Efficiency of Instrumental Analytical Approaches at the Control of Bacterial Infections in Water, Foods and Feeds

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Abstract Bacterial agents are those that are pathogenic to humans, plants and animals either by causing infectious diseases or by producing toxins. The review provides the description of some pathogenic bacteria, which are a threat to human health and life and must be strictly controlled in the food, water and the environment. With increasing reports on bioterrorism and other bio-threats, rapid and real time detection methods for various pathogens are warranted. Nowadays is important to develop strategies for early detection and monitoring bacterial agents under any conditions that warrant their recognition, including clinical-based diagnostics and biological warfare applications. The review is devoted to the microorganism's indication methods. A study of methods of exposure and authentication of biological agents is important design of biosensors and automatic microorganism's indication. In this study the authors compare the characteristics of the immune biosensors based on the SPR, TIRE, quartz crystal acoustic wave, amperometry, chemiluminescence and on the ISFETs with CeO_x gate surface and conclude that they have similar sensitivity. Special attention is paid to biosensors and last tendencies of their creation based on nanostructures such as deposited nanorods, quantum dots and graphene nanostructures. The achievements of the authors in this field and other researching groups all over the world are described.

Keywords Microroganisms · Identification · Control level · Biosensors · Bioterrorism

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D.P. Nikolelis and G.-P. Nikoleli (eds.), *Biosensors for Security and Bioterrorism Applications*, Advanced Sciences and Technologies for Security Applications,
DOI 10.1007/978-3-319-28926-7_10

1 Introduction

The world we live in has been dealt with key security challenges for years and it seems that nowadays, human security as well as national security level interchangeable. Terrorism by itself is a shadow in almost every part of our lives; however, it is also a reason for all of us to work on preventing and preempting possible consequences of its strike. That could be done through addressing the threat and forming responsible policy regarding it on a timely basis.

Foodborne infections and intoxications are a global problem. Changed agricultural production methods and consumption habits as well as modern harvesting, processing and packaging technologies promote the development of new and emerging pathogens. Travel and border crossing trade facilitate their global expansion. Diseases considered beaten are on the rise again. Bioterrorism emerges as a new threat to food safety [1].

"Food security is a huge issue for businesses, governments and society. As populations grow and climate change and competition for land use are taken into consideration, the problem of securing future food supplies is only going to get worse" says Neil Smith, Lloyd's Emerging Risks & Research Manager. He continues, "Insurance is likely to play a key role in mitigating some of the risks relating to food security, including agroterrorism" [4].

Agro-terrorism is a new term for one kind of ecological terrorism, which means terrorists attempts to destroy or damage agriculture in a country by misusing plant or animal pathogens to cause disruption in affected field. However, the term is relatively new, while the practice is not.

Biological warfare agents can be classified as microorganisms and toxins, these can be extremely toxic to humans and animals and the substances work to kill or incapacitate a population. Biological weapons may be used to target living organisms such as humans, animals or vegetation as well to contaminate essential materials such as air, water and soil. Massive epidemic rooted by pathogenic bacteria have been reported throughout human history. Thus, the increased threat of bioterrorism attacks to the public has stimulated growing demand for rapid detection of biological warfare agents (BWAs) in order to take effective countermeasures [98].

Bacterial agents are those that are pathogenic to humans, plants and animals either by causing infectious diseases or by producing toxins. Antibiotic medications have been successful in controlling some bacterial infections; however, bacteria can gain antibiotic resistance, either naturally or through genetic modification [69].

Nowadays is important to develop strategies for early detection of pathogens responsible for emerging foodborne diseases, and re-evaluate pathogens that are gaining importance for food safety. The rapid detection of foodborne pathogens is of vital importance to keep the food supply rid of contamination. The review provides the description of some pathogenic bacteria, which are a threat to human health and life and must be strictly controlled in the food, water and the environment. One of the global challenges is to provide both rapid and highly sensitive determination of pathogens. High-specific and rapid methods of bacteria detection

are urgently needed in all segments such as medical, environmental, food, and military. Furthermore, over the past few years, there has been a shift toward more pathogen detection on-site e.g. in food-processing plants and this demands portability and non-laboratory technics. We believe that the problem can be solved using new methods of analysis based on principles of biosensors. In the review, we provide overview of the most common pathogenic bacteria that pose a threat for human health and describe the most frequently used conventional and rapid methods. Special attention is paid to biosensors and last tendencies of their creation based on nanostructures such as deposited nanorods, quantum dots and graphene nanostructures. The achievements of the authors in this field and other researching groups all over the world are described.

2 Pathogens

The presence of microorganisms in a commodity is not necessarily an indicator of hazard to a consumer or of inferior quality. Moulds, yeasts, and bacteria are almost always found in food and water unless they are sterilized. When these are contaminants, microorganisms may be innocuous, others may cause spoilage, and still others may cause disease. The possibility of commodities becoming hazardous to consumers increases significantly if sanitation or hygiene is compromised. As a result, many international organizations such as the International Commission on Microbiological Specifications for Foods (ICMSF) and the Joint FAO/WHO Codex Alimentarius Commission as well as regional/country jurisdictions (e.g., European Commission Regulation [EC] No. 2073/2005) have established hygienic practices, sampling plans and microbiological specifications as well as other composite programs such as the Hazard Analysis Critical Control Point System (HACCP) to help prevent food- and water-borne diseases [31].

In recent years public concern about the safety of foods of animal origin has heightened due to problems that have arisen with bovine spongiform encephalopathy (BSE), dioxin contamination, outbreaks of foodborne bacterial infections, as well as growing concern about veterinary drug residues and microbial resistance to antibiotics. These problems have drawn attention to feeding practices within the livestock industry and have prompted health professionals and the feed industry to closely scrutinize food quality and safety problems that can arise in foods of animal origin as a result of animal feeding systems [104].

While the World Organization for Animal Health (OIE) has attempted to include animal diseases that present public health risks in the compilation of these lists (e.g., anthrax, brucellosis), it is clear that the primary focus has been, until recently, on animal diseases of economic importance. Zoonotic pathogens associated with waterborne and foodborne diseases such as Salmonella enterica, *E. coli* O157:H7 and other enterohaemorrhagic *E. coli*, *Campylobacter jejuni*, *Giardia duodenalis*, and *Cryptosporidium parvum* are not included in these lists. Leptospirosis is a list B 386 Waterborne Zoonoses disease. The lack of representation of many zoonotic

waterborne and foodborne pathogens in these lists is explained by the fact that they are not frequently associated with severe animal disease. However, the OIE has recently recognized the increasing importance of food safety programmes to member countries in satisfying national and international trade requirements and the fundamental importance of controlling zoonotic pathogens in food animal populations as a cornerstone of these efforts [101].

The gathering, identification of pathogens and bacteriological defense measures in advance in order to be effective, is very difficult. The environment (water, air, soil) reside in different amounts pathogenic microbes and various organic compounds, so when identifying bacteriological pathogens difficulties arise.

2.1 Foodborne Bacterial Infections

2.1.1 *Salmonella* Species

Salmonella spp. is one of the most frequently occurring food borne pathogens affecting the microbial safety of food and causes great concern in the food industry. Representatives of this species can cause different pathogenic diseases which provide a harmful influence to all body systems and are hardly treated. Majority of *Salmonella* is pathogenic for human and animals but in epidemiological meaning only a few of them have especially significant part. Infection spreads very fast and can afflict large quantity of people or animals. For example, it is estimated that about 1.4 million *Salmonella* infections occur each year in the United States, and cause more than 500 deaths annually [91]. Another big concern is *Salmonella spp.* antibiotic resistance. Among 180 raw food samples, including chicken, beef, pork, and shellfish samples about half (50.5 %) of the isolates is resistant to at least one antibiotic. Furthermore, from all food types are isolated multiresistant *Salmonella* isolates resistant to at least three different classes of antibiotics [93]. This fact complicates treatment of diseases caused by *Salmonella* representatives, and makes it possible to use resistant *Salmonella spp*. as a biological weapon [78].

Many species of *Salmonella* have been implicated in diseases of farm animals. Of these, *S. typhimurium* is universally distributed while *S. enteriditis* has emerged as a regular pathogen of poultry and contaminant of eggs and chicken meat. Animal feeds are thought to be an important source of these bacteria. Meat and bone meal and fishmeal are frequently contaminated with *Salmonella*. Intensive pasture utilization provides an additional source through contamination of faeces from infected animals. Furthermore, the practice of spreading cattle slurry on to pastures in conventional and organic farms is another potentially significant source of infection.

In many parts of the world poultry manure is used as a feed for ruminants. For example in the USA, two poultry waste products are available for such use: dried poultry waste and dried poultry litter [45]. Dried poultry waste represents undiluted excreta generally derived from caged layer flocks, whereas dried poultry litter is a mixture of excreta and litter. These products are heated to reduce bacterial

contamination but are, nevertheless, not sterile. It is reassuring to note that in the trail of [45] that all samples of processed poultry litter collected from 13 dairy farms were totally free of *Salmonella* contamination even though virtually all samples contained Enterobacteriacea, non-glucose fermenting Gram-negative and Gram-positive bacteria.

Numerous analytical methods were developed for the detection of Salmonella antibodies including Widal agglutination test, enzyme linked immunosorbent assay [12, 68], antiglobulin haemagglutination test [19], indirect haemagglutination test [65], latexagglutination test [67] and counter immunoelectrophoresis [40]. However, these methods require complex sample pretreatment procedures and expert personnel and also they are time consuming. Among the serological tests, Widal test is commonly employed for reactive diagnosis of typhoid fever but it is not very reliable [52, 73].

2.1.2 *Escherichia Coli* Including *E. Coli* O157

It is widely recognised that cattle feeds contain *E. coli* through contamination with faeces. There is particular concern over the occurrence of *E. coli* O157 since this form has been definitively linked with specific outbreaks of illness in humans.

The application of slurry on to pastures means that there is potential for the transfer of faecal *E. coli* to grazing animals, a practice that has caused some disquiet among those concerned with food safety. It is important to distinguish the notion of `reservoir' from that of an incidental host as regards the source of *E. coli* O157. They were not able to support the thesis that cattle are the reservoir species of this form of the bacterium. The impact of spreading slurry on land still requires evaluation but proceeded to recommend the introduction of precautionary measures with this practice. Thus, the contamination of pasture grass with *E. coli* O157 from slurry remains a contentious issue.

Other forms of *E. coli* occur extensively in cattle feeds. Thus, it was reported that just over 30 % of cattle feed samples collected from 13 dairies and four feed mills in USA were found to be contaminated with *E. coli*, although *E. coli* O157 was not detected in any of these samples. In five dairies, concentrations of *E. coli* exceeded 1000 colony forming units/g feed. These authors suggested that attention should be focused on the replication of *E. coli* in moist feeds and duration of storage in feed bunks. Other studies in the USA also demonstrated the occurrence of *E. coli* (non-O157) in cattle feeds. Jeffrey et al. [45] reported that 13 non-O157 *E. coli* bacteria were isolated from 52 samples of dried poultry litter destined for dairy feeds [17].

2.2 Waterborne Bacterial Infections

The environmental burden of excreta from domestic animals, wildlife, and human beings will increase in coming decades, and excreta are likely the largest source of

pathogens for the environment. The potential for transfer of these pathogens to surface water and groundwater is evident. Current water treatment systems have been designed to address some of the well known waterborne diseases (e.g., cholera). However, pathogens that are important causes of waterborne illness today and those that represent potential emerging threats present significant challenges for current strategies to prevent waterborne illness [26].

Management of water quality has been applied mainly to receiving waters contaminated by point sources of human pollution. Waters affected by point sources of pollution are usually subject to regulation, because the pollution frequently impacts bathing beaches or shellfish harvesting waters, as well as drinking-water supplies. Water resources contaminated by dispersed, unidentified sources of pollution, the type usually associated with animals, have not been given special attention until fairly recently. This is the result of a greater awareness of emerging waterborne pathogenic zoonotic microorganisms and improved technical methods to measure water quality. This, in turn, has led to some technical problems that were not anticipated when microbial indicators of faecal contamination were first proposed as a means of monitoring water quality. These problems include the methodology and microbes traditionally.

The monitoring of water quality and the recognition of emerging zoonotic pathogens as a waterborne risk, resulting, in some instances, from changing host population behaviour patterns. At the beginning of the 20th century, Escherich noted that harmless, easily cultivated bacteria occurred in faeces. The organism was suggested for use as an indicator of the presence of faeces from warm-blooded animals in water. The practice of using *Bacillus coli* (later named *Escherichia coli*) as a measure of water quality was adopted, and, over the years, it was used in many forms, dependent on the practical methodology available. Coliforms, faecal coliforms, and *E. coli* were measured, respectively, over time as new specific methods became available. The measurement of *E. coli* in water served as evidence of the presence of faeces and even as a guide to how much faecal contamination was present. This approach appeared to work, because it was applied to waters near relatively small urban centres with poor infrastructure, little water treatment, and populations with little mobility. However, this approach to measuring water quality has been shown to have many inadequacies in identifying public health risk, no doubt related to extending its practical use to unintended applications that have never been validated. Urban centres have expanded beyond their central confines, to the extent that suburban spread is encroaching on feral animal habitat. Another pressure not envisioned when microbial indicators, such as *E. coli*, were proposed is the commercialization of aquatic food resources harvested from waters that were frequently contaminated with human and animal faecal wastes. Similarly, the practice of confining large numbers of animals to feedlots has created a situation where very large volumes of faecal waste frequently reach waterways, leading to pollution of aquatic food sources and water resources used for recreation. In addition, much of the increased leisure time available to humans is spent on recreational water activities far from urban centres and nearer to natural animal habitats. This population movement has led to regulations attempting to govern the

safety of all surface waters, including those with non-anthropogenic sources of faecal contamination. However, the means by which we monitor surface waters to provide evidence that they are free of faeces—and, therefore, zoonotic enteric pathogens—has changed little in the last 100 years. The microbial indicator of faecal contamination has not changed, and the methodology for measuring these faecal indicator organisms has changed very little. The methods still rely on bacterial growth in culture media to enumerate their presence in water samples. This usually requires a 24-h period of growth before results are obtained, presenting a situation where the potential risk associated with a water body is detected long after a risk activity has occurred [59].

2.2.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a common environmental organism and can be found in faeces, soil, water and sewage. It can multiply in water environments and also on the surface of suitable organic materials in contact with water. *Pseudomonas aeruginosa* is a recognized cause of hospital-acquired infections with potentially serious complications. It has been isolated from a range of moist environments such as sinks, water baths, hot water systems, showers and spa pools [59].

The widespread habitat of *P. aeruginosa* in nature, which includes soil, water, food and the surfaces of plants and animals, makes very difficult controlling the organism in hospital settings, and prevention of contamination is almost impossible. The list of materials from which this bacterium can be isolated is almost endless and, despite the recent development of different detection methods with no need for target amplification and enrichment, no sufficiently quick and reliable diagnostic systems for *P. aeruginosa* detection are available to date [22].

The risk of illness associated with exposure to foods harvested from animal contaminated waters or the risk of illness due to direct exposure to these waters is real, but largely unquantified. Although we are fully aware of waterborne zoonotic illnesses through individual cases and outbreaks of illness, we have no way of predicting illness associated with animal-contaminated waters. Our current system for monitoring water quality is designed to protect human health from human-derived pathogens. Unfortunately, the source of faecal indicator bacteria used to monitor water quality is not specific to humans. Currently used indicator methodology cannot distinguish an *E. coli* from animals from an *E. coli* whose source is human.

This shortcoming has led many countries to treat animal-contaminated water as if it were water contaminated by humans and therefore of equal risk. Some solutions to these problems are presented in the chapters of this section. The means to define risk associated with waterborne zoonoses, techniques for identifying sources of animal pollution, and techniques for measuring the safety of animal-contaminated water are discussed with respect to providing tools and information for managing water resources [26].

Detection of contaminated water by pathogenic microorganism is an important concern for ensuring water safety, security and public health. A clean and treated

water supply to each house may be the norm in Europe and North America, but in developing countries, access to both clean water and sanitation are not in the prime focus thus waterborne infections are common. Two and a half billion people have no access to improved sanitation, and more than 1.5 million children die each year from water borne diarrheal diseases [59].

In regard to waterborne pathogen detection, there are several important issues to consider. These chips are designed to detect minute quantities of target molecules. The target molecules must be free from contaminants before being applied to the chips. In order to utilize these chips, water samples must go through extensive cell amplification (growth in culture) or sample concentration by filtration, separation, absorption, or centrifugation. Particles in a water sample will easily block the channels used in the microfluidic biochips. The necessity of preparing a water sample prior to analysis will not allow the biochips to provide "real-time" detection of pathogens in water at this time. Another concern is viability of the pathogens to be detected by biochips. Monitoring the presence of some target molecule will provide evidence only for the presence or absence of the target pathogen, not for the viability of the pathogen in question. Some form of culture enrichment to ensure growth is still needed in order to obtain meaningful results. The potential of biochips and microarrays for waterborne pathogen detection is great; at this time, however, much more research is needed to make this technology a reality in applied water microbiology [26, 59].

2.3 Animal Feed Pathogens

Potential bacterial pathogens found in the feed are *Cl. Perfringens, Cl. Botulinum, Listeria* s. (*L. monocytogenes*), *Escherichia coli, Salmonella* spp. Feed, therefore, may contain a diverse microflora originating from soil that is characterized by its ability to survive under conditions of desiccation and a wide range of microenvironments. The dissemination of bacteria may be assisted by boring insects that invade seeds. Low water activity limits bacterial grown in stored grain. When animal feed is stored under moist, anaerobic conditions such as silage or haylage, bacterial diversity is inhibited by low pH. However, bacteria in feed have received relatively little attention compared to moulds and fungi [60].

2.4 Approaches for Microbial Diversity Characterization

Researchers have investigated microbial population behavior in complex environments by examining DNA sequences that are conserved across pathogen species, plasmids, or by using restriction enzymes to characterize genomes based on sequences vulnerable to digestion. [16] used small subunit (ss) rRNA genes to examine microbial population diversity in pasture soil. [42] used a combination of

16S rDNA analysis and BOX-PCR (targeting repetitive intergenic sequence elements of *Streptococcus* spp.) to investigate nitrogen-fixing *Azoarcus* spp. in soil. Restriction fragment length polymorphism (RFLP) analysis, in which population diversity is measured by variations in the length of an amplified 16S rDNA gene, has been used in environmental samples such as bioreactor sludge, termite guts, and seagrass (*Halophila stipulacea*), and to determine bacterial population differences in cow versus human fecal populations [13, 57, 103]. If specific microorganisms could be tracked throughout the environment, a clearer understanding of transmission cycles may lead to new advances in slowing disease spread.

Molecular analysis has also been applied as a surveillance method to detect microbial contamination in animal feed samples [62]. These techniques have an advantage over accepted methods involving the isolation of bacteria by enrichment, selective culturing, and biochemical or serological identification is that molecular assays are more rapid. Traditional culturing methods can take up to 5–7 days to produce results versus 24–36 h for molecular analysis. Thus, a feed mill that produces several tons of feed per hour can monitor its production in a more timely manner. There are numerous commercial PCR kits available. A sampling of PCR kits for the detection of *Salmonella* spp. include: BAXTM (Qualicon, Wilmington, DE.), ProbeliaTM (Sanofi Diagnosic Pasteur, Marnes La Coquette, France), and TaqManTM (PE Applied Biosystems, Foster City, CA). All of these assays have reported greater than 95 % accuracy in detecting the bacterium in various matrices. Part of the difficulty with molecular assays lies with the problem of extracting and recovering representative samples from feeds for molecular analyses. Several methods for the extraction of microbial nucleic acids have been tested to create a sampling strategy that may be used to isolate and amplify microbial DNA from animal feeds using polymerase chain reaction (PCR). A detergent-based initial detachment of microorganisms from feeds followed by the modified procedure of Widmer et al. [21, 103] was found to be the most robust extraction technique for removing inhibitory compounds [61]. However, the extraction requires an overnight incubation step, which complicates its use as a rapid method. Incubation at lower temperatures (−70 °C instead of −20 °C) may be able to shorten this incubation. As more commercial assays become available standard protocols for extraction, enrichment and PCR can be more systematically evaluated to determine the efficacy, detection limits, and the minimum enrichment time required for routine analyses of animal feeds [60].

3 Traditional Methods for Detection of Waterborne and Foodborne Pathogens

The effective testing of bacteria requires methods of analysis that meet a number of challenging criteria. Analysis time and sensitivity are the most important limitations related to the usefulness of bacterial testing. An extremely selective detection

methodology is also required, because low numbers of pathogenic bacteria are often present in a complex biological environment along with many other non-pathogenic bacteria [27].

Many advances have been made in rapid methods and automation in microbiology in the past 20 years. Fung comprehensively reviewed the subject in the inaugural issue of the journal Comprehensive Review in Food Science and Food Safety, published by the Institute of Food Technologists. The history and key developments in the field were described. In addition, advances in sample preparation and treatments, total viable cell count methodologies, miniaturization and diagnostic kits, immunological testing, instrumentation and biomass measurements, genetic testing, and biosensors were described. Finally, US and world market and testing trends and predictions of future developments were included. Of particular interest are the developments in genetic testing and biosensors, which can be used in water microbiology. Traditional microbiological methods rely on growth characteristics of organisms at various temperatures, colony morphology in various cultural media, biochemical reactions in various carbohydrates, and immunological antibody–antigen reactions. These characteristics are influenced by environmental factors and growth conditions of cells [21, 26, 84, 88, 105].

Existing and prospective microbiological control methods may be inherent physical, chemical and biological principles detection of biological agents [98]. Biological methods to control bacterial contamination lies in the fact that the studied samples (water, air, soil, food, swabs techniques, etc.). secrete microorganisms, sow them in culture media and after the incubation temperature count the number of colonies that grew [32]. During testing samples for the presence of microorganisms prefer membrane filtration method. This method air, water or suspension of the sample is passed through a special membrane bacterial filter. More filter placed in a nutrient medium, incubated with the appropriate temperature and count the number of colonies that grew [29]. This method makes it possible to confirm the presence of viable microorganisms in the sample with the highest possible probability. The main factors affecting the efficiency of determining the degree of bacterial contamination, is the object of the sample for analysis, engineering planting, nutrient composition, time and temperature crops, etc. [29, 102].

Microscopic method is used for the detection of the morphologically similar bacteria in the selected material to reveal the suspected agents of disease. This method provides a basis for the approximate diagnosis and usually requires further confirmation with the help of the bacteriological and serological methods. The last ones include the following reactions: agglutination, precipitation, bakteriolysis, haemolysis, complement fixation and others. Bacteriological method is particularly important in the diagnostics of most infectious diseases and it demands the obtaining a pure culture of the causative agent of disease and the further investigations. This method is a key in the diagnosis of bacterial diseases [82].

Existing biological methods of bacterial analysis, despite the high efficiency, usually quite labor-intensive, requiring a lot of time to get results and expensive media for culturing microorganisms [27]. All this prevents use these methods for permanent monitoring and rapid analysis.

In pathogenic bacteria more dangerous are products of their decomposition or life. For food characterized by the presence of toxins and for injectable solutions—lipopolysaccharide (pyrogens). Pyrogens—substances of different origin (mostly biological) that cause a feverish condition of the body with a sharp increase in body temperature [27]. It is possible that with pyrogenic reactions and endotoxins [102]. In this case, pyrogenicity test can be used to determine the presence of the samples. Wide application acquired control method for pyrogenicity by raising body temperature in response to an investigational intravenous drugs tested on rabbits. This method is recorded only in the State Pharmacopoeia of our country but also in the pharmacopoeias of other countries [64].

However, years of experience defining pirogenicity of rabbits revealed shortcomings of this method: the high cost of purchasing and operating the equipment, keeping animals and care for them, the dependence of the results on the individual animal, the need to test the sample for three or more animals to obtain reliable results [64].

At this time in the US Pharmacopoeia and Canada introduced a new method for determining the biological pyrogenicity which is to measure the optical density of the gel amebocyte lysate crab Limulus polyphemus influenced by Gram-negative bacteria endotoxin. This method has been tested on different types of bacteria *Escherichia coli*, *Proteus vulgaris*, *Klebsiella aerogenes*, *Salmonella thyphimurium*, *Pseudomonas aeruginosa*. Its sensitivity is in the range: *E. coli*—750, *P. vulgaris*—200, *P. aeruginosa*—12,000 cells. By A. M. Greek was developed the method of pyrogenicity were asked to determine water for injections largest ratio of fluorescence intensity excimer to monomer pyrene. Also the possibility of determining liposacharyds using photoacoustic spectroscopy, thermal-lens spectrometry.

If microbial samples before testing process solution ethylenediaminetetraacetic acid or methyl thymol blue [74], the reaction threshold is increased and the test gives a positive result for the presence of *E. coli* 125 cells, 50 cells of *P. vulgaris* and *P. aeruginosa* 400 cells per unit volume [25]. This method, despite its advantages, has some significant disadvantages: difficulty obtaining amebocyte lysate, the need for special storage conditions, instability lysate, complexity catching, transportation, holding crabs, lobsters and Xiphosura. That lack of classic biological analyzes the driving force behind the development of methods specific indication and diagnosis of infectious diseases.

Methods for determining the presence of microorganisms in the samples using probes—dyes. The essence of it is in the studied sample injected rhodamine 6G, record its fluorescence intensity on the wavelength of 555 nm in the presence of bacteria that is compared with the intensity of fluorescence standard solution indicator on the same wavelength. The results of measurements are concluding the presence of microorganisms. For research took 3 types of microorganisms—*E. coli*, *Bacillus subtilis*, *P. aeruginosa*. The magnitude of changes in fluorescence intensity depended on the type of indicator bacteria and their strength. The resazurin as indicator, example you can use to determine the total microbial number during the microbiological control of foodstuffs. Experiments were performed cultures: *Bacillus cereus*, *B. subtilis*, *E. coli*, *Staphylococcus aureus*, *Candida utilis* [27].

Most of the microorganisms have the property of absorbing light and intrinsic fluorescence in the ultraviolet area by tryptophan and tyrosine residues of protein toxins [34]. On this basis, it was proposed to use the said property to identify microorganisms in aqueous solutions and in dry soil samples [95], and the fluorescence and light scattering to determine the presence of pathogens in the air [46].

Microorganisms have the ability to not only the absorption in the ultraviolet (UV), but also in the infrared (IR) section. With the shift of the maximum absorption of infrared-spectral range in 1600–1700 cm^{-1} can determine the degree of cell viability [49].

In connection with the development of sensitive equipment to register superweak luminescence (chemiluminescence, bioluminescence and electrochemiluminescence) of various biological objects the ability to use this feature to test the presence of microorganisms in different environments. Some types of microorganism's bioluminescence observed in the presence of ions Mg [30].

The glow is due to the addition of oxygen to the complex luciferase luciferin adenosine monophosphate. The source adenosine diphosphate serves as adenosine triphosphate (ATP) microbes. Sensitivity of 10^3 microbial cells in 1 ml.

Bioluminescence method can be used to control the presence of microorganisms in different environments [18]. In addition, microorganisms can decompose hydrogen peroxide using catalysis in the presence of chemiluminescent compound. It was used during the development of the method of determination of the total concentration and the presence of live microorganism's cells. Applying as oxidizing agent of potassium dihydrogen phosphate, can increase the sensitivity chemiluminescence method [49].

The most sensitive radiometric methods are that allow the definition based on the number $C^{14}O_2$ that emerged in the course of microorganisms with C^{14} labeled carbohydrate nutrient medium, to identify individual cells *E. coli* (even one in the water) [8]. The calculation was performed on a liquid scintillation spectrometer. The presence of the negatively charged bacterial membrane allows to determine their presence in different environments. Technically method is carried out as follows: in the cooking chamber with liquid nutrient and studied a model containing two electrodes—measuring and standard. Standard covered electrode material, permeable to microorganisms.

When the camera reached some cell concentration, measured potential difference proportional to their number [105]. Possible areas of application: determine the intensity of contamination of samples machinery, food products, evaluation of the effectiveness of antibacterial agents.

We also consider a method that allows the change in the electrical conductivity of the sample slurry to conclude that the degree of growth of microorganisms in the medium and their number. The sensitivity of this method is low. Changing the voltage or current to the electrode is recorded only when the concentration of microorganisms 10^5–10^6 cells per 1 ml [89].

Create a constant electric potential on the graphite electrode leads to inhibition of respiratory activity of microbial cells of *Saccharomyces cerevisiae*, *B. subtilis*, *E. coli*, placed directly on this electrode.

The degree of respiratory activity depends on the electrode potential and the type of bacteria. Fall respiratory activity correlated with a decrease in the number of viable cells. All this suggests the possibility of creating an electrochemical method indicating the presence of microorganisms.

Some interest electric method of identifying and counting the bacteria in suspensions based on determining the change in the polarization of the laser beam that occurs after its passage through the bacteria suspension, oriented in an electric field [58].

There is a way to identify *Enterobacteriaceae* based on tryptychnomu solubilization and decomposition of proteins by their laser mass spectrometry desorption ionization with matrix [75].

One of the methods of high definition and identification of bacteria is to compare the outer membrane protein with a database full set of proteins. For a complete set of tabulations masses used proteins laser mass spectrometry method with liquid chromatography [94, 100]. Identification of proteins on the surface of the outer membrane of bacterial cells is carried out by ligands that fluorescing and have a greater propensity to bind to proteins [28]. This method can be used to indicate and to differentiate between pathogenic and non-pathogenic strains [53].

The surface-enhanced Raman spectroscopy [23, 37] of electromagnetic waves in the optical range sorted on metals chemicals and biological agents (viruses, bacteria, toxins) can also be used to determine small concentrations of highly toxic substances. Two-dimensional spectral resonance scattering in the ultraviolet region allowing identification of bacteria and identify similar genetic samples [10].

In 1985 Karl Myullis with team developed a method for cloning DNA sequences in vitro, known as polymerase chain reaction (PCR). This method of amplification in vitro, by which for a few hours can be identified and multiplying a sequence of DNA in excess of 108 times the output in [10]. PCR can be made fast and highly sensitive determination of Mycobacterium tuberculosis [36, 63, 97], human immunodeficiency virus (HIV), papilloma virus [35] and so on. It is not necessary to work with radioactive isotopes, as amplified viral DNA segment appears immediately after electrophoretic separation of DNA and dye coloring it accordingly [35].

Genotypic characteristics of a cell are far more stable than phenotypic expressions. Genetic methods have been developing rapidly in the past two decades. First, DNA and RNA hybridization techniques were perfected and widely used to rapidly detect organisms such as *Salmonella*, *Listeria*, *Campylobacter*, etc. More recently, PCR has been widely used for the detection of a great variety of microorganisms in clinical, food, industrial, and water microbiology. Basically, a DNA molecule (double helix) of a target pathogen (e.g., *Salmonella*) is first denatured at about 95 °C to form single strands, then the temperature is lowered to about 55 °C for two primers (small oligonucleotides specific for *Salmonella*) to anneal to specific regions of the single-stranded DNA. The temperature is then increased to about 70 °C for

a special heat-stable polymerase, the TAQ enzyme from *Thermus aquaticus*, to add complementary bases (A, T, G, or C) to the single-stranded DNA and complete the extension to form a new double strand of DNA. This is called a thermal cycle. After this cycle, the tube is heated to 95 °C again for the next cycle. After one thermal cycle, one copy of DNA will become two copies. After about 21 cycles and 31 cycles, 1 million and 1 billion copies of the DNA will be formed, respectively. This entire process can be accomplished in less than an hour in an automatic thermal cycler. After PCR reactions, one still needs to detect the presence of the PCR products to indicate the presence of the pathogen to be detected. In the original PCR procedure, PCR products were detected by electrophoresis, which is time consuming and laborious. Several new PCR protocols have recently been developed to efficiently report successful PCR reactions. It was described an automatic system for screening a family of PCR assays for pathogens, which combines DNA amplification and automated homogeneous detection to determine the presence or absence of specific targets. All primers, polymerase, and deoxyribonucleotide bases necessary for PCR as well as a positive control and an intercalating dye are incorporated into the single tablet. The system works directly from an overnight enrichment of the target organisms. No DNA extraction is required. Assays are available for *Salmonella*, *E. coli* O157:H7, *Listeria*, and *L. monocytogenes*. The system uses an array of 96 blue light emission diodes as the excitation sources and a photomultiplier tube to detect the emitted fluorescent signal indicating successful PCR reactions. This integrated system improves the ease of use of the assay. The inclusivity and exclusivity of this system reach almost 100 %, meaning that false-positive and false-negative rates are almost zero. Additionally, this automated system can now be used with assays for the detection of *Cryptosporidium parvum* and *Campylobacter jejuni/coli* and for the 372 Waterborne Zoonoses quantitative and qualitative detection of genetically modified organisms in soy and corn described a self-contained PCR system that can report the successful PCR reaction by measuring fluorescence reaction in the experimental chambers. A special molecule is annealed to the single-stranded DNA to report the linear PCR amplification. The molecule has the appropriate sequence for the target DNA. It also has two attached particles. One is a fluorescent particle, and the other is a quencher particle. When the two particles are close to each other, no fluorescence occurs. However, when the TAQ polymerase is adding bases to the linear single strand of DNA, it will break this molecule away from the strand. As this occurs, the two particles will separate from each other, and fluorescence will occur. By measuring fluorescence in the tube, a successful PCR reaction can be determined. A new system using molecular beacon technology has been developed. In this technique, all of the reactions are in the same tube. A molecular beacon is a tailor-made hairpin-shaped hybridization probe. The probe is used to attach to target PCR products. On one end of the probe, a fluorophore is attached; on the other end is a quencher of the fluorophore. In the absence of the target PCR product, the beacon is in a hairpin shape and there is no fluorescence. However, during PCR reactions and the generation of target PCR products, the beacons will attach to the PCR products and cause the hairpin molecule to unfold. As the quencher moves away from the fluorophore, fluorescence will occur, and this can

be measured. By using molecular beacons containing different fluorophores, one can detect different PCR products in the same reaction tube and thus perform "multiplex" tests of several target pathogens (Bio-Rad 2003). One of the major problems of PCR systems is that of contamination of PCR products from one test to another. A system developed by the Pasteur Institute attempts to eliminate PCR product contamination by substituting the base uracil for thymine in the entire PCR protocol. Thus, in the reaction tube, there are adenine, uracil, guanine, and cytosine, but not thymine. During the PCR reaction, the resultant PCR products will be AUGC pairing and not the natural ATGC pairing. After one experiment is completed, a new sample is added to another tube for the next experiment. That tube contains an enzyme called uracil-D-glycosylase (UDG), which will hydrolyse any DNA molecules that contain a uracil base. Therefore, if there is contamination from a previous run, the AUGC-DNA will be destroyed before the beginning of the new run. Before a new PCR reaction, the tube with all reagents is heated to 56 °C for 15 min for UDG to hydrolyse any contaminants. During the DNA denaturation step, the UDG will be inactivated and will not act on the new AUGC-PCR products. This Detection/enumeration methods 373 system can detect *Salmonella*, *Listeria monocytogenes*, and other. The nucleic acid sequence-based amplification (NASBA) technique has been perfected in recent years for water microbiology. It has advantages over the PCR technologies. Since the target is RNA, it can be used to detect RNA viruses and functional mRNA targets. It is isothermal and thus does not require a thermal cycler for the reaction. It is rapid and sensitive for detection of target molecules. PCR, NASBA, and related genetic technologies can be powerful tools for water microbiology once the existing problems are solved and the systems are automated. Also, analysts have to be convinced to invest time and money to convert to these technologies for the routine analysis of water microbiology [17].

4 Biosensors for Detection of Waterborne and Foodborne Pathogens

There are now a large number of biosensors available for detection of target microorganisms in a variety of food, water, clinical, and industrial samples. [43] provided a comprehensive overview of different physicochemical instrumental techniques for direct and indirect identification of bacteria, including infrared and fluorescence spectroscopy, flow cytometry, chromatography, and chemiluminescence techniques, as a basis for biosensor construction [43]. Biosensor development and application are exciting fields in applied microbiology. The basic idea is simple, but the actual operation is quite complex and involves much instrumentation. Basically, a biosensor is a molecule or a group of molecules of biological origin attached to a signal recognition material. When an analyte comes in contact with the biosensor, the interaction will initiate a recognition signal that can be reported in an instrument. Many types of biosensors have been developed, including a large variety

of enzymes, polyclonal and monoclonal antibodies, nucleic acids, and cellular materials. In some applications, whole cells can also be used as a biosensor. Analytes detected include toxins (e.g., staphylococcal enterotoxins, tetrodotoxins, saxitoxin, and botulinum toxin); specific pathogens (e.g., *Salmonella*, *Staphylococcus*, and *Escherichia coli* O157:H7); carbohydrates (e.g., fructose, lactose, and galactose); insecticides and herbicides; ATP; antibiotics (e.g., penicillins); and others. The recognition signals used include electrochemical (e.g., potentiometry, voltage changes, conductance and impedance, and light addressable); optical (e.g., ultraviolet, bioluminescence, chemiluminescence, fluorescence, laser scattering, reflection and refraction of light, surface plasmon resonance, and polarized light); and miscellaneous transducers (e.g., piezoelectric crystals, thermistor, acoustic waves, and quartz crystal). Recently, much attention has been directed to the development of "biochips" and "microchips," which may detect a great variety of molecules associated with waterborne and foodborne pathogens.

Due to the advancements in miniaturization technology, as many as 50 000 individual spots (e.g., DNA microarrays), each spot containing millions of copies of a specific DNA probe, can be immobilized on a specialized microscope slide. Fluorescent labelled targets can be hybridized to these spots and detected. Biochips can also be designed to detect all kinds of waterborne bacteria by imprinting a variety of antibodies, or DNA molecules, against specific pathogens on the chip for the simultaneous detection of pathogens such as *Salmonella*, *Listeria, E. coli*, and *Staphylococcus aureus* on the same chip. Biochips are an exceedingly important technology in life sciences, and the market value is estimated to reach $5 billion by the middle of this decade. This technology is especially important in the rapidly developing field of proteomics, which requires massive amounts of data to generate valuable information. The development of these biochips and microchips provides an impressive method for obtaining a large amount of information for biological sciences [17, 26].

4.1 SPR Based Biosensors

Optical biosensors are powerful analytical tools for detection of microorganisms, toxins and other substances and represent the largest group among sensor devises. Due to their high sensitivity, ease of use and short time of analysis some of them were successfully commercialized and have been applied in numerous important fields such as medical diagnostics, environmental monitoring, food safety and security. Phenomenon of surface plasmon resonance (SPR) in different modifications is widely used for biosensors creating. SPR phenomenon was first described by Kretschmann in 1971 and then the proposed scheme for SPR was widely used for detection of organic mono- and multi-layers on metal surfaces. After [56] used it in the purposes of biosensing SPR was deeply studied and became a powerful tool for label-free studying of interactions between biological sensing elements and target molecules. By far, the most common SPR sensor platforms are based on

prism scheme [41] and angular modulation, but recently, a lot of attention have been paid to the study of waveguides with optical phase detection technique since it demonstrates high sensitivity for detection of bio-reactions [66]. As a biological sensing elements the proteins (e.g., antibodies) and peptides are the most frequently used. In addition, it was shown that immobilization of biomolecules to the bare transducer surfaces has negative impact to their reactivity therefore different methods of previous surface preparation are used. The main aim of surface modification is to provide maximum interaction between biomolecule (ligand) and analyte. Alternative to physical adsorption is covalent binding between amino groups of the protein and activated carboxyls on self-assembled monolayers (SAM) of alkanethiolates or within a dextran matrix. Avidin (or streptavidin)-biotin interactions are widely used for providing effective immobilization of biomolecules, such as oligonucleotides or for site-specific binding of antibodies. In case of antibodies immobilization to get a biomolecular layer with high level of site-oriented molecules intermediate binding molecules are used such as Fc-related proteins A [81, 83], G [72] and hybrid immunoglobulin highly-specific binding proteins LG and LA [47, 90]. Covalent binding of antibodies with SAM-molecules or Langmuir-Blodgett films via thiol groups increases the activity of antibodies up to 70 % [5]. The sensitivity of analysis strongly depends on the algorithms of detection fulfillment. There are next variants: (1) the "direct" way when the specific antibodies, which were immobilized on the transducer surface interact with solution contented different concentration of antigen; (2) the "competitive" way-1 when antigen to be analyzed compete with its conjugate (covalent linked complex antigen with enzyme or some protein) for the specific antibodies which were immobilized on the transducer surface; (3) the "competitive" way-2 when antigen in form of conjugate (covalent linked complex antigen with enzyme or some protein) are immobilized on the transducer surface and it competes with free antigen to be analyzed for the specific antibodies in solution; (4) the "to saturate" way when the specific antibodies which were immobilized on the transducer surface interact at first with free antigen to be analyzed and then with antigen in form of conjugate (covalent linked complex antigen with the enzyme or some protein); (5) the "sandwich method" when antigen interacts at first with specific antibodies which were immobilized on the transducer surface and then with second portion of specific labeled antibodies.

In recent years, the interest and application of biosensors based on SPR for detection and control of pathogens has highly increased. For this purpose immune reaction and direct way of analysis is commonly used. As it was shown before by the numerous studies antibodies immobilization via physical adsorption is ineffective for antigen binding, therefore previous modification of transducer surfaces for providing oriented binding of antibodies has wide application. Koubova´ et al. demonstrated *Salmonella enteritidis* detection with SPR based biosensor using antibodies immobilized to the bare gold surface via physical adsorption. Analysis was performed in a direct way. Concentration of detected cells of bacteria was on the level 10^6 CFU/ml [48]. Barlen et al. attempted to detect *Salmonella typhimurium* and *Salmonella enteritidis* simultaneously in phosphate buffer firs and then in milk using

SPR biosensor "Plasmonic". Detection was performed using "sandwich-method" and polyclonal antibodies were immobilized to the hydrophobic surface. Detection level for *S. typhimurium* in phosphate buffer was 1.25×10^5–2.5×10^6 cells/ml and for *S. enteritidis* it was 2.5×10^8–2.5×10^9 cells/ml. After milk analysis contaminated by given microorganisms, detection level was 2.5×10^5–5×10^5 and 2.5×10^8–3×10^9 cells/ml respectively [11].

Oh with group carried out a series of experiments for *S. typhimurium* and *S. paratyphi* detection using SPR biosensor. For *S. typhimurium* detection the working surface of biosensor was covered by SAM of 11-mercapto-undecanoic acid, then the layer of protein G was formed and after that, the monoclonal antibodies were immobilized. The range of device sensitivity was 10^2–10^9 CFU/ml. For *S. paratyphi* detection protein G was immobilized to the sensing surface using 2-iminohiolan and then the monoclonal antibodies to bacteria were added. Using described method the sensor sensitivity was on the range of 10^2–10^7 CFU/ml [71, 72].

Bokken et al. detected *Salmonella* of group A, B, D and E using commercial SPR based biosensor Biacore. Antibodies were immobilized to the layer of carboxymethylated dextran and for *Salmonella* serotype identification the "sandwich-method" was applied. As a result the detection level of the devise was 1.7×10^3–10^7 CFU/ml even in the presence of the other bacteria cells in quantity of 10^8 CFU/ml [15].

There are numerous articles dedicated to *Salmonella* detection using SPR biosensors based on commercial Spreeta modules. Lan et al. have determined *S. typhimurium* in a quantity of 1×10^6 CFU/ml [50]. Son with group detected *S. enteritidis*. Antibodies specific to *S. enteritidis* were immobilized to the gold surface via neutravidin. The smallest number of detected cells was about 10^5 CFU/ml [79].

Starodub with group also studied the possibility of different substances detection using SPR based biosensors and *S. typhimurium* was among them (Table 1). For the study two SPR biosensors were used: one was based on commercial Spreeta module and the other one "Plasmonotest" was designed V.M. Glushkov Institute of Cybernetics of NAS of Ukraine. As reactive part antigen-antibody interactions were used and previous working surface preparation was occurred which included several sequential steps: (a) covering of surface by polyalylamine hydrochloride (PAA) (b) immobilization of protein A from *Staphylococcus aureus*; (c) the oriented binding of the specific antibodies; (d) bovine serum albumin immobilization (BSA) for blocking free non-specific binding centers on the gold surface. For biological material immobilization on the gold surface polyelectrolytes are widely used [86]. Thin films obtained using small charged organic molecules are common since this molecules form insoluble polymer which electrostatically sorbs molecules with opposite charge [85]. In particular polyallylamin hydrochloride (PAA) immobilization on the transducer surface provides the acquisition of positive charge. To overcome the blocking of active sites of antibodies (antigen-binding sites) because of their interaction with gold surface the protein A from *Staphylococcus aureus* was previously immobilized [85]. The reason is that this protein has affinity to Fc-fragments of immunoglobulin G (IgG) and can bind its molecules without active sites participation. High quantity of free antigen-binding

Table 1 The comparison of the sensitivity of SPR based biosensors depending on the method of working surface preparation and biosensors with different types of transducer

Item	The type of biosensor and transducer modification	Analyte	Level/range of sensitivity
1.	SPR immune biosensor, physical adsorption [48]	*S. enteritidis*	10^6 CFU/ml
2.	SPR immune biosensor "Plasmonic", hydrophobic surface [11]	*S. typhimurium, S. enteritidis*	In phosphate buffer: 1.25×10^5–2.5×10^6 cells/ml, 2.5×10^8–2.5×10^9 cells/ml.
			In milk: 2.5×10^5–5×10^5 cells/ml.
			2.5×10^8–3×10^9 cells/ml
3.	SPR immune biosensor; 11-mercapto-undecanoic acid, protein G^1; 2-iminohiolan, protein G^2 [71, 72]	*S. typhimurium*[1], *S. paratyphi*[2]	10^2–10^9 CFU/ml
			10^2–10^7 CFU/ml
4.	SPR immune biosensor "Biacore", carboxymethylated dextran [15]	*Salmonella* of group A, B, D and E	1.7×10^3–10^7 CFU/ml
5.	SPR "Spreeta" module based immune biosensor [50]	*S. typhimurium*	1×10^6 CFU/ml
6.	SPR "Spreeta" module based immune biosensor, neutravidin [79]	*S. enteritidis*	10^5 CFU/ml
7.	SPR "Spreeta" module based immune biosensor; PAA, protein A [70, 81, 83–84]	*S. typhimurium*	10^3–10^7 cells/ml
8.	SPR immune biosensor "Plasmonotest"; PAA, protein A [70, 81, 83–84]	*S. typhimurium*	10^2–10^6 cells/ml.
9.	TIRE based biosensor; PAA, protein A [81]	*S. typhimurium*	Several cells (less than 5) in 10 ml
10.	PhL of ZnO nanorods [96]	*S. typhimurium*	10^2–10^6 cells/ml
11.	Au and magnetic Fe_2O_3 nanoparticles modified with polyaniline [99]	*E. coli* O157:H7	10^1–10^6 CFU/ml
12.	Graphene magnetic nanosheet decorated with chitosan [2]	*P. aeruginosa, S. aureus*	In suspension, using fluorescence: 4.5–5.0×10^2 CFU/ml— using MALDI: 4.5–5.0×10^2 CFU/ml
			In blood: using fluorescence: 1.0–4.0×10^2 CFU/ml— using MALDI: 5.0–6.0×10^2 CFU/ml.
13.	ISFETs based immune biosensor; glutaraldehyde, protein A [81]	*S. typhimurium*	2–5×10^5 cells/ml.

sites improves antigen binding and provides increasing of biosensor sensitivity. In the case of Spreeta based biosensor, it has been defined that device sensitivity was on the level 10^3–10^7 cells/ml. "Plasmonotest" detection level was within 10^2–10^6 cells/ml [70]. After analyzing the above results one can conclude that the lowest limit of sensitivity of SPR based biosensors in case of *Salmonella* detection is on the level of 10^2 cells/ml. But considering the fact that the infectious dose of a pathogen such as *E. coli* O157:H7 or *Salmonella* is as low as 10 cells and the existing coliform standard for *E. coli* in water is 4 cells/100 ml [43] demonstrated technique it is not sufficient for all practice situations. The current situation makes scientists to continue their work in this field and develop more sensitive methods for bacteria detection.

4.2 TIRE Based Biosensors

One of the promising optical methods for biosensors creating is total internal reflection ellipsometry (TIRE). Ellipsometry is an optical method for studying of surfaces and environments, which is based on analysis of amplitude and phase changes of a light wave during its interaction with the investigated object. Later it was studied the possibility of using phase change of reflected light in terms of total internal reflection. This method was called total internal reflection ellipsometry and was first described in 1976 [3]. Getting ellipsometry parameters in terms of total internal reflection significantly increased sensitivity and detection level comparing with conventional ellipsometry and SPR technology. Thus, the method of TIRE provides a level of determination within 5×10^{-7} RIU while for ellipsometric measurements this index is 10^{-5} RIU [44]. The reflected wave is formed on the edge of optically contrasting environments therefore ellipsometric measurements provide the information about optical structure of surface area and these processes that affect to its optical properties [7, 9].

In the work of Starodub et al. TIRE method was applied for *S. typhimurium* detection in model solutions. For the experiment prism scheme sensor platform in combination with specific antibodies as a reactive part were used and previous surface preparation was occurred as described in [70, 81]. Biosensor based on TIRE showed higher sensitivity than the SPR based. Maximal sensitivity was on the level of several cells (less than 5) in 10 ml [70, 81].

TIRE has several advantages that make it attractive for further study and application in field of biosensing. First of all TIRE provides high sensitivity. Thus, sensitivity of biosensors based on ellipsometry can be different for different substances but usually it reaches the level of a few nanograms. At the same time, analysis does not require large amounts of reagents and can be satisfied by using of microliters of samples. Other advantages of this method are: no need of reagents labeling; no damaging effects on the object of study; the possibility of real-time analysis; large measuring range (from nM to μM) and short time of analysis [24, 33, 76].

4.3 PhL of Nanomaterials for Biosensor Application

In recent years, there is a growing interest in studying and obtaining photoluminescent nanomaterials such as nanoscale structures and quantum dots of metal oxides. This is because nanoparticles of metal oxides acquire new qualitative changes of physical and chemical properties, catalytic ability and reactivity, which are not observed in microscopic bodies of the same chemical nature. One of these properties is photoluminescence (PhL). PhL is a powerful technology for development of optical biosensors since it does not require any procedures of bioreceptors preparation, complex electrical circuits and expensive equipment. The principle of PhL based biosensor operating is to measure changes of photoluminescence spectra of nanoparticles (intensity and peak position) caused by interaction of biological components. Many different substances were successfully detected by means of nanoparticle PhL such as ions, DNA molecules,dopamine, carbohydrate antigen, bovine leukemia virus and *S. typhimurium* etc. [38, 39, 54, 77, 96].

Viter et al. described a novel approach of *S. typhimurium* detection in model solutions using biosensor based on room temperature PhL of ZnO nanorods. The metal oxide nanopowder wos dispersed in solution and then deposited on glass substrate. Immobilization of biomolecules on metal oxide surface was performed due to coordination bond and van der-Waals interaction between biomolecules and surface. As a biorecognition element specific polyclonal antibodies to *S. typhimurium* were used. Anti-salmonella antibodies were immobilized on ZnO surface via physical adsorption. To prevent non-specific adsorption of antigen BSA solution was applied after immobilization of the anti-salmonella antibodies. After washing and drying ZnO nanorod layer wos immersed for 20 min into the 0.85 % of NaCl solution containing controlled concentration of *S. typhimurium* cells. On each step PhL of ZnO nanorods was measured. It was shown that PhL intensity of ZnO nanorods increased due to interaction between adsorbed proteins (specific antibodies and BSA) and ZnO surface. After adding of *S. typhimurium* cells and Ag-Ab interaction, decrease of PhL intensity was observed (Fig. 1). The biosensor showed

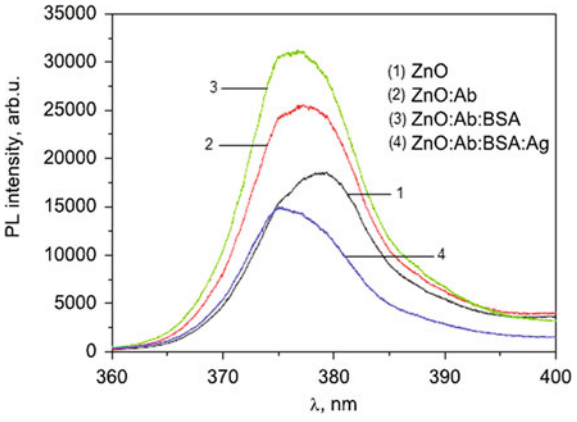

Fig. 1 PhL spectra of ZnO nanorods, after immobilization of anti-*Salmonella* Ab (ZnO:Ab), after BSA (ZnO:Ab:BSA), and after immune reaction with *Salmonella* Ag (ZnO:Ab:BSA:Ag)

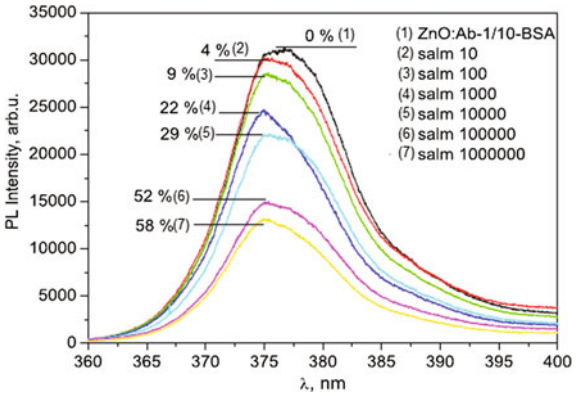

Fig. 2 Sensitivity of the ZnO biosensor to *Salmonella* Ag at different concentrations [96]

the changes of the signal in the range of 10^1–10^6 cells/ml (Fig. 2). The detection limit of biosensor was about 10^2 cells/ml [96]. Chances are to increase sensor sensitivity when surface functionalization will be provided for covalent binding of antibodies.

Gold nanoparticles in combination with magnetic nanoparticles of Fe_2O_3 were successfully combined by Wang et al. for *Escherichia coli* O157:H7. Fe_2O_3 nanoparticles were modified with polyaniline. Magnetic nanoparticles were conjugated with monoclonal antibodies to separate target *E. coli* O157:H7 cells from broth samples. Gold nanoparticles were conjugated with polyclonal Abs, and then were added to solution to form sandwich of magnetic nanoparticles- *E. coli*- gold nanoparticles. The amount of gold nanoparticles was measured through electrochemical method and the amount of the target bacteria were determined. Sensitivity of the described method was 10^1 CFU/ml with a linear range of 10^1–10^6 CFU/ml [99].

Graphene is a novel allotropic member of carbon-based nanomaterials and it has attracted worldwide interest due to excellent features such as high specific surface area, electronic conductivity, thermal conductivity, mechanical strength, cheap and easy to undergo functionalization via π–π interactions, covalent bonding, polymer blending and electrostatic interactions and it has been used for biosensing and fluorescence imaging [2]. Graphene nanostructures with their microscale area, sensitive electrical properties, and modifiable chemical functionality are excellent candidates for such biodevices at both biocellular and biomolecular scale. Graphene has already been successfully applied in biosensors creating for numerous substances detection such as DNA and proteins [106, 107] and pathogenic bacteria are among them. Abdelhamid et al. described graphene magnetic nanosheet decorated with chitosan as a promising biosensor for fluorescence spectroscopy and it can be also applied for matrix assisted laser desorption/ionization mass spectrometry (MALDI) for sensitive pathogenic bacteria detection. *P. aeruginosa* and *S. aureus* were detected in cell suspension and in blood. Limit of detection of *P. aeruginosa* and *S. aureus* in suspension using fluorescence was 5.0×10^2, 4.5×10^2 CFU/ml and for blood it was 4.0×10^2, 1.0×10^2 CFU/ml for each bacteria respectively.

MALDI provided sensitivity on the level of 5.0×10^2, 4.5×10^2 CFU/ml in suspension and 6.0×10^2, 5.0×10^2 CFU/ml in blood samples [2].

4.4 ISFETs Based Biosensors

Ion-sensitive field-effect transistors (ISFETs) represent the group of semiconductor potentiometric devices and are widely applied in biosensors. The interest to these devices only grows since 70 s of past century when P. Bergvald and T. Matsuo have proposed pH-FET as promising transducers for biosensors [20, 80, 92] and reported that these structures are suitable for highly sensitive detection of protons generated during the biochemical reaction and as elements for integrated multifunctional and multiparametric on-chip biosensors. Lately ISFET biosensors were adapted for different purposes including detection of glucose and urea, with the further use in clinical analyses. This tendency can be explained by the numerous advantages of ISFTs such as: miniature, possibility to put several electrodes on a single chip of semiconductor, low cost in mass production. The most often for biosensor creation pH-sensitive FETs are used. The numerous biological substances can be applied for modification of FETs surface to provide its selectivity depending of the task, but the largest number of works is dedicated to the use of enzymes, immunoglobulins and DNA molecules therefore biosensing FET systems are divided to enzymatic, immune and DNA FETs [14, 51]. Immune ISFET based biosensor was successfully applied by Starodub et al. for detection herbicide simazine. To the gate surface of ISFET the protein A from *Staphylococcus aureus* was immobilized to provide site-oriented binding of polyclonal antibodies specific to simazine. Detection of simazine was carried out in two ways such as "competitive" and "to saturate" way. In the first case detection level of simazine was 1,25 ng/ml and the use of "to saturate" method improved the biosensor sensitivity up to 0,65 ng/ml. The paper says that ISFET can be reused several times without signal reducing after it treatment with acid solution and water. The duration of analysis taking into account all preparatory steps was about 50 min. [80].

Starodub and Ogorodnijchuk [81] developed new type of immune biosensor based on ISFETs with CeO_x instead of Si_3N_4 gate surface, which provides high sensitivity and stability of the analysis (Figs. 3 and 4).

The biosensor was used for *S. typhimurium* detection in model solutions using immune reaction. The surface was activated twice by water solution of glutaraldehyde (GA). Next step was the protein A from *Staphylococcus aureus* immobilization and the solution of glycine was applied for blocking of GA non-bonded groups. At last, the working chip was covered by specific antiserum. Analysis was fulfilled by the "sandwich" method in the way when the immobilized specific antibodies interact with *Salmonella* cells diluted in 0.85 % solution of NaCl. Then, bounded cells on the transducer surface were treated by the specific antibodies labeled by horseradish peroxidase (HRP) which activity was registered. For HRP activity registration special buffer was used which contained 5 mM

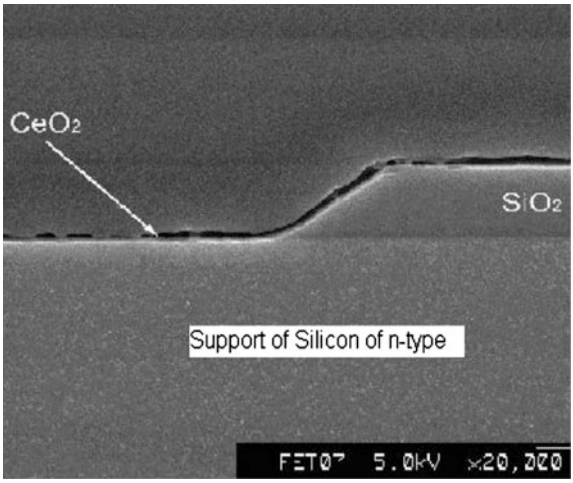

Fig. 3 Scanning electron microscopy of ISFET gate surface

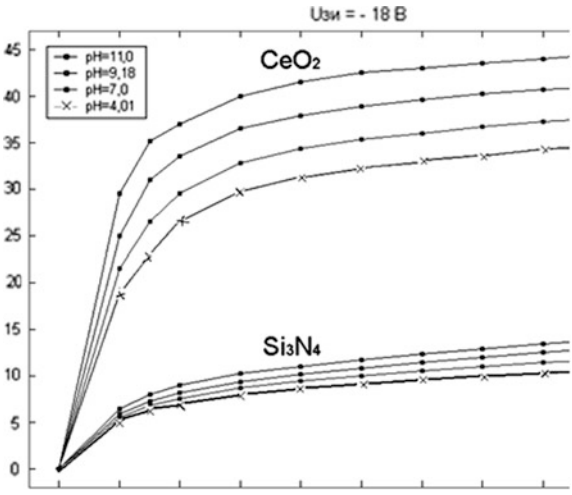

Fig. 4 Influence of pH on the output characteristics of ISFETs with the Si_3N_4 and CeO_2 dielectric structures (Starodub and Ogorodnijchuk [81]

tris-HCl (pH 7.5), 100 mM NaCl, 15 mM ascorbic acid and 5 or 10 mM H_2O_2. Substrate reaction provided local change of pH as a result forming of dehydroascorbic acid that is more alkaline than ascorbic acid. The antigen was in form of suspension of autoclaved cells of *S. typhimurium* diluted in 0.85 % NaCl with initial concentration of 10^6 cells/ml. The conjugate of specific antibodies with HRP have been prepared using previously described method [87]. It was demonstrated that the sensitivity of the analysis of salmonella was about 2–3 cells/ml with the maximal response up to 5×10^5 cells/ml. The total time of the measurements can be reduced to 15–20 min if the transducer surface was previously prepared and if one part of the specific antibodies are immobilized on it and the other one labeled by horseradish peroxidase is added to the analyzed solution. Each chip may be reused up to

5 times without signal decreasing after its treatment by 0.1 M HCl for 5 min followed by washing with 5 mM phosphate buffer, pH 7,5 [81].

The authors compare the characteristics of the immune biosensors based on the SPR, TIRE, quartz crystal acoustic wave, amperometry, chemiluminescence and on the ISFETs with CeO_x gate surface and conclude that they have similar sensitivity but the last approach may provide to achieve low cost of analysis [81]. To have the sensitivity of analysis in the respect of *Salmonella* and others bacteria on the level of infection dose [43] there is necessary to have a special system of the analyzed sample and in particular, accumulation of cells through application of bioaffinic columns as it was early demonstrated by some authors [55]. The comparison of the sensitivity of biosensors with different types of transducer is presented in Table 1.

5 Conclusions

Nowadays the need to develop rapid and highly sensitive methods for pathogens diagnostic is relevant in the world. Thousands of people die and millions illnesses occur each year in developed and developing countries caused by foodborne pathogens. High-specific and rapid methods of bacteria detection are urgently needed in all segments such as medical, environmental, food, and military. The United States Department of Agriculture (USDA) estimated that US food industry alone performs in total more than 144 million microbial tests annually. Known today methods of pathogens detection can be divided into the categories of conventional and rapid methods. Conventional methods involve enriching the food sample and performing various media-based metabolic tests (agar plates or slants). Depending on pathogen and method, tests typically require 3-7 days to obtain a result. Rapid methods, however, are based on immunochemical or nucleic acid technologies. Commercially available rapid tests can provide results in 8-48 h but results from these screening tests are presumptive and require further isolation of organism as proof of contamination. Thus, the combination of speed and sensitivity is the key for any rapid detection method. Furthermore, over the past few years, there has been a shift toward more pathogen detection on-site e.g. in food-processing plants and this demands portability and non-laboratory technics [6]. We believe that all these parameters can be successfully combined in modern instrumental devices based on the principles of biosensorics. As it shown above optical biosensors are portable and provide real-time analysis, which makes them very attractive for rapid-test systems creation. However, the lowest limit of sensitivity of SPR based biosensors for *Salmonella spp.* representatives is on the level of 10^2 cells/ml, which is insufficient since single-cell detection is required. Practical demands can satisfy TIRE based biosensors, which provides sensitivity on the level of several cells (less than 5) in 10 ml. Nanostructure-based techniques are very promising for biosensor creation and as it is shown above provide quite high sensitivity for pathogen detection both in form of deposited structures and quantum dots as well as graphene nanostructures. At the same time, these techniques are

quite new in biosensorics and need further studying. ISFETs with CeO_x gate surface are the best suited for use in industrial scale such as provide high sensitivity on the level of 2–3 cells/ml, can be reused up to 5 times without signal decreasing and provide low cost of analysis comparing with other types of biosensors. In order to obtain the sensitivity on the level of infectious dose it is necessary to pay attention to the effective methods of previous food samples preparation especially to the methods of bacteria cells concentration in the solution, which will be analyzed.

Acknowledgements This work was partially supported by EU funded BIOSENSORS-AGRICULT project (Development of nanotechnology based biosensors for agriculture FP7-PEOPLE-2012-IRSES, contract N. 318520) and NATO multi-year Science for Peace Project NUKR.SFPP 984637—"Development of Optical Bio-Sensors for Detection of Bio-Toxins" as well as National University of Life and Environmental Sciences of Ukraine, projects "Development of new generation of the instrumental analytical approaches based on the principles of nano-biotechnology and biosensorics for providing systems of biosafety" and "Creation of effective methods for the construction of artificial selective sites for the instrumental analytical devices of new generation based on the principles of biosensorics", N 110/476-Appl. and №110/71-fund., respectively.

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