

### MONITORING FOR LEGIONELLA IN BUILDING WATER SYSTEMS:

## Best Practices and Role in Risk Assessments

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Drinking Water: What Ben Franklin Had to Say ...



"With wine comes wisdom, with beer comes freedom,

with water comes *Legionella*."



### How Are We Doing?

### PREVENTING LEGIONNAIRES' DISEASE

### Legionella Is Winning



### 217% Increase in Cases (2011)

## Centers for Disease Control and Prevention Weekly / Vol. 60 / No. 32

Morbidity and Mortality Weekly Report August 19, 2011

#### Legionellosis — United States, 2000–2009

Legionnaires disease (LD), a serious, sometimes lethal pneumonia, and Portiac fever (PF), an influenza-like, selflimited illness, are the two most common forms of legionellosis, which is caused by *Legionella* bacteria. Legionellosis cases are reported to CDC through the National Notifiable Disease Surveillance System (NNDSS) and a Supplemental Legionnaires Disease Surveillance System (SLDSS) designed to manage surveillance data on travel-related cases and enhance outbreak detection. For this report, cases reported to NNDSS during 2000–2009 from the 50 states and the District of Columbia (DC) were assessed, and crude and age-adjusted incidence rates per 100,000 persons were calculated. U.S. legionelosis cases reported annually increased 217%, from 1,110 in 2000 to 3,522 in 2009, and the crude national incidence rate increased Census divisions.\* Only cases considered corfirmed under the 2005 Council of State and Territorial Epidemiologists' (CSTE) legionelosis case definitions are described in this report.<sup>†</sup> To be classified as confirmed, cases must be clinically compatible with legionellosis (i.e., fever, myalgia, cough, and/or clinical or radicgraphic evidence of pneumonia) and meet at least one of the confirmatory laboratory criteria (i.e., recovery of *Legionella* sp. in culture, detection of *Legionella pneumophila* serogroup 1 antigen in urine, or fourfold or greater rise in *L. pneumophila* serogroup 1-specific serum antibodies).

States also are encouraged to report cases to SLDSS to enhance detection of travel-related outbreaks and to provide information on additional legionellosis case variables not captured by NNDSS.<sup>§</sup> Legionellosis cases ideally should be reported

### Conclusions

- Legionellosis cases have increased substantially, particularly in the eastern U.S. and among middle-aged adults
- Public health professionals should focus on prevention of this important and increasing public health problem

### What You Need to Know

- 1. Why test? What will I learn?
- Methods: Collection and Processing
   *Legionella* bacteria in building water systems
  - Where do I test?
  - How (common errors)
  - Interpretation and relation to risk

### Environmental Monitoring for *Legionella:* Key to Prevention

### WHY TEST?

### **Proactive Approach**

#### **Proactive: ACHD**

- Initiate environmental testing now!
- if >30% sites positive for *L. pneumophila*, intensify clinical surveillance for cases
- If cases identified, then consider disinfection



ACHD Guideline Approach to Disinfection



### Proactive Approach Reduced Legionnaires' Disease



A proactive approach to prevention of health care-acquired Legionnaires' disease: The Allegheny County (Pittsburgh) experience

Cheryl L. Squier, RN, CIC, <sup>b,c</sup> Janet E. Stout, PhD,<sup>c,d</sup> Sharon Krsytofiak, MS, MT(ASCP), CIC,<sup>b</sup> Joan McMahon, RN, MPH,<sup>a</sup> Marilyn M. Wagener, MS,<sup>d</sup> Bruce Dixon, MD,<sup>a</sup> and Victor L. Yu, MD<sup>c,d</sup> Pittsburgh, Pennsylvania

#### Am. J. Infection Control 2005; 33(6):360-367

### **Testing Can Be Revealing**

Hospitals performing *Legionella* environmental testing are more likely to prevent cases of hospital-acquired Legionnaires' disease

Squier CL, Stout JE, Krystofiak S, McMahon J, Wagener MM, Dixon B. Yu VL. Am. J. Infect. Control 2005; 33(6): 360-367.



### **Questions to Ask**

- Was *Legionella* found in the water system?
- Pathogenic species?
- Extent of colonization?
  - Colonization Rate of >30% (Percent Positivity) = Increased Risk (Especially if L. pneumophila serogroup 1 present)
- Colonization in sensitive areas?

# Evaluating Risk of Exposure to Legionella

WHERE TO TEST?

### Approach to Environmental Sampling

- Select a minimum of 10 distal sites (faucets or showers) that roughly represent the water distribution system, plus hot water tanks or recirc. line.
- Include sites on multiple floors and wings, high risk areas like oncology, transplant units, medical surgical units.

**Calculating Percent Positivity** 

- Number of OUTLETS positive for Legionella of 10 distal sites (faucets or showers) that were tested
- Estimating the probability of a patient "bumping into" *Legionella (Percent Positive)*

### VHA Directive: More Proactive Prevention

Department of Veterans Affairs Veterans Health Administration Washington, DC 20420 VHA DIRECTIVE 2008-010

February 11, 2008

#### **PREVENTION OF LEGIONELLA DISEASE**

1. PURPOSE: This Veterans Health Administration (VHA) Directive establishes guidelines for the annual evaluation of *Legionella* risk at VHA inpatient facilities.

#### 2. BACKGROUND

a. The Gram-negative bacterium, *Legionella*, causes respiratory diseases including *Legionella* pneumonia (traditionally known as Legionnaires' disease), hereafter abbreviated as "LD" for "*Legionella* disease." Disease is primarily caused by *Legionella pneumophila*; however other species of *Legionella* can be pathogenic, particularly in transplant and other immunocompromised patients. The bacteria, found naturally in water, have been associated with man-made reservoirs, such as building water distribution systems and cooling towers. Disease occurs after inhalation or aspiration of contaminated water, followed by an average incubation period of 2 to 10 days. The disease is not transmitted from person-to-person.

h Health care facilities have been connected with the transmission of Contenant to anti-

### **Risk Assessment: Environment**

Annual environment testing for *L.* pneumophila SG 1

- Sample at least 10 distal water sites
  - >500 beds, increase sample size by 2 distal sites per 100 beds over 500

 Select sites that include all water distribution systems and high risk areas VHA Directive Key Elements: Environmental Testing

Determine if exceed "threshold level" (>30%) positive for *L. pneumophila* serogroup 1

- If yes, proceed to "Action Plan"
- If no, assessment complete

### Interpretation – Potable Water

Risk of nosocomial Legionnaires' disease was better predicted by the proportion of water system sites testing positive for *Legionella* than by the concentration of *Legionella* bacteria.

Kool J L, et al. Infect. Control Hosp. Epid. 1999 20:797-805

### Whack-a-Mole



### Water Safety Plan





1 Drop of Water Can Contain Thousands of *Legionella* 

### Legionella Is Not Alone

- Legionella bacteria are part of a community of microorganisms—a consortium.
- They live synergistically—one benefiting the other for mutual survival.

### Many Other Microbes in Water Help *Legionella* Grow



#### **Bacteria Provide Nutrients**



# Eliminate (Kill) the Competition

### **Collection and Processing**

### **METHODS**

### Legionella Testing: Sample Collection



"That's not quite the stool sample we had in mind, Mr. O'Donnell."

### "That's not quite the stool sample we had in mind"

## WHAT TO TEST? What Is the Best Sample to Collect?

### Water Sample Collection and Processing

- Water samples are plated directly and after concentration by filtration (10X)
- 100 mL of water is filtered and re-suspended in 10 mL of the original sample



What Volume To Collect/ Concentrate?

- Filter Concentrate Comparison (Percentage of samples positive)
  - Volume 100 98%
  - Filter 200 100%
  - Filter 1000 100%

### Flush/No Flush?

- No Flush
  - Collect immediately after opening faucet or shower valve
  - Flushing reduces recovery

- If CFU>0.5 log in the sample:
  - Immediate Draw 97.7% (42/43)
  - 2 min. flush
     69.1% (29/43)

### **Swab Samples**

- A swab sample is acid-pretreated by inserting the swab into 2.0 mL of the acid buffer.
- Allow to react for 3 min. and plate 0.1mL/plate



### **Equipment for Sample Collection**

- Sterile bottle with neutralizer (sodium thiosulfate) and/or sterile swab
- Insulated shipping pouch or box protects samples
- Ship to lab within 48hr of collection
  - Longer accepted, but not recommended

Recommendation to Ship Next Day Delivery

No significant increase or decrease in *Legionella* viability when cultured within the time recommended from collection to processing, i.e. 48 hr of collection.

- Shaffer A. Mietzner, et al 2013 APIC
- Flanders, W., et al Water Research (2014),doi:10.1016/j.watres.
   2014.05.025.




#### What Happens in the Lab?

#### **Detection Methods**

- Culture isolation
- Direct visualization
- Molecular methods

#### **Culture Is Gold Standard**

- Culture is more reliable than "rapid tests"
  - DFA
  - ICT
  - PCR
  - Dip slide

 Laboratory-based and validated culture method is the industry standard

#### **Culture Method**

- Industry standard/best practice
  - Proficiency Programs
  - Standards based

     ISO 11731 (1&2)
     ASTM D 5952
     International HSE L8 ACP 2013

#### **Culture Method**

Proficiency Programs- Limited
 Public Health of England (PHE) is an international proficiency program

 Environmental Legionella Isolation Techniques Evaluation Program (ELITE) is offered by CDC

#### **Methods**

- Culture isolation
- Direct visualization
- Molecular methods

#### Fluorescent Antibody Stain

- Polyvalent and monovalent reagents can be used
- Do Not Use Monoclonal antibody for direct testing of environmental samples



#### **Methods**

- Direct visualization
  - Direct Fluorescent Antibody Stain
    - Non-specific
    - Low sensitivity
    - Not recommended for environmental testing

**Reagents for Identification** 

- Remel Legionella Poly-ID Test Kit (not currently available – production problem)
- Bio-Rad Monofluo Legionella pneumophila IFA Test Kit
- m-Tech/ Monoclonal Technologies, Inc. monospecific and panvalent FITC Conjugate Reagents
- Pro-Lab Diagnostics Legionella Reagents for Direct Fluorescent Antibody Test

#### **Culture Is Gold Standard**

- Laboratory-based and validated culture method is the industry standard
- Culture is more reliable than "rapid tests"
  - DFA
  - ICT
  - PCR
  - Dip slide

#### ICT Not Sensitive: Same Water Sample —Different Results



## Media for Isolation of *Legionella* spp. from Environmental Samples



Media for Isolation of *Legionella* spp. from Environmental Samples

• DGVP –

Dyes 10 ug/ml

Glycine 3.0 mg/ml

Vancomycin 1.0 ug/ml

Polymyxin B 50 U/mI

• CCVC –

Colistin 16 mg/L

Cephalothin 4 mg/L

<u>V</u>ancomycin

0.5mg/L

Cycloheximide 80mg/L Pretreatment

- Acid buffer is a HCI-KCI solution at a pH of 2.2
  - 0.2M hydrochloric acid plus 0.2 M potassium chloride, adjusted to pH 2.2
- Heat treatment 50°C 30 min

#### Culture Isolation of Legionella

- View plate under dissecting microscope
- Identify colonies with morphology consistent with Legionella spp.



#### Legionella Colony Morphology

Colonies of *Legionella pneumophila* have a typical ground-glass, opalescent morphology



#### Legionella Colony Morphology

- Non-pneumophila species may not have this typical morphology
- L. micdadei may appear as grayish mucoid protuberant colonies



#### Legionella Culture Isolation

- Some Legionella species autofluoresce either blue-white or red under long-wave (365nm) UV light.
- These species include L. dumoffii, L. bozemanii, L. gormanii, L. erythra and L rubrilucens



#### Legionella Identification

- Suspected Legionella isolates are confirmed by either slide agglutination or by direct fluorescent antibody staining
- Latex agglutination for *L. pneumophila* serogroups 1-14 and L. species.



#### Sources "Fingerprinted" by DNA Typing (PFGE)



The isolate from the patient can be compared to isolates recovered from environmental sources.

#### **Methods**

- Culture isolation
- Direct visualization
- Molecular methods

#### **Molecular Methods**

- Polymerase Chain Reaction (PCR)
   Long history
- Loop-mediated Isothermal Amplification (LAMP)
  - Relatively New
- DNA microarray
  - Experimental

#### **Molecular Methods**

- The CDC does not currently recommend the routine use of genetic probes or PCR for detection of *Legionella*.
  - "Use of PCR for the identification of Legionella spp. is not recommended until more data regarding sensitivity and specificity of procedures are available"

#### Molecular Assay False-Positivity

- PCR-based assays for Legionella detection in environmental samples have shown PCR positivity when culture results are negative
  - Termed "False-Positivity"
  - Thought to be due to: viable non-culturable Legionella, non-viable Legionella, or Legionella living within amoebae
- Organisms exhibiting cross-reaction are commonly found in water and may interact with previously designed PCR primers for *Legionella* detection

#### **NEW FINDINGS**

False positives may be due to 86% 16S rRNA sequence similarity with *Legionella* and these organisms\*



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#### The Microbiome

#### **The Future of Monitoring?**

#### Phylogenetic Methods for Studying Microbial Communities

# PNAS

### Opportunistic pathogens enriched in showerhead biofilms

Leah M. Feazel<sup>a</sup>, Laura K. Baumgartner<sup>a</sup>, Kristen L. Peterson<sup>a</sup>, Daniel N. Frank<sup>a</sup>, J. Kirk Harris<sup>b</sup>, and Norman R. Pace<sup>a,1</sup>

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Contributed by Norman R. Pace, July 29, 2009 (sent for review June 25, 2009)

The environments we humans encounter daily are sources of exposure to diverse microbial communities, some of potential readily cultured with standard methods (13, 14). Consequently,

Feasel et al. PNAS September 22, 2009 vol. 106 no. 38 16393-16399

#### Relative Abundance Genus-level Phylogenetic Analysis



454 Pyrosequencing **A Phylogenetic** Method for Comparing Microbial Communities

- Julianne Baron, J. Kirk Harris, Eric P. Holinger, Norman R. Pace, Janet E. Stout
  - University of Pittsburgh, Pittsburgh, PA, USA.
  - University of Colorado, Boulder, CO, USA.

Hospital Water System Microbiome Project

How does monochloramine alter the diversity of the microbes found in the water system and the relative abundance of *Legionella*?

## Water Treatment Caused a Shift in Microbial Community



#### **Changes in Microbial Populations**

- Significant reduction in *Legionella*
- No increase in other pathogens (*Pseudomonas*, *Stenotrophomonas*, or non-tuberculous mycobacteria)

Baron JL, Vikram A, Duda S, Stout JE, Bibby K (2014) Shift in the Microbial Ecology of a Hospital Hot Water System following the Introduction of an On-Site Monochloramine Disinfection System. **PLoS ONE 9(7): e102679.** doi:10.1371/journal.pone.0102679

#### Is the Sky Falling if I Find Some *Legionella*?



# What is the acceptable amount of *Legionella*?

#### **INTERPRETING RESULTS**
# IS ZERO NECESSARY?

## **Establish Targets**

- Action Levels
- Minimum Levels
- Goals

# Food and Drug Administration

Product	Type of insect contamination	Action Level
Canned sweet corn	Insect larvae (corn ear worms or corn borers)	2 or more 3 mm or longer larvae

EPA Maximum Contaminant Regulatory Levels

- Maximum
- Contaminant
- Level

- Maximum
- Contaminant
- Level
- Goal

## **Environmental Protection Agency**

Regulated	MCL (mg/L)	MCL Goal
Arsenic	0.010 milligrams per Liter	zero
Cyanide	0.2 milligrams per Liter	0.2 mg/L

Legionella

# Zero Is the goal, but not necessary to avoid outbreaks

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Defining the Risk Associated with the Presence o *Legionella* in Water

What is the acceptable upper limit of *Legionella* in potable water and cooling towers?

#### **Potential Health Concern**

- If a water sample from a cooling tower (or a whirlpool spa) has >1000 cfu/mL of *L. pneumophila*, particularly serogroup 1:
  - We notify immediately
  - Take action to reduce



# Risk Criteria Based on Environmental Monitoring Results

 Concentration-based thresholds that establish target values in CFU per litre or millilitre

#### Colonization rate

or proportion of distal sites in the water system that are positive for *Legionella* 

- OK for cooling towers
- Best for hospitals and building water systems

## Overview

- Potable Water, especially in hospitals (and other buildings) with complex hot water systems, is the most important source of *Legionella* transmission.
- Transmission via aerosolization and aspiration
- Currently, ISO culture-based method is the "Gold Standard" for *Legionella* detection
- Environmental testing for *Legionella* is a key indicator for disease risk

# Lots of ... Legionella Anxiety

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# MEDICATION

## Dr. Stout's Legionella Chill Pills

- For treatment of Legionella-related anxiety.
- Take 2 tablets 1
   hour before
   *Legionella* testing
   or as needed.





# THANK YOU

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