



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

071102

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

PDX-SIB

manufactured by

**Paradigm Diagnostics, Inc.
800 Transfer Road, Ste 12
Saint Paul, MN 55114
USA**

This method has been evaluated in the AOAC[®] Performance Tested MethodsSM 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 TestedSM 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 (November 06, 2019 – December 31, 2020). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

November 06, 2019

Date

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KIT NAME(S) PDX-SIB	CATALOG NUMBERS 26003-25, 26009-50, 26005-100
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INDEPENDENT LABORATORY Q-Labs 1400 Harrison Avenue Cincinnati, Ohio 45214 USA	AOAC EXPERTS AND PEER REVIEWERS Yi Chen ¹ , Maria Cristina Fernandez ² , Wendy McMahon ³ ¹ US FDA CFSAN, College Park, MD, USA ² University of Buenos Aires, Buenos Aires, 1419 Argentina ³ Silliker Inc., South Holland, IL, USA
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APPLICABILITY OF METHOD Target organism – <i>Salmonella</i> Matrices – Ceramic tile, stainless steel, plastic, and sealed concrete Performance claims - The overall sensitivity relative to the reference method across all four surfaces was >100% (it is possible bacterial growth occurred during the study period). There were no significant differences between PDX-SIB and the reference method on any of the surfaces tested.	REFERENCE METHOD U.S. Food and Drug Administration (2011) <i>Bacteriological Analytical Manual</i>, Chapter 5, www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM070149. (5)
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ORIGINAL CERTIFICATION DATE July 29, 2011	CERTIFICATION RENEWAL RECORD Renewed annually through December 2020
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METHOD MODIFICATION RECORD NONE	SUMMARY OF MODIFICATION NONE
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Under this AOAC® <i>Performance Tested</i>SM License Number, 071102 this method is distributed by: NONE	Under this AOAC® <i>Performance Tested</i>SM License Number, 071102 this method is distributed as: NONE
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PRINCIPLE OF THE METHOD (1)

The principle of PDX-SIB utilizes two operating conditions, the first selective enrichment of the *Salmonella* population from the background microflora and secondly the simultaneous metabolism of a very specific *Salmonella* substrate. PDX-SIB is a balanced blend of proprietary selective agents highly restrictive to non-*Salmonella* bacteria and combining a highly specific metabolic substrate for *Salmonella*. As the selected population grows out the media becomes acidified and an incorporated pH indicator detects the pH change by a color shift from purple to yellow.

DISCUSSION OF THE VALIDATION STUDY (1)

PDX-SIB is an easy to use and interpret screening test for *Salmonella* species in environmental samples. Inclusivity and exclusivity studies revealed that PDX-SIB is very comprehensive for the detection of *Salmonella* species at very low levels (10-100 CFU/sample). Two strains, *S. Cubana* and *S. Gallinarum*, were originally negative in the inclusivity study. An additional isolate of *S. Cubana* was obtained from a different source, the University of Pennsylvania *Salmonella* Reference Center. This particular isolate was positive in fermenting the indicator compound in SIB in contrast to the isolate obtained from the University of Minnesota's culture collection. These data suggest that the Minnesota isolate was likely defective in a metabolic pathway for fermentation of the indicator compound. In regard to specificity, high levels of some *Citrobacter* species remain to be a possible source of false positive results. False positive results, although not desired by the typical end user, still tells a great deal about the overall microbial cleanliness of the areas sampled. Learning about the presence of *Citrobacter* species is important for another aspect, since many *Citrobacter* species occupy similar niches to *Salmonella* species and arise as contamination sources from the GI tracts of warm blooded animals. This information is potentially useful when monitoring food processing surfaces intended to be free of microflora after sanitation operations. These results demonstrate a cross reaction in two of three *Citrobacter* species tested underscoring the need to confirm all SIB-positive results by the traditional biochemical or genetic methods. PDX-SIB was found to be at least as sensitive as the reference method in all the surfaces studied. In fact PDX-SIB was slightly more sensitive than the reference method in one of the method comparison studies: five positives for PDX-SIB versus three positives for the FDA-BAM method in the stainless steel study. Regarding the ruggedness studies, recommended parameters have been studied for PDX-SIB. Results of the ruggedness studies suggested that for the most part, selected deviations from test parameters did not interfere with the true detection of microorganisms selected with the exception of *Salmonella* Anatum, which was not detected at 40°C at 24 hours incubation. Lot-to-lot variability studies showed that there was no difference between the production lots. Shelf life studies documented in table 4b, revealed that PDX-SIB is shelf-stable at 3 months of refrigerated storage.

Table 1a. Results of Inclusivity Test for PDX-SIB (1)

Serovar	Source	Origin	SIB Medium Color	Presumptive Result	Sero-group
S. Adelaide	U of MN 94679420	Meat meal	Yellow	+	O
S. Agona	U of MN inv 95650951	Soybean meal	Yellow	+	B
S. Albany	U of MN 2009595	Frozen fish paste	Yellow	+	C3
S. Anatum	U of MN 95645854	Chicken feed	Yellow	+	E1
S. Bovismorbificans	U of MN 3064124	Vietnam	Yellow	+	C2
S. Carrau	U of MN 2003413	Frozen shrimp	Yellow	+	H
S. Cerro	U of MN 94713965	Poultry feed	Yellow	+	K
S. Cubana	U of MN 94679421	Swine feed	Blue	-	G2
S. Chester	U of MN 3063650	Frozen tilapia fish	Yellow	+	B
S. Emek	U of MN 3063892	Frozen catfish	Yellow	+	C3
S. Enteritidis	U of MN 95657613	Ice cream	Yellow	+	D1
S. Give	U of MN 1829352	Lobster tail	Yellow	+	E1
S. Gloucester	U of MN 1676771	Sesame seeds	Yellow	+	B
S. Hvittingfoss	U of MN 200373	Frozen frog legs	Yellow	+	I
S. Infantis	U of MN 2015422	Frozen lobster tail	Yellow	+	C1
S. Javiana	U of MN 1842147	Frozen shrimp	Yellow	+	D1
S. Kentucky	U of MN 95-690-012	Cottonseed meal	Yellow	+	C3
S. Lille	U of MN 95-713-959	Chicken feed	Yellow	+	C1
S. Mbandaka	U of MN 95690014	Soybean meal	Yellow	+	C1
S. Meleagridis	U of MN 1949345	Frozen shrimp	Yellow	+	E1
S. Montevideo	U of MN 95573493	Raw eggs	Yellow	+	C1
S. Muenchen	U of MN 1842204	Frozen shrimp	Yellow	+	C2
S. Newbrunswick	U of MN 1842304	Frozen shrimp	Yellow	+	E1
S. Nashua	U of MN 2006036	Poultry feed	Yellow	+	M
S. Newport	U of MN 2006038	Frozen lobster tail	Yellow	+	C2
S. Penilla	U of MN 1949289	Frozen shrimp	Yellow	+	M
S. Poona	U of MN 1103174	White pepper	Yellow	+	G1
S. Sterrenbos	U of MN 1842082	Frozen shrimp	Yellow	+	C3
S. Thompson	U of MN 95657618	Ice cream	Yellow	+	C1
S. Weltevreden	U of MN 1950358	Dried ling shrimp	Yellow	+	E1
S. Typhimurium	U of MN	Salted dune egg	Yellow	+	B

	3019907				
S. Worthington	U of MN 95-713-958	Chicken feed	Yellow	+	G2
S. Kumasi	U of MN 1929854	Frozen crab meat	Yellow	+	N
S. Rubislaw	U of MN 2004976	Frozen shrimp	Yellow	+	F
S. Goodwood	U of MN	Faeces	Yellow	+	E4
S. Senftenberg	U of MN	Sewage	Yellow	+	E4
S. Ohio	U of MN	Animal feed	Yellow	+	C1
S. Limete	U of MN		Yellow	+	B
S. Tennessee	U of MN	Soybean meal	Yellow	+	C1
S. Newington	U of MN	Wild poultry	Yellow	+	B
S. Aberdeen	NCTC 5791	Infantile diarrhea	Yellow	+	F
S. Aequatoria	NCTC 7891	African zoonosis	Yellow	+	C1
S. Alabama	NCTC 9868	Human faeces	Yellow	+	B
S. Altendorf	NCTC 10546		Yellow	+	B
S. Austin	NCTC 8447		Yellow	+	C1
S. Ball	NCTC 9870		Yellow	+	B
S. Berkeley	NCTC 8260	Diseased turkey	Yellow	+	U
S. Brookfield	NCTC 10946		Yellow	+	O66
S. California	NCTC 6018	Animal feed	Yellow	+	B
S. Canastel	NCTC 6948	Animal feed	Yellow	+	D1
S. Carmel	NCTC 9872	Infantile diarrhea	Yellow	+	O17
S. Champaign	NCTC 6851	Hen liver	Yellow	+	Q
S. Chicago	NCTC 9873		Yellow	+	M
S. Colombo	NCTC 9922	Sheep	Yellow	+	P
S. Ealing	NCTC 11949	Dried baby milk	Yellow	+	O
S. Dahlem	NCTC 9949	Cattle	Yellow	+	Y
S. Gallinarum	NCTC 10532	Poultry	Blue	-	D1
S. Houten	NCTC 10401	Reptile	Yellow	+	O43
S. Kottbus	NCTC 5753	Faeces	Yellow	+	C2
S. Illinois	NCTC 8498	Poults	Yellow	+	E3
S. Lexington	NCTC 6244	Soybean	Yellow	+	E1
S. Manchester	NCTC 7372		Yellow	+	C2
S. Minnesota	NCTC 5800	Swine	Yellow	+	L
S. Mississippi	NCTC 6487	Faeces	Yellow	+	G2
S. Napoli	NCTC 6853	Food handlers	Yellow	+	D1
S. Pensacola	NCTC 6946		Yellow	+	D1
S. Pretoria	NCTC 6234	Meat	Yellow	+	F
S. Shanghai	NCTC 9791		Yellow	+	I
S. Sunsvall	NCTC 9787	Dried egg	Yellow	+	H
S. Waycross	NCTC 7401	Urine	Yellow	+	S
S. Alachua	U Penn STS 6	Swine	Yellow	+	O
S. Choleraesuis	ATCC 10708	Fish	Yellow	+	C

S. Arkansas	U Penn STS 11		Yellow	+	B
S. Blockley	U Penn STS 15	Environment	Yellow	+	C2
S. Brandenburg	U Penn STS 18	Swine	Yellow	+	B
S. Derby	U Penn STS 22	Polluted water	Yellow	+	B
S. Dublin	U Penn STS 27	Cattle	Yellow	+	D1
S. Hadar	U Penn STS 45	Turkey	Yellow	+	C2
S. Heidelberg	U Penn STS 48	Poultry	Yellow	+	B
S. London	U Penn STS 64	Polluted water	Yellow	+	E1
S. Manhattan	U Penn STS 65	Avian	Yellow	+	C2
S. Oranienburg	U Penn STS 83	Egg	Yellow	+	C1
S. Panama	U Penn STS 86	Infantile diarrhea	Yellow	+	D1
S. Paratyphis	ATCC 13314	Sewage	Yellow	+	A
S. Saint Paul	U of MN	Milk powder	Yellow	+	B
S. Schwarzengrund	U Penn STS 95	Chicken	Yellow	+	B
S. Stanley	U Penn STS100	Reptile	Yellow	+	B
S. Urbana	U Penn STS110	Reptile	Yellow	+	N
S. Johannesburg	U Penn STS 56	Meat meal	Yellow	+	R
S. Thomasville	U Penn STS103	Poultry meal	Yellow	+	E3
S. Virchow	U Penn STS 112	Basil	Yellow	+	C1
S. Abaetetuba	ATCC 35640	Fresh water	Yellow	+	F
S. Choleraesuis var. Kunzendorf	ATCC 12011	Swine	Yellow	+	B
S. Vallore	ATCC 15611		Yellow	+	B
S. Paratyphis	U of MN 2014696	Frozen frog legs	Yellow	+	B
S. Tallahassee	ATCC 12002		Yellow	+	C3
S. Salford	U of MN 2009532	Oregano turkey	Yellow	+	I
S. Birmingham	U of MN DI95764802	Alfalfa seed	Yellow	+	E1
S. Brunei	U of MN 1680318	Frozen Shrimp	Yellow	+	C3
S. Ikeja	U of MN 3019543	Frozen Shrimp	Yellow	+	E1
S. Cubana	UPenn	Swine	Yellow	+	G2

Table 1b. Results of Exclusivity Test for PDX-SIB (1)

Species	Source	Origin	SIB Result
<i>Klebsiella pneumoniae</i>	NCTC 9633	Sputum	-
<i>Proteus mirabilis</i>	ATCC 12453	GI tract	-
<i>Citrobacter freundii</i>	NCTC 9750	Soil	-
<i>Escherichia coli</i>	ATCC 13706	GI tract	-
<i>Escherichia coli</i>	ATCC 14948	GI tract	-
<i>Hafnia alvei</i>	ATCC 700025	Brewery fermentation samples	-
<i>Serratia liquefaciens</i>	ATCC 27592		-
<i>Morganella morganii</i> subsp. <i>morganii</i>	ATCC 25829		-
<i>Pseudomonas aeruginosa</i>	ATCC 10145		-
<i>Providencia rettgeri</i>	ATCC 9250		-
<i>Enterobacter amnigenus</i>	ATCC 51816		-
<i>Enterobacter aerogenes</i>	ATCC 13048		-
<i>Shigella sonnei</i>	ATCC 25931		-
<i>Shigella flexneri</i>	ATCC 9199		-
<i>Staphylococcus epidermidis</i>	ATCC 14990		-
<i>Staphylococcus aureus</i>	ATCC 700699		-
<i>Serratia marcescens</i>	ATCC 13880	Polenta	-
<i>Enterobacter cloacae</i> subsp. <i>cloacae</i>	ATCC 23355		-
<i>Enterobacter gergoviae</i>	ATCC 33028		-
<i>Klebsiella oxytoca</i>	ATCC 13182		-
<i>Providencia wickerhamii</i>	ATCC 16529		-
<i>Shigella boydii</i>	ATCC 9207		-
<i>Staphylococcus aureus</i>	NCTC 12973		-
<i>Yersinia enterocolitica</i> subsp. <i>enterocolitica</i>	ATCC 23715		-
<i>Yersinia ruckerii</i>	ATCC 29473		-
<i>Citrobacter freundii</i>	ATCC 8090		+
<i>Citrobacter braakii</i>	ATCC 43162		-
<i>Citrobacter koseri</i>	ATCC 27156		+
<i>Escherichia coli</i>	NCIMB 11943		-
<i>Escherichia coli</i>	NCTC 10538		-
<i>Listeria monocytogenes</i>	ATCC 13932		-
<i>Listeria innocua</i>	ATCC 33090		-
<i>Pasteurella multocida</i> subsp. <i>multocida</i>	ATCC 12945		-
<i>Providencia stuartii</i>	ATCC 33672		-
<i>Edwardsiella tarda</i>	ATCC 15947		-

Table 2. Summary of Method Comparison Studies of SIB at 48 Hour Incubation. (1)

Matrix	Strain	N ^a	PDX-SIB		FDA -BAM	Chi Square ^b	Relative Sensitivity ^c
			Presumptive Pos.	Confirmed Pos.	Positive		
Plastic	S. Newport	5	0	0	0	-	-
		20 Low level	11	11	13	0.406	84.6%
		20 High level	20	20	20	0	100%
Sealed concrete	S. Anatum	5	0	0	0	-	-
		20 Low level	7	7	8	0.104	87.5%
		20 High level	20	20	20	0	100%
Ceramic tile	S. Abaetetuba	5	0	0	0	-	-
		20 Low level	10	10	7	0.898	142.9%
		20 High level	20	20	20	0	100%
Stainless steel ^d	S. Typhimurium : 10X C. freundii	5	0	0	0	-	-
		20 Low level	0	0	2	2.05	0
		20 High level	5	5	3	0.609	166.7%

^aN = Number of test portions
^bChi Square = Mantel-Haenszel: $\chi^2 = (n-1)(ad-bc)^2 / [(a+b)(a+c)(b+d)(c+d)]$, where n = total number of samples

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