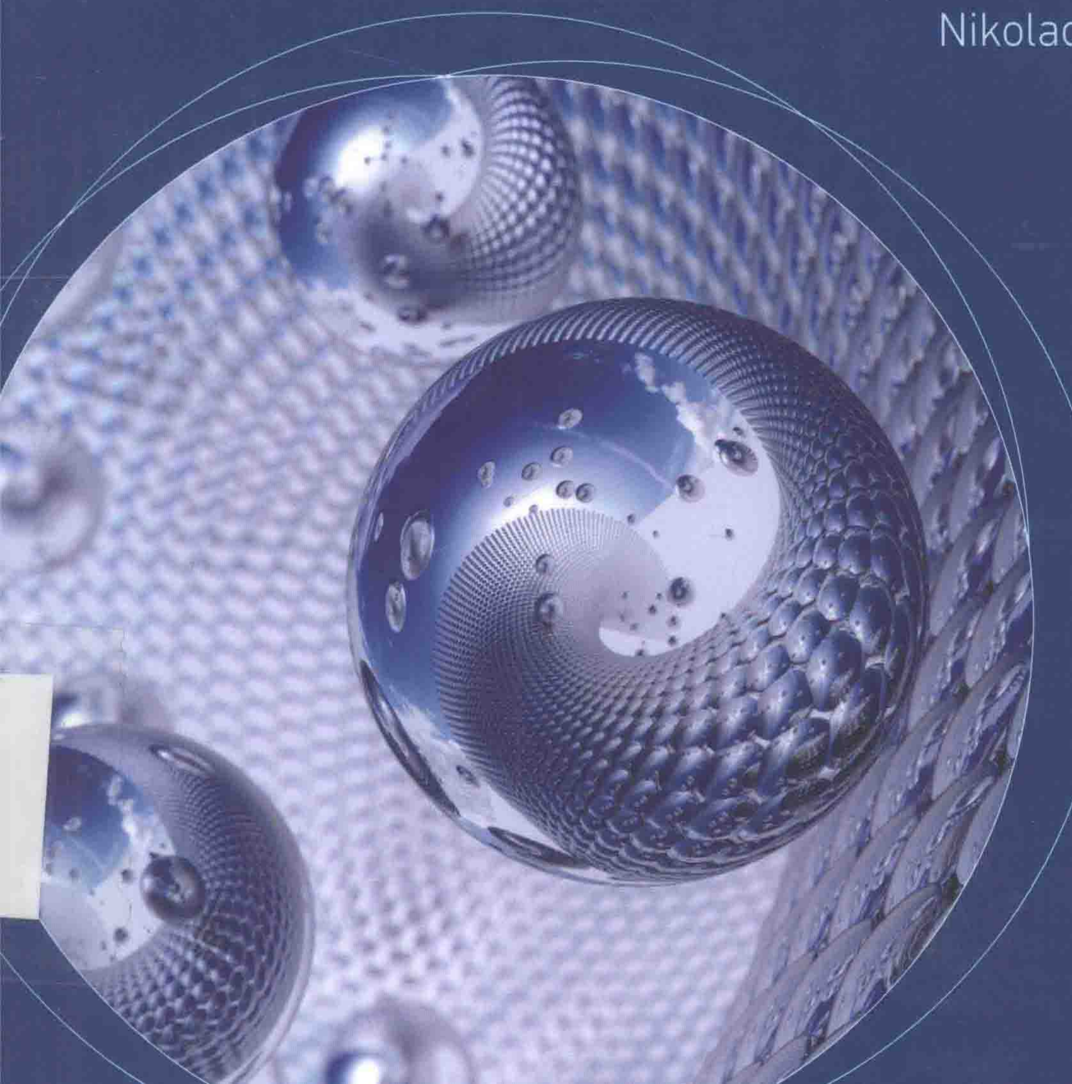
Detection of Pathogens in Water Using Micro and Nano-Technology

Giampaolo Zuccheri and
Nikolaos Asproulis



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Preface

The microbiological safety of drinking-water is one of the key environmental determinant of health. Assurance of drinking-water quality has been a pillar of primary prevention for more than 150 years and continues to be the foundation for the prevention and control of waterborne diseases. Roughly 1 every 8 people in the world still lack access to safe drinking water, according to the World Health Organization and UNICEF. Meanwhile, water use has increased by more than twice the rate of the world population growth during the past century.

The population of the industrialized world trusts the quality of the drinking water that distribution systems provide, but it is now becoming a fact that the microbiological safety on drinking water can no longer be taken for granted. Waterborne diseases are one of the major world-wide threats to public health, despite significant advances in water and wastewater treatment technology (World Health Organization, 2003). Waterborne diseases are estimated to be responsible for 4% of all deaths and 5.7% of the total disease burden worldwide. The occurrences of natural waterborne disease outbreaks as result of a failure in the conventional water treatment barriers were documented. Outbreaks have occurred in developed countries such as the United States (Cryptosporidium, Milwaukee, 1993) and Canada (*Escherichia coli* O157:H7, Walkerton, Ontario, 2000) and also in the United Kingdom and Europe. In Europe, in 2007, 17 waterborne outbreaks were reported by 8 countries (European Food Safety Authority, 2009), probably under-reporting the true number. These involved 10912 cases, with 232 hospitalisations. The main biological risks involved were Campylobacter, Norovirus, Giardia and Cryptosporidium.

Erratic and extreme precipitation events can overwhelm water treatment facilities and lead to Cryptosporidium outbreaks due to oocysts infiltrating drinking-water reservoirs from springs and lakes and persisting in the water distribution system for a long time despite vigorous and repetitive flushing of the system. A study from England and Wales found that 20% of waterborne outbreaks in the past century were associated with a sustained period of low rainfall, compared with 10% associated with heavy rainfall. Droughts or extended dry spells can reduce the volume of river flow possibly increasing the concentration of effluent pathogens posing a problem for the clearance capacity of treatment plants.

In Europe, flooding has rarely been associated with an increased risk of waterborne disease outbreaks, but a few exceptions exist in the UK, Finland, the Czech Republic, and Sweden. An outbreak of

Cryptosporidium hominis in November 2010 in northern Sweden (in Östersund) is held responsible for about 12700 cases: in samples from 174 cases, Cryptosporidium was confirmed; the water supply tested positive for Cryptosporidium, both in raw and potable water, very likely due to sewage water being released to the lake serving as reservoir for drinking water. The recommendation to boil drinking water was lifted only in February 2011 after an upgrade of the water treatment plant (ECDC, 2011).

Deliberate sabotage of large municipal water supplies is possible while difficult, especially due to the large amount of biological agents needed. Nevertheless, criminal acts perpetrated on smaller water supplies have been recorded and taking into account the complexity of water systems many possible access points for deliberate contamination acts can be identified. Breakdown in water supply safety may lead to large scale contamination and potentially to detectable disease outbreaks. Other breakdowns and low-level, potentially repeated contaminations may lead to significant sporadic diseases, but it is unlikely for these to be associated with the drinking water source by public health surveillance.

The consequences of the use of water of non-potable quality may be severe on food processing facilities and public health and they will depend not only on the direct use of the water but also on the subsequent processing of potentially contaminated materials. Water of a quality that may be tolerated occasionally in drinking water supply may be unacceptable for some uses in the food industry. Inefficient management of the water quality may result in a significant financial impact on food production, for example, through product recalls. Currently, there is no single method to collect, process, and analyze a water sample for all pathogenic microorganisms of interest. In fact, water is currently monitored through infrequent batch measurements procedures, which unfortunately, can miss transient, but problematic water safety events. Some of the difficulties in developing a universal method include the physical differences between the major pathogen groups (viruses, bacteria, protozoa), efficiently concentrating large volume water samples to detect low target concentrations of certain pathogen groups, removing co-concentrated inhibitors from the sample, and standardizing a culture-independent endpoint detection method. Integrating the disparate technologies into a single, universal, simple method and detection system would represent a significant advance in public health and microbiological water quality analysis. Recent advances in sample collection, on-line sample processing and purification, and DNA microarray technologies may form the basis of a universal method to detect known and emerging waterborne pathogens. Thus, specific detection methods are still required in order to trace the origin of etiological agents, identify lapses in water treatment, and identify new quality control processes and procedures.

By the time the results of microbiological assays on the water supply are available, thousands of people may have consumed the water and become sick. As also conveyed by the Technical Task Force of the WHO/UNICEF (Villié-Morgon, France 16–18 November 2010) new tools should be used for rapid assessments. The need of a fast response to some of the threats related to drinking water pushes towards the development and validation of molecular-based and biosensor-based methods which are expected to speed up significantly the analysis with respect to state-of-the-art cell culturing. Still, technological challenges must be overcome. Microtechnology and nanotechnology can come in help: the speed and performance of microelectronics, the versatility of microfluidics, the self-assembly of nanostructures for the making and functioning of biosensing surfaces.

In this context, the DINAMICS EU FP6 Collaborative Research Project put together the efforts of university laboratories, research institutes and private companies to deliver technology for point-of-need automated networkable microbiological analysis systems. The end-result was the development of technology for a prototype of a fully automatic system that could collect a large volume of water from the supply (also directly from the tap), concentrate the nano- and micro-particulate comprising the pathogens, lyse the cells, extract the nucleic acids and expose them to the sensing surface of an electronic (multi-pathogen) biosensor. The software managing the entire analytical process could then send a message exploiting the mobile telephone network, in the case of positive detection of pathogens.

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This book reports on some of the results and technologies of DINAMICS, through a number of chapters written by the project participants. Sabine Müller and Jonathan Loeffler (Steinbeis Europa Zentrum) give an overview of the European regulations for drinking water. Christian Mittermayr (Lambda, GmbH) describes the intricacy of microbiological risk assessment, the mathematical modelling that can turn analytical results in the input for decision making. Miloslava Prokšová and collaborators (at the Slovak Water Research Institute) tell about the sound procedures for water sampling. Christoph Zeis (Provenion Engineering) describes an automatic system for concentrating pathogens out of large volumes of water. Hunor Santha and co-workers (University of Technology and Economy of Budapest) illustrate their cell-lysis device. Theo Veenstra (LioniX, BV) zooms in on microfluidics devices designed to do wonders such as DNA extraction, on-chip PCR, mixers and valves, hybridization chambers with electrodes. Daniele Gazzola and co-workers (University of Bologna) review the characteristics of electrochemical biosensors and report on the type of such biosensor developed within DINAMICS. Alessandra Vinelli and collaborators (University of Bologna) reviews some of the modern nucleic acids technologies that can be used to enhance the signal coming from the recognition of pathogenic nucleic acids. Dimitris Mantzalis and collaborators provide an overview of water modelling approaches in both continuum and molecular framework. Nikolaos Asproulis discusses the various numerical techniques employed for simulating transport phenomena within micro- and nano-fluidic devices.

To make the picture more complete, researchers who did not participate in DINAMICS were invited to contribute their views and their results. Many enriched this book. Sophie Courtois (Suez Environment) describes the process from water concentration to microarrays developed within the HealthyWater EU Project. Joseph Faulkinham (Virginia Polytechnic Institute and State University) tell about quantitating Micobacteria. Vicente Catalan and co-workers (LabAqua) tells about new methods for the detection of Legionella. Johan Nordgren and co-workers (Linköping University) tell about how to detect viruses in water. Richard Christen and co-workers (Université de Nice) tells about how to design PCR primers to detect waterborne bacteria. Nikolai Priezjev provides fruitful insights on the boundary slippage in nanoscale liquid films. Theodoros Karakasidis and Antonios Liakopoulos discuss about the slip phenomena noticed within micro and nano-fluidic devices along with the contributing factors.

Of course, the technology and knowledge described in this book alone are not enough to revolutionize the microbiological safety testing of drinking water. Still, the process has started and we would not be surprised if micro- and nanotechnology will soon lead the molecular detection of pathogens into a mature technology-driven field. In a similar way as chemical analysis takes full advantage of automation nowadays, our cities and homes might be protected in the future against waterborne infections. We trust we and the authors of this book contributed to this goal.

Giampaolo Zuccheri
(Bologna, Italy)
Nikolaos Asproulis
(Cranfield, UK)

Contributors

Chapter 1

Sabine Müller
Jonathan Loeffler
Steinbeis-Europa-Zentrum der Steinbeis Innovation gGmbH
Haus der Wirtschaft
Erbprinzenstrasse 4-12
D-76133 Karlsruhe
Germany

Chapter 2

Christian Mittermayr
Lambda GmbH
Gewerbepark 2
Rainbach
Austria, A-4261

Chapter 3

Miloslava Prokšová
Marianna Cíchová
Lívia Tóthová
Water Research Institute
Slovak National Water Reference Laboratory
Arm.gen.L.Svobodu 5
812 49 Bratislava – Slovak Republic

Chapter 4

Christoph Zeis
Provenion GmbH
Spannleitenberg 1
85614 Kirchseeon
Germany

Chapter 5

Hunor Sántha
Budapest University of Technology and Economics
Department of Electronics Technology
H-1521, Hungary
P.O. box: 91

Chapter 6

Theo T. Veenstra
LioniX BV
PO Box 456
7500 AL Enschede
The Netherlands

Chapter 7

Daniele Gazzola
Università degli Studi di Bologna
Centro Interdipartimentale di Ricerca
Industriale Scienze della Vita e Tecnologie per la Salute
Via Tolara di Sopra
50 – Ozzano Emilia
Bologna
Italy 40064

Simone Bonetti
Università degli Studi di Bologna
Dipartimento di Biochimica "G. Moruzzi"
Via Irnerio
48 – Bologna
Italy 40126

Giampaolo Zuccheri
Università degli Studi di Bologna
Dipartimento di Biochimica "G. Moruzzi"

(currently: Università degli Studi di Bologna,
Department of Pharmacy and Biotechnology)
Via Irnerio
48 – Bologna
Italy 40126

Chapter 8

Alessandra Vinelli
Università degli Studi di Bologna
Dipartimento di Biochimica "G. Moruzzi"
Via Irnerio
48 – Bologna
Italy 40126

Manuele Onofri
Università degli Studi di Bologna
Centro Interdipartimentale di Ricerca Industriale Scienze della
Vita e Tecnologie per la Salute
Via Tolara di Sopra
50 – Ozzano Emilia
Bologna
Italy 40064

Giampaolo Zuccheri
Università degli Studi di Bologna
Dipartimento di Biochimica "G. Moruzzi"
(currently: Università degli Studi di Bologna
Department of Pharmacy and Biotechnology)
Via Irnerio
48 – Bologna
Italy 40126

Chapter 9

Dimitrios Mantzalis
Konstantinos Karantonis
Nicolaos Asproulis
László Könözsy
Dimitris Drikakis
Dept. of Fluid Mechanics and Computational Sciences
Cranfield University
Bld. 83
Cranfield University
Cranfield
Beds MK43 0A

Chapter 10

Nicolaos Asproulis
Dept. of Fluid Mechanics and Computational Sciences
Cranfield University
Bld. 83, Cranfield University
Cranfield, Beds MK43 0A

Chapter 11

Sophie Courtois
Anne Cajon
Aurore Romey
Fanny Poyet
Claude Mabilat
SUEZ ENVIRONNEMENT
38, Rue du President Wilson
78230 Le Pecq – France

Chapter 12

Joseph O. Falkinham III
Department of Biological Sciences
Virginia Polytechnic Institute and State University
Derring Hall
Blacksburg, VA, 24061-0406, U.S.A.

Chapter 13

Elena Soria
M. Adela Yáñez
Raquel Múrtula
Vicente Catalán
Labaqua
C/Dracma, 16-18
Polígono Industrial Las Atalayas
03114 Alicante, Spain

Chapter 14

Johan Nordgren
Div. Molecular Virology
Linkoping University
581 85 Linköping, Sweden

Lennart Svensson
Div. Molecular Virology
Linkoping University
580 85 Linköping, Sweden

Chapter 15

Julien Gardès
Institute of Signaling
Developmental Biology and Cancer Centre de Biochimie
Faculté des Sciences
Université de Nice, France
6109 Nice cedex 2, France

Richard Christen
Institute of Signaling, Developmental Biology and Cancer Centre de Biochimie
Faculté des Sciences
Université de Nice, France
06108 Nice cedex 2, France

Chapter 16

Nikolai V. Priezjev
Dept. of Mechanical Engineering, Michigan State University
2465 Engineering Building
East Lansing, MI 48824-1226

Chapter 17

Theodoros E. Karakasidis
Athanassios Liakopoulos
Department of Civil Engineering
School of Engineering
University of Thessaly
Pedion Areos, 38334 Volos, Greece

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