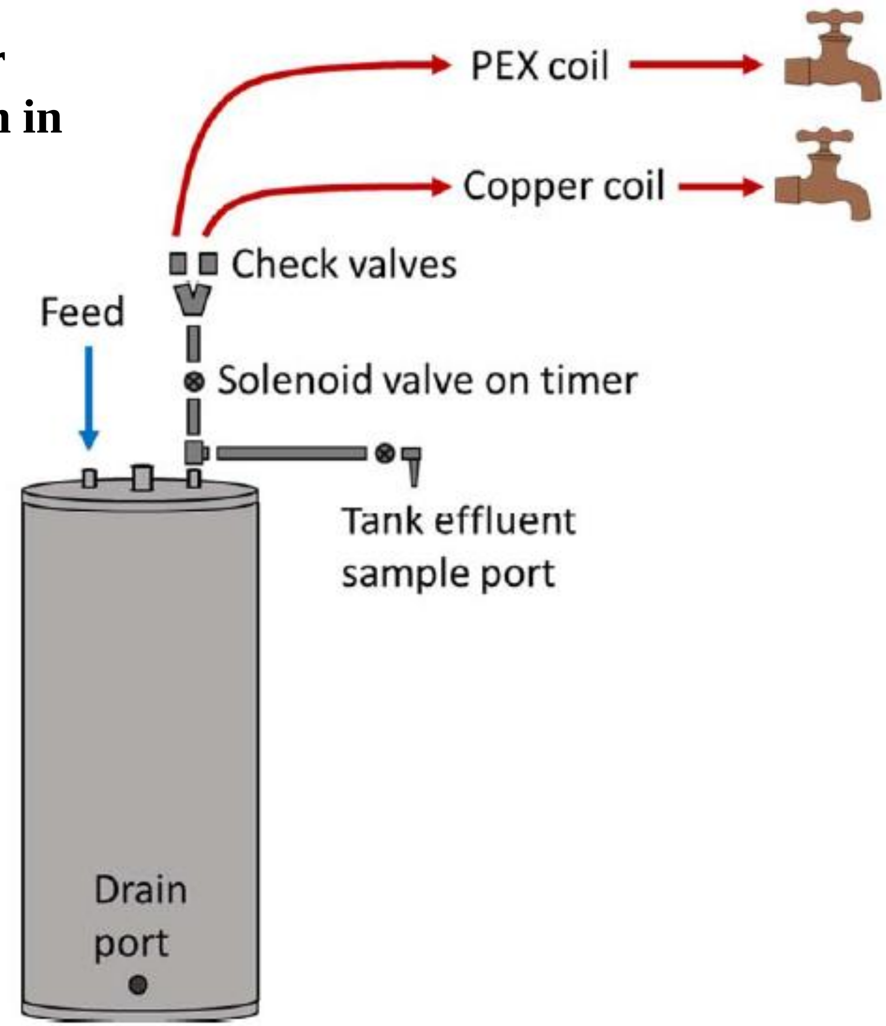


Figure 4. Hot Water Experiment Plumbing

University of Colorado Boulder

THANK YOU

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L. pneumophila sg1
drinking water isolate
grown on BCYE agar plates