



Review of methods for the rapid identification of pathogens in water samples

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identification of pathogens in water samples

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1. ABSTRACT

Microbiological water contaminants represent an acute health risk in drinking water. There are a wide variety of bacteria and viruses that can potentially be found in drinking water resulting from either an attack or a natural contamination incident. Whatever the origin of the contamination, rapid identification is needed to ensure water quality and subsequent citizen safety.

Currently, various detection and identification methods exist, but they are mostly time-consuming and unsuited to emergent harmful micro-organisms. New developments are occurring to deal with this concern.

In this desk study, the main basic technologies to identify pathogens (such as immunological and genetic methods as well as mass spectrometry, microarrays and physical approaches) are reported, as well as their applications in the drinking water area. Then, some promising technologies under development are presented, especially integrated tools or new concepts in mass spectrometry. However, bottlenecks still exist, such as sample preparation or live and dead pathogen discrimination.

Additionally, different projects founded by the European Commission are briefly reported in this study, as they allow a clear vision of the scientific teams and networks working on this concern.

Finally, European standards are being established as well as national initiatives that currently remain unofficial.

2. INTRODUCTION

Proper management of water resources is associated with human health, because water is used for many purposes. Infectious waterborne diseases acquired through water networks because of natural or intentional reasons are of paramount importance. Consequently, a rapid identification of biological contaminants is essential to prevent a large dissemination, to delimitate a security area and to warn people and health services in order to initiate appropriate prophylaxis.

Until now, these measures have been based mainly on the principle of end-point monitoring for indicators and a small number of selected pathogens. This kind of monitoring involves detection of bacteria (i.e. indicators and *Salmonella*), viruses (i.e. enteroviruses) and microscopic observation (i.e. *Cryptosporidium*).

In September 2009, the US EPA (United States Environmental Protection Agency) published the final CCL-3, which is a drinking water priority contaminant list for regulatory decision-making and information collection. The listed contaminants are known or anticipated to occur in drinking water systems and will be considered for potential regulation. This final CCL-3 contains 10⁴ chemicals or chemical groups and 12 microbial contaminants (<http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm>). However, significant numbers of other human microbial pathogens or toxins could be present in water networks.

Monitoring of biological contamination in water is currently performed by enumerating bacteria and can require more than a day for laboratory analysis. This is too slow to provide full protection from exposure to waterborne pathogens. The limiting factor for the current methods is that they rely on culturing techniques that either measure a metabolic endpoint or determine growth of a microorganism after an extended incubation period. Moreover, precise identification is needed to differentiate pathogenic versus apathogenic microorganisms and represents a challenge, especially for emerging biological contaminants.

Technologies reducing the measurement period and performing accurate identification of pathogens are available. This is what this report deals with, by reviewing the methods addressing these concerns.

3. STATE OF THE ART METHODS FOR PATHOGEN IDENTIFICATION

3.1 Water microbiology: counting, colourimetric and fluorimetric methods

The **HPC** test (Standard Plate Count) is widely used to measure the heterotrophic microorganisms (which require an external source of organic carbon for growth) in drinking water.

There is no universal 'HPC measurement'. Although standardised methods have been formalised, HPC test methods involve a wide variety of test conditions (temperatures employed range from around 20 °C to 40 °C, incubation times from a few hours to seven days or a few weeks...) that lead to a wide range of quantitative and qualitative results. Even if high plate counts do not necessarily indicate that water is a danger for health, a rising plate count may give the earliest sign of pollution and would call for immediate investigation. However, the test itself does not specify the organisms that are detected.

Total coliforms are indicators of organic pollution in surface water, groundwater or supply sources of potable water. For instance, *Escherichia coli* (*E. coli*) is an indicator of pollution of human or animal faecal origin. The presence of this microorganism in drinking water represents a significant risk.

Enzyme/substrate methods are currently approved methods based on colourimetric or fluorimetric assays relying on specific enzymatic activities (i.e. Colilert® or Enterolert® from IDEXX Laboratories Inc) and give an answer within 18 hours. These technologies have been improved in conjunction with high-sensitivity fluorescence detection instruments to reduce the time required for the assay: upon growth, specific bacterial enzymatic activity cleaves the fluorophore from the substrates, causing fluorescence to increase. Detection can be done by a number of instruments.

Some are automated (TECTA™ B16 from Veolia, etc) and the sensitivity is directly related to the time of analysis: the shorter the time, the less the sensitivity.

3.2 Immunological methods

Antibody-based (Ab) approaches take advantage of the specific binding affinities of Abs to specific antigens. Once produced and tested for specificity, Abs are typically mounted onto a support system (nylon supports, cantilevers, magnetic beads, plastic, etc.). Different methods exist, such as **ELISA** (Enzyme-Linked ImmunoSorbent Assay), **lateral flow tests** (immunochromatographic assays), **SPR** (Surface Plasmon Resonance),

Western blots and **chips**, etc. Each are associated with specific devices and applications but, whatever the method, the sensitivity and the specificity depend on the antibody: for example, the detection limit is usually around 10^5 bacteria per mL in Elisa versus 10^7 bacteria per mL using a lateral flow assay. Similarly, the time of analysis can range from 10 minutes (lateral flow tests) to several hours (ELISA). In the CBRNE area, the KDTB Gold® kit enables fast-and-easy field biological detection by loading the sample onto a strip in order to detect the presence of toxins such as *Botulinum* toxin A, B & E, Ricin and SEB *Staphylococcus enterotoxin*. Within less than 15 min a colourimetric reaction allows a visualisation of whether the test is positive or negative. An electronic reader can be associated in order to enhance the reliability of the results and archives the samples made (<http://preprod.nexter-group.fr/en/products/item/350-kdtb-gold%C2%AE-field-biological-detection?tmpl=component&print=1>). An equivalent approach is under development for entire pathogens such as bacteria/spores and toxic algae (Gas F, 2010).

Another option is flow cytometry (**FCM**) paired with immunomagnetic capture to concentrate cells which are physically analysed based upon characteristics such as natural fluorescence or light scattering (Veal DA, 2000). However, flow cytometers are generally not portable or robust enough, and require advanced training to operate.

However, these immunological methods are unable to indicate the viability of organisms. The successful use of these techniques in the water field currently relies on their combination with conventional culturing/genetic/microscopic methods.

3.3 Genetic methods

These approaches rely upon the affinity of specific nucleic acid sequences to 'fish' for a complementary sequence of interest, allowing these methods to be highly specific. The most frequently used techniques are based on Polymerase Chain Reaction (PCR) methods, isothermal nucleic acid amplification or microarrays.

3.3.1 Quantitative PCR (Polymerase Chain Reaction)

Quantitative real-time PCR (**qPCR**) follows the general principle of PCR, but its key features are that (i) the amplified DNA is detected as the reaction progresses in real time and (ii) a sequence-specific DNA probe consisting of

oligonucleotides labelled with a fluorescent reporter allows highly specific detection. In the case of RNA detection (i.e. viruses), a reverse transcription step may be added before amplification (RT-qPCR). Thus, (RT)-qPCR enables both detection and quantification. With correctly designed probes/primers and proper controls, (RT)-qPCR can be one of the most sensitive, efficient, fast and reproducible methods for detecting, identifying and quantifying pathogens: this genetic approach can detect as few as one genome copy per reaction in less than one hour.

In the field of water monitoring, various detection kits (dedicated or not to specific devices) targeting waterborne pathogens are available (non-exhaustive list): iQ-Check Legionella Real-Time PCR kits from BioRad; AquaScreen® qPCR kits for quantitative detection of *Legionella pneumophila*, *Legionella* species, *Pseudomonas aeruginosa* and *Escherichia coli* from Minerva Biolabs GmbH; Enterovirus Real Time PCR kit from Diagenode, etc. The main differences between these kits are based on the degrees of standardisation of the three critical steps: DNA extraction, PCR preparation and data analysis. For example, US-EPA can approve some procedures: Method 1615 — Measurement of Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR.

Quantitative PCR is easy to automate and it can be used in the detection of pathogens that may be present below the limit of detection of other assays. However, one restriction of genetic methods is the sample preparation as the quality of the sample strongly impact on the sensitivity of the detection. Moreover, the presence of inhibitors still represents a limitation in the analysis of environmental samples. This makes direct comparison of absolute gene numbers between studies extremely problematic. Consequently, the protocols used need to be improved and standardised.

3.3.2 Immuno-PCR (IPCR)

IPCR is an interesting approach as it combines the versatility of enzyme-linked immunosorbent assays (ELISAs) with the amplification power and sensitivity of the PCR or qPCR (qIPCR). As a consequence, the limit of detection of a given ELISA is, in general, enhanced 100-1000-fold by the use of PCR as a signal amplification system (Adler M, 2008). The most prominent obstacle of IPCR is the high background signals often prohibiting meaningful results. This trouble can often be overcome by simply diluting the matrix using appropriate buffers, without significantly decreasing the sensitivity in the overall assay.

A number of variations of the initial IPCR method have been introduced in order to fit with today's requirements of commercially available kits for routine diagnostics and food monitoring such as those commercialised by Chimera Biotech (<http://www.chimera-biotech.com>). Most of the diagnostics assays reported in diagnostics can easily be adapted to water monitoring (i.e. Rotavirus, *E. coli* β -glucuronidase, toxins, etc.) (Chang TC, 1997) (Chao HY, 2004).

3.4 DNA sequencing

DNA sequencing could offer an alternative for making routine species-level identifications, even though related methods are not quantitative. DNA sequences can be easily obtained and are highly accurate for identifying pathogens. However, until recently it has not been feasible to use sequencing for routine monitoring. Even with automated extraction, PCR and sequencing, species would need to be individually sorted which is laborious, expensive and time-consuming.

In 2003, Paul Hebert proposed '**DNA barcoding**' as a way to identify species (Hebert PDN, 2003). Barcoding uses a very short genetic sequence from a standard part of the genome in the same way that a supermarket scanner distinguishes products. The primary utility of DNA barcoding is to identify unknown specimens at the species level by comparing the query sequence to a DNA barcode reference library built based on known species. However, it is not a feasible approach for tackling bulk environmental samples because these samples can contain thousands of individuals from hundreds of species ranging from bacteria to higher eukaryotes. The bottleneck in this case may not only be at the DNA sequencing step but can also occur at the collection, sorting and preparation steps. Hopefully, this is becoming conceivable thanks to **NGS** technologies.

The high demand for low-cost sequencing has driven the development of high-throughput sequencing or next-generation sequencing (NGS) technologies that parallelise the sequencing process, producing thousands or millions of sequences concurrently. With NGS, as many as 500 000 sequencing-by-synthesis operations may be run in parallel.

Thus, NGS has the potential to be used for routine environmental monitoring. In environmental monitoring, NGS technologies are of great interest (Carew ME, 2013) and have been used to realise phylogenetic and metagenomic analyses (Kisand V, 2012). NGS has also been used to improve the barcoding

approach (Hajibabaei M, 2011) or to estimate biodiversity, especially in fresh water (Logares R1, 2013).

3.5 Mass spectrometry

The principle idea of identifying microorganisms by mass spectrometry analysis of proteins stems from the 1970s. It detects an intrinsic physical property, the mass-to-charge (m/z) ratio of an analyte, leading to robust and precise analyses. Environmental microbial diversity can be considered by means of exhaustive comparative proteomic surveys where thousands of proteins are detected and quantified (Armengaud J, 2013).

3.5.1 Mass spectrometers

Different mass spectrometry methods exist and various combinations of mass analysers can be assembled. Tandem mass spectrometry (**MS/MS**) possesses distinct advantages such as: (i) higher selectivity resulting in less interference of co-eluting compounds and matrix, (ii) better Signal-to-Noise allowing quantitation with lower limits of quantitation, (iii) a wider linear range of quantitation and better accuracy and reproducibility especially at low concentrations.

Tandem MS (LC-MS/MS) is an alternative proteomics-based method that has been used to identify bacteria, including Biosafety Level 3 (BSL-3) select agents. Moreover, nanoscale liquid chromatography coupled with MS/MS (nano LC-MS/MS) has become an essential tool in the field of proteomics. In fact, its sensitivity has advantages over conventional LC-MS/MS that allow the analysis of peptide mixtures in sample-limited situations (Gaspari M, 2011). Bacterial pathogens can rapidly be identified by shotgun proteomics: peptide sequence data from nano-LC-MS/MS is searched against a database represented by concatenated proteomes of completed genome sequences (Tracz DM, 2013).

3.5.2 Bioinformatic data

Mass spectra can be easily digitalised, which enables straightforward data storage and exchange. For microbial identification and taxonomic classification user-friendly commercially software packages such as the MALDI Biotyper provided by Bruker Daltonics (<http://www.bdal.com>) and Samaris developed by AnagnosTec (<http://www.anagnostec.eu>) are currently mainly used. Besides these two widely proven software packages, recent data analysis tools such as Andromas (Bille E., et al.) provide algorithms for reliable identification and classification of protein mass patterns. Numerous MS-based methods currently used for the analyses of pathogens are used (Ho YP, 2011; see also the Sara

Rodriguez-Mozaz report). Adding reference spectra of microorganisms that are either under- or not-at-all represented and increasing the number of available mass spectra per species will steadily improve the performance of the method.

However, viral detection remains difficult as viruses have relatively lower protein content in general.

3.6 Microarrays

A microarray is a multiplex lab-on-a-chip that assays large amounts of biological material using high-throughput screening methods. The concept and methodology of microarrays was first introduced in antibody microarrays in 1983. As the 'gene chip' industry started to grow in the 1990s, the technology of DNA microarrays has become the most sophisticated and the most widely used. Different types of microarrays exist (tissue microarrays, cellular microarrays also called transfection microarrays and glycoarrays, etc.). The most compatible with biological monitoring and pathogens identification are reported below.

3.6.1 DNA microarrays

In **DNA microarrays**, probe molecules are short single-stranded nucleic acid (DNA or RNA) or oligonucleotides with known sequences whereas target molecules are usually prepared from PCR amplification of genomic extracts. Various providers propose either catalogue or customised DNA-chips with associated readers and software, especially the Affymetrix and Agilent companies.

High-throughput microarray technology has already been applied to studies of complex microbial communities in various environments using different types of microarray probes, such as oligonucleotides, cDNAs and microbial genomes. He et al. developed a comprehensive microarray called GeoChip — a functional gene array that comprises more than 24 000 oligonucleotide probes that target thousands of functional genes (He Z, 2007). The GeoChip has been applied to the analysis of functional microbial communities in deep-sea hydrothermal vents (Wang F, 2009).

The main advantage of DNA microarrays is the multiplex capacity which is particularly interesting for complex samples.

3.6.2 [Protein microarrays](#)

A **protein microarray** is a high-throughput method used to track the interactions and activities of proteins on a large scale. Probe molecules, protein typically labelled with a fluorescent dye, are added to the array. Protein microarrays are rapid, automated, economical and sensitive, consuming small quantities of samples and reagents. One of the emerging protein array technologies is the magneto-nanosensor array, where giant magnetoresistive sensors are used to quantitatively measure the analytes of interest which are labelled with magnetic nanoparticles (Lee JR, 2013). This kind of approach is of great interest to detect the biomarkers specific for contamination, however no environmental application has been reported yet.

3.6.3 [Antibody microarrays](#)

An **antibody microarray** is a specific form of protein microarray: a collection of capture antibodies are spotted and fixed on a solid surface for the purpose of detecting antigens. Recently, a 200-antibody microarray for environmental monitoring through immunoprofiling was realised allowing bacterial cell and spore detection and identification (Rivas LA, 2008). Moreover, Lian et al. have developed a fluorescent antibody microarray system for the detection of bioterrorism agents exemplified by ricin, and SEB toxins. Sensitivity, specificity, and reproducibility were achieved by using their antibody biosensor array (Lian W1, 2010). They found that substituting monoclonal antibodies with highly purified polyclonal antibodies, even though having similar titer by ELISA, could dramatically improve the microarray performance. The limit of detection is around 10^4 - 10^5 CFU/mL for bacteria and 10-100 pg/mL for toxins, depending on the antibody features.

3.7 [Physical methods](#)

3.7.1 [Infrared and Raman spectroscopy](#)

Fourier transform infrared spectroscopy (**FT-IR**) analyses the total composition of all components of the cell using infrared spectroscopy (Helm D, 1991) and has been established as a method for identification of bacteria, yeasts and other microorganisms. The FT-IR method is rapid and reliable and therefore can be easily adapted to routine analysis. However, precise identification (especially pathogenic versus apathogenic strains) remains to be developed.

Raman spectroscopy is currently used for the reliable classification of complex biological samples due to its non-destructive and fast methodology. Moreover, it has been shown that very reliable recognition results can be

obtained without time-consuming cultivation steps, since it is possible to extract informative spectra from single cells. Due to these advantages, Raman spectroscopy is becoming a tool used for a variety of tasks such as food control or microorganism identification (Rösch P, 2005), pathogen identification (Hamasha K, 2013) or general bacteria classification (Krause M, 2008).

3.7.2 LIBS

Laser-induced breakdown spectroscopy (**LIBS**) is an outgrowth of atomic emission spectroscopy in which elemental composition was determined by placing samples in a flame or laser plasma, and observing the resulting spectrum. LIBS has been used to differentiate the type of bacteria (*E. coli* or *S. enterica*) along with the metabolic state (viable or heat killed) at a concentration of 1 °CFU/100µL (Multari RA, 2013). The main advantages of LIBS-based technology for this application will be the speed of analysis, minimal sample preparation, use of few consumables and the ability to detect pathogens on all types of surfaces. Once the algorithm has been developed and loaded into a LIBS instrument, a sample could be analysed in about three minutes. However precise identification is not yet accessible.

4. BOTTLENECKS

4.1 Sample processing

4.1.1 General impact

Most detection technologies are based on measuring sample volumes of less than 1 ml. However, for example, EPA's recommended marine bathing water standard is 35 *enterococci* per 100 mL, which equates to less than one cell per mL. Thus, detectors measuring only a 1 ml volume, even if they are capable of detection of one cell per ml, will necessarily produce unacceptable sensitivity and poor precision at concentrations near the standard.

The option is pre-concentration, which can enhance sensitivity several times by increasing the number of target organisms per unit volume. Several available modes of pre-concentration are being used, including filtration, size-fractionation, centrifugation and immune-magnetic separation or combinations of these methods. For example, an immuno-concentration step can be added upstream of any detection technique. This will allow the tester to specifically concentrate the target and to eliminate potential chemical or biological contaminants which could inhibit or interfere with the reaction. One advantage of some Ab-based approaches is that captured bacteria can be still viable and further studied. However, the biggest drawback to pre-concentration is the additional time it requires. Depending on the method used, pre-concentration could also result in partial loss of target organisms or the unintended concentration of environmental contaminants: water contains substantial amounts of organic matter, humic and fulvic acids and tannins, as well as organic substances resulting from human activity (e.g. detergents, pesticides, hydrocarbons and pharmaceuticals). These compounds vary in both type and concentration, depending on the water source. Also, if samples are concentrated to detect the microorganisms present at low densities, then minerals, organic components and biomass will also be concentrated, thus altering the detection. The water composition (inorganic, organic and biomass content) and the variability of composition between samples make it difficult to optimise standard concentration methods.

Pre-processing to separate microorganisms or molecular targets in microorganisms from matrix constituents may involve chemical precipitation, solvent extraction, adsorption to charged surfaces, chelation or binding through immunomagnetic separation. Thus, pre-concentration developments will play an important role in advancing the field.

4.1.2 [Genetic detection](#)

Concerning genetic detection methods, sample preparation is the main bottleneck as the quality of the sample strongly impacts on the sensitivity of the detection (Jofre J, 2010). Consequently, the genomic DNA or RNA to be detected has to be extracted and purified before amplification in order to remove potential inhibitors that could interfere with the reaction and cause false negative signals. The purification of nucleic acids extracted from microorganisms previously concentrated from water samples is also a challenge because samples may contain particles to which nucleic acids adsorb and ions that favour nucleic acid adsorption. Although many commercial kits for nucleic acid extraction are available, their efficiency varies when low concentrations of nucleic acids are involved (Sen K, 2007). However, DNA extraction methods and commercial kits for environmental samples have been improved during recent years (Horáková K, 2008).

4.1.3 [Mass spectrometry](#)

Although mass spectrometry is a powerful method for microorganism identification, proper sample preparation is critical because the quality and reproducibility of sample extraction and preparation significantly impact results.

Although a single bacterial colony is usually sufficient for analysis, in many cases culturing of bacteria is required to obtain enough material. A broad range of (selective or non-selective) solid or liquid media known from conventional procedures can be applied to various microorganisms but may have to be optimised from case to case. In general sub-culturing requires at least one additional day. Sample preparation for mass spectrometry analysis also depends on ion sources with different requirements for volume, concentration and composition of the analyte solution. In order to concentrate the microbial target, antibodies can be used. For example, the association of the signal amplification property of gold nanoparticles, monoclonal antibody recognition and the high sensitivity of ICP-MS, enables the specific detection of as few as 500 *E. coli* O157:H7 cells in a 1 mL sample.

Differential mass spectra of the same bacteria can occur owing to dramatically varying preparation methods or culturing conditions, standardised sample preparation procedures are required to enable the generation of reproducible mass spectra for routine bacterial identification. For inactivating highly pathogenic samples (bacteria and spores, etc.), fast pre-treatment steps that use for example ethanol or trifluoro acetic acid have been developed (Lasch P, 2008).

4.2 Dissociate live and dead pathogens

Current methods used to consider the viability of pathogens mainly rely on culturing samples on solid media. These methods involve several manual steps which make them labour-, space- and time-intensive. Validated rapid-viability test protocols are therefore needed.

Concerning biothreat agents (for which detection/identification methods address the same challenge as for waterborne pathogens) an approach, referred to as rapid-viability (RV)-PCR, has been reported and uses accepted methods including culturing and real-time PCR analysis to allow rapid and specific analysis (two to three hours for relatively clean samples). This approach has even been validated on bacterial spores. High-throughput sample processing is enabled by commercial automation in combination with 96-well real-time PCR analysis; the analysis can be completed in less than 12 hours with a detection level of 10 to 99 CFU/sample (Létant SE, 2011). On the other hand, quantification of viable but non-culturable (VBNC) *E. coli* O157:H7 was the focus of a new method using quantitative PCR targeting the *rpoS* mRNA (Liu Y, 2010). However, to evaluate whether viable cells, and not only DNA, are detected in the samples, molecular methods must be adapted. Various approaches have been evaluated; for example, propidium monoazide has been used to discern whether a cell is alive or dead, in combination with qPCR (Nocker A, 2009).

Ratiometric pre-rRNA analysis (RPA) detects the replenishment of rRNA precursors that occurs rapidly upon nutritional stimulation of bacterial cells. In contrast to DNA detection by PCR, RPA distinguishes viable from inactivated bacteria. Real-time quantitative PCR (RT-qPCRs) targeted the 5' pre-rRNA leader region are used to assess the time course of pre-rRNA replenishment upon nutritional stimulation. Bacteria were suspended in the culture medium and RNA isolated before RT-qPCR assays. Pre-rRNA stimulation was very rapid as less than four hours were adequate for near-maximum stimulation depending on the bacteria. Ratios of RT-qPCR values in stimulated and control samples were calculated following normalisation to genomic DNA standard curves. In its present form, RPA is not quantitative but it is promising as a molecular viability test for pathogens in water and other environmental samples (Cangelosi GA, 2010).

4.3 Identification of unknown biothreats

As previously mentioned, identification methods for unknown or emergent pathogens is needed. Currently, only a few technologies can answer this point.

Microarrays (especially DNA microarrays) could enable new pathogen identification in samples. With thousands of molecular probes on a chip, unknown but relatively similar genomic sequences could hybridise and be detected. This approach could be used to detect and further identify initially untargeted microorganisms only if they are genetically related to known pathogens. Identification will have to be confirmed with another technology such as sequencing. Toxin identification cannot be considered with DNA microarrays.

Sequencing is the only way to precisely identify and characterise unknown pathogens even if informatics data analysis requires specialised operators. However, there is huge investment in improving this technology. Thus, even if this is not currently conceivable, one can imagine that in the near future this technology will be reachable for routine monitoring.

Mass spectrometry: in contrast to the identification of microorganisms in pure culture samples, which relies on matching protein fingerprints against specific databases, the new approach developed by the team of Jean Armengaud does not restrict the search to predefined species or protein subsets. This approach is based on the analysis of extensive shotgun proteomics data from the most abundant proteins of organisms in the sample, coupled with bioinformatic extraction of taxonomical information. Deconvolution of signals arising from each organism in the sample is performed using a specific algorithm, applicable regardless of the organisms considered. The method proved to be fast and accurate with confident identification currently better than the species level. The method allows the quantification of the ratio of organisms in the mixture, using either spectral count or Extracted Ion Chromatogram-based label-free quantification (CEA/DSV France/personal communication — ongoing patents).

5. PROMISING TECHNOLOGIES FOR PATHOGEN IDENTIFICATION

In the field of drinking water monitoring, some commercial kits allowing contamination detection in water samples are available (cf. the Legionella Systems Test Kit which is for detecting *Legionella*: the test operates via a Lateral Flow Immuno-Chromatographic Assay — Orbeco-Hellige). However, most of the tests performed by the water industry correspond to conventional approaches (enzymatic activity, culturing, etc.) that are either not highly specific or time consuming. Optimisation and innovation are being developed to assess this concern and are presented below.

5.1 Immuno-detection approaches

➤ An immunochromatographic test using a monoclonal antibody labelled with fluorescent liposomes (called **immunoliposomes**) as tracers has been developed to allow the detection of a large number of microcystins and nodularin variants in water samples. The fluorescent signal generated by these immunoliposomes can be measured and quantified using a small transportable, easy-to-use fluorometer. This method allows the sensitive detection of microcystins (0.06 ng/ml) which satisfies the strictest World Health Organisation standard in drinking water (1 ng/mL). Entire pathogens detection/identification is under development (Khreich N, 2010). See Annex 1, Figure 1.

➤ A 3D microfluidic paper-based electrochemical immunodevice (**3D- μ PEID**) based on functionalised 3D μ PADs (microfluidic Paper-based Analytical Devices) is being developed. 3D μ PADs are particularly useful because they permit fluid movement in the x-, y- and z-directions, and therefore, they can accommodate more assays on a smaller footprint than the typical 2D, lateral-flow devices. A 3D μ PAD can distribute a sample from a single entry point to hundreds of test regions. It is a simple, inexpensive, portable, rapid (less than 15 min) and multiplex point-of-care testing (POCT). No environmental applications on toxins or pathogens have been reported yet but one can imagine that it is workable (Zang D, 2012). See Annex 1, Figure 2.

➤ The Single-Walled carbon NanoTubes (**SWNTs**) are based on materials obtained on impregnation of common filtration papers with carbon-nanotubes and antibodies. The mechanism of sensing is predicated on the formation of antibody-antigen complex between carbon nanotubes forming a dense percolation network. The conductivity of this network strongly depends on the presence of analytes. The change in conductivity of the paper was used to

sense the microcystin in the water rapidly with a limit of detection of 0.6 ng/mL and is comparable to the detection limit of the traditional ELISA (Wang L, 2009).

One can imagine that with the simultaneous development of these approaches, disposable and user-friendly sensing platforms are just a matter of time, but as previously mentioned, technical performance will always depend on the quality of the Abs to avoid false positives.

5.2 Genetic methods

To detect bacteria or viruses by PCR a previous sample preparation step is required and is time-consuming. This step aims to purify the genomic DNA. Several DNA purification kits already exist, but have to be used separately and necessitate manual and expert handling.

➤ Currently, only one commercial device has this sample preparation step integrated which was commercialised by Cepheid (GeneXpert, Cepheid; <http://www.cepheid.com/us/cepheid-solutions/systems/genexpert-systems/genexpert-xvi>). The GeneXpert System is available in a 1-, 2-, 4-, 16-, 48- or 80-module configuration. All GeneXpert modules use the same patented cartridge technology for every Xpert[®] test. This technology is well-suited for environmental and water applications and analysis duration is around 1 hour. See Annex 1, Figure 3.

➤ A transportable system is under development which satisfies the requirements: (i) fully autonomous, (ii) complete protocol integration from sample collection to final analysis and (iii) detection of diluted molecules or biological species in a large real-life environmental sample volume. To do so, a two-step concentration protocol based on magnetic beads is automated in a reusable macro-to-micro fluidic system. The detection module is a PCR-based miniaturised platform using digital micro-fluidics, where reactions are performed in 64 nL droplets handled by electrowetting on dielectric (EWOD) actuation (Delattre G, 2012). See Annex 1, Figure 4.

➤ On the other hand, isothermal DNA amplification is a good alternative to PCR-based amplification. In fact, isothermal DNA amplification does not require a thermal cycler (the denaturation step is performed by enzymes that are efficient at the same temperature as polymerases). Consequently, no huge device is required and isothermal PCR is well-suited for field applications. Moreover, in most cases, it is cost effective, easy-to-use and more tolerant to

inhibitory elements from a crude sample compared with PCR, showing equivalent or higher sensitivity and reliability. Detection time is less than 15 minutes. An integrated system including the DNA purification step is being developed by HSG-IMIT (Institut für Mikro- und Informationstechnik, Germany) using RPA (Recombinase Polymerase Amplification) isothermal technology. The whole system would allow full genetic detection within one hour. (<http://www.loac-hsg-imit.de/index.php?id=486&L=1>). See Annex 1, Figure 5.

5.3 NGS (third generation)

Third-generation sequencing is coming out with new insight in the sequencing. For example, the Single-molecule real-time (SMRT) developed by Pacific Bioscience and makes use of modified enzyme and direct observation of the enzymatic reaction in real time. The advantages are the following: (i) the sample preparation is very fast; it takes four to six hours instead of days and it does not need a PCR step in the preparation step, which reduces bias and error, (ii) the turnover rate is quite fast; runs are finished within a day and (iii) the average read length is longer than that of any second-generation sequencing technology (<http://www.pacificbiosciences.com/products/smrt-technology>).

However, continued effort is needed to improve NGS and also to determine the detection limits for species in mixed and complex samples along with potential error rates. Furthermore, there is a need for simple analysis pipelines to deal with NGS data that use new bioinformatic tools and software. In fact, although NGS makes genome sequences handy, the subsequent data analysis are still the bottle-neck in sequencing genomes. Moreover, as with all the genetic methods reported in this paragraph, matrix complexity and consequent sample preparation (need for pre-concentration or pre-culturing, elimination of chemical pollutants, etc.) have to be carefully considered in order to obtain reliable results.

6. EUROPEAN PROJECTS FOR PATHOGEN (OR TOXIN) IDENTIFICATION

Various projects related to water monitoring are funded by the European Commission (http://cordis.europa.eu/fp7/home_en.html). Some of them dealing with pathogens (and toxins) identification are reported below.

6.1 Aquavalens

(<http://aquavalens.org/>)

This project develops and uses new molecular techniques to allow the routine detection of waterborne pathogens and improve the provision of safe, hygienic water for drinking and food production throughout Europe. Funded by European Union FP7, the Aquavalens, or 'healthy water' consortium brings together 39 partners from small businesses, universities and research institutes (13 countries across Europe).

New knowledge on the molecular genetics of viral, bacterial and parasitic waterborne pathogens such as *Cryptosporidium*, *Campylobacter* and Norovirus will be generated. Subsequently, new technologies that integrate sample preparation and detection into a single platform will be developed before field studies. The key focus will be to adopt and, where appropriate, adapt existing technologies to develop these detection systems.

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6.2 MicroAqua

(<http://microaqua.eu/project>)

μAQUA aims to design and develop a universal microarray chip for high-throughput detection in water of known and emerging pathogens (bacteria, viruses, protozoa and cyanobacteria) and to assess the water quality monitoring for the presence of select bioindicators. A chip able to detect cyanobacterial toxins will also be developed in order to obtain efficient, sensitive, robust, rapid and inexpensive tests to monitor various aspects of water quality for control and prevention of diseases caused by waterborne pathogens and by algal toxins. μAQUA also aims to identify cyanophages potentially capable of controlling and mitigating the periodical blooming of toxic cyanobacteria in drinking water reservoirs.

Molecular biological tools have now greatly enhanced the ability to investigate biodiversity by identifying species and to estimate gene flow and distribution of species in time and space. These innovative molecular tools should be amenable to automation so that they could be deployed on moorings for routine semi-continuous monitoring of water quality. This project plans to adapt DNA

and antibody microarrays for water monitoring and will be completed at the end of 2014. It includes the participation of eight countries (10 partners).

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Camerino (MC), Italy

6.3 Healthy Water

(www.helmholtz-hzi.de/healthy-water)

The overall goal of the project is to advance knowledge on the pathogenesis of emergent microbial pathogens in drinking water and to understand their transmission to humans. The project will focus on all major types of pathogens, i.e. viruses, bacteria and protozoa, and will concentrate on a representative set of European drinking water supply systems and source waters of specific sensitivity to human health. To reach the overall goal, the objectives are: (i) validation and application of detection technologies for emerging microbial pathogens based on nucleic acids, (ii) molecular survey and comparative detailed study of emerging pathogens in European drinking water sources and supply systems, (iii) understanding the human health impact of emerging pathogens by primary epidemiological studies targeted at specific systems and pathogens and (iv) determination of epidemiological correlations with molecular and environmental data and assessment of risk for waterborne microbial infections in Europe.

An integrated research approach will be pursued to achieve these objectives by combining molecular and classical detection, activity assessment and epidemiological understanding of emerging pathogens in a specific set of drinking water systems from different European regions. Six countries are involved in this project.

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6.4 BIOMONAR

(http://www.sdu.dk/en/Om_SDU/Institutter_centre/fysik_kemi_og_farmaci/forskning/forskningsgrupper/raewyn_m_town/biomonar)

This project develops multiplexed nanoarray biosensors for selective and sensitive detection of environmental targets, i.e. pollutants and pathogens. The sensor platforms probe different aspects in the 'exposure to effect' chain of processes: each responds to a certain proportion of the total target concentration and has a characteristic dynamic window. The sensor signals are

quantitatively interpreted and represented in terms of the spectra of reactivities and fluxes of target compounds. This level of sophistication allows a coherent elucidation of the link between dynamic target speciation and predicted toxicological impact.

Biomonar will end this year (2014) and involves six countries (10 partners).

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6.5 QUANDHIP

(<http://www.quandhip.info/Quandhip>)

The Joint Action QUANDHIP combines two previously existing European networks, aiming to link and consolidate their objectives. The 'European Network for Highly Pathogenic Bacteria' (ENHPB), coordinated by the Robert Koch Institute and the 'European Network of P4 Laboratories' (ENP4Lab), coordinated by the National Institute for Infectious Diseases (INMI), Italy.

The primary aim of these combined two networks, dealing with high threat bacteria (Risk Group 3) on the one hand and with highly infectious viruses (Risk Group 4) on the other, is to create a stabilised permanent consortium that links up and unites 38 highly specialised and advanced laboratories from 23 European countries. This is meant to ensure a universal exchange of best diagnostic strategies to support a joint European response to outbreaks of highly pathogenic infectious agents, including the generation of a biodiverse repository of reference materials. The project will provide a supportive European infrastructure and strategy for external quality assurance exercises (EQAE), training and biosafety/biosecurity quality management.

Even if this joint action mainly concerns diagnostics, pathogens as well as detection and identification methods could be closely related with water monitoring.

Coordinator: Robert Koch-Institut (RKI), Germany

Co-coordinator: National Institute for Infectious Diseases (INMI), Italy

6.6 SecurEau

(<http://www.secureau.eu/>)

The main objectives of SecurEau is to launch an appropriate response for rapidly restoring the use of the drinking water network after a deliberate contamination: (i) design of methodologies to identify new relevant contaminants, (ii) modelling of the contaminants distribution throughout the network and identification of the origin point of the contamination, (iii) adaptation and integration of various sensors in a surveillance system in an

optimal configuration and (iv) development of methods to decontaminate a polluted drinking network and installations including the neutralisation of contaminated water and residues.

This project mainly targeted chemical contaminations, but bacteria and spores surrogates were also tested.

This project is currently complete and involved six countries and 14 partners.

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Nancy, France

6.7 The RiSKWa

(<http://www.bmbf.de/en/index.php>)

The funding measure, Risk Management of Emerging Compounds and Pathogens in the Water Cycle (RiSKWa), aims to develop an innovative and dynamic risk management system accompanied by on-site demonstrations in Germany. In this framework, 12 joint research projects are working on the identification of risks as well as the development of technologies and strategies to avoid or reduce the appearance of emerging compounds and pathogens in the water cycle. A main issue of the projects is the identification and classification of pollutants and pathogens relevant for the aquatic environment.

➤ For example, the research projects PRiMaT, RiMaTH and Tox-Box address the risk management of emerging compounds and pathogens focusing on drinking water supplies.

➤ PRiMaT aims at the development of a risk-based master plan for handling trace pollutants and pathogens in drinking water supplies. Risk analysis will focus on a description of sources and propagation scenarios for emerging pollutants and pathogens in water catchment areas. Therefore molecular biological methods for microorganisms as well as concepts for the characterisation of trace pollutants, nano materials and pathogens will be developed and validated.

➤ The aim of the research project RiMaTH is to develop fast, miniaturised, chip-based methods for detection, classification and activity testing of relevant microorganisms (*Legionella* und *Pseudomonas*). Therefore these chip-based molecular-biological methods will be implemented and evaluated against traditional culture techniques. A proof-of-concept study on the use of Raman spectroscopy for pathogens detection will be implemented. These methods shall enable a pathogen classification at species level but also a live/dead discrimination.

➤ The evaluation of pollutants and pathogens for the protection of the drinking water supply is a central issue of the research project Tox-Box. The

project aims at a harmonised testing strategy, laid down in a guideline, concerning an exposure-based and hazard-based risk management of anthropogenic trace substances. This approach is a key element of health-related environmental policy.

6.8 EQuATox

(http://equatox.net/equatox_project/index.html)

EQuATox intends to create a network of expert laboratories among the EU-27 and associated countries, focusing on the detection of biological toxins such as ricin, *botulinum* toxins, *staphylococcal* enterotoxins and saxitoxin. These toxins, at the interface of classical biological and chemical agents, could be used for terrorist attacks on the basis of their availability, ease of preparation, high toxicity and/or lack of medical countermeasures. Generally, proficiency tests and certified reference materials for the mentioned toxins are lacking.

Four large EU-wide proficiency tests on the mentioned toxins will be organised with 32 laboratories from 20 countries worldwide so far being interested in participating and joining the network. The task will include the generation and characterisation of toxin reference materials which in the future can be further developed into ISO-compliant certified reference materials. This project will be completed at the end of 2014.

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6.9 GEFREASE

(<http://www.agence-nationale-recherche.fr/>)

This project is funded by the French National Research Agency (ANR) and is realised in collaboration with Germany. The GERman FRENch Equipment for Analysis and Surveillance of biothreats in the Environment (Gefrease) proposes to answer the main issues associated with the detection of potential biological warfare agents.

On the basis of previous experience gained by the four project partners, an integrated diagnostic approach will be developed, combining on-site detection systems (provisional detection) based on immunoassays and confirmed and precise identification methods based on state-of-the-art mass spectrometry. Both toxins (ricin, *botulinum* toxin serotypes A, B, C, D, E, F, *staphylococcal* enterotoxin A and B, abrin) and microorganisms (*Francisella tularensis*, *Bacillus anthracis*, *Yersinia pestis*, *Vibrio cholerae* and the pox virus) are targeted. The technologies will be able to detect and identify the presence of these potential

agents in different environmental media (air, environmental and consumable waters, and drinks such as milk). Although there are various initiatives at state level there is no European accepted/established commercial technology which aims at reliably detecting a broad range of all different relevant biological agents (bacteria, viruses and toxins).

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6.10 COMBITOX

<http://www.agence-nationale-recherche.fr/en/anr-funded>

The objective of this French project is the conception of a multi-parametric instrument for continuous measurement of toxic compounds. Biosensor modules will be developed and optimised starting from technologies developed in our academic research laboratories and will be transferred to an in-line measurement device in collaboration with AP2E company. The detection is based on the reporter gene technology (for the detection of metals), on the antigen-antibody interaction (for the detection of toxins) and the specific infection of bacteria by *bacteriophage* (for pathogenic microorganisms). In each case, the signal is photochemical (fluorescence, bio-luminescence or chemi-luminescence), leading to a high sensitivity of the measure.

This ANR funded project will be completed at the end of 2015.

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In conclusion, funding of such a number of projects dealing with the biological risks in the water sector and associated detection methods demonstrates that this area is a great concern for all European countries. Some projects aim to adapt or improve existing identification technologies, but others intend to develop new rapid and sensitive methods. As the majority of these projects is not completed yet, upcoming scientific data (reports, bibliography, communications, etc.) will have to be checked carefully.

From the analysis of the partners participating in these projects, it appears that public institutes, as well as private industries, working in a very wide range of thematic (water, biotechnologies, physics, informatics, health, etc.) are invested. See Annex 1, Table 1.

7. CONCLUSIONS

7.1 The methods

Few devices can detect microorganisms in a continual manner and the ones that are available typically do not perform species identification (pathogenic vs non-pathogenic strains). Microchip-based devices using antibodies can perform the required levels of detection, but their operation is typically expensive, especially for a continual-monitoring system, although some have promise for reusability (Han JH, 2009). Detection limits for some of these automated systems (Yacoub-George E, 2007) are reported to be on the order of 10^4 CFU/mL; lower detection limits (more sensitive measurement) will be needed especially for measurement of viral contaminants having low requirements for infectivity. Antibody arrays provide detection of multiple targets at one time; however, the broad-based microbial population of drinking water makes their implementation difficult. Quantitative PCR methods have high specificity and sensitivity and require a fairly short time for detection. Unfortunately, PCR methods can be limited for environmental analyses in that very small sample volumes are applied (which may lead to non-representative testing), the polymerase enzymes can be inhibited by waterborne substances including humic acids and the DNA has to be purified for sensitive detections. Thus, particular attention has to be paid to sample preparation to overcome some of these points.

On the other hand, new sequencing approaches are promising but the challenge has now shifted from sequencing DNA to managing and understanding the extraordinary mass of data that is produced from each DNA sequence. Most sequencing centres take between 5-14 days to perform basic analysis of the raw reads to produce a list of annotated variants. So, we are witnessing a new wave of innovation about this concern.

Mass spectrometry technology is also of interest but one of its major challenges is the detection of pathogens from complex samples. Also, improvement of the detection limits for microbial cells will continue to be a major task in the years to come. To this end, cell enrichment through affinity techniques will play an increasingly important role.

Thus, nowadays, the solution for detecting waterborne pathogens does not rely on a single method, but on the combination of at least two of them.

7.2 The biosafety laboratories

There is a deficiency in biosafety laboratories at level 4 (BSL-4) in Europe. Even if a large number of European countries are equipped with BSL-3, work on highly infectious pathogens such haemorrhagic fever viruses is not possible. The EU currently has eight civil BSL-4 labs in six countries — UK, Germany, Sweden, France, Italy and Hungary — plus one in Switzerland and at least four

more are in the planning stage or under construction in the Netherlands, Italy and Germany (although the Italian facility will replace the existing one there). The issue is currently being tackled by a three-year study (due to finish in 2014) funded by the European Commission which will bring all EU BSL-4 labs into a single network and which looks set to determine the future of the European BSL-4 landscape. The ERINHA (European Research Infrastructure on Highly Pathogenic Agents) infrastructure will provide open access to state-of-the-art BSL-4 facilities for the European scientific community to enhance basic and finalised research activities (<http://www.erinha.eu>). The infrastructure will promote the harmonisation of biosafety and biosecurity procedures, will develop standards for the management of biological resources, diagnosis of group 4 pathogens and training of BSL4 labs users. Meanwhile, detection and/or identification can be performed either in BSL-3 frequently present in European states, or in BSL-1 with inactivated samples (although the inactivation process can interfere with the detection method).

7.3 Directives and standards

National and international standards or quality systems set the requirements for the reliability of measurement results.

7.3.1 International recommendations

The WHO (World Health Organisation) and EPA (Environmental Protection Agency, US) reported guidelines with the concern of microbial contaminants. However, although chemicals are precisely identified, only very little information corresponding to biological pollutants is available. See Annex 2.

7.3.2 European Directives

Existing European Directives dealing with water quality and monitoring are listed in Annex 3. Information can be obtained via the following link:

http://ec.europa.eu/environment/water/water-framework/index_en.html

One can note that although some chemical pollutants are identified, no information concerning biological contaminants is reported. Only the US EPA listed microbial contaminants (see Introduction).

However, the **4th European Water Conference** will take place in Brussels on the 23-24 March 2015. The main focus of the conference will be the implementation of the Water Framework Directive and the Floods Directive. The timing of the Conference will coincide with the public consultations of the draft

River Basin Management Plans and (some of) the draft Flood Risk Management Plans. The Conference will also feature the links to other related Directives and policies.

7.3.3 JRC-IRMM

The Institute for Reference Materials and Measurements (IRMM) is one of the seven institutes of the Joint Research Centre (JRC), a Directorate-General of the European Commission (<http://irmm.jrc.ec.europa.eu>). The mission of this institute is to support industrial competitiveness, quality of life, safety and security in the EU by developing advanced measurement standards and providing state-of-the-art scientific advice concerning measurements and standards for EU policies. The prime objective is to reinforce comparability of measurements by:

- ✓ setting up EU-wide standards for testing, routine procedures and reliable methods;
- ✓ organizing comparative tests;
- ✓ training analysts from national laboratories;
- ✓ providing scientific and technical assistance to the European Commission, especially if a Member State contest the results of analyses or trans-boundary disputes;
- ✓ coordinating a network of national reference laboratories.

One can imagine that this institute could be involved in evaluating and validating pathogen identification methods (through the production of reference tools or international comparisons) in the field of biological contaminations in the water sector.

7.3.4 European Mandate M/487

In May 2011 the European Commission launched Mandate M/487 to establish a Roadmap for Security Standards. While this analysis only concerns the Chemical, Biological, Radiological, Nuclear and Explosives (CBRNE) area, common standards could concern biological risks in water.

Mandate M/487 requested a study to analyse the current standardisation 'landscape' in the field of security standards and recommend priority sectors (Phase 1) and subsequently, after approval by the European Commission, the development of a proposed work programme (Phase 2). Relevant stakeholders participated to this work, including representatives from national authorities, standardisation bodies, academia, technology providers and integrators of systems, as well as regulatory bodies and user groups.

The following proposal was highlighted: 'To develop standard testing and evaluation (T&E) methodologies to assess the performance of CBRNE Sampling and Detection equipment'. It was estimated relevant and included in the proposed standardisation roadmap.

This topic perfectly fit with the problems of water security and waterborne pathogen identification. Similar guidelines for standardisation and certification could be considered, as some detection methods are the same.

7.3.5 National initiatives

In France, a group including industries, end-users and research laboratories, etc. has been working with AFNOR (the French agency for standardisation) in order to define guidelines in the CBRNE area to homogenise an assessment methodology for detection techniques for identifying biological pathogens. These guidelines should be published during 2014 and could be an example for the methods used in the water sector.

8. List of acronyms

Ab: antibody
ANR: National Research Agency (France)
BSL-3/4: Biosafety Laboratory levels 3/4
CBRNE: Chemical, Biological, Radiological, Nuclear and Explosive risks
CFU: colony-forming unit
DNA: Deoxyribonucleic acid
ELISA: Enzyme-Linked ImmunoSorbent Assay
ESI-MS: Electrospray Ionisation-Mass Spectrometry
EPA: Environmental Protection Agency
FCM: Flow Cytometry
FTICR: Fourier transform ion cyclotron resonance
FT-IR: Fourier transform infrared spectroscopy
GC-MS: Gas Chromatography-MS
HPC: Heterotrophic plate count
IMS: Ion mobility-MS
IPCR: Immuno-PCR
IRMM: Institute for Reference Materials and Measurements
LC-MS: Liquid Chromatography MS
LIBS: Laser-induced breakdown spectroscopy
MALDI: Matrix-Assisted Laser Desorption Ionisation
MEMS: micro-electromechanical systems
MS: Mass Spectrometry
NGS: Next-Generation Sequencing
PCR: Polymerase Chain Reaction
qPCR: quantitative PCR
RNA: Ribonucleic acid
RPA: Ratiometric Pre-rRNA Analysis
RT-qPCR: Reverse Transcription qPCR
RV-PCR: rapid-viability PCR
SEB: Staphylococcal Enterotoxin B
SELDI: Surface Enhanced Laser Desorption Ionisation
SPR: Surface Plasmon Resonance
SNP: Single-Nucleotide Polymorphism
SWNTs: Single-Walled carbon NanoTubes

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10. ANNEX 1: FIGURES

Figure 1: Microcystin (MC) detection by immunochromatographic assay optimised with immunoliposomes

Related to Part 5.1, Immuno-detection approaches

Source: Khreich N, 2010

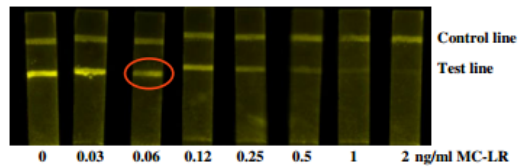


Figure 2: Schematic diagram of the concept of the 3D μ PAD.

Related to Part 5.1, Immuno-detection approaches

Source: Zang D, 2012

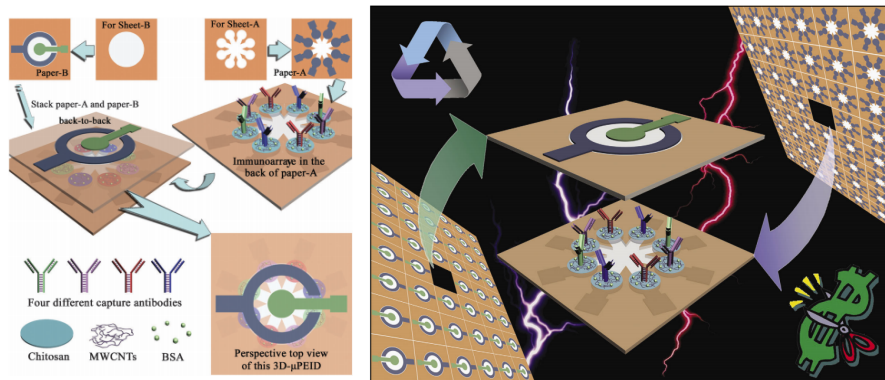


Figure 3: GeneXpert® XVI (Cepheid). Fully integrated and automated on-demand molecular diagnostic system (US).
 Related to Part 5.2, Genetic methods
 Source: www.cepheid.com



Figure 4: 'All in One' Pathogen Detection System (CEA/DRT/Leti, France).
 Related to Part 5.2, Genetic methods
 Source: <http://www-leti.cea.fr>

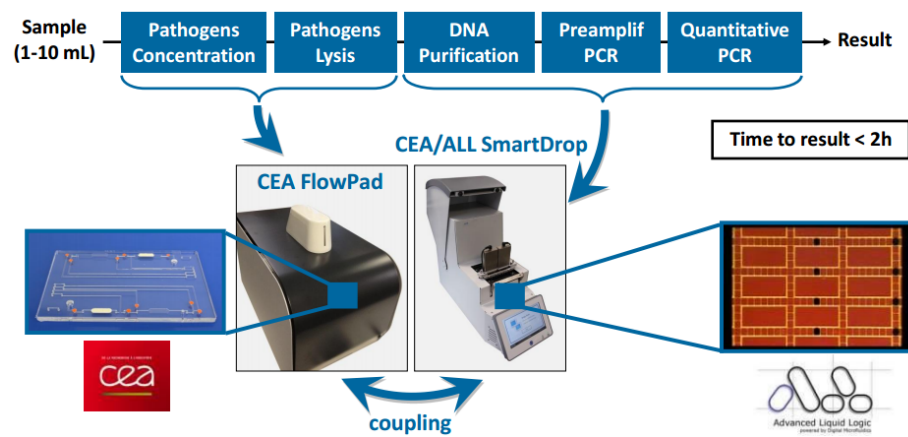


Figure 5: LabDisk and LabDisk Player prototypes, Germany
 Related to Part 5.2, Genetic methods
 Source: www.loac-hsg-imit.de



Table 1: List of main public institutes and private industries invested in the water security thematic (including water, biotechnologies, physics, informatics, health, etc. ...).

Related to Part 6, European projects for pathogen (or toxin) identification

Austria	AGES	Detection of highly infectious pathogens	Alexander INDRA: Alexander.Indra@ages.at http://www.ages.at/
	mbOnline GmbH mbOnline	Develop, produce, market and sell novel devices for microbial online-monitoring	http://www.mbonline.at/ Thomas Lendenfeld
	Medical University Vienna (MUW)	Detection of pathogenic microorganisms based on epifluorescence microscopy and solid phase cytometry	Alexander K.T. Kirschner: alexander.kirschner@meduniwien.ac.at http://www.meduniwien.ac.at/
Denmark	Nordvand A /S (NV)	Water supply and wastewater management in north Copenhagen	Bo Lindhardt: nordvand@nordvand.dk http://www.nordvand.dk/
France	Ceeram	Detection of emerging microbial agents (viruses, bacteria, parasites and fungi) in food production and process, environment, public health, animal health, pharmaceutical and cosmetic industries.	Fabienne Loisy-Hamon: fabienne.loisy@ceeram.com http://www.ceeram.com/
	SUEZ Environnement	Molecular detection of waterborne pathogens (generic concentration, micro-array technology, real-time PCR)	Sophie Courtois: sophie.courtois@suez-env.com http://www.suez-environnement.com/
	Veolia Environnement Recherche et Innovation	Management of water and wastewater services for municipal and industrial clients — design/build of technological solutions and facilities necessary to deliver water and wastewater services	Karim Helmi: karim.helmi@veolia.com http://www.veolia.com/fr/innovation/recherche-innovation/
Germany	DVGW Technologiezentrum Wasser Karlsruhe	Contact point for authorities, ministries and associations in all questions concerning surface and ground water, drinking water and water technology and provides a link between with basic research at the universities and the water utilities	Beate Hamsch: beate.hamsch@tzw.de http://www.dvgw.de/
	IWW	Chemical and microbiological water quality — biofilm-related problems in drinking water and process water	Hans-Curt Flemming: HansCurtFlemming@Compuserve.com http://www.iww-online.de/index.php/en/

	Ribocon GmbH (Ribocon)	Bioinformatics services and solutions provider for Microbiology, serving Academia and Industry	Jörg Peplies: contact@ribocon.com http://www.ribocon.com/
	SCIENION AG	Ultra-low volume liquid handling and microarray technologies. Improvement of multiparallel bioanalytics, high-throughput screening and high-throughput production of microarrays in the genomics and proteomics field	Wilfried Weigel: weigel@scienion.de http://www.scienion.de/
Ireland	City Analysts Ltd	Leading environmental analysis company- analytical services in the expanding areas of parasitology and biomonitoring	Miriam Byrne: miriamb@cityanalysts.ie http://www.cityanalysts.ie/
Italy	Institute for health and Consumers protection JRC	NanoBiotechnology Laboratory: bio interface Engineering, Nanotoxicology and molecular detection applied in the field of life science	francois.rossi@jrc.it http://ihcp.jrc.ec.europa.eu/our_labs/nanob_lab
The Netherlands	LioniX	Co-development of products and manufacturing of components based on cutting-edge micro/nano technology for its (OEM) customers. The main focus markets include Life Sciences, Telecom, Datacom, Industrial Process Control and Space	René Heideman info@lionixbv.nl
Spain	Genetic PCR Solutions (GPS)	Development of comprehensive validated procedures to detect and identify microorganisms by using fast and reliable genetic technologies	Antonio Martínez Murcia: info@geneticpcr.com www.geneticpcr.com
	University of Barcelona	Molecular detection of microorganisms without cultivation	Albert Bosch: abosch@ub.edu http://www.ub.edu/microbiologia/viruse/index.htm
UK	Heriot-Watt University	Microsystems, rapid prototyping, bio-MEMS, advanced manufacturing technologies and systems integration	Helen Bridle: h.l.bridle@hw.ac.uk http://www.hw.ac.uk/
	University of Southampton	Development of new technologies to detect low levels of contaminants, assessment and improvement of existing decontamination protocols and the use of sensors for surveillance/ biofilms	Bill Keevil: cwk@soton.ac.uk http://www.southampton.ac.uk/biosci

11. ANNEX 2: STANDARDS, GUIDELINES

WHO (World Health Organisation):

Guidelines for drinking-water quality — Volume 1: Recommendations

Third edition, incorporating first and second addend (2008)/Chapter 7: Microbial aspects.

ISBN: 978 92 4 154761 1

http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/

EPA (Environmental Protection Agency):

National Primary Drinking Water Regulations (NPDWRs or primary standards) are legally enforceable standards that apply to public water systems. Primary standards protect public health by limiting the levels of contaminants in drinking water.

EPA 816-F-09-0004 (May 2004): Drinking Water Contaminants/List of Contaminants and their (MCLs)

<http://water.epa.gov/drink/contaminants/index.cfm#Microorganisms>

12. ANNEX 3: EU/EC DIRECTIVES

- **Directive 2000/60/EC** of the European Parliament and of the Council establishes a framework for the Community action in the field of water policy (or EU Water Framework Directive, WFD).
Official Journal L 327, 22/12/2000 p. 0001-0073

- **Directive 2008/105/EC** is a directive on Environmental Quality Standards. The EQSD establishes limits on concentrations of the priority substances in surface waters of 33 priority chemical substances and eight other pollutants (Annex I).

- **Directive 98/83/EC** is the Drinking Water Directive on the quality of water intended for human consumption. Its objective is to protect human health from the adverse effects of any contamination of water intended for human consumption by ensuring that it is wholesome and clean. The Drinking Water Directive laid out minimum requirements as regards the monitoring programmes in its Annexes II (Monitoring) and III (Specifications for the analysis of parameters).

European Commission

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Abstract

Microbiological water contaminants represent an acute health risk in drinking water. There are a wide variety of bacteria and viruses that can potentially be found in drinking water resulting from either an attack or a natural contamination incident. Whatever the origin of the contamination, a rapid identification is needed to ensure water quality and subsequent citizens security. Currently, various detection and identification methods exist, but they are mostly time-consuming and unsuited to emergent harmful microorganisms. New developments are being performed in order to deal with this concern. In this desk study, the main basic technologies to identify pathogens (such as immunological and genetic methods as well as mass spectrometry, microarrays and physical approaches) are reported as well as their applications in the drinking water area. Then, some promising technologies under development are presented, especially integrated tool or new concepts in mass spectrometry. However, bottlenecks still exist such as sample preparation or live and dead pathogens discrimination. Alternatively, different projects founded by the European Commission are briefly reported in this study, as they allow a clear vision of scientific teams and networks working on this concern. Finally, European standards are being established as well as national initiatives that currently remain unofficial.

As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle. Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new methods, tools and standards, and sharing its know-how with the Member States, the scientific community and international partners.

