Detection, Quantification and Identification of *Legionella, L. pneumophila* and *L. pneumophila* serogroup 1 in Water Samples

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Introduction

Legionella, ubiquitous bacterium in aquatic environments, is an opportunistic pathogen of significant public health concern, especially the species *Legionella pneumophila* among which *L. pneumophila* serogroup 1 (Lp1) accounts for about 84 % of human infections worldwide. Hence, identification and quantification from environmental sources is crucial for tracking outbreak, risk assessment and disease prevention. The two most widely used and accepted methods are culture and real-time polymerase chain reaction (qPCR). In order to circumvent limits of the culture method enabling to detect culturable bacteria only, Pall® method offers a range of qPCR-based methods for the detection, the quantification and the identification of *Legionella* in water samples.

Material and Methods

Sample preparation relies on bacterial concentration by filtration of the water samples (100 mL – 1 L) through 0.45 µm polycarbonate membrane, mechanical bacterial lysis by sonication and heating, and optional DNA purification onto silica column (figure 1).

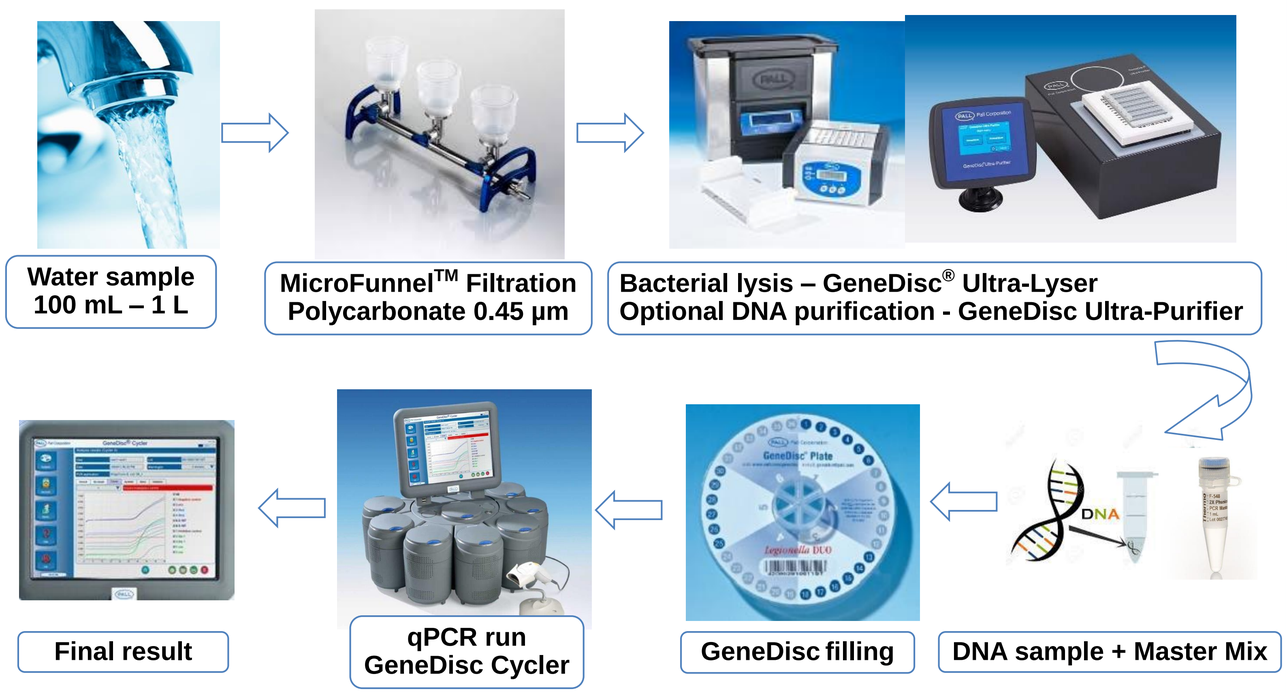

Figure 1: Workflow of the GeneDisc* Legionella *method

PCR analyses are performed with the GeneDisc Cycler, by using 3 different GeneDisc plates: GeneDisc *Legionella pneumophila* for the quantification of *L. pneumophila*, GeneDisc *Legionella* DUO for the simultaneous quantification of *Legionella* spp. and *L. pneumophila*, and GeneDisc *Legionella* ID for the detection/identification of the genus, the species and *L. pneumophila* serogroup 1. Development of the GeneDisc Plates was realized according to requirements of the NF T90-471 (2015) standard and the MIQE[1] guidelines. Table 1 summarizes the workflow of GeneDisc plate for *Legionella* methods.

Table 1: GeneDisc methods for* Legionella *detection/quantification in water samples

Protocol		All types of water	Clean water
Filtration		Polycarbonate 0.45 µm – 47 mm	
DNA Extraction		Extraction Pack Environment 1	Extraction Pack Environment 3
Bacterial Lysis		Sonication (8 min, 230 W) + heating (19 min, 110°C)	
DNA purification and/or concentration		Silica column	Optional NanoSep® 30K
GeneDisc Plates	*Legionella* DUO	Simultaneous quantification of *Legionella* spp. and *L. pneumophila*	
	Legionella spp.	Quantification of *Legionella* spp.	
	L. pneumophila	Quantification of *Legionella pneumophila*	
	Legionella ID	Detection and Identification of *Legionella* spp., *L. pneumophila* and *L. pneumophila* serogroup 1	
Performance	**LOD (1L sample)** **LOQ (1L sample)**	190 GU/L 940 GU/L	420 or 83 GU/ (1) 2,100 or 420 GU/L(1

(1) with NanoSep™

[1]Huggett JF, Foy CA, Benes V, Emslie K, Garson JA, Haynes R, et al. The digital MIQE guidelines : minimum information for publication of quantitative digital PCR experiments. Clin Chem 2013 ; 59 : 892-902.

Results

Specificity of the PCR assays

Specificity of the *Legionella* PCR assays was evaluated with 48 strains (table 2). Inclusivity tests were carried out with 100 Genomic Units (GU) per PCR well while exclusivity tests were performed with 10^5 GU/PCR wells. As expected, any cross-reaction was observed and all *Legionella* strains were detected.

Table 2: Strains tested to evaluate specificity of the 3* Legionella *PCR assays

PCR assay	List of strains tested
Legionella spp. n = 11	*L. anisa, L. bozemanae, L. cincinnatiensis, L. dumoffii, L. jordanis, L. lansingensis, L. longbeachae, L. maceachernii, L. micdadei, L. oakridgensis, L. parisiensis*
L. pneumophila n = 18	*L. pneumophila* serogroup 1 - 17, *L. pneumophila* subsp. *pascullei* serogroup 5
***L. pneumophila* serogroup 1** n = 8	*L. pneumophila* strain Philadelphia 1, *L. pneumophila* strain Uppsala 3, *L. pneumophila* strain Camperdown, *L. pneumophila* strain Lorraine, *L. pneumophila* strain Lens, *L. pneumophila* strain Olda, *L. pneumophila* strain Bellingham, *L. pneumophila* strain Pontiac
Other strains n = 11	*Aeromonas sobria, Aeromonas hydrophila, Bacillis subtilis, Burkholderia cepacia, Flavobacterium flavobacter, Klebsiella oxytoca, Listeria monocytogenes, Pseudomonas putida, Proteus vulgaris, Serratia marcescens, Stenotrophomonas maltophilia,*

Performance of the qPCR assays

All PCR analyses were realized with standard *L. pneumophila* DNA from the strain WDCM 00107. The results in table 3 show that all results fulfill the NF T90-471 requirements.

Table 3: Performance of the 3* Legionella *PCR assays

PCR assay	LOD at 5 GU/PCR n = 30	LOQ at 25 GU/PCR n = 30	Linearity from 250,000 to 25 GU/PCR well n = 5 independent DNA ranges		
			E_{lin}	Slope	Intercept
***Legionella* spp.**	100 % Presence	$E_{LOQ} = 0.15$	$0.10 < E_{lin} < 0.12$	-3.2 ± 0.3	38 ± 1.5
L. pneumophila		$E_{LOQ} = 0.13$	$0.02 < E_{lin} < 0.12$	-3.3 ± 0.3	39 ± 1.5
***L. pneumo* sg 1**		-	-	--	-

Performance of the global mEthod

The global method including the sample preparation and the RT-PCR analysis was evaluated with 3 different types of water samples, artificially contaminated with the *L. pneumophila* WDCM 00107 strain, at 2 different levels: 1,000 and 100,000 CFU/sample. Ten samples are analyzed at each spiking dose, under reproducibility conditions (different days, different operators). The results are reported in the table 4.

Table 4: Performance of the global method with different types of water samples

Parameters	Mineral Water		Hot Sanitary Water		Cooling Tower Water	
	PENVI1	PENVI3	PENVI1	PENVI3	PENVI1	PENVI3
$Mean_{Bias}$ $LogQ_{calculated}/LogQ_{theoretical}$	0.15	0.40	0.24	0.48	0.08	Not Applicable
SD_{Bias}	0.25	0.10	0.23	0.08	0.16	
Uncertainty $(2x\sqrt{(Mean_{Bias}^2 + SD_{bBas}^2)}$	**0.58**	**0.82**	**0.66**	**0.97**	**0.18**	

Whatever the sample type and the sample preparation protocol, *i.e.* with or without DNA purification through silica column (PENVI3), the mean bias and the standard deviation were inferior to 0.5 and 0.25 Log, respectively, so that uncertainty of the global method is inferior to 1 Log.

Conclusion

The GeneDisc technology provides a range of flexible methods enabling to monitor *Legionella* contamination in water samples, either by detection or quantification of as low as 80 GU/L and 400 GU/L, respectively. This quick method enabling to get results in less than 3 hours and its very friendly use, can be easily implemented in quality control laboratory for routine analysis.