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Detection of *Salmonella enterica* serovar Enteritidis using real time PCR, immunocapture assay, PNA FISH and standard culture methods in different types of food samples

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ABSTRACT

Several methods for the rapid and specific detection of *Salmonella* in food samples have been described. Here, we compare 4 of those methods in terms of assay time, procedure complexity, detection limit, sensitivity, specificity and accuracy. Milk, eggs and mayonnaise samples were artificially contaminated with *Salmonella enterica* serovar Enteritidis cell concentrations ranging from 1×10^{-2} to 1×10^{2} CFU per 25 g or ml of food. Samples were then pre-enriched and analyzed by either: i) real-time PCR, using the iQ-Check Salmonella kit; ii) immunocapture, using the RapidChek SELECT Salmonella; iii) a peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) method and iv) the traditional bacteriological method ISO 6579:2002. All methods were able to detect *Salmonella* in the different types of food matrixes and presented a similar detection level of 1 CFU per 25 g or ml of food sample. The immunocapture and the PNA FISH methods proved to be very reliable, as their results were 100% in agreement with the ISO method. However, real-time PCR presented a significant number of false positives, which resulted in a specificity of 55.6% (CI 95%, 31.3–77.6) and an accuracy of 82.2% (CI 95%, 63.2–91.4) for this method. Sensitivity was 100% since no false negative results were observed. In conclusion, the implementation of these molecular techniques, mainly the immunocapture and PNA-FISH methods, provides a reliable and less time-consuming alternative for the detection of *Salmonella* spp. in food samples.

1. Introduction

Despite the close monitoring and control measures available to limit *Salmonella* spp. contamination, these microorganisms are still a leading cause of foodborne illness worldwide (Pui et al., 2011a,b; Scallan et al., 2011). According to the Center for Disease Control and Prevention (CDC), approximately 40,000 cases of salmonellosis are reported in the United States every year (Scallan et al., 2011). Since milder cases are usually not diagnosed or reported, CDC estimated that the real number of infections may be thirty or more times greater. Worldwide, there are annually 3 million deaths due to *Salmonella* infections (Bhunia, 2008; Pui et al., 2011a,b). *Salmonella* serotype Typhimurium and *Salmonella* serotype Enteritidis are the most prevalent throughout the world (Herikstad et al., 2002), but more than 2500 *Salmonella* serovars have been identified to date, with most of them being capable of infecting a wide variety of animal species and humans (Popoff et al., 2003; Pui et al., 2011a,b). Foodborne sources of *Salmonella* are very diverse and include a wide variety of fresh fruits and vegetables and food from animal sources such as beef, poultry meat, eggs and milk (Berger et al., 2010; Gillespie et al., 2003; Jamshidi et al., 2010; Pui et al., 2011a,b).

The standard culture method currently used for *Salmonella* detection in food samples is described in ISO 6579:2002 (Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp). As most culture methods it is time-consuming (it usually takes 4 to 7 days to complete) and laborious (Uyttendaele et al., 2003). It includes non-selective pre-enrichment, followed by a selective enrichment and plating on selective and differential agars. Suspect colonies are then confirmed by biochemical and serological tests. The implementation of faster and equally reliable identification tools would assist in infection control and would also represent important cost savings for the food industry, since for some cases stocks may not be released until the final results of microbiological tests are known.

Recently, a number of rapid methods have been developed for the detection of *Salmonella* in foods (Fratamico, 2003; Li et al., 2000; Lofstrom et al., 2010; Nde et al., 2008; Uyttendaele et al., 2003). For instance, real-time PCR is currently being applied using different fluorescent-based detection systems (Lofstrom et al., 2009; Malorny et al., 2004; Malorny et al., 2007; Nde et al., 2008; Oliver et al., 2005; Olsen et al., 2009). Recently, the iQ-Check Salmonella real time PCR (Bio-Rad), based on the amplification of the iagA gene (involved in

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the bacteria invasion process), was introduced (Miras et al., 1995). Some authors have investigated its performance in artificially contaminated samples and have found similar sensitivity to culture-based methods (Fakhr et al., 2006; Liming and Bhagwat, 2004; Nde et al., 2008; Patel et al., 2006; Uyttendaele et al., 2003). However, some authors also reported the occurrence of false negative and false positive results (Fakhr et al., 2006; Uyttendaele et al., 2003).

Several immunoassay systems are also commercially available for food products (De Paula et al., 2002; Gonzalez et al., 2009; Uyttendaele et al., 2003). The RapidChek Salmonella immunoassay (Strategic Diagnostics Inc., SDIX) uses a lateral flow format for the detection of *Salmonella* through an antibody which is specific for a target pathogen protein. Fakhr et al. have shown that a considerable number of positive samples were not detected by the RapidCheck (Fakhr et al., 2006). Similar results have been reported for other commercially available immunoassay (BioMérieux) (Uyttendaele et al., 2003). However, recently SEDIX presented the SELECT system, which includes phage supplemented primary media that presents strong selectivity against other microbial competitors, hence promoting *Salmonella* growth (Gonzalez et al., 2009). Recently this SELECT system was validated in different food samples and was found to perform as well as the culture-based method (Gonzalez et al., 2009; Muldoon et al., 2007; Muldoon et al., 2011).

Our group has recently developed a new fluorescence *in situ* hybridization (FISH) method using peptide nucleic acid probes (PNA) for the specific detection of *Salmonella* in a broad range of samples (Almeida et al., 2010). The method is based on the specific binding of a PNA probe, a synthetic DNA analogue capable of hybridizing to complementary nucleic acid targets, to a conserved RNA sequence. The method proved to be very specific and sensitive, being able to detect as few as 1 CFU/10 ml of sample in less than 24 h.

The main goal of this work was to compare three emerging technologies (real time PCR, immunoassay and PNA FISH) with the traditional bacteriological method (ISO 6579:2002) for the detection of a prevalent *Salmonella* serotype, *S.* Enteritidis, in three different food matrixes: milk, eggs and mayonnaise. To the best of our knowledge, this is the first report comparing the four technologies.

2. Materials and methods

2.1. Bacterial strain and media

Salmonella enterica serovar Enteritidis, strain ATCC 13076, was used in the study. The strain was maintained in tryptic soy agar (Liofilchem, Italy) for 24 h at 37 °C, and streaked on to fresh plates every 24 h. Following overnight incubation at 37 °C, a loopful of biomass was transferred to 20 ml of LB Miller broth (Liofilchem) and incubated overnight (~18 h) at 37 °C and 120 rpm. Cells were then suspended in phosphate buffered saline (PBS) and adjusted to a cell density corresponding to approximately 1×10^8 cells/ml. The relationship between OD and total cell counts was previously established by performing CFU counts and OD readings at several cell dilutions. Cells were further diluted in PBS to obtain desired cell concentration for inoculation into food samples. Cell concentrations were confirmed by plating on LB agar (Liofilchem).

2.2. Samples inoculation

Methods were tested in three different types of food samples (eggs, milk and mayonnaise), all obtained from Pingo Doce, Braga. Initially, 25 g or ml of each type of food were mixed with 225 ml of pre-warmed buffered peptone water, BPW (for culture, PCR and PNA FISH techniques), or primary medium supplemented with 5 ml of phage solution (for the immunocapture method, RapidChek). The samples were then artificially contaminated with *Salmonella* concentrations ranging from 0.01 CFU/25 g or ml to 100 CFU/25 g or ml of food. A non-inoculated food sample was included with each experiment to ensure that samples were *Salmonella*-free. Three different enrichment protocols were followed according to each detection method (see below). Three independent experiments were performed for each method.

2.3. Conventional bacteriological method

For the detection of *Salmonella* using conventional culture-based methods, the ISO 6579:2002 was followed. Briefly, artificially contaminated samples prepared as described above, were incubated overnight at 37 °C at 120 rpm. After pre-enrichment, 100 μl and 1 ml samples were taken and mixed with 10 ml of Rappaport Vassialidis soya (RVS) broth (Liofilchem) and Muller Kauffmann tetrathionate-novobiocin (MKTTn) broth (Liofilchem), respectively. Cultures were also incubated overnight at 37 °C for MKTTn broth and at 42 °C for RSV broth. After the selective enrichment step, a loopful of each enriched sample was streaked on differential medium, such as MacConkey, xylose lysine desoxyscholate (XLD), or brilliant green phenol red agar (BGA). Finally, 5 presumptive *Salmonella* colonies from each selective agar medium were also confirmed biochemically.

2.4. Real time PCR method using the iQ-Check kit

Real time PCR was performed according to the manufacturer instructions (http://www.bio-rad.com/webroot/web/pdf/fsd/literature/357-8123_iQ-Check_Salmonella_II_user_guide.pdf). Artificially contaminated samples were incubated for 21 h ± 1 h at 37 °C without shaking. Following the incubation period, 100 μl samples were taken from the top without disturbing the food debris. For mayonnaise, samples were taken under the lipid layer formed on the top of the culture media. The 100 μL samples of the enriched suspension were mixed with 100 μL of the lysis buffer. Then, lysis was performed by incubating the suspension at 95 °C for 15 min in a dry-block heater (CH-100, BIOSAN). Subsequently, lysed samples were vortexed and centrifuged at 12,000 g for 5 min. The PCR mix, containing the usual expected constituents as well as a molecular beacon probe and an internal control, was prepared according to the kit instructions and 45 μl was mixed with 5 μl of the supernatant obtained in the previous step. Beyond the normal compounds of a PCR reaction, the mix contains a Molecular Beacon probe labeled with carboxyfluorescein (FAM) at the 5′ end and DABCYL at the 3′ end that acted as a quencher. The probe from iQ-Check Salmonella kit targets the iagA (invasion associated gene) which is highly specific to *Salmonella* species (Miras et al., 1995). To monitor for successful DNA amplification in each reaction tube, the kit provides synthetic DNA as part of the reaction mixture, which works as an 'internal control.' This control is detected with a specific probe at the same time as the *Salmonella* DNA sequence. For positive and negative controls, 5 μL of sample or reagent D (negative control) or reagent E (positive control) were also mixed with 45 μL of theamplification mix. At least one positive and one negative control were included in each PCR run. Next, the PCR plate was placed in a thermocycler (model CFX96,C1000, Bio-Rad Laboratories) and run under the following cycling conditions: 50 °C for 2 min, 95 °C for 5 min, (95 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s) × 50 cycles, and 72 °C for 5 min. Results were interpreted according to the instructions in the real-time PCR system user guide for iQ-Check kits. Briefly, in each PCR assay a Ct value > 10 for the iagA probe was considered positive and Ct values of 0 (non-amplified, NA) were considered negative if the internal control presented a Ct value ≥ 28. If the Ct value for both probes was zero, the PCR assay was repeated with a tenfold diluted DNA template suspension to avoid PCR inhibitors.

2.5. PNA FISH method

For the PNA FISH method, samples were incubated for 18 to 21 h at 37 °C and then 20 μl samples were placed directly on glass slides.

PNA FISH was performed as previously described (Almeida et al., 2010). Briefly, smears were immersed in 4% (wt/vol) paraformaldehyde (Sigma) followed by 50% (vol/vol) ethanol for 10 min each and allowed to air dry. Smears were then covered with 20 μl of hybridization solution containing 10% (wt/vol) dextran sulfate (Sigma), 10 mM NaCl (Sigma), 30% (vol/vol) formamide (Sigma), 0.1% (wt/vol) sodium pyrophosphate (Sigma), 0.2% (wt/vol) polyvinylpyrrolidone (Sigma), 0.2% (wt/vol) Ficol (Sigma), 5 mM disodium EDTA (Sigma), 0.1% (vol/vol) Triton X-100 (Sigma), 50 mM Tris–HCl (pH 7.5; Sigma), and 200 nM PNA probe. Samples were covered with coverslips, placed in moist chambers, and incubated for 45 min at 57 °C. Subsequently, the coverslips were removed, and the slides were submerged in a pre-warmed (57 °C) washing solution containing 5 mM Tris base (Sigma), 15 mM NaCl (Sigma), and 1% (vol/vol) Triton X (pH 10; Sigma). Washing was performed at 57 °C for 30 min, and the slides were allowed to air dry. The smears were mounted with one drop of non-fluorescent immersion oil (Merck) and covered with coverslips. Visualization was performed using an Olympus BX51 (Olympus Portugal SA, Porto, Portugal) epifluorescence microscope equipped with one filter sensitive to the Alexa Fluor 594 molecule attached to the PNA probe (excitation, 530 to 550 nm; barrier, 570 nm; emission long-pass filter, 591 nm). Other filters present in the microscope were used in order to confirm that cells did not autofluoresce. All images were acquired using the Olympus CellB software with a magnification of 900×.

2.6. Immunocapture method using RapidChek

For the immunocapture assay (ICA) using the RapidChek SELECT Salmonella kit (SEDIX), artificially contaminated samples were prepared in primary medium as described above. Then samples were incubated for 16 to 21 h at 42 °C. Subsequently, 100 μl samples were transferred to the 1 ml of pre-warmed secondary medium and incubated for another 16 to 21 h at 42 °C. After the secondary enrichment, a strip was placed on each tube and results were read after 10 min according to the manufacturer instructions. Each experiment was repeated three times and a non-inoculated sample was simultaneous used to check for any indigenous contamination in the food samples selected.

3. Results

Three different food matrixes were selected and artificially contaminated with *S. enterica* serovar Enteritidis. After the corresponding enrichment procedures (Fig. 1), the presence of the bacterium was determined using 4 different technologies: traditional culture media, real time PCR (iQ-Check), ICA (RapidChek) and PNA FISH. All four methods were able to detect bacterial concentrations as low as 1 CFU per 25 g or ml of sample (Table 1 and Table 2). It was noted that the results obtained with RapidChek *Salmonella* and with PNA FISHwere easily interpreted since difference between positive and negative samples were obvious. The RapidChek test is based on the appearance of either one or two bars in the strip for negative or positive samples, respectively. In the case of PNA FISH the microscopic visualization shows a clear distinction between positive and negative samples in the different matrixes, even at the lower concentrations of *Salmonella* (Fig. 2). This is due to the fact that bacteria concentration reached Log 8 to Log 9 after an overnight enrichment, even for 1 CFU per 25 g orml of sample.

It was also observed that PNA FISH and RapidChek results were always concordant with culture (Table 1 and Table 2), resulting in sensitivity, specificity and accuracy values of 100%, respectively, for these two molecular methods (Table 3). However, with the real time PCR results, 8 samples, which theoretically should be negative, gave positive results using the parameters defined by the manufacturer (Table 2). In fact, iQ-check kit instructions state that any Ct value (which is defined as the number of PCR cycles required for the fluorescent signal to cross the threshold, i.e. exceed the background noise level) higher than 10 should be considered positive. But for these samples we have noticed that the Ct values were always very high, ranging between 34 and 40 (Table 1). The appearance of these high values was not related to any type of sample or with any particular assay, since we found this variation in all samples, within the same assay or between assays. In Fig. 3, A and B, we show an example of this variation for egg samples in two different assays.

These findings were investigated using DNA from two egg samples, with an initial concentration of 0.01 CFU/25 g, that gave different results of Ct, 37.55 and no amplification, respectively, in separate PCR assays. Five replicates of each DNA extract were run simultaneously in the real time PCR. Both samples gave similar results, with some replicates giving high Ct values and others not amplifying at all (Fig. 3C and D). For the NA sample we obtained two NA results and 3 high Ct values, while for the 37.55 sample we observed one NA result and 4 high Ct values. This indicates that high Ct values are not usually reproducible and this maybe because they might be situated near the detection limit of the technique, a behavior already reported by Uyttendaele et al. (2003) for false positive samples. However, using the manufacturer instructions, the occurrence of 8 false positive results (Table 2) decreased specificity and accuracy values for iQ-Check real time PCR (Table 3).

Regarding the time required to obtain the final results, the iQ-Check real time PCR and PNA FISH methods were very similar requiring less than 24 h to achieve the final results (Table 3). However, real time PCR was much more technically demanding since it required sample treatment before the PCR procedure and a trained technician on quantitative PCR procedures. It is also important to mention that repetitions are needed for some samples, which could

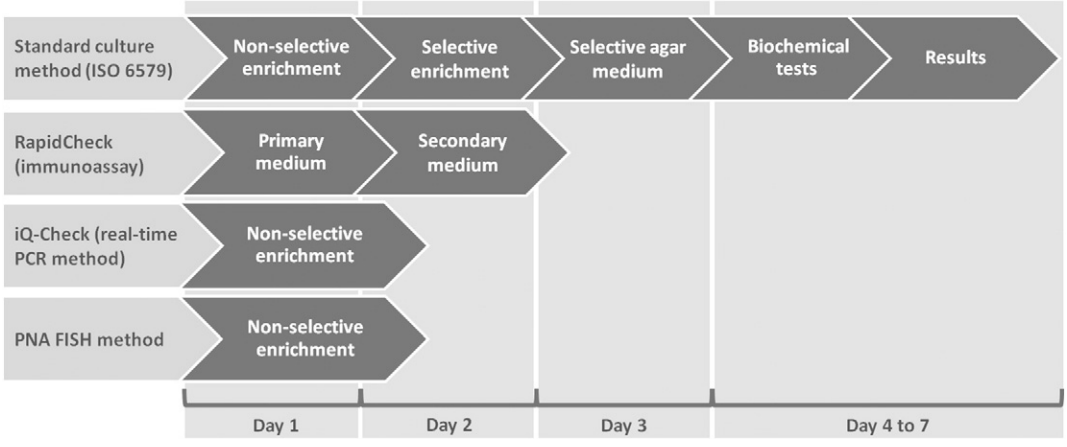

Fig. 1. Timeline for the 4 methodologies tested: immunocapture (RapidCheck), real time PCR (iQ-Check), PNA FISH and culture-based method ISO 6579:2002.

Table 1
RapidChek (immunocapture assay, ICA), iQ-Check (real-time PCR assay), PNA FISH and culture (ISO 6579:2002) results obtained for the detection of *S.* Enteritidis with different food matrixes inoculated with concentrations ranging between 0.01 and 100 CFU per 25 g or ml. Results for each independent assay are presented and Ct values for real time PCR are also provided.

	Milk				Eggs				Mayonnaise			
CFU/25 g or ml (real value)	ICA	PCR (Ct)	PNA FISH	Culture	ICA	PCR (Ct)	PNA FISH	Culture	ICA	PCR (Ct)	PNA FISH	Culture
1st assay												
100 (107.7 ± 8.7)	+	+(17.92)	+	+	+	+(16.28)	+	+	+	+(17.55)	+	+
10 (12.3 ± 2.1)	+	+(17.21)	+	+	+	+(16.42)	+	+	+	+(18.01)	+	+
1 (1.6 ± 0.6)	+	+(17.51)	+	+	+	+(16.63)	+	+	+	+(18.18)	+	+
0.1 (0)	-	-(NA)	-	-	-	-(NA)	-	-	-	-(NA)	-	-
0.01 (0)	-	-(NA)	-	-	-	-(NA)	-	-	-	+(39.02)*	-	-
2nd assay												
100 (127.0 ± 6.6)	+	+(18.11)	+	+	+	+(16.29)	+	+	+	+(18.56)	+	+
10 (13.0 ± 2.6)	+	+(17.88)	+	+	+	+(16.24)	+	+	+	+(17.58)	+	+
1 (1.3 ± 0.6)	+	+(18.03)	+	+	+	+(16.48)	+	+	+	+(17.99)	+	+
0.1 (0)	-	-(NA)	-	-	-	+(37.50)*	-	-	-	-(NA)	-	-
0.01 (0)	-	-(NA)	-	-	-	+(37.55)*	-	-	-	-(NA)	-	-
3rd assay												
100 (110.3 ± 5.9)	+	+(17.54)	+	+	+	+(16.07)	+	+	+	+(17.63)	+	+
10 (12.0 ± 1.7)	+	+(17.55)	+	+	+	+(17.18)	+	+	+	+(17.82)	+	+
1 (1.3 ± 0.6)	+	+(17.42)	+	+	+	+(16.74)	+	+	+	+(17.79)	+	+
0.1 (0)	-	+(35.08)*	-	-	-	- (NA)	-	-	-	+(39.66)*	-	-
0.01 (0)	-	+(38.57)*	-	-	-	+(34.95)*	-	-	-	+(39.03)*	-	-

* iQ-check kit user guide considers a Ct value ≥ 10 a positive *Salmonella* sample. However, these high Ct values are usually indicative of minimal amounts of target nucleic acid.

increase the overall assay time. On the other hand, PNA FISH also requires a technician with at least some experience of fluorescence microscopy for the assessment of results. RapidChek immunocapture method was the easiest to perform since it did not require any specific equipment or trained technician, but it takes almost 48 h to provide a result. Culture-based method was by far the more time consuming, taking several days to give a definitive result (Fig. 1).

4. Discussion

In the last decade several real time PCR and immunological methods have been developed and widely evaluated on different types of samples for the detection of a great variety of foodborne pathogens. Some of these approaches are actually available as commercial systems. For *Salmonella* detection there is for instance: iQ-Check kit from Bio-Rad Laboratories, BAX systems from Dupont Qualicon, MicroSEQ from Applied Biosystems, VIDAS from BioMerieux and RapidChek SELECT from SDIX. However, as mentioned below in more detail, the literature shows that studies comparing PCR and immunological technologies with standard bacteriological methods were not always concordant. Recently, PNA FISH methods on microbial identification have emerged and a variety of assays for a wide range of microorganisms are now available [see (Cerqueira et al., 2008) for a review]. Our group has recently developed a PNA FISH method for the detection of *Salmonella* (Almeida et al., 2010). In this study, 4 distinct technologies were compared with respect to their detection limit, sensitivity, specificity, accuracy, labor performance time and overall ease of performance. All methods performed well with the different food matrices giving a similar detection limit of 1 CFU per 25 g or ml of food sample (Table 3). This is probably due to the presence of an enrichment step in all four methods (Fig. 1). We also observed that culture, immunocapture method and PNA FISH were concordant, providing results with high accuracy, sensitivity and specificity (Table 3). Real time PCR results were discrepant and this may have been due to the Ct interpretation advice provided by the manufacturer which resulted in lower values for specificity and accuracy (Table 3). In real time PCR detection assays negative samples without target pathogen, should not give a Ct value, since no amplification should occur. However, in our experiments some samples that, in theory, should have been negative gave high Ct values (Fig. 2). These Ct values ranged between 34 and 39, while those for positive samples ranged between 16 and 19 (Table 1). This can be interpreted in two ways: either PCR is more sensitive than culture or these are actually false positive results. Considering: (i) the negative results obtained using the gold standard (ISO6579:2002) and the other two molecular methods (Table 2), (ii) the high Ct values observed (Table 1) and (iii) the lack of reproducibility for these samples (Fig. 3C and D), it is rational to consider these values as false positive results. However, in literature, both hypotheses have been considered, but the common absence of the Ct values makes the results comparison difficult.

Some works have used iQ-Check kit in different artificially contaminated food samples (vegetables, fruits, poultry and meat products) and also found that the kit is as sensitive as the conventional culture methodology in detecting *Salmonella*, with reported detection levels of 2 and 4 CFU/25 g (Liming and Bhagwat, 2004; Patel et al., 2006). However, other authors obtained different results, especially for naturally contaminated samples. For instance, Fakhr et al. compared the iQ-check *Salmonella* kit with bacteriological techniques in turkey meat samples and found that the PCR method failed to detect 3 of 49 samples that were positive by the conventional culture method (Fakhr et al., 2006). However, when carried out after a selective enrichment step, the real-time PCR detected all 49 samples recovered by the culture method and additionally detected the presence of *Salmonella* in some samples that were not recovered by the culture method. However, Ct

Table 2
Comparison between culture (ISO 6579:2002, considered as the gold standard), ICA, real time PCR and PNA FISH results regarding *S.* Enteritidis detection in 45 food samples.

			ISO 6579:2002 result		
			Present	Absent	Total
Test outcome	ICA	Test positive	27	0	27
		Test negative	0	18	18
		Total	27	18	45
	PCR	Test positive	27	8	35
		Test negative	0	10	10
		Total	27	18	45
	PNA FISH	Test positive	27	0	27
		Test negative	0	18	18
		Total	27	18	45

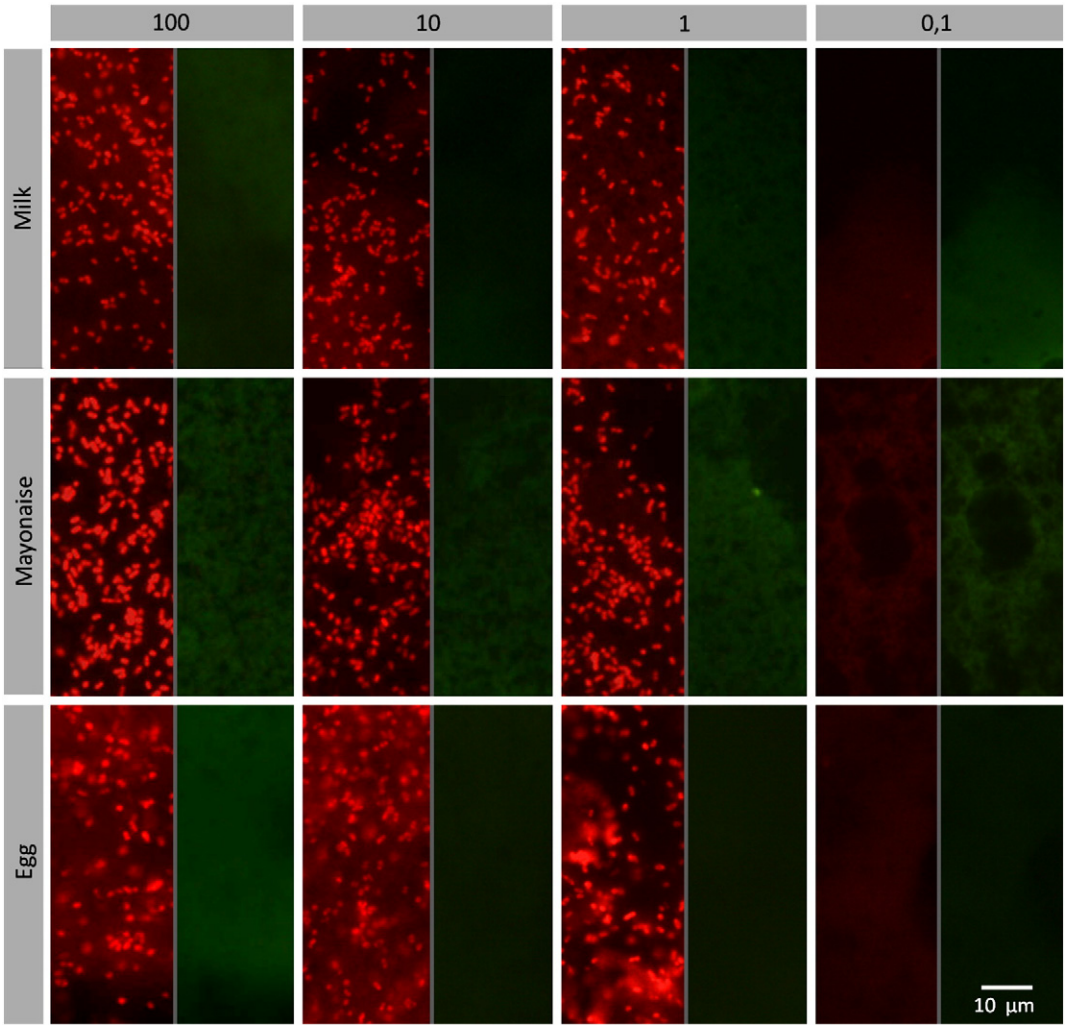

Fig. 2. PNA FISH outcome for the three food matrixes tested (eggs, mayonnaise and milk) artificially inoculated with 100, 10, 1 and 0,1 CFU of S. *Enteritidis* per 25 g or ml of food sample.

values for these samples are not presented, which would be important to evaluate the significance of these results. Nde et al. also compared the iQ-Check performance with an invA-PCR [gene involved in invasion of epithelial cells (Darwin and Miller, 1999)] for the detection of *Salmonella* in premarket and retail turkey samples. They also found a high number of false negative and false positive samples in both PCR-based methods (Nde et al., 2008).

Table 3
Performance parameters observed for each molecular test.

Parameters	ICA	Real time PCR	PNA FISH
Detection limit (CFU/25 g)	1	1	1
Sensitivity[1] (CI, 95%)	100 (84.5–100)	100 (84.5–100)	100 (84.5–100)
Specificity[2] (CI, 95%)	100 (78.1–100)	55.6 (31.3–77.6)	100 (78.1–100)
Accuracy[3] (CI, 95%)	100 (81.9–100)	82.2 (63.2–91.4)	100 (81.9–100)
Total time (h)	~48	~24	~24
Ease of use	Easy	Difficult	Medium

[1] Sensitivity as determined as TP/(TP + FN) × 100, where TP stands by the number of true positive results and FN is the number of false negative results.
[2] Specificity was determined as TN/(TN + FP) × 100, where TN stands for the number of true negative results and FP is the number of false positive results.
[3] Accuracy was determined as the number of correct results divided by the number of all returned results.

Other studies have also been published in which the use of a PCR assays is described as more sensitive than the culture method for detecting *Salmonella* in food, especially in poultry, meat, and poultry-related products (Bennett et al., 1998; Eyigor et al., 2002; Fratamico, 2003; Oliveira et al., 2002; Whyte et al., 2002). Some possible explanations presented by those authors were as follows: i) *Salmonella* found in natural samples may show atypical biochemical profiles and may not be detected using bacteriological methods and ii) the presence of *Salmonella* cells in a viable but non-culturable state (Bennett et al., 1998; Eyigor and Carli, 2003; Eyigor et al., 2002; Knight et al., 1990). Unfortunately, once again, most part of the studies lack the Ct values that are fundamental to perform results comparison. In order to understand the results discrepancy observed between studies, a comprehensive work in naturally contaminated samples, comparing the different technologies, would bring more conclusive evidences about the real time PCR value for food microbiological analyses.

Regarding the RapidChek SELECT *Salmonella* assay, we also observed different results from those previously reported. Fakhr et al. compared the RapidChek kit with the culture method and found that the use of this immunoassay alone might underestimate the *Salmonella* presence in turkey meat products, as it it failed to detect 11 out of 49 samples (Fakhr et al., 2006). Some other studies using related immunoassays, such as VIDAS and Tansia Card, reached similar results. Uyttendaele et al. reported that the sensitivity of the Vidas method was 93% compared with the conventional culture method (Uyttendaele et al., 2003). Tansia Card *Salmonella* immunoassay was

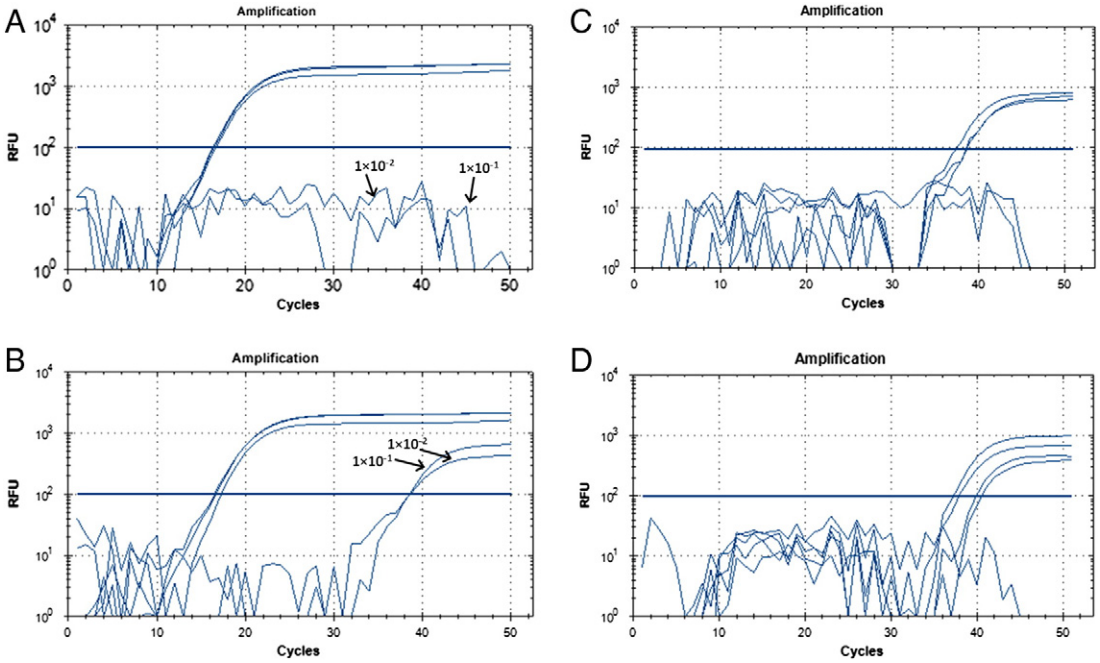

Fig. 3. - Real-time PCR results obtained for pre-enriched eggs samples artificially contaminated with concentrations raging from 1×10^{2} to 1×10^{-2} CFU of S. *Enteritidis*/25 g. Assay 1 (A) and 2 (B) presented different behavior for the lower concentrations (1×10^{-1} and 1×10^{-2} CFU/25 g). Lack of reproducibility for the 1×10^{-2} CFU/25 g pre-enriched egg samples. Panel (C) presents 5 replicates for a sample that did not amplify (from assay 1) and (D) presents replicates for a sample with a Ct of 37,55 (from assay 2).

also described as less specific than culture and PCR-based assays for the detection of *Salmonella* in ground chicken, turkey, and beef (Fratamico, 2003). However, it might be possible that the new version of RapidChek, the SELECT RapidCheck (which includes an enrichment medium supplemented with phage), may present superior characteristics and thus may present a similar performance to bacteriological methods, as reported by Muldoon et al. (Muldoon et al., 2007; Muldoon et al., 2011) and Gonzalez et al. (2009), and as observed in this study.

There is a lack of PNA FISH studies for *Salmonella* detection in food stuffs, but we have found a similar detection limit to that previously reported for powdered infant formula samples (Almeida et al., 2010). Here, the method has shown to be as sensitive, specific and accurate as bacteriological methods. An additional advantage is that it may be performed simultaneously with the culture method without requiring any additional sample preparation, since pre-enrichment is similar, this is not so with the other two technologies. Nonetheless, all 3 technologies can drastically decrease the detection time and performed well in different food matrixes with adequate detection levels. Major differences lie on the procedure complexity and in the assay time (Table 3).

5. Concluding remarks

This work shows that new rapid approaches for *Salmonella* detection in food samples can be reliable alternatives to culture based techniques. Imunocapture (RapidChek) and PNA FISH methods have presented the higher specificity and accuracy. RapidChek proved to be the easier method to use. However, when rapid results are needed, PNA FISH would be a more suitable option.

Regarding real time PCR, special attention should be taken when interpreting Ct values as high Ct values may not necessarily imply low concentrations of *Salmonella*. Defining a range of Ct values for interpreting positive results when using PCR detection assays may assist in their standardization. Nevertheless, all assays presented a detection limit of 1 CFU/25 g or ml, allowed a rapid identification, were quite easy to use, performed well in different food matrixes and are also less time- and technically-demanding than culture. These important features may assist public health authorities, in the management of *Salmonella* outbreaks, and food industry in the stocks screening for an earlier liberation of the food products.

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