Matrix Extension Study of the iQ-Check *Salmonella* II and iQ-Check STEC VirX Real-Time PCR Methods for Hemp Flower

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Introduction

Escherichia coli is a bacterium that is commonly found in the human gastrointestinal tract. Some strains can be pathogenic to humans: STEC, EPEC, EHEC, etc. STEC are *E. coli* that produce Shiga toxins encoded by *stx* genes. STEC are not necessarily associated with human disease. The main modes of transmission of STEC infections to humans is in the consumption of contaminated food such as meat products, milk and dairy products made with pasteurized or unpasteurized cow's milk or goat's milk, consumption of raw vegetables contaminated by animal feces, and ingestion of contaminated water and contact with animals (particularly bovines).

Salmonella has been recognized as a primary cause of foodborne illness worldwide. This genus of bacteria is classified into two species: *S. enterica* and *S. bongori*, the former containing the majority of serotypes associated with human disease. *Salmonella* can contaminate a wide range of foods including poultry, meat, eggs, dairy, fruit and vegetables as well as pet food.

The Bio-Rad iQ-Check PCR Detection Kits are based on gene amplification and detection using real-time polymerase chain reaction (PCR) technology. Each kit contains ready-to-use PCR reagents: oligonucleotides (primers and probes) specific for the target analytes, and DNA polymerase and nucleotides. Detection and data analysis are optimized for use with a Bio-Rad real-time PCR instrument, such as the CFX96 Touch Deep Well system.

Purpose

This study evaluated the effectiveness of the iQ-Check *Salmonella* II PCR Detection Kit and iQ-Check STEC VirX PCR Detection Kit methods to screen for the presence of *Salmonella* species and STEC in dried hemp flower samples at a 25g test portion size.

Highlights/Conclusion

The iQ-Check *Salmonella* II and STEC VirX PCR Detection Kits provide presumptive positive results of *Salmonella* and STEC spp. in roughly 2.5-3 hours following 20 hours of incubation in 25g test portion sizes of dried hemp flower. The use of iQ-Check Free DNA Removal Solution (FDRS) in sample preparation ensured higher concordance of presumptive results against confirmed results when comparing data to the samples processed without FDRS.

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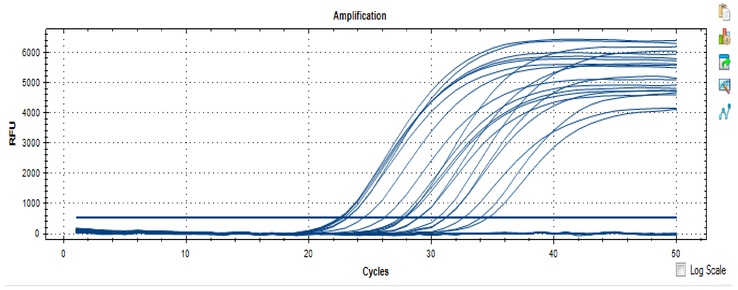
Method

The dried hemp flower was co-inoculated using *Salmonella* Typhimurium and *E. coli* O157 in the form of a lyophilized pellet crushed into Non-fat Dry Milk (NFDM). Serial dilutions were made of the lyophilized pellet and NFDM mixture to determine the CFU/pellet. A large container of dried hemp flower was inoculated with the NFDM mixture and allowed to equilibrate for two weeks at room temperature (20-25°C) prior to testing. An MPN was performed at a time point in the two-week hold time to determine the inoculum concentration in the matrix prior to validation testing.

Sample preparation, analysis, and presumptive positive confirmations were performed according to SMPR 2020.002 and SMPR 2020.012.

The matrix was enriched and incubated, and after incubation, all test portions were processed by the Bio-Rad iQ-Check *Salmonella* II and iQ-Check STEC VirX methods. All samples, regardless of presumptive result, were confirmed according to the corresponding SMPRs.

Samples are enriched 1:10 with BPW and incubated at 37°C for 20 hours. Following incubation, a single lysis step is performed and transferred to wells with PCR reagents for *Salmonella* and STEC analysis.



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Results

APC results for the dried hemp flower were 1.9 x 10^2. At the low level of inoculation, fractional positive results were achieved. The POD comparisons between the candidate method presumptive and confirmed results indicated there was no significant difference with all ranges of the 95% confidence intervals containing the zero point.

MPN analysis values were obtained using the Least Cost Formulations MPN calculator. For *Salmonella*, the resulting low inoculation was 1.45 CFU/test portion, and 4.65 CFU/test portion for the high inoculation level. For STEC, the resulting low inoculation was 1.28 CFU/test portion and 7.19 CFU test portion for the high inoculation level.

Results obtained for this Independent Laboratory study met the statistical requirements in AOAC Appendix J. Only one on false positive result was seen in the STEC analysis for the non-FDRS treated samples, although all other confirmations matched presumptive results for both *Salmonella* and STEC analysis.

Table 1: Bio-Rad iQ-Check *Salmonella* II and iQ-Check STEC VirX
Presumptive vs Confirmed Results (Paired) – POD Results Hemp 25 g

Matrix and Inoculum	Test Kit and Parameters	MPN_a / Test Portion	N^b	x^c	Presumptive		x	Confirmed		$dPOD_{cp}{}^f$	95% CI[g]
					$POD_{cp}{}^d$	95% CI		$POD_{cc}{}^e$	95% CI		
Dried hemp flower 25 g (*Salmonella* Typhimurium ATCC 14028 and E. coli O157:H7 ATCC 43895)	iQ-Check *Salmonella* II	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.45 (0.88, 2.60)	20	14	0.70	0.48, 0.86	14	0.70	0.48, 0.86	0.00	(-0.13, 0.13)
		4.65 (1.80, 12.0)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
	iQ-Check *Salmonella* II – FDRS treated	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.45 (0.88, 2.60)	20	14	0.70	0.48, 0.86	14	0.70	0.48, 0.86	0.00	(-0.13, 0.13)
		4.65 (1.80, 12.0)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
	iQ-Check STEC VirX	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.28 (0.74, 2.18)	20	14	0.70	0.48, 0.86	13	0.65	0.43, 0.82	0.05	(-0.11, 0.21)
		7.19 (2.51, 20.6)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
	iQ-Check STEC VirX – FDRS treated	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
		1.28 (0.74, 2.18)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	(-0.13, 0.13)
		7.19 (2.51, 20.6)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

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