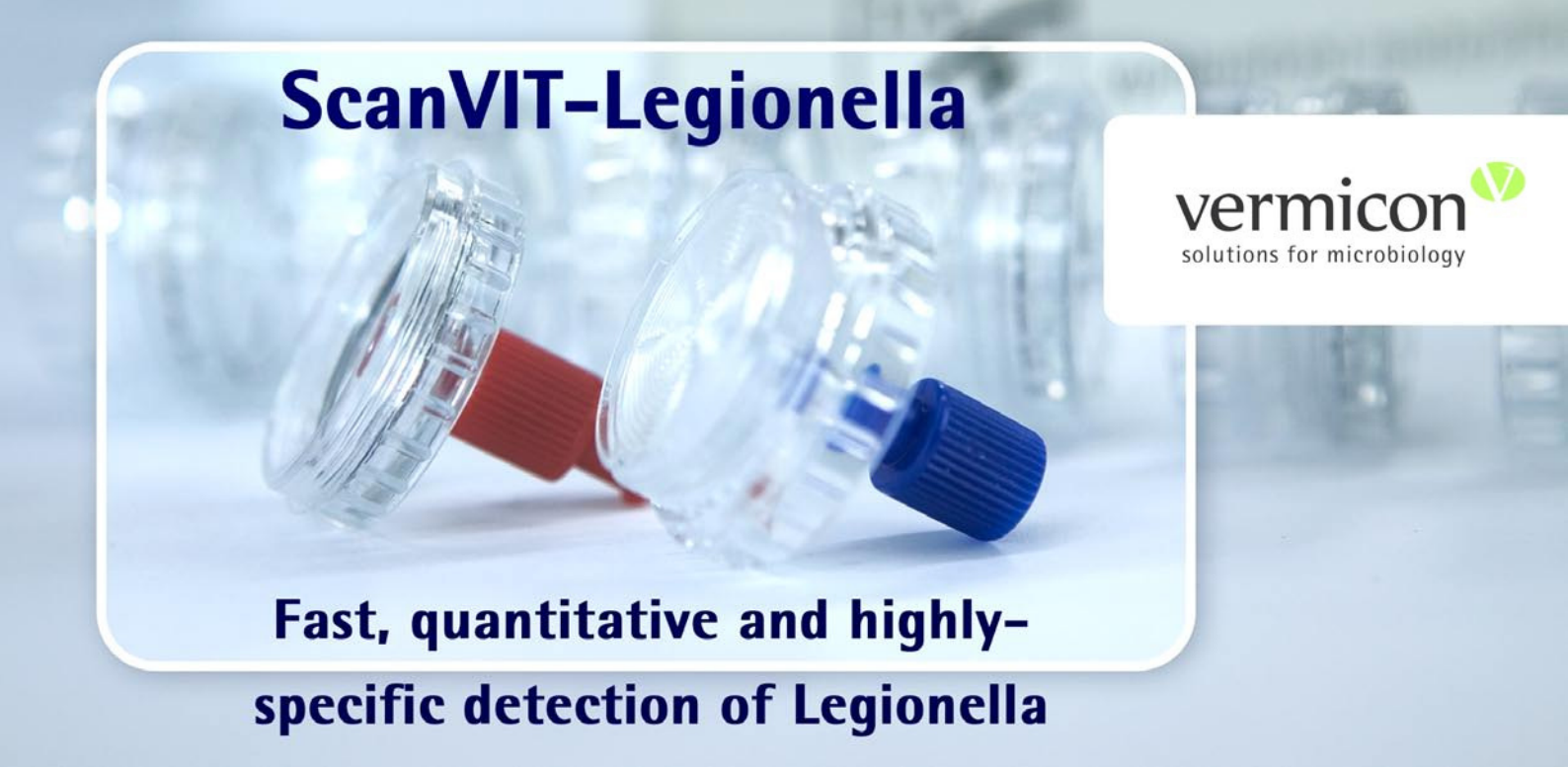


ScanVIT-Legionella

Reliable results guaranteed

Fast and quantitative detection

ScanVIT-Legionella allows the fast and reliable identification and quantification of viable *Legionella* species in drinking and cooling water. The fact that *Legionella* proliferate especially in warm water, causes a high risk of infection mainly in showers, humidifiers and air-conditioners.

Periodical control of water pipes and installations with ScanVIT-Legionella allows in case of a *Legionella* contamination an earlier reaction. Adequate counter measures can be carried out quicker and the effectivity of disinfection measures can be controlled faster. Only a fast and effective disinfection can reduce and eliminate *Legionella* in water installations and thus prevent the proliferation of these bacteria. Furthermore the exact quantification of only viable bacteria is a requirement for a correct evaluation of the situation.

The conventional method takes 10 to 14 days for the detection of *Legionella*. Despite this long analysis time, the conventional method is often not specific enough. With ScanVIT-Legionella harmful *Legionella* bacteria can be identified and quantified precisely in water samples within only 3 days. The detection of *Legionella* takes 75 hours from sampling until the quantitative results are available.

The test is based on the highly specific vermicon identification technology (VIT®).

The detection of the bacteria takes place on a filter membrane, which is incubated for only 3 days after the filtration of the water sample.

After incubation, the Scan-VIT analysis is carried out directly on the filter membrane. The results are read using a fluorescence microscope. All the micro-colonies on the filter are screened by specific gene probes and all *Legionella* and *Legionella pneumophila* micro-colonies are identified and quantified distinctly in the same test.

ScanVIT-Legionella revolutionises the detection of *Legionella* due to its rapidness and precision.

vermicon identification technology VIT

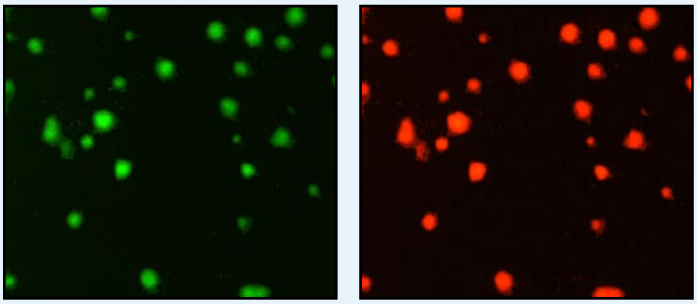
vermicon
solutions for microbiology

How does VIT work?

VIT® is a reliable and highly specific gene probe technology. DNA-molecules marked with a dye have been specially programmed by vermicon AG. They only match to distinctive, genetic material of viable microorganisms. During the VIT-analysis these gene probes penetrate the bacterial cells and bind to their specific target sites. Caused by excitation light, the dye illuminates the whole cell and makes it shine.

Easy Evaluation:

Identical microscopic view of micro-colonies on a filter membrane:



Micro colonies of *Legionella* light up green.
Micro colonies of *L. pneumophila* light up green and red.

We provide attractive offers for fluorescence microscopes on request.

Specifications for ScanVIT-Legionella

Content:	100 analyses
Enrichment:	72 hours, GVPC-Agar
Detection:	- *Legionella* - *Legionella pneumophila*
Required hardware:	- drying oven (46°C +/- 2°C) - Filtration unit with filter funnels suitable for membranes with a diameter of 25 mm - Fluorescence microscope with VIT-adapted filters

Advantages of ScanVIT-Legionella

Specific:	All micro-colonies on the filter membrane are analysed in one single test. The whole genus *Legionella* and the most harmful species *Legionella pneumophila* are identified and quantified unequivocally.
Relevant:	Only viable *Legionella* cells are detected.
Rapid:	After a short incubation time, the micro-colonies are detected within 3 hours. The result is available after only 3 days which generates a decisive advantage in rapidness and precision. This allows quick countermeasures, their prompt control and a fast release of the examined installations.
Easy:	The test is easy to carry out and requires a minimum of hands-on time.
Unequi-vocal:	VIT® is based on the detection of specific genetic signatures in the *Legionella* cells. Results are reliable and unequivocal.

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ORIGINAL ARTICLE

Inter-laboratory validation of a rapid assay for the detection and quantification of *Legionella* spp. in water samples

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Keywords
gene probes, inter-laboratory comparison, *Legionella* spp, ScanVIT-Legionella, standard culture method, water samples.

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Abstract

Aims: To compare the standard culture method with a new, rapid test (ScanVIT-Legionella™) using fluorescently labelled gene probes for the detection and enumeration of *Legionella* spp. The new technique was validated through experiments conducted on both artificially and naturally contaminated water and through an inter-laboratory comparison.
Methods and Results: All samples were processed by the ScanVIT test according to the manufacturer's instructions and by a culture method (ISO 11731). ScanVIT detected significantly more positive samples, although concentrations were similar and a strong positive correlation between the two methods was observed ($r = 0{\cdot}888$, $P < 0{\cdot}001$). The new test was more accurate in identifying the co-presence of *Legionella pneumophila* and *Leg.* non-*pneumophila*. ScanVIT showed a slightly higher *Legionella* recovery from water samples artificially contaminated with *Leg. pneumophila* alone or together with *Pseudomonas aeruginosa*. Lastly, the inter-laboratory comparison revealed that the ScanVIT test exhibits a lower variability than the traditional culture test (mean coefficient of variation 8·7 *vs* 16·1%).
Conclusions: The results confirmed that the ScanVIT largely overlaps the reference method and offers advantages in terms of sensitivity, quantitative reliability and reduced assay time.
Significance and Impact of the Study: The proposed method may represent a useful validated alternative to traditional culture for the rapid detection and quantification of *Legionella* spp. in water.

Introduction

Legionella spp. are waterborne pathogen bacteria. Currently, the genus includes 52 species and 71 distinct serogroups (SG) (http://www.bacterio.cict.fr/l/legionella.html, last accessed on 17 June 2010). Up to now, only 20 species have been associated with human disease, and *Legionella pneumophila* appears responsible for more than 90% of reported cases of Legionnaires' disease (Yu *et al.* 2002; Doleans *et al.* 2004; Den Boer *et al.* 2008).

Legionella spp. are ubiquitous in natural and man-made aquatic environments (Albert-Weissenberger *et al.* 2007) and are frequently isolated from hot water plants, shower heads, cooling towers, spas, whirlpools, humidifiers, evaporative condenser, eyewash stations, dental devices and other aquatic sources (Atlas 1999).

At temperatures between 20 and 50°C, *Legionella* spp. frequently colonize water distribution systems. The presence of sediment, sludge, scale, rust and other materials within the system, together with biofilms, is thought to play an important role in harbouring and providing favourable conditions in which the legionella bacteria may grow (EWGLI 2005). These bacteria may be able to survive as intracellular parasites of protozoa (e.g. amoebae, ciliates) or within biofilms or sediments also in chlorinated water (Kuchta *et al.* 1993; Diederen 2008).

To prevent *Legionella* infection, routine maintenance of water distribution systems and suitable disinfection actions are requested, under national and international guidelines (Italian Guidelines 2000; EWGLI 2005; WHO 2007). In addition, monitoring for the presence of *Legionella* is generally mandatory in hospital wards with patients whose immune system is severely compromised and may be requested in other risk settings such as dental units, spas, tourist accommodation with persisting risk factors and/or in cases of an outbreak. On these occasions, a rapid detection of contamination is needed to minimize the risk of case appearance and/or to check the effectiveness of any corrective measures. Although the culture technique represents the gold standard, it takes up to 10 days before results are available and has a limited sensitivity, and inter-laboratory variations have been reported in diagnostic field, but not on water samples (Boulanger and Edelstein 1995; Ballard *et al.* 2000; Tronel and Hartemann 2009). Thus, a more rapid, simple and reproducible technique for legionellae detection in environmental samples may be of special interest for routine laboratory applications.

The objective of this study was a validation of the ScanVIT-Legionella™ (Vermicon, Munchen, Germany), a rapid commercial test using fluorescently labelled gene probes, according to the VIT® technology. This method was recently tested by Ditommaso *et al.* (2010) for the monitoring of hospital water systems. We conducted further investigations to validate the method in different settings and to evaluate its inter-laboratory reproducibility. We demonstrated that the method is comparable with the conventional bacterial culture to detect viable *Leg. pneumophila* and *Legionella* spp. in both naturally and artificially contaminated water, showing also a good inter-laboratory reproducibility.

Materials and methods

Experimental study

Tap water samples negative for *Leg. pneumophila* were artificially contaminated with serial dilutions of *Leg. pneumophila* SG 1 grown on BCYE (Buffered Charcol Yeast Extract) medium. The experiments were performed by adding 2 ml of each dilution in 2 l of water to obtain a concentration from 10 to 10^6 colony-forming units per litre (CFU l^{-1}). Five aliquots of 100 μl of each serial dilution were spread on plates of BCYE medium to evaluate the real concentration of the experimental contamination, which was expressed as a logarithmic interval. The experiments were conducted on samples contaminated with *Leg. pneumophila* alone or together with *Pseudomonas aeruginosa* (10^3 CFU l^{-1}) and replicated four and three times, respectively. Each artificially contaminated sample was processed with both culture and ScanVIT-Legionella™ method (Vermicon). With culture method, *Leg. pneumophila* was counted on untreated plates, whereas heat treatment was needed in co-contaminated experiments because recovery of *Leg. pneumophila* from untreated sample was 50% because of inhibiting effect of *Pseudomonas* on legionellae growth (Leoni and Legnani 2001).

Natural water samples

Three geographically diverse, accredited research laboratories participated in the study, two public university departments and the National Institute for Occupational Prevention and Safety (ISPESL). In total, 113 hot waters from different structures (hospital, spa and sporting club) were analysed. A strict protocol was established between the participants to standardize the procedures for sample collection, transport, handling and storage until analysis, according to ISO 19458 (ISO 2006). Samples were taken from distal outlets (showers or taps), storage tank or return line in sterile glass bottles containing sodium thiosulfate as a chlorine-neutralizing agent without flaming and after 1 min of flushing. *Legionella* analysis was performed on each sample with both the reference and the trial method.

Inter-laboratory reproducibility

Twenty of the 113 natural waters were examined with both methods by the three participant laboratories according to ISO 17994 (ISO 2004). They were selected on the basis of previous knowledge on *Legionella* spp. contamination and thus the likelihood of scoring a zero count was small. For each sample, 4 l of water was collected and then divided into three identical portions, immediately shipped and analysed the day after by the participant laboratories, according to the ISO/TR 13843 (ISO 2000).

Culture method

Culture and identification of *Legionella* spp. were carried out by using the ISO 11731 (ISO 1998) method. Briefly, 1 l of water was filtered (0·2-μm pore-size polyamide filter, Millipore, Billerica, MA, USA), resuspended in 10 ml of the original sample water by vortexing for 10 min, and 5 ml heat-treated (50°C for 30 min in a water bath) to reduce contamination by other micro-organisms (Leoni and Legnani 2001; Borella *et al.* 2004). Two aliquots of 100 μl of the original and concentrated specimens (heat-treated and untreated, diluted 1:10 and undiluted) were plated onto GVPC (Glycine Vancomycin Polymixin

Cyclohexamide) selective medium (Oxoid Ltd, Basingstoke, UK). The plates were incubated at 36 ± 1°C with 2·5% CO_2 for 10 days and read from day 4 with a dissecting microscope. Presumptive *Legionella* colonies were subcultured on BCYE (with cysteine) and CYE (cysteine-free) media (Oxoid) to test their inability to grow in the absence of this amino acid and incubated at 36 ± 1°C for 48 h. Colonies growing only on BCYE were subsequently identified by an agglutination test (*Legionella* latex test, Oxoid). Results were given according to the best culture procedure able to give the highest number of legionellae and expressed as CFU l^{-1}.

Throughout the entire study period, the three laboratories routinely participated in external quality control programme (*Legionella* EQA Scheme, Health Protection Agency, UK) to verify the proficiency of the reference culture method. An internal quality control gave a coefficient of variation (CV) <5% performing counts by two different persons**.**

ScanVIT-Legionella™

The ScanVIT-Legionella™ (Vermicon) test was performed according to the manufacturer's instructions. Briefly, 50 ml of water was filtered through the 0·45-μm ScanVIT membrane (25 mm diameter) that has a grid for colony counts. A suitable filter holder manifold with a funnel for membrane filters with a diameter of 25 mm needed to be purchased to avoid problems with the vacuum filtration. During the decontamination phase, despite not being requested, we closed the end of funnel to assure that the acid-buffer (0·2 mol l^{-1} HCl/KCl, pH 2·2) remained in contact with the filter for 5 min. After decontamination, the filter was incubated on a selective medium (GVPC agar, Oxoid) for 72 h at 36 ± 1°C, and then brought into contact with the gene probes in reactors provided in the kit. This method is based on the principle that gene probes specific for the genus *Legionella* spp. and for the species *Leg. pneumophila*, labelled with two different fluorescent dyes, penetrate the bacteria and bind to the ribosomal RNA at the target points. After a 90 -min incubation, the membrane is then transferred to a slide and examined under a fluorescence microscope (Axioskop 40; Carl Zeiss, Gottingen, Germany) equipped with two separated filter sets (09 and 15 from Zeiss) for both the blue and green excitation. All bacteria that show up as green belong to the genus *Legionella* and all those that show up as both green and red belong to *Leg. pneumophila*. The number of *Legionella* spp. and *Leg. pneumophila* colonies are counted separately and the results expressed in CFU l^{-1}.

Preliminarily, training sessions on the use of ScanVIT-Legionella™ (Vermicon) were conducted within each laboratory under the supervision of experts sent by the manufacturer, as recommended by ISO/TR 13843 (ISO 2000) and ISO (2004). They consisted of repeated analyses on the same samples conducted by the same operator as well as by two different operators to check their repeatability and reproducibility, respectively. An average CV <5% on the same slides and <10% on repeated evaluations was accepted for repeatability. The inter-operator reproducibility gave a mean CV of between 5·3 and 14·7%, depending on bacteria concentration and laboratory performance.

Statistical analysis

Statistical analysis was performed according to the principles outlined in Sections 6 and 7 of ISO (2004). The bacteriological data were converted into $\log_{10}$ $(x+1)$ before statistical analyses; results are presented as log CFU l^{-1} and expressed as geometric mean. Tests for normality using the Kolmogorov–Smirnov test were applied to both untransformed and transformed data. The log-transformed data were normally distributed ($P > 0{\cdot}05$), consequently parametric statistics were applied on these sets of data (paired *t*-test, analysis of variance). In addition, chi-square analysis was applied to evaluate difference in positive/negative results (2×2 contingency table), and Pearson's correlation coefficient between the two methods was calculated. Differences at $P < 0{\cdot}05$ were considered significant. In addition, the relative difference between trial and reference method was calculated for each sample according to the formula:

$$x = 100[\ln(a)–\ln(b)]$$

where

$\ln(a)$ = the normal logarithm of the count by the trial method (ScanVIT)

$\ln(b)$ = the normal logarithm of the count by the reference method (culture)

Prior to statistical analysis, results were excluded from the data analyses when both methods yielded counts of zero. The relative performance between the trial and the reference method was then computed using the mean relative difference according to ISO description. A value of 10% difference between methods was used to determine the statistical significance. All statistical analyses were made using SPSS ver. 17.0 (SPSS Inc., Chicago, IL).

Results

Experimental study

Figure 1 presents the results of experimental study. The ScanVIT test allowed to count slightly higher

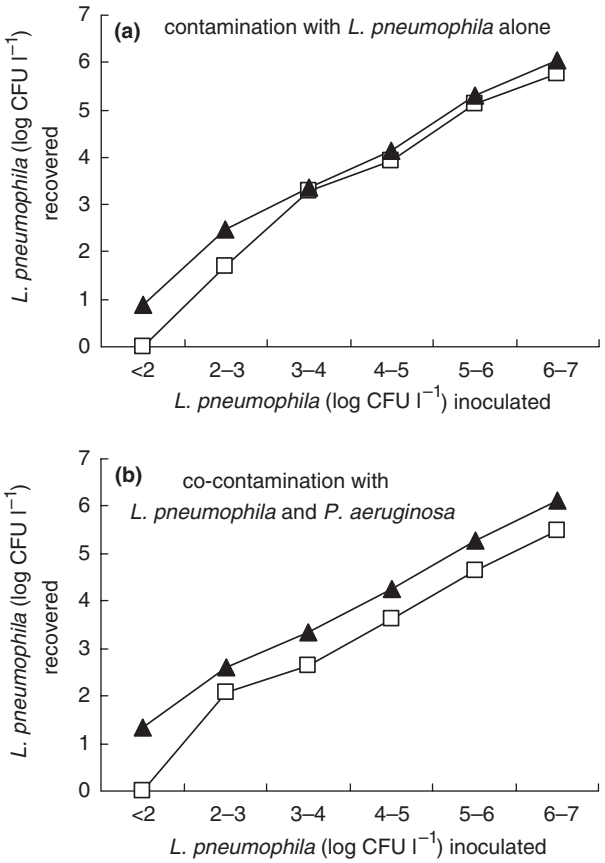

Figure 1 Quantification of *Legionella pneumophila* by ScanVIT (▲) and standard culture method (□) in artificially contaminated water samples. Data are means of (a) four and (b) three experiments.

concentrations of legionellae compared to standard culture method, both in water samples contaminated with *Leg. pneumophila* alone (Fig. 1a) and in the presence of co-contamination with *Ps. aeruginosa* (Fig. 1b). In samples added with *Leg. pneumophila* alone, 94·4% were positive by ScanVIT *vs* 83·3% by the traditional method, and the false negatives were at concentration <60 and <600 CFU l^{-1}, respectively. In water co-contaminated with *Ps. aeruginosa*, *Leg. pneumophila* was detected in all samples by both methods except one <100 CFU l^{-1} negative by the culture method.

Table 1 ScanVIT test *vs* standard culture method for the detection of *Legionella* species in natural water samples

Assay evaluated	Culture-positive *N* (%)	Culture-negative *N* (%)	Total
ScanVIT-positive *N* (%)	60 (53·1)	12 (10·6)	72 (63·7)
ScanVIT-negative *N* (%)	4 (3·5)	37 (32·8)	41 (36·3)
Total	64 (56·6)	49 (43·4)	113 (100)

Natural water samples

Table 1 shows the detection of *Legionella* spp. in natural water samples by the two methods. Sixty samples (53·1%) were positive and 37 (32·8%) were negative by both methods (agreement 85·9%). Of the 60 samples positive by both methods, *Legionella* concentrations did not differ: $3{\cdot}7 \times 10^3$ CFU l^{-1} (range 20–$1{\cdot}1 \times 10^6$ CFU l^{-1}) by ScanVIT *vs* $3{\cdot}2 \times 10^3$ CFU l^{-1} (range 50–$2{\cdot}6 \times 10^5$ CFU l^{-1}) by cultural method. Among the discordant 16 samples, ScanVIT was able to detect another 12 positive (range 20–800 CFU l^{-1}) compared to 4 by the cultural method (range 25–1000 CFU l^{-1}). The difference in isolation frequencies was statistically significant ($\chi^2 = 59{\cdot}4$, $P < 0{\cdot}001$). Regression analysis on total samples revealed a strong positive correlation between the two methods ($r = 0{\cdot}888$, $P < 0{\cdot}001$).

Legionella pneumophila was detected in all the 72 ScanVIT-positive samples, among which six were also positive for *Leg.* non-*pneumophila*. By the traditional method, 62 samples were positive for *Leg. pneumophila* and two for *Leg.* non-*pneumophila*.

The combined paired results data from the three participant laboratories were compared using the mean relative difference procedure of ISO (2004), and results are presented in Table 2. As the objective of the study was to compare a trial method with an established reference method in terms of being 'at least as reliable' (European Union, 1998), the 'one-sided' comparison was taken being appropriate for the acceptance of the trial method (Sartory *et al.* 2008). According to this statement, the two methods did not differ, with the values of the confidence interval (x_L, x_H) being either side of zero.

Inter-laboratory reproducibility

Table 3 shows the inter-laboratory reproducibility of the two methods on natural water samples. Only *Leg. pneumophila* was detected in the examined water, and a non-significant difference among the three laboratories was

Table 2 Mean relative difference analysis (trial method–reference method) of paired sample results from the trial method (ScanVIT) and the reference method (culture) for water samples analysed according to ISO 17994 (ISO 2004)

	(*N* = 76)
Mean relative difference	55·2
Standard deviation	242·2
Expanded uncertainty, *U*	55·5
x_L	−0·3
x_H	110·9
Outcome (one-sided test)	Not different

Table 3 Inter-laboratory reproducibility of the two methods on natural water samples. Values in the parentheses represent the coefficient of variation (CV) between the three laboratories and are expressed in percentage

	Legionella pneumophila (log CFU l^{-1})											
	ScanVIT						Culture					
	Laboratory						Laboratory					
	L1	L2	L3	Mean	SD	(CV)	L1	L2	L3	Mean	SD	(CV)
1	3·01	3·23	3·04	3·09	0·12	(3·6)	3·56	3·24	3·90	3·57	0·33	(9·2)
2	1·32	1·79	1·61	1·57	0·23	(15·1)	2·68	2·15	2·00	2·28	0·36	(15·6)
3	4·05	3·52	3·26	3·61	0·40	(11·1)	3·69	3·60	4·11	3·80	0·27	(7·2)
4	4·25	4·35	4·43	4·34	0·09	(2·1)	4·48	4·24	4·30	4·34	0·12	(2·9)
5	4·16	4·5	4·11	4·26	0·21	(5·0)	4·36	4·57	4·23	4·39	0·17	(3·9)
6	4·72	4·65	4·28	4·55	0·24	(5·2)	4·75	4·95	4·40	4·70	0·28	(5·9)
7	4·19	4·48	4·3	4·32	0·15	(3·4)	4·33	4·35	3·98	4·22	0·21	(4·9)
8	5·17	5·30	4·76	5·08	0·28	(5·5)	5·41	5·00	4·84	5·08	0·29	(5·8)
9	3·02	2·90	3·26	3·06	0·18	(6·0)	3·18	2·88	3·00	3·02	0·15	(5·0)
10	2·95	2·73	2·82	2·83	0·11	(3·9)	3·18	2·48	2·55	2·74	0·38	(14·1)
11	2·99	2·99	1·78	2·59	0·70	(27·0)	2·70	2·85	2·60	2·72	0·12	(4·6)
12	0	0	0				0	0	0			
13	1·79	1·32	1·61	1·57	0·24	(15·1)	1·71	1·95	0	1·22	1·06	(87·0)
14	1·61	1·91	1·32	1·61	0·29	(18·3)	1·41	1·52	0	0·98	0·85	(86·7)
15	2·78	2·53	2·66	2·66	0·12	(4·7)	2·78	2·33	2·74	2·62	0·25	(9·5)
16	2·15	2·00	2·30	2·15	0·15	(7·0)	2·00	1·90	2·00	1·97	0·06	(2·9)
17	2·00	2·58	2·66	2·41	0·36	(14·9)	3·24	2·41	2·70	2·78	0·42	(15·1)
18	3·09	3·11	2·81	3·00	0·17	(5·6)	3·37	3·12	3·6	3·36	0·24	(7·1)
19	3·76	4·00	4·34	4·03	0·29	(7·2)	4·34	4·54	4·26	4·38	0·14	(3·3)
20	3·43	3·62	3·73	3·59	0·15	(4·2)	3·2	3·4	4·3	3·63	0·58	(16·1)

observed by paired *t*-test *(data not shown)*. A mean CV of 8·7% was obtained by ScanVIT ranging from 2·1 to 18·3%, whereas the culture method had a mean CV of 16·1% with a range from 2·9 to 87·0%. According to the ISO/TR 13843, the inter-laboratory reproducibility was also evaluated in terms of root square relative variation (RSD %, relative standard deviation): the dispersion of the results around the mean value was 10·8% for ScanVIT and 29·5% for the standard culture method.

Discussion

The availability of a more rapid and sensitive alternative to the traditional culture method for the detection and quantification of viable *Legionella* spp. is of special relevance for water monitoring. Recently, many PCR-based methods have been proposed as attractive alternative procedures (Levi *et al.* 2003; Yánez *et al.* 2005; Dusserre *et al.* 2008), but they do not discriminate between live and dead bacteria, while the information on viable legionellae is fundamental to assess health risks. Furthermore, Joly *et al.* (2006) recently reported that quantitative real-time PCR is influenced by the type of water sample and that results may be laboratory dependent. Lastly, quantitative real-time PCR gives the number of genome units per litre, but an equivalence with CFU has not been definitively established (Morio *et al.* 2008; Bonetta *et al.* 2010).

In this study, we compared the conventional culture to the ScanVIT test, a rapid method aimed at detecting and enumerate viable *Leg. pneumophila* and other *Legionella* spp. in water samples. The peculiarity of this molecular fast method is represented by the capability of fluorescently labelled gene probes to link bacterial rRNA. Only vital *Leg. pneumophila* have a sufficiently high rRNA content per cell to be detected by this method (Stephan *et al.* 2003). The application of this highly specific gene probe technology has also been reported as a useful tool for the fast and specific detection of different food pathogens (Stephan *et al.* 2003; Schmid *et al.* 2005) and food spoilers (Thelen *et al.* 2003).

One of the advantages of the ScanVIT test compared to the standard culture is the reduction in the analysis time (3 *vs* 10 days), allowing a prompt application of corrective actions aimed at reducing infection risks. In this study, the new technique was validated through experiments conducted on both artificially and naturally contaminated tap water and through an inter-laboratory comparison. The results confirmed that the ScanVIT technique largely overlapped the standard cultural method and offered a series of advantages. These included

qualitative benefits such as fewer false negatives in the lowest interval of concentration and more accuracy in identifying the co-presence of *Leg. pneumophila* and *Leg.* - non-*pneumophila*. Furthermore, the ScanVIT test provided excellent quantitative results compared to the traditional culture on natural samples, represented here by hot waters collected from structures frequently involved in disease risk such as hospitals and spas. The mean relative difference analyses (one-sided test) indicate that the new method was equivalent to the reference method. In addition, the ScanVIT test was less influenced by co-contamination, showing a higher recovery of *Legionella* from water artificially contaminated with *Ps. aeruginosa*. In contrast with our results, Ditommaso *et al.* (2010), monitoring hospital water supplies, reported consistently higher *Legionella* concentration from the culture technique. These contrasting results could be because of differences in the examined water in terms of higher/lower level of contamination, presence/absence of concomitant microbial flora, supply and structure type, all factors possibly influencing the bacteria detection by the culture method (Leoni and Legnani 2001).

These observations also highlight the need to evaluate inter-laboratory reproducibility for the traditional culture method. Recent inter-laboratory comparison on culture, in fact, has been conducted for *Legionella* diagnostic tests (Tronel and Hartemann 2009) but not for *Legionella* isolation in water. In the present study, we studied the inter-laboratory reproducibility of the *Legionella* culture method using a standardized procedure (ISO 11731) and demonstrated a higher variability compared to ScanVIT, despite the latter method requiring trained personnel to count colonies under a fluorescence microscope. Limited disadvantages such as some technical issues in the filtration and decontamination phase (see specifications in the materials and method section) can easily be solved.

In conclusion, the major practical advantage associated with a more rapid, but sensitive and comparable test is the opportunity to repeat the analyses, for instance to frequently check the efficacy of preventive measures in at-risk structures. Obviously, the isolation of the *Legionella* spp. remains the gold standard procedure for linking clinical strains (when detected) to environmental sources. However, we stress that routine water monitoring may benefit from utilizing this new rapid technique as a validated alternative to traditional culture methods.

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