

## Detection of Different Serotypes of *Salmonella enterica* in Experimentally Inoculated Equine Fecal Samples by Commercially Available Rapid Tests

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**Background:** *Salmonella enterica* can significantly impact management of animal facilities. Comprehensive screening is essential for effective control in high-risk populations. Availability of reliable point-of-care diagnostic tests would facilitate these efforts.

**Hypothesis/Objectives:** Compare the ability of commercially available rapid diagnostic assays (2 lateral flow immunoassays [LFIs], DNA hybridization [DNAH], real-time PCR [qPCR]), and culture to detect common serotypes of *S. enterica* in feces.

**Animals:** n/a.

**Methods:** In an experimental study, 112 *S. enterica* isolates were randomly selected from the 10 most common serotypes recovered at a veterinary hospital. Archived isolates were amplified in broth and standardized inocula (100 colony forming units) were incubated with equine feces in tetrathionate broth (TET). Cultures were tested in a blinded fashion by using LFIs, DNAH, qPCR, and culture.

**Results:** The LFIs detected 84% and 67% of isolates, respectively, but reactivity varied among serotypes. Both reacted poorly with serotype Cerro (Group K) isolates, and 1 LFI did not react with any serotype Mbandaka (Group C1) or Montevideo (Group C1) isolates. DNAH detected 94% of isolates, whereas culture and qPCR most reliably detected all serotypes. False-positive results were obtained for 4 negative controls by using DNAH and 1 negative control by using qPCR, but LFIs and culture had no false-positive results.

**Conclusions and Clinical Importance:** Culture, qPCR, and DNAH were effective in detecting most *Salmonella* isolates, but have limited application at point-of-care settings. LFIs are appealing as point-of-care tests because of low cost and ease of use, but limited detection of some serotypes needs to be evaluated with samples obtained from naturally infected animals.

**Key words:** Culture; Horse; Lateral flow immunoassay.

**S**almonella enterica can have a significant impact on the management of animal facilities. In a survey of accredited Veterinary Teaching Hospitals, *S. enterica* was reported to be the most common cause of outbreaks of nosocomial infections at equine hospitals with 71% of affected facilities restricting admissions and 39% closing completely to aid mitigation efforts.<sup>1</sup> Comprehensive screening and rapid detection of *S. enterica* in fecal and environmental samples are extremely important aids for effective control of this agent in high-risk animal populations (ie, populations which congregate at veterinary hospitals, boarding facilities, and equestrian events). Unfortunately, there are limitations with the most common detection methods (aerobic culture and real-time polymerase chain reaction [qPCR]) including cost, limited sensitivity, time needed to obtain results, and

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### Abbreviations:

BPW	buffered peptone water
cfu	colony forming units
CSU-VTH	Colorado State University Veterinary Teaching Hospital
Ct	cycle threshold
DNAH	DNA hybridization
LFIs	lateral flow immunoassays
OD	optical density
qPCR	real-time PCR
TET	tetrathionate
TSA	trypticase soy agar with 5% sheep blood
USDA	United States Department of Agriculture
XLT4	xylose-lysine-tergitol 4

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laborious testing methodologies. These factors limit availability of tests and limit the ability of veterinary practitioners to employ comprehensive testing and surveillance programs in at-risk animal populations. With these limitations in mind, we propose that an ideal test for the rapid detection of *S. enterica* in field applications may include the following: (1) availability as a point-of-care test, (2) minimal need for expensive equipment and specialized training, (3) providing results within 24 hours, (4) applicable in a variety of settings and regions, and (5) be applicable for use with specimens relevant to clinical settings (ie, fecal and environmental samples in equine practice).

There are several commercially available assays that are marketed in North America for rapid detection of *S. enterica* in food safety and other applications, including lateral flow immunoassays (LFIs),<sup>2,3</sup> DNA

hybridization (DNAH) assays,<sup>4</sup> and real-time PCR (qPCR) assays.<sup>5</sup> However, sample matrices relevant to food safety generally have a low background bacterial burden. In contrast, equine feces has high numbers of background bacteria and other substances (eg, PCR inhibitors) that can interfere with target organism detection, especially when hosts are shedding very low numbers of *Salmonella* bacteria. In prior investigations, we identified an optimized culture technique for use with LFIs which allows for the detection of ~4 cfu/g in experimentally inoculated equine fecal samples within 24 hours.<sup>6</sup> However, limited preliminary assessment of LFI assay reactivity with 1 strain (isolate) from each of 5 different *S. enterica* serotypes (Typhimurium—serogroup B; Montevideo—serogroup C1; Newport—serogroup C2; Muenster—serogroup E1; Cerro—serogroup K) suggested that the performance of LFIs might vary by strain, serotype, or both (results not shown). Variable ability to detect different *Salmonella* serotypes would be an important factor when considering use in veterinary practices.

Given the ease of use, low cost, and the potential utility LFIs demonstrated in prior investigations,<sup>6</sup> we believe they may have greater applicability in practice than other commercially available rapid diagnostic tests such as DNAH and qPCR. However, thus far, it has not been shown that LFIs are more or less useful than DNAH, qPCR, or standard culture at detecting variable *S. enterica* serotypes. Therefore, the goal of this study was to evaluate variability in the ability to detect a variety of different clinical isolates of *S. enterica* by using 4 different commercially available rapid tests (2 LFIs, a DNAH test, and a qPCR assay) and aerobic culture when inoculated into equine feces.

## Materials and Methods

### Study Summary

The ability to detect different strains of *S. enterica* was evaluated by using 4 commercially available rapid diagnostic testing systems (2 different LFIs, DNA hybridization, and real-time qPCR), and results were compared to aerobic culture. A total of 112 isolates were randomly selected from archived strains of the 10 most commonly isolated serotypes that had been recovered in a veterinary hospital. Standardized inocula were incubated with broth media and equine feces at a concentration which is consistent with low-level shedding in naturally infected horses and then tested using all tests. An additional 25 equine fecal samples that were not inoculated with *Salmonella* served as negative controls that underwent identical processing to aid in blinding. Random assignment of identification numbers to all samples and blinding were used to ensure that investigators were not aware of isolate identification when classifying test results. This study was approved by the Colorado State University Institutional Animal Care and Use Committee, and owner consent was obtained to collect samples that yielded *S. enterica* isolates and equine feces used in this study.

### Commercially Available Rapid Tests

Four commercially available rapid diagnostic testing systems were used in this study: 2 LFIs (LFI-A, Reveal for *Salmonella* Test System<sup>a</sup> and LFI-B<sup>b</sup>), a DNAH test (GeneQuence for

*Salmonella*<sup>a</sup>), and a real-time PCR assay (MicroSEQ *Salmonella* spp. Detection Kit).<sup>c</sup> Both of these LFIs are sold in a small strip format, employing gold-labeled antibody specific to *S. enterica* subsp. *enterica*. As the broth cultures flow through the test strip, an antibody-coated colloidal gold reagent, which provides a visible signal in the test, is rehydrated. If *Salmonella* antigens are present in the sample, they bind to the antibody-gold conjugate forming an antigen-antibody complex that is subsequently captured by a zone of anti-*Salmonella* antibody on the test strip forming a visible line (the sample test line). A second zone captures antibody-gold conjugate that is not bound in the first zone, thus forming a second visible line on the test strip (the control line). The DNA hybridization test is a multistep test which lyses bacteria in the broth cultures, allowing the ribosomal RNA to bind to a horseradish peroxidase-labeled indicator probe and a capture probe specific for *S. enterica*. The presence of *Salmonella* will result in a color change which is measured as a change in optical density (OD) by means of a 96-well plate reader. The qPCR test amplifies and detects a *S. enterica*-specific DNA sequence with specialized thermocycler equipment which contains sensors for measuring the fluorescence of the probe that is released during polymerization of DNA primers. The presence of *Salmonella* in the sample is determined based on a threshold level of detection which is reached during the exponential phase of DNA amplification.

### *Salmonella* Isolates Used in Testing

*Salmonella* isolates used in this study were randomly selected from all isolates that had been recovered and archived as part of long-term surveillance conducted at the Colorado State University Veterinary Teaching Hospital (CSU-VTH). Briefly, in addition to testing clinical suspects, active surveillance has been used to identify subclinical *Salmonella* shedding in large animal inpatients hospitalized at the CSU-VTH. Further, environmental samples are collected monthly from approximately 60 sites to identify environmental contamination that might be present in the Veterinary Teaching Hospital. Isolate serotyping was conducted at the USDA National Veterinary Services Laboratory (Ames, IA) and susceptibility testing, by the Kirby-Bauer disk diffusion method, was performed by the Colorado State University Veterinary Diagnostic Laboratory (Fort Collins, CO) (Table 1). Since 2002, more than 1,300 different *Salmonella* isolates have been collected and archived at -80°C. A formal random process was used to select 112 isolates from the 10 most common serotypes that have been saved in this archive since 2002 (these serotypes represent approximately 80% of all isolates in the archive). The sample size was selected arbitrarily. Probability sampling was used for selection of individual isolates with a maximum of 15 and a minimum of 5 isolates selected per serotype. Thus, serotypes with greater representation in the archive had greater probability of selection, but each isolate had an equal probability of selection (a simple random sample). A random number generator was used to assign a unique number to each isolate for selection and testing purposes. Only isolates from these 10 serotypes were eligible for selection, and isolates included in this study were limited to 1 per animal and only 1 unique isolate from the same environmental sampling date.

### *Salmonella* Serotypes Tested

Isolates included in the study included Serogroup B: Typhimurium (n = 10), Typhimurium var. 5- (formerly Typhimurium var Copenhagen; n = 12); Serogroup C1: Mbandaka (n = 9), Montevideo (n = 15); Serogroup C2: Muenchen (n = 5), Newport (n = 10); Serogroup C3: Kentucky (n = 9); Serogroup E:

**Table 1.** Phenotypes of isolates used for inoculation of equine feces used in testing commercially available rapid tests and aerobic culture.

Resistance Pattern	Serogroup B		Serogroup C1			Serogroup C2		Serogroup C3		Serogroup E1		Serogroup K
	Typhimurium	Typhimurium var. 5-	Mbandaka	Montevideo	Muenchen	Newport	Kentucky	Meleagridis	Muenster	Cerro		
No Resistance	2		3	12	3	2	5	14	11	10		
Amo, Amp, Cefi, Ceph, Chlor, Str, Sul, Tet	4	2				8	1			2		
Amo, Amp, Chlor, Str, Sul, Tet	2	3										
Amp, Chlor, Str, Sul, Tet	2								1			
Amp, Chlor, Str, Sul, Tet, Tri	1	2										
Amp, Str, Sul, Tet	1	3										
Amik, Amp			2								1	
Str, Sul, Tet			1									
Sul, Tet			2	3	2		1	1				
Sul			1				2		1			
Tet			9	15	5	10	9	15	13	14		
Total	10	12										

Amik, amikacin; Amo, amoxicillin; Amp, ampicillin; Cefi, ceftiofur; Ceph, cephalothin; Chlor, chloramphenicol; Str, streptomycin; Sul, sulfonamide; Tet, tetracycline; Tri, trimethoprim-sulfamethoxazole. Susceptibility determined by Kirby-bauer disk diffusion.

Meleagridis (n = 15), Muenster (n = 13); and Serogroup K: Cerro (n = 14) (Table 1). Seventy-four isolates included in these evaluations were originally recovered from animal feces (including 63 that were isolated from cattle, 8 from horses, and 3 from New World camelids) and 38 isolates were originally recovered from environmental samples (including 19 recovered from the Live-stock Hospital, 12 from the Equine Hospital, and 7 from core facilities or the Small Animal Hospital).

**Salmonella Isolate Testing Using Lateral Flow Immunoassays and a DNA Hybridization Test**

Archived *Salmonella* isolates stored in glycerol solution at -80°C were thawed and streaked for isolation on tryptic soy agar plates with 5% sheep blood (TSA).<sup>d</sup> For each isolate, a standardized inoculating dose of approximately 100 cfu/mL was developed by first creating a stock solution using sterile saline with approximately 10<sup>8</sup> cfu/mL based upon OD (equivalent to a 0.5 McFarland standard), then performing 10-fold dilutions to achieve the desired concentration of approximately 100 cfu/mL of solution. The concentration of *Salmonella* in these samples was confirmed by enumerating colony counts after plating each dilution on TSA and incubating at 43°C for 18 hours. A composite pool of feces was used in testing all samples to provide a uniform sample matrix and background microbiome. This was created by collection of approximately 100 g of feces from each of 5 adult horses that were part of an isolated horse herd (housed in a dry paddock and having a history of being culture-negative for *Salmonella* with periodic testing), and mixed thoroughly to create 1 composite fecal pool. Uninoculated fecal samples were also tested to ensure that the original fecal pool was negative for *Salmonella*, or at least had a very low likelihood of containing detectable quantities of this target bacterium.

For each isolate, approximately 100 cfu in 1 mL culture broth and a 1 g sample of the composite fecal pool were added to 9 mL tetrathionate broth (TET)<sup>e</sup> and incubated at 43°C for 18 hours.<sup>6</sup> After incubation, samples of TET broth were evaluated in parallel by using the LFI-A and LFI-B. Both LFIs have a test line and control line; for the purposes of this study, results were classified as positive if the test line was at least as intense (ie, darkly colored) as the control line on the test strip.

In addition, because the color of TET broth interferes with evaluation of OD, 1 mL of enriched sample was inoculated into 9 mL buffered peptone water (BPW) and incubated at 43°C for an additional 6 hours before evaluation with the DNAH test. The OD of each sample was determined with an automated plate reader at 450 nm, with a positive test defined as an absorbance value ≥ 0.10, per manufacturer’s recommendation.

**Salmonella Isolate Testing Using Aerobic Culture**

All TET and BPW broth cultures that were previously described were plated on xylose-lysine-tergitol 4 (XLT4)<sup>f</sup> agar plates and incubated at 43°C for 18–24 hours. In addition, to help rule-out the possibility of contamination, 10% of isolates recovered from BPW culture broth were randomly selected for serogroup confirmation using commercially available grouping antisera. A 1 mL aliquot of all TET broth cultures was frozen at -80°C, then thawed and recultured on the day that qPCR testing was performed (within 2 weeks of being frozen).

**Salmonella Isolate Testing Using Real-time PCR**

For logistical reasons, samples were batch tested by qPCR after testing samples with the other rapid tests (within 2 weeks).

As described, aliquots of all TET cultures were frozen at  $-80^{\circ}\text{C}$ . After thawing, samples were processed using a commercially available DNA extraction kit (PrepSEQ Nucleic Acid Extraction Kit<sup>c</sup>) and qPCR kit<sup>c</sup> per manufacturer's directions.<sup>5</sup> This qPCR utilizes an internal positive control for inhibition detection. A positive qPCR result was defined as a test having a cycle threshold (Ct) number  $\leq 35$ . If results suggested that PCR inhibition had occurred, as evidenced by a negative sample Ct value in conjunction with a negative internal positive control Ct value, extracted DNA samples were diluted 1 : 10 in PCR-grade water and retested, based on manufacture's recommendation. Thawed TET broth cultures were also recultured on the day that qPCR testing was performed to help confirm that frozen samples were comparable to those previously tested with the other rapid tests.

### Uninoculated Control Samples

Twenty-five 1-g aliquots of the pooled feces were not inoculated with *Salmonella*, but were processed in a manner identical to that described for testing *S. enterica* isolates. Inclusion of these uninoculated control samples served several purposes, including aiding in the blinding process, allowing assessment of false-positive rates for the assays, and to confirm that the pooled feces was actually free of *Salmonella* before laboratory inoculation.

### Data Analysis

After recording interpretations for all tests, randomly assigned study identification numbers were decoded, results were tabulated, and descriptive statistics calculated. While traditional, reference-based estimates of sensitivity and specificity might be calculated, these estimates would lack external validity because of the use of experimentally inoculated samples and therefore were not determined.

## Results

Overall, all experimental and control samples were correctly identified by at least 1 of the 4 commercially available tests evaluated in this study (Table 2). Of 112 *Salmonella* isolates tested, 39% ( $n = 44$ ) were negative on at least 1 rapid test (28 were negative on 1 test, 15 negative on 2 tests, and 1 isolate [serotype Cerro] was negative on 3 tests; Table 2). LFI-A detected 84% of isolates and LFI-B detected 67%, but reactivity varied among serotypes. A majority of the misclassification was related to isolates from 2 serogroups. Both immunoassays reacted poorly with serotype Cerro (serogroup K) isolates, and LFI-B did not react with any serotype Mbandaka (serogroup C1) or Montevideo (serogroup C1) isolates (Table 2). DNAH detected 94% of isolates, and qPCR detected 99% of isolates tested. Culture most reliably detected *Salmonella* of all serotypes, as all samples of BPW were culture positive, as were all TET broth samples that were cultured on 2 occasions (at the time of original processing and also after thawing frozen aliquots of the original enrichments). When isolates were not detected with 2 tests, 86% (12/14) of the time they were undetected with both LFIs, and isolates that were not detected with  $\geq 2$  tests were most commonly serotypes Cerro (9 isolates) or Montevideo (4 isolates). When comparing to aerobic culture results, 4 uninoculated control samples were test positive (false positive) when evaluated with DNAH and 1 control sample had false-positive results by using qPCR, but there were no false-positive results with the LFIs.

One uninoculated control sample was test positive on both LFIs, DNAH, qPCR, and on culture. The iso-

**Table 2.** Results of testing equine fecal samples experimentally inoculated with different *Salmonella enterica* isolates using commercially available rapid tests and aerobic culture.

Serogroup	Serotype	Total Isolates	Positive Test Results						
			LFI-A <sup>a</sup>	LFI-B <sup>b</sup>	DNAH <sup>c</sup>	qPCR <sup>d</sup>	TET Culture 1 <sup>e</sup>	TET Culture 2 <sup>e</sup>	BPW Culture <sup>f</sup>
B	Typhimurium	10	10	10	9	10	10	10	10
	Typhimurium var. 5-	12	12	11	12	12	12	12	12
C1	Mbandaka	9	8	0	9	8	9	9	9
	Montevideo	15	13	0	12	15	15	15	15
C2	Muenchen	5	5	5	5	5	5	5	5
	Newport	10	9	10	10	10	10	10	10
C3	Kentucky	9	7	9	7	9	9	9	9
E1	Meleagridis	15	14	14	15	15	15	15	15
	Muenster	13	12	13	13	13	13	13	13
K	Cerro	14	4	3	13	14	14	14	14
Total		112	94	75	105	111	112	112	112

Inoculating dose and culture method:  $\sim 100$  cfu in 1 g of feces with 9 mL of TET broth, incubated for 18 hours at  $43^{\circ}\text{C}$ .

BPW, buffered peptone water; DNAH, DNA hybridization; TET, tetrathionate.

<sup>a</sup>Reveal® for *Salmonella* Test System, Neogen® Corporation, Lansing, MI.

<sup>b</sup>RapidChek® SELECT™ *Salmonella* Test System, SDIX, Newark, NE.

<sup>c</sup>GeneQuence® for *Salmonella*, Neogen® Corporation, Lansing, MI.

<sup>d</sup>MicroSEQ® *Salmonella* spp. Detection Kit, Applied Biosystems™, Carlsbad, CA.

<sup>e</sup>Culture performed on different aliquots of same TET enrichment, plating on XLT-4 agar that was incubated for 24 hours at  $43^{\circ}\text{C}$ .

<sup>f</sup>Aliquots of TET enrichment were incubated in BPW for 6 hours at  $43^{\circ}\text{C}$ , then plated on XLT-4 agar and incubated for 24 hours at  $43^{\circ}\text{C}$ .

late recovered from this culture was phenotypically evaluated (serotype and susceptibility profile) and found to match an environmental isolate included in the study. Evaluation of unique sample identification numbers and processing sequence allowed determination that the sample inoculated with this environmental isolate was handled immediately before processing of the uninoculated control sample. Thus, these results suggest that this individual sample was inadvertently contaminated during the study. The remaining 24 uninoculated control samples were culture negative on 2 occasions, and tested negative with both LFIs and qPCR, suggesting that horses that provided feces used in this study were truly uninfected and that cross-contamination was not a significant issue in this study. Evaluation of the serogroup for 10% (12/112) of isolates randomly selected from those recovered from BPW broth cultures found that all 12 isolates matched the phenotype of isolates that were used to inoculate fecal samples, suggesting that contamination was unlikely to have complicated interpretation of positive test results from inoculated samples.

## Discussion

The 4 rapid tests that were evaluated in this study were able to reliably detect the most common serotypes that have been recovered from large animal inpatients and their housing environment at the CSU-VTH by using a 24-hour enrichment technique with experimentally inoculated equine feces. Lateral flow immunoassay-A appears to have the greatest promise for point-of-care testing in equine practice, when considering all factors (eg, cost, ease of use, applicability in field settings, and reliability). Culture and qPCR were effective at detecting most *Salmonella* isolates, but these tests have limited potential for point-of-care use in private practice settings. These tests are also technically challenging to perform without specialized training. In addition, the DNAH and qPCR tests require relatively expensive, specialized equipment, as compared to aerobic culture. While costs for materials and labor used to conduct aerobic culture are modest, they are substantial for DNAH and qPCR relative to the number of tests that need to be performed in a comprehensive surveillance system.

In contrast, immunoassays can be easily adapted for use as rapid point-of-care tests, and tests similar to LFI-A and LFI-B have been marketed for use in clinical practice and even as over-the-counter diagnostics sold for human health conditions. Subjectively, the LFIs were much easier to perform than any of the other rapid tests or culture. The method that was developed for use of these tests in veterinary practice is simple, and does not require extensive training or specialized equipment other than a modest one-time purchase of a small incubator.<sup>6</sup> High-quality culture media (TET broth) can be purchased in 10-mL volumes that would be ready to use with 1-g fecal samples as demonstrated in this study. All other materials (including disposable pipettes and vials) are

sold with the test kits. The kits are relatively inexpensive, and personnel time needed to conduct testing with the LFIs is quite reasonable (15 minutes for LFI-A and 10 minutes for LFI-B). Combined, these factors make LFIs a practical alternative to other methods of *Salmonella* testing in veterinary practice settings as they are easy to use and relatively inexpensive.

Limited detection of some *Salmonella* serotypes is an important consideration regarding the practical utility of LFIs in clinical practice. While isolates of several serotypes were consistently detected with the LFIs used in this study, some strains were less reliably detected, especially with LFI-B. These findings confirm our preliminary observations that serotypes can have different reactivity in commercial immunoassays. As isolates tested were randomly selected from an isolate bank derived from long-term surveillance, we believe it likely that selected isolates represent different strains, although this was not confirmed through genetic evaluation. To our knowledge, variable ability of these tests to detect different serotypes has not been previously reported, even in literature regarding use of these products in food safety applications.<sup>2-4</sup> This may be due in part to differences in application and also in test interpretation. In food safety applications, manufacturers' of the LFIs used in this study recommend interpreting any visible color at the sample test line, no matter how intense, as indication of a positive test result. In previous work optimizing the culture method and interpretation of these LFIs for use in veterinary applications, we found that to minimize the false-positive rate, the color of the sample test line should be as intense as the control test line.<sup>6</sup> This method of interpretation not only improved test specificity in samples relevant to veterinary medicine but also provides an internal reference for classifying test results. This difference may also be related to differences in background microbiome, as sample matrices tested in food safety applications have relatively low background microbial contamination which is quite different from animal feces which can contain  $10^{10}$ – $10^{11}$  bacteria per gram.<sup>7</sup>

This variability in detection of different serotypes is critically important to implementation of LFIs in clinical veterinary practice as their practical value will depend upon which serotypes are most likely to be detected in a particular population or region. Cumulatively, from 2009 to 2011, the USDA National Veterinary Services Laboratory reported that equine isolates most commonly submitted for serotyping included (in descending order of frequency): Javiana (group D1), Typhimurium (B), Newport (C2), Braenderup (C1), Anatum (E), Infantis (C1), 4,5,12:i:- (B), Typhimurium var 5- (B), Muenchen (C2), and Mbandaka (C1).<sup>8-10</sup> These 10 serotypes represent about 65% (1367/2069) of all isolates submitted to USDA-NVSL during those 3 years. Five of these serotypes are included in the 10 serotypes that were selected for inclusion in this study based upon frequency of recovery at the CSU-VTH, which is a referral hospital for all species of animals.

Because of differences in recovery in different regions as well as the frequency of *Salmonella* shedding in dairy cattle at the time they are admitted to this hospital, complete overlap in the 2 lists is not expected. It is relevant to note the particularly poor recognition of C1 strains when tested with LFI-B, and serotype Cerro was not detected well by either of the LFIs. Serotype Cerro has become a predominant strain recovered from cattle, but this trend has not been mirrored in other species, thus would not be a serotype generally expected to be isolated from horses.<sup>11–13</sup> At this time, we are making no recommendations for the use of LFIs on cattle feces for the detection of *S. enterica*. Preliminary investigations found that both LFIs used in this study showed very poor specificity when used to detect *Salmonella* in feces collected from dairy and feedlot cattle that were being fed high concentrate rations (results not shown). However, if used in equine populations, LFI-A appears to have better reliability, though it will be important to test this assumption in practice using specimens from naturally infected horses. This becomes critically important as the distribution of serotypes has been shown to vary by geographic location and can change over time in the same geographic location.<sup>14,15</sup>

Overall, there was a low occurrence of false-positive test results. Both LFIs had correct results for all negative control samples and also correctly detected *Salmonella* in 1 negative control sample that was contaminated by an isolate with the same phenotype as an environmental isolate being used in this test assessment. However, both the DNAH and qPCR tests had lower specificity given the 4 false-positive test results and 1 false-positive test result, respectively. While aerobic culture results were repeatedly negative, increasing our confidence that these samples did not contain viable *Salmonella*, we cannot rule-out the possibility that these samples may have contained DNA fragments or nonviable organisms which resulted in a positive test.

While this study evaluated the occurrence of false-positive and false-negative test results under realistic conditions that mimicked an appropriate sample matrix and background microbiome, it is not possible to obtain a relevant estimation of test accuracy from this study. A more valid evaluation of the diagnostic (epidemiologic) sensitivity and specificity would require application of tests as they would be employed in practice using specimens from a variety of naturally infected and uninfected animals, as opposed to inoculating a standard number of cfu from laboratory amplified strains into a common pool of feces. Test sensitivity and specificity will need to be evaluated for these rapid tests using studies that are relevant to practice settings before the true value to veterinarians can be fully understood.

Results of this study suggest that LFIs could be useful alternatives to traditional aerobic culture, DNAH, and qPCR methods for detection of *Salmonella*. As discussed, culture, qPCR, and DNAH have significant limitations which restrict their ability to be used as point-of-care tests in veterinary practice. In addition,

current pricing suggests that LFIs could be utilized in practice to obtain test results within 24 hours for approximately 3–5 times lower cost per test. Even if limitations in serotype reactivity for LFIs lead to some false-negative test results, lower costs per test would allow testing of more total samples which should improve the overall sensitivity of the surveillance system. However, it is important that aerobic culture be part of any surveillance system for *Salmonella* to provide phenotypic and genotypic information that inform us about the epidemiology of infections and antimicrobial susceptibility that can affect treatment decisions for patients. Thus, it is recommended that any LFI test-positive sample be submitted to a veterinary diagnostic laboratory for culture to allow phenotypic testing, and in special circumstances genotypic comparisons to aid in epidemiologic investigations.

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## Footnotes

- <sup>a</sup> Reveal® for *Salmonella* Test System, Neogen Corporation, Lansing, MI  
<sup>b</sup> RapidChek SELECT *Salmonella* Test System; SDIX, Newark, NE  
<sup>c</sup> Applied Biosystems, Carlsbad, CA  
<sup>d</sup> BD Diagnostic Systems, Sparks, MD  
<sup>e</sup> Becton Dickinson and Co, Cockeysville, MD  
<sup>f</sup> Hardy Diagnostics, Santa Maria, CA
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*Conflict of Interest Declaration:* The authors disclose no conflict of interest.

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