Toxin immunosensors and sensor arrays for food quality control

Simone S. Moises · Michael Schäferling

Abstract The global efforts to improve consumer protection and public health lead to an increasing number of analytical approaches applicable to food analysis and process control. Biosensor systems are efficient analytical tools to monitor production processes or storage of nutrition and to control contamination outbreaks as they are easy-to-use, fast, and with minimal effort on sample preparation. Relevant targets of immunosensors implemented to food safety are prevalent bacterial toxins (staphylococcal enterotoxins and clostridial toxins), plant toxins (Ricin), mycotoxins (aflatoxins and ochratoxin A), marine toxins, and other pathogenic bacterial contaminations (*Listeria*, *Salmonella*, *Staphylococcus aureus*, or *Escherichia coli*). These cause acute intoxication and also chronic diseases in humans consuming contaminated food. Promising approaches for the determination of different types of toxins in food matrices will be outlined. The corresponding sensor systems use immunological receptor units such as antibodies or antigens and include optical (fluorescence and surface plasmon resonance), electrochemical, or acoustical readout methods. This review is focused on recent developments of sensor formats devoted to food safety control and is structured according to the type of toxin or contaminant that is recognized. It is intended to give an overview on emerging sensor technologies and their potential applications for the rapid analysis of the most important food poisoning agents.

Abbreviations

AP	Alkaline phosphatase
ATR	Attenuated total reflection
BoNT	Botulinum neurotoxin
BSA	Bovine serum albumin
CCD	Charge-coupled device
CWC	Chemical Weapons Convention
DON	Deoxynivalenol
DPV	Differential pulse voltammetry
EAPM	Electrically active polyaniline-coated magnetic
ECL	Electrochemoluminescence
EDC	N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
ELFA	Enzyme-linked fluorescent assay
ELIMCL	Enzyme-linked immunomagnetic chemiluminescence
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
FIA	Flow injection analysis
FITC	Fluorescein isothiocyanate
HPLC	High-pressure liquid chromatography
HRP	Horseradish peroxidase
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization
LD50	Lethal dose (50% of the tested organisms die)
LED	Light-emitting diode
LOD	Limit of detection
MED	Multichannel electrochemical detection
MS	Mass spectrometry
NHS	N-hydroxysuccinimide
NPP	Nitrophenylphosphate
OTA	Ochratoxin A

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OWLS   Optical waveguide lightmode spectroscopy
PCR   Polymerase chain reaction
PDMS   Polydimethylsulfoxide
PEG   Polyethylene glycol
PEMC   Piezoelectric-excited, millimeter-sized cantilever
PMMA   Polymethylmethacrylate
PZT   Lead zirconate titanate
QCM   Quartz crystal microbalance
RPLA   Reversed passive latex agglutination assay
SPE   Screen-printed electrode
SPR   Surface plasmon resonance
TMB   Tetramethylbenzidine
TPA   Tripropylamine
WHO   World Health Organization

Introduction

Foodborne intoxications are an enduring risk for public health, and therefore, the feasibility of producing and consuming safe foods is considered as one of the major achievements of the last century. Over 200 known diseases are transmitted via food consumption [1]. The spectrum of foodborne pathogens includes a variety of viruses, fungi and fungal toxins, chemicals, heavy metals, parasites, bacterial toxins, and bacteria, whereas bacteria-related poisoning is the most prevalent. Only less than 20 different bacteria act as originators. Every year, *Staphylococcus aureus*, *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and enteropathogenic *Escherichia coli* are causing more than 90 % of all food poisonings that are related to known pathogens. These bacteria are mainly found in raw foods [2, 3].

The Centers for Disease Control and Prevention [4] in the USA are collecting data on foodborne disease outbreaks from all states and territories through the Foodborne Disease Outbreak Surveillance System to quantify the impact of these diseases on health. The estimated number of food-related diseases causes approximately 76 million illnesses, 323,914 hospitalizations, and 5,194 deaths. Only 14 million illnesses, 60,000 hospitalizations, and 1,500 deaths are caused by known pathogens, while unknown agents are responsible for the remaining numbers [3].

Outbreak data reported internationally for source attribution were collected by Greig and Ravel [5]. Public report sources reveal that 4,093 outbreaks have been registered and analyzed between 1988 and 2007. According to this study, 2,168 cases are allotted to the USA, 1,287 to the European Union (EU), 246 to Australia and New Zealand, 208 to Canada, and 184 to other countries. Based on a study from the European Commission Rapid Alert System for Food and Feed, a total of 11,403 reports were published between July 2003 and June 2007 [6].

Controlling bodies and guidelines are necessary due to the large number of outbreaks and their impact on public health. The EU parliament assumes legislation in the form of directives and regulations for EU member states and is consulted on food safety affairs by the European Food Safety Authority [7]. The Food and Drug Administration of the USA publishes the Food Code which represents a model set of guidelines and procedures that assists food control jurisdictions. It provides a scientifically sound technical basis for regulating the retail and food service industry [8]. In 2003, the WHO and FAO published the *Codex Alimentarius*, which serves as a guideline to food safety [9]. In addition, *Outbreak Alert Databases* [10] and information tools for current outbreaks [11] in the USA are available online.

As food safety concerns consumers, food producers, and regulatory agencies, widespread concepts through the whole feed and food chain—farm, transport, supply, and consumption—are required to protect consumers from pathogen ingestion. Hazard Analysis and Critical Control Points is a systematic preventive approach to food and pharmaceutical safety which addresses physical, chemical, and biological hazards [12–14]. In Europe and the USA, a considerable number of research projects aiming for new tools for food safety were funded. Most of the projects unify research and development topics such as improved analytical and sampling methods with modeling and the compilation of databases. Some projects have also a strictly food chain-dominated structure. The large number of generated data sets affords refined statistical informatics. An introduction to practical biotraceability is given by Barker et al. [15].

The high relevance attributed to consumer protection is documented by the substantial number of integrated EU projects such as BIOTRACER [16]. It has 46 project partners from 24 countries, including four INCO countries, and has a total budget of 15 million euro. Its objective is to provide tools and computer models for the improvement of tracing accidental and deliberate microbial contaminations of feed, food, and bottled water. PathogenCombat [17] is another large integrated project that aims for better control and prevention of merging pathogens at cellular and molecular level throughout the food chain. PathogenCombat includes 20 industrial partners and 24 research partners from 17 countries. The main objective of the TRACE-BACK [18] project states the development of a functioning generic system for traceability of proliferations and information processing within food chains. It involves 28 project partners from 11 countries. Finally, CHILL-ON [19] researches for the improvement of quality, safety, and transparency of the food supply chain. Further information

on more related projects within different Framework Programmes of the EU is available online [20].

The Food Safety Project [21] in the USA provides resources for consumers, food service operators, students, and educators to access research-based, unbiased information on food safety and quality. Its goal is to develop educational materials that give the public helpful tools for minimizing the risk of foodborne illness and disseminate information about food safety.

Rapid and reliable detection methods are essential tools to process a large amount of samples that accumulate if a consistent food control shall be achieved. Customary microbiological methods such as cell culture techniques are often laborious and ineffective due to their incompatibility with the speed of the production chain and the distribution of food, its endurance, and the operational costs. Furthermore, bacterial strains can fail regular growth processes and lead to false analysis results.

Quantitative polymerase chain reaction (PCR) is an accurate, rapid, specific, and sensitive method for detection of small amounts of pathogen deoxyribonucleic acid (DNA) in food samples. Unfortunately, DNA-based assays can only detect the presence of toxin-producing organisms and do not quantify the amount of effective toxins. Online detection with PCR methods is also expensive and requires well-trained personnel [22].

Typical methods of instrumental analytical chemistry such as mass spectrometry, liquid chromatography, infrared, or UV/Vis spectrometry are powerful tools for a precise determination of pathogens, but they require time-consuming sample preparation and are usually not transportable devices, thus inapplicable for online monitoring, e.g., in the production process.

Sensor-based bioassays and microarray techniques are rapid and sensitive tools for online detection and automated process control during food production and the supply chain. They can also be used in extensive research studies, mass tests, or to generate supporting data for modeling programs. The results can be used to create new ISO or DIN norms that are significant for a huge number of food producers.

This review focuses on new developments in the field of sensors and arrays for the detection of food pathogens. It is structured corresponding to the type of targeted toxin to provide an easier overview on already evaluated sensor approaches for a certain type of contaminant. The article is focused on the most relevant targets accounted for in food safety. It considers only antibody- and cell-based technologies for the detection of food-contaminating bacteria and their corresponding toxins. Thus, it particularly includes immunochemical recognition units. In this regard, it should be noted that the term "immunosensor" is frequently used to illustrate the operating mode, but strictly speaking, these devices are not sensors as their responses are not reversible and they are not suited for continuous measurements [23]. Other types of biomolecular targets (viral or bacterial DNA) or recognition systems (e.g., molecular imprints) are out of the scope of this review.

Common ELISA methods and commercially available assay kits

Many established analytical methods for a straightforward and fast determination of toxins applicable to food, clinical, or environmental samples are based on enzyme-linked immunosorbent assays (ELISAs). These can be performed with a high throughput of tested samples in microwell plate formats; hence, commercially available ready-to-use assay kits originate from the classical sandwich-type ELISA.

ELISAs for toxin determination

Common techniques of instrumental analysis such as mass spectrometry, gas chromatography, high-pressure liquid chromatography (HPLC), or gel electrophoresis are often time-consuming, expensive, and their processing demands professionally trained personnel. Immunochemical methods such as ELISA fulfill the requirements of modern high-throughput analysis, including rapidness, specificity, sensitivity, multiplicity, and the availability of simple and cost-effective equipment. Moreover, the whole analysis can be carried out in portable instruments. These advantages turned ELISAs into well-established, high-throughput assays with low sample volume requirements and often less extensive sample preparation steps compared to conventional instrumental methods such as quantitative HPLC. Numerous assays targeting on the most frequent toxin contaminations in food matrices have been developed as summarized in Table 1. Figure 1 depicts a schematic overview over the basic ELISA formats.

A variant of the classical ELISA is the dipstick sandwich assay (Fig. 2) which has been established for the detection of staphylococcal enterotoxins (SEs). Capture antibodies for SE B are deposited on a polystyrene stick which is dipped in homogenized cheese samples contaminated with SE B. A typical sandwich assay is performed by subsequent addition of primary antibodies conjugated to horseradish peroxidase (HRP). The assembly is transferred to a tube containing H_2O_2 and o-diaminobenzene dihydrochloride. This substrate is converted enzymatically into a blue-colored reaction product in the presence of HRP. The optical density of the solution can be read out at 490 nm with a commercial fiber-optic probe after 5–10 min reaction time [33].

Table 1 ELISAs developed for toxin detection in food

| Analyte	ELISA type	Food matrix	LOD	Ref.
Ochratoxin A	Direct competitive	Maize, barley, soy	0.5 ng/g	[24]
Listeria monocytogenes	Sandwich (direct, indirect, biotin-amplification)	Low-fat milk	1×10^3 cells/mL; 3×10^3 cells/mL	[25]
C. perfringens A enterotoxin	Immunomagnetic separation (IMS)	Meat	2.5 ng/mL	[26]
SEA	Sandwich and competitive[a]	Ham paté, turkey paté, milk, sausage, potato mayonnaise, cheese and lemon cake	0.5 ng/g	[27]
Aflatoxin M1	Indirect competitive	Milk, milk based-confectionery	0.5 ng/mL	[28]
Streptomycin, S-derivate, Gentamycin, neomycin	Different	Milk	ns	[29]
SEA	Microsphere-packed capillary/Sandwich	Ham, cheese, chicken, bean sprouts	1 ng/g	[30]
SEH	Sandwich	Mashed potato (raw milk), sausage, bulk milk	55.5 ng/g	[31]
SEA-SED	Indirect double sandwich	Cheese, chicken, salad, milk, cream	1 ng/g	[32]
SEB	Dipstick sandwich	Cheese	0.5–1 ng/mL	[33]
Aflatoxin M1	Supermagnetic nanoparticles (direct competitive)	Milk	4 ng/mL	[34]
C. botulinum BoNT A, B, E,F	Amplified/sandwich	Soft drinks, juices, bottled water, vanilla extract, ice cream, honey, milk, legumes, spices, turkey, sausages, beef	2 ng/mL	[35]
C. botulinum BoNT B	BoNT/B endopeptidase	Pate, cheese, cod, mince, sausage	10 pg/mL	[36]
C. botulinum BoNT B	BoNT/B endopeptidase	Pate, cheese, cod, mince, sausage, yoghurt	5 pg/mL	[37]
Fumonisin B1	Indirect competitive	Corn	5 ng/g	[38]
Deoxynivalenol, Nivalenol, T-2, H-2	Competitive	Wheat kernels	80 ng/g DON 30 ng/g T-2	[39]

LOD limit of detection, *ns* not specified

[a] Sandwich and competitive ELISA or used for different matrices

The immunomagnetic separation assay (Fig. 3) utilizes dispersed magnetic particles (Magnetic Dynabead M280) [40] pre-coated with polyclonal capture antibodies specific for *C. perfringens* enterotoxin A. For performance of the assay, target enterotoxins included in the sample were placed into microwell plates blocked with bovine serum albumin (BSA) and incubated therein with the specific antibody-coated magnetic particles. After binding of toxins to the particles, they were concentrated to the bottom of the microtiter plates with a magnetic concentrator. Following concentration of loaded particles and subsequent washing steps, a biotinylated enterotoxin-specific target antibody was added, building the base of the enzyme anti-enterotoxin complex. In order to ensure quantitative complex formation, a preformed avidin–biotin alkaline phosphatase complex (AP) or streptavidin–biotin HRP complex was added and incubated. To quantify the amount of toxin in the sample, *p*-nitrophenylphosphate or azino-benzthiozoline sulfonate substrate was used following particle concentration to the bottom of the wells with a
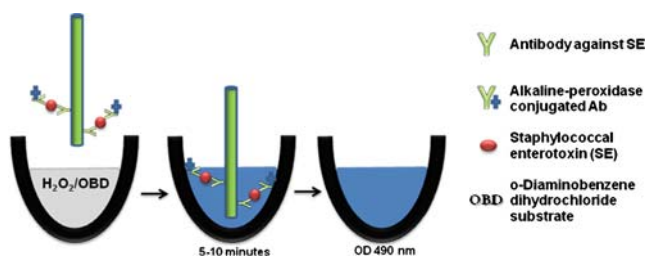
Fig. 1 Different types of ELISA assays
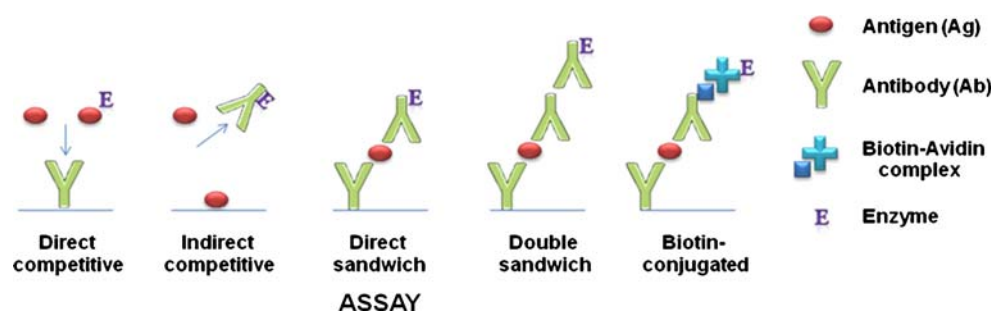
Fig. 2 Dipstick sandwich assay scheme [33]

magnetic concentrator. The colored reaction mixture was transferred to a new microtiter plate, and absorbance was measured at 414 nm [26].

Sensitive assays for *Botulinum* neurotoxins use their high endoprotease activity. These enzymes cleave different isoforms of neuronal proteins, controlling the docking of the synaptic vesicles to the synaptic membrane. Synthetic peptide substrates immobilized on a solid phase of a column can be used for the determination of *Clostridium botulinum* neurotoxin B (BoNT B) in food samples. The toxin cleaves the peptides, which are additionally biotinylated at the terminal position, after addition to the column. The eluate containing the released peptide fragment is transferred to streptavidin-coated microwell plates and detected with specific antibodies conjugated to HRP [36].

Commercialized detection kits

The necessity of very rapid standardized tests raises the demand for commercially available (semi-)automated ready-to-use assay kits. These are typically based on ELISAs or related methods such as the enzyme-linked fluorescent assay (ELFA) or the reversed passive latex agglutination assay (RPLA). There are only a few examples where they were designed to distinguish between different types of toxins of one species (e.g., the different *Staphylococcus* enterotoxins). The results of the RPLA can be read out by the naked eye. The agglutination of latex can be monitored in case of toxin attendance. ELISAs and ELFAs are quantified by means of photodetectors, measuring the resulting absorbance or fluorescence intensity, respectively [41].
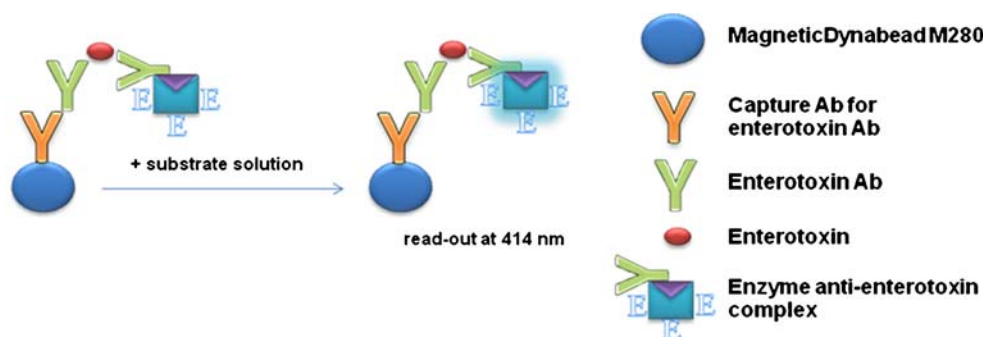
The widely used TECRA, RIDASCREEN, Veratox, AGRAQUANT, or Transia kits are generally derived from the ELISA principle, whereas the VIDAS system affiliates the category of ELFAs (Table 2).

Other typical sensor formats

Definitions, classifications, and essential components of biosensors are summarized by Wolfbeis [56]. In this chapter, the most important technologies applied in sensor technology will be outlined.

Fiber-optic sensors

Fiber-optic sensors use optical waveguides connected to a biofunctionalized interface as sensing element or as signal processing element (transducer) receiving signals from a remote sensor. Fibers are preferentially used because of their small size, and they do not call for electrical power at the point of measurement. Furthermore, many sensors can be multiplexed along the fiber length using different wavelengths for each sensor.

Waveguides with integrated optical frequency filters can be used as sensor elements to measure temperature, pressure, and other physical quantities. Thermal or physical deformation of the fiber leads to a modulation of the wavelength, intensity, polarization, phase, or transit time of the light that leaves the fiber.

Fiber-optic sensors can also be coated with chemical sensor layers, e.g., for oxygen or pH measurement [57], or biological recognition elements such as proteins, antibodies, or DNA. Their binding to target molecules from the sample can be monitored by fluorescence or ELISA-based methods or by the change in the optical properties (e.g., the refraction index) of the sensor interface [56, 58].

Surface plasmon resonance

If the surface of a glass substrate is coated with a thin metal film, a part of the incident light can refract into the metallic film. Typical coatings consist of noble metals such as silver,

Fig. 3 Immunomagnetic separation assay scheme [26]

Table 2 Examples of toxin determination in food with commercially available assay kits

Analyte	KIT type	Food matrix	LOD	Ref.
Staphylococcal enterotoxins	VIDAS SET2; Transia plate SET; SET-RPLA	Cake, liquid milk, cheeses from cow, goat, ewe, unspecific species	62.5–500 pg/mL	[42]
SEA-SEC	TECRA; RPLA	Ham, cheese, mushrooms, salami, pasta	0.75–1.25 ng/mL	[43]
SEA-SEE	RIDASCREEN SET; TECRA EIA	Noodle, ham, cheese, turkey, salami	200–750 pg/g	[44]
SEA-SEE	VIDAS SET; VIDAS SET2; Transia plate; Staphylococcal enterotoxins Diffchamb	Cooked food, canned food, liquid food, raw meat, dehydrated food, Delicatessen meat	< 0.5–1 ng/g	[45]
Salmonella (typhimurium, derbi, enteritidis, typhi)	BAX system; TECRA unique Salmonella	Raw pork, raw poultry, 9 major ready-to-eat food groups	10 cfu/25 g sample	[46]
Ochratoxin A, T-2	Collaborative study kits	Maize, wheat, barley, rye	4 mg/kg OTA 50 mg/kg T-2	[47]
Ochratoxin A	RIDASCREEN OTA ELISA	Coffee beans	25 ng/kg	[48]
E. coli O157	TECRA E. coli O157 visual immunoassay	Cheese, milk powder casein, whey products	ns	[49]
E. coli O157	VIDAS E. coli; Dynabeads	Raw milk cheeses, poultry, raw sausage, ground beef	ns	[50]
Listeria spp.	TECRA Listeria visual immunoassay	Lettuce, ice cream, fish fillets, cooked chicken, cooked ground turkey	ns	[51]
Listeria spp.	Palcam; VIDAS	Raw dairy, raw egg, raw fish, raw meat, raw vegetables	ns	[52]
Bacillus cereus enteroroxin	BCET-RPLA; TECRA	Noodles, spices, grains, legumes, legumes products, cooked foods	ns	[53]
Deoxynivalenol	RIDASCREEN DON; Veratox5/5 DON; Deoxynivalenol EIA; AgraQuant DON Assay 0.25/5.0 test kit	Beer, wheat	ns	[54]
Aflatoxin B1, B2, G1, G2	AgraQuant 4-40 ppb	Corn, corn meal, corn gluten feed, corn germ, corn gluten meal, corn/soy blend, popcorn, sorghum, wheat, milled rice, soybeans, peanuts, cottonseed	2.2–16.4 ppb	[55]

ns not specified

gold, copper, titanium, and chromium. In this assembly, a second critical angle exists which is greater than the angle of total reflection. At this angle, the surface plasmon resonance angle, a loss of light appears and the intensity of reflected light reaches a minimum. This results from the interaction of the incident light with oscillation modes of mobile electrons at the surface of the metal film. These oscillating plasma waves are called the surface plasmons. The electron oscillations resonate if the wave vector of the incident light aligns this wavelength (surface plasmon resonance). This resonance of incident light with the plasmons reduces the intensity of reflected light due to a loss of energy. The resonance frequency of the surface plasma and the critical surface plasmon resonance (SPR) angle depend on the dielectric coefficient of an adjacent layer on the metal surface. If this metal surface is coated by a thin layer of affinity ligands, the binding of biomolecules,

e.g., proteins, causes a change of the refractive index. This is detected by a shift in the resonance angle.

The frequently used Kretschmann configuration (Fig. 4) is based on a metal film which is evaporated on one face of a glass prism. The light is coupled into the prism above the critical angle of total reflection, and the resulting evanescent wave penetrates the metal film. The plasmons are excited at the outside of the film. SPR reflectivity measurements can be used for the detection of specific molecular interactions of bound receptor molecules on the metal surface with their corresponding targets (e.g., DNA or proteins) [58, 59].

Evanescent wave methods

If a light beam traverses from a material with a high index of refraction such as glass into one with a low index (water
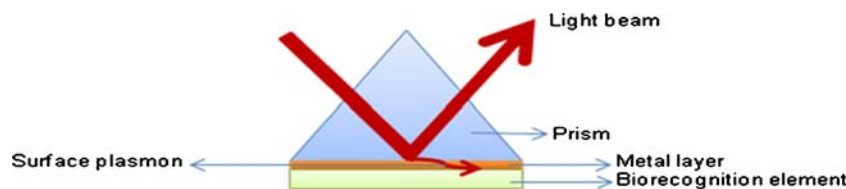
Fig. 4 Kretschmann configuration for SPR sensors

or liquids), a part of the light is reflected from the surface. If the light strikes the interface at a certain angle that is greater than the critical angle, it is completely reflected (total internal reflection). In this case, an evanescent wave propagates perpendicular to the interface into the surrounding medium of the lower refractive index. Its field strengths decay exponentially with penetration depths typically in the magnitude of the wavelengths of the incident light [58].

Evanescent wave sensors can be applied in different manners. Total internal reflection fluorescence uses the evanescent field for the excitation of fluorophores bound on the sensor surface [58]. The absorbance of the evanescent light by molecules close to the sensor surface leads to attenuated total reflection (ATR). This can be measured by the loss of the intensity of the reflected light coupled out from the ATR substrate. The resonance angle for the generation of evanescent waves is also an indicator of the changes of the refractive index of the sensor surface.

The optical waveguide lightmode spectroscopy (OWLS) technique bases on the measurement of the resonance angle of polarized laser light coupled into a thin wave guide. The incoupling resonance occurs at precise angles which depend on the optical parameters of the sensor chip and the refractive index of the covering sample medium. The sensor consists of an optical waveguide layer on top of a glass support which includes a fine optical grating. When a polarized laser beam strikes the grating, it is diffracted. The diffraction angle does not only depend on the optical parameters of the sensor but also on the refractive index of the cover medium. The waveguide is rotated around its axis, and when the diffracted beam is incoupled into the waveguide, it is propagating toward the edge of the sensor through multiple internal reflections. A photodiode measures the intensity of the incoupled light (Fig. 5). Light intensity versus angle of incoupling is following a sharp, resonant peak profile. A change in the effective refractive index due to the binding of analyte molecules on the sensor surface results in a shift of the incoupling resonance angle. Similar to the SPR technique, association and dissociation of analyte molecules on the sensor surface can be followed in real time without labeling by continuous measurement of change in the incoupling angle. The method can be applied for direct sensing of

various types of biomolecules if appropriate probes or receptors are immobilized on the waveguide.

Piezoelectric sensors

Quartz crystal microbalance Piezoelectric sensors represent another option to build comparatively cheap sensor instruments. These are based on the piezoelectric effect that appears in anisotropic crystals like quartz. They provide a label-free detection of molecular binding events in real time. The crystals generate oriented dipoles by means of pressure application. Accordingly, if external voltage is applied, a mechanical deformation can be monitored. This allows probing an acoustic resonance by electrical power. Application of an alternating current to the crystal induces oscillations, and a standing shear wave can be generated if a special cut crystal is placed between two disc electrodes. The oscillation frequency depends on the crystal thickness. Mass deposition on the surface of the crystal increases its thickness and the oscillation frequency decreases. The change of the frequency can be correlated with the mass change using the Sauerbrey equation:

$$\Delta f = \frac{-2f_0^2 \Delta m}{A\sqrt{\rho_q \mu_q}} = -2.3 \times 10^6 f_0^2 \frac{\Delta m}{A}.$$
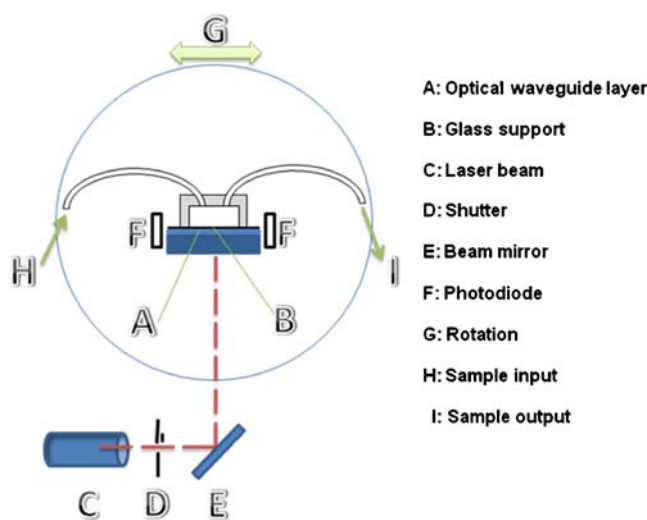

A: Optical waveguide layer
B: Glass support
C: Laser beam
D: Shutter
E: Beam mirror
F: Photodiode
G: Rotation
H: Sample input
I: Sample output

Fig. 5 Schematic arrangement of an optical waveguide lightmode spectroscopy immunosensor adapted from [160]

Accordingly, the change of the frequency, Δf, is direct proportional to the crystal's fundamental mode, f_0, and the bound mass, Δm, on the crystal. Furthermore, it is indirectly proportional to the piezoelectrically active area, A. ρ_q represents the density of quartz (2.648 g/cm^3) and μ_q the shear modulus of quartz (2.947×10^{11} g/cm×s^2) [60, 61].

PEMC sensors Cantilever biosensors exhibit the possibility of label-free protein and pathogen detection with high sensitivity. They can be divided into two categories: static bending or dynamic resonating cantilevers. Using the bending mode, the binding of antigens to the surface of the cantilever changes its surface stress and results in a deflection response proportional to the analyte concentration. In the dynamic mode, the attachment of antigen is causing a decrease in resonance frequency of the oscillating cantilever due to an increase in mass. Damping occurs if this mode is used in liquids. A less damped response in fluidic experiments can be achieved with millimeter-wide cantilevers. The piezo-excited, millimeter-sized cantilever sensor is a macro-cantilever comprising a piezoelectric layer (lead zirconate titanate, PZT) that is connected to a glass layer. The piezoelectric effect is used for the excitation of the cantilever vibration, and the piezo film also senses the response. The sensor is modified by antibodies or antigens in the case of biosensor applications. The resonance frequency decreases as target analytes bind to these receptors, and this can be measured by means of an impedance analyzer. The significant advantages of piezoelectric-excited, millimeter-sized cantilevers (PEMCs) are the employment of label-free reagents, the high sensitivity (in the range of femtograms (protein) per liter) in complex matrices, and that only little sample preparation is required [62, 63].

Electrochemiluminescence sensors

Electrochemiluminescence (ECL) is based on the production of light near an electrode surface by the generation of species that are capable of undergoing highly energetic electron transfer reactions. The process involves the formation of electronically excited states via electron transfer of electrochemically generated species. Customarily, ECL is originated from the annihilation due to an electron transfer reaction between a reduced and an oxidized species that are produced at an electrode by alternating pulsing (~3 V) of the electrode potential. It can be formed in a single step reaction with a co-reactant. Ruthenium complexes such as Ru(bpy)$_3$$^{2+}$ (bpy: 2,2'-bipyridine) are applied as light-emitting molecules, and tripropylamine (TPA) serves as co-reactant (Fig. 6). After its deprotonation, TPA acts as a reducing agent. In the following reaction of TPA with Ru(bpy)$_3$$^{3+}$, the Ru dye enters an excited state by high-energy electron light transfer from TPA.

ECL is a very attractive method for the detection of biomolecular interactions because of its high sensitivity. The ECL luminophore is bound to a receptor molecule such as an antibody, thus resembling commercially available radioactive or fluorescent labels. The luminophore does not require an excitation light source, and therefore, ECL is not interfered by luminescent impurities and scattered light [64]. Only the molecules that are in close proximity to the electrode surface are stimulated to luminescence.

Biosensors and sensor arrays for screening toxins and bacterial or fungal contaminants in food products

The applicability and acceptance of a sensor system is mainly determined by its robustness, the simple handling of the instrumental setup, and rapid sample preparation. It is of common interest in industry, governmental food control, and research to be able to resort to sensor devices which provide reliable measurement results. This includes that the sensor has been thoroughly evaluated and standardized operation protocols are available. When glancing on the multiplicity of different kinds of reported sensors, many results are obtained from calibrations in buffered aqueous solutions. A majority of the described assays have not been carried out in real food samples or still require some additional development or evaluation steps before they are applicable to real samples. Additionally, the study of matrix effects, cross-contaminations, and other interferences are crucial points that have not always been

Fig. 6 Mechanism of ECL generated from a ruthenium complex and TPA
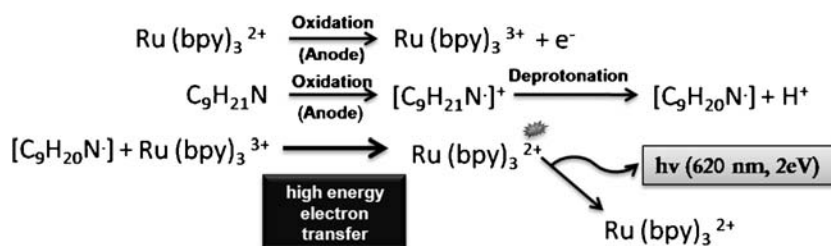
considered consequently. The stability and specificity of the used receptor systems is also a major problem. Finally, any new sensor system has to overcome a critical comparison with established analytical methods.

Sensors for bacterial toxins and spores

This section gives an overview of recent approaches for the detection of bacterial toxins and spores with significant impact on food safety and health using biosensors. These include *Bacillus anthracis* spores, *C. botulinum* neurotoxins (BoNTs), SEs, and aminoglycosides. Bacterial toxins are often the cause of foodborne illness. They particularly occur in dairy and fruit products. They can be produced in purified form and be used as food additives in terms of biological weapons. There is an urgent demand for rapid detection and quantification methods due to public health risks and the high number of samples.

B. anthracis spores

Bioterrorism which uses microorganisms as biological weapons has already been reported in history. It is reported that the dissemination of *Yersinia pestis* was used in the fourteenth century as one of the first bioweapons during the Mongolian siege of Kaffa on the Crimea peninsula. *B. anthracis* spores were deliberately released in 2001 through the United States postal system, which resulted in 22 cases of anthrax causing five cases of death. *B. anthracis* is a large Gram-positive (1–1.5×3– 10 μm), aerobic spore-bearing bacteria. It is an ideal organism to be employed as biological weapon. It forms spores that can be aerolized easily and survive under harsh conditions. Spores are not formed in host tissues unless infected body fluids are exposed to air. When nutrients are exhausted, resistant spores form that can survive in soil for decades. These spores then germinate when exposed to a nutrient-rich environment (blood or tissue of human or animal). Inhalation of the spores causes mortality rates near 100% [65–70]. The lethal dose LD_{50} is estimated to be about 2,500–5,000 inhaled spores [71]. Gastrointestinal anthrax results from ingestion of undercooked food from animals infected with *B. anthracis* (incubation period, 2– 5 days).

Fiber-optic immunosensor A portable evanescent-wave fiber-optic biosensor, the Research International Analyte 2000 fluorometer [72], can be used for the detection of *B. anthracis* spores in different products [73]. Biotinylated capture antibodies for *B. anthracis* were attached to a tapered streptavidin-coated polystyrene fiber-optic waveguide. The target analyte can be determined by means of a Cy5-labeled detection antibody. The instrumentation requires minimal sample preparation steps and an overall assay time of less than 1 h. No false positive results and a limit of detection (LOD) of 3.2×10^5 spores per milligram were obtained using this commercially available device.

Immunomagnetic sensor Lower LODs can be obtained by electrically active polyaniline-coated magnetic particles (EAPM). These can be applied as transducers in a biosensor that enables LODs of 4.2×10^2 spores per milliliter in lettuce or beef and 4.2×10^3 spores per milliliter in milk samples [74]. The sensor is based on a sandwich immunoassay with capture antibodies immobilized on a nitrocellulose membrane pad and a detector antibody conjugated to EAPMs with a diameter of 100 nm. The particles are used for immunomagnetical concentration of spores in the food matrices and applied to the sample admission pad subsequently. The antigen–antibody–EAPM conjugate flows to the capture pad and the sandwich complex is formed. The conductive EAPM nanoparticles in the sandwich act as a voltage-controlled "ON" switch, resulting in decreased resistance across the silver electrode, which is recorded electrically. The mode of operation of the biosensor is summarized in Fig. 7.

Piezoelectric sensors In comparison to fluorescent or electrochemical detection modes, cantilever sensors are devoid of unfavorable effects like fluorophore quenching, noise, or high background signals. Monitoring of *B. anthracis* spores in water was achieved by means of a microresonator mass sensor (microcantilever sensor) functionalized with capture antibodies. These enable a selective absorption of the analyte on the surface [75]. The quantification is based on the measurement of the decrease of the resonance frequency as a result of the added mass of the bound spores by means of a laser Doppler vibrometer. A minimum of 50 spores (139 pg) can be determined using a cantilever of 20-μm length, 9-μm width, and 200-nm thickness.

A complete sensing assay of Anthrax spores in liquids was done in 20 min with an antibody-modified PEMC sensor in a flow cell [76]. PEMC immunosensors provide mass change sensitivities in the range of picograms to subfemtograms [77, 78]. The glass substrate is modified with antibodies using silane monolayers and EDC-NHS coupling. It is exposed to a pre-concentrated sample in phosphate-buffered saline, and the resonance frequency is measured directly in the sample containing the toxin. The resonance frequency decreases linearly with the bound mass in the case of small mass quantities. Quantitative determination of bound spores can be carried out by measuring the change in resonance frequency with an impedance analyzer. A detection limit of 38 spores per liter sample was attained.
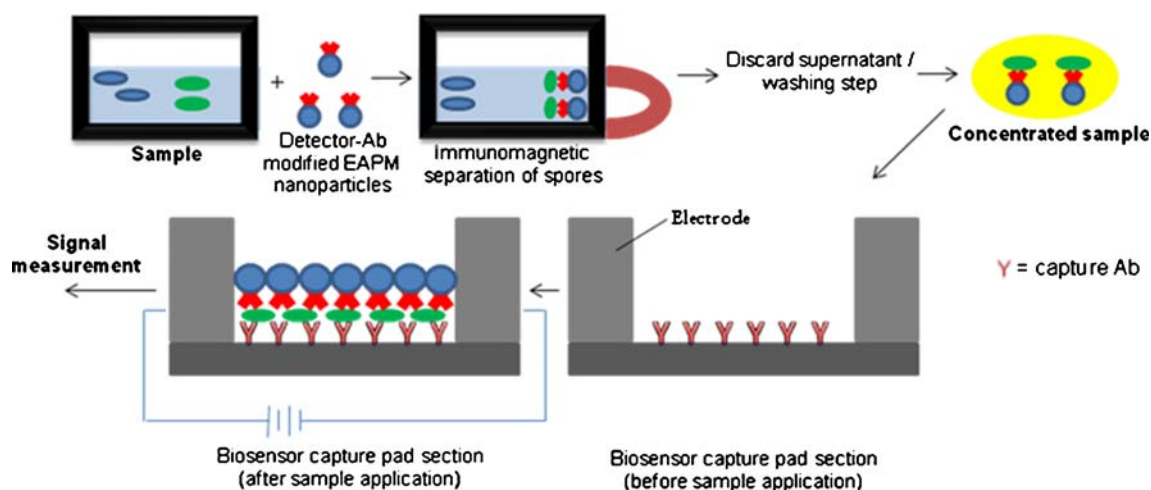
Fig. 7 Biosensor architecture (*A*) and schematic representation of the biosensor system (*B*) adapted from [74]

C. botulinum toxins

BoNTs are considered as one of the most hazardous types of toxins for humans with a lethal dose of 1 μg toxin per kilogram body mass. The seven serologically dissimilar toxins A–G are produced by *C. botulinum* bacteria [79]. They selectively target cholinergic nerve endings and act as zinc-dependent endoproteases. BoNTs cleave proteins involved in the release of acetylcholine, resulting in flaccid paralysis or death [80].

The majority of bacterial strains only produce one type of toxin, but some strains are capable of multiple BoNT release [81]. Botulism has been involved in many food contamination cases. Products like meat, fish, dairy, sautéed onions, chopped garlic in oil, and preserved vegetables are mostly affected. Estimated 58 cases of botulism are documented in the USA per year. Among these, 80% of all cases require hospitalization. Hence, foodborne botulism is rare, but fatal. Contamination of food or drink with microbial toxins is not only a form of a bioterrorism but also a global public health problem in general [82]. Rapid detection of exposure to BoNTs is considered as an important public health goal [83]. A LOD of 40 pg/mL or less has been recommended because of the high toxicity of BoNTs [84, 85].

Surface plasmon resonance sensor The SPR technique allows a quantitative real-time detection of biomolecular interactions including immunological assays. It is a label-free method and therefore avoids unwanted interferences of the fluorescent label with the antibody-antigen recognition. SPR is an appropriate alternative to ELISA methods capable of multi-analyte and multiplexed sample screening. A four-channel SPR biosensor employing wavelength modulation has been reported for the determination of BoNT A, B, and F in honey solutions [86]. A mixed self-

assembled monolayer of biotinylated alkanethiol and oligo (ethylene glycol)thiol is formed on a gold surface which serves as binding matrix for streptavidin and, subsequently, biotinylated capture antibodies. The assay provides an LOD of 5 ng/mL for each type of toxin after 1-h incubation time.

Hydrogel sensor Toxin-responsive hydrogel sensors are an additional alternative to common detection methods used in order to improve the sensitivity of *Botulinum* neurotoxin A determination. The response of the hydrogels relies on the enzymatic activity of the toxin and provides a high selectivity as the toxin includes a specific substrate cleavage site. The sensor is based on a toxin-sensitive, peptide-modified hydrogel layer and requires only 20 μL of contaminated sample for visual analysis of buffer, milk, and cream samples. Peptide-modified hydrogels can dissolve in the presence of proteolytic enzymes. They represent an optimized matrix for sensing a toxin possessing enzymatic activity because of their simple fabrication and application. Toxin contamination is indicated in visually evident gel dissolution. The degradation of the hydrogel can be monitored by stereoscope imaging after removal of the supernatant solution [87].

Immunomagnetic–electrochemical sensor Novel assays using a paramagnetic bead-based ECL technology were used for the detection of botulinum neurotoxins A, B, E, and F in milk, apple juice, ground beef, pastry, and raw eggs [88]. Biotinylated serotype-specific antibodies bound to streptavidin-coated beads act as solid support and capture the target from sample solutions. Analysis was carried out with a BioVeris M-Series M1R analyzer [89] based on the use of ruthenium chelate-labeled antibodies. On the surface of the working electrode, photon-emitting species are generated from stable precursors by anodic oxidation in

the presence of TPA. LODs of 50 pg/mL of BoNT A and 800 pg/mL of BoNT F could be obtained.

S. aureus enterotoxins

S. aureus are Gram-positive bacteria that produce enteric toxins and grow in animal food products at ambient temperatures [90]. S. aureus preferentially grows in foods with low water activity (cooked or strong salted food) where they outcompete other microorganisms [91]. Outbreaks are frequently traced back to situations where food preparers did not comply with hygiene and safety regulations or to incidences where food, that has to stay frozen, was exposed to room temperature. Unfortunately, alignments containing enterotoxins usually taste, smell, and look unsuspicious and not adulterated [92]. SEs cause severe gastroenteritis with symptoms like diarrhea and vomiting within 1–6 h after ingestion and act as superantigens that stimulate non-specific T cell proliferation [93]. Enterotoxins are very heat-stable and resistant to cooking, heating, and proteolytic decomposition; thus, only the bacterial cells will be inactivated or killed during meal digestion [94–96]. Ten different SEs have been identified (A, B, C1, C2, C3, D, E, H, I, G, and J) to date [97–99]. Among these, SEA-SED are the most common food pathogens. Particularly, enterotoxin A is involved in most of the disease outbreaks, whereas staphylococcal enterotoxin B (SEB) is a potential bioterrorism agent. Their physical and chemical properties were summarized by Jay et al. [100]. SE levels of 1 ng/g food were reported to cause gastroenteritis in the case of adult persons [101, 102]. Thus, assays which are able to determine enterotoxin concentrations below 1 ng/g (1 ppb) reliably are required to guarantee the safety of food.

Fluorescent immunosensors Dong et al. [103] have shown that microfluidic immunosensors for SEB can be integrated in polydimethylsiloxane (PDMS) chips. Supported phosphatidylcholine bilayer membranes were generated in oxidized PDMS microchannels by vesicle fusion. They minimize the non-specific binding of biomolecules. A streptavidin layer was attached to the membranes which enables the immobilization of biotinylated antibodies. This functionalization results in reinforced supported bilayer membranes including the immobilized antibodies as recognition units for SEB. Their detection occurs via primary and, subsequently, Cy3-labeled secondary antibodies. A linear range of 1–1,000 ng/mL with a LOD of 1 ng/mL was obtained in milk samples.

The rapid automated particle immunosensor from Sapidyne [104] can be applied to analyze SEB contaminations in cream. Polymethylmethacrylate (PMMA) beads conjugated to antibodies were applied as the solid phase of the flow-through device and trapped by a filter. Reagents for the assay could float the beads by means of a rotary valve. Fluorescein-labeled target antibodies are used for detection. The device is capable of determining 5 ng/g of SEB in approximately 10-min incubation time [105].

Surface plasmon resonance sensors The immunological determination of SEB in milk can be carried out with a wavelength modulation-based SPR sensor using two different operation modes, either by direct toxin detection or a sandwich assay. The sandwich assay mode uses secondary antibodies that bind to the captured target toxins in order to amplify the attained response. SEB concentrations of as low as 0.5 ng/mL could be determined in milk samples [106]. The BIACORE SPR biosensor [107] can be adapted for the analysis of SEA and SEB in various matrices and numerous conjunctions.

The multitoxin bioapplication interaction mass spectrometry (BIA-MS) is a method which has been adapted for the detection of SEB, e.g., in milk and mushrooms [108]. A BIACORE X instrument was combined with a sensor chip in a flow cell. Capture antibodies were immobilized on the carboxymethyl dextran-derivatized surface of the sensor chip to determine the toxin concentration in the sample via SPR, which is followed by the identification of the bound toxin by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. A LOD of 1 ng/mL SEB in milk and mushrooms can be obtained.

An extended BIA-MS with two flow cells containing anti-SEB or anti-toxic shock syndrome toxin-1 was performed with the BIACORE X instrument and an antibody-derivatized sensor chip with an LOD of 1 ng/mL SEB in mushrooms [109].

Competitive immunoassays are also feasible with an upgraded BIACORE device employing two different sensor chips. SEB was covalently linked to the chip surface. The surface-bound toxins compete with the free toxins in the sample for the antibody binding sites. Antibodies bound to toxin molecules from the sample are washed away and the mass increase on the surface measured. A LOD of 312 pg/mL of SEB in fresh whole milk and skimmed milk could be achieved [110]. SEA was verifiable in a similar approach in spiked raw egg supernatants with an LOD of 1 ng/mL and a dynamic range of 1–40 ng/mL within 15-min of assay time [111].

Portable fiber-optic sensors Portable fiber-optic biosensors (Analyte 2000, see "*B. anthracis* spores") can also be applied for SEB determination [112]. This compact and lightweight biosensor system is capable of running four tests simultaneously by means of using four individual fiber probes. Light from a 635-nm diode laser is coupled into a tapered 600-μm fiber. Fluorescent molecules within 100-

nm distance from the fiber can be excited evanescently (see "Evanescent wave methods") and a portion of the fluorescence emission recouples into the fiber. The fibers were silanized and a heterobifunctional linker was attached for binding the rabbit anti-SEB capture antibody. Cy5-labeled sheep anti-SEB detection antibodies were added after incubating the fiber with aqueous ham extracts. The sandwich assay forms a fluorescent complex at the surface of the fiber, generating only signals in the reach of the evanescent field. The assay can be performed within 45 min with a LOD of 0.5 ng/mL of SEB in aqueous ham extract.

Piezoelectric sensors An approach by Lin and Tsai [113] uses an AT-cut quartz crystal (8 mm in diameter) which was coated on both sides with gold electrodes (4 mm in diameter) as transducer element. The crystal surface was covered with a polyethylenimide layer and anti-SEB capture antibodies were subsequently cross-linked with glutaraldehyde. The piezoelectric sensor was exposed to samples containing SEB for 1 h and the resonance frequency measured. A LOD of 2.5 μg/mL of SEB in spiked milk samples was attained.

PEMC (see "Piezoelectric sensors") cantilever sensors that are excited by piezoelectric actuators are reliable and sensitive devices for the quantification of SEB. They were applied to beverage samples such as apple juice and milk. The LOD in milk was reported to be 10 fg of toxin without any sample preparation steps. The sensor device is composed of PZT cantilevers on glass slides. These were consecutively coated with polyurethane, gold, and protein G for selective anti-SEB binding. The assays can be performed in a flow cell [114].

Chemiluminescence sensor A new integrated sensor combining three detection elements—carbon nanotubes as support for the immobilization of primary antibodies, enhanced chemiluminescence excitation for signal generation, and a simple cooled charge coupled device (CCD) detector—has been described by Yang et al. [115]. Antibodies against SEB attached to nanotube surfaces were immobilized onto a polycarbonate surface. SEB was detected by a sandwich-type ELISA. This combination of sensor device and chemiluminescent ELISA provides a LOD of 10 pg/mL of SEB in soy milk, apple juice, and meat baby food.

Immunomagnetic–electrochemical sensors Sensitive and rapid detection of SEB in 5% skim milk in PBS can be performed by a combination of immunomagnetic separation and electrochemiluminescent detection using the ORIGEN system [89]. An assembly of biotinylated antibodies immobilized on streptavidin-coated magnetic beads was used for capturing the antigens from the sample solution.

The magnetic beads were fixed in the electrochemical flow cell of the ORIGEN system. The detector antibody was conjugated to ruthenium(II)-tris-bipyridine and binds to the immunocomplex in the presence of toxin antigens. Light is generated electrochemically after addition of an excess of TPA which is oxidized at the surface of the anode (see "Electrochemiluminescence sensors"). The resulting chemiluminescent emission of photons can be detected at 620 nm with a linear range of 7 logs using a photomultiplier tube. This method requires a sample incubation time of 30 min and an assay time of 1 min. It assures fast results with 100% specificity, 98% sensitivity, and an LOD of 1 pg/mL [116]. The reaction scheme is illustrated in Fig. 6.

Evanescent wave biosensor The evanescent wave biosensor IAsys [117] was applied to analyze SEA in potato salad, canned mushrooms, hot dogs, and dry milk in order to develop a real-time detection system for the identification of foodborne toxins. Carboxymethyl dextran-coated cuvettes were functionalized with capture antibodies. A sandwich-type assay can be carried out within 4 min measuring the change of the refractive index of the sensor surface. The sensitivity is in the range of 10–100 ng/g depending on the type of food sample [118]. An overview of sensor formats for bacterial toxins and spores is shown in Table 3.

Sensors for contaminations by aminoglycosides

Aminoglycosides are broad-spectrum antibiotics most commonly used in veterinary drug medicine for the treatment of infections such as mastitis [119]. Gentamycin (GTM) is used for the therapy of bacterial infections in pigs and cattle, while neomycin (NEO) is indicated to treat gastrointestinal infections of sheep, pigs, goats, cattle, and poultry and applied in case of mastitis. Streptomycin and its derivate, dihydrostreptomycin (DHS), are used to treat bacterial diseases in sheep, pigs, poultry, and cattle. DHS is also administered in combination with benzyl penicillins. Unfortunately, all aminoglycosides present some toxicological adverse reactions like nephrotoxicity and ototoxicity. Additionally, allergic reactions can be initiated by means of aminoglycoside residues [120]. Injections of these substances in cattle produce aminoglycoside residues that are a potential cause of food contamination [121, 122]. Maximum residue limits (MRLs) have been constituted due to the consumer risk connected with the presence of antibiotics in food. The MRLs of aminoglycosides in food defined by the European Union range from 100 ng/mL (GTM) to 500 ng/mL (NEO).

Generally, screening of these substances is mainly performed by microbial tests [123], immunoassays [124,

Table 3 Overview of sensor methods for the detection of bacterial toxins and spores

Analyte	Sensor type	Food matrix	LOD	Ref.
Anthrax spores	Portable evanescent wave fiber-optic sensor	Talc-based baby powder, cornstarch-based baby powder, sugar, baking soda	3.2×10^5 spores/mg	[73]
Anthrax spores	Biosensor with electrically active polyaniline coated magnetic (EAPM) nanoparticles	Lettuce, ground beef, whole milk	4.2×10^2 spores/mL (lettuce, beef) 4.2×10^3 spores/mL (milk)	[74]
Anthrax spores	PEMC	Air, water	50 spores/139 pg	[75]
Anthrax spores	PEMC	Air, liquid	38 spores/L	[76]
BoNT A, B, F	SPR	Honey	5 ng/mL	[86]
BoNT A	Toxin-responsive hydrogel sensor	Milk, cream	Visual	[87]
BoNT A, B, E, F	Paramagnetic bead ECL	Milk, apple juice, ground beef, pastry and raw eggs	50–800 pg/mL	[88]
SEB	Microfluidic immunosensor	Milk	1 ng/mL	[102]
SEB	Fluorescence-based particle sensor (Sapidyne)		5 ng/g	[103]
SEB	Wavelength-modulated SPR	Milk	0.5 ng/mL	[105]
SEB	SPR-BIA-MS (BIACORE X)	Milk, mushrooms	1 ng/mL	[93]
SEB	SPR-BIA-MS (BIACORE X)	Mushrooms	1 ng/mL	[107]
SEB	SPR (BIACORE 1000)	Milk	0.312 ng/mL	[108]
SEA	SPR (BIACORE 1000)	Raw egg	1 ng/mL	[109]
SEB	Portable fiber-optic sensor	Ham extract	0.5 ng/mL	[110]
SEB	Piezoelectric crystal immunosensor	Milk	2.5 μg/mL	[111]
SEB	PEMC	Milk, juice	10 fg (juice) 100 fg (milk)	[112]
SEB	Carbon nanotube ECL sensor	Soy milk, apple juice, meat baby food	0.01 ng/mL	[113]
SEB	IMS-ECL-ORIGEN system	5% skimmed milk	1 pg/mL	[114]
SEB	IAsys	Potato salad, canned mushrooms, hot dogs, dried milk	10–100 ng/g	[116]

125], and liquid chromatography [126]. A review of current methodologies for the detection of aminoglycosides is presented by Stead [127].

Surface plasmon resonance sensors Different approaches for the detection of streptomycin and other aminoglycosides have been performed on the BIACORE SPR biosensor system (see last section). Competitive immunoassays for the detection of streptomycin and dihydrostreptomycin residues in whole milk, honey, pig muscle, and kidney have been described. A functionalized sensor chip containing a covalently bound carboxylated dextrane matrix on a gold surface was coated with a streptomycin derivative. A fixed concentration of antibody was mixed with the sample and injected over the sensor chip surface. Cross-reactivities were evaluated by means of samples spiked with structurally related compounds and commonly used antibiotics such as dihydrostreptomycin, neomycin, gentamycin, virginiamycin, kanamycin, penicillin G, sulfmethazine, or chlortetracycline. No significant cross-sensitivity higher than 0.1% could be observed, except with dihydrostreptomycin. The LODs were determined from spiked samples.

They range between 30 and 70 μg/kg for the different food matrices surveyed [128]. Furthermore, the BIACORE SPR systems were evaluated in an interlaboratory study with 14 participants. The analysis results of 12 samples spiked with streptomycin and dihydrostreptomycin were compared to results obtained by commercial ELISA kits [129].

Another competitive immunoassay using SPR sensors with four flow channels and a mixture containing four specific antibodies was applied to reconstituted skimmed milk [130]. Five relevant aminoglycosides (streptomycin, gentamycin, neomycine, kanamycin, and a streptomycin derivative) were determined in four single inhibition assays on a sensor chip containing immobilized haptens in different flow channels. All detection limits were found to be below the maximum residue limits (100–500 ng/mL) varying between 15 and 60 ng/mL.

Hapten microarray A regenerable immunochip for the rapid determination of 13 different antibiotics (among others sulfamethazine, streptomycin, penicillin G, gentamycin, enrofloxacin, and tetracyclin) in raw milk was presented by Kloth et al. [131] using an automated

microarray platform reader. The chips consist of epoxylated glass slides which are coated with polyethylene glycol diamine and a diepoxy-polyethylene glycol layer. The latter couples antibiotic haptens to the chip surface. Antibiotic levels can be quantified by means of a competitive immunoassay where free antibodies were added to the sample. Signal generation occurs via chemiluminescent reaction of HRP-labeled secondary antibodies with a luminol-based substrate. The detection of the 13 analytes can be performed by means of a CCD camera in less than 6 min. An overview of amino-glycoside biosensors is given in Table 4.

Sensors for mycotoxins

Mycotoxins represent a group of rather chemically different compounds produced by fungi, particularly *Penicillium*, *Fusarium*, and *Aspergillus* species, which grow on organic matter. Nuts, cereals, oil seeds, or fruits are mainly affected because of their high moisture content and richness of nutrients. The major exposure routes are the intake of contaminated food, inhalation of spore-borne toxins, or dermal contamination by skin contact. The extent of contaminations by toxigenic mold species depends on several factors, e.g., temperature, storage conditions, or humidity [132]. The most significant toxins are aflatoxins, deoxyniva-lenol, ochratoxin, and fumonisins. An overview of their chemical structures is given in Fig. 8.

The appearance of aflatoxins is quite common while other mycotoxins are rare. They are all highly toxic metabolites produced by *Aspergillus* species. Up to now, 18 different types are identified, with aflatoxin B1, B2, G1, and G2 (AFB1, AFB2, AFG1, and AFG2) as the most important ones [133, 134]. Aflatoxins are polycyclic
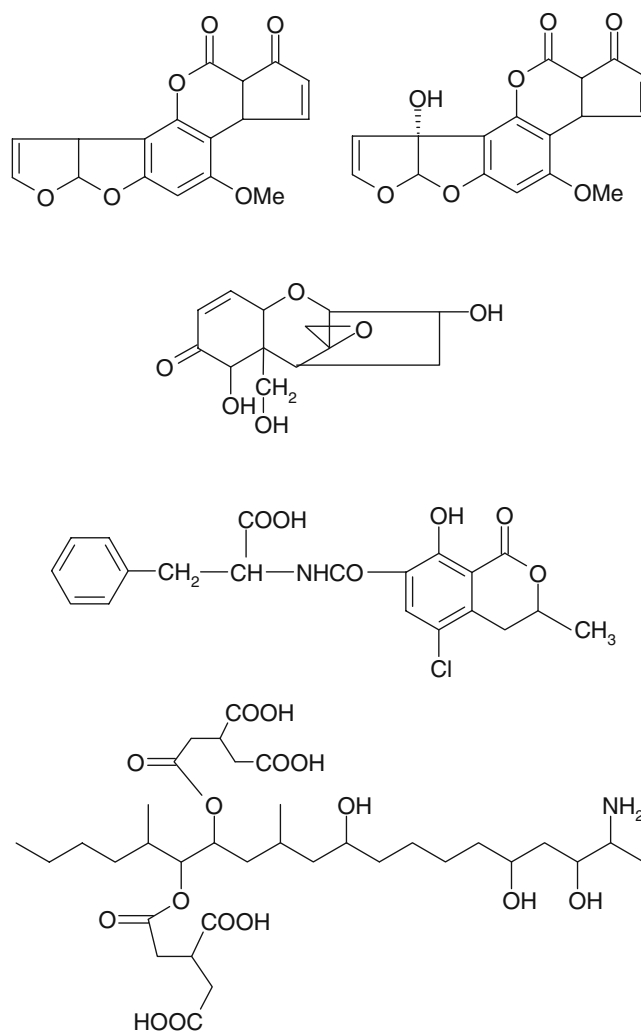

Fig. 8 Chemical structures of the most important mycotoxins AFB1, AFM1, OTA, and DON

Table 4 Overview of sensor methods for the detection of amino-glycosides

Analyte	Sensor type	Food matrix	LOD	Ref.
Streptomycin	BIACORE Q	Milk, honey, kidney, muscle	30, 15, 50, and 70 μg/kg	[126]
Streptomycin S-Derivate	BIACORE Q	Bovine milk	ns	[29]
Streptomycin Gentamycin Kanamycin Neomycin S-Derivate	BIACORE 3000	Skimmed milk	15–60 ng/mL	[127]
Antibiotics	Munich chip reader platform MCR3	Raw milk	ns	[128]

ns not specified

compounds which fluoresce strongly in UV light. The International Agency for Research on Cancer (IARC) has classified all four aflatoxins as group 1 carcinogens [133]. The maximum levels for aflatoxins set by the European Commission are 2 ng/g for AFB1 and 4 ng/g for total aflatoxins in corn, groundnuts, nuts, dried fruit, and cereals [135]. AFB1 is the most prevalent and biologically active toxin, and its carcinogenicity is related to the possibility of forming adducts with DNA [136].

When aflatoxin B1 is ingested by cows, excretion of the hydroxylated product aflatoxin M1 (AFM1) in milk occurs. Although it exhibits lower toxicity, this toxin is hazardous because of its hepatotoxic and carcinogenic effect. Unfortunately, it is relatively heat-stable and therefore resistant during pasteurization and dairy production processes. The legal limit defined by the European Union is 0.05 mg/kg for AFM1 in milk [137–141].

Ochratoxin A (OTA), a coumarin derivate, is produced by several *Aspergillus* and *Penicillium* molds [142]. It contaminates mainly cereals, but also coffee, dried fruits, grape juice, wine, beer, and nuts. OTA is considered as a nephrotoxin, hepatotoxin, carcinogen, and teratogen. The IARC has classified OTA as possibly carcinogenic in humans (group 2B) [143]. The admissible limit for OTA in cereal products for human consumption is defined by the European Commission as 3 µg/kg.

Another relevant group of mycotoxins, Fumonisins, are released by *Fusarium* species. They also exhibit a potential risk for humans and animals [144]. Twelve Fumonisins are identified; among these, Fumonisin B1 (FB1, macrofusin) is the most prevalent and toxic compound. Fumonisins are hepatotoxic and classified as potential carcinogenic agents. The European Union prescribes a maximum tolerable daily intake of 2 µg/kg body mass [145]. Deoxynivalenol (DON) is another hazardous substance produced by *Fusarium* in moderate climate areas. Its tolerable daily intake is proposed by the European Scientific Committee on Food as 0.5 µg/kg bodyweight [146].

Electrochemical sensors A simple and fast disposable electrochemical immunosensor using differential pulse voltammetry (DPV) has been described for the detection of AFB1 in barley. A graphite working electrode was coated with a BSA-AFB1 conjugate following a blocking step. Monoclonal AFB1 antibodies were added to the sample which is exposed to the electrode, forming a competitive assay. A secondary AP-labeled antibody was used to detect the bound target antibodies. 1-Naphthol was detected by means of DPV in a typical three-electrode cell, which is a product of the enzymatic conversion of 1-naphthylphosphate. A LOD of 2 ng of AFB1 in 1 g of barley could be obtained [147].

A similar approach includes disposable screen-printed electrode systems (SPEs) for the quantification OTA in wheat extract. A direct electrochemical ELISA is approached using a typical three-electrode assembly. Therefore, an OTA-AP conjugate is added to the sample. These compete for the binding to the monoclonal capture antibodies immobilized on the electrode surface. The AP activity again was measured by DPV recording the enzymatic formation of 1-naphthol. The method exhibits a detection limit of 400 ng/kg wheat sample [148].

An indirect competitive electrochemical immunosensor for OTA uses avidin-coated microwell plates. These were incubated with biotinylated OTA and blocked with skimmed milk. The competition reaction was carried out by incubation of OTA-contaminated samples with anti-OTA. This mixture was disposed to the wells followed by addition of AP-conjugated secondary antibodies. Their activity was measured by means of SPEs as described

above using DPV or chronoamperometry. Concentrations of 10 ng/mL of OTA in wine samples could be monitored [149].

Multichannel electrochemical detection systems can also be integrated into microwell plates. These immunosensor arrays have turned out to be a useful tool for the determination of mycotoxins such as AFB1 in corn samples [150]. Measurements were performed with an indirect ELISA scheme in 96-well, screen-printed plates. The surfaces of the SPEs were coated with AFB1-BSA conjugate. A mixture of polyclonal antibodies and samples containing AFB1 is poured into the wells followed by the addition of a secondary AP-labeled antibody. In this case, the amount of the enzymatically formed 1-naphthol was detected by means of intermittent pulse amperometry. AFB1 levels of 30 pg/mL can be determined in a dynamic range between 0.05 and 2 ng/mL.

Volatile components of mycotoxin contaminations can be detected with the help of electronic noses. These can quantify DON and OTA, e.g., in barley grains. A volatile compound mapper is composed of ten metal oxide semiconductor field effect transistor sensors, six SnO_2-based gas sensors, and a Gascard O_2 monitor. The artificial nose requires only 90 s of effective measurement time and can be considered as a real alternative to conventional single sensors if only semiquantitative results are required (e.g., if the OTA concentration is below or above a certain threshold) [151].

Surface plasmon resonance sensors A SPR-based screening technique for AFB1 and AFG1 in maize is demonstrated by Cuccioloni et al. [152]. This biosensor represents a new approach for the detection of aflatoxins based on the reversible interaction between elastase, a proteolytic enzyme, and the soluble toxin ligand in the sample. Elastase was immobilized on functionalized cuvettes in a thermostated sensing chamber and AFB1 and AFG1 reference standards were added in different concentrations. Association kinetics were followed up to equilibrium. After regeneration of the sensor surface, aflatoxin-containing samples were added and each response was routinely followed and analyzed. The major advantages of this method are its relative rapidity, the reusability of the surface, and the low cost per single test. The LOD for AFB1 was 970 ng/kg.

Tudos et al. [153] used a BIACORE SPR system to monitor DON in contaminated wheat and dry wheat powder. The sensor surface was modified by a casein–DON conjugate. An excess of DON antibodies were added to the sample to capture DON. Subsequently, the mixture was exposed to the sensor to perform a competitive assay. Binding of free antibody is recorded by means of SPR. The sensor could be reused more than 500 times without

significant loss of sensitivity, providing a dynamic range of 2.5–30 ng/mL for DON determination.

The BIACORE system [107] was also adapted for multiplexed analysis of mycotoxins in food. A competitive assay format is capable of determining DON, FB1, AFB1, and OTA in different samples in one measurement with the help of four flow channels. After sample extraction and incubation with a mixture of the corresponding antibodies, the results can be obtained within 25 min. LODs of 0.5, 0.2, 50, and 0.1 ng/g could be achieved for DON, FB1, AFB1, and OTA, respectively [154].

Fiber-optic and portable immunosensors A portable immunosensor for the quantification of AFM1 in milk samples was developed by Sibanda et al. [155]. It consists of a nylon membrane modified with a secondary antibody. After immobilization of capture antibodies and incubation of the sample, a detector antibody conjugated to HRP was added. TMB is used as enzyme substrate, and colorimetric readout yields a LOD of 0.05 ng/mL of AFM1 in milk samples.

Fiber-optic waveguides can be modified with monoclonal FB1 capture antibodies. Fluorescein-labeled FB1 was added to the sample to carry out a competitive assay. The sensor works in a dynamic range from 10 to 1 μg/mL of FB1, and a LOD of 10 ng/mL was attained in ground corn [156].

Fiber-optic fluorescent sensors based on evanescent wave excitation were demonstrated by means of FB1 and AFB1 determination in maize. Again, a competitive format was applied. It uses monoclonal capture antibodies attached to a silica fiber. In this case, FITC-conjugated AFB1 was added to the sample and its fluorescence is measured after exposure to the fiber. The LOD in methanolic extracts was 3.2 μg of FB1 in 1 g maize. AFB1 was detected in parallel with a direct assay via its intrinsic fluorescence [157].

The details of the Naval Research Laboratory (NRL) multi-analyte array biosensor have been already introduced in the last sections. It provides also a powerful platform for the screening of mycotoxins. Barley, wheat pasta, corn-flakes, ground barley, cornmeal, red wine, and roasted coffee were screened for OTA contaminations without extensive sample pretreatment. The NeutrAvidin-coated surface is used for the immobilization of biotinylated Ochratoxin A molecules. These compete with the free toxin in the sample extract for binding to the added Cy5-conjugated OTA antibodies. Quantification occurs by the measurement of the formation of fluorescent OTA–anti-OTA–Cy5 complexes on the waveguide by CCD imaging. The net assay time was approximately 30–40 min. The LODs in different food matrices range from 3 to 100 ng/g [158]. The same type of assay can be performed for the determination of DON [159].

Intrinsic fluorescence sensors A label-free method for the optical determination of OTA contaminations is based on silica sensor spots with integrated photodiodes as detectors. These monitor silica-bound OTA by means of its intrinsic fluorescence under UV radiation. The sensor assembly consists of a thin-layer chromatography plate which is placed on a transparent conductive oxide material with integrated a-Si:H photodiodes. The intrinsic fluorescence of the mycotoxin can be excited by UV radiation at 253 nm. The device is capable of detecting 0.25 ppb of toxin in a sample volume of 10 mL (Fig. 9) [160].

The fiber-optic immunosensor for AFB1 described by Carter et al. [161] utilizes either its intrinsic fluorescence or competitive assays with labeled capture antibodies. Intrinsic fluorescence detection is carried out after binding of AFB1 to an antibody-functionalized polymer membrane. A LOD of 50 pg/mL of AFB1 in corn and peanut extracts could be detected. The excitation and emission wavelengths were 362 and 425 nm, respectively. The competitive assay requires enzyme or fluorescent-labeled capture antibodies. In the latter case, AFB1 probes were immobilized on a cellulose membrane. These compete with the free toxins in the sample for the binding to the added FITC-labeled antibodies. This assay has a LOD of 1 ng/mL of AFB1 in peanut extracts.

Immunosensors integrated in FIA systems The analysis of mycotoxins in milk can be performed using a flow injection

Fig. 9 Schematic view of a sensor for detection of intrinsic fluorescence with an integrated photodiode [157]
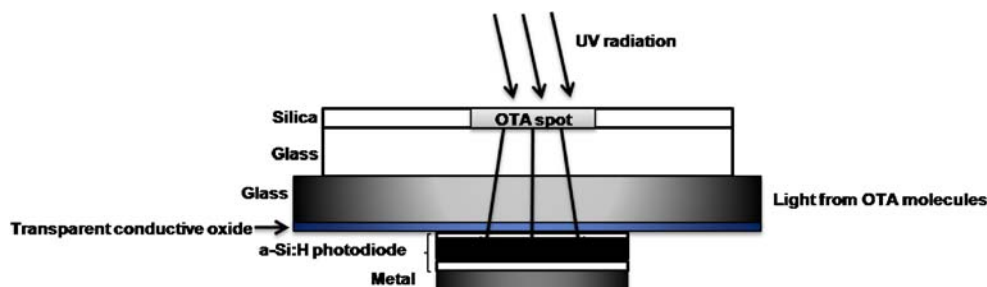
immunoassay (FIA) method with amperometric detection. Badea et al. [162] mixed milk samples with defined amounts of anti-AFM1 and AFM1-HRP conjugate. AFM1-HRP acts as tracer in a competitive assay format. The mixture was injected to the flow system where the antibody-bound tracer is separated from the free tracer via a protein G-modified column. The activity of the eluted enzyme label was detected amperometrically using TMB as substrate.

Optical waveguide lightmode spectroscopy (see "Evanescent wave methods") was also shown as a promising approach to determine AFB1 and OTA in food samples such as barley and wheat flour [163]. OWLS uses the evanescent field technique for in situ and label-free studies of surface processes at molecular levels (see Fig. 5). The OWLS sensor chip can be integrated in a flow injection analysis system after immobilization of antigen conjugate. The sample was applied with antibodies and incubated for 1 min following injection to the system. A competitive assay format can be used for the simultaneous detection of OTA and AFB1. Hapten-BSA conjugates are immobilized on the waveguide as capture probes. Standards and samples containing the mycotoxin of interest are mixed with monoclonal antibodies and injected in the OWLS system.

Hapten microarray A chemiluminescence-based, flow-through microarray system for the detection of OTA and aflatoxins was presented by Cervino et al. [164]. A hapten microarray combined with highly selective monoclonal detector antibodies and an automated microfluidic system with chemiluminescence readout enable the identification of aflatoxins in the sub-micrograms per kilogram range in complex raw food extract matrices without prior cleanup. A self-constructed microfluidic platform was used for the automated competitive immunoassay sensor. The haptens were bound to PEGylated and epoxy-modified glass slides. The detection of the bound primary antibody was performed by means of an enzymatic chemiluminescent reaction. The luminescence intensity was measured with a CCD camera. The different sensor approaches for mycotoxin detection are summarized in Table 5.

Sensors for plant toxins

Ricin is a highly toxic plant toxin which can be extracted from the beans of the castor oil plant, *Ricinus communis*. This toxic lectin is thousand times more poisonous than cyanide and 30 times more potent than nerve gases and can be used to contaminate food or water. Ricin is a glycoprotein with a molecular weight of 60–65 kDa and consists of two glycoprotein chains. Its ribotoxic subunit inhibits protein synthesis in mammalian cells. [165–173].

With an estimated lethal dose of 1–10 μg/kg body mass, ricin leads to death within approximately 60 h [174]. Ricin is significant as a potential chemical warfare agent and a weapon of mass destruction for everyone because of its high availability and listed by the Chemical Weapons Convention (CWC).

The detection of ricin in water was carried out by a magnetoelastic sensor integrated in a sealed disposable container [175]. The sensor consists of $Fe_{40}Ni_{38}Mo_4B_{18}$ coated with Cr and Au layers. The latter was modified with cystamine and the cross-linker glutaraldehyde which enables the covalent coupling of anti-ricinus capture antibodies on the surface. A sandwich assay with AP-conjugated anti-ricinus detector antibodies is used in combination with a precipitation reaction. AP converts 5-bromo-4-chloro-3-indolyl phosphate into an insoluble product that precipitates on the sensor surface. This amplifies the mass change associated with antigen–antibody binding events on the sensor surface. Accordingly, a linear shift of the resonance frequency of the magnetoelastic sensor occurs for ricin concentrations ranging from 10 to 100 μg/mL with a LOD of 5 ng/mL.

An alternative approach makes use of an indirect competitive ELISA followed by amperometric detection. The assay is carried out on single-use polystyrene–graphite SPEs. These sense the formation of 1-naphthol from 1-naphthyl phosphate in the presence of a detector antibody conjugated to AP [176]. The response of the amperometric sensor is proportional to the logarithmic of the ricin concentration from 100 to 3.2 μg/mL. The sensor provides a LOD of 40 ng/mL of ricin which can be quantified in 90 min of assay time. The assay flowchart is presented in Fig. 10.

Sensors for marine toxins

Marine shellfish is a source of paralytic shellfish poisons. These are harmful because they block sodium channels and evoke poisoning of humans. Particularly, these fish species can comprise the toxic agents tetrodotoxin (TTX), saxitoxin (STX), and gonyautoxin (GTX). Their chemical structures have been revealed and their threat for food safety has been ascertained [177–180]. These substances are water-soluble, acid- and heat-stable, and not decomposed by ordinary cooking.

Nanomolar concentrations of TTX suffice to block the fast voltage-activated sodium channel in neurons. TTX is one of the most potent non-protein toxins with a mortal dose of 10 μg/kg body mass. GTX and STX bind to the same site of sodium channels. Moreover, STX is listed in schedule 1 of the CWC, and the lethal dose in a single application is 200 μg [181]. The chemical structures of STX and TTX are presented in Fig. 11.

Table 5 Sensors for the detection of mycotoxins

Analyte	Sensor type	Food matrix	LOD	Ref.
Aflatoxin B1	Electrochemical immunosensor	Barley	2 ng/g	[144]
Ochratoxin A	Electrochemical immunosensor	Wheat extract	400 ng/kg	[145]
Ochratoxin A	Electrochemical immunosensor	Wine	10 ng/mL	[146]
Aflatoxin B1	Electrochemical sensor	Corn	30 pg/mL	[147]
Deoxynivalenol Ochratoxin A	Electronic nose	Barley grains	ns	[148]
Aflatoxin B1/G1	SPR	Maize	0.97 ng/g	[149]
Deoxynivalenol	SPR (BIACORE Q)	Wheat, wheat powder	ns	[150]
Deoxynivalenol Aflatoxin B1	SPR (BIACORE 2000)		500 pg/g 20 pg/g	[151]
Fumonisin B1			50 ng/g	
Ochratoxin A			100 pg/g	
Aflatoxin M1	Portable field immunosensor	Milk	50 pg/mL	[152]
Fumonisin B1	Fiber-optic immunosensor	Ground corn	10 ng/mL	[153]
Fumonisin B1 Aflatoxin B1	Evanescent wave-based fiber-optic immunosensor	maize	400 ng/g	[154]
Ochratoxin A	Fluorescence-based NRL sensor	Wheat pasta, cornmeal, cornflakes, barley, coffee Wine	7–100 ng/g 38 ng/mL	[155]
Deoxynivalenol	Fluorescence-based NRL sensor	Oats, barley, cornflakes, cornmeal, meat	1–50 ng/g	[156]
Ochratoxin A	Amorphous silicon sensor	Wine	100 pg	[157]
Aflatoxin B1	Fiber-optic immunosensor	Peanut meal, corn meal	1 ng/mL	[158]
Aflatoxin M1	Amperometric immunosensor	Milk	ns	[159]
Aflatoxin B1 Ochratoxin A	Optical waveguide lightmode spectroscopy sensor (OWLS 1000)	Barley flour, wheat flour	ns	[160]
Aflatoxins Ochratoxin A	Flow-through luminescence-detection microarray	Raw food extracts	ns	[161]

ns not specified

Diarrheic shellfish poisoning is caused by the toxic substance ocadaic acid (OA). This fatty acid derivative is cytotoxic and inhibits certain protein phosphatases [182]. OA is responsible for a notable number of intoxications every year, and its quick detection during food production and processing is highly demanded. The critical limit for OA defined by the EU is 40–60 μg/100 g mussel tissue [183]. The structure of OA is shown in Fig. 12.

Amnesic shellfish poisoning can be traced back to domoic acid (DA), an amino acid associated with harmful algal blooms [184]. In mammals, including humans, domoic acid acts as a neurotoxin. Most countries have adopted a legal upper limit of 20 mg DA per gram shellfish for consumer protection [185]. The structure of DA is presented in Fig. 13.

Electrochemical sensors The electrochemical immunosensor presented by Micheli et al. [186] was adapted for the analysis of DA. The sensor is based on a competitive indirect ELISA and involves the use of disposable SPEs. The signal readout is performed by DPV. The working electrode was modified with a BSA-DA conjugate. DA antibodies were added to the sample. Enzyme-amplified detection with a secondary antibody conjugated to AP enabled a LOD of 5 ng/mL of DA in spiked mussels tissue and shellfish in a linear range of 5–70 ng/mL.

Immunosensors integrated in FIA systems Semi-automated analysis of OA in mussels can be carried out by means of a chemiluminescent immunosensor integrated to a FIA system [187]. The indirect competitive assay is carried out on commercially available polyethersulfon membranes using immobilized haptens. The competing OA in the sample is captured by the addition of HRP-labeled anti-OA. An overall measurement time of 20 min and a dynamic measurement range from 200 ng to 200 mg OA/100 g mussel homogenate assure a high applicability. A reproducible response was obtained during the first 34 measurements.

Fig. 10 Electrochemical immunoassay for ricin determination (adapted from [173])

1. Immobilisation of ricin sample on the electrode surface with subsequent BSA blocking.

2. Incubation of rabbit anti-ricin antibody.

3. Incubation of detection antibody conjugated to alkaline phosphatase on the electrode.

4. Connection of the electrode to a potentiostat and addition of substrate.
   The product formed by enzymatic reaction is oxidised and the oxidation current is measured.

◇ Ricin antigen

Y Rabbit anti-ricin antibody (ab)

Y Alkaline peroxidase-conjugated detection ab

Potentiostat

Counter electrode   Reference electrode
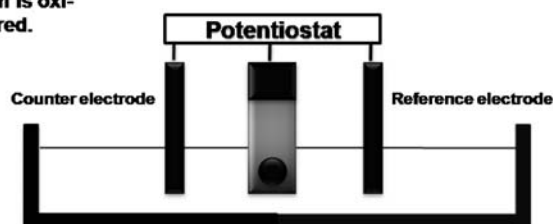
Tissue biosensor An integrated tissue biosensor for measuring sodium channel blockers such as TTX and STX was described by Cheun et al. [188]. Muscle, testis, skin, liver, and ovary of *Takifugu niphobles* and muscle and skin of *Takifugu parudale* (swellfish) have been examined for TTX and *Zosims aeneus* (crab) for STX. The tissue sensor consists of a Na^+-sensitive electrode in a flow cell. The electrode tip is covered with frog bladder membrane (*Rana catesbeiana* membrane section from frog bladder) sandwiched between two sheets of cellulose acetate. The distinctive features of this tissue biosensor are its long life cycle (250-h reproducibility of analysis results), the short assay times (5 min), and the low limit of detection (86 fg of toxin). Measurements with an advanced tissue sensor were carried out to determine the paralytic shellfish toxins such as GTX [189]. GTX was determined in different seafood samples. Linear ranges of sensor response were achieved between 86 and 1,000 fg for TTX and 5 and 1,000 fg for STX.
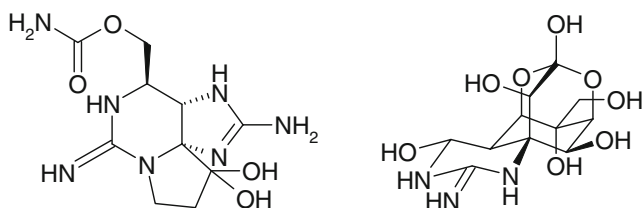

Fig. 11 Chemical structures of the marine toxines saxitoxin (*left*) and tetrodotoxin (*right*)

Sensors for major interesting food-contaminating bacteria

As the bacterial pathogens *Salmonella* spp., *L. monocytogenes*, *Campylobacter jejuni*, and *E. coli* O157:H7 have been assessed to be responsible for approximately 67% of lethal food poisonings, their rapid and reliable detection is a major task in consumer protection [190]. Water- and foodborne infections are a problem that particularly affects developing countries. *C. jejuni* is the most prevalent cause of diarrheal and intestinal disease [191, 192]. Food, in particular poultry, is the predominant source of its growth. An average of 65–70% of retail poultry carcasses and 80–95% of carcasses from processing plants is contaminated. Infection typically results from ingestions of non-pasteurized milk and milk products, undercooked meat, or eggs. Major symptoms of intoxication are diarrhea, fever, abdominal and muscle pain, nausea, and headache. The minimum infective dose in clinical studies on humans was found to be 500 bacteria for *C. jejuni* [193].

E. coli O157:H7 is an enteropathogenic bacterium which releases Shiga toxins. Infection often leads to bloody diarrhea, painful abdominal cramps, and, occasionally, to kidney failure [194–196]. Most cases of contamination have been associated with undercooked ground meat or raw milk. Infection can also occur after swimming in or drinking sewage-contaminated water [194, 197, 198]. The US Food Safety Inspection Service established a zero tolerance threshold for *E. coli* O157:H7 in raw meat products [199]. The infectious dosage is ten cells, and the Federal EPA standard in water is 40 cells per liter [200].

**Fig. 12** Chemical structure of okadaic acid
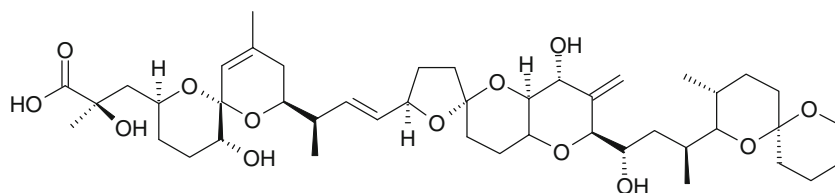
L. monocytogenes has been regarded an important foodborne pathogen since its discovery in 1926 and was the cause of some examined food poisoning outbreaks [201–204]. Infections with *Listeria* cause Listeriosis which can induce spontaneous abortions, premature delivery, stillbirths, and infection of newborns [205]. Symptoms are diarrhea, nausea, vomiting, slight headache, and mild fever [206]. Listeriosis has been associated mainly with dairy products or chicken. The intoxication is accompanied by a high mortality rate, but its incidence is low compared to other food-associated infections.

Shigella dysenteriae are another non-spore forming type of bacteria. They can cause shigellosis (bacillary dysentery) with a minimum human effective dose of ten bacteria [207–209]. Illness arises through the ingestion of fecally contaminated food and water, and therefore, insufficient personal hygiene and sanitation are the prevalent sources. *S. dysenteriae* causes the most severe dysentery because of its potent and deadly Shiga toxin [210]. Infection symptoms include diarrhea, fever, abdominal and muscle pain, nausea, and headache.

A number of foodborne illness outbreaks have occurred where the source of contamination has been traced to *Salmonella* species. *Salmonella* is an enterobacterium that causes typhoid fever, paratyphoid fever, and salmonellosis. Contamination can occur via a wide range of food products [211, 212]. Although established microbial methods are predominantly used for the determination of bacteria due to their simple applicability and standardized operation protocols, there is also a demand for rapid and straightforward sensing techniques.

Immunomagnetic sensors The combination of immunomagnetic separation and ECL detection is a promising approach for an efficient screening of bacterial contaminations in food samples as cell culture-based methods are relatively slow. A commercially available sensor [213] was evaluated for the determination of *E. coli* O157 and *Salmonella typhimurium* in food matrices [214]. The immunomagnetic separation was carried out in milk, juices,

Fig. 13 Chemical structure of domoic acid
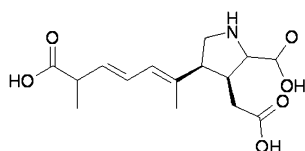
supernatants from ground beef, chicken, and fish suspensions followed by ECL detection with the ORIGEN analyzer. The assay was processed as described in "*S. aureus* enterotoxins" [117] in less than 1 h. Antibody-coated magnetic beads are capable of binding high cell amounts. A limit of 100–1,000 bacteria per milliliter could be detected.
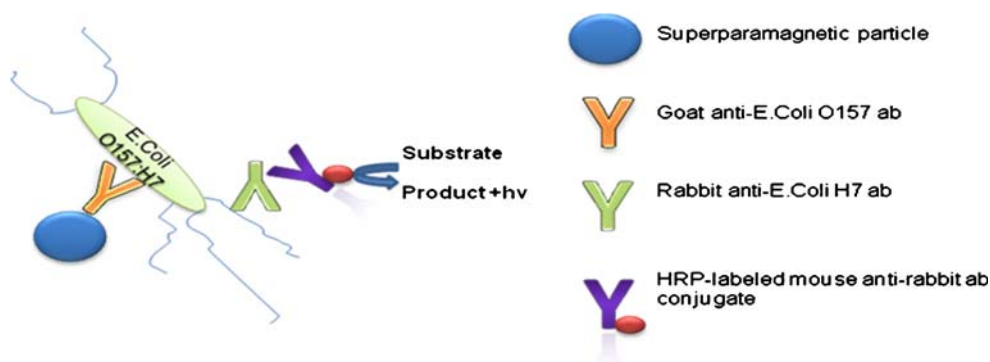
An improved enzyme-linked immunomagnetic chemiluminescence (ELIMCL) method has been developed to lower incidences of false positive results of *E. coli* O157:H7 in mixed cultures. Highly specific combinations of anti-O157 and anti-H7 antibodies in the sandwich immunoassay format and a semi-automated version of ELIMCL are providing reliable results. The total assay time is 6.5 h. A detection limit of 400 cfu/g of *E. coli* cells in inoculated ground beef was attained [215]. The method scheme is presented in Fig. 14.

Fiber-optic immunosensors An antibody-based fiber-optic immunosensor for the determination of *Listeria* cells was presented by Geng et al. [216]. Its principle relies on a sandwich assay format and uses polyclonal capture antibodies which are immobilized on a polystyrene waveguide via biotin–strepavidin binding. Cy5-conjugated detector antibodies were incubated along with the sample. The complete assay could be performed in less than 24 h from sampling including an enrichment step. Since *Listeria* is problematic in ready-to-eat meats, spiked hot dog and Bologna sausage samples were tested after 20 h of enrichment. Results showed that *L. monocytogenes* cells are detectable directly from the enrichment broth even with low levels of inoculations (10 to 1,000 cells per gram).

The occurrence of *Listeria* in sausages can also be revealed with the RAPTOR automated fiber-optic sandwich immunosensor system [217]. It is based on an evanescent wave excitation generated by four 635-nm diodes coupled to four optical fibers. These are assembled in a disposable coupon with a fluidic channel flow system for automated operation. The fluorescence emission of Cy5-conjugated monoclonal detector antibodies is measured by means of photodiodes at 670-nm wavelength. After inoculation of sausage samples with *Listeria* cells and 20 h of cell enrichment, a minimum of 5×10^5 cfu/mL of *Listeria* cells could be determined.

Another evanescent wave fiber-optic biosensor with high potential for rapid, sensitive, and real-time microbial

Fig. 14 Scheme of the ELIMCL method for *E. coli* detection adapted from [211]

Superparamagnetic particle

Goat anti-E.Coli O157 ab

Rabbit anti-E.Coli H7 ab

HRP-labeled mouse anti-rabbit ab conjugate

analysis was developed by Geng et al. [218]. They were able to detect low levels of *E. coli* O157:H7 in ground beef. Their sensor is also based on a direct sandwich immunoassay. Polyclonal capture antibodies specific for *E. coli* were immobilized on polystyrene fiber waveguides via biotin–streptavidin coupling. Indicator antibodies labeled with AlexaFluor 647 were used for cell detection. The analysis is performed by means of an Analyte-2000 system [219] employing evanescent field excitation of the fluorophores. The emitted light is transmitted by the fiber and quantified by a photodiode. A detection limit of 1 cfu/mL of *E. coli* in spiked ground beef could be attained after pre-enrichment.

Piezoelectric sensors Campbell et al. [220] presented a piezoelectric millimeter-sized cantilever sensor for *E. coli* analysis in raw or sterilized ground beef. An *E. coli* O157:H7 specific antibody was immobilized on a PZT-layer bonded on borosilicate glass. Binding of target cells induces a significant resonance frequency change. In this way, 50–100 cells per milliliter could be determined in a 3-mL broth sample containing meat particles.

A piezoelectric quartz crystal microbalance sensor with a gold-plated AT-cut surface was presented by Minunni et al. [221]. In this case, the surface was initially coated with protein A or G. This facilitates the immobilization of heat-killed *Listeria* cells for a competitive assay. *Listeria* cells in the sample compete for antibodies with immobilized cells on the crystal surface. The binding of antibodies on the sensor can be monitored. This very fast assay (15 min) provides a sensitivity of 3.19×10^6 cells/crystal.

Amperometric sensors Listeria cells in low-fat and infant formula milk can be determined by means of an immunosensor SPE device. Sandwich assays with immobilized capture antibodies were employed. After the binding of target cells, a second primary antibody was incubated followed by the addition of a secondary AP-conjugated antibody. The enzymatic formation of *p*-aminophenol from *p*-aminophenyl phosphate can be monitored amperometrically as the former can be oxidized at the electrode. The

assay provides a dynamic range of 1.3×10^6 to 1.3×10^3 cells per milliliter with a LOD of 9.3×10^3 cells per milliliter milk and can be carried out within 3.5 h [222].

Surface plasmon resonance sensors The Plasmonic® surface plasmon resonance device (HS Systeme, Wallenfels, Germany) [223] was adapted for a rapid, simple, and specific screening of *S. typhimurium* in milk [224]. A cuvette was placed on a gold-coated alkylsilane-modified prism with Kretschmann ATR configuration. The chamber was subdivided into eight small channels to enable measurements of eight different samples on one single chip. A laser diode emits an elliptical light beam which is then converted into a divergent beam with the help of a cylindrical lens. Therefore, it is possible to cover all possible angles of incidence required for the real-time determination of the SPR angle (Fig. 15). It was possible to determine 1.25×10^5 cells per milliliter milk without the call for sample preparation or enrichment.

The miniSPR dual-channel sensor platform SPREETA™ (Texas Instruments, Dallas, TX, USA) [225] was used for the analysis of *C. jejuni* [226]. The miniaturized sensor is a fully integrated plasmon resonance device based on Kretschmann geometry in a flow cell. It is configured of an AlGaAs light emitting diode (LED, 840 nm) with a polarizer, a temperature sensor, two photodiode arrays, and a reflecting mirror. The LED illuminates the gold-coated glass in a range of different angles after passing a polarizer. After reflection by the gold layer, a mirror directs the light beams toward the two independent linear 256-pixel Si photodiodes. The gold surface was pretreated with a neutravidin layer and subsequently incubated with biotinylated capture antibodies. The assay provides a good sensitivity using commercially available anti-*Campylobacter* (with a LOD of 10^3 cfu/mL in chicken rinse).

Salmonella contaminations could be identified with the SPREETA™ (Ref) sensor platform. Biotinylated *Salmonella* antibodies were immobilized on a Neutravidin-coated gold sensor surface. Salmonella could be detected at concentrations as low as 10^6 cfu/mL in chicken extract [227].
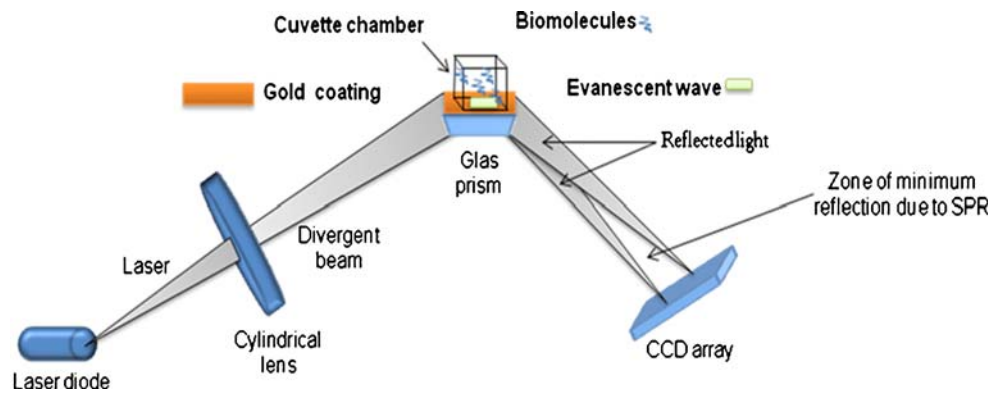
The BIACORE 3000 SPR sensor system [114] was established for *L. monocytogenes* in a subtractive inhibition assay [228]. The dextran surface of the sensor chip was functionalized by Fab polyclonal antibodies. Samples containing *Listeria* cells were incubated together with the corresponding polyclonal secondary antibody. Subsequently, unbound antibody was separated stepwise by centrifugation and passed over the Fab-coated sensor chip surface. The received response is inversely proportional to the cell concentration. An assay time of below 30 min and a LOD of 10^5 cells per milliliter sample were achieved with a minimum effort in sample preparation.

Fluorescence-based array sensor Culturing food matrices is an established method for the detection of *E. coli* O157: H7 to investigate food contamination. A sandwich assay was developed, optimized, and tested with spiked food matrices using the NRL array biosensor (see "*C. botulinum* toxins") [229]. Fluorescently labeled detector antibodies were used for quantification of the cells and led to detection limits of $1–5×10^4$ cells per milliliter in different food matrices. A similar sandwich immunoassay for the detection of *Salmonella enterica* serovar Typhimurium was reported by Taitt et al. [230]. A wide variety of food samples (fruit, egg, meat, and feed) as screened for *Salmonella* contaminations with minimal sample preparation yielding LODs in the magnitude of $10^4–10^5$ cfu/mL. An overview of different sensors for the detection of food-contaminating bacteria is presented in Table 6.

Multitoxin sensor arrays

Protein or hapten arrays are perfectly suited platforms for the screening of several toxins simultaneously because of their multiplexing capacities. Multitoxin sensor arrays exhibit an excellent opportunity for online tracking if they can be integrated in microfluidic systems. They are less expensive and time-consuming in comparison to classical analytical methods and enable the detection of multi-intoxication events.

Fluorescent immunosensors The array biosensor developed by the NRL [231] is applicable to a multiplicity of sample matrices and analytes. It is capable of analyzing multiple targets rapidly and simultaneously on the surface of a single waveguide. It uses sandwich as well as competitive fluoroimmunoassay formats for the detection of different toxins. Biotinylated capture antibodies can be patterned on Neutravidin-functionalized slides using a PDMS flow cell. Ready-to-use slides contain two columns for positive controls, five specific for *C. botulinum* toxin A (BoNT A) and five specific for SEB using immunoassays in a sandwich-type format. Screenings of canned food (e.g., corn, fish, and vegetable) can be performed within 20 min after simple sample preparation steps and an incubation time of 2 h of the spiked sample. Detection limits for BoNT A and SEB range from 20 to 500 ng/mL (BoNT A) and 100 to 500 pg/mL (SEB) for different food samples using a "cocktail" of fluorescently labeled tracer antibodies [232].

Capture molecules such as antibodies or toxins (in the case of indirect competitive assays) can be immobilized on specific locations (stripes) on the surface of a planar optical waveguide, forming a "barcode." The resulting patterned array with regions targeting at different analytes (e.g., BoNT A and B, SEB, Ricin, *C. jejuni*, *S. typhimurium*, *E. coli*, *S. dysenteriae*, *B. anthracis*, *L. monocytogenes*, and other targets) was used to read out up to 12 samples. Solutions and reagents were loaded onto the waveguide by means of PDMS flow chambers with sample application perpendicular to the "bar code" columns. Binding of fluorescently labeled detector molecules can be monitored by a CCD camera. Fluorescence determination and background correction of the evanescently excited spots was accomplished with automated image analysis. The compact optics of the sensor system enables the fabrication of an automated instrument of low size and weight. For instance, SEB could be determined in carnation non-fat milk,

Table 6 Overview of sensor methods for the detection of food-contaminating bacteria

Analyte	Sensor type	Food matrix	LOD	Ref.
Salmonella typhimurium E.coli	Immunomagnetic separation–electro-chemiluminescence sensor (IMS-ECL)	Milk, juices, Supernatant from ground beef, minced chicken, fish suspensions	100–1000 bacteria/mL	[210]
E. coli	Enzyme-linked immunomagnetic chemiluminescence sensor (ELIMCL)	Ground beef	400 cfu/g	[211]
Listeria monocytogenes	Fiber-optic immunosensor	Hot dog, Bologna	2.3×10^8–3.9×10^9 cfu/mL	[212]
Listeria monocytogenes	Fiber-optic RAPTOR sensor	Frankfurters	5×10^5 cfu/mL	[213]
E. coli	Fiber-optic immunosensor	Ground beef	1 cfu/mL	[214]
E. coli	PEMC sensor	Ground beef	50–100 cells/mL	[216]
Listeria monocytogenes	QCM sensor	Milk	3.19×10^6 cells/crystal	[217]
Listeria monocytogenes	Amperometric screen-printed electrodes (SPE) sensor	Milk	9.3×10^3 cells/mL	[25]
Salmonella typhimurium	SPR sensor	Milk	1.25×10^5 cells/mL	[219]
Campylobacter jejuni	SPREETA SPR sensor	Broiler	10^3 cfu/mL	[221]
Salmonella enteritidis	SPREETA SPR sensor	Chicken extract	10^6 cfu/mL	[222]
Listeria monocytogenes	SPR (BIACORE 3000)		10^5 cells/mL	[223]
E. coli	Fluorescence-based NRL sensor	Ground beef, turkey sausage, chicken carcass wash, apple juice	$1 - 5 \times 10^4$ cells/mL	[224]
Salmonella typhimurium	Fluorescence-based NRL Sensor	Cantaloupe, liquid eggs, chicken carcass rinse, national brand pork sausage, Alfalfa sprout rinse	1.6×10^4–3.6×10^6 cfu/g	[225]

cantaloupe, and ham using monoclonal biotinylated capture antibodies immobilized on the neutravidin sensor surface and Cy5-labeled tracer antibodies in a sandwich assay. It is detectable with an LOD of 1 ng/mL in milk and 100–500 pg/mL in food [233].

Finally, the NRL array biosensor was also applied to determine *C. jejuni* and *S. dysenteriae* in poultry and milk products [234]. The sandwich immunoassay can be carried out within 25 min using biotinylated capture antibodies. The 12-channel system is capable of analyzing different food samples without the need for extensive sample preparation.

In a related approach, ground turkey ham, ground turkey sausage, carnation non-fat dried milk, and Vanilla fat-free yoghurt were examined for contamination with *C. jejuni* cells and aflatoxin B1 [235]. Sandwich and competitive assay types were combined on one sensor. *Campylobacter* cells were determined by a direct sandwich assay, whereas a competitive format was applied for the determination of aflatoxin B1. The fluorescent analysis was carried out as described above.

Current improvements of biochemical and engineering aspects of the NRL sensor enabled the expansion of the repertoire of analytes detected. *Salmonella* spp. and *Listeria*

Fig. 16 A PMMA microfluidics cube in combination with the NRL sensor array (*S* sample channel, *T* tracer channel) [231]
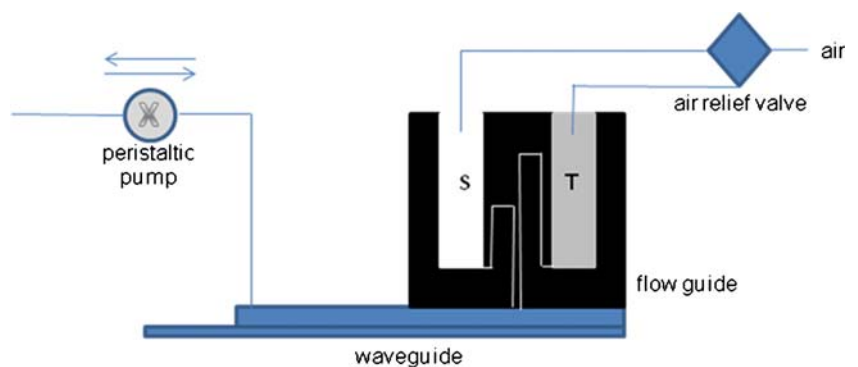
Table 7 Determination of food pathogens with the NRL sensor array

Analyte	LOD	Linear range
Deoxynivalenol (DON)	0.2 ng/g	1–10 ng/g
Ochratoxin A (OTA)	0.8 ng/g	3.8–100 ng/g
Aflatoxin B1 (AFB1)	0.3 ng/g	0.6–5.1 ng/g
Botulinum toxin A (BoNT A)	20 ng/mL	20–500 ng/mL
Staphylococcal enterotoxin B (SEB)	0.1 ng/mL	100–500 pg/mL
Salmonella typhimurium	8×10^4 cfu/mL	8×10^4–4×10^5 cfu/mL
Shigella dysenteriae	5×10^4 cfu/mL	5×10^4–8×10^5 cfu/mL
Campylobacter spp.	1×10^3 cfu/mL	2×10^3–3×10^3 cfu/mL
E. coli	5×10^3 cfu/mL	1×10^4–5×10^4 cfu/mL

spp. are applicable to the system now. A new "plug-and-play" methodology, the fluidics cube, was installed. It permits that all parts of the assay can be prepared in advance. Ricin, SEB, and cholera toxin were identified simultaneously using the novel fluidics cube module that limits the number of operations to only the initial sample loading. Listeria cells were analyzed with an LOD of 2.4×10^5 cfu/mL. A schematic overview of the cube is given in Fig. 16.
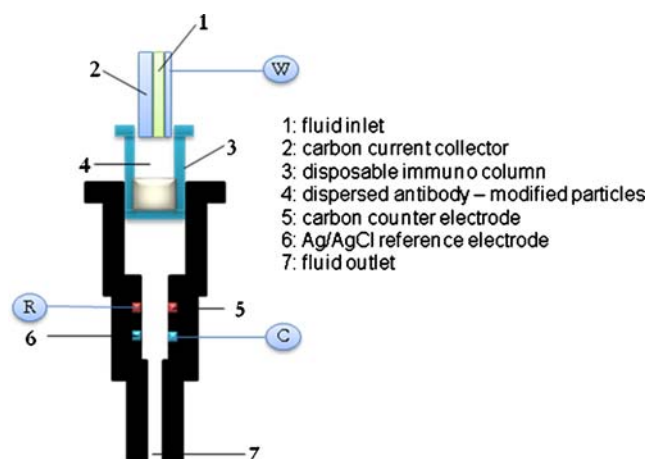
The PMMA fluidics cube includes holes and/or grooves that form a three-dimensional network of channels and reservoirs. This implies six reservoirs for samples and six for fluorescent tracer antibodies and was attached to a six-channel flow guide fabricated from a PMMA matrix. Each channel was connected to a pair of sample and tracer reservoirs via milled conduits. Stainless steel tubes were installed on the opposite side of each channel to form outlet ports for a pumping system. An aliquot of tracer antibody solution was added and the cube was frozen at −20 °C and freeze-dried. The antibody can be rehydrated by addition of PBS buffer and the cube is ready for sample loading [236]. A user manual for the practical application of the NRL

array biosensor to the detection of SEB, Campylobacter, and Salmonella in different food matrices is available [237].

A recent work compiles an application list of the NRL sensor regarding toxins in food [238]. LODs and linear ranges of analyzed targets in different matrices are reproduced in Table 7.

The Hanson Technologies' Leopard [239] and the Constellation Technologies Array Biosensor [240] are commercially available versions of the NRL biosensor for food pathogen screening.

Electrochemical sensors Carbon nanoparticles were used to identify *C. jejuni*, *L. monocytogenes*, and *E. coli* O157:O7 in milk and chicken extract via a flow through immunoassay system combined with amperometric detection (Fig. 17) [241]. The carbon particles were functionalized with the corresponding capture antibodies and placed on a disposable centrifugal filter. Pathogenic Campylobacter cells were captured by the modified particles and indicated with HRP-labeled antibodies. The disposable element at the hollow carbon rod acts as working electrode, a second hollow carbon rod as counter electrode, and a hollow Ag/AgCl

1: fluid inlet
2: carbon current collector
3: disposable immuno column
4: dispersed antibody – modified particles
5: carbon counter electrode
6: Ag/AgCl reference electrode
7: fluid outlet

Fig. 17 Schematic presentation of the flow-through immunosensor adapted from [236]
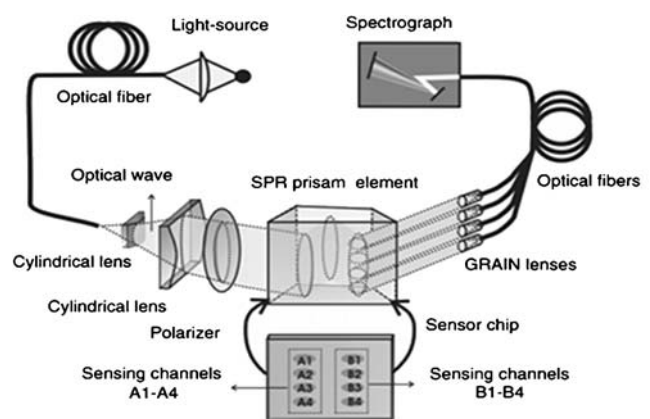
Fig. 18 Schematic of the eight-channel SPR sensor based on wavelength division multiplexing (reprinted from [237] with permission from Elsevier)

electrode as reference electrode. An amperometric signal results from the conversion of hydrogen peroxide due to the peroxidase reaction which is recorded at the carbon counter electrode and is proportional to the number of captured cells. Quantification of *Campylobacter*, *E. coli*, and *Listeria* was demonstrated with detection limits of 10–50 cells per milliliter milk within 30 min.

Multichannel SPR A multi-analyte device for *E. coli* O157: H7, *Salmonella cholerasius* serotype Typhimurium, *L. monocytogenes*, and *C. jejuni* was reported by Taylor et al. [242]. It is based on an eight-channel SPR system

(Fig. 18). The LODs for the four bacteria species ranged from 3.4×10^3 to 1.2×10^5 cfu/mL in apple juice samples.

Cell-based sensors The CANARY B lymphocyte-based pathogen sensor (cellular analysis and notification of antigen risks and yields sensor) is a promising alternative for the identification of more than one target per assay. The sensor uses B lymphocytes due to their efficient identification of pathogens. B lymphocytes have been engineered to emit light within seconds if they are exposed to certain bacteria and viruses. B cell lines specific for *E. coli* strain O157:H7 were engineered to express cytosolic aequorin, a

Table 8 Overview of multitoxin sensor methods

Analyte	Sensor type	Food matrix	LOD	Ref.
BoNT A	Fluorescence-based NRL Sensor	Canned food Corn, green beans and whole tomatoes, mushrooms, tuna	20–500 ng/mL	[227]
SEB	Fluorescence-based NRL Sensor	Carnation non-fat milk, ham, Cantaloupe	1 ng/mL milk, 100–500 pg/mL food	[233]
Campylobacter jejuni	Fluorescence-based NRL Sensor	Chicken carcass, lettuce leaf wash, ground turkey meat, carnation non-fat dried milk	$1,6\times10^3$–7.8×10^5 cfu/mL	[229]
Shigella dysenteriae *Campylobacter jejuni*	Fluorescence-based NRL Sensor	Ground turkey ham, ground turkey sausage, carnation non-fat dried milk, vanilla fat-free yoghurt	4.7×10^2–9×10^3 cfu/mL	[230]
SEB *Listeria* *Salmonella* Ricin Cholera toxin	Fluorescence-based NRL Sensor		2.4×10^5 cfu/mL (*Listeria*)	[231]
SEB DON OTA AFB1 BoNT Campylobacter *E. coli* Shigella Salmonella	Fluorescence-based NRL Sensor	Various	100–500 pg/mL 200 pg/g 800 pg/g 300 pg/g 20 ng/mL 1×10^3 cfu/mL 5×10^3 cfu/mL 5×10^4 cfu/mL 8×10^4 cfu/mL	[233]
Campylobacter jejuni *Listeria monocytogenes* *E. coli* O157:H7	Carbon naoparticle sensor with amperometric detection	Milk, chicken extract	10–50 cells/mL	[236]
Salmonella typhimurium *E. coli* *Listeria monocytogenes* *Campylobacter jejuni*	SPR sensor	Apple juice	3.4×10^3–1.2×10^5 cfu/mL	[237]
E. coli	CANARY B lymphocyte based sensor	Lettuce	500 cfu/g	[238]

calcium-sensitive protein from *Aequoria victoria* as well a membrane-bound antibodies for this pathogen. Cross-linking of antibodies even with low levels of *E. coli* cells elevated intracellular calcium concentrations within seconds and the aequorin emits light. Detection of *E. coli* levels of 500 cfu/g in lettuce could be attained within 5 min including sample preparation time [243].

An overview of multitoxin sensors is presented in Table 8.

Conclusion

Quantitative real-time or online analysis of food samples with respect to composition and concentration of toxic contaminations is one of the major attributes for promising sensor methods. This aim evokes academic as well as industrial research and development. Low sample preparation times, reduced staff requirements, and the possibility to develop ready-to-use sensors and kits for complex analytical matrices are the main goals. Practicable solutions for problems like the lack of stability and reversibility of immobilized biological receptor units, their limited selectivity, and an inappropriate sensitivity are still demanded.

Conventional methods for the analysis of microbial pollutants and their toxins are rather sensitive and provide qualitative and quantitative results. An obvious disadvantage is denoted by their extensive sample preparation and overall analysis times. Sensors or microarrays with receptor units are an excellent alternative in comparison to classic microbiological methods. They are capable of performing multiple analyses very fast and allow the application of complex food matrices. Problems concerning stability, sensitivity, selectivity, and unspecific background signals will be circumvented by extending the disposability of high-quality biological recognition elements (e.g., monoclonal antibodies) or improved techniques of surface modification to prevent unspecific binding. The application of precise and effective cleanup and preconcentration steps is another promising road to success.

In the case of sandwich assays using labeled antibodies, the interpretation of the binding event and the corresponding signal achieved can be problematic. There is more than one step in the assay that might fail and thus be falsely interpreted. Matrix effects are not excludable in the process of measurement, so they have to be studied very intensively before new tests can be commercialized and used in a highly sensitive field such as food safety.

The SPR technique allows real-time detection and monitoring of binding analytes onto monolayer-coated surfaces without any label and background from the sample solution [244]. Although being a very sensitive and reliable method, commercially available SPR devices provoke still too high costs for mass testing. Less expensive fluorescent or electrochemical sensors are still the most promising approaches for high-throughput as well as multianalyte analysis. Reducing the costs per assay and the progress in miniaturization will increase their attractivity for screening applications on-site. Fiber-optic sensors will still play an important role for online monitoring of food processing and to control the quality of food during storage, transport, and distribution.

A predominant number of published sensor approaches have been tested up-to-date only in a proof of principle manner or in spiked model sample matrices. Only a part of them was applied to real samples with natural contaminations and assessed with established reference methods. The survey should highlight the current progress in straightforward sensor methods and devices that have already been evaluated in food matrices and could be applied to standard food analysis to achieve a better and closer control of health risks.

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