



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
060701

The AOAC Research Institute hereby certifies the method known as:

RAPID'E. coli O157:H7 Agar

Corporate Location
Bio-Rad Laboratories
2000 Alfred Nobel Drive
Hercules, CA 94547
USA

Manufacturing Location
Bio-Rad Laboratories
Route de Cassel
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France

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

Scott Coates, Senior Director
Signature for AOAC Research Institute

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SUBMITTING COMPANYBio-Rad Laboratories
2000 Alfred Nobel Drive
Hercules, CA 94547 USA**METHOD NAME**RAPID'*E. coli* O157:H7 Agar**CATALOG NUMBER**

356-4748

INDEPENDENT LABORATORYrtech Laboratories
4001 Lexington Ave. North
Arden Hills, MN 55112**APPLICABILITY OF METHOD**Target organism – *E. coli* O157:H7.

Matrixes – (25 g) - raw ground beef and fresh spinach

Performance claims - RAPID'*E. coli* O157:H7 is a chromogenic medium for isolation and detection of *E. coli* O157:H7 in food and found to be comparable to the reference methods.**REFERENCE METHODS***Bacteriological Analytical Manual Online* (2006) 8th Ed., U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Chapter 4A. (2)*Microbiology Laboratory Guidebook* (2004) U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Chapter 5.03. (3)**ORIGINAL CERTIFICATION DATE**

June 26, 2007

CERTIFICATION RENEWAL RECORD

Renewed annually through December 2024.

METHOD MODIFICATION RECORD

1. January 2020 Level 1
2. January 2020 Level 1
3. October 2021 Level 1
4. October 2023 Level 1

SUMMARY OF MODIFICATION

1. Editorial/clerical changes and reformatting of insert.
2. Editorial/clerical changes.
3. Editorial changes and addition of user information in French, German, Spanish, Portuguese, and Italian.
4. Editorial/clerical changes.

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NONE**PRINCIPLE OF THE METHOD (1)**

RAPID'*E. coli* O157:H7 is a selective chromogenic plating medium for the rapid isolation and presumptive identification of *E. coli* O157:H7. Characteristic *E. coli* O157:H7 colonies appear as dark blue with a black precipitate around the edge after 24 hours incubation at 37°C. This chromogenic medium differentiates *E. coli* O157:H7, including atypical β-glucuronidase positive or sorbitol positive *E. coli* O157:H7 isolates, from other *E. coli* strains. The cultural properties of the medium are based on a balance of careful selected growth-promoting nutrients and classical selective ingredients. Potassium tellurite and novobiocin are used in addition to enhance the selectivity of the medium by inhibiting interfering flora. The presumptive identification system relies on differential characteristics driven by proprietary chromogenic substrates coupled with both carbohydrate fermentation and pH modification. Background flora, if not inhibited, give rise to red, green or colorless colonies.

DISCUSSION OF THE VALIDATION STUDY (1)

Method comparison studies demonstrated excellent correlation between the RAPID'*E. coli* O157:H7 method and the USDA/FSIS MLG and the FDA/BAM methods. No statistical differences were observed between the methods. However, RAPID'*E. coli* O157:H7 identified more confirmed positive samples than the USDA/FSIS MLG method for ground beef in the internal and independent method comparison studies. The USDA/FSIS MLG reference method specifies that a screening test be performed prior to culture confirmation. RAPID'*E. coli* O157:H7 results matched this screen (lateral flow) test results. To further investigate these discrepant samples from the tests on ground beef, two PCR tests were employed to determine if these samples were "true" or "false" positive samples. These discrepant ground beef samples were confirmed by both PCR tests. An addendum to the USDA/FSIS MLG method recommends the use of PCR for screening. The difficulty with the reference method was attributed to the lawn of bacteria that grew on the Rainbow Agar plates after IMS. Isolated colonies could not be selected from the IMS product, the dilution or the concentrated sample. The reference method specifies a spread plate technique to inoculate plates. This could have also attributed to the inability to select colonies. The RAPID'*E. coli* O157:H7 agar method involves a sample volume of 50µl and plating technique of streaking for isolation. This proved to be a more efficient method for isolating colonies from ground beef samples.

Table 1 – Inclusivity Results (1)

Strain	ID	Source	Result
<i>Escherichia coli</i> O157:H7	Ad485	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad486	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad487	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad488	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad489	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad552	Slaughterhouse environment	+
<i>Escherichia coli</i> O157:H7	Ad553	Slaughterhouse environment	+
<i>Escherichia coli</i> O157:H7	Ad554	Slaughterhouse environment	+
<i>Escherichia coli</i> O157:H7	Ad555	Slaughterhouse environment	+
<i>Escherichia coli</i> O157:H7	Ad556	Sewage purification station	+
<i>Escherichia coli</i> O157:H7	Ad557	Sewage purification station	+
<i>Escherichia coli</i> O157:H7	Ad558	Sewage purification station	+
<i>Escherichia coli</i> O157:H7	Ad559	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad560	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad561	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad562	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad563	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad564	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad565	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad566	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad567	Slaughterhouse environment	+
<i>Escherichia coli</i> O157:H7	Ad568	Slaughterhouse environment	+
<i>Escherichia coli</i> O157:H7	Ad569	Slaughterhouse environment	+
<i>Escherichia coli</i> O157:H7	Ad570	Slaughterhouse environment	+
<i>Escherichia coli</i> O157:H7	Ad571	Stool sample	+
<i>Escherichia coli</i> O157:H7	Ad572	Stool sample	+
<i>Escherichia coli</i> O157:H7	Ad573	Stool sample	+
<i>Escherichia coli</i> O157:H7	Ad574	Stool sample	+
<i>Escherichia coli</i> O157:H7	Ad575	Bovine feces	+
<i>Escherichia coli</i> O157:H7	Ad576	Bovine feces	+
<i>Escherichia coli</i> O157:H7	Ad577	Bovine feces	+
<i>Escherichia coli</i> O157:H7	Ad578	Bovine feces	+
<i>Escherichia coli</i> O157:H7	Ad579	Stool sample	+
<i>Escherichia coli</i> O157:H7	Ad580	Stool sample	+
<i>Escherichia coli</i> O157:H7	Ad581	Stool sample	+
<i>Escherichia coli</i> O157:H7	Ad582	Stool sample	+
<i>Escherichia coli</i> O157:H7	Ad583	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad584	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad585	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad586	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad587	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad588	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad589	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad590	Ground beef	+
<i>Escherichia coli</i> O157:H7	Ad591	Ground beef	+
<i>Escherichia coli</i> O157:H7	ATCC 35150	Human feces	+
<i>Escherichia coli</i> O157:H7	ATCC 43888	Human feces	+
<i>Escherichia coli</i> O157:H7	ATCC 700728	NCTC	+
<i>Escherichia coli</i> O157:H7	EF187	Bovine feces	+
<i>Escherichia coli</i> O157:H7	EF190	Bovine feces	+

Ad = Culture collection, Adria Developpement Laboratory, France

ATCC = American Type Culture Collection, USA

EF = Culture collection, Institute Pasteur Lille, France

NCTC = National Collection of Type Cultures, United Kingdom

Table 2 – Exclusivity Results (1)

Strain	ID	Origin	Result	Colony Color
<i>Aeromonas sobria</i>	ATCC 43979	Fish	-	no growth
<i>Bacillus cereus</i>	ATCC 14579	Milk	-	light blue, atypical
<i>Candida albicans</i>	ATCC 10231	Human isolate	-	red
<i>Citrobacter freundii</i>	Ad25	Frozen spinach	-	blue green
<i>Citrobacter freundii</i>	Ad104	Chopped steak	-	blue gray
<i>Edwardsiella tarda</i>	ATCC 15947	Human feces	-	no growth
<i>Enterobacter aerogenes</i>	ATCC 13048	Sputum	-	white, yellow halo
<i>Enterococcus faecalis</i>	ATCC 29212	Urine	-	no growth
<i>Escherichia coli</i> O3:H2	Ad504	Infant diarrhea	-	blue green
<i>Escherichia coli</i> O6:H6	Ad506	Human feces	-	green gray
<i>Escherichia coli</i> O6:H10	Ad507	Human isolate	-	blue, translucent edge
<i>Escherichia coli</i> O18:K1:H7	Ad522	Neonate spinal fluid	-	blue green, blue halo
<i>Escherichia coli</i> O26:H11	Ad510	Human isolate	-	no growth
<i>Escherichia coli</i> O44:H18	Ad519	Infant stool	-	gray green
<i>Escherichia coli</i> O55:H6	Ad521	Infant isolate	+	dark blue
<i>Escherichia coli</i> O55:H7	Ad518	Human isolate	-	blue green, blue halo
<i>Escherichia coli</i> O78:H11	ATCC 35401	Human feces	-	blue green
<i>Escherichia coli</i> O78:K80:H12	ATCC 43896	Infant diarrhea	-	blue green
<i>Escherichia coli</i> O86:H43	Ad509	Elephant	-	blue, blue halo
<i>Escherichia coli</i> O92:H33	Ad503	human diarrhea	+	dark blue
<i>Escherichia coli</i> O104:H21	Ad516	Infant isolate	-	blue green, blue halo
<i>Escherichia coli</i> O111:H2	Ad513	Infant isolate	-	no growth
<i>Escherichia coli</i> O111:H8	Ad511	Human isolate	-	blue green
<i>Escherichia coli</i> O111:H21	Ad508	Infant isolate	-	no growth
<i>Escherichia coli</i> O127:H6	Ad520	infant diarrhea	-	dark blue, blue halo
<i>Escherichia coli</i> O128:H2	Ad512	Human isolate	-	blue green
<i>Escherichia coli</i> O128:H7	Ad514	Human isolate	-	blue gray, green halo
<i>Escherichia coli</i> O157:H43	Ad517	Pig	-	no growth
<i>Escherichia coli</i> O157:H-	Ad535	Unknown	-	gray green
<i>Escherichia coli</i> O157:H-	Ad536	Unknown	-	gray green
<i>Escherichia coli</i> O157:H7-	Ad524	Dairy environment	-	green turquoise with halo
<i>Escherichia coli</i> O157:H-	Ad525	Bovine feces	-	green turquoise with halo
<i>Escherichia coli</i> O157:H7-	Ad526	Bovine feces	-	green turquoise with halo
<i>Escherichia coli</i> O157:H-	Ad527	Clinical isolate	-	green turquoise with halo
<i>Escherichia vulneris</i>	Ad127	Raw milk	-	green turquoise with halo
<i>Escherichia vulneris</i>	Ad134	Pork liver	-	white, yellow halo
<i>Flavobacterium indologenes</i>	Ad26	Raw egg	-	no growth
<i>Hafnia alvei</i>	Ad88	Pastry	-	blue, translucent edge
<i>Hafnia alvei</i>	Ad167	Sausage	-	red with gray center
<i>Klebsiella pneumonia</i>	ATCC 13883	NCTC	-	yellow
<i>Lactobacillus delbruckii</i>	ATCC 10697	Unknown	-	no growth
<i>Listeria monocytogenes</i>	ATCC 19112	Spinal fluid	-	no growth
<i>Mircococcus luteus</i>	ATCC 10240	Air	-	no growth
<i>Morganella morganii</i>	CIP A236	Pasteur Institute	-	no growth
<i>Proteus vulgaris</i>	Ad43	Ham	-	no growth
<i>Providencia rettgeri</i>	Ad112	Egg whites	-	yellow
<i>Psuedomonas aeruginosa</i>	ATCC 27853	Blood	-	pink
<i>Salmonella landau</i>	Ad499	Pasteur Institute	-	white, yellow halo
<i>Salmonella sternnauze</i>	Ad500	Pasteur Institute	-	white, yellow halo
<i>Salmonella urbana</i>	Ad501	Pasteur Institute	-	white, yellow halo
<i>Salmonella wayne</i>	Ad502	Pasteur Institute	-	white, yellow halo
<i>Serratia proteomaculans</i>	Ad340	Food sample	-	no growth
<i>Shigella sonnei</i>	ATCC 29930	Unknown	-	blue green, pinpoint
<i>Staphylococcus aureus</i>	ATCC 25923	Clinical isolate	-	no growth

Table 3 – Method Comparison Results (1)

Matrix	Inoc Level	MPN/25g	# samples	RAPID'E. coli O157:H7	Reference method	Method	X ²
				# positive	# positive	Agreement	
Raw ground beef (internal)	Control	< 0.075	5	0	0	100%	-
	Low	0.35	20	11	9	90%	0.25
Fresh spinach	Control	< 0.075	5	0	0	100%	-
	Low	3.325	20	9	9	100%	-
Raw ground beef (independent)	Control	< 0.075	5	0	0	100%	-
	Low	0.525	20	11	10	95%	0.00

REFERENCES CITED

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3. *Microbiology Laboratory Guidebook* (2004) U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Chapter 5.03. Online at <http://www.fsis.usda.gov/ophs/Microlab/Mlg5.03.pdf>. Accessed on May 1, 2007.