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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https://doi.org/

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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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Declarations of interest: none
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 asymptptomatically 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).
The ability to test dog food products for *Salmonella* quickly and efficiently is essential to preventing salmonellosis in dogs and their handlers. The gold standard for bacterial detection is traditional culture-based methods; however, these methods are time-consuming, generally requiring at least 5 days for confirmed results (Andrews, Wang, Jacobsen, & Hammack, 2016).

Real-time polymerase chain reaction (PCR) is a commonly used method that can reduce the time to detection to 1-2 days and is sensitive to low levels of bacteria. However, it is susceptible to inhibitors commonly found in food products (Margot et al., 2013).

Loop-mediated isothermal amplification coupled with bioluminescent assay in real-time (LAMP-BART), as used by the 3M Molecular Detection System [(MDS) (St. Paul, MN)], is a novel, rapid method for pathogen detection that combines isothermal DNA amplification with bioluminescence detection (Gandelman et al., 2010; Yang, Domesle, Wang, & Ge, 2016). Isothermal DNA amplification does not require thermal cycling and has shown greater tolerance to assay inhibitors compared to PCR (Margot et al., 2013, Yang et al., 2016; Wang, Shi, Alam, Geng, & Li, 2008). Previous studies have reported LAMP and LAMP-BART to be precise, rapid, and sensitive for the detection of *Salmonella* in a variety of food products (Yang et al., 2015; Yang et al., 2016; Wang et al., 2008). Yang et al. (2015) found LAMP-BART was able to detect 1.1-2.9 CFU/25g of several different serovars of *Salmonella* in inoculated produce when paired with a 6-8 h enrichment period, whereas the same results were obtained with PCR after a 24-h pre-enrichment. Later, Yang et al. (2016) reported the ability to detect *Salmonella* Infantis in dry dog food at concentrations of $10^0$-$10^1$ CFU/25 when a 24-h enrichment step was used in combination with LAMP-BART. In the absence of the enrichment step, the detection limit was 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 10⁴ 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
temperature. Immediately before performing microplate IC, the blocking agent was removed and
the wells were washed twice more with 200 µL 1X PBS.

2.3. Microplate IC for broth samples

The experimental conditions for microplate IC of S. Infantis were first optimized in the
absence of a food matrix. The bacterial culture was prepared as described above, followed by
serial dilution in TSB to allow for concentrations of $10^0$ to $10^4$ CFU/ml. Then, 1.6 mL of each
dilution was distributed across an 8-well strip of the prepared microplate for a total of 200 µL of
sample per well. An un-inoculated broth sample was included as a negative control.

Optimization of microplate IC was conducted using an Eppendorf ThermoMixer® C (Hamburg,
Germany) using the minimum shaking speed of 300 rpm. The procedure was optimized for
incubation temperature (30-37°C), fill cycles (1-4), and cycle incubation time (30-120 min), as
shown in Table 1. Each fill cycle involved addition of 1.6 mL of bacteria to the corresponding 8-
well microplate strip, incubation and shaking on the Thermomixer for a given cycle incubation
time, and removal of the concentrated bacteria.

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

<table>
<thead>
<tr>
<th>Trial</th>
<th>Microplate incubation temperature (°C)</th>
<th>No. of fill cycles</th>
<th>Fill cycle incubation time (min)</th>
<th>Total incubation time (min)</th>
<th>Minimum detection on XLD (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>3</td>
<td>60</td>
<td>180</td>
<td>10^2</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>4</td>
<td>60</td>
<td>240</td>
<td>10^6</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>4</td>
<td>45</td>
<td>180</td>
<td>10^6</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>3</td>
<td>45</td>
<td>135</td>
<td>10^2</td>
</tr>
<tr>
<td>5</td>
<td>37</td>
<td>4</td>
<td>30</td>
<td>120</td>
<td>10^1</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>3</td>
<td>60</td>
<td>180</td>
<td>10^2</td>
</tr>
<tr>
<td>7^a</td>
<td>37</td>
<td>1</td>
<td>120</td>
<td>120</td>
<td>10^6</td>
</tr>
</tbody>
</table>

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

2.4. Microplate IC for food samples

An 11-kg bag of chicken-flavored, dry dog food and a 4.5-kg bag of pig ear dog treats were purchased from a local retail outlet in Orange, CA. The dog food products were confirmed negative for the presence of Salmonella according to the conventional culture method described in the Bacteriological Analytical Manual (BAM) (Andrews et al., 2016). A bacterial culture of S. Infantis was prepared as described above, followed by serial dilution in buffered-peptone water (BPW). Dog food/treat samples (25 g) were spot-inoculated with S. Infantis with concentrations of 10^0 to 10^4 CFU/25g. An un-inoculated sample was used as a negative control for each trial. The samples were dried in sterile plastic bags inside a biosafety cabinet for 2 h at room temperature, then 225 mL of pre-warmed (35°C) BPW was added to each sample. The inoculated dog treats were mixed by swirling and the dry dog food samples were homogenized in...
Microplate IC optimization for dog food samples included testing of short pre-enrichment periods (0-3 h) at 37°C prior to running microplate IC. Additionally, the effectiveness of scraping only 1 well of the microplate for each sample was compared to the effectiveness of scraping 8 wells. After microplate IC was completed, the well(s) of the microplate were scraped with a sterile inoculating loop and streaked to XLD agar. The plates were examined for typical Salmonella growth after incubation for 18-24 h at 37°C. The procedure was considered to be successful if all bacterial concentrations were confirmed by growth on XLD. The duration of the pre-enrichment period was selected based on the shortest period that consistently produced more ≥ 3 colonies at the lowest bacterial concentration (10^0 CFU/25 g). The optimized conditions for dog food were used for pig ear treats without any further optimization. The optimized procedure (Table 2) was tested in three independent trials with both XLD agar and the 3M MDS (described below) for dog food and treat samples. The inoculated dog food/treat samples were also tested three times using a traditional 24-h enrichment in BPW at 37°C (Yang et al. 2016), with no microplate IC step. After the 24-h enrichment, each sample was streaked onto to XLD agar and 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.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Pre-enrichment time (min)</th>
<th>No. of microplate wells used</th>
<th>Microplate incubation temperature (°C)</th>
<th>No. of fill cycles</th>
<th>Fill cycle incubation time (min)</th>
<th>Total pre-enrichment + incubation time (min)</th>
<th>Minimum detection on XLD (CFU/25 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>8</td>
<td>37</td>
<td>1</td>
<td>120</td>
<td>120</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>8</td>
<td>37</td>
<td>1</td>
<td>120</td>
<td>240</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>1</td>
<td>37</td>
<td>1</td>
<td>120</td>
<td>240</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>1</td>
<td>37</td>
<td>1</td>
<td>120</td>
<td>210</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>180</td>
<td>1</td>
<td>37</td>
<td>1</td>
<td>120</td>
<td>300</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Pre-enrichment at 37 °C.

<sup>b</sup> Minimal growth observed for trials 2-3 at 10<sup>9</sup> CFU/25 g (≤ 2 colonies/plate).

<sup>c</sup> 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
Salmonella in dog food as low as $10^1$ CFU/25g using XLD agar (Table 2), while a pre-
enrichment period of 2-3 h followed by microplate IC enabled detection of Salmonella in dog
food at the lowest level tested ($10^0$ CFU/25g). While a 2-h pre-enrichment period yielded
positive results at concentrations of $10^0$ CFU/25g, only 1-2 colonies were observed on each plate,
as opposed to $\geq 5$ colonies per plate when a 3-h pre-enrichment period was used. Therefore, the
3-h pre-enrichment period was selected for testing of food samples.

The TSB trials were completed using 8 wells of the microplate for each concentration,
but later optimization with dog food showed that scraping only 1 well of the microplate resulted
in comparable growth on XLD. These results were unexpected, as it was thought that there
would be a greater chance of capturing bacteria if more wells were scraped. However, the use of
a 3-h pre-enrichment step combined with the 2 h microplate incubation time likely increased the
number of bacteria sufficiently to enable detection based on just one microplate well.

3.2. Microplate IC trials

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

<table>
<thead>
<tr>
<th>Matrix</th>
<th><em>Salmonella Infantis</em> concentration&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Rate of detection (no. positive samples/total no. samples)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Microplate IC&lt;sup&gt;b&lt;/sup&gt; + XLD</td>
<td>24-h enrichment + XLD</td>
<td>Microplate IC + LAMP-BART</td>
</tr>
<tr>
<td>TSB</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3/3</td>
<td>N/A</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3/3</td>
<td>N/A</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3/3</td>
<td>N/A</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3/3</td>
<td>N/A</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;0&lt;/sup&gt;</td>
<td>3/3</td>
<td>N/A</td>
<td>3/3</td>
</tr>
<tr>
<td>Overall</td>
<td>15/15 (100%)</td>
<td>N/A</td>
<td>15/15 (100%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Dry dog food</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
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<tr>
<td></td>
<td>10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
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<tr>
<td></td>
<td>10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
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<td>3/3</td>
<td>2/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Overall</td>
<td>15/15 (100%)</td>
<td>15/15 (100%)</td>
<td>14/15 (93%)</td>
<td>15/15 (100%)</td>
</tr>
<tr>
<td>Pig ear treats</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3/3</td>
<td>3/3</td>
<td>2/2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3/3</td>
<td>3/3</td>
<td>2/2</td>
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<tr>
<td></td>
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<tr>
<td></td>
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<td>3/3</td>
<td>1/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Overall</td>
<td>15/15 (100%)</td>
<td>15/15 (100%)</td>
<td>9/10 (90%)</td>
<td>10/10 (100%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Concentration units are CFU/mL for TSB and CFU/25 g for dry dog food and pig ear treats.

<sup>b</sup>Microplate IC includes a 3-h pre-enrichment step.

<sup>c</sup>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/25 g 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 $10^9$ 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 $10^9$ 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.

**Acknowledgements**

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

**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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