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Highly sensitive and rapid determination of *Escherichia coli* O157:H7 in minced beef and water using electrocatalytic gold nanoparticle tags

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ABSTRACT

A simple, highly sensitive and specific immunosensing assay for rapid detection and quantification of *Escherichia coli* O157:H7 in meat and water samples based on the electrocatalytic properties of gold nanoparticles (AuNPs) towards hydrogen evolution reaction and superparamagnetic microbeads (MBs) as pre-concentration/purification platforms without the need of broth enrichment is developed for the first time. Minced beef and water samples inoculated with different concentrations of *E. coli* O157:H7 have been tested using anti-*E. coli* O157–magnetic beads conjugate (MBs–pECAb) as a capture platform and sandwiching afterwards with AuNPs modified with secondary antibodies (AuNPs–sECAb) and detected using chronoamperometric measurement with screen-printed carbon electrodes (SPCEs). Detection limits (LOD) of 148, 457 and 309 CFU/mL were obtained in buffer solution, minced beef and tap water samples respectively, with a broad detection range of 10^2–10^5 CFU/mL in all cases. Recoveries percentages after spiking of 5 different samples of both minced beef and tap water with 10^3 and 10^4 CFU/mL were 94.7 and 90.4 (in beef) and 91.3 and 94.8% (in water), respectively. Specificity, reproducibility and comparison with a commercial lateral flow kit in terms of LOD and detection range were also studied showing clear advantages of the electrochemical method performance. The successful application of this AuNPs based technology in minced beef and tap water indicates the possibility of its using in various food items and other water resources.

1. Introduction

Escherichia coli O157:H7 is a type of enterohemorrhagic, Shiga toxin producing *E. coli* (EHEC, STEC) first discovered in 1982 which normally lives in the intestines of humans and other animals. It has emerged as an important enteric highly infective food and water borne pathogen posing a tremendous challenge to public health. Illnesses caused by *E. coli* O157:H7 can range from mild, watery diarrhea to life-threatening conditions, such as hemolytic uremic syndrome (in 2–7% of cases) and hemorrhagic colitis especially in children and elderly (Jay, 2000; World Health Organization, 2006). It has been stated that the presence of *E. coli* O157:H7 at or above 1 CFU/25 g constitutes adulterated and dangerous ground beef, which corresponds to a dose of *E. coli* O157:H7 of 10–100 cells (FDA, 2011).

High levels of competing microflora in food and water samples compound the difficulty of isolating *E. coli* O157:H7. The common isolation methods using selective culturing media can take more than 48 h to complete and still require expert judgment and further testing to confirm its presence (Johnson et al., 1998). Immunoassay methods which yield a presumptive positive or negative screening result in 24 h or less have been developed. Typical sensitivities of immunoassays are approximately 10^6 CFU/mL, which may lead to false-negative results, particularly when initial levels of competing flora reach 10^5–10^6 CFU/g (Firstenberg-Eden and Sullivan, 1997) a situation not uncommon in ground beef. Since standard methods of identifying *E. coli* O157:H7 require at least a full day (to rule out a negative sample) and up to several days (to confirm a positive result) and employ highly qualified persons in highly specialized laboratories. Consequently, sensitive, rapid and simple methods are required.

Biosensor technologies play an increasingly important role in the detection of pathogenic bacteria because they have great potential to satisfy the practical need for rapid, sensitive, easy to use, portable, and low-cost detection (Liébana et al., 2009; Afonso

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et al., 2013). Biosensor technology coupled with the use of nanoparticles (NPs) tags and other nanomaterials offers benefits compared to traditional methods in terms of time of analysis, sensitivity and simplicity (Mao et al., 2011; Chen et al., 2009; Liu et al., 2009).

The objective of this work is to develop a rapid electrochemical biosensing strategy for *E. coli* O157:H7 quantification in real food samples (minced beef and tap water) using specific antibodies labeled with gold nanoparticles (AuNPs) and magnetic beads (MBs) capture platforms in liquid suspensions. AuNPs have been shown as excellent labels in both optical (e.g., ELISA) and electrochemical (e.g., differential pulse voltammetric) detection of DNA (Pumera et al., Langmuir 2005) and proteins (Ambrosi et al., 2007; Ambrosi et al., 2010). The use of the electrocatalytic properties of the AuNPs on hydrogen formation from hydrogen ions (hydrogen evolution reaction, HER) also enables an enhanced quantification of nanoparticles (Maltez-da Costa et al., 2010) allowing the detection of proteins (De la Escosura-Muñiz et al., 2009a, 2010) and cells (De la Escosura-Muñiz et al. 2009b; Maltez-da Costa et al., 2012a, 2012b) of clinical interest through their labeling with nanoparticle conjugates. However, this technology has not been used yet for the screening of any kind of bacteria in real samples.

2. Experimental section

2.1. Materials

Dynabeads anti-*E. coli* O157 (superparamagnetic polystyrene microscopic beads modified with *E. coli* O157 primary antibody) (MBs-pECAb) 2.8 μm sized (Prod. no.710.03) were purchased from Invitrogen Dynal Biotech AS (Oslo, Norway). *E. coli* O157:H7 (CECT 4783) and *Salmonella enterica* subsp. enterica serovar typhimurium LT2 (CECT 722 T) strains were obtained from "Colección Española de Cultivos Tipo (CECT)". Both bacterial strains and inocula were prepared following a standard procedure, as detailed in Supplementary material.

20 nm gold nanoparticles (AuNPs) were synthesized by reducing tetrachloroauric acid with trisodium citrate, a method pioneered by Turkevich (Turkevich et al., 1951) and were modified with antibodies according a procedure previously optimized by our group (Ambrosi et al., 2007). The detailed experimental procedure for AuNPs synthesis and bioconjugation, together to TEM images is available in Supplementary material.

Antibodies, general reagents and apparatus and electrodes used in this work are also detailed in the Supplementary material.

2.2. Methods

2.2.1. Preparation of minced beef samples

Minced beef samples were obtained from local retail butcher shop and analyzed by a standard culturing method (International Organization for Standardization, 2001) for the presence of *E. coli* O157:H7, and only negative samples were selected to be spiked by bacteria. 10 g of *E. coli* O157:H7 free minced beef were homogenized with 90 mL of sterile PBST in a stomacher (Lab Blender 400, Seward, UK) during 3 min. Beef extract was purified by centrifugation (1000 rpm/5 min) to remove large particles then used as a diluent to prepare different bacterial concentrations.

2.2.2. Spike and recovery protocol

Spike and recovery experiment is important method for validating and assessing the accuracy of an analytical technique for particular sample types. It was done to determine whether *E. coli* O157:H7 detection is affected by a difference between the diluent used to prepare the standard curve (PBST) and the real sample matrix (minced beef and tap water). This experiment was performed by spiking of PBST, minced beef and tap water with 10^3 and 10^4 CFU/mL of *E. coli* O157:H7 ($n=3$ for each sample), then the spiked samples were evaluated under the same condition in a single experiment. After that the recovery % of bacteria from minced beef and tap water samples was obtained as comparing with PBST results.

2.2.3. Magnetosandwich assay using gold nanoparticle (AuNP) labels and electrocatalytic detection

The magnetosandwich assay was performed following a methodology previously optimized in our group (Ambrosi et al. 2007; Afonso et al. 2013), with some variations. Briefly, 15 μL stock solution of previously washed MBs–pECAb was incubated for 5 h/25 °C under gentle stirring in a thermoShaker, with 135 μL casein sodium salt 5% then kept overnight in the fridge. The blocked MBs–pECAb were incubated with 150 μL of heat killed *E. coli* O157:H7 (EC; different concentrations) for 30 min/25 °C/650 rpm. After that MBs–pECAb/EC complex was magnetically separated from solution, and washed with WB and PBS buffer. A blank PBST was used as blank for this assay. The last incubation step with the previously prepared AuNPs–sECAb conjugate, was performed under the same conditions of the previous incubation with subsequently washing steps, resulting in complete magnetosandwich that is ready to be analyzed (Fig. 1A).

Quantitative analyses were carried out following the hydrogen evolution reaction (HER) catalyzed by the AuNPs, taking advantage of the chronoamperometric mode (Maltez-da Costa et al., 2010). Chronoamperograms were obtained by placing a mixture of 25 μL of 1 M HCl and 25 μL of the magnetosandwich solution (performed for PBST, beef extract and tap water containing different concentrations of the target bacteria) onto the surface of the electrodes (Fig. 1B) and, subsequently, holding the working electrode at a potential of +1.35 V for 1 min and then applying a negative potential of −1.00 V for 100 s, recording the cathodic current generated which constitutes the analytical signal. A new electrode was employed for each measurement. This value of potential applied during a fixed time (−1.00 V; 100 s) was previously optimized through cyclic voltammetric studies (Fig. 1C; see detailed explanation in Supplementary material).

3. Results and discussion

3.1. Electrochemical detection of E. coli O157:H7

MBs–pECAb and MBs–pECAb/EC conjugates were first characterized by SEM analysis. Fig. 1D illustrates the immunological attachment of *E. coli* O157 to the MBs–pECAb forming MBs–pECAb/EC conjugates (more SEM figures for the conjugate are available in Supplementary material).

After optimization of the adequate antibody to form the specific sandwich with the MBs/EC complex (goat polyclonal antibody ab30521) and of the blocking agent for avoiding unspecific absorption (5% casein) (see detailed optimization studies in Supplementary material), *E. coli* O157:H7 containing samples were analyzed by the electrochemical method. Fig. 2A,B shows the results of electrochemical determination of *E. coli* O157:H7 in PBST. The mean currents obtained for increasing concentrations of bacteria (10^2–10^7 CFU/mL) show a gradual increasing (from 10^2 to 10^5 CFU/mL) with a logarithmic response from 10^2 to 10^4 (correlation coefficient, $r \approx 1$). From this logarithmic relationship it can be estimated a detection limit (LOD) of 148 CFU/mL (calculated as the CFU/mL which gives a signal equal to the mean signal of the blank+3 times its standard deviation).

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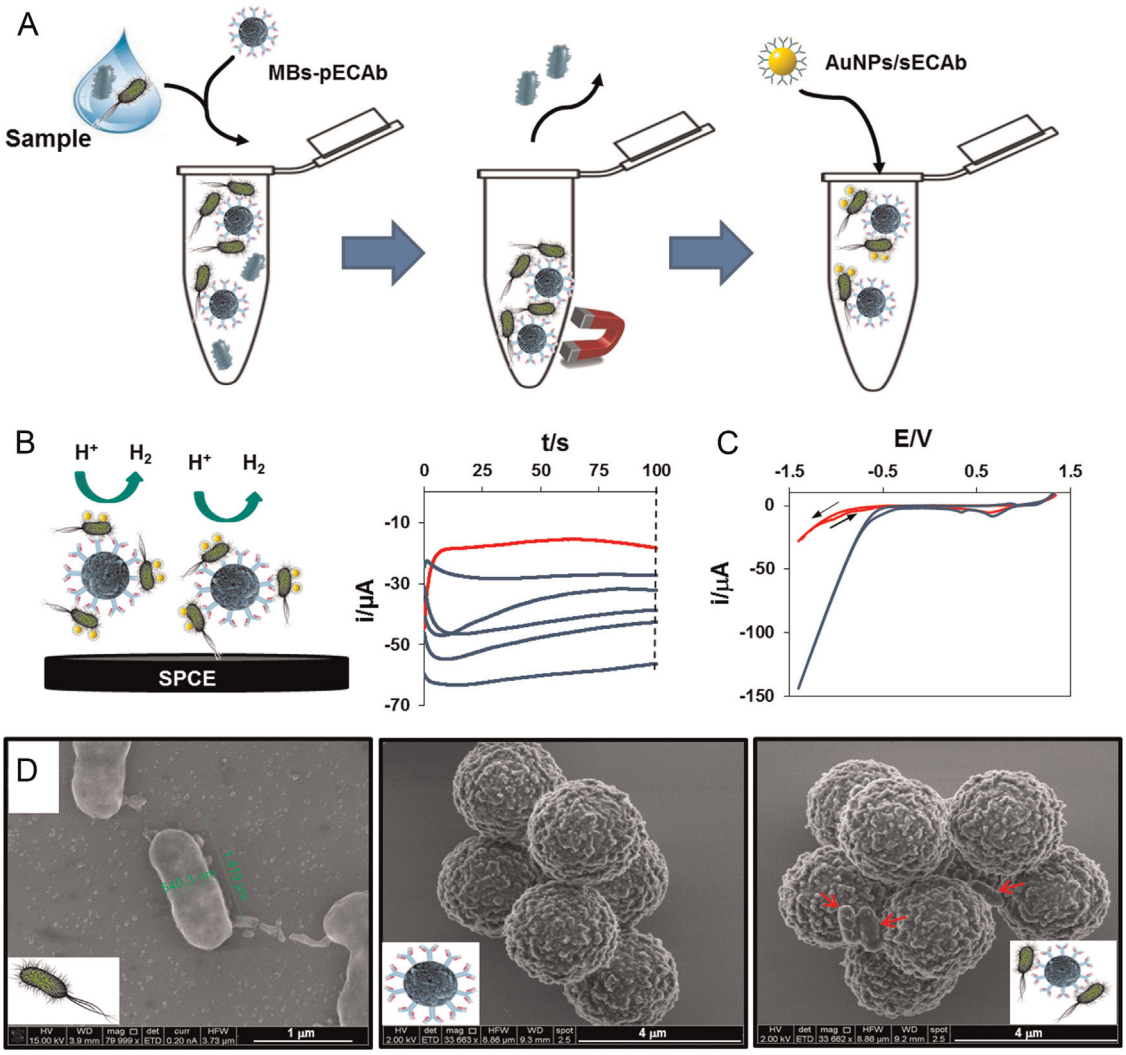

Fig. 1. Schematic experimental design. (A) *E. coli* O157:H7 was captured by MBs–pECAb and subsequently labelled with AuNPs–sECAb in the presence of control bacteria (*Salmonella typhimurium*). (B) Left: detection of labeled *E. coli* O157:H7 through the Hydrogen Evolution Reaction (HER) electrocatalyzed by the AuNP labels. Right: Chronoamperograms registered in 1 M HCl, during the HER applying a constant voltage of −1.0 V for increasing *E. coli* O157:H7 concentrations (from top to bottom: 0, 10^2, 5×10^2, 10^3, 10^4 and 10^5 CFU/mL). (C) Cyclic voltammograms recorded from +1.25 V to 0 V in 1 M HCl for 0 (red curve) and 10^5 (blue curve) CFU/mL; scan rate: 50 mV/s. (D) SEM image for heat-killed E. coli O157:H7 (left) and MBs–pECAb before (center) and after (right) incubation with 10^5 CFU/mL of *E. coli* O157:H7 (bacteria are pointed by red arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

This decline in the signal for bacterial concentrations higher than 10^5 could be related to the blocking of the electrode surface by the bacteria, hindering the electrocatalytic reaction. It could be concluded from the behavior of this dose-response relationship that the quantification of *E. coli* O157:H7 in unknown sample using this logarithmic range could be done by preparation of two tenfold serial dilutions from the original sample; increasing of the current by dilution indicates that *E. coli* O157:H7 concentration in the original sample is $>10^5$, while decreasing of the current by dilution indicates that it is $\leq 10^5$ CFU/mL and consequently it can be quantified. This kind of decrease in the signal for saturated concentrations of bacteria was also observed using commercial available kits based on Lateral Flow Assays, as we explain at Section 3.2 (see also Fig. SM-5 and SM-7 in Supplementary material). To evaluate the precision of the developed technique, five different samples of PBST spiked with 5×10^2 CFU/mL of E. coli O157:H7 were tested under the same conditions in a single experiment. It was found a main value of the current of 27.9 μA and a RSD of 4.7% which indicates good reproducibility of the developed system.

The specificity of the electrochemical immunoassay *versus* another bacterial spp. of the same "Enterobacteriaceae family" such as *Salmonella typhimiurium* LT2 was demonstrated. For this study, we selected 10^4 CFU/mL which is the highest concentration of *E. coli* O157:H7 within the dynamic range of response of our system (see Fig. 2A and B). As shown in Fig. 2C the mean current values obtained by *E. coli* O157:H7 were higher than two folds of that obtained by *Salmonella typhimurium* (22 ± 1.5 and 46.7 ± 3.5 μA, respectively), while the later was approximately similar to that of blank PBST (17 ± 4.9 μA) which demonstrates that this method is highly selective for the target bacteria. Furthermore, the mean current value produced by the *E. coli* O157:H7 was almost similar to that of the sample containing both bacterial spp. which clarifies that the electrochemical signal is not affected by the presence of other competing bacterial spp. Regarding these slight differences in produced signal between blank PBST and *Salmonella* containing sample and also between *E. coli* containing sample and sample of the mixture can be related to a certain degree of cross reactivity and non-specific binding of the used antibody, due to the antigenic similarity between some bacterial spp. these antibodies could cross-react and bind to a

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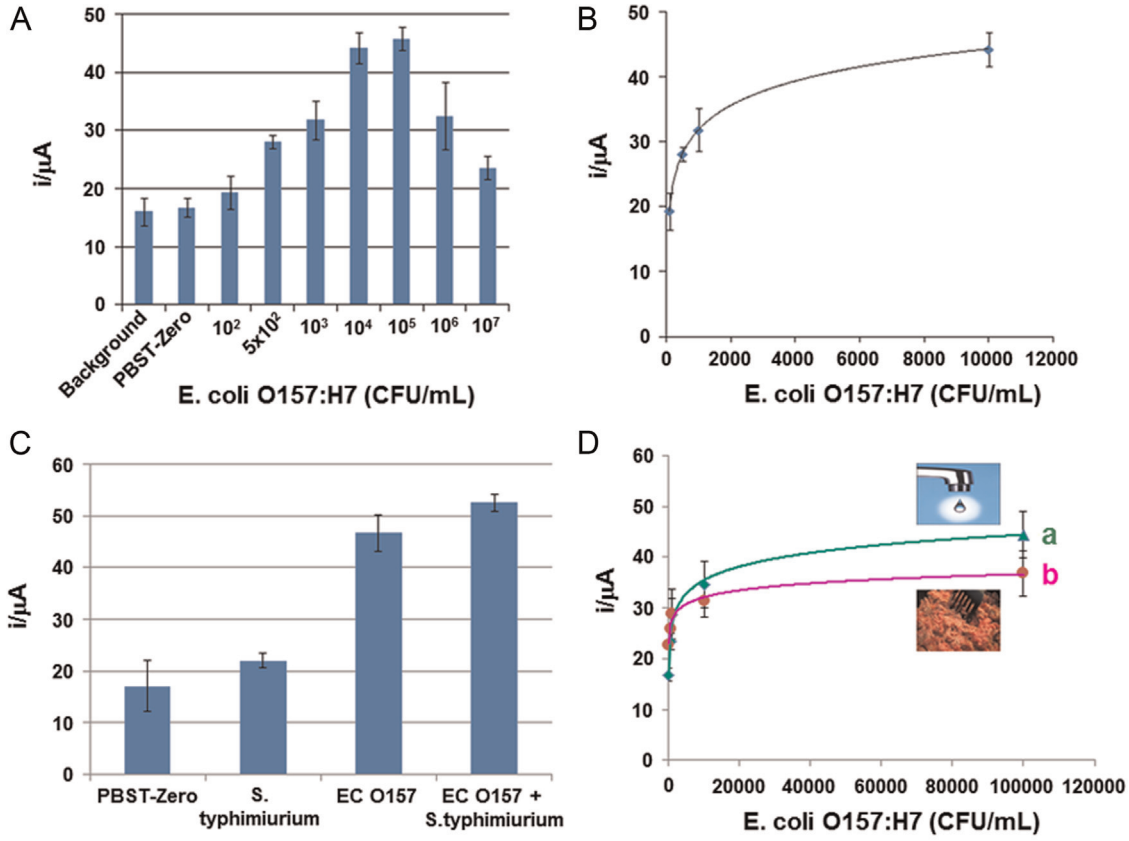

Fig. 2. (A) Electrochemical results obtained by blank PBST and different concentrations of *E. coli* O157:H7 in PBST (10^2 to 10^7 CFU/mL). (B) Logarithmic range of the dose-response relationship is from 10^2 to 10^4 CFU/mL ($r=1$). (C) Specificity study of the immunoassay in blank PBST and PBST inoculated by *Salmonella typhimurium* (10^4 CFU/mL), *E. coli* O157:H7 (10^4 CFU/mL), and a mixture of *E. coli* O157:H7 and *Salmonella typhimurium* (10^4 CFU/mL for each bacteria). (D) Logarithmic response of the current produced by 10^2, 5×10^2, 10^3, 10^4, and 10^5 CFU/mL in tap water (a) and minced beef extract (b). The error bars show standard deviation for three replicates.

limited extent with some spp. of *Salmonella*, *Proteus* and *Escherichia hermanii* (Invitrogen, 2008). Nevertheless this level of interference does not affect the feasibility of the *E. coli* O157:H7 detection.

3.2. *E. coli O157:H7 analysis in real samples: spike and recovery*

While analysis in buffer solution is important, analysis in real samples with minimal sample preparation is the main goal. Different concentrations of *E. coli* O157:H7 (10–10^7 CFU/mL) were prepared in minced beef extract and tap water as described previously. As shown in Fig. 2D, the obtained results for increasing concentrations of the target analyte in minced beef showed a logarithmic range (from 10^2 to 10^5 CFU/mL with $r=0.979$). Similar results to those of minced beef were obtained in tap water samples (logarithmic range from 10^2 to 10^5 CFU/mL with $r=0.985$). The estimated LOD were 457 and 309 CFU/mL, respectively, which are lower than the reported using alternative rapid advanced technologies (see Table SM-1 at the Supplementary material).

Table 1
Spike and recovery assay data. The study was done by spiking 10^3 and 10^4 CFU/mL of *E. coli* O157:H7 in PBST, minced beef and tap water ($n=3$ for each sample) and the recovery % of bacteria from real samples was obtained as comparing with PBST.

Samples	Spiked *E. coli* O157:H7 (CFU/mL)	Current in PBST (μA)	Current in real samples (μA)	Recovery (%)
Minced beef	10^3	30.4	28.8	94.7
	10^4	38.4	34.7	90.4
Tap water	10^3	30.4	27.7	91.3
	10^4	38.4	36.3	94.8

In order to compare the limit of detection of the developed assay with available rapid methods, RapidChek® E. *coli* O157 (including H7) Lateral Flow Assay was used for this comparison. The performance of these kits was reviewed by AOAC Research Institute and approved as a tool for detection of E. *coli* O157:H7 in food samples (Sdix. RapidChek, 2011). The limit of detection of the commercial test assay was evaluated in PBST, minced beef and tap water samples using the same samples used for the electrochemical immunoassay. It was found that LOD by the commercial kits was 10^5 CFU/mL in each of PBST, minced beef and tap water samples (see Figs. SM-5, 6 and 7 at the Supplementary material), it means that LOD was improved by about 1000, 200 and 300-fold by the electrochemical immunoassay, respectively. Furthermore, a decrease in the signal for saturating concentrations of E. *coli* O157:H7 was also observed (Figs. SM-5 and SM-7), which is in concordance with the electrochemical observations, as mentioned in Section 3.1.

Spike and recovery experiment was performed to determine whether E. *coli* O157:H7 detection is affected by the real sample matrix of both minced beef and water. The data showed in Table 1 summarizes the results of spike and recovery experiment of E. *coli* O157:H7 in both samples.

It is clear that recovery rate of the target bacteria was 94.7 and 90.4% from minced beef samples and 91.3 and 94.8% from tap water samples for spiked amount of 10^3 and 10^4 CFU/mL, respectively. These high recovery rates demonstrate that this technology

is a promising alternative for determination of E. *coli* O157:H7 in such samples.

In order to determine E. *coli* O157:H7 in real unknown non-spiked minced beef samples using this technology, it is recommended that 25 g of sample should be homogenized with 225 mL of sterile TSB using a stomacher for 3 min, then two tenfold serial dilutions are prepared from the original dilution and incubated at 37 °C/8 h, then centrifuge the samples at 1000 rpm/5 min to remove the solid particles. Finally, 150 μL of each dilution could be used directly for immunosandwich preparation. Regarding the application in water samples, the same as in minced beef but without homogenization is recommended.

In contrast, the time required to carry out the electrochemical technique (from sampling until quantification) is approximately 9–10 h (8 h for enrichment + 30 min for each incubation with MBs–pECAb and AuNPs–sECAb + 30 min for magnetic separation and washing). This is a great advantage over the standard method, which requires 24 h even for an initial positive/negative result (FDA, 2011). Moreover, this assay time (70–90 min) is competitive in comparison with other electrochemical detection methods (120 min for IMS then label-free amperometeric detection (Pérez et al., 1998) and IMS, enzymatic label and amperometeric detection (Ruan et al., 2002)).

Another important advantage of this biosensor is its portability. The handheld potentiostat can be paired with a pocket PC, battery-operated, and transported easily. All the other necessary equipment and reagents can also be transported and operated remotely, which enables the possibility of field analysis using this biosensor.

Because of the low infective dose of E. *coli* O157:H7 and low levels naturally present in foods with uneven distribution, enrichment stage is still required for the detection of this organism by any technology. The estimated LOD in this study without broth enrichment by our assay confirms the possibility of detecting E. *coli* O157:H7 at much lower concentrations within the infective dose (10–100 CFU) reported by FDA (FDA, 2011) after 8 h of sample enrichment.

4. Conclusions

In conclusion, the electrocatalytic properties of AuNPs towards HER in combination with magnetic beads as a pre-concentrator showed a high sensitivity rate in detection of E. *coli* in beef and water samples. The developed assay can detect E. *coli* O157:H7 in both beef and water samples without broth enrichment at a much lower concentration (457 and 309 CFU/mL respectively) than other previously reported advanced technologies within shorter time (70–80 min). The selection of the optimum antibody for specifically recognizing the bacteria strain after the magnetic capturing together with the adequate blocking agent for avoiding unspecific absorptions are key findings for the success of the analytical method. Furthermore, this method showed a high specificity rate when tested against *Salmonella typhimurium*, in addition to high sensitivity rate in the presence of other competing bacteria. The accuracy of the designed immunosensor was good with a variation coefficient equals 6.7%, moreover a high recovery rates (94.7 and 90.4% from minced beef samples and 91.3 and 94.8% from tap water samples for spiked amount of 10^3 and 10^4 CFU/mL, respectively) were obtained. Several advantages were observed in this technology compared to the previously reported ones (see Table SM1 at the Supplementary material) regarding to limit of detection, simplicity, rapid assay duration, high accuracy, selectivity and high specificity in the presence of other competing bacterial spp. The high complexity of beef as a food matrix indicates the possibility of using this technology in various food items also other water resources. The possibility to avoid broth enrichment together with the use of portable handheld potentiostat paired with a pocket PC enables rapid in-field analysis of food and water samples without the need for highly equipped and specialized laboratories.

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Appendix A. Supplementary Information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.09.019

References

Afonso, A.S., Pérez-López, B., Faria, R.C., Mattoso, L.H.C., Hernández-Herrero, M., Roig-Sagués, A.X., Maltez-daCosta, M., Merkoçi, A., 2013. Biosens. Bioelectron. 40, 121–126.
Ambrosi, A., Castañeda, M.T., Killard, A.J., Smyth, M.R., Alegret, S., Merkoçi, A., 2007. Anal. Chem. 79, 5232–5240.
Ambrosi, A., Airò, F., Merkoçi, A., 2010. Anal. Chem. 82, 1151–1156.
Chen, X., Zhang, J.J., Xuan, J., Zhu, J.J., 2009. Nano Res 2 (3), 210–219.
De la Escosura-Muñiz, A., Maltez-da Costa, M., Merkoçi, A., 2009a. Biosens. Bioelectron. 24, 2475–2482.
De la Escosura-Muñiz, A., Sánchez-Espinel, C., Díaz-Feitas, B., González-Fernández, A., Maltez-da Costa, M., Merkoçi, A., 2009b. Anal.Chem. 81, 10268–10274.
De la Escosura-Muñiz, A., Maltez-da Costa, M., Sánchez-Espinel, C., Díaz-Freitas, B., Fernández-Suarez, J., González-Fernández, A., Merkoçi, A., 2010. Biosens. Bioelectron. 26, 1710–1714.
Firstenberg-Eden, R., Sullivan, N.M., 1997. J. Food Prot. 60, 219–225.
International Organization for Standardization, ISO 16654:2001: Horizontal method for the detection of *Escherichia coli* O157. Available on: ⟨http://www.iso.org/⟩.
Invitrogen™. 2008. Dynabeads® anti-*E. coli* O157. Available on: ⟨http://www.invitrogen.com⟩.
Jay, J.M., 2000. Modern Food Microbiology 6th ed.: Microorganisms in foods. Van Nostrand Reinhold, New York.
Johnson, J.L., Brooke, C.L., Fritschel, S.J., 1998. Appl. Environ. Microbiol. 64, 4390–4395.
Liébana, S., Lermo, A., Campoy, S., Barbé, J., Alegret, S., Pividori, M.I., 2009. Anal. Chem. 81, 5812–5820.
Liu, Z., Tabakman, S., Welsher, K., Dai, H.J., 2009. Nano Res 2 (2) (285-219).
Maltez-da Costa, M., de la Escosura-Muñiz, A., Merkoçi, A., 2010. Electrochem. Commun. 12, 1501–1504.
Maltez-da Costa, M., De la Escosura-Muñiz, A., Nogués, C.,, Barrios, L., Ibáñez, E., Merkoçi, A., 2012a. . Small 8, 3605–3612.
Maltez-da Costa, M., De la Escosura-Muñiz, A., Nogués, C., Barrios, L., Ibáñez, E., Merkoçi, A., 2012b. Nano Lett. 12, 4164–4171.
Mao, S., Yu, K.H., Lu, G.H., Chen, J.H., 2011. Nano Res. 4 (10), 921–930.
Pérez, F.G., Mascini, M., Tothill, I.E., Turner, A.P.F., 1998. Anal. Chem. 70, 2380–2386.
Pumera, M., Castañeda, M.T., Pividori, M.I., Eritja, R., Merkoçi, A., Alegret, S., 2005. Langmuir 21, 9625–9629.
Ruan, C., Wang, H., Li, Y., 2002. Trans. ASAE 45, 249–255.
Sdix. RapidChek *E. coli* O157 (including H7). 2011. Available on: ⟨http://www.sdix.com⟩.
Turkevich, J., Stevenson, P., Hillier, J., 1951. Discuss. Faraday Soc. 11, 55–75.
World Health Organization (WHO). 2006. Guidelines for Drinking-Water Quality, (3rd ed. Yang et al.).