

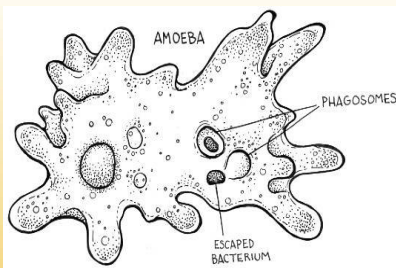
Evaluation of IDEXX Legiolert for In-house Testing of Cooling Tower Water Samples

Jennifer Hunter, Chemist 3 Microbiology

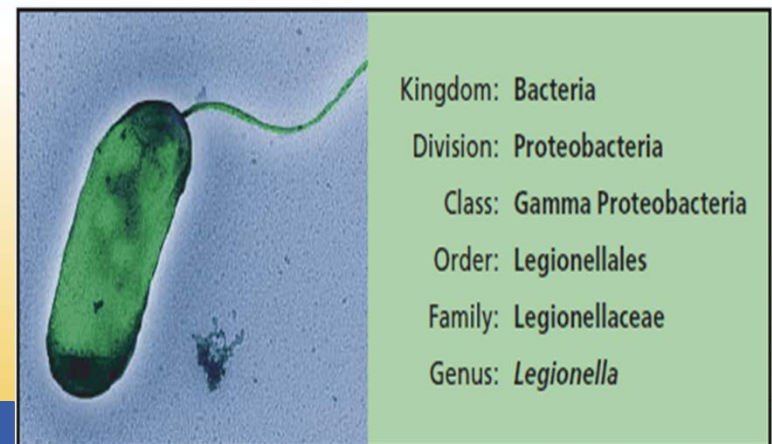
Pinellas County Utilities Laboratory

What is *Legionella*?

- Gram negative, aerobic motile rods (58 species, 3 subspecies, 70 serogroups)
- Growth between 20°C to 42°C. Optimal: 35°C to 37°C
- Ecology: most aquatic and soil environments
- Built environment: biofilms or free flowing water
- Opportunistic pathogen
- Facultative intracellular parasite of amoebae in both natural and built environments



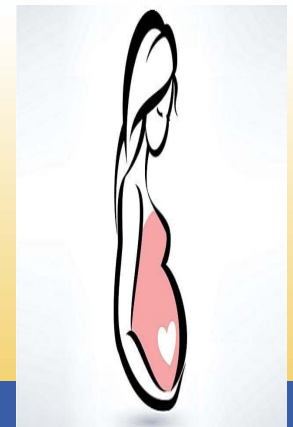
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Legionellosis



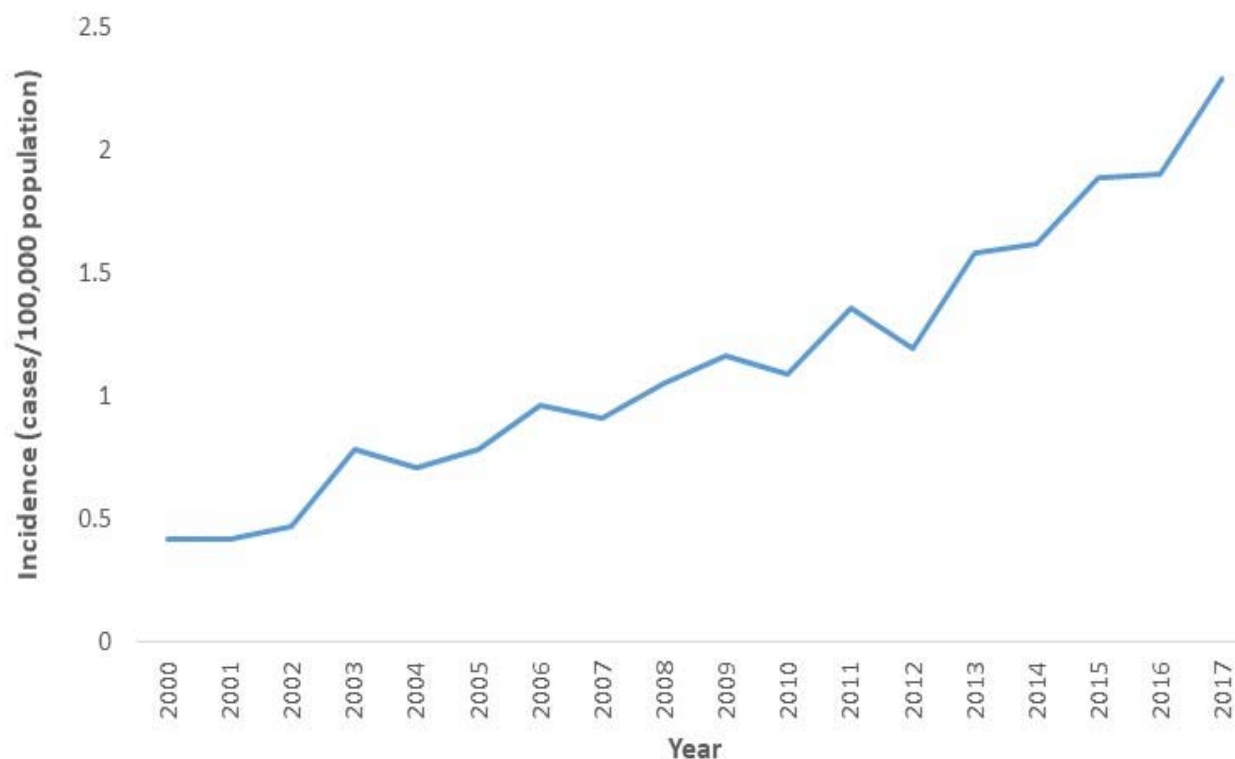
- Legionellosis = Legionnaires' disease & Pontiac fever
- Legionnaires' disease: pneumonia
- Pontiac fever: self limiting mild flu
- Nationally notifiable diseases
- Transmission → Inhalation of fine mist
- Human to human transmission rare
- 90% cases *L. pneumophila* (95% = serogroup 1)



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<http://hcinfo.com/about/outbreaks/>

Legionnaires' disease is on the rise in the United States



Rate of reported cases increased 5.5 times (2000–2017)

Source: National Notifiable Diseases Surveillance System

Centers for Disease Control and Prevention (CDC)

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Legionella can grow and spread in many areas of a building.

Effective water management programs can **REDUCE** the risk of Legionnaires' disease.

Legionella can make people sick when the germs grow in water and spread in droplets small enough for people to breathe in.

Legionella grows best in warm water that is not moving or that does not have enough disinfectant to kill germs.



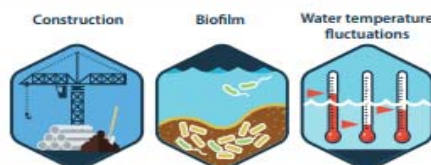
SOURCE: Vital Signs, June 2016

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<https://www.cdc.gov/vitalsigns/legionnaires/infographic.html>

How *Legionella* affects building water systems and people

1. Internal and external factors can lead to *Legionella* growth in building water systems.



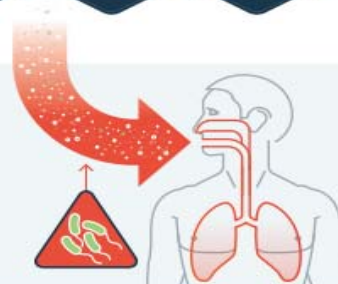
2. *Legionella* grows best in large, complex water systems that are not adequately maintained.



3. Water containing *Legionella* is aerosolized through devices.



4. People can get Legionnaires' disease when they breathe in mist or accidentally swallow water into the lungs containing *Legionella*. Those at increased risk are adults 50 years or older, current or former smokers, and people with a weakened immune system or chronic disease.



www.cdc.gov/legionella

01/12/2018

4x

The number of people with Legionnaires' disease grew by nearly 4 times from 2000–2014.

1 in 10

Legionnaires' disease is deadly for about 10% of people who get it.

9 in 10

CDC investigations show almost all outbreaks were caused by problems preventable with more effective water management.



Outbreaks



Date	Location	Cases (fatalities)	Cause
March 2019	Wisconsin Dells, WI	3 (1)	Domestic water (resort)
December 2018	Wisconsin	14 (3)	Domestic water (hospital)
February 2018	Sarasota County, FL	13	Spa & pool (residence)
September 2017	Disneyland, CA	12 (1)	Cooling towers (theme park)
March 2017	Mihara, Japan	39 (1)	Hot spring (bathing facility)
September 2016	Seattle, WA	5 (2)	Domestic water (hospital)
January 2016	Manzanares, Spain	230 (3)	Decorative fountain
July 2015	New York City, NY	120 (12)	Cooling tower (hotel)
June 2014 – Nov 2015	Flint, MI	88 (10)	Multiple factors

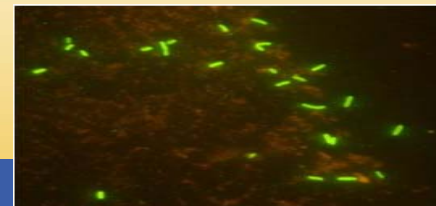
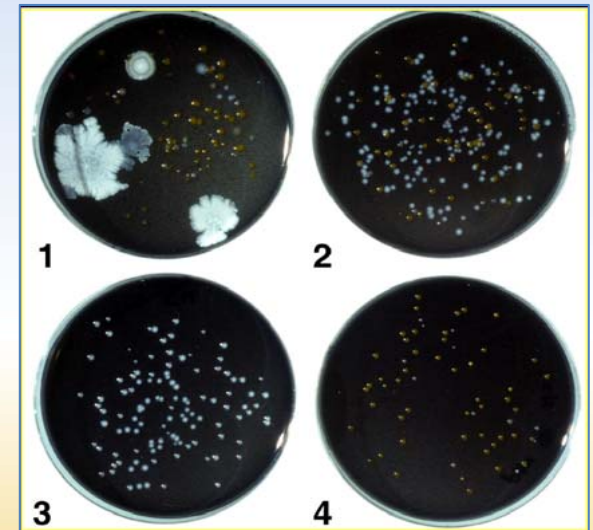
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<http://hcinfo.com/about/outbreaks/>

Detection methods



- Culture based
 - CDC method - Environmental *Legionella* Isolation Techniques Evaluation (ELITE) program
 - ISO method
 - IDEXX Legiolert
- Quantitative PCR
- Urine ELISA
- Direct Fluorescent Antibody



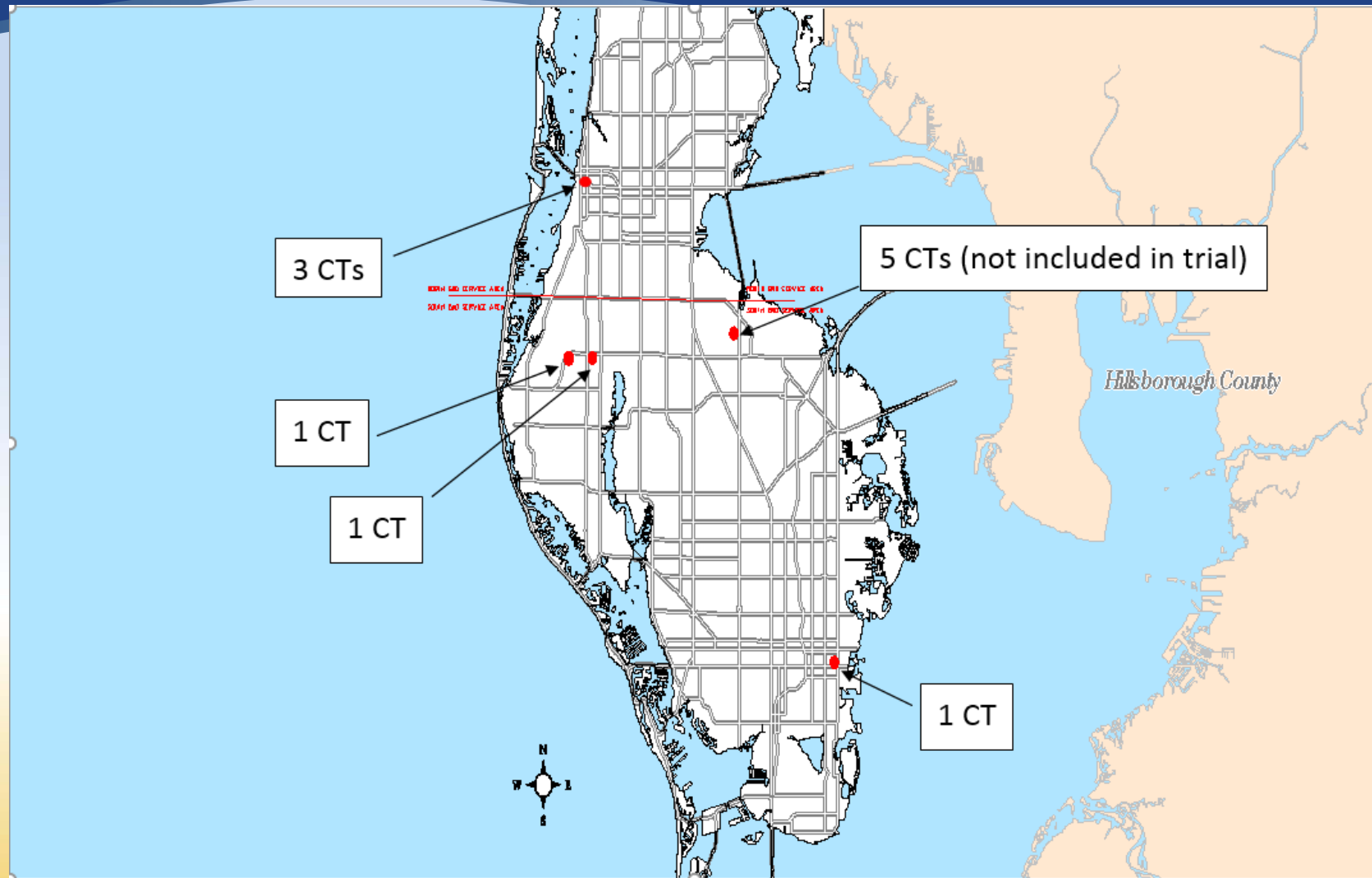
PCU cooling tower monitoring



- Monitoring Real Estate Management CTs since before 2006
- EMS, ME, Courthouses, Jails, Central Energy Plant
- Samples collected once per month
- Contract labs for sample processing
- Remedial actions for positive results



Cooling tower locations

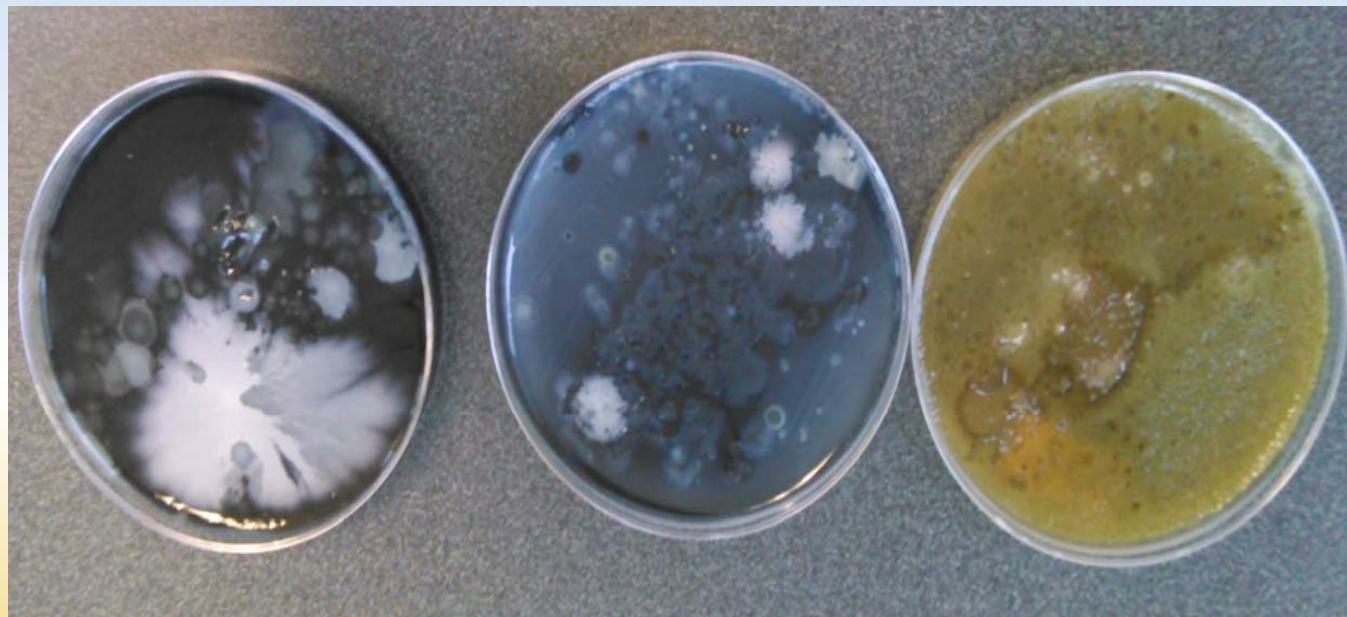


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Why consider Legiolert?



- Different contract lab every 3 years – bid process
- 3 consecutive years of negative results from 8 towers



Methods comparison



	CDC method	Legiolert (NP)
Organism detected	All culturable <i>Legionella</i> species	Only <i>Legionella pneumophila</i>
Methodology	Culture based	Culture based
Units of measurement	CFU/mL	MPN/100mL
Incubation period	7 to 14 days	Up to 7 days
Volume tested	5 mL	1 mL
Detection Limit	20 CFU/mL	100 MPN/100mL
Confirmation	Using staining and/or serotyping	No confirmation required

Legiolert advantages



- Equivalent or greater sensitivity
- Ease of sample processing
- Reduced ambiguity of test results
- Elimination of confirmation steps
- Faster turnaround time



<https://www.idexx.com/en/water/water-products-services/legiolert/>



Treat nonpotable sample with Legiolert pretreatment.



Add pretreated sample to reagent mixture.



Pour sample into Quanti-Tray/Legiolert.



Seal using the Quanti-Tray Sealer PLUS and incubate for 7 days.

Results



		CDC method		Total
		Positive	Negative	
Legiolert	Positive	3	13	16
	Negative	1	58	59
Total		4	71	75

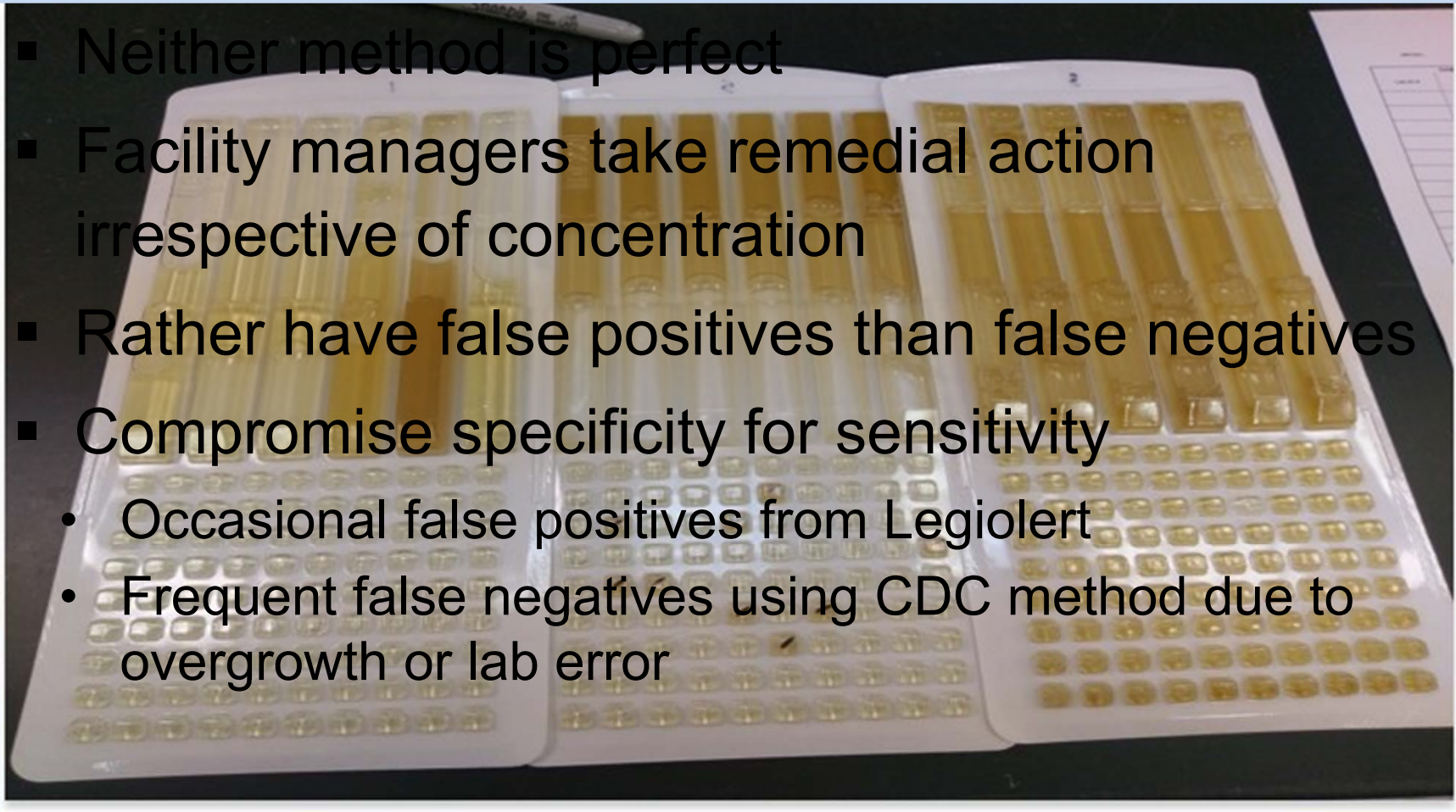
- Six cooling towers, 1 year study
- One incidence CDC method positive, Legiolert negative
 - *L. pneumophila* serogroup 1 (DFA confirmation)
- One Legiolert positive was confirmed as false positive using PCR and serotyping

CDC method (CFU/100mL)	Legiolert (MPN/100mL)
0	100
0	110
2000	0
0	390
0	230
0	5340
0	110
40	230
0	110
440	470
0	390
0	580
0	110
76000	110
0	227000
0	14900
0	110

Conclusions



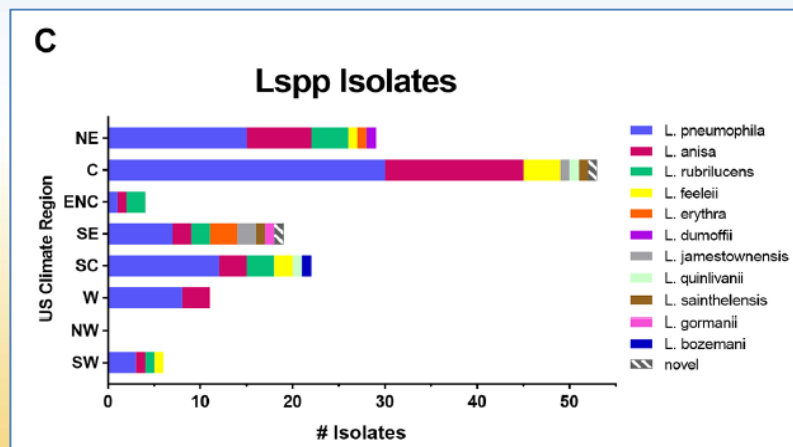
- Neither method is perfect
- Facility managers take remedial action irrespective of concentration
- Rather have false positives than false negatives
- Compromise specificity for sensitivity
 - Occasional false positives from Legiolert
 - Frequent false negatives using CDC method due to overgrowth or lab error



Other considerations



- Legiolert certification
- Evaluate Legiolert for potable water
- UCMR5: *Legionella* monitoring in drinking water distribution system samples?
- Other related studies





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