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# A New Real-Time PCR Assay for the Specific Detection of *Salmonella* spp. Targeting the *bipA* Gene

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**Abstract** A straightforward real-time polymerase chain reaction (PCR)-based assay was designed and evaluated for the detection of *Salmonella* spp. in food and water samples. This new assay is based on the specific detection of the *bipA* gene of *Salmonella*, which encodes a protein of the guanosine triphosphate (GTP)-binding elongation family that displays global modulating properties, by regulating a wide variety of downstream processes. The new method correctly identified all 48 *Salmonella* strains used in the inclusivity test, and did not detect all 30 non-*Salmonella* species tested. The method was evaluated by analyzing 120 diverse food and water samples enriched in buffered peptone water. The *bipA*-based real-time PCR assay showed 100% efficiency, sensitivity, and specificity compared to the *invA*-based method previously published, which was developed as a part of a European project for the standardization of PCR methods in food microbiology. The assay includes an independent internal amplification control (IAC) in each reaction to control false negative results.

**Keywords** *Salmonella* spp. · BipA · Real-time PCR · Food Analysis

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## Introduction

Salmonellosis is a major public health concern causing millions of human cases worldwide every year and resulting in thousands of deaths. This food-borne disease is by the bacteria *Salmonella*, a rod-shaped, gram-negative non-spore-forming bacterium. This genus is a member of the family Enterobacteriaceae, and encompasses three species: *Salmonella enterica*, *Salmonella subterranea*, and *Salmonella bongori*. *S. enterica* includes six subspecies of clinical importance for humans, and over 2,500 known types, or serotypes. Environmental sources of *Salmonellae* include water, soil, insects, factory and kitchen surfaces, animal feces, raw meats, raw seafood, raw poultry, eggs, milk and dairy products, sauces and salad dressings, etc. (Tirado and Schmidt 2001).

Up to 5 days are required to detect *Salmonella* using traditional culture-based methods (Anonymous, ISO 6539 2000). The need for new, quick, and sensitive methods to detect *Salmonella* is, therefore, a major concern in food safety.

Many PCR-based assays have been described to date. Both conventional and modern real-time PCR protocols have been implemented, targeting phylogenetic genes such as the 16S ribosomal subunit (Lin and Tsen 1996). However, functional genes involved in virulence and infectivity are currently the markers of choice for most PCR procedures. The most widely used gene to date to detect *Salmonella* spp. is *invA* (invasion A) (Malorny et al. 2003a, 2004). The *invA* gene encodes for an essential component of the invasion-associated protein secretion apparatus, and is the first gene of the *inv* locus (Chiu and Ou 1996). This locus allows *Salmonella* spp. to enter epithelial cells and cause an infection (Galan et al. 1992). Other authors use genes like *tyv, prt, viaB*, *flic-d*, or *flic-a*,

**Table 1** *Salmonella* and non-*Salmonella* strains used in inclusivity/exclusivity tests and results obtained by *bipA*-targeted real-time PCR

| Organism | *bipA*-targeted real time PCR[a] |
|---|---|
| *S. enterica* subsp. *arizonae* CECT 4395 | + |
| *S. enterica* subsp. *enterica* serovar Dublin CECT4152 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis CECT4371 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis TE 232 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis TE 31154 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis TE 31327 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis TE 31888 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis TE 32271 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis TE 32302 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis TE 32337 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis TE 32395 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis TE 64752 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis TE 75108 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis TE 85230 | + |
| *S. enterica* subsp. *enterica* serovar Enteritidis (var. chaco) CECT4155 | + |
| *S. enterica* subsp. I *Enteritidis* lisotype 1 | + |
| *S. enterica* subsp. I *Enteritidis* lisotype 1 | + |
| *S. enterica* subsp. I *Enteritidis* lisotype 1 | + |
| *S. enterica* subsp. I *Enteritidis* 9.12 lisotype 1B | + |
| *S. enterica* subsp. I *Enteritidis* 9.12 lisotype 4 | + |
| *S. enterica* subsp. *enterica* serovar Gallinarum CECT4182 | + |
| *S. enterica* subsp. *enterica* serovar Gallinarum | + |
| *S. enterica* subsp. *enterica* serovar Paratyphi CECT4139 | + |
| *S. enterica* subsp. *enterica* serovar Saint Paul CECT4153 | + |
| *S. enterica* subsp. *enterica* serovar Typhi CECT409 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium CECT4296 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium CECT443 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium CECT4594 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium TT 30018 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium TT 31658 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium TT 31980 | + |
| *S. enterica* subsp. enterica serovar Typhimurium TT 32050 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium TT 55402 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium TT 64472 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium TT 67090 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium TT 88301 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium TT 98881 | + |
| *S. enterica* subsp. *enterica* serovar Typhimurium TT54336 | + |
| *S. enterica* subsp. *enterica* serovar Urbana CECT4151 | + |
| *S. enterica* subsp. *enterica* serovar Virchow CECT4154 | + |
| *S. enterica* subsp. *enterica* strain LT2 CECT 878 | + |
| *S. enterica* subsp. *salamae* CECT 4000 Type strain | + |
| *Salmonella* spp. Isolate 06/162 LSPG | + |
| *Salmonella* spp. Isolate 04/01 LSPG | + |
| *Salmonella* spp. Isolate 05/2069 LSPG | + |
| *Salmonella* spp. Isolate 05/2070 LSPG | + |
| *Salmonella* spp. Isolate 05/2071 | + |
| *Salmonella* spp. Isolate 06/299-D1 LSPG | + |
| *Arthrobacter* spp. strain VPI | − |
| *Bacillus cereus* | − |
| *Bacillus cereus* CECT 148 | − |
| *Bacillus megaterium* | − |
| *Bacillus subtilis* | − |
| *Citrobacter freundii* CECT 401 | − |
| *Clostridium perfringens* CECT 376 | − |
| *Escherichia coli* CECT 434 | − |

(continued)

| Organism | *bipA*-targeted real time PCR[a] |
|---|---|
| *Escherichia coli* O157:H7 CECT 4267 | − |
| *Enterobacter aerogenes* | − |
| *Enterobacter cloacae* | − |
| *Enterobacter cloacae* CECT 194 | − |
| *Enterococcus faecalis* ATCC 10541 | − |
| *Enterococcus faecalis* CECT 795 | − |
| *Legionella pneumophila* ATCC 33152 | − |
| *Listeria innocua* CECT 910 | − |
| *Listeria monocytogenes* CECT 4032 | − |
| *Micrococcus luteus* | − |
| *Proteus mirabilis* strain 170 | − |
| *Pseudomonas aeruginosa* | − |
| *Pseudomonas aeruginosa* CECT 108 | − |
| *Pseudomonas fluorescens* | − |
| *Serratia marcescens* | − |
| *Shigella sonnei* CECT 457 | − |
| *Shigella sonnei* CECT 4631 | − |
| *Shigella* spp. | − |
| *Staphylococcus aureus* ATCC9144 | − |
| *Staphylococcus aureus* CECT 435 | − |
| *Staphylococcus epidermidis* CECT 232 | − |
| *Yersinia enterocolitica* biovar CECT 4315 | − |

[a] + positive test result, − negative test result

which are O, H, and Vi antigen genes (Hirose et al. 2002) to detect and identify *Salmonella enterica* serovars Typhi and Paratyphi. Finally, genes in the ttrRSBCA locus, which is required for tetrathionate respiration and located near the pathogenicity island 2 of *Salmonella*, are also used as a target to detect *Salmonella* in food by real-time PCR (Malorny et al. 2004).

Most of these genes, however, present either nonspecific amplifications or inclusivity problems such as the failure in the detection of the serovar Saint Paul of *Salmonella* by the *invA*-targeted PCR (Malorny et al. 2004; Cohen et al. 1996). Furthermore, virulence genes are normally subjected to strong variability, mainly caused by silent mutations in the third base of the codon (Friis et al. 2000; Yang et al. 2000). Considering the high specificity of probes used in real-time PCR, a single mismatch can result in a dramatic decrease in the efficiency of the reaction and might therefore give rise to a false negative result.

Regulatory genes are less subjected to spontaneous silent mutations than regular functional genes and an inverse correlation has been demonstrated between the rate of evolution of transcription factors and the number of genes they regulate (Rajewsky et al. 2002). Therefore, we sought to develop a PCR tool to detect *Salmonella* targeting one of these genes. The *bipA* (or *typA*) gene is a member of the "GTP-binding elongation" family of genes, and belongs to the category N. This gene exhibits global modulating properties and acts as an essential translation factor for the efficient expression of *fis*, thus regulating a wide variety of downstream processes, such as type III secretion and DNA metabolism (Owens et al. 2004). BipA was first revealed as a strongly upregulated protein when *Salmonella enterica* was exposed to the host defense protein BPI (Qi et al. 1995). Nevertheless, further studies showed that null *Escherichia coli* mutants of *bipA* (or *typA*) are pleiotropic, with deficiencies in crucial processes such as the expression of the K5 capsule system (Rowe et al. 2000), poor growth at low temperatures (Grant et al. 2003; Pfennig and Flower 2001), defects in flagella-mediated cell motility (Grant et al. 2003; Farris et al. 1998) and loss of resistance to some antimicrobial peptides (Qi et al. 1995; Barker et al. 2000). *BipA* binds to ribosomes at a site that concurs with that of elongation factor G, and has a GTPase activity that is sensitive to high guanosine diphosphate (GDP):guanosine triphosphate (GTP) ratios and stimulated by ribosomes programmed with mRNA and aminoacylated tRNAs.

Results obtained with this new *bipA* real-time PCR-based method have been compared to those obtained by the *invA*-based PCR method described by Hoorfar (1999) and Malorny et al. (2003a), which was developed as a part of a European project for the standardization of PCR methods in food microbiology. This new assay displays the requirements of a diagnostic PCR assay, and meets the requirements to become a standardized method for the quick identification of *Salmonella* spp.-contaminated water and food samples.

## Material and Methods

### Bacterial Reference Strains Used in this Study

A total of 48 *Salmonella* strains were used for inclusivity tests (Table 1). These strains were either obtained from the Spanish Type Culture Collection (CECT), or isolated by the Public Health Laboratory of Girona, and the Laboratory of Microbiology of the Hospital Universitari Dr. Josep Trueta of Girona. In addition, 30 different bacterial species belonging to all major phylogenetic lineages were used for exclusivity tests. Some of them were specifically selected because of the close phylogenetic relation to *Salmonella* spp. or because they grow in the same environments and conditions (Table 1).

### DNA Purification

For inclusivity and exclusivity tests, bacterial strains were grown in nutritive medium as previously described (Miller 1972). Isolated colonies were resuspended in bidistilled sterile water and DNA was extracted and purified using the NucleoSpin® Tissue kit (Macherey-Nagel, Düren, Germany) as specified by the manufacturer. DNA from food and water samples was extracted from 1-ml aliquot of a pre-enriched culture in buffered peptone water using the same kit.

DNA concentration was determined by the PicoGreen™ method (Molecular Probes, Carlsbad, CA, USA), which compares fluorescence values with those of a calibration curve built from a dilution series of salmon sperm DNA (Sigma Chemicals, Madrid, España).

Genomic copy numbers were calculated based on the *Salmonella enterica* serotype Typhimurium genome size (4,857 kb) (McClelland et al. 2001), and considering that the average base pair weight is 652 g $mol^{-1}$.

### Primer Design

All sequences of the *bipA* gene from *Salmonella* spp. available in the databases were retrieved and aligned using ClustalX version 1.81 (Thompson et al. 1997). The consensus sequence was then used to design a set of primers and a probe for the quantitative determination of *Salmonella* spp. by real-time PCR using the software Primer Express™ v. 2.0 (Applied Biosystems, Foster City, CA). Both forward SAL1410f and reverse SAL1494r primers, and the probe SAL1441pr (Table 2) were evaluated with the NetPrimer software (PREMIER Biosoft International, Palo Alto, CA, USA) for the formation of primer-dimer structures and hairpins. The oligonucleotides for the amplification of the internal amplification control were manually designed based on the IAC sequence (Table 2).

**Table 2** Primers, probes, and IAC sequence used for the *bipA*-targeted real-time PCR assay

| Designation | Sequence (5′–3′) |
|---|---|
| SAL1410f | GGTCTGCTGTACTCCACCTTCAG |
| SAL1494r | TTGGAGATCAGTACGCCGTTCT |
| SAL1441pr | 6FAM – TTACGACGATATTCGTC CGGGTGAAGTG – TAMRA |
| IACf | TCCAGGGCGAAAGTAAACGT |
| IACr | GGCGAGCCGTACGAACAC |
| IACpr | VIC – CCCAGTTGGCTGATCACTTTCG – TAMRA |

The *Salmonella* and IAC probes were labeled at the 5′ end with 6-FAM and VIC, respectively. TAMRA was used as a quencher at the 3′ end in both cases.

### Real-Time PCR Conditions

Real-Time PCR was carried out in 20-µl (total volume) reaction mixtures using an ABI Prism 7300 SDS RTi-PCR system (PE Applied Biosystems, Foster City, CA, USA). A positive reaction and a non-template reaction were included in all tests.

An artificial, chimeric 65-bp DNA fragment was designed and used as an internal amplification control (IAC) in all reactions (Table 2). This DNA fragment was synthesized by TIB MOLBIOL (Syntheselabor GmbH, Berlin, Germany) and used at an optimal concentration of $10^3$ amplicon copies per reaction.

PCR conditions were 95°C for 10 min, then 50 cycles consisting of 95°C for 15 s and 60°C for 1 min. Reaction mixtures contained 1× Universal Master Mix (PE Applied Biosystems, Foster City, CA, USA), 1-µl DNA template, 300 nM of SAL1410f, 300 nM SAL1494r, 100 nM of SAL1441pr, 300 nM of IACf, 300 nM of IACr, 50 nM of IACpr, and $10^3$ copies of IAC.

Fluorescence values in PCR reactions were analyzed using the ABI Prism 7300 System SDS software v1.2.3 (PE Applied Biosystems, Foster City, CA, USA). An automatic threshold setting of 0.2 was used for all samples.

### Determination of the Linearity and Efficiency of the Reaction

The linearity and the efficiency of the PCR assay were determined using purified DNA from the *S. enterica* subsp. *enterica* LT2 CECT 878 strain within a concentration range of $10^1$ to $10^6$ genomic DNA copies per reaction. One microliter of each dilution was added to five replicas in PCR tubes and run in the presence of $10^3$ copies of the IAC as described above. The linearity of the PCR reaction

was assessed by plotting the obtained threshold cycle (Ct) against the logarithm of the number of genomes in the reaction. The efficiency of the *bipA*-based real-time PCR assay was calculated using the formula: Efficiency = $[10^{(-1/\text{slope})}] - 1$.

### Food and Water Samples

A total of 120 samples including 15 fish samples (monkfish, hake, smoked salmon), 10 egg samples (fresh egg, potato omelet, pasteurized egg, boiled egg, refrigerated omelet), 26 meat samples (lamb, minced beef and pork, chicken and minced chicken, hamburger meat, duck meat, and sausages), 20 dairy products (milk, cream, powdered milk, sour cream, cheese, *fromage frais*, yogurt), 26 prepared food samples (tuna pies, salads, lasagna, puff pastry, muffins, black pepper), and 23 water samples (bottled, from water supply systems, from a well) were analyzed using both the *invA*-based PCR method (Friis et al. 2000; Rowe et al. 2000) and the *bipA*-based real-time PCR. The samples were chosen to find the highest number of naturally positive samples. All samples were enriched in BPW for 16–20 h at 36±2°C, prior to being processed.

## Results

### Selectivity Test

Inclusivity and exclusivity tests were performed with a total of 48 *Salmonella* and 30 non-*Salmonella* strains (Table 1). All 48 tested strains of *Salmonella* were correctly identified, and all non-*Salmonella* strains presented a threshold cycle number (Ct) above 30, whereas the IAC threshold cycle number (Ct) was around 28–29 in all reactions.

### Linearity and Efficiency of the Reaction

Genomic DNA of the strain *S. enterica* subsp. *enterica* LT2 CECT 878 was used as a template for linearity and efficiency tests The real-time PCR described here detected as little as $10^2$ genomes of *Salmonella* per reaction in all cases, whereas in only two of the five replicas $10^1$ genomes of *Salmonella* were detectable (Table 3). The linearity of the assay was calculated using the equation Ct=38.268 – 3.3224 log (*N* of genomes) with a $R^2$ correlation value of 0.9993.

The efficiency of the *bipA*-based real-time PCR was 99.98%.

### Detection Limit of the Assay

To calculate the detection limit of our real-time PCR method, enrichments of 22 food and water negative samples were inoculated with *S. enterica* subsp. *enterica* Serovar Typhimurium CECT 4594. Food samples were inoculated with 6 cfu/25 g, whereas bottled water samples were inoculated with 3 cfu $l^{-1}$, and well or water supply system samples with 3 cfu/100 ml. The *Salmonella* suspension used for the inoculation was quantified by plate counting in nutritive agar plates.

The probability of detection of *Salmonella* in the samples was calculated as a percentage, resulting in a 100% in the case of water samples contaminated with 3 cfu, whereas in the case of food samples, the probability of detecting the *Salmonella* in a sample inoculated with 6 cfu/25 g was 95.45%.

**Table 3** Mean Ct and end point fluorescence (*F*), and standard deviations of serial 10-fold-diluted concentrations of *S. enterica* subsp. *enterica* strain LT2 CECT878 genome equivalent

| No. of genomes *Salmonella* per reaction | Reporter | | | |
|---|---|---|---|---|
| | FAM (*Salmonella*) | | VIC (IAC) | |
| | Ct±SD | F±SD | Ct±SD | F±SD |
| $10^6$ | 18.35±0.05 | 4.26±0.24 | Undet.[A] | 0.09±0.02 |
| $10^5$ | 21.68±0.20 | 4.33±0.17 | 31.51±0.26 | 0.58±0.02 |
| $10^4$ | 25.11±0.07 | 4.32±0.06 | 28.86±0.12 | 0.75±0.02 |
| $10^3$ | 28.38±0.13 | 3.87±0.13 | 28.80±0.10 | 0.78±0.02 |
| $10^2$ | 31.49±0.67 | 2.98±0.32 | 28.84±0.10 | 0.82±0.02 |
| $10^1$ | 35.63±0.70[B] | 0.75±0.27 | 28.97±0.08 | 0.87±0.01 |

IAC is not amplified when high amounts of *Salmonella* DNA are in the sample. Three of the five replicas were undetectable. These data have not been included in further calculations.
This assay was performed in the presence of $10^3$ copies of IAC.

Efficiency of the Assay Using Food and Water Samples

Sensitivity, specificity, and efficiency of the *bipA*-based real-time PCR assay were calculated after analyzing 97 diverse food samples and 23 water samples by both the *invA*-based (Malorny et al. 2004; Hoorfar 1999) PCR, and the real-time PCR method presented here (Table 4). To increase the number of positive samples to analyze, some food and water samples initially yielding a negative result for *Salmonella* with both PCR methods were spiked with 50 cfu of *S. enterica* subsp. *enterica* Serovar Typhimurium CECT 4594 per analytical portion (food 25 g, water 100 ml or 1 l) and then analyzed again.

Results were in agreement in all cases, which resulted in a sensitivity, specificity, and efficiency of the *bipA*-based real-time PCR assay of 100% for all types of food matrices and waters. Accordingly, neither false positives nor false negatives were obtained in the 120 samples analyzed (Table 4).

To avoid PCR inhibition, 1:10 and 1:100 dilutions were used.

## Discussion

The new real-time PCR-based assay for the detection of *Salmonella* spp. in food and water samples through the detection of the *bipA* gene of *Salmonella* was designed and evaluated against the procedure of the European project FOOD-PCR based on the detection of the *invA*-gene (Friis et al. 2000; Rowe et al. 2000). As it happens with conventional microbiological analysis, this method requires previous enrichment incubation in buffered peptone water. After genomic DNA extraction using a column-based extraction kit, a *bipA*-targeted real-time PCR is performed in the presence of an internal amplification control. The described procedure detects *Salmonella* in food and water samples in less than 24 h, drastically reducing the time needed by the conventional culture-based methods. Most PCR-based methods for the detection of *Salmonella* used to date target either phylogenetic or functional genes. However, most of the assays used until now present either specificity or inclusivity problems (Malorny et al. 2004; Cohen et al. 1996). For instance, the S. *enterica* subsp. *enterica* serovar Saint Paul is not detected by the *invA*-based assay. As previously mentioned (Table 1), this strain was satisfactorily tested with our system. Moreover, critical variations in the target DNA, which can hamper the specificity and accuracy of the analysis, are produced by either the codon usage or silent mutations (Cohen et al. 1996). This means that, for example, in a 21-bp oligonucleotide up to seven positions are at risk of being nonspecific because of the natural genetic variability of bacterial populations, which can compromise the selectivity of the PCR assay. For some reason, the *bipA* (or *typA)* gene does not seem to show this variability, which makes it an ideal target for a PCR. Besides, another pitfall of some of the PCR-based methods reported so far is that they do not include an internal amplification control in the reaction.

**Table 4** Results of the *bipA*-based real-time PCR method compared to the *invA*-based method (Hoorfar 1999)

| | | *invA*-based PCR (Malorny et al. 2003a; Hoorfar 1999) | | |
|---|---|---|---|---|
| | | Presence | Absence | |
| *bipA*-based PCR | Presence | 57 | 0 | 57 |
| | Absence | 0 | 63 | 63 |
| | | 57 | 63 | 120 |

The use of an internal amplification control (IAC) in the reactions helps to control false negatives resulting from PCR inhibition or reaction malfunction because of either spoiled reactives or a damaged thermocycler (Hoorfar et al. 2003). In these reactions, the IAC consists of an artificially constructed DNA sequence, which is detected by two primers and a probe labeled with the VIC reporter. The optimal IAC concentration was set as low as $10^3$ copies per reaction. The IAC was amplified with Ct values of 28–29 in all the samples except those containing high amounts of genomic DNA of *Salmonella,* in which the IAC was usually undetectable or appeared at Ct values above 30 (Table 3) because of template competence.

Selectivity of the *bipA*-targeted primers and probe was first tested using the NCBI Blastn program (Altschul et al. 1997). An inclusivity/exclusivity test was then performed using 48 *Salmonella* and 30 non-*Salmonella* strains, revealing 100% selectivity of the primers and probe presented in this work. Besides, no false negatives or false positives were detected when comparing the results obtained in the analysis of 120 food and water samples with the results obtained by the PCR method described by the European Project Food-PCR (Malorny et al. 2003a, b Hoorfar 1999).

The real-time PCR presented here allows detection of as few as $10^2$ genomes of *Salmonella* per reaction in the presence of an internal amplification control when using a quantified *Salmonella* suspension. Besides, the detection probability of 6 and 3 cfu in food and water sample rinses was 95.45% and 100%, respectively. Considering that the samples are subjected to an overnight BPW enrichment before DNA extraction, an initial contamination of one viable cell of *Salmonella* in a given sample would normally produce at least $10^5$–$10^7$ cfu $ml^{-1}$, assuming 16–20 h at doubling times of 0.45 to 1 h, which is detectable by the

*bipA*-based real-time PCR. Typically, in real analyses, a BPW overnight incubation of a 10-cfu inoculum would produce a positive real-time PCR result at Ct values between 25 and 28.

The *bipA*-based real-time PCR assay presented in this work is linear and highly selective, specific, and efficient. It is also fast because it permits a sample for the presence of *Salmonella* to be analyzed in no more than 24 h. Therefore, this method might substitute the previous *invA*-based method for the rapid detection of *Salmonella* in food and water samples.

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