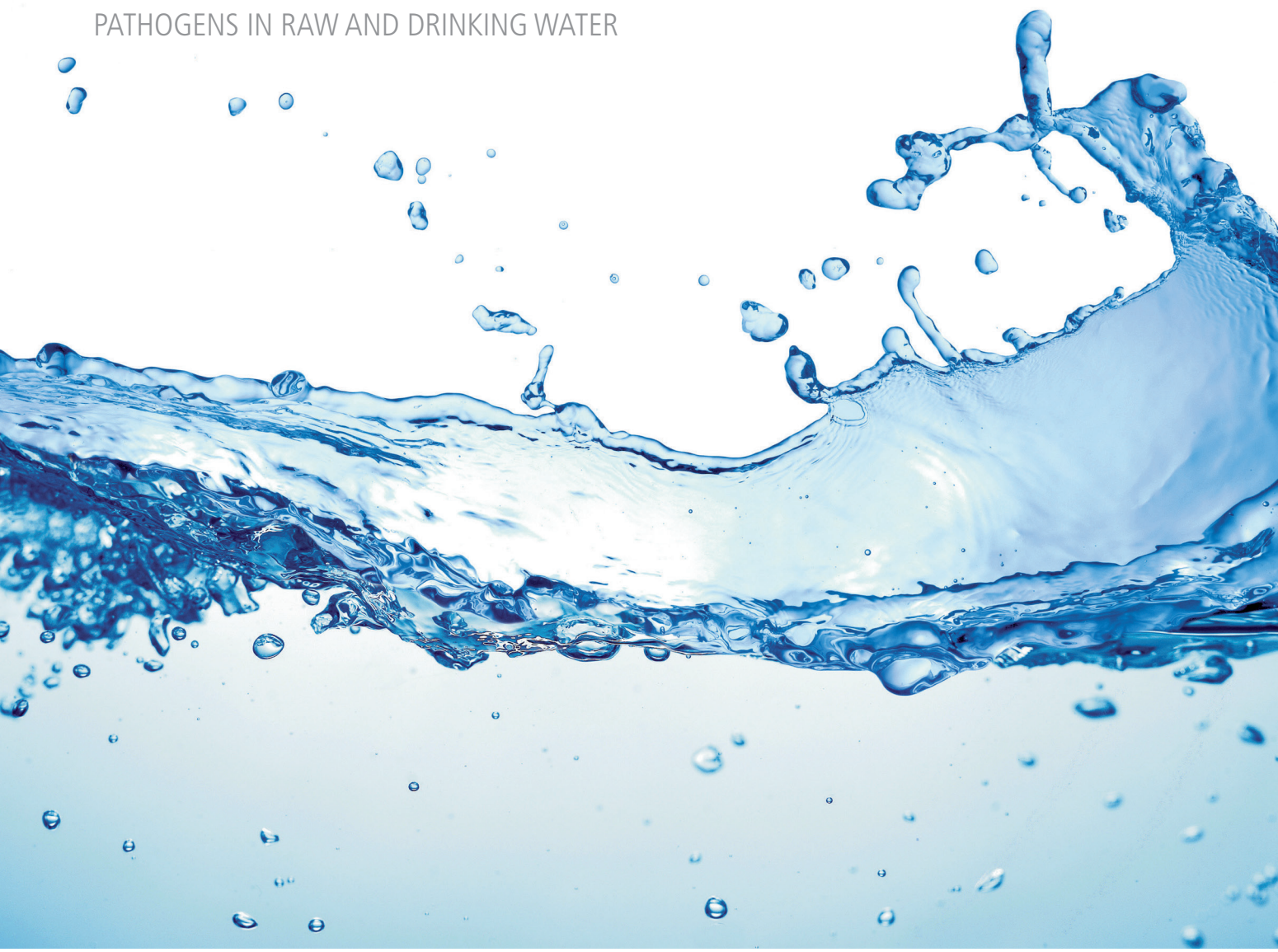
DEVELOPMENT AND IMPLEMENTATION OF A CONCENTRATION AND DETECTION SYSTEM FOR INLINE MONITORING OF WATERBORNE PATHOGENS IN RAW AND DRINKING WATER



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PROJECT PROFILE

DEVELOPMENT AND IMPLEMENTATION OF A CONCENTRATION AND DETECTION SYSTEM FOR INLINE MONITORING OF WATERBORNE PATHOGENS IN RAW AND DRINKING WATER

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Photo: André Künzelmann, UFZ

CONTENTS

1 INTRODUCTION

Laboratory for the surveillance of drinking water quality, Photo: André Künzelmann, UFZ

The provision of hygienically safe drinking water was one of the greatest societal advances of the 20th century in the industrialized countries. In the early 21st century, however, ageing infrastructures as well as climate and demographic changes constitute new challenges for public water supply.

The project "Development and Implementation of a Concentration and Detection System for the Inline Monitoring of Waterborne Pathogens in Raw and Drinking Water" (EDIT) aimed at the development and pilot testing of a rapid detection system for bacteria and viruses that is suitable for application in the water industry. The system was designed to reduce the time needed for pathogen detection in raw and drinking water and to screen samples for more microbiological parameters from larger volumes than is possible with established methods. It is expected that in the future, there will be an increasing international demand for such systems – not only for drinking water surveillance but also for the monitoring of wastewater treatment or bathing water quality.

The EDIT project was supported by the German Federal Ministry of Education and Research in the context of the funding initiative "Smart and Multifunctional Infrastructural Systems for Sustainable Water Supply, Sanitation and Stormwater Management" (INIS) within the "Sustainable Water Management" (NaWaM) program.

2 PROJECT CONSORTIUM

Technische Universität München

Chair of Analytical Chemistry and Institute of Hydrochemistry (IWC), Technical University of Munich

IMTEK

Institute for Microsystems Technology, University of Freiburg

TZW Technologiezentrum Wasser

Technologiezentrum Wasser (TZW, Technology Center for Water) of the German Technical and Scientific Association for Gas and Water, Karlsruhe

Fraunhofer IOSB Institutsteil Angewandte Systemtechnik AST

Application Center for Systems Technology, Fraunhofer Institute of Optronics, System Technologies and Image Exploitation (IOSB), Ilmenau

HELMHOLTZ ZENTRUM FÜR UMWELTFORSCHUNG UFZ

Helmholtz Centre for Environmental Research GmbH – UFZ, Magdeburg

MHB MEDIZINISCHE HOCHSCHULE BRANDENBURG

Institute of Microbiology and Virology, Brandenburg Medical School Theodor Fontane, Senftenberg

Berliner Wasserbetriebe

Municipal Water Supply Company of Berlin

gwk GmbH

GWK Präzisionstechnologie GmbH, Munich

r-biopharm

R-Biopharm AG, Darmstadt

ASSOCIATED PARTNERS:

Municipal water supply company of Magdeburg – TWM | Municipal water supply company of Marburg – SWM
Department of Geography, Georg-August University, Göttingen

Preparing samples for water quality surveillance, Photo: André Künzelmann, UFZ

3 BACKGROUND

Samples for microbiological examination, Photo: André Künzelmann, UFZ

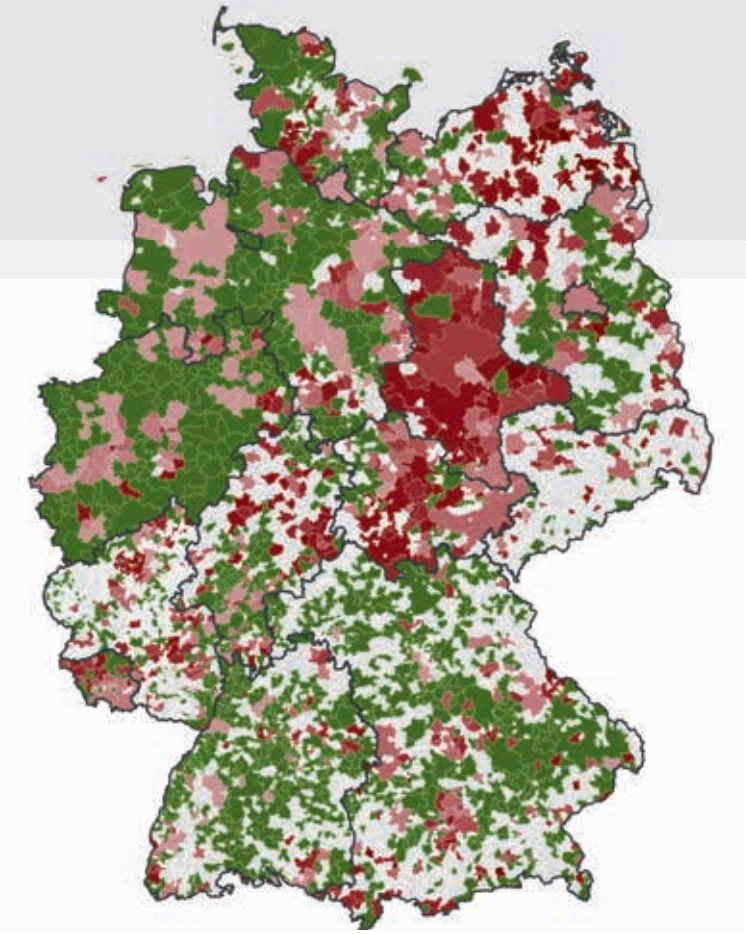
The drinking water supply in Germany is considered to be of very high standard. Supply shortfalls and impairments of drinking water quality occur only in exceptional situations. However, the relatively old age of supply infrastructures, climate change and demographic change result in new challenges which are relevant for public water supply.

IMPACTS OF CLIMATE CHANGE

Observed and expected climate changes in Germany have significant but spatially heterogeneous impacts on regional hydrology and water availability. A predicted increase in the frequency and severity of hydro-meteorological extremes (i.e. floods and droughts) is likely to lead to reduced raw water quality and potentially even the contamination of drinking water supply and distribution systems. Moreover, increasing water temperatures can also favor the survival and reproduction of hygienically relevant microorganisms. The challenges resulting from climate change tend to be aggravated by demographic changes.

IMPACTS OF DEMOGRAPHIC CHANGE

In the aftermath of Germany's reunification, some parts of eastern Germany experienced massive population shrinkage. Low birthrates and internal migration towards dynamically developing urban regions continue to lead to a depopulation of smaller cities and peripheral rural areas. The reduction in water demand in affected regions is exacerbated by a declining per capita consumption, with negative impacts on the degree of capacity utilization in water works and distribution systems. Without countermeasures, the resulting increase in water transit times in pipelines and intermediate storage tanks may also promote the growth of harmful microorganisms.



Relative frequency that hygienic norms were exceeded

up to 0,1	0,3 to 0,4	1 to 5	WSZ without contamination
0,1 to 0,2	0,4 to 0,5	5 to 10	No major water suplier
0,2 to 0,3	0,5 to 1	over 10	

The map shows the total number of cases that any hygienic parameter exceeded the maximum limit per 10,000 people. Only such water supply areas are shown that have more than 5000 inhabitants or a daily supply of more than 1000 m³

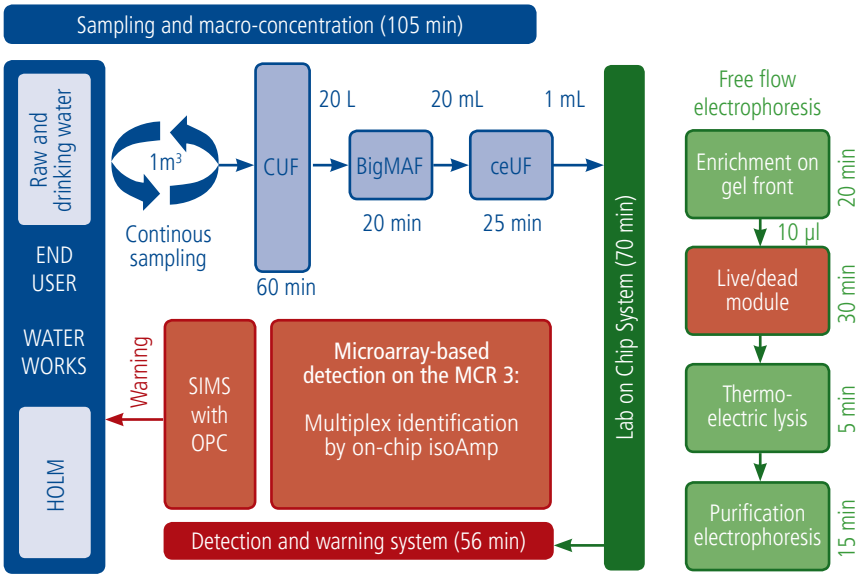
Cartography: Niklas Rehkopp (2015)
Source: Federal Agency for Carthography and Geodesy (2013)
Federal Ministry of Health and Federal Enviroment Agency (2011, 2015)

Kilometers
0 100 200 400

NEED FOR INNOVATIVE SURVEILLANCE METHODS

In the light of these challenges, rapid and reliable methods for the surveillance of drinking water hygiene could help to ensure a safe water supply in the future. Currently used methods are based on the cultivation of indicator bacteria. The main disadvantage is the time needed for detection and quantification of these bacteria (typically at least 18 hours). Moreover, even negative test results cannot completely rule out a contamination with different micro-organisms, especially viruses.

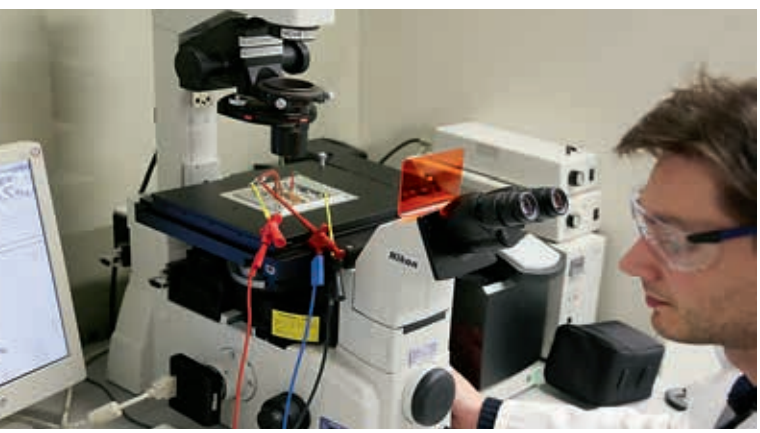
Time and labor-intensive cultivation techniques are still the standard for surveillance of water hygiene, Photo: Dr. Daniel Karthe, UFZ



Flowchart of the hygiene online monintoring, Source: EDIT Consortium

CUF: Crossflow ultrafiltration
MAF: Monolithic adsorption filtration
ceUF: Centrifugal ultrafiltration
isoAmp: isothermal amplification
MCR3: Microarray chip reader 3
SIMS: Systemically integrated management system
OPC: OLE for Process Control (standardized software interface)

4 TECHNICAL APPROACH AND WORKFLOW



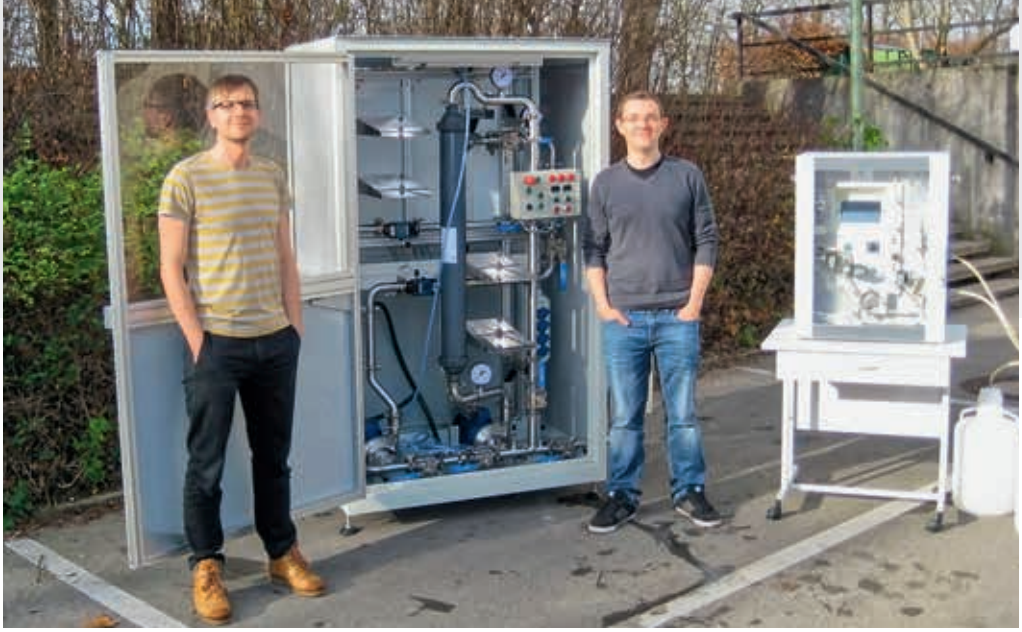
Upper photo: Development of the lab-on-chip system
Lower photo: Demonstration of the CUF system to end users at BWB, Photos: Dr. Daniel Karthe, UFZ

The EDIT project aimed at the development of an online monitoring system for hygiene which allows for the continuous surveillance and rapid detection of harmful microorganisms in raw and drinking water.

As compared to state-of-the-art techniques in which a small sample volume (100 ml) is placed on selective cultivation media, the EDIT approach is based on the combination of multiple concentration steps and the subsequent detection of bacteria and viruses using molecular biology.

FILTRATION, PREPARATION AND DETECTION STEPS:

- concentration of samples in four steps from several hundred/ thousand liters to about 10 µl;
- sample purification and extraction of microorganisms;
- module for discrimination between active and inactive microorganisms and viruses (in order to mark harmless microorganisms such as those inactivated by sterilization);
- detection of (harmful) microorganisms and viruses by molecular methods (based on DNA or RNA);
- data interpretation and communication of results.



CUF and MAF concentration systems being tested at TUM, Photo: PD Dr. Michael Seidel, TUM

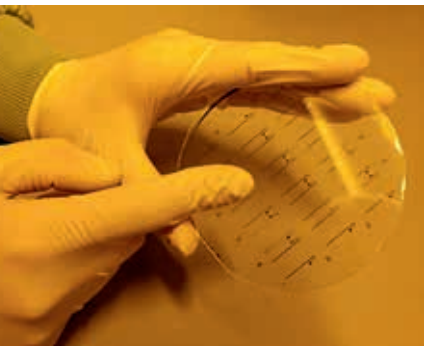
5 CONCENTRATION AND EXTRACTION OF MICROORGANISMS

The fact that exposure to even a small number of pathogenic microorganisms or viruses can have detrimental health effects is a major challenge for the surveillance of drinking water. In order to permit a reliable risk assessment, water samples of a sufficient volume need to be analyzed. Because state-of-the-art detection systems cannot handle very large volumes, the EDIT project established a method to concentrate samples from several hundred to several thousand liters to a few milliliters (macro-concentration) and subsequently reach a volume of a few microliters in a lab-on-chip system (micro-concentration). The lab-on-chip system also allows for the extraction of DNA or RNA of target pathogens, which can then be amplified and detected on the microarray analysis system MCR 3.

MACROCONCENTRATION OF BACTERIA AND VIRUSES

Jointly with GWK Precision Technology, the Institute of Hydrochemistry at Technical University of Munich developed several sequential concentration modules for raw and drinking water. In a first step, bacteria and viruses are concentrated via crossflow ultrafiltration (CUF) at a rate of about 1000 l/h from several hundred to thousand liters of raw or drinking water to about 20 l.

Lab-on-chip system, Photo: Matthias Hügle, MHB

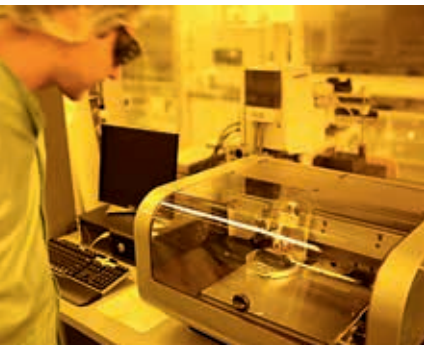


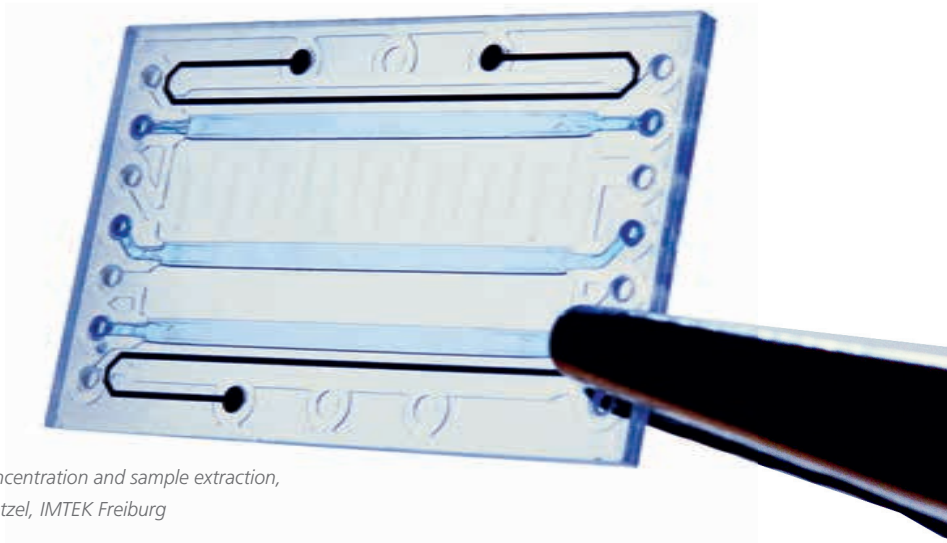
Development and production of the lab-on-chip system, Photos: Dr. Daniel Karthe, UFZ

The next step consists of a large system for monolithic adsorption filtration (BigMAF), which concentrates bacteria and viruses from 20 l samples to about 20 ml, thereby removing most of the matrix components. This is followed by a centrifugal ultrafiltration (ceUF), which further reduces the sample volume to about 1 ml. The time needed for these two steps is about 45 min.

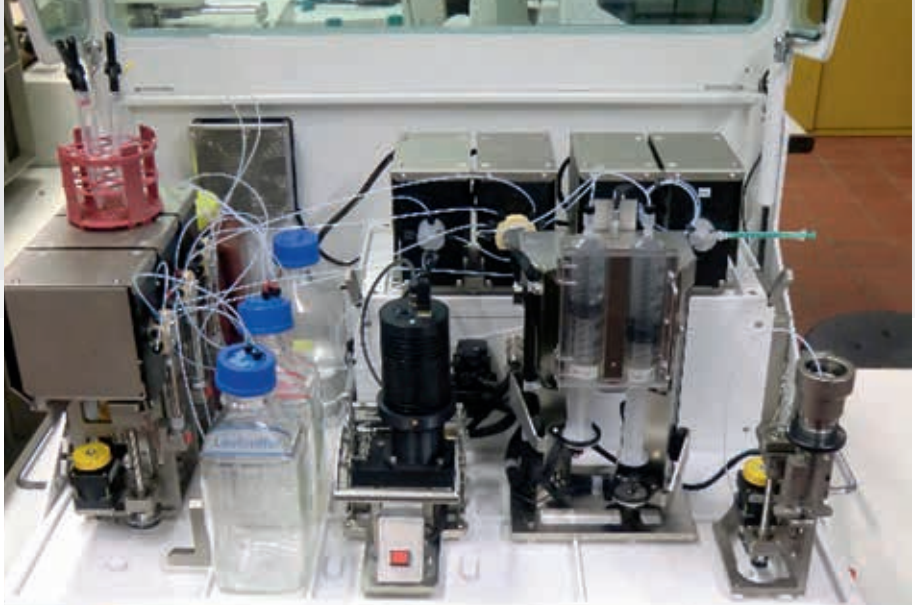
LAB-ON-CHIP MICROCONCENTRATION AND EXTRACTION OF NUCLEIC ACIDS

The subsequent micro-concentration and extraction of nucleic acids from the macro-concentrated sample were implemented on a single lab-on-chip system. In this system, suspended bacteria and viruses are concentrated at a specific gel front with high efficiency using the principle of free flow electrophoresis. The volume in the chip is reduced to about 10 µl after accumulation of microorganisms. Lysis of micro-organisms and the extraction of nucleic acids are performed in the same lab-on-chip system. In the next step the released nucleic acids are purified via gel electrophoresis. After this the extract is transmitted to the automated analytical microarray (MCR3).





Chip for micro-concentration and sample extraction, Photo: Richard Rietzel, IMTEK Freiburg



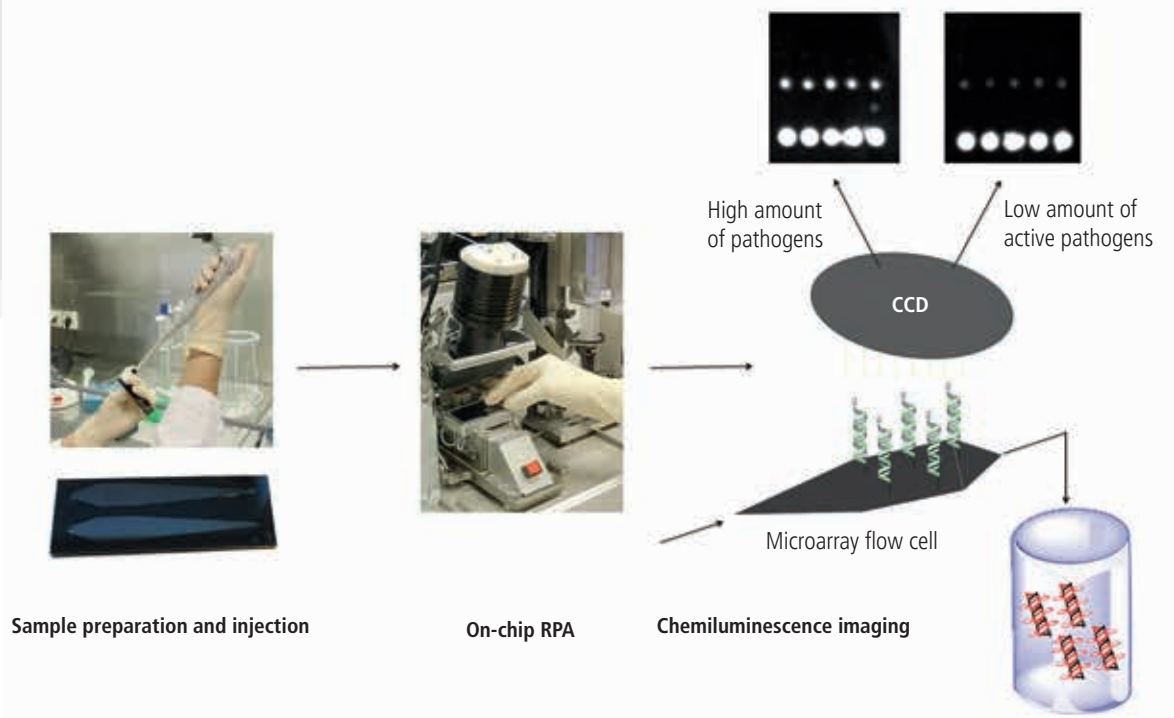
DNA Microarray System,
Photo: GWK & TUM

6 DETECTION OF PATHOGENS

Currently used methods for the assessment of drinking water hygiene are based on the cultivation of indicator bacteria in the laboratory. By contrast, the hygiene online monitoring (HOLM) system developed in EDIT is based on molecular biology. Due to the multiplex amplification and detection approach, several indicators and pathogens can be identified simultaneously and within much shorter time than conventional methods (about 5 hours instead of 18 hours).

In the project, two methods for nucleic acid amplification systems were considered: polymerase chain reaction (PCR) and an isothermal amplification technique (Recombinase Polymerase Assay, RPA). While applications for RPA in medical diagnostics have increased significantly in recent years, the technique has the additional advantage of combining a high amplification (and therefore detection) speed with relatively inexpensive and less complex technology as compared to PCR. This facilitated the integration into the HOLM system. Despite advances, the simultaneous amplification of different DNA sequences (i.e. multiplexing) that are useful for the identification of waterborne bacteria and viruses remains challenging.

For the detection of the specific DNA of pathogens and indicators, a DNA microarray was designed for the HOLM system. With species specific DNA oligomers generated by isothermal amplification the pathogen nucleic acids are detected *in situ* via chemiluminiscence on the analytical microarray, allowing for the direct detection of multiple pathogenic microorganisms and viruses.



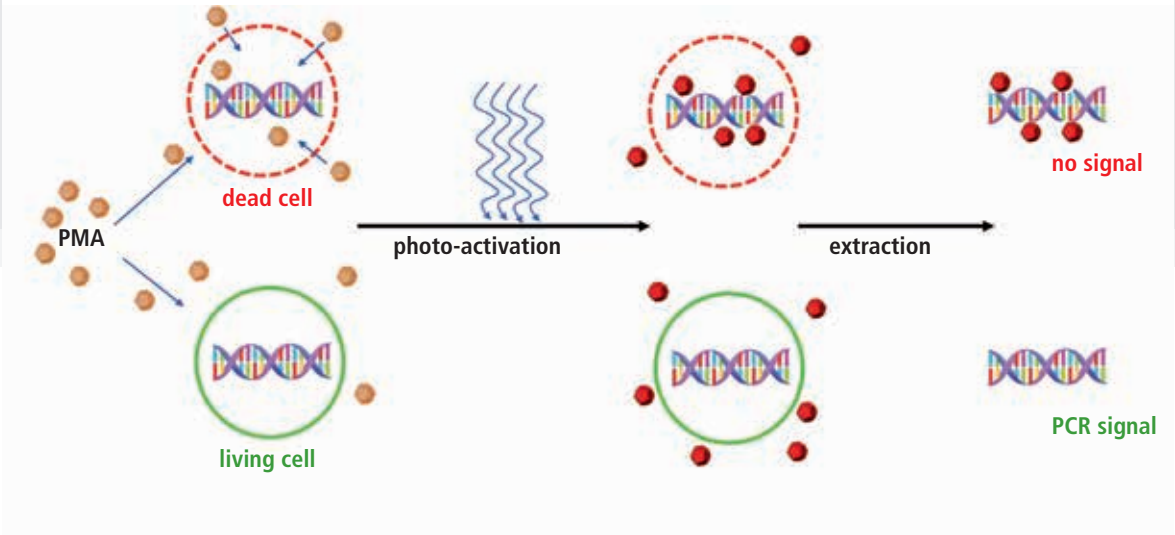
Pathogen detection on the MCR3,
Source: Dr. Andreas Kunze, TUM

MICROORGANISMS CONSIDERED IN EDIT

Based on the simultaneous detection of several microorganisms, the HOLM system developed in EDIT can identify a wide range of bacteria, viruses and phages (see table 1) that exceeds the requirements of the German Drinking Water Ordinance. Viruses are particularly relevant in this context because of the large amounts excreted in feces, low infective doses and the high environmental stability of viruses may promote their occurrence in raw water used for drinking water or food production.

Bacteria	Viruses	Bacteriophages
• Escherichia coli[1]	• Norovirus GI, II	• MS2
• Enterococcus faecalis[1]	• Adenovirus 40, 41, 52	• PhiX174
• Pseudomonas aeruginosa[1]	• Enteroviruses	
• Legionella pneumophila[1]		
• Campylobacter jejuni		
• Klebsiella pneumoniae[2] and Klebsiella oxytoca		

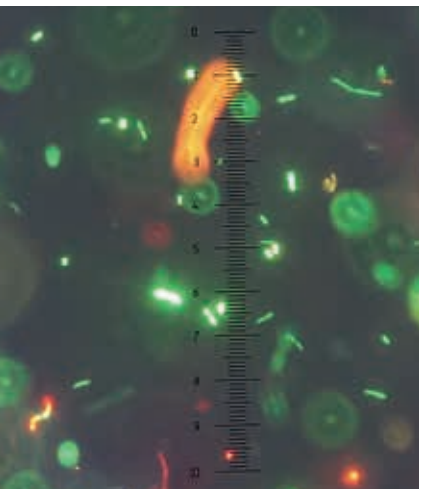
Table 1: Target organisms of the amplification and detection module
[1]Regulated by the German Drinking Water Ordinance, [2]Coliform bacteria



Color marking of inactive microorganisms,
Source: Dr. Johannes Ho, TZW

7 DISCRIMINATION BETWEEN ACTIVE AND INACTIVE MICROORGANISMS

Drinking water treatment sometimes relies on physical or chemical disinfection methods to inactivate microorganisms and thus ensure hygienic water safety. Such treatments can damage microorganisms in various ways. For instance, bacteria or viruses can no longer replicate or infect a person after their membranes or capsids are damaged. However, the genome may be still undamaged and can thus be detected by molecular methods. The (positive) detection of bacteria or viruses which are no longer infectious would constitute an undesirable, false-positive result for drinking water surveillance.



Color marking of inactive microorganisms, Photo: Dr. Johannes Ho, TZW

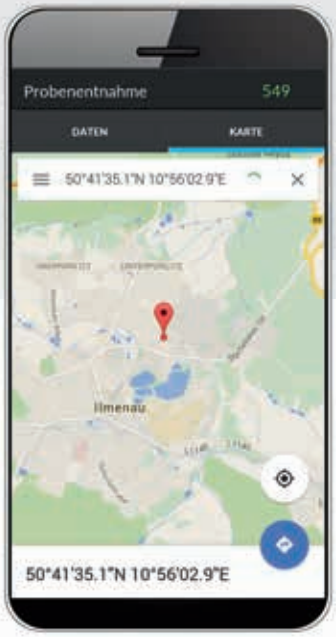
MARKING DAMAGED CELLS BY PROPIDIUM MONOAZIDE (PMA)

PMA is adsorbed to the nucleic acids of damaged cells or virus particles and in this way inhibits their detection by molecular methods. By contrast, PMA cannot penetrate undamaged cells and viruses. In this way, only active microorganisms are detected while inactive organisms are excluded. The left picture shows a water sample with dead (red, PMA) and living (green, SYBR Green) cells.

Due to their complex structure, bacteria and viruses can be damaged in different ways by disinfection. Depending on the detection method, different characteristics are used: reproduction (culture methods), genomes (PCR and RPA), membrane or capsid damages (PMA-PCR), amount of DNA and size of cells (flow cytometry). Different methods differ in their test results when cells or virus particles are damaged. The definitions for live and dead (active or inactive) micro-organisms are, however, not unambiguous and currently the subject of scientific discussion. In the EDIT project, different detection methods were compared to increase the information about microorganisms exposed to different disinfection methods.

INTEGRATION INTO THE HOLM SYSTEM

The treatment with PMA is implemented after the concentration of water samples and prior to the sample preparation for amplification and detection. The application of PMA was therefore integrated into the lab-on-chip system.



Right picture: Automatic position marking during sampling

Left picture: Data entry during sample concentration, Source: Dipl.-Ing. Thomas Westerhoff, Fraunhofer AST

8 DATA MANAGEMENT

The hygiene online monitoring system (HOLM) developed in EDIT consists of several modules for unique steps of sample processing. These subsystems had to be connected to each other not only for the physical transfer of samples, but also in a way that for the complete workflow of any sample all results and operational parameters are documented. This is not only a prerequisite for quality-assurance, but also for system-internal plausibility and validity checks.

DATA CAPTURE

For the capture, storage and analysis of data at different process steps, the Fraunhofer AST created an online database using PostgreSQL. Data can be entered both automatically and manually via an app for mobile devices. For security reasons, all data are transported in an encrypted form. Individual samples can be identified by scanning QR code on sample containers with a smartphone camera. Location data can automatically added by the smartphone's GPS functionality. For the direct and automated transmission of analytical data and operational parameters to mobile devices, the implementation of interfaces such as NFC or Bluetooth is planned for the future. Results obtained on the analytical microarray are automatically evaluated. When relevant microoranisms are detected, an alarm is automatically transmitted to the waterworks' control system via a standardized OPC interface. A subsequent software-based simulation of the spread of pathogens in the distribution network can further help to plan intervention strategies.

INTELLIGENT DATA MANAGEMENT

Seamless communication from sensors to an online database used by end users is a good example for Industry 4.0, which is an important element of the high-tech strategy of the German Federal government which aims at the creation of added value by intelligent application of IT in industry.

Demonstration of the lab-on-chip system and MCR 3 at TUM
Photo: Dr. Daniel Karthe, UFZ

9 PROJECT RESULTS, PUBLICATIONS, OUTLOOK

In the EDIT project, a modular hygiene online monitoring system was developed and tested both under laboratory conditions and by future end users in the water industry. The feasibility and potential of combining multiple concentration steps, further sample pretreatment and DNA extraction on a lab-on-chip system, and RPA-based amplification and detection on an analytical microarray could be demonstrated. However, future research is needed to validate the technique under real-life and laboratory conditions and to further improve the system components in order to develop a prototype that is cost-effective and widely acceptable by end-users.

PUBLICATIONS

HAKENBERG, S.; HÜGLE, M.; MEYER, P.; BEHRMANN, O.; DAME, G. & URBAN, G.A. (2015): Fenton fragmentation for faster electrophoretic on chip purification of amplifiable genomic DNA. Biosensors and Bioelectronics 67:59-52. doi: 10.1016/j.bios.2014.06.003

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OUTLOOK

A need for rapid and automated systems for the surveillance of pathogens in water exists not only in Germany but worldwide. However, future research is still needed to come to a prototype that is cost-effective and widely acceptable by end-users. In particular, the following aspects need to be addressed:

- validation of the technique under real-life and laboratory conditions;
- improvement of the system components and complete automation of the system;
- optimization of size and handling of the system according to end-user requirements;
- minimization of maintenance and operational costs

Ultimately, such systems have a potential for the hygienic monitoring not only of raw and drinking water, but also in specific water cycles (e.g. in large buildings or on cruise ships), in the food industry or for bathing waters.

CONTACT

Helmholtz-Centre for Environmental Research GmbH – UFZ

Department Aquatic Ecosystem Analysis and Management

Brückstraße 3a | 39114 Magdeburg | Dr. Daniel Karthe | daniel.karthe@ufz.de

Brandenburg Medical School Theodor Fontane, Senftenberg

Institute of Microbiology and Virology

Universitätsplatz 1 | 01968 Senftenberg | Dr. Gregory Dame/Prof. Dr. Frank Hufert | dame@mhb-fontane.de

Technical University of Munich

Chair of Analytical Chemistry and Institute of Hydrochemistry

Marchioninistraße 17 | 81377 München | PD Dr. Michael Seidel/Prof. Dr. Reinhard Niessner | michael.seidel@ch.tum.de

German Technical and Scientific Association for Gas and Water - DVGW

Technologiezentrum Wasser (TZW)

Karlsruher Straße 84 | 76139 Karlsruhe | Prof. Dr. Andreas Tiehm | andreas.tiehm@tzw.de

Fraunhofer Institute of Optronics, System Technologies and Image Exploitation – IOSB

Application Center for Systems Technology (AST)

Am Vogelherd 50 | 98693 Ilmenau | Dr.-Ing. Buren Scharaw | buren.scharaw@iosb-ast.fraunhofer.de

Albert-Ludwigs-University, Freiburg

Institute of Microsystems Technology (IMTEK)

Georges-Köhler-Allee 103 | 79110 Freiburg | Prof. Dr. Gerald Urban | urban@imtek.de

Municipal Water Supply Company of Berlin (Berliner Wasserbetriebe) – BWB

Neue Jüdenstraße 1 | 10179 Berlin | Dipl.-Ing. Fereshte Sedehizade | fereshte.sedehizade@bwb.de

GWK Präzisionstechnik GmbH

Gollierstraße 70 | 80339 München | Christian Heese | christian.heese@gwk-munich.com

R-Biopharm AG

An der neuen Bergstraße 17 | 64297 Darmstadt | Dr. Silvia Vosseler | s.vosseler@r-biopharm.de

https://nawam-inis.de/en/inis-projects/edit