

## FAST DETECTION OF LEGIONELLA PNEUMOPHILA IN COOLING TOWERS BY ESTAPOR<sup>®</sup> IMMUNOMAGNETIC MICROSPHERES

Guillermo Rodríguez<sup>a</sup>, Fabrice Sultan<sup>b</sup>, Begoña Bedrina<sup>a</sup>, and Inmaculada Solís<sup>c</sup>

<sup>a</sup>. Biótica, Bioquímica Analítica, S.L., Parque Científico Tecnológico y Empresarial de la Universidad Jaume I- Edificio de Investigación I, 2<sup>a</sup> Planta, Castellón-Spain

<sup>b</sup>. Merck Chimie SAS, Estapor Microspheres - 201 rue Carnot - 94126 Fontenay sous Bois Cedex - France - estapor.info@merck.fr

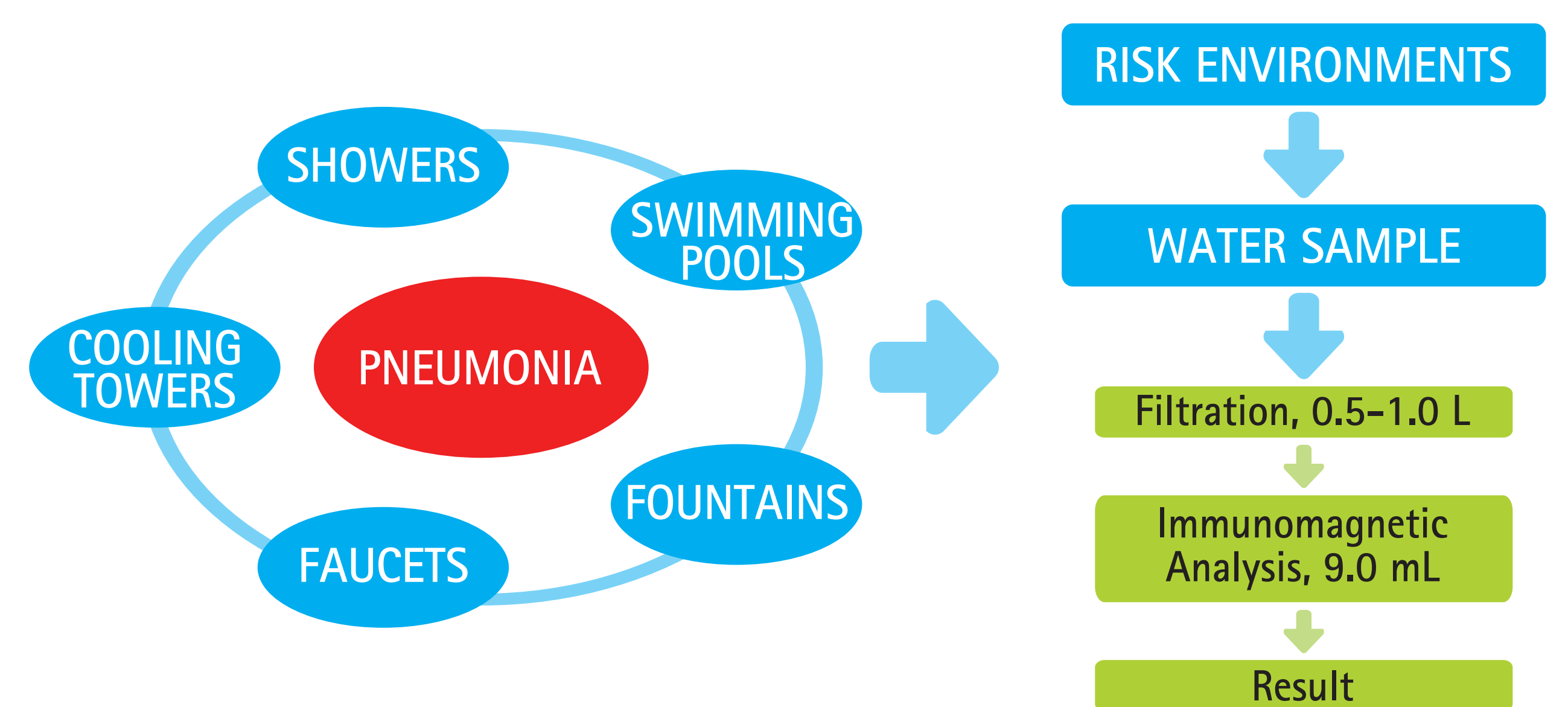
<sup>c</sup>. Iproma, S.L., Camino de la Raya, No. 46, Castellón-Spain

### ABSTRACT

Legionnaires' disease is a severe form of pneumonia caused by bacteria of the genus *Legionella*. It is a notifiable disease worldwide, with relevant clinical aspects ( fatality rate of 12-15 %, easily extended up to 30-50% in immuno-compromised patients). Predominant species responsible for illness is *L. pneumophila*, a virulent pathogen which is responsible of 90-98 % of the cases. Rapid, cost-effective, reliable and simple test is a key issue for monitoring water quality and prevention of the outbreaks of *Legionella* infections. Cooling towers are known to be one of potential sources of harboring, amplifying and disseminating *L. pneumophila*. Composition of water in cooling towers can be very complex, causing a loss of culturability in the cells, so they can not be recovered by culture technique. Moreover, this water can contain toxic substances, such as heavy metals, inhibiting polymerase reaction in PCR. Immuno-sensing techniques addressed to detection of intact cell can be good approaches to restrict detection to the viable cells.

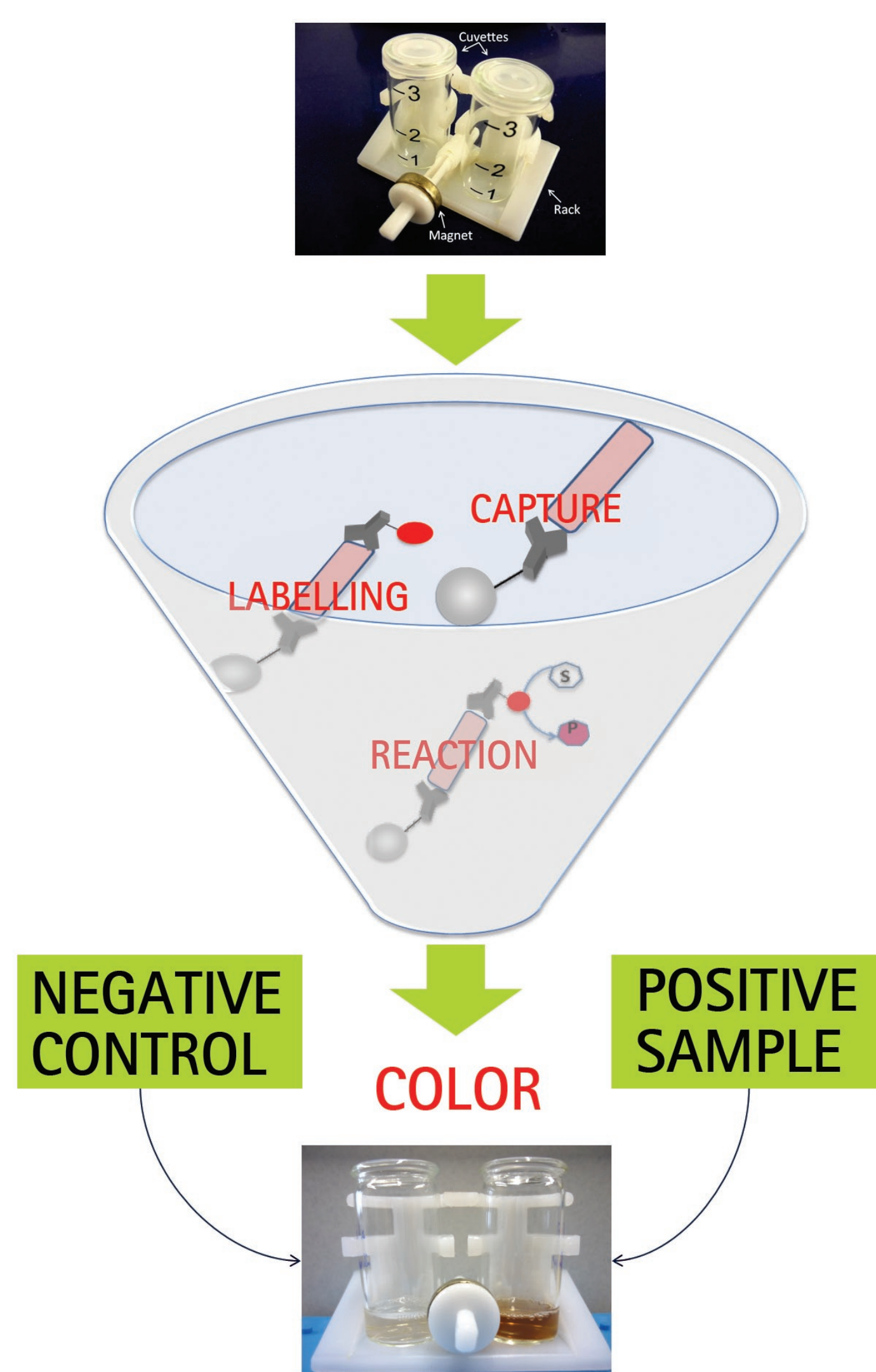
### INTRODUCTION

In this study, kit Bioalarm Legionella based on Estapor<sup>®</sup> magnetic microspheres is applied to environmental monitoring of cooling towers, overcoming drawbacks of the above mentioned techniques. Methodology used is enzyme-linked immunomagnetic colorimetry.



### RESULTS

The procedure entails the capture of *L. pneumophila* by immunomagnetic microspheres (IMM), labeling with enzyme-conjugated antibody in a sandwich format, and subsequent colorimetric analysis after a brief reaction with enzyme substrates. This kit enhancing both procedure and compositions, to obtain reduced non-specific binding, high colloidal stability, low bacterial aggregation, and inhibited bacterial enzymes that may interfere with reading reaction. If the basin of the sample turns out to be more colored than the basin of the control, the result is considered positive. Then, this color is visually compared to a color card to estimate the order of magnitude of the quantity of *L. pneumophila* that would expect to be obtained by culture: 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup> or more than 10<sup>4</sup> CFU/L. In this way, the user know his fulfillment of the in force legislation, based on a rapid screening of the samples. Approximately in 50 minutes, one person can analyze between 10-20 samples, without special training or expensive instrumentation.



Six environmental samples collected from different cooling towers, were examined. Each sample was divided into three portions, a first portion analyzed by culture method, a second portion analyzed by PCR method, and a third portion analyzed by Kit Bioalarm. This third portion was divided into ten identical portions as replicates. Detection limit was considered the level of *L. pneumophila* in which nine portions of the ten ones were visually positive.

Water samples form cooling towers		Kit Bioalarm (*)		
Culture (cfu/L)	PCR	No. of Positives	No. of Negatives	Positives (%)
280	Not defined	9	1	90
340	+	10	0	100
1600	+	10	0	100
2800	+	10	0	100
3400	+	10	0	100
27800	+	10	0	100

(\*) Ten replicates for each sample

Under our conditions, the detection limit of the Kit Bioalarm obtained by comparison with culture was 280 CFU/L. It is interesting that this detection limit allows the use of this method to rapidly monitoring the degree of the mandatory regulations.

In other experiment, a water sample systematically positive by PCR but negative by culture was also analyzed by the three methods: culture, PCR and Kit Bioalarm. Culture revealed the presence of background organisms, inhibiting growth of Legionellae in the plate. PCR was positive, suggesting that *L. pneumophila* was present but in a form that is not detectable by culture technique. Recovery by culture of *L. pneumophila* in spiked samples of this water was not possible, confirming loss of cultivability. Chlorine disinfection treatment did not alter the qPCR results, confirming that PCR assay lack the ability to discriminate between living and not living *L. pneumophila* cells. This water "culture - / PCR +" was also analyzed by the Kit Bioalarm. The result was positive, with a color corresponding to an order of magnitude of 100 CFU/L.

Finally, chlorine disinfection treatment did reduce obtained color to 50%, but did not reduce PCR result, demonstrating that this kit may be used as a good indicator of the risk associated with *L. pneumophila*.

Parameters	Plate Culture	PCR	Kit Bioalarm Legionella
Time	10-15 Days	3-6 Hours	50 Minutes
Cost	Medium	High	Low
Specificity	Medium	High	High
Utility	Verification	Verification	Verification And Prevention,
Specific Instrument	No	Yes	No
Training	Medium	High	Low

### CONCLUSION

Based on Estapor<sup>®</sup> magnetic microspheres, Kit Bioalarm can avoid false-positives results compared to PCR because it is based on antigen-antibody interaction which is depending on surface integrity, so damaged surfaces are less reactive than non-damaged surfaces, and give rapid results compared to the current time-consuming culture method, also minimizing false-negatives due to the detection of viable but non-cultivable cells.