LEGIONELLA FAST DETECTION









biotica®

Here at **Biótica** we offer fast microorganism detection techniques. We combine immunomagnetic capture with enzyme immunoassay (C.E.I.A.), with a view to enhancing the capacity to evaluate the risk associated with pathogen bacteria, quickly and reliably. This is the only technology capable of delivering efficient control, thereby reducing the cost of non-prevention. Our procedures are simple and fast, capable of determining the quantity of microorganisms associated with the risk, in a user- friendly, low-cost format and without any instrumental requirements.

Biótica is the first biotechnology firm located in the Science, Technology and Business Park of Jaime I University in Castellón de la Plana, Spain. It has the best facilities for research, development and the production of innovative techniques, along with real scenarios to design and test products (living lab), with the direct participation of the end user. It delivers products with an endgame vision which are the result of applying intensive know-how.

Biótica manufactures Legipid® Legionella Fast Detection, a patent-protected product (PCT/ES2008/000613) for the swift and simple detection of whole Legionella pne____op_ila cells.





Legionella

Would you like to know much *Legionella pneumophila* is present in your facilities/equipment? Have you calculated how much it will cost you not to prevent this risk? Prevention improves with the use of **Legipid**[®].

Legionella pneumophila is a gram-negative pathogen bacterium, an obligate aerobe, which is coccoid or bacillary in form. A strong survivor, it is undemanding in its natural environment, water, and is very persistent. It reaches buildings and facilities through the water supply and distribution network. Its arrival is inevitable and it is a natural coloniser. In the water present in facilities and equipment, it cannot cause harm, but these are the conditions in which it proliferates. It then swells to large numbers. So, in the aerosols generated in a shower head, in a hospital or a hotel, in a car wash or in an industrial cooling system, there might be small droplets brimming with this bacterium. This is how it reaches us, through inhalation. *Legionella pneumophila* can travel great distances. Cases of infection have been recorded in a radius of up to 10 km around a point of origin. Near or far, if *Legionella pneumophila* reaches our lungs it will behave in a very similar way, either colonising or invading. In a few days, we develop pneumonia. Legionellosis is a systemic infectious disease that primarily affects the lungs and has a mortality rate of between 5 and 30%.

Of all the cases recorded, 95-98% can be attributed to *Legionella pneumophila*. This disease is a very hot topic in the field of Public Health, since the average mortality rate is 12-15% and can easily reach 30-50% in patients with a poor immune system or who do not receive antibiotics promptly.



The main cause of this infection is the strain *Legionella pneumophila* which is responsible for 95.5% of diagnosed cases. 80% of these cases are due to serogroup 1. This bacterium is found naturally in water and colonises facilities and equipment that use water throughout the world. These biofilms act as time bombs which unpredictably release free forms of the bacteria in water. In just 2-3 days, it can transform from a non-infectious to an infectious form. These are reservoirs of bacteria which reinfect facilities.







High-risk facilities

60% of the time a person spends at work and home takes place in proximity to different types of highrisk facilities or equipment. Legionellosis is a worldwide health topic, for example, in Europe, 6,000 cases are registered every year and between 8000 and 18,000 people are hospitalised every year in USA. The proportion of deaths reported every year ranges from 6% to 15%, but this is deemed to be an underestimation, since many countries are unable to provide mortality figures.

High-risk facilities and equipment include:

- Humidifiers.

- Ornamental fountains.

- Sprinkler watering systems in urban environments.

- Fire extinguishing systems that use water.

- Outdoor aerosol cooling elements.

- Other apparatus that accumulates water and could produce aerosols.

- Therapeutic respiratory equipment.

- Respirators.

- Nebulisers.

- Other.

- Cooling towers and evaporative condensers.

- Hot water systems with water tanks and back circuits.

- Heated water systems with constant movement and recirculation through high speed water jets or air injection.

- Industrial humidifiers.

 Interior systems for cold water intended for human consumption (pipes, water tanks), cisterns or mobile tanks and hot water systems without a back circuit.

- Evaporative cooling equipment which sprays water.



Legipid® Legionella Fast Detection

The **Legipid**[®] Legionella Fast Detection kit is a validated technique capable of detecting Legionella pneu op ila in water samples in just one hour. No specific laboratory equipment is required. It is the only fast system in the world capable of detecting *Legionella pneumophila* in water samples which has been awarded an international certificate by the AOAC Research Institute.



Acknowledgements:

- Validation by ISO 17025 laboratories - Intercolaborative study - AOAC Certification (by ISO 11731 comparison)









Parameters	Petri Dish Cultivation	Molecular Techniques (Pcr)	Legipid [®] Legionella Fast Detection
Result Time	10-15 days	3-6 hours	50 Minutes
Operator's Level of Qualification	Medium	High	Low
Specific Instrumentation	Yes	Yes	No
Average Cost	Medium	High	Low



Why use Legipid®?

a result in just one hour, as opposed to 10-15 days, which is the length of time taken using the traditional method (culture method), allowing corrective measures to be taken in line with the real risk present, thanks to the speed with which a result is obtained.

Strategy. Legionella is not distributed uniformly through the entire water circuit and can be found in very different concentrations depending on the point tested. With the Legipid® test, you can apply a representative sampling of the entire facility, by testing different points and identifying critical control points for efficient preventive maintenance.

Simplicity. As simple as following the step-by-step instructions that come with the kit. You will be able to carry out the test and get a useful and reliable result.

Validated and internationallity certified method. The Legipid® Kit is the only quick test for Legionella pneumophila in the world that has been certified by the AOAC Research Institute.

Economic. The Kit contains everything you need to carry out the test. Fewer requirements in terms of equipment, staff and a competitive price make this kit an ideal method for ensuring prevention in your facilities.

Operability. By working in batches, you will be able to carry out up to 15 tests in 1 hour.

The **Legipid**® Legionella Fast Detection Kit is a test that can quickly and effectively detect the amount of free and intact *Legionella pneumophila* in water, with a view to preventing this bacterium from reaching dangerous and sustained levels for human contagion, with a high probability of cases or outbreaks.

The measures taken in the event of an outbreak entail costs through the closure of facilities, economic sanctions, healthcare costs, as well as reputational costs and sick leave taken. Cases involving hotels, hospitals and care homes have a high media impact which causes social panic and leads to a loss of customers and even the closure of a facility.

The possibility of eradicating Legionella completely from facilities is very difficult because not only is Legionella present in the planktonic phase (aqueous), but it also lives in the biolayer generated in the water-mechanical container interface (sessile phase), which, with different densities (thicknesses), is present in the entire water distribution system.

Given this situation, it is important to ensure environmental supervision which capitalises on the main characteristics of the test:

Speed. *Legionella*, a bacterium which is regularly present in water, can reach infectious levels in 2-3 days. The Legipid® Kit enables the user to obtain



Technology

This technique is based on the use of magnetic particles and antibodies. We coat particles with anti-Legionella pneumophila antibodies. So, when the presence of this intact bacterium is detected in a sample of water, it will stick to the particle, owing to the antigen-antibody interaction, forming a particlebacterium complex. This interaction will depend on the integrity of the cell envelope, on which depends the bacterium infectivity. A second tagged antibody will develop a final colour intensity proportional to the amount of Legionella pneumophila present in the sample, and so the user can take any action required in just one hour.











The volume of water examined is filtered with a view to preconcentrating the microorganism present in the sample, which is retained by the filter. It is then eluted using the reactive included in the test before being analysed. This is a series of stages that encompasses the incubation of the sample with a view to carrying out the selective capture of the microorganism. Thanks to the formation of bacteria-particle complexes and the properties of these particles, these complexes can be retained magnetically. After the sample is washed, the complexes formed are tagged using an antibody combined with an enzyme. The addition of substrate to develop a colorimetric reaction will allow the user to analyse the sample.

Inmunomagnetic

analysis (kit)

> During the process, an analysis is carried out in parallel in relation to a negative control. Following the intuitive testing protocol, the reaction is stopped after 2 or 10 minutes, according to the instructions provided. The interpretation of results is as simple as comparing the colour of the samples with the colour chart (comparison after 2 minutes) or with the negative control (after 10 minutes). The kit's detection limit is 93 CFU/volumen examined.

Result









Analysis



~3 -2 APPENDIX A - Color cha min from the indicated in the To estimate the level c beginning of color reac eration of following color chart. numeration of ganization for Results in cfu / volume examined resis (PFGE), Fields, D F. Art 30 years 103 104 tates viable and Other

biotica[®]

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