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Electrochemical biosensors for *Salmonella*: State of the art and challenges in food safety assessment

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ABSTRACT

According to the recent statistics, *Salmonella* is still an important public health issue in the whole world. Legislated reference methods, based on counting plate methods, are sensitive enough but are inadequate as an effective emergency response tool, and are far from a rapid device, simple to use out of lab. An overview of the commercially available rapid methods for *Salmonella* detection is provided along with a critical discussion of their limitations, benefits and potential use in a real context. The distinguished potentialities of electrochemical biosensors for the development of rapid devices are highlighted. The state-of-art and the newest technologic approaches in electrochemical biosensors for *Salmonella* detection are presented and a critical analysis of the literature is made in an attempt to identify the current challenges towards a complete solution for *Salmonella* detection in microbial food control based on electrochemical biosensors.

1. Introduction

Foodborne diseases are caused by ingestion of water or food contaminated by pathogenic microorganisms, like bacteria and virus, pesticides residues or other toxins(Xihong Zhao et al., 2014). Despite the legislation and control methods developed to preserve food nutritional quality and prevent contamination, a significant increase in foodborne diseases has been observed since 1980 and it continues to be an emerging public health theme in whole world(2009; Brandão et al., 2015; Thakur and Ragavan, 2013). According to World Health Organization (WHO) the consumption of food and water contaminated by pathogenic microorganisms causes 1.8 millions of deaths per year worldwide (Shen et al., 2014), and the various *Salmonella* serotypes are the more predominant cause of alimentary infection (Dong et al., 2013; Lee et al., 2015).

In Europe, as reported in the Rapid Alert System for Food and Feed (RASFF) in 2013, the priority vehicles of contaminations were animal products (meet, eggs, milk, and sea products), vegetables and water. *Salmonella* is one of the most common pathogens in meat (Chemburu et al., 2005; Farabullini et al., 2007; Lee et al., 2015). *Salmonella* is a Gram-negative bacterium, from *Enterobacteriaceae* family. *S. (Salmonella) enterica* and *S. bongori* are the species that can cause illness in humans producing numerous symptoms like diarrhea, vomiting, gastroenteritis, severe dehydrating (Bula-Rudas et al., 2015; Dong et al., 2013; Yang et al., 2009) and other sickness stages as typhoid fever. These two species were divided into 2500 known serotypes based on the Kaufmann-White typing scheme (Brenner et al., 2000; Bula-Rudas et al., 2015). The *S. enterica* serotype *typhi* is the bacteria responsible for most of the foodborne diseases and along with serotype *paratyphi,* it can be found only in humans. The *S. paratyphi* causes typhoid salmonellosis, which according to the Food and Drug Administration (FDA), if not treated can result in a mortality rate of 10%. In this case, the infection dose is 1000 Colony Forming Unit (CFU), which is much higher than the infection dose required to occur the symptoms associated with a non-typhoid salmonellosis - which are as low as 1 CFU - although the dangerousness of the side-effects is higher for typhoid salmonellosis (Administration, 2012; Dong et al., 2013; Dungchai et al., 2008).

Due to the extremely low infection limits, 1 CFU, the associated side effects and the high *Salmonella* susceptibility for dissemination in perishable and semi-perishable products, the limits imposed by law have been tightened over the years. In the European Commission (EC) regulation No 2073/2005 on microbiological criteria for foodstuffs, the *Salmonella spp*. are considered a group of pathogens which its presence by itself in ready-to-eat food (portion of 25 g), is enough to be considered a risk factor for human health. Consequently, if this pathogen is detected the food product is classified as unsatisfactory. The absence of *Salmonella* spp. is a figurative quantification, since

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"zero" in analytical measures is unreal, because each method has a limit of detection and there are always errors associated. Even the conventional culture methods recommended by International Organization for Standardization (ISO) (ISO standard 6579:2002) due to their exceptional sensitivity (Melo et al., 2016) are only capable to detect 1 CFU/25 g of foodstuffs.

These regulations are compatible with the Hazard Analysis and Critical Control Point (HACCP) approach, which are used in most of the countries, including the European Union (EU) and United States of America (USA), to establish adequate controls for the identification of *Salmonella* in ready-to-eat foods to assure that it is absent when it is taken by the consumers(Lawley, 2012). Additionally, some countries have specific rules for products like eggs and fresh daily products. For instance, the FDA has a specific rule to prevent S. *enteritis* in eggs, because it is one of the largest contamination vehicles for infection dissemination in the country. This rule is a set of measures which are implemented in the production (for example, the pasteurization implementation), storage and transportation of shell eggs(Lawley, 2012). The effect of more control and the sanctions for non-compliant producers has recently shown positive effects in the statistics of salmonellosis outbreaks in EU. Indeed, between 2004 and 2009 the human cases reduced almost for one-half (EFSA 2014). Counterbalancing these encouraging statistics from the European Food Safety Authority (EFSA), in the USA it was estimated from 2 to 4 million cases of salmonellosis annually, being already considered one of the major causes of hospitalization and dead (Elaine et al., 2011; Oliver et al., 2005; Xihong Zhao et al., 2014).

Because of these alarming statistics, it is still necessary to develop new simple methods and technologies for *Salmonella spp*. detection with the ability to provide valid results at the time of consumption of perishable foods, thus avoiding mass contaminations. Nowadays there are several methods purposely designed to accelerate the pathogen detection but most of them have difficulties to get validated and enter to the market, because they have a high probability of false negative results, sometimes are restricted to a specific type of food or considered expensive by the food industries (Valderrama et al., 2016). In the future, the best approaches for rapid *Salmonella* detection in food control will be designed for application outwards the laboratory and may involve disruptive innovations to minimize the pre-enrichment and sample preparation steps.

The purpose of this review is to give an overview of current methods for *Salmonella* detection in microbial food control and to present the authors view about the most promising route to develop new rapid methods. A critical survey of rapid commercial methods is presented aiming to identify current needs for further development in rapid practical food control. Among several existing methods, which have already been recently reviewed (Lee et al., 2015; Rahman et al., 2016; Su et al., 2011; Valderrama et al., 2016), the biosensors were chonse as an emerging tool for *Salmonella spp*. control due to the increasing interest in the scientific community, as shown by the increasing number of publications using this technology, and their characteristics, namely the operational simplicity, sensitivity, readiness and real-time analysis potential. Among all existing biosensors for *Salmonella spp*., the electrochemical biosensors are reviewed because they show distinguished advantages like the low cost of the equipment, miniaturization capacity and inherent sustainability, due to the use of a few solvents and low sample volumes, both in its development and application. Considering that the acceptance by the industry for novel rapid methods depends not only on speed but also on the initial investment, cost, technical support, and ease of use, electrochemical biosensors are specially well suited to fulfill these requirements[1].

[1] This review is not intended to endorse or recommend any commercial product, and any omission of a commercial product is not intentional.

2. Commercial rapid methods for *Salmonella spp*. detection in food products

Conventional methods for bacteria detection rely on standard culture methods that involve the use of different enrichment and selective broths for the isolation of each bacteria, in which large amounts of sample are used in a complex sequencing of steps (Lee et al., 2015). Beyond their sensitivity and high accuracy, the conventional methods require at least 1 week for trusted results (2–3 days for results and 7–10 days for confirmation)(Farabullini et al., 2007; Yang et al., 2009). Besides these time consuming methods recommended by ISO, it is already possible to obtain similar results in 24–48 h using nucleic acid-based assays or even in less than 24 h with some immunologically-based methods like Enzyme Linked Immunosorbent Assay (ELISA), which together with the biosensors belongs to the rapid methods for pathogen detection in food samples (Valderrama et al., 2016).

In the last years, various devices for rapid detection of *Salmonella spp*. were developed, tested and commercialized (Brandão et al., 2015; Law et al., 2015; Lee et al., 2015; Melo et al., 2016; Valderrama et al., 2016). According to current regulation for food control parameters, commercial methods should accomplish several requirements: the devices have to be able to detect a single *Salmonella* CFU in 25 g of food; they must have a sensitivity and specificity of at least 99%; and operational personnel ideally must need no special skills to perform the analysis (Eijkelkamp et al., 2009). Besides these general requirements, the analysis time of rapid methods preferably must be in the range of hours to a limit of 24 h (Valderrama et al., 2016).

Commercial rapid detection methods should be validated by the competent authorities for example the HACCP, the FDA and the Association of Official Analytical Chemists (AOAC) in the United States of America, and the European Certification Organization (ECO) for the validation and approval of alternative methods for the microbiological analysis of food and beverages (MicroVal) in the EU. The validity of a method depends upon its sensitivity and specificity. Sensitivity is the probability of the test to detect a true positive, while specificity is the probability of the test to detect a true negative. A schematic overview of current rapid methods for salmonella detection in food products is provided in Fig. 1. They can be divided into several categories including miniaturized culture assays - modified or adapted from conventional procedures, but using new selective culture media - immunologically-based assays, nucleic acid-based assays and biosensors. It is difficult to make an accurate comparative analysis about the performance of commercial rapid methods because it depends on several experimental factors, such as sampling, sample matrix, enrichment processes and it lacks normalization of the evaluation schemes (Lee et al., 2015). Comparative studies for the test kits should be set up under identical test conditions to better compare and evaluate the test results from different laboratories. Information about the performance (sensitivity, analysis time, advantages and limitations) of validated commercial methods were obtained from the producer's brochures and websites, or scientific papers (Barthelmebs et al., 2010; Cheung et al., 2007; Eijkelkamp et al., 2009; Oxoid Limited; RomerLabs 2013b; SM, 2004/, 2005a) and it is organized in Tables 1–4, according to their methodology.

2.1. Immunologically based methods

The immunologically based methods for *Salmonella* spp. detection explore the specificity of the antibodies (monoclonal or polyclonal) for specific antigens, normally located at *Salmonella* cellular membrane surface. There are several formats for these assays but the commercially available methods are mainly based on agglutination, immunoprecipitation, immunodiffusion and enzyme immunoassay (EIA) / ELISA, which includes several lateral flow devices.

The agglutination and immunoprecipitation methods use particles