Evaluation and implementation of commercial antibodies for improved nanoparticle-based immunomagnetic separation and real-time PCR for faster detection of *Listeria monocytogenes*

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Revised: 6 April 2020 / Accepted: 15 April 2020 / Published online: 24 April 2020
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Abstract *L. monocytogenes* continues to be a major health issue in Europe, as well as worldwide. Faster methods, not only for detection, but also for sample preparation are of great interest particularly for this slow-growing pathogen. Immunomagnetic separation has been previously reported to be an effective way to concentrate bacteria, and remove inhibitors. In the present study, different commercial antibodies were evaluated to select the most appropriate one, in order to develop a highly specific method. Additionally, magnetic nanoparticles, instead of microparticles, were selected due to their reported advantages (higher surface-volume ration and faster kinetics). Finally, the separation protocol, with a calculated capture efficiency of 95%, was combined with real-time PCR for highly sensitive detection of the concentrated bacteria. The optimized IMS-qPCR allowed to reduce hands-on time in the sample treatment, without affecting the overall performance of the method as a very low limit of detection was still obtained (9.7 CFU/25 g) with values for sensitivity, specificity, accuracy, positive and negative predictive values of 100%, resulting in a kappa index of concordance of 1.00. These results were obtained in spiked food samples of different types (chicken, fish, milk, hard and fresh cheese), further demonstrating the applicability of the optimized methodology presented.

Keywords Rapid methods · Magnetic nanoparticles · IMS-qPCR · *hly* · *Listeria monocytogenes*

Introduction

Listeria monocytogenes is one of the most serious food-borne pathogens due to its high mortality rate and ubiquity (Zilelidou et al. 2016). Data from EU surveillance of human listeriosis, mainly focused on severe, invasive forms of the disease, indicates that it affects several risk groups including elderly, immunocompromised people as well as pregnant women and infants, causing high hospitalization and mortality, particularly among the elderly. Invasive listeriosis has shown a significant increasing trend since EU surveillance was initiated in 2008 and continued this trend in the last surveilled 5 years (2013–2017) (EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control) 2018).

Currently, official methods for the detection of bacterial foodborne pathogens are based on classical microbiology, which require several days for bacterial isolation and identification. Particularly for *L. monocytogenes* which is considered a slow growing microorganism, particularly due to the fact that antimicrobial agents are used to suppress the native flora of foods that may hamper the development of *L. monocytogenes* (Zilelidou et al. 2016). Faster, but at the same time reliable, analytical methods are needed by both, the industry and control laboratories. Such methods should allow food industry to ensure the health of consumers, to easily determine whether a food product has been contaminated, and if possible, identify how and when this contamination occurred (Garrido-Maestu et al. 2017a). Molecular biology based techniques have been implemented on foodborne pathogen detection due to their

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advantages and capability of overcoming the limitations associated with culture based methods being polymerase chain reaction (PCR) and real-time PCR (qPCR) the most widely used and accepted (Valderrama et al. 2016; Garrido-Maestu et al. 2017a).

While PCR and qPCR based methods are highly specific, their successful use in foods may be limited by low pathogen levels (Suh et al. 2013). Bacterial enrichment is normally used before DNA extraction, purification, and qPCR detection, with the objective of generating enough number of cells to allow their detection, which is particularly relevant for certain food products such as Ready-to-Eat (RTE) food products where the required microbiological criteria are particularly strict (Bergis and Lombard 2018; Ricci et al. 2018). However, this step is considered as a bottleneck on the objective of developing faster methods for foodborne pathogen detection due to the time required for microorganism growth (Cho and Ku 2017).

Alternative methods are needed for capture, concentration and purification of microorganisms before DNA purification and amplification (Hice et al. 2018) in order to reduce the time required for microorganisms enrichment, or even to eliminate this step, with the objective of having much faster analytical approaches without losing the required sensitivity. Cell concentration can be enabled by filtration and centrifugation sample preparation, however clogging of filters and isolation of other particles, particularly from complex samples such as food sample hinder their use as optimal alternative method (Suh et al. 2013).

Immunomagnetic separation (IMS) has demonstrated to be an effective sample pre-treatment to separate and concentrate different pathogens from complex food matrices, decrease the time required for detection, removal of inhibitory compounds and significantly reduce background microorganisms (Välimaa et al. 2015; Mao et al. 2016; Day and Hammack 2019; Park et al. 2020). Other concentration strategies such as centrifugation and filtration present the limitation of being non-specific. In this sense, IMS has been applied in combination with other techniques, for the detection of different pathogens including *L. monocytogenes*. Many of these studies utilized antibodies (Ab), either monoclonal (mAb) or polyclonal (pAb), that react either with all *Listeria* spp., or even with other non-*Listeria* species, leading to unsatisfactory results (Koo et al. 2011).

In addition to the improvement of the Abs selected, the use of nanoparticles provides additional advantage for the analytical procedure. Recent advances in nanoscience and nanotechnology are greatly impacting the development of analytical methods (Gómez-Hens et al. 2008). Traditionally, large microbeads (> 1 µm) have been used for this type of assays. One of the most interesting features of the use of Magnetic Nanoparticles (MNPs) versus the use of larger particles such as microbeads, includes the larger

surface-to-volume ratio which allows higher capture efficiency of the microorganisms of interest (Prado et al. 2016), faster binding kinetics and minimal sample preparation (Zeng et al. 2014; Auvolat and Besse 2016; Cho and Ku 2017).

The aim of the present study was to develop, and evaluate, a novel qPCR method in combination with MNPs for the specific detection of *L. monocytogenes* in food samples. To this end, commercial Abs were evaluated attending to their specificity, and the best candidate was selected for the functionalization of MNPs, and implemented in the final IMS-qPCR targeting *L. monocytogenes*. Finally, the novel methodology was evaluated in spiked food samples.

Materials and methods

Bacterial strains and culture media

In the present study *L. monocytogenes* WDCM 00021, *L. innocua* WDCM 00017 and *Salmonella enterica* serovar Typhimurium WDCM 00031 (World Data Center for Microorganisms) were selected as the reference strains for all inoculation/ specificity tests experiments. Fresh cultures were prepared by suspending 1 single colony in 5 mL of Buffered Peptone Water (BPW, Biokar Diagnostics S.A., France) and incubated overnight at 37 °C. To determine bacterial concentration of the pathogen, after incubation, ten-fold serial dilutions were performed in NB and plated in Tryptic Soy Yeast Extract Agar (TSYEA) with the formulation described in the ISO 11290-1/ 2 for *Listeria* spp. The plates were incubated overnight at 37 °C overnight.

Enrichment of food samples

Twenty-five g of food sample were weighted, mixed with 225 mL of Half-Fraser broth (HF, Biokar Diagnostics S.A., France) and homogenized for 2 min at 230 rpm in a Stomacher 400 Circulator (Seward Limited, West Sussex, UK). Finally, the matrixes (mixture of food sample and HF) were incubated at 30 °C for 24 h. A detailed list of the samples, along with the spiking concentration is provided in Table 1. All spiked samples were inoculated before homogenization. The food types included in the study were: meat (chicken breast), dairy (hard and fresh cheese), and fish (anchovies). For confirmation purposes, after enrichment a loop-full was streaked on COMPASS *Listeria* agar (Biokar Diagnostics S.A., France), the plates were incubated up to 48 h at 37 °C and examined for typical colonies.

Table 1 Samples analyzed

Foodstuff	N	Inoculum (cfu/ 25 g)	IMS-qPCR	Plate
Milk	2	–	–	NG
Milk	1	$< 1.0 \times 10$	+	+
Milk	1	1.0×10 – 1.0×10^2	+	+
Milk	1	1.0×10^2 – 1.0×10^3	+	+
Hard cheese	1	–	–	NG
Hard cheese	4	$< 1.0 \times 10$	+	+
Hard cheese	1	1.0×10 – 1.0×10^2	+	+
Hard cheese	2	1.0×10^2 – 1.0×10^3	+	+
Hard cheese	1	$> 1.0 \times 10^3$	+	+
Anchovy	3	–	–	NG
Anchovy	1	$< 1.0 \times 10$	+	+
Anchovy	1	1.0×10 – 1.0×10^2	+	+
Chicken ^b	4	1.0×10 – 1.0×10^2	+	+
Chicken ^b	2	$< 1.0 \times 10$	+	+
Chicken	2	$< 1.0 \times 10$	+	+
Chicken	2	1.0×10 – 1.0×10^2	+	+
Chicken	2	1.0×10^2 – 1.0×10^3	+	+
Fresh cheese ^a	10	$< 1.0 \times 10$	+	+
Fresh cheese ^a	1	–	–	NG

NG no growth

^aReference samples for the determination of the LoD. Plate counts indicated that the actual value was 9.7 cfu/ 25 g

^bThese samples were not originally inoculated, but were naturally contaminated with *L. monocytogenes*

Antibody evaluation

In the present study, four different antibodies were evaluated attending to their purity and specificity. The ones providing the best results were further evaluated attending to their capture efficiency (CE) in the functionalized magnetic nanospheres (MNP). The selected Abs were two polyclonal and another two monoclonal. Information about all antibodies used in the study, including secondary Abs used for indirect ELISA test, is provided in Table 2.

Purity

The purity of the selected antibodies was analyzed by performing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) under reduced conditions, using a protocol based on the method of Laemmli et al., (Laemmli 1970). Three dilutions of each Ab were prepared rendering final concentrations of 100, 10 and 1 µg/mL. These were reduced in the same volume of Laemmli sample buffer (Bio-Rad Laboratories, Inc., USA) supplemented with 5% (V/V) of 2-mercaptoethanol and incubated at 95 °C during 5 min. Electrophoresis was performed using 4–15% Mini-PROTEAN® TGX™ Pre-cast gel (Bio-Rad Laboratories, Inc., USA), where 5 µL of ruler and 20 µL of reduced samples was loaded and run first 5 min, at 50 V, and after, approximately 1 h at 100 V. The detection of the protein in the polyacrylamide gel was achieved by Silver Stain Plus™ Kit (Bio-Rad Laboratories, Inc., USA). The protein gels were visualized by Gel Doc™ EZ Imager, using 4.1 Image LAB™ Software (Bio-Rad Laboratories, Inc., USA).

Specificity

To evaluate the specificity of each Ab, an indirect ELISA was performed, against *L. monocytogenes* WDCM 00021, *L. innocua* WDCM 00017 and *S. Typhimurium* WDCM 00031, in a 96 well Nunc MaxiSorp™ plate (Thermo Fisher Scientific, USA). The plate was first coated with 200 µL per well of a fresh bacterial culture prepared as detailed above (expected concentration of 10^8 CFU/mL) and incubated overnight, at 50 °C, to evaporate the medium. The following day, 100 µL of test Ab, diluted in PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, pH 7.4) to a final concentration of 10 µg/mL, was incubated 1 h at room temperature (RT) with agitation. The plate was then washed three times with 200 µL of PBS, followed by a blocking step with 5% BSA in PBS, for 1 h, at RT with agitation. A new washing step was performed before the deposition of the respective secondary Ab

Table 2 Commercial antibodies evaluated

Name	Company	Clone name	Class	Isotype	Host
MA1-20271	ThermoFisher	LZF7	Monoclonal	IgG2a	Mouse
PA1-7230	ThermoFisher	–	Polyclonal	IgG	Rabbit
MAB8953	Abnova	3a15	Monoclonal	IgG2b	Mouse
MD-05-0329	RayBiotech	–	Polyclonal	Not defined	Goat
Anti-Mouse IgG-HRP ^a	Santa Cruz Biotechnology	–	Polyclonal	IgG	Goat
Anti-Rabbit IgG, Human ads-HRP ^a	SouthernBiotech	–	Polyclonal	IgG	Goat
Anti-Goat IgG, HRP ^a	Novus Biologicals	–	Polyclonal	IgG	Chicken

^aSecondary Abs used for the indirect ELISA

diluted 1:500 in PBS, 100 µL/well was also incubated for 1 h at RT with agitation. Finally the plate was washed one last time, and the measurement of the chemiluminescence was performed, using the Microplate Reader Synergy H1 Multi-Mode (BioTek, USA).

Magnetic beads functionalization

AbraMag® Magnetic Nanospheres (MNPs, average size of 500 nm) coated with protein A, were purchased from Abraxis Inc. (Warminster, PA, USA). The MNPs were functionalized with the Ab with the best specificity results. To do this, first the MNPs were washed twice with 1 mL of 0.1 M sodium phosphate buffer with Tween®20 (PBT, 19 mM NaH₂PO₄, 81 mM Na₂HPO₄, 0.05% Tween®20, pH 7.4), being recovered with a magnetic particle concentrator (Dynal® MPC (Invitrogen, Carlsbad, CA, USA)) for 2 min. An Ab concentration of 60 µg/mL of MNPs was added, in a final volume 10 times higher, to allow the distribution of the Ab in the particles. The solution was incubated for 1 h at RT in a Mini Tube Rotator (Fisher Scientific) set at 10 rpm. Finally, the MNPs were washed again twice as described above.

Capture efficiency (CE)

The evaluation of the CE was based on the protocol described by Varshney et al., (Varshney et al. 2005). In this sense, pure cultures of *L. monocytogenes* were diluted to reach a theoretical concentration of 10⁴ CFU/mL, from this dilutions three aliquots were treated with the IMS protocol (*C_b* cells bound to the MNPs) and another three aliquots were not (*C₀* total number of cells). DNA was extracted from all aliquots with the protocol described for food samples (see M&M 2.4), and the bacterial concentration was calculated by qPCR as described below.

To do so a pure culture of *L. monocytogenes* was ten-fold serially diluted and 1 mL of each dilution was used for DNA extraction as described in M&M 2.4. In parallel, these dilutions were plated in TSYEA in order to plot CFU/mL vs Cq values for the construction of a standard curve. This approach was used for the optimization of the IMS protocol. In addition to the protocol detailed for food samples, the inclusion of three additional washing steps were also tested as recommended by the supplier. The CE was calculated with the following formula:

$$CE (\%) = (C_b/C_0) \times 100.$$

DNA extraction

After IMS, the DNA was extracted from the bacteria captured by the MNPs following the protocol described by Kawasaki et al., (Kawasaki et al. 2005) modified by

Garrido et al., (Garrido et al. 2013). The particles were resuspended in 200 µL of enzymatic solution (1 mg/mL lysozyme and 1 mg/mL achromopeptidase) and incubated with constant agitation (1000 rpm) for 1 h at 37 °C. This was followed by the addition of 300 µL of guanidium isothiocyanate, and 400 µL of the mixture was transferred to a tube containing another 400 µL of isopropanol 100%. The samples were centrifuged for 10 min at 16,000 × g for 10 min. The resulting pellets were rinsed with 1 mL of isopropanol 75%, resuspended in 160 µL of sterile milliQ water, incubated at 70 °C for 3 min with constant agitation (1400 rpm) and finally centrifuged at 16,000 g for 5 min at 4 °C.

***L. monocytogenes* IMS and detection**

One mL of 24 h enriched HF broth was taken and 20 µL of MNPs was added. These were incubated at room temperature for 15 min under constant mixing in a Mini Tube Rotator at 10 rpm. After incubation the MNPs were separated with a MPC for 3 min, the buffer was removed and the MNPs were resuspended in the enzymatic solution to proceed with the DNA extraction following the protocol described in M&M 2.4.2.

The qPCR was performed in a final reaction volume of 20 µL containing 2 µL of template, 10 µL of Maxima Probe/ROX qPCR Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA), 200 nM primers and 150 nM probe. The thermal profile selected consisted in 2 min at 50 °C for Uracil-DNA Glycosylase (UDG) treatment (avoid carryover contamination), followed by 10 min at 95 °C hot start polymerase activation, and 40 cycles of dissociation at 95 °C for 15 s and annealing-extension at 63 °C for 30 s. All reactions were run in a StepOne Plus™ Real-Time PCR system (Applied Biosystems™, Foster City, CA, USA).

Gene and primers for qPCR

Detection of *L. monocytogenes* was accomplished targeting the *hlyA* gene. To this end, the method described by Garrido-Maestu et al., was applied (Garrido-Maestu et al. 2018). The primers and probes used were: hly-P3F GCAACAACTGAAGCAAAGGAT, hly-P3R CGATTG GCGTCTTAGGACTTGC, hly-P3P^{5'FAM} CATGGCAC C/ZEN/ACCAGCATCTCCG^{IABkFQ3'}.

Evaluation of IMS-qPCR method

The complete evaluation of the new method developed was performed assessing the parameters detailed by Garrido-Maestu et al., Tomás et al., and Anderson et al., (Anderson et al. 2011; Garrido-Maestu et al. 2017a; Tomas et al.

2009). According to these studies, first the Limit of Detection (LoD) was lowest concentration of starting bacteria which could be reliably detected. To determine this parameter 10 samples (25 g each) were inoculated with less than 10 CFU of *L. monocytogenes*, and after analysis (enrichment, IMS, DNA extraction and qPCR) at least 9 had to be positive (90% presences). To perform these analysis, fresh cheese was selected as the reference food type (one extra non-inoculated sample was also analyzed to assure absence of *L. monocytogenes* in the original matrix). After the determination of the LoD, the relative sensitivity (SE), specificity (SP) and accuracy (AC), positive and negative predictive values (PPV/ NPV), and the index kappa of concordance (κ) were also calculated. To do so, all samples analysed were classified according to the expected and obtained results as positive or negative agreement (PA/ NA) and positive or negative deviations (PD/ ND).

Results

Ab evaluation

The evaluation of the different commercial antibodies was based on their purity and specificity, and the one with the best results was selected for the functionalization of the MNS to determine the CE.

Purity of the Abs

The SDS-PAGE results, Fig. 1, show two main bands, one about 20–25 kDa and another one at 55 kDa. These correspond to the light chain and heavy chains of the Abs. No

additional bands are present in the two mAbs (MA1-20271 and MAB8953). In the pAbs, a common contaminant protein is present, which can correspond to BSA, with molecular weight around 66 kDa. Another protein is present in the goat pAb (MD-05-0329) with higher molecular weight between 70–100 kDa.

Specificity of the Abs

Evaluation of the specificity was based on the results obtained by indirect ELISA with other microorganisms. Both pAbs exhibited cross-reactivity with other species than *L. monocytogenes*. Regarding the mAbs, MA1-20271 also presented cross-reactivity, in addition to low signal with *L. monocytogenes*. As can be observed in Fig. 2, mAb MAB8953 obtained the best results in terms of specificity and signal intensity, thus was selected for further experiments.

Capture efficiency (CE)

CE was calculated based on the quantification results obtained by qPCR (expressed as log CFU/mL). It was observed that the inclusion of three washing steps, recommended by the supplier of MNPs, greatly reduces the CE, as direct analysis of the MNPs provides a CE of 95% ($C_b = 3.8$; $C_o = 4.0$), while extensive washing reduces this value to 75% ($C_b = 3.6$; $C_o = 4.8$). Thus no extensive MNPs washing was included in the final protocol. The correlation between CFU/mL vs Cq values can be observed in Fig. 3.

Fig. 1 SDS-PAGE results obtained for the evaluation of the purity of the evaluated Ab. Three concentrations of each Ab were loaded: 100, 10 and 1 μ g/mL

Fig. 2 Specificity of the Abs tested. PA1-7230 and MD-05-0329 are pAbs, while MA1-20271 and MAB8953 are mAbs. Signal intensity is presented in Relative Luminescence Units (RLU)

Fig. 3 Quantification performed by qPCR for the determination of the CE. The values for the standard IMS protocol (three washing steps with PBS) are represented in green (dark green for the direct quantification and light green after the MNPs treatment), against

optimized IMS (direct separation from sample enrichment) represented in blue (dark blue for the direct quantification and light blue after the MNPs treatment)

Evaluation of the IMS-qPCR method

The analysis of all the spiked samples was performed with the threshold fixed at 0.35 ΔR_n (value obtained after subtracting the baseline to the normalized reporter (R_n , ratio of the fluorescence emission intensity of the reporter dye to the fluorescence emission intensity of the passive reference dye (Rodríguez-Lázaro et al. 2005)). With this setup, all positive samples obtained C_q values lower than 38 regardless the type of food type and the initial bacterial concentration spiked. The non-inoculated negative control fresh cheese sample included in the LoD determination, resulted negative. Regarding the 10 spiked samples with low bacterial concentration (plate counts indicated that samples were inoculated with 9.7 CFU) obtained 100% positive results, thus 9.7 CFU/ 25 g was established as the LoD of the IMS-qPCR method. The analysis of a non-contaminated control was confirmed as negative.

In addition to these, another 31 assorted samples were spiked and analyzed with the described methodology. Likewise with the determination of the LoD, the results obtained by IMS-qPCR matched perfectly (100% for SE, SP, AC, PPV, NPV, with a κ of 1.00) with those expected, and confirmed after plating the enrichment cultures on COMPASS Listeria agar. The results are summarized in Table 3.

It was observed that 6 non-inoculated chicken samples, from two different batches, resulted positive by IMS-qPCR. As mentioned above, the results were confirmed by plating the enriched samples on COMPASS agar, thus were not considered as deviations, but as natural contamination of those samples. Additionally, enumeration of *L. monocytogenes* was performed in triplicate for these two batches being obtained values of $< 1.0 \times 10$ CFU/ g for one batch, and $2.6 \times 10 \pm 9.2$ CFU/ g.

Discussion

The combination of IMS with different detection strategies has been previously evaluated to reduce the time needed for the detection of *L. monocytogenes* (Garrido-Maestu et al. 2019). However, some studies have reported reduced sensitivity of this approach when compared to conventional

methods. In addition to this, even though the inclusion of specific antibodies should provide enhanced specificity, cross-reactivity with non-target bacterial species has also been described (Kaclíková et al. 2001). In the present study, four different commercial antibodies (two polyclonal and two monoclonal) were evaluated, in terms of purity and specificity, in order to select the most appropriate one for the development of the IMS protocol. In this sense, as expected, all the antibodies tested were relatively pure, but exhibited great differences in terms of specificity. It was observed that the mAb MAB8953 was the most specific for *L. monocytogenes*, and thus selected for nanoparticle functionalization.

The IMS protocol had to be optimized, as it was observed that the CE obtained with the standard protocol recommended by the supplier of the MNPs, and most IMS kits, obtained lower CE values. These recommended approaches included up to three bead washing steps prior to further analysis. Our results are in agreement to those reported by Hudson et al., who highlighted that extensive washing of the particles reduces the amount of bacteria captured (Hudson et al. 2001), and obtained similarly high CE values to those recently reported in other studies (Bi et al. 2020). After optimization, the implementation of the IMS step in the method allowed to reduce time and hands-on for the sample treatment prior to DNA extraction from the standard approach, which included several centrifugation, filtration and washing steps to effectively remove food particles and chemicals which may interfere in the DNA extraction of amplification (Kawasaki et al. 2005; Garrido-Maestu et al. 2015).

The evaluation of the LoD obtained an expected value below 1.0×10 CFU/25 g of sample (9.7 CFU/25 g) but lower concentrations may be reached as plate counts obtained from the other spiked samples obtained values comprised among 4.6 to 7.1 CFU/25 g. These values are comparable to those previously reported for *L. monocytogenes* and other pathogens using different DNA amplification methodologies such as loop-mediated isothermal amplification, recombinase polymerase amplification or even PCR/ qPCR with IMS (Uyttendaele et al. 2000; Hudson et al. 2001; Ohtsuka et al. 2010) or without it (Cho et al. 2014; Kim and Lee 2016; Rossmanith et al. 2006),

Table 3 Summary of the results obtained in the evaluation of the IMS-qPCR

Method	N	PA	PD	NA	ND	SE	SP	AC	PPV	NPV	κ
IMS-qPCR	41	34	0	7	0	100	100	100	100	100	1.00

N total number of samples, *PA* Positive Agreement, *PD*: positive deviation. *NA* negative agreement, *ND* negative deviation, *SE* relative sensitivity, *SP* relative specificity, *AC* relative accuracy, *PPV* positive predictive value, *NPV* negative predictive value, κ index kappa of concordance

and even improve others previously reported (Wei et al. 2019).

Finally, the evaluation of the performance parameter (SE, SP, AC, PPV and NPV) obtained values of 100% for all them. The calculation of the κ (value of 1.00) fell in the range of 0.81–1.00, what is interpreted as “almost complete concordance” (Altman 1991; Anderson et al. 2011). These values are similar to those obtained in other studies without IMS, demonstrating that the novel method is highly reliable, but allowing a significant hands-on reduction during the sample treatment (Garrido-Maestu et al. 2017a, b).

The presented approach demonstrated to be suitable in a wide different range of foodstuffs, and adequate for the analysis of complex matrixes where sometimes the complete removal of interfering food debris is difficult. This was demonstrated by the correct detection of *L. monocytogenes* in six chicken samples which were not artificially inoculated, but presented different concentrations of natural *L. monocytogenes*, which were all correctly identified by IMS-qPCR and classical microbiology methods. Yang et al., reported improved performance of MNPs over conventional microspheres, for the detection of *L. monocytogenes* (Yang et al. 2007). This is in agreement with our results which also resulted more sensitive than other studies previously published using microspheres (Kacľíková et al. 2001). The reasons behind this improvement have already been mentioned, such as higher surface-to-volume ratio of the nanomaterial, resulting in higher CE, and faster binding kinetics, among others.

Conclusion

The evaluation of commercial Abs allowed to develop a highly specific IMS-qPCR method for the detection of *L. monocytogenes* in food samples applying magnetic nanoparticles instead of conventional microparticles. Additionally, the new method permitted to reduce time (up to four days respect to ISO method), and sample manipulation before DNA extraction without affecting the overall performance of the method. The extensive evaluation of the novel method in different foodstuffs demonstrates the applicability of the proposed methodology.

Acknowledgements This work was supported by the project Nanotechnology Based Functional Solutions (NORTE-01-0145-FEDER-000019), supported by Norte Portugal Regional Operational Programme (NORTE2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF), and by NANOIMMUNOTECH S.L. in the framework of the project Smart Factory for Safe Foods (SF4SF) supported by FEDER Interconecta 2015 (EXP-00082964/ ITC-20151195).

Compliance with ethical standards

Conflict of interest Alejandro Garrido-Maestu*, Sarah Azinheiro, Joana Carvalho, Begoña Espiña and Marta Prado declare that they do not have conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent (in case humans are involved) Not applicable.

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