ARPA FVG
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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

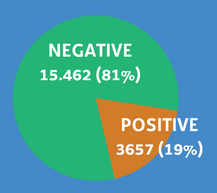
## 2002 - 2016

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**
2002/02/19 - 2016/12/28

**SAMPLES**
19.119

**AVERAGE SAMPLES PER YEAR**
1.275

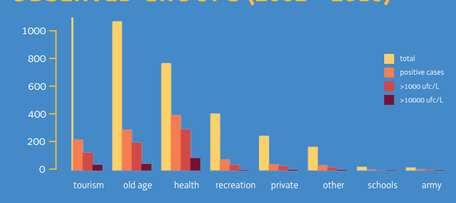



**(0-1000] CFU/L**
1.748 (9%)

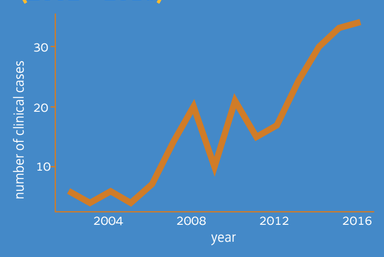
**(1000-10000] CFU/L**
1.529 (8%)

**>10000 CFU/L**
370 (2%)

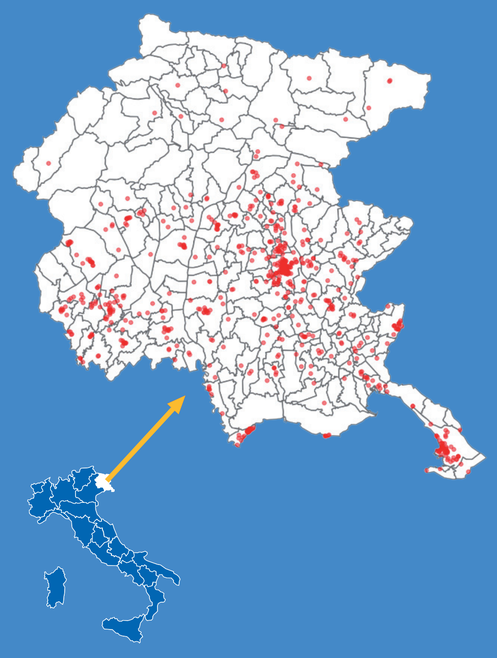
**OBSERVED GROUPS (2002 – 2016)**




**NUMBER OF SAMPLES FROM STRUCTURES RELATED WITH CASES OF LEGIONNAIRES' DISEASE (2002 – 2016)**



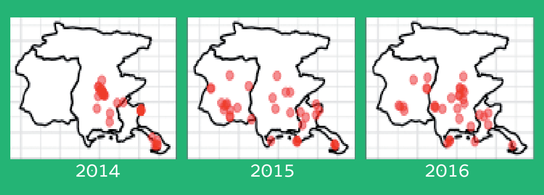

**GEOGRAPHIC DISTRIBUTION OF SITES (2002 -2016)**



## 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)**

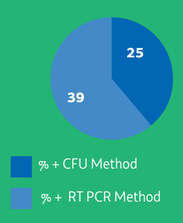



## RESULTS

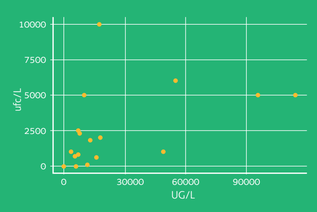
From 2014 -2016, 308 samples have been analyzed applying the two methods.

| 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**



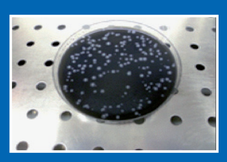
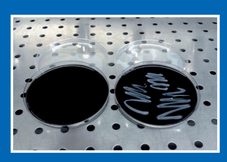

## DISCUSSION

- 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*".

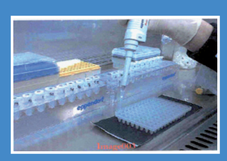



- The method is recognized in the **Guidelines**.
- The levels of action indicated in Italian and European legislation are expressed in CFU/ L.
- Results in **more than 10 days**.
- Determine live bacteria such as CFU / L (ISO 11731).
- **Low cost**.

## 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.

- The method is **recommended** in the 2015 Guidelines 79 / CSR for a quick analysis, in particular in legionellosis cases or clusters. Positive samples should however be analyzed with the culture method.
- 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.

## BIBLIOGRAPHY

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