Evaluation of commercial molecular screening platforms for the detection of food-borne bacterial pathogens by FSIS Field Service Laboratories

Abstract

Four commercially available molecular screening platforms were evaluated against the FSIS/OPHS 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 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 by FSIS/OPHS Field Service Laboratory (FSL) personnel to analyze a total of 440 *Salmonella* inoculated samples, 120 *L. monocytogenes* inoculated samples, 120 *Campylobacter* inoculated samples, and 180 Shiga toxin-producing *E. coli* (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 FSL high-throughput microbiology screening environment. All platforms evaluated had a high degree of concordance when compared to the FSIS MLG reference cultural methods, and met the performance expectations for predictive value, sensitivity, and selectivity.

Introduction

USDA 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* serotype O157:H7 and non-O157 (STEC), *Listeria monocytogenes*, and *Campylobacte*r spp.

Based upon the criteria devised by FSIS for the selection of the screening technology, for each targeted microorganism, USDA/FSIS tested a minimum of three (3) food product matrices with sixty (60) samples per method-. 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 alternative molecular screening method associated with the tested commercial instruments. Laboratory evaluations included a review of limit of detection, exclusivity and inclusivity panels, and a review of each technology for fitness-for-use as defined by the bench analysts at the three FSIS Field Services Laboratories

The USDA FSIS Field Service Laboratories (FSLs) laboratories evaluated the performance of each platform compared to the cultural methods described in the FSIS MLG (reference method[s]). For each microorganism, USDA FSIS tested a minimum of three food product types with sixty (60) samples per method. Food product samples were inoculated with the target

microorganisms at fractional recovery levels. A paired-study design was used to compare the performance of each platform (predictive value, sensitivity, selectivity) to the MLG reference cultural methods.

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

Each instrument was assigned a letter designation 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 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), ground beef, chicken rinses, and swabs. Data was not be 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 S*almonella*, *Listeria*, and *STECs,* the stock inoculum was plated on Petrifilm™ APC in duplicate and incubated at 35 ± 1°C for 24 ± 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 ± 2 hours at 42 ± 1.0^{O}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, *stx*2e, *stx*2g, *Salmonella enterica* subspecies III, non-β 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. 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. The LOD was the inoculum dilution at which 100% detection was no longer achieved. The lowest pure culture inoculum was plated in triplicate on plating medium or Petrifilm to determine the 95% Confidence Interval (CI) for LOD.

Results

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 are listed in **Tables 1-5** by microorganism and matrix along with the final results of the MLG reference cultural method. 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 – < 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**). Statistical analysis was also performed on the data using McNemar 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. A p- value of ≥ 0.05 indicates results are statistically similar to the MLG reference culture method.

Inclusivity/exclusivity evaluation

Inclusivity and exclusivity panels were gathered from pure culture DNA from bacterial strains collected from USDA FSIS 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 limits of detection for the microorganisms of interest were examined for each screening instrument. Matrices were enriched and incubated according to the appropriate MLG method. A 0.5 McFarland saline solution (~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**).

During the evaluation the technologies provided results described as inhibited, indeterminate or unidentifiable. Following the current business rules of the USDA 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.

Discussion

The USDA FSIS laboratories are high throughput laboratories requiring technologies that are reliable and high-throughput, in order to protect the safety of the consumer while minimizing the commercial impact for the producers. The USDA FSIS laboratories seek to utilize technologies that show a high degree of accuracy in a qualitative screen coupled with ease of use and robustness in a high-throughput setting. According to the data collected during extensive comparisons by the FSIS Field Service Laboratories, the performance of each molecular screening instrument was not statistically significantly different from the MLG reference cultural methods. The Kappa values indicated a good to very good agreement of all instruments to each MLG reference cultural method. Accuracy calculations, which took into account the false positives and false negatives were greater than 90% for a majority of the instruments. Overall, the molecular screening technologies and associated screening methods correctly classified the organisms tested in the exclusivity and inclusivity panels.

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

Table 2: Comparison of Positive *L. monocytogenes* 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 8**
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

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

Matrix	**Sample Number**	**Inoculum Level (CFU)**	**C**	**D**	**Cultural Method MLG 41**
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

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

Matrix	Sample Number	Inoculum Level (CFU)	B	C	D	Cultural Method MLG 5 & 5B
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

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

Table 6: Statistical Comparison of Microorganism Recovery from Each Screening Technology (A-D) against the MLG Reference Cultural Methods

			Test Positive	Test Negative	Kappa	McNemar p-value
Salmonella* *(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%)		

			Test Positive	Test Negative	Kappa	McNemar p-value
Listeria monocytogenes* *(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%)		

			Test Positive	Test Negative	Kappa	McNemar p-value
Campylobacter* *(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%)		

			Test Positive	Test Negative	Kappa	McNemar p-value
STEC* *(MLG 5 and 5B)	**B**	Positive	89 (49.44%)	1 (0.56%)	.88	0.0067
		Negative	10 (5.56%)	80 (44.44%)		

			Test Positive	Test Negative	Kappa	McNemar p-value
	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%)		

			Test Positive	**Test Negative**	**Kappa**	**McNemar p-value**
***E. coli* 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

			Test Positive	Test Negative
Listeria monocytogenes	**Technology A**	Inclusivity (Positive)	30	0
		Exclusivity (Negative)	0	20
	Technology B	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	19
	Technology C	Inclusivity (Positive)	30	0
		Exclusivity (Negative)	0	20
	Technology D	Inclusivity (Positive)	30	0
		Exclusivity (Negative)	6*	14

Notes: * Late Ct value false positives (>35 cycles)

			Test Positive	Test Negative
Salmonella	**Technology A**	Inclusivity (Positive)	52	0
		Exclusivity (Negative)	0	20
	Technology B	Inclusivity (Positive)	52	0
		Exclusivity (Negative)	5*	15
	Technology C	Inclusivity (Positive)	52	0
		Exclusivity (Negative)	7*	13
	Technology D	Inclusivity (Positive)	52	0
		Exclusivity (Negative)	2*	18

Notes: * Late Ct value false positives (>35 cycles)

			Test Positive	Test Negative
Campylobacter	**Technology C**	Inclusivity (Positive)	30	0
		Exclusivity (Negative)	0	20
	Technology D	Inclusivity (Positive)	30	0
		Exclusivity (Negative)	0	20

			Test Positive	Test Negative
***E. coli* O157:H7 (O-group)**	**Technology A**	Inclusivity (Positive)	28	1
		Exclusivity (Negative)	0	20

	Technology B	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
	Technology C	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
	Technology D	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
E. coli* O157:H7 *(stx)	**Technology B**	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
	Technology C	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
	Technology D	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
E. coli* O157:H7 *(eae)	**Technology B**	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
	Technology C	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20
	Technology D	Inclusivity (Positive)	29	0
		Exclusivity (Negative)	0	20

			Test Positive	**Test Negative**
***E. coli* non-O157 (O-group)**	**Technology B**	Inclusivity (Positive)	24**	0
		Exclusivity (Negative)	N/A**	N/A**
	Technology C	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	0	20
	Technology D	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	0	20
E. coli* non- O157 *(stx)	**Technology B**	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	2*	18
	Technology C	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	3*	17
	Technology D	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	0	20
		Inclusivity (Positive)	64	0

E. coli* non-O157 *(eae)	**Technology B**	Exclusivity (Negative)	0	20
	Technology C	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	2	18
	Technology D	Inclusivity (Positive)	64	0
		Exclusivity (Negative)	1	19

Notes: * Late Ct value false positives (>35 cycles)
** **Technology** Failure, no remaining kits

Table 8: Limit of Detection Evaluation of Evaluated Screening Technologies using MLG methods

Organism	Matrix	Technology A	Technology B	Technology C	Technology D
Salmonella	**Raw Beef**	10^4	10^4	10^4	10^4
	RTE Catfish	10^5	10^5	10^4	10^4
	Cecal	10^4	10^3	10^3	10^4
	Turkey Sponges	10^4	10^3	10^2	10^4
L. monocytogenes	**RTE Catfish**	10^4	10^5	10^4	10^3
***E. coli* O157:H7**	**Raw Beef**	10^4	10^4	10^2	10^4
STEC	**Raw Beef**	**NA***	10^5	10^5	10^4
Campylobacter	**Ground Chicken**	**NA***	**NA***	10^3	10^4
	Turkey Sponges	**NA***	**NA***	10^2	10^4

*NA=organism is not applicable for technology