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Electrochemical DNA biosensor for rapid detection of *Listeria monocytogenes* in meat samples

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Abstract

Introduction: Listeriosis, a zoonosis caused by the consumption of food contaminated with *L. monocytogenes*, shows the highest fatality rate in Europe. Official methods are laborious and time-consuming; moreover, an underestimation of the microorganism number is possible due to a viable but non-culturable (VBNC) state. *Vibrio cholerae*, *E. coli*, *Campylobacter jejuni*, *Salmonella* spp., *Listeria monocytogenes*, and *Yersinia enterocolitica* have been identified in the VBNC state, highlighting the importance of specific detection of *L. monocytogenes*.

Materials and methods: A biosensor based on a screen-printed gold electrode coupled to voltammetry analysis of the redox couple ferrocyanide/ferricyanide was used. The bio-recognition element was a DNA probe (ListE) designed to detect *L. monocytogenes* and previously tested with the dot blot technique. The differential pulse voltammetry technique was adopted for the analysis of genomic DNA, raw meat and industrial environmental samples. The AFNOR (French Association for Standardization) validated Listeria Precis™ method (Thermo Scientific, Basingstoke, UK) was also used to verify the presence of the target microorganism.

Results: The electrochemical biosensor showed a good linear response between signal decrement and the amount of target hybridized on the electrode both in ListE complementary sequence, used as a control, and in DNA extracted from pure cultures. The system showed a good performance when utilized for analyses on raw meat and industrial environmental samples comparing the outcomes with the results produced with the AFNOR validated Listeria Precis™ method.

Conclusions: Considering these preliminary results, the biosensor represents a promising tool for the detection of *L. monocytogenes* that is rapid, easy to use and cheap, although some aspects need to be further investigated.

Keywords: *differential pulse voltammetry, Listeria monocytogenes, screen-printed electrodes, food pathogen detection, electrochemical genosensor*

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1. Introduction

The last report published by the European Food Safety Authority (EFSA) reported a total of 251,679 cases of zoonosis in Europe in 2023, promoting food safety as a widespread issue that receives particular attention in research trends [1]. A total of 2952 cases of listeriosis, a zoonosis induced by food contaminated with *Listeria monocytogenes*, were identified with an increasing trend compared to the previous EFSA report in 2019. Special attention is given to ready-to-eat products [2, 3], with the European regulation EC No 2073/2005 [4], which establishes different limits for *L. monocytogenes* presence in this type of product. Two criteria are applied: the qualitative method requires the absence of *L. monocytogenes* in 25 g, while the quantitative method poses a limit of 100 colony-forming units per gram (CFU/g). Based on ready-to-eat foods, some countries, like the United States (U.S.), apply zero tolerance for this product category according to the Code of Federal Regulations "current good manufacturing practice, hazard analysis, and risk-based preventive controls for human food" [5]. The official detection methods for *L. monocytogenes,* such as the Listeria Precis™ method validated by AFNOR according to [6], are culture-based techniques, and they require providing samples to a laboratory, laborious steps for sample preparation, and a long time (up to 5–6 days) to obtain the results.

Food industries require alternative tools for rapid detection and quantification of pathogens to reduce health risks and possible recalls [7–9]. In the last decade, nanobiotechnologies have promoted the adoption of biosensors as a possible solution for rapid tests in food analysis thanks to a broad range of fields of application and the capability of detecting several targets in one test [10–12]. Food industries focus their attention on sensors for pathogen detection thanks to their quick response, low cost, and high specificity [13–15]. Among the different methods that are being developed, electrochemistry has demonstrated its advantages as rapidity, specificity and simplicity in the detection of various substances [16]. Electrochemical biosensors based on gold screen-printed electrodes (SPAuEs) use a modified electrode for the analyte detection and are commonly used for the analysis of small volume liquid samples. The response signal depends on the oxidation/reduction in electro-active compounds evaluated using voltammetry or amperometry [17, 18]. The differential pulse

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voltammetry (DPV) technique is widely recognized for its ability to minimize background currents, thereby enhancing sensitivity when compared to other voltammetric methods [19]. In this approach, the electric potential is superimposed on the electrodes in an impulse using a staircase shape ramp, and the output current is the difference between the current measured immediately at the beginning and at the end of pulse imposition [20]. In the current study, the reversible mono electronic redox couple ferrocyanide/ferricyanide is used [21–23].

The aim of this work was to evaluate the possibility to apply an electrochemical biosensor that uses DPV combined with a specific DNA probe for the routine detection of *L. monocytogenes* in raw ham and environmental samples from a ham factory.

2. Materials and methods

Summary of the steps and materials involved in this work:

(1) Various microorganisms were selected to obtain DNA to be used as positive and negative controls (**Table 1**). Raw ham samples (raw meat of pork) and environmental samples (surfaces from the cutting and transport area, located in the receiving part of the factory) were gathered from local ham factories to be analyzed. (2) An ssDNA probe, was designed, synthesized and tested with dot blot technique. (3) Screen-printed gold electrodes were purchased and functionalized with the selected ssDNA probe. (4) Voltammetry measurements were performed using a computer-controlled μ-AutoLab type II potentiostat run by Nova 2.1 software (EcoChemie, Utrecht, The Netherlands).

Table 1 • List of microorganisms used to test the ListE probe with dot blot immunoassay.

Species	Reference number
L. monocytogenes 1/2c	* ATCC 7644
L. monocytogenes 1/2a	# DSM 112143
L. monocytogenes 1/2b	# DSM 19094
L. monocytogenes 4b	# DSM 15675
L. innocua	# DSM 20649
L. ivanovii subsp. *londoniensis*	# DSM 12491
Salmonella enterica	# DSM 9145
Escherichia coli	# DSM 1103
Bacillus cereus	# DSM 2301
Campylobacter jejuni	# DSM 49943
Lactiplantibacillus plantarum	* ATCC BAA-793
Lacticaseibacillus rhamnosus	* ATCC 53103
Lacticaseibacillus paracasei	# DSM 5622
Levilactobacillus brevis	# DSM 20054

* ATCC: American Type Culture Collection (Manassas, VA, USA); # DSM: German Collection of Microorganisms and Cell Cultures GmbH (DSM, Braunschweig, Germany).

2.1. Microorganisms and DNA

All of the strains used positive and negative controls that were cultured at 37 °C on BHI (Brain Heart Infusion) agar, under aerobic conditions, for 48 h, except for *Campylobacter jejuni* DSM 49943, which was cultured under microaerophilic conditions generated with a Sachet Oxoid™ CampyGen™ 2.5 l (Oxoid, Italy) at 37 °C for 48 h.

A sterile gauze (3 × 7 cm), after rehydration with 3 ml of peptone water was used on surface areas of 10 × 10 cm. In the case of fresh meat, samples were taken from the thighs. The DNA was extracted from 2 ml ONE Broth Listeria Base after 24 h incubation at 30 °C. The 2 ml were centrifuged at 13,000× *g* and the pellet suspended in 300 μl breaking buffer in the presence of glass beads of 0.1 mm diameter [24].

The integrity of the DNA extracted from the bacteria listed in **Table 1** was assessed by running an electrophoresis in a 1% agarose gel (Sigma-Aldrich, St. Louis, MO, USA) in TBE (tris-borate ethylenediaminetetraacetic acid) buffer 0.5X (Sigma-Aldrich, St. Louis, MO, USA) for 1 h. After the electrophoresis evaluation, the extracted DNA was diluted in PBS (phosphate buffer solution) 1X to reach the concentrations required for the optimization of the protocol to use for the food and environmental analyses.

2.2. Probe design and test with the dot blot enzyme immunoassay

A DNA probe (ListE probe) (5′- TAC ACC ATC TAA AAA TAC CAA TAC TAA TAC AAA CTC CAA TAC G -3′) of 43 bp was designed in the sequence of the *iap* gene to specifically detect *L. monocytogenes*. After *in silico* testing with basic local alignment search tool for nucleotides (BLASTn) [25] and Integrated DNA Technologies (IDT) OligoAnalyzer [26], the probe was tested with a dot blot enzyme immunoassay [27] on DNA extracted from the reference strains listed in **Table 1** to evaluate specificity. A sequence complementary to the probe synthesized by Eurofins Genomic (Ebersberg, Germany) was used as a positive control (SPC).

An amount of 1 μl aliquots of diluted SPC was spotted onto a positively charged Zeta Probe-GT nylon membrane (Bio-Rad, Irvine, CA, USA) and revealed using the digoxigenin-labeled probe (ListE-dig) at 200 ng/μl. Nucleic acids were cross-linked to the air-dried membranes by exposure to UV light for 10 min and the hybridization with the probe was carried out overnight at 40 °C. The membrane was washed twice with SSC (saline-sodium citrate) buffer 2X (Promega, Milan, Italy) with 0.1% sodium dodecyl sulfate (SDS, *w/v*) and then washed again with 1X washing buffer (Roche Diagnostics, Mannheim, Germany) at room temperature. Then, 1X detection buffer (Roche Diagnostics, Mannheim, Germany) was used to color the membrane, which was incubated with a color solution (NBT (p-nitro blue tetrazolium chloride)/BCIP (5-bromo-4-chloro-3-indolyl phosphate toluidine salt), Roche Diagnostics, Mannheim, Germany) for 30 min. Finally, the membrane was washed with sterile distilled water and photographed using the bio-imaging system GeneGenius (Syngene, Cambridge, UK).

2.3. Screen-printed functionalization

A computer-controlled μ-AutoLab type II potentiostat run by Nova 2.1 software (EcoChemie, Utrecht, The Netherlands) with DropSens 250AT Screen-Printed Gold Electrodes (SPAuEs) purchased from Metrohm-Dropsense (Asturias, Spain) was used to detect *Listeria monocytogenes*. Each SPAuE consisted of a three-electrode setup: a gold (Au) working electrode (WE), a silver (Ag) reference electrode (RE), and a platinum (Pt) counter electrode (CE). The electrodes were supported on a ceramic substrate with dimensions of 3.4 × 1.0 × 0.05 cm.

The SPAuEs were immersed for the electrochemical cleaning in 8 ml sulfuric acid solution (H_2SO_4 0.5 M) and conditioned by applying ten voltammetry cycles from 0 to +1.3 V with a scan rate of 0.1 V/s. Washing procedures between each step were performed twice with 500 μl of bi-distilled water to remove organic material that may have adsorbed to the surface and dried under a laminar flow hood. The ListE probe obtained from MWG-Biotech (Ebersberg, Germany) was added with a thiol group (-SH) at the 5′ end (ListE-SH probe) for utilization in the biosensor development. ListE-SH probe (capture probe) was diluted in PBS 1X (Sigma-Aldrich, Milan, Italy) to obtain a final concentration of 10 ng/μl. The SPAuE functionalization process was performed with 12 μl of solution, which was deposited by drop-casting on the working electrodes (WEs) of the SPAuE. The electrodes were incubated overnight at room temperature (RT), rinsed twice with 500 μl of sterile bi-distilled water (S-ddwater) and dried before the blocking step was performed by deposing 12 μl of 6-Mercapto-1-hexanol (MCH) (Sigma-Aldrich, Milan, Italy) at 1 mM (solution in PBS 1X on the WE surface (functionalization step)). After 60 min at RT, it was rinsed twice with 500 μl of S-ddwater and dried.

2.4. DNA target hybridization

A short sequence (ssDNA) complementary to the probe (SPC) was used as a template to optimize the conditions of DPV measurements. Serial decimal dilutions of the SPC were prepared in PBS 1X to achieve the following final concentrations: 10 ng/μl, 1 ng/μl, 100 pg/μl, 10 pg/μl and 1 pg/μl. Each dilution was denatured at 95 °C for 10 min, 12 μl deposited on the WE, incubated for 60 min at 40 °C, and finally measured with DPV. The different dilutions were tested starting from the lower concentration (1 pg/μl) to the higher one (10 ng/μl) using the same SPAuE, rinsing twice the WE with S-ddwater and drying before adding another concentration.

The same protocol was adopted for the genomic DNA extracted from *L. monocytogenes* ATCC 7644 (positive control), used at concentrations from 1 pg/μl to 10 ng/μl to build the calibration curve.

The DNA from the samples collected in the ham factory were tested with the SPAuEs functionalized with a ListE-SH capture probe using the conditions described previously.

2.5. Voltammetry measurements

The DPV measurements were carried out in a 10 mM $K_4[Fe(CN)_6] \times 3H_2O$ (Sigma-Aldrich) PBS 1X solution with a scan potential from −0.2 V to +0.4 V at 0.01 V/s. The data were collected with a computer-controlled μ-AutoLab type II potentiostat run by Nova 2.1 software (EcoChemie, Utrecht, The Netherlands). For each measurement, the current peak (ipa sample) obtained after the hybridization of the SPC or genomic DNA was evaluated after the application of DPV scan potential. The anodic peak current obtained from the gold electrode functionalized with the ListE-SH probe was used as blank (ipa blank). Current decrement (Δipa) was calculated as the difference between ipa sample and ipa blank. Finally, this difference was expressed as a percentage of signal suppression using the following equation:

$$\Delta\text{ipa } (\%) = \frac{\text{ipa sample} - \text{ipa blank}}{\text{ipa blank}} \times 100$$

The linear correlation between the current decrement caused by target DNA hybridization and the logarithm ($\log_{10}$) of DNA concentrations was estimated to obtain the final calibration curve for the genomic DNA. The points considered represent the mean values derived from the results achieved with three different SPAuEs applying the above-mentioned protocol. The limit of detection (LOD) was calculated using the following formula:

$$\text{LOD} = \frac{3\text{SD}}{\text{m}}$$

where SD is the standard deviation of the blank (functionalized surface) and m is the slope of the calibration curve obtained with the genomic DNA.

2.6. Food and environmental samples

Six different samples were collected from different points of production in a ham factory in the Friuli Venezia Giulia Region in Italy. Using sterile gauzes of 3 × 7 cm, rehydrated with 3 ml peptone water, the surfaces of 10 × 10 cm of three raw pork meat (RPM1, RPM2, RPM3) samples and three surfaces from the cutting and transport area (environmental samples; ES1, ES2, ES3) were analyzed. Then, 10 ml of sterile peptone water was added to each tube containing the gauze used for the RPM samples and 8 ml of peptone water was added in the tubes containing the gauzes used for testing the working surfaces (ES samples). ONE Broth Listeria Base (Oxoid, Milan, Italy) was inoculated with the peptone water used for sample analyses in a ratio of 1:10 as described in the AFNOR validated Listeria Precis™ method [6]. After incubation at 30 °C for 24 h, 10 μl of ONE broth was streaked on Brilliance™ Listeria Agar plates (Thermo Scientific, Basingstoke, UK) and incubated for 24 h at 37 °C. Finally, the supposed *L. monocytogenes* colonies grown on Brilliance™ Listeria Agar medium (Thermo Scientific, Basingstoke, UK) were tested with the O.B.I.S mono test (Thermo Scientific, Basingstoke, UK) for confirmation.

3. Results

3.1. Dot blot enzyme immunoassay

The ListE probe specificity was previously assessed with the dot blot technique on DNA extracted from the bacteria previously listed in **Table 1**, and the results achieved are reported in Figure S1. As expected, an intense blue spot can be observed for the short positive control complementary to the ListE probe. Moreover, blue spots appeared only in the first row, indicating positivity only for DNA extracted from *L. monocytogenes*. No further halos are

presented in the other lines, confirming the probe specificity at the optimized conditions used for hybridization (40 °C).

3.2. Biosensor test

The average signal registered for the functionalized surface was 373 ± 46 μA considering all the electrodes used (N = 6) to realize the calibration curves of *L. monocytogenes* ATCC 7644 genomic DNA and short positive control. **Figure 1** reports an example of the voltammogram obtained after the hybridization of different concentrations of genomic DNA of *L. monocytogenes*. As expected, the current peaks decreased as the DNA concentration increased.

Data collected from the voltammogram of *L. monocytogenes* DNA samples are reported in **Table 2**. The lowest target concentration reduced the current by around 53.9 μA while the highest concentration generated a current decrement of 119.2 μA. This signal variation led to Δipa values that ranged from −15.5 to −34.3%.

Figure 2A,B show the current decrement as percentage (Δipa%) registered at different concentrations of SPC and *L. monocytogenes* ATCC 7644 genomic DNA. As can be observed, for both targets a clear trend was reached with a signal that decreased when the target concentration increased. The Δipa values obtained were different between the two targets, with a greater increment registered for the genomic DNA. **Figure 2C** reports the calibration curve calculated from the current decrement registered and the target concentration used, expressed with a logarithmic scale. The limit of detection (LOD) was estimated using the formula provided in the Materials and Methods section and is around 9 pg/μl, which corresponds to a Δipa of −18.5%.

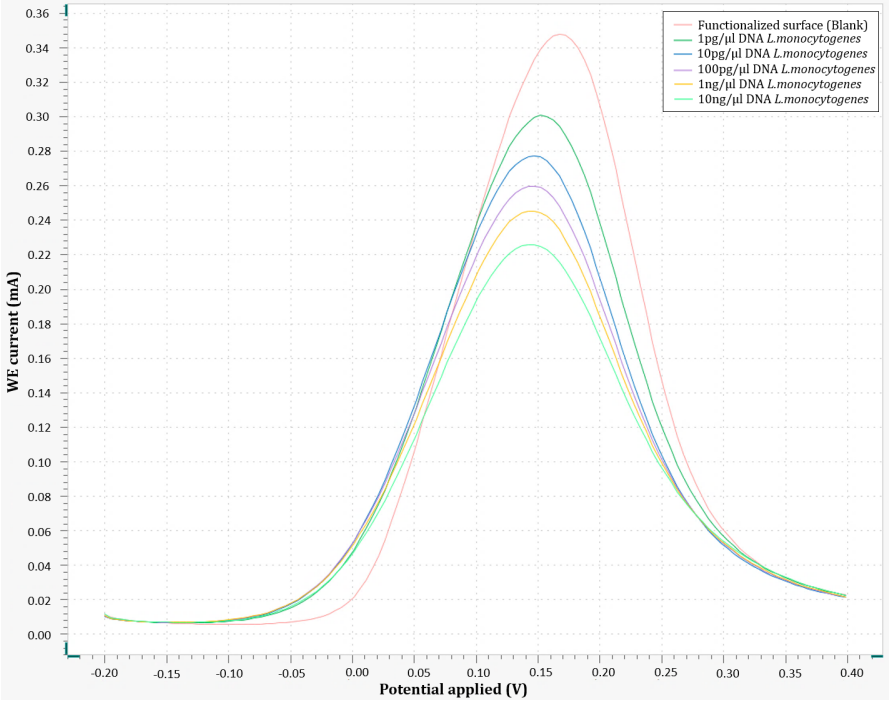

Figure 1 • Example of a voltammogram generated from different concentrations of genomic DNA of *L. monocytogenes* ATCC 7644. The DNA concentrations ranged from 1 pg to 10 ng/μl. The test was performed in DPV mode at the following conditions: start potential −0.2 V, stop potential +0.4 V, step 0.005 V, modulation amplitude 0.15 V, modulation time 0.05 s, interval time 0.5 s, scan rate 0.01 V/s. Blank: no DNA addition; WE: working electrode.

Table 2 • Signal reduction obtained for the hybridization of genomic DNA of *L. monocytogenes* (ATCC 7644) after target hybridization. Values were reported as means of three replicates. Signal reduction is expressed as percentage of the blank signal (Δipa (%) = ([Signal_DNA (μA) − Signal_blank (μA)]/Signal_blank (μA)) × 100).

***L. monocytogenes* DNA concentration**	**Ipa sample (μA)**	**Current decrement (μA)**	**St.dev**	**Δipa%**
1 pg/μl	293.3	−53.9	±8.92	−15.5
10 pg/μl	274.6	−72.6	±3.06	−20.9
100 pg/μl	261.8	−85.4	±3.77	−24.6
1 ng/μl	251.2	−95.9	±9.18	−27.6
10 ng/μl	228.0	−119.2	±4.29	−34.3

Ipa: anodic peak current; Δipa: the difference between ipa sample and ipa blank.

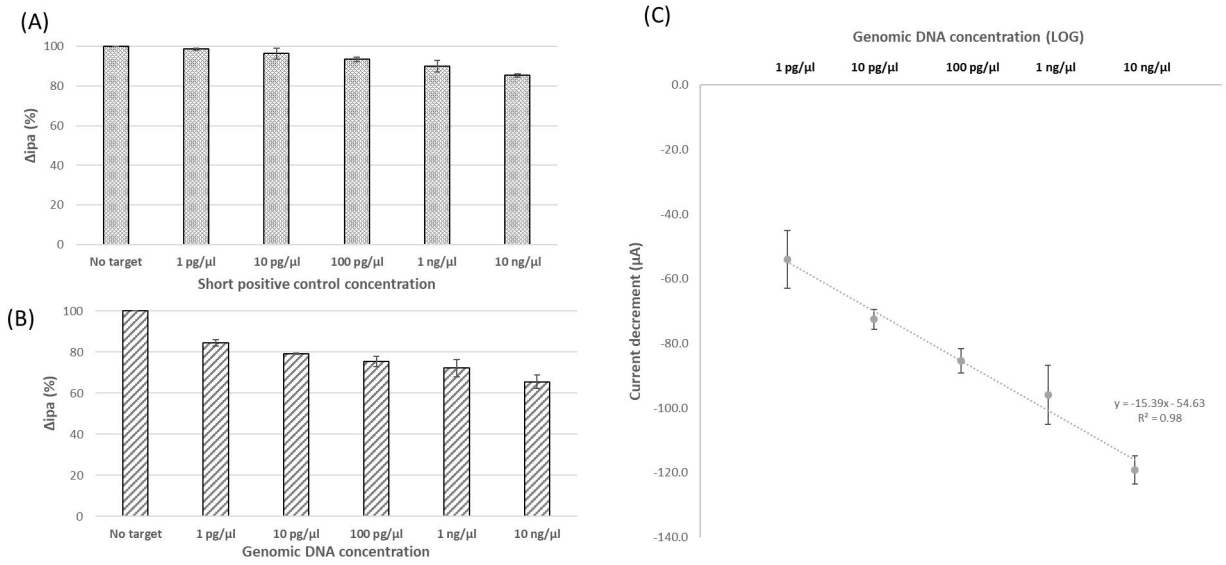

Figure 2 • Bar plot of current decrement (Δipa%) obtained by DPV measurements for short positive control (**A**) and *L. monocytogenes* (ATCC 7644) genomic DNA (**B**). Values are reported as average values ± standard deviation of three replicates. The height of the bar represents the current decrement as percentage (Δipa%) registered at different concentrations of SPC and *L. monocytogenes* ATCC 7644 genomic DNA. In (**C**) is the regression line obtained for the genomic DNA of *L. monocytogenes* ATCC 7644, fitting the logarithm of target concentration and the current decrement (µA) detected after target hybridization. Values are reported as mean values ± standard deviation (N = 3).

3.3. Official and electrochemical analyses of samples

The results for the samples analyzed with the AFNOR Listeria Precis™ method were positive for the presence of *L. monocytogenes*. Samples RPM2, ES2 and ES3 were positive while samples RPM1, RPM3, and ES1, resulted negative. The obtained Δipa and the results from the AFNOR method are reported in **Table 3**. It is possible to see that the values obtained from the collected samples have a different range compared to the genomic DNA. In fact, values range from −9.04 to −49.9% with values higher than those obtained with *Listeria monocytogenes* genomic DNA that range from −15.5 to −34.3%. Positive samples show a current decrement above −21.3%, while negative samples reached values below −10% except for ES1.

Table 3 • Results of raw meat and environmental samples tested with the electrochemical biosensor and with the official AFNOR Listeria Precis™ method. The signal reduction was expressed as percentage of blank signal (Δipa (%) = ([Signal_DNA (µA) − Signal_blank (µA)]/Signal_blank (µA)) × 100).

Sample	Δipa%	AFNOR results
RPM1	−7.34	Negative
RPM2	−21.3	Positive
RPM3	−9.04	Negative
ES1	−49.9	Negative
ES2	−33.7	Positive
ES3	−45.3	Positive

Δipa: the difference between ipa sample and ipa blank; AFNOR: French Association for Standardization.

4. Discussion

The increased number of cases of listeriosis in Europe has induced EFSA and European Center for Disease Prevention and Control (ECDC) to give more attention to this zoonosis [1]. Foods (milk, meat, eggs, fish, fruit, fruit juice, etc.) and work environments subjected to contamination have increased and control methods/protocol need to be developed to speed analyses. Time reduction and analysis simplification can help the laboratories to control this zoonosis. Our electrochemical method is a non-destructive method, and it reduces the analyses' time compared to the AFNOR Listeria Precis™ method, in fact, it requires few hours compared to the days of the official method. The method is based on the detection of the DNA of *L. monocytogenes* (when present), which hybridizes to the ListE-SH probe immobilized on the gold screen-printed electrode, producing a decrease in the current peak that is proportional to the concentration of the DNA hybridized, in agreement with data reported by other authors [22, 23, 28, 29]. As expected, the hybridization of the DNA of the target used in the development of the test produced a decrease due to DNA steric hindrance on the gold surface, reducing the electron exchange on the WE surface. The reduction was higher for the genomic DNA extracted from cells grown in pure cultures compared to the reduction produced by the hybridization of the sequence complementary to the ListE probe due to the higher steric hindrance of the genomic DNA. A reduction in the signal was also obtained using the DNA recovered with the sterile gauzes utilized for sampling, indicating the viability of this non-destructive method for food and surfaces analyses.

Results achieved with the electrochemical method agree with those obtained with the AFNOR validated Listeria Precis™ method for 5 of the 6 analyzed samples, suggesting a general agreement between the two methods. Despite a small current

decrement in RPM1 and RPM3 reported in **Table 3**, the results obtained with the sensor can be considered negative since the current decrement is under the LOD, and consequently, the difference detected can be due to a small fluctuation in the current baseline. These results agree with the official method used for comparison. A different situation occurs for samples RPM2, ES1, ES2 and ES3. The values obtained for the Δipa of the samples RPM2, ES2 and ES3 indicate the positivity of these samples for the presence of *L. monocytogenes,* results in agreement with the AFNOR validated Listeria Precis™ method; the Δipa obtained for sample ES1 indicates positivity for *L. monocytogenes,* while the official method was negative. Although the signal for ES1 and ES3 was outside the range covered by the calibration curves, for ES3 there was agreement with the AFNOR Listeria Precis™ method; on the contrary, sample ES1 was considered positive with the electrochemical analyses and negative with the official method used for comparison. This discrepancy can be justified by the possible failure of a validated rapid microbiological method. This failure can be due to a high level of competitive microbial content growing as background associated with a low level of *L. monocytogenes* in the sample. The incubation broth and the agar medium allow the growth of all the *Listeria* species; thus, the presence of non-*L. monocytogenes* species can affect the results. Moreover, a high level of non-*Listeria* bacteria on the surfaces used for acceptance of the pork thighs and for the first steps of production can worsen the specificity of the analyses, which could have happened for the ES1 sample. The presence of a high microbial background does not affect the results of the electrochemical method proposed thanks to the specificity of the ListE probe giving a positive result. The Δipa of the sample ES1 was close to the Δipa of sample ES3, which was also confirmed to be positive via the AFNOR Listeria Precis™ method. The LOD estimated for the proposed set up is around 9 pg/μl, and considering an average DNA content of around 10 fg/cell, the obtained value in terms of cells corresponds to about 900 cells. Consequently, this LOD is not compatible with the current limits imposed by regulation in the EU for products that do not support *L. monocytogenes* growth (100 CFU/g). However, zero tolerance is applied to products that support *L. monocytogenes* growth or products that will be exported to some states (ex. USA) and consequently it is important to develop a rapid, non-destructive and cheap method for the analyses. At the current stage, the sensor allows us to discriminate the presence or absence of this pathogen in foods where the analysis is usually conducted with the AFNOR validated Listeria Precis™ method or Official ISO method [30], which require an enrichment step.

Our results indicate the need for further tests to evaluate the behavior of the biosensor in a wider range. This work represents a preliminary study to investigate the system response to the genomic target, and consequently the results obtained could be evaluated positively.

Further efforts are needed to improve its sensitivity so that it can be applied to a greater number of foodstuffs including those with a limit of 100 CFU/g. Moreover, the correlation between target concentration and signal decrement has to be further investigated because they might not be linear out of the range tested.

5. Conclusions

Since *Listeria monocytogenes* has relevant economic consequences for food industries and a significant impact on the costs of public health, the development of a rapid and simple method for its detection is beneficial. The electrochemical biosensor based on SPAuEs showed a good linear correlation between the current decrement and the amount of target hybridized on the electrode in both cases, the short sequence and the genomic DNA. The ListE probe showed good performance when utilized for analyses on meat and environmental samples. However, further insights are needed to study the sensor response outside the concentration interval considered in the current study.

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Author contributions

Conceptualization, M.M. and R.T.; methodology, M.B.; formal analysis, M.B.; investigation, M.B., P.V. and E.B.; writing—original draft preparation, M.B.; writing—review and editing, M.M., R.T. and R.S.; supervision, M.M. and R.T. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

Data availability statement

The data supporting the findings of this publication can be made available upon request.

Institutional review board statement

Ethical review and approval were not required for this study per the regulations of the University of Udine, due to the use of commercially produced pork meat samples obtained from a ham factory and the absence of any live animal procedures, animal handling, or animal experimentation.

Supplementary materials

The supplementarymaterials are available at https://doi.org/10.20935/AcadBiol8300.

Additional information

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