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DETECTION ASPECTS OF LEGIONELLA IN WATER BY ALTERNATIVE METHODS

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Introduction

Legionella pneumophila is a microorganism dangerous to human health and can be identified, more difficultly than right, even in water used for drinking purposes. *Legionella pneumophila* is responsible for 90% of cases of legionellosis, inapparent infections or foci of fibrin-purulent bronchopneumonia - which lead to abscess in immunocompromised patients. *Legionella* species live in a wide variety of aquatic environments. They can store carbon and energy in the form of polyhydroxybutyrate granules, which allows the bacterial cell to survive without the substrate for a long time. The natural habitat for Legionella is mainly the aquatic environment, including running water, lakes, thermal waters, these microorganisms being able to withstand variations in temperature and pH.

In most European countries, *Legionella* is isolated in water samples by analyzing a liter of sample from the point of exit, the methods used being established according to different rules. The standard method for identifying the bacterium *L. pneumophila* is the method of insemination developed by the Centers for Disease Control and Protection (CDC), the time interval of the analysis being between 10 days and 2 weeks. Instead, there are other methods, even quantitative, in which the working time is shorter and the results are more accurate.

Depending on the rules followed, selective or non-selective media should be sown. To obtain optimum sensitivity, the samples should always be concentrated, preferably by filtration. To prevent the proliferation of other microorganisms present in the water, a pretreatment by heating or acidification is applied.

In correlation with the filtration procedure, in which Legionella bacteria are retained in the filter from where they will be transferred to culture medium, the pretreatment is relatively aggressive, being responsible for the loss of a variable number of microorganisms, for which the final result may be underestimated relative to the actual *Legionella spp.* number in the sample analyzed. The isolation limit in most laboratories is 50-100 CFU / L.

Materials and methods

To demonstrate the effectiveness of two distinct methods for determining *Legionella spp*, 4 synthetic water samples were used in the membrane filtration method and the MPN (Most Probable Number) (Idexx) method. For the membrane filtration method, the samples were seeded on GVPC medium and incubated for 10 days at 37°C. The characteristic grey-blue colonies were transferred to BYCE without L-Cysteine and BYCE with L-Cysteine medium (Oxoid) and incubated for 2 days at 37°C. the confirmation test was performed with an agglutination kit using monoclonal serum.
In the same time, the samples were seeded in Legiolert medium (Idexx) and dispensed in Quanti-Tray bags and incubated at 37°C for 7 days.
All samples were duplicated and negative controls were permanently used.

Results and conclusions

The results of the membrane filtration method indicated a 98% efficiency in the qualitative detection of *Legionella*, compared to the MPN method which showed an efficiency of 99%. The accuracy of the quantitative determination of CFU / 100 mL was higher for the MPN method compared to the membrane filtration method, the difference between them being about 12%.
The characteristic colonies developed on the membrane and repeated on selective media with and without cysteine proved not to be entirely *Legionella* (Figure 1), while by the MPN method the test did not require the selectivity stage (Figure 2).

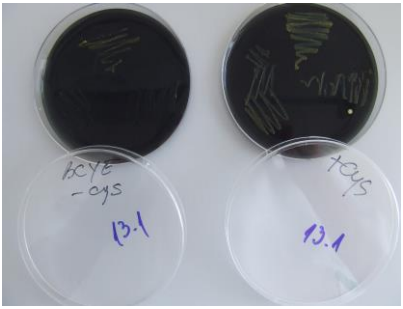

Fig.1. Characteristic colonies of *Legionella* isolated on specific media (BCYE without L-Cysteine – left side and BCYE with L-Cysteine – right side).

Fig.2. The single step of counting the CFU / mL Legionella on the Quanti-tray wells – MPN method.

From the analysis performed, the filter membrane method is more sensitive and can be applied for water samples with low bacterial content, while the MPN method can be used more effectively for contaminated water samples.
In conclusion, both methods have high UFC detection rate of Legionella / mL with advantages and disadvantages. the filter membrane method involves more time and potential human errors due to the successive stages of manipulation, and the MPN method is performed in a shorter time with a final result in a single stage.