



Australian Government

Department of Agriculture, Fisheries and Forestry

Approved methods for microbiological testing of meat and meat products

(Amended August 2022)

The following is a list of Department of Agriculture, Fisheries and Forestry (DAFF) approved test methods for meat and meat products. From 31 January 2006 all testing of product relating to export certification, including carcass testing under National Carcass Microbiology Monitoring Program (formerly known as ESAM), must be by one of the methods listed with the modifications and options specified; no other modifications are permitted. Laboratory manuals and protocols must reflect the above and will be subject to audits to ensure compliance.

[Aerobic Plate Count/Total Viable Count \(TVC\)](#)
[Escherichia coli O157:H7](#)
[Shiga-toxin producing Escherichia coli \(STEC\)](#)
[Generic Escherichia coli](#)
[Listeria monocytogenes](#)
[Salmonella](#)

Aerobic Plate Count/Total Viable Count (TVC)

➤ AS 5013.5-2016	Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Colony count at 30°C by the pour plate technique
➤ AOAC 990.12	TVC Petrifilm™
➤ AOAC 2008.10	TEMPO TVC Method: Automated Enumeration of Total Viable Count in Food
➤ AOAC 010404	Compact Dry TC
➤ AOAC 091702 and MicroVal 2015LR52	MC-Media Pad AOAC 091702 is a validation study for incubation of MC-Media Pad at 35 ± 1°C for 24 -48 h and applies only to 50 g raw meats and other foods. MicroVal 2015LR52 is a validation study for 10 g samples, incubated at 30 ± 1°C for 72 h
➤ Other methods	Any method that has been validated by an internationally recognised certification body using ISO 16140 (or equivalent i.e. AOAC guidelines) for the enumeration of total viable count in meat and meat products Note where specific market access requirements exist for the methodology used to determine total viable count these requirements must be met

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Escherichia coli O157:H7

- **ISO 16654:2001** Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157
Note - when analysing frozen or chilled samples, the temperature of broth and samples must be at $41.5 \pm 1^\circ\text{C}$ for a minimum of 6 h and subsequently for a further 12 to 18 hours.
 - **FSIS MLG 5** Detection, isolation, and identification of *Escherichia coli* O157:H7 from meat products
Note the method has been updated changing the initial enrichment to mTSB and the definition of *E. coli* O157:H7. When analysing frozen or chilled samples, the temperature of broth and samples must be at $42 \pm 1^\circ\text{C}$ for a minimum of 15 hours.
 - **FDA BAM Chapter 4A(K)** Diarrheagenic *Escherichia coli* - Enrichment and isolation of *E. coli* Serotype O157:H7 from Foods
With the following modification; must use the IMS option and a sample size of 325 g for ground beef, analysed as five separate 65 g portions
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Rapid methods

Where positive confirmation is required such confirmation must be by ISO 16654:2001, FDA BAM 4A(K) or FSIS MLG 5

Note all modifications/notes listed for each method must be followed

- **FSIS MLG 5A** FSIS procedure for the use of *Escherichia coli* O157:H7 screening tests (15-22h PCR based screening test using DuPont BAX MP)
Note the method has been updated and only mTSB should be used for enrichment of samples and the definition of *E. coli* O157:H7 has been changed. Note - temperature of broth and samples must be at $42 \pm 1^\circ\text{C}$ for a minimum of 15 hours.
 - **AOAC 031002** DuPont Qualicon BAX® System PCR Assay for Real-Time *E. coli* O157:H7
This method is approved for 375 g composite samples in 1.5 L of BAX® System *E. coli* O157:H7 MP medium and incubation for 10-24 h at 42°C . Note – temperature of broth and sample must be at 42°C for a minimum of 10 hours
 - **AOAC 2005.04** Assurance GDS for *Escherichia coli* O157:H7 in Selected Foods and Assurance GDS *E. coli* O157:H7 Tq
This method is approved for using 375 g composite samples in 1.2 L mEHEC medium and incubation for 8-18 h at 42°C . Note – temperature of broth and samples must be at 42°C for a minimum of 8 hours.
 - **AOAC 071001** MicroSEQ^(R) Real-Time PCR System for Detection of *E. coli* O157:H7 in raw ground beef and beef trim
This method is approved for 375 g composite samples in 1.5 L of BPW and incubation for 16 h at 42°C . Note – temperature of broth and sample must be at 42°C for a minimum of 16 hours
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- **AOAC 2017.01** 3M™ Molecular Detection Assay (MDA) 2 – *E. coli* O157 (including H7) Method
 This method is approved for 375 g sample in 975 mL of ISO BPW and incubation for 10 – 18 h at 41 ± 1°C. Note – temperature of broth and sample must be at 41± 1°C for a minimum of 10 hours

 - **AOAC 022002** BACGene *E. coli* O157:H7 Workflow
 This method is approved for 375 g composite samples in 750 mL (1:2) of mTSB and incubation for 10-24 h at 41.5 ± 1°C. Note temperature of broth and sample must be at 41 ± 1°C for a minimum of 10 hours.

 - **AOAC 2019.03** GENE-UP *E. coli* O157:H7 2 (ECO 2)
 This method is approved for 375 g composite samples in 1,125 mL of buffer peptone water and incubation for 10-24 h at 42. ± 1°C. Note temperature of broth and sample must be at 42 ± 1°C for a minimum of 10 hours. The GENE-UP® *E. coli* O157:H7 2 kit (REF 423108) must be used in conjunction with the GENE-UP® Lysis kit (REF 414057).
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The following rapid methods are not to be used for the routine testing of export meat and meat products for *E. coli* O157. They are approved as backup methods for use when PCR methods are temporarily unavailable. They may be used for the testing of product under commercial arrangements when a methodology is not specified under that arrangement:

- **AOAC 996.09** BioControl VIP (8-12 h and 18-28 h options)
 With the following modification: disregard plating steps for confirmation, confirmation must be by ISO 16654:2001, FSIS MLG 5.05 or FDA BAM using the IMS option. 8 h enrichment is carried out in mEHEC media
 Note 18-28h option for 375g samples incubated in 1L of mTSB+n media has been validated and is approved, 8-12h option for 375g in 1L of mEHEC media has been validated and approved. Temperature of broth and samples must be at 36 ±1°C for a minimum of 18 hours (for 18-28 h protocol) or at 42°C for a minimum of 8 hours (8-12 h protocol).

 - **AOAC 2000.13** Reveal (8-hours)
 With the following modification: disregard plating steps for confirmation, confirmation must be by ISO 16654:2001, FSIS MLG 5.04 or FDA BAM using the IMS option.
 Note longer incubation times (12-14h) are required for large samples (375g) diluted less than 1:10 in initial enrichment media i.e. in one litre. Temperature of broth and samples must be at 42°C for a minimum of 8 hours.

 - **AOAC 2000.14** Reveal (20-hours)
 With the following modification: disregard plating steps for confirmation, confirmation must be by ISO 16654:2001, FSIS MLG 5.04 or FDA BAM using the IMS option.

 - **AOAC 070201** Rapid√ for *Escherichia coli* O157 Lateral Flow Assay
 With the following modifications, mTSB+n must be used for selective enrichment and enrichment can only be for 15 to 22h at 42±1°C (as specified in MLG 5.05). The 8 hour enrichment option is not approved. Note – temperature of broth and samples must be at 42 ± 1°C for a minimum of 15 hours.
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Shiga-toxin producing *E. coli* (STEC)

- **FSIS MLG 5B** Detection and isolation of non-O157 Shiga-toxin Producing *Escherichia coli* (STEC) from meat products
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Rapid methods

Where positive confirmation is required such confirmation must be by FSIS MLG 5B

- **AOAC 071301** Assurance GDS® MPX Top 7 STEC for detection of top 7 pathogenic STEC in beef trim
Samples (375 g) are diluted in 1.5 L of pre-warmed (42°C) mEHEC medium. Incubation is carried out for 10 h at 42°C. Note – temperature of broth and samples must be 42 ± 1°C for a minimum of 10 hours
 - **AOAC 091301** DuPont Qualicon BAX® System Real-Time PCR Assays for detection of selected STEC in beef trim
Samples (375 g) are diluted in 1.5 L pre-warmed (45-46°C) Bax® System MP enrichment broth. Samples are incubated at 39-42°C for 12-24 h. Note – temperature of broth and samples must be at 39-42°C for a minimum of 12 hours.
 - **AOAC 0100701** IEH *E. coli* Test System for detection of non-O157 Shiga-toxin producing *E. coli* and *E. coli* O157 in raw ground beef
Samples (375 g) are diluted in 750 mL of pre-warmed IEH enrichment medium. Incubation at 42 ± 1°C for 9-48 hours. Note – temperature of broth and samples must be 42°C for a minimum of 9 hours
 - **AOAC 061602** RapidFinder™ STEC Detection Workflow for detection of top 7 STEC serogroups in beef products.
Samples (375 g) are diluted in 1.0 L of pre-warmed (48°C) Trypticase Soy Broth. Incubation at 42°C for 8 hours. Note – temperature of broth and samples must be at 42°C for a minimum of 8 hours
 - **AOAC 031401** Pall GeneDisc^(R) Plate STEC Top 6 methods for detection of O157 and top 6 non-O157 Shiga toxin producing *E. coli* in raw ground beef and beef trim
Samples (375 g) are diluted in 1.5 L of pre-warmed (41.5 ± 1°C) BPW Broth. Incubation at 41.5 ± 1°C for 10-20 hours. Note – temperature of broth and samples must be at 41.5 ± 1°C for a minimum of 10 hours
 - **AOAC 101502** Assurance GDS® MPX ID for Top 6 STEC
Detection of Top 6 Shiga toxin-producing *E. coli* (O26, O45, O103, O111, O121 and O145) in beef trim as a secondary screening method following a positive result using the Assurance GDS® MPX Top 7 STEC assay (AOAC 071301). All screen positive samples must be confirmed by MLG 5B.
 - **AOAC 022003** BACGene STEC Top 7 Workflow
This method is approved for 375 g composite samples in 750 mL (1:2) of mTSB and incubation for 10-24 h at 41.5 ± 1°C. Note temperature of broth and sample must be at 41 ± 1°C for a minimum of 10 hours.
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➤ AOAC 2020.06	GENE-UP EHEC Detection Method This method is approved for 375 g composite samples in 1,125 mL of buffer peptone water and incubation for 10-24 h at 41.5 ± 1°C. Note temperature of broth and sample must be at 41 ± 1°C for a minimum of 10 hours. This method is approved for STEC confirmatory testings
➤ AOAC 071902	3M Molecular Detection Assay (MDA) 2 – STEC Gene Screen (stx and eae) GENE
➤ AOAC 081901	NeoSeek STEC - This method is approved for STEC confirmatory testings.
➤ AOAC 012102	SureTect™ E. coli O157:H7 and STEC Screening PCR Assay

Generic *Escherichia coli*

➤ AS 5013.15	General guidance for enumeration of presumptive <i>Escherichia coli</i> - Most probable number technique Note this is an update from the 2004 standard, the main change being that the temperature of incubation is now 44±1°C
➤ AOAC 991.14	<i>E. coli</i> PetriFilm™ Butterfields or buffered peptone water or 0.1% Peptone Salt Solution must be used as diluent
➤ AOAC 998.08	<i>E. coli</i> PetriFilm™ Butterfields or buffered peptone water or 0.1% Peptone Salt Solution must be used as diluent
➤ AOAC 2005.03	SimPlate® Colour Indicator: Detection and Quantitation of Coliforms and <i>E. coli</i> in foods
➤ AOAC 2009.02	Tempo® EC AFNOR Bio 12/13 – 02/05 for testing of generic <i>E. coli</i>
➤ AOAC 110402	Compact Dry EC
➤ AOAC 070901 and MicroVal 2017LR71	MC-Media Pad <i>E. coli</i> AOAC 070901 is a validation study that applies only to 50 g raw meats and other foods. MicroVal 2017LR71 is a validation study that applies to sample diluted 1:10. This method is approved for 50 g sample in 450 mL diluent
➤ AOAC 2018.13	3M Petrifilm Rapid <i>E. coli</i>/Coliform Count Plate

Listeria monocytogenes

➤ AS 5013.24.1	Food and animal feeding stuffs – Horizontal method for the detection and enumeration of <i>Listeria monocytogenes</i>. Detection method
➤ FSIS MLG 8	Isolation and identification of <i>Listeria monocytogenes</i> from red meat, poultry, egg, and environmental samples Note alternative secondary enrichment has been included

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Rapid methods

Where positive confirmation is required such confirmation must be by Australian Standard AS 5013.24.1 or FSIS MLG 8

Note the following bio-chemical test systems can be used for confirmation for all methods MICRO-ID® *Listeria* or API®-*Listeria* or VITE® 2 Compact, β-lysin. CAMP factor discs (Remel #21-120, or equivalent) can be used instead of the traditional CAMP test procedure

➤ FDA BAM Ch 10	Detection and Enumeration of <i>Listeria monocytogenes</i> in Foods
➤ FSIS MLG 8A	FSIS procedure for the use of <i>Listeria monocytogenes</i> BAX screening test Note the method has been updated to include testing of liquid egg products
➤ AOAC 2003.12	Automated BAX System for Detection of <i>Listeria monocytogenes</i> in Foods
➤ AOAC 999.06 & AOAC 2004.06	VIDAS LIS Assay for <i>Listeria</i> in Food
➤ AOAC 997.03	BioControl <i>Listeria</i> Visual Immunoprecipitate (VIP) Assay
➤ AOAC 996.14	BioControl Assurance <i>Listeria</i> Immunoassay
➤ AOAC 070401	Foodproof <i>Listeria monocytogenes</i> Detection Kit, 5'Nuclease and Hybridization Probes
➤ AOAC 031204	Pall GeneDisc [®] method for the detection of <i>Listeria monocytogenes</i> in food and environmental samples
➤ AOAC 011002	MicroSEQ [®] Real-Time PCR System for Detection of <i>Listeria monocytogenes</i> in food
➤ NF BIO 12/33-05/12	VIDAS UP <i>Listeria</i> method (VIDAS LPT)
➤ NF SOL 37/02-06/13	Solus <i>Listeria</i> ELISA
➤ AOAC 071304	Thermo Scientific™ SureTect™ <i>Listeria</i> species PCR Assay - AOAC 071304 (AFNOR UNI 03/09 - 11/13)
➤ AOAC 121402	DuPont™ BAX® System Real-Time PCR Assay for <i>Listeria monocytogenes</i> - AOAC 121402
➤ AOAC 2016.08	3M™ Molecular Detection Assay (MDA) 2 – <i>Listeria monocytogenes</i> Method
➤ AOAC 061703	BACGene <i>Listeria monocytogenes</i>

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Salmonella

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- **AS 5013.10** Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp
- The following options are required: Second agar choice must be capable of detecting H₂S negative *Salmonella* (internationally validated *Salmonella* media e.g. BGA, BGS, Rambach, ChromAgar)
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- **FSIS MLG 4** Isolation and identification of *Salmonella* from meat, poultry and egg products
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Rapid methods

When positive confirmation is required such confirmation must be by Australian Standard AS 5013.10-2009 or FSIS MLG 4.04

- **FSIS MLG 4C** FSIS procedure for the use of the BAX system PCR assay for screening *Salmonella* in raw meat, carcass sponge samples, whole bird rinses, ready-to-eat meat and poultry products and pasteurised egg products
- Note procedure to follow when a PCR indeterminate or signal-error occurs has been updated
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- **AOAC 2003.09** BAX Automated System for Screening *Salmonella* in foods
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- **AOAC 992.11** BioControl Assurance EIA
- With instructions specified for pre-enrichment in table 999.08 C (ie use of BPW + novobiocin)
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- **AOAC 999.08** BioControl Assurance Gold
- pre-enrichment with BPW + novobiocin as per instructions
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- **AOAC 999.09** BioControl VIP
- With instructions specified for pre-enrichment in table 999.08 C (ie use of BPW + novobiocin)
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- **AOAC 996.08** VIDAS *Salmonella* (SLM) Assay
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- **AFNOR BIO 12/16-09/05** VIDAS EASY *Salmonella* method – AFNOR
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- **AOAC 071101** VIDAS UP *Salmonella* method (VIDAS SPT)
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- **AOAC 2001.09** VIDAS Immuno Concentration *Salmonella* (ICS)
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- **AOAC 2009.03** Assurance GDS™ *Salmonella* method for foods and Assurance GDS *Salmonella* Tq method
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- **AOAC 100701** IEH PCR assay for detection of *Salmonella* in carcass and environmental sponges or swabs
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- **AOAC 120301** foodproof *Salmonella* Detection Kit, 5'Nuclease and Hybridization Probes
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- **AOAC 100201** DuPont Qualicon BAX^(R) System *Salmonella*2 PcR Assay
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➤ AOAC 2013.02	DuPont Qualicon BAX ^(R) System real-time PCR assay for <i>Salmonella</i>
➤ AOAC 031001	MicroSEQ ^(R) Real-Time PCR System for Detection of <i>Salmonella</i> in food
➤ AOAC 050602	Assurance GDS for <i>Salmonella</i>
➤ AOAC 2014.01	3M Petrifilm <i>Salmonella</i> Express
➤ AOAC 011404	Veriflow TM <i>Salmonella</i> Species (SS)
➤ NF SOL 37/01-06/13	Solus <i>Salmonella</i> ELISA
➤ AOAC 051303	Thermo Scientific TM SureTect TM <i>Salmonella</i> spp PCR Assay - AOAC 051303 (AFNOR UNI 03/07 – 11/13)
➤ AOAC 2016.01	3M TM Molecular Detection Assay (MDA) 2 – <i>Salmonella</i> Method
➤ AOAC 121501	BACGene <i>Salmonella</i> spp.

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