CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

022202

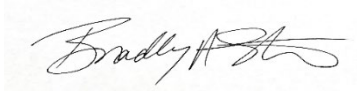
The AOAC Research Institute hereby certifies the method known as:

PathoSEEK® Salmonella and STEC E. coli Multiplex Assay

manufactured by

Medicinal Genomics Corp.
100 Cummings Center, Suite 406L
Beverly, MA 01915 USA

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.



Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date October 26, 2024
Expiration Date December 31, 2025

METHOD NAME	CATALOG NUMBERS	ORIGINAL CERTIFICATION DATE
PathoSEEK® Salmonella and STEC E. coli Multiplex Assay	420120, 420001, 420004, 420201, 420332	August 10, 2021

PRINCIPLE OF THE METHOD

The PathoSEEK Salmonella & STEC E. coli Multiplex Assay is used to detect a wide variety of species classified as *Salmonella* and STEC. The assay targets STEC using the FAM fluorophore and *Salmonella* using the ROX fluorophore. Positive controls for each target are recommended for use with each analysis.

The PathoSEEK Microbial Safety Testing Platform utilizes a novel, contamination-free, PCR-based assay and provides an internal plant DNA control for every reaction. It is a two-step protocol (DNA extraction followed by RT-PCR analysis) which is flexible and automation compatible. The PathoSEEK assay is used in combination with the SenSATIVAx extraction kit (assay version 2) or the MaGiC Lysis Kit (assay version 3) and qPCR Master Kit.

An internal cannabis control (ICC) is introduced at the lysis step to ensure accurate detection of multiple species of *Salmonella*, Shiga-Toxin producing *E. coli*, and internal control DNA in every reaction. Unlike other techniques, this multiplexing strategy verifies the performance of the assay when detecting pathogens, resulting in the minimization of false-negative results due to reaction setup errors or failing experimental conditions. The process to add ICC at the lysis step ensures that the entire process is carried out correctly, not just the qPCR analysis.

CERTIFIED CLAIM STATEMENT: The PathoSEEK® Salmonella and STEC E. coli Multiplex Assay is certified for the detection of *Salmonella* and Shiga-toxin producing *E. coli* (STEC) within the scope of Tables 1 and 2 and with the modifications indicated in Table 3.

Certified method includes:

1. PathoSEEK® Salmonella & STEC E. coli Multiplex Assay v2 with SenSATIVAx® Extraction Kit:
    a. Bio-Rad CFX96 Touch™ Real-Time System, standard
    b. Agilent AriaMx Real-Time PCR System G8830A, standard
2. PathoSEEK® Salmonella & STEC E. coli Multiplex Assay v3 with MaGiC Lysis Kit:
    a. Bio-Rad CFX96 Touch™ Real-Time System, software version 10.0.26100, standard
    b. Agilent AriaMx Real-Time PCR System G8830A, software version 3.1.2306.0602, standard
    c. Bio Molecular Systems (BMS) Mic 4-Channel PCR Instrument, software version 1.4.10, standard

Table 1. Method Performance Claims

Matrix[a]	Test Portion	Enrichment Conditions				SMPR[c,d]	Claim[e]
		Broth[b]	Volume	Temperature	Time		
Salmonella							
Dried cannabis flower (>0.3%THC)	10 g	TSB	90 mL	37 ± 2°C	16-24 h	2020.002	Eq
THC-infused oil (>0.3%THC)	5 g	TSB	12 mL[f]	37 ± 2°C	16-24 h	2020.002	Eq
	5 g	TSB	45 mL	37 ± 2°C	16-24 h	2020.002	Eq

THC-infused chocolate bar (>0.3%THC)	25 g	TSB	60 mL[f]	37 ± 2°C	16 h	2020.002	NSDD[g]
	25 g	TSB	60 mL[f]	37 ± 2°C	24 h	2020.002	Eq
	25 g	TSB	225 mL	37 ± 2°C	16-24 h	2020.002	Eq
STEC							
Dried cannabis flower (>0.3%THC)	10 g	TSB	90 mL	37 ± 2°C	16 h	2020.012	Eq/SDD-R[h]
	10 g	TSB	90 mL	37 ± 2°C	24 h	2020.012	Eq/NSDD[h]
THC-infused oil (>0.3%THC)	5 g	TSB	12 mL[f]	37 ± 2°C	16-24 h	2020.012	Eq
	5 g	TSB	45 mL	37 ± 2°C	16-24 h	2020.012	Eq
THC-infused chocolate bar (>0.3%THC)	25 g	TSB	60 mL[f]	37 ± 2°C	16-24 h	2020.012	Eq
	25 g	TSB	225 mL	37 ± 2°C	16-24 h	2020.012	Eq

[a] All matrixes were co-inoculated with *Salmonella* and STEC.

[b] TSB = Tryptic Soy Broth.

[c] AOAC *Standard Method Performance Requirements* (SMPR) 2020.002 for Detection of *Salmonella* in Cannabis and Cannabis Products.

[d] AOAC *Standard Method Performance Requirements* (SMPR) 2020.012 for Detection of Shiga Toxin-Producing *Escherichia coli* in Cannabis and Cannabis Products.

[e] Eq = Equivalence of candidate method presumptive and confirmed results demonstrated by 90% confidence interval on $dPOD_{CC}$ meeting the criteria according to TR364 (Least Cost Formulations, Virginia Beach, VA). For cannabis matrixes, comparison is only between presumptive and confirmed (paired) candidate method results. NSDD = No statistical difference detected using SLV study design from OMA Appendix J (2012). The SLV qualitative method comparison study design from OMA Appendix J (2012) is not intended to demonstrate statistical equivalence. Expert opinion is that the method is appropriate for its intended use. SDD-R = Statistical difference detected with a positive bias for the reference (confirmation) procedure.

[f] Enrichment volume specific to SenSATIVAx® Extraction protocol.

[g] No significant difference detected at 16 h using AriaMx and CFX96 at the method developer laboratory with SenSATIVAx® Extraction only.

[h] Significant difference detected at 16 h using CFX96 at the independent laboratory with SenSATIVAx® Extraction only. No significant difference detected at 24 h using CFX96 at the independent laboratory with SenSATIVAx® Extraction only.

Table 2. Method Selectivity

	Enrichment		Inclusivity Strains		Exclusivity Strains	
Target	Broth[a]	Temperature	No. Tested	No. Positive	No. Tested	No. Positive
Salmonella	TSB	37 ± 1°C	102[b]	102	45[c]	0
STEC	TSB	37 ± 1°C	51[d]	51	45[c]	1[e]

[a] TSB = Tryptic Soy Broth.

[b] Comprised of 2 strains of *S. bongori*, 3 strains *S.* enterica subsp *arizonae*, 3 strains *S. enterica* subsp. *diarizonae*, 3 strains *S. enterica* subsp. *indica*, 3 strains *S. enterica* subsp. *houtenae*, 4 strains *S. enterica* subsp. *salamae*, and the remaining 82 strains represent 80 serovars of *S. enterica* subsp. *enterica*.

[c] Comprised of 45 species.

[d] Comprised of 5 strains O26, 5 strains O45, 6 strains O103, 5 strains O111, 5 strains O121, 6 strains O145, 8 strains O157:H7 and 11 strains from 5 other serogroups.

[e] *Shigella dysenteriae* contains the Shiga-toxin gene *stx* and was expected to be detected by the assay on the STEC channel.

Table 3. Method History

No.	Date	Summary	Supporting Data
1	March 2024	Original Certification.	Certification Report
2	April 2025	Level 3 Modification: new lysis procedure (the MaGiC Lysis Kit), the use of a lyophilized qPCR Amplification Mix, and the addition of a new qPCR instrument.	Modification Report 1