

Chapman University

Chapman University Digital Commons

Food Science Faculty Articles and Research

Science and Technology Faculty Articles and
Research

2-28-2020

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

Danielle K. Rosen
Chapman University

Miguel Gallardo
Chapman University

McClain Vail
Chapman University

Rosalee S. Hellberg
Chapman University, hellberg@chapman.edu

Follow this and additional works at: https://digitalcommons.chapman.edu/food_science_articles



Part of the [Aquaculture and Fisheries Commons](#), [Food Biotechnology Commons](#), [Food Microbiology Commons](#), [Food Processing Commons](#), and the [Other Food Science Commons](#)

Recommended Citation

Rosen, D.K., Gallardo, M., Vail, M., Hellberg, R.S. (2020). 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. *Journal of Microbiological Methods*, 172, 105881. <https://doi.org/10.1016/j.mimet.2020.105881>

This Article is brought to you for free and open access by the Science and Technology Faculty Articles and Research at Chapman University Digital Commons. It has been accepted for inclusion in Food Science Faculty Articles and Research by an authorized administrator of Chapman University Digital Commons. For more information, please contact laughtin@chapman.edu.

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

Comments

NOTICE: this is the author's version of a work that was accepted for publication in *Journal of Microbiological Methods*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *Journal of Microbiological Methods*, volume 172, in 2020.
<https://doi.org/>

The Creative Commons license below applies only to this version of the article.

Creative Commons License



This work is licensed under a [Creative Commons Attribution-Noncommercial-No Derivative Works 4.0 License](https://creativecommons.org/licenses/by-nc-nd/4.0/).

Copyright

Elsevier

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

4
5 **Authors:** Danielle K. Rosen, Miguel Gallardo, McClain Vail, and Rosalee S. Hellberg*

6
7 Chapman University, Schmid College of Science and Technology, Food Science Program, One
8 University Drive, Orange, CA USA 92866

9

10

11

12

13 ***Corresponding Author**

14 Rosalee S. Hellberg, Ph.D.

15 Ph: 714-628-2811

16 Email: hellberg@chapman.edu

17

18

19

20

21 **Declarations of interest: none**

22

23

24 **ABSTRACT**

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

38
39
40
41
42
43
44
45 **Keywords:** Microplate immunocapture; LAMP-BART; pet food; selective plating; *Salmonella*
46 *Infantis*

47 **1. Introduction**

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

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

91 A potential means for shortening the enrichment period for the detection of low
92 concentrations of *Salmonella* is through the use of microplate immunocapture (IC). Microplate
93 IC utilizes an antibody-coated microtiter plate to concentrate bacterial cells for greater detection
94 efficiency when used with PCR and/or selective plating (Arbault, Desroche, & Larose, 2014ab;
95 Fakruddin, Hossain, & Ahmed, 2017; Rogers, Calicchia, & Hellberg, 2018). Although
96 microplate IC is not as widely used as immunomagnetic separation, it is considerably less
97 expensive because it does not require production of antibody-coated beads. Previous studies have
98 reported use of microplate IC to concentrate bacterial cells for detection with PCR and/or
99 selective plating (Rogers et al. 2018; Fakruddin et al. 2017). For example, Fakruddin et al.
100 (2017) found that coupling microplate IC with PCR allowed for detection of *Salmonella* Typhi in
101 62.7% of food samples inoculated with concentrations of 10^1 - 10^5 CFU/25 g, as compared to 56%
102 detection for samples that underwent traditional enrichment plus PCR. Rogers et al. (2018) found
103 that microplate IC coupled with PCR could detect *L. monocytogenes* at levels of 10^0 , 10^2 , and
104 10^4 CFU/25g at rates of 88.9%, 94.4%, and 100% respectively, but microplate IC with selective
105 plating yielded 0% recovery at 10^0 CFU/25g and 44.4% at 10^2 CFU/25g. Rogers et al. (2018) did
106 not use a pre-enrichment step prior to conducting microplate IC and selective plating which
107 could explain the limited recovery of bacteria at low concentrations.

108 The goal of this study was to evaluate the use of microplate IC to reduce the enrichment
109 time required for detection of *Salmonella* in pet food with the 3M MDS and selective plating.
110 The specific aims of this study were to: 1) optimize the microplate IC parameters to enable
111 detection of *Salmonella* in dog food and treats within 1 working day (8 h) when combined with
112 LAMP-BART with the 3M MDS, 2) determine the ability of microplate IC combined with the
113 3M MDS or selective plating to consistently detect low levels of *S. Infantis* (10^0 - 10^4 CFU/25 g)

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

116 **2. Materials and methods**

117 **2.1. Bacterial isolation and preparation**

118 *Salmonella enterica* serovar Infantis ATCC® 51741 was obtained from American Type
119 Culture Collection® [(ATCC) (Manassas, VA)]. All media used in this study were from Becton,
120 Dickinson and Company [(BD) (Franklin Lakes, NJ)], unless otherwise specified. Bacterial
121 isolation was conducted by streaking the stock culture of *S. Infantis* onto tryptic soy agar (TSA)
122 and incubating for 48 h at 37°C. An isolated colony was transferred from TSA to 10 mL of
123 tryptic soy broth (TSB) and incubated at 37°C until the bacteria reached the desired
124 concentration of 10⁴ colony-forming units (CFU)/mL.

125 **2.2. Microplate preparation**

126 *Salmonella* Polyclonal Antibody PA1-7244 (Invitrogen™, Carlsbad, CA) was diluted to 1
127 µg/mL in carbonate-bicarbonate buffer, pH 9.6. The microplate was prepared according to a
128 protocol adapted from Abcam (<http://www.abcam.com/protocols/sandwich-elisa-protocol-1>).
129 First, 200 µL of the antibody solution were added to individual wells of a 96-well polystyrene
130 microtiter microplate separated into 8-well strips. The plate was covered with sterile
131 polyethylene sealing films (Excel Scientific, Victorville, CA) and incubated at 4°C for 24 ± 2 h.
132 Plates that were not used immediately were stored at -20°C with the antibodies in each well, and
133 then prepared according to the following procedure. The wells were washed twice with 200 µL
134 1X phosphate buffered saline [(PBS) (Fisher Scientific, Hampton, NH)]. Next, 200 µL of 5%
135 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 μ L 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 μ L 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 10⁰ to 10⁴ 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 ≥ 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 10⁰ CFU/25 g (≤ 2 colonies/plate).

^c Optimized conditions selected for further testing are indicated with gray shading.

188

189 2.5. 3M Molecular Detection System

190 Samples were tested on the 3M MDS using the 3M Molecular Detection Assay
 191 2 – *Salmonella* kit according to Protocol 2. For microplate IC samples, the microplate wells were
 192 scraped with an inoculating loop and transferred to a sterile tube containing the pre-mixed lysis
 193 solution. For the TSB trials, 8 wells were scraped, whereas only 1 well was scraped for the food
 194 samples (based on the results of microplate IC optimization). Next, 20 µL of the liquid portion of
 195 the sample was added to the same tube. For the 24-h enrichment samples, 20 µL of the liquid
 196 portion of the sample was added to each sample tube. For the negative control and reagent
 197 (positive) control for the 3M MDS, 20 µL of sterile, pre-warmed (35°C) BPW was added to the
 198 corresponding tubes of lysis solution. All lysis tubes were held in a dry heat block for 15 min at
 199 100°C and then cooled in a chilling block at ambient temperature for 5 min. Next, 20 µL of each
 200 sample or control was transferred to its corresponding reagent tube. The reagent control was
 201 provided with the 3M Molecular Detection Assay 2-*Salmonella* kit to serve as a positive control,

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

206 **2.6 Statistical analysis**

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

213 **3. Results and discussion**

214 **3.1. Microplate IC optimization**

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

221 When the optimized conditions for TSB were applied to dog food samples, *S. Infantis*
222 could not be detected at concentrations of 10^0 - 10^1 CFU/25 g (Table 2). Therefore, a short pre-
223 enrichment incubation (1.5-3 h) at 37°C was incorporated into the protocol prior to microplate
224 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	<i>Salmonella</i> <i>Infantis</i> 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%)

^aConcentration units are CFU/mL for TSB and CFU/25 g for dry dog food and pig ear treats.

^bMicroplate IC includes a 3-h pre-enrichment step.

^cData from the third trial of pig ear treats was not used because the negative control tested positive for *Salmonella*

248 In contrast to the current study, previous research has reported limited recovery of
249 foodborne pathogens when microplate IC was combined with selective plating (Rogers et al.,
250 2018; Fakruddin et al., 2017). Rogers et al. (2018) achieved 0% recovery of *Listeria* from cheese
251 and milk samples inoculated at 10^0 CFU/25 g, and only 44.4% recovery at 10^2 CFU/25 g.
252 Similarly, Fakruddin et al. (2017) detected *Salmonella* Typhi in only 13.1% of minced beef
253 samples inoculated at a level of 10^1 CFU/25 g. However, neither of the previous studies used a
254 pre-enrichment step prior to conducting microplate IC. The 3-h pre-enrichment step employed in
255 the current study likely provided sufficient time for *Salmonella* to grow to detectable levels when
256 combined with microplate IC and selective plating on XLD.

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

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

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

285 The use of a 24-h enrichment period prior to the 3M MDS enabled *Salmonella* detection
286 in 100% of the dog food samples (15/15) and pig ear treats (10/10) tested. These results are
287 consistent with those reported by Yang et al. (2016), which found that after a 24-h period, the
288 3M MDS positively detected 1-3 CFU/ 25 g in dry dog food. Although the detection rates in the
289 current study were greater after the 24-h enrichment, microplate IC coupled with 3M MDS
290 allowed for *S. Infantis* to be consistently detected in food samples at levels down to 10^1 CFU/25
291 within one working day (8 h). Extending the pre-enrichment time to 4 h could potentially
292 increase the sensitivity of this method while still allowing for detection within one working day.

293 **3.3 Time to detection**

294 In accordance with the aims of this study, the optimized conditions for testing of *S.*
295 *Infantis* in TSB and dog food samples can be completed within one working day using
296 microplate IC combined with 3M MDS. Testing of TSB samples required a total time to
297 detection of 4.25 h with LAMP-BART and 27 h with XLD, while dog food samples required a
298 total time to detection of 7.25 h with LAMP-BART and 30 h with XLD. These times include
299 sample preparation (0.5 h), pre-enrichment (0-3 h), microplate IC (2 h), and LAMP-BART (1.75
300 h including DNA extraction) or XLD (24.5 h including transfer to plates). It is important to point
301 out that the time to detection does not include the preparation of the media or the microplates
302 because these materials can be prepared in bulk ahead of time. Microplate preparation requires
303 24 ± 2 h to incubate the antibody coating on each plate prior to freezing at -20° C for later use.
304 A 2-h washing step is required immediately before use; however, this can be completed
305 simultaneously with pre-enrichment period of food samples, keeping the time to detection within
306 one working day (8 h). Despite the reduced time to detection, it is important to point out that the
307 microplate IC method does require additional laboratory steps and hands-on work as compared
308 to traditional enrichment.

309 **4. Conclusions**

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

317 90-93%. These results indicate that microplate IC combined with the 3M MDS can be used to
318 detect *Salmonella* at low levels within 1 working day, as opposed to 2 days using a 24-h
319 enrichment combined with the 3M MDS. However, further research is needed to verify the
320 specificity, sensitivity and repeatability of the method using a range of food types and
321 *Salmonella* strains.

322 **Acknowledgements**

323 This work was supported by the National Science Foundation, Division of Earth Sciences NSF-
324 EAR #1659892 and Chapman University, Schmid College of Science and Technology.

325 **Author Contributions**

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

330 **References**

331 Andrews, W.H., Wang, H., Jacobson, A., Hammack, T. (2016). *Salmonella*. Bacteriological
332 Analytical Manual (pp. 1-21).

333 Arbault, P., Desroche, N., Larose, D. (2014a). *14710330*. Methods for immunocapture and
334 concentration of bacteria in a sample.

335 Arbault, P., Larose, D., Nexidia, J.G. (2014b, April). *Microplate Immunocapture (IMC): A New*
336 *Solution for the Isolation/Concentration of Escherichia coli O157:H7 from Food*
337 *Samples*. Presented at the International Association for Food Protection Annual Meeting,
338 Indianapolis, IN.

339 CDC. (2017, September 27). Pet Food Safety. Retrieved from
340 <https://www.cdc.gov/features/salmonelladrypetfood/>

341 CDC. (2019, October 30). Outbreak of multidrug-resistant *Salmonella* infections linked to
342 contact with pig ear dog treats. Retrieved from [https://www.cdc.gov/salmonella/pet-](https://www.cdc.gov/salmonella/pet-treats-07-19/index.html)
343 [treats-07-19/index.html](https://www.cdc.gov/salmonella/pet-treats-07-19/index.html)

344 Fakruddin, M., Hossain, M.N., Ahmed, M.M. (2017). Evaluation of microplate immunocapture,
345 method for detection of *Vibrio cholerae*, *Salmonella* Typhi and *Shigella flexneri* from
346 food. *BMC Microbiology* 17(189), 1-9. <https://doi.org/10.1186/s12866-017-1099-y>

347 FDA. (2012). *Salmonella* species. In *Bad Bug Book, Foodborne Pathogenic Microorganisms and*
348 *Natural Toxins* (pp. 9-13).

349 FDA. (2018, September 5). Foodborne Illnesses: What You Need to Know. Retrieved from
350 <https://www.fda.gov/Food/ResourcesForYou/Consumers/ucm103263.htm>.

351 FDA. (2019, July 26). Recalls and Withdrawals. Retrieved from
352 <https://www.fda.gov/AnimalVeterinary/SafetyHealth/RecallsWithdrawals/default.htm>.

353 Gandelman, O.A., Church, V.L., Moore, C.A., Kiddle, G., Carne, C.A., Parmar, S., Jalal, H.,
354 Tisi, L.C., Murray, J.A.H. (2010). Novel bioluminescent quantitative detection of nucleic
355 acid amplification in real-time. *PLoS One* 5:e14155.
356 <https://doi.org/10.1371/journal.pone.0014155>.

357 Imanishi, M., Rotstein, D.S., Reimschuessel, R., Schwensohn, C.A., Woody Jr., D.H., Davis,
358 S.W., Hunt, A.D., Arends, K.D., Achen, M., Cui, J., Zhang, Y., Denny, L.F., Phan, Q.N.,
359 Joseph, L.A., Tuite, C.C., Tataryn, J.R., Behravesh, C.B. (2014) Outbreak of *Salmonella*
360 *enterica* serotype *Infantis* infection in humans linked to dry dog food in the United States

361 and Canada, 2012. *Journal of the American Veterinary Medical Association* 244(5), 545-
362 53. <https://doi.org/10.2460/javma.244.5.545>

363 Lambertini, E., Mishra, A., Guo, M., Cao, H., Buchanan, R.L., Pradhan, A.K. (2016). Modeling
364 the long-term kinetics of *Salmonella* survival on dry pet food. *Food Microbiology* 58:1-6.
365 <https://doi.org/10.1016/j.fm.2016.02.003>

366 Margot, H., Stephan, R., Guarino, S., Jagadeesan, B., Chilton, D., O'Mahoney, E., Iversen, C.
367 (2013). Inclusivity, exclusivity, and limit of detection of commercially available real-
368 time PCR assays for detection of *Salmonella*. *International Journal of Food Microbiology*
369 165(3), 221-26. <https://doi.org/10.1016/j.ijfoodmicro.2013.05.012>

370 Rogers, S.A., Calicchia, M., Hellberg, R.S. (2018). Concentration of *Listeria monocytogenes* in
371 skim milk and soft cheese through microplate immunocapture. *Journal of*
372 *Microbiological Methods* 153: 54-59. <https://doi.org/10.1016/j.mimet.2018.09.005>

373 Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones,
374 J.L., Griffin, P.M. (2011). Foodborne illness acquired in the United States-major
375 pathogens. *Emerging Infectious Diseases* 17(1), 7-15.
376 <https://doi.org/10.3201/eid1701.P11101>.

377 Wang, L., Shi, L., Alam, M.J., Geng, Y., Li, L. (2008). Specific and rapid detection of
378 foodborne *Salmonella* by loop-mediated isothermal amplification method. *Food*
379 *Research International* 41(1), 69-74. <https://doi.org/10.1016/j.foodres.2007.09.005>

380 Yang, Q., Wang F., Jones, K.L., Meng, J., Prinyawiwatkul, W., Ge, B. (2015). Evaluation of
381 loop-mediated isothermal amplification for the rapid, reliable, and robust detection of
382 *Salmonella* in produce. *Food Microbiology* 46(2015), 485-493.
383 <http://dx.doi.org/10.1016/j.fm.2014.09.011>

384 Yang, Q., Domesle, K.J., Wang, F., Ge, B. (2016). Rapid detection of *Salmonella* in food and
385 feed by coupling loop-mediated isothermal amplification with bioluminescent assay in
386 real-time. BMC Microbiology 16(112), 1-10. <https://doi.org/10.1186/s12866-016-0730-7>