https://doi.org/10.22319/rmcp.v13i3.5997

Article

Evaluation of real-time polymerase chain reaction coupled to immunomagnetic separation (rtPCR-IMS) as an alternative method for the routine detection of *Salmonella* spp. in beef in Mexico

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Abstract:

Salmonella is a pathogenic bacterium considered a threat to the food industry, its timely detection being relevant. The objective of the study was to evaluate the real-time polymerase chain reaction coupled to immunomagnetic separation (rtPCR-IMS) as an alternative method to the Official Mexican Standard (NOM-114-SSA1-1994) for the detection of *Salmonella* in beef. The parameters evaluated were limit of detection, sensitivity, specificity, selectivity (inclusivity and exclusivity) and degree of agreement between both methods for the detection of *Salmonella* in presumptive and artificially contaminated beef samples. The incidence of *Salmonella* in presumptive beef samples (n= 60) ranged from 20.0 to 21.6 % by both methods. In the inoculated samples (n= 60), the detection rate of *Salmonella* by rtPCR-IMS (93.3 %) and NOM-114-SSA1-1994 (98.3 %) showed a match of 56 occasions with a negative deviation. The comparison of rtPCR-IMS and NOM-114-SSA1-1994 in beef

reported an accuracy of 98.3 %, sensitivity of 98.2 %, specificity of 100 % and selectivity of 100 %. The limit of detection for both methods was 1-5 CFU·25 g^{-1} of beef. The statistical analysis indicates that the rtPCR-IMS is equivalent to the reference method for the detection of *Salmonella* in beef. These results warn of the high incidence of *Salmonella* in beef and propose rtPCR-IMS as an ideal and fast method for the control of *Salmonella* in the meat industry.

Key words: Beef, Polymerase chain reaction (PCR), *Salmonella*, Immunomagnetic separation (IMS).

Received: 28/05/2021

Accepted: 03/02/2022

Introduction

Salmonella is a group of Gram-negative bacteria that comprises >2,600 serotypes classified into two species, *S. enterica* (includes six subspecies) and *S. bongori*. Among these, *Salmonella enterica* subsp. *enterica* (>1,500 serotypes) is the main group responsible for diseases in man called "salmonellosis"[(1)]. The main clinical manifestations are enteric fever (typhoid and paratyphoid) and gastroenteritis caused by typhoidal and nontyphoidal serotypes of *Salmonella*, respectively[(2)]. The annual global estimate of salmonellosis is >25 million cases of enteric fever and 153 million cases of gastroenteritis with ~300 thousand deaths, which are mostly associated with the consumption of contaminated foods[(3)]. Salmonellosis is currently one of the four major foodborne diseases (FBDs) worldwide[(4)].

Salmonella is widely distributed in nature and can survive in a wide variety of foods (animal origin and vegetables), which have been identified as vehicles of transmission of the bacterium[(2,4,5)]. The zoonotic nature of nontyphoidal serotypes of *Salmonella* points to animals as the main risk factor for exposing the bacterium to the environment and transferring the pathogen to humans during the production, handling or consumption of foods[(6)]. *Salmonella* surveillance should be based on reliable detection methods that favor food safety[(7)].

Detecting *Salmonella* in foods can be complex because the bacteria are often found in low concentrations in foods. Another aspect that hinders the detection of the microorganism in a food is the process of production, the background microorganisms and the type of food matrix[(8)]. This can warn of a health risk and justify the need for timely methods for the detection of relevant pathogens such as *Salmonella*[(7)].

The culture method is considered the gold standard for the isolation and detection of pathogenic microbes in foods[9]. The culture method for the detection of *Salmonella* involves a stage of nonselective pre-enrichment, followed by selective enrichment and seeding on selective agars, and subsequent biochemical and serological characterization of presumptive colonies, allowing a negative or positive result to be obtained in 4 and 6-7 d, respectively[9]. The consumption of time, labor and reagents involved in this method are an inconvenience for the rapid detection of *Salmonella* in the food industry[7].

Currently, rapid molecular methods have been developed, such as the polymerase chain reaction (PCR) method and its real-time variant (rtPCR), which allow the detection of *Salmonella* in a short time (24-72 h) in various foods[10-12]. Immunological, biochemical tests and biosensors have also been proposed as rapid methods[8]. Molecular methods are useful as detection tools, reducing labor and response time compared to culture methods[12]. However, the accuracy of molecular methods can be limited by the presence of inherent substances of the food (bile salts, bilirubin, hemoglobin, urea, polysaccharide, feces) and antigens and DNA, which can interfere with the results[13].

Immunomagnetic separation (IMS) has been used for the isolation of *Salmonella* in different food matrices[8,14-16]. IMS improves the sensitivity and specificity of detection of *Salmonella* in foods due to the anti-*Salmonella* polystyrene beads that capture the bacterium, and this can be identified by culture, immunological or molecular methods[17]. Recently, methods that combine IMS and rtPCR technologies have been developed, which dispense with DNA extraction and concentrate the microorganism by IMS after a primary enrichment of <24 h[10,11,18].

In Mexico, *Salmonella* represents an issue concerning public health and the food industry. The General Directorate of Epidemiology of Mexico annually reports 28,815 cases of typhoid fevers and 78,681 cases of other salmonellosis nationwide[19]. But the identification of these cases as FBDs is not clarified. Despite the fact that, in Mexico, *Salmonella* has been identified as a contaminant of chicken meat[20,21], pork[21], beef [21-23], vegetables[24] and eggs[25].

The timely detection of *Salmonella* in beef is of special interest given its high frequency (28.9 to 32.4 %) in this food[21-23] and the relevance of production (1,960 t) and annual per capita consumption (14.8 kg) of beef in Mexico[26]. In addition, livestock and birds have been identified as reservoirs of *Salmonella*[27]. In Mexico, the culture method is the gold standard for the detection of *Salmonella* in foods, whose process can be laborious and require a lot of time (4 to 7 days) to obtain final results[28]. Therefore, the objective of this study was to perform an internal validation of the commercial method of rtPCR-IMS for the detection of *Salmonella* in beef, and to compare its efficiency with the reference culture method used in Mexico.

Material and methods

Description of the study

The internal validation study of the real-time polymerase chain reaction method coupled to immunomagnetic separation (rtPCR-IMS) for the detection of *Salmonella* in beef was evaluated with respect to the reference culture method in Mexico "NOM-114-SSA1-1994"[28], as specified in the manual for the validation of alternative microbiological methods proposed by ISO16140:2003[29]. The validation of the rtPCR-IMS method was performed on presumptive and artificially contaminated beef samples. The parameters evaluated were limit of detection, sensitivity, specificity, selectivity, and the degree of agreement between the methods.

Bacterial strains

The *Salmonella* strain ATCC 35664 was used as a reference control for the validation assay. One pure colony was transferred to 30 mL of trypticasein soy broth (TSB; Becton Dickinson) and incubated for 4 h in a water bath at 35± 2 °C with constant stirring. An aliquot of the culture was used to inoculate 50 mL of TSB to obtain an absorbance (OD) of 0.1 at 600 nm. This subculture was incubated under the same conditions until obtaining an OD 1.0 (λ= 600 nm). Serial dilutions and plate count in Hektoen enteric agar (Becton Dickinson) were performed to standardize the concentrations of 1-5, 6-10, 1-15 and 16-30 CFU·100 μL^{-1}.

A list of 60 strains classified as *Salmonella* (n= 30) and non-*Salmonella* (n= 30) were used for the selectivity parameter (Table 1). The selection of non-*Salmonella* strains was based on the biochemical characteristics they share with *Salmonella* or because they are considered contaminants of the meat. Most of the non-*Salmonella* strains corresponded to collection cultures and were obtained commercially. *Salmonella* strains were obtained from the laboratory, whose identification was previously made with molecular tests. All strains were grown in TSB at 37 ºC for 24 h.

Table 1: Microorganisms used for the selectivity test

Inclusivity test			**Exclusivity test**		
Salmonella strains	**No**	**Reference**	**Non-*Salmonella* strains**	**No**	**Reference**
S. Agona	1	CIAD A.C	*Bacillus subtilis*	1	Ambiental
S. Albany	1	CIAD A.C	*Candida Albicans*	1	ATCC 10231
S. Anatum	1	CIAD A.C	*Citrobacter freundii*	1	LEM 04001
S. B monofásica	1	CIAD A.C	*Citrobacter freundii*	1	LEM-04001
S. C1 monofasica	1	CIAD A.C	*Enterobacter aerogenes*	1	LEM-04003
S. Cayar	1	CIAD A.C	*Enterobacter aerogenes*	1	ATCC 13048
S. Cholerasuis	1	CIAD A.C	*Enterobacter cloacae*	1	LEM-04013
S. Enteritidis	1	CIAD A.C	*Enterococcus faecalis*	1	LIBM-01003
S. F	1	CIAD A.C	*Escherichia coli*	1	LEM-01005
S. Gaminara	1	CIAD A.C	*Escherichia coli*	1	ATCC 25922
S. Give	1	CIAD A.C	*Eschrichia* coli *O157:H7*	1	CENAPA700728
S. Haviana	1	CIAD A.C	*Klebsiella pneumoniae*	1	ATCC 13883
S. Infantis	1	CIAD A.C	*Listeria innocua*	1	ATCC 33090
S. Luciana	1	CIAD A.C	*Listeria ivanovvi*	1	ATCC 19119
S. Meliagridis	1	CIAD A.C	*Listeria monocytogenes*	1	Ambiental
S. Minnesota	1	CIAD A.C	*Listeria monocytogenes*	1	ATCC 7694
S. Montevideo	1	CIAD A.C	*Listeria monocytogenes*	1	ATCC 7644
S. Muenchen	1	CIAD A.C	*Listeria monocytogenes*	1	Ambiental
S. Newport	1	CIAD A.C	*Proteus mirabilis*	1	ATCC 12453
S. Oranienburg	2	CIAD A.C	*Proteus mirabilis*	1	LEM-03011
S. Saintpaul	2	CIAD A.C	*Proteus vulgaris*	1	LEM-06070
S. San Diego	1	CIAD A.C	*Pseudomonas aeroginosa*	1	ATCC 27853
S. Senftenberg	1	CIAD A.C	*Pseudomonas aeruginosa*	1	LEM-01002
S. Sohanina	1	CIAD A.C	*Rhodococcus equi*	1	LEM-01019
Salmonella sp	1	CIAD A.C	*Rhodococcus equi*	1	ATCC 6939
S. Typhimurium	1	CIAD A.C	*Shigella flexneri* Gpo. B	1	ATCC 12022
S. Thompson	1	CIAD A.C	*Shigella flexnieri*	1	LEM-04004
S. Weltevreden	1	CIAD A.C	*Shigella sonnei*	1	ATCC 9290
		CIAD A.C	*Staphylococcus aereus*	1	ATCC 25923
		CIAD A.C	*Staphylococcus epidermis*	1	ATCC 12228

The acronyms stands for: CIAD A.C. (*Centro de Investigación en Alimentación y Desarrollo A.C.*), CENAPA (*Centro Nacional de Servicios de Diagnóstico en Salud Animal*), ATCC (American Type Culture Collection), LEM (internal code), LIBM (internal code).

Collection of beef samples

For the study, 60 samples of raw beef consisting of ground beef (n= 20), inside round (n= 20) and peeled knuckle (n= 20) were collected from three markets located in the city of Culiacán, Sinaloa, Mexico. The number of beef samples was assigned according to the minimum recommendation suggested by ISO16140:2003[29]. Samples of 500 g of beef were taken, which were placed in sterile plastic bags and transported in refrigeration to the laboratory for their further analysis. The study contemplated performing the analysis on presumptive samples to simulate the actual effects of contamination occurring in nature, and on artificially contaminated beef samples.

Analysis of presumptive beef samples

Fifty grams of beef were homogenized with 50 ml of sterile distilled water in a grinder at 230 rpm for 2 min. Subsequently, the sample was divided equally into 25 mL portions for the method of rtPCR-IMS and NOM-114-SSA1-1994.

Analysis of artificially contaminated beef samples

Samples of beef classified as negative for *Salmonella* by both methods were used. A 100 g portion of beef was homogenized with 100 mL of sterile distilled water for 2 min at 230 rpm and separated equally into two 50 mL portions; one portion was used as a negative control and the remaining portion for contamination of the beef with *Salmonella*. The contaminated portion was homogenized for 2 min at 230 rpm, and subsequently the homogenate was divided into two portions of 25 mL for their analysis by both methods. In parallel, a negative control under the same conditions was included. The selection of the inoculum corresponded to the limit of detection that allows a fractional recovery of the bacterium by any of the methods.

Limit of detection

To establish the relative detection limit, five levels of inoculum of *Salmonella* were prepared: 0, 1-5, 6-10, 1-15 and 16-30 CFU·25 mL^{-1}. From the beef samples classified as negative for *Salmonella*, 250 g of sample were taken and homogenized with 250 mL of sterile distilled water for 2 min at 230 rpm in an automatic homogenizer (Stomacher 400 Circulator). The homogenate was divided into 10 portions of 25 mL for their inoculation with *Salmonella*: portion 1-2 (0 CFU·25 mL^{-1}), portion 3-4 (1-5 CFU·25 mL^{-1}), portion 5-6 (6-10 CFU·25 mL^{-1}), portion 7-8 (1-15 CFU·25 mL^{-1}) and portion 9-10 (16-30 CFU·25 mL^{-1}). The levels of inoculum added were corroborated by the method of plate count in Hektoen enteric agar, and the types of portions were evenly distributed for their analysis for both methods. The

limit of detection will correspond to the smallest concentration of the inoculum that can be detected in the sample 50 % of the time by the methods[29].

Selectivity test

The selectivity test of the methods was performed *in vitro* without the use of beef samples and the selection of the bacterial inoculum was performed according to the specifications of ISO16140:2003[29]. For each bacterium (Table 1), an inoculum was standardized at an absorbance of 1.0 (λ= 600 nm) in both assays. For the exclusivity assay, a concentration of 10^4 CFU 100 μL^{-1} was adjusted in 225 mL of peptone water. Whereas, in the inclusivity assay, the concentration of 100 CFU·100 μL^{-1} was adjusted in 225 mL of the enrichment media used by the rtPCR-IMS (peptone water) and NOM-114- SSA1-1994 (lactose broth) methods, and the subsequent addition of 100 µL from a pool of the strains used in the exclusivity test. All cultures were evaluated by the methods of rtPCR-IMS and NOM-114-SSA1-1994.

Reference culture method (NOM-114-SSA1-1994)

Beef samples (25 mL) were enriched with 225 mL of lactose broth (Becton Dickinson) and incubated at 35 ± 2 °C for 24 ± 2 h. Subsequently, aliquots of 1 mL of the culture were transferred to 10 mL of Selenite Cystine broth (Becton Dickinson) and 10 mL of Rappaport Vassiliadis broth (Becton Dickinson), for their incubation at 35 ± 2 °C for 24 h. Once incubation was complete, 10 µL aliquots of the previous cultures were inoculated into Hektoen enteric agar (Becton Dickinson), Xylose Lysine Deoxycholate Agar (Becton Dickinson) and *Salmonella-Shigella* agar (Becton Dickinson) and incubated at 35 ± 2 °C for 24 h. From the agars, presumptive colonies (n= 3) of *Salmonella* were selected for their identification by primary biochemical tests (triple sugar iron agar, lysine and iron agar and urea broth), API 20E (Biomeriux NC) and serological tests based on the detection of polyvalent O antigen (InDRE)[28]. In parallel, a negative control (medium without bacteria) and a positive control (*Salmonella* ATCC 35664) were included.

rtPCR-IMS method

The detection of *Salmonella* was carried out according to the conditions of the supplier (www.biocontrolsys.com). All reagents necessary for the preparation of the rtPCR reaction for *Salmonella* (GDS *Salmonella* Tq 71008) are commercially available by Assurance GDS®, BioControl System Inc. A 25 mL portion of sample was enriched with 225 mL of buffered peptone water (Becton Dickinson) and incubated at 35 ± 2 °C for 24 h. Subsequently, 1 mL of the previous enrichment was transferred to the concentration plate containing 20 µl of concentration reagent (magnetic beads coated with anti-*Salmonella* antibodies) and mixed

for 10 min in an automatic homogenizer (Vortex Mixed Biocontrol Bellevue System). The magnetic beads were removed with a magnetic pipette (Pick Pen™ Biocontrol System Bellevue), washed with 1 ml of the buffer for 7 sec and transferred to a plate containing 35 µL of the resuspension reagent. With a multichannel pipette, 20 µL of the resuspension buffer was transferred with the immunomagnetic beads and deposited in the PCR tubes, which previously contained the probe, oligonucleotides and Taq DNA polymerase (5-Prime). Finally, the tubes were placed in the rtPCR machine. In parallel, a negative control (medium without bacteria) and a positive control (*Salmonella* ATCC 35664) were included. All samples analyzed by the rtPCR-IMS assay were re-evaluated from the original enrichment using the reference method to verify the detection of *Salmonella*.

Statistical analysis

The validation parameters of the rtPCR-IMS method were based on the comparison of positive agreements (PAs), negative agreements (NAs), positive deviations (PDs) and negative deviations (NDs) of the results obtained in the detection of *Salmonella* in the beef samples compared to the NOM-114-SSA1-1994 method. The parameters were calculated with the following formulas:

$$\text{Accuracy} = \frac{\text{PAs+NAs}}{\text{PAs+NAs+PDs+NDs}} \text{ x } 100\% \quad (1)$$

$$\text{Specificity} = \frac{\text{NAs}}{\text{NAs+PDs}} \text{ x } 100\% \quad (2)$$

$$\text{Sensitivity} = \frac{\text{PAs}}{\text{PAs+NDs}} \text{ x } 100\% \quad (3)$$

$$\text{False positives} = 100\ \% - \%\ \text{sensitivity} \quad (4)$$

$$\text{False negatives} = 100\ \% - \%\ \text{specificity} \quad (5)$$

$$\text{Discordant values (Y)} = \text{PDs} + \text{NDs} \quad (6)$$

The degree of agreement between the methods (rtPCR-IMS and NOM-114-SSA1-1994) was determined by the kappa index and the McNemar test (χ^2) with significance of 5 %.

Results

Limit of detection

Table 2 shows the capacity of the rtPCR-IMS and NOM-114-SSA1-1994 methods for the detection of different levels of *Salmonella* contamination in beef. The limit of detection for the rtPCR-IMS method was 1-5 CFU·25 g^{-1}. No PCR products were obtained from the non-inoculated samples.

Sensitivity, specificity and accuracy

Table 3 summarizes the agreements (PAs and NAs), deviations (PDs and NDs) and relative validation parameters of the rtPCR-IMS assay for the detection of *Salmonella* in the beef samples. The detection rate of *Salmonella* in presumptive samples (n= 60) by the rtPCR-IMS (21.6 %) and NOM-114-SSA1-1994 (20.0 %) analyses was not sufficient to calculate the validation parameters.

In the 60 artificially contaminated beef samples (1-5 CFU·25 mL^{-1}), the rtPCR-IMS and NOM-114-SSA1-1994 assays detected *Salmonella* in 56 (93.3 %) and 59 (98.3 %) times, respectively. The methods coincided in the detection of *Salmonella* on 59 occasions: 56 PAs and 3 NAs. Only one ND (detected by NOM-114 but not by rtPCR-IMS) was obtained. The rtPCR-IMS had a sensitivity of 98.2 % (56/57), specificity of 100 % (3/3) and accuracy of 98.3 % (59/60). The concordance indices (k= 0.85 and χ^2= 1.0) and the discordant value (Y= 1) indicated that the rtPCR-IMS assay and the reference method (NOM-114-SSA1-1994) coincide in the statistical criteria (Table 3). All non-inoculated beef samples were negative for *Salmonella* by both methods.

Selectivity

The rtPCR-IMS and NOM-114-SSA1-1994 methods had 100 % exclusivity and 100 % inclusivity. None of the methods reported cross-reactions. Figure 1 shows the amplifications of the rtPCR-IMS method corresponding to the inclusivity and exclusivity tests.

Discussion

Salmonella represents a threat to public health and the food industry worldwide[5], and in Mexico it is no exception[19,20-25]. The results of this work show the high persistence of *Salmonella* in presumptive beef samples (20 to 21.6 %), as previously shown by some studies in Mexico[21-23]. Conventional microbiological methods serve as the basis for routine analysis in many food safety and public health laboratories due to the ease of use, reliability of the results, high sensitivity and specificity[8]. However, the analysis time (5 to 7 d) of the culture methods is observed as a limitation. The incorporation of rapid molecular methods for the detection of *Salmonella* in foods allows early intervention and makes possible the preventive protection of the consumer[10,11,18].

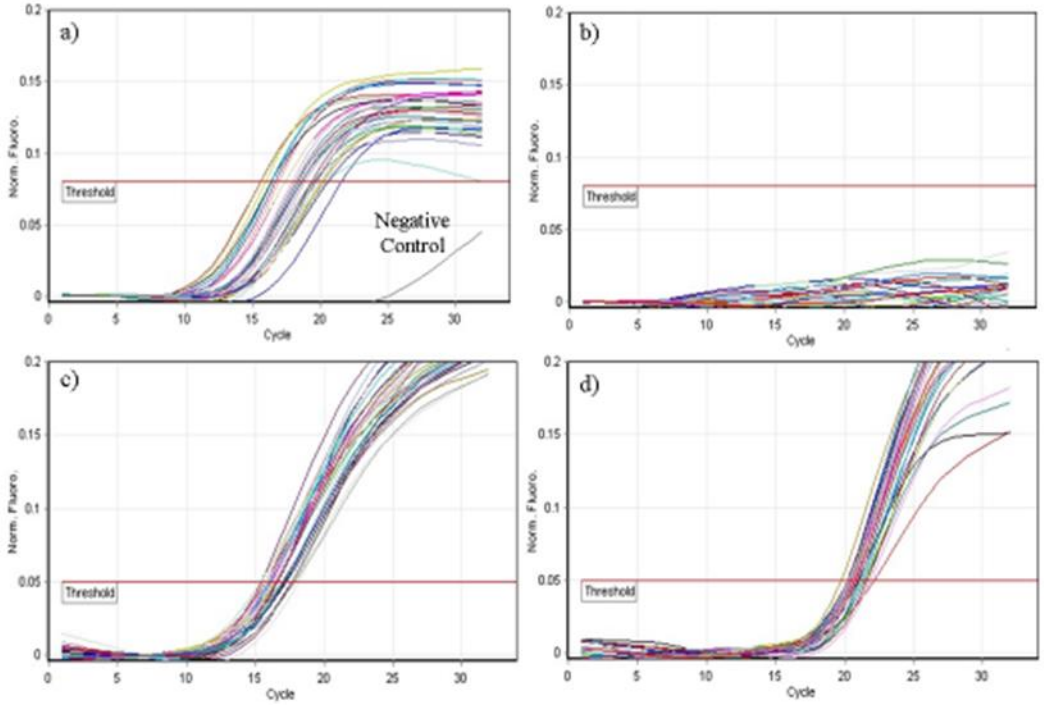
The limit of detection of the rtPCR-IMS and NOM-114-SSA1-1994 methods was 1-5 CFU·25 g^{-1} in beef, which corresponds to the lowest concentration evaluated. Of the total samples inoculated with 1-5 CFU·25 g^{-1}, the alternative method yielded three negative repetitions, of which only two were confirmed as truly negative by the reference method

(Table 2). The non-detection by the rtPCR-IMS method can be explained by the small amount (1-5 CFU·25 g^{-1}) of *Salmonella* in the non-selective enrichment, probabilistic inoculation considerations or the effect of enrichment[30]. Widjojoatmodjo *et al*[31] highlight the importance of the pre-enrichment prior to PCR detection to increase its sensitivity, given that most PCR methods require high concentrations of microorganisms for adequate detection. Similar to these results, Notzon *et al*[30] and O'Regan *et al*[32] reported a limit of detection of the rtPCR-IMS method in beef of 10-100 CFU·25 g^{-1}, and in chicken meat of 1-10 CFU·25 g^{-1}, respectively. These methods used an enrichment of 6 h[30] or 24 h[32] prior to detection by PCR. On the other hand, it is mentioned[10] that IMS is an alternative to avoid secondary enrichment, allowing the detection of 1-10 cells in an incubation period of 12-24 h. The limit of detection (1-5 CFU·25 g^{-1}) observed with rtPCR-IMS would allow alignment with the national regulatory requirements (NOM-213-SSA1-2002) that require zero tolerance of *Salmonella* in 25 g of raw beef[33].

The data obtained in the presumptive samples did not allow the determination of the validation parameters, because the calculations are made on a series of negative results obtained by the reference method, which cannot exceed twice the number of positive results as stipulated in the validation manual[29]. So, this study explains the validation of the rtPCR-IMS method for *Salmonella* analysis based on artificially contaminated beef samples. The degree of sensitivity, specificity, accuracy and agreement of the commercial method of rtPCR-IMS with the reference culture method validates its use for the analysis of *Salmonella* in beef, generating ideal results in a time of 24 h (Table 2). In addition, the rate of false positives (0 %) and false negatives (1.8 %) of the method are low. It is important to note that molecular methods do not replace culture techniques, since positive results must be confirmed by the reference method[34].

Some previous studies have exposed the concordance of rtPCR-IMS protocols with the reference culture methods for the detection of *Salmonella* in beef[30] and chicken[32,35], highlighting their high degree of sensitivity (94-100 %), specificity (80-94 %) and accuracy (89-100 %). These characteristics determined in the commercial method of rtPCR-IMS (Table 3) can be attributed to the fact that the immunomagnetic beads contain antigens that allow the microbe of interest to be concentrated from non-selective enrichments, reducing the analysis time[35]. In addition, the oligonucleotides used are able to detect different types of *Salmonella* strains (Figure 1).

Figure 1: Amplifications of the rtPCR-IMS method for the inclusivity and exclusivity test



Figures a and b show the amplification of the 30 strains of *Salmonella* (25 different serotypes) and the 30 strains of non-*Salmonella*, respectively. The position of the lines respect to the threshold indicates a positive (upper) or negative (lower) result. Figures c and d show the amplification of the internal controls of the reaction (ICA) of the strains included in the inclusivity (a) and exclusivity (b) assay. Those of ICA lines exceeded the threshold so it is considered a valid reaction.

The negative deviation (ND) observed between the methods (Table 2) can be explained by the fact that the culture method contains several enrichment stages that favor the recovery of damaged cells and the growth of the microorganism of interest compared to the rapid methods[12]. Also, the presence of *Proteus*, *E. coli*, *Klebsiella aerogenes* and *Enterobacter* in mucoid state in the enrichment broth can bind to the antibodies of the pearls, causing cross-reactions and preventing the detection of *Salmonella*[35]. It has been widely described that the type of matrix and its chemical components can affect the results of molecular methods[12-13]. As for the three negative agreements between the methods, it can be attributed to the extremely low amount of the microorganism after enrichment or that there were no cells in the initial inoculum.

The McNemar value (χ^2= 1.0, *P*= 0.317) obtained in this study meets the non-significance parameter (χ^2< 3.84)[29], and demonstrates that there is no difference between the rtPCR-IMS methods and the NOM-114-SSA1-1994 method for the detection of *Salmonella* in beef. In addition, the Kappa index reveals a high concordance (0.85 or 85 %) between the methods. Notzon *et al*[30] inferred the comparability of the alternative method of rtPCR-IMS with a

concordance of 85 % (k= 0.85) and 87 % (k= 0.87) for the detection of *Salmonella* in artificially and naturally contaminated beef, respectively.

A selective method is one that allows detecting the analyte being examined, and that can guarantee that the detected signal can only be a product of that specific analyte[29]. In this sense, the rtPCR-IMS method was able to discriminate against *Salmonella*, since it detected the 30 strains of *Salmonella* corresponding to 25 different serotypes even in the presence of other microorganisms, and not generate interference with the strains other than *Salmonella*. Mercanoglu & Griffiths[36] have reported that the combination of rtPCR and IMS for the detection of *Salmonella* have a selectivity of 100 %, attributing this property to the effect of the immunomagnetic beads and the selection of the oligonucleotides used.

Conclusions and implications

The results propose rtPCR-IMS as an efficient method for the rapid detection of *Salmonella* spp. in beef since it did not present differences with the reference method (NOM-114-SSA1-1994), providing the advantage of detecting the microorganism in a short time (24 h) and in a minimum concentration (1 CFU·25 g^{-1}) and without causing cross-reactions with other microorganisms found as natural microbiota in beef. The incorporation of this type of methods in the food industry and microbiological laboratories will allow a rapid response to ensure food safety and prevent the risk of diseases. Additionally, health authorities are alerted to the high incidence of *Salmonella* in raw beef in order to include controls along the food chain.

Acknowledgements

Thanks to QFB. Jesús Héctor Carrillo Yáñez, person in charge of the Laboratory of Molecular Biology and Functional Genomics, for his technical support, and to Fund S0007-2006-1. SAGARPA-CONACYT. Project No. 48134 for financing the development of the research.

Conflict of interest

The authors declare that they have no conflicts of interest, financial or otherwise.

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Table 2: Limit of detection of *Salmonella* in beef samples by rtPCR-IMS and NOM-114-SSA1-1994 methods

No. of replication	rtPCR-IMS					NOM-114-SSA1-1994				
	1-5 CFU	**6-10 CFU**	**11-15 CFU**	**16-30 CFU**	**0 CFU**	**1-5 CFU**	**6-10 CFU**	**11-15 CFU**	**16-30 CFU**	**0 CFU**
1	+/+/+	+/+/+	+/+/+	+/+/+	-/-/-	+/+/+	+/+/+	+/+/+	+/+/+	-/-/-
2	+/+/+	+/+/+	+/+/+	+/+/+	-/-/-	+/+/+	+/+/+	+/+/+	+/+/+	-/-/-
3	+/+/+	+/+/+	+/+/+	+/+/+	-/-/-	+/+/+	+/+/+	+/+/+	+/+/+	-/-/-
4	+/+/-*	+/+/+	+/+/+	+/+/+	-/-/-	+/+/+	+/+/+	+/+/+	+/+/+	-/-/-
5	+/+/+	+/+/+	+/+/+	+/+/+	-/-/-	+/+/ -	+/+/+	+/+/+	+/+/+	-/-/-
6	+/ -/ -	+/+/+	+/+/+	+/+/+	-/-/-	+/+/+	+/+/+	+/+/+	+/+/+	-/-/-

+ = *Salmonella* sp. was detected in the sample; – = *Salmonella* sp. was not detected in the sample.

*The assay was negative, but confirmation by the reference method was positive from the original enrichment.

Table 3: Comparison of rtPCR-IMS and NOM-114-SSA1-1994 methods for the detection of *Salmonella* in beef

Sample	Results*				Y	Sensitivity	False negative	Specificity	False positive	Accuracy	χ^2	Kappa
	PA	PD	NA	ND	No	(%)	(%)	(%)	(%)	(%)		
Presumptive	5	7	41	7	14	NDe	NDe	NDe	NDe	NDe	NDe	NDe
Contaminated**	56	0	3	1	1	98.2	1.8	100	0	98.3	1.0 (*P*=0.317)	0.85

*PA (positive agreement): Detection of the pathogen by both methods. NA (negative agreement): No detection of the pathogen by both methods. PD (positive deviation): Detection of the pathogen by the alternative method, but not by the reference method. ND (negative deviation): Detection of the pathogen by the reference method, but not by the alternative method. NDe (not determined).

**The results correspond to beef samples inoculated with a concentration of 1-5 CFU·25 ml^{-1} of *Salmonella* ATCC 35664.