

# **PathoSEEK<sup>®</sup> Salmonella & STEC Multiplex Detection Assay with MaGiC Lysis for Detection in Cannabis Flower and MIP Matrices**

## **Method Developer Validation**

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## **Abstract**

### ***Background:***

Current regulations require cannabis flower and cannabis products to be free of species of *Salmonella* and shiga-toxin producing *E. Coli*. The PathoSEEK<sup>®</sup> Salmonella & STEC *E. coli* Multiplex Detection Assay v3 with MaGiC Lysis Kit will detect these species using a qPCR (Quantitative Polymerase Chain Reaction) assay in cannabis flower, cannabis concentrates, infused edibles and infused non-edibles.

### ***Objective:***

To evaluate the PathoSEEK<sup>®</sup> Salmonella & STEC *E. coli* Multiplex Detection Assay v3, using the MaGiC Lysis protocols for the presence/absence detection of *Salmonella* and *Shiga Toxin producing E.coli* species in cannabis flower (delta 9-tetrahydrocannabinol >0.3%; 1g) and marijuana-infused products (MIP).

### ***Results:***

Inclusivity and exclusivity results showed the PathoSEEK<sup>®</sup> Salmonella & STEC *E. coli* Multiplex Detection Assay method is highly specific in discriminating target organisms found in cannabis flower and infused products from non-target organisms. The MaGiC Lysis kit and PathoSEEK<sup>®</sup> Salmonella & STEC *E. coli* Multiplex Detection Assay was successfully validated through an internal matrix study showing equivalency to the current AOAC approved version, PTM Certificate number 22022. The kit was further verified by running NSI Certified Reference Materials, part numbers CMQC 025, 027, 029 and 030. Results show that the MaGiC Lysis kit and PathoSEEK<sup>®</sup> Salmonella & STEC *E. coli* Multiplex Detection Assay accurately detected *Salmonella* & *STEC E. coli* when present.

## **Materials**

PathoSEEK<sup>®</sup> Salmonella & STEC E. coli Multiplex Detection Assay v3 with MaGiC Lysis Kit, P/N **420517** (Kit contains sufficient reagents for 200 reactions)

Kit Components:

Component Name	Qty Provided	Storage Conditions
MaGiC Lysis Reagent	1 Bottle (12 mL)	RT
MaGiC Stabilization Buffer	1 Bottle (24 mL)	RT
PathoSEEK <sup>®</sup> Amplification Mix	3 Vials (67 rxns/each)	RT / -20 °C*
PathoSEEK <sup>®</sup> Salmonella & STEC E. coli Multiplex Detection Assay v3	1 Tube (200 µL)	-20 °C

Additional Required Reagents Not in Kit:

Item P/N	Item Name	Qty Provided	Storage Conditions
420337	Internal Cannabis Control	1 Tube (50 µL)	-20 °C
420322	PathoSEEK <sup>®</sup> Salmonella & STEC Positive Control	1 Tube (50 µL)	-20 °C
420205	Tryptic Soy Broth	CS/10 x 500mL bottles	2-25°C
420184	PCR Grade Water	500 mL Bottle	2-25°C

## **Method Developer Validation**

### ***Methodology***

For inclusivity evaluation, 100 strains of Salmonella and 51 strains of STEC were tested. Strains were grown for 16 hours at  $37 \pm 1^\circ\text{C}$  in TSB. The cultures were then diluted to 100 x the Limit of Detection (LOD) of the method. For the exclusivity evaluation, a total of 45 non-targeted strains were tested. Exclusivity organisms were cultured under optimal conditions to achieve growth at the stationary phase. Inclusivity and exclusivity strains were randomized and blind coded prior to analysis.

### ***Results***

Detailed results for the inclusivity and exclusivity evaluations are presented in Tables 1-3. For the inclusivity evaluation, all 100 strains of Salmonella and all 51 strains of STEC were detected by the assay using MaGiC lysis on the CFX-96, AriaMx, and the BMS MIC qPCR instruments. For the exclusivity, 45 of the organisms were correctly excluded on the Salmonella channel and 44 of the organisms were correctly excluded on the STEC channel. One organism, *Shigella dysenteriae*, contains the shiga-toxin genes stx and is expected to be detected by the assay on the STEC channel.

**Table 1: Salmonella Inclusivity Results, PathoSEEK<sup>®</sup> Salmonella & STEC E. Coli Multiplex**

<b>Organism</b>	<b>Species/ subspecies</b>	<b>Serovar</b>	<b>Source</b>	<b>Origin</b>	<b>Salmonella</b>
<i>S. enterica</i>	<i>arizonae</i>	Not Available	ATCCc 33952	Not Available	Positive
<i>S. enterica</i>	<i>arizonae</i>	Not Available	ATCC 29933	Not Available	Positive
<i>S. enterica</i>	<i>arizonae</i>	Not Available	ATCC BAA-731	Tissue of snake	Positive
<i>S. enterica</i>	<i>diarizonae</i>	IIIb 35:i:z	ATCC BAA-216	Blood	Positive
<i>S. enterica</i>	<i>diarizonae</i>	47:i:z53:z57	ATCC 12325	Not Available	Positive
<i>S. enterica</i>	<i>diarizonae</i>	Not Available	ATCC 29934	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Aberdeen	NCTCd 5791	Not Available	Positive

<i>S. enterica</i>	<i>enterica</i>	Abortusequi	NCTC 5727	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Abortusovis	ATCC 31684	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Adelaide	NCTC 6586	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Agona	ATCC 51957	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Anatum	ATCC 9270	Pork liver	Positive
<i>S. enterica</i>	<i>enterica</i>	Bareilly	ATCC 9115	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Berta	ATCC 8392	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Bispebjerg	ATCC 9842	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Braenderup	ATCC 700136	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Bredeney	ATCC 10728	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Breukelen	ATCC 15782	Cuscus	Positive
<i>S. enterica</i>	<i>enterica</i>	Bristol	ATCC 700136	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Caracas	NCTC 9937	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Cerro	NCC 5801	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Champaign	NCTC 6851	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Chester	ATCC 11997	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Choleraesuis	ATCC 10708	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Crossness	NCTC 11059	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Cubana	ATCC 12007	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Dahlem	NCTC 9949	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Derby	ATCC 6960	Tank water/pork pies	Positive
<i>S. enterica</i>	<i>enterica</i>	Deversoir	NCTC 9792	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Dublin	ATCC 15480	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Enteritidis	ATCC 13076	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Essen	ATCC 6961	Not Available	Positive

<i>S. enterica</i>	<i>enterica</i>	Gallinarum	NCTC 10532	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Gaminara	ATCC 8324	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Give	ATCC 9268	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Hadar	NCTC 9877	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Hartford	NCTC 6802	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Havana	NCTC 6086	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Heidelberg	ATCC 8326	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Hillingdon	ATCC 9184	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Indiana	NCTC 11304	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Infantis	NCTC 10679	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Inverness	NCTC 6591	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Javiana	NCTC 6495	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Jerusalem	ATCC 700137	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Johannesburg	NCTC 8272	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Kentucky	NCTC 5799	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Krefeld	NCTC 9884	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Lille	NCTC 9885	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	London	ATCC 8389	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Matadi	NCTC 9887	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Mbandaka	ATCC 51958	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Meleagridids	NCTC 6023	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Menden	ATCC 15992	Feces	Positive
<i>S. enterica</i>	<i>enterica</i>	Mgulani	NCTC 8492	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Minnesota	NTCT 5800	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Montevideo	ATCC 8387	Not Available	Positive

<i>S. enterica</i>	<i>enterica</i>	Muenchen	NCTC 6246	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Newport	ATCC 6962	Food poisoning	Positive
<i>S. enterica</i>	<i>enterica</i>	Nottingham	NCTC 7832	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Oranienburg	ATCC 9239	Illinois	Positive
<i>S. enterica</i>	<i>enterica</i>	Panama	ATCC 7378	Baby	Positive
<i>S. enterica</i>	<i>enterica</i>	Paratyphi A	ATCC 9150	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Paratyphi B	ATCC 8759	Gall bladder	Positive
<i>S. enterica</i>	<i>enterica</i>	Pomona	NCTC 6589	Turkey intestine	Positive
<i>S. enterica</i>	<i>enterica</i>	Poona	NCTC 5792	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Potsdam	ATCC 25957	Child	Positive
<i>S. enterica</i>	<i>enterica</i>	Pullorum	ATCC 9120	Clinical isolate	Positive
<i>S. enterica</i>	<i>enterica</i>	Reading	ATCC 6967	Guinea pig	Positive
<i>S. enterica</i>	<i>enterica</i>	Saintpaul	ATCC 9712	Cystitis	Positive
<i>S. enterica</i>	<i>enterica</i>	Sandiego	ATCC 231999	Bladder, turtle	Positive
<i>S. enterica</i>	<i>enterica</i>	Schwarzengrund	NCTC 6756	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Senftenberg	ATCC 8400	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Sloterdijk	ATCC 15791	Family outbreak	Positive
<i>S. enterica</i>	<i>enterica</i>	Stanley	ATCC 7308	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Sundsvall	NCTC 6758	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Tennessee	ATCC 10722	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Thompson	ATCC 8391	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Typhi	ATCC 19430	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Typhimurium	ATCC 13311	Feces	Positive
<i>S. enterica</i>	<i>enterica</i>	Typhisuis	ATCC 8321	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Urbana	NCTC 6248	Not Available	Positive



<i>S. enterica</i>	<i>enterica</i>	Utrecht	NCTC 10077	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Vellore	ATCC 15611	Rectal swab	Positive
<i>S. enterica</i>	<i>enterica</i>	Virchow	ATCC 51955	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Waycross	NCTC 7401	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Weltevreden	NCTC 6534	Not Available	Positive
<i>S. enterica</i>	<i>enterica</i>	Zwickau	ATCC 15804	Not Available	Positive
<i>S. enterica</i>	<i>houtenae</i>	45:g,z51:-	ATCC 43974	Not Available	Positive
<i>S. enterica</i>	<i>houtenae</i>	Not Available	ATCC BAA-1580	Not Available	Positive
<i>S. enterica</i>	<i>houtenae</i>	11:z4,z23: -	ATCC 15788	Not Available	Positive
<i>S. enterica</i>	<i>indica</i>	45:a:e,n,x	ATCC BAA-1578	Not Available	Positive
<i>S. enterica</i>	<i>indica</i>	1,6,14,25:a:e,n,x	ATC 43976	Not Available	Positive
<i>S. enterica</i>	<i>indica</i>	6, 14, 25:a:e,n,x	NCTC 10458	coconut	Positive
<i>S. enterica</i>	<i>salamae</i>	56:b:-	ATCC 700149	Not Available	Positive
<i>S. enterica</i>	<i>salamae</i>	1,9,12:l,w:e,n	ATCC 6959	Urine	Positive
<i>S. enterica</i>	<i>salamae</i>	55:k:z39	ATCC 700148	Not Available	Positive
<i>S. enterica</i>	<i>salamae</i>	1,9,12:l,w:e,n,x	ATCC 43972	Not Available	Positive
<i>S. bongori</i>		66:z41:-	ATCC 43975	Not Available	Positive
<i>S. bongori</i>		48:z35	NCTC 14392	Not Available	Positive

**Table 2: STEC Inclusivity Results, PathoSEEK<sup>®</sup> Salmonella & STEC E. Coli Multiplex**

Organism	Source	Orgin	Result
<i>Escherichia coli</i> O3	TW01413c	Germany	Positive
<i>Escherichia coli</i> O3	TW01414	Germany	Positive
<i>Escherichia coli</i> O5	TW00021	Cow (MI)	Positive
<i>Escherichia coli</i> O5	TW05097	Cow (CA)	Positive

<i>Escherichia coli</i> O26:H11	ATCCd BAA-2205	Feces	Positive
<i>Escherichia coli</i> O26:H11	ATCC BAA-2196	Feces	Positive
<i>Escherichia coli</i> O26:H11	ATCC BAA-2204	Feces	Positive
<i>Escherichia coli</i> O26:H11	ATCC BAA-2181	Not Available	Positive
<i>Escherichia coli</i> O26:H11	ATCC BAA-2188	Feces	Positive
<i>Escherichia coli</i> O45:H2	ATCC BAA-2193	Feces	Positive
<i>Escherichia coli</i> O45:H2	ATCC BAA-2189	Feces	Positive
<i>Escherichia coli</i> O45:H2	ATCC BAA-2185	Not Available	Positive
<i>Escherichia coli</i> O45:H2	ATCC BAA-2202	Feces	Positive
<i>Escherichia coli</i> O45:H2	ATCC BAA-2198	Feces	Positive
<i>Escherichia coli</i> O91:H21	ATCC 51434	Not Available	Positive
<i>Escherichia coli</i> O91:H21	ATCC 51435	Clinical Isolate	Positive
<i>Escherichia coli</i> O103:H11	ATCC BAA-2215	Not Available	Positive
<i>Escherichia coli</i> O103:H11	ATCC BAA-2200	Not Available	Positive
<i>Escherichia coli</i> O103:H11	NJDPhE 151297-1	Not Available	Positive
<i>Escherichia coli</i> O103:H11	NJDPh 130928-3	Not Available	Positive
<i>Escherichia coli</i> O103:H11	TW08869	Human Isolate	Positive
<i>Escherichia coli</i> O103:H11	TW08872	Human Isolate	Positive
<i>Escherichia coli</i> O111:H8	ATCC BAA-2201	Feces	Positive
<i>Escherichia coli</i> O111:H8	ATCC BAA-2180	Feces	Positive
<i>Escherichia coli</i> O111:H8	ATCC BAA-184	Feces	Positive
<i>Escherichia coli</i> O111:H8	ATCC BAA-180	Feces	Positive
<i>Escherichia coli</i> O111:H8	ATCC BAA-181	Feces	Positive
<i>Escherichia coli</i> O113	ATCC BAA-177	Feces	Positive
<i>Escherichia coli</i> O113	ATCC BAA-176	Feces	Positive
<i>Escherichia coli</i> O113	ATCC BAA-183	Urine	Positive
<i>Escherichia coli</i> O118	TW08644	Feces	Positive
<i>Escherichia coli</i> O118	TW08134	Cow Feces	Positive
<i>Escherichia coli</i> O121:H19	ATCC BAA-2219	Feces	Positive

<i>Escherichia coli</i> O121:H19	ATCC BAA-2203	Feces	Positive
<i>Escherichia coli</i> O121:H19	ATCC BAA-2187	Not Available	Positive
<i>Escherichia coli</i> O121:H19	ATCC BAA-2220	Feces	Positive
<i>Escherichia coli</i> O121:H19	TW08868	Feces	Positive
<i>Escherichia coli</i> O145:NM	TW09356	Human Isolate	Positive
<i>Escherichia coli</i> O145:NM	TW07596	Feces	Positive
<i>Escherichia coli</i> O145:NM	ATCC BAA-2223	Feces	Positive
<i>Escherichia coli</i> O145:NM	ATCC BAA-2192	Feces	Positive
<i>Escherichia coli</i> O145:NM	NJDPH 17257-1	Not Available	Positive
<i>Escherichia coli</i> O145:NM	NJDPH 161296-1	Not Available	Positive
<i>Escherichia coli</i> O157:H7	ATCC 35150	Feces	Positive
<i>Escherichia coli</i> O157:H7	ATCC 43889	Feces	Positive
<i>Escherichia coli</i> O157:H7	ATCC 43894	Feces	Positive
<i>Escherichia coli</i> O157:H7	ATCC 700599	Salami	Positive
<i>Escherichia coli</i> O157:H7	TW00116	Feces	Positive
<i>Escherichia coli</i> O157:H7	TW00975	Human Isolate	Positive
<i>Escherichia coli</i> O157:H7	TW02302	Human Isolate	Positive
<i>Escherichia coli</i> O157:H7	TW04863	Feces	Positive

**Table 3: Exclusivity Results, PathoSEEK<sup>®</sup> Salmonella & STEC E. Coli Multiplex**

Organism	Source	Origin	Results	
			STEC	Salmonella
<i>Aeromonas bestiarum</i>	ATCCc BAA-231	Cake	N	N
<i>Aeromonas hydrophila</i>	ATCC 7966	Milk	N	N
<i>Burkholderia multivorans</i>	ATCC 17616	Soil	N	N
<i>Bacillus subtilis</i>	ATCC 11774	Not Available	N	N
<i>Campylobacter jejuni</i>	ATCC 29428	Feces	N	N

<i>Candida tropicalis</i>	ATCC 13803	Not Available	N	N
<i>Citrobacter braakii</i>	ATCC 3037	Urine	N	N
<i>Citrobacter farmerii</i>	ATCC 51112	Feces	N	N
<i>Citrobacter freundii</i>	ATCC 8090	Not Available	N	N
<i>Citrobacter koseri</i>	ATCC 25408	Throat	N	N
<i>Citrobacter murliae</i>	ATCC 51118	United States; Illinois	N	N
<i>Citrobacter youngae</i>	ATCC 29935	Meat scraps	N	N
<i>Edwardsiella tarda</i>	ATCC 23672	Not Available	N	N
<i>Enterobacter aerogenes</i>	ATCC 13048	Sputum	N	N
<i>Enterobacter cloacae</i>	ATCC 13047	Spinal fluid	N	N
<i>Enterobacter gergoviae</i>	ATCC 33028	Urine	N	N
<i>Enterobacter sakazakii</i>	ATCC BAA-894	Clinical specimen	N	N
<i>Enterobacter amnigenus</i>	ATCC 51818	Milk	N	N
<i>Enterobacter cancerogenus</i>	ATCC 35318	Cerebrospinal fluid	N	N
<i>Erwinia rhapontici</i>	ATCC 29290	English pink wheat grains	N	N
<i>Escherichia coli</i>	ATCC 25922	Clinical isolate	N	N
<i>Escherichia fergusonii</i>	ATCC 35469	Feces	N	N
<i>Escherichia hermannii</i>	ATCC 700368	Not Available	N	N
<i>Escherichia vulneris</i>	ATCC 33821	Wound	N	N
<i>Hafnia alvei</i>	ATCC 51873	Feces	N	N
<i>Klebsiella oxytoca</i>	ATCC 51983	Blood	N	N
<i>Klebsiella pneumonia</i>	ATCC BAA-2146	Urine	N	N
<i>Listeria monocytogenes</i>	ATCC 7647	Bovine	N	N
<i>Morganella morganii</i>	ATCC 25829	Stool	N	N
<i>Pantoea agglomerans</i>	ATCC 43348	Gypsophila paniculata galls	N	N
<i>Proteus hauseri</i>	ATCC 13315	Not Available	N	N
<i>Proteus mirabilis</i>	ATCC 43071	Rectum	N	N
<i>Proteus vulgaris</i>	ATCC 8427	Inner ear infection	N	N
<i>Pseudomonas aeruginosa</i>	ATCC 15442	Water bottle in animal room	N	N
<i>Pseudomonas fluorescens</i>	ATCC 13525	Pre-filter tanks	N	N

<i>Pseudomonas putida</i>	ATCC 47054	Not Available	N	N
<i>Rahnella aquatilis</i>	ATCC 33991	Soil	N	N
<i>Ralstonia insidiosa</i>	ATCC 49129	Clinical isolate	N	N
<i>Serratia marcescens</i>	ATCC 27137	Not Available	N	N
<i>Shigella dysenteriae</i>	ATCC 13313	Foreign seaman	N	N
<i>Shigella flexneri</i>	ATCC 12022	Not Available	N	N
<i>Shigella sonnei</i>	ATCC 9290	Not Available	N	N
<i>Trichoderma harzianum</i>	ATCC 60850	Soil	N	N
<i>Vibrio vulnificus</i>	ATCC 29307	Blood	N	N
<i>Yersinia ruckeri</i>	ATCC 29473	Rainbow trout	N	N

### **Limit of Detection**

The method is able to detect down to 1 CFU per test portion

## Proficiency Testing/Certified Reference Material Results

NSI Certified Reference Materials were used to compare the detection of *Salmonella* and *Shiga Toxin E. coli* in the presence of different matrices. NSI part numbers used were CMQC 025, 027, 029 and 030.

Table 4 contains the resulting data.

**Table 4: NSI CRM Data**

CRM Name	Present?	MaGiC Lysis				SenSATIVax DNA Purification			
		Target	Cq (ΔR)	Target	Cq (ΔR)	Target	Cq (ΔR)	Target	Cq (ΔR)
Qualitative Salmonella in Hemp	Y	FAM/STEC	No Cq	ROX/SAL	<b>19.27</b>	FAM/STEC	No Cq	ROX/SAL	<b>14.81</b>
Qualitative Salmonella in Hemp	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative Salmonella in Hemp	Y	FAM/STEC	No Cq	ROX/SAL	<b>20.00</b>	FAM/STEC	No Cq	ROX/SAL	<b>14.78</b>
Qualitative Salmonella in Hemp	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative Salmonella in Hemp	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative Salmonella in Oil	Y	FAM/STEC	No Cq	ROX/SAL	<b>23.94</b>	FAM/STEC	No Cq	ROX/SAL	<b>23.79</b>
Qualitative Salmonella in Oil	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative Salmonella in Oil	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative Salmonella in Oil	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative Salmonella in Oil	Y	FAM/STEC	No Cq	ROX/SAL	<b>22.58</b>	FAM/STEC	No Cq	ROX/SAL	<b>22.38</b>
Qualitative STEC in Edible	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative STEC in Edible	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative STEC in Edible	Y	FAM/STEC	<b>26.73</b>	ROX/SAL	No Cq	FAM/STEC	<b>20.13</b>	ROX/SAL	No Cq
Qualitative STEC in Edible	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative STEC in Edible	Y	FAM/STEC	<b>26.61</b>	ROX/SAL	No Cq	FAM/STEC	<b>20.25</b>	ROX/SAL	No Cq
Qualitative STEC in Oil	Y	FAM/STEC	<b>22.17</b>	ROX/SAL	No Cq	FAM/STEC	<b>22.96</b>	ROX/SAL	No Cq
Qualitative STEC in Oil	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative STEC in Oil	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative STEC in Oil	N	FAM/STEC	No Cq	ROX/SAL	No Cq	FAM/STEC	No Cq	ROX/SAL	No Cq
Qualitative STEC in Oil	Y	FAM/STEC	<b>21.72</b>	ROX/SAL	No Cq	FAM/STEC	<b>22.59</b>	ROX/SAL	No Cq
POS C		FAM/STEC	11.93	ROX/SAL	16.44				
NTC		FAM/STEC	No Cq	ROX/SAL	No Cq				

***Conclusions:***

The MaGiC Lysis kits in conjunction with the PathoSEEK<sup>®</sup> Salmonella & STEC E.coli Multiplex Detection Assay is a rapid, alternative method to traditional plating procedures for accurate detection of *Salmonella sp.* and Shiga Toxin producing *E.coli* in cannabis flower and cannabis infused products.

## REVISION HISTORY

Version	Date	Description
v1	August 2024	Validation for MaGiC Lysis with Salmonella and STEC Multiplex Method

## DISCLAIMER

This test was developed, and its performance characteristics determined by Medicinal Genomics Company, for laboratory use. Any deviations from this protocol are not supported by MGC.

This test has not been validated on remediated (irradiated, ozone treated, acid treated, hydrogen peroxide treated, etc.) samples. Samples that have undergone remediation may cause discordant results between plating methods and PathoSEEK methods. When remediated samples produce a result above the action limit on qPCR, we recommend confirming viability with an approved plating method.

Results may vary based on laboratory conditions. Altitude and humidity are factors known to affect the growth of bacterial and fungal species.

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