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The "Rapid'*Salmonella*" Method: Estimation of the Limit of Detection for *Salmonella* Strains Typhimurium and Enteritidis Isolated from Frozen Poultry Meat

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Abstract

As a part of evaluation the surveillance system of *Salmonella* in frozen imported poultry meat into Jordan, we conducted a study to estimate the limit of detection ($LOD_{50\%}$ and $LOD_{95\%}$) of *Salmonella* Typhimurium and *Salmonella* Enteritidis based on chromogenic media of Rapid'*Salmonella* method. *Salmonella*-free chicken meat samples was inoculated with 1 to 100 CFU of 11 wild strains that originated from frozen imported poultry meat and 2 reference strains. In the experiment, the observed lowest concentration for *Salmonella* Typhimurium and *Salmonella* Enteritidis using Rapid'*Salmonella* method were from 1 to 50 CFU/25 g. Based on these results, probability of detection (POD) curve was estimated according to the model described in EN ISO 16140-4. From the estimated POD functions, the $LOD_{50\%}$ and $LOD_{95\%}$ was determined for the Rapid'*Salmonella* method. The $LOD_{50\%}$ of the different strains varied from 0.9 to 21.2 CFU/25 g. The two reference strains and 9 wild strains had a $LOD_{50\%}$ less than 2 CFU/25 g, one wild strain of *Salmonella* Enteritidis had a $LOD_{50\%}$ of 6.8 CFU/25 g and another one had a $LOD_{50\%}$ of 21.2 CFU/25 g. The majority of *Salmonella* strains has a $LOD_{50\%}$ of 1-4 CFU/25 g in poultry meat, but also that there are some *Salmonella* strains which will first be detected at 10 CFU/25 g and higher.

Keywords: $LOD_{50\%}$, $LOD_{95\%}$, POD, poultry meat, Rapid'*Salmonella*, *Salmonella* Typhimurium, *Salmonella* Enteritidis, surveillance

1. Introduction

Non-Typhoidal *Salmonella* (NTS) including *Salmonella* Typhimurium and Enteritidis are the most frequent causes of foodborne salmonellosis in the Middle East and North Africa (MENA). In the MENA countries including Jordan, the presence of NTS strains Typhimurium and Enteritidis in domestic and imported poultry meat is one of the main concerns of the food safety authorities (Malaeb, Bizri, Ghosn, Berry, & Musharrafieh, 2016; Nimri, Abu AL- Dahab, & Batchoun, 2014; Osaili et al., 2014).

According to the Jordan Food and Drug Administration (JFDA) guidelines, all imported frozen poultry meat at customs ports requires a scheduled sampling to test for *Salmonella* strains Typhimurium and Enteritidis (Jordan Food and Drug Administration, 2015). From each batch, one sample is collected. A batch of poultry meat is equivalent to one type of product produced on a specific date in one establishment (Jordan Food and Drug Administration, 2015). Sample units are transported to the JFDA laboratories where 25 g is apportioned, thawed

and analyzed according to the Rapid'*Salmonella* method (Bio-Rad, Marnes-la-Coquette, France). This method was introduced in 2015 as an alternative to the reference method "ISO 6579:2017" for rapid detection of *Salmonella* spp. including strains of *Salmonella* Typhimurium and Enteritidis and followed by real time (RT)-PCR (Maurischat, Baumann, Martin, & Malorny, 2015) for confirmation and identification.

The Rapid'*Salmonella* method has been certified by Association Francaise de Normalisation (AFNOR), Nordic System for Validation of Alternative Microbiological Methods (NordVal), and Association of Official Analytical Chemist (AOAC) as alternative to reference method "ISO 6579:2017", for the detection of *Salmonella* spp., according to the ISO 16140 protocol (ADRIA Development, 2017; Anynomous, 2016, 2017; Lauer, 2009; Norli & Nielsen, 2018).

In 2015, with the use of Rapid'*Salmonella* method, 29 batches of poultry meat (representing approximately 200 tons) out of 3,109 examined (representing approximately 50,000 tons) were rejected at the Jordan border because they were found positive for *Salmonella* strains Typhimurium and Enteritidis. However, it is expected that a number of contaminated batches of poultry meat were not detected by the method used. The likelihood to detect a contaminated batch depends on the actual occurrence of *Salmonella* in the batch (prevalence of contaminated items and concentration of *Salmonella* in those items) and limit of detection (LOD) of laboratory method used. This likelihood can be described by the probability of detection (POD) function (Wilrich & Wilrlich, 2009), and is a useful quantitative measurement of the overall performance of a surveillance program.

However, the LODs of the Rapid'*Salmonella* method for *Salmonella* strains Typhimurium and Enteritidis in frozen poultry contaminated with relevant field strains for poultry meat imported to Jordan has never been studied.

The objective of this study was to determine the lowest number of cells of different *Salmonella* Typhimurium and Enteritidis strains isolated from imported frozen poultry meat that can be detected using the commercial laboratory method Rapid'*Salmonella*. This was done in a spiking experiment using a serial dilution of concentration of several field strains. Subsequently, a probability function of detection (POD) was fitted to the observed LOD values, from where $LOD_{50\%}$ and $LOD_{95\%}$ was determined.

The overall aim of the border control is to protect the consumers against salmonellosis attributable to imported poultry meat. The POD functions estimated in this study will be important input for subsequent assessment of the border control using quantitative risk assessment. In addition, the experimental setup and the estimation of the POD function can be used when assessing the effect of improved laboratory methods and sampling strategies used at border control.

2. Method

In the spiking experiment known numbers of different strains of *Salmonella* Typhimurium and Enteritidis were duplicate inoculated on *Salmonella*-free chicken meat samples, and subsequently the samples were analyzed using the Rapid'*Salmonella* method for detection. The observed data from the spiking experiment (concentration and positive/negative) was used for estimating the POD, $LOD_{50\%}$, and $LOD_{95\%}$.

2.1 Chicken Meat Samples

The *Salmonella*-free chicken meat samples (whole chicken carcasses and boneless chicken breast fillet with skin) used in this study were equally brought from Denmark and Brazil in 2018. The European Commission regulation 2018/307 declared Danish broiler meat as *Salmonella*-free. Before conducting the study, the Brazilian chicken meat samples were collected from a batch of boneless breast fillet chicken meat with skin of 2.5-kg packages. From this batch, five samples of 25 g were collected and tested for the presence of *Salmonella* using the ISO 6579:2017 method (Anynomous, 2017). All five samples were negative.

The *Salmonella*-free chicken meat was cut into 25-g portions representing samples, and these samples (n=132) were stored at -18°C for a maximum of 30 days. The samples were thawed at 4°C for 24 h before use.

2.2 Bacterial Strains and Inoculum Preparation

The samples were spiked with 13 strains of *Salmonella* Typhimurium and *Salmonella* Enteritidis from the JFDA surveillance collection (see Table A1 in Appendix). These strains were grown in nutrient broth (Oxoid, Basingstoke, UK) and incubated at 37°C ± 1°C for 24 h to obtain expected bacterial concentrations 10^9 CFU/ml. Using 10-ml volumes, serial dilutions established five levels with expected bacterial concentrations of 100, 50, 10, 5, and 1 CFU/ml. The number of cells in each established level of inoculation were enumerated and recorded to calculate the initial bacterial concentrations as described below.

2.3 Total Count of Inocula

One ml of each of the above five levels of established serial dilutions was poured on duplicate plates of aerobic Plate Count Agar (PCA, Scharlau, Barcelona, Spain). These two plates were used for counting the total count of the bacteria after incubation at 37°C ± 1°C for 48 h. Colony counts from 0-250 CFU/plate were used for estimating the total viable count. The same person throughout the study performed the counting. The estimated total count was used to determine the apparent concentration of *Salmonella* in the 25-g of spiked samples (see Table 1). The total count of bacterial concentration in each inoculum was estimated according to the formula:

$$\textit{Total count (CFU/ml)} = \frac{\sum_{plate=1}^{plate=n} number\ of\ enumarated\ colonies\ [CFU]}{\sum_{plate=1}^{plate=n} (dilution\ factor \times volume\ plated\ [ml])} \quad (1)$$

Note. Plate=1...n is the plates with colony numbers between 0-250 CFU for a specific strain.

2.4 Spiking Samples

For each dilution and strain, we performed duplicate spikes on two separate chicken meat samples. Each 25-g sample was spiked individually with 1 ml of each of the 13 strains of *Salmonella* Typhimurium and *Salmonella* Enteritidis with the established five levels of expected bacterial concentrations 100, 50, 10, 5, and 1 CFU/25 g. In addition, two samples were not spiked and served as negative controls. All samples were analyzed for *Salmonella* presence as described below.

2.5 Laboratory Procedure

The method "Rapid'*Salmonella*" was used in this study to detect the presence of *Salmonella* in the spiked samples. Buffered Peptone Water (225 ml, BPW, Oxoid, Basingstoke, UK) was added to each of the spiked 25-g sample portions in a stomacher bag along with 1 ml of Rapid'*Salmonella* capsule-prepared solution (Bio-Rad, Marnes-la-Coquette, France). After homogenization in a stomacher device (BagMixer, Interscience, Saint-Nom-la-Bretèche, France) at high speed for 1 min, samples were incubated at 41.5°C ± 1°C for 20 h. One hundred µl of each incubated samples were streaked onto Rapid'*Salmonella* chromogenic agar plates (Bio-Rad, Marnes-la-Coquette, France). Cultured plates were incubated at 37°C ± 1°C for 24 h. Suspected colonies were picked, streaked to Nutrient Agar (NA, Oxoid, Basingstoke, UK) and incubated at 37°C ± 1°C for 24 h to control for non-specific growth and as a measurement of purity. Pure colonies were picked for presumptive *Salmonella* spp. detection and confirmation via *Salmonella* polyvalent O (somatic) antiserum (Remel, Dartford, UK), as an agglutination test. Identification of *Salmonella* strains Typhimurium and Enteritidis were carried out using real time (RT)-PCR (Maurischat et al., 2015).

Table 1. Rapid'*Salmonella* method detection results in chicken samples artificially contaminated with *Salmonella* strains

Expected	*S.*Typhimurium	*S.*Enteritidis	*S.*Typhimurium (*n* = 7)					*S.*Enteritidis (*n* = 4)					
Inoculum	ATCC 12048	ATCC 13076	1	2	3	4	5	6	7	1	2	3	4
Concentration	Detection ([a] 0,1,2 / [b] 0,1,2)												
100	2/0	2/2	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0
50	2/0	2/2	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0
10	2/0	2/2	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	0/2
5	2/0	2/2	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	0/2	2/0	0/2
1	1/1	1/1	0/2	0/2	1/1	1/1	1/1	1/1	1/1	0/2	0/2	0/2	0/2

Note. [a]=a number of samples results indicate *Salmonella* strain presence; [b]=a number of samples results indicate *Salmonella* strain absence

2.6 Determining the Limit of Detection

The observed lowest concetration that was detected from the inoculation experiment by comparing the observed results in terms of presence/absence of growth with the number of *Salmonella* in the 25-g spiked samples. We used two measures for "the number of *Salmonella* in the spiked samples": the number based on the total count as calculated in formula 1, and the expected number of bacteria based on the dilution series. Based on the qualitative results, a probability function for detecting the strain at different concetrations (*d*) was estimated under the statistical analysis was carried out by application of the EXCEL sheet PODLOD.xls (Wilrich & Wilrlich, 2009). This model described in EN ISO 16140-4, using a program in Excel, freely available on the Internet, version 9, dated 2017-09-23 (Anonymous, 2016). The LOD_p was defined as the lowest contamination

level (CFU/25 g) where the Rapid'*Salmonella* method is positive with specified probability, *p*.

Based on the function, the $LOD_{50\%}$ and $LOD_{95\%}$ was calculated for each strain, specifying the lowest concentration of *Salmonella* in the meat matrix that can be detected with a probability of 50% or 95%, respectively. The $LOD_{50\%}$ and $LOD_{95\%}$ with confidence limits for each strain were calculated (Table 2 and Table 3). Finally, The obtained estimates is used to express the POD function as $p(d)$ of wide range of assumed known contamination d according to inoculated levels from 0 to 100 CFU/25 g, and as the following formula:

$$p(d) = 1 - exp(-A_0 F_i d) \quad (2)$$

Where A_0 is the sample size =25-g, Fi is the matrix effect that is < 1 (estimated the deviation of the POD curve from the ideal POD curve that has estimated LOD =1 by application of the EXCEL sheet PODLOD.xls (Wilrich & Wilrlich, 2009)), and d the contamination in CFU/25 g.

3. Results and Discussion

3.1 Concentrations of Bacterial Inocula

The validity of the estimted LOD_p is strongly depending on that the number of bacteria in the inocula is known. In this study, we performed the estimation of the probability function using both expected number of bacteria and apparent number of bacteria. The total counts of bacteria were about 50%-100% of the expected bacterial concentration that was established for *Salmonella* pure cultures and spiked chicken meat samples (see Table 1). The relatively low apparent counts may be due to bacterial clustering features, and some organisms may have been stressed and died during handling of the sample (Capozzi, Fiocco, Amodio, Gallone, & Spano, 2009; Sutton, 2011). Most likely, the actual bacterial concentrations in this study were in-between the total apparent concentrations and the expected bacterial concentrations based on the dilution series. Accordingly, the observed lowest concentration was assigned to both apparent and expected bacterial concentration. The differences bewteen LOD_p based on apparent and expected bacterial concentration were negligible (see Table A3 and Table A4 in Appendix).

3.2 Limit of Detections

The observed lowest concentration for *Salmonella* Typhimurium and *Salmonella* Enteritidis using Rapid'*Salmonella* method were from 1 to 50 CFU/25 g for spiked chicken meat samples (Table 1). The estimatd $LOD_{50\%}$ for *Salmonella* Typhimurium were from 0.9 to 1.8 CFU/25 g (Table 2) and for *Salmonella* Enteritidis were from 0.8 to 21.2 CFU/25 g (Table 3). The $LOD_{95\%}$ for *Salmonella* Typhimurium were from 3.7 to 7.6 CFU/25 g (Table 2) and for *Salmonella* Enteritidis were from 3.7 to 91.7 CFU/25 g (Table 3). The $LOD_{50\%}$ combined results for *Salmonella* Typhimurium and *Salmonella* Enteritidis were 1.1 CFU/25 g (95% CI: 0.6-1.8 CFU/25 g) and 4.2 CFU/25 g (95% CI: 2.3-7.3 CFU/25 g), respectively, indicating a significant difference in general between *Salmonella* Typhimurium and *Salmonella* Enteritidis detection level.

Table 2. Expected count of *Salmonella* Typhimurium calculation of $LOD_{50\%}$ in CFU/25 g

Salmonella strains	$LOD_{50\%}$	confidence interval (95%)	$LOD_{95\%}$	confidence interval (95%)
Salmonella Typhimurium ATCC	0.9	(0.2-4.0)	3.7	(0.8-17.4)
Salmonella Typhimurium isolate no. 1	1.8	(0.5-6.2)	7.6	(2.1-26.8)
Salmonella Typhimurium isolate no. 2	1.8	(0.5-6.2)	7.6	(2.1-26.8)
Salmonella Typhimurium isolate no. 3	0.9	(0.2-4.0)	3.7	(0.8-17.4)
Salmonella Typhimurium isolate no. 4	0.9	(0.2-4.0)	3.7	(0.8-17.4)
Salmonella Typhimurium isolate no. 5	0.9	(0.2-4.0)	3.7	(0.8-17.4)
Salmonella Typhimurium isolate no. 6	0.9	(0.2-4.0)	3.7	(0.8-17.4)
Salmonella Typhimurium isolate no. 7	0.9	(0.2-4.0)	3.7	(0.8-17.4)
Combined results	1.1	(0.6-1.8)	4.6	(2.8-7.7)

Table 3. Expected count of *Salmonella* Entritidis calculation of $LOD_{50\%}$ in CFU/25 g

Salmonella strains	$LOD_{50\%}$	confidence interval (95%)	$LOD_{95\%}$	confidence interval (95%)
Salmonella Enteritidis ATCC	0.9	(0.2-4.0)	3.7	(0.8-17.4)
Salmonella Enteritidis isolate no. 1	1.8	(0.5-6.2)	7.6	(2.1-26.8)
Salmonella Enteritidis isolate no. 2	6.8	(1.9-24.3)	29.5	(8.3-105.2)
Salmonella Enteritidis isolate no. 3	21.2	(6.7-66.4)	91.5	(29.1-287.1)
Salmonella Enteritidis isolate no. 4	1.8	(0.5-6.2)	7.6	(2.1-26.8)
Combined results	4.2	(2.3-7.3)	18.4	(10.6-31.7)

The laboratory American Type Culture Collection (ATCC) strains have the lowest detection level (Table 1). The reason for this is probably because these strains are fit to culture under laboratory conditions (de Moraes et al., 2016). Some wild strains have the same detection level as the laboratory ATCC strains, whereas the LOD_p of some wild strains were higher. The highest $LOD_{50\%}$ (21.2 CFU/25 g) was obtained from an isolate of *Salmonella* Enteritidis sampled from imported chicken legs. A part of the observed variation in values of LOD_p is probably due to a random variation between experiments, but the results indicate that the LOD_p is varying between *Salmonella* strains.

The estimated POD of different concentrations of *Salmonella* Typhimurium and *Salmonella* Enteritidis giving *d* values of LOD_p using the Rapid'*Salmonella* method to detect *Salmonella* strains in imported poultry meat to Jordan is presented in figure 1. Given by the estimated POD curve, the lowest $LOD_{50\%}$ was 1 CFU/25 g (95% CI: 0.2-4.0 CFU/25 g); and 85% of studied *Salmonella* strains has a $LOD_{50\%}$ equal or lower than 2 CFU/25 g (95% CI: 0.5-6.2 CFU/25 g). The results shows that the majority of these *Salmonella* strains will be detected at concentrations of few cells that around 1-5 CFU/25 g, but there is also some *Salmonella* strains which will first be detected at concentrations around 50 CFU/25 g (Table 1).

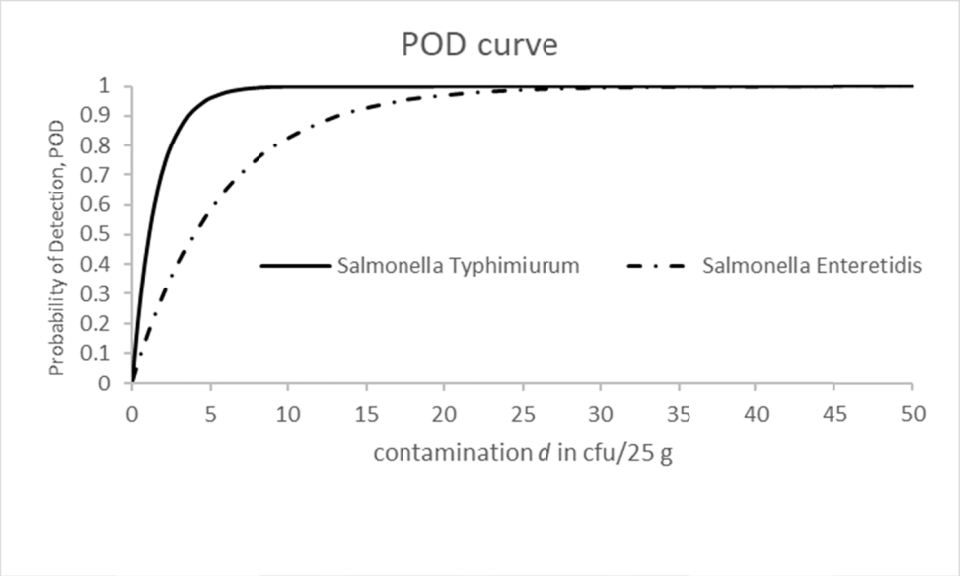

Figure 1. The estimated Probability of Detection (POD) for different concentrations of studied *Salmonella* strains in poultry meat. The solid line represent of the general POD for seven strains of *Salmonella* Typhimiurum and dashed line for four *Salmonella* Enteretidis strains

That the Rapid'*Salmonella* method had different observed detection limits for different *Salmonella* strains recovered from examined poultry meat samples can be explained by the fact that the strains has different growth rates during the enrichment phase (Löfström, Hansen, Mansdal, & Hoorfar, 2012). The actual detection method afterwards depends on that enrichment phase has resulted in a detectable concentration. The bacterial growth rate during the enrichment phase depends on characteristics of the strain of *Salmonella* and stress introduced by storage conditions, growth inhibitors from the meat and competing microflora (Lammerding, 2006).

The use of non-frozen isolates in our experiment might result in unrealistic low detection levels compared with

realistic situations where the bacteria in the imported meat has been frozen, and thereby, they are stressed and injured causing a lag in the growth. However, due to controlled conditions in the enrichment, the lag-phase can be assumed to last for only 1-2 h (Oscar, 1998), which is equivalent with 3-6 generations of growth for *Salmonella* in optimal growth conditions in broth at 37 ℃, assuming a generation time of 20-30 min. The loss of 3-6 generations due to lag-phase is proportionally a low number compared with the approximately 100 generations that can be expected for *Salmonella* in 18-20 h given experimental conditions (Oscar, 1998). Thereby, the effect of using non-frozen isolates on the estimated LOD_p is expected to be minor. Contemporary, by not freezing the samples after spiking, we know how many viable bacteria that are actually present in the sample, which strengthens the validity of the estimated LOD_p.

In the spiking experiment, one strain of each *Salmonella* Typhimurium and Enteritidis from the ATCC and 11 from the JFDA's isolates in imported frozen poultry meat were used. Even though, the estimated LOD_p cannot be generalized to all strains of *Salmonella* in all types of food items, the estimated LOD_p in this study indicate the LOD_p that can be expected when using of Rapid'*Salmonella* method at the border control of frozen poultry meat.

In the spiking study performed by AFNOR, they used 152 *Salmonella* strains at contamination levels between 5-25 CFU/sample, but only 5 of those represent *Salmonella* Typhimurium and Enteritidis spiked into poultry meat (ADRIA Development, 2017). In our study, 12 out of 13 *Salmonella* strains are at observed lowest concentration ≤5 CFU/25 g, which is in alignment with AFNOR spiking study finding approximately 70% of tested *Salmonella* strains with the same observed lowest concentration (ADRIA Development, 2017). In addition, they compare the performances of reference method and alternative method by estimating $LOD_{50\%}$, which were between 0.1-5.6 and 0.1-1.8 CFU/25 g, respectively (Norli & Nielsen, 2018). In our study, the $LOD_{50\%}$ for *Salmonella* Typhimurium and *Salmonella* Enteritidis were between 0.6-7.3 CFU/25 g, which is in alignment with AFNOR validation certification (Norli & Nielsen, 2018).

There are many surveillance programs that employ rapid immunoassays and PCR methods instead of conventional culture methods for detecting *Salmonella* in poultry meat to cope with the enormous volume of samples (Brooks, Lutze-Wallace, Devenish, Elmufti, & Burke, 2012; Cheung & Kam, 2012; Hitchins, 2012; Tomás Fornés, McMahon, Moulin, & Klijn, 2017). The observed detection level of the conventional pre-enrichment step that directly coupled with the PCR methods is 100-200 CFU/25 g of *Salmonella* Typhimurium and Enteritidis in poultry meat (Mohd Afendy & Son, 2015; Paião et al., 2013; Siala et al., 2017). Compared to this, the Rapid'*Salmonella* method can be considered relatively sensitive for most strains of *Salmonella* Typhimurium and Enteritidis.

4. Conclusion

In the spiking experiment, we found that the observed level of detecting *Salmonella* Typhimurium and *Salmonella* Enteritidis from poultry meat using the commercial Rapid'*Salmonella* method varies between 1 and 50 CFU/25 g. The most natuarally wild *Salmonella* strains and laboratory-adapted ones have $LOD_{50\%}$ between 1 and 4 CFU/25 g. However, due to the studied *Salmonella* strains are limited in numbers and serotypes, their results can't be generalized to all *Salmonella* spp. without caution. Future studies should focus on including more serotypes that representing different countries of origin and interlaboratory comparison for robustness.

Referring to the POD curves in figure 1, it can be concluded that most studied *Salmonella* strains has a $LOD_{50\%}$ of 1-4 CFU/25 g in poultry meat, but also that there are some *Salmonella* strains which will be detected at concentrations around 10 CFU/25 g and higher. The concetration in the matrix, which gives a 95% likelihood for detection ($LOD_{95\%}$) was for most *Salmonella* Typhimiurm strains around 5 CFU/25 g, whereas for most *Salmonella* Enteretidis strains it was around 50 CFU/25 g.

This study is the intial step in evaluating and optimizing current *Salmonella* surveillance of poultry meat in the MENA region.

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Appendix

Table A1. *Salmonella* strains Typhimurium and Enteritidis used for Inoculation

Inoculating organism	Source
*S.*Typhimurium (n = 1)	ATCC®[a] 12048
*S.*Enteritidis (n = 1)	ATCC® 13076
*S.*Typhimurium (n = 7)	Chicken Breast fillet, Chicken Mechanically deboned meat
*S.*Enteritidis (n = 4)	Chicken legs, boneless chicken carcass

Note. [a] = ATCC, American Type Culture Collection

Table A2. Estimated count of *Salmonella* Typhimurium strains calculation of $LOD_{50\%}$ in CFU/25 g

Salmonella strains	$LOD_{50\%}$	confidence interval (95%)	$LOD_{95\%}$	confidence interval (95%)
Salmonella Typhimurium ATCC	6.7	(1.5 -32.3)	29.8	(6.4-139.5)
Salmonella Typhimurium isolate no. 1	1.7	(0.5 -6.2)	7.6	(2.1-26.8)
Salmonella Typhimurium isolate no, 2	1.7	(0.5-6.2)	7.6	(2.1-26.8)
Salmonella Typhimurium isolate no. 3	0.8	(0.2-4.0)	3.7	(0.8-17.4)
Salmonella Typhimurium isolate no. 4	0.8	(0.2-4.0)	3.7	(0.8-17.4)
Salmonella Typhimurium isolate no. 5	0.7	(0.1-3.2)	3.0	(0.6-14)
Salmonella Typhimurium isolate no. 6	0.3	(0.1-1.6)	1.5	(0.3-7)
Salmonella Typhimurium isolate no. 7	0.3	(0.1-1.3)	1.2	(0.3-5.8)
Combined results	1.1	(0.7-1.9)	5.0	(3.0-8.2)

Table A3. Estimated count of *Salmonella* Eneteritidis calculation of $LOD_{50\%}$ in CFU/25 g

Salmonella strains	$LOD_{50\%}$	confidence interval (95%)	$LOD_{95\%}$	confidence interval (95%)
Salmonella Enteritidis ATCC	6.9	(1.5-32.3)	29.8	(6.4-139.5)
Salmonella Enteritidis isolate no. 1	3.0	(0.8-10.5)	12.9	(3.6-45.5)
Salmonella Enteritidis isolate no, 2	7.5	(2.1-26.8)	32.5	(9.1-115.7)
Salmonella Enteritidis isolate no. 3	7.0	(2.2-21.9)	30.2	(9.6-94.7)
Salmonella Enteritidis isolate no. 4	0.8	(0.2-2.7)	3.3	(0.9-11.8)
Combined results	4.1	(2.3-7.3)	17.8	(10.0-31.7)