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 (LOD50% and LOD95%) 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 LOD50% and LOD95% was determined for the Rapid’Salmonella method. The LOD50% of the different strains varied from 0.9 to 21.2 CFU/25 g. The two reference strains and 9 wild strains had a LOD50% less than 2 CFU/25 g, one wild strain of Salmonella Enteritidis had a LOD50% of 6.8 CFU/25 g and another one had a LOD50% of 21.2 CFU/25 g. The majority of Salmonella strains has a LOD50% 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: LOD50%, LOD95%, 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<sub>50%</sub> and LOD<sub>95%</sub> 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<sub>50%</sub>, and LOD<sub>95%</sub>.

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<sup>5</sup> 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:

\[
\text{Total count (CFU/ml)} = \frac{\sum \text{number of enumerated colonies (CFU)}}{\sum \text{plate}=1 \text{dilution factor} \times \text{volume plated (ml)}}
\]

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).

| Expected | *S.* Typhimurium (n = 7) | *S.* Enteritidis (n = 4) |
|----------|--------------------------|-------------------------|
| Inoculum | ATCC 12048 | ATCC 13076 | ATCC 12048 | ATCC 13076 |
| Concentration | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 |
| 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 |
| 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 |
| 10 | 2/0 | 2/2 | 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 | 2/2 | 2/0 | 2/0 | 2/0 | 2/0 | 2/0 | 2/0 | 2/0 | 2/0 | 2/0 |
| 1 | 1/1 | 1/1 | 0/2 | 0/2 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 0/2 | 0/2 |

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

2.6 Determining the Limit of Detection

The observed lowest concentration 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 concentrations (d) was estimated under the statistical analysis was carried out by application of the EXCEL sheet PODLOD.xls (Wilrich & Wilrich, 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 LODp 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:

\[
p(d) = 1 - \exp (-A_0 F_i d)
\]

Where \( A_0 \) is the sample size =25-g, \( F_i \) 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 estimated LOD, 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 between 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 estimated 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)                        |
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 Morais et al., 2016). Some wild strains have the same detection level as the laboratory ATCC strains, whereas the LODₜ of some wild strains were higher. The highest LODₜ (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 LODₜ is probably due to a random variation between experiments, but the results indicate that the LODₜ is varying between Salmonella strains.

The estimated POD of different concentrations of Salmonella Typhimurium and Salmonella Enteritidis giving $d$ values of LODₜ 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 LODₜₚ was 1 CFU/25 g (95% CI: 0.2-4.0 CFU/25 g); and 85% of studied Salmonella strains has a LODₜₚₜ 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 Typhimurium and dashed line for four Salmonella Enteritidis 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, & Hoornfar, 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

| Salmonella strains               | LODₜₚₜ confidence interval (95%) | LODₜₚₜ 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)                |

Table 3. Expected count of Salmonella Enteritidis calculation of LODₜₚₜ in CFU/25 g
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 °C, 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 LODp is expected to be minor. Contemporarily, 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 LODp.

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 LODp cannot be generalized to all strains of Salmonella in all types of food items, the estimated LODp in this study indicate the LODp 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 LOD50%, which were between 0.1-5.6 and 0.1-1.8 CFU/25 g, respectively (Norli & Nielsen, 2018). In our study, the LOD50% 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 naturally wild Salmonella strains and laboratory-adapted ones have LOD50%, 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 LOD50% 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 concentration in the matrix, which gives a 95% likelihood for detection (LOD95%) was for most Salmonella Typhimurium strains around 5 CFU/25 g, whereas for most Salmonella Enteritidis strains it was around 50 CFU/25 g.

This study is the initial 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\textsuperscript{a} 12048               |
| *S.* Enteritidis (n = 1)   | ATCC\textsuperscript{b} 13076               |
| *S.* Typhimurium (n = 7)   | Chicken Breast fillet, Chicken Mechanically deboned meat |
| *S.* Enteritidis (n = 4)   | Chicken legs, boneless chicken carcass      |

\textit{Note.} \textsuperscript{a} = ATCC, American Type Culture Collection

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

| *Salmonella* strains       | LOD\textsubscript{50\%} | confidence interval (95\%) | LOD\textsubscript{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* Enteritidis calculation of LOD\textsubscript{50\%} in CFU/25 g

| *Salmonella* strains       | LOD\textsubscript{50\%} | confidence interval (95\%) | LOD\textsubscript{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)                |

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