Comparison of two methods for the enumeration of *Legionella pneumophila* from potable water samples

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BACKGROUND

Legionella pneumophila is the causative agent of the human respiratory disease legionellosis and is responsible for a significant percentage of waterborne disease outbreaks in the US. These outbreaks are frequently associated with the premise plumbing systems of large buildings.

Detection methods: The availability of better detection methods for these bacteria is needed to aid facility managers in implementing building water management plans.

Research motivation: Culturing techniques for the isolation of *Legionella*, such as using Buffered Charcoal Yeast Extract (BCYE) Agar, have been developed and improved upon since the late 1970's. However, these methods can be labor intensive, prone to overgrowth by non-target bacteria that obscure the *Legionella* colonies, and require additional analysis to determine whether the *Legionella* recovered are *L. pneumophila*.

A newer MPN based method called Legiolert has recently been introduced that promises to resist the growth of non-target bacteria while specifically recovering and quantifying *L. pneumophila* from water samples.

Research objective

In this study, we investigated the **Legiolert** method by comparing its performance using environmental water with **the standard culture method (GVPC agar)**.

MATERIALS & METHODS

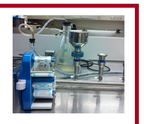
Sampling → **Membrane Filtration**

- **204** water samples form a simulated home plumbing system
  - ✓ Hot (n=100), Cold (n=104)
  - ✓ Free Cl: Hot (<0.1), Cold (0.2-0.5)
- Sample vol collected: **1 liter**
- Equivalent vol analyzed: **10 mL** for both methods

Standard Culturable Method

- Step 1: Spread plating on BCYE agar
- Step 2: Incubate at 35°C for 5-7 days
- Step 3: Counting colonies

1. **BCYE w/o L-cysteine**
2. **Latex agglutination**



Legiolert

qPCR

BCYE vs Legiolert in drinking water
<Literature review>

- **Sartory et al. (2017, Letters in Appl. Microbiol.): EU**
  - ISO 11731-2 membrane filtration method: 100 mL sample volume analyzed, GVPC or BCYE agar followed by serological confirmation, incubated at 36°C for 10 days
  - Legiolert yielded on average higher counts and had a high specificity of 96.4%.
- **Petrisek and Hall (2017, J. of Water and Health): USA**
  - Standard Method 9260J: 5-20 mL sample volume analyzed, CCVC and BCYE agar followed by a sub-culturing confirmation test, incubated at 35 ± 2°C for 7 days
  - The Legiolert method was found to be more sensitive and had a high specificity with no false positive signals.
- **Our study**
  - CDC Manuel: Approximately 20 mL sample volume analyzed, GVPC agar followed by a sub-culturing confirmation test, incubated at 35 ± 2°C for 7 days

Statistical Analyses

- McNemar's binomial analysis
- Pearson correlation analysis
- Regression analysis

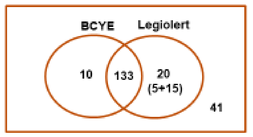
RESULTS & DISCUSSION

1. Qualification: Prevalence

GVPC vs. Legiolert
<McNemar's binomial analysis>

Presence/absence statistics

		Legiolert +	Legiolert -	
GVPC	+	133	10	143
	-	5	41	46
	TNTC	15	NA	15
		153	51	204



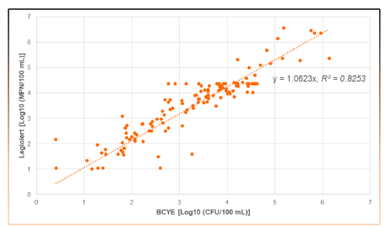
Binomial test
(McNemar chi-square with Yates' correction = 2.7, P = 0.100348)

Both methods showed no significant difference for determining presence/absence of *L. pneumophila*.

- Qualification (presence/absence) by McNemar: no difference
- Quantification by regression analysis: more counts by Legiolert

2. Quantification: *Legionella* counts

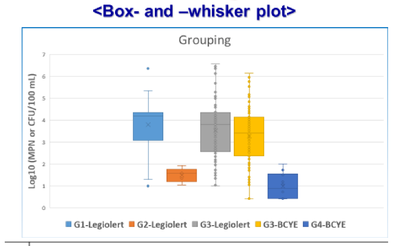
GVPC (BCYE) vs. Legiolert
<Regression analysis>



– A slope of the regression equation is 1.06, indicating slightly more counts by Legiolert. Other researchers reported that Legiolert exhibited statistically greater sensitivity for quantifying *L. pneumophila* from potable waters (Sartory et al., 2017; Petrisek and Hall, 2017).

3. Quantification: Groups

GVPC vs. Legiolert
<Box- and –whisker plot>



	G1-Legiolert (BCYE-TNTC)	G2-Legiolert (BCYE -)	G3-Legiolert (BCYE +)	G3-BCYE (Legiolert +)	G4-BCYE (Legiolert -)
n=	15	5	133	133	10

4. Confirmation test (for Legiolert)

Specificity

- 3.5% false positives from 254 positive wells
- 0% false negative from 82 negative wells

→ Further confirmation for false positives is needed

Preliminary test

- against non-*Legionella* environmental isolates from BCYE agar
- False positives with *Pseudomonas aeruginosa*

G1: The greatest average concentration was measured by the Legiolert method, whereas GVPC (BCYE) method failed to count *Legionella* colonies due to overgrowth of non-Legionella bacteria.

G2: Legiolert (+) and BCYE (-)
G3: Legiolert (+) and BCYE (+)
G4: Legiolert (-) and BCYE (+)

SUMMARY

- Of 204 samples, 163 (80%) were positive for presence of *L. pneumophila* using both methods.
- The BCYE culture method identified 10 (4.9%) samples as *L. pneumophila* positive however these were negative using Legiolert. Conversely, 20 (9.8%) of the samples were positive only by Legiolert.
  - ✓ The McNemar's statistics showed no statistical difference (P=0.1), indicating both methods are equally sensitive for determining the prevalence of *L. pneumophila*.
- Of the 20 samples positive by Legiolert only, 15 samples resulted in failing to identify any *Legionella* using the BCYE method due to the overgrowth of non-target microorganisms.
- The log transformed quantities determined by both methods showed a high cross-correlation (Pearson's r of 0.9149), and the slope of the regression equation was near one.
- The Legiolert method had a relatively high specificity (i.e., 3.5% false positives from 254 positive wells and 0% false negatives from 82 negative wells) which is comparable to other published studies.

The new Legiolert method performed as well or better than the standard agar-based method in qualitative and quantitative detection of *L. pneumophila* in premise plumbing water samples.

Further Study

- There was no evidence of interference by non-target microorganisms when processing the water samples using the Legiolert method. These samples, however, were collected from one premise plumbing water system. Moreover, in preliminary studies on further evaluation of specificity against other non-*Legionella* environmental isolates, it appears *Pseudomonas aeruginosa* may give a false positive result (unpublished).
- **For further study,** 1) more diverse sources of environmental water and 2) more rigorous evaluation of specificity with various non-*Legionella* bacteria need to be tested.

ACKNOWLEDGMENT

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