



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

030202

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

PATHATRIX Pooling System for *E. coli* O157 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[®] *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 (October 28, 2018 – December 31, 2019). 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 28, 2018

Date

METHOD AUTHORS

ORIGINAL VALIDATION: Adrian Parton & Michael Scott
MODIFICATION DECEMBER 2012: Kathy Latham
MODIFICATION OCTOBER 2015: V. Zepnickaite, A. Markina, & S. Mantipragada

SUBMITTING COMPANY

Matrix MicroScience Ltd.
 Lynx Business Park
 Fordham Road
 Cambridgeshire, CB8 7NY
 United Kingdom

CURRENT SPONSOR

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

KIT NAME(S)

PATHATRIX Pooling System for *E. coli* O157 spp.

CATALOG NUMBERS

APE500SDP, APE250SDP, APE50SD, PATHATRIXAUTO

INDEPENDENT LABORATORY

Original Validation:
 Campden & Chorleywood Food Research Association
 Chipping Campden
 Gloucestershire, GL55 6LD
 United Kingdom

AOAC EXPERTS AND PEER REVIEWERS

Thomas Hammack^{1,5}, Edward Richter^{2,5}, Don Warburton^{3,5}, Yi Chen^{4,6}
¹ U.S. Food and Drug Administration, College Park, MD 20740
² Richter Consulting, Columbus, OH, USA
³ Health Canada, Canada
⁴ U.S. Food and Drug Administration, College Park, MD
⁵ Original validation and June 2005 Modification
⁶ December 2012 Modification

APPLICABILITY OF METHOD

Target organism – *Escherichia coli* O157 (including H7)

Matrices – (25 g) - raw ground beef

Performance claims - PATHATRIX allows the detection and isolation of *E. coli* O157 from raw ground beef at low levels (1-10cfu/25g).

REFERENCE METHOD

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

ORIGINAL CERTIFICATION DATE

April 09, 2002

CERTIFICATION RENEWAL RECORD

Renewed Annually through December 2019

METHOD MODIFICATION RECORD

1. December 2012
2. October 2015
3. December 2017 Level 1

SUMMARY OF MODIFICATION

1. Location change from Newmarket, UK to Austin, TX.
2. Location change from Austin, TX to Vilnius, Lithuania.
3. Editorial changes for clarity and updates

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 NONE

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 NONE

PRINCIPLE OF THE METHOD (1)

The PATHATRIX *E. coli* O157 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 *E. coli* O157. The whole food sample is homogenised in a non-selective enrichment medium (Buffered Peptone Water) in a sterile stomacher bag containing a mesh liner. The bag is then placed on the PATHATRIX in a thermally controlled pot at 37°C and magnetic beads, coated with antibodies to *E. coli* O157, 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, containing a metal grid, which becomes magnetised and captures the beads onto the grid as they pass. After continuously circulating the sample around the system and through the phase for 180 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 Sorbitol MacConkey agar supplemented with Cefixime and Potassium Tellurite (CT-SMAC). These plates are incubated at 37°C for 16-24h and examined for presumptive colonies of *E. coli* O157. Having used the system to isolate *E. coli* O157, the laboratory would then take a selection of typical colonies and subject these to confirmation of O157 antigen and biochemical identification of *E. coli*.

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 USDA FSIS method for the detection *E. coli* O157 in food matrices and in particular raw ground beef.

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 3 hours.

The CT-SMAC plates showed a significant 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 *E. coli* O157 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 18-24 hours and if serological tests are used e.g. agglutination, results can be confirmed within 24 hours of commencement of the test. This represents a significant improvement by comparison to the USDA FSIS method and other methods which typically require 48 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 *E. coli* O157 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”.

Table 1: Results from Inclusivity Study of 40 strains of *Escherichia coli* O157 (1)

<i>Escherichia coli</i>	Strain (CRA Code)	CFU added to 250ml BPW	PATHATRIX Result	O157 Latex Agglutination Result	API 20E Result
O157:H-	14603	5.1 x 10 ²	+	+	+
O157	13378	2.6 x 10 ²	+	+	+
O157:H7	13543	4.6 x 10 ²	+	+	+
O157	13380	4.9 x 10 ²	+	+	+
O157	13473	3.2 x 10 ²	+	+	+
O157	13471	2.8 x 10 ²	+	+	+
O157	13377	3.7 x 10 ²	+	+	+
O157:H7	13459	4.1 x 10 ²	+	+	+
O157:H7	13539	8.1 x 10 ²	+	+	+
O157	13472	3.5 x 10 ²	+	+	+
O157	13479	4.1 x 10 ²	+	+	+
O157	13618	4.3 x 10 ²	+	+	+
O157	8263	5.4 x 10 ²	+	+	+
O157	13464	8.6 x 10 ²	+	+	+
O157	13465	3.7 x 10 ²	+	+	+
O157	13478	2.1 x 10 ²	+	+	+
O157:H7	14602	1.1 x 10 ³	+	+	+
O157	13466	6.4 x 10 ²	+	+	+
O157:H7	13457	9.5 x 10 ²	+	+	+
O157:H7	13456	1.1 x 10 ³	+	+	+
O157:H7	13544	8.9 x 10 ²	+	+	+
O157:H7	13536	1.3 x 10 ³	+	+	+
O157	13479	1.9 x 10 ³	+	+	+
O157	13379	1.6 x 10 ²	+	+	+
O157:H-	13538	1.8 x 10 ²	+	+	+
O157:H7	13461	2.5 x 10 ²	+	+	+
O157	13467	2.3 x 10 ²	+	+	+
O157:H7	13458	2.3 x 10 ²	+	+	+
O157	13470	2.7 x 10 ²	+	+	+
O157	13477	1.4 x 10 ²	+	+	+
O157	13474	9.5 x 10 ¹	+	+	+
O157	13476	1.7 x 10 ²	+	+	+
O157	13475	2.6 x 10 ²	+	+	+
O157:H7	13460	3.7 x 10 ²	+	+	+
O157	13376	1.4 x 10 ²	+	+	+
O157	13372	3.1 x 10 ²	+	+	+
O157	13374	4.9 x 10 ²	+	+	+
O157	13622	8.2 x 10 ²	+	+	+
O157	13373	5.8 x 10 ²	+	+	+
O157	13375	5.1 x 10 ²	+	+	+
TOTAL			40/40	40/40	40/40

Table 2: Results of Exclusivity Study (Campden CCFRA) of the PATHATRIX E. coli O157 Test (1)

CRA Code		CFU added to 250ml BPW	PATHATRIX Result	O157 Latex Agglutination Result
4110	<i>Bacillus cereus</i>	5.9×10^4	-	NT
5502	<i>Bacillus cereus</i>	5.0×10^2	+	- (Auto)
193	<i>Bacillus cereus</i>	1.5×10^3	-	NT
4112	<i>Bacillus subtilis</i>	5.0×10^2	-	NT
15736	<i>Enterobacter aerogenes</i>	4.4×10^4	-	NT
4108	<i>Enterobacter aerogenes</i>	2.8×10^4	+	- (Auto)
6804	<i>Lactobacillus gasseri</i>	4.7×10^4	-	NT
166	<i>Lactobacillus plantarum</i>	1.8×10^5	+	- (Auto)
7834	<i>Pseudomonas aeruginosa</i>	9.95×10^4	+	-
8299	<i>Pseudomonas aeruginosa</i>	3.2×10^4	-	NT
7268	<i>Pseudomonas fragi</i>	2.1×10^4	-	NT
1356	<i>Salmonella</i> Dublin	3.4×10^4	-	NT
5449	<i>Salmonella</i> Ealing	4.8×10^4	+	- (Auto)
1385	<i>Salmonella livingstone</i>	3.9×10^4	-	NT
1216	<i>Staphylococcus aureus</i>	5.0×10^2	+	- (Auto)
5518	<i>Aeromonas hydrophila</i>	2.8×10^4	-	NT
8392	<i>Edwardsiella tarda</i>	3.4×10^4	-	NT
4113	<i>Enterococcus faecalis</i>	1.6×10^4	-	NT
7057	<i>Erwinia herbico</i>	3.4×10^4	-	NT
8389	<i>Pasteuralla avium</i>	7.5×10^3	-	NT
8391	<i>Pasteuralla bettii</i>	5.1×10^4	-	NT
130	<i>Serratia marcescens</i>	3.7×10^4	-	NT
324	<i>Shigella boydii</i>	9.0×10^3	-	NT
325	<i>Shigella flexneri</i>	1.3×10^4	+	-
326	<i>Shigella sonnei</i>	5.5×10^3	-	NT
4105	<i>Staphylococcus aureus</i>	1.3×10^4	-	NT
11018	<i>Staphylococcus aureus</i>	4.0×10^3	-	NT
7115	<i>Streptococcus agalactiae</i>	5.5×10^3	-	NT
5492	<i>Streptococcus thermophilus</i>	1.1×10^4	-	NT
6351	<i>Vibrio mimicus</i>	2.5×10^3	-	NT
15737	<i>Vibrio parahaemolyticus</i>	5.5×10^3	-	NT
4103	<i>Yersinia enterocolitica</i>	5.5×10^3	-	NT

Table 3: Results of "In-House" (MATRIX) Exclusivity Study of the PATHATRIX *E. coli* O157 Test (1)

Source code	Bacterial Species	Spike Level cfu/250ml	Detected	Confirmed Agglutination NT = Not tested
CRA 5998	<i>Proteus mirabilis</i>	1.8 x 10 ⁴	-ve	NT
Campden cat	<i>Serratia liquifaciens</i>	1.8 x 10 ⁴	-ve	NT
CRA 40	<i>Citrobacter freundii</i>	5.6 x 10 ⁴	-ve	NT
CRA 7460	<i>Eschericia hermanii</i>	1.2 x 10 ⁴	-ve	NT
CRA 8387	<i>Klebsiella aerogenes</i>	2.3 x 10 ⁴	-ve	NT
Severn Trent	<i>Klebsiella pneumoniae 18</i>	7.4 x 10 ⁴	-ve	NT
Severn Trent	<i>Klebsiella oxytoca 17</i>	6.2 x 10 ⁴	-ve	NT
CRA 7469	<i>Pseudomonas alcalifasciens</i>	8.4 x 10 ⁴	-ve	NT
CRA 1891	<i>Eschericia coli</i>	7.2 x 10 ⁴	-ve	NT
Severn Trent	<i>Eschericia coli 01</i>	6.8 x 10 ⁴	-ve	NT
Severn Trent	<i>Eschericia coli 37</i>	4.4 x 10 ⁴	-ve	NT
Severn Trent	<i>Eschericia coli 39</i>	3.3 x 10 ³	-ve	NT
Severn Trent	<i>Eschericia coli 77</i>	2.2 x 10 ⁴	-ve	NT
Severn Trent	<i>Eschericia coli 83</i>	3.6 x 10 ⁴	-ve	NT
Severn Trent	<i>Enterobacter aeglomerans 38</i>	3.3 x 10 ⁴	-ve	NT
Severn Trent	<i>Enterobacter sakazaki 35</i>	1.3 x 10 ⁴	-ve	NT
Severn Trent	<i>Enterobacter cloacae 31</i>	1.1 x 10 ³	-ve	NT
Severn Trent	<i>Enterobacter aerogenes 06</i>	9.6 x 10 ⁴	-ve	NT
CRA 1988	<i>Salmonella heidelberg</i>	7.2 x 10 ⁴	-ve	NT
CRA 1012	<i>Salmonella virchow</i>	3.9 x 10 ³	-ve	NT
CRA 12265	<i>Salmonella gallinarum</i>	1.2 x 10 ⁴	-ve	NT
CRA 1042	<i>Salmonella newport</i>	3.8 x 10 ³	-ve	NT
CRA 1019	<i>Salmonella hadar</i>	4.2 x 10 ⁴	-ve	NT
CRA 1099	<i>Salmonella braenderup</i>	2.6 x 10 ⁴	-ve	NT
CRA 9280	<i>Salmonella phoenix</i>	3.4 x 10 ⁴	-ve	NT
CRA 9275	<i>Salmonella poona</i>	2.9 x 10 ⁴	-ve	NT
CRA 3654	<i>Salmonella enteritidis</i>	6.8 x 10 ⁴	-ve	NT
CRA 1974	<i>Salmonella typhimurium</i>	3.4 x 10 ⁴	-ve	NT

Table 4: Exclusivity Study (Campden CCFRA) for the PATHATRIX *E. coli* O157 Test using Strains of Non O157 Verocytotoxin-producing *Escherichia coli* (1)

<i>Escherichia coli</i>	Isolate Origin	CRA Code	CFU added to 250ml BPW	PATHATRIX Result	O157 Latex Agglutination Result
O26:H11	Clinical	12512	1.24 x 10 ⁵	-	NT
O26:H11	Clinical	14604	6.0 x 10 ⁴	-	NT
O26:H11	Clinical	3490	5.3 x 10 ⁴	-	NT
O91:H NM	Clinical	3475	1.34 x 10 ⁵	-	NT
O91:H NM	Environmental	9910	9.55 x 10 ⁴	-	NT
O91:H NM	Food	12623	1.10 x 10 ⁵	-	NT
O111:H8	Unknown	12753	6.55 x 10 ⁴	-	NT
O111:H8	Unknown	12847	8.4 x 10 ⁴	-	NT
O118:H12	Food	12624	4.95 x 10 ⁴	-	NT
O145:H NM	Environmental	9895	1.23 x 10 ⁵	-	NT

Summary of Results Comparing the Detection of *E. coli* in Raw Ground Beef by the USDA FSIS Method and PATHATRIX *E. coli* O157 Test (1)

Level of Contamination	PATHATRIX O157	USDA FSIS Culture Method
Low (5.25 cells/25g)	13/20	3/20
Intermediate (27.5 cells/25g)	20/20	10/20
Intermediate (23.5 cells/25g)	20/20	11/20
Total	53/60	24/60

REFERENCES CITED

1. Parton, A., & Scott, M., Evaluation of the PATHATRIX *E. coli* Test, AOAC® Performance TestedSM certification number 030202.
2. AOAC Research Institute Validation Outline for PATHATRIX *E. coli* Test, Approved – April 2002.
3. USDA/FSIS Microbiology Laboratory Guidebook 3rd Edition 1998 (Revision # 1; 9-6-99)
4. BAM Bacteriological Analytical Manual 8th Edition 1998
5. CCFRA Microbiological Methods Guidebook 2001