# *Salmonella* detection in poultry meat and meat products by the Vitek immunodiagnostic assay system easy *Salmonella* method, a LightCycler polymerase chain reaction system, and the International Organization for Standardization method 6579

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**ABSTRACT** This study was conducted to evaluate the capability of the Vitek immunodiagnostic assay system easy *Salmonella* (VIDAS ESLM) method and a specific real-time PCR system (LightCycler, LCPCR) to complement the International Organization for Standardization Method 6579 (ISO) in detecting *Salmonella* from a total of 105 naturally contaminated samples comprised of poultry meat and poultry meat products. The detection limit of ISO and LCPCR was 9 cfu/mL for both poultry meat and poultry meat products, whereas that of VIDAS ESLM with both sample types was determined to be 90 cfu/mL. Twelve (33.33%), 11 (30.55%), and 18 (50.00%) out of 36 poultry meat samples were positive for *Salmonella* by ISO, VIDAS ESLM, and LCPCR, respectively. *Salmonella* detection rates from poultry meat products were 5.80% for ISO and 8.69% for LCPCR, whereas none of these products tested positive by VIDAS ESLM. In poultry meat samples, VIDAS ESLM and LCPCR detection results were in substantial agreement with ISO, with the relative accuracy, sensitivity, and specificity rates of 97.2, 91.7, and 100%, respectively, for VIDAS ESLM and 83.3, 100, and 75%, respectively, for LCPCR. This is the first report on the evaluation of both VIDAS ESLM and LCPCR to complement ISO for the rapid detection of *Salmonella* in poultry meat and meat products. We determined that both VIDAS ESLM and LCPCR have the potential to complement the ISO standard culture method in the rapid screening of *Salmonella* from naturally contaminated poultry meats. For the poultry meat products, VIDAS ESLM and LCPCR can be used for rapid primary screening, and they should be complemented absolutely by ISO. Although LCPCR can preferentially be used for initial screening poultry meat products, the results should definitely be confirmed by ISO. Also, the VIDAS ESLM did not seem to be a suitable method for detecting *Salmonella* in poultry meat products.

**Key words:** *Salmonella*, Vitek immunodiagnostic assay system, polymerase chain reaction, poultry meat, poultry meat product

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## INTRODUCTION

The presence of *Salmonella* in poultry meat and poultry meat products, primarily due to cross-contamination and undercooking (Luber, 2009), is of major concern to both developed and developing countries, because *Salmonella* causes food-borne disease in humans (Bell and Kyriakides, 2002; ISO, 2002; Chen et al., 2010).

The detection of this pathogen in poultry meat and its products, both at the production level and before consumption, can play a significant role in the prevention of food-borne salmonellosis. Different methods have been developed to reduce the time required for the detection of this pathogen, because standard culture methods, such as the International Organization for Standardization Method 6579 (**ISO**) and the United States Food and Drug Administration's Bacteriological Analytical Manual Chapter 5: *Salmonella* (**FDA**), require up to 5 d (including biochemical and serological confirmations; ISO, 2002; FDA, 2007) and are not efficient in the routine monitoring of large numbers of samples. In this context, rapid, accurate, and economical methods, which require less technical expertise in the detection of *Salmonella* in these types of foods, are crucial both for the industry and for laboratories reporting results to governmental authorities for taking legal actions.


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One alternative to these culture methods is the use of the Vitek immunodiagnostic assay (**VIDAS**; Biomérieux, Marcy L'Etoile, France), an automated enzyme-linked fluorescent assay-based system that allows for the accurate and rapid screening of large numbers of samples for the presence of *Salmonella* by the Vitek immunodiagnostic assay system *Salmonella* (**VIDAS SLM**) method (Yeh et al., 2002; Eriksson and Aspan, 2007). The VIDAS SLM method is particularly useful for detecting *Salmonella* from food matrices heavily contaminated with competitive background flora (Korsak et al., 2004). Although several studies in the current literature report the successful use of VIDAS SLM for detecting *Salmonella* in poultry meat (McMahon et al., 2004; Reiter et al., 2007; Ivic Kolevska and Kocic, 2009) and in poultry meat products (Smith et al., 2008), recent advancements to this method have resulted in a more current version of this system, known as the Vitek immunodiagnostic assay system easy *Salmonella* (**VIDAS ESLM**) method. This method, which has been validated by the Association Française de Normalization and certified by the AOAC, has reduced the detection time to 2 d by using a single selective enrichment in *Salmonella* Xpress 2 Broth (**SX2**) and by reducing the incubation periods both in nonselective and selective enrichments. Jasson et al. (2011) recently reported the successful use of VIDAS ESLM in a study where they used the method to detect low numbers of healthy and sublethally injured *Salmonella enterica* in chocolate.

In recent years, real-time PCR has been used for the rapid, reliable, and feasible detection of *Salmonella*, particularly from poultry meat and poultry meat products (Kawasaki et al., 2005; Fakhr et al., 2006; Patel et al., 2006; D'Aoust et al., 2007; Malorny et al., 2007; Eglezos et al., 2008; Krascsenicsová et al., 2008; Nde et al., 2008; O'Regan et al., 2008; Löfström et al., 2009; Suo et al., 2010). LightCycler PCR (**LCPCR**) is a specific real-time PCR system, comprised of a high throughput capillary air-thermo cycler, allowing rapid and reliable detection and quantification of the specific PCR product with probe-based technology and high sensitivity (Ellingson et al., 2004; Perelle et al., 2004; Bohaychuk et al., 2007). However, complementation of this PCR approach by standard culture is required for the elimination of possible false negatives and variable PCR results related to inhibitory substances within the process (Wilson, 1997) and for avoiding false-positive results due to the amplification of target DNA from dead/nonculturable/injured *Salmonella* cells in the sample (Knight et al., 1990).

In the literature, there are several studies on the detection of *Salmonella* by real-time PCR and VIDAS SLM from poultry meat (Uyttendaele et al., 2003; Cheng et al., 2009; Elizaquível et al., 2009), and there are also reports on the use of LCPCR in the detection of *Salmonella* from artificially contaminated poultry meats (Cheung et al., 2004), from naturally contaminated poultry carcasses (Bohaychuk et al., 2007), and from poultry meats (Eyigor et al., 2010). However, to our knowledge, there have been no studies designed to evaluate the efficiencies of both the VIDAS ESLM and LCPCR systems for the detection of *Salmonella* in potentially naturally contaminated poultry meat and poultry meat products. Therefore, the aim of this study was to determine the capability of these 2 systems to detect *Salmonella* from a total of 105 naturally contaminated poultry meat and poultry meat products and to evaluate these methods for potential use as rapid, primary screening tests to complement the ISO standard culture method.

## MATERIALS AND METHODS

### *Salmonella* Strains

*Salmonella enterica* ssp. *enterica* serovar Enteritidis (*S.* Enteritidis) 64K (M. Y. Popoff, Institut Pasteur, Paris Cedex 15, France) and *Salmonella enterica* ssp. *enterica* serovar Typhimurium NCTC 12416 (Refik Saydam, National Public Health Agency, Ankara, Turkey) were used as positive controls in the ISO, VIDAS ESLM, and LCPCR tests.

### Detection Limit of ISO and VIDAS ESLM with Artificially Contaminated Samples

***Determining Salmonella-Free Poultry Meat and Meat Product.*** To prepare artificially contaminated samples of poultry meat and meat product, adequate quantities of chicken meat and packages of schnitzel samples were purchased from a retail market, and were individually examined for the presence/absence of *Salmonella* by standard bacteriology as described in ISO 6579 (ISO, 2002) as follows. Briefly, 25 g of chicken meat/product sample was aseptically placed into a sterile stomacher bag with a filter that contained 225 mL of buffered peptone water (**BPW**; Biomeriéux, Marcy l'Etoile, France) and was incubated at 37°C for 18 h after homogenization by hand massage from the outer surface of the bag for 2 min. After pre-enrichment, 0.1 and 1 mL from the BPW culture were transferred into Rappaport Vassiliadis soy peptone broth (**RV**; CM0866, Oxoid, Ottowa, Canada) and into Muller-Kauffmann tetrathionate-novobiocin broth (**MK**; CM1048, Oxoid,), respectively, and these were incubated for 24 h for primary enrichment at 37°C and 41.5°C, respectively. After incubation, 20 μL of these cultures were plated onto Chrom ID Salmonella (43621, Biomeriéux) and xylose lysine deoxycholate agar (**XLD**; 278850, Becton Dickinson, Franklin Lakes, NJ). The absence of typical colonies for *Salmonella* on plates examined after incubation at 37°C for 24 h indicated that the samples were free from *Salmonella*.

***Calculation of S. Enteritidis 64K Number in Stock Culture.*** Serial and 2 parallel 10-fold dilutions of 18-h

RV primary enrichment culture of *S.* Enteritidis 64K stock was performed up to $10^{-9}$ with PBS and 100 μL from each dilution was plated onto XLD agar. After 24 h of incubation at 37°C, colonies were counted from the appropriate plates and the mean bacterial count in the stock culture was calculated as $9 \times 10^8$ cfu/mL.

***Preparation of Artificially Contaminated Samples.*** Ten different parts of the previously identified *Salmonella*-free poultry meat/product samples, each weighing 25 g, were placed into 10 individual sterile stomacher bags that contained 225 mL of BPW and were homogenized for 2 min. One-hundred microliters from serial 10-fold dilutions of the pure *S.* Enteritidis 64K culture was inoculated into the homogenates and were analyzed both with ISO and with VIDAS ESLM as follows. 1) For ISO, all cultures were incubated at 37°C for 18 h for pre-enrichment. Then, 0.1 and 1 mL from the pre-enrichment culture were transferred into RV and into MK, respectively. The RV and MK cultures were incubated for 24 h for primary enrichment at 37°C and 41.5°C, respectively. After incubation, 20-μL inocula from the cultures were plated onto XLD agar and incubated at 37°C for 24 h. 2) For VIDAS ESLM, after taking samples for ISO, pre-enrichment cultures were incubated for an additional 4 h at 37°C to complete the total incubation time of 22 h. Then, 0.1 mL of each pre-enrichment culture was transferred into 10 mL of SX2 and incubated at 41.5°C for 22 h for enrichment. Following this incubation, the SX2 broth cultures were stored at 4°C for selective plating, after taking 1 mL aliquots from each dilution sample (10 meat and 10 meat product) for LCPCR template preparation (which was stored at −20°C until DNA isolation) and 1.5 mL aliquots from each dilution sample for heat treatment at 100°C for 15 min in a block heater (FBD02DD, Techne Corp., Minneaspolis, MN). After cooling to room temperature, 500 μL from each heat-treated SX2 broth culture was placed into a VIDAS SLM strip (VIDAS Salmonella, 30702, Biomeriéux). Then, they were put into a miniVIDAS (Biomeriéux) instrument for 45 min in the VIDAS SLM solid-phase receptacle and analyzed according to the manufacturer's instructions. For the dilution samples giving a relative fluorescence value of ≥0.23 in VIDAS, a 20-μL inoculum from the SX2 cultures was plated onto XLD agar and incubated at 37°C for 24 h.

***Detection Limit Determination of ISO.*** Twenty microliters from each RV and MK primary enrichment culture was plated onto XLD agar and incubated at 37°C for 24 h. The growth observed corresponding to *Salmonella* count in the highest dilution plate was accepted as the detection limit of ISO.

***Detection Limit Determination of VIDAS ESLM.*** Twenty microliters from the SX2 primary enrichment culture was plated onto XLD agar and incubated at 37°C for 24 h. The growth observed corresponding to *Salmonella* count in the highest dilution plate was accepted as the detection limit of VIDAS ESLM.

## DNA Isolation from Artificially Contaminated Samples for LCPCR

One milliliter of SX2 primary culture sample dilutions was taken into sterile Eppendorf tubes and stored at −20°C, as indicated in the Preparation of Artificially Contaminated Samples section. The DNA from each sample was isolated from a 1-mL aliquot of the SX2 broth using the Foodproof sample preparation kit I (1.20473.0001, Biotecon Diagnostics Gmbh, Berlin, Germany). Concentrations and purity determinations of the template DNA were performed with a NanoDrop spectrophotometer (ND1000, Thermo Scientific, Waltham, MA).

## Detection Limit Determination of LCPCR with Artificially Contaminated Samples

Template DNA prepared from the sample dilutions as explained in the DNA Isolation from Artificially Contaminated Samples section were used in the LCPCR, which was performed using a Foodproof *Salmonella* detection kit (1.20453.0001, Biotecon). The positive result obtained from the highest dilution was accepted as the detection limit of LCPCR.

## Poultry Meat and Poultry Meat Product Samples

Thirty-six poultry meat samples (33 chicken meat: whole chicken, drumstick, boneless thigh, thigh, wing, and 3 turkey meat: neck and chops), 79 poultry meat product samples (69 chicken meat products: burger, cordon bleu, croquette, ham, nugget, salami, sausage, schnitzel, and soudjouk), and 10 turkey meat product samples (ham, cordon bleu, salami, and sausage) were randomly purchased from local food stores during 2009–2010, and they were transferred to the laboratory on ice. Samples in their original packages were individually repacked in polyethylene bags to prevent cross-contamination during purchase and transfer. The analysis of all samples was initiated immediately after transfer to the laboratory.

## Analysis of Samples by VIDAS ESLM and ISO

Twenty-five grams of the meat/product sample was aseptically placed into a sterile stomacher bag with a filter that contained 225 mL of BPW, and these samples were incubated at 37°C for 18 h (this was extended to 22 h for VIDAS ESLM) for pre-enrichment after homogenization by hand massage from the outer surface of the bag for 2 min. Next, the same sample was analyzed both with VIDAS ESLM and with ISO as follows. 1) For VIDAS ESLM, 0.1 mL of the 22-h BPW pre-enrichment culture was transferred into 10 mL of

SX2 and incubated at 41.5°C for 22 h for enrichment. Following this incubation, the SX2 broth culture was stored at 4°C until confirmation, after taking a 1 mL aliquot for LCPCR template preparation and 1.5 mL for heat treatment at 100°C for 15 min in a block heater (FBD02DD, Techne). After cooling to room temperature, 500 μL from the heat-treated SX2 broth culture was placed into a VIDAS SLM strip (VIDAS Salmonella, 30702, Biomeriéux) and then into a miniVIDAS (Biomeriéux) instrument for 45 min in the VIDAS SLM solid-phase receptacle, and then analyzed according to the manufacturer's instructions. A relative fluorescence value of ≥0.23 for a sample was considered a presumptive positive result. The results were expressed as presence or absence of *Salmonella* in 25 g of sample. For the presumptive positive samples, confirmation was performed by streaking 10 μL of the SX2 broth culture onto the selective agar plate Chrom ID *Salmonella*, indicated in the VIDAS ESLM protocol, and onto an alternative XLD plate that we selected. After incubation at 37°C for 24 h, 5 typical colonies were picked for further biochemical identification by API 20 E (20100, Biomeriéux) and serological identification using *Salmonella* group-specific antisera (Becton Dickinson). A culture from one of the selected *Salmonella* reference strains (indicated as *Salmonella* strains above) were used as a positive control in each VIDAS ESLM sample test run. 2) For ISO, 0.1 and 1 mL from 18-h BPW preenrichment culture were transferred into RV, and into MK, respectively, and these were incubated for 24 h for primary enrichment at 37°C and 41.5°C, respectively. Selective plating, biochemical identification, and serological identification were performed as indicated in the VIDAS ESLM section.

### DNA Isolation and LCPCR

The DNA from each sample was isolated from a 1-mL aliquot of the SX2 broth using the Foodproof sample preparation kit I (1.20473.0001, Biotecon). The isolated DNA was then used as a template in the LCPCR, which was performed using a Foodproof *Salmonella* detection kit (1.20453.0001, Biotecon) after concentration and purity determination of the template DNA with a NanoDrop spectrophotometer. The total PCR reaction volume was 20 μL and it was comprised of 5 μL of template DNA added into 15 μL of PCR mix: 13 μL of Foodproof *Salmonella* master mix (ready-to-use primer and hybridization probe mix), 1 μL of Foodproof *Salmonella* enzyme solution (FastStart *Taq* DNA polymerase and uracil-DNA glycosylase, heat labile, for prevention of carry-over contamination), and 1 μL of Foodproof *Salmonella* internal control. The Foodproof *Salmonella* control template DNA and DNA from one of the selected *Salmonella* reference strains, indicated above, were used as positive controls, and PCR-grade water was used as a negative control in the PCR. The amplification protocol included an initial denaturation step at 95°C for 10 min, 55 cycles of denaturation at 95°C for 0 s, annealing at 59°C for 30 s, and 5 s of primer extension at 72°C. Fluorescence values of the internal control and of each sample were automatically measured at 705/back 530 nm (channel F3/back-F1) and at 640/back 530 nm (channel F2/back-F1) at the end of each annealing step. Data analysis was automatically performed by the LightCycler software version 4.1.

### Statistical Analysis

Relative accuracy, sensitivity, and specificity of VIDAS ESLM and LCPCR versus the ISO reference method were calculated according to a protocol described in ISO 16140 (ISO, 2003) and were complemented with Cohen's kappa test to evaluate the significant similarities between the 1) ISO and VIDAS ESLM and 2) ISO and LCPCR results.

## RESULTS

The detection limit of ISO and LCPCR was 9 cfu/mL with both poultry meat and poultry meat product, whereas that of VIDAS ESLM with both sample types was determined to be 90 cfu/mL.

In total, 36 poultry meat samples were tested for *Salmonella* and 33.33% and 30.55% were found to be positive by ISO and VIDAS ESLM, respectively. All of the ISO-positive poultry meat samples except one were also positive by VIDAS ESLM (Table 1), showing almost perfect agreement between the 2 methods. The slightly lower *Salmonella* detection rate in poultry meats by VIDAS ESLM, as compared with ISO, resulted in one false negative in VIDAS ESLM, which did not have a substantial negative effect on the relative accuracy, sensitivity, and specificity rates of this method relative to ISO reference method, which were determined to be as high as 97.2, 91.7, and 100%, respectively (Table 2).

There was no *Salmonella* detected by VIDAS ESLM in the 69 poultry meat products we tested, whereas ISO detected *Salmonella* in 4 (5.80%) of these samples (Table 3). The 4 false-negative results of VIDAS ESLM, as compared with ISO, significantly reduced the sensitivity of this method for the detection of *Salmonella*, particularly in these types of products (Table 2).

Eighteen out of 36 (50.00%) poultry meat samples were found to be positive for *Salmonella* by LCPCR, whereas only 12 of these meats were found to harbor *Salmonella* by ISO (Table 1), indicating a substantial agreement between these methods for this sample type, with high sensitivity (Table 2).

For the poultry meat products, 4 and 6 samples were found to be positive for *Salmonella* by ISO and LCPCR, respectively. However, none of the ISO positive samples were positive by LCPCR (Table 3). This disagreement was due to false negatives and false positives by LCPCR in the poultry meat products, which substantially affected the sensitivity of this method relative to ISO (Table 2).

**Table 1.** Poultry meat results for the detection of *Salmonella* using the International Organization for Standardization method 6579 (ISO, 2002), the Vitek immunodiagnostic assay system easy *Salmonella* method (VIDAS ESLM), and a LightCycler PCR system (LCPCR)

| | Test result (no. of positives) | | | |
|---|---|---|---|---|
| | | VIDAS ESLM | | |
| Sample type (n) and ID | ISO 6579 | Presumptive | Confirmed | LCPCR |
| Chicken meat (33) | | | | |
| Whole chicken (1) | | | | |
| 9 | – | – | – | – |
| Drumstick (10) | | | | |
| 1, 3, 4, 5, 23, 26 | + (6) | + (6) | + (6) | + (6) |
| 6 | – | – | – | + (1) |
| 2, 16, 21 | – | – | – | – |
| Boneless thigh (6) | | | | |
| 8, 13, 24, 25 | + (4) | + (4) | + (4) | + (4) |
| 7 | – | + (1) | – | + (1) |
| 17 | – | – | – | – |
| Thigh (5) | | | | |
| 15 | – | – | – | + (1) |
| 30, 31, 32, 33 | – | – | – | – |
| Wing (11) | | | | |
| 29 | + (1) | + (1) | + (1) | + (1) |
| 22 | + (1) | + (1) | – | + (1) |
| 28 | – | + (1) | – | + (1) |
| 10, 14 | – | – | – | + (2) |
| 11, 12, 18, 19, 20, 27 | – | – | – | – |
| Subtotal positive/tested (%) | 12/33 (36.36) | 14/33 (42.42) | 11/33 (33.33) | 18/33 (54.54) |
| Turkey meat (3) | | | | |
| Neck (2) | | | | |
| 34, 35 | – | – | – | – |
| Chop (1) | | | | |
| 36 | – | – | – | – |
| Subtotal positive/tested (%) | 0/3 (0.00) | 0/3 (0.00) | 0/3 (0.00) | 0/3 (0.00) |
| Total positive/tested (%) | 12/36 (33.33) | 14/36 (38.88) | 11/36 (30.55) | 18/36 (50.00) |

## DISCUSSION

The aim of this study was to determine the capability of 2 rapid detection systems, VIDAS ESLM and LCPCR, to detect *Salmonella* in a total of 105 naturally contaminated poultry meat and poultry meat products, to assess their potential use as rapid, primary screening tests to complement the ISO standard culture method.

The results of VIDAS ESLM in detecting *Salmonella* from poultry meat samples in this study were similar to the results of ISO, whereas we determined the detection limit of ISO with artificially contaminated samples to be 10 times higher than that of VIDAS ESLM. One explanation for this case can be the relatively high number (>90 cfu/mL, which is higher than the detection limit of both methods) of *Salmonella* in the naturally contaminated poultry meat samples, which enabled the detection of this pathogen by both methods. Several previous studies compared VIDAS and various culture methods for the detection of *Salmonella* in poultry meats, and these studies observed similar results to ours as follows: De Medici et al. (1998) indicated full agreement between VIDAS immuno-concentration *Salmonella* and ISO; Reiter et al. (2007) found an even higher percentage of *Salmonella*-positive samples by VIDAS SLM versus the FDA method; McMahon et al. (2004) indicated that VIDAS SLM performed equally well as the FDA method; and Uyttendaele et al. (2003)

**Table 2.** Relative accuracy, sensitivity, and specificity results of the Vitek immunodiagnostic assay system easy *Salmonella* method (VIDAS ESLM) and a LightCycler PCR system (LCPCR)

| | Reference method | | Alternative method | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sample type (n) | Positive | Negative | False $_{negative}$ | False $_{positive}$ | Accuracy (%) | Sensitivity (%) | Specificity (%) | Cohen's kappa value |
| Poultry meat (36) | ISO 6579 | | VIDAS ESLM | | | | | |
| | 11 | 24 | 1 | 0 | 97.2 | 91.7 | 100 | 0.93 |
| | ISO 6579 | | LCPCR | | | | | |
| | 12 | 18 | 0 | 6 | 83.3 | 100 | 75 | 0.66 |
| Poultry meat product (69) | ISO 6579 | | VIDAS ESLM | | | | | |
| | 0 | 65 | 4 | 0 | 94.2 | 0 | 100 | 0 |
| | ISO 6579 | | LCPCR | | | | | |
| | 0 | 59 | 4 | 6 | 85.5 | 0 | 90.8 | −0.07 |

**Table 3.** Results for the detection of *Salmonella* in poultry meat products using the International Organization for Standardization method 6579 (ISO, 2002), the Vitek immunodiagnostic assay system easy *Salmonella* method (VIDAS ESLM), and a LightCycler PCR system (LCPCR)

| | Test result (no. of positives) | | | |
|---|---|---|---|---|
| | | VIDAS ESLM | | |
| Sample type (n) and ID | ISO 6579 | Presumptive | Confirmed | LCPCR |
| Chicken meat product (59) | | | | |
| Burger (12) | | | | |
| 15, 36 | + (2) | – | – | – |
| 37 | – | – | – | + (1) |
| 13, 14, 38, 39, 49, 56, 57, 58, 69 | – | – | – | – |
| Cordon bleu (1) | | | | |
| 55 | – | – | – | – |
| Croquette (6) | | | | |
| 1, 47 | – | – | – | + (2) |
| 2, 3, 4, 53 | – | – | – | – |
| Ham (1) | | | | |
| 30 | – | – | – | – |
| Nugget (9) | | | | |
| 42 | + (1) | – | – | – |
| 41 | – | – | – | + (1) |
| 9, 10, 11, 12, 40, 50, 68 | – | – | – | – |
| Salami (10) | | | | |
| 29 | – | – | – | + (1) |
| 18, 19, 20, 21, 22, 31, 32, 51, 62 | – | – | – | – |
| Sausage (6) | | | | |
| 17 | – | + (1) | – | – |
| 16, 33, 34, 35, 59 | – | – | – | – |
| Schnitzel (11) | | | | |
| 5, 6, 7, 8, 43, 44, 45, 48, 54, 66, 67 | – | – | – | – |
| Soudjouk (3) | | | | |
| 60, 61, 64 | – | – | – | – |
| Subtotal positive/tested (%) | 3/59 (5.08) | 1/59 (1.69) | 0/59 (0.00) | 5/59 (8.47) |
| Turkey meat product (10) | | | | |
| Ham (1) | | | | |
| 24 | + (1) | – | – | – |
| Cordon bleu (1) | | | | |
| 63 | – | – | – | – |
| Salami (6) | | | | |
| 23, 26, 46, 52, 65 | – | – | – | – |
| 25 | – | – | – | + (1) |
| Sausage (2) | | | | |
| 27, 28 | – | – | – | – |
| Subtotal positive/tested (%) | 1/10 (10.00) | 0/10 (0.00) | 0/10 (0.00) | 1/10 (10.00) |
| Total positive/tested (%) | 4/69 (5.80) | 1/69 (1.44) | 0/69 (0.00) | 6/69 (8.69) |

found a 95% agreement between VIDAS SLM and DIASALM, as a standard culture method.

For the poultry meat products, *Salmonella* was only detected by the ISO method, resulting in a reduced sensitivity of VIDAS ESLM compared with ISO for these types of products. One explanation for this can be the presence of *Salmonella* counts in some samples below the detection limit of VIDAS ESLM (<90 cfu/mL) but within the detection limit of ISO (9 to <90 cfu/mL). Another possible explanation for this difference is that there may have been heat-injured or freeze-stressed cells in the samples we analyzed, which would not have been sufficiently recovered by the enrichment steps of the VIDAS ESLM method. The severe heat applications, such as frying, cooking, and cooling (bringing the core temperature of the product to a maximum 72°C and minimum 4°C) in the processing stages of these poultry meat products could have sublethally injured the *Salmonella* cells in these products. To reduce the number of false negatives in poultry meat products, further studies, such as revisions in the specific enrichment protocol, are required to improve this alternative method. There have been several reports describing false negatives by VIDAS, although not for the same sample types, and these reports suggest that severely freeze-stressed bacteria account for the false negatives (Uyttendaele et al., 2003; Fakhr et al., 2006; Jasson et al., 2011).

The LCPCR method detected *Salmonella* with high sensitivity in the poultry meat samples, which indicated a substantial agreement with the ISO method, as observed in the compatibility of the detection limits in both methods. Similar high sensitivity results with LCPCR from red meat (Ellingson et al., 2004), artificially contaminated raw chicken meats (Cheung et al., 2004), naturally contaminated poultry carcasses (Bohaychuk et al., 2007), and poultry meats (Eyigor et al., 2010) have previously been reported, supporting our

findings in this study. However, sensitivity of LCPCR was relatively lower than ISO in detecting *Salmonella* from the poultry meat products, which led to disagreements between the 2 methods. The false positives in our LCPCR results, which had previously been observed in other poultry meat-related studies, including studies using real-time PCR (Fakhr et al., 2006; Nde et al., 2008; O'Regan et al., 2008), and specifically, with LCPCR by Bohaychuk et al. (2007) and Eyigor et al. (2010), could possibly be related to 2 factors. First, high numbers of nonculturable or dead *Salmonella* cells in the sample(s) could have resulted in positive results by PCR but negative results in the culture-based method or culture-confirmed method used in VIDAS ESLM. Second, insufficient recovery of sublethally injured cells, despite the optimized specific enrichment steps in the culture-based or culture-confirmed method (VIDAS ESLM), which could have resulted in positive detection by PCR but could lead to negative results in the culture-based or culture-confirmed method (VIDAS ESLM). The sublethally injured cells could in fact be the false negative of the culture method but was misreported as the false positive of PCR (Eyigor et al., 2002; Bohaychuk et al., 2007). The false negatives in LCPCR for poultry meat product samples, as were detected by VIDAS ESLM, could be the result of insufficient outgrowth of thermally injured (cooking/cooling) *Salmonella* cells in the restricted enrichment period or environment. This type of a false-negative result by real-time PCR had been reported in poultry carcasses and parts by Uyttendaele et al. (2003), who related this finding to the increased lag time of freeze-stressed cells during enrichment.

Although not included as a primary aim of this study, our results demonstrated a considerably higher prevalence of *Salmonella* in chicken meat samples. These rates were comparably lower than those reported recently by Ivic Kolevska and Kocic (2009), Eyigor et al. (2010), and Suo et al. (2010) but higher than those reported by D'Aoust et al. (2007), O'Regan et al. (2008), and Patel and Bhagwat (2008). The absence of *Salmonella* that we observed in the turkey meat samples was also observed by Patel and Bhagwat (2008), although at much higher rates, ranging between 24 to 67%, were previously reported by others (Fakhr et al., 2006; D'Aoust et al., 2007; Nde et al., 2008; Eyigor et al., 2010; Suo et al., 2010). Moreover, much higher *Salmonella* detection rates from raw and frozen chicken meat products were reported by Bucher et al. (2007) and Eglezos et al. (2008), compared with our relatively lower rates in these types of products. Additionally, there was only one study by Patel and Bhagwat (2008), who reported no *Salmonella* detection in turkey meat products, which is in contrast to our relatively high detection rate of *Salmonella* in this type of product. Among many contributing factors, the differences in the detection rates of *Salmonella* between our study and the results reported in other studies can be linked to 1) differences in the detection methods used in the studies, 2) the strictness of hygiene and biosecurity policies used at the various sampling locations, and 3) the sample type and product processing technology.

## Conclusion

In this study, we determined that both VIDAS ESLM and LCPCR systems have the potential to complement the ISO standard culture method in the rapid and reliable screening of *Salmonella* from naturally contaminated poultry meats. For poultry meat products, VIDAS ESLM and LCPCR can be used as rapid primary screening methods, and they should be complemented absolutely by ISO. Although LCPCR also can preferentially be used for initial screening poultry meat products, the LCPCR results should definitely be confirmed by ISO. Apart from that all, we thought that the VIDAS ESLM did not seem to be a suitable method for detecting *Salmonella* in poultry meat products.

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