Verification Testing of iQ-Check™ and dd-Check STEC Kits with Onions, Carrots, and Cucumbers

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Abstract

While fresh produce is nutritionally beneficial, it can pose a risk of foodborne outbreaks due to bacterial contamination, especially because it is often consumed raw. Food safety standards and preventive measures are needed to protect the safety of these commodities. The purpose of this study was to perform verification testing on onions, carrots, and cucumbers with iQ-Check PCR Detection Kits and dd-Check STEC Kits (onions only) to determine their fit for purpose for these matrices.

Introduction

Salmonella spp. and *Escherichia coli* are two of the most common bacterial pathogens responsible for foodborne outbreaks. Both can contaminate a wide range of foods, leading to significant public health concerns. In general, they can be present in raw fruits and vegetables due to contamination during farming, harvesting, or processing or due to contaminated water sources.

In a survey of foodborne illness from 1998 to 2022, the Centers for Disease Control and Prevention (CDC) attributed 66.8% and 39.4% cases of *E. coli* and *Salmonella* illness, respectively, to a fruit or vegetable food matrix. Over the last few months of 2024, there were outbreaks in the United States associated with organic carrots, cucumbers, and onions, with the last being linked to a popular fast-food chain. These three outbreaks alone involved 265 reported cases and led to 82 hospitalizations.

The use of molecular methods for pathogen detection are useful for rapid determination of results, particularly for produce matrices that have a short shelf life. Real-time PCR (qPCR) has the added advantage of increased sensitivity for the target organism and selectivity against competing bacteria. Droplet Digital™ PCR (ddPCR™) technology is a powerful tool in which a sample is divided into thousands of nanoliter-sized droplets. This approach enables direct colocalization, which is useful for detection and confirmation of, for example, Shiga toxin–producing *E. coli* (STEC), ensuring the target virulence genes *stx1* and *stx2*, as well as *eae*, are detected as physically present on the genome of a single bacterial cell and each not merely present in different cells in the sample.

In this study, we performed verification testing to determine the viability of iQ-Check PCR Kits for detecting pathogens in onions, carrots, and cucumbers. Onion samples were also tested with the dd-Check STEC Kit.

Methods

Matrices

Fresh chopped onions, sliced carrots, and whole cucumbers were purchased from a local grocery store.

Test Strains

Cultures were prepared by inoculating 9 ml Tryptic Soy Broth (TSB; Hardy Diagnostics, catalog #88) and incubating for 18 hr at 37 ± 1°C, followed by refrigeration at 4 ± 1°C. Cultures were serial diluted and plated on Tryptic Soy Agar (TSA; Hardy Diagnostics, #G60) for 18 hr before inoculating matrices to determine titers for target inoculation. Cultures were plated on TSA again at the time of inoculation. Target strains were paired with competing organisms for inoculation (Table 1).

Enrichment Conditions and PCR/ddPCR Methods

The onion and carrot samples were enriched with Buffered Peptone Water Standard (BPW Standard; Bio-Rad Laboratories, Inc., #12013259) prewarmed to 37 ± 1°C at a 1:9 ratio (25 g sample + 225 ml medium). Selective STEC Supplement (Bio-Rad, #3564005) was added to half of the onion enrichments. Samples were incubated for 18 hr at 37 ± 1°C. The iQ-Check Easy II DNA extraction protocol was used for samples tested with the iQ-Check *E. coli* O157:H7 PCR Detection Kit (Bio-Rad, #3578114) and iQ-Check STEC VirX PCR Detection Kit (Bio-Rad, #3578139).

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Table 1. Target organisms matched with competing strains.

Food Matrix	Target Organism	Strain ID	Competing Organism	Strain ID
Onion	*E. coli* O157:H7	FDA ESC1177	*E. coli*	ATCC 25922
Carrot	*E. coli* O157:H7	FDA ESC1177	*E. coli*	ATCC 25922
Cucumber	*S.* Typhimurium	ATCC 14028	*E. coli*	ATCC 25922

The tube filtration and centrifugation protocols were used for samples tested with the dd-Check STEC Kit (Bio-Rad, #12009999). PCR was run on either the CFX96 Touch Deep Well or the CFX Opus Deepwell (Bio-Rad, #17007991) Real-Time PCR System. For Droplet Digital PCR, droplets were generated with the QX200™ Droplet Generator (Bio-Rad, #17005227) and read with the QX200 Droplet Reader (Bio-Rad, #17005228).

Whole cucumbers were sliced and enriched with BPW Standard prewarmed to 37 ± 1°C at a 1:9 ratio (25 g sample and 225 ml medium). Samples were incubated for 18 hr at 37 ± 1°C. The iQ-Check Easy I DNA extraction protocol was used, and samples were tested with the iQ-Check *Salmonella* II Kit (Bio-Rad, #3578123).

Test Protocol

Cultures were prepared based on the 18 hr–incubation plate count, and the final count was calculated by plating the refrigerated culture used for inoculation. For high level–inoculum samples, the targeted bacteria range was 100–1,000 colony forming units (CFU), and the competing bacteria range was >1,000 CFU. For low level–inoculum samples, the targeted bacteria range was 5–10 CFU, and the competing bacteria range was 100–500 CFU. Any deviation from these values was noted.

For each matrix tested, three samples were spiked with high-level inoculum of target bacteria and competing bacteria. In addition, three samples were spiked with low-level inoculum of target and competing bacteria; one sample not inoculated was used as a matrix control.

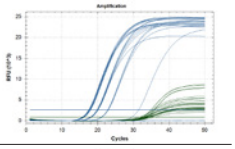
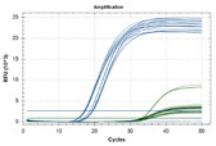
After 18 hr of enrichment, samples were removed from the incubator and tested with iQ-Check PCR and dd-Check STEC Kits.

Results

Detection of *E. coli* O157:H7 in Onions

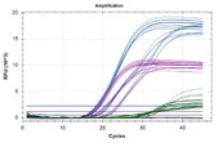
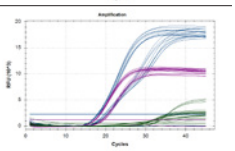
When tested in triplicate with the iQ-Check *E. coli* O157:H7 PCR Kit, positive results were obtained for all inoculation levels after 18 hr of incubation, with or without STEC Supplement (Table 2). All inoculation levels also yielded positive results after 18 hr of incubation, with or without STEC Supplement, when tested with the iQ-Check STEC VirX PCR Kit (Table 3). When tested with dd-Check STEC Solution, all inoculation levels showed strong positive results after 18 hr of incubation. The samples were run with dithiothreitol (DTT; Bio-Rad, #12012171), and dilution from 1:100 to 1:1,000 was needed to ascertain linkage (Table 4, Figure 1).

Table 2. iQ-Check *E. coli* O157:H7 PCR Kit results for onions.

Matrix	Inoculum	Sample	Rep 1 FAM	Rep 1 HEX	Rep 1 Result	Rep 2 FAM	Rep 2 HEX	Rep 2 Result	Rep 3 FAM	Rep 3 HEX	Rep 3 Result	PCR Curves
Onions, fresh chopped 18 hr incubation BPW	No inoculum	0	N/A	33.5	Negative	N/A	32.98	Negative	N/A	33.66	Negative	
	E. coli O157:H7: 560 CFU *E. coli:* 3,794 CFU	H1	18.03	34.25	Positive	17.85	34.55	Positive	17.88	34.45	Positive	
		H2	17.95	34.14	Positive	17.74	34.14	Positive	17.61	34.49	Positive	
		H3	17.51	34.39	Positive	17.48	35.96	Positive	17.46	34.87	Positive	
	E. coli O157:H7: 6 CFU *E. coli:* 379 CFU	L1	19.81	33.84	Positive	19.82	33.36	Positive	20.12	32.86	Positive	
		L2	22.80	31.68	Positive	22.94	31.85	Positive	23.02	32.50	Positive	
		L3	20.07	33.03	Positive	20.07	33.08	Positive	19.98	33.50	Positive	
Onions, fresh chopped 18 hr incubation BPW + STEC Supplement	No inoculum	0	N/A	33.34	Negative	N/A	33.01	Negative	N/A	33.16	Negative	
	E. coli O157:H7: 560 CFU *E. coli:* 3,794 CFU	H1	17.19	35.26	Positive	17.20	35.38	Positive	17.30	36.06	Positive	
		H2	17.11	35.52	Positive	17.33	34.88	Positive	17.39	34.10	Positive	
		H3	17.12	35.01	Positive	17.36	35.39	Positive	17.20	34.31	Positive	
	E. coli O157:H7: 6 CFU *E. coli:* 379 CFU	L1	18.67	34.01	Positive	18.75	34.18	Positive	18.70	33.70	Positive	
		L2	19.36	33.59	Positive	19.53	33.23	Positive	19.54	33.45	Positive	
		L3	19.36	34.01	Positive	19.25	33.65	Positive	19.42	34.01	Positive	

FAM (—), *E. coli* O157:H7 detection; HEX (—), internal control detection. BPW, buffered peptone water; CFU, colony forming units; STEC, Shiga toxin–producing *E. coli*.

Table 3. iQ-Check STEC VirX PCR Kit results for onions.

Matrix	Inoculum	Sample	Rep 1 FAM	Rep 1 Cy5	Rep 1 HEX	Rep 1 Result	Rep 2 FAM	Rep 2 Cy5	Rep 2 HEX	Rep 2 Result	Rep 3 FAM	Rep 3 Cy5	Rep 3 HEX	Rep 3 Result	PCR Curves
Onions, fresh chopped 18 hr incubation BPW	No inoculum	0	N/A	N/A	32.15	Negative	N/A	N/A	31.94	Negative	N/A	N/A	32.44	Negative	
	E. coli O157:H7: 560 CFU *E. coli:* 3,794 CFU	H1	18.77	18.24	27.71	Positive	18.63	18.07	26.83	Positive	18.53	17.96	27.19	Positive	
		H2	18.72	18.18	28.25	Positive	18.54	18.03	27.01	Positive	18.39	17.69	26.86	Positive	
		H3	18.22	17.79	28.01	Positive	18.28	17.81	27.29	Positive	18.29	17.62	N/A	Positive	
	E. coli O157:H7: 6 CFU *E. coli:* 379 CFU	L1	20.63	19.9	30.26	Positive	20.53	19.9	29.97	Positive	20.99	20.05	29.64	Positive	
		L2	23.35	22.67	30.09	Positive	23.70	22.9	30.08	Positive	23.56	22.77	30.03	Positive	
		L3	20.94	20.42	30.82	Positive	20.73	20.29	30.67	Positive	20.63	20.23	30.33	Positive	
Onions, fresh chopped 18 hr incubation BPW + STEC Supplement	No inoculum	0	N/A	N/A	32.19	Negative	N/A	N/A	32.06	Negative	N/A	N/A	31.86	Negative	
	E. coli O157:H7: 560 CFU *E. coli:* 3,794 CFU	H1	18.23	17.51	26.39	Positive	17.93	17.26	25.89	Positive	18.01	17.39	30.91	Positive	
		H2	17.94	17.22	27.46	Positive	18.11	17.41	26.79	Positive	18.12	17.46	N/A	Positive	
		H3	17.85	17.27	26.45	Positive	17.83	17.28	33.43	Positive	17.97	17.30	26.20	Positive	
	E. coli O157:H7: 6 CFU *E. coli:* 379 CFU	L1	19.68	19.08	29.82	Positive	19.77	19.17	29.85	Positive	19.64	19.15	29.93	Positive	
		L2	20.37	19.52	30.14	Positive	20.42	19.73	30.18	Positive	20.14	19.55	29.49	Positive	
		L3	20.14	19.5	30.08	Positive	20.16	19.28	30.14	Positive	20.38	19.57	30.13	Positive	

FAM (—), *stx1/stx2* detection; Cy5 (—), *eae* detection; HEX (—) internal control detection. BPW, buffered peptone water; CFU, colony forming units; Cy, cyanine; STEC, Shiga toxin–producing *E. coli*.

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Table 4. dd-Check STEC ddPCR results for onions.

Matrix	Inoculum	Sample	Accepted Droplets	*stx* Value, copies	*eae* Value, copies	Linkage, %	Sample Result
Onions, fresh chopped 18 hr incubation BPW with DTT 1:100 dilution	No inoculum	0	19,679	0	0		Negative
	E. coli O157:H7: 560 CFU *E. coli:* 3,794 CFU	H1	17,108	16,406	16,014	88	Positive: linked targets
		H2	18,459	35,281	32,900	77.8	Positive: linked targets
		H3	18,982	35,492	32,780	75	Positive: linked targets
	E. coli O157:H7: 6 CFU *E. coli:* 379 CFU	L1	17,296	11,308	10,989	85.5	Positive: linked targets
		L2	18,795	791	730	82.3	Positive: linked targets
		L3	18,009	7,683	6,928	78.6	Positive: linked targets
Onions, fresh chopped 18 hr incubation BPW with DTT 1:1,000 dilution	No inoculum	0	19,549	0	0		Negative
	E. coli O157:H7: 560 CFU *E. coli:* 3,794 CFU	H1	19,866	3,216	3,105	87.7	Positive: linked targets
		H2	18,995	2,951	2,759	79.7	Positive: linked targets
		H3	17,869	3,592	3,176	75.4	Positive: linked targets
	E. coli O157:H7: 6 CFU *E. coli:* 379 CFU	L1	18,786	1,146	1,084	86.5	Positive: linked targets
		L2	18,042	64	66.6	74	Positive: linked targets
		L3	16,675	709	689	78.1	Positive: linked targets

BPW, buffered peptone water; CFU, colony forming units; DTT, dithiothreitol.

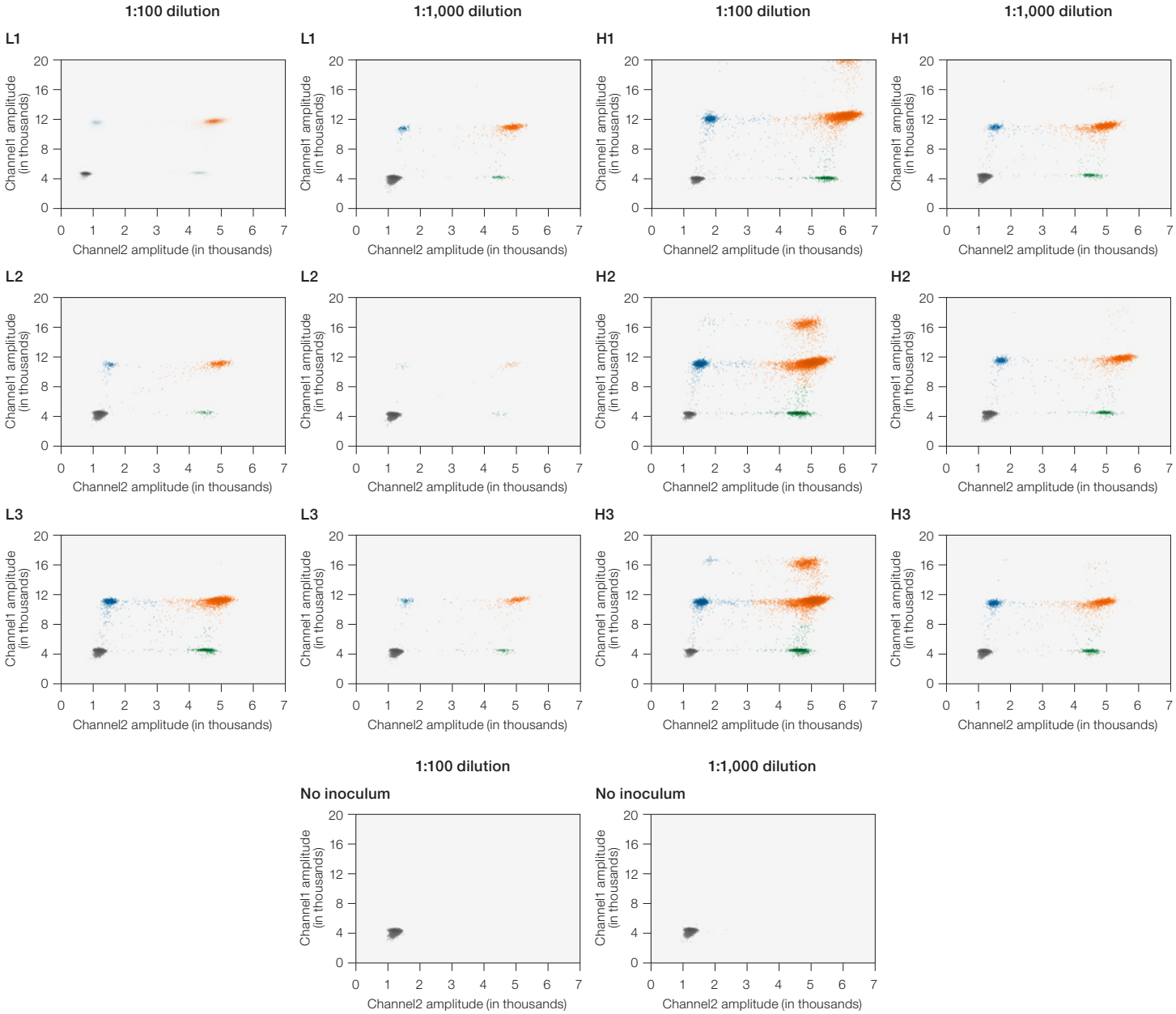
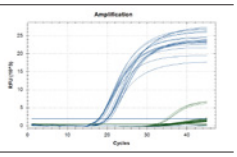


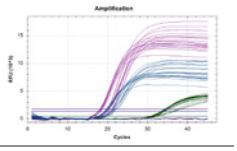
Fig. 1. 2-D amplitude graph of *E. coli* O157:H7 in onions when tested with dd-Check STEC Solution. The plots show droplets negative for both targets (■), droplets positive for *stx* only (■), droplets positive for *eae* only (■), and droplets positive for both *stx* and *eae* on the same genome (■).

Table 5. iQ-Check *E. coli* O157:H7 PCR Kit results for carrots.

Matrix	Inoculum	Sample	Rep 1 FAM	Rep 1 HEX	Rep 1 Result	Rep 2 FAM	Rep 2 HEX	Rep 2 Result	Rep 3 FAM	Rep 3 HEX	Rep 3 Result	PCR Curves
Carrots, fresh sliced 18 hr incubation BPW	No inoculum	0	N/A	33.20	Negative	N/A	33.08	Negative	N/A	32.52	Negative	
	E. coli O157:H7: 618 CFU *E. coli:* 3,564 CFU	H1	17.83	N/A	Positive	17.88	38.94	Positive	17.82	38.35	Positive	
		H2	17.93	38.07	Positive	17.94	39.68	Positive	17.76	39.47	Positive	
		H3	18.53	37.54	Positive	18.27	38.40	Positive	18.04	38.19	Positive	
	E. coli O157:H7: 6 CFU *E. coli:* 356 CFU	L1	20.08	36.82	Positive	19.95	37.48	Positive	19.78	35.84	Positive	
		L2	20.59	36.45	Positive	20.57	35.36	Positive	19.96	36.62	Positive	
		L3	19.93	36.49	Positive	20.02	N/A	Positive	19.34	39.71	Positive	

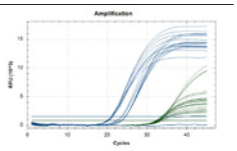
FAM (—), *E. coli* O157:H7 detection; HEX (—), internal control detection. BPW, buffered peptone water; CFU, colony forming units.

Table 6. iQ-Check STEC VirX PCR Kit results for carrots.

Matrix	Inoculum	Sample	Rep 1 FAM	Rep 1 Cy5	Rep 1 HEX	Rep 1 Result	Rep 2 FAM	Rep 2 Cy5	Rep 2 HEX	Rep 2 Result	Rep 3 FAM	Rep 3 Cy5	Rep 3 HEX	Rep 3 Result	PCR Curves
Carrots, fresh sliced 18 hr incubation BPW	No Inoculum	0	N/A	N/A	32.04	Negative	N/A	N/A	32.11	Negative	N/A	N/A	31.85	Negative	
	E. coli O157:H7: 618 CFU *E. coli:* 3,564 CFU	H1	19.3	18.05	31.4	Positive	19.14	18	31.16	Positive	19.12	18.02	31.20	Positive	
		H2	19.17	18.21	31.08	Positive	19.2	18.15	31.24	Positive	19.36	18.43	31.83	Positive	
		H3	19.51	18.65	30.76	Positive	19.41	18.51	31.10	Positive	19.36	18.32	32.85	Positive	
	E. coli O157:H7: 6 CFU *E. coli:* 56 CFU	L1	20.94	20.24	31.23	Positive	20.77	20.12	30.82	Positive	20.48	19.87	31.75	Positive	
		L2	21.48	21.06	30.97	Positive	21.55	21.16	30.7	Positive	21.37	20.74	31.91	Positive	
		L3	21.1	20.27	30.56	Positive	21.17	20.35	31.08	Positive	20.91	19.80	31.80	Positive	

FAM (—), *stx1/stx2* detection; Cy5 (—), *eae* detection; HEX (—) internal control detection. BPW, buffered peptone water; CFU, colony forming units; Cy, cyanine.

Table 7. iQ-Check *Salmonella* PCR Kit results for cucumbers.

Matrix	Inoculum	Sample	Rep 1 FAM	Rep 1 HEX	Rep 1 Result	Rep 2 FAM	Rep 2 HEX	Rep 2 Result	Rep 3 FAM	Rep 3 HEX	Rep 3 Result	PCR Curves
Cucumbers, fresh sliced 18 hr incubation BPW	No Inoculum	0	N/A	33.07	Negative	N/A	33.08	Negative	N/A	33.20	Negative	
	S. Typhimurium: 534 CFU *E. coli:* 3,564 CFU	H1	21.04	34.4	Positive	21.15	33.62	Positive	21.01	33.29	Positive	
		H2	21.46	33.61	Positive	21.22	33.58	Positive	21.03	32.70	Positive	
		H3	21.36	33.49	Positive	21.18	33.17	Positive	21.15	32.98	Positive	
	S. Typhimurium: 7 CFU *E. coli:* 356 CFU	L1	24.50	32.82	Positive	24.61	33.02	Positive	24.57	32.30	Positive	
		L2	23.82	33.06	Positive	23.68	32.55	Positive	23.46	32.75	Positive	
		L3	24.47	32.59	Positive	24.51	32.35	Positive	24.52	32.38	Positive	

FAM (—), *Salmonella* spp. detection; HEX (—) internal control detection. BPW, buffered peptone water; CFU, colony forming units.

Detection of *E. coli* O157:H7 in Carrots

When tested in triplicate with the iQ-Check *E. coli* O157:H7 PCR Kit, all inoculation levels showed positive results after 18 hr of incubation (Table 5). All inoculation levels also yielded positive results after 18 hr of incubation when tested with the iQ-Check STEC VirX PCR Kit (Table 6).

Detection of *Salmonella* in Cucumbers

When tested in triplicate with the iQ-Check *Salmonella* PCR Kit, positive results after 18 hr of incubation were obtained for all inoculation levels, with or without STEC Supplement (Table 7).

Conclusion

Onions, carrots, and cucumbers tested with iQ-Check and dd-Check PCR Kits were verified at the target enrichment ratio and incubation time for *E. coli* O157:H7 (onions and carrots) and *Salmonella* (cucumbers). Selective STEC Supplement was not required for the enrichment of onions. The dd-Check STEC Kit was able to determine that the strain used to inoculate onions contained both *stx* and *eae* virulence genes on the same genome. Samples were detected after 18 hr of incubation, which allows for a rapid response in the event of a contamination event.

Further Reading

Center for Disease Control and Prevention. *E. coli* Outbreak Linked to Onions Served at McDonald's. cdc.gov/ecoli/outbreaks/e-coli-O157.html accessed January 13, 2025.

Center for Disease Control and Prevention. *E. coli* Outbreak Linked to Organic Carrots. cdc.gov/ecoli/outbreaks/e-coli-o121.html accessed January 13, 2025.

Center for Disease Control and Prevention. *Salmonella* Outbreak Linked to Cucumbers. cdc.gov/*salmonella*/outbreaks/cucumbers-11-24/index.html accessed January 13, 2025.

Center for Disease Control and Prevention. Foodborne Illness Source Attribution Estimates – United States, 2022. cdc.gov/ifsac/php/data-research/annual-report-2022.html accessed January 13, 2025.

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