Application Note

Detection of Salmonella and Shiga-toxin producing *E.coli* (STEC) in Beverage and Topical Matrices using the PathoSEEK® Salmonella & STEC E. coli Multiplex Detection Assay

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Introduction

Colorado cannabis testing regulations require cannabis flower and cannabis products to be free of Salmonella species and shiga toxin producing *E. coli* (STEC *E.coli*). The PathoSEEK® Salmonella and STEC *E. coli* Multiplex Assay with SenSATIVAx® Extraction Protocol is designed to detect a wide variety of species classified as Salmonella and STEC *E. coli* in cannabis and hemp flower, concentrates, and infused products using a single quantitative polymerase chain reaction (qPCR).

Cannabis testing labs in Colorado must produce additional validation data in their lab in order to test a matrix that was not included in a manufacturer's AOAC PTM (Association of Official Analytical Chemists Performance Tested Method). In this application note, Minova Labs shows that The PathoSEEK® Salmonella and STEC E. coli Multiplex Assay with SenSATIVAx® Extraction Protocol can be used to test cannabis-infused beverages and topical matrices.

Materials and Methods

Salmonella enterica (NSI Lab Solutions, Flash Pellet, PN 61-00031) and *E. coli* (NSI Lab Solutions, E.coli Serotype O26 STEC flash pellet, PN FM-716) pellets were re-hydrated separately as outlined by the manufacturer's instructions. They were then plated on Hardy Diagnostics ETB (Salmonella) and EC (STEC E.coli) plates. After 24 hours of incubation at 37°C, a single colony from each plate was selected and inoculated into 2 mL of Tryptic Soy Broth (TSB) in a 50 mL conical tube. The inoculated cultures were incubated at 37C for 24 hours, then combined into 1 mixed culture for a total of 4 mL.

Cells were counted using a cell stain and hemocytometer counting chamber method to determine CFU/uL. to allow for low level (2 cfu) and high level (20 CFU) spikes. Next a total of 20 inoculations were prepared for each matrix: 5 uninoculated, 10 low level (2 CFU) and 5 high level (20 CFU).. The samples were then homogenized and enriched for 24 hours at 37C.

In adherence to the Medicinal Genomics Salmonella and STEC Detection Assay User Guide and also the State of Colorado CDPHE compliance guidelines, 25 g of a beverage sample and lotion sample were weighed into 2 separate 8 oz sterilized glass bottles. Next, 60 mLs of TSB were added to each bottle before enriching the samples for 24 hours at 37C.

After 24 hours, the samples were removed from the incubator and 115 mLs of MIP Solution A was added to each, followed by vortexing. 1mL of each sample was transferred into a 1.5 mL snap cap tube. The internal SCCG control was added, and the sample was centrifuged. A portion of this sample was transferred into a fresh 1.5 mL snap cap tube, and an equal volume of chloroform was added. After completing the chloroform wash, the sample was centrifuged again, and a portion of the supernatant was transferred to a 96-well extraction plate. This process was performed in parallel for each of the samples being tested. Using SenSATIVAx MIP DNA Extraction Kit reagents, the remainder of the DNA extraction was processed. Samples were then tested for Salmonella and shiga-toxin-producing *E. coli* with the PathoSEEK Salmonella and STEC detection assay.

Results

Table I: Uninoculated, low and high live organism spiked beverage samples

Matrix Type	Sample Name	Live Organism Spike Level	Target	Cq (ΔR)	Target	Cq (ΔR)	Target	Cq (ΔR)
Beverage	T4406	Uninoculated	Salmonella	No Cq	STEC	No Cq	IC	26.24
Beverage	T4407	Uninoculated	Salmonella	No Cq	STEC	No Cq	IC	27.13
Beverage	T4408	Uninoculated	Salmonella	No Cq	STEC	No Cq	IC	27.09
Beverage	T4409	Uninoculated	Salmonella	No Cq	STEC	No Cq	IC	26.95
Beverage	T4410	Uninoculated	Salmonella	No Cq	STEC	No Cq	IC	27.3
Beverage	T4391	Low	Salmonella	16.41	STEC	16.57	IC	No Cq
Beverage	T4392	Low	Salmonella	16.96	STEC	17.1	IC	No Cq
Beverage	T4393	Low	Salmonella	16.89	STEC	16.32	IC	No Cq
Beverage	T4394	Low	Salmonella	17.28	STEC	17.22	IC	No Cq
Beverage	T4395	Low	Salmonella	15.85	STEC	16.54	IC	No Cq
Beverage	T4396	Low	Salmonella	16.67	STEC	16.22	IC	No Cq
Beverage	T4397	Low	Salmonella	17.14	STEC	17.01	IC	No Cq

Beverage	T4398	Low	Salmonella	16.33	STEC	17.22	IC	No Cq
Beverage	T4399	Low	Salmonella	16.44	STEC	18.42	IC	No Cq
Beverage	T4400	Low	Salmonella	15.19	STEC	16.31	IC	No Cq
Beverage	T:4376	High	Salmonella	16.26	STEC	16.29	IC	No Cq
Beverage	T:4377	High	Salmonella	15.57	STEC	15.95	IC	No Cq
Beverage	T:4378	High	Salmonella	15.56	STEC	16.3	IC	No Cq
Beverage	T:4379	High	Salmonella	15.64	STEC	15.93	IC	No Cq
Beverage	T:4380	High	Salmonella	15.88	STEC	16.57	IC	No Cq

Table 2: Uninoculated, low and high live organism spiked topical samples

Matrix Type	Sample Name	Live Organism Spike Level	Target	Cq (ΔR)	Target	Cq (ΔR)	Target	Cq (ΔR)
Topical	T4401	Uninoculated	Salmonella	No Cq	STEC	No Cq	IC	26.97
Topical	T4402	Uninoculated	Salmonella	No Cq	STEC	No Cq	IC	26.49
Topical	T4403	Uninoculated	Salmonella	No Cq	STEC	No Cq	IC	26.77
Topical	T4404	Uninoculated	Salmonella	No Cq	STEC	No Cq	IC	25.72
Topical	T4405	Uninoculated	Salmonella	No Cq	STEC	No Cq	IC	27.28
Topical	T4381	Low	Salmonella	18.46	STEC	19.55	IC	No Cq
Topical	T4382	Low	Salmonella	19.06	STEC	19.95	IC	No Cq
Topical	T4383	Low	Salmonella	19.53	STEC	18.65	IC	39.22
Topical	T4384	Low	Salmonella	17.2	STEC	16.93	IC	No Cq
Topical	T4385	Low	Salmonella	15.94	STEC	17.76	IC	No Cq
Topical	T4386	Low	Salmonella	17.09	STEC	18.15	IC	No Cq
Topical	T4387	Low	Salmonella	17.53	STEC	18.38	IC	No Cq
Topical	T4388	Low	Salmonella	15.8	STEC	17.63	IC	No Cq
Topical	T4389	Low	Salmonella	16.03	STEC	17.36	IC	No Cq
Topical	T4390	Low	Salmonella	18.72	STEC	18.75	IC	No Cq
Topical	T:4371	High	Salmonella	16.91	STEC	18.73	IC	No Cq
Topical	T:4372	High	Salmonella	16.21	STEC	18.41	IC	No Cq

Topical	T:4373	High	Salmonella	16.02	STEC	18.74	IC	No Cq
Topical	T:4374	High	Salmonella	16.49	STEC	18.56	IC	No Cq
Topical	T:4375	High	Salmonella	17.14	STEC	18.58	IC	No Cq
Control	MATRIX SPIKE	MCT Oil + TSB + SAL/STEC	Salmonella	18.24	STEC	17.14	IC	30.11
Control	REAGENT BLANK	MCT Oil + TSB	Salmonella	No Cq	STEC	No Cq	IC	27.85
Control	POSITIVE CONTROL	Assay Positive Control	Salmonella	13.21	STEC	8.53	IC	No Cq
Control	NEGATIVE CONTROL	RNase/DNase Free Water	Salmonella	No Cq	STEC	No Cq	IC	No Cq

Discussion

PathoSEEK® Salmonella and STEC *E. coli* Multiplex Assay offers a faster and more accurate microbial detection alternative to traditional plating methods. The SenSATIVAx MIP DNA Extraction Kit used in conjunction with the Salmonella & STEC E. Coli Multiplex Detection Assay is suitable to extract and detect Salmonella and STEC *E. coli* in alternative matrices such as beverages and topicals as seen in Table I and Table II.

Ordering information

Product	Part Number
PathoSEEK® Salmonella & STEC E. Coli Multiplex Detection Assay (200 Rxn) V2	SKU: 420120
PathoSEEK® Salmonella & STEC E. Coli Multiplex Positive Control (50 Rxn) V2	SKU: 420322
SenSATIVAx® MIP DNA Extraction Kit (200 rxn)	SKU: 420004