

Environmental sampling for LD: When and how?

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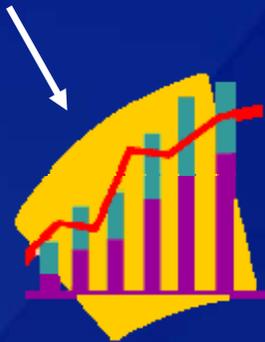


Outline

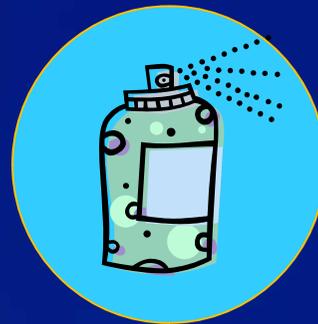
- *Ecology of Legionella*
- Determining when and where to sample
- Collecting samples
- Processing Samples
- Characterizing Isolates
- References and Resources

Events leading to Legionnaires' Disease

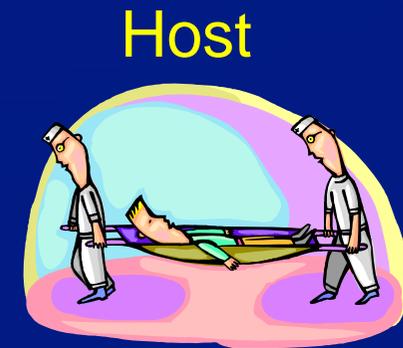
Water Supplies



Amplification



Aerosolization



Host

Transmission

The Bacterium

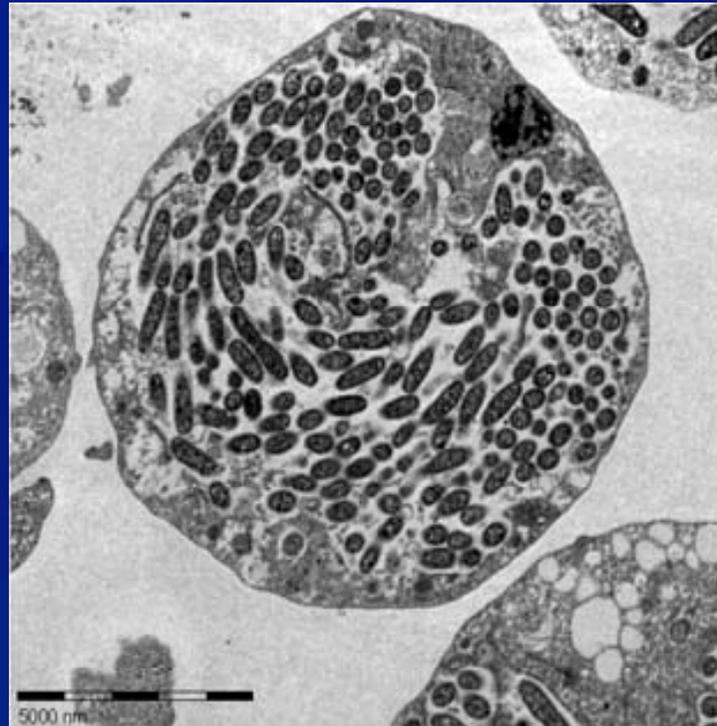


- Gram negative, aerobic rod
- Single polar flagellum
- Require L-cysteine for growth on artificial media
- Facultative intracellular parasite
- *L.pneumophila* sg 1 most common etiological agent

Temperature range of *Legionella* growth

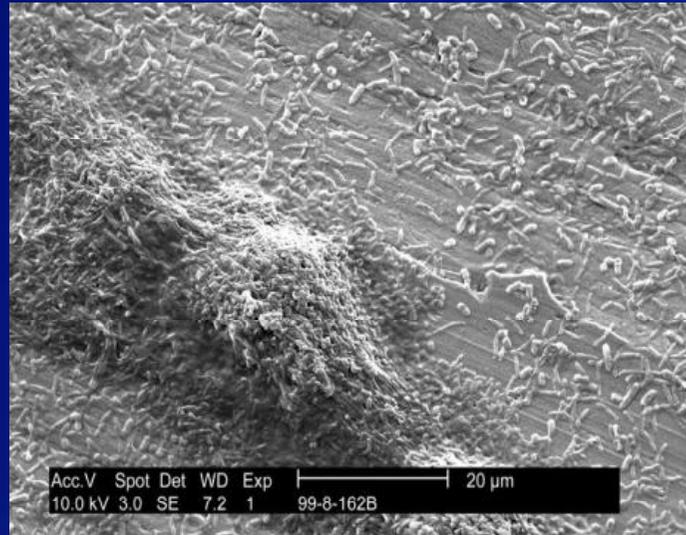


Amoebae shelter *Legionella* from adverse environmental conditions

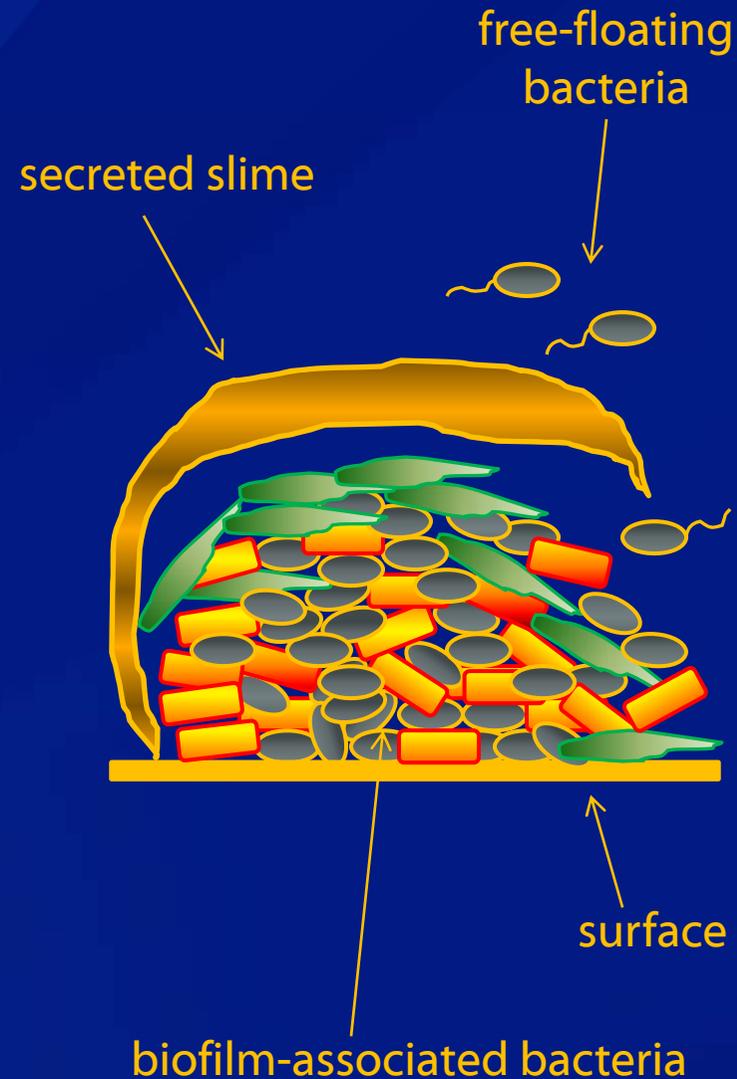


Holland/Özel, Robert Koch-Institut

Legionella are biofilm associated



Biofilm - a community of microorganisms surrounded by the slime they secrete, attached to either an inert or living surface.



Favorable conditions for *Legionella* amplification:

- Temperature 25°C - 42°C
- Stagnation
- Scale and sediments
- Protozoa
- Biofilms
- Natural rubbers, wood and some plastics

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When to take environmental samples:

- 1+ confirmed case of legionellosis
- 2+ suspect cases of legionellosis
- Post remediation
- Transplant facilities
- No recommendations for routine environmental monitoring
- There is no acceptable level of legionellae

Where to take environmental samples:

- Review epidemiological data
- Perform an environmental assessment
 - Cooling towers
 - Potable water (showers and faucets)
 - Whirlpool spas and hot tubs

Environmental Assessment of Water Systems

www.cdc.gov/legionella/files/EnvironmentalAssessmentInstrument.pdf

Environmental Assessment of Water Systems

Assessor's Name: _____ Facility Name: _____
Assessor's Title: _____ Facility Address: _____
Assessor's Organization: _____
Assessor's Address: _____
Assessor's Telephone Number: _____
Date of assessment: _____ Type of Assessment: *(Circle one)*
Time of assessment: _____ On-site assessment
Time needed to complete assessment: _____ Telephone assessment

Note to Assessor:

This environmental assessment instrument may be used where a thorough understanding of a facility's water system is needed to assist facility management in minimizing the risk of legionellosis either in the presence or absence of disease transmission. It should be completed in as much detail as possible. Not all the information specified may be available for or applicable to every facility.

For very large, complex facilities, completion of the form may take several hours. Please keep in mind that this initial investment of time is important. If reassessment is needed in subsequent months or years, the information contained in this document will be very valuable. Do not leave sections blank. If a question does not apply, write "N/A". If a question cannot be answered, explain why. Where applicable, specify the units of measurement being used (e.g., ppm). It is recommended that if the form is being completed electronically, a different font and/or italics should be used. This will make the information much easier to read if additional information is added in the future.

A. Facility Characteristics

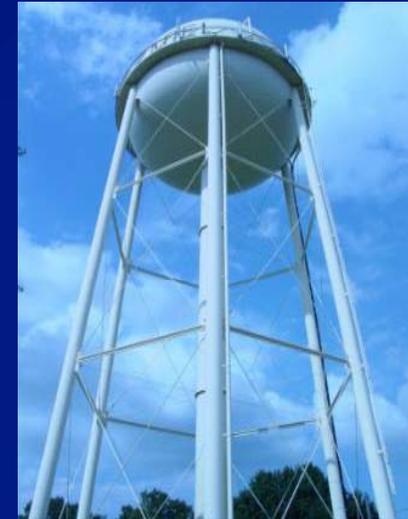
1. Type of facility *(Circle one)*:
 - a. Healthcare facility
 - Hospital with bone marrow or solid organ transplant patients
 - Hospital without bone marrow or solid organ transplant patients
 - Outpatient facility with bone marrow or solid organ transplant patients
 - Outpatient facility without bone marrow or solid organ transplant patients
 - Long-term care facility
 - Outpatient surgical center
 - b. Hotel, motel
 - c. Residential building (e.g., apartment, condominium)
 - d. Office building
 - e. Manufacturing facility

General Facility Characteristics

- Size of facility
- Type and number of guests
- Water usage patterns
- Presence of aerosol generating devices
- Previous association with LD

Water Supply

- What is the source?
- How is the water disinfected?
- Has treatment changed in the last six months?



Recent or ongoing construction

- Interruptions in service
- Changes in water pressure
- Activities which cause the ground to shake
- Changes in color, taste, or smell



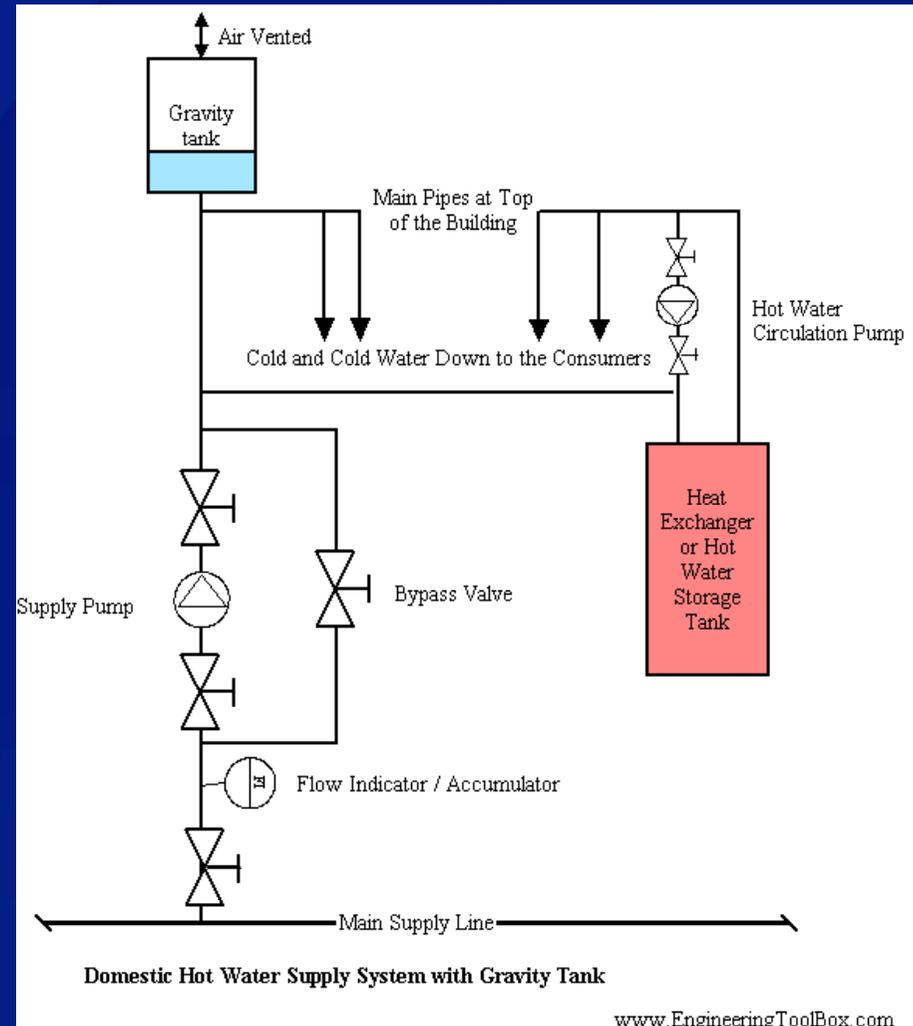
Cooling towers

- Location
- Maintenance
- Modifications or repairs
- Recently turned on or off



Design of the existing potable water system(s)

- Where does the water go?
- How long is it held?
- What is the lowest **HOT** temperature?
- What is the highest **COLD** temperature?



Whirlpool spas and hot tubs

- Location
- Maintenance
- Super-halogenation
- What type of filter



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Who Samples?

The Sampling Team

- A multidisciplinary team of experts
- Should understand design and operation of water system
- Have knowledge of repairs and alterations
- Have knowledge of factors which encourage amplification and transmission of *Legionella*

Top 10 items for sampling

1. Sterile plastic 1 L bottles
2. Sodium thiosulfate – 0.1 N solution
3. Pipettes and bulbs
4. Dacron-tipped swabs
5. Sterile plastic screw top tubes
6. pH test kit
7. Chlorine test kit
8. Thermometer
9. Labels
10. Pads and pens to document samples



Transporting Samples

1. Cooler may be assembled and stored up to 1 year
2. One cooler holds 30 bottles, tubes, and swabs
3. <48 hours transport samples at ambient temperature
4. >48 hours storage refrigerate
5. Never freeze environmental samples



Most commonly sampled locations in a hospital

- Central distribution point (hot water heater)
- Rooms where patients were housed (potable)
- Aerosol generating devices (cooling towers, humidifiers, etc.)
- Medical devices (whirlpool spas, CPAP machines, etc.)

Sampling: Hot Water Heaters



- Look for drain valve from which to take 1 L water sample
- Always check water quality at the HWH
- Rarely obtain biofilm sample

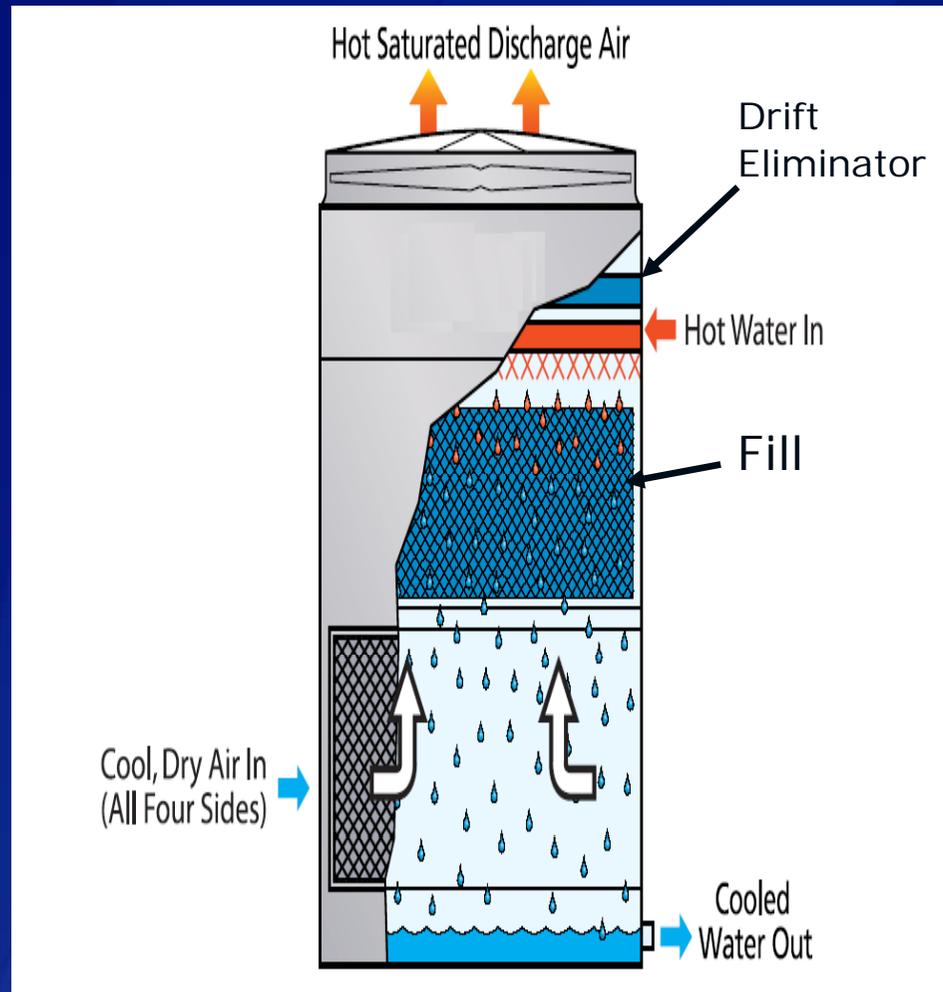
Sampling: Faucets and Showers

➤ Biofilm swab



1. Remove the aerator or showerhead
2. Swab the pipe, pressing firmly
3. Place swab in sterile tube with 3-5 ml water
4. Break off stem so that cap fits securely
5. Take 1 L bulk water sample
6. Add sodium thiosulfate

Sampling: Cooling Towers



- Look for an exit/overflow port to take 1 L water sample
- Always check water quality from the cooling tower
- Biofilm samples at water line and other moist surfaces
- Be aware of personal safety!

Sampling: Whirlpool Spas and Hot Tubs

- Bulk water from the untreated pool
- If pool is empty check compensatory tank
- Biofilm from water line
- Filter sample



Filter Samples:

- Diatomaceous Earth, DE
- Sand
- Cartridge



Sampling: Diatomaceous earth filter



Sampling: Sand filter



Filter samples

diatom
powder



sand

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Processing Samples: Potable



Processing Samples

- Concentration methods:
 - Filtration
 - Centrifugation
- Enrichment methods:
 - Acid Treatment
 - Heat enrichment
 - Co-culture
- Physical separation:
 - Serial dilutions
 - Gradient separation

Note that a single sample may require multiple treatments .

Selecting *Legionella* Isolates

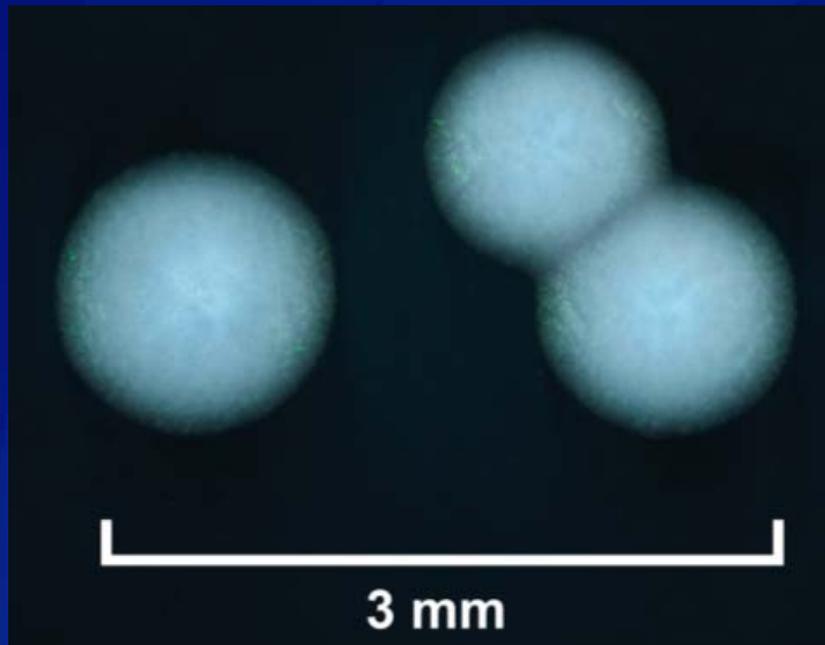


Buffered Charcoal Yeast Extract
(BCYE)

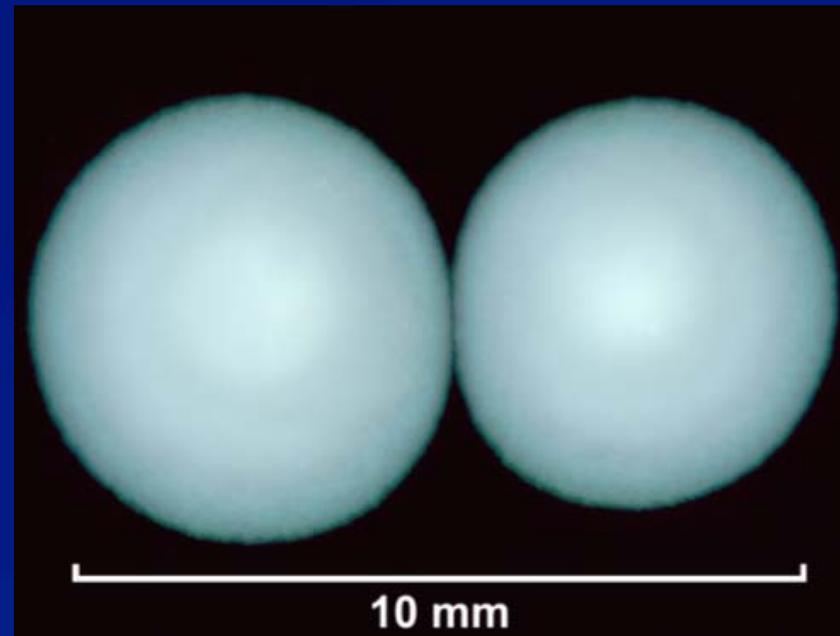
PCV = BCYE + Polymyxin B,
Cyclohexamide, and
Vancomycin

GPCV = PCV + Glycine

Selecting *Legionella* Isolates



3 day *L. pneumophila*



7 day *L. pneumophila*

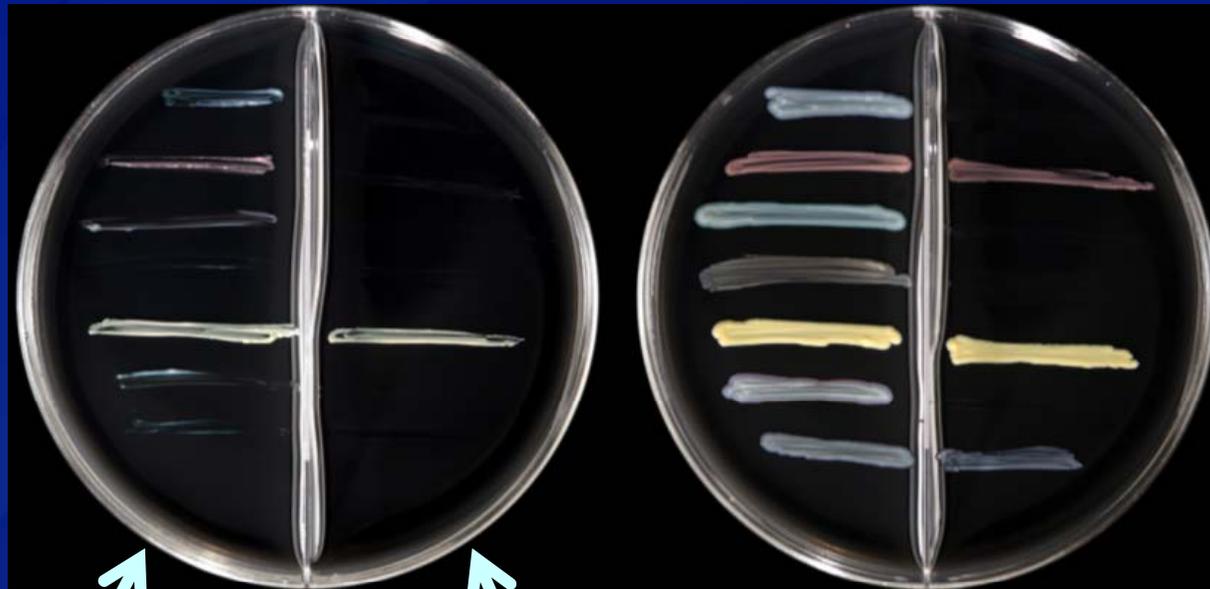
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Confirming *Legionella* Isolates to Genus Level

24 Hours

48 Hours

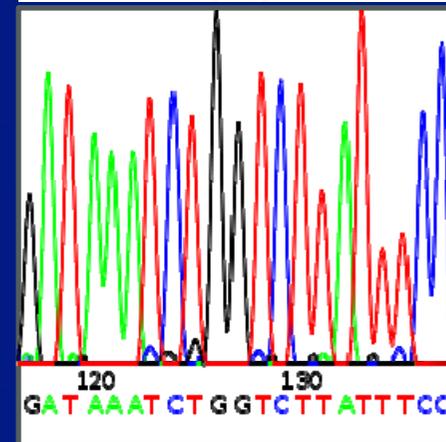
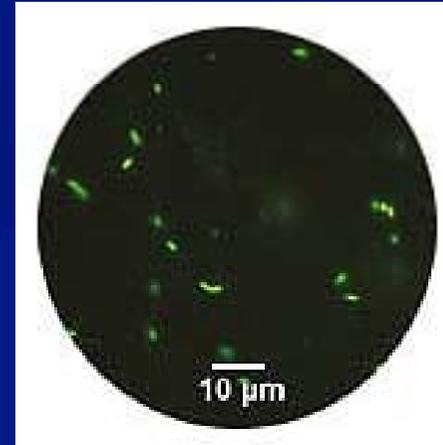


BCYE

BCYE without
L-cysteine

Characterizing Isolates to Species Level

- Serological:
 - DFA
 - Slide Agglutination
 - Dot blot / ELISA
- Molecular:
 - Sequence

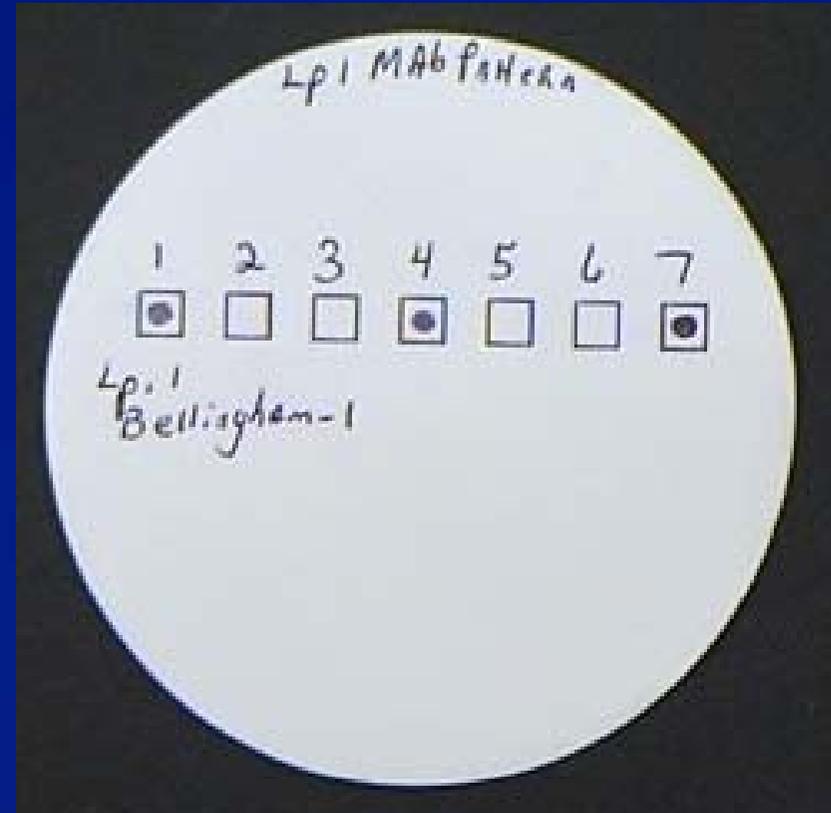


Typing Isolates Beyond Species: Why?

- Match clinical and environmental isolates
- Confirm eradication of outbreak strain post-remediation
- Determine likely outbreak strain(s) when multiple isolates are recovered

Monoclonal antibody typing

International panel of seven MAbs for *L. pneumophila* established in 1986



PFGE

- Genomic DNA is cut with restriction enzymes
- Fragments separated by gel electrophoresis
- Direction of voltage periodically switched

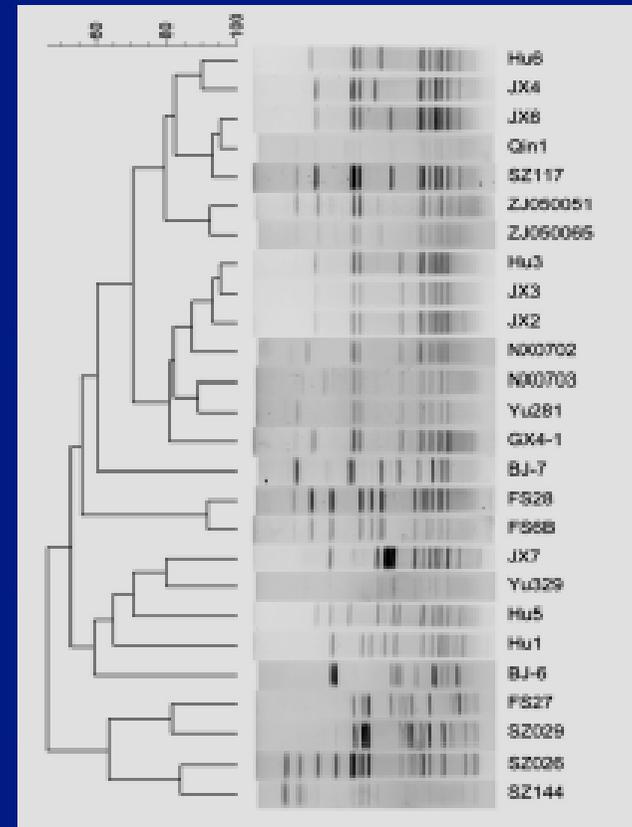
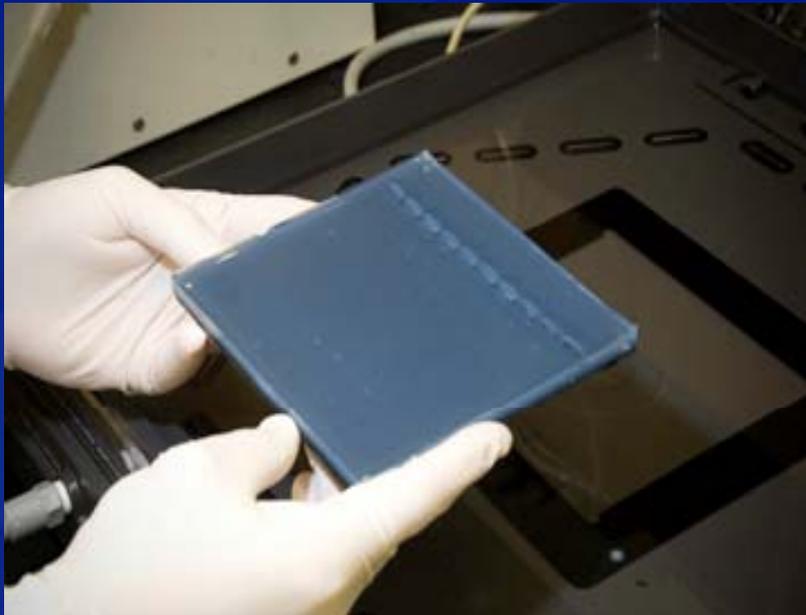
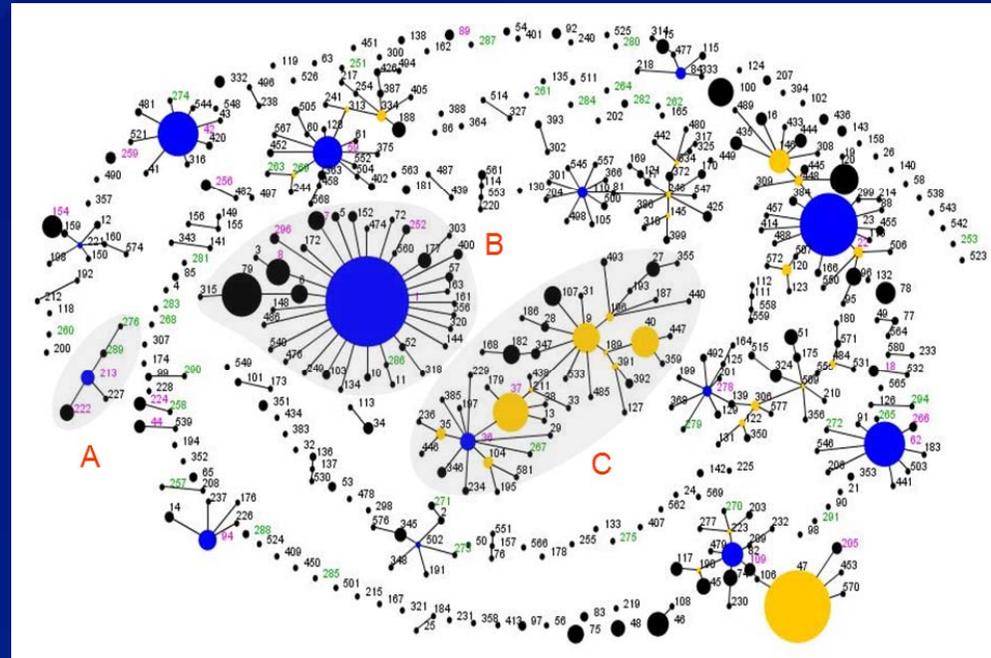


Figure from Zhou, H. *et al.* 2010. *Appl. Environ. Microbiol.* **76**: 1334-1340

Sequence Based Typing (SBT)

- PCR-amplification and sequencing of 7 *L. pneumophila* gene fragments
- The combination of alleles determines the sequence type (ST)
- ST lineages may be compared geographically and temporally
- Supported by the European Working Group for *Legionella* Infection (EWGLI)



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Website and Downloads:

<http://www.cdc.gov/legionella/>

Information for Specific Groups and Settings:

Clinicians and Health Care Setting

Clinical evaluation and management

- [Top ten things for clinicians](#)
- [Patient fact sheet](#)
- [Case report form](#)  21KB

References and helpful links

- [References](#)
- [European Working Group for Legionella Infections \(EWGLI\)](#)

ELITE Program

Environmental Legionella Isolation Techniques Evaluation (ELITE)

- [Overview](#)
- [Frequently Asked Questions](#)
- [ELITE Application](#)

Health Departments

- [Frequently Asked Questions](#)

- [Patient fact sheet](#)

Epidemiologic investigation tools

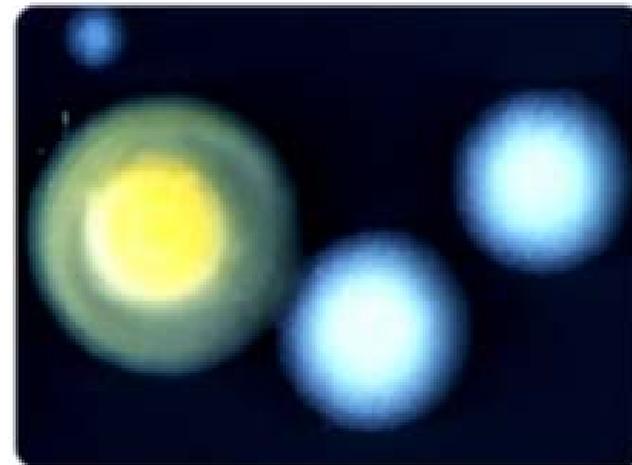
- [Sample questionnaires](#)  10KB
- [Sample Legionella interview](#)  21KB
- [Sample letter to hotels](#)
- [Case report form](#)  21KB
- [Sample Environmental Assessment of Water Systems](#)  148KB

Specimen collection and management

[Environmental testing](#)  3.7MB

- [Download: Procedures for the Recovery of Legionella from the environment](#)
- [Sampling Protocol](#)  901KB
- [Procedures for collecting and processing](#)
- [Potential Sampling Sites](#)

Procedures for the Recovery of *Legionella* from the Environment



CDC ELITE Program

<https://wwwn.cdc.gov/elite/Public/MemberList.aspx>

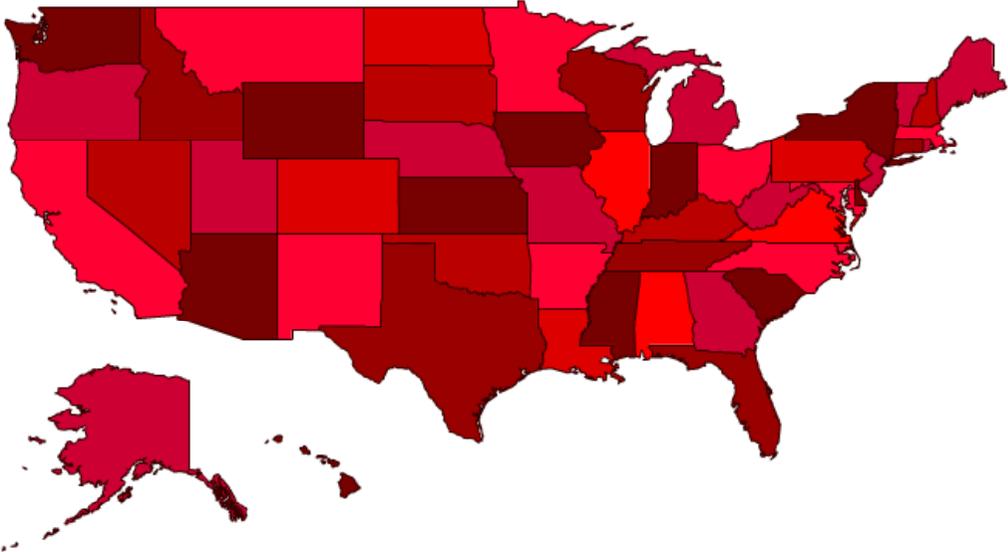
Environmental Legionella Isolation Techniques Evaluation (ELITE) Program

ELITE Overview [ELITE Overview](#) Text size: S

- Login
- Enroll Program
- Member List**
- Contact Us

Related Link
[Legionella Homepage](#)

Member List



Filter by State: Filter by Member Since:

Typing References:

- Joly, J.R. et al. 1986. Development of a standardized subgrouping scheme for *Legionella pneumophila* serogroup 1 using monoclonal antibodies. *J. Clin. Microbiol.* 23: 768-771
- Fry, N.K. et al. 1999. A multicenter evaluation of genotypic methods for the epidemiologic typing of *Legionella pneumophila* serogroup 1: results of a pan-european study. *Clin. Microbiol. Infect.* 5: 462-477
- *Legionella pneumophila* Sequence-Based typing homepage:
http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php

LD Prevention References:

- Centers for Disease Control and Prevention. **Legionellosis Resource Site:** www.cdc.gov/legionella/index.htm
- American Society of Heating, Refrigeration, and Air-Conditioning Engineers. 2000. **ASHRAE Guideline 12-2000 – Minimizing the risk of legionellosis associated with building water systems.** www.ashrae.org; www.baltimoreaircoil.com, or www.marleyct.com/publications.asp.
- European Surveillance Scheme for Travel Associated Legionnaires' Disease and European Working Group for Legionella Infection. 2005. **European Guidelines for Control and Prevention of Travel Associated Legionnaires' Disease**
http://ewgli.org/data/european_guidelines/european_guidelines_jan05.pdf
- ***Legionella* and the Prevention of Legionellosis**, edited by Jamie Bartram *et al.* 2007, WHO.

Thank you!

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

National Center for Immunization & Respiratory Diseases
Division of Bacterial Diseases

