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Research Paper

Rapid Detection and Classification of *Salmonella enterica* Shedding in Feedlot Cattle Utilizing the Roka Bioscience Atlas *Salmonella* Detection Assay for the Analysis of Rectoanal Mucosal Swabs

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ABSTRACT

With an increasing focus on preharvest food safety, rapid methods are required for the detection and quantification of foodborne pathogens such as *Salmonella enterica* in beef cattle. We validated the Atlas *Salmonella* Detection Assay (SEN), a nucleic acid amplification technology that targets *Salmonella* rRNA, for the qualitative detection of *S. enterica* with sample enrichment using immunomagnetic separation as a reference test, and we further evaluated its accuracy to predict pathogen load using SEN signal-to-cutoff (SCO) values from unenriched samples to classify animals as high or nonhigh shedders. Rectoanal mucosal swabs (RAMS) were collected from 238 beef cattle from five cohorts located in the Midwest or southern High Plains of the United States between July 2015 and April 2016. Unenriched RAMS samples were used for the enumeration and SEN SCO analyses. Enriched samples were tested using SEN and immunomagnetic separation methods for the detection of *Salmonella*. The SEN method was 100% sensitive and specific for the detection of *Salmonella* from the enriched RAMS samples. A SEN SCO value of 8, with a sensitivity of 93.5% and specificity of 94.3%, was found to be an optimum cutoff value for classifying animals as high or nonhigh shedders from the unenriched RAMS samples. The SEN assay is a rapid and reliable method for the qualitative detection and categorization of the shedding load of *Salmonella* from RAMS in feedlot cattle.

Key words: Atlas; Beef cattle; Immunomagnetic separation; Rectoanal mucosal swab; *Salmonella*; Shedding

The preharvest food animal environment presents a number of complex challenges in the environment-pathogen-host triad, particularly with regard to *Salmonella enterica* control in cattle destined for human consumption. Cattle are asymptomatic carriers of the foodborne pathogen *S. enterica (7, 16, 20, 28),* which has been demonstrated in recent years to colonize the cattle's peripheral lymph nodes *(4, 10, 23–25)* as well as to be shed in the feces at various levels *(1, 15, 17, 20, 21, 47)*. *Salmonella* shedding in bovine feces is associated with hide contamination and downstream carcass contamination in beef cattle processing *(3, 5, 11)*. Postharvest carcass intervention strategies have been in place for many years and have been demonstrated to be effective in reducing *Escherichia coli* O157 and *Salmonella* contamination via multihurdle approaches *(48)*. Although not currently classified as an adulterant by the U.S. Department of Agriculture Food Safety and Inspection Service, *Salmonella* has resulted in outbreaks associated with and recalls of ground beef products, highlighting a continual challenge in mitigating the risk associated with this foodborne pathogen *(12, 13, 31, 35)*. It is noteworthy that the preharvest colonization of bovine peripheral lymphatic tissues by *Salmonella* has been recently implicated as a source of ground beef product contamination through downstream comminuted processing techniques *(4, 23, 24, 27)*. Given that inhabiting the lymphatic tissue protects *Salmonella* from processing interventions and that it is not possible for processors to remove all lymphatic tissues from carcasses, the efficacy of intervention and pathogen control programs is limited. As a result, food safety controls targeting this pathogen source are needed in the preharvest environment.

Numerous preharvest pathogen reduction strategies have been proposed in recent years for *S. enterica, E. coli* O157:H7, and non-O157 Shiga toxin–producing *E. coli*. Such strategies, which have varying degrees of commercial use and reduction efficacy, include direct-fed microbials or ration modifications, vaccination, hide washes, and feedlot management practices *(18, 33, 34, 36, 41, 42)*. In general,

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culture-based qualitative and quantitative methods are used to measure the prevalence and concentrations of the pathogen as a means of determining the efficacy of the intervention or treatment. Culture-based techniques, although generally highly accurate, are often laborious and can require many days before the confirmed final results are received. Many rapid methods for pathogen detection and enumeration are available in the food safety market, covering a wide range of chemistries and platform technologies *(43, 45)*. In the preharvest environment, the sample matrices are highly complex and generally have high levels of background microbial flora as well as a variety of potential physical and chemical inhibitors of rapid diagnostic methods. As the commercial production of meat animals shifts to a preharvest food safety focus, food safety professionals and researchers alike will benefit from rapid microbiological methods that are accurate and perform robustly in complex sample matrices, such as rectoanal mucosal swabs (RAMS), feces, soil, and hide swabs. Furthermore, such methods could lead to the development of rapid testing strategies that could affect preharvest management practices for reducing risk downstream at harvest.

Developing tools to investigate the relationship among *S. enterica,* cattle, and their environment requires, foremost, sample matrices that are consistent in nature (to the degree possible) and represent the population accurately. For *Salmonella* testing in cattle, fecal grabs have long been a recognized standard for measuring both qualitative *Salmonella* and the quantitative load per gram of feces, which is an indication of the individual animal shedding status; however, other sampling methods have been evaluated *(40, 42)*. In addition to defining a high-shedding threshold approximating 3.4 log CFU per swab, recent work *(1)* has demonstrated that RAMS are a diagnostic sample type equivalent to fecal grab. However, only culture-based methods have been evaluated for this specific sample type. RAMS sampling also offers a number of advantages over fecal grab as a sample matrix for routine sample collection and is associated with improved animal handling and laboratory processing considerations. Such considerations also may be conducive to the development of preharvest testing programs.

The Atlas *Salmonella* Detection Assay (SEN; Roka Bioscience, Warren, NJ) is a rapid nucleic acid amplification technology that uses rRNA from *S. enterica* as a target for transcription-mediated amplification *(29)*. Prepared samples are pipetted into an alkaline lysis reagent contained in a sample tube, which stabilizes the released nucleic acid. The sample tubes are then placed into the instrument, in which the assay processing sequence is entirely automated. The sequence begins with the magnetic capture of target nucleic acid onto paramagnetic microbeads using a specific probe for *S. enterica* rRNA. The initial nucleic acid capture is followed by a series of washings to remove potential inhibitors that could interfere with downstream reactions. The captured rRNA analyte is then amplified using isothermal transcription-mediated amplification, followed by product detection using an acridinium ester–labeled probe *(26)*. During detection, relative light units resulting from the probe-target hybridizations in the chemiluminescent detection step are measured using a luminometer for the samples as well as for the internal control. The ratio of the analyte relative light units to the calibrated relative light units cutoff (from a positive calibration control) results in a signal-to-cutoff (SCO) value ranging from 0 to 16 *(29)*. This technology both confers the advantage of performing accurately and robustly in complex preharvest sample matrices and demonstrates the broad inclusivity of *S. enterica* detection through the conserved rRNA target *(29)*.

The objectives of this study were (i) to validate the SEN assay for the qualitative detection of *S. enterica* from RAMS via paired comparisons to an immunomagnetic separation (IMS) culture method *(1)* and (ii) to evaluate a specific, rapid application of the assay that could be used to differentiate individual animals as high-level *Salmonella* shedders or nonhigh-level shedders.

MATERIALS AND METHODS

Animals. The RAMS samples were collected from 238 adult cattle representing five cohorts that were sampled at various times from three different commercial cattle feedlot operations located in the Midwest and the southern High Plains of the United States. The samples were collected from June 2015 to April 2016. The animals were sampled while they were restrained in a squeeze chute for the administration of a growth promoter implant, approximately 90 days preharvest. This study was approved by the U.S. Meat Animal Research Center Institutional Animal Care and Use Committee *(2)*.

RAMS sample collection, and *Salmonella* enumeration and qualitative detection. Using foam-tipped applicator swabs (VWR International, Buffalo Grove, IL), RAMS were collected by swabbing an area of 3 to 5 cm inside the anal canal of each animal. After collection, the swabs were immediately placed in a 15-mL conical tube containing 5 mL of tryptic soy broth (Sigma, St. Louis, MO) with 2.13 g/L KH_2PO_4 and 12.54 g/L K_2HPO_4 (TSB-PO_4) (final pH of 7.2). Gloves were changed in between sampling of each animal. All the collected RAMS were placed in coolers with ice packs and transported to the laboratory for processing within 24 h of the original collection.

In the laboratory, the swab samples were individually vortexed for 10 s (vortex mixer, Fisher Scientific, Hampton, NH), and the debris was allowed to settle for approximately 5 min. To enumerate the *Salmonella* present, 0.5 mL of the vortexed TSB-PO_4 was removed and placed into a 2-mL cluster tube (Simport, Beloeil, Canada) for spiral plate analysis (50 μL per plate) on xylose lysine desoxycholate agar (Remel, Lenexa, MO) plus 4.6 mL/L Tergitol, 15 mg/L novobiocin, and 10 mg/L cefsulodin (XLD_{tnc}). After plating, we incubated the XLD_{tnc} plates at 37°C for 24 h and enumerated the presumptive colonies, which appeared as black colonies with a clear outer ring, characteristic of *Salmonella (6, 9)*. The SEN assay also was evaluated to estimate the *Salmonella* levels directly in the unenriched samples. Using the reported *(1)* RAMS high-shedding threshold of approximately 2.7 log CFU/mL in the prepared 5-mL TSB-PO_4 sample, we estimated that an aliquot of 200 μL of the solution diluted 1:1 with fresh TSB-PO_4 (400 μL total) was the analytical volume that would approximate the SEN assay's reported *(29)* analytical limit of detection. For all samples, 200 μL of the unenriched TSB-PO_4 was transferred into SEN sample transfer tubes along with 200 μL of sterile TSB-PO_4 and was

subsequently prepared for the SEN assay, as per the manufacturer's instructions.

To determine the individual qualitative *Salmonella* prevalence in the RAMS, the remaining TSB-PO_4 in the conical tube was enriched for 8 h at 42°C and transitioned into 4°C holding for processing the following day. The following day, the enriched TSB-PO_4 samples were subjected to a parallel assessment of *Salmonella* detection via continued culture-based analysis, incorporating the IMS method for the selective isolation of *Salmonella* and the rapid molecular SEN assay method. For the IMS culture method, the enrichments were subjected to IMS using anti-*Salmonella* beads (Dynabeads, Invitrogen, Carlsbad, CA). The recovered beads were transferred to 3 mL of Rappaport-Vassiliadis broth (Remel) and incubated at 42°C for 18 to 20 h, at which time the samples were streak plated onto XLD_{tnc} and incubated at 37°C for 18 h, as previously described *(1, 8, 11)*. For the SEN assay, the standard analytical volume of 400 μL of the enriched TSB-PO_4 was placed in a SEN sample transfer tube and prepared for assay according to the manufacturer's instructions. The incubated XLD_{tnc} plates that presented morphologically typical *Salmonella* colonies were used to select isolates for confirmation of both the SEN and IMS methods. Isolates were confirmed as *Salmonella* using a PCR assay to detect the *Salmonella*-specific portion of the *invA* gene, as previously described *(37, 38)*.

Each RAMS sample, and therefore each individual animal sampled, had four test results: culture-based enumeration result, IMS detection method result, SEN unenriched sample assay result, and SEN enriched (qualitative) sample assay result. For the samples found to contain *Salmonella* at levels >1.3 log CFU/mL, or 20 CFU/mL (the limit of quantification of the spiral plate enumeration assay), we calculated the *Salmonella* concentration for each sample, which was log transformed and reported as log CFU per milliliter. As previously noted, we chose log CFU per milliliter as the unit of measurement, as opposed to the log CFU per swab, for the RAMS because of the possible variation among the individual samples due to swab weight. In addition, this unit creates uniformity with other methods, such as fecal grab; both methods result in liquid media sample suspension *(1)*. To the samples found to be positive for *Salmonella* using the enrichment IMS method but negative using the enumeration assay, we assigned a value of 1 CFU/mL to avoid zero counts prior to log-transforming the data. Last, we determined a shedding classification for individual animals based on previously reported findings in which a high-shedding animal was defined as having a RAMS yielding ≥2.7 log CFU/mL *(1)*.

All the SEN samples in the study across the five feedlot cattle cohorts were run on one instrument at the U.S. Meat Animal Research Center laboratory and used three different production master lots of the SEN assay. Within each sampling round and across the three kit master lots, the assay calibrations were found to be within acceptable tolerances. The SEN assay results are automatically interpreted qualitatively as positive or negative by the instrument and assay software.

SEN application for shedding classification. SEN postassay amplification and detection SCO values from the unenriched RAMS TSB-PO_4 samples (the ones that were analyzed in parallel with the direct plating enumeration method) were recorded and analyzed as a semiquantitative outcome against the corresponding enumeration values.

Statistical analyses. We compared the qualitative detection and prevalence of *Salmonella* following RAMS sample enrichment of the IMS culture method and the SEN assay method using Bowker's test and kappa in JMP 11.0.0 (SAS, Cary, NC). The kappa values were interpreted using the Landis and Koch *(30)* method as follows: <0 (poor agreement), 0 to 0.2 (slight agreement), 0.21 to 0.4 (fair agreement), 0.41 to 0.6 (moderate agreement), 0.61 to 0.8 (substantial agreement), and 0.81 to 1.0 (almost perfect agreement). In addition, we calculated the standard diagnostic performance criteria, including method agreement, sensitivity, specificity, positive and negative predictive values, and area under the receiver operating characteristics curve (AU-ROC), using the EpiTools (AusVet, Orange, New South Wales, Australia) and MedCalc (MedCalc Software, Ostend, Belgium) online statistical calculators and STATA13 (Stata Corp., College Station, TX).

For the rapid classification of individual sample or animal RAMS *Salmonella* shedding status, we plotted individual SEN SCO values against paired enumeration data according to our four arbitrarily defined shedding status classifications. We performed an AU-ROC analysis *(19, 22, 39)* to determine the optimal SCO value that differentiated high-shedding from nonhigh-shedding animals based on the 2.7 log CFU/mL threshold. From the AU-ROC analysis, we determined that the optimal SCO value differentiating animals as high or nonhigh shedders is the value at which the squared difference between sensitivity and specificity of the SEN assay for the binary shedding classification (high and nonhigh shedder) is minimized, or the SCO value at which the curves intersect on the sensitivity-specificity plot. The optimal cutoff value for the SEN assay is therefore the one that yields the highest combination of sensitivity and specificity for the shedding classification.

RESULTS AND DISCUSSION

Comparison of qualitative *Salmonella* outcomes by detection method in RAMS. In total, the prevalence of *Salmonella* across all cohorts of cattle sampled using the IMS culture method with RAMS was 60.1% and significantly ($P < 0.001$) varied by cattle cohort. The prevalence using the SEN detection assay in the paired samples was in 100% agreement for all samples, with a detection prevalence of 60.1%, and also varied significantly ($P < 0.001$) by cattle cohort (Table 1). In all, 143 *Salmonella*-positive samples were detected using both methods (Table 2). Using the IMS culture method as a reference test for the RAMS analysis, the SEN assay had a sensitivity of 100% (95% confidence interval [CI], 97.5 to 100%) and a specificity of 100% (95% CI, 96.2 to 100%). The positive predictive value was 100% (95% CI, 97.5 to 100%), and the negative predictive value was 100% (95% CI, 96.2 to 100%). The Bowker's test (equivalent to the McNemar χ^2 test) χ^2 value was 0, indicating the equivalence of the methods ($P = 1.00$), and the kappa coefficient value of 1 indicates perfect agreement as interpreted by the Landis and Koch *(30)* method. The SEN assay had AUC value of 1.

Unenriched application of SEN SCO as a rapid method for the *Salmonella* shedding classification. The mean *Salmonella* load for an assay positive response on an unenriched RAMS sample preparation using the SEN assay was 2.6 log CFU/mL (95% CI, 2.4 to 2.8) compared with the assay negative response using SEN (mean of 0.09; 95% CI,

TABLE 1. *Summary statistics for the prevalence and quantification of* S. enterica *in beef cattle using culture and SEN methods*[a]

Cohorts sampled	No. of samples collected	IMS culture prevalence (%)	SEN assay prevalence (%)	Samples enumerable (%)[b]	RAMS, mean (95% CI) (log CFU/mL)	SEN SCO, mean (95% CI)
1	48	89.6	89.6	52.1	1.31 (0.97–1.64)	3.81 (2.95–4.66)
2	66	72.7	72.7	45.5	1.04 (0.75–1.32)	3.15 (2.42–3.88)
3	25	100.0	100.0	96.0	4.13 (3.66–4.59)	9.73 (8.55–10.92)
4	30	90.0	90.0	40.0	0.94 (0.51–1.36)	2.48 (1.40–3.56)
5	69	0.0	0.0	0.0	0.00 (−0.28–0.28)	0.00 (−0.71–0.71)
Total	238	60.1	60.1	38.2	1.10	2.98

[a] CI, confidence interval; SCO, signal-to-cutoff value.
[b] Lower limit of quantification = 1.3 log CFU/mL.

–0.09 to 0.27), as determined by the enumerable samples. This is noteworthy given previous observations of *Salmonella* high shedders with RAMS samples containing ≥2.7 log CFU/mL *(1)*.

Of all the RAMS samples ($n = 238$), 38.2% were enumerable using the culture-based spiral plating method (Table 1). The overall mean *Salmonella* RAMS load across all sample cohorts was 1.1 log CFU/mL, and the mean load significantly ($P < 0.0001$) varied by sample cohort. For the cohorts sampled, the mean *Salmonella* loads were 1.31 (95% CI, 0.97 to 1.64), 1.04 (95% CI, 0.75 to 1.32), 4.13 (95% CI, 3.66 to 4.59), 0.94 (95% CI, 0.51 to 1.36), and 0.00 (95% CI, –0.28 to 0.28) log CFU/mL for cohorts 1 through 5, respectively (Table 1). Overall, the mean SEN SCO value was 2.98, and the within-cohort means were 3.81 (95% CI, 2.95 to 4.66), 3.15 (95% CI, 2.42 to 3.88), 9.73 (95% CI, 8.55 to 10.92), 2.48 (95% CI, 1.40 to 3.56), and 0.00 (95% CI, –0.71 to 0.71) for cohorts 1 through 5, respectively, with significant ($P < 0.0001$) variation among the cohorts sampled (Table 1).

TABLE 2. *Diagnostic characteristics of the SEN assay after sample enrichment with reference to the IMS culture method for qualitative detection of* S. enterica *in feedlot cattle from RAMS samples*

	SEN assay		
IMS culture method	Positive	Negative	Total
Positive	143	0	143
Negative	0	95	95
Total	143	95	238
Parameters	Estimate	95% confidence interval	
Observed agreement	100.00		
Kappa	1.00	1.00–1.00	
Prevalence (IMS culture method) (%)	60.08	53.90–66.30	
Prevalence (SEN method) (%)	60.08	53.90–66.30	
Sensitivity (%)	100.00	97.5–100.00	
Specificity (%)	100.00	96.2–100.00	
Positive predictive value (%)	100.00	97.5–100	
Negative predictive value (%)	100.00	96.2–100	
Area under the curve	1.0	1.0–1.0	

A data-driven shedding classification scheme for individual animals based on the enumeration and IMS culture results revealed that the mean SCO values of the SEN assay followed a linear increase as defined by four ordinal categories (Fig. 1). The categories were (i) negative shedding (IMS *Salmonella* negative and nonenumerable); (ii) nonenumerable shedding (IMS *Salmonella* positive but nonenumerable); (iii) low shedding ($<1.0 \times 10^3$ CFU/mL), and (iv) high shedding ($\geq 1.0 \times 10^3$ CFU/mL).

Using the enumeration values reported here and the previously defined *Salmonella* RAMS load thresholds for high and nonhigh shedders of ≥2.7 log CFU/mL *(1)* and <2.7 log CFU/mL, respectively, we assessed the individual SEN SCO values as a binary shedding classification system using AU-ROC analysis. We found that the optimum SCO value (i.e., the cutoff point that minimizes the difference between sensitivity and specificity) for distinguishing between high shedders and nonhigh shedders was a SCO of 8 with a sensitivity of 93.5 and a specificity of 94.3 (Fig 2 and Table 3). At a SCO of 8, 94% of the animals were correctly classified either as high shedders or nonhigh shedders. The AU-ROC was 0.98 (95% CI of 0.96 to 0.99), interpreted as the probability that a randomly selected high shedder will have a greater SCO value than a randomly selected nonhigh shedder.

The reduction of *Salmonella* in preharvest food animal environments is a significant challenge. Although the efficacy of carcass interventions and processing aids has been demonstrated and these methods have been used for years in the beef industry, recent research demonstrating peripheral lymphoid tissue colonization by *Salmonella* and comminuted processing contamination presents new challenges for mitigating *Salmonella* risk *(23, 24, 32)*. Effective means of controlling *Salmonella* exposure at preharvest are not well understood, but vaccination and direct-fed microbials appear to be viable options for *Salmonella* reduction *(14, 44)*. Nonetheless, cattle will continue to be colonized and to shed *Salmonella* in their feces. The rapid identification of *Salmonella*-positive cattle, especially those shedding the organism at high levels, may allow for novel management practices, such as the segregation of animals based on their shedding status. A potential preharvest food safety management option would be to use rapid testing at the animal, pen, or feedlot level and to use the results to influence management

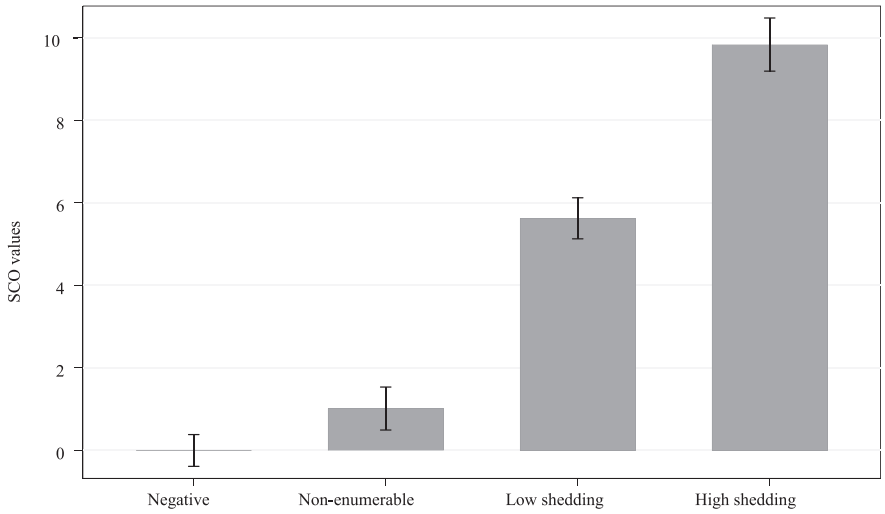

FIGURE 1. *Mean SCO values by category of* Salmonella *shedding.*

practices, harvest ordering, or downstream harvesting actions. A similar approach has been used in Denmark in the pork industry, where pig herds are routinely tested for *Salmonella* and subjected to herd level reduction actions or are harvested under increased hygienic conditions, with the products potentially diverted to cooking processes *(46)*. Key to these preharvest management schemes is the development and validation of rapid diagnostic tools, such as the method described here.

The analysis of preharvest sample matrices, such as RAMS, requires detection methods that are robust and not easily inhibited by the intrinsic components of the sample matrix. Notably, the current "gold standard" IMS method takes more than 3 days before a *Salmonella* isolate can be confirmed because of the necessary primary and secondary enrichments and selective plating incubation periods. The SEN method used in this study provided results that were 100% concordant with a single 8-h enrichment and <4-h instrument run time. As such, this method can provide a robust *Salmonella* result for qualitative RAMS analysis within 12 to 24 h. Notably, the earlier sample collection times from enrichments were not evaluated in this study, but the time needed may potentially be reduced to <8 h depending on the specific objectives of the testing, as in the case of unenriched high-shedding detection. The described difference in the time to result could provide a number of advantages in how cattle are handled just prior to harvest and in other settings such as animal health, in which veterinary intervention may be needed for clinical salmonellosis.

In addition to understanding the prevalence of RAMS *Salmonella* and therefore the bovine colonization status, the concentration of *Salmonella* in the sample is another factor that should be considered. As the shedding concentrations increase, the pen level burden of *Salmonella* increases, which may lead to the increased exposure of noncolonized animals and the cross-contamination of hides, water sources, feed, and so on *(17, 28, 40)*. The current RAMS enumeration standard requires dilution plating onto agar plates, followed by incubation and counting, a process that takes approximately 2 days *(1)*. As such, we explored the use of the SEN SCO values as a nonenriched, rapid means of categorizing animal shedding. The nonenriched SEN method has the potential to provide an indication of the

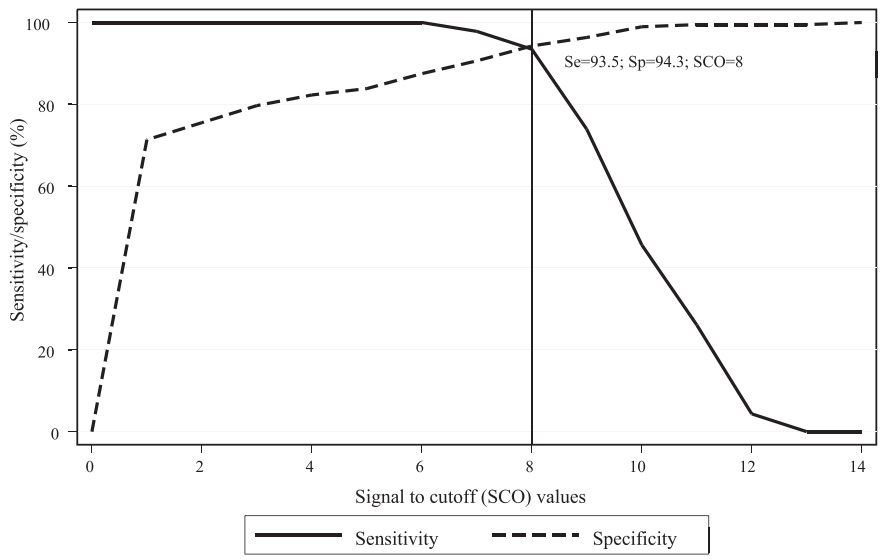

FIGURE 2. *Sensitivity-specificity plot of SEN SCO to predict the shedding status of an animal as a high shedder or nonhigh shedder. Se, sensitivity; Sp, specificity.*

TABLE 3. *Diagnostic parameters of the SEN assay to classify feedlot cattle as high shedders or nonhigh shedders using culture-based enumeration data*[a]

SCO values	Sensitivity (%)	Specificity (%)	Correctly classified (%)	LR+	LR−
≥0	100.0	0.0	19.3	1	
≥1	100.0	71.4	76.9	3.5	0
≥2	100.0	75.5	80.1	4.1	0
≥3	100.0	79.7	83.6	4.9	0
≥4	100.0	82.3	85.7	5.6	0
≥5	100.0	83.6	87.0	6.2	0
≥6	100.0	87.5	89.9	8.0	0
≥7	97.8	90.6	92.0	10.4	0.02
≥8	93.5	94.3	94.1	16.3	0.07
≥9	73.9	96.4	92.0	20.3	0.27
≥10	45.7	99.0	88.7	43.8	0.55
≥11	26.1	99.5	85.3	50.1	0.74
≥12	4.4	99.5	81.1	8.3	0.96
≥13	0.0	99.5	80.3	0.0	1.01
>13	0.0	100.0	80.7	—	1.0

[a] SCO, signal-to-cutoff value; LR+, likelihood ratio positive (the probability of a positive test result in a high-shedding animal relative to the probability of the same test result in a nonhigh-shedding animal); LR−, likelihood ratio negative (the probability of the negative result in a high-shedding animal relative to the probability of the same test result in a nonshedding animal).

shedding load in a matter of hours. By simply collecting the RAMS sample, diluting the swabs in TSB, vortexing the sample, and transferring the sample to the SEN sample transfer tube, collection could be done in the field and the samples could be taken directly to the laboratory for results the same day.

In this study, we found a strong positive correlation between the *Salmonella* enumeration result and the SEN SCO values. Therefore the SEN SCO values from unenriched samples can be used to predict the *Salmonella* shedding status of cattle. Using this technique removes the need for culture-based enumeration and has the potential to yield results the same day as the sample collection. The SEN SCO value of 8 was found to be an optimum cutoff point, with a sensitivity and specificity of 94%, for categorizing feedlot cattle as high or nonhigh shedders, based on a previously established RAMS *Salmonella* load of 2.7 log CFU/mL. We note that the SEN method has 100% analytic specificity, meaning it is specific only to *S. enterica* and does not detect any of the 30 non-*Salmonella* strains, as shown by the validation study conducted by Kwong et al. *(29)*. In the present study, we also found that the SEN method had 100% diagnostic specificity, meaning no animals tested positive using the SEN method that tested negative using the IMS method (i.e., zero false-positive rate). This 100% diagnostic specificity also was evident in cattle cohort 5, in which cattle from a feedlot operation with a documented history of no *Salmonella* infection were sampled, tested, and found to be *Salmonella* negative by both the SEN and IMS culture methods. The 100% perfect agreement between SEN and IMS in the enriched RAMS samples shows that these methods can be used interchangeably, with SEN providing a significantly faster time to result.

In summary, we found the SEN assay to be a rapid, sensitive, and specific molecular test, both for the detection of *Salmonella* and the categorization of shedding load in RAMS samples from beef cattle. Although the SEN system does not provide information that may be important for epidemiological or public health purposes, such as the *Salmonella* serotype or antimicrobial resistance information, it can be used as a screening tool. Moreover, this limitation can be overcome by culturing the SEN positive samples, with subsequent serotyping and antimicrobial susceptibility testing of the resulting *Salmonella* isolates. Thus, the ease of use and decreased turnaround time to results compared to the traditional culture-based methods suggest that the use of this or similar assays holds the potential to revolutionize on-farm surveillance of cattle herds for *Salmonella* carriage and to provide valuable data needed for the development of *Salmonella* preharvest mitigation strategies.

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