



# CERTIFICATION

**AOAC<sup>®</sup> Performance Tested<sup>SM</sup>**

Certificate No.

**090201B**

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

**PATHATRIX Pooling System for *Listeria* spp.**

manufactured by

**Life Technologies, part of Thermo Fisher Scientific  
Wade Road  
Basingstoke, Hampshire, RG24 8PW  
United Kingdom**

This method has been evaluated in the AOAC<sup>®</sup> *Performance Tested Methods*<sup>SM</sup> Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC<sup>®</sup> Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*<sup>SM</sup> 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 (October 29, 2018 – December 31, 2019). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

*Scott Coates*

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

October 29, 2018

\_\_\_\_\_  
Date

**METHOD AUTHORS**

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**MODIFICATION JUNE 2005:** Adrian Parton, John Murray, Nicole Prentice, & Michael Scott  
**MODIFICATION DECEMBER 2012:** Kathy Latham  
**MODIFICATION OCTOBER 2015:** V. Zepnickaite, A. Markina, & S. Mantipragada

**SUBMITTING COMPANY**

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**CURRENT SPONSOR**

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**KIT NAME(S)**

PATHATRIX Pooling System for *Listeria* spp.

**CATALOG NUMBERS**

APL50, APL250P, ZBLQCAP, ZBLQCA

**INDEPENDENT LABORATORY**

Original Validation:  
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<sup>4</sup> Original validation and June 2005 Modification  
<sup>5</sup> December 2012 Modification

**APPLICABILITY OF METHOD**

**Target organism – *Listeria* species**

**Matrices – soft cheese, single cream, cooked chicken, ready meal, frozen prawns, soft cheese\*, cream\*, cooked chicken\***

**Performance claims - PATHATRIX allows the detection and isolation of *Listeria* species from a range of foods at low levels (1-10cfu/25g).**

**\*Modification validated June 2005 for PATHATRIX AUTO and November 29, 2009 for Auto 5 Pooling**

**REFERENCE METHODS**

**Original Validation:**

USDA/FSIS Microbiology Laboratory Guidebook 3<sup>rd</sup> Edition 1998 (Revision # 1; 9-6-99) (3)

BAM Bacteriological Analytical Manual 8<sup>th</sup> Edition 1998 (4)

**MODIFICATION**

U.S. Food & Drugs Administration. 2003. Bacteriological Analytical Manual (online). <http://www.cfsan.fda.gov/~ebam/bam-toc.html> (7)

Modification June 2005:

U.S. Food and Drug Administration, FDA *Bacteriological Analytical Manual*, <http://www.cfsan.fda.gov/~ebam/bam-10.html> (8)

United States Department of Agriculture/Food Safety Inspection Services

*Microbiological Laboratory Guidelines*,

[http://www.fsis.usda.gov/PDF/MLG\\_8\\_06.pdf](http://www.fsis.usda.gov/PDF/MLG_8_06.pdf) (9)

**ORIGINAL CERTIFICATION DATE**

September 05, 2002

**CERTIFICATION RENEWAL RECORD**

Renewed Annually through December 2019

**METHOD MODIFICATION RECORD**

1. November 2005
2. December 2012 Level 1
3. October 2015 Level 2
4. December 2017 Level 1

**SUMMARY OF MODIFICATION**

1. Validation of PATHATRIX AUTO instrument
2. Kit acquired by Life Technologies. Manufacturing location change from Newmarket, UK to Austin, TX.
3. Location change from Austin, TX to Vilnius, Lithuania.
4. Editorial changes on insert and labels

Under this AOAC® *Performance Tested*<sup>SM</sup> License Number, 090201B this method is distributed by:

NONE

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NONE

**PRINCIPLE OF THE METHOD (1)**

The PATHATRIX Test System is a novel patented method that comprises of a pre-programmed workstation and a consumable pack and employs magnetic beads coated with antibodies specific to the target organism, which for this test was *Listeria*. The whole food sample is homogenised in a non-selective enrichment medium (Buffered Peptone Water) in a sterile stomacher bag (that may or may not containing a mesh liner according to the manufacturers instruction – see protocol). The stomacher bag is then incubated overnight at 30°C. After incubation the bag is then placed on the PATHATRIX in a thermally controlled pot at 30°C and magnetic beads, coated with antibodies to *Listeria*, are added to the sample homogenate. The consumable pack is then loaded into the PATHATRIX, and the pre-programmed run started. The liquid sample is then continuously re-circulated over the phase from the bag by a peristaltic pump via tubing (Figure 1). Within this closed loop system is a plastic phase, there is a wedge shaped capture surface that projects into the centre of the phase. A permanent magnet is placed on the opposite side of this wedge (external to the phase) and this enables beads to be captured as they pass over internal sloped face of the phase.

After continuously circulating the sample around the system and through the phase for 30 minutes, the target organisms are bound to the magnetic beads onto the phase. Any residue and food debris is removed from the phase by a subsequent wash step. The beads from the capture phase are then eluted into a wash vessel and concentrated using a magnetic rack.

After completion of the capture step the sample can then be directly plated onto Oxford or PALCAM plates. These plates are incubated at 30°C for 16-24h and examined for presumptive colonies of *Listeria*. Having used the system to isolate *Listeria*, the laboratory would then take a selection of typical colonies and subject these to confirmation by agglutination and/or biochemical identification of *Listeria*.

**DISCUSSION OF THE VALIDATION STUDY (1)**

It is clear from the data presented in the Internal and External validation studies that the PATHATRIX system represents a significant improvement on the current FDA BAM method for the detection *Listeria* in a range of food matrices.

The PATHATRIX system is fundamentally different from other detection systems in that the entire 250ml sample is actually analysed rather than looking at 1ml (or less) fractions of enrichment cultures, that other methods rely on. Therefore a greater degree of sensitivity is achieved, which enables the effective enrichment and purification stage of the process to be reduced to only 16 hours.

The OXFORD & PALCAM plates showed a reduction in the number of background contamination by comparison to other methods. This produced clearer isolated colonies that enable more accurate reading and ease of confirmation of *Listeria* organisms.

An additional benefit of the PATHATRIX system is speed. Presumptive results i.e. “typical” colonies on a plate can be achieved in as little as 24 hours (after plating) and if serological tests are used e.g. agglutination, results can be confirmed within 40 hours of commencement of the test. This represents a significant improvement by comparison to the FDA BAM method and other methods which typically require 72 hours or more to obtain a presumptive result.

Other considerations are “ease of use” of methods and here again the PATHATRIX system has been shown in external validation studies to be significantly easier to use involving less manipulation by the operator and a lower skill level to operate the test. Clearly these factors are highly significant to the laboratories that conduct *Listeria* testing, and could lead to more widespread testing in the industry as the tests become more accessible and less expensive than current methods.

The pre-programmed nature of the PATHATRIX instrument removes areas of concern relating to operator error and therefore makes the instrument more robust to use than by comparison to conventional methods which require a greater degree of “skill”/ “operator technique”.

**Original Validation Data (1)****Table 1: Species / strains used for Inclusivity Study of 60 strains of *Listeria***

No	Organism	CCFRA code	Serogroup	Source	From
1	<i>Listeria monocytogenes</i>	6600	4b	NCTC 11994	NCTC
2	<i>Listeria monocytogenes</i>	6601	1/2b	NCTC 10887	NCTC
3	<i>Listeria monocytogenes</i>	1100	1/2a	Stilton cheese	CPHL*
4	<i>Listeria monocytogenes</i>	1101	1/2a	Coleslaw salad	CPHL
5	<i>Listeria monocytogenes</i>	1102	1/2a	Lettuce	CPHL
6	<i>Listeria monocytogenes</i>	1103	1/2a	Pate	CPHL
7	<i>Listeria monocytogenes</i>	1104	1/2a	Soft cheese	CPHL
8	<i>Listeria monocytogenes</i>	1105	1/2a	Raw milk	CPHL
9	<i>Listeria monocytogenes</i>	1108	1/2b	Pate	CPHL
10	<i>Listeria monocytogenes</i>	1149	1/2b	Pork liver pate	CPHL
11	<i>Listeria monocytogenes</i>	1150	1/2b	Fish fingers	CPHL
12	<i>Listeria monocytogenes</i>	1152	1/2c	Cooked turkey	CPHL
13	<i>Listeria monocytogenes</i>	1155	1/2c	Sliced ham	CPHL
14	<i>Listeria monocytogenes</i>	1158	3a	Cooked chicken	CPHL
15	<i>Listeria monocytogenes</i>	1164	3b	Chicken	CPHL
16	<i>Listeria monocytogenes</i>	1169	3c	Unknown	CPHL
17	<i>Listeria monocytogenes</i>	1172	3c	Cooked ham	CPHL
18	<i>Listeria monocytogenes</i>	1173	3c	Chicken & lettuce sandwich	CPHL
19	<i>Listeria monocytogenes</i>	1175	4b	Cooked turkey	CPHL
20	<i>Listeria monocytogenes</i>	1176	4b	Cheese	CPHL
21	<i>Listeria</i>	1177	4b	Ice cream	CPHL

	<i>monocytogenes</i>				
22	<i>Listeria monocytogenes</i>	1178	4b	Chicken salad roll	CPHL
23	<i>Listeria monocytogenes</i>	1179	4b	Cheese	CPHL
24	<i>Listeria monocytogenes</i>	1180	4b	Soft cheese	CPHL
25	<i>Listeria monocytogenes</i>	1181	4	Cheese	CPHL
26	<i>Listeria monocytogenes</i>	1182	4	Egg mayonnaise sandwich	CPHL
27	<i>Listeria monocytogenes</i>	1183	4	Pasteurised egg	CPHL
28	<i>Listeria monocytogenes</i>	1184	4	Pate	CPHL
29	<i>Listeria monocytogenes</i>	1186	4	Cheese	CPHL
30	<i>Listeria monocytogenes</i>	1187	4a	Unknown	CPHL
31	<i>Listeria innocua</i>	1110		Pate	CPHL
32	<i>Listeria innocua</i>	1111		Cheese	CPHL
33	<i>Listeria innocua</i>	1112		Lettuce	CPHL
34	<i>Listeria innocua</i>	1113		Beefburger	CPHL
35	<i>Listeria innocua</i>	1114		Black pudding	CPHL
36	<i>Listeria innocua</i>	1115		Celery salad	CPHL
37	<i>Listeria innocua</i>	1117		Salami	CPHL
38	<i>Listeria innocua</i>	1118		Raw chicken	CPHL
39	<i>Listeria innocua</i>	1119		Egg mayonnaise	CPHL
40	<i>Listeria innocua</i>	6602		NCTC 11288	NCTC
41	<i>Listeria welshimeri</i>	1130		Salami	CPHL
42	<i>Listeria welshimeri</i>	1132		Raw chicken	CPHL
43	<i>Listeria welshimeri</i>	1134		Chicken	CPHL
44	<i>Listeria welshimeri</i>	1135		Smoked mackerel	CPHL
45	<i>Listeria welshimeri</i>	1138		Food	CPHL
46	<i>Listeria seeligeri</i>	1139		Lettuce	CPHL
47	<i>Listeria seeligeri</i>	1142		Pork loaf	CPHL
48	<i>Listeria seeligeri</i>	1146		Pate	CPHL
49	<i>Listeria seeligeri</i>	1147		Chicken roll	CPHL
50	<i>Listeria seeligeri</i>	6603		NCTC 11856	NCTC
51	<i>Listeria ivanovii</i>	6599		NCTC 11007	NCTC
52	<i>Listeria ivanovii</i>	1120		Radish	CPHL
53	<i>Listeria ivanovii</i>	1121		Belgian salami	CPHL
54	<i>Listeria ivanovii</i>	1123		Soft cheese	CPHL
55	<i>Listeria ivanovii</i>	1129		Unknown	CPHL
56	<i>Listeria grayi</i>	9298		NCTC 10815	NCTC
57	<i>Listeria grayi</i>	12561		LMG 16794	BCCM LMG Belgium
58	<i>Listeria grayi</i>	12524 A		NCTC 10812	NCTC
59	<i>Listeria grayi</i>	12526 A		NCTC 10812	NCTC
60	<i>Listeria grayi</i>	12527 A		NCTC 10841	NCTC

\* CPHL Central Public Health Laboratory London

Table 2: Species/Strains of competitors used for Exclusivity Study of the PATHATRIX *Listeria* Test (1)

No	Organism	CCFRA code	Source/Strain Reference
1	<i>Aeromonas hydrophila</i>	5518	NCTC 8049
2	<i>Bacillus cereus</i>	4110	ATCC 10876
3	<i>Bacillus cereus</i>	5502	NCIMB 9373
4	<i>Bacillus cereus</i>	193	NCIMB 3329
5	<i>Bacillus subtilis</i>	4112	ATCC 6633
6	<i>Edwardsiella tarda</i>	8392	NCTC 10391
7	<i>Enterobacter aerogenes</i>	4108	ATCC 13048
8	<i>Enterobacter aerogenes</i>	15736	NCTC 10006
9	<i>Enterococcus faecalis</i>	4113	NCTC 775
10	<i>Erwinia herbico</i>	7057	NCIMB 11521
11	<i>Escherichia coli</i>	11017	NCTC 12241
12	<i>Escherichia coli</i>	11626	NCTC 5933
13	<i>Lactobacillus gasseri</i>	6804	NCIMB 13081
14	<i>Lactobacillus plantarum</i>	166	NCTC 6376
15	<i>Pasteuralla avium</i>	8389	NCTC 11297
16	<i>Pasteuralla bettii</i>	8391	NCTC 10535
17	<i>Pseudomonas aeruginosa</i>	8299	NCIMB 10753
18	<i>Pseudomonas aeruginosa</i>	7834	NCIMB 10548
19	<i>Pseudomonas fragi</i>	7268	NCTC 10476
20	<i>Salmonella Typhimurium</i>	11634	ATCC 14028
21	<i>Serratia marcescens</i>	130	NCTC 10211
22	<i>Shigella boydii</i>	324	NCTC 11321
23	<i>Shigella flexneri</i>	325	NCTC 9950
24	<i>Shigella sonnei</i>	326	NCTC 10352
25	<i>Staphylococcus aureus</i>	1216	NCTC 10655/ATCC 19095
26	<i>Staphylococcus aureus</i>	4105	ATCC 25923
27	<i>Staphylococcus aureus</i>	11018	NCTC 6571
28	<i>Streptococcus agalactiae</i>	7115	ATCC 13813
29	<i>Streptococcus thermophilus</i>	5492	NCIMB 8510
30	<i>Vibrio mimicus</i>	6351	NCTC 11435
31	<i>Vibrio parahaemolyticus</i>	15737	NCTC 11344
32	<i>Yersinia enterocolitica</i>	4103	NCTC 10460

Comparison of PATHATRIX *Listeria* vs FDA BAM methods for a range of foods (1)

Food Sample	MPN LEVEL	No +ve Samples PATH'X LOW	No +ve Samples CONV LOW	MPN LEVEL	No +ve Samples PATH'X HIGH	No +ve Samples CONV HIGH
Raw Ground Beef	7.5cfu	18	19	14cfu	20	20
Cooked sliced Ham	4.3cfu	18	18	15cfu	20	20
Milk Powder	0.74cfu	16	16	35cfu	20	20
Orange Juice	4.3cfu	20	19	27cfu	20	20
Black Ground Pepper	2.3cfu	19	9	12cfu	20	18
Chocolate	4.3cfu	19	15	24cfu	19	19
Soft Cheese	6.4cfu	20	19	27cfu	20	20
Lettuce	2.3cfu	20	19	28cfu	20	20
Raw Fish	0.92cfu	16	19	46cfu	20	20
Lasagne Ready Meal	9.3cfu	20	19	28cfu	20	20

## Modification Matrix Extension using Auto "5" Pooling November 2005 (10)

## Comparison of PATHATRIX AUTO to FDA BAM Chapter 10/USDA FSIS MLG 8.06 Food Matrices Study

Matrix	Test Portion	MPN / 25g	No. test portions for each method	Reference Method	Pathatrix		$\chi^2$ <sup>e</sup>	Pathatrix	
				Positive	Presumptive Positive	Confirmed Positive		False Negative	False Positive
Soft Cheese	Individual	0.08	20	13	13	13	0	0	0
	Pooled	0.08	20	-	13	13	0	0	0
	Control	0	20	0	0	0	-	-	-
Cooked Chicken	Individual	0.0368	20	15	16	16	0.104	0	0
	Pooled	0.0368	20	-	16	16	0.104	0	0
	Control	0	20	0	0	0	-	-	-
Cream	Individual	0.092	20	12	13	13	0.104	0	0
	Pooled	0.092	20	-	13	13	0.104	0	0
	Control	0	20	0	0	0	-	-	-

## REFERENCES CITED

- Parton, A., & Scott, M., Evaluation of the PATHATRIX Pooling System for *Listeria* spp., AOAC® Performance Tested<sup>SM</sup> certification number 090201B.
- AOAC Research Institute Validation Outline for PATHATRIX Pooling System for *Listeria* spp., Approved – September 2002.
- USDA/FSIS Microbiology Laboratory Guidebook 3<sup>rd</sup> Edition 1998 (Revision # 1; 9-6-99)
- BAM Bacteriological Analytical Manual 8<sup>th</sup> Edition 1998
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- Parton, A., Murray, J., Prentice, N., Scott, M., Evaluation of the PATHATRIX® AUTO *Listeria* species 5 Pooling TEST (APL250-P), AOAC® Performance Tested<sup>SM</sup> certification number 090201B, Approved June 2005.
- Latham, K., Pathatrix Beed Manufacturing Move Validation Report, AOAC® Performance Tested<sup>SM</sup> certification number 090201B. Approved December 2012
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