

agenzia regionale per la **PROTEZIONE DELL'AMBIENTE** Del Friuli venezia giulia





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CULTURAL AND RT-PCR COMPARISON METHOD: UDINE ARPA FVG LABORATORY EXPERIENCE

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arpa FVG

The ARPA FVG Udine Lab is the **Regional Reference Center for** the Environmental Legionellosis Diagnosis

2002 - 2016

From 2002 to 2016, **19119** samples from **707** different sites were analyzed.

In particular, from 2014 to 2016, 308 samples from Legionnaires' disease related structures were analyzed using both the CULTURE METHOD and REAL TIME PCR

PERIOD SAMPLES 2002/02/19 19.119 **OBSERVED GROUPS (2002 – 2016)**



GEOGRAPHIC DISTRIBUTION OF SITES (2002 -2016)









AIMS

To compare "classical" **CULTURAL METHOD** and the **REAL TIME PCR** method, both applied to the Legionella pneumophila identification in drinking water. Those samples are taken from structures in which positives cases of Legionnaires' disease were found

A correlation between the results of the two methods has been evaluated in order to demonstrate **CFU and GU equivalence**

LEGIONNAIRES'DISEASE-RELATED SITES (2014 - 2016)



RESULTS

AVERAGE

SAMPLES PER

From 2014 -2016, 308 samples have been analyzed applying the two methods.

YEAR	NUMBER OF SAMPLES ANALYZED	CULTURAL METHOD: % POSITIVE SAMPLES	PCR REAL TIME METHOD: % POSITIVE SAMPLES
2014	43	25	39
2015	159	32	41
2016	106	20	40
TOTAL SAMPLES	308	25%	39%

POSITIVE **SAMPLES**

CORRELATION COEFFICIENT R IS 0,58



DISCUSSION

The results confirmed that **PCR is more sensitive** and specific than the conventional method for the L. pneumophila detection, but this analysis can't distinguish between living and dead cells.

This technique can be used for preventive screening and complementary method because permits to analyze a **significant number** of samples in a **short time**.

The correlation analysis did **not reveal any** significant relationship between the two **methods**, as reported in other studies.

From the observation of the data there is **no** evidence of equivalence between GU and CFU.





CULTURAL METHOD

Udine Laboratory is certified ISO 17025:2005. Material and methods used in this work are described in the ISO 11731:1998 "Water quality – enumeration of Legionella".













The method is recognized in the **Guidelines**. The levels of action indicated in Italian and European legislation are expressed in CFU/ L.

Results in **more than 10 days**.



Low cost.

REAL TIME PCR METHOD

The Real time PCR method is reported in the guideline "Allegato 6, 79/CSR/2015". Quantification of DNA has been made according to ISO/TS 12869 : 2012 "Water quality- Detection" and quantification of L. peumophila by concentration and amplification by qPCR".





Correlation was checked by means of linear regression. Computed positive results are expressed in CFU/1000 ml e GU/1000 ml units from 16 samples.



There are no levels of action indicated in Italian and European legislation expressed in GU/ L.

- Results in a **short time** (less than 24 hours).
- Determines the DNA of living, dead or damaged bacteria or vital non-cultivated (VNBC) cells (ISO 12869: 2012).

High cost.

Greater sensitivity: samples analyzed by this method show a higher number of positive samples than cultural method.

- **Greater specificity**: the specific sequence of DNA target bounded by primers.
- The fluctuations of a *Legionella* population could be monitored faster by PCR than by culture, potentially leading to reduced biocide costs and ecological benefits.



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