

# Technical evaluation of commercial molecular screening platforms for the detection of food-borne bacterial pathogens by FSIS Laboratories

## Abstract

The performance of four commercially available molecular screening platforms was compared to that of the Food Safety and Inspection Service Microbiology Laboratory Guidebook (MLG) reference cultural methods for *Salmonella* sp., *Listeria monocytogenes*, *Campylobacter* sp., and Shiga toxin-producing *Escherichia coli* (STEC) composed of *E. coli* O157 and adulterant non-O157 STECs. A variety of representative food product samples were inoculated with target microorganisms at a fractional recovery range of 20-80%. Each screening platform was used at each FSIS/OPHS Laboratory to analyze a total of 440 *Salmonella*-inoculated samples, 120 *L. monocytogenes*-inoculated samples, 120 *Campylobacter* inoculated-samples, and 180 STEC-inoculated samples. Sixty of the STEC-specific samples were inoculated with *E. coli* O157. Limits of detection, inclusivity, and exclusivity panels were examined along with an internal evaluation of the strengths and weaknesses of each technology during use in the FSIS microbiology laboratory screening environment. Inoculated samples were identified with a range of 89% to 94% across vendor and matrix. Most technologies erred towards false positives. Specifically, with *L. monocytogenes*, all technologies had agreement with the cultural method, except with smoked fish matrix where all technologies missed about one third of the inoculated samples, except Bax<sup>®</sup>, that identified all but one. Two technologies were evaluated for *Campylobacter* sp. detection, Bax<sup>®</sup> and GENE-UP<sup>®</sup>. Both correctly identified all positive inoculated samples. Detection of non-O157:H7 STECs was evaluated for three vendors. iQ-check<sup>®</sup> correctly identified a higher proportion, of samples as compared to Bax<sup>®</sup> or GENE-UP<sup>®</sup>. Detection of *E. coli* O157:H7 was evaluated on all platforms. Bax<sup>®</sup> had more false negative results than the other vendors with raw beef matrix. Overall, no single platform was consistently superior for all pathogens and all matrices. Additional considerations for a molecular screen platform in a high-throughput food testing laboratory include fitness-for-purpose, ease of use by analysts, quality of customer support, and cost scenarios as sampling needs change.

## Introduction

The FSIS laboratories utilize a rapid molecular screen step in each of their foodborne pathogen isolation and identification methods. In late 2017, the existing vendor contract for molecular screening instruments was ending, creating an opportunity for the FSIS laboratories to evaluate and update their pathogen screening capabilities. In order to modernize the established and successful pathogen screening approach, FSIS assessed several high-throughput, multi-analyte molecular screening platforms for the detection of bacterial pathogens in food products and food producing environments regulated under the Poultry Inspection Act (PIA), Egg Products Inspection Act (EPIA), and the Federal Meat Inspection Act (FMIA), including *Siluriformes* fish, as added under the 2008 and 2014 Farm Bills. The targeted microorganisms included: *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC) serotype O157:H7, the top six non-O157 STEC serotypes with the most public health significance, *Listeria monocytogenes*, and *Campylobacter* species.

## Materials and Methods

Microbiology Laboratory Guidebook (MLG):

<https://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook>

The four analytical platforms evaluated were:

A = 3M™ Molecular Detection System

B = BioMérieux GENE-UP® Test System

C = Bio-Rad iQ-Check® Real-Time PCR Solution

D = Hygiena BAX® System

This lettering scheme is followed throughout this report.

Based upon the criteria devised by FSIS for the selection of the screening technology, for each targeted microorganism, FSIS tested a minimum of three (3) food product matrices with sixty (60) samples per method for each applicable pathogen. Samples were artificially inoculated with the target microorganism at fractional recovery levels (20-80% recovery). A paired-study design was used to compare the MLG reference method to the performance of the alternative molecular screening method. Laboratory evaluations included sensitivity, specificity, predictive value, and precision as well as a review of limit of detection, exclusivity and inclusivity panels, and an assessment of each technology for fitness-for-use as defined by the bench analysts at the three FSIS Laboratories.

Each analytical platform was assigned a letter designation (as indicated above) prior to the start of the evaluation to prevent any secondary bias when assessing the results. The instruments were evaluated with food product matrices inoculated with target microorganisms at fractional recovery levels or the reference cultural methods (FSIS MLG Chapters 4, 5, 5B, 8 and 41). Fractional recovery is defined as 20-80% positive cultural reference MLG method results from the inoculated samples. If a set of inoculated samples did not meet an acceptable fractional recovery range of 20-80% based on the MLG cultural reference method, a new set of samples were inoculated.

Evaluated instruments were identified using a lettering scheme consistent in each FSIS laboratory. Each laboratory was assigned a matrix to evaluate along with the appropriate number of spiked sample (see **Table 1** for a full list of food product and other included matrices). In addition, at least twenty units of each sample type from the field were analyzed by the laboratories for the analyses appropriate to the food product matrix: ready-to-eat (RTE) product, ground beef, chicken rinses, and swabs. Data was not used from any spiked set of samples that did not meet the appropriate fractional recovery range for the MLG cultural reference methods.

Each set of 10 samples included an un-inoculated food product matrix control, a positive control, a media sterility control, and in some cases a negative control as outlined in the MLG chapter for each microorganism: *Salmonella enterica* Typhimurium (Microbiologics Sal54 01223 UV-V FDA Sal 5694); *Escherichia coli* O157:H7 (Microbiologics EC43 01227 UV-V FDA ESC 1177); *E. coli* O26:H2 (USDA, ARS #TB285); *E. coli* O45:H2 (USDA, ARS #96-3285); *Listeria monocytogenes* (Microbiologics 01248UV-V); *L. innocua* (ATCC 33090, negative control); and *Campylobacter jejuni* (ATCC 33291).

### **Sample Preparation.**

Food product sample sizes and enrichment volumes were consistent with MLG protocols. For MLG 8 (*L. monocytogenes*), samples were enriched with UVM, and carried forward to MOPS-BLEB. All MOPS-BLEB tubes are plated onto MOX and carried forward to HL; no direct plating to MOX was performed.

### **Inoculum preparation**

Samples were spiked by culturing each organism 18-24h on TSA/SB. See MLG chapters 4, 5, 8, and 41 for control culture preparation. Suspensions of the target microorganisms were prepared in 0.85% saline of 0.5 McFarland (approximately  $10^8$ - $10^9$  cells per mL). Serial dilutions were prepared to attain stock suspensions used to inoculate the food product matrix at the appropriate concentration to achieve fractional recovery. For *Salmonella*, *Listeria*, and *STECs*, the stock inoculum was plated on Petrifilm™ APC in duplicate and incubated at  $35 \pm 1^\circ\text{C}$  for  $24 \pm 2$  hours to determine actual levels. For *Campylobacter*, 0.1 ml of serial dilutions was plated from the stock solution in duplicate on TSA/SB plates and incubated for  $24 \pm 2$  hours at  $42 \pm 1.0^\circ\text{C}$  under microaerobic conditions to determine the actual level.

### **MLG cultural method endpoint**

The MLG cultural reference comparison terminated for each sample set at the following points in each method; MLG Chapter 4: DMLIA and BGS plating; MLG Chapter 5 and 5B: latex agglutination; MLG Chapter 8: HL plating; MLG Chapter 41: microscopic examination and latex agglutination. These points of termination were chosen as the best points to assure recovery of the microorganisms to determine the false negative and false positive rates of each technology compared to the reference methods.

### **Inclusivity/Exclusivity determination**

To evaluate inclusivity of each instrument and screening method, at least 30 strains of the target microorganism for each method were chosen and tested using pure culture. To evaluate exclusivity of each technology, at least 20 strains of the target analyte for each method were chosen and tested using pure culture. When available, strains were chosen that have non-typical characteristics (for example, *stx2e*, *stx2g*, *Salmonella enterica* subspecies III, non- $\beta$  hemolytic *Listeria monocytogenes* strains, *stx*-negative *E. coli* O157:H7). The pure culture work

was performed directly from colonies on the plating medium as well as from DNA templates prepared from pure culture.

### Limits of Detection

The limit of detection (LOD) was evaluated for each assay by spiking incubated, screen-negative enrichment media with estimated target levels prepared from serially diluted inoculum. Matrices were enriched and incubated according to the appropriate MLG method. A 0.5 McFarland saline solution ( $\sim 10^8$  cfu/mL) was made for each organism. Each of these solutions was used to prepare a 1:10 dilution series in saline buffer from  $10^7$  to  $10^2$  concentrations. The solutions were plated in triplicate for the  $10^4$  to  $10^2$  dilution tubes. The  $10^7$  tube for each organism was then used to make a  $10^6$ - $10^1$  dilution series in triplicate using the enriched matrices. The dilution series were then analyzed on each screening instrument along with the appropriate controls to provide the LOD data (**Table 8**). The enrichment medium was inoculated with levels ranging from  $10^2$ - $10^7$  cells/ml and tested using the assay kit appropriate for each target. The test at each inoculum level was performed in triplicate. All samples within each triplicate had to be positive to indicate the lowest detectable level. The lowest pure culture inoculum was plated in triplicate on plating medium or Petrifilm™ to determine the 95% Confidence Interval (CI) for LOD.

### Results and Discussion

#### Instrument/molecular screen comparison.

Four molecular screening instruments were evaluated against the applicable MLG reference cultural methods. Technology A was evaluated for *Salmonella*, *L. monocytogenes*, *E. coli* O157, and *Campylobacter*. Technology B was evaluated for *Salmonella*, STEC, and *L. monocytogenes*. Technologies C and D were evaluated for *Salmonella*, STEC, *L. monocytogenes*, and *Campylobacter*. Samples were prepared from a variety of representative food products (“matrices”) (**Tables 1 through 5**) and inoculum levels (CFU) for each matrix were at fractional recovery levels for each method. Each result was compared against the MLG reference cultural method to evaluate false positive and false negative rates at fractional recovery levels. Any inoculated samples that did not meet fractional recovery of 20-80% as verified by the MLG reference cultural method were discarded and a new set of samples were prepared. If a technology provided a result described as indeterminate, inhibited, or error it was treated as a positive for the purposes of this evaluation.

Positive results for each technology compared to the results of the cultural method are listed in **Tables 1-5** by microorganism and matrix. Statistical analysis was performed on the data using Kappa Coefficient, a statistical test of agreement, to evaluate recovery for each technology against the existing MLG reference cultural methods for similarity. Kappa values of  $0.80 \leq 1.00$  were interpreted as very good agreement. The Kappa Coefficient values of each method are listed below for each instrument by microorganism (**Table 6**). With one exception, for technology D when testing for *E. coli* O157, all Kappa statistic results were  $> 0.80$ . Statistical

analysis was also performed on the data using McNemar's test for Dependent/Paired Samples, comparing each technology with the culture reference method (**Table 6**). A p-value of < 0.05 indicates results from the technology are statistically different than the MLG reference culture method. As expected, there was not a perfect concordance for cultural method and analytical platforms. Most of the statistically significant differences, were driven by a false positive classification. This is an expected bias observed with most screening technologies. At the same time, because we were working at fractional recovery levels, it was expected that none of the platforms would correctly identify all the positive samples as detected by the cultural method. That said, when looking at the performance for detecting contamination in each pathogen/matrix combination, there were some statistical differences (Table 6). For *L. monocytogenes*, all technologies had agreement with the cultural method, except with smoked fish matrix, where all technologies missed about one third of the inoculated samples, except for the Bax<sup>®</sup> platform, that identified all but one sample. Two technologies were evaluated for *Campylobacter sp.* detection, Bax<sup>®</sup> and GENE-UP<sup>®</sup>. Both correctly identified all positive inoculated samples. Detection of *E. coli* non-O157:H7 STECs was evaluated for three vendors. iQ-check<sup>®</sup> correctly identified a higher proportion, of samples as compared to Bax<sup>®</sup> or GENE-UP<sup>®</sup>. Detection of *E. coli* O157:H7 was evaluated on all platforms. Bax<sup>®</sup> had more false negative results than the other vendors with raw beef matrix. Overall, no single platform was consistently superior for all pathogens and all matrices.

### **Inclusivity/exclusivity evaluation**

Inclusivity and exclusivity panels were gathered from pure culture DNA from bacterial strains collected from tested products. Each technology was evaluated using the strains to assess the ability of the technology to distinguish between a known positive (True Positive) and a known negative (True Negative). Overall, the technologies identified True Positives equally well across the organisms tested. Any of the False Positives (i.e. screen positive result for a known negative) had late cycle threshold (Ct) values (>35 cycles) and were likely artifacts or potential carry over (**Table 7**). As the isolates were being tested from purified DNA, it is expected that the isolate should be positive well before 35 cycles for a real-time PCR assay.

### **Limits of detection evaluation**

The limit of detection for the microorganisms of interest was examined for each analytical platform. . The LOD was the inoculum dilution at which 100% detection was no longer achieved (Table 8).

### **Inhibited or indeterminate results**

During the evaluation some of the technologies provided results described as “inhibited”, “indeterminate” or “unidentifiable”. Following the current business rules of the FSIS laboratories, these inhibited, indeterminate or unidentifiable results cannot be used to rule out the presence of the target analyte in the sample. Samples with these results would be carried forward like a positive result, using the cultural method for confirmation. Inhibited,

indeterminate or unidentifiable results could indicate matrix interference, questionable test kit performance, or that random errors should be expected using a technology.

## **Conclusion**

In our assessment of performance, some differences were observed between the four platforms evaluated and based on the predetermined performance metrics, such as limit of detection, sensitivity, specificity, predictive value, inclusivity and exclusivity, all were deemed to perform comparable to the MLG reference method. Because no single technology was superior in all substrates and for all pathogens, other considerations factored into the selection of screening technology for use in FSIS Laboratories for detection of a specific pathogen. These considerations include the laboratory's analytical process, which includes number of samples, instrument footprint, ease-of-use, sample flow, cost considerations, and staffing.

FSIS Laboratories require reliable and high-throughput technologies, to assess the Agency's public health initiatives while minimizing the commercial impact to producers. The FSIS laboratories seek to utilize technologies with a high degree of accuracy in a qualitative screen coupled with ease of use, reproducibility and robustness in a setting where a large volume of regulatory samples are analyzed year-round.

**Table 1: Comparison of Positive *Salmonella* Results for Each Technology Compared Against the MLG Reference Culture Method Using Samples Inoculated for Fractional Recovery**

Matrix	Sample Number	Inoculum Level (CFU)	A	B	C	D	Cultural Method MLG 4
Raw Beef Products*	60	0.40-0.80	27	25	29	27	31
Raw Poultry Products*	60	0.69-0.74	27	29	28	27	30
Ready to Eat (RTE) Products*	60	0.52-0.59	23	23	23	24	23
Turkey Sponges	20	0.63-1.1	8	7	9	7	7
Ground Chicken	20	0.50-1.3	7	5	5	5	9
Smoked Catfish	20	0.50	7	7	6	8	8
Environmental Sponges	20	0.69	7	7	7	7	7
Meat Carcass Sponges	20	0.69-0.71	6	5	5	5	5
Whole Eggs	20	0.60-0.73	7	7	14	5	7
Raw Catfish	20	0.43-0.52	9	9	12	10	10
Raw Pork	20	1.8	16	15	15	11	15
Cecal Beef	30	0.40-1.0	14	16	16	17	16
Cecal Pork	30	0.40-0.63	16	15	15	11	15
Cecal Turkey	20	0.40-1.0	5	9	8	7	9
Cecal Chicken	20	0.60-0.90	14	6	15	15	15

\*Raw Beef Products were composed of raw ground beef and raw beef trim

Raw Poultry Products were composed of ground turkey, chicken rinses, and chicken part rinse

RTE Products were composed of hot dogs and chicken nuggets

A = 3M™ Molecular Detection System

B = BioMérieux GENE-UP® Test System

C = Bio-Rad iQ-Check® Real-Time PCR Solution

D = Hygiena BAX® System

**Table 2: Comparison of Positive *L. monocytogenes* Results for Each Technology Compared Against the MLG Reference Culture Method Using Samples Inoculated for Fractional Recovery**

<b>Matrix</b>	<b>Sample Number</b>	<b>Inoculum Level (CFU)</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>Cultural Method MLG 8</b>
RTE Products*	60	1.2-1.5	36	36	36	38	36
Environmental Sponges	20	0.63-0.72	4	4	4	4	4
Smoked Catfish	20	1.3	10	10	10	14	15
Whole Eggs	20	4.7-5.6	10	10	9	11	10

\*RTE Products were composed of hot dogs and chicken nuggets

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B = BioMérieux GENE-UP® Test System

C = Bio-Rad iQ-Check® Real-Time PCR Solution

D = Hygiena BAX® System

**Table 3: Comparison of Positive *Campylobacter* Results for Each Technology Compared Against the MLG Reference Culture Method Using Samples Inoculated for Fractional Recovery**

<b>Matrix</b>	<b>Sample Number</b>	<b>Inoculum Level (CFU)</b>	<b>C</b>	<b>D</b>	<b>Cultural Method MLG 41</b>
Raw Poultry Products*	60	1.5-3.5	29	25	27
Turkey Sponges	20	0.34-3.0	10	9	8
Ground Chicken	20		6	6	6
Environmental Sponges	20		7	7	7

\*Raw Poultry Products were composed of ground turkey, chicken rinses, and chicken part rinse

C = Bio-Rad iQ-Check® Real-Time PCR Solution

D = Hygiena BAX® System

**Table 4: Comparison of Positive STEC Results for Each Technology Compared Against the MLG Reference Culture Method Using Samples Inoculated for Fractional Recovery**

<b>Matrix</b>	<b>Sample Number</b>	<b>Inoculum Level (CFU)</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>Cultural Method MLG 5 &amp; 5B</b>
Raw Beef Products*	60	0.70-1.4	22	27	23	28
Raw Pork	60	0.97-1.0	34	35	31	37
Environmental Sponges	60	0.69-0.71	34	30	34	34

\*Raw Beef Products were composed of raw ground beef and raw beef trim

B = BioMérieux GENE-UP® Test System

C = Bio-Rad iQ-Check® Real-Time PCR Solution

D = Hygiena BAX® System



**Table 5: Comparison of Positive *E. coli* O157 Results for Each Technology Compared Against the MLG Reference Culture Method Using Samples Inoculated for Fractional Recovery**

Matrix	Sample Number	Inoculum Level (CFU)	A	B	C	D	Cultural Method MLG 5
Raw Beef Products*	60	0.70-1.4	8	10	10	5	10
Raw Pork	60	0.97-1.0	14	10	12	8	13
Environmental Sponges	60	0.63-0.71	11	11	11	11	11

\*Raw Beef Products were composed of raw ground beef and raw beef trim

A = 3M™ Molecular Detection System

B = BioMérieux GENE-UP® Test System

C = Bio-Rad iQ-Check® Real-Time PCR Solution

D = Hygiena BAX® System

Table 6: Statistical Comparison for Microorganism Recovery Between MLG Reference Method and Each Screening Technology the MLG Reference Cultural Methods A = 3M™ Molecular Detection System, B = BioMérieux GENE-UP® Test System, C = Bio-Rad iQ-Check® Real-Time PCR Solution, D = Hygiene BAX® System

	Technology	Screen Results	Culture Positive	Culture Negative	Kappa	McNemar's p-value
<i>Salmonella sp.</i> (MLG 4)	A	Positive	189 (42.95%)	5 (1.14%)	.89	0.0043
		Negative	19 (4.32%)	227 (51.59%)		
	B	Positive	181 (41.14%)	5 (1.14%)	.85	0.00010
		Negative	27 (6.14%)	227 (51.59%)		
	C	Positive	195 (44.32%)	15 (3.41%)	.87	0.71
		Negative	13 (2.95%)	217 (49.32%)		
	D	Positive	185 (42.05%)	7 (1.59%)	.86	0.0035
		Negative	23 (5.23%)	225 (5.23%)		
<i>Listeria monocytogenes</i> (MLG 8)	A	Positive	60 (50.00%)	0 (0.00%)	.92	0.025
		Negative	5 (4.17%)	55 (45.83%)		
	B	Positive	60 (50.00%)	0 (0.00%)	.92	0.025
		Negative	5 (4.17%)	55 (45.83%)		
	C	Positive	59 (49.17%)	2 (1.67%)	.87	0.16
		Negative	6 (5.00%)	53 (44.17%)		
	D	Positive	64 (53.33%)	1 (0.83%)	.97	1
		Negative	1 (0.83%)	54 (45.00%)		
<i>Campylobacter sp.</i> (MLG 41)	C	Positive	46 (38.33%)	6 (5.00%)	.86	0.16
		Negative	2 (1.67%)	66 (55.00%)		
	D	Positive	43 (35.83%)	4 (3.33%)	.84	0.74
		Negative	5 (4.17%)	68 (56.67%)		
<i>E. coli</i> non-O157 STEC (MLG 5 and 5B)	B	Positive	89 (49.44%)	1 (0.56%)	.88	0.0067
		Negative	10 (5.56%)	80 (44.44%)		
	C	Positive	91 (50.56%)	1 (0.56%)	.90	0.020
		Negative	8 (4.44%)	80 (44.44%)		
	D	Positive	88 (48.89%)	0 (0.00%)	.88	0.00090
		Negative	11 (6.11%)	81 (45.00%)		
<i>E. coli</i> O157:H7 (MLG 5)	A	Positive	32 (53.33%)	1 (1.67%)	.90	0.56
		Negative	2 (3.33%)	25 (41.67%)		
	B	Positive	29 (48.33%)	1 (1.67%)	.80	0.10
		Negative	5 (8.33%)	25 (41.67%)		
	C	Positive	32 (53.33%)	1 (1.67%)	.90	0.56
		Negative	2 (3.33%)	25 (41.67%)		
D	Positive	24 (40.00%)	0 (0.00%)	.68	0.0016	
	Negative	10 (16.67%)	26 (43.33%)			

Table 7: Exclusivity and Inclusivity Results by Pathogen Type for the Four Technologies: A = 3M™ Molecular Detection System, B = BioMérieux GENE-UP® Test System, C = Bio-Rad iQ-Check® Real-Time PCR Solution, D = Hygiene BAX® System

	Technology	Screen Results	Culture Positive	Culture Negative
<i>Listeria monocytogenes</i>	A	Inclusivity (Positive)	30	0
		Exclusivity (Negative)	0	20
	B	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	19
	C	Inclusivity (Positive)	30	0
		Exclusivity (Negative)	0	20
	D	Inclusivity (Positive)	30	0
		Exclusivity (Negative)	6*	14
<i>Salmonella</i>	A	Inclusivity (Positive)	52	0
		Exclusivity (Negative)	0	20
	B	Inclusivity (Positive)	52	0
		Exclusivity (Negative)	5*	15
	C	Inclusivity (Positive)	52	0
		Exclusivity (Negative)	7*	13
	D	Inclusivity (Positive)	52	0
		Exclusivity (Negative)	2*	18
<i>Campylobacter</i>	C	Inclusivity (Positive)	30	0
		Exclusivity (Negative)	0	20
	D	Inclusivity (Positive)	30	0
		Exclusivity (Negative)	0	20
<i>E. coli</i> O157:H7 (O-group)	A	Inclusivity (Positive)	28	1
		Exclusivity (Negative)	0	20
	B	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
	C	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
	D	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
<i>E. coli</i> O157:H7 (stx)	B	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
	C	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
	D	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
<i>E. coli</i> O157:H7 (eae)	B	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
	C	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20

		Exclusivity (Negative)	0	20
	<b>D</b>	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
<i>E. coli</i> non-O157 (O-group)	<b>B</b>	Inclusivity (Positive)	24**	0
		Exclusivity (Negative)	N/A**	N/A**
	<b>C</b>	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	0	20
	<b>D</b>	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	0	20
<i>E. coli</i> non-O157 ( <i>stx</i> )	<b>B</b>	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	2*	18
	<b>C</b>	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	3*	17
	<b>D</b>	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	0	20
<i>E. coli</i> non-O157 ( <i>eae</i> )	<b>B</b>	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	0	20
	<b>C</b>	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	2	18
	<b>D</b>	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	1	19
* Late Ct value false positives (>35 cycles)				
** Technology Failure, no remaining kits				

**Table 8: Limit of Detection by Evaluated Screening Technologies Following MLG methods Performed in Triplicate.** A = 3M™ Molecular Detection System, B = BioMérieux GENE-UP® Test System, C = Bio-Rad iQ-Check® Real-Time PCR Solution, D = Hygiene BAX® System

Organism	Matrix	Technology A	Technology B	Technology C	Technology D
<i>Salmonella sp.</i>	Raw Beef	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>4</sup>
	RTE Catfish	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>4</sup>
	Cecal	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>4</sup>
	Turkey Sponges	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>4</sup>
<i>L. monocytogenes</i>	RTE Catfish	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>
<i>E. coli</i> O157:H7	Raw Beef	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>2</sup>	10 <sup>4</sup>
Non-O157 STEC	Raw Beef	NA*	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>4</sup>
<i>Campylobacter sp.</i>	Ground Chicken	NA*	NA*	10 <sup>3</sup>	10 <sup>4</sup>
	Turkey Sponges	NA*	NA*	10 <sup>2</sup>	10 <sup>4</sup>

\*NA=organism is not assessed for technology