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Microplate Immunocapture Coupled with the 3M Molecular Detection System and Selective Plating for the Rapid Detection of *Salmonella* Infantis in Dry Dog Food and Treats

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ABSTRACT

The objective of this study was to use microplate immunocapture (IC) to reduce the enrichment time required for detection of *Salmonella* in pet food with the 3M Molecular Detection System (MDS) or selective plating on XLD. Dog food and pig ear treats were inoculated with *Salmonella* Infantis at concentrations of 10^0-10^4 CFU/25 g, followed by a 3-h enrichment, then microplate IC and 3M MDS or microplate IC and selective plating on XLD. Another set of samples underwent a traditional 24-h enrichment followed by 3M MDS or selective plating. Based on the results of three independent trials, microplate IC followed by selective plating enabled detection of *Salmonella* in 100% of dog food and treat samples tested, including at levels as low as 10^0 CFU/25 g. Microplate IC coupled with 3M MDS enabled detection of *Salmonella* in dog food and treat samples down to levels of 10^0 CFU/25 g, with an overall detection rate of 92%. These results indicate high potential for microplate IC to be used in place of the traditional 24-h enrichment step, enabling detection of *Salmonella* in complex matrices when coupled with 3M MDS or selective plating.

Keywords: Microplate immunocapture; LAMP-BART; pet food; selective plating; *Salmonella* Infantis

1. Introduction

Salmonella enterica is the leading bacterial cause of foodborne illness in the United States, responsible for approximately 1.2 million infections, 23 thousand hospitalizations and over 400 deaths each year (Scallan et al., 2011). The infection is typically self-limiting in otherwise healthy individuals, but in severe cases an untreated *Salmonella* infection can lead to death due to dehydration and electrolyte imbalance (FDA, 2012). *Salmonella* is a highly resilient bacterium that can survive well in low moisture foods, including dry dog food and treats (Lambertini et al., 2016). The main ingredients in dry dog food and treats (e.g., poultry, beef, and animal products) are also common sources of *Salmonella* (FDA, 2018). *Salmonella* infection can lead to illness and death in pets (Imanishi et al., 2014). Pets can also asymptomatically carry the bacteria for months and spread it to other animals. Humans can contract salmonellosis from handling contaminated pet foods or fecal eliminations from pets that have ingested contaminated food (CDC, 2017).

Over the course of 2018-2019, 28 pet food products were removed from the market due to potential or confirmed *Salmonella* contamination (FDA, 2019). Several outbreaks of *Salmonella* in recent years have been traced back to dog foods and treats. For example, an outbreak of multi-drug resistant salmonellosis in the United States affected 154 people in 34 states and caused 35 hospitalizations from 2015 to 2019 (CDC, 2019). The outbreak was linked to pig ear dog treats contaminated with *Salmonella enterica* strains, including serotypes Cerro, Derby, London, Infantis, Newport, Rissen, and I 4,[5],12:i:-. In 2012, a multistate outbreak in the United States linked to *Salmonella* Infantis in dry dog food was associated with 53 human illnesses and 31 dog illnesses (Imanishi et al., 2014).

69 The ability to test dog food products for *Salmonella* quickly and efficiently is essential to
70 preventing salmonellosis in dogs and their handlers. The gold standard for bacterial detection is
71 traditional culture-based methods; however, these methods are time-consuming, generally
72 requiring at least 5 days for confirmed results (Andrews, Wang, Jacobsen, & Hammack, 2016).
73 Real-time polymerase chain reaction (PCR) is a commonly used method that can reduce the time
74 to detection to 1-2 days and is sensitive to low levels of bacteria. However, it is susceptible to
75 inhibitors commonly found in food products (Margot et al., 2013).
76 Loop-mediated isothermal amplification coupled with bioluminescent assay in real-time
77 (LAMP-BART), as used by the 3M Molecular Detection System [(MDS) (St. Paul, MN)], is a
78 novel, rapid method for pathogen detection that combines isothermal DNA amplification with
79 bioluminescence detection (Gandelman et al., 2010; Yang, Domesle, Wang, & Ge, 2016).
80 Isothermal DNA amplification does not require thermal cycling and has shown greater tolerance
81 to assay inhibitors compared to PCR (Margot et al., 2013, Yang et al., 2016; Wang, Shi, Alam,
82 Geng, & Li, 2008). Previous studies have reported LAMP and LAMP-BART to be precise,
83 rapid, and sensitive for the detection of S*almonella* in a variety of food products (Yang et al.,
84 2015; Yang et al., 2016; Wang et al., 2008). Yang et al. (2015) found LAMP-BART was able to
85 detect 1.1-2.9 CFU/25g of several different serovars of *Salmonella* in inoculated produce when
86 paired with a 6-8 h enrichment period, whereas the same results were obtained with PCR after a
87 24-h pre-enrichment. Later, Yang et al. (2016) reported the ability to detect *Salmonella* Infantis
88 in dry dog food at concentrations of 10^0-10^1 CFU/25 when a 24-h enrichment step was used in
89 combination with LAMP-BART. In the absence of the enrichment step, the detection limit was
90 reported to be 10^5-10^6 CFU/25 g.

A potential means for shortening the enrichment period for the detection of low concentrations of *Salmonella* is through the use of microplate immunocapture (IC). Microplate IC utilizes an antibody-coated microtiter plate to concentrate bacterial cells for greater detection efficiency when used with PCR and/or selective plating (Arbault, Desroche, & Larose, 2014ab; Fakruddin, Hossain, & Ahmed, 2017; Rogers, Calicchia, & Hellberg, 2018). Although microplate IC is not as widely used as immunomagnetic separation, it is considerably less expensive because it does not require production of antibody-coated beads. Previous studies have reported use of microplate IC to concentrate bacterial cells for detection with PCR and/or selective plating (Rogers et al. 2018; Fakruddin et al. 2017). For example, Fakruddin et al. (2017) found that coupling microplate IC with PCR allowed for detection of *Salmonella* Typhi in 62.7% of food samples inoculated with concentrations of 10^1-10^5 CFU/25 g, as compared to 56% detection for samples that underwent traditional enrichment plus PCR. Rogers et al. (2018) found that microplate IC coupled with PCR could detect *L. monocytogenes* at levels of 10^0, 10^2, and 10^4 CFU/25g at rates of 88.9%, 94.4%, and 100% respectively, but microplate IC with selective plating yielded 0% recovery at 10^0 CFU/25g and 44.4% at 10^2 CFU/25g. Rogers et al. (2018) did not use a pre-enrichment step prior to conducting microplate IC and selective plating which could explain the limited recovery of bacteria at low concentrations.

The goal of this study was to evaluate the use of microplate IC to reduce the enrichment time required for detection of *Salmonella* in pet food with the 3M MDS and selective plating. The specific aims of this study were to: 1) optimize the microplate IC parameters to enable detection of *Salmonella* in dog food and treats within 1 working day (8 h) when combined with LAMP-BART with the 3M MDS, 2) determine the ability of microplate IC combined with the 3M MDS or selective plating to consistently detect low levels of *S.* Infantis (10^0-10^4 CFU/25 g)

in dog food and treats, and 3) compare the results obtained with microplate IC to those obtained with a traditional 24-h enrichment process.

2. Materials and methods

2.1. Bacterial isolation and preparation

Salmonella enterica serovar Infantis ATCC® 51741 was obtained from American Type Culture Collection® [(ATCC) (Manassas, VA)]. All media used in this study were from Becton, Dickinson and Company [(BD) (Franklin Lakes, NJ)], unless otherwise specified. Bacterial isolation was conducted by streaking the stock culture of *S.* Infantis onto tryptic soy agar (TSA) and incubating for 48 h at 37°C. An isolated colony was transferred from TSA to 10 mL of tryptic soy broth (TSB) and incubated at 37°C until the bacteria reached the desired concentration of 10^4 colony-forming units (CFU)/mL.

2.2. Microplate preparation

Salmonella Polyclonal Antibody PA1-7244 (Invitrogen™, Carlsbad, CA) was diluted to 1 µg/mL in carbonate-bicarbonate buffer, pH 9.6. The microplate was prepared according to a protocol adapted from Abcam (http://www.abcam.com/protocols/sandwich-elisa-protocol-1). First, 200 µL of the antibody solution were added to individual wells of a 96-well polystyrene microtiter microplate separated into 8-well strips. The plate was covered with sterile polyethylene sealing films (Excel Scientific, Victorville, CA) and incubated at 4°C for 24 ± 2 h. Plates that were not used immediately were stored at -20°C with the antibodies in each well, and then prepared according to the following procedure. The wells were washed twice with 200 µL 1X phosphate buffered saline [(PBS) (Fisher Scientific, Hampton, NH)]. Next, 200 µL of 5% non-fat dry milk prepared in 1X PBS was added to each well and incubated for 2 h at room

136 temperature. Immediately before performing microplate IC, the blocking agent was removed and
137 the wells were washed twice more with 200 µL 1X PBS.

138 **2.3. Microplate IC for broth samples**

139 The experimental conditions for microplate IC of *S.* Infantis were first optimized in the
140 absence of a food matrix. The bacterial culture was prepared as described above, followed by
141 serial dilution in TSB to allow for concentrations of 10^0 to 10^4 CFU/ml. Then, 1.6 mL of each
142 dilution was distributed across an 8-well strip of the prepared microplate for a total of 200 µL of
143 sample per well. An un-inoculated broth sample was included as a negative control.
144 Optimization of microplate IC was conducted using an Eppendorf ThermoMixer® C (Hamburg,
145 Germany) using the minimum shaking speed of 300 rpm. The procedure was optimized for
146 incubation temperature (30-37°C), fill cycles (1-4), and cycle incubation time (30-120 min), as
147 shown in Table 1. Each fill cycle involved addition of 1.6 mL of bacteria to the corresponding 8-
148 well microplate strip, incubation and shaking on the Thermomixer for a given cycle incubation
149 time, and removal of the concentrated bacteria.

150 Following microplate IC, the 8 wells corresponding to each sample and negative control
151 were scraped with a sterile inoculating loop and streaked onto xylose lysine deoxycholate (XLD)
152 agar. The plates were incubated at 37°C for 18-24 h and then examined for typical *Salmonella*
153 growth. The procedure was considered successful if all bacterial concentrations had positive
154 growth on XLD. The optimized procedure (Table 1) was then tested in three independent trials
155 with both XLD agar and the 3M MDS (described below).
156
157
158

Table 1. Microplate IC optimization trials for detection of *S.* Infantis in TSB with selective plating on XLD. All trials were carried out using 8 microplate wells and a plate shaker at 300 rpm.

Trial	Microplate incubation temperature (°C)	No. of fill cycles	Fill cycle incubation time (min)	Total incubation time (min)	Minimum detection on XLD (CFU/mL)
1	30	3	60	180	10^2
2	37	4	60	240	10^0
3	37	4	45	180	10^0
4	37	3	45	135	10^2
5	37	4	30	120	10^1
6	37	3	60	180	10^2
7 [a]	37	1	120	120	10^0

[a] Optimized conditions selected for further testing are indicated with gray shading.

159

160 **2.4.Microplate IC for food samples**

161 An 11-kg bag of chicken-flavored, dry dog food and a 4.5-kg bag of pig ear dog treats
162 were purchased from a local retail outlet in Orange, CA. The dog food products were confirmed
163 negative for the presence of *Salmonella* according to the conventional culture method described
164 in the Bacteriological Analytical Manual (BAM) (Andrews et al., 2016). A bacterial culture of *S.*
165 Infantis was prepared as described above, followed by serial dilution in buffered-peptone water
166 (BPW). Dog food/treat samples (25 g) were spot-inoculated with *S.* Infantis with concentrations
167 of 10^0 to 10^4 CFU/25g. An un-inoculated sample was used as a negative control for each trial.
168 The samples were dried in sterile plastic bags inside a biosafety cabinet for 2 h at room
169 temperature, then 225 mL of pre-warmed (35°C) BPW was added to each sample. The
170 inoculated dog treats were mixed by swirling and the dry dog food samples were homogenized in

171 a Stomacher 400C (Seward Laboratory Systems Inc., Bohemia, NY) for 2 min at 260 rpm (Yang
172 et al., 2016).
173 Microplate IC optimization for dog food samples included testing of short pre-enrichment
174 periods (0-3 h) at 37°C prior to running microplate IC. Additionally, the effectiveness of
175 scraping only 1 well of the microplate for each sample was compared to the effectiveness of
176 scraping 8 wells. After microplate IC was completed, the well(s) of the microplate were scraped
177 with a sterile inoculating loop and streaked to XLD agar. The plates were examined for typical
178 *Salmonella* growth after incubation for 18-24 h at 37°C. The procedure was considered to be
179 successful if all bacterial concentrations were confirmed by growth on XLD. The duration of the
180 pre-enrichment period was selected based on the shortest period that consistently produced more
181 $\geq$ 3 colonies at the lowest bacterial concentration (10^0 CFU/25 g). The optimized conditions for
182 dog food were used for pig ear treats without any further optimization. The optimized procedure
183 (Table 2) was tested in three independent trials with both XLD agar and the 3M MDS (described
184 below) for dog food and treat samples. The inoculated dog food/treat samples were also tested
185 three times using a traditional 24-h enrichment in BPW at 37°C (Yang et al. 2016), with no
186 microplate IC step. After the 24-h enrichment, each sample was streaked onto to XLD agar and
187 run with the 3M MDS (described below).

Table 2. Microplate IC optimization trials for detection of *S.* Infantis in dry dog food with selective plating on XLD. All trials were carried out with a plate shaker at 300 rpm.

Trial	Pre-enrichment time (min) [a]	No. of microplate wells used	Microplate incubation temperature (°C)	No. of fill cycles	Fill cycle incubation time (min)	Total pre-enrichment + incubation time (min)	Minimum detection on XLD (CFU/25 g)
1	0	8	37	1	120	120	10^2
2	120	8	37	1	120	240	10^0 [b]
3	120	1	37	1	120	240	10^0 [b]
4	90	1	37	1	120	210	10^1
5 [c]	180	1	37	1	120	300	10^0

[a] Pre-enrichment at 37 °C.
[b] Minimal growth observed for trials 2-3 at 10^0 CFU/25 g (≤ 2 colonies/plate).
[c] Optimized conditions selected for further testing are indicated with gray shading.

2.5. 3M Molecular Detection System

Samples were tested on the 3M MDS using the 3M Molecular Detection Assay 2 – *Salmonella* kit according to Protocol 2. For microplate IC samples, the microplate wells were scraped with an inoculating loop and transferred to a sterile tube containing the pre-mixed lysis solution. For the TSB trials, 8 wells were scraped, whereas only 1 well was scraped for the food samples (based on the results of microplate IC optimization). Next, 20 µL of the liquid portion of the sample was added to the same tube. For the 24-h enrichment samples, 20 µL of the liquid portion of the sample was added to each sample tube. For the negative control and reagent (positive) control for the 3M MDS, 20 µL of sterile, pre-warmed (35°C) BPW was added to the corresponding tubes of lysis solution. All lysis tubes were held in a dry heat block for 15 min at 100°C and then cooled in a chilling block at ambient temperature for 5 min. Next, 20 µL of each sample or control was transferred to its corresponding reagent tube. The reagent control was provided with the 3M Molecular Detection Assay 2-*Salmonella* kit to serve as a positive control,

while the negative control contained only sterile enrichment medium (BPW). Detection of *S.* Infantis for each sample was signified by an output of either a red positive symbol if *Salmonella* was detected, or a green negative symbol if *Salmonella* was not detected. The detection results were only accepted if the results of all controls were valid.

2.6 Statistical analysis

Detection rates obtained with the optimized methods in food samples were compared statistically with the McNemar Test using a significance level of $p < 0.05$. Specifically, the results of microplate IC + XLD were compared to the results of traditional (24-h) enrichment + XLD and the results of microplate IC + 3M MDS were compared to the results of traditional (24-h) enrichment + 3M MDS. The analyses were carried out with IBM SPSS Statistics 23 (Armonk, NY).

3. Results and discussion

3.1. Microplate IC optimization

Microplate IC was successfully optimized for detection of *S.* Infantis in TSB (Table 1) and dog food (Table 2) with selective plating on XLD. The optimal microplate incubation time and temperature were found to be 2 h at 37°C. It was expected that the use of multiple fill cycles would increase the sensitivity of the assay by allowing more bacteria to adhere to the antibodies coated onto microplate wells; however, it was found that just one fill cycle resulted in comparable growth on XLD, in addition to reducing the labor and time needed for microplate IC.

When the optimized conditions for TSB were applied to dog food samples, *S.* Infantis could not be detected at concentrations of 10^0-10^1 CFU/25 g (Table 2). Therefore, a short pre-enrichment incubation (1.5-3 h) at 37°C was incorporated into the protocol prior to microplate IC. A pre-enrichment period of 1.5 h followed by microplate IC resulted in detection of

225 *Salmonella* in dog food as low as 10^1 CFU/25g using XLD agar (Table 2), while a pre-
226 enrichment period of 2-3 h followed by microplate IC enabled detection of *Salmonella* in dog
227 food at the lowest level tested (10^0 CFU/25g). While a 2-h pre-enrichment period yielded
228 positive results at concentrations of 10^0 CFU/25g, only 1-2 colonies were observed on each plate,
229 as opposed to ≥5 colonies per plate when a 3-h pre-enrichment period was used. Therefore, the
230 3-h pre-enrichment period was selected for testing of food samples.
231 The TSB trials were completed using 8 wells of the microplate for each concentration,
232 but later optimization with dog food showed that scraping only 1 well of the microplate resulted
233 in comparable growth on XLD. These results were unexpected, as it was thought that there
234 would be a greater chance of capturing bacteria if more wells were scraped. However, the use of
235 a 3-h pre-enrichment step combined with the 2 h microplate incubation time likely increased the
236 number of bacteria sufficiently to enable detection based on just one microplate well.

237 **3.2. Microplate IC trials**

238 As shown in Table 3, the optimized conditions determined for microplate IC enabled
239 detection of *S.* Infantis in 100% of the TSB samples tested across three trials. *S.* Infantis in the 15
240 samples was detected with the 3M MDS and confirmed with selective plating on XLD at all
241 concentrations tested (10^0-10^4 CFU/mL). Similarly, *S.* Infantis was detected in 100% (30/30) of
242 dry dog food samples and pig ear treats tested with microplate IC combined with selective
243 plating on XLD, even at the lowest detection level (10^0 CFU/25 g). These results are consistent
244 with those found when the food samples underwent a 24-h enrichment period followed by
245 selective plating on XLD (Table 3), indicating that microplate IC could be used to shorten the
246 time required for confirmation of *Salmonella* using selective plating to 27-30 h as opposed to 48
247 h using the traditional 24-h enrichment.

Table 3. Detection rates for *S.* Infantis in broth and food samples following microplate IC or a 24-hr enrichment step. There were no significant differences ($p > 0.05$) between the detection rates in food samples for XLD or LAMP-BART when comparing microplate IC to 24-h enrichment, according to the McNemar Test.

Matrix	***Salmonella* Infantis concentration[a]**	**Rate of detection (no. positive samples/total no. samples)**			
		Microplate IC[b] + XLD	24-h enrichment + XLD	Microplate IC + LAMP-BART	24-h enrichment + LAMP-BART
TSB	10^4	3/3	N/A	3/3	N/A
	10^3	3/3	N/A	3/3	N/A
	10^2	3/3	N/A	3/3	N/A
	10^1	3/3	N/A	3/3	N/A
	10^0	3/3	N/A	3/3	N/A
	Overall	15/15 (100%)	N/A	15/15 (100%)	N/A
Dry dog food	10^4	3/3	3/3	3/3	3/3
	10^3	3/3	3/3	3/3	3/3
	10^2	3/3	3/3	3/3	3/3
	10^1	3/3	3/3	3/3	3/3
	10^0	3/3	3/3	2/3	3/3
	Overall	15/15 (100%)	15/15 (100%)	14/15 (93%)	15/15 (100%)
Pig ear treats	10^4	3/3	3/3	2/2[c]	2/2
	10^3	3/3	3/3	2/2	2/2
	10^2	3/3	3/3	2/2	2/2
	10^1	3/3	3/3	2/2	2/2
	10^0	3/3	3/3	1/2	2/2
	Overall	15/15 (100%)	15/15 (100%)	9/10 (90%)	10/10 (100%)

[a]Concentration units are CFU/mL for TSB and CFU/25 g for dry dog food and pig ear treats.
[b]Microplate IC includes a 3-h pre-enrichment step.
[c]Data from the third trial of pig ear treats was not used because the negative control tested positive for *Salmonella*

In contrast to the current study, previous research has reported limited recovery of foodborne pathogens when microplate IC was combined with selective plating (Rogers et al., 2018; Fakruddin et al., 2017). Rogers et al. (2018) achieved 0% recovery of *Listeria* from cheese and milk samples inoculated at 10^0 CFU/25 g, and only 44.4% recovery at 10^2 CFU/25 g. Similarly, Fakruddin et al. (2017) detected *Salmonella* Typhi in only 13.1% of minced beef samples inoculated at a level of 10^1 CFU/25 g. However, neither of the previous studies used a pre-enrichment step prior to conducting microplate IC. The 3-h pre-enrichment step employed in the current study likely provided sufficient time for *Salmonella* to grow to detectable levels when combined with microplate IC and selective plating on XLD.

Compared to the results of microplate IC and selective plating, a slightly lower detection rate of 93% (14/15) was observed for *S.* Infantis in dry dog food samples when microplate IC was combined with the 3M MDS. This method showed detection of *S.* Infantis in 100% of samples at levels down to 10^1 CFU/25 g; however, one of the three samples tested at 10^0 CFU/25 g could not be detected. Along these lines, the pig ear treats also showed a reduced detection rate of 90% when microplate IC was combined with the 3M MDS as compared to microplate IC and selective plating (100% detection rate). Microplate IC and 3M MDS showed 100% detection in *Salmonella* at levels at low as 10^1 CFU/25 g, but one of the two samples tested at 10^0 CFU/25 g could not be detected. Although the pig ear treats were tested in a series of three trials, data from the third trial could not be used due to the negative control testing positive for *Salmonella*. The results of the McNemar Test showed no significant differences ($p > 0.05$) between the detection rates in food samples for the 3M MDS when comparing microplate IC to traditional (24-h) enrichment. Consistent detection at 10^0 CFU/25g was anticipated to be difficult due to the combination of a low bacterial concentration with the small amount of sample utilized (20 µL)

for LAMP-BART with the 3M MDS. Similarly, Yang et al. (2015) was unable to detect *Salmonella* in 100% of produce samples inoculated at levels of 1.1-2.9 CFU/25g when combining a 6-8 h enrichment period with LAMP.

The overall rates reported here for detection of *S.* Infantis in food samples using microplate IC combined with the 3M MDS (90-93%) were greater than the rate reported by Fakruddin et al. (2017) for detection of *S.* Typhi in minced beef samples (62.7%) using microplate IC combined with PCR. Fakruddin et al. (2017) tested bacterial concentrations of 10^1 CFU/25 to 10^5 CFU/25 and reported detection of only 20% (3/15) of samples at the lowest concentration. In contrast, the current study reported the ability to detect *S.* Infantis at levels as low as 10^0 CFU/25 with rates of 50-66%. The greater detection rates reported in the current study may be due, in part, to the enhanced specificity and sensitivity of the 3M MDS as opposed to PCR. Additionally, the current study utilized a 3-h pre-enrichment period, a 2-h microplate incubation, and did not discard the sample from the microplate prior to testing as was done by Fakruddin et al. (2017).

The use of a 24-h enrichment period prior to the 3M MDS enabled *Salmonella* detection in 100% of the dog food samples (15/15) and pig ear treats (10/10) tested. These results are consistent with those reported by Yang et al. (2016), which found that after a 24-h period, the 3M MDS positively detected 1-3 CFU/ 25 g in dry dog food. Although the detection rates in the current study were greater after the 24-h enrichment, microplate IC coupled with 3M MDS allowed for *S.* Infantis to be consistently detected in food samples at levels down to 10^1 CFU/25 within one working day (8 h). Extending the pre-enrichment time to 4 h could potentially increase the sensitivity of this method while still allowing for detection within one working day.

3.3 Time to detection

In accordance with the aims of this study, the optimized conditions for testing of *S.* Infantis in TSB and dog food samples can be completed within one working day using microplate IC combined with 3M MDS. Testing of TSB samples required a total time to detection of 4.25 h with LAMP-BART and 27 h with XLD, while dog food samples required a total time to detection of 7.25 h with LAMP-BART and 30 h with XLD. These times include sample preparation (0.5 h), pre-enrichment (0-3 h), microplate IC (2 h), and LAMP-BART (1.75 h including DNA extraction) or XLD (24.5 h including transfer to plates). It is important to point out that the time to detection does not include the preparation of the media or the microplates because these materials can be prepared in bulk ahead of time. Microplate preparation requires 24 ± 2 h to incubate the antibody coating on each plate prior to freezing at -20° C for later use. A 2-h washing step is required immediately before use; however, this can be completed simultaneously with pre-enrichment period of food samples, keeping the time to detection within one working day (8 h). Despite the reduced time to detection, it is important to point out that the microplate IC method does require additional laboratory steps and hands-on work as compared to traditional enrichment.

4. Conclusions

Overall, the results of this study suggest that microplate IC combined with the 3M MDS or selective plating could be used to shorten the time to detection for *Salmonella* in food samples. Microplate IC followed by selective plating on XLD enabled consistent detection of *Salmonella* in all dog food and pet treat samples tested, including at levels of 10^0 CFU/25 g. This reduced the time to detection to 27 h, compared to 48 h using traditional enrichment combined with selective plating. Microplate IC coupled with the 3M MDS enabled detection of *Salmonella* in dog food and pet treat samples down to levels of 10^0 CFU/25 g, with overall detection rates of

90-93%. These results indicate that microplate IC combined with the 3M MDS can be used to detect *Salmonella* at low levels within 1 working day, as opposed to 2 days using a 24-h enrichment combined with the 3M MDS. However, further research is needed to verify the specificity, sensitivity and repeatability of the method using a range of food types and *Salmonella* strains.

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Author Contributions

D. Rosen conducted experimental optimization, full experimental testing, and drafted the manuscript. M. Gallardo conducted experimental optimization and experiment material preparation. M. Vail assisted in experiment material preparation. R. Hellberg designed the study, interpreted results, procured all experiment materials, and drafted the manuscript.

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