A DNA CHIP FOR SIMULTANEOUS DETECTION OF MICROORGANIMS IN WATER SAMPLES: COLIFORM BACTERIA, NON-MANDATORY BACTERIA, HEPATITIS A VIRUS AND NOROVIRUSES

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The use of surveillance systems demonstrates that waterborne pathogens are responsible for several diseases associated either with either consuming of contaminated drinking water, or contacting polluted recreational waters. To assure water quality not only mandatory microorganisms should be screened for. Emergent pathogens that represent danger to public health should also be analysed. Culture methods used routinely in water microbiology (bacteriology) laboratories are long time consumable. Moreover some of the emergent pathogens are not represented by conventional indicator bacteria, which limit the screening power of the current methodology. The present study aims at developing a rapid method to simultaneously detect several microorganisms (bacteria, protozoa, and virus) in water samples by the use of a DNA chip (AQUACHIP©). Species specific DNA probes will be implemented on a chip, allowing detection of which sample is positive for which microorganism.

The first selected microorganisms were the Portuguese and European Community mandatory microbiological parameters and indicator parameters required to water intended for human consumption, delivered by public distribution systems, trucks or cistern-ships, or used in the alimentary industry. These are the coliform bacteria group, *Escherichia coli*, Enterococcus and *Clostridium perfringens* (including spores). For this microorganism we have developed 9 probes, 1 for total coliform, 5 for *E. coli*, 2 for Enterococcus, and 1 for *Clostridium perfringens* (figure 1A). All these probes have been amplified by PCR amplified from pure cultures and validated against artificial samples.

The group of non-mandatory selected microorganisms were selected according to their impact on public health. These are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Legionella pneumophila*, *E. coli* O157, *Campylobacter coli* and *C. jejuni*, *Salmonella* spp. and *Shigella* spp., each with one selected probe (Figure 1B). All these probes have been amplified by PCR from pure cultures and will be validated against artificial samples.

The analysis of the viral genomes in study (we have started with hepatitis A virus and noroviruses genogroup 1) revealed highly conserved sequences, which were amplified by RT-PCR, using specific primers (one for each viral group), in order to obtain the probes for viral detection (Figure 1C). These probes were both cloned and hepatitis A virus probe was validated by dot blot using artificial samples. The hepatitis A virus probe and the Noroviruses (GI) probes which were cloned into pBS KS were further sequenced to confirmed their sequence. The BLAST results confirm that these probes are specific for their viral groups.

In the future, further tests are needed to evaluate the probes efficiency in water samples of the water supply network. These species specific DNA probes will be implemented on

a chip, and for that future work will include chip support (e.g. glass or silica) and an appropriate label detection method selection.

References:
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A. Mandatory microorganisms

	E. coli				*E. faecalis*	*E. faecium*	Total coliform	*C. perfringens*
Probe number	4	5	7	8	2	3	1	6
pb	162	186	139	187	140	161	147	184
Ratio sequenced genomes detected	6/13	4/13	13/13	13/13	1/1	-/-	*Escherichia, Klebsiella Enterobacter Citrobacter Moellerella Morganella Salmonella Shigella Yersinia Hafnia Erwinia Proteus Serratia Among othres*	1/1

B. Non-mandatory microorganisms

	S. aureus	*L. pneumophila*	*E. coli* O157	*C. jejuni*	*C.* coli	*Shigella* spp.	*Faecal coliforms*	*Salmonella* spp.	*P. aeruginosa*
Probe number	9	10	11	12	13	15	16	17	18
pb	128	128	152	107	134	111	164	110	141
Ratio sequenced genomes detected	14/14	4/4	2/2	5/5	-/-	6/6	13/13	7/7	3/3

C. Virus

	HAV	**NV (GI)**
Probe number	19	20
pb	222	118

Figure 1. Identification of probes selected for each microorganism group. A. Mandatory microorganisms. B. Non-mandatory microorganisms. C. Virus.