

New method for the quantification and identification in three days of *Legionella* spp. and *Legionella pneumophila* cultivable on GVPC

Garrelly Laurent, Elodie Gemrot, Minervini Céline and Boissezon Bernadette, Bouisson Bertrand Laboratories, Montpellier, France



Background

Regular monitoring for the presence of *Legionella* in water systems is necessary action to prevent outbreaks of *Legionella* infections. Traditional cultured-based methods for the detection of *Legionella* spp. and *Legionella pneumophila* are performed in accordance with ISO 11731. These methods are too time-consuming and not sensitive enough. Rapid, simple and sensitive methods are required for routine laboratory applications.

The new, ready-to-use method ScanVIT-Legionella™ is based on gene probe technology, which enables the quantification as well as simultaneous detection of cultivable *Legionella* and *Legionella pneumophila* within three days. The detection of bacteria using ScanVIT-Legionella™ takes place on a filter membrane, which after filtration of a water sample and 72 hours of cultivation on GVPC-agar is brought into contact with the gene probes. Real cfu numbers are obtained using this method.

Objectives

The primary objective of this study was to compare this new ScanVIT-Legionella™ method with the standardised 10-day culture method in different types of water sample, with and without interferent flora. The secondary objective was to evaluate the specificity of the ScanVIT-Legionella™.

Methods

Ninety-six water samples were tested for the detection and quantification of *Legionella* spp. and *Legionella pneumophila* using ScanVIT-Legionella™ and culture reference method ISO 11731. The water samples are from different origins (cooling tower water, hot sanitary water, drinking water and thermal water).

One litre was used for the culture method and 1 ml or 20 ml was used for ScanVIT-Legionella™. Culture *Legionella* followed the ISO 11731 standard protocol. ScanVIT-Legionella™ followed the above method and for the result interpretation the number of microcolonies was counted using a fluorescence microscope. For the specificity study, spiked samples with collections of strains from the Pasteur Institute or ATCC were used.

Results

Table 1: Comparative analysis of the results obtained using the culture method and the ScanVIT-Legionella™ method.

Culture method \	Positive (+)	Negative (-)	Total
ScanVIT	%	%	%
Positive (+) %	50	15.2	65.2
Negative (-) %	5.4	29.4	34.8
Total %	55.4	44.6	100

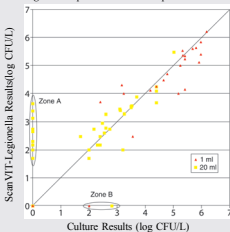
Specificity for ScanVIT-Legionella™.

The specificity of ScanVIT-Legionella™ method was verified on 15 *Legionella* species:

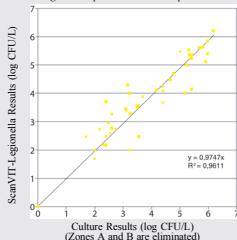
L. anisa, *L. birminghamensis*, *L. cherrii*, *L. cincinnatiensis*, *L. dumofii*, *L. erythra*, *L. feeleii*, *L. hackeliae*, *L. jordanis*, *L. lansingensis*, *L. longbeachii*, *L. micdadei*, *L. oakridgensis*, *L. pneumophila* pSI, *L. sainthelensis*

The ScanVIT-Legionella™ method detected all these *Legionella* strains.

Graph 1 Comparison of the culture method and the ScanVIT-Legionella™ methods for the enumeration of *Legionella* species in water samples.



Graph 2 Revised comparison of the culture method and the ScanVIT-Legionella™ methods for the enumeration of *Legionella* species in water samples.



Discussion

Zone A (Graph 1): this difference in results may be due to interferent flora issue in the standard method. Zone B (Graph 1): the difference in the results may be due to a combination of too low a *Legionella* concentration and sample volume for ScanVIT-Legionella™.

The ScanVIT-Legionella™ method offers easier and more reliable interpretation by counting highlighted microcolonies rather than visual characteristic colonies.

This method also gives simultaneous results regarding the presence of *Legionella* spp and/or *Legionella pneumophila*.

ScanVIT-Legionella™ does not allow to isolate for further investigation of epidemic strains.

Conclusion

ScanVIT-Legionella™ demonstrates a time saving of more than 50% compared with the standard method.

ScanVIT-Legionella™ on water samples shows more positive results than the standardised method.

This study did not show any statistically significant difference between the two methods for the enumeration of *Legionella* spp in water sample.

No false negative was found in the specificity part of this study in either of the methods.

ScanVIT-Legionella™ is a trademark of Vermicon AG, Munich, Germany.