



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
090203C

The AOAC Research Institute hereby certifies the method known as:

PATHATRIX Pooling System for *Salmonella* species

manufactured by

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

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.

A handwritten signature in black ink, appearing to read 'Bradley A. Stawick'.

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

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METHOD NAME PATHATRIX Pooling System for <i>Salmonella</i> species	CATALOG NUMBERS AP550, APS250P, APS500P, 4403930, PATHATRIXAUTO
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INDEPENDENT LABORATORY Original Validation: Campden & Chorleywood Food Research Association Chipping Campden Gloucestershire, GL55 6LD United Kingdom
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APPLICABILITY OF METHOD Target organism – <i>Salmonella</i> species. Matrixes – (25 g) – pasteurized liquid egg, raw ground beef, cooked sliced ham, milk powder, orange juice, black ground pepper, chocolate, soft cheese, produce, raw fish, lasagna (ready meal) MODIFICATION 2005 – (25 g) – cooked ham, raw whole egg, chocolate, milk powder, frozen prawns Performance claims – PATHATRIX allows the detection and isolation of <i>Salmonella</i> species from a range of foods at low levels (1-10cfu/25g).	REFERENCE METHODS USDA/FSIS Microbiology Laboratory Guidebook 3 rd Edition 1998 (Revision # 1; 9-6-99) (3) BAM Bacteriological Analytical Manual 8 th Edition 1998 (4) U.S. Food & Drugs Administration. 2003. Bacteriological Analytical Manual (online) (8)
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ORIGINAL CERTIFICATION DATE September 02, 2002	CERTIFICATION RENEWAL RECORD Renewed annually through December 2025.
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METHOD MODIFICATION RECORD 1. June 2005 2. December 2012 Level 2 3. May 2013 Level 2 4. October 2015 Level 2 5. December 2017 Level 1 6. December 2022 Level 1 7. January 2024 Level 1	SUMMARY OF MODIFICATION 1. Addition of pooling samples. 2. Kit acquired by Life Technologies. Manufacturing location change from Newmarket, UK to Austin, TX. 3. MicroSEQ® <i>Salmonella</i> spp. Linked to PTM 090203C Pathatrix® Pooling System <i>Salmonella</i> spp. Kit for fresh diced tomatoes, chocolate, and deli ham. 4. Manufacturing location change from Austin, TX to Vilnius, Lithuania. 5. Editorial/clerical changes. 6. Editorial/clerical changes. 7. Editorial/clerical changes.
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PRINCIPLE OF THE METHOD (1)

The PATHATRIX *Salmonella* 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 *Salmonella*. 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 manufacturers guidelines – see protocol). The stomacher bag is then incubated overnight at 37°C. After incubation the bag is then placed on the PATHATRIX in a thermally controlled pot at 37°C and magnetic beads, coated with antibodies to *Salmonella*, 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 that incorporates a sloped face, which becomes magnetised and captures the beads onto the face's surface as they pass.

After continuously circulating the sample around the system and through the phase for 30 minutes, the target organisms are bound to the magnetic beads on the phase. Any residue and food debris are 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, by streaking, onto XLD, Brilliant Green and Hektoin agar plates, which are incubated at 37°C overnight in an incubator.

DISCUSSION OF THE VALIDATION STUDY (1)

It is clear from the data presented in the Internal and External validation studies that the PATHATRIX system is at least equivalent to the USDA /BAM methods for the detection *Salmonella* in a range of different 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 use of non-selective enrichment broths that do not inhibit growth in any way.

The agar plates showed a significant reduction in the number of background contamination by comparison to other methods (with the exception of raw ground chicken). This produced clearer isolated colonies that enable more accurate reading and ease of confirmation of *Salmonella* 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 18 hours from 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 USDA FSIS and FDA-BAM methods 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 *Salmonella* testing. 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".

Table 15.2.2.1: Results of Inclusivity Study for PATHATRIX (1)

Number	Organism	CCFRA code	O-antigen group	Source/Strain
1	<i>Salmonella arizonae</i>	1571	51	NCTC 8297
2	<i>Salmonella</i> Treforest	1413	51	NCTC 10075
3	<i>Salmonella</i> Utrecht	1417	52	NCTC 10077
4	<i>Salmonella</i> Uccle	1416	54	NCTC 10251
5	<i>Salmonella</i> Tranaroa	1412	55	NCTC 10252
6	<i>Salmonella</i> Locarno	1386	57	NCTC 10272
7	<i>Salmonella</i> Basel	1292	58	NCTC 10310
8	<i>Salmonella</i> Abony	11632	B	NCTC 6017
9	<i>Salmonella</i> California	1319	B	NCTC 6018
10	<i>Salmonella</i> Derby	1352	B	NCTC 5721
11	<i>Salmonella</i> Essen	1370	B	NCTC 5723
12	<i>Salmonella</i> Altendorf	1278	B	NCTC 10546
13	<i>Salmonella</i> Cairo	1318	B	NCTC 8274
14	<i>Salmonella</i> Typhimurium	1974	B	ATCC 13311 NCTC 74
15	<i>Salmonella</i> Typhimurium	11634	B	ATCC 14028
16	<i>Salmonella</i> Schwarzengrund	1408	B	NCTC 6756
17	<i>Salmonella</i> San Diego	1407	B	NCTC 6024
18	<i>Salmonella</i> Reading	1405	B	NCTC 5720
19	<i>Salmonella</i> Chester	1329	B	NCTC 5718
20	<i>Salmonella</i> Budapest	1314	B	NCTC 5724
21	<i>Salmonella</i> Banana	1289	B	NCTC 8718
22	<i>Salmonella</i> Ball	1288	B	NCTC 9870
23	<i>Salmonella</i> Java	1378	B	NCTC 5706
24	<i>Salmonella</i> Bareilly	1291	C ₁	NCTC 5745
25	<i>Salmonella</i> Oranienbury	1402	C ₁	NCTC 5743
26	<i>Salmonella</i> Birkenhead	1297	C ₁	NCTC 7744
27	<i>Salmonella</i> Tennessee	1411	C ₁	NCTC 6388
28	<i>Salmonella</i> Norwich	1401	C ₁	NCTC 7077
29	<i>Salmonella</i> Menden	9279	C ₁	ATCC 15992
30	<i>Salmonella</i> Hartford	1375	C ₁	NCTC 6802
31	<i>Salmonella</i> Eschweiler	1369	C ₁	NCTC 8442
32	<i>Salmonella</i> Edinburgh	1364	C ₁	NCTC 7407
33	<i>Salmonella</i> Denver	1351	C ₁	NCTC 8445
34	<i>Salmonella</i> Amersfoort	1280	C ₁	NCTC 5749
35	<i>Salmonella</i> Livingstone	1385	C ₁	NCTC 9125

36	<i>Salmonella</i> Jerusalem	1380	C ₁	NCTC 8146
37	<i>Salmonella</i> Austin	1286	C ₁	NCTC 8447
38	<i>Salmonella</i> Fayed	1372	C ₂	NCTC 7371
39	<i>Salmonella</i> Emek	1367	C ₂	NCTC 8485
40	<i>Salmonella</i> Bronx	1313	C ₂	NCTC 9903
41	<i>Salmonella</i> Brovis-morbificans	1306	C ₂	NCTC 5754
42	<i>Salmonella</i> Bonariensis	1304	C ₂	NCTC 6481
43	<i>Salmonella</i> Banalia	1290	C ₂	NCTC 8242
44	<i>Salmonella</i> Amherstiana	1281	C ₂	NCTC 6385
45	<i>Salmonella</i> Dublin	1356	D ₁	NCTC 9676
46	<i>Salmonella</i> Alabama	1273	D ₁	NCTC 9868
47	<i>Salmonella</i> Miami	1393	D ₁	NCTC 7112
48	<i>Salmonella</i> Javiana	1379	D ₁	NCTC 6495
49	<i>Salmonella</i> Canastel	1321	D ₁	NCTC 6948
50	<i>Salmonella</i> Antarctica	1282	D ₁	NCTC 11342
51	<i>Salmonella</i> Gallinarum	15831	D ₁	NCTC 10532
52	<i>Salmonella</i> Pullorum	15832	D ₁	NCTC 10706
53	<i>Salmonella</i> Give	1374	E ₁	NCTC 5778
54	<i>Salmonella</i> Muenster	1397	E ₁	NCTC 5780
55	<i>Salmonella</i> Amager	1279	E ₁	NCTC 5782
56	<i>Salmonella</i> London	1387	E ₁	NCTC 5777
57	<i>Salmonella</i> Uganda	5109	E ₁	NCTC 6015
58	<i>Salmonella</i> Shangani	1409	E ₁	NCTC 5784
59	<i>Salmonella</i> Lexington	5110	E ₁	NCTC 6244
60	<i>Salmonella</i> Meleagridis	1392	E ₁	NCTC 6023
61	<i>Salmonella</i> Elisabethville	1366	E ₁	NCTC 8703
62	<i>Salmonella</i> Butantan	1316	E ₁	NCTC 7831
63	<i>Salmonella</i> Clerkenwell	1333	E ₁	NCTC 8462
64	<i>Salmonella</i> Cambridge	1320	E ₂	NCTC 8256
65	<i>Salmonella</i> Senftenbury	9281	E ₄	ATCC 8400
66	<i>Salmonella</i> Krefeld	1383	E ₄	NCTC 9884
67	<i>Salmonella</i> Chittagong	1331	E ₄	NCTC 7374
68	<i>Salmonella</i> Abaetetuba	1268	F	NCTC 8244
69	<i>Salmonella</i> Solt	1569	F	NCTC 6757
70	<i>Salmonella</i> Pretoria	1404	F	NCTC 6234
71	<i>Salmonella</i> Maastricht	9273	F	ATCC 15789
72	<i>Salmonella</i> Rubislaw	1406	F	NCTC 6016
73	<i>Salmonella</i> Aberdeen	1269	F	NCTC 5791
74	<i>Salmonella</i> Clifton	1334	G	NCTC 9599
75	<i>Salmonella</i> Poona	725	G	NCTC 4840
76	<i>Salmonella</i> Albuquerque	1276	H	NCTC 8262
77	<i>Salmonella</i> Sundsvall	1410	H	NCTC 6758
78	<i>Salmonella</i> Ferlac	13737	H	NCTC 10458
79	<i>Salmonella</i> Caracus	1323	H	NCTC 8715
80	<i>Salmonella</i> Brazil	1309	I	NCTC 8446
81	<i>Salmonella</i> Carmel	1324	J	NCTC 9872
82	<i>Salmonella</i> Minnesota	1394	L	NCTC 5800
83	<i>Salmonella</i> Pomona	1403	M	NCTC 6589
84	<i>Salmonella</i> Ezra	1371	M	NCTC 9917
85	<i>Salmonella</i> Urbana	1414	N	NCTC 6248
86	<i>Salmonella</i> Adelaide	9766	O	ATCC 10718
87	<i>Salmonella</i> Alachua	1274	O	NCTC 8261
88	<i>Salmonella</i> Ealing	5449	O	NCTC 11948
89	<i>Salmonella</i> Inverness	9274	P	ATCC 10720
90	<i>Salmonella</i> Emmastad	1368	P	NCTC 9921
91	<i>Salmonella</i> Allandale	1277	R	NCTC 7898
92	<i>Salmonella</i> Duval	1361	R	NCTC 9875
93	<i>Salmonella</i> Bulawayo	1315	R	NCTC 9948
94	<i>Salmonella</i> Waycross	1885	S	NCTC 7401
95	<i>Salmonella</i> Houten	1376	U	NCTC 10401
96	<i>Salmonella</i> Berkeley	1295	U	NCTC 8260
97	<i>Salmonella</i> Clovelly	1335	V	NCTC 10436
98	<i>Salmonella</i> Dugbe	1357	W	NCTC 10347
99	<i>Salmonella</i> Deversoir	1353	W	NCTC 9792
100	<i>Salmonella</i> Phoenix	9280	X	ATCC 29931
101	<i>Salmonella</i> Dahlem	1345	Y	NCTC 9949
102	<i>Salmonella</i> Wassenaar	1415	Z	NCTC 7318

Table 15.2.2.2: Results of Exclusivity Study of the PATHATRIX (1)

Number	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>Listeria monocytene</i> s	6600	NCTC 11994
16	<i>Pasteuralla avium</i>	8389	NCTC 11297
17	<i>Pasteuralla bettii</i>	8391	NCTC 10535
18	<i>Pseudomonas aeruginosa</i>	8299	NCIMB 10753
19	<i>Pseudomonas aeruginosa</i>	7834	NCIMB 10548
20	<i>Pseudomonas fragi</i>	7268	NCTC 10476
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
33	<i>Citrobacter freundii</i>	40	NCTC 9750
34	<i>Proteus vulgaris</i>	1581	Poultry

Comparative Study of PATHATRIX to Standard Methods in a Variety of Food Matrixes (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	20	18	20cfu	20	20
Cooked sliced Ham	2.3cfu	18	19	11cfu	20	20
Milk Powder	2.3cfu	12	4	36cfu	20	20
Orange Juice	9.3cfu	19	18	20cfu	20	20
Black Ground Pepper	4.3cfu	17	15	12cfu	20	20
Chocolate	6.1cfu	20	19	46cfu	20	20
Soft Cheese	7.2cfu	20	19	27cfu	20	20
Carrots	1.1cfu	19	16	36cfu	20	20
Raw Fish	7.5cfu	20	18	20cfu	20	20
Lasagne Ready Meal	4.3cfu	20	19	46cfu	20	20

DISCUSSION OF THE MODIFICATION STUDY 2005 (9)

It is clear from the data presented in the Internal validation studies that the PATHATRIX *Salmonella spp* Pooling system represents a valid method for the detection of *Salmonella* in a range of food matrices.

The PATHATRIX system is fundamentally different from other detection systems in that the entire 250ml sample is analysed rather than looking at 1ml (or less) fractions of enrichment cultures, that other methods typically rely on. Thus with the Pathatrix pooling approach where 1/5th (50ml) of the standard Pathatrix sample size (250ml) is taken the sensitivity of the assay is not compromised. The 50ml sub-sample still represents a sample size that is typically between 50 & 5,000 times larger than other assay systems.

The selective media 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 *Salmonella* 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 20 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 Pooling 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 *Salmonella* testing and could lead to more widespread testing in the industry as the tests become more accessible and significantly 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".

Modification of pooling samples 2005 (9)

Sample Type		MPN Inoculum Level/25g	No +ve Pooled Samples	No +ve Individual Samples	No -ve Individual Samples
Cooked Ham	Pathatrix	1.5cfu	10	10	40
Cooked Ham	FDA/BAM	1.5cfu	10	10	40
Milk Powder	Pathatrix	3.8cfu	10	10	40
Milk Powder	FDA/BAM	3.8cfu	10	10	40
Raw whole Egg	Pathatrix	1.5cfu	10	10	40
Raw whole Egg	FDA/BAM	1.5cfu	10	10	40
Chocolate	Pathatrix	3.8cfu	10	10	40
Chocolate	FDA/BAM	3.8cfu	10	10	40
Frozen prawns	Pathatrix	4.3cfu	10	10	40
Frozen prawns	FDA/BAM	4.3cfu	10	10	40

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