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Characterization of Airborne Legionella Exposure

Laurence Mathieu, Thi Lan Ha, Magali Deloge-Abarkan, Enric Robine

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Mathieu L.¹, Ha T.L.², Deloge-Abarkan M.³, Robine E.²

¹ Ecole Pratique des Hautes Etudes, Laboratoire d'Hydroclimatologie Santé Environnement, Faculté de médecine, 54505 Vandoeuvre-lès-Nancy, France

² Centre Scientifique et Technique du Bâtiment, Laboratoire de Microbiologie des Environnements Intérieurs, 77447 Marne La Vallée cedex 2, France

³ Université Henri Poincaré, Département Environnement et Santé Publique, INSERM ERI n°11, Faculté de médecine, 54505 Vandoeuvre-lès-Nancy, France

Introduction

First described in 1977, *Legionella* are still of topical interest, and constitute a significant problem of public health. The human contamination occurred exclusively by inhalation of an aerosol contaminated by this hydro-telluric bacteria. In France, the management of the risk associated with *Legionella* consists of preventing its proliferation in water and keeping its concentrations $< 10^3$ CFU/L in hot-water networks. This approach is unreliable due to the underestimation of the culture-based method because of the presence of viable/active but non culturable bacteria, and *Legionella* within biofilms or intra-amoebas that are not accessible with the standardized cultural technique. In addition, no direct relation between *Legionella* concentrations in water and in aerosols has been established to date.

It is therefore difficult to assess the *Legionella* health risk based only on water measurements.

To have a more representative exposure assessment, it would be helpful to determine airborne *Legionella* bacteria generated from suspicious aquatic environments. Therefore, new approaches in the field of the metrological aspects of *Legionella* bioaerosols are required.

Objective

Our objective was to perform an environmental study on the feasibility of the detection of airborne *Legionella* while showering. This monitoring needed efficient sampling and adequate methods for analysis.

Step 1 - Laboratory trials in well-controlled *Legionella* aerosols aimed to identify the methodologies to be used, by testing:

- 3 bioaerosol collection principles
- evaluate their influence on the culturability of aerosolized *Legionella*,

Step 2 - Field trials in 34 health institutions (nursing homes) to appreciate:

- the abundance of airborne *Legionella* generated by hot-water showers,
- the relevance of the collection principles and the methods for airborne *Legionella* detection

Methods

Bioaerosol collection principles

Impaction onto solid media



Detection by culture using a specific agar media for *Legionella*

Impaction in liquid media



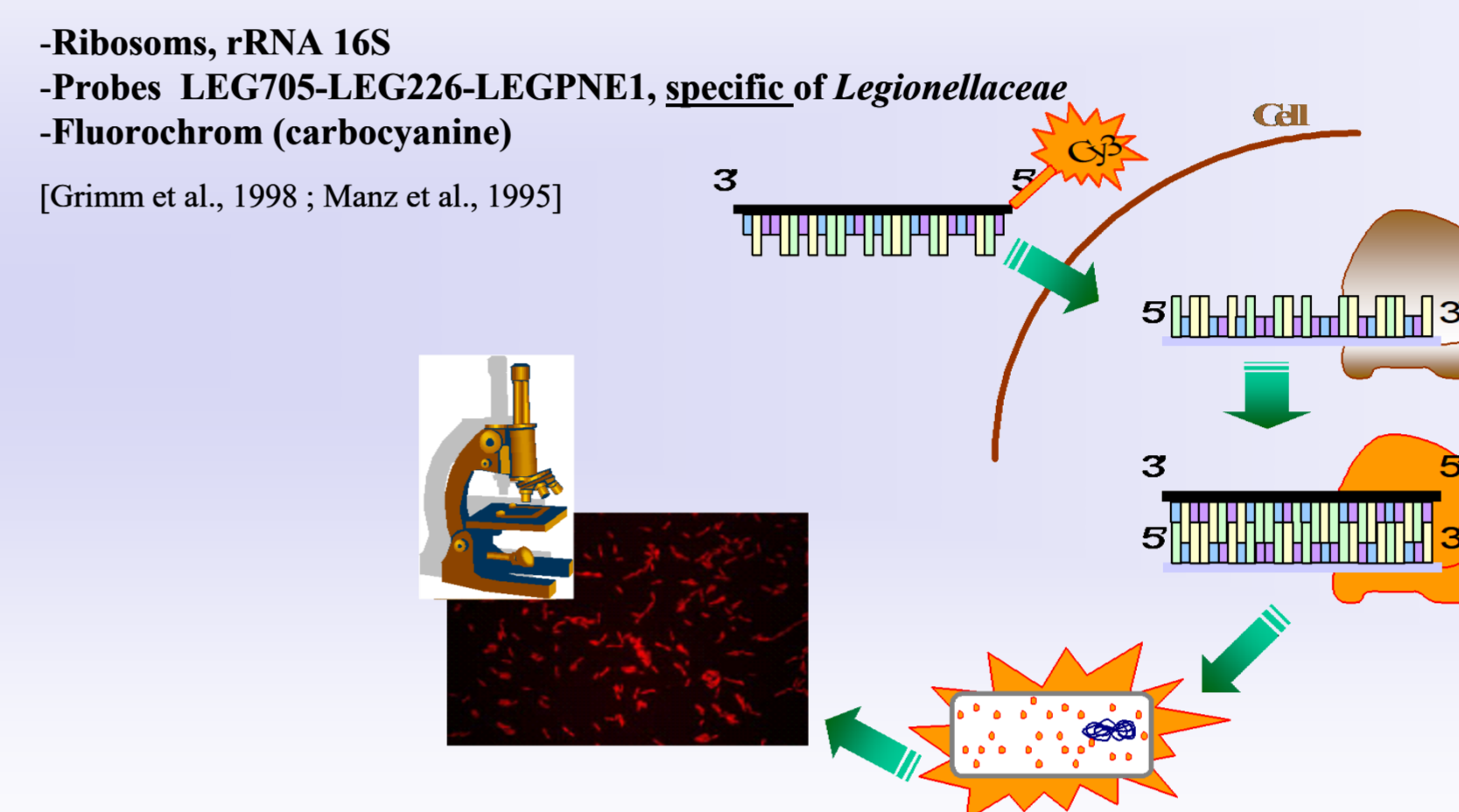
Legionella detection by culture and FISH methods

Filtration

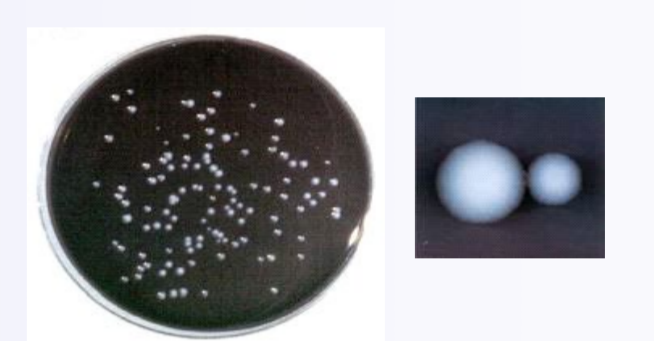


Legionella detection

In situ Hybridization (FISH) technique



Culture method (standardized method)



Laboratory trials

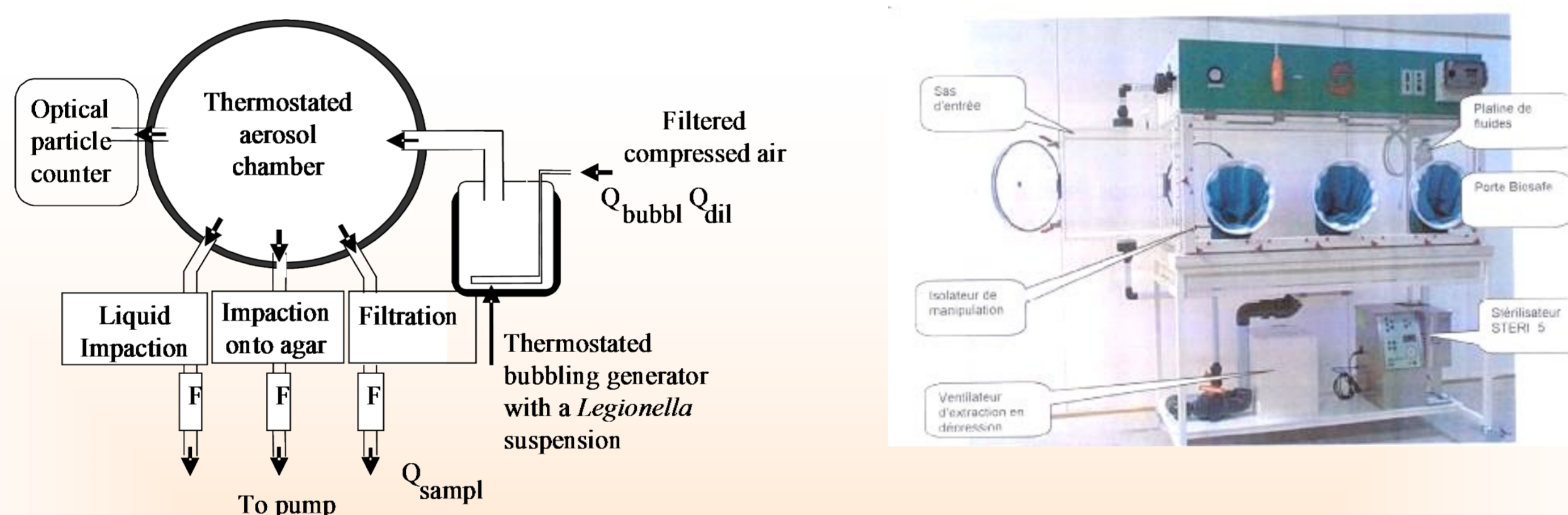


Fig. 1 The aerosolization chamber housed into a biological safety zone class III

The bioaerosol collection principles influence the culturability of *Legionella*

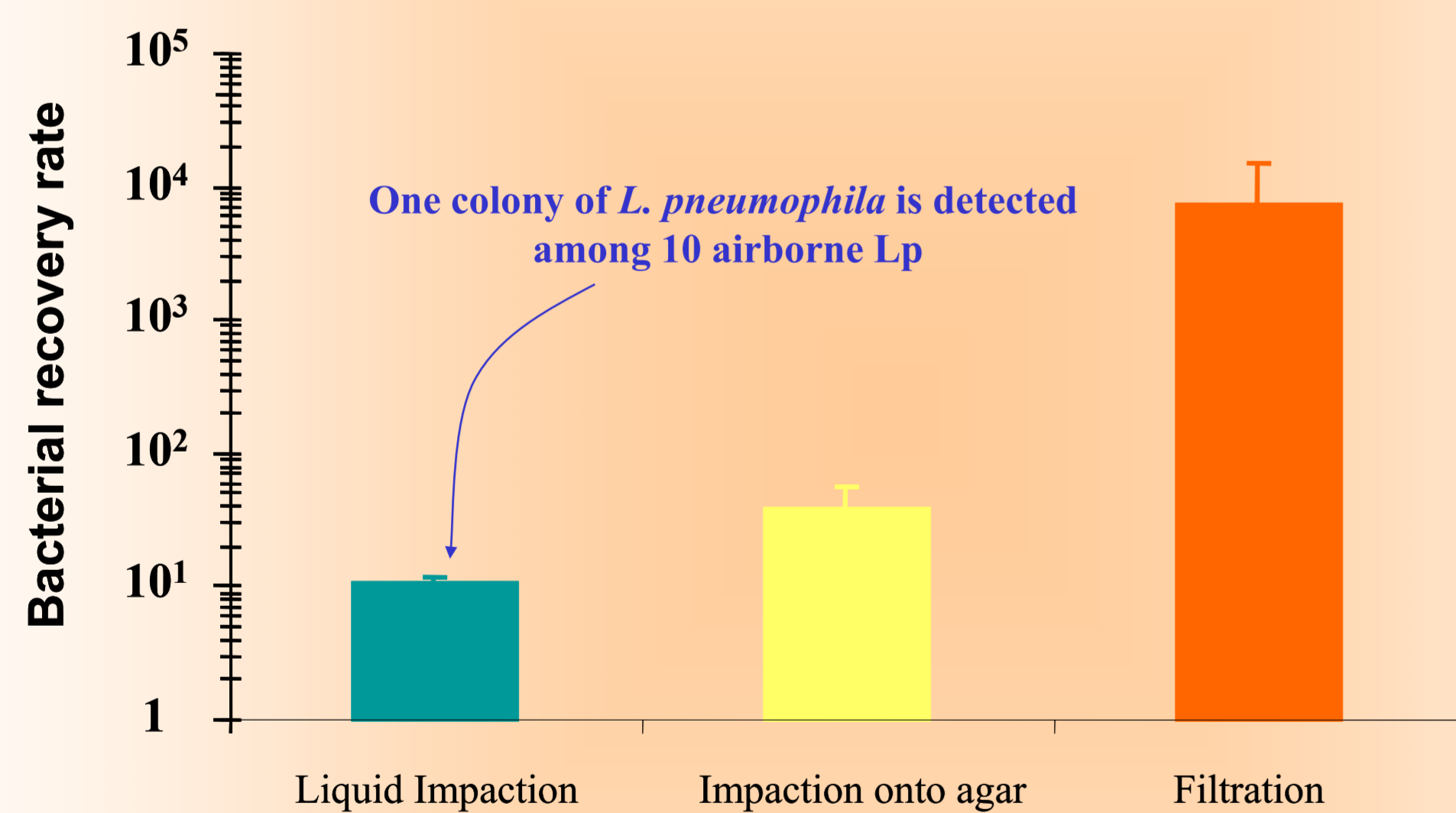
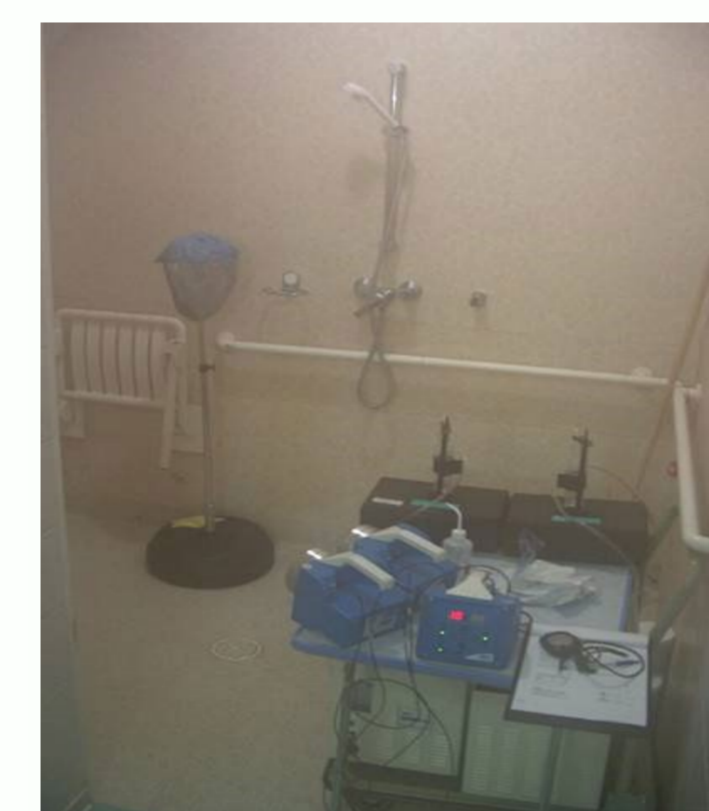


Fig. 2 Bacterial recovery rates between average *L. pneumophila* concentrations in the aerosol and their culturable fraction, according to the 3 collection methods used in the laboratory trials (n = 3).

Liquid impingement is suitable for airborne *Legionella* detection

Field trials



Hot water faucet open to its maximum pressure and temperature during 7 minutes. Aerosols samplings began only after the faucet of hot water was closed. Registration of the relative humidity and temperature of air

Water temperature: 37.4 ± 3.4 °C RH: 89.3 ± 12.3 % Air temperature: 25 ± 2°C

In hot water

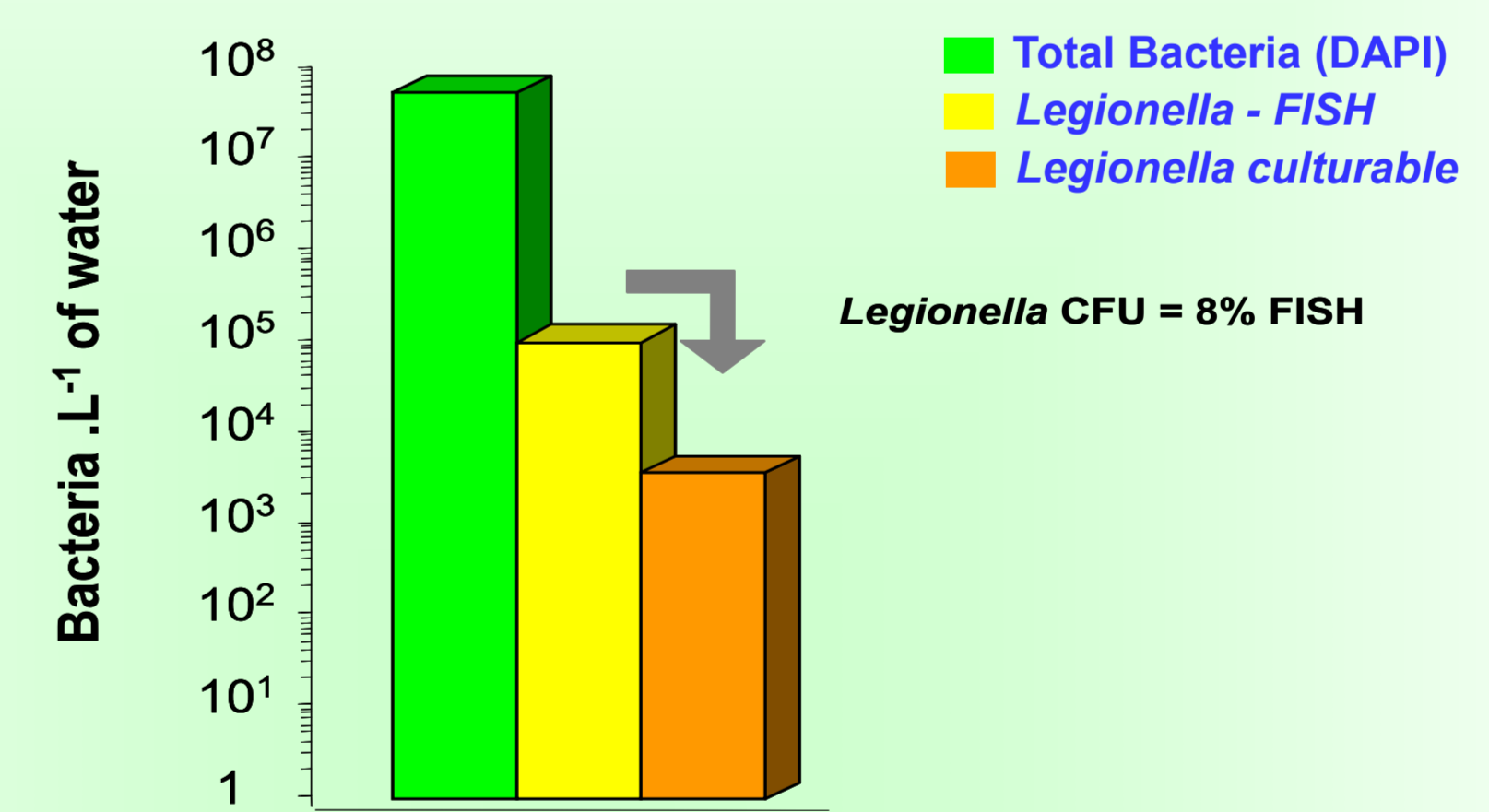


Fig. 3 Concentration of bacteria (DAPI measurement) and *Legionella* (FISH and CFU methods) in hot water while showering (n = 164).

In bioaerosol

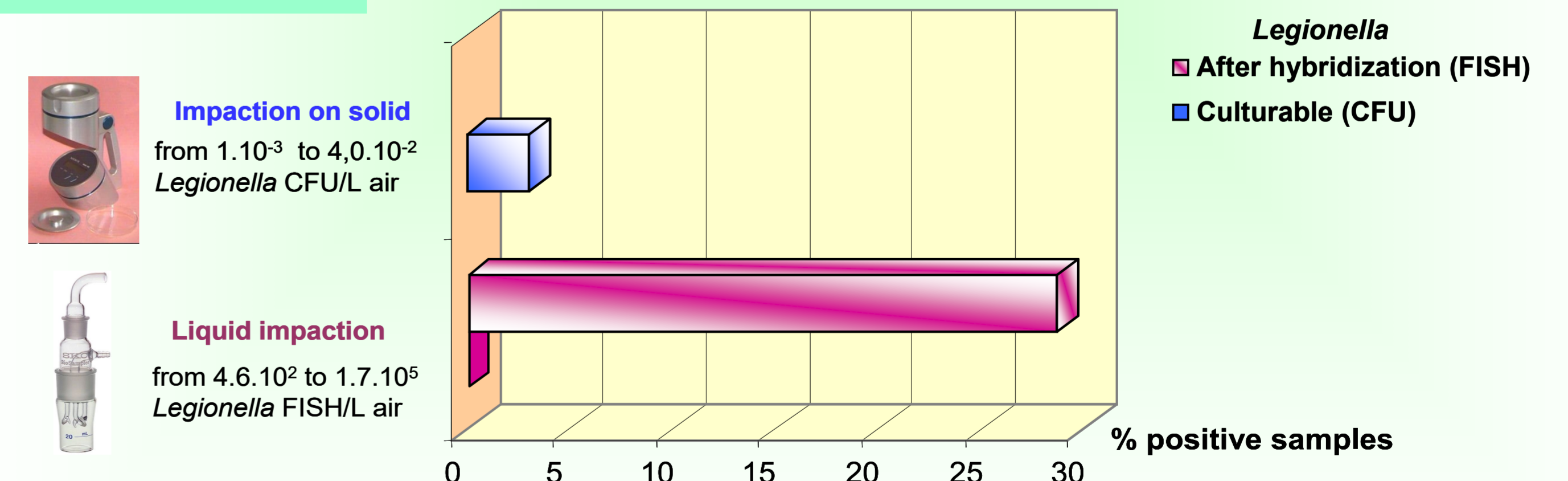


Fig. 4 Percentage of aerosol samples in which *Legionella* was detected by culture using solid impaction and FISH using liquid impingement (n = 164).

Improvement of the *Legionella* detection using FISH and liquid impingement

Conclusion

Our objective was to perform an environmental study on the feasibility of the *Legionella* detection while showering, using the three main principles of bioaerosol collection. The liquid impingement technique has been identified as the most appropriate method for collecting airborne *Legionella* in well controlled conditions (aerosolization chamber) and during field trials (hot water shower aerosols). Moreover, this study showed an improved assessment of airborne *Legionella* using the impingement technique combined with the FISH detection method. It may provide a more accurate characterization of aerobiocontamination by this pathogenic bacteria.