

***Development and validation of a DNA-chip technology for the assessment of the bacteriological quality of bathing and drinking water***

**Project acronym: AQUA-CHIP**  
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**AQUA-CHIP final project summary**

**Objectives:**

The overall objective of the AQUA-CHIP project was to develop and validate a DNA-chip technology to assess rapidly and accurately potential human health risks arising from bacterial contamination in drinking and bathing waters. This main objective can be divided in three parts: i) Develop molecular methods to identify and quantify the presence of important pathogenic and indicator bacteria in any given water sample and to assess their state of activity and pathogenicity. ii) Compare laboratory and field data to assess health risks in aquatic environments and design a multiparametric assay based on molecular probes. iii) Combine quantitative PCR and DNA-chip technology to validate a DNA-array suitable for mass application that leads to scientifically sound new procedures for the assessment of the hygienic quality of drinking and bathing water.

**Results and Milestones:**

During the project the joint efforts of the AQUA-CHIP consortium concentrated on reaching several milestones to achieve the objectives stated above. The following milestones could be met:

*1. Development of a simple and robust procedure for extraction of environmental RNA/DNA from water samples suitable for mass application*

The extraction procedure consists of filtration of several 100 ml of water through a specific membrane filter that can be stored frozen until extraction. Following extraction, the nucleic acids (DNA/RNA) are cleaned up and ready for molecular analyses such as PCR and DNA arrays. The new procedure was validated against established extraction procedures.

*2. Provision of primers and probes for housekeeping and pathogenicity genes of the targeted pathogenic and indicator bacteria*

Specificity and validity of the primers and probes for target genes was assessed *in silico*. Specific primers and probes for the 2 housekeeping genes and about 30 different pathogenicity factors were designed for 26 target species. About 120 primer pairs and 100 probes were made available to the consortium. Many of these primers and probes were validated using a set of nucleic acids extracted from bacterial reference strains. Also, PCR conditions were optimised and sensitivity was assessed.

*3. Design and production of DNA-chips for the assessment of waterborne bacterial pathogens*  
Based on the *in vitro* screening results of the primers and probes several prototype DNA-chips for the detection of eleven target taxa were produced and tested using reference nucleic acids. Several DNA-chips were validated for all major waterborne bacterial pathogens using reference DNA and RNA from test strains. In combination with the developed rapid extraction protocol for nucleic acids from drinking and natural bathing water samples these technologies enabled validation of the AQUA-CHIP technology with real samples from the European test sites.

*4. Molecular assessment of activity and abundance of pathogenic bacteria in water*  
Two novel methods were developed to quantify DNA and RNA in water samples based on real-time PCR and RT-PCR, respectively. The DNA quantification can be even used for uncultured bacteria if a signature sequence is known. The RNA quantification was validated with *Salmonella* spp. but enables quantification of any targeted RNA even without an internal reference gene because a general, artificial RNA standard was designed and the whole procedure validated in natural water samples. The quantitative PCR methodology was sensitive enough to detect single cells if appropriate amounts (10 fg) of environmental genomic DNA or RNA were provided.

*5. Assessing the overall microbial community structure and composition of bathing and drinking water*

This analysis was done using community fingerprints of 16S rDNA amplicons based on 'Single Strand Conformation Polymorphism' (SSCP) analysis followed by sequencing of important bands of the fingerprints. It could be shown with a variety of drinking and bathing water samples that each habitat had its own characteristic fingerprint with underlying seasonal variations. These community fingerprints could function as indicators of the quality of drinking water from the supplier to the end user. An analysis of the microflora of a complete drinking water supply system from source to tap was done. These molecular analyses demonstrated that the various treatment processes had a significant impact on the overall microbial community structure and composition of the drinking water microflora. On the other hand, the drinking water microflora was rather stable for several months at the tap end of the supply system. Therefore, community fingerprints and composition analyses could function as a new monitoring tool for the quality of drinking water from the supplier to the end user.

*6. Comparison of classical and molecular analyses for assessment of water-borne pathogens*  
More than 500 water samples were collected and analysed for pathogenic and indicator bacteria using conventional culture-based methods. In addition, nucleic acids were extracted from a carefully selected subset of these environmental samples to assess the abundance of specific pathogens and indicator bacteria using the developed quantitative PCR methodologies and the DNA-chip technology. Real-time PCR quantification of indicator bacteria (*E. coli*, *Enterococcus* spp.) correlated well with the quantification of the respective taxa by conventional plate counts. Such a correlation could not be demonstrated for pathogens, such as *Salmonella*, due to lack of sensitivity of the molecular quantification and lack of high enough incidences for the pathogens in the water samples analysed.

### **Benefits and Beneficiaries:**

The concept of the AQUA-CHIP project was to quantify and identify pathogenic and indicator bacteria directly from environmental DNA using a combined approach with highly

selective PCR and DNA-chip detection and not to rely on the cultivation of indicator bacteria. The current detection technology is dependent on cultivation and therefore rather slow and unreliable. It does not provide directly information about the taxonomic position of the detected organism. The technology developed can be faster, more reliable and provide taxonomic information about the detected bacteria. It is open to automation and mass application. Potential end users are public health authorities, water authorities, drinking water industries and tourism information systems.

**Future Actions:**

A DNA-chip based technology for the detection of pathogenic and indicator bacteria in bathing and drinking water was developed. This technology includes: i) a kit for the extraction of nucleic acids from water samples, ii) several DNA-chips for specific waterborne pathogens and indicators, and iii) a computer-based analytical systems that quantifies and identifies the response of the aquatic DNA on the chip. Future activities will be: i) market the developed extraction kits, ii) market the chip technology for specific target bacteria, e.g. *Legionella* and *Salmonella*, and iii) seek new funds for general validation of the chip technology and approval as new regulation for the assessment of the hygienic quality of bathing and drinking water.