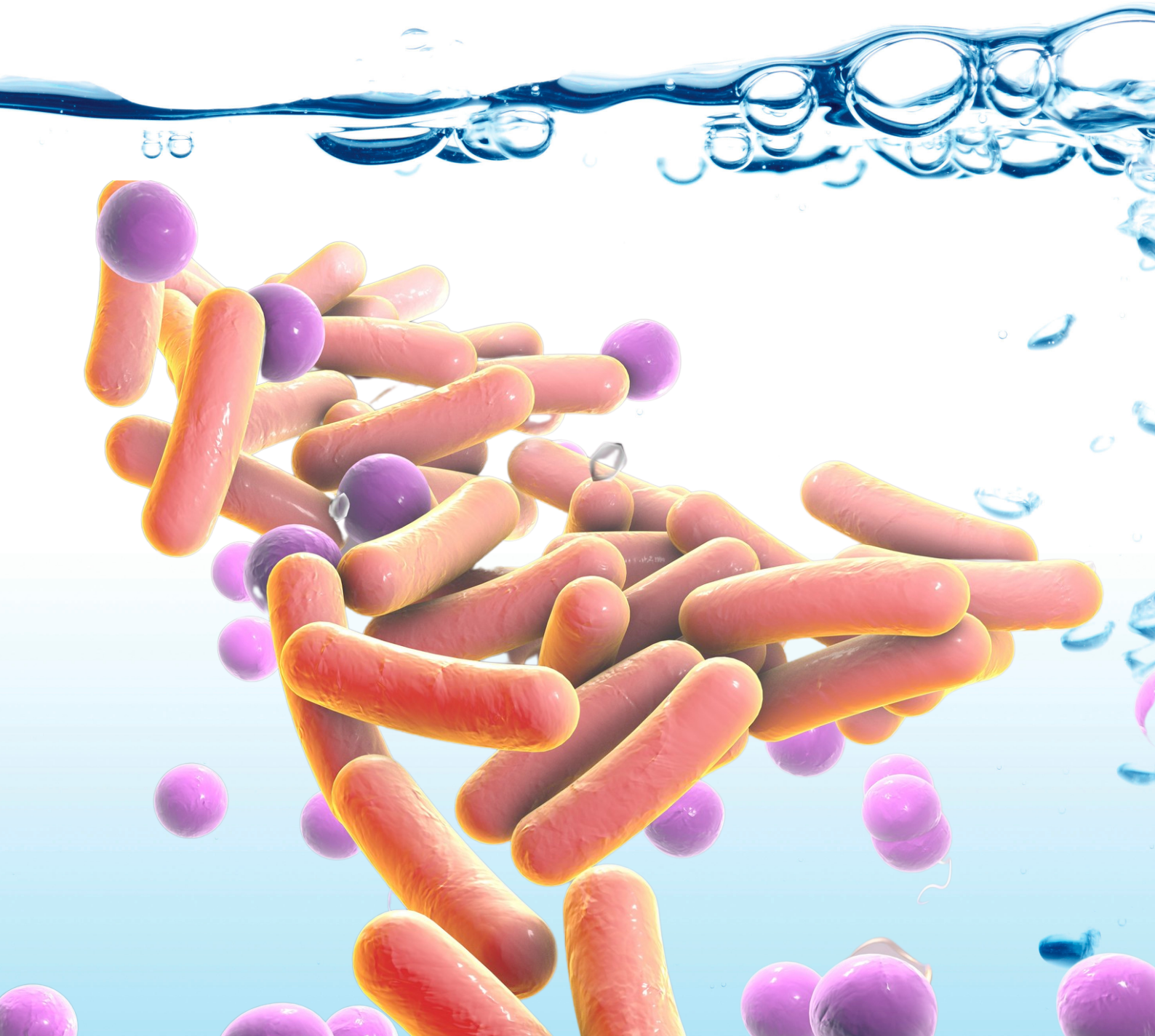
DI-Check Legionella pneumophila method as screening test of negative water samples

- DIATHEVA offers an alternative method to the culture method for *Legionella pneumophila* results based on Real Time PCR technology which allows you to obtain the result in less than 4 hours. The DIATHEVA DI-Check Legionella pneumophila molecular method complies with ISO 12869 and is **AFNOR** certified

- DI-Check Legionella pneumophila represents an excellent solution for the screening of all types of water allowing to exclude negative samples in a very short time and identify positive ones



Introduction

Evaluation of the potential risk associated with the presence of *Legionella pneumophila* in water samples is currently determined using culture-based methods. The ISO 11731: 2017 method, "Water quality - Enumeration of Legionella " is a very complex protocol that requires different culture media, long incubation time (up to 10 days for a negative result) and requires a high degree of technical skill in the manual operation and in the ability to recognize characteristic Legionella colonies (a).

This method is also characterized by an error in assessing which leads to an underestimation of the bacterial count by approximately 10 to 60% (b).

Legionella can be detected by other methods including Real-Time PCR according to ISO / TS 12869: 2019. In this context, Diatheva's DI-Check Legionella pneumophila method allows to detect and quantify *L. pneumophila* in all types of water samples in less than four hours, in compliance with AFNOR XP T90-471 and ISO / TS 12869: 2019.

The method is based on 3 steps: filtration of the water sample, DNA extraction and real-time PCR amplification. The amplification kit offers a ready-to-use PCR mix that is highly resistant to PCR inhibitors and with an extremely rigorous automatic hot-start system.

This robust PCR mix contains an internal control to evaluate the efficiency of the amplification reaction by detecting the presence of inhibitors in the sample.

The aim of this work is to evaluate the possibility to use the DI-Check Legionella pneumophila method to quickly detect the presence of *Legionella pneumophila* in water samples and to evaluate the prognostic significance of negative results, minimizing time to result and optimizing human and material resources.

Materials and Methods

Water samples (n = 101) screened for *L. pneumophila* contamination were collected by a local hospital institution within the institution's building. The sanitary water samples came from several sites undergoing continuous hydrogen peroxide sanitation and all samples were analyzed for possible contamination by *L. pneumophila* with the culture method according to ISO 11731: 2017 and with the DI-Check Legionella method pneumophila of Diatheva.

The DNApure Water Isolation kit (MBK0081, Diatheva) was used for the extraction of microbial DNA from Gram negative bacteria present in the water samples. The purified DNA was amplified in Real-Time PCR using the DI-Check Legionella pneumophila kit.

Statistical analysis to examine culture results and Real-Time PCR was performed using GraphPad 5 software, via $\chi 2$ test to examine whether the results of the two methods (culture and real-time PCR) were independent or less.

The small number of samples was compensated for by Yates' correction. For the molecular method, positive and negative predictive values (PPV and NPV respectively) were calculated, corresponding to the ratio between the number of positive (or negative) samples for both methods and the number of positive (or negative) samples by PCR .

Results and Discussions

101 sanitary water samples were analyzed by culture and molecular method. 3 samples (2.9%) were culture positive and the remaining 98 (97.1%) were negative. The 3 positive cultured samples were successfully disseminated using the DI-Check Legionella pneumophila method. In addition, the molecular method showed 5 positive samples which tested negative for the culture method. As reported in the literature, using the culture method there is the possibility of obtaining an underestimation of *L. pneumophila*, with the possibility of false negative results (b).

$\chi 2$ was calculated considering 1 degree of freedom and a p value <0.0001 ($\alpha = 0.001$). According to the $\chi 2$ distribution, for 1 degree of freedom and $\alpha = 0.001$, the critical value is 10.83. In this study, $\chi 2$ was calculated as 24,109, so these results clearly showed that culture and molecular method are related.

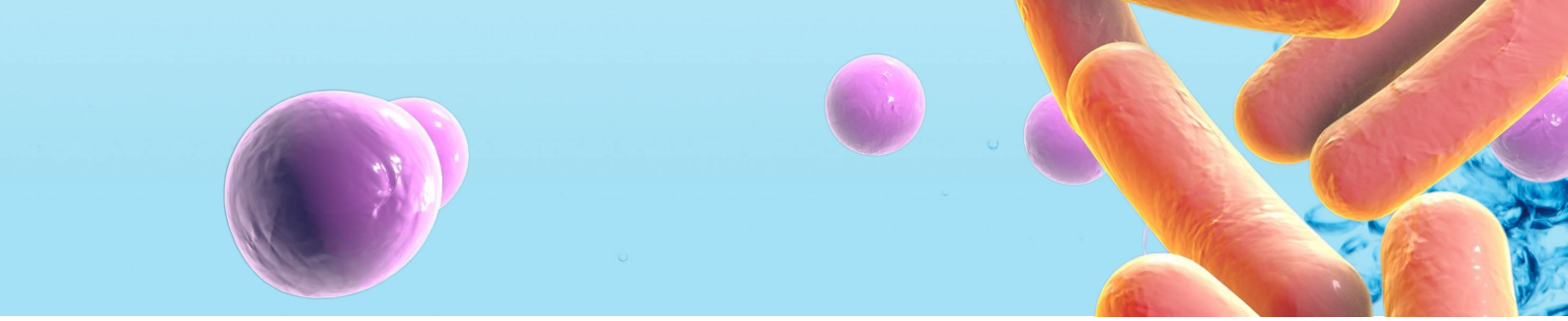
The PCR PPV and NPV for the sanitary water samples were 100% and 94.8%, respectively.

Conclusion

Diatheva's DI-Check Legionella pneumophila method detected a total of 5 positives discordant with the results obtained with the culture method, out of a total of 101 water samples tested.
The high NPV value (94.8%) obtained with the molecular method indicates that this method can be used for the screening of negative samples, facilitating the timely control of *L. pneumophila*, which plays a fundamental role in protecting health public.
The molecular method can be used for sample screening, being able to quickly identify negative samples and identify positive ones. In case of obtaining positive results, the microbiologist is encouraged to carry out the confirmation of the positive result in culture according to the ISO 12869 guidelines with a more specific research on legionella through culture examination. In this context and confirming this, the use of Real Time PCR is in fact also encouraged by the main Italian reference centers for Legionella (c).

CULTURE	Real-time PCR		TOTAL
	Positive	Negative	
Positive	**3**	**0**	3
Negative	**5**	**93**	98
Total	**8**	**93**	101

Table 1: Contingency table for the DI-Check Legionella pneumophila method



References

a Bartie C., Venter S.N. and Nel L.H. (2001) Evaluation of detection methods for Legionella species using seeded water samples. Water SA, 24:523-527.
b Musumeci R., Martinelli M., Signorini L., Calaresu E., Pecovela A., Pastoni F. and Cocuzza C. (2017) Legionella pneumophila in water: performance of culture method detection carried out by Italian laboratories participating to UNICHIM proficiency testing and validation of three molecular methods for quantitative detection. ECCMID, the congress of ESCMID, Vienna 22-25 April 2017.
c Notiziario dell'Istituto Superiore di Sanità Volume 31 - Numero 9 Settembre 2018

Ordering information

PRODUCT CODE	PRODUCT DESCRIPTION	SIZE
MBK0058	**DI-Check Legionella spp. Kit**	**100 tests**
MBK0081	**DI-Check Legionella pneumophila Kit**	**100 tests**
MBK0080	**DNA Pure Water Isolation Kit**	**100 extractions**

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Visit the page **https://www.diatheva.com/it/?s=legionella&post_type=product&type_aws=true**

Contact us at **info@diatheva.com**

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