

PathoSEEK[®] Salmonella Detection Assay v3 with MaGiC Lysis Kit

Method Developer Validation

Real Time PCR (qPCR) assay for the detection of *Salmonella sp.* in dried cannabis flower and infused products matrices

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Abstract

Background

Current regulatory standards mandate the absence of *Salmonella* species in cannabis flower and derived products. The PathoSEEK® *Salmonella* Detection Assay v3, in conjunction with the MaGiC Lysis Kit, facilitates the detection of these species via a quantitative Polymerase Chain Reaction (qPCR) assay, applicable to cannabis flower, cannabis concentrates, infused edibles, and infused non-edible products.

Objective

To evaluate the PathoSEEK® *Salmonella* Detection Assay v3, using the MaGiC Lysis protocol for the presence/absence detection of *Salmonella* species in cannabis flower (delta 9-tetrahydrocannabinol >0.3%; 1g) and marijuana-infused products (MIPs).

Results

Inclusivity and exclusivity results showed the PathoSEEK® *Salmonella* Detection Assay with MaGiC Lysis method is highly specific in discriminating target organisms found in cannabis flower and infused products from non-target organisms. The kit was further verified by running NSI Certified Reference Materials, part numbers FM-605 and FM-732. Results show that the PathoSEEK® *Salmonella* Detection Assay with MaGiC Lysis Kit accurately detected *Salmonella* when present.

Materials

PathoSEEK® Salmonella Detection Assay v3 with MaGiC Lysis Kit - P/N 420532

(Kit contains sufficient reagents for 200 reactions)

Kit Components

Component Name	Qty Provided	Storage Conditions
MaGiC Lysis Reagent	1 Bottle (12 mL)	RT (20-28 °C)
MaGiC Stabilization Buffer	1 Bottle (24 mL)	RT (20-28 °C)
PathoSEEK Amplification Mix <i>Includes 2 tubes nuclease free water for resuspension</i>	4 Vials (50 rxns/each)	RT (20-28 °C)/ -15 to -20 °C*
PathoSEEK® Salmonella Detection Assay v3	1 Tube (200 µL)	-15 to -20 °C

Note: Actual fill volumes include overage

*The PathoSEEK Amplification Mix can be stored lyophilized at Room Temperature for up to 2 years. Once re-hydrated it must be stored at -15 to -20 °C for up to 3 months.

Method Developer Validation

Inclusivity and Exclusivity

For inclusivity evaluation, 100 strains of *Salmonella* were tested. Strains were grown for 16 hours at 37 ± 1°C in TSB. 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* were detected by the assay using MaGiC Lysis on the

CFX-96, AriaMx, and the BMS Mic qPCR instruments. For the exclusivity all 45 of the organisms were correctly excluded.

Table 1: Inclusivity Results

Organism	Species/ subspecies	Serovar	Source	Origin	Salmonella
<i>S. enterica</i>	<i>arizonae</i>	Not Available	ATCC 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>	Abortus Equi	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 9855	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>	Meleagridis	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	Gallbladder	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>	Saint Paul	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	ATCC 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: Exclusivity Results

Organism	Source	Origin	Results
<i>Aeromonas hydrophila</i>	7966	Tin of milk with fishy odor	Negative
<i>Aspergillus Niger</i>	66295	Jerusalem artichoke tuber	Negative
<i>Aspergillus Tubingensis</i>	1004	N/A	Negative
<i>Bacillus amyloliquefaciens</i>	23350	Soil	Negative
<i>Bacillus subtilis</i>	11774	N/A	Negative
<i>Bacillus subtilis</i>	23857	N/A	Negative
<i>Botrytis cinerea</i>	11542	Azalea flowers	Negative
<i>Burkholderia multivorans</i>	17616	Soil enriched with anthranilate at 41°C	Negative
<i>Candida albicans</i>	10321	Man with bronchomycosis	Negative
<i>Candida glabrata</i>	26512	bovine placenta	Negative
<i>Citrobacter braakii</i>	BAA-3037	Urine	Negative
<i>Citrobacter koseri</i>	25408	Throat	Negative
<i>Edwardsiella tarda</i>	23672	N/A	Negative
<i>Erwinia rhapontici</i>	29290	English pink wheat grains	Negative
<i>Escherichia hermannii</i>	700368	N/A	Negative
<i>Klebsiella oxytoca</i>	51983	Blood	Negative
<i>Klebsiella pneumoniae</i>	BAA-2146	Urine	Negative
<i>Lactobacillus leichmannii</i>	4797	N/A	Negative

<i>Listeria monocytogenes</i>	7647	Bovine	Negative
<i>Meyerozyma guilliermondii</i>	6260	Sputum from patient with bronchomycosis	Negative
<i>Morganella morganii</i>	25829	Stool from Providence City Hospital	Negative
<i>Paecilomyces variotii</i>	26820	N/A	Negative
<i>Pantoea agglomerans</i>	45340	Knee laceration	Negative
<i>Penicillium chrysogenum</i>	10106	Cheese	Negative
<i>Penicillium citrinum</i>	9849	N/A	Negative
<i>Penicillium venetum</i>	16025	Hyacinthus sp. bulb	Negative
<i>Pichia membranifaciens</i>	24249	N/A	Negative
<i>Pityrosporum ovale</i>	12078	N/A	Negative
<i>Proteus mirabilis</i>	43071	Rectum	Negative
<i>Pseudomonas aeruginosa</i>	35554	N/A	Negative
<i>Pseudomonas aeruginosa</i>	15442	Water bottle in animal room	Negative
<i>Pseudomonas fluorescens</i>	13525	Pre-filter tanks	Negative
<i>Pseudomonas putida</i>	47054	N/A	Negative
<i>Purpureocillium lilacinum</i>	10114	Soil	Negative
<i>Ralstonia insidiosa</i>	49129	Clinical isolate	Negative
<i>Rhizopus oryzae</i>	52748	N/A	Negative
<i>Rhizopus stolonifer</i>	2276	N/A	Negative
<i>Saccharomyces cerevisiae</i>	18824	Top yeast	Negative
<i>Saccharomyces kudriavzevii</i>	2601	N/A	Negative
<i>Scopulariopsis acremonium</i>	58636	Chicken house soil	Negative
<i>Sphingomonas paucimobilis</i>	29837	Hospital respirator	Negative
<i>Staphylococcus aureus</i>	12600	Pleural fluid	Negative
<i>Staphylococcus haemolyticus</i>	29970	Skin	Negative
<i>Trichosporon mucoides</i>	204094	N/A	Negative
<i>Wickerhamomyces anomalus</i>	2349	N/A	Negative
<i>Yersinia enterocolitica</i>	9610	Tissue; glanders-like infection of the face	Negative

Limit of Detection

Serial dilutions, ranging from 1:10 to 1:1,000,000, were generated from a saturated overnight culture. For each dilution, a 10 μ L aliquot was prepared using the MaGiC lysis protocol and immediately tested while an equivalent volume (10 μ L) was subsequently plated onto 3M RAC (Rapid Aerobic Count) petrifilm. The plates were incubated at 37°C for 24 hours and then counted. The data is presented in Table 3.

Table 3: LOD qPCR vs 3M RAC Comparison Results

Sample	Dilution	Cq Fam	Cq Hex	3M Plate RAC CFUs
<i>Salmonella</i> Culture	1:10	19.95	26.57	TNTC
<i>Salmonella</i> Culture	1:100	23.07	26.54	TNTC
<i>Salmonella</i> Culture	1:1K	26.82	27.10	TNTC
<i>Salmonella</i> Culture	1:10K	30.14	26.81	TNTC
<i>Salmonella</i> Culture	1:100K	33.30	27.06	96
<i>Salmonella</i> Culture	1:1M	37.67	26.91	10

*TNTC stands for Too Numerous To Count

Proficiency Testing/Certified Reference Material Results

To assess the efficacy of the PathoSEEK® *Salmonella* Detection Assay with MaGiC Lysis, NSI Certified Reference Materials FM-605 and FM-732 were reconstituted in 9 mL of Tryptic Soy Broth (TSB) and incubated for 24 hours at 37°C. Following the enrichment period, each sample was processed according to protocol and then subjected to analysis. The data is presented in Table 4.

Table 4: *Salmonella* CRM Results

Sample	Catalog #	Lot #	Cq Fam	Cq Hex
<i>Salmonella</i> in Chocolate	FM-605	220513	26.89	27.28
<i>Salmonella</i> in Chocolate	FM-605	220513	26.96	27.39
<i>Salmonella</i> in Chocolate	FM-605	220513	26.97	27.11
<i>Salmonella</i> in Hemp	FM-732	240828	20.28	22.92
<i>Salmonella</i> in Hemp	FM-732	240828	20.24	23.30
<i>Salmonella</i> in Hemp	FM-732	240828	20.26	22.75

Conclusion

The PathoSEEK[®] Salmonella Detection Assay v3 with MaGiC Lysis Kit is a rapid, alternative method to traditional plating procedures for accurate detection of *Salmonella sp.* in cannabis flower and cannabis infused products.

REVISION HISTORY

Version	Date	Description
v1	April 2025	Validation of MaGiC Lysis with <i>Salmonella</i> Detection Assay v3 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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