DNA ARRAY BASED DETECTION OF LEGIONELLA SPECIES REVEALS LIMITED DETECTION CAPABILITY OF THE CONVENTIONAL CULTURE BASED DETECTION METHOD

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A DNA array was developed to simultaneously detect and identify a comprehensive set of *Legionella* species associated with human disease. Each diagnosis can be achieved within 36 hours of sampling and provides a substantial improvement from the conventional culture plating diagnosis time of seven days. Validation of the assay using environmental samples revealed consistent differences compared to the results obtained by classical plating on GVPC agar. All isolates obtained by culturing were identified as *L. pneumophila* or *L. anisa*. Using the DNA array, a variety of additional *Legionella* species were detected, including *L. birminghamiensis*, *L. bozemanii*, *L. cincinnatiensis*, *L. gormanii*, *L. longbeachae* and *L. sainthelensii*. Consequently, this study illustrates the usefulness of the novel DNA array technique in legionellae monitoring.

Background and aims

Legionnaires' disease is a form of pneumonia caused by legionellae, which are ubiquitous in aquatic environments (Fig 1). Out of more than 50 *Legionella* species, *L. pneumophila* (sg1) is reported as the most common cause of legionellosis. Nevertheless, in addition to *L. pneumophila*, 19 other species have been reported as human pathogens. The aim of this study was to develop and validate a DNA array for the simultaneous detection and identification of all *Legionella* species that have been associated with human infections and to compare the developed array with the standard plating method of accreditated laboratories.
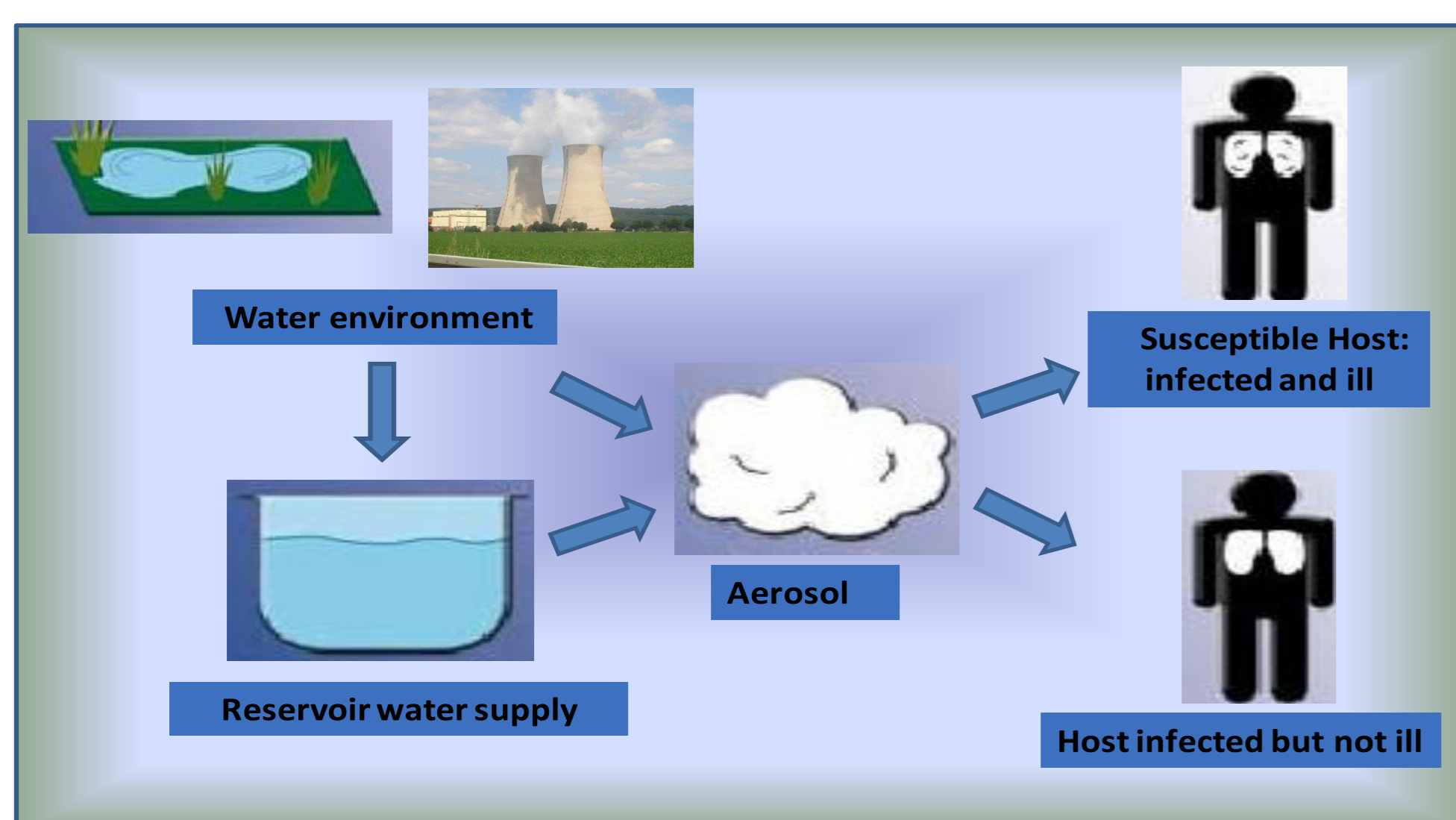

Fig 1. *Transmission of Legionaires' disease*

Materials and Methods

Based on macrophage infectivity potentiator (mip) and 16S rRNA gene sequences a DNA array was developed for 20 *Legionella* spp. associated with human disease (Table 1). Specificity and sensitivity of the assay was evaluated using a large collection of reference cultures.

Table1. *Legionella targets*

Specificity	Target Gene	Specificity	Target Gene
Legionella sp.	16S rRNA gene	*L. lansingensis*	mip gene
L. anisa	mip gene	*L. longbeachae*	mip gene
L. birminghamensis	mip gene	*L. maceachernii*	mip gene
L. bozemanii	mip gene	*L. micdadei*	mip gene
L. cincinnatiensis	mip gene	*L. oakridgensis*	mip gene
L. dumofii	mip gene	*L. pariensis*	mip gene
L. erythra	mip gene	*L. pneumophila*	mip gene
L. feeleii	mip gene	*L. sainthelensi*	mip gene
L. gormanii	mip gene	*L. tucsoniensis*	mip gene
L. hackeliae	mip gene	*L. wadsworthii*	mip gene
L. jordanis	mip gene		

In addition, 183 water samples were processed using the array and classical plating on a charcoal yeast extract agar containing antibiotics (GVPC agar) as described in the ISO 11731 standard protocol. Genomic DNA was extracted and the target regions were amplified and simultaneously labeled with alkaline-labile digoxigenin using *Legionella* specific 16S rRNA and mip gene prime*rs* (Ratcliff et al., 1998. JCM). Hybridization was conducted overnight. Detection was performed using anti-digoxigenin alkaline phosphatase conjugate and CDP-Star substrate (Fig 2).
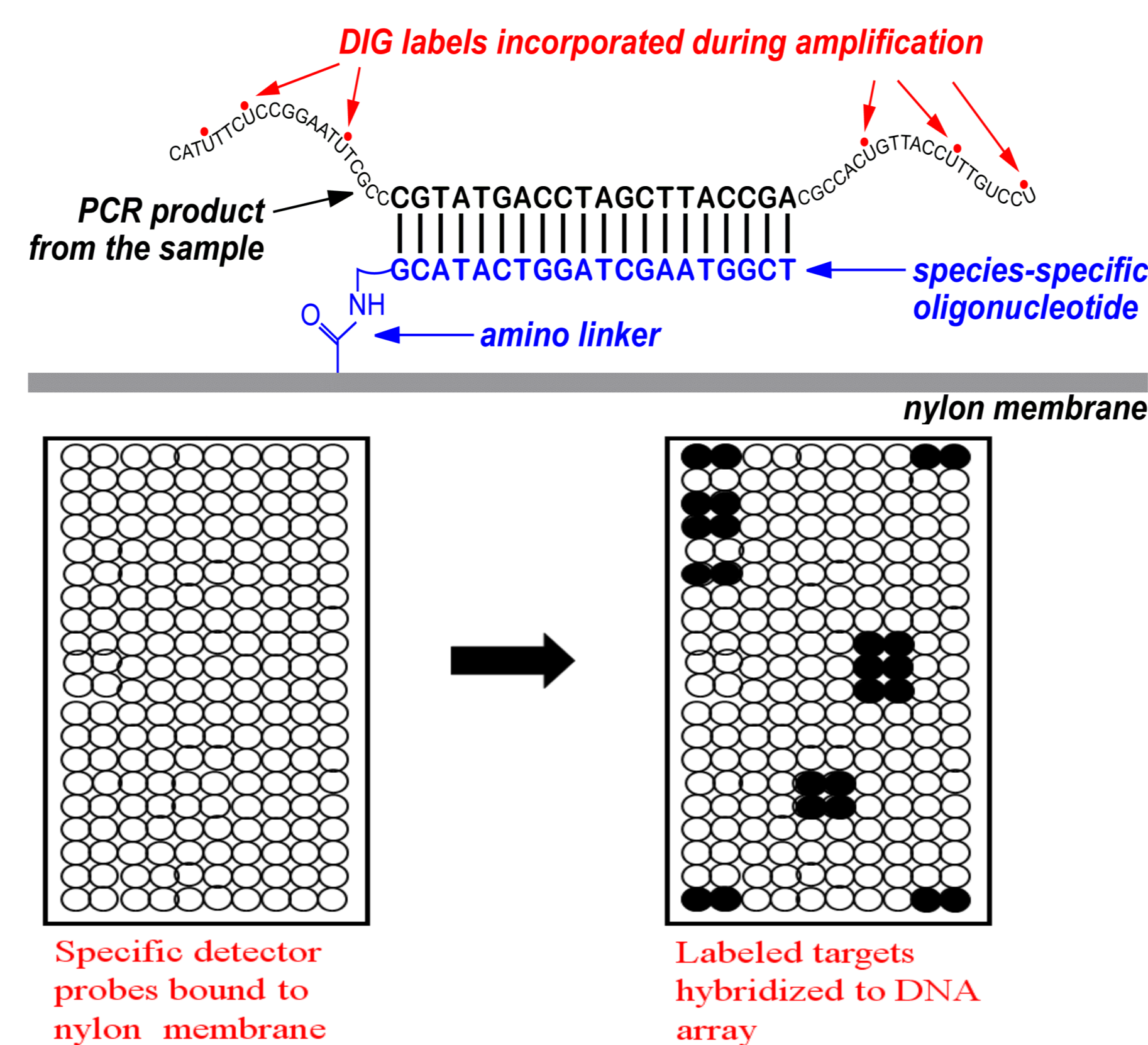

Fig 2. *Principle of the DNA array*

Results and Discussion

Specificity and sensitivity of the DNA array

The developed mip oligonucleotides are very specific and capable of discriminating between the different species. Nevertheless, detection of the 16S rDNA is more sensitive as less than 10 cfu can be detected; using the mip gene the detection limit was found to be 10^3 cfu ml^{-1}. However, this lower sensitivity still allows detection of excessive levels of these species.

Validation of the DNA array

Analysis of environmental samples revealed consistent differences between classical plating and the molecular assay. Whereas *Legionella* DNA was detected in approximately 80 % of the samples, classical plating recovered *Legionella* colonies in only 32 % of the samples (Fig 3). All isolates obtained by culturing were identified as *L. pneumophila* or *L. anisa*. In addition to these two species, the DNA array detected a variety of *Legionella* species including *L. birminghamiensis*, *L. bozemanii*, *L. cincinnatiensis*, *L. gormanii*, *L. longbeachae* and *L. sainthelensii* (Fig 3).
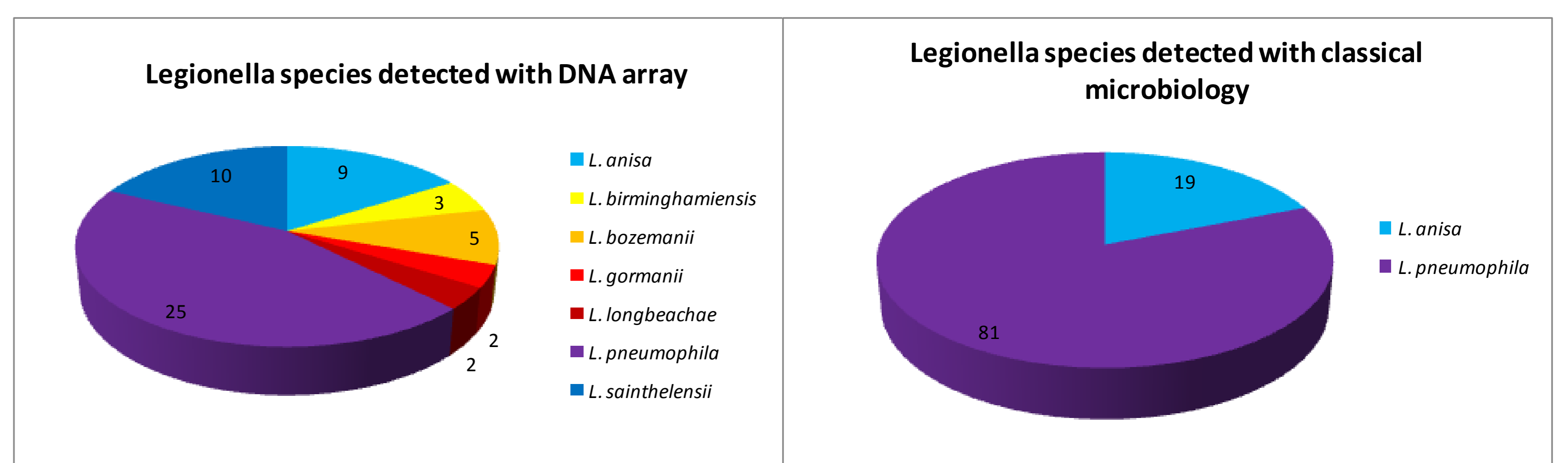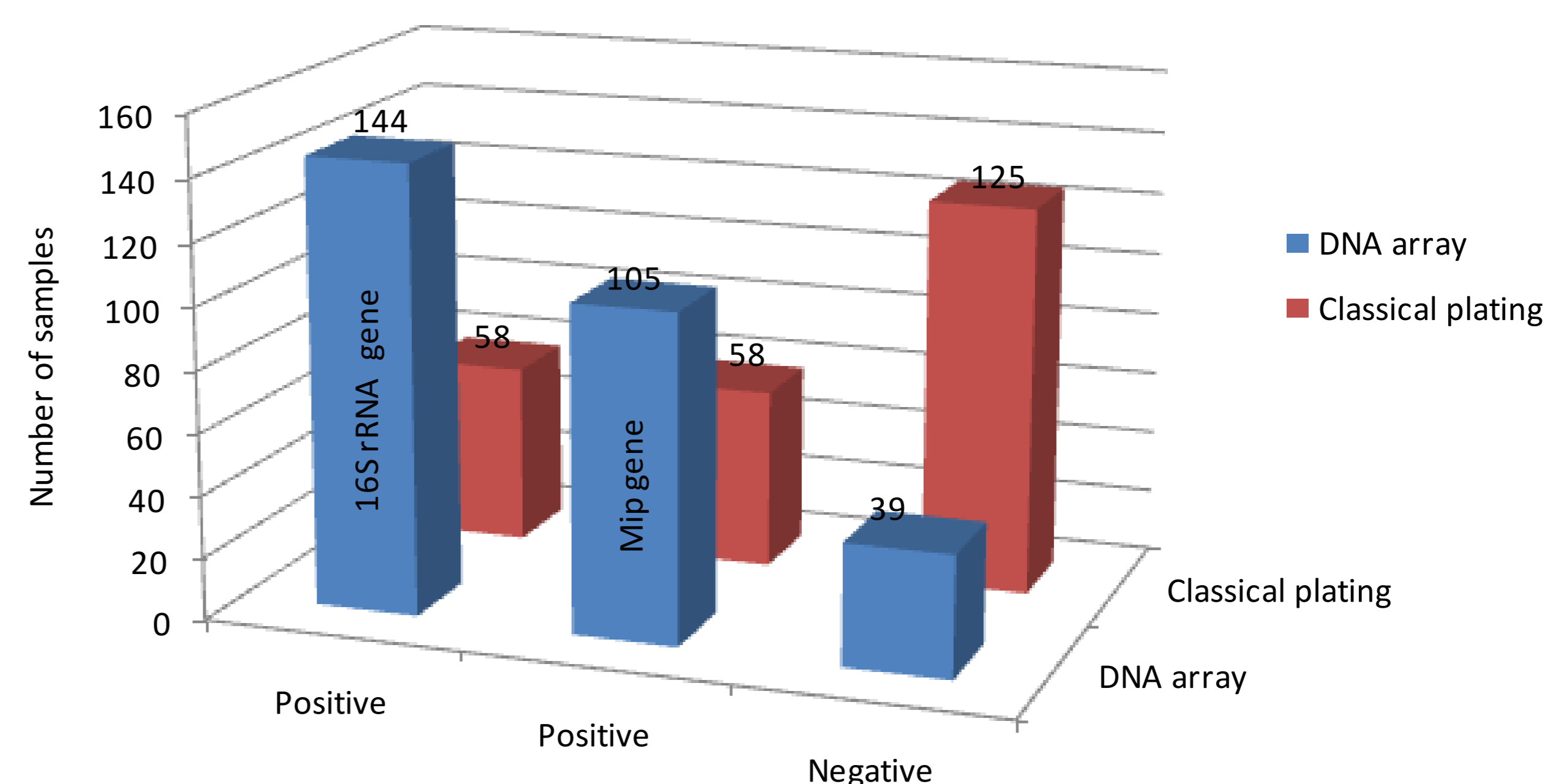

Fig 3. *Results of 183 water samples analyzed with the DNA array on the one hand and classical plating on the other hand*

Conclusions:

The currently used GVPC agar plating method for detection of *Legionella* spp. selectively detects *L. anisa* and *L. pneumophila*. Screening of a large number of water samples using the DNA array revealed the occurrence of other potentially pathogenic *Legionella* spp. Consequently, this study emphasizes the importance of novel detection tools in legionellae monitoring.

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