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# Rapid Detection Technologies for Monitoring Microorganisms in Water

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## Abstract

The evaluation of microbial water quality in drinking water is necessary to protect consumers from water-borne or water-based illnesses caused by pathogens, such as bacteria, viruses, and protozoa. In the past, microbial water quality was determined through the use of indicator organisms, whose presence indicated the potential incidence of pathogenic microorganisms in water. However, there has been a great debate among scientists, engineers, public health officials and water utilities regarding the use of indicators to determine microbial water quality. In addition, outbreaks due to contamination of drinking water have occurred regardless of the presence or absence of indicator organisms. However, consumers still demand safe drinking water that meets health quality standards, and aesthetic aspects such as color, turbidity, taste and odor. As a result, most water utilities have developed quality management and on-line monitoring systems because of i) lower costs; ii) real-time monitoring (not require laboratory measurements); and iii) recent security concerns against bioterrorism. On-line monitoring sensors are installed as early warning systems for monitoring treated water quality and microbial contamination in the distribution systems. In this paper, we review strategies for rapid detection of microorganisms in water. These technologies allow improveddetection sensitivity, and also provide important early warning data to decision-makers to protect public health.

**Keywords:** Online monitoring; Biosensors; Drinking water; Water quality

## Introduction

Microbial contamination of drinking water is a major issue worldwide because it is still a major source of water-borne or water-based ill-nesses in developing as well as developed countries, and can cause mortality as illustrated by recorded outbreaks. In the United States, a total of 833 waterborne disease outbreaks (WBDOs), 577,991 cases of illness, and 106 deaths were reported from 1971 to 2006. During the 36-year period, a total of 854 deficiencies were identified in the 833 WBDOs, 97.8% with single deficiencies, 2.2% with two or more deficiencies and 38 outbreaks with unknown deficiencies [1]. From 2003 through 2004, 36 WBDOs associated with drinking water in the U.S. were caused by microbial pathogens including protozoa, viruses, and bacteria, resulting in 2,760 reported cases of illness and 4 deaths. Approximately 42% of those were due to source water contamination, treatment inadequacies, or microbial intrusion in the municipal distribution system (DS) [2]. During 2007-2008, 36 drinking water--associated outbreaks caused illness among at least 4,128 persons and were linked to three deaths. In the last decade, the overall number of reported outbreaks associated with community water systems has decreased; however, the number of outbreaks associated with DSs has increased [3]. This trend is partly due to the rapid deterioration of DS pipes, because DS networks in the U.S. are aging and most of them have reached and even exceeded their estimated life span limit [4,5].

Detection of pathogenic bacteria in drinking water is an important issue for water utilities because they pose critical impact on public health.The annual number of endemic acute gastrointestinal illness cases associated with consumption of public drinking water in the U.S. has been estimated to range from 4.3 to 11.7 million cases [6] and from 5.5 to 32.8 million cases [7]. Although traditional microbiological methods, such as plate counting and cell culture, are the gold standards to confirm the presence of pathogens, it often takes 24-48 hours to obtain the results [8,9]. For cell culture detection of viruses, cytopathogenic effect may require 7-10 days to occur [10]. In addition, water distribution systems are highly vulnerable to contamination and reliability of supply as a result of many factors including natural, accidental, and intentional intrusion events. Rapid recognition of such intrusion events is vital to protect the integrity of the water supply, safeguard consumers from potentially pathogenic microbial contaminants, and ensure compliance with environmental regulations. Thus, both private and public sectors are strongly trending towards online monitoring using a biosensor that can detect pathogens rapidly and precisely [11]. Therefore, to ensure the public health, an effective sensor-based monitoring and management system is required to detect potential water borne pathogens in the water supply including source water, treatment process and distribution system. Table 1 provides potential waterborne pathogens which have significances of human health effect, persistence in drinking water distribution.

New software and real time instrumentation and monitoring systems provide the tools that allows for the design and developmentof early warning systems for microbial contaminants [12]. Proper integration of hydraulic modeling systems, online monitoring sensors for the water distribution network, and the installation of a supervisory control and data acquisition (SCADA) system for both water treatment as well as the monitoring of critical points within the distribution system are an invaluable resource that allow water utilities to overcome accidental or intentional contamination [13]. Furthermore, after September 11, 2001, research accelerated in an effort to determine if conventional water quality sensors could be deployed for extended periods of time, and if water quality parameters would change in response to chemical and biological contamination. Research using online water quality monitors to detect contamination events has

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| Pathogens | Diseases |
|---|---|
| **Viruses** | |
| *Enteroviruses* | |
| *Poliovirus* | Paralysis, aseptic meningitis |
| *Norwalk* | Gastroenteritis |
| *Hepatitis A* | Infectious hepatitis |
| *Adenovirus* | Respiratory disease, eye infections, diarrhoea |
| *Rotavirus* | Gastroenteritis |
| *Parvovirus* | Gastroenteritis |
| *Reovirus* | Respiratory disease, gastroenteritis |
| *Astrovirus* | Gastroenteritis |
| *Calicivirus* | Gastroenteritis |
| *Coronavirus* | Gastroenteritis |
| **Bacteria** | |
| *Shigella* | Dysentery |
| *Salmonella* | Typhoid fever, Paratyphoid fever, gastroenteritis |
| *Vibrio cholera* | Cholera |
| *Escherichia coli (E. coli)* | Gastroenteritis |
| *E.coli 0157:H7* | Gastroenteritis |
| *Yersina enterocolitica* | Diarrhoea and septicaemia |
| *Legionella pneumophila* | Legionnaire's disease, Pontiac fever |
| *Campylobacter jejuni* | Gastroenteritis |
| *Pseudomonas aeruginosa* | Skin infections, dermatitis |
| *Helicobacter pylori* | Abdominal pain, Peptic ulcers, Gastric cancer |
| *Mycobacterium* | Pneumonia, gastrointestinal illness |
| **Protozoa** | |
| *Entamoeba histolytica* | Amoebic dysentery, liver abscess, colonic ulceration |
| *Giardia lamblia* | Giardiasis |
| *Balantidium coli* | Dysentery, diarrhoea, colonic ulceration |
| *Cryptosporidumparvum* | Cryptosporidiosis, diarrhoea, fever |
| *Naegleria Fowleri* | Primary amoebic meningo encephalitis |
| *Entamoeba coli* | Mild diarrhoea, colonic ulceration |

**Table 1:** Waterborne pathogens in water and associated diseases.

been performed and sponsored primarily by manufacturers of water quality monitors as described in Table 2. The goal of an early warning monitoring system is to reliably identify low probability and high impact contamination events (chemical or microbial) in source water or distribution systems rapidly to allow for an effective local response that reduces or avoids entirely the adverse impacts. This requires real-time detection and decision making [14]. Some of the advantages of an ideal early warning system over traditional assays would include detection in sufficient time for action and minimal false positive or negative results Table 3. In addition, the ideal sensor should be robust, reproducible, verifiable, and durable.

To date, there are a limited number of studies that have evaluated the use of commercial water quality sensors for real-time monitoring in DSs [15-21]. Sensors monitoring the DS have mostly focused on chemical contaminants or basic non-specific water quality parameters. Meanwhile, there are only a few studies on sensors that detect microorganisms in real-time. Byer and Carlson [22] performed a study evaluating the impact of arsenic, cyanide, and two pesticides on water quality parameters such as residual chlorine, turbidity, pH, conductivity, and total organic carbon (TOC). Their results showed that cyanide had a measurable influence on all the sensors, whereas arsenic's effect was primarily on sensors measuring electrical conductivity and turbidity. Overall, this study demonstrated that sensors monitoring several general water quality parameters could detect contamination events in a DS [23]. Hall et al. [24] evaluated six single parameter sensors and three multi-parameter sensors that measured free chlorine, turbidity, pH, specific conductivity, TOC, oxidation reduction potential (ORP), chloride, ammonia, and nitrate. This study assessed the response to contaminants qualitatively using non-chlorinated secondary wastewater effluent. The sensors were challenged with potassium ferricyanide, a pesticide formulation, an herbicide formulation, arsenic trioxide, and nicotine, as model chemical contaminants. *E. coli* K-12 strain in growth media was also tested as a model microbial contaminant. Results showed that no single sensor was able to respond to all the contaminants tested, although the specific conductivity, TOC, free chlorine, chloride, and the ORP sensors did respond to a large number of contaminants [25].

The U.S. EPA's National Exposure Research Laboratory has developed several new detection methods for measuring microorganisms in water (www.epa.gov/nerlcwww) that overcome most of the disadvantages associated with conventional techniques in terms of sensitivity, specificity, and required time for detection. Traditional methods used for the detection of microorganisms include cell culture, immunological methods, polymerase chain reaction (PCR), and microscopic identification. Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry are newer methods that are also now being accepted [26]. Many commercial companies have developed either single or multi-parameter in-line sensors that measure water quality parameters (e.g., YSI 6920DW & 600DW-B, Hydraclam, Censar, Intellisonde™, etc.). Sensors that detect and specifically identify microorganisms in a large scale application can be difficult, and thus there are a limited number of biological sensor technologies available for real time detection, such as immunoassays, detection of bacterial adenosine triphosphate (ATP), flow cytometry or micro-flow based technology, and multi-angle light scattering technology. Additionally, there are some emerging sensor technologies that can detect biological activity in water, such as the lateral flow assay, labels, magnetic beads, flow-through columns, Raman spectroscopy, microelectrode arrays, DNA microarrays, and photo luminescent biochips [27].

## Technologies to Detect Microbial Contaminants

The challenge in managing urban water systems is to detect and identify any potential pathogen in the presence of many other non-pathogenic microbes. Concentration techniques to increase microbial density to detectable level are essential for sensitive detection and identification. Sample pretreatment to improve recovery rate and minimize the time for concentration is still a major challenge [28]. Laboratory as well as on-site detection methods have been developed and improved for the identification and quantification of a wide range of microorganisms (i.e., viruses, bacteria, protozoa) in water samples, but not in real-time. Characteristics of various technologies with advantages and disadvantages are shown in Tables 3 and 5.

Examples of these technologies used are described briefly in the following sections:

### Physical detection principles

There is an increasing interest in utilizing the physical characteristics of microorganisms as a means to detect them [10-17]. This area of research is still developing, and the methodologies include turbidity measurement, vibrational spectroscopy, and multi-angle light scattering technologies.

**Turbidity:** Turbidity is caused by suspended particles or impurities that interfere with the clarity of the water. Increased turbidity level may indicate anomaly of water quality, and turbidity measuring is

| Sensor | Approximate Purchase Price(US $) | Target | Description |
|---|---|---|---|
| JMAR BioSentry™ Technology: Light Scattering (JMAR, 10905 Technology Place, San Diego, CA 92127) | 40,000 | Bacteria | The Multi-Angle Light Scattering (MALS) technologies use laser beams to strike individual cells or particles in water within a distribution system, resulting in unique light scattering patterns. Such patterns depend on the morphological characteristics of the target particle. Comparisons of obtained patterns with a computerized database of patterns from known pathogens allows for continuous real-time monitoring and classification of microbial contaminants. |
| SCAN spectro lyser Technology: Light Scattering (scan Measuring Systems LLC, P.O. Box 36402, Cincinnati, OH 45236) | 25,000 | TOC, DOC | The SCAN uses UV-spectroscopy to generate a broadband picture of overall water quality. The assumption is that any new contaminant in the water will be detected as a deviation from the baseline or reference signal. The reference signal is normally generated from historical samples that allow for the system to be trained to a specific type of water. This training is essential for real-time monitoring to reduce the incidence of false alarms. |
| HACH Monitoring Platforms: Non-Specific Sensors (HACH, P.O. Box 389, Loveland, CO 80539) | 80,000 | pH, TOC, chlorine, turbidity, electrical conductivity | This unit utilizes in-line sensors that would likely change following a change in water quality that result from a chemical or microbial intrusion. The parameters include pH, total organic carbon (TOC), free chlorine, turbidity, and electrical conductivity.TOC is measured with a non-dispersive infrared (NDIR) method by adding 0.6 M phosphoric acid and 0.6 M sodium per sulfate to the water sample to produce TOC. Subsequently, the TOC is oxidized by UV light to convert it to $CO_2$. This gas/liquid mixture is separated and the gas read by an NDIR detector. The output is directly proportional to the original TOC in the sample. |
| Real Tech Real UVT (1375 Hopkins Street Whitby ON L1N 2C2 Canada) | 5,000 | UV 254 | The Real UVT Online monitor is a continuous UV 254nm testing monitor. The UV 254 wavelength provides an estimate of organic content in test water. The instrument measures UV transmittance referenced to a test water sample. The UVT online monitor uses two different path lengths to overcome this parameter's typical problems with lamp drift or flow-cell fouling. |
| GE 5310 Online Total Organic Carbon (TOC) unit (GE Analytical Instruments, 6060 Spine Rd., Boulder, CO 80301) | 25,000 | TOC | The GE TOC unit is a single parameter sensor that measures TOC with selective membrane conductimetric technology. This process separates organic molecules into CI, $CO_2$, and $SO_4$ by an ultraviolet light reactor. These molecules pass into a $CO_2$ transfer module containing a membrane that only allows $CO_2$ to pass through. The $CO_2$ can then be further separated into $H^+$ and $HCO_3$. Thereafter the TOC is measured as it accumulates in the conductivity cell. |

**Table 2:** Commercial Sensor Characteristics.

| Characteristics of Test Method | Online sensors | Traditional Microbiology | Molecular Techniques (PCR) | ATP Luminescence | Immunoassays |
|---|---|---|---|---|---|
| Sample Type | Continuous flow | Grab sample | Grab sample | Grab sample | Grab sample |
| Assay time | Minutes | Hours to days | Hours | Minutes | Hours |
| Performance Approach | Full Automation | Manual | Semi-automated | Semi-automated | Semi-automated |
| Remote Operation | √ | × | × | × | × |
| Automated Notification | √ | × | × | × | × |
| Customized Thresholds | √ | × | × | × | × |
| SCADA compatible | √ | × | × | × | × |
| Consumable/ Reagent costs | Low Cost | Low Cost | High Cost | Low Cost | High Cost |

**Table 3:** Comparison of Detection Systems for Microbial Pathogens in Water.

| Signal transduction method | Recognition element | Nanoparticle identity | Organisms | Detection limit | References |
|---|---|---|---|---|---|
| Electrochemical | Antibody<br>Antibody | Gold nanoparticle<br>Magnetic nanoparticle | *S.typhi*<br>*Adenovirus* | 98.9cfu/ml (PBS)<br>$10^3$pfu/ml(PBS) | [62]<br>[63] |
| fluorescence | Antibody<br>Antibody<br>Antibody | Quantum dot<br>Quantum dot<br>Quantum dot | *S.typhi*<br>*E.coli* O157:H7<br>*C.parvum, G. lamblia* | $10^3$ cells/ml<br>$10^6$ cells/ml(PBS)<br>Not reported | [64]<br>[65]<br>[66] |
| magnetic | Antibody | Magnetic nanoparticle | *Mycobacterum avium spp. Paratuberculosis* | 15.5 cfu/ml | [67] |
| Surface plasmon resonance | Antibody<br>Antibody | Gold nanorod<br>Silver nanoshell | *E.coli* O157:H7<br>*E.coli* | 1-10 cfu/ml(PBS)<br>3-5 cells (water) | [68]<br>[69] |
| Surface enhanced Raman | Antibody<br>Antibody<br>Antibody | Gold nanoparticle<br>Gold nanoparticle<br>Silver nanoparticle | *Feline calicivirus*<br>*Cryptosporidium parvum*<br>*E.coli* | $10^6$ pfu/ml<br>Not reported<br>Not reported | [70]<br>[71]<br>[72] |

**Table 4:** Nano material enabled sensor applications.

sometimes used to monitor microbial contamination; in fact, the Japanese Potable Water Quality Standard specifies turbidity level of 0.1 mg/L (kaolin turbidity standard) in finished drinking wateras the standard turbidity value to prevent *Cryptosporidium* contamination. Turbid meter technology is currently used by numerous water utilities to monitor water quality. Companies such as HACH and SCAN have developed water quality monitors which can measure physical parameters such as turbidity (Table 2). Real-time monitoring of the turbidity coupled to the use of "intelligent interpretive algorithms" can be used to establish baseline conditions for acceptable water quality, where, changes in these baseline conditions are indicative of altered water quality.

| Technique | Advantages | Disadvantages |
|---|---|---|
| Nucleic-acid based methods | o<br>• High sensitivity, qPCR provides quantitative information | o<br>• Labor intensive and time-consuming<br>• Need to extract nucleic acids from samples<br>• Cannot distinguish viable and non-viable cells<br>• Sample matrix inhibition<br>• Not real-time, takes more than 2 hours |
| Biosensors | • Low sample and reagents volume<br>• High sensitivity<br>• High assay detectability and selectivity<br>• Low-cost and reduced assay times | • Complex instrumentation and high capital cost<br>• Near real-time |
| Immunoassays | • High specificity<br>• Recombinant antibodies can be used<br>• Some assays are applicable to on-site measurements | • Low sensitivity<br>• Specificity and sensitivity are largely dependent on the quality of antibodies<br>• Long assay times<br>• Viable and non-viable cells are not distinguished<br>• Antibodies might be unstable in complex sample matrices |
| Vibrational Spectroscopy | • Low reagents cost<br>• Ability to distinguish between viable and non-viable cells | • Matrix interference while measurement<br>• High cost instrumentation<br>• Not applicable in point-of-use settings<br>• Spectra changes with metabolic state of microbe |
| MALDI/TOF mass spectrometry | • Relatively simple instrumentation<br>• Based on proteomic analysis | • High cost instrumentation<br>• Near real-time<br>• Sample matrix interference may lead to ionization suppression phenomena |
| Adenosine Tri-phosphate assay | • Measures all living organisms<br>• Results can be obtained within minutes<br>• User-friendly and affordable tests | • Cannot distinguish viable and non-viable cells<br>• Expensive than heterotrophic plate counts<br>• Within minutes |

**Table 5:** Advantages and Disadvantages of different detection techniques.

**Vibrational spectroscopy:** This technology involves interpreting the spectra that are emitted from transitions between vibrational levels of a molecule following excitation by laser light. Molecules such as nucleic acids, cytoplasmic proteins, membrane lipids or cell wall components are the building blocks of micro-organisms, and their exact composition and distribution is unique for each organism. Vibrational spectroscopy is a non-invasive and reagent-free method. It has been successfully applied to identify, differentiate and classify pathogenic microorganisms based on their unique spectroscopic signatures [29-31]. The following sections briefly describe two methods:

(a) Raman spectroscopy

The Raman effect is defined as light excitation due to elasticity of scattered light, and Raman spectroscopy utilizes laser wavelengths ranging from ultra-violet through visible to near infra-red. It has recently been applied to two technologies for microbial detection: (1) surface enhanced Raman-spectroscopy (SERS) and (2) optical tweezer. The SERS identifies micro-organisms from the spectra produced due to the surface of the organism following reaction with antibodies. The combination of antibody and Raman spectroscopy increases the specificity of the identification Since all molecules have their unique Raman spectroscopic signatures, the reservoir for SERS labels is greater than that of fluorescent labels. The optical tweezer is used to attach to a micro-organism, followed by irradiation of laser light that produces a Raman spectrum [32]. Using this technique, the discrimination between different strains of bacteria (*Bacillus cereus,Enterobacteraerogenes, Escherichia coli, Streptococcus pyrogenes, Enterococcus faecalisandStreptococcussalivarius*) [33] and the germination of a single *Bacillus* spore [34] have been reported.

(b) Fourier transform infrared spectroscopy (FT-IR spectroscopy) The mid-IR region covers the wavelength range of 4000 to 400 cm. The basic principle of this IR technique is that various organic functional groups absorb infrared light at specific wavelengths. Thus, since every organic molecule has a unique chemical structure, it also has a unique infrared spectrum. Biological samples are mainly composed of proteins, carbohydrates, lipids and nucleic acids. Since these molecules contain different functional organic groups, the IR spectrum produced consists of bands from each of these components.

Infrared spectra are very complex and contain large amounts of information. To evaluate the data requires multivariate statistical analysis; Yu et al. [20] reported the identification of eight different micro-organisms in an apple juice matrix based on FT-IR spectroscopy.

A general drawback of vibrational spectroscopy is that the molecular composition of a micro-organism depends on metabolic and environmental factors. Thus potentially any microbe can have multiple spectra. These technologies can only be developed further in the future if spectrum deviations caused by metabolic or environmental factors are less than the spectral deviations between strains. However, the ability to distinguish viable from non-viable cells is of great importance in evaluating microbial water safety. Conventional cultural microbiological analysis is confounded when the water is contaminated with viable but non culturable (VBNC) microorganisms [35] and so, rapid spectroscopic screening of drinking water for the presence of pathogenic microorganisms has the potential to become a powerful tool for determining microbial water safety and public health security.

**Multi-angle light scattering (MALS) technology:** MALS is a variation of turbidity measurements but instead of one light source, several light sources and angles of refraction are used. With proprietary algorithms, the shape, size, refraction index and internal structure of a particle can be deduced from the light scattering patterns. With this technique, microorganisms can be accurately identified.

MALS identification of micro-organisms is less reliable than identification by vibrational spectroscopy. However, MALS technology is beginning to mature and commercial equipment is available in the market such as BioSentry®, which is an in-line sensor that allows for continuous real-time monitoring of microbial contaminants Table 2. The sensor contains a laser beam that strikes individual cells or particles

in water, resulting in unique light scattering patterns. Such patterns depend on the size and morphological characteristics of the target particles. Data obtained are compared to patterns with a computerized database of patterns from known pathogens, which are then placed into 4 identifying categories: rods, spores, protozoa, and unknown. Based on Sherchan et al., [36] and USEPA [25] evaluation results, sensitivity and threshold levels of these devices need to be further improved before implementing into a SCADA system in a large-scale water quality monitoring program. However, BioSentry® can also be utilized as a real-time trigger that informs the operator that the water quality is degrading, and that the situation warrants investigation. This is the case when microbial counts in the water increase rapidly.

## Biosensors

This sensor group uses biological components, such as a protein (antibody, enzyme, receptor or DNA), other cell components, or the whole cell or organism. Rodriguez-Mozaz et al., [37] suggested that new biosensors must be evaluated with environmental samples since there may be problems with selectivity and sensitivity in these real-world samples. The function of a pathogenic biosensor is to transduce receptor recognition towards the target pathogen into a detectable signal [38]. Biosensors can be subdivided into two classes based on the type of biorecognition molecule; catalytic or affinity biosensors. In catalytic biosensors, these biomolecules catalyze a reaction to give a product for further quantification. And in affinity biosensors, these biomolecules bind a target analyte with high affinity without any further reaction.

Pathogenic sensing relies on either immunosensing or nucleic acid detection. Immunosensors are based on the interaction between antigens presented on the target cells and antibodies immobilized on surfaces [39-41]. The resulting conjugates have been detected via various sensing methods [42-45], including fluorescence, electrical or electrochemical impedance [46], cantilever [47], quartz crystalline microbalance (QCM) [48], or surface plasmon resonance (SPR) [49]. SPR is one of the most popular optical transduction techniques using light reflection characteristics. This is sensor chip occurring biological binding reaction. The reflected light is dependent on the mass of material at the surface. This change in SPR angle means mass change, and we're able to monitor the analyte concentration in this plot of resonance signal versus time.There are also electrochemical transduction techniques which measure the electron transfers by potentiometric, amperometric, and conductometric methods. QCM is a kind of piezoelectric sensor. Because Piezo is Greek meaning to squeeze or press, piezoelectricity means electricity resulting from pressure. In QCM, changes in mass on the quartz sensor surface are related to changes in frequency of the oscillating crystal. Another popular piezoelectric sensor is using cantilever like as AFM. In bending-mode, the cantilever deflects as surface stress changes, while in resonant-mode resonant frequency decreases when target of interest attaches [50].

Moreover, biosensor performance is evaluated a show sensitively detect, how selectively or specifically distinguish the target, and how low, how wide detectable with linear relationship and accurate and precise data. And rapid response time, stability, and life span of sensor are important parameters to evaluate sensor performance [51].

In today's world, the genetically engineered whole cell-based bacteria have usually utilized to generate and amplify the luminescence signal. Bacteria incorporate reporter genes that code for signaling elements that emit bioluminescent, fluorescent, or colorimetric endpoints. There are two types of luminescence whole cell-based biosensors; light off or light on mode. In light off mode, toxic compounds resulted in turning off the luminescence signal by inhibition of normal activity. In light on mode, target compounds activate the reporter gene and turn on the generation of luminescent materials [52].

And there is another approach for whole cell-based electrochemical biosensor using screen printed electrode. Microorganisms are immobilized with carbon nanotube and alginate to form a very thin biofilm on the surface of electrode by screen printing technique. The thin biological coating gives very rapid response time. In this example, green algae produce oxygen by photosynthesis, and consequently generate the electrochemical signal as current. But, in the presence of toxic chemicals such as herbicide, the current rapidly decrease [53].

In contrast, nucleic acid-based sensors detect DNA or RNA originating from target cells [54]. Because cells contain a low copy number of nucleic acids, the sensor generally requires the step of amplifying target nucleic acids using polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR). In addition, there are several intricate strategies for amplifying signals that report the hybridization between probe and target DNA [54-58]. Pathogen sensors based on nucleic acid detection includes several steps comprising lysis, extraction of nucleic acids, purification, and detection. While a lab on-a-chip sensor can be an attractive platform for real-time sensing, it has been challenging to integrate PCR with other required steps for this technology [59-61]. In addition, these technologies require at least 2 hours to obtain the results and are therefore not real-time.

Micro- and nano-scale sensors are suitable for detecting waterborne pathogens, and common nano-scale materials such as carbon nanotubes and quantum dots are now extensively applied for quantitative detection of microorganisms including bacteria and protozoa [62-66]. Vikesland and Wigginton [67] recently provided a review on nanomaterial-enabled biosensors. Only a few studies have been successful using nanomaterial enabled bioassays to detect waterborne pathogens. There are still problems, such as nonspecific binding, particle size variation, nanoparticle aggregation, nanoparticle stability and most importantly, these techniques do not differentiate viable from nonviable cells or organisms in VBNC state. In another review, the pros and cons of biosensors for detection of pathogens in water. They summarized that while DNA biosensors and immunosensors have the potential to reduce sampling time and sensitivity of detection, there are still other issues including specificity and sensitivity with these techniques.

## Molecular Biological Methods

Molecular biological methods are based on recognition of specific nucleotide (DNA or RNA) sequences of microorganisms. The major advantages of the molecular methods are high sensitivity and specificity, as well as relatively rapid response although it is still not rapid enough to be implemented in real-time microbial detection. Currently, quantitative PCR (qPCR) is widely available, low cost, and provides as good as or better sensitivity for the detection of molecular targets than all other currently available detection strategies, including newly developed assays such as microarrays and pyrosequencing [68-72]. However, there are still issues including the inability to differentiate viable and non-viable organisms, the presence of target microorganisms at low numbers in the environment, and the presence of PCR-inhibitory substances [73]. These assays need to be improved since they overestimate the viable pathogen numbers in disinfected water [74].

To overcome the problems associated with conventional cell culture and PCR assays, Reynolds et al. [75] developed a new method combining cell culture with PCR (ICC-PCR). Rodriquez et al. [37] discussed the pros and cons of this ICC-PCR approach. Advantages include reduced time needed for detection of infectious viruses in environmental samples, and detection via this technique implies that the virus were infective since growth in cell culture prior to PCR is a prerequisite. Similarly, the use of propidiummonoazide (PMA) in conjunction with end-point PCR and denaturing gradient gel electrophoresis (DGGE) can also discriminate between live and dead cells (94-96). The development of protein nucleic acids (PNA) and nucleic acid sequence based amplification (NASBA) assays increases the possibilities for rapid detection of microorganisms in water samples [76].

A real-time PCR assay was developed by Yanez et al. [21] for rapid detection of *Helicobacter pylori* in water. This method was based on the amplification of a fragment of a gene specific to *H. pylori* and the use of an external standard for quantification. However, Janzon et al. [29] failed to detect *H. pylori* in drinking and environmental water samples using highly sensitive qPCR assays. Recently, the needto further reduce the PCR run time led to the development of "fast PCR" chemistry using a fast enzyme, such as the *Thermusaquaticus* (*Taq*) polymerase engineered for fasteramplification capability and/orquicker reactivation, which reduces the total reaction time up to 50%. Additionally, multiplexing qPCR reactions may further reduce the cost and time required per sample.

## ATP Measurements

Adenosine triphosphate (ATP) is the energy transporter of living organisms and as soon as an organism dies ATP concentrations rapidly decrease. The concentration of ATP in micro-organisms depends on species, strain, metabolic activity and environmental factors. The determination of ATP using a bioluminescence assay is based on a reaction between the enzyme luciferase, the substrate luciferin and ATP. Light is emitted during this reaction and can be measured quantitatively to estimate the amount of bacteria present in a water sample [77]. ATP measurements are rapid, and on-site analyses are available within a couple of minutes. However, disadvantages of ATP measurements include the cost and the ambiguous relation between the measured signal and the numbers and activity of the organisms present. The enzymes required for the analysis are very expensive. Separation of cell-bound ATP from free ATP and removal of any eukaryotic cells would provide a more reliable estimate of bacterial numbers, particularly if this was an automated process. This may yield a powerful tool in the future, when combined with specific microbial identification techniques [78].

## Antibody Assays

Antibody assays are less selective than molecular biology assays due to the differences in specificity of proteins and carbohydrates. Antibodies can be manufactured to recognize specific protein and carbohydrate structures, a principle that can be exploited to identify micro-organisms. The exterior of any micro-organisms consists of multiple protein and carbohydrate molecules and some of these are species-or strain-specific. The main drawbacks to antibody assays are the requirements for reagents and vulnerability to matrix effects of the sample. Highly selective and sensitive antibodies are readily available for many pathogens, and there are a number of well-established methods to conjugate antibodies to nanomaterials. For these reasons, immunological recognition by antibodies continues to be the most widely used tool for the selective capture and labeling of microorganisms. Antibody-based methods have been used extensively to detect bacteria, virus, toxins, and spores as shown in Table 4.

## Immunomagnetic Separation Methods and Flow Cytometry

Flow cytometry methods are rapid and quantitative and can be versatile since many methods can be combined such as nucleic acid probes and immune fluorescence to monitor viability [79]. The use of the flow cytometry instrument in the water industry has been evaluated for the identification of *Escherichia coli* O157:H7 and *Cryptosporidium parvum* [80-82]. A portable hand-held fluorescence detector was developed by Wildeboer et al., [62] for the rapid detection of *E. coli* in water. This method used a 4-methyl-umbelliferone-β-D-glucuronide as a substrate and results obtained could be easily compared to other traditional methods.

MALDI-MS has also been used to identify and characterize microorganisms. Glassmeyer et al., [60] applied MALDI-time of flight mass spectrometry (TOF/MS) to identification of *Cryptosporidium parvum*oocysts. This is not real-time since oocysts of *C. parvum* and the matrix have to be mixed and held for at least 45 min. In another study, Li et al. [13] developed a new ICP-MS method using gold nanoparticle labeling to measure *E. coli* O157:H7 in water. This technique was very rapid with high specificity and sensitivity, but didn't distinguish viable and non-viable cells. However, a new method developed by Liu et al., [83] was capable of quantifying viable but non culturable (VBNC) *E.coli* O157:H7.

## Conclusion

Currently, the development of various sensors and on-line monitoring systems is progressing rapidly. These technologies can have clear and multiple benefits for water utilities, such as lower costs and real-time detection. However, many of these technologies need to be improved further and have not been tested in real world scenarios. With most new technologies, there are still problems with robustness, sensitivity, precision, reproducibility, and reliability. On the other hand, most on-line sensors are utilized for physicochemical parameters such as TOC, turbidity, pH, and water temperature. Other parameters include free chlorine, fluoride, spectral adsorption, and conductivity. Sensors for microbial contaminants are less frequently utilized by water utilities. Following are major issues related to continuous online sensors: false negatives, false positives, limit of detection, identification of treatment failures, integration of software data management, e.g., Labview, development of SCADA system, sensor maintenance & cost evaluation.

Water distribution networks are a major area of vulnerability for microbial contamination. Water systems require the ability to track the transport of microbial contaminants and therefore, these online sensors have to be placed at points throughout the distribution system. This is an extremely complex process that will require sophisticated distribution water quality modeling for real-time monitoring. While it is not reasonable or feasible to use real-time monitoring for every biological agent that could be introduced deliberately into a water system, it is practical to monitor overall microbial water quality parameters where a change in numbers can signal in real-time, the potential for the presence of other pathogenic microorganisms in water. Most importantly, these monitors must be capable of remote operation, maintainable, sensitive, quality assured, and last but not least

affordable. In summary, proper design and integration of the hydraulic modeling systems, online monitoring sensors and SCADA system can allow water utilities to plan for and rapidly react to an accidental or intentional contamination of water distribution systems.

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