LEGIONELLA

Environmental and Clinical Challenges

Janet E. Stout, PhD
President, Director, Special Pathogens Laboratory
Research Associate Professor, University of Pittsburgh
PUZZLED BY LEGIONELLA?
The National Academies of Sciences, Engineering, and Medicine

- The *ad hoc* committee of the Academies will review the state of science with respect to *Legionella* contamination of water systems and issue a report.

- The report will identify gaps in research and needs for additional research.
Important Questions

• Are there important differences in occurrence and management of *Legionella* with respect to:
  - Cold water vs. hot water systems?
  - Healthcare vs. community-acquired?
  - *Legionella pneumophila* vs. other species?
  - Routine testing for *Legionella* vs. public health investigations?
  - What defines controlled?
Today’s Topics

• Our evolving understanding of *Legionella pneumophila* as a waterborne pathogen
  ▪ Environmental and clinical challenges

• Evaluating risk – environmental testing for *Legionella*

• Approaches to prevention: water treatment and risk management

• Why *Legionella* is my favorite microbe!
1976 Philadelphia

- 58th convention of the American Legion held July 21-24
- Mysterious illness effects 221 and kills 34
- Causative agent of pneumonia would not be identified until 1977
- *Legionella pneumophila*, serogroup 1
My Research Begins at the Pittsburgh VA In 1980

- Over 100 nosocomial cases identified in 5 years in one hospital
- The source of the outbreak was the hospital water system – not a cooling tower!

Janet E. Stout and Victor L. Yu
UBIQUITOUSNESS OF LEGIONELLA PNEUMOPHILA IN THE WATER SUPPLY OF A HOSPITAL WITH ENDEMIC LEGIONNAIRES’ DISEASE

Janet Stout, M.S.,
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Michele Best, B.A.,
Arnold Brown, M.D.,
Robert B. Yee, Ph.D.,
and Robert Wadowsky, M.S.
Paradigm Shift: Not Cooling Towers

- NOT a common source for hospital-acquired cases
- More commonly associated with large community outbreaks
What We Know Now

Potable water especially in hospitals (and other buildings) with complex hot water systems, is the most important source of *Legionella* transmission.
Lessons Learned

• More than 30 years studying Legionnaires’ disease
  ▪ I’m a Legionellologist

• Mission: What I’ve learned can help you more effectively address *Legionella* risk.
THE CHALLENGE OF LEGIONNAIRES’ DISEASE
Consider This…

Legionellosis cases have increased substantially – over 200% in last 10 years
Why Increase in Reported Cases?

- Increase in diagnostic testing = more cases identified
- Environmental factors?
Rain, flooding likely led to spike in Legionnaires' disease

Reports of Legionnaires' disease follow heavy rains.

By Tim Darragh, Of The Morning Call
11:42 P.M. EDT, OCTOBER 17, 2011

All that rainfall in September may have left more than wet basements here and flood-wrecked communities in northeast Pennsylvania.

It also appears it was at least partly responsible for a record spike in Legionnaires' disease, a water-borne bacterial pneumonia that can be fatal to some people.
Increased rainfall is associated with increased risk for legionellosis

L. A. HICKS¹,²*, C. E. ROSE Jr.³, B. S. FIELDS¹, M. L. DREES²,⁴, J. P. ENGEL⁵, P. R. JENKINS⁵, B. S. ROUSE⁶, D. BLYTHE⁷, A. P. KHALIFAH¹, D. R. FEIKIN¹ AND C. G. WHITNEY¹
Weather
and Legionnaires’ Disease

Weather-Dependent Risk for Legionnaires’ Disease, United States

Jacob E. Simmering, Linnea A. Polgreen, Douglas B. Hornick, Daniel K. Sewell, Philip M. Polgreen

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 23, No. 11, November 2017
Weather and Legionnaires’ Disease

• Community-acquired cases associated with warmer temperatures (60-80°F) and higher humidity
• Higher rainfall (80.4mm vs. 61.7mm controls)
• No seasonality for healthcare-acquired cases
Risk of Acquiring Disease Low

Exposure Alone Doesn’t = Disease Acquisition

Legionella in water (reservoir) + Transmission to the host (exposure) + Susceptible Host

Risk of Acquiring Disease Low
Legionnaires’ Disease

- In the U.S. approximately 600,000 adults are diagnosed with community-acquired pneumonia requiring hospitalization annually.

- 2 – 5% are caused by *Legionella*, as many as 30,000 cases/year.
Modes of Transmission for Legionnaires’ disease

• Aerosolization

• Aspiration

• Direct instillation into the patient (tap water rinsing of nasogastric tubes, respiratory tubing, etc)
Letter to the Editor

Two cases of Legionnaires’ disease associated with continuous positive airway pressure therapy

To the Editor

Sporadic cases and outbreaks of Legionella pneumophila usually are related to exposure to water-containing Legionella bacterium. Legionnaires’ disease is transmitted by contaminated water in medical equipment, including oxygen humidifiers, nebulizers, and face masks, and has been extensively reported [1,2]. Neverthe-

...
Who’s at Risk?

• Elderly (>50)
• Smokers
• Immunocompromised
  ▪ Transplant patients
  ▪ High-dose steroids for lung disease
  ▪ Diabetes
  ▪ Cancer
• Approx. 25% cases no known risk factors
Centers for Disease Control and Prevention Statistics

• 77% >50 years
• 62% male
• Hospitalizations occurred in 98%
  ▪ ICU admission in 39%
  ▪ Death in 10%–30%
Clinical Notes

• 40%–50% of Legionnaires' disease patients have neurological symptoms
  ▪ Some with encephalopathy (brain inflammation) triggered by antibodies that target brain cells (www.legionella.org)

• Risk of death highest for community-acquired infection in immunocompromised followed by healthcare-acquired infections
Many Cases Still Missed

- Diagnostic tests for *Legionella* not routine – often not done
- Many studies have demonstrated under reporting/missed diagnosis
How often is a work-up for *Legionella* pursued in patients with pneumonia? A retrospective study

Brian Hollenbeck¹, Irene Dupont² and Leonard A Mermel²,³*

**Abstract**

**Background:** It is unclear how often patients with pneumonia are assessed for *Legionella* in endemic areas. Additionally, the sensitivity of the IDSA/ATS criteria for recommended *Legionella* testing is undefined.
41% of *Legionella* cases were missed when following current IDSA-ATS recommendations for *Legionella* testing
Diagnostic Methods

- Urine antigen (UA) tests confirm approximately 95% of U.S. resident cases
  - Disadvantage = No *Legionella* bacteria available for further study
- Only 5% of cases confirmed by culture
  - Advantage = *Legionella* bacteria available for further epidemiologic study
Dangers of Dependency on Urine Antigen Testing for Diagnosis

• Urine antigen specific for *L. pneumophila*, serogroup 1 only

• If used to screen for healthcare-acquired LD, you’d better know what’s in your water!
  ▪ If Lp-6 in the water, diagnosis will be missed
Clinical Utility of Urinary Antigen Detection for Diagnosis of Community-Acquired, Travel-Associated, and Nosocomial Legionnaires’ Disease

Jürgen H. Helbig,¹ Søren A. Uldum,² Sverker Bernander,³ Paul Christian Lück,¹ Günther Wewalka,⁴ Bill Abraham,⁵ Valeria Gaia,⁶ and Timothy G. Harrison⁷
Healthcare-acquired Cases Missed

- Community-acquired cases sensitivity of Binax EIA was 86.5%
- Healthcare-acquired cases sensitivity only 44%
- Difference due to different disease-causing strains/subtypes of *L. pneumophila*
  - Mab-2 negative strains more common among hospital-acquired and often not detected
All Urine Antigen Tests Are Not Created Equal
Sensitivity and Specificity Among Seven *Legionella* Urinary Antigen Test Kits: An International Collaborative Study

JOHN D. RIHS, SOPHIE JARRAUD, CHRISTAN LUCK, CHUEN-SHEUE CHIANG, PAOLA BORELLA, JOSE DOMINGUEZ, JANET E. STOUT, AND VICTOR L. YU
Study Design

• 103 urines tested from 5 countries
• 28 cases of Legionnaires’ disease
  ▪ 26 L. pneumophila, serogroup 1
  ▪ 2 L. pneumophila, serogroup 5 and 12
• 24/26 culture-confirmed
  (Legionella pneumophila, serogroup 1)
• 75 negative urines
Seven Urinary Antigen Tests

- BinaxNOW *Legionella* (Alere)
- *Legionella V-Test*
- RapiCard InstaTest
- Uni-Gold *Legionella*
- *Xpect Legionella*
- SAS *Legionella* Test
- Accusay *Legionella*
Variable Sensitivity / Specificity

- BinaxNOW Legionella – 100% / 100%
- Legionella V-Test – 92% / 100%
- RapiCard InstaTest – 92% / 79%
- Uni-Gold Legionella – 85% / 100%
- X/pect Legionella – 85% / 100%
- SAS Legionella Test – 85% / 100%
- Accusay Legionella – 77% / 98.7%
Another Study Shows Poor Sensitivity of Urinary Antigen Test

Evaluation of Two New Immunochromatographic Assays (Rapid U Legionella Antigen Test and SD Bioline Legionella Antigen Test) for Detection of *Legionella pneumophila* Serogroup 1 Antigen in Urine

Bram M. W. Diederens* and Marcel F. Peeters
Prolonged Duration of Positivity

Sequential Urines from 21 Culture Positive Patients

- 0 - 10d (n=14) *: 71%
- 11 - 30d (n=14): 78%
- 31 - 60d (n=13): 46%
- 61 - 120d (n=7): 0%
- 121 - 240d (n=13): 7%

*n = no. patients

Days Post Onset

© Special Pathogens Laboratory
Diagnosis: CDC Recommends Culture and Urine Antigen!

What Clinicians Need to Know about LEGIONNAIRES’ DISEASE

Legionnaires’ disease is a sometimes fatal form of pneumonia that is on the rise in the United States. Unfortunately, this disease is also underrecognized and underdiagnosed. Clinicians are in a unique position to make sure cases are detected, allowing rapid investigation by public health officials and prevention of additional cases.

Diagnosis and Testing
Clinical features of Legionnaires’ disease include cough, fever, and radiographic pneumonia. Signs and symptoms for Legionnaires’ disease are similar to pneumonia caused by other pathogens; the only way to tell if a pneumonia patient has Legionnaires’ disease is by getting a specific diagnostic test. Indications that warrant testing include:

- Patients who have failed outpatient antibiotic therapy for community-acquired

Order both a culture of a lower respiratory specimen and a urinary antigen test when testing patients for *Legionella*.

https://www.cdc.gov/legionella/clinicians.html
Preventing Legionnaires’ Disease

How are we doing?
Progress Has Been Slow…
Legionellosis in the U.S.

Outbreaks continue to occur (building warm water systems, cooling towers, fountains)
2016 CDC Report on Legionnaires’ Disease Outbreaks

Morbidity and Mortality Weekly Report

Vital Signs: Deficiencies in Environmental Control Identified in Outbreaks of Legionnaires’ Disease — North America, 2000–2014

Laurel E. Garrison, MPH¹; Jasen M. Kunz, MPH²; Laura A. Cooley, MD¹; Matthew R. Moore, MD¹; Claressa Lucas, PhD¹; Stephanie Schrag, DPhil¹; John Sarisky, MPH²; Cynthia G. Whitney, MD¹

On June 7, 2016, this report was posted as an MMWR Early Release on the MMWR website (http://www.cdc.gov/mmwr).
Outbreak Sources: More Than Healthcare

• 27 Outbreaks - Sources
  ▪ 44% Hotels and resorts
  ▪ 19% Long-term care facilities
  ▪ 15% Hospitals
  ▪ 21% Senior living facilities, workplaces, and the community
Cases Linked to Water Systems

- Warm water distribution in:
  - Hospitals
  - Nursing homes
  - Rehabilitation centers
  - Office buildings
  - Apartment buildings
  - Hotels

- Other water systems:
  - Spas and hot tubs
  - Decorative fountains
  - Humidifiers
  - Cooling towers
Legionella Growth

BUILDING WATER SYSTEMS
How does *Legionella* get into our water?
Legionella in Source Water (1987)

• “Public water supplies may contaminate the plumbing systems of hospitals and other large buildings.”

“Results of this study confirm the ubiquity of Legionella in aquatic environments, even ground water.”

Origins of Legionella in Building Water Systems?
Building Risk Assessment

• How to assess the risk for Legionnaires’ disease from building water systems.

• Understand conditions within water systems that contribute to *Legionella* growth in building water systems
3 Factors Needed For *Legionella* Growth in Building Water Systems

*Legionella* are in 30–50% of water systems

- Warm water (optimal near 98.6)
- Nutrients in the water (soil, organics)
- Other microbes (bacteria and Protozoa/amoebae)
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

Amoebae

Bacteria Provide Nutrients
Flow and Legionella

ORIGINAL ARTICLE

Effect of flow regimes on the presence of Legionella within the biofilm of a model plumbing system

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4 Department of Civil and Environmental Engineering, University of Pittsburgh, Pittsburgh, PA, USA

Liu et al 2006 J Appl Microbiol
Study of the Effects of Flow on Legionella

- Model water system constructed
- Flow regimes by Reynolds number
  - Laminar <1000
  - Turbulent >10,000
- Legionella cultures of biofilm & bulk water
Results and Conclusions

• Results failed to show that stagnation promoted growth
• Increasing flow velocity leads to higher biofilm density due to mass transfer
• Higher transfer rate results in greater particle deposition
Materials of Construction (Piping) and Effect of Temperature
Influence of Temperature and Plumbing Material Selection on Biofilm Formation and Growth of * Legionella pneumophila * in a Model Potable Water System Containing Complex Microbial Flora

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Received 24 November 1993/Accepted 10 March 1994

Survival and growth of * Legionella pneumophila * in both biofilm and planktonic phases were determined with a two-stage model system. The model used filter-sterilized tap water as the sole source of nutrient to culture a naturally occurring mixed population of microorganisms including virulent * L. pneumophila *. At 20°C, * L. pneumophila * accounted for a low proportion of biofilm flora on polybutylene and chlorinated polyvinyl chloride, but was absent from copper surfaces. The pathogen was most abundant on biofilms on plastics at 40°C, where it accounted for up to 50% of the total biofilm flora. Copper surfaces were inhibitory to total biofouling and included only low numbers of * L. pneumophila * organisms. The pathogen was able to survive in biofilms on the surface of the plastic materials at 50°C, but was absent from the copper surfaces at the same temperature. * L. pneumophila * could not be detected in the model system at 60°C. In the presence of copper surfaces, biofilms forming on adjacent control glass surfaces were found to incorporate copper ions which subsequently inhibited colonization of their surfaces. This work suggests that the use of copper tubing in water systems may help to limit the colonization of water systems by * L. pneumophila *.
Results

• Growth most abundant on plastics at 40°C (104°F). *Warm preferred*

• No growth on copper at 20°C *Too cold*

• Survived in biofilm on plastic at 50°C (122°F)

• Not detected at 60°C (140°F) *Too hot*

• Copper inhibitory – new copper may help limit *Legionella* growth
Biofilm formation and multiplication of *Legionella* in a model warm water system with pipes of copper, stainless steel and cross-linked polyethylene

Dick van der Kooij\textsuperscript{a,*}, Harm R. Veenendaal\textsuperscript{a}, Will J.H. Scheffer\textsuperscript{b}

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Pipe Materials

RESEARCH ARTICLE

Microbial diversities (16S and 18S rRNA gene pyrosequencing) and environmental pathogens within drinking water biofilms grown on the common premise plumbing materials unplasticized polyvinylchloride and copper

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¹Dynamac Corp c/o US EPA, Cincinnati, OH, USA; ²National Exposure Research Laboratory, US EPA, Cincinnati, OH, USA; ³Department of Biological Sciences, Kent State University, Kent, OH, USA; and ⁴School of Public Health, University of Alberta, Edmonton, AB, Canada

Buse H.Y. et al. FEMS Microbiol Ecol 2014; 88:280-95
Study Design

- 16s and 18s rRNA gene pyrosequencing
- Characterized the microbial community structure within biofilms established on unplasticized polyvinyl chloride (uPVC) and copper (Cu) surfaces
- Evaluated the impact of introducing *Legionella pneumophila* (Lp) and *Acanthamoeba polyphaga*
Results

• Drinking water biofilms host complex communities that vary based on substratum
• Clear differences were observed between uPVC and copper
• Introduction of *Legionella* alone or with *A. polyphaga* had no effect on bacterial community profiles (P > 0.05)
Study of 28 High-rise Buildings

WAS LEGIONELLA UBIQUITOUS?
Legionella Positivity

• 53.5% (15/28) were positive

• 73.3% (11/15) of positive buildings had *Legionella pneumophila*, serogroup 1 detected
Positive vs. Negative Buildings

• No significant difference in:
  ▪ Cold water free chlorine level (0.38 mg/L vs. 0.36 mg/L)
  ▪ Outlet temperature, chlorine or TOC
  ▪ pH
  ▪ Calcium, zinc, copper, magnesium, manganese
  ▪ No correlation between distal HPC and distal positivity
Hot Water Return Line

• Significant association between hot water return line positivity and outlet positivity (p=0.002)
  ▪ 91.7% (11/12) systems with positive hot water return lines had at least one positive outlet
  ▪ 27.3% (9/33) systems with negative hot water return lines had at least one positive outlet
What Next Generation Sequencing Analysis of Bacterial Community Structure Can Tell Us About *Legionella* Risk in Building Premise Plumbing

Xiao Ma, PhD¹; David Pierre¹; Kyle Bibby, PhD²; Janet Stout, PhD¹,²

1. Special Pathogens Laboratory
2. University of Pittsburgh
- Incoming cold water
- Cold water from storage tank
- Hot water return line
- Distal site hot water

- DNA isolation
- 16S rRNA gene amplicon library

- High throughput sequencing

- Culture testing for *Legionella* spp.
Differences in the Microbial Community (Phyla) by Sample Location

- Cold Water
- Hot Water Return Line
- Outlet

- Other phyla
- Chlorobi
- Firmicutes
- Cyanobacteria
- Actinobacteria
- [Thermi]
- Planctomycetes
- Gemmatimonadetes
- Acidobacteria
- Bacteroidetes
- Proteobacteria
Conclusions

• Bacterial community structure changes correlated with the presence of culturable *Legionella*;
  ▪ Certain phyla (Bacteroidetes, Gemmatimonadetes, and Chloracidobacteria) were significantly enriched in *Legionella* culture positive water samples (p-values < 0.05)
  ▪ Proteobacteria were significantly enriched in *Legionella* culture negative water samples (p < 0.05).
Conclusions

• Findings highlight the need for further research of ecological relationships between premise plumbing bacterial communities and *Legionella* colonization.
Manuscript In Preparation

Bacterial Community Structure Correlates with *Legionella* Colonization of High Rise Building Premise Plumbing Systems

Xiao Ma¹, David Pierre¹, Kyle Bibby²,³, Janet E. Stout¹,²,*

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³Department of Computational and Systems Biology, University of Pittsburgh Medical School, Pittsburgh, PA 15261, USA
Some Areas of Concern

OTHER WATER SOURCES
Water-Saving Low Flow Faucets

Manual Faucet

Sensor Faucet
Sensor Faucets

• Used because...
  ▪ Meets code
  ▪ Saves water (low flow)
  ▪ Automatically turns off
  ▪ Tempered water
  ▪ Non-touch fixture

• Published reports of bacterial contamination
Disturbing Findings


Non-touch fittings in hospitals:
a possible source of Pseudomonas aeruginosa and Legionella spp.

M. Halabi*, M. Wiesholzer-Pittl†, J. Schöberl† and H. Mittermayer‡
Electronic-Eye Faucets: *Legionella* Species Contamination in Healthcare Settings

Emily R. M. Sydnor, MD, MHS; Gregory Bova; Anatoly Gimburg, BEE; Sara E. Cosgrove, MD, MS; Trish M. Perl, MD, MSc; Lisa L. Maragakis, MD, MPH

Infection Control Hospital Epid 2012
• Johns Hopkins Study
  ▪ Newly installed electronic faucets found to have greater risk for \textit{Legionella} than existing standard fixtures.
Anatomy of an Electronic Faucet

A = aerator
B = solenoid valve
C = check valve
D = inline filter
Decorative Fountains

Water Wall

Sponge-like foam material
1.2 million CFU *Legionella*
Ice/Water Dispensers

• Cause for concern
  ▪ Cold water feed
  ▪ Increased internal water temperatures

• Location
  ▪ Service to at-risk individuals
Epidemiology and Infection Prevention

HEALTHCARE-ACQUIRED CASES
Vital Signs: Deficiencies in Environmental Control Identified in Outbreaks of Legionnaires’ Disease — North America, 2000–2014

Laurel E. Garrison, MPH\(^1\); Jasen M. Kunz, MPH\(^2\); Laura A. Cooley, MD\(^1\); Matthew R. Moore, MD\(^1\); Claressa Lucas, PhD\(^1\); Stephanie Schrag, DPhil\(^1\); John Sarisky, MPH\(^2\); Cynthia G. Whitney, MD\(^1\)
Hospitals at Significant Risk According to Recent CDC Report

Legionella in hospitals – accounted for 57% of all cases and 85% of deaths
Whose Case Is It?

Was it community or hospital-acquired?
Hospital-Acquired or Community-Acquired?

• Incubation period for Legionnaires’ disease is typically 2-10 days, but can be longer

• **Definite Case**: a patient admitted at least 10 days prior to onset of illness.

• **Probable Case**: admitted for a portion of the incubation period prior to onset, including patients discharged and re-admitted within the incubation period.
Problem

• The average length of stay for pneumonia in the U.S. was 5.4 days.*

• Very few cases will fulfill the definition for “Definite” healthcare-acquired Legionnaires’ disease

*According to the Healthcare Cost and Utilization Project Nationwide Inpatient Sample from the Agency for Healthcare Research and Quality
If the Health Department Comes

• Initial environmental testing (case patient exposure areas) plus assess building colonization status
• Water restrictions (bottled water, no showering)
• Emergency disinfection – was it successful?
• Sample for *Legionella* every 2 weeks for 3 months, then monthly for 3 more months
• If *Legionella* detected, sequence begins again
• Frequent communication with the Health Department
• Long-term disinfection measures?
Approaches to Prevention

HEALTHCARE-ACQUIRED LEGIONNAIRES’ DISEASE
Denial

Most wait to address the problem until after a case of Legionnaires’ disease is diagnosed.
Approaches to Prevention

**REACTIVE**
- **After** cases identified
  - Case investigation and environmental testing/investigation
  - *Legionella* source identified = decontaminate

**PROACTIVE**
- **Before** cases occur, perform environmental testing
  - *Legionella* source identified = decontaminate
Denial & Legionella Testing

If you don’t look for it, you won’t find it.

If you don’t find it, you don’t think you have a problem.

If you don’t think you have a problem, you don’t do anything about it.

−Bruce Dixon, M.D.
Director, Pittsburgh ACHD
CDC’s Position on Testing

“We are not against testing water for the presence of *Legionella*…

We think it has its place, particularly in healthcare facilities.”

Cynthia Whitney, MD, Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, CDC. June 8, 2016 Pittsburgh Post Gazette
Test to Find Out Where You Stand

Myth – Legionella is not everywhere (ubiquitous)

• Healthcare facilities – greater risk
  ▪ Is your facility in the 50% of buildings with or without *Legionella*?

• Only 30-50% of cooling towers positive for *Legionella*
Why Test for *Legionella*?

- **Assess the risk**
- **Control the risk** (through engineering controls or water treatment)
- **Before cases occur**
- **Prevent and End Legionnaires’ disease!**
Is the Sky Falling?

If I Find Some *Legionella*?
What is the acceptable amount of *Legionella*?
IS ZERO NECESSARY?
Preventing Legionnaires’ Disease Through *Legionella* Control

Zero Cases Is The Goal, Not Zero Legionella
Don’t Chase Zero

Zero *Legionella* is virtually impossible to achieve in complex water systems.
Preventing Legionnaires’ Disease

• Controlling *Legionella* is about preventing disease not about reaching zero *Legionella* in water.
Testing Is Revealing

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

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

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

Pittsburgh, Pennsylvania

Pittsburgh Proactive Approach

Culture hot water systems. Was *Legionella* found?

- YES
  - Prior cases of legionellosis observed?
    - YES
      - Colonization of distal sites >30%?
        - YES
          - Prospective clinical surveillance detected legionellosis?
        - NO
          - Consider Secondary Disinfection
    - NO
      - Continue Environmental Surveillance
  - NO

Reference:
*Approaches to Prevention and Control of Legionella Infection in Allegheny County Health Care Facilities.* 1997.
Study Findings

- Reviewed 487 reported cases from 1991-2001 (Pre- and-post guideline)
- Proportion of cases hospital-acquired:
  - Pre-guideline = 33%
  - Post-guideline = 9%
- Significant decrease in the post guideline period (p<0.0001)
Our Study Results of 48 Hospitals

- Proportion of hospitals performing environmental surveillance = 65%

- Proportion that started water treatment (disinfection) = 44%
New York Is Being Proactive!

First state-wide regulation requires testing and water management plans for cooling towers AND potable water systems of healthcare facilities.
New New York State Regulation for Legionella Prevention

• Requires that all covered healthcare facilities adopt and implement:
  ▪ a sampling and management plan for their potable water systems by December 1, 2016, and
  ▪ new covered facilities must adopt such plan prior to providing services.
## Interpretation of Routine *Legionella* Culture Results from Covered Facilities

<table>
<thead>
<tr>
<th>Percentage of Positive <em>Legionella</em> Test Sites</th>
<th>Response</th>
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<tbody>
<tr>
<td>&lt;30%</td>
<td>Maintain environmental assessment and <em>Legionella</em> monitoring in accordance with the sampling and management plan</td>
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</table>
| >30%                                          | Institute short-term control measures and notify the department.  
  • Re-sample no sooner than 7 days and no later than 4 weeks after disinfection  
  • If retest is ≥ 30% positive, repeat short-term control measures.  
  • If results < 30% positive, resume monitoring in accordance with the sampling and management plan. |
Where Did the 30% Target Come From?
EVIDENCE-BASED ORIGINS: 30% DISTAL SITE POSITIVITY AS RISK INDICATOR

THE LANCET, AUGUST 6, 1983

LEGIONELLACEAE IN THE HOSPITAL WATER-SUPPLY

Epidemiological Link with Disease and Evaluation of a Method for Control of Nosocomial Legionnaires’ Disease and Pittsburgh Pneumonia

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Correlation Between Disease and Distal Site Positivity

• Whenever monthly site positivity exceeded 30%, cases of Legionnaires’ disease appeared in those months.

• Similarly, when positivity fell to 20% or less, no case of disease were observed.
Risk of Legionnaires’ disease was better predicted by the proportion of water system sites testing positive for *Legionella* than by the concentration of *Legionella* bacteria.

Role of Environmental Surveillance in Determining the Risk of Hospital-Acquired Legionellosis: A National Surveillance Study With Clinical Correlations

Janet E. Stout, PhD; Robert R. Muder, MD; Sue Mietzner, MS; Marilyn M. Wagener, MS; Mary Beth Perri, BS; Kathleen DeRoos, MSN; Dona Goodrich, BS; William Arnold, MS; Theresa Williamson, MS; Ola Ruark, MSN; Christine Treadway, MSN; Elizabeth C. Eckstein, MSN; Debra Marshall, RN; Mary Ellen Rafferty, MS; Kathleen Sarro, RN; Joann Page, MS; Robert Jenkins, BA; Gina Oda, MS; Kathleen J. Shimoda, RN, BS; Marcus J. Zervos, MD; Marvin Bittner, MD; Sharon L. Camhi, MD; Anand P. Panwalker, MD; Curtis J. Donskey, MD; Minh-Hong Nguyen, MD; Mark Holodniy, MD; Victor L. Yu, MD; and the Legionella Study Group
Risk Assessment and Environmental Monitoring Results

- **Concentration-based** thresholds that establish target values in CFU per liter or milliliter
  - OK for cooling towers as a performance-based target (not health-based)

- **Colonization rate** or proportion of distal sites in the water system that are positive for *Legionella*
  - Best for hospitals and building water systems
SAMPLE COLLECTION:
FIRST DRAW HOT WATER
Do Not Flush Prior to Sample Collection

• No Flush
  ▪ Collect immediately after opening faucet or shower valve
  ▪ Flushing reduces recovery
  ▪ Immediate draw 97.7% positive reduced to 69.1% after 2 min. flush
BIOFILM AT THE PERIPHERY

PRE FLUSH (Positive)

FLUSH TO WASTE

POST FLUSH (Negative)

Courtesy M. Weinbren
How Do You Measure *Legionella* Risk From Your Cooling Towers?
SAMPLE COLLECTION: FROM THE BASIN
You Can’t Tell by Looking

Automated dosing of chemical biocides and clean → >3000 CFU/mL *Legionella pneumophila* serogroup 1
LEGIONELLA UNDER THE MICROSCOPE
Colonies of *Legionella pneumophila* have a typical ground-glass, opalescent morphology.
Legionella Testing According to CDC

- CDC recommends using a testing method capable of detecting all members of the Legionella genus but also one that provides material for typing.
- At the moment, this means culture.
- Particularly true during an investigation and in the immediate aftermath
Legionella Testing

• Culture is more reliable (sensitive & specific) than other “rapid tests”

• Newer methods/approaches
  - Molecular (qPCR and microarray)
  - Most Probable Number (L. pneumophila only)
  - Immunochromatographic (ICT) test
Genus-specific PCR: Reactions With Non-Legionella Bacteria

Cross-reacting 16s rRNA sequences (false positive) were identified for *Aeromonas, Pseudomonas*, and *Mycobacteria*

Am. Society for Microbiology 2016,
Are All Legionella Alike?
Not All *Legionella* Are Dangerous
Not All Legionella Are Pathogenic

• If you find *Legionella* – what type did you find?

• There are over 60 species and serogroups
  - Not all *Legionella* have the same risk
  - *L. pneumophila* serogroup 1 has highest risk for disease
  - Many species common in water, but rarely cause infection (much less risk)
Legionella Species That Fluoresce Under UV Light

- Some fluoresce red and some fluoresce blue-white
- Blue-white species
  - *L. anisa*
  - *L. dumoffii*
  - *L. gormanii*
  - *L. bozemanii*

*Legionella rubrilucens*
Approximately Half of the 60 Named Species Have Been Implicated In Human Disease

(But We Should Assume All Can Equally Cause Illness?)
Species and Virulence

CONCISE COMMUNICATION

Distribution of *Legionella* Species and Serogroups Isolated by Culture in Patients with Sporadic Community-Acquired Legionellosis: An International Collaborative Survey

Victor L. Yu,¹ Joseph F. Plouffe,² Maddalena Castellani Pastoris,⁵ Janet E. Stout,¹ Mona Schousboe,⁶ Andreas Widmer,⁸ James Summersgill,⁴ Thomas File,³ Christopher M. Heath,⁹ David L. Paterson,¹,¹⁰ and Annette Chereshsky⁷,¹¹

¹Veterans Affairs Medical Center, Pittsburgh, Pennsylvania; ²Ohio State University, Columbus, and ³Northeastern College of Medicine, Akron, Ohio; ⁴University of Louisville, Louisville, Kentucky; ⁵Instituto Superiore di Sanita, Rome, Italy; ⁶Canterbury Health Laboratories, Christchurch, and ⁷Communicable Disease Centre, Porirua, New Zealand; ⁸Kantonsspital, Basel, Switzerland; ⁹Royal Perth Hospital, Perth, and ¹⁰Princess Alexandra Hospital, Brisbane, Australia

Yu, et al. 2002 18886: 127-8
508 Culture-confirmed Cases

- 91.5% *L. pneumophila*
- 84.2% *L. pneumophila*, serogroup 1
- 7.4% other serogroups
- Other *Legionella* species
  - *L. longbeachae* 3.9%
  - *L. bozemanii* 2.4%
  - All others 2.2%
What’s In Your water?
Lots of Bacteria In Our Water
Microbial Communities

The Microbiome
Microbiome

The collective genetic material of microorganisms that reside in an environmental niche
Millions of Bacteria In Our Pipes
Microbiological Testing: HPC (Total) Bacteria

What Do You Learn From Testing For HPC?
Table 2
HPC genera commonly found in drinking water

<table>
<thead>
<tr>
<th>Acinetobacter</th>
<th>Methylomonas</th>
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</thead>
<tbody>
<tr>
<td>Actinomycetes</td>
<td>Micrococcus</td>
</tr>
<tr>
<td>Alcaligenes</td>
<td>Mycobacterium</td>
</tr>
<tr>
<td>Aeromonas</td>
<td>Morexella</td>
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<tr>
<td>Aeromonas hydrophila</td>
<td>Nitrospira</td>
</tr>
<tr>
<td>Arthrobacter</td>
<td>Proteus</td>
</tr>
<tr>
<td>Bacillus</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>Beggiatoa</td>
<td>P. cepacia</td>
</tr>
<tr>
<td>Citrobacter</td>
<td>P. fluorescens</td>
</tr>
<tr>
<td>Co.</td>
<td>P. maltophilia</td>
</tr>
<tr>
<td>Enterobacter agglomerans</td>
<td>Serratia liquefaciens</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>Sphaerotilus</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Sphingomonas</td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>Staphylococcus</td>
</tr>
<tr>
<td>Flavobacterium meningosepticum</td>
<td>Streptococcus</td>
</tr>
<tr>
<td>Gallionella</td>
<td>Streptomyces</td>
</tr>
<tr>
<td>Hafnia alvei</td>
<td>Yersinia enterocolitica</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td></td>
</tr>
</tbody>
</table>
Heterotrophic Plate Count (HPC) Bacteria Testing

What do we know?

- HPC method does not detect Legionella
- HPC cannot be used to predict presence or absence of *Legionella*
- HPC counts can be **low** when *Legionella* is **high**
EPA Surface Water Treatment Rule (SWTR)

- Public water systems under the influence of surface water must maintain a detectable disinfectant level in the distribution or
- Maintain HPC <500 CFU/mL in the distribution system
  - This criteria is now being applied by some consultants incorrectly to building water systems
Total Bacterial (HPC) Counts Often >500 CFU/mL

Fig. 7. HPC bacteria concentration in hot water samples decreased significantly after ClO₂ treatment (t-test, p<0.05).

Lack of correlation between *Legionella* colonization and microbial population quantification using heterotrophic plate count and adenosine triphosphate bioluminescence measurement

Scott Duda · Julianne L. Baron · Marilyn M. Wagener · Radisav D. Vidic · Janet E. Stout
Water Is a Source of Infection Especially for High Risk Patients – Including Neonates
High Risk Patients

- Should have water that meets a higher standard for microbiological quality than normal tap water
Who’s at Greatest Risk

- Immunocompromised
  - Transplant patients (bone marrow and solid organ)
  - High-dose steroids for lung disease
  - Diabetes
  - Cancer (Hematology/oncology units)
  - Neonates (NICU)
Healthcare-acquired Pediatric LD

- CDC study showed 72% pediatric cases had healthcare exposure
- Mortality rate 22%

Alexander NT, et al 2008 ICAAC
We Filter the Air…
But Let Them Drink Tap Water?

PROTECTING TRANSPLANT, HIGH RISK PATIENTS, NEONATES
Efficacy of new point-of-use water filter for preventing exposure to *Legionella* and waterborne bacteria

Patricia J. Sheffer, MPM, a Janet E. Stout, PhD, a,b Marilyn M. Wagener, MPH, b and Robert R. Muder, MD a,b
Pittsburgh, Pennsylvania

*Background:* *Legionella* species cause health care-acquired infections in which immunocompromised patients are disproportionately affected. Epidemiologic studies have demonstrated that point-of-use water fixtures are the reservoirs for these infections. The current approach to prevention is system-wide chemical disinfection of the hospital water system. These methods affect both low-risk and high-risk areas. A more effective approach to prevention may be a targeted approach aimed at protecting high-risk patients. One option is the application of a physical barrier (filter) at the point-of-use water fixture.

Field evaluation of a new point-of-use faucet filter for preventing exposure to *Legionella* and other waterborne pathogens in health care facilities

Julianne L. Baron PhD\textsuperscript{a,b}, Tammy Peters RN, BSN, COHN-S\textsuperscript{c}, Raymond Shafer\textsuperscript{c}, Brian MacMurray\textsuperscript{c}, Janet E. Stout PhD\textsuperscript{b,d,*}

AJIC 2014; 42: 1193-1196
Filters Prevent Recovery of *Legionella* for 12 weeks

Results are the average of 5 sites.

Blue bars = Control Faucets/no filter
Point-of-Use Filtration

- High Risk Patients
- Bone marrow and solid organ transplant units
- Hematology/oncology units
- NICU
I Have Legionella in My Building… Now What?

DISINFECTION APPROACHES
Hospitals are often required to perform a supplemental disinfection of their water systems to protect individuals from hospital-acquired Legionnaires' disease. The authors of this article recently studied one hospital where three cases of hospital-acquired Legionnaires' disease were detected in less than two years. These cases were linked to Legionella contamination of the hospital's water system. Chlorine dioxide (ClO₂) was considered a cost-effective approach to disinfection given that ClO₂ generators could treat the 11 buildings comprising the hospital complex from one central location.

The authors evaluated the efficacy of maintaining a residual of 0.55 to 0.68 mg/L of ClO₂ for Legionella control in the secondary distribution system of this 471-bed hospital over a six-year period. Monthly monitoring showed mean Legionella positivity at hot water outlets and cold building source water sites decreased from 22 to 15%, and 0 to 0%, respectively (p < 0.05). ClO₂ residuals decreased with increasing distance from the application point and temperature. Mean ClO₂ concentrations were found in hot water outlets (0.89 mg/L) followed by cold water outlets (0.30 mg/L) and reservoirs (0.09 mg/L). Colony enumeration (0% positivity) of Legionella was achieved after 1.79 years, and no cases of Legionnaires' disease were reported during this time.

keeping Legionella out of water systems

DISINFECTION OPTIONS
Secondary Disinfection Methods

- Thermal shock treatment (heat & flush)
- Shock chlorination (>10 mg/L residual), may require water tanks to be 20-50 mg/L
- Continuous chlorination (2-4 mg/L)
- Copper-silver ionization (continuous and short-course)
- Chlorine Dioxide (ClO2)
- Monochloramine
- Point-of-use filtration
EPA: Safe Drinking Water Act

Safe Drinking Water Act (SDWA)

The Safe Drinking Water Act (SDWA) is the main federal law that ensures the quality of Americans' drinking water. Under SDWA, EPA sets standards for drinking water quality and oversees the states, localities, and water suppliers who implement those standards.

SDWA was originally passed by Congress in 1974 to protect public health by regulating the nation's public drinking water supply. The law was amended in 1986 and 1996 and requires many actions to protect drinking water and its sources: rivers, lakes, reservoirs, springs, and ground water wells. (SDWA does not regulate private wells which serve fewer than 25 individuals.) For more information see:

http://water.epa.gov/lawsregs/rulesregs/sdwa/index.cfm
# Building Disinfection Options

<table>
<thead>
<tr>
<th>Municipal Water Treatment</th>
<th>Building Water Treatment</th>
</tr>
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<tbody>
<tr>
<td>Chlorine</td>
<td>Chlorine</td>
</tr>
<tr>
<td>Chlorine Dioxide</td>
<td>Chlorine Dioxide</td>
</tr>
<tr>
<td>Monochloramine</td>
<td>Monochloramine</td>
</tr>
<tr>
<td>UV</td>
<td>UV</td>
</tr>
</tbody>
</table>

* Copper-Silver Ionization
* Point of Use Filters

* Not Covered in SDWA
States Vary In Interpretation and Enforcement of SDWA

• Hot vs. cold water application

• Requirements for certified operators

• What disinfection methods will be permitted
How to Choose?
Evidence-based Approach to Evaluating Efficacy

• Four Steps

  1. In vitro laboratory testing
  2. Reports of anecdotal experience of efficacy
  3. Controlled field studies
  4. Confirmatory reports from multiple hospitals with prolonged duration of follow-up
Evaluation of A New Monochloramine Generation System for Controlling Legionella In Building Hot Water Systems

Scott Duda, MS; Sheena Kandiah, MD, PhD; Janet E. Stout, PhD; Julianne L. Baron, BS; Mohamed Yassin, MD, PhD; Marie Fabrizio, BSN, CIC; Juliet Ferrelli, MS, MT (ASCP) CIC; Rahman Hariri, PhD; Marilyn M. Wagener, MS; John Goepfert; James Bond; Joseph Hannigan, CWT; Denzil Rogers
Significant Reduction in Legionella

>30% Cases

<30% No Cases
Copper-silver Ionization

Distal Site Positivity in Copper-silver Treated Building

- 90% on 11/1/2015
- 40% on 4/1/2016
- 10% on 8/1/2016
- 0% on all other dates from 1/1/2016 to 3/1/2018

© Special Pathogens Laboratory
Chlorine Dioxide

Hospitals are often required to perform a supplemental disinfection of their water systems to protect individuals from hospital-acquired Legionnaires' disease. The authors of this article recently studied one hospital where three cases of hospital-acquired Legionnaires' disease were detected in less than two years. These cases were linked to Legionella colonization of the hospital’s water system. Chlorine dioxide (ClO₂) was considered a cost-effective approach to disinfection given that ClO₂ generators could treat the 22 buildings comprising the hospital complex from one central location. The authors evaluated the efficacy of maintaining a residual of 0.5 to 0.8 mg/L of ClO₂ for Legionella control in the secondary distribution system of this 437-bed hospital over a two-year period. Monthly monitoring showed mean Legionella positivity at hot water outlets and cold building source water areas decreased from 22 to 12% and 9 to 0%, respectively (p < 0.05). ClO₂ residuals decreased with increasing distance from the application point and temperature. Mean ClO₂ concentrations were lowest in hot water outlets (0.08 mg/L) followed by cold water outlets (0.32 mg/L) and reservoirs (0.88 mg/L). Complete eradication (0%) positivity of Legionella was achieved after 1.75 years, and no cases of Legionnaires’ disease were reported during this time.


Significant Reduction in *Legionella*
Required More Than 1 Year

*Figure 2.* Declining monthly *Legionella* positivity in hot water over time.
Disinfection Methods Review

Controlling *Legionella* in Hospital Drinking Water: An Evidence-Based Review of Disinfection Methods

Yusen E. Lin, PhD, MBA; Janet E. Stout, PhD; Victor L. Yu, MD

Choice of method must include infection control in addition to engineering
Disinfection Review

Maintaining *Legionella* control in building water systems

This article reviews how *Legionella* and other waterborne pathogens can present a risk to consumers of potable water. In particular, building hot water systems have been established as the primary reservoir for bacteria linked to cases of Legionnaires’ disease (LD). These systems provide ideal conditions for *Legionella* proliferation because of their elevated temperature and lack of disinfection residual. Control of *Legionella* in potable water systems has become a focus for health care facilities because they serve a population that is particularly susceptible to LD from underlying health conditions, such as suppressed immune systems.

Journal Am Water Works Assoc 2014; 106(10): 24-32
EPA Issues Review of Legionella Control & Disinfection Methods

Technologies for *Legionella* Control in Premise Plumbing Systems:
Scientific Literature Review
Legionella
A HIGHLY SUCCESSFUL MICROBE
Legionella Bumper Sticker

Proud of My Highly Successful Microbe!
Measures of Success

• Numbers up?
• In the news?
• Famous people talking about you?
• Getting too famous – industry and government agencies after you?
Successful Microbe: Disease Incidence Increasing?

Legionnaires’ Disease Is On the Rise
2000–2015*

*National Notifiable Diseases Surveillance System

© Special Pathogens Laboratory
Legionnaires' death toll rises to 10 in western Illinois

Published September 09, 2015

QUINCY, Ill. – At least 10 people in western Illinois have now died from Legionnaires' disease, after a state veterans home reported two new fatalities among its residents.

An outbreak first identified in late August after an initial case was detected several weeks earlier has sickened 53 residents at the Quincy home, nine of whom died. Four others in Quincy have been diagnosed with the illness, one of whom died. Officials say those cases aren't connected to the larger outbreak.
Hotel Outbreaks

Legionellosis Outbreak at the Aria Hotel, 2009-2011 --Las Vegas, Nevada

Public Health Investigation Final Report
Hospital Construction

• New $135M 12-story patient tower

• Media coverage and legal action

• $61k for outbreak management
South Bronx Outbreak 2015

- 138 cases, 16 deaths
- 26 were culture-confirmed with *Legionella pneumophila*, serogroup 1 isolated.
Outbreak Linked to Hotel Cooling Tower
Emergency Regulation

- Cooling towers must be registered
- *Legionella* testing every 90 days
- Inspection
- Certification
- Maintenance program

**Department of Health**

**EMERGENCY RULE MAKING**

Protection Against Legionella

*Action taken:* Addition of Part 4 to Title 10 NYCRR.

*Statutory authority:* Public Health Law, section 225(5)(a)

*Finding of necessity for emergency rule:* Preservation of public health, public safety and general welfare.

*Specific reasons underlying the finding of necessity:* Improper maintenance of cooling towers can contribute to the growth and dissemination of *Legionella* bacteria, the causative agent of legionellosis. Legionellosis causes cough, shortness of breath, high fever, muscle aches, headaches and can result in pneumonia. Hospitalization is often required, and between 5-30% of cases are fatal. People at highest risk are those 50 years of age or older, current or former smokers, those with chronic lung diseases, those with weakened immune systems from diseases like cancer, diabetes, or kidney failure, and those who take drugs to suppress the immune system during chemotherapy or after an organ transplant. The number of cases of legionellosis reported in New York State between 2005-2014 increased 323% when compared to those reported in the previous ten year period.
I Love New York!
Flint, Michigan

Flint water crisis likely the cause of deadly Legionnaires outbreak

By Sara Ganim, CNN  Mar 30, 2017
Measures of Success

✓ Increasing ratings?
✓ In the news?
  • Famous people talking about you?
  • Getting too famous – industry and government agencies after you?
Successful *Legionella*

**FAMOUS PEOPLE TALKING ABOUT YOU?**
Erin Brockovich and *Legionella*?

- “Legionnaires’ disease cases are on the rise in New York because of poor enforcement of clean water regulations.”
Hollywood Walk of Fame for *Legionella*?
Measures of Success

✓ Increasing ratings?
✓ In the news?
✓ Famous people talking about you?
  • Getting too famous – industry and government agencies after you?
Legionella Has Their Attention

- NASEM
- ASHRAE
- CDC
- CMS
- EPA
- NSF
- Industry organizations
  AWT, CTI, NSF
Important Questions

• Are there important differences in occurrence and management of *Legionella* with respect to:
  - Cold water vs. hot water systems? [Warm]
  - Healthcare vs. community-acquired? [Different]
  - *Legionella pneumophila* vs. other species?
    • *Legionella pneumophila*, serogroup 1!
Important Questions

• Routine testing for *Legionella* vs. public health investigations?
  ▪ Need clear distinction (different criteria) for sample collection and results interpretation

• What defines controlled?
  ▪ Not zero *Legionella*, zero cases!
  ▪ Be careful using HPC and ATP – not predictive and could trigger expensive (unnecessary) action
Legionella Anxiety?
Treatment for Legionella anxiety

MEDICATION
Dr. Stout’s Legionella Chill Pills

• For treatment of Legionella-related anxiety.

• Take 2 tablets 1 hour before testing or starting your ASHRAE 188 Water Management Program
SPL’s Mission:
End Legionnaires’ Disease

• No one should die from a preventable disease caused by a bacteria in water.

• Legionnaires’ disease can and should be prevented.
Let’s End LD Together
THANK YOU

Dr. Janet E. Stout
President, Microbiologist

info@specialpathogenslab.com

WWW.SPECIALPATHOGENSLAB.COM