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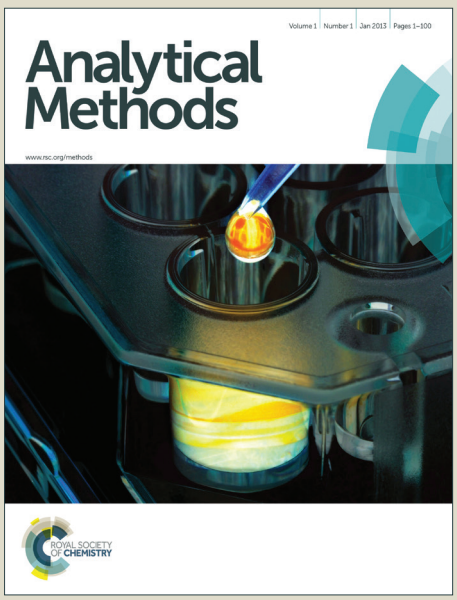

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Electrochemical immunosensors, genosensors and phagosensors for *Salmonella* detection

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Abstract

This review discusses the current state of the art of *Salmonella* detection. In this perspective, emphasis is given to the recent developments in biosensors, in particular to electrochemical immunosensors, genosensors and phagosensors. Different aspects of the biosensors development has been summarised and discussed in detail. The integration of new materials into biosensors such as magnetic particles is also fully revised. More importantly, the advantages of using magnetic particles in magnetic separation of the bacteria coupled with different detection techniques are also reviewed. This article also deals with the latest developments in simultaneous detection of several foodborne pathogenic bacteria. Accordingly, research opportunities and future development trends in these areas are finally discussed.

Keywords: Immunosensor, genosensor, phagosensor, magnetic separation, magnetic particle, electrochemical biosensor.

Introduction

Over the last fifty years, microbiologists have developed reliable culture-based techniques to detect pathogens. Although these are considered to be the "gold-standard," they remain cumbersome and time-consuming. More recently, standard culture-based pathogen detection methods have been refined and even improved, with an eye towards reducing time to detection. This is generally done by replacing the selective and differential plating step with more rapid immunological or molecular-based assays. Among these, the enzyme-linked immunosorbent assay (ELISA), DNA hybridisation, and polymerase chain reaction (PCR) methods should be highlighted. These "rapid" assays must include a number of essential features. Firstly, they must exhibit a high degree of sensitivity, defined as the ability to detect the pathogen when it is actually present in the sample. This is required to prevent false negative results and hence assure that a contaminated unit is accurately identified. Routinely, detection limits of a single viable cell of contaminating pathogen per sample unit are required. A high level of test specificity, or the ability to classify a sample as negative if the pathogen is absent, is no less important as it reduces the likelihood of having to spend additional time and resources confirming results on products which do not represent a risk to public health. Although rapid methods can be highly accurate (some are over 98 % in agreement with a reference culture method [1]), they are not considered definitive because they usually do not produce an isolate. Rapid methods that exhibit both high specificity and high sensitivity can be used as a screening tool when they are performed in tandem with the culture method.

Figure 1 displays, as an example, the comparison of time needed with different methods for *Salmonella* spp. detection [2]. In detail, classical cultural techniques are highly labour-intensive and require skilled microbiologist to carry out the analysis. Negative results are obtained after approximately 4 – 5 days and confirmation of presumptive-positives requires further 2 days. In the case of ELISAs, negative results are obtained as early as 24 – 30 h and confirmation of presumptive-positive requires further 2 days. Although the reagents and material costs in cultural techniques are generally lower than ELISAs, the labour costs are likely to be higher. Among the different methods, immunological techniques are promising because of their sensitivity and rapidity. However, even rapid detection tests normally require enrichment of the target bacteria to the level of the assay's detection limit. A major disadvantage of the immunological techniques is that every step in the assay requires a rigorous washing procedure, which is labour-intensive and difficult to automate.

Concerning molecular methods based on nucleic acid probes and PCR, the total time frame of the analysis is still several hours. However, these are generally an order of magnitude more sensitive and exhibit better specificity than the immunological techniques, and the results obtained are usually definitive, with no requirement for confirmation by classical cultural techniques. A major drawback is that molecular techniques require high levels of technical skill, special laboratory facilities to avoid PCR contamination problems, generally high capital equipment costs and are prone to PCR inhibition depending on the matrix analysed.

Preferred position for Figure 1

All the current generation of tests, both molecular and immunological, usually requires at least an overnight pre-enrichment step before analysis. Despite the advent of these rapid detection methods, it is clear that reduction and/or elimination of cultural enrichment will be essential in the quest for truly real-time detection methods. The development of new methods that provide confirmed results in 1 day are still necessary for the consumer protection, and the quality of these results should be at least as reliable as those of the reference method. Rapid methods still require 1 to 3 days and often lack specificity or sensitivity. In recent years, some developments became accepted to reduce the time for gaining a result while enhancing sensitivity and specificity. However, there is still a need of finding a way to improve them, in terms of reducing their time assay and complexity [1, 3-5].

Over the recent years, a lot of effort has gone into the study and development of biosensors of the most diverse nature as an alternative to classical and rapid methods. Most of the currently developed biosensors for pathogenic bacteria detection are based on the specific antigen-antibody binding reactions, where the antibody is immobilised on the sensor platform to capture the bacteria that are of interest. Then, the bacteria detection is measured through electrochemical, optical, or piezoelectric signals [6, 7]. Moreover, the genetic biorecognition is also widely used in biosensing, as well as the biorecognition through bacteriophages, virus specific for bacteria [8, 9]. The need of more flexible, reliable and sensitive targeting of pathogens has promoted research on the potential of nanomaterials, such as carbon nanotubes, gold nanoparticles, quantum dots or magnetic particles and their incorporation into biosensor systems [10, 11]. In this article, an overview of electrochemical biosensors for bacterial detection is presented focusing on aspects of genosensors,

immunosensors and phagosensors development. *Salmonella* has been selected as a representation of pathogenic bacterial for being among the most studied pathogens that account for over 90 % of estimated food-related deaths: *Salmonella* (31 %), *Listeria* (28 %), *Toxoplasma* (21 %), Norwalk-like viruses (7 %), *Campylobacter* (5 %), and *E. coli* O157:H7 (3 %) [12]. Finally, an overview of the recent developments towards the simultaneous detection of pathogenic bacteria is also discussed.

Rapid methods for *Salmonella* detection

Several rapid methods have been developed for testing food for the presence of *Salmonella* spp. In this section, approaches based on immunological, genetic and bacteriophage-based biorecognition, as well as commercial kits available are discussed. Tables 1 – 4 show an extended compilation of the main bibliography reported since 2009 onward and some outstanding previous works. Earlier work was reported in detail in several reviews [1, 13-15].

Antibodies are common bioreceptors used in immunosensors. Accordingly to production strategy they can be classified in polyclonal, monoclonal or recombinant. In any case, antibodies are generally immobilised on a substrate, which can be the detector surface, its vicinity, or a carrier. An antigen-specific antibody fits its unique antigen in a highly specific manner, so that the three-dimensional structures of antigen and antibody molecules are matching. This unique property of antibodies is the key that makes the immunosensor a powerful analytical tool and their ability to recognise molecular structures allows the development of antibodies that bind specifically to

chemicals, biomolecules, or microorganisms. Many immunological methods involve the use of labelled antibodies. Enzymes, biotin, fluorophores and radioactive isotopes are commonly used labels to provide a detection signal in biological assays. As outlined in Table 1 the most used optical readout system were absorbance and fluorescence, and regarding electrochemical techniques amperometry or impedance. Concerning colorimetric assays, based on ELISA format, the pathogen detection sensitivity was improved from 10^5 CFU mL^{-1} obtained by standard ELISA assays to 10^3 CFU mL^{-1} obtained with the incorporation of nanomaterials, such as single-walled carbon nanotubes (SWCNTs) [16]. Improved LOD of 10 CFU mL^{-1} was obtained by using novel matrixes for the immunoassay such as polyacrylonitrile (PAN) fibers showed in Figure 2 [17]. Similar LOD was obtained for an electrochemical approach using screen-printing technique (21 CFU mL^{-1}) [5]. A significant shortening of the time assay was obtained for all the assays, being able to detect the target in less than 30 minutes for some approaches.

Preferred position for Figure 2

In the case of the approaches based on genetic biorecognition, the identification of a target nucleic acid is achieved by matching the complementary base pairs that are the genetic components of an organism. The classical nucleic acid biosensors measure the hybridisation of single stranded DNA present in the sample to a complementary probe immobilised onto the sensor chip surface. Biosensors based on nucleic acid as biorecognition element are simple, rapid, and inexpensive and hence it is widely used in pathogen detection. In contrast to enzyme or antibodies bioreceptors, nucleic acid recognition layers can be readily synthesised and regenerated. DNA damage is one of

the most important factors to be considered when nucleic acid bioreceptor are used. Hundreds of compounds bind and interact with DNA. Detection of chemicals may cause irreversible damage to DNA by changing the structure of DNA and the base sequence, which in turn disturbs the DNA replication. DNA hybridisation microarrays have been suggested as a platform for the parallel detection of multiple pathogenic microorganisms in food in a relatively short time. Recent advances in nucleic acid recognition, like the introduction of peptide nucleic acid (PNA) and aptamer technology, have opened up exciting opportunities for DNA biosensors. Due to their high binding affinity, simple synthesis, easy storage, and wide applicability, nucleic acid biorecognition elements have gained popularity and can substitute the commonly used antibody as bioreceptor in biosensor. Rapid approaches based on genetic biorecognition recently reported are outlined in Table 2. The majority of the methods developed were based on nucleic acid amplification techniques, such as Polymerase Chain Reaction (PCR) coupled with hybridisation techniques. Except some works based on Surface Plasmon Resonance (SPR) detection technique, the vast majority of methods were based on electrochemical detection and in particular on Differential Pulse Voltammetry (DPV). Figure 3 shows an example of the transducer fabrication procedure. Although most methods were tested only with synthetic oligonucleotides, the limits of detection determined for inoculated bacteria were ranged from 10 to 10^4 CFU mL^{-1}.

Preferred position for Figure 3

On the other hand, rapid approaches based on bacteriophage biorecognition are summarised in Table 3. The reported methods for bacteria detection using

bacteriophages include (i) expression of bacteriophage-encoded bioluminescent genes which produce visible products within the specific target cells (lux-bacteriophage strategy), (ii) fluorescence-labelled phage, which can be combined with immunomagnetic separation (labelled phage strategy), (iii) detection of bacteria by the intracellular replication of specific bacteriophages (named "phage amplification" strategy), and the (iv) detection of the phage-mediated bacterial lysis and release of host enzymes (e.g., adenylate kinase) or ATP (termed "lysin-release ATP bioluminescence strategy") [35]. Bacteriophages recognise the bacterial receptors through their tail spike proteins. This biorecognition is highly specific and has been employed for the typing of bacteria. This level of specificity and selectivity opens avenues for the development of specific pathogen detection technologies and for the creation of biosensing platforms. Biosensing approaches based on quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) as transduction platform were reported [36]. These early reports relied on physical adsorption of the bacteriophage on the sensor surface. Other studies reported the electrostatically-facilitated physisorption on silica particles [37]. Single-point, oriented, covalent attachment of the bacteriophages on different materials and transducers was also reported in order to yield better coverage and to improve the performance of these devices. Streptavidin-mediated attachment of bacteriophages that were genetically modified to directly express biotin on their capsid was reported [38]. Covalent immobilisation of bacteriophages on gold [39], carbon [40], and glass substrates [41] for biosensor application was also reported. Other approaches are addressed towards chemical modification of the viral capsid, such as biotinylation for further immobilisation on biosensor surfaces [42]. In particular for *Salmonella* detection, the

LODs obtained with rapid approaches based on bacteriophage biorecognition were ranged from 10^2 to 10^5 CFU mL^{-1}, and the time assay was less than 16 hours (Table 3).

Concerning commercial available kits, PCR and ELISA systems are the most frequently commercialised, as well as immunodiffusion, hybridisation and dip-stick technology (Table 4). The majority of the methods are based on optical detection (fluorescence or absorbance). To the best of our knowledge, the only electrochemical commercial kit is the QFast™ *Salmonella* from iMICROQ, Tarragona, Spain. Methods based on nucleic acid hybridisation are on the market for several organisms including *Salmonella* spp. However, the detection level of nucleic acid hybridisation methods is about 10^5 – 10^6 CFU mL^{-1}, and enrichment steps are therefore needed for food samples. In general for food testing, there are other available kits based on PCR. These include Bax2 from Qualicon, Wilmington, DE, USA; TaqMan2 from Perkin Elmer Applied Biosystems, Foster City, CA, USA, and Probelia2 from Sano® Diagnostics Pasteur, Marnes La Coquette, France [2]. Currently, there are several ELISA-based assay systems for the detection of *Salmonella* spp. Some of these tests have the advantage of being able to process numerous samples at once in 96 well microtitre plates, and some such as the Tecra™ *Salmonella* Visual Immunoassay (3M), provide a visual indication of detection without the use of colorimetric equipment. In addition, ELISA systems have been automated to facilitate routine laboratory testing such as the EIAFoss (Foss Electronics) and the VitekImmuno Diagnostic Assay System (VIDAS) (BioMerieux). Nevertheless, ELISA methods are not without disadvantages, some of which include poor LODs (over 10^5 CFU mL^{-1}) or cross reactivity. Novel ELISA-based techniques are based on fluorogenic, electrochemiluminescent, and real-time PCR reporters to create quantifiable signals.

Electrochemical biosensing of *Salmonella* based on magnetic separation

Although there are new types of transducers being developed for use in biosensors, the most popular are the optical, electrochemical and mass-based transduction methods. An ideal biosensing device for the rapid detection of food contaminants should be fully automated, inexpensive, and able to be used routinely in the field as well as in the laboratory. As analytical systems, electrochemically based transduction devices are more robust, easy to use, portable, and inexpensive [53]. The electrochemical measurement system is highly sensitive, quite cheap and already exists in portable formats. Even the advanced pulsed, voltammetric and galvanostatic techniques are available in hand-held instruments from several companies: PalmSens and EmStat (Palm Instruments), µStat (DropSens), PG581 (Uniscan Instruments), 910 PSTAT mini (Metrohm), as well as other prototypes designed in laboratories [54]. As the measuring element, the screen-printed electrodes (SPE) are widely applied due to an easy and reproducible fabrication at both laboratory and mass production scales [55, 56]. The suppliers of SPEs include companies as BVT Technologies, DropSens and The Gwent Group; however, researchers can print the sensing patterns themselves using commercial inks and pastes or even using custom mixtures containing carbon nanotubes [57] and metal nanoparticles [58] for enhanced response.

In the last decade, extensive research has been done on the integration of micro- and nanomaterials into electrochemical biosensors. Of particular interest is the integration of magnetic particles for a novel generation of biosensors. Magnetic particles have been commercially available for many years (*e.g.* BioMag®, Dynabeads®, Adembeads® and SiMAG®) and are widely used in laboratories to extract desired biological

components, such as cells, organelles or DNA, from a fluid. As showed in Figure 4, they consist of an inorganic core of magnetic materials such as iron, nickel, cobalt, neodymium-iron-boron, samarium-cobalt or magnetite coated with polymer to confer stability (such as polystyrene, dextran, polyacrylic acid or silica), which can be modified with functional groups (such as amino and carboxylic acids) to make subsequent conjugations easy. Hence, magnetic particles can carry diverse ligands, such as peptides, small molecules, proteins, antibodies and nucleic acids. Magnetic particles can have any size from a few nanometres up to a few micrometres. Nano-sized particles (5 – 50 nm) are usually composed of a single magnetic core with a polymer shell around it. Larger particles (30 nm – 10 μm) can be composed of multiple magnetic cores inside a polymer matrix. These particles can be used for efficient transport, faster assay kinetics, improved binding specificity and as labels for detection [59].

Preferred position for Figure 4

In particular, superparamagnetic particles are highly attractive for use in biosensors due to their capability to magnetise under an applied magnetic field. Thus, the particles can be separated easily from the liquid phase with a small magnet, but can be redispersed immediately after the magnet is removed [60]. They confer a number of benefits, including easy separation and suitability for automation. When coated with recognition molecules, magnetic spheres are ideal for an efficient capture and separation of target. Unwanted sample constituents may be washed away, following a simple magnetic separation step. In particular, antibody-coated superparamagnetic particles are used for the immunomagnetic separation (IMS) of nucleic acids, proteins,

viruses, bacteria and cells, being for this reason the basis of several tests. Immunomagnetic separation has proved to be a very efficient method for separating target organisms from food materials and background flora. Several procedures may be used for subsequent final detection, such as conventional culturing, microscopy, impedance technology, ELISA, latex agglutination or DNA hybridisation involving amplification techniques. In addition to the short separation and concentration time, IMS technology also overcomes the problem associated with unwanted inhibition due to selective media components. Since IMS can be used in conjunction with different readouts technologies, it is expected that several automated analytical procedures will make use of this potent technique in the near future [61, 62].

This capacity has led to the use of biorecognition agent functionalised magnetic particles for the separation and pre-concentration of whole organisms from complex media [63]. The most commonly used commercial IMS bead for the recovery of *Salmonella* from food is Captivate *Salmonella* (Lab M), Tecra *Salmonella* Unique (3M), as well as for specific serovars, such as *S. enteritidis*, via Rapidchek Confirm *S. enteritidis* IMS kit (SDIX). IMS can also be automated using automated IMS separators such as the BeadRetriever (Invitrogen), Kingfisher IMS separator (Thermofisher) or Mag Max (Life Technologies) capable of processing up to 100 samples with the capability of re-suspending the IMS target complex in microtitre plates for further testing by PCR, or ELISA. Another IMS variation was also developed by Pathatrix (Matrix MicroScience Ltd) combining IMS and a recirculation step (Flow Through Immunocapture or FTI), to further increase the sensitivity of detection since larger enrichment volumes can be reacted with IMS beads [64].

The combination of ELISA with IMS step with aiming to pre-concentrate cells from mixed cultures has been previously used in some works, nevertheless, the detection sensitivity was considered close to that of a conventional ELISA (10^5 – 10^6 CFU mL^{-1}). In Table 5, a compilation of the main enzyme-linked immunomagnetic assays (IMS-ELISA) is showed. Among the advantages of using magnetic particles, one of the most important is the capability of being separated easily from the liquid phase with a magnetic field, while being dispersed immediately after removed it. Higher reproducibility and improved LODs are thus achieved by the use of magnetic particles that can easily bind the target while being dispersed in solution avoiding sensitivity and precision problems resulting from more desorption of antibodies during the assay or less diffusion of the analyte to the surface of the solid support, such as microplates. The integration of nanomaterials such as quantum dots (QDs) or gold nanoparticles (AuNPs), as well as the coupling of the magneto-immunoassay with electrochemical detection in what is named ELIME (Enzyme-Linked Immunomagnetic Electrochemical method) improved the typical sensitivity up to 1 – 10^2 CFU mL^{-1} in a considerably reduced time.

Previous reviews reported how magnetic micro/nano particles have made significant contributions in the developments of electrochemical biosensors [10, 73]. Table 6 highlights the improvement that involves the use of IMS in rapid approaches for the detection of *Salmonella* spp. with immunological, genetic and bacteriophage-based biorecognition. Approaches based on IMS coupled with QDs or AuNPs labelling have been developed obtaining excellent limits of detection (10^2 CFU mL^{-1}) in less than 2 hours of assay. IMS in conjunction with PCR was evaluated for detection of *Salmonella* spp., the limit of detection demanded by legislation (1 – 10 CFU 25 g^{-1}) was reached in

all cases by using this combination coupled with agarose gel electrophoresis detection technique. This limit of detection was also achieved in a considerably reduced time (9 h) by combining the IMS with electrochemical genosening and immunosensing.

This latest development that combines the use of magnetic particles and electrochemical detection is of particular interest due to the considerable improvement achieved on the analytical features such as assay time and limit of detection. Figure 5 displays the scheme of three different strategies based on magnetic separation coupled with electrochemical genosensing and immunosensing. In these approaches, magnetic particles have the dual function of (i) pre-concentrating the bacteria from complex matrix, using different biorecognition reactions (immunomagnetic (IMS) and phagomagnetic (PMS) separations) and also (ii) improving the analytical features of both electrochemical genosensing and immunosensing of the bacteria. In detail, the first approach, "IMS/double-tagging PCR/m-GEC electrochemical genosensing" [13], was based on a double biorecognition of the bacteria, in this case immunological followed by genetic biorecognition. The bacteria were captured and pre-concentrated from food samples with magnetic particles through the immunological reaction with the specific antibody against *Salmonella*. After the immunomagnetic separation, the bacteria were lysed and further amplification of the genetic material by Polymerase Chain Reaction with a double-tagged set of primers was performed to confirm the identity of the bacteria. The double-tagged amplicon was then detected by electrochemical magneto-genosensing. The second strategy, "PMS/double-tagging PCR/m-GEC electrochemical genosensing" [82], was based on the use of bacteriophages, which offer several analytical advantages as biorecognition element for the magnetic separation of pathogenic

bacteria. The phage capabilities as biorrecognition element were explored by using the model phage nanoparticle P22 towards *Salmonella*. P22 bacteriophages were immobilised on tosyl-activated magnetic particles in an oriented way. The bacteria were then captured and pre-concentrated by the phage-modified magnetic particles throughout the phage-host interaction. To confirm the identity of the bacteria, further double-tagging PCR amplification of the captured bacteria DNA and electrochemical magneto-genosensing of the amplicon were performed. In the third strategy, "IMS/m-GEC electrochemical immunosensing" [81], the detection of the bacteria was performed by a double immunological recognition. The bacteria were captured from food samples and pre-concentrated by immunomagnetic separation. After the IMS, the enzymatic labelling of the bacteria was also performed using a specific antibody against *Salmonella* labelled with HRP, performing thus the electrochemical magneto-immunosensing.

Preferred position for Figure 5

In the detailed strategies, magnetic separation based on different affinity biorecognition principles was evaluated, *i.e.* immunomagnetic and phagomagnetic separation. Although similar analytical performance were obtained (LOD of 1 CFU mL^{-1} in 3 h assay time), the use of bacteriophages as a biorecognition element offers additional advantages, such as low-cost, rapidity and animal-friendly production of the bacteriophages, among others. It must be highlighted that for the first time non-modified bacteriophages were covalently coupled to magnetic particles, as showed in Figure 6. Improved LODs (1 CFU mL^{-1}) were obtained in both cases if compared with the IMS and PMS followed by conventional gel electrophoresis (10^2 and 10^3 CFU mL^{-1},

respectively), as well as a significant reduction of the assay time if compared with IMS and PMS followed by microbiological culture method (3 h *vs*. 18 – 24 h). The accuracy of the magnetic separation step coupled with microbiological culture is not measurable since agglomeration of particles often occurs and several target bacteria bound to the same particle give rise to only one colony forming unit (CFU) on the plating media. Therefore, by coupling IMS or PMS with double-tagged PCR amplification and electrochemical magneto-genosensing quantitative methods were achieved, due to the fact that a single cell is detected and these methods were not affected by the formation of aggregates. The double-tagging PCR also allows the amplification of the analytical signal by the amplification of the bacterial genome in a rapid way, instead of the multiplication of the bacteria number by growing in traditional culturing methods. The magnetic separation and the double-tagging PCR provide specificity, as well as versatility to the assay, by selecting different capture antibodies, bacteriophages or tagged primers. Therefore, the models described can be widening to other bacterial targets.

Preferred position for Figure 6

The third strategy discussed represents a simplification of the analytical methodology, in which the detection of the bacteria was performed by a double immunological recognition. After the IMS, the bacteria was detected by a second immunological biorecognition, reducing considerably the assay time from 3 h to 60 min, as well as the complexity of the procedure compared with the electrochemical magneto-genosensing strategy that is PCR-dependent. On the other hand, worse LOD and a slight matrix effect were obtained with this strategy (5 x 10^3 CFU mL^{-1} in LB broth and 7.5 x 10^3 CFU

mL^{-1} in skimmed milk diluted 1/10 in LB broth). On the contrary, this method presents better features for being implemented in microfluidic systems or in portable devices to measure in field, due to its simplicity. In terms of specificity, both genosensing and immunosensing approaches, result in good performance due to the magnetic separation, however, it must be emphasised that although the fact of being PCR-dependent increases the complexity of the assay the selection of specific primers in the genosensing approach gives greater selectivity to the strategy.

Despite the differences, a real shortening of the analytical time is obtained for both genosensing and immunosensing approaches by the IMS or PMS followed by the double-tagging PCR with electrochemical magneto-genosensing, or by the serological confirmation with electrochemical magneto-immunosensing for the confirmation of the bacteria, as an alternative for the gold-standard microbiological culture method, in which the whole procedure (selective enrichment, differential plating culture, biochemical and serological confirmation testing) are time consuming. All the strategies strategies fulfil the LOD required by the legislation (absence of *Salmonella* in 25 g of sample). Comparing with traditional methodologies, a significant improvement in total assay time has been achieved from 3 – 5 days to 9 hours in both cases, when the pre-enrichment step is included. In spite of the higher LOD obtained for the "IMS/m-GEC electrochemical immunosensing" approach compared with the "IMS/double-tagging PCR/m-GEC electrochemical genosensing" (10^3 *vs*. 1 CFU mL^{-1}), after the pre-enrichment step, the same LOD demanded by legislation (1 CFU in 25 mL) was achieved in approximately 9 hours of total assay time for both strategies, having in this manner no differences among them in terms of assay time.

All the approaches discussed, are more rapid and show better LODs than other rapid antibody-based and nucleic acid-based PCR methods previously reported (Tables 1 – 6). As an example, enzyme-linked electrochemical detection coupled with IMS generally gave detection limits of 10^3 CFU mL^{-1}, whereas PCR methods could achieve LODs ranging from 10^1 to 10^4 CFU mL^{-1} depending on the efficiency of the DNA extraction, with or without enrichment step, and the nature of the food samples. Comparing with other commercial PCR assays for the detection of *Salmonella* without magnetic separation the main advantage of the "IMS/double-tagging PCR/m-GEC electrochemical genosensing" and "PMS/double-tagging PCR/m-GEC electrochemical genosensing" procedures is that free DNA coming from death or injured cells during food processing are not detected with this strategy, because of the IMS or PMS, which separate and pre-concentrate whole bacteria cells but not DNA from food samples. Moreover, as the bacteria are pre-concentrated and separated from the original matrix, the PCR inhibitors are also avoided, overcoming thus one of the most important issues of PCR-based assays. The amplicon detection with the electrochemical magneto-genosensing strategies demonstrated improved sensitivity than other approaches for detecting DNA. Regarding other rapid approaches based on genetic recognition, most of them are demonstrated with synthetic oligonucleotides, and only few procedures are based on inoculated bacteria detection obtaining LODs ranged from 10 to 10^4 CFU mL^{-1} (Table 2). Other rapid approaches based on immunological recognition coupled with electrochemical impedance spectroscopy or fluorescence detection are able to detect the bacteria faster (ranging from 6 min to 2.5 h), but with significantly higher LODs (from 10^2 to 10^5 CFU mL^{-1}) (Table 3). To the best of our knowledge, only

detection techniques based on fluorescence are able to obtain similar features in terms of sensitivity to the approaches presented in this section.

These discussed procedures are suitable for the rapid and sensitive on-site screening-out of *Salmonella* in HACCP. Since screening assays are used on large sample populations, often with the aim of determining which samples require further investigation and confirmation of the results, these approaches are promising strategies to screen-out negative samples and thereby to isolate negative from presumptive contaminated samples. Positive test results should be always considered presumptive and must be confirmed by an approved microbiological method, which is still considered the gold-standard for bacteria detection.

Simultaneous electrochemical biosensing of pathogenic bacteria

The development of novel strategies for simultaneous detection of different foodborne pathogens presents a cost effective and time saving strategy, reducing substantially the assay times and costs. These strategies are mostly based on traditional PCR methods, real-time PCR, classical immunological techniques, biosensors, microarrays and multi-channel platforms. The most developed methodology for simultaneous bacterial detection is the multiplex Polymerase Chain Reaction (mPCR). In spite of its high sensitivity, PCR-based methodologies still have some drawbacks such as price, sensitivity to matrix interference and no live/dead cell differentiation. Recently, some papers review last developments in bioanalytical multiplex technologies [83, 84]. Microarrays and multi-channel platforms offer high multiplexing

capabilities for the biological binding assays. Other methods based on electrochemical sensors incorporate other platforms such as screen-printed electrodes. These devices usually involve antibody-antigen and DNA hybridisation specific interactions. Table 7 shows a brief summary of some rapid approaches for simultaneous detection of pathogenic bacteria. The most prominent detection methods are the optical, mostly fluorescence and chemiluminescence. Better LODs were obtained when immunoassays were combined with IMS and multiplexed PCR for lateral flow or optical detection ($10 – 10^3$ CFU mL^{-1}).

Examples of multiplexed electrochemical biosensing of pathogenic bacteria with different detection platforms are showed in Table 8. Several methodologies were reported using screen printed electrode based arrays, especially for the design of electrochemical genosensors. A bio-barcoded DNA assay based on gold and magnetic nanoparticles in a screen-printed carbon electrode chip was able to detect as low as 0.5 ng mL^{-1} of *Salmonella enteritidis* and 50 pg mL^{-1} of *Bacillus anthracis* in 2.5 h. Screen-printed carbon arrays were also coated with multi-walled carbon nanotubes, sodium alginate and carboxymethyl chitosan composite films to enhance the sensitisation of the electrode. Multiplexed electrochemical biosensing approaches are still in an earlier stage, being mostly supported on electrode arrays platforms. The widespread development of novel materials for integration in electrochemical biosensors presents an important alternative, offering unlimited possibilities for the design of novel assays for multiplex pathogens detection. At present, the commercially available test kits are designed for a single pathogen, thus to test a product for multiple bacteria, multiple assay kits must be used. Therefore, a long path for

improvement in multiplexed pathogenic microorganism detection methods still needs to be done.

Conclusions and future trends

For the past several decades, significant advancements in the microbial analysis of food and environmental samples have been made. All advancements are aimed at achieving sensitive and specific detection of pathogens, but despite these efforts, the methods still require lengthy cultural enrichment steps. In fact, the main hurdle in the development of more rapid detection methods is the dependency on culture. In the near term, techniques such as immunomagnetic separation and alternative bioaffinity ligands such as bacteriophage are promising approaches to explore for improved target capture and sample preparation. This sort of methods opens avenues for rapid microbial detection from farm-to-table using simple, integrated platforms contained in automated, miniaturised and portable devices. Publications from 2009 to 2014, which devoted to the development of biosensors for *Salmonella* detection were summarised in this review. Special emphasis was given to the integration of magnetic particles into biosensors and the discussion of three different strategies based on magnetic separation and electrochemical detection. The integration of micro- and nanostructured materials within biosensing devices is providing a significant improvement of the analytical performances in the detection of pathogens. Despite such advances on the field there are still challenges to explore new strategies for improving the analytical features such as sensitivity, specificity and time assay of the

bacterial detection. New trends are addressed towards not only the integration of new materials in biosensing but also the design of portable platforms incorporating all the necessary preparation and fluidic processes, rapid diagnostic tests, low-cost instrumentation and point-of-care devices for the rapid and simultaneous detection of pathogens.

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TABLES

Table 1. Main features of rapid approaches based on immunological biorecognition for the detection of *Salmonella* spp.

Assay format	Detection technique	Test matrix	Pre-enrichment	Total assay time	LOD	Ref.
Immunosensing on screen-printed gold electrodes	Amperometry	PBS and chicken breast (inoculated)	18 – 24 h	27 h	21 CFU mL^{-1}	[5]
Direct and sandwich ELISA with SWCNTs labelling platform	Absorbance	PBS, UHT milk (inoculated)	Not-performed	4 h	10^3 – 10^4 CFU mL^{-1}	[16]
ELISA using modified polyacrylo-nitrile fibers (PAN)	Absorbance	PBS, milk and juice matrix (inoculated)	Not-performed	2 h 30 min	10 CFU mL^{-1}	[17]
Solid-phase sandwich ELISA	Absorbance	PBS (inoculated)	Not-performed	21 h	2 x 10^3 CFU mL^{-1}	[18]
Sandwich immunoassay	Ion-Sensitive Field-Effect Transistor (ISFET)	NaCl solution (inoculated)	Not-performed	30 min	2 – 3 CFU mL^{-1}	[19]
Screen-printing technique immunosensing	Electrochemical Impedance Spectroscopy (EIS)	PBS (inoculated)	Not-performed	6 min	5 x 10^2 CFU mL^{-1}	[20]
Immunosensing by using macroporous silicon trapping array	EIS	PBS (inoculated)	Not-performed	30 min	10^3 CFU mL^{-1}	[21]
Fibre-optic immunosensor	Evanescent wave, Time-Resolved Fluorescence (TRF)	Egg and chicken breast (inoculated)	2 – 6 h	< 8 h	10^4 CFU mL^{-1}	[22]
Multichannel electrochemical immunosensor (MEI) using screen-printed sensor array	Intermittent Pulse Amperometry (IPA)	NaCl solution (inoculated)	Not-performed	3 h	2 x 10^6 CFU mL^{-1}	[23]
Array-Based Immunosensor	Fluorescence	PBS, poultry, chicken excretal samples (inoculated)	Not-performed	1 h	10^3 – 10^6 CFU mL^{-1}	[24]
Electrochemical ELISA	Electrochemical Flow Injection Analysis (FIA) and IPA	PBS, pork, chicken and beef (inoculated)	5 h	8 h	1 – 10 CFU 25 g^{-1}	[25]

Table 2. Main features of rapid approaches based on genetic biorecognition for the detection of *Salmonella* spp.

Assay format	Detection technique	Test matrix	Pre-enrichment	Total assay time	LOD	Ref.
Gene-based electrochemical DNA biosensor based on thin-film gold electrodes	DPV	PBS (Synthetic oligonucleotides)	Not-performed	1 h	0.2 µmol L^{-1}	[26]
PCR and gene-based electrochemical DNA biosensor	DPV	Luria-Bertani broth (inoculated)	Not-performed	3.5 h	0.5 pmol L^{-1}/ 10 CFU mL^{-1}	[27]
AuNP-DNA biosensor using Screen-Printed Carbon Electrodes	DPV	Luria-Bertani broth (inoculated), 2 % milk, 100 % orange juice	Not-performed	6 h	100 ng mL^{-1} / 10^4 CFU mL^{-1}	[28]
PCR and DNA biosensor label-free	SPR	Luria-Bertani broth (inoculated)	Not-performed	4.5 h	0.5 nmol L^{-1}/ 10^2 CFU mL^{-1}	[29]
DNA biosensor based on polystyrene-modified glassy carbon electrodes	Osteryoung Square Wave Voltammetry (OSWV)	PBS (Synthetic oligonucleotides)	Not-performed	12 h	0.55 µmol L^{-1}	[30]
DNA biosensor based on SWCNTs modified electrode	EIS	Phosphate buffer solution (Synthetic oligonucleotides)	Not-performed	20 min	1 nmol L^{-1}	[31]
PCR and optical thin-film DNA biosensor	Human eye	Luria-Bertani broth and pork (inoculated)	18 – 24 h	21.5 /28.5 h	8.5 x 10^1 CFU mL^{-1} (LB) / 0.4 CFU g^{-1} (pork)	[32]
PCR and hybridisation in screen-printed gold electrodes	DPV	PBS (Synthetic oligonucleotides)	Not-performed	3 h	5 nmol L^{-1}	[33]
PCR and hybridisation in screen-printed electrodes	DPV	PBS (Synthetic oligonucleotides)	Not-performed	3 h	0.3 nmol L^{-1}	[34]

Table 3. Main features of rapid approaches based on bacteriophage biorecognition for the detection of *Salmonella* spp.

Phage	Assay format	Detection technique	Test matrix	Pre-enrichment	Total assay time	LOD	Ref.
E2	Phage-coated sensor	Magnetoelastic (ME) biosensor	Eggshells (inoculated)	Not-performed	30 min	160 CFU cm^{-2}	[43]
E2	Phage-coated sensor	ME biosensor	Fresh tomato (inoculated)	Not-performed	30 min	5×10^2 CFU mL^{-1}	[44]
P22	Phage-based biosorbent (genetically engineered tailspike proteins (TSPs) on gold surface)	SPR	Luria-Bertani broth (inoculated)	Not-performed	30 min	10^3 CFU mL^{-1}	[45]
E2	Phage-coated sensor	ME biosensor	Water (inoculated)	Not-performed	1 h	5×10^3 CFU mL^{-1}	[46]
P22	Phage-based biosorbent (monolayer) and ELISA	Absorbance	Luria-Bertani broth (inoculated)	Not-performed	2.5 h	-	[41]
P22	Recombinant P22::luxAB phage	Bioluminiscence	Luria-Bertani broth and poultry and feed samples (inoculated)	12 – 14 h	16 h	1.65×10^3 CFU mL^{-1}	[47]
E2	Phage-based biosorbent (physical adsorption)	ME biosensor	Skimmed milk and water (inoculated)	Not-performed	20 min	5×10^3 CFU mL^{-1}	[48]
E2	Piezoelectric platform (physical adsorption)	Quartz Cristal Microbalance (QCM)	PBS (inoculated)	Not-performed	-	10^2 CFU mL^{-1}	[49]
SJ2	Phage-mediated cell lysis	Bioluminiscence	Trypticase Soy Broth (inoculated)	Not-performed	2 h	10^3 CFU mL^{-1}	[50]
SJ2	Phage-based biosorbent (streptavidin magnetic beads and biotinylated phage)	Bioluminiscence	Luria-Bertani broth (inoculated)	Not-performed	40 min	4×10^3 CFU mL^{-1}	[51]
Sapp-hire	Phage-based biosorbent (passive immobilisation on polystirene strips)	PCR / Agarose gel electrophoresis	Luria-Bertani broth (inoculated)	Not-performed	2 h	10^5 CFU mL^{-1}	[52]

Table 4. Main features of commercial kits available for the detection of *Salmonella* spp.

Commercial kit	Assay format	Applicable to	Pre-enrichment	Total assay time	LOD	Company
VIDAS® Easy SLM	Sandwich immunoassay (fluorescence)	Environmental sampling	16 – 22 h (x2)	45 h	1 – 5 CFU / 25 g	BioMérieux
VIDAS® UP *Salmonella*	Sandwich immunoassay using phage recombinant protein (fluorescence)	Food, feed, environmental sampling	18 – 24 h	25 h	1 – 5 CFU / 25 g	BioMérieux
TRANSIA® PLATE *Salmonella* Gold	ELISA Sandwich immunoassay (LPS detection)	Food, feed, environmentals ampling	18 – 20 h	24 h	1 – 5 CFU / 25 g	BioControl
RIDASCREEN® *Salmonella* ELISA	ELISA Sandwich immunoassay	Food, feed, environmentals ampling	16 – 20 h	< 23 h	1 – 5 CFU / 25 g	R-Biopharm
LOCATE® *Salmonella* ELISA	ELISA monoclonal antibody (O somatic antigen detection)	Food commodities	46 h	< 48 h	1 – 5 CFU / 25 g	R-Biopharm
TECRA® ULTIMA™ *Salmonella*	ELISA	Raw meats and carcass swabs	16 – 20 h (x2)	42 h	1 – 5 CFU / 25 g	3M
3M™ Tecra™ *Salmonella* Visual Immunoassay	ELISA	Raw materials, finished products and environmental surfaces	16 – 20 h (x2)	42 h	1 – 5 CFU / 25 g	3M
LightCycler ® foodproof *Salmonella* Detection	Real-time PCR	>100 tested food matrices and environmental samples	16 – 20 h	18 – 22 h	1 – 5 CFU / 25 g	Roche Diagnostics GmbH Roche Applied Science
Foodproof® *Salmonella* PCR	Real-time PCR	Food, environmental samples and beverages	16 – 20 h	< 24 h	1 – 5 CFU / 25 g	Merck-Millipore
MicroSEQ® *Salmonella* spp. Detection Kit	Real-time PCR	Food, beverage and animal feed	16 – 24 h	18 – 27 h	1 – 5 CFU / 25 g	Applied Biosystems, Life Technologies
HybriScan™D *Salmonella*	RNA sandwich hybridisation and enzyme-linked optical detection	Food samples	18 h – 24 h	44.5 h	1 – 5 CFU / 25 g	Sigma-Aldrich
RapidChek® SELECT™ *Salmonella* enteritidis	Test strip, sandwich immunoassay using colloidal gold	Chicken house drag swabs, egg pool samples and chicken rinse samples	16 – 22 h (x2)	32 – 48 h	1 – 5 CFU / 25 g	SDIX
QFast™ *Salmonella*	IMS and electrochemical detection	Skin and chicken meat, raw materials (cereals, nuts, extracts)	20 – 24 h	< 24 h	1 – 5 CFU / 25 g	iMICROQ

Table 5. Main features of rapid approaches based on enzyme-linked immunomagnetic assay for the detection of *Salmonella* spp.

Assay format	Detection technique	Test matrix	Pre-enrichment	Total assay time	LOD	Ref.
IMS with immuno-AuNP network	Absorbance	PBS, fat milk, ground beef, pineapple juice (inoculated)	2 h	4 h 30 min	3 CFU mL^{-1}	[65]
IMS with immuno-QDs	Fluorescence	PBS (inoculated)	Not-performed	30 min	500 CFU mL^{-1}	[66]
IMS-ELIME	IPA	Pork, chicken, beef, and turkey (real and inoculated)	6 h	8 h	1 – 10 CFU 2 g^{-1}	[67]
IMS-ELISA	Absorbance	Skimmed milk powder in buffered peptone water (BPW) (inoculated)	18 – 24 h	24 h	10^5 – 10^6 CFU mL^{-1}	[68]
IMS-ELIMC / IMS-ELIME	Absorbance / Osteryoung square wave voltammetry (OSWV)	PBS (inoculated)	Not-performed	80 min	2 x 10^4 CFU mL^{-1} (ELIMC) / 8 x 10^3 CFU mL^{-1} (ELIME)	[69]
IMS-ELISA	Absorbance	Eggs	18 – 24 h	24 h	10^5 – 10^6 CFU mL^{-1}	[70]
IMS-ELISA	Absorbance	Eggs and chicken meat	24 h	26 h	10^5 CFU mL^{-1}	[71]
Automated IMS and Enzyme Immunoassay (EIA)	Absorbance	Poultry environmental samples	18-24 h	48 h	10^4 – 10^6 CFU mL^{-1}	[72]

Table 6. Main features of rapid approaches for the detection of *Salmonella* spp. based on immunomagnetic separation

Assay format	Detection technique	Test matrix	Pre-enrichment	Total assay time	LOD	Ref.
IMS with AuNP labelling	Differential Pulse Voltammetry (DPV)	PBS-Tween (inoculated)	Not-performed	1 h 30 min	143 CFU mL^{-1}	[74]
IMS with Magnetic Nanobeads (MNBs) and QDs labelling	Fluorescence	PBS, ground beef, chicken carcasses, fresh-cut broccoli and lettuce (inoculated)	Not-performed	2 h	20 – 50 CFU mL^{-1}	[75]
IMS screen-printing technique and enzymatic detection	EIS and amperometry	Peptone water (inoculated)	Not-performed	1 h	10^2 – 10^5 CFU mL^{-1}	[76]
IMS and label free detection	IR fingerprinting	2 % milk and spinach extract (inoculated)	Not-performed	30 min	10^5 CFU mL^{-1}	[77]
IMS and phage amplification assay (SJ2)	Fluorescence or optical density	Luria-Bertani broth (inoculated)	Not-performed	4 – 5 h	10^4 CFU mL^{-1}	[78]
IMS and PCR	Agarose gel electrophoresis	Brain Heart Infusion broth, minced beef, pork and chicken meats (inoculated)	16 – 18 h and 6 h post-enrichment after IMS	26 h	1 – 10 CFU / 25 g	[79]
IMS and PCR	Agarose gel electrophoresis	Chicken meats (inoculated)	12 h	16 h	1 – 10 CFU / 25 g	[80]
IMS, PCR and electrochemical genosensing	Amperometry	Skimmed milk (inoculated)	6 h	9 h	1 – 10 CFU / 25 g	[13]
IMS and electrochemical immunosensing	Amperometry	Skimmed milk (inoculated)	8 h	9 h	1 – 10 CFU / 25 g	[81]

Table 7. Main features of rapid approaches for simultaneous detection of pathogenic bacteria.

Target	Assay format	Detection technique	Test matrix	Total assay time	LOD	Ref.
B. anthracis *E. coli* O157:H7 *F. tularensis* *Listeria* sp. *Salmonella* sp. *Shigella* sp. *Y. pestis* Cholera toxin Ricin toxin SEB toxin	Immunoassay using fluorescent coded microspheres	Microflow cytometer/ Fluorescence	PBS, serum and nasal wash (inoculated)	< 2 h	$10^4 - 10^6$ CFU mL^{-1}	[85]
Listeria, E. coli and *Salmonella*	Immunoassay with fluorescent antibodies reporters	Evanescent-based fibre optic sensor/ Fluorescence	BHI, beef, chicken and turkey meats (inoculated)	22 h	10^3 CFU mL^{-1}	[86]
Salmonella and *Cronobacter*	DNA array, PCR, hybridisation	DVD driver	Powder skimmed milk (inoculated)	2 h	$10^0 - 10^2$ CFU mL^{-1}	[87]
E. coli, *Bacillus subtilis* and *Salmonella*	IMS and bioactive paper strips	Lateral flow colorimetric	Milk, orange juice, lettuce (inoculated)	8 h	1 CFU 100 mL^{-1}	[88]
E. coli and *Salmonella*	IMS-mPCR	Agarose gel electro- phoresis	Ground beef and whole milk (inoculated)	< 24 h	$10^3 - 10^4$ CFU mL^{-1}	[89]
E. coli and *Salmonella*	IMS-mPCR	Agarose gel electro- phoresis	Minced chicken meat and peach juice (inoculated)	2 h	10^4 CFU mL^{-1}	[90]
Listeria, E. coli and *Salmonella*	Sandwich immunoassay. IMS and QDs labelling	Fluorescence	BHI, chicken carcasses, ground beef, fresh cut broccoli, and fresh-cut lettuce (inoculated)	1 h 30 min	20 – 50 CFU mL^{-1}	[75]
Campylobacter, *E. coli*, *Listeria*, *Salmonella*, *Shigella*, and *Tularemi.* Cholera, ricin, and SEB toxins	Sandwich immunoassay using fluorescent coded microspheres	Microflow cytometer/ Fluorescence	PBS (inoculated)	1 h 15 min	10^5 CFU mL^{-1}	[91]
E. coli, Listeria, and *Salmonella.* Cholera, ricin, and SEB toxins	Sandwich immunoassay. Microspheres and signal amplification	Microflow cytometer/ Fluorescence	PBS (inoculated)	2 h 30 min	10^4 CFU mL^{-1}	[92]
E. coli, Yersinia, Salmonella and *Listeria*	Sandwich EIA	Chemilumi-nescence	Human fecal and bovine meat samples (inoculated)	10 h	$10^4 - 10^5$ CFU mL^{-1}	[93]
E. coli and *Salmonella*	Sandwich immunoassay with IMS	Electrochemi-luminescence	Milk, juices, serum, ground beef, chicken, fish and freshwater	< 1 h	$10^2 - 10^3$ CFU mL^{-1}	[94]

Table 8. Main features of rapid approaches for simultaneous electrochemical biosensing of pathogenic bacteria.

Target	Assay format	Detection technique	Test matrix	Total assay time	LOD	Ref.
Escherichia coli O157:H7 *Campylobacter* and *Salmonella*	Sandwich immunoassay	Square wave anodic stripping voltammetry	Milk (inoculated)	1 h	400 – 800 CFU mL^{-1}	[95]
Escherichia coli O157:H7 and *Enterobacter sakazakii*	Immunosensor arrays	Cyclic voltammetry	Not-performed	2 – 3 h	10^3 – 10^4 CFU mL^{-1}	[96]
Staphylococcus aureus and *Salmonella choleraesuis*	Glucose determination	Flow injection amperometry	Not-performed	7 h	6.5 CFU mL^{-1}	[97]
Salmonella spp., *Listeria monocytogenes*, *E. coli 0157:H7* and *Staphylococcus aureus*	Screen-printed gold electrode arrays, PCR, hybridisation	Differential pulse voltammetry	Not-performed	1 h	5 nmol L^{-1}	[98]
Protective antigen A (*pagA*) gene of *B. anthracis* and the insertion element (*Iel*) gene of *S. enteritidis*	Nanoparticle-based, bio-barcoded electrochemical biosensor	Square wave anodic stripping voltammetry	Not-performed	2 h 30 min	50 pg mL^{-1} (B. *anthracis)* 0.5 ng mL^{-1} (S. *enteritidis*)	[99]
E. coli, P. *mirabilis*, P. *aeruginosa*, *Enterococcus* spp., *Serratia*, *Providencia*, *Morganella* and *Staphylococcus* spp.	Integrated nucleic acid and protein biosensor assay	Amperometry	Urine samples	1 h	10^4 CFU mL^{-1}	[100]

FIGURES

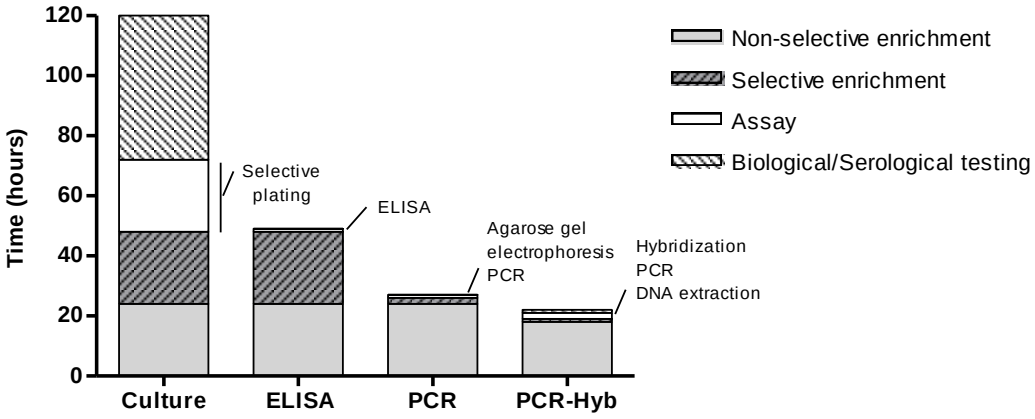

Figure 1. Comparison of maximum assay times for the detection of *Salmonella* spp.

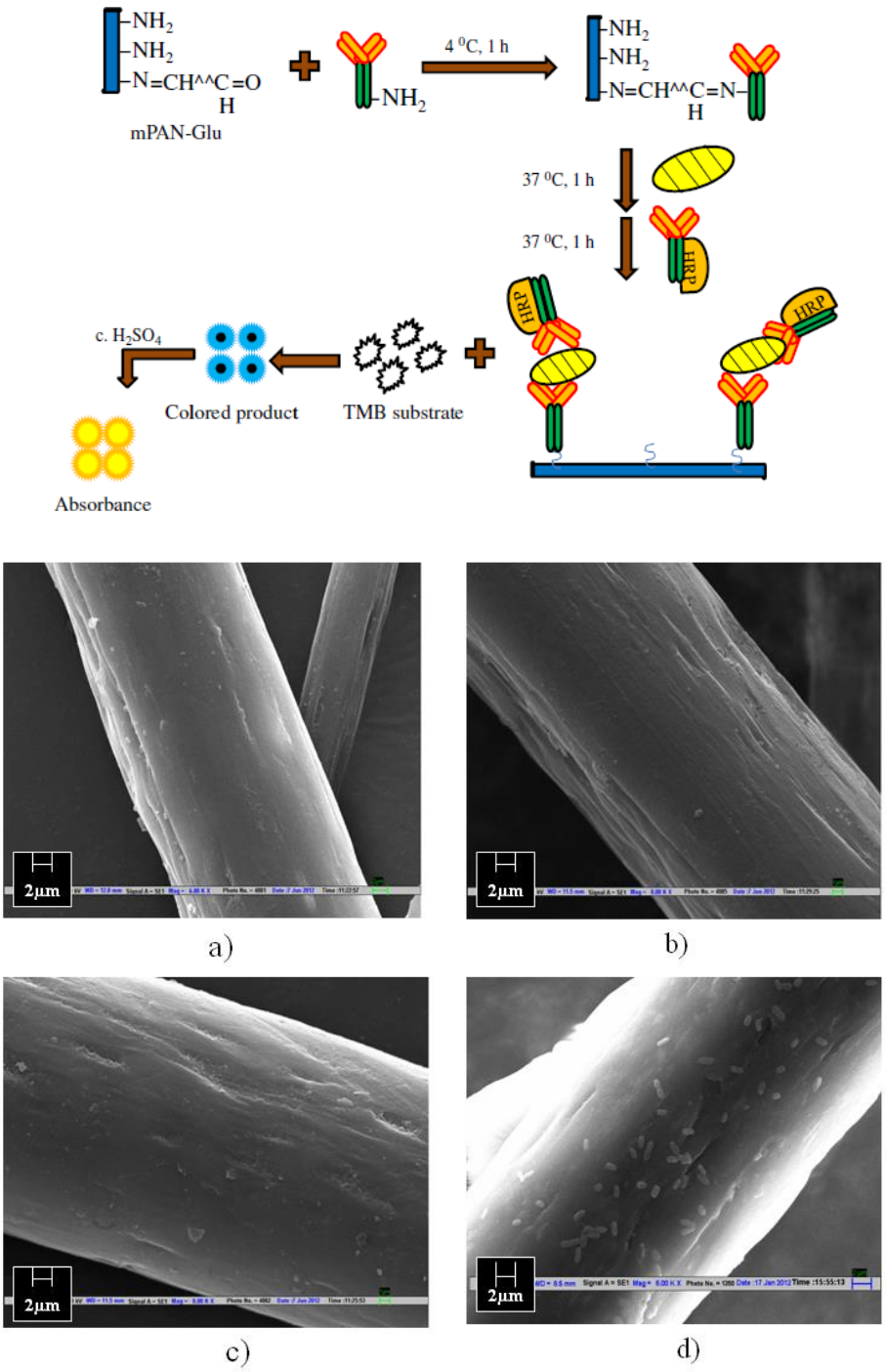

Figure 2. Up: Schematic representation of immunoassay for the detection of *S. typhimurium* bacteria developed on modified PAN fibers. Down: Scanning electron micrographs of (a) virgin, (b) surface aminated, (c) CSA-1-Ab immobilized and (d) *S. typhimurium* captured fibers (Reprinted with permission from ref. 17)

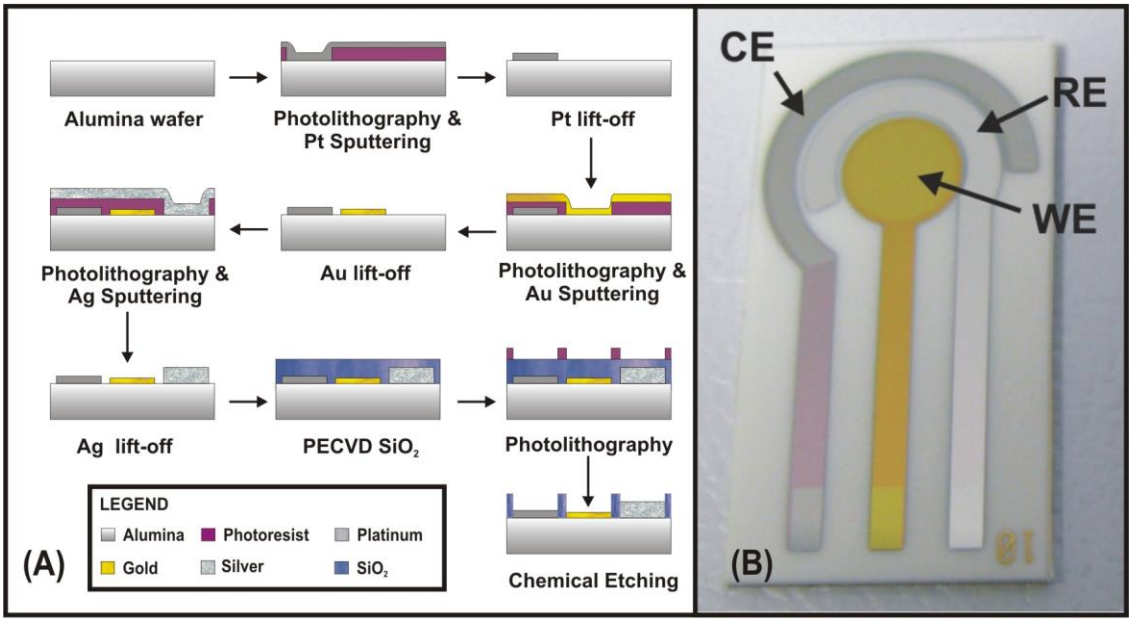

Figure 3. (A) Schematic horizontal flowchart of the transducer fabrication procedure. (B) Picture of the developed transducer consisting of a three-electrode configuration, where WE, CE and RE denote the working, the counter and the reference electrodes respectively. (Reprinted with permission from ref. 26)

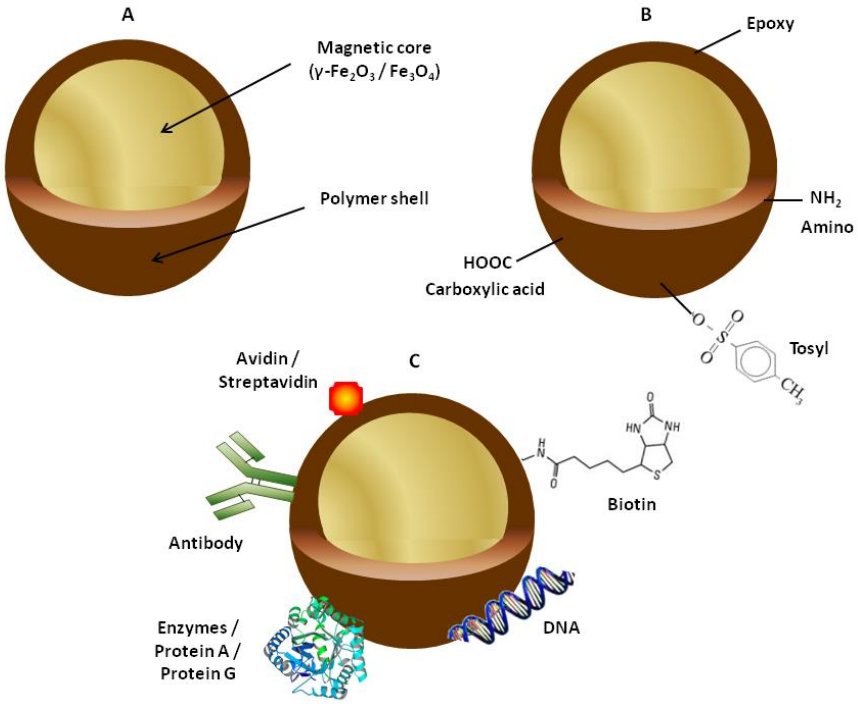

Figure 4. Schematic representation of magnetic particles (A), activated with functional groups (B) and conjugated to biological molecules (C).

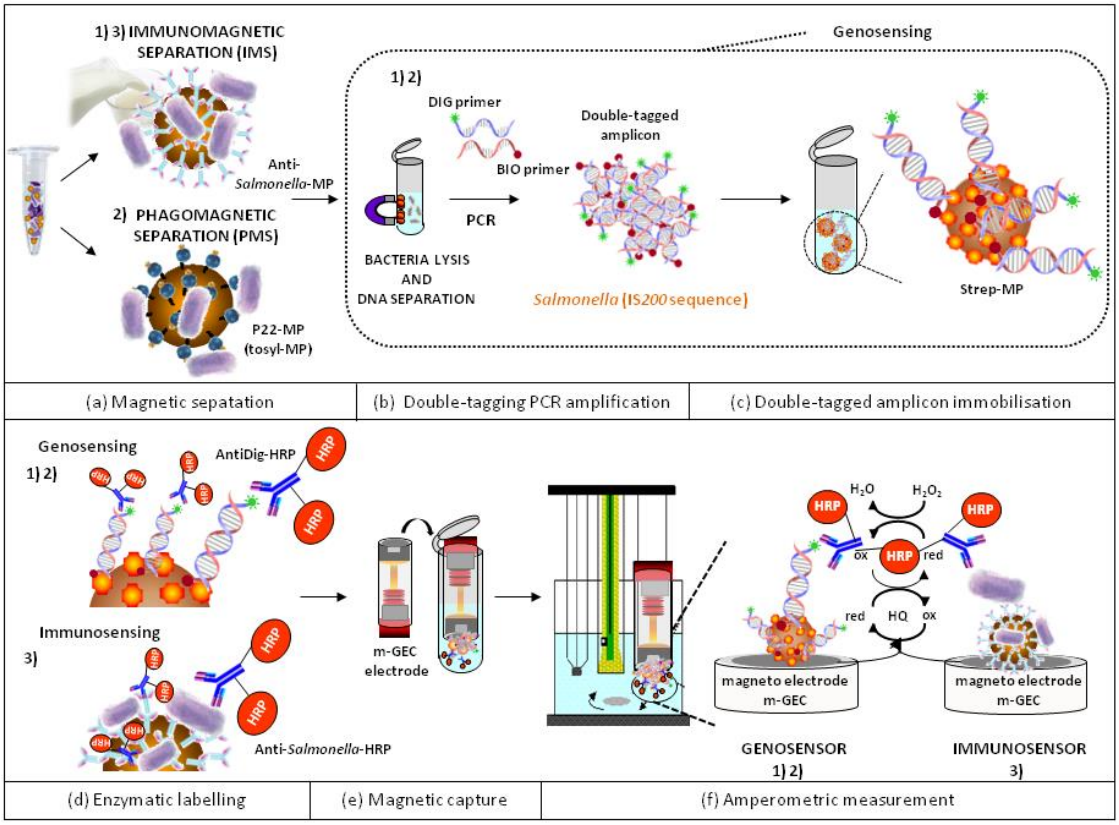

Figure 5. Schematic representation of the electrochemical strategies for *Salmonella* spp. detection: 1) "IMS/double-tagging PCR/m-GEC electrochemical genosensing" [13], 2) "PMS/double-tagging PCR/m-GEC electrochemical genosensing" [82], and 3) "IMS/m-GEC electrochemical immunosensing" [81].

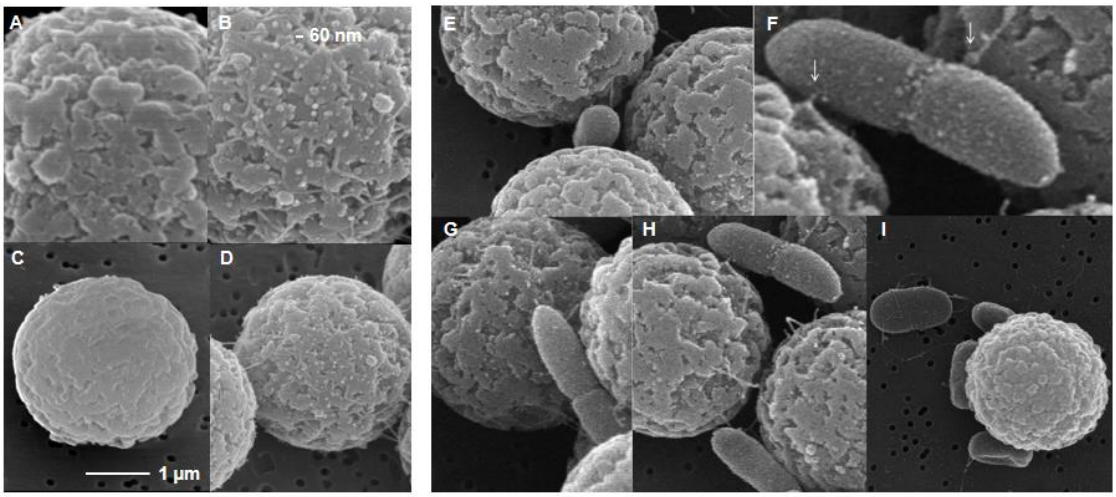

Figure 6. Left: Evaluation of the P22 bacteriophage immobilised on magnetic particles by SEM (2000 PFU/MP) at different resolution levels (panels B and D). Panels A and C show the magnetic particle without modification as a negative control. Right: Evaluation of the phagomagnetic separation (PMS) by SEM at a *Salmonella* concentration of 2.9×10^7 CFU mL^{-1}. Images E – I show the *Salmonella* cells attached to the magnetic particles through the tail spikes. In all cases, identical acceleration voltage (15 KV) was used.

References

1. H. P. Dwivedi and L. A. Jaykus, *Crit Rev Microbiol,* 2011, **37**, 11, 40.
2. K. G. Maciorowski, S. D. Pillai, F. T. Jones and S. C. Ricke, *Crit Rev Microbiol,* 2005*,* **31**, 45.
3. H. Y. Tsen, in R. K. Robinson, C. A. Batt and P. D. Patel (Eds.) 1999, *Encyclopedia of Food Microbiology*, Elsevier Science Ltd., Amsterdam, 640.
4. T. S. Hammack and W. H. Andrews, W. H., in R. K. Robinson, C. A. Batt and P. D. Patel (Eds.) 1999, *Encyclopedia of Food Microbiology*, Elsevier Science Ltd., Amsterdam, 1937.
5. F. Salam and I. E. Tothill, *Biosens Bioelectron,* 2009, **24**, 2630.
6. F. Ricci, G. Volpe, L. Micheli and G. Palleschi, *Anal Chim Acta*, 2007, **605**, 111.
7. P. Durand Skottrup, M. Nicolaisen and A. Fejer Justesen, *Biosens Bioelectron*, 2008, **24**, 339.
8. S. Cagnin, M. Caraballo, C. Guiducci, P. Martini, M. Ross, M. SantaAna, D. Danley, T. West and G. Lanfranchi, *Sensors,* 2009, **9**, 3122.
9. B. Van Dorst, J. Mehta, K. Bekaert, E. Rouah-Martin, W. De Coen, P. Dubruel, R. Blust and J. Robbens, *Biosens Bioelectron,* 2010, **26**, 1178.
10. N. Sanvicens, C. Pastells, N. Pascual and M. P. Marco, *Trends Analyt Chem,* 2009, **28**, 11, 1243.
11. F. Ricci, G. Adornetto and G. Palleschi, *Electrochim Acta,* 2012, **84**, 74.
12. P. S. Mead, L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin and R. V. Tauxe, *Emerg Infect Dis,* 1999, **5**, 5, 607.
13. S. Liébana, A. Lermo, S. Campoy, J. Barbé, S. Alegret and M. I. Pividori, *Anal Chem,* 2009, **81***,* 5812.
14. P. Arora, A. Sindhu, H. Kaur, N. Dilbaghi and A. Chaudhury, *Appl Microbiol Biotechnol,* 2013, **97**, 1829.
15. A. E. Smartt, T. Xu, P. Jegier, J. J. Carswell, S. A. Blount, G. S. Sayler and S. Ripp, *Anal Bioanal Chem,* 2012, **402**, 3127.
16. W. Chunglok, D. K. Wuragil, S. Oaew, M. Somasundrum and W. Surareungchaia, *Biosen Bioelectron,* 2011, **26**, 3584.
17. S. Chattopadhyay, A. Swati Jain and H. Singh, *Biosens Bioelectron,* 2013, **45**, 274.
18. S. Jain, S. Chattopadhyay, R. Jackeray, C. K. V. Z. Abid, G. S. Kohli and H. Singh, *Biosens Bioelectron,* 2012, **31**, 37.

19. N. F. Starodub and J. O. Ogorodnijchuk, *Electroanalysis,* 2012, **24**, 3, 600.

20. V. Nandakumar, D. Bishop, E. Alonas, J. LaBelle, L. Joshi and T. L. Alford, *IEEE Sens J,* 2011, **11**, 1, 210.

21. R. D. Das, C. RoyChaudhuri, S. Maji, S. Das and H. Saha, *Biosens Bioelectron,* 2009, **24**, 3215.

22. A. M. Valadez, C. A. Lana, S.-I. Tu, M. T. Morgan and A. K. Bhunia, *Sensors,* 2009, **9**, 5810.

23. E. Delibato, G. Volpe, D. Stangalini, D. De Medici, D. Moscone and G. Palleschi, *Anal Lett,* 2006, **39**, 1611.

24. C. R. Taitt, Y. S. Shubin, R. Angel and F. S. Ligler, *Appl Environ Microbiol,* 2004*,* **70**, 152.

25. L. Croci, E. Delibato, G. Volpe and G. Palleschi, *Anal Lett,* 2011, **34**, 15, 2597.

26. T. García, M. Revenga-Parra, L. Añorga, S. Arana, F. Pariente and E. Lorenzo, *Sens Actuators B Chem,* 2012, **161**, 1030.

27. Q. Li, W. Cheng, D. Zhang, T. Yu, Y. Yin, H. Ju and S. Ding, *Int J Electrochem Sci,* 2012, **7**, 844.

28. S. A. Vetrone, M. C. Huarng and E. C. Alocilja, *Sensors,* 2012, **12**, 10487.

29. D. Zhang, Y. Yan, Q. Li, T. Yu, W. Cheng, L. Wang, H. Ju and S. Ding, *J Biotechnol,* 2012, **160**, 123.

30. M. D. Serrano, A. Rosado, J. del Pilar, M. Arias and A. R. Guadalupe, *Electroanalysis,* 2011, **23**, 8, 1830.

31. J. E. Weber, S. Pillai, M. K. Ram, A. Kumar and S. R. Singh, *Mater Sci Eng C Mater Biol Appl,* 2011, **31**, 821.

32. S. Bai, J. Zhao, Y. Zhang, W. Huang, S. Xu, H. Chen, L.-M. Fan, Y. Chen and X. W. Deng, *Appl Microbiol Biotechnol,* 2010, **86**, 983.

33. F. Farabullini, F. Lucarelli, I. Palchetti, G. Marrazza and M. Mascini, *Biosens Bioelectron,* 2007, **22**, 1544.

34. M. L. Del Giallo, D. O. Ariksoysal, G. Marrazza, M. Mascini and M. Ozsoz, *Anal Lett*, 2005, **38**, 2509.

35. A. E. Smartt and S. Ripp, *Anal Bioanal Chem,* 2011, **400**, 991.

36. S. Balasubramanian, I. Sorokulova, V. I. Vodyanoy and A. L. Simonian, *Biosens Bioelectron,* 2007, **22**, 948.

37. R. Cademartiri, H. Anany, I. Gross, R. Bhayani, M. W. Griffiths and M. A. Brook, *Biomaterials,* 2010, **31**, 1904.

38. M. Tolba, O. Minikh, L. Y. Brovko, S. Evoy and M. W. Griffiths, *Appl Environ Microbiol,* 2010, **76**, 528.

39. A. Singh, N. Glass, M. Tolba, L. Brovko, M. Griffiths and S. Evoy, *Biosens Bioelectron,* 2009, **24**, 3645.

40. A. Shabani, M. Zourob, B. Allain, C. A. Marquette, M. F. Lawrence and R. Mandeville, *Anal Chem,* 2008, **80**, 9475.

41. H. Handa, S. Gurcynski, M. J. Jackson, G. Auner, J. Walker and G. Mao, *Surf Sci,* 2008, **602**, 1392.

42. L. Gervais, M. Gel, B. Allain, M. Tolba, L. Brovko, M. Zourob, R. Mandeville, M. Griffiths and S. Evoy, *Sens Actuators B Chem,* 2007, **125**, 615.

43. Y. Chai, S. Li, S. Horikawa, M. K. Park, V. Vodyanoy and B. A. Chin, *J Food Prot,* 2012, **75**, 4, 631.

44. S. La, Y. La, H. Chen, S. Horikawa, W. Shen, A. Simonian and B. A. Chin, *Biosens Bioelectron,* 2010, **26**, 1313.

45. A. Singh, S. K. Arya, N. Glass, P. H. Moghaddam, R. Naidoo, C. M. Szymanski, J. Tanha and S. Evoy, *Biosens Bioelectron,* 2010, **26**, 131.

46. S. Huang, H. Yang, R. S. Lakshmanan, M. L. Johnson, J. Wan, I.-H. Chen, H. C. Wikle, V. A. Petrenko, J. M. Barbaree and B. A. Chin, *Biosens Bioelectron,* 2009, **24**, 1730.

47. G. Thouand, P. Vachon, S. Liu, M. Dayre and M. W. Griffiths, *J Food Prot,* 2008, **71**, 2, 380.

48. R. S. Lakshmanan, R. Guntupalli, J. H. Valery, A. Petrenko, J. M. Barbaree and B. A. Chin, *Sens Actuators B Chem* 2007, **126**, 544.

49. E. V. Olsen, I. B. Sorokulova, V. A. Petrenko, I. H. Chen, J. M. Barbaree and V. J. Vodyanoy, *Biosen Bioelectron,* 2006, **21**, 1434.

50. Y. Wu, L. Brovko and M. W. Griffiths, *Lett Appl Microbiol*, 2001, **33**, 311.

51. W. Sun, L. Brovko and M. W. Griffiths, *J Ind Microbiol Biotechnol,* 2001, **27**, 126.

52. A. R. Bennett, F. G. C. Davids, S. Vlahodimou, J. G. Banks and R. P. Betts, *J Appl Microbiol,* 1997*,* **83**, 259.

53.F. Ricci, G. Adornetto and G. Palleschi, *Electrochim Acta,* 2012, **84**, 74.

54. P. Skládal, D. Kovář, V. Krajíček, P. Šišková, J. Přibyl and E. Švábenská, *Int J Electrochem Sci,* 2013, **8**, 1635.

55. R. M. Pemberton, T. Cox, R. Tuffin, I. Sage, G. A. Drago, N. Biddle, J. Griffiths, R. Pittson, G. Johnson, J. Xua, S. K. Jackson, G. Kenna, R. Luxton and J. P. Hart, *Biosens Bioelectron*, 2013, **42** 668.

56. J. P. Metters, R. O. Kadara and C.E. Banks, *Analyst,* 2011, **136**, 1067.

57.J. P. Metters, M. Gomez-Mingot, J. Iniesta, R. O. Kadara and C. E. Banks, *Sens Actuators B Chem*, 2013, **177**, 1043.

58. A. F-Y Kong, S-X Gu, W-W Li, T-T Chen, Q. Xu and W. Wang, *Biosens Bioelectron*, 2014 , **56**, 77.

59. E. Sharif, J. Kiely and R. Luxton, *J Immunol Methods,* 2013, **388**, 78.

60. S. Solè, A. Merkoçi and S. Alegret, *Trends Anal Chem* 2001, **20**, 2, 102.

61. J. W. Austin and F. J. Pagotto, in B. Caballero, L. C. Trugo and P. M. Finglas (Eds.) 2003, *Encyclopedia of Food Science and Nutrition*, Elsevier Science Ltd., Amsterdam 3886.

62. M. Upmann and C. Bonaparte, in R. K. Robinson, C. A. Batt and P. D. Patel (Eds.) 1999, *Encyclopedia of Food Microbiology*, Elsevier Science Ltd., Amsterdam, 1887.

63. P. Vikesland and A. Wigginton, *Environ Sci Technol,* 2010, **44**, 3656.

64. J. A. Odumeru and C. G. León-Velarde, in Mahmoud, B. S. M. (Ed.) 2012, *Salmonella – A Dangerous Foodborne Pathogen*, InTech, Croatia, 373.

65. I. H. Cho and J. Irudayaraj, *Int J Food Microbiol*, 2013, **164**, 70.

66. H. Kuang, G. Cui, X. Chen, H. Yin, Q. Yong, L. Xu, C. Peng, L. Wang and C. Xu, *Int J Mol Sci,* 2013*,* **14**, 8603.

67. E. Delibato, G. Volpe, D. Romanazzo, D. de Medici, L. Toti, D. Moscone and G. Palleschi, *J Agric Food Chem,* 2009, **57**, 7200.

68. L. P. Mansfield and S. J. Forsythe, *Lett App Microbiol,* 2000, **31**, 279.

69. A. G. Gehring, C. G. Crawford, R. S. Mazenko, L. J. van Houten and J. D. Brewster, *J Immunol Methods,* 1996, **195,** 15.

70. P. S. Holt, R. K. Gast and C. R. Greene, *J Foof Prot*, 1995*,* **58**, 9, 967.

71. K. S. Cudjoe, T. Hagtvedt and R. Dainty, *Int J Food Microbiol,* 1995, **27**, 11.

72. C. G. Leon-Velarde, L. Zosherafatein and J. A. Odumeru, *J Microbiol Methods,* 2009, **79**, 13.

73. Y. Xu and E. Wang, *Electrochim Acta,* 2012, **84**, 62.

74. A. S. Afonso, B. Pérez-López, R. C. Faria, L. H. C. Mattoso, M. Hernández-Herrero, A. X. Roig-Sagués, M. Maltez-da Costa and A. Merkoçi, *Biosens Bioelectron,* 2013, **40**, 121.

75. H. Wang, Y. La, A. Wang and M. Slavik, *J Food Prot,* 2011, **74**, 12, 2039.

76. D. Mata, D. Bejarano, M. L. Botero, P. Lozano, M. Constantí and I. Katakis, *Electrochim Acta,* 2010, **55**, 4261.

77. S. P. Ravindranath, L. J. Mauer, C. Deb-Roy and J. Irudayaraj, *Anal Chem*, 2009, **81**, 2840.

78. S. Favrin, S. Jassim and M. W. Griffiths, *Appl Environ Microbiol*, 2001, **67**, 217.

79. A. N. Moreira, F. R. Conceição, R. de C. S. Conceição, C. N. Dias, J. B. Carvalhal, O. A, Dellagostin and J. A. G. Aleixo, *J Food Safety,* 2009, **29**, 59.

80. B. M. Taban and S. A. Aytac, *Eur Food Res Technol,* 2009, **229**, 623.

81. S. Liébana, A. Lermo, S. Campoy, M. P. Cortés, S. Alegret and M. I. Pividori, *Biosens Bioelectron*, 2009, **25**, 510.

82. S. Liébana, D. A. Spricigo, M. P. Cortés, J. Barbé, M. Llagostera, S. Alegret and M. I. Pividori, *Anal Chem*, 2013, **85**, 3079.

83. M. Pedrero, S. Campuzano and J. M. Pingarrón, *Sensors,* 2009, **9**, 5503.

84. S. R. Raz and W. Haasnoot, *Trends Analyt Chem,* 2011, **30** (9), 1526.

85. L. C. Shriver-Lake, J. Golden, L. Bracaglia and F. S. Ligler, *Anal Bioanal Chem,* 2013, **405**, 5611.

86. S. H. Ohk and A. K. Bhunia, *Food Microbiol,* 2013, **33**, 166.

87. T. Arnandis-Chover, S. Morais, L. A. Tortajada-Genaro, R. Puchades, A. Maquieira,J. Berganza and G. Olabarria, *Talanta,* 2012, **101**, 405.

88. S. M. Zakir Hossain, C. Ozimok, C. Sicard, S. D. Aguirre, M. M. Ali, Y. Li and J. D. Brennan, *Anal Bioanal Chem,* 2012, **403**, 1567.

89. C. C. Tsai, H. Y. Hsih, C. H. Tsai and H. Y. Tsen, *J Food Safety,* 2012, **32**, 246.

90. V. C. Ozalp, G. Bayramoglu, M. Y. Arica and H. A. Oktem, *Appl Microbiol Biotechnol,* 2013, **97**, 9541.

91. A. L. Thangawng, J. S. Kim, J. P. Golden, G. P. Anderson, K. L. Robertson, V. Low and F. S. Ligler, *Anal Bioanal Chem,* 2010, **398**, 1871.

92. J. S. Kim, G. P. Anderson, J. S. Erickson, J. P. Golden, M. Nasir and F. S. Ligler, *Anal Chem,* 2009, **81***,* 5426.

93. M. Magliulo, P. Simoni, M. Guardigli, E. Michelini, M. Luciani, R. Lelli and A. Roda, *J Agric Food Chem,* 2007, **55**, 4933.

94. H. Yu and J. G. Bruno, *Appl Env Microbiol,* 1996, **62**, 587.

95. S. Viswanathan, C. Rani and J. A. Ho, *Talanta*, 2012, **94**, 315.

96. W. Dou, W. Tang and G. Zhao, *Electrochim Acta*, 2013, **97**, 79.

97. M. D. Morales, B. Serra, A. Gúzmán-Vázquez de Prada, A. J. Reviejo and J. M. Pingarrón, *Analyst*, 2007, **132**, 572.

98. F. Farabullini, F. Lucarelli, I. Palchetti, G. Marrazza and M. Mascini, *Biosens Bioelectron*, 2007, **22**, 1544.

99. D. Zhang, M. C. Huarng and E. C. Alocilja, *Biosens Bioelectron*, 2010, **26**, 1736.

100. R. Mohan, K. E. Mach, M. Bercovici, Y. Pan, L. Dhulipala, P. K. Wong and J. C. Liao, *PLoS One*, 2011, **6** (10), e26846.