A DNA CHIP FOR THE DETECTION OF MICROORGANISMS IN WATER SAMPLES – DESIGN AND PRELIMINARY RESULTS

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Waterborne pathogens are responsible for several diseases, either due to the consumption of contaminated drinking water or due to the contact with polluted recreational waters. There is an increasing awareness that emergent and viral pathogens should also be monitored for determining water quality. Conventional detection methodologies present several shortcomings, such as reliance in indicator species, low throughput and increasing resources as more species are to be detected. DNA chips have the potential to serve as surveillance systems for the simultaneous detection of pathogens, overcoming these limitations. In the present study, a rapid method for the detection of multiple waterborne pathogens (bacteria and viruses) was developed, using a DNA chip (AQUACHIP®). Species and group specific probes were implemented on a DNA chip, both for mandatory and non-mandatory microorganisms. Considerations regarding the AQUACHIP® design (probe layout, replicates and dilutions), DNA labeling and amplification, and preliminary results of the application of the chip are presented.

The probes which were previously developed and validated were cloned into pBS KS for sequencing and to develop an accessible source of the DNA for implementation on the AQUACHIP®. Probe identity and target microorganisms are described in detail elsewhere [1], and are divided in three main groups: probes for the detection of the groups and species of microorganisms that are currently enforced by the Portuguese and European legislation (the coliform bacteria group, *Escherichia coli*, Enterococci and *Clostridium perfringens*); a group of probes for non-mandatory microorganisms, selected according to their impact on public health (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Legionella pneumophila*, *E. coli* O157, *Campylobacter coli* and *C. jejuni*, *Salmonella* spp. and *Shigella* spp.); and two probes for pathogenic virus detection (Hepatitis A virus and Norovirus genogroup I).

The chip was designed in order to maximize the cost-effectiveness, replicability of the results, and to allow the determination of the sensitivity limits of the technology. Additionally, the chip was designed to accommodate future expansion to a greater number of species. Each AQUACHIP® was printed in quadruplicate in glass slides, allowing for the processing of a maximum of four water samples simultaneously; in each AQUACHIP®, three dilutions of nine replicates of each probe were distributed semi-randomly in different zones, to avoid probe location biases.

Before hybridization, sample DNA was labeled and amplified in a single step using the Roche-Nimblegen One-Color DNA kit, to increase the detection sensitivity [2] and reduce processing time.

Preliminary results show the detection capabilities of the AQUACHIP® for *E.coli* DNA.

Future work will include the validation of the AQUACHIP® with all the target species, alone and in combination, with DNA extracted from pure cultures and from artificially contaminated water samples, and finally with DNA extracted from complex environmental water samples.

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References:

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