CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

092001

The AOAC Research Institute hereby certifies the test kit known as:

Enviro X-F

manufactured by

PathogenDx
9375 E. Shea Blvd., Ste. 100
Scottsdale, Arizona 85260
USA

This method has been evaluated in the AOAC® *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (September 24, 2020 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

October 08, 2020
Date

2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 Fax: +1-301-924-7089
Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

METHOD AUTHORS
Benjamin A. Katchman, Cory S. Newland, Rick Eggers, Fushi Wen, and Kevin O'Brian

SUBMITTING COMPANY
PathogenDx
9375 E. Shea Blvd., Ste. 100
Scottsdale, AZ 85260
USA

KIT NAME(S)
Enviro[X-F]

CATALOG NUMBERS
Enviro[X-F] EF-100

INDEPENDENT LABORATORY
Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204
USA

AOAC EXPERTS AND PEER REVIEWERS
Yi Chen[1], Michael Brodsky[2], Mark Carter[3]
[1]FDA CFSAN, College Park, MD, USA
[2]Brodsky Consultants, Thornhill, Ontario, Canada
[3]MC Squared, Chattanooga, TN, USA

APPLICABILITY OF METHOD
Analytes – *Listeria* spp. (*L. monocytogenes, Listeria innocua, Listeria ivanovii, Listeria seeligeri, Listeria welshimeri,* and *Listeria grayi*), *L. monocytogenes*, and *Salmonella* spp.

Matrices – WorldBio PUR-Blue™ Swabs in 5 mL of Hi-Cap broth - Environmental Surface Swabs (4in x 4in): Stainless Steel, Plastic (polystyrene), Rubber, and Sealed Concrete

Performance claims - The PathogenDx Enviro[X-F] assay is considered not statistically different than the FDA BAM Chapter 10: *Detection of L. monocytogenes in Foods and Environmental Samples, and Enumeration of L. monocytogenes in Foods* (2) and FDA BAM Chapter 5, *Salmonella* (3) for stainless steel, plastic (polystyrene), rubber, and sealed concrete environmental surface swabs.

REFERENCE METHODS
US Food and Drug Administration Bacteriological Analytical Manual, Chapter 10 *Detection of L. monocytogenes in Foods and Environmental Samples, and Enumeration of L. monocytogenes in Foods* (2)
US Food and Drug Administration Bacteriological Analytical Manual, Chapter 5 *Salmonella* (3)

ORIGINAL CERTIFICATION DATE
September 24, 2020

CERTIFICATION RENEWAL RECORD
New Approval 2020

METHOD MODIFICATION RECORD
NONE

SUMMARY OF MODIFICATION
NONE

Under this AOAC® *Performance Tested*[SM] License Number, 092001 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*[SM] License Number, 092001 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
The PathogenDx Enviro[X-F] assay consists of sample DNA amplified via a tandem Polymerase Chain Reaction (PCR) as a crude lysate which avoids purification. The Cy3 labeled PCR product is used without amplicon clean-up, quantitation, or normalization prior to hybridization. The Cy3 labeled tandem PCR product is diluted in hybridization buffer, which is then hybridized to the microarray. The hybridized and washed microarray is then imaged to yield a Cy3 hybridization pattern distributed among the probe spots. The PathogenDx software analysis tool, Augury©, automatically finds the hybridized spots in the image and then calculates the median Cy3 intensity of each hybridized spot.

DISCUSSION OF THE VALIDATION STUDY (1)
In the validation study, the PathogenDx Enviro[X-F] Assay successfully detected *Listeria* spp., *L. monocytogenes,* and *Salmonella* spp. on stainless steel, plastic (polystyrene), rubber, and sealed concrete environmental surface samples. The method proved to correctly identify all target organisms and correctly exclude all non-target organisms When comparing results obtained from the PathogenDx Enviro[X-F] assay to the confirmation results, no false positives or false negatives were observed. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the reference method and the PathogenDx Enviro[X-F] Assay. The product stability and lot to lot study showed that there is no significant impact on the performance of the assay over time. The robustness evaluation demonstrated that minor changes to the operational parameters has no significant impact on the performance of the test and that the assay is robust. The instrument variation study clearly demonstrates that there is no impact on the detection of target organisms based on the use of different slide imagers
The method allows the user to swab a surface and within the same day, get results for the presence of *Listeria* spp., *L. monocytogenes, and Salmonella* spp. This allows for faster turnaround time for client testing by eliminating overnight incubation as the method has a limit of detection (LOD) of 1 CFU. The package insert is professionally written, and the software is also easy to use as well as obtaining the final analysis through the Augury© software.

Table 22. Stainless Steel Candidate vs. Reference Method – POD Results (1)

Matrix	Strain	CFU[a]/ Test Area	N[b]	Candidate			Reference			dPOD$_C$[f]
				x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI	
Stainless Steel[h] (4" x 4")	*Salmonella* Typhimurium ATCC 14028 & *Citrobacter freundii* ATCC 8090	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00
		48 &110	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05
		& 1200	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00
	L. monocytogenes 4b ATCC 13932 & *Enterococcus faecalis* ATCC 29212		5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00
		58 & 191	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15
		450 & 1100	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00
Plastic[h] (Polystyrene) (4" x 4")	*Salmonella* Heidelberg ATCC 8326	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00
		63	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05
		160	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00
	L. innocua ATCC 33091	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00
		46	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00
		130	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00
	L. monocytogenes 4b ATCC 51780	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00
		52	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15
		170	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00
Rubber[h] (4" x 4")	*Salmonella* Enteritidis ATCC 13076	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00
		61	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05
		130	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00
	L. welshimeri ATCC 35897	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00
		70	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00
		180	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00
	L. monocytogenes 1/2a ATCC 15313	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00
		60	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05
		190	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00
Sealed Concrete[h] (4" x 4")	*Salmonella* Newport ATCC 6962	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00
		47	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00
		220	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00
	L. seeligeri ATCC 11289	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00

		45	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00
		180	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00
	L. monocytogenes 1/2a FSL J1-129	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00
		73	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05
		210	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[h]No difference in results were observed between samples analyzed with and without the Live/Dead Protocol

Table 23: Stainless Steel Presumptive vs. Confirmed – POD Results (1)

Matrix	Strain	CFU[a]/ Test Area	N[b]	Candidate			Reference			$dPOD_C$[f]	95% CI[g]
				x[c]	POD_C[d]	95% CI	X	POD_R[e]	95% CI		
Stainless Steel[h] (4" x 4")	*Salmonella* Typhimurium ATCC 14028 & *Citrobacter freundii* ATCC 8090	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		48 &110	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		& 1200	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.47, 0.47
	L. monocytogenes 4b ATCC 13932 & *Enterococcus faecalis* ATCC 29212		5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		58 & 191	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		450 & 1100	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.47, 0.47
Plastic[h] (Polystyrene) (4" x 4")	*Salmonella* Heidelberg ATCC 8326	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		63	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		160	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.47, 0.47
	L. innocua ATCC 33091	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		46	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		130	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.47, 0.47
	L. monocytogenes 4b ATCC 51780	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		52	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		170	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.47, 0.47
Rubber[h] (4" x 4")	*Salmonella* Enteritidis ATCC 13076	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		61	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		130	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	L. welshimeri ATCC 35897	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		70	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		180	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.47, 0.47
	L. monocytogenes 1/2a ATCC 15313	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		60	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13

		190	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.47, 0.47
Sealed Concrete[h] (4" x 4")	*Salmonella* Newport ATCC 6962	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		47	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		220	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.47, 0.47
	L. seeligeri ATCC 11289	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		45	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		180	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.47, 0.47
	L. monocytogenes 1/2a FSL J1-129	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		73	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		210	5	5	1.00	0.57, 1.00	5	1.00	0.57,1.00	0.00	-0.47, 0.47

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for the matrix in triplicate
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[h]No difference in results were observed between samples analyzed with and without the Live/Dead Protocol