Comparison of 2 culture methods and PCR assays for *Salmonella* detection in poultry feces

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ABSTRACT The present work compared 2 culture methods and the combinations of pre-enrichment and enrichment culture methods with PCR assays [buffered peptone water-PCR and tetrathionate-PCR or modified semisolid Rappaport-Vassiliadis (MSRV)-PCR] for motile and nonmotile *Salmonella* strain detection using artificially contaminated poultry feces. The specificity and positive predictive values were equal to one in both culture methods. Specificity and positive predictive values, accuracy, sensitivity, and negative predictive values were higher for motile than nonmotile *Salmonella* strains in culture methods. Only *Salmonella enterica* serovar Gallinarum was detected by the MSRV method with low accuracy, sensitivity, and negative predictive value. The detection level of motile strains was 2×10^0 to 22×10^2 cfu per 25 g for these methods, whereas it was 6.9×10^2 cfu per 25 g in culture methods for *Salmonella* Gallinarum. Extending the incubation time of the enrichment medium to 6 d in the TT method did not improve the isolation rates. In general, all selective plating media did not show any statistical differences in the parameters of performance studied. On the other hand, accuracy and sensitivity values were higher in MSRV-PCR and tetrathionate-PCR methods than in the buffered peptone water-PCR method. Specificity and positive predictive values were equal to one in most of the cases. In terms of detection limits, motile *Salmonella* strains were recovered from 5×10^0 cfu per 25 g in MSRV-PCR and tetrathionate-PCR methods, whereas the detection limit was better for nonmotile *Salmonella* in MSRV-PCR methods than in the tetrathionate-PCR method. Kappa coefficients showed that there was a very good agreement between tetrathionate and MSRV methods for motile *Salmonella* strains, whereas these methods did not show any concordance for nonmotile *Salmonella* strains. When buffered peptone water-PCR was compared with both tetrathionate-PCR and MSRV-PCR, agreement was poor for motile *Salmonella* strains and slight to fair for nonmotile *Salmonella* strains. The difference in isolation rate obtained with the methods used for motile and nonmotile *Salmonella* strains must be taken into account when a poultry fecal sample is considered negative for the presence of *Salmonella*.

Key words: *Salmonella*, poultry fecal sample, culture method, polymerase chain reaction

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INTRODUCTION

Salmonella enterica is a diverse bacterial species that is currently divided into 6 subspecies and more than 2,400 serotypes. Certain serotypes of *Salmonella* can be important bacterial pathogens in humans and animals, with different serotypes having varying levels of host specificity (Singer et al., 2009). Poultry can be infected with many different serovars of *Salmonella* (Betancor et al., 2010). Fowls are the specific host of *Salmonella enterica* serovar Gallinarum biovar Pullorum and Gallinarum, which cause pullorum disease and fowl typhoid, respectively.These avian-adapted serotypes (nonmotile) lack flagella and associated motility (Guard-Petter, 2001). Other serotypes with no specific host, such as Typhimurium and Enteritidis, may infect chickens and persist in the final poultry product, inducing (or not) clinical disease in these animals during rearing. Thus, the control of *Salmonella* in poultry flocks is crucial for poultry industry success (Gama et al., 2003).

Like many other bacterial diseases, *Salmonella* can be transmitted in several ways. The infected bird (reactor and carrier) is by far the most important means of perpetuation and spread of the organism (Shivaprasad, 2003). Colonization of the intestinal tract is the initial event in the infection sequence that can result in the invasion of reproductive tissues (Gast et al., 2005, 2007).

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Vertical transmission of *Salmonella* can result in the internal or external contamination of eggs. Eggshells can be contaminated with *Salmonella* by fecal contamination during oviposition. Horizontal transmission can be mediated by mechanisms including direct bird-to-bird contact, ingestion of contaminated feces or litter, contaminated water, or by personnel and equipment. Therefore, feces from infected birds are a source of bacteria, important in horizontal and vertical transmission (Shivaprasad, 2003; Gast et al., 2005; Gast, 2008). Recently, García et al. (2011) compared the *Salmonella* contamination on a laying hen farm with *Salmonella* presence in the hen eggs. They followed the annex D of ISO method 6579:2002 to isolate *Salmonella* Enteritidis, and they reported that feces were the most positive sample (92%), followed by eggshells (34%) and cloacal swabs (4%), whereas no *Salmonella* spp. were found in the egg contents.

Routinely used methods for isolating and identifying *Salmonella* rely on pre-enrichment in nonselective media, selective enrichment, plating in selective and diferential media, and biochemical and serological identification. No one method has superiority over another, and the sensitivity and specificity of the method depends on the sample type as well as the isolation conditions (Rybolt et al., 2004). A wide range of culture methods and PCR assays are available, and several studies had been developed to test their ability to detect *Salmonella* in different matrices (Rybolt et al., 2004; Myint et al., 2006; Eriksson and Aspan, 2007; Love and Rostagno, 2008; Singer et al., 2009). Most of the studies concentrate on isolation and detection of motile serotypes because they represent zoonotic disease but do not take into account nonmotile *Salmonella* strains. Therefore, the present work was conducted to compare 2 culture methods and combinations of PCR with them to know their ability to detect motile and nonmotile *Salmonella* strains in artificially contaminated poultry fecal samples. Furthermore, the accuracy (**AC**), sensitivity (**Se**), specificity (**SP**), positive predictive value (**PPV**), and negative predictive value (**NPV**) of each method and the agreement among methods were investigated.

MATERIALS AND METHODS

Fecal Samples

Poultry fecal samples were provided by laying-hen farms from the state of Entre Rios, Argentina. Each sample was analyzed by the tetrathionate (**TT**) method, described below, before carrying-out assays to ensure the absence of *Salmonella* spp. This method was used in this case because it is commonly used in our laboratory. Furthermore, total bacteria and *Enterobacteriaceae* counts of fecal samples were determinated in tryptic soy agar (Acumedia, Lansing, MI) and Mac Conkey agar (Acumedia), respectively.

Salmonella Strains and Culture

As summarized in Table 1, a total of 6 *Salmonella* strains was selected to assay, 4 of them were motile *Salmonella* (*Salmonella* Enteritidis, Typhimurium, Coeln, and Orion) and the others were nonmotile *Salmonella* (*Salmonella* Gallinarum and Pullorum). The strains belong to American Type Culture Collection and the collection from the Poultry Health Laboratory of the Estación Experimental Agropecuaria Instituto Nacional de Tecnología Agropecuaria in Entre Rios, Argentina. Each *Salmonella* strain was activated from nutrient agar (Acumedia) and was grown for 24 h in tryptic soy broth (Merck, Darmstadt, Germany) at 37°C. Purity of cultures was confirmed by streaking onto Mac Conkey agar and tryptic soy agar. The number of viable microorganisms was estimated by the method of Miles and Misra (1938) and was expressed as cfu per milliliter. Cells were pelleted by centrifugation in a tabletop centrifuge at 302 × *g* for 15 min at room temperature (25 ± 2°C). The supernatant was discarded and the pellet cell was resuspended to the original volume (5 mL) with PBS (pH 7.4).

Preparation of Salmonella spp. Inocula in Poultry Fecal Samples

Twenty-five grams of a *Salmonella*-free poultry fecal sample was introduced into a sterile plastic bag. Each *Salmonella* strain was grown as described above. After that, serial dilutions were made in peptone water (0.1%) to inoculate between 2.2×10^0 and 1.8×10^8 cfu/25 g for motile *Salmonella* strains and between 6.9×10^0 and 8.4×10^7 cfu/25 g for nonmotile strains, respectively. All treatments were performed in triplicate, so 3 samples of each dose for each *Salmonella* strain were considered in the assays. Altogether 138 spiked samples were constructed in the study. For each trial set, 3 nonseeded samples were analyzed as negative controls.

Recovery of Salmonella spp. Strains from Poultry Fecal Samples

Figure 1 shows a flowchart diagram for the detection of *Salmonella* in poultry fecal samples by TT and modified semisolid Rappaport-Vassiliadis (**MSRV**) methods. *Salmonella*-free poultry fecal samples contaminated with different concentrations of *Salmonella* strains were pre-enriched in 225 mL of buffered peptone water (**BPW**; Merck, Darmstadt, Germany). The mixture was incubated at 35 ± 2°C for 24 h. One milliliter of incubated broth was transferred to 10 mL of tetrathionate broth base (Acumedia), in addition to 20 mL/L of iodine potassium iodide solution (6 g of iodine; 5 g of potassium iodide; 20 mL of demineralized water), brilliant green 0.1% (Sigma, Steinheim, Germany), and 40

Table 1. *Salmonella* strains used in the comparison of different methods to detect this bacterium in poultry fecal samples

Salmonella strain	Source
Salmonella Enteritidis ATCC 13076	American Type Culture Collection
Salmonella Typhimurium ATCC 13311	American Type Culture Collection
Salmonella Coeln CUB 21/08	Avian cloacal swab, EEA INTA Concepcion del Uruguay[1]
Salmonella Orion CUB 28/08	Avian cloacal swab, EEA INTA Concepcion del Uruguay
Salmonella Pullorum ATCC 13036	American Type Culture Collection
Salmonella Gallinarum CUB 55/10	Poultry, EEA INTA C. del Uruguay

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mg/mL of novobiocin (Sigma) and incubated at 35 ± 2°C for 6 d (TT method). At d 1 (TT1) and 6 (TT6), a loopful of tetrathionate broth was streaked onto xylose lysine desoxicholate agar (Oxoid, Basingtoke, UK), with or without tergitol 4 (4.6 mL/L, Sigma-Aldrich, St. Louis, MO), and EF-18 (Acumedia) agar and incubated at 35 ± 2°C for 24 h. On the other hand, 30 μL of incubated BPW culture were inoculated in MSRV medium (Acumedia) agar plates supplemented with 1 mL/L of a 2% novobiocin solution, which were incubated at 41.5 ± 1°C for 24 h and subsequently streaked onto the same selective media listed above (MSRV method). This last method was based on Draft Amendment 1 Annex D: Detection of *Salmonella* spp. in animal feces and in samples from the primary production stage, which is suggested as a new addendum to ISO 6579 (ISO, 2002). Colonies of presumptive *Salmonella* were inoculated onto triple-sugar iron agar (Acumedia) and lysine iron agar (Merck). Further confirmation was done based on *o*-nitrophenyl-β-galactoside tests and agglutination reaction with somatic (O) polivalent antisera (Difco, Beckton Dickinson, Sparks, MD).

DNA Extraction

For detection of *Salmonella* sp. from poultry fecal samples, 1 mL of bacterial cells were recovered from BPW pre-enrichment broth and TT enrichment broth. In addition, a loopful of cultures on MSRV was resuspended in 1 mL of sterile demineralized water (Figure 1). From each of these samples, DNA extraction was based on the protocol suggested by Perez et al. (2008).

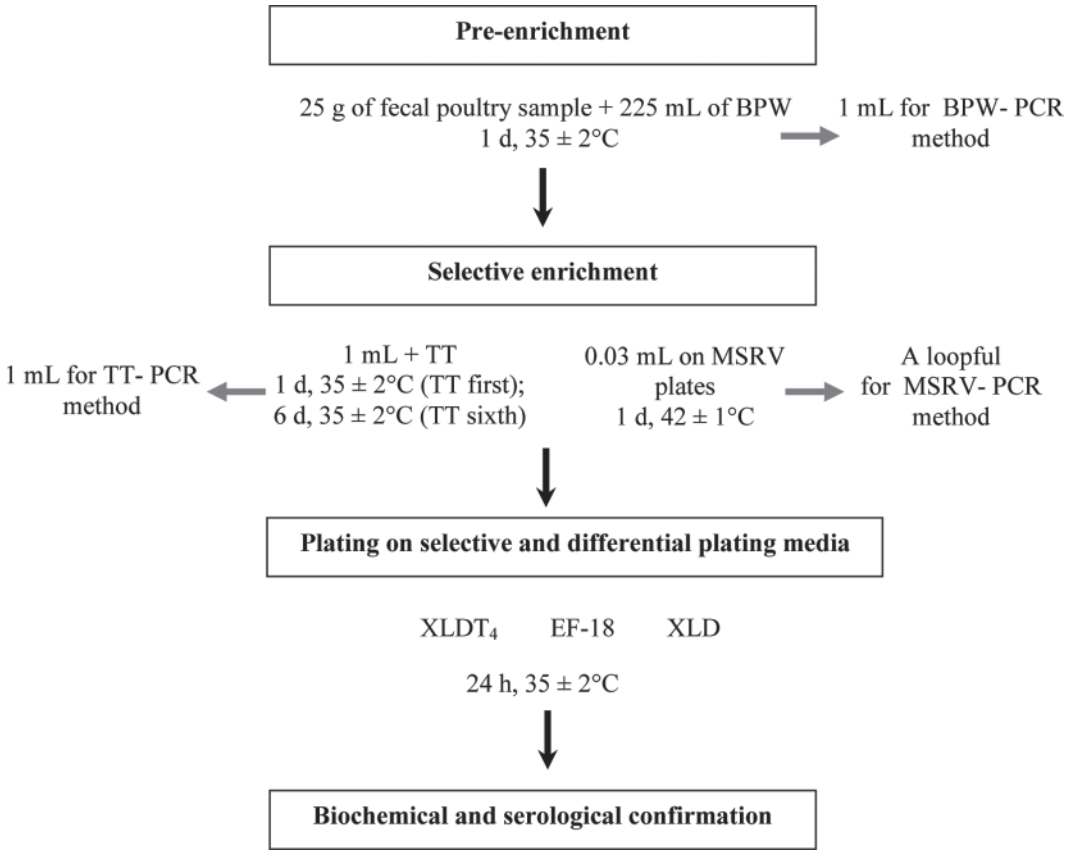

Figure 1. Flowchart diagram for the detection of *Salmonella* in poultry fecal samples by Tetrathionate broth (TT), Rappaport Vassiliadis medium semisolid modified (MSRV), and PCR methods. BPW = buffered peptone water; $XLDT_4$ = xylose lysine desoxicholate agar with tergitol 4; and XLD = xylose lysine desoxicholate agar.

Samples were centrifuged at 10,400 × *g* for 5 min at room temperature. The supernatant was carefully discarded, and the cell pellet was suspended in 1 mL of lysis buffer containing 0.2% Triton 100× (Sigma) on Tris-HCl (Bio-rad, Hercules, CA) and EDTA (pH 8.0; Biomedicals, Illkirch, France) and it was centrifuged at 13,400 × *g* for 6 min at room temperature. This step was repeated once. Then, the pellet was resuspended on 1 mL of sterile demineralized water and centrifuged at 13,400 × *g* for 1 min at room temperature. The step was repeated once. After that, the pellet was resuspended in 0.5 mL of sterile demineralized water and the microcentrifuge tubes were incubated at 100°C for 10 min on a hot block (Labnet, D1100, Labnet International Inc., Edison, NJ). The cellular debris was pelleted by centrifugation at 9,300 × *g* for 1 min and the clear supernatant fluid containing nucleic acids was fractioned in eppendorf tubes and conserved at −70°C until it was used in subsequent PCR assays.

PCR Assay

The DNA samples (5 μL) were amplified in an optimized 25-μL reaction mixture consisting of 0.25 μL of each 0.1 m*M* primer, 2.5 μL of buffer 1× (Fermentas Inc., Hanover, MD), 1.5 μL of 1.5 m*M* $MgCl_2$ (Fermentas), 0.5 μL of each 0.2 m*M* dNTP (Fermentas), 0.2 μL of 5 U/μL *Taq* DNA polymerase (Fermentas), and double-distilled water to 25 μL. The reaction mixture was incubated in a programmable DNA thermal cycler (model Mastercycler Gradient, Eppendorf, Germany). *Salmonella* genus-specific primers 139 and 141 (Operon Biotechnologies GmbH, Cologne, Germany) based on the *invA* gene of *Salmonella* were used in the PCR assay. They have the following nucleotide sequences: 5′-GTGAAATTATCGCCACGTTCGGGCAA-3′ and 5′-TCATCGCACCGTCAAAGGAACC-3′, respectively. A reagent blank containing all the components of the reaction mixture, with the exception of the template DNA (which was replaced by sterile distilled water), was included with every PCR assay. Furthermore, negative and positive DNA controls were included, which were prepared from *Citrobacter* sp. (isolated from poultry feed) and *Salmonella* sp., respectively. The cycling parameters used were initial denaturation at 95°C for 1 min followed by 38 cycles of amplification of 30 s at 95°C, 30 s at 64°C, and 30 s at 72°C. The reaction was completed by a final 3-min extension at 72°C. Then, PCR tubes were held at 4°C.

Detection of PCR Products

The PCR products were analyzed by gel electrophoresis. Ten microliters of each sample were loaded onto a 2.0% agarose gel in 0.5× Tris-borate-EDTA buffer at 120 V/cm for 1 h. The gel was stained with 0.5 μg/mL ethidium bromide, and electrophoresed products were visualized with a UV transilluminator (model M-20, UVP inc., Upland, CA). A 100-bp ladder (PB-L Productos Bio-Lógicos, Buenos Aires, Argentina) was used as a molecular weight marker.

Analysis of Performance Criteria

The detection limit of the methods was considered and it was defined as the lowest concentration (cfu/25 g) of the *Salmonella* strain inoculum that could be recovered. The AC, Se, SP, PPV, and NPV were calculated for each method. The assumption was that all nonspiked samples were negative for *Salmonella* and only those samples that were spiked with *Salmonella* were true positives (**TP**). Samples being positive on at least one selective agar plate (xylose lysine desoxicholate, xylose lysine desoxicholate plus tergitol 4, or EF-18) were considered positive. Based on this, the AC, Se, SP, PPV, and NPV rates were obtained by using the following definitions and equations:

Table 2. Sensitivity (Se), accuracy (AC), and negative predictive value (NPV) of tetrathionate (TT) and modified semisolid Rappaport-Vassiliadis (MSRV) methods for each *Salmonella* strain in artificially contaminated poultry fecal samples[1]

Salmonella enterica Serovar[2]	Se		AC		NPV	
	TT	MSRV	TT	MSRV	TT	MSRV
Typhimurium ATCC 13311	$0.75^{A,a}$ (0.43–0.93)	$0.92^{A,a}$ (0.68–0.69)	$0.80^{A,a}$ (0.46–0.91)	$0.93^{A,a}$ (0.74–0.99)	$0.50^{A,a}$ (0.01–0.81)	$0.75^{A,a}$ (0.28–0.94)
Enteritidis ATCC 13076	$0.83^{A,a}$ (0.54–0.96)	$1.00^{A,a}$ (0.75–0.99)	$0.87^{A,a}$ (0.65–0.95)	$1.00^{A,a}$ (0.79–0.99)	$0.60^{A,a}$ (0.22–0.88)	$1.00^{A,a}$ (0.79–0.99)
Coeln CUB 21/08	$0.92^{A,a}$ (0.73–0.99)	$0.96^{A,a}$ (0.79–0.99)	$0.93^{A,a}$ (0.76–0.97)	$0.96^{A,a}$ (0.81–0.99)	$0.60^{A,a}$ (0.22–0.88)	$0.75^{A,a}$ (0.28–0.94)
Orion CUB 28/08	$1.00^{A,a}$ (0.86–0.99)	$1.00^{A,a}$ (0.86–0.99)	$1.00^{A,a}$ (0.87–0.99)	$1.00^{A,a}$ (0.87–0.99)	$1.00^{B,a}$ (0.67–0.99)	$1.00^{A,a}$ (0.67–0.99)
Pullorum ATCC 13036	$0.04^{B,a}$ (0.00–0.20)	$0.00^{B,a}$ (0.04–0.28)	$0.15^{B,a}$ (0.06–0.32)	$0.11^{B,a}$ (0.04–0.28)	$0.12^{C,a}$ (0.04–0.29)	$0.11^{B,a}$ (0.04–0.28)
Gallinarum CUB 55/10	$0.00^{B,a}$ (0.00–0.13)	$0.63^{C,b}$ (0.42–0.78)	$0.11^{B,a}$ (0.04–0.28)	$0.67^{C,b}$ (0.47–0.81)	$0.11^{C,a}$ (0.04–0.28)	$0.25^{B,a}$ (0.09–0.51)

[a,b]Values with different superscripts in the same row are significantly different ($P < 0.05$).

[A–C]Values with different superscripts in the same column are significantly different ($P < 0.05$).

[1]Values in parentheses indicate a 95% confidence interval for the respective parameter.

[2]ATCC = American type culture collection; CUB = Concepcíon del Uruguay-Bacteriology.

- TP: *Salmonella* was detected in a sample where *Salmonella* had been added.
- True negative (**TN**): *Salmonella* was not detected in a sample where *Salmonella* had not been added.
- False positive (**FP**): *Salmonella* was detected in a sample where *Salmonella* had not been added.
- False negative (**FN**): *Salmonella* was not detected in a sample where *Salmonella* had been added.

Accuracy is a measure for the ability of a method to correctly classify samples containing *Salmonella* as positive for *Salmonell*a and samples not containing *Salmonella* as negative for *Salmonella*:

$$AC = \frac{TP + TN}{TP + TN + FP + FN}.$$

Sensitivity is a measure for the ability of a method to classify a sample containing *Salmonella* as positive for *Salmonella*:

$$SE = \frac{TP}{TP + FN}.$$

Specificity is a measure for the ability of a method to classify a sample not containing *Salmonella* as negative for *Salmonella*:

$$SP = \frac{TN}{TN + FP}.$$

Positive predictive value is a measure for the probability of the samples with positive test results for *Salmonella* that are correctly determined:

$$PPV = \frac{TP}{TP + FP}.$$

Negative predictive value is a measure for the probability of the samples with negative test results for *Salmonella* that are correctly determined:

$$NPV = \frac{TN}{TN + FN}.$$

On the other hand, agreement between culture- and PCR-based methods for the detection of *Salmonella* was evaluated by the use of the kappa coefficient (Martin, 1977). This was calculated to test how well the methods agreed in classifying the samples as positive or negative. The kappa statistic measures the agreement between 2 tests that is beyond chance (Dawson and Trapp, 2005). Kappa coefficients were summarized as excellent agreement (0.93 to 1.00), very good agreement (0.81 to 0.92), good agreement (0.61 to 0.80), fair agreement (0.41 to 0.60), slight agreement (0.21 to 0.40), poor agreement (0.01 to 0.20), and no agreement (<0.01).

Table 3. Sensitivity (Se), accuracy (AC), and specificity (SP) of buffered peptone water (BPW)-PCR, modified semisolid Rappaport-Vassiliadis (MSRV)-PCR, and tetrathionate (TT)-PCR methods for each *Salmonella* strain in artificially contaminated poultry fecal samples[1]

Salmonella enterica Serovar[2]	Se			AC			SP		
	BPW-PCR	MSRV-PCR	TT-PCR	BPW-PCR	MSRV-PCR	TT-PCR	BPW-PCR	MSRV-PCR	TT-PCR
Typhimurium ATCC 1331	$0.00^{A,a}$ (0.00–0.24)	$0.83^{A,b}$ (0.54–0.96)	$0.50^{A,b}$ (0.25–0.96)	$0.20^{A,a}$ (0.07–0.45)	$0.80^{A,b}$ (0.54–0.92)	$0.53^{A,b}$ (0.29–0.75)	$1.00^{A,a}$ (0.39–0.99)	$0.67^{A,a}$ (0.19–0.93)	$0.67^{A,a}$ (0.19–0.93)
Enteritidis ATCC 13076	$0.00^{A,a}$ (0.00–0.24)	$1.00^{A,b}$ (0.75–0.99)	$1.00^{B,b}$ (0.75–0.99)	$0.20^{A,a}$ (0.07–0.45)	$0.93^{A,b}$ (0.74–0.99)	$0.93^{B,b}$ (0.74–0.99)	$1.00^{A,a}$ (0.39–0.99)	$0.67^{A,a}$ (0.11–0.98)	$0.67^{A,a}$ (0.11–0.98)
Coeln CUB 21/08	$0.29^{B,a}$ (0.21–0.55)	$0.92^{A,b}$ (0.73–0.97)	$0.75^{A,b}$ (0.54–0.87)	$0.37^{AB,a}$ (0.21–0.55)	$0.93^{A,b}$ (0.76–0.97)	$0.78^{A,b}$ (0.59–0.89)	$1.00^{A,a}$ (0.67–0.99)	$1.00^{A,a}$ (0.67–0.99)	$1.00^{A,a}$ (0.67–0.99)
Orion CUB 28/08	$0.43^{B,a}$ (0.24–0.61)	$1.00^{A,b}$ (0.84–0.99)	$1.00^{B,b}$ (0.86–0.99)	$0.50^{B,a}$ (0.30–0.66)	$1.00^{A,b}$ (0.86–0.99)	$1.00^{B,b}$ (0.87–0.99)	$1.00^{A,a}$ (0.67–0.99)	$1.00^{A,a}$ (0.67–0.99)	$1.00^{A,a}$ (0.67–0.99)
Pullorum ATCC 13036	$0.00^{A,a}$ (0.00–0.13)	$0.29^{B,b}$ (0.14–0.49)	$0.13^{C,b}$ (0.04–0.31)	$0.11^{A,a}$ (0.04–0.28)	$0.37^{B,b}$ (0.21–0.55)	$0.22^{C,ab}$ (0.10–0.40)	$1.00^{A,a}$ (0.67–0.99)	$1.00^{A,a}$ (0.67–0.99)	$1.00^{A,a}$ (0.67–0.99)
Gallinarum CUB 55/10	$0.33^{B,a}$ (0.17–0.53)	$0.92^{A,b}$ (0.73–0.97)	$0.50^{C,a}$ (0.31–0.97)	$0.41^{B,a}$ (0.24–0.59)	$0.93^{A,b}$ (0.76–0.97)	$0.56^{A,a}$ (0.37–0.97)	$1.00^{A,a}$ (0.67–0.99)	$1.00^{A,a}$ (0.67–0.99)	$1.00^{A,a}$ (0.67–0.99)

[a,b]Values with different letters in the same row are significantly different ($P < 0.05$).
[A–C]Values with different numbers in the same column are significantly different ($P < 0.05$).
[1]Values in parentheses indicate a 95% confidence interval for the respective parameter.
[2]ATCC = American type culture collection; CUB = Concepción del Uruguay-Bacteriology.

Statistical Analysis

To compare the results of all assays, a hypothesis test for a difference of proportions was made. The Se, AC, PPV, and NPV of the test were reported at the shortest confident intervals, under the assumption that all values are equally probable. The calculations were performed using the Octave Program, developed by the Group of Numerical Method (GMN), from the National Technological University of Concepcion del Uruguay, Entre Rios, Argentina, Projects 25D041. The values reported define the boundaries of an interval that, with 95% certainty, contains the true value of AC, Se, PPV, or NPV. The results were only considered to be statistically different at $P < 0.05$. In relation to agreement, the 3 methods were treated as raters and the Z-test was used to test the statistical significance of kappa coefficients.

RESULTS

Fecal samples had an average of 6.6×10^7 cfu/g of total bacteria and 1.8×10^4 cfu/g of *Enterobacteriaceae*. In relation to the performance of the methods, SP was one for all strains studied for both bacteriological method assays. We found significant differences between motile and nonmotile *Salmonella* strains in reference to Se, AC, and NPV (Table 2). The Se was from 0.75 to 1 for MSRV and TT methods in motile S*almonella* strains, respectively, whereas it was from 0 to 0.63 for nonmotile *Salmonella* strains. On the other hand, AC was from 0.80 to 1 and from 0.11 to 0.67 for motile and nonmotile *Salmonella* strains, respectively. In reference to NPV, motile *Salmonella* strains showed values from 0.50 to 1.00 for the TT method, whereas in the MSRV method, they were from 0.75 to 1.00. Nonmotile S*almonella* strains showed NPV between 0.11 and 0.25 for both bacteriological methods. The PPV was 1 for motile *Salmonella* strains. Because of the abscense of TP and FP samples, PPV could not be calculated (0/0 = indeterminate) on MSRV and TT methods for *Salmonella* Pullorum and *Salmonella* Gallinarum, respectively.

In reference to PCR methods, in general, Se and AC were lower in the BPW-PCR method than in the other PCR methods (MSRV-PCR and TT-PCR) for most of the strains studied (Table 3). The SP was one for all strains, except for *Salmonella* Typhimurium and *Salmonella* Enteritidis, due to some FP samples. The NPV and PPV are shown in Table 4. The PPV was one for most of the *Salmonella* strains tested and it was indeterminate (0/0) in BPW-PCR for *Salmonella* Typhimurium, Enteritidis, and Pullorum. All strains showed NPV less than 0.21 for BPW-PCR. On the other hand, MSRV-PCR and TT-PCR showed NPV values from 0.25 to 1 and 0.13 to 0.60 for motile and nonmotile *Salmonella* strains, respectively.

When the detection limit of the MSRV and TT methods was studied (Table 5), all motile *Salmonella* strains were recovered in the lowest dilutions tested for all methods (2–18 $\times 10^0$ cfu/25 g), except *Salmonella* Typhimurium that showed a detection limit of 2.2×10^2 cfu/25 g of fecal sample in the TT method. In reference to nonmotile *Salmonella* strains, only *Salmonella* Pullorum could be recovered at the initial inoculum of 8.4×10^7 cfu/25 g in the TT method. On the other hand, *Salmonella* Gallinarum could be isolated from 6.9×10^2 cfu/25 g in the MSRV method. In relation to molecular methods, BPW-PCR only detected *Salmonella* Orion and *Salmonella* Coeln from 1.8×10^4 cfu/25 g and 4.6×10^3 cfu/25 g, respectively. Both MSRV-PCR and TT-PCR detected motile *Salmonella* strains at the lowest concentrations tested. For nonmotile *Salmonella* strains, *Salmonella* Gallinarum was detected from

Table 4. Positive predictive value (PPV) and negative predictive value (NPV) of buffered peptone water (BPW)-PCR, modified semisolid Rappaport-Vassiliadis (MSRV)-PCR, and tetrathionate (TT)-PCR methods for each *Salmonella* strain in artificially contaminated poultry fecal samples[1]

Salmonella enterica serovar[2]	PPV			NPV		
	BPW-PCR	MSRV-PCR	TT-PCR	BPW-PCR	MSRV-PCR	TT-PCR
Typhimurium ATCC 1331	IND[3]	$0.91^{A,a}$ (0.61–0.97)	$0.86^{A,a}$ (0.47–0.96)	$0.20^{A,a}$ (0.07–0.45)	$0.50^{A,a}$ (0.14–0.85)	$0.25^{A,a}$ (0.07–0.60)
Enteritidis ATCC 13076	IND	$0.92^{A,a}$ (0.66–0.98)	$0.92^{A,a}$ (0.66–0.98)	$0.20^{A,a}$ (0.07–0.45)	$1.00^{BC,b}$ (0.29–0.99)	$1.00^{B,b}$ (0.29–0.99)
Coeln CUB 21/08	$1.00^{A,a}$ (0.63–0.99)	$1.00^{A,a}$ (0.85–0.99)	$1.00^{A,a}$ (0.82–0.89)	$0.15^{A,a}$ (0.05–0.36)	$0.60^{AC,a}$ (0.22–0.88)	$0.33^{A,a}$ (0.22–0.88)
Orion CUB 28/08	$1.00^{A,a}$ (0.71–0.99)	$1.00^{A,a}$ (0.84–0.99)	$1.00^{A,a}$ (0.86–0.99)	$0.19^{A,a}$ (0.06–0.99)	$1.00^{C,b}$ (0.67–0.99)	$1.00^{B,b}$ (0.67–0.99)
Pullorum ATCC 13036	IND	$1.00^{A,a}$ (0.63–0.99)	$1.00^{A,a}$ (0.67–0.99)	$0.11^{A,a}$ (0.04–0.28)	$0.15^{A,a}$ (0.05–0.36)	$0.13^{A,a}$ (0.04–0.31)
Gallinarum CUB 55/10	$1.00^{A,a}$ (0.66–0.99)	$1.00^{A,a}$ (0.85–0.99)	$1.00^{A,a}$ (0.75–0.99)	$0.16^{A,a}$ (0.05–0.37)	$0.60^{A,a}$ (0.22–0.88)	$0.20^{A,a}$ (0.07–0.45)

[a,b]Values with different superscripts in the same row are significantly different ($P < 0.05$).

[A–C]Values with different superscripts in the same column are significantly different ($P < 0.05$).

[1]Values in parentheses indicate a 95% confidence interval for the respective parameter.

[2]ATCC = American type culture collection; CUB = Concepcíon del Uruguay-Bacteriology.

[3]IND = indeterminate (0/0).

Table 5. Results obtained when *Salmonella* strains were inoculated in poultry fecal samples and were isolated following tetrathionate (TT), modified semisolid Rappaport-Vassiliadis (MSRV), and PCR [buffered peptone water (BPW)-PCR, MSRV-PCR, TT-PCR] methods[1,2]

Salmonella enterica serovar[3]	cfu/25 g	Methodology to detect *Salmonella* from poultry fecal samples					
		TT1	TT6	MSRV	BPW-PCR	MSRV-PCR	TT-PCR
Typhimurium ATCC13311	0	0/3	0/3	0/3	0/3	0/3	0/3
	2.2×10^0	0/3	0/3	2/3	0/3	1/3	0/3
	2.2×10^2	3/3	3/3	3/3	0/3	3/3	2/3
	2.2×10^3	3/3	3/3	3/3	0/3	3/3	2/3
	2.2×10^4	3/3	3/3	3/3	0/3	3/3	2/3
Enteritidis ATCC 13076	0	0/3	0/3	0/3	0/3	1/3	1/3
	5.0×10^0	1/3	0/3	3/3	0/3	3/3	3/3
	4.6×10^1	3/3	3/3	3/3	0/3	3/3	3/3
	4.6×10^2	3/3	3/3	3/3	0/3	3/3	3/3
	4.6×10^3	3/3	3/3	3/3	0/3	3/3	3/3
Orion CUB 28/08	0	0/3	0/3	0/3	0/3	0/3	0/3
	1.8×10^1	3/3	3/3	3/3	0/3	3/3	3/3
	1.8×10^2	3/3	3/3	3/3	0/3	3/3	3/3
	1.8×10^3	3/3	3/3	3/3	0/3	3/3	3/3
	1.8×10^4	3/3	3/3	3/3	1/3	3/3	3/3
	1.8×10^5	3/3	3/3	3/3	2/3	3/3	3/3
	1.8×10^6	3/3	3/3	3/3	1/3	3/3	3/3
	1.8×10^7	3/3	3/3	3/3	3/3	3/3	3/3
	1.8×10^8	3/3	3/3	3/3	3/3	3/3	3/3
Coeln CUB 21/08	0	0/3	0/3	0/3	0/3	0/3	0/3
	5.0×10^0	3/3	3/3	3/3	0/3	2/3	1/3
	4.6×10^1	3/3	3/3	3/3	0/3	3/3	2/3
	4.6×10^2	3/3	3/3	3/3	0/3	3/3	3/3
	4.6×10^3	3/3	3/3	3/3	1/3	3/3	3/3
	4.6×10^4	3/3	3/3	3/3	1/3	3/3	2/3
	4.6×10^5	3/3	3/3	3/3	2/3	3/3	1/3
	4.6×10^6	3/3	3/3	3/3	1/3	3/3	3/3
	4.6×10^7	3/3	3/3	3/3	2/3	2/3	3/3
Pullorum ATCC 13036	0	0/3	0/3	0/3	0/3	0/3	0/3
	8.4×10^0	0/3	0/3	0/3	0/3	0/3	0/3
	8.4×10^1	0/3	0/3	0/3	0/3	0/3	0/3
	8.4×10^2	0/3	0/3	0/3	0/3	0/3	0/3
	8.4×10^3	0/3	0/3	0/3	0/3	0/3	0/3
	8.4×10^4	0/3	0/3	0/3	0/3	0/3	0/3
	8.4×10^5	0/3	0/3	0/3	0/3	2/3	0/3
	8.4×10^6	0/3	0/3	0/3	0/3	2/3	1/3
	8.4×10^7	1/3	0/3	0/3	0/3	3/3	2/3
Gallinarum CUB 51/10	0	0/3	0/3	0/3	0/3	0/3	0/3
	6.9×10^0	0/3	0/3	0/3	0/3	0/3	0/3
	6.9×10^1	0/3	0/3	0/3	0/3	0/3	0/3
	6.9×10^2	0/3	0/3	1/3	0/3	3/3	0/3
	6.9×10^3	0/3	0/3	3/3	0/3	3/3	1/3
	6.9×10^4	0/3	0/3	3/3	0/3	3/3	3/3
	6.9×10^5	0/3	0/3	3/3	2/3	3/3	3/3
	6.9×10^6	0/3	0/3	2/3	3/3	3/3	2/3
	6.9×10^7	0/3	0/3	3/3	3/3	3/3	3/3

[1]The TT method was separated considering the different time of streaking in the selective plating media at d 1 (TT1) or 6 (TT6) of incubation of the selective broth.

[2]The data are presented as the number of positive samples/number of total samples.

[3]ATCC = American type culture collection; CUB = Concepcíon del Uruguay-Bacteriology.

6.9×10^5, 6.9×10^2, and 6.9×10^3 for BPW-PCR, MSRV-PCR, and TT-PCR methods, respectively. The BPW-PCR did not detect *Salmonella* Pullorum in any concentration tested. This strain was detected from 8.4×10^5 cfu/25 g by MSRV-PCR method and 8.4×10^6 cfu/25 g by TT-PCR method.

For all *Salmonella* strains, the 3 selective plating media did not show any significant differences among them in terms of Se and AC in both bacteriological methods (Table 6). The 3 media performed high Se and AC in the MSRV method for motile strains, with values of greater than 0.82. However, these parameters had a value less than 0.22 for nonmotile *Salmonella* strains. In relation to the 2 times of incubation in the TT method (TT1 and TT6), Se and AC were similar in TT1 and TT6 for *Salmonella* strains studied. The Se was from 0.33 to 1.00 and the AC was from 0.47 to 1 for motile *Salmonella* strains. On the other hand, these parameters were between 0 and 0.15 for nonmotile *Salmonella* strains, with an Se of 0 in the 3 selective plating media used.

Table 7 shows PPV and NPV for each agar medium studied for the bacteriological methods in motile and nonmotile *Salmonella* strains, with details of the TT

method. The PPV was one for all motile *Salmonella* strains. On the other hand, PPV was indeterminate (0/0) in the nonmotile *Salmonella* strains. The NPV was between 0.27 and 1 for motile *Salmonella* strains and it was very low (0.11–0.23) for nonmotile *Salmonella* strains.

Analysis of the data using kappa coefficients showed that there was a very good agreement between the TT and MSRV methods for motile *Salmonella* strains, whereas these methods did not show concordance for nonmotile *Salmonella* strains (Table 8). In relation to PCR methods, when BPW-PCR was compared with both TT-PCR and MSRV-PCR, agreement was poor for motile *Salmonella* strains and slight to fair for nonmotile *Salmonella* strains. However, kappa coeficients showed fair agreement between TT-PCR and MSRV-PCR for both motile and nonmotile *Salmonella* strains.

DISCUSSION

We studied the performance of 2 culture and PCR methods for motile and nonmotile *Salmonella* detection in poultry feces, using artificially contaminated samples. Voogt et al. (2001) tested 1,022 fecal samples from poultry layer flocks. They found that 92% of those samples containing *Salmonella* could be detected by MSRV. This result is consistent with our study, where high Se and AC values for MSRV method for motile *Salmonella* strains studied were found. Similar results were found by Eriksson and Aspan (2007) in the same kind of sample for that media. For the TT method and motile *Salmonella* strains, the Se and AC were equally high as MSRV in our assay.

When performance of culture methods was compared between motile and nonmotile *Salmonella* strains, we

Table 6. Sensitivity (Se) and accuracy (AC) values of selective plating media in tetrathionate (TT) and modified semisolid Rappaport-Vassiliadis (MSRV) methods for *Salmonella* strains[1,2]

Salmonella enterica serovar[3]	Media[3]	Se			AC		
		TT1	TT6	MSRV	TT1	TT6	MSRV
Typhimurium ATCC 13311	XLD	$0.75^{A,a}$ (0.43–0.93)	$0.50^{A,a}$ (0.25–0.74)	0.92^{A} (0.68–0.99)	$0.80^{A,a}$ (0.43–0.93)	$0.60^{A,a}$ (0.43–0.93)	0.93^{A} (0.43–0.93)
	EF18	$0.50^{A,a}$ (0.25–0.24)	$0.42^{A,a}$ (0.19–0.68)	0.92^{A} (0.68–0.99)	$0.60^{A,a}$ (0.35–0.80)	$0.53^{A,a}$ (0.29–0.75)	0.93^{A} (0.74–0.99)
	$XLDT_4$	$0.42^{A,a}$ (0.19–0.68)	$0.33^{A,a}$ (0.13–0.61)	0.92^{A} (0.68–0.99)	$0.53^{A,a}$ (0.54–0.92)	$0.47^{A,a}$ (0.24–0.60)	0.93^{A} (0.74–0.99)
Enteritidis ATCC 13076	XLD	$0.83^{A,a}$ (0.54–0.96)	$0.67^{A,a}$ (0.38–0.86)	0.92^{A} (0.68–0.99)	$0.87^{A,a}$ (0.65–0.95)	$0.73^{A,a}$ (0.46–0.91)	0.93^{A} (0.74–0.99)
	EF18	$0.75^{A,a}$ (0.43–0.93)	$0.67^{A,a}$ (0.38–0.86)	0.92^{A} (0.68–0.99)	$0.80^{A,a}$ (0.54–0.92)	$0.73^{A,a}$ (0.46–0.91)	0.93^{A} (0.74–0.99)
	$XLDT_4$	$0.67^{A,a}$ (0.38–0.86)	$0.50^{A,a}$ (0.25–0.74)	0.83^{A} (0.54–0.96)	$0.73^{A,a}$ (0.46–0.91)	$0.60^{A,a}$ (0.5–0.80)	0.87^{A} (0.65–0.95)
Coeln CUB 21/08	XLD	$0.92^{A,a}$ (0.73–0.97)	$0.96^{A,a}$ (0.79–0.99)	0.96^{A} (0.79–0.99)	$0.93^{A,a}$ (0.76–0.97)	$0.96^{A,a}$ (0.81–0.99)	0.96^{A} (0.81–0.99)
	EF18	$0.83^{A,a}$ (0.64–0.95)	$0.88^{A,a}$ (0.68–0.95)	0.92^{A} (0.73–0.97)	$0.88^{A,a}$ (0.71–0.95)	$0.88^{A,a}$ (0.71–0.95)	0.92^{A} (0.76–0.99)
	$XLDT_4$	$0.91^{A,a}$ (0.71–0.97)	$0.92^{A,a}$ (0.73–0.97)	0.96^{A} (0.79–0.99)	$0.92^{A,a}$ (0.67–0.93)	$0.93^{A,a}$ (0.76–0.97)	0.96^{A} (0.81–0.99)
Orion CUB 28/08	XLD	$1.00^{A,a}$ (0.86–0.99)	$1.00^{A,a}$ (0.86–0.99)	1.00^{A} (0.86–0.99)	$1.00^{A,a}$ (0.87–0.99)	$1.00^{A,a}$ (0.87–0.99)	1.00^{A} (0.87–0.99)
	EF18	$0.96^{A,a}$ (0.79–0.99)	$1.00^{A,a}$ (0.86–0.99)	1.00^{A} (0.86–0.99)	$0.96^{A,a}$ (0.81–0.99)	$1.00^{A,a}$ (0.87–0.99)	1.00^{A} (0.87–0.99)
	$XLDT_4$	$1.00^{A,a}$ (0.86–0.99)	$1.00^{A,a}$ (0.86–0.99)	1.00^{A} (0.86–0.99)	$1.00^{A,a}$ (0.87–0.99)	$1.00^{A,a}$ (0.87–0.99)	1.00^{A} (0.87–0.99)
Pullorum ATCC 13036	XLD	$0.00^{A,a}$ (0.00–0.13)	$0.00^{A,a}$ (0.00–0.13)	0.00^{A} (0.00–0.13)	$0.11^{A,a}$ (0.04–0.28)	$0.11^{A,a}$ (0.04–0.28)	0.11^{A} (0.04–0.28)
	EF18	$0.00^{A,a}$ (0.00–0.13)	$0.00^{A,a}$ (0.00–0.13)	0.00^{A} (0.00–0.13)	$0.11^{A,a}$ (0.04–0.28)	$0.11^{A,a}$ (0.04–0.28)	0.11^{A} (0.04–0.28)
	$XLDT_4$	$0.00^{A,a}$ (0.00–0.13)	$0.00^{A,a}$ (0.00–0.13)	0.00^{A} (0.00–0.13)	$0.15^{A,a}$ (0.06–0.32)	$0.15^{A,a}$ (0.06–0.32)	0.15^{A} (0.06–0.32)
Gallinarum CUB 55/10	XLD	$0.00^{A,a}$ (0.00–0.13)	$0.00^{A,a}$ (0.00–0.13)	0.58^{A} (0.38–0.75)	$0.11^{A,a}$ (0.04–0.28)	$0.11^{A,a}$ (0.04–0.28)	0.63^{A} (0.44–0.78)
	EF18	$0.00^{A,a}$ (0.00–0.13)	$0.00^{A,a}$ (0.00–0.13)	0.29^{B} (0.14–0.75)	$0.11^{A,a}$ (0.04–0.28)	$0.11^{A,a}$ (0.04–0.28)	0.37^{B} (0.21–0.55)
	$XLDT_4$	$0.00^{A,a}$ (0.00–0.13)	$0.00^{A,a}$ (0.00–0.13)	0.29^{B} (0.14–0.49)	$0.11^{A,a}$ (0.04–0.28)	$0.11^{A,a}$ (0.04–0.28)	0.37^{B} (0.21–0.55)

[a,b]Values with different superscripts in the same row are significantly different ($P < 0.05$).

[A,B]Values with different superscripts in the same column are significantly different ($P < 0.05$).

[1]The TT method was separated considering the different time of streaking in the selective plating media at d 1 (TT1) or 6 (TT6) of incubation of the selective broth.

[2]Values in parentheses indicate a 95% confidence interval for the respective parameter.

[3]ATCC = American type culture collection; CUB = Concepcíon del Uruguay-Bacteriology.

[4]XLD = xylose lysine desoxicholate agar; and $XLDT_4$ = xylose lysine desoxicholate agar plus 4.6 mL/L of tergitol 4.

Table 7. Positive predictive value (PPV) and negative predictive value (NPV) of selective plating media in tetrathionate (TT) and modified semisolid Rappaport-Vassiliadis (MSRV) methods for *Salmonella* strains[1,2]

Salmonella enterica serovar[3]	Media[4]	PPV			NPV		
		TT1	TT6	MSRV	TT1	TT6	MSRV
Typhimurium ATCC 13311	XLD	1.00 [1 a] (0.89–0.99)	1.00 [1 a] (0.89–0.99)	1.00 [1] (0.91–0.99)	0.50 [1 a] (0.43–0.93)	0.33 [1 a] (0.43–0.93)	0.75 [1] (0.43–0.93)
	EF18	1.00 [1 a] (0.59–0.99)	1.00 [1 a] (0.59–0.99)	1.00 [1 a] (0.91–0.99)	0.33 [1 a] (0.12–0.65)	0.30 [1 a] (0.10–0.60)	0.75 [1] (0.28–0.24)
	$XLDT_4$	1.00 [1 a] (0.54–0.99)	1.00 [1 a] (0.54–0.99)	1.00 [1 a] (0.91–0.99)	0.30 [1 a] (0.10–0.60)	0.27 [1 a] (0.09–0.57)	0.75 [1] (0.28–0.94)
Enteritidis ATCC 13076	XLD	1.00 [1 a] (0.71–0.99)	1.00 [1 a] (0.66–0.99)	1.00 [1] (0.91–0.99)	0.60 [1 a] (0.22–0.88)	0.43 [1 a] (0.09–0.53)	0.75 [1] (0.11–0.98)
	EF18	1.00 [1 a] (0.71–0.99)	1.00 [1 a] (0.66–0.99)	1.00 [1] (0.91–0.99)	0.50 [1 a] (0.01–0.81)	0.43 [1 a] (0.09–0.53)	0.75 [1] (0.11–0.98)
	$XLDT_4$	1.00 [1 a] (0.66–0.99)	1.00 [1 a] (0.59–0.99)	1.00 [1] (0.71–0.99)	0.43 [1 a] (0.09–0.53)	0.33 [1 a] (0.12–0.65)	0.60 [1] (0.22–0.88)
Coeln CUB 21/08	XLD	1.00 [1 a] (0.85–0.99)	1.00 [1 a] (0.85–0.99)	1.00 [1] (0.85–0.99)	0.60 [1 a] (0.22–0.88)	0.75 [1 a] (0.28–0.94)	0.75 [1] (0.28–0.94)
	EF18	1.00 [1 a] (0.83–0.99)	1.00 [1 a] (0.84–0.99)	1.00 [1] (0.85–0.99)	0.50 [1 a] (0.18–0.81)	0.50 [1 a] (0.18–0.81)	0.60 [1] (0.22–0.88)
	$XLDT_4$	1.00 [1 a] (0.83–0.99)	1.00 [1 a] (0.85–0.99)	1.00 [1] (0.85–0.99)	0.60 [1 a] (0.22–0.88)	0.60 [1 a] (0.22–0.88)	0.75 [1] (0.28–0.88)
Orion CUB 28/08	XLD	1.00 [1 a] (0.86–0.99)	1.00 [1 a] (0.86–0.99)	1.00 (0.86–0.99)	1.00 [1 a] (0.67–0.99)	1.00 [1 a] (0.67–0.99)	1.00 [1] (0.67–0.99)
	EF18	1.00 [1 a] (0.85–0.99)	1.00 [1 a] (0.86–0.99)	1.00 (0.86–0.99)	0.75 [1 a] (0.28–0.97)	1.00 [1 a] (0.67–0.99)	1.00 [1] (0.67–0.99)
	$XLDT_4$	1.00 [1 a] (0.85–0.99)	1.00 [1 a] (0.86–0.99)	1.00 (0.86–0.99)	1.00 [1 a] (0.67–0.99)	1.00 [1 a] (0.67–0.99)	1.00 [1] (0.67–0.99)
Pullorum ATCC 13036	XLD	IND[5]	IND	IND	0.11 [1 a] (0.04–0.31)	0.11 [1 a] (0.04–0.28)	0.11 [1] (0.04–0.28)
	EF18	IND	IND	IND	0.11 [1 a] (0.04–0.31)	0.11 [1 a] (0.04–0.28)	0.11 [1] (0.04–0.28)
	$XLDT_4$	IND	IND	IND	0.12 [1 a] (0.04–0.29)	0.11 [1 a] (0.04–0.28)	0.11 [1] (0.04–0.28)
Gallinarum CUB 55/10	XLD	IND	IND	IND	0.11 [1 a] (0.04–0.28)	0.11 [1 a] (0.04–0.28)	0.23 [1] (0.08–0.50)
	EF18	IND	IND	IND	0.11 [1 a] (0.04–0.28)	0.11 [1 a] (0.04–0.28)	0.15 [1] (0.05–0.36)
	$XLDT_4$	IND	IND	IND	0.11 [1 a] (0.04–0.28)	0.11 [1 a] (0.04–0.28)	0.15 [1] (0.05–0.36)

[1]The TT method was separated considering the different time of streaking in the selective plating media at d 1 (TT1) or 6 (TT6) of incubation of the selective broth.

[2]Values in parentheses indicate a 95% confidence interval for the respective parameter.None of the data were significant.

[3]ATCC = American type culture collection; CUB = Concepcíon del Uruguay-Bacteriology.

[4]XLD = xylose lysine desoxicholate agar; and $XLDT_4$ = xylose lysine desoxicholate agar plus 4.6 mL/L of tergitol 4.

[5]IND = indeterminate (0/0).

found a significance difference. The Draft Amendment 1 Annex D (ISO, 2002) reports that nonmotile *Salmonella* strains (Gallinarum and Pullorum) do not seem to survive long in environmental samples and will therefore rarely be detected in fecal samples, independent of the method. Besides, this amendment and Poppe et al. (2004) refer to MSRV as a less-appropriate selective enrichment media for the detection of nonmotile *Salmonellae*. However, we could recover *Salmonella* Gallinarum in the MSRV method from 6.9×10^2 cfu/25 g of poultry fecal samples. Furthermore, MSRV-PCR and TT-PCR methods could detect nonmotile *Salmonella* with a better detection limit than bacteriological methods.

Waltman et al. (1993) studied the optimum times for incubating enrichment cultures for isolating *Salmonella* from diagnostic and environmental samples. They found that inoculation of the enrichment broths onto plating media after 24 h of incubation followed by a 5-d delayed secondary enrichment made possible the detection of 96 to 98% of the *Salmonella*-positive samples, and it was the best combination of conditions. We incubated tetrathionate broth at 35 ± 2°C for 6 d (TT Method) and we did not find any difference in *Salmonella* isolation between TT1 and TT6.

On the other hand, Petersen (1997) reported that the combination of 2 media (EF-18 agar and modified brilliant green agar with lutensit) clearly would reduce the number of FN results, although with a little extra cost. They also obtained high sensitivity using EF-18 agar for fecal samples and suggested that this media is more specific for this kind of sample. We used 3 selective plating media in our assay, included EF-18 agar, and in general, there were not any significant differences among them.

The type of sample, and especially the composition of the background flora, is of considerable importance for the efficiency of a specific plating media. Growth

Table 8. Kappa coefficient values showing agreement between modified semisolid Rappaport-Vassiliadis (MSRV), tetrathionate (TT), and PCR methods for poultry fecal samples

Salmonella strain	Comparison	Kappa coefficient
Motile	TT/ MSRV	0.89*
	MSRV-PCR/ BPW-PCR	0.02*
	TT-PCR/ BPW-PCR	0.12*
	TT-PCR/ MSRV-PCR	0.59*
Nonmotile	TT/ MSRV	−0.03
	MSRV-PCR/ BPW-PCR	0.23*
	TT-PCR/ BPW-PCR	0.52*
	TT-PCR/ MSRV-PCR	0.47*

*Indicates that kappa is significantly nonzero ($P < 0.05$).

of non-*Salmonella* may disturb the reading of plates, because well-isolated colonies of *Salmonella* may not be obtained (Busse, 1995). Van Hoorebeke et al. (2009) and Huneau-Salaün et al. (2009) considered that fecal sampling underestimated the actual prevalence of *Salmonella* in laying hen flocks. We had a similar result with nonmotile *Salmonella* strains.

Berchieri et al. (1995) discussed that, unlike other *Salmonella* serotypes, Pullorum and Gallinarum are not excreted extensively in the feces. In fact, later, Berchieri et al. (2000) showed that excretion depended on the susceptibility of the fowl line to *Salmonella* infection. On the other hand, Proux et al. (2002) reported that if surveillance is based on bacteriologic analysis of feces and environmental swabs, it will not be easy to isolate *Salmonella* Pullorum and Gallinarum. They could only isolate these bacteria from the liver or spleen of an inoculated chicken. Feces and environmental swabs from the room of the inoculated chickens were never found to be contaminated.

It is known that feces contain large amounts of phenolic and metabolic compounds and polysaccharides that are inhibitory for PCR. Thus, sample treatment should be assesed before evaluating the primer selectivities on target and nontarget strains. Besides, the detection of S*almonella* at low levels by PCR methods requires an enrichment culture step for the multiplication of the cells before the PCR assay (Malorny and Hoofar, 2005). In our study, we followed a protocol developed by Perez et al. (2008) that consists of a simple technique of DNA extraction designed to avoid inhibition of PCR. Besides, we compared PCR methods with samples from pre-enrichment (BPW-PCR) and enrichment (MSRV-PCR and TT-PCR) media. Both MSRV-PCR and TT-PCR showed higher values of Se and AC than BPW-PCR. Carli et al. (2001) found that tetrationathe broth was not inhibitory to PCR assays, which is in agreement with our results. On the other hand, Eriksson and Aspan (2007) showed that a combination of MSRV media followed by a PCR test had near 100% of AC, Se, and SP. We obtained similar results to MSRV-PCR for most of the *Salmonella* strains. In this sense, it should be noted that incubation in an enrichment broth increases the number of viable organisms in the sample to allow detection by PCR (Carli et al., 2001).

The TT and MSRV methods are similar in terms of AC, Se, SP, PPV, and NPV for different *Salmonella* strains in poultry fecal samples. Although MSRV is considered better for motile *Salmonella*-strain isolation, our study shows that this medium can detect *Salmonella* Gallinarum from fecal samples. The use of the PCR method after the enrichment step improves the parameters described before in this matrix. The difference in isolation rate obtained with the methods used for motile and nonmotile *Salmonella* strains must be taken into account when a poultry fecal sample is considered negative for the presence of *Salmonella*.

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