Validation of single sample enrichment for microbial detection of *Salmonella* spp./STEC in cannabis products using real-time PCR

Authors: Sean Peschisolido, Mike Clark, Amrita Puri, Christophe Quiring
In collaboration with Weck Laboratories, 14859 East Clark Avenue City of Industry, CA 91745

Introduction

With increasing state-level adoption of medicinal and recreational marijuana legislation across the United States and federal legalization internationally, there has emerged a need for microbial pathogen detection in cannabis. Consumers now have access to cannabis edibles and inhalables for consumption prompting a need for strict monitoring of finished goods analogous to those methods adopted in food manufacturing. *Salmonella* and STEC (Shiga-toxin producing *E. coli*) bacteria are of particular concern with regard to cannabis products. These bacteria can cause illnesses and infections to users and pose serious risk to those with compromised immune systems. Due to the concerning health impacts relating to pathogen exposure, laboratories are challenged to find effective and efficient screening methods. Bio-Rad has established itself as a global leader in food safety with a portfolio of microbial detection methods that have received accreditations from AOAC and AFNOR. These methods can be successfully adapted to screen cannabis for both *Salmonella* and STEC.

Traditionally, screening methods for these pathogens have utilized time consuming independent enrichments. In the present study, the use of iQ-Check *Salmonella* and STEC kits demonstrates a fast, easy to use, reliable way to yield accurate results with only a single enrichment. This harmonized enrichment combined with iQ-Check's easy to use protocol delivers a simple solution with ONE sample, ONE enrichment, and ONE extraction.

The Bio-Rad iQ-Check kits are based on gene amplification and detection by the use of real-time PCR. Ready-to-use PCR reagents contain everything needed to detect targeted pathogens with high sensitivity and specificity. The iQ-Check *Salmonella* II Kit uses primers and patented double stranded probes specific for *Salmonella* species while the iQ-Check STEC VirX Kit uses primers and double stranded probes specific for STEC virulence genes *stx1, stx2,* and *eae*. An internal control is included in the reagents to validate any negative results in each well to monitor PCR inhibition.

Inoculum Preparation

Inoculum used for this study was *E. coli* O45:H2 (CDC 00-3039) and *Salmonella* Pullorum (ATCC 1303). Stock culture was serially diluted, then plated in triplicate between 10^{-4} to 10^{-9} and incubated overnight at 37 ± 1° C to achieve a target inoculation of 1-10 CFU per sample size for the low inoculum level and 10-100 CFU per sample size for the high inoculum level. Streaking was performed on Standard Methods Agar (SMA) with a thin layer overlay of MacConkey's agar for *E. coli* identification and XLD agar for *Salmonella* identification.

Seeding of inoculum

An aliquot of inoculum for *Salmonella*, *E. coli*, and a cocktail of both target organisms was administered drop-wise to each 1 g sample size of dried cannabis flower and allowed to dry for 1 hr. The artificially spiked samples were then refrigerated at 2–8 °C overnight to allow the inoculum to equilibrate to the matrix.

Table 1. Inoculum levels achieved per test portion

Inoculating Organism	*S.* Pullorum ATCC 1303	*E. coli* O45:H2 CDC 00-3039
Low-Inoculum Level CFU/Test Portion	6.4	5.4
High-Inoculum Level CFU/Test Portion	13.5	16.0

Table 2. Replicates for low and high inoculum levels

Matrix	Inoculating Organism	Number of Replicates
Cannabis Flower	*S.* Pullorum ATCC 1303	1 Negative 10 Low 5 High
Cannabis Flower	*E. coli* O45:H2 CDC 00-3039	1 Negative 10 Low 5 High
Cannabis Flower	Cocktail (S. Pullorum + *E. coli* O45:H2)	1 Negative 10 Low 10 High

iQ-Check Protocol

Fig. 1. iQ-Check Protocol

Each 1 g inoculated and uninoculated sample was aseptically weighed and added to 9 ml of Buffered Peptone Water (BPW), homogenized for 2 min and incubated for 21 ± 2 hr at 37 ± 1°C. After incubation, DNA extraction was performed following Bio-Rad's Easy I extraction protocol. During extraction, separate PCR mix was prepared for both target organisms as instructed in the iQ-Check user manuals and distributed to the appropriate sample well. A single PCR plate was used for both *Salmonella* and STEC assays. After DNA extraction, 5 µl of extract was added to the appropriate well and the PCR plate sealed. The PCR plate was immediately placed in the CFX 96 Touch Deep Well Real-Time PCR instrument and for PCR amplification step. Upon completion of the PCR program, results are automatically analyzed and interpreted by Bio-Rad's user friendly CFX Manager IDE Software.

Following PCR analysis, samples that yielded negative results were culturally confirmed to have no growth using selective agar plates for each targeted organism.

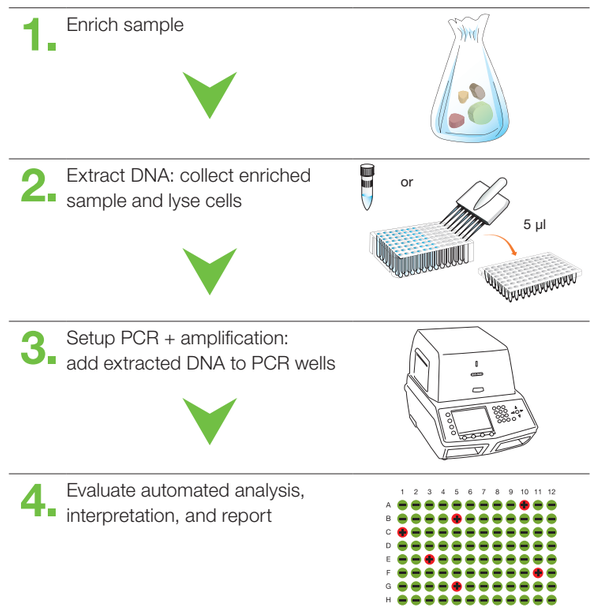

Fig. 2. Four-step Protocol

Results

All uninoculated samples tested negative by PCR and confirmed negative by culture as well (Tables 3 and 4). For the samples inoculated with low levels of *S.* Pullorum, 8 of 10 samples tested positive by PCR and 2 samples that tested negative were confirmed negative culturally. All 5 samples of high levels of *S.* Pullorum tested positive by PCR. Samples inoculated with low and high level of *E. coli* O45:H2 tested positive on all samples. Cannabis flower inoculated with the cocktail (*S.* Pullorum + *E. coli* O45:H2), tested positive for the target bacteria for all samples.

Table 3. Results of iQ-Check *Salmonella* II kit from harmonized enrichment

Inoculated Cannabis Flower	*S.* Pullorum only	Cocktail (*S.* Pullorum + *E. coli* O45:H2)
Result	PCR Positive	PCR Positive
Uninoculated	0/1*	0/1*
Low	8/10*	10/10
High	5/5	10/10

Table 4. Results of iQ-Check STEC VirX kit from harmonized enrichment

Inoculated Cannabis Flower	*E. coli* O45:H2 only	Cocktail (*S.* Pullorum + *E. coli* O45:H2)
Result	PCR Positive	PCR Positive
Uninoculated	0/1*	0/1*
Low	10/10	10/10
High	5/5	10/10

*Negative PCR results culturally confirmed as negative.

Conclusions

The data generated by this study demonstrates that using a single enrichment of cannabis flower in BPW incubated for 21 ± 2 hr at 37 ± 1°C followed by PCR screening with iQ-Check *Salmonella* II and iQ-Check STEC VirX kits, allows for the detection of *Salmonella* and STEC from 1 sample using 1 extraction and analyzed in 1 PCR plate. The use of a single enrichment media for the detection of two bacterial pathogens, also significantly reduces the environmental impact of pathogen testing by reducing the need for consumables and multiple incubators. This protocol provides a simple, cost effective way to screen cannabis products for both *Salmonella* and STEC.

Further studies are underway to validate more sample types of both inhalable and edible products.

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