Evaluation of automated nucleic acids extraction system for *Legionella pneumophila* detection in water samples

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Introduction

The increasing number of deaths associated with Legionnaire's disease has resulted in growing public interest in problems associated with intracellular bacteria. Since these bacteria are present in water, they can be found in several artificial ecosystems, such as boilers, air conditioning units, and nuclear plants.

Legionella contamination can impact on the public in two ways:

- Health: For example, in hospitals, *Legionella* can rapidly infect immunocompromised people.
- Economy: Rigorous cleaning requirements can lead to financial losses in industry.

Therefore, rapid and highly sensitive detection of *Legionella* is required for detection and confirmation of clearance.

Real-time PCR assays for Legionella have shown high sensitivity. In France, a guideline procedure (AFNOR XP T90-471) drives the procedure for *Legionella pneumophila* detection and quantification using real-time PCR.

Evaluation principle

According to AFNOR XP T90-471, efficiency of the *Legionella* DNA extraction method should be calculated using *Legionella*-free water artificially supplemented with a solution containing a known quantity of *Legionella pneumophila* ATCC 33.152 (CIP 103 854T).

Performance of the EZ1® system was evaluated over five days and with two different handlers, in order to generate 32 independent calculations for each of two levels of doping.

Performance was calculated in two stages:

- Preparation and evaluation of the concentration of a solution (or doping solution).
- Determination of the *Legionella* concentration in doped water, and hence, the yield from the extraction and purification procedure.

Since the assay was used for COFRAC accreditation, it was performed following GLP.

The BioRobot EZ1.

Materials and methods

- The BioRobot® EZ1 system uses automated magnetic-particle technology to rapidly purify high-quality DNA from 1–6 samples in as little as 20 minutes.
- The extraction reagents for one sample are packaged in a single-use cartridge that limits the possibility of cross-contamination.

Pre-packaged reagents.

Simple reagent handling.

Materials and methods

Preparation of doping solution:

- Doping solutions were prepared by mixing 3–4 bacterial colonies in physiological serum. A direct lysis followed by qPCR allows determination of the number of *Legionella* genomes/100 µl of doping solution (Genome Unit/100 µl; or GU/100 µl).

EZ1 extraction and calculation of yield:

- Calibrated doping solutions were added to 1 liter of spring water at a concentration of 10,000 UG/l and 100,000 UG/l. Each sample was filtered through a 0.45 µm membrane.
- 2 ml of Buffer ATL was added to the surface of the membrane (30 min, 37°C) with orbital agitation.
- 200 µl of this mixture was then purified using the EZ1 and the DNA Bacteria protocol card.
- 5 µl of eluate was then tested in duplicate for *Legionella* species and specifically *L. pneumophila*.

Results

- A total of 64 experiments gave two average yields (sample results are shown).
- Both yields were significantly higher than the 25% dictated by the guidelines.

Sample of raw data used for calculation of yield

Day	Technician	Batch	Doping solution	Concentration	Yield (%)
16/01/2007	DE	124119349	20070116DE1	10,000	81.69
16/01/2007	DE		20070116DE2	10,000	33.57
16/01/2007	ND	124119349	20070116ND1	10,000	71.95
16/01/2007	ND		20070116ND2	10,000	33.31
17/01/2007	DE	124119349	20070117DE1	10,000	48
17/01/2007	DE		20070117DE2	10,000	47.4
18/01/2007	DE	124119349	20070118DE1	10,000	41.54
18/01/2007	DE		20070118DE2	10,000	122.05
18/01/2007	ND	124119349	20070118ND1	10,000	74.04
18/01/2007	ND		20070118ND2	10,000	40.58
23/01/2007	DE	124119349	20070123DE1	10,000	47.07
23/01/2007	DE	124119349	20070123DE2	10,000	31.36
23/01/2007	ND	124119349	20070123ND1	10,000	40.08
23/01/2007	ND	124119349	20070123ND2	10,000	23.33
25/01/2007	DE	124119349	20070125DE1	10,000	71.46
25/01/2007	DE	124119349	20070125DE2	10,000	31.97
25/01/2007	ND	124119349	20070125ND1	10,000	52.92
25/01/2007	ND	124119349	20070125ND2	10,000	88.32

DNA yield recovered from doping solution after EZ1 extraction

Concentration (GU/l)	Yield (%)
10,000	50.13
100,000	45.70

Discussion and conclusions

- With potential health and economic problems, rapid sensitive detection of *Legionella* is required – PCR provides a potential solution.
- Detection is the critical step. *Legionella* is difficult to process. An effective and reliable extraction/purification procedure is needed.
- Results obtained using the EZ1 meet COFRAC needs.
- All results were compliant with COFRAC requests and LDA 50 was the first French public laboratory accredited for *Legionella* detection using PCR with an automated system.
- First results with the EZ1 (not shown) on real samples with high PCR inhibitor concentrations show good results, with very few PCR inhibitions compared with silica-membrane–based columns.
- The EZ1 Advanced XL includes new features, such as a UV lamp, barcode reader, and LIMS connectivity.

The EZ1 Advanced XL.

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1058386 09/2009