Development of rapid *Legionella* viability qPCR detection assay to reduce risk of disease outbreaks associated with cooling towers

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INTRODUCTION

Legionellosis has been an increasing public health concern in New Brunswick. The severe disease condition is predominantly caused by the bacterium *Legionella pneumophila (LP)* which thrives in moist environments such as cooling towers. Traditional culture-based detection approaches are time-consuming, since results take 7–9 days. These approaches also lack the ability to detect "viable but non-culturable" *Legionella* bacteria that are often residually present after common disinfection treatments (e.g., chlorination or heat). Quantitative real time PCR (qPCR) based detection is a rapid molecular method that directly addresses these issues for accurate identification of *LP*. The main drawback of existing qPCR approaches is the lack of live-dead discrimination of the bacterium. We've developed a *Legionella* viability qPCR detection method which combines the rapidity of qPCR with the ability to discriminate between viable and non-viable cells (Figure 1). Our objective is to develop a high-throughput, rapid qPCR detection method for *LP*, *LP* serogroup 1 (LPSG1) and other *Legionella* species.

MATERIALS & METHODS

Water samples were spiked with known quantities (High – 10^3 to 10^4 MPN/mL; Medium – 10^2 to 10^3 MPN/mL; Low – 10^1 to 10^2 MPN/mL) of target (*LP*) and non-target (*E.coli*) viable and non-viable pathogens. The spikes were filter concentrated and subjected to DNA extraction followed by qPCR analysis[1] on the Bio-Rad Opus CFX-96 platform. The method contains a cell-permeable intercalating crosslinker that exclusively binds to DNA from membrane-compromised dead, dying, or lysed cells[2,3] (Figure 1). Once bound, the molecule irreversibly crosslinks DNA, making it un-amplifiable in PCR reactions. As a result, only the signal exclusively from viable cells is detected. The data was analyzed using Bio-Rad CFX Maestro software (Figures 2a, b) and qPCR results were compared with traditional methods[4]. Relative quantification was performed using a DNA standard with known copies of target DNA.

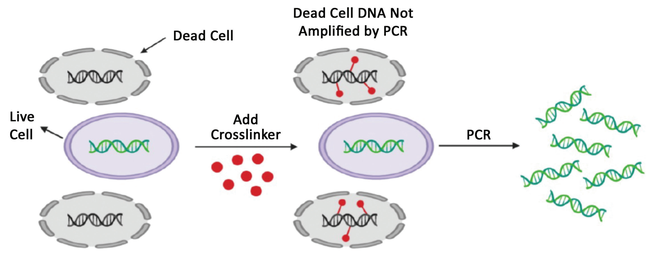

Figure 1: Viability qPCR Overview

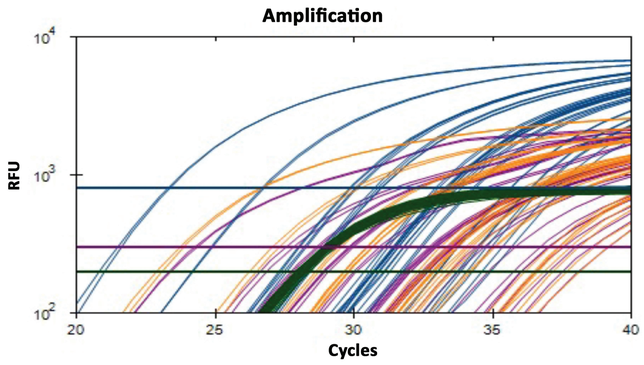

Figure 2a: qPCR Amplification Curve

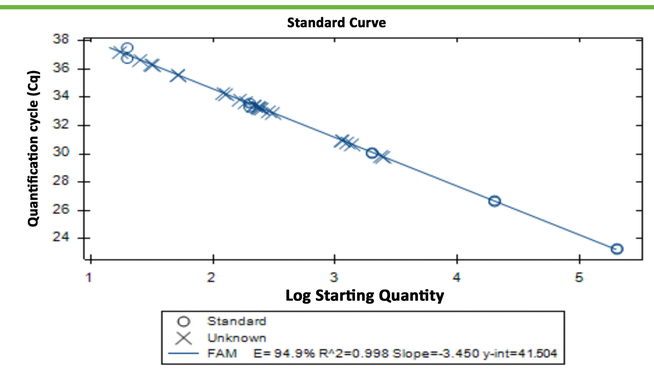

Figure 2b: qPCR Standard Curve

RESULTS AND DISCUSSION

The qPCR protocol was highly specific to *Legionella* spp. (Table 1) and differentiated from non-target (*E.coli*) and negative controls (no amplification was observed). The results based on inclusivity and exclusivity show that the method can accurately distinguish between viable and non-viable cells (Figure 3).

Acceptable Range	Efficiency (86-115%)	Slope (-3.0 to -3.7)	R^2 (>0.970)
Observed range	89.57 to 100.07	-3.32 to -3.6	>0.995

Table 1: Evaluation of the qPCR Standard Curve

Spike	MPN±RSD/mL	vGE ±RSD/mL
High	> 2272.6	1791.4 ± 28.4
Medium	970.9 ± 23.3	116.5 ± 33.0
Low	114.1 ± 40.9	24.3 ± 113.5

Table 2: Comparison of Culture-Based Enumeration of *LP* via Most Probable Number (MPN/mL) and Viable Genomic Equivalent qPCR vGE/mL

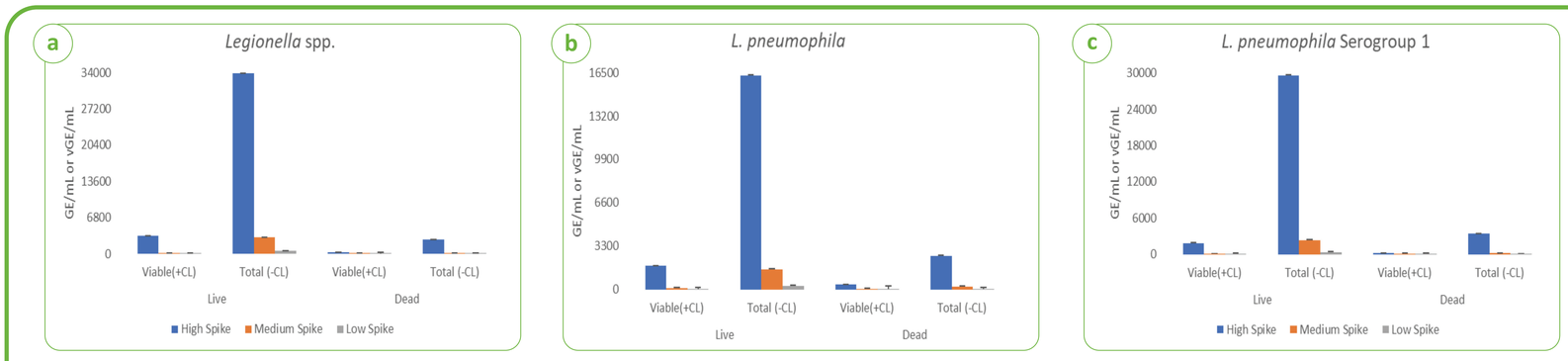

Figure 3: Viable (treated with crosslinker (CL)) and total (without CL) gene copies in live and dead samples of a) *Legionella* spp.; b) *L. pneumophila*; and c) *L. pneumophila* Serogroup 1 in high (blue), medium (orange) and low (grey) spikes. GE/mL – Genomic equivalent/mL; vGE/mL – viable genomic equivalent/mL. Error bars represent RSD%.

The limit of detection and quantification of the assay was 20 copies/reaction. Based on the results, vGU/mL can be used as an accurate, rapid and informative alternative to the more commonly used culture-based methods.

CONCLUSIONS

- The viability qPCR method is a rapid and reliable alternative to traditional culture-based methods for the detection of *Legionella* spp., *L. pneumophila* and *L. pneumophila* Serogroup 1.
- This rapid detection method (~3-4 hours) can be used to assist public health/environmental professionals in performing risk assessment and allows for rapid response to mitigate outbreaks.

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