

**Department of Agriculture, Fisheries and Forestry** Australian Quarantine and Inspection Service

AQIS MEAT NOTICE			
NUMBER: 2010/03 NSFS Ref 16		<i>Escherichia coli</i> O157:H7 testing of raw ground beef components destined for export to the US and US Territories	
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Distribution		n Categories	Last notice on this issue
<ul> <li>Central &amp; Regional Office</li> <li>OIC Inspection Staff Meat Establishments</li> </ul>		☑ Managers, Export Meat Establishments	2008/05
<b>IMPLEMENTATION SCHEDULE</b> (to be completed by the On Plant Supervisor on the AQIS file copy)			
Date Received:Date Discussed With Management:			
Initial Implementation Date:Date Completed:			
Management Representative Initials:   AQIS OPS Initials:			

# PURPOSE

To advise establishments of revised requirements for *Escherichia coli* O157:H7 testing in raw ground beef components destined for export to the United States (US) and its Territories.

# SCOPE

All export registered, US listed meat establishments that produce and / or store raw ground beef components and all laboratories undertaking testing of such products for *E. coli* O157:H7. Raw ground beef components intended for grinding and export to the USA must be tested for *E coli* O157:H7 under the requirements of this notice prior to export.

This Notice supersedes AQIS Meat Notice 2008/05.

# BACKGROUND

Studies have shown that detection of *E. coli* O157:H7 on carcases and derived meat is most likely associated with rare and random occurrences of individual cattle that are highly contaminated at the time of slaughter. When *E. coli* 0157:H7 is detected within the lot, contamination does not appear to be widespread. Minimisation of hide contamination and good dressing practices are possibly the most effective way of reducing the likelihood of contamination of meat. The Australian industry currently samples and tests (under AQIS supervision) trim and manufacturing beef exported to the US.

Recent events in the US have highlighted a need for modification of Australia's approach in order to limit the impact of a positive test for *E. coli* O157:H7 at port of entry (POE) testing. The US Food Safety and Inspection Service (FSIS) have advised that in the event of a positive test for *E. coli* O157:H7 they will require the recall of all products from the "sampled lot" and that this lot will be treated as adulterated. If the "sampled lot" is split across containers the recall will apply to each part of that lot and companies will need to maintain control until the totality of the lot has cleared US import inspection. If the entire sampled lot is present in the container tested at port of entry then action taken by FSIS will be limited to that sampled lot.

This notice is based upon an equivalence arrangement with FSIS. Equivalence is based on applying an AQIS monitored "sampled lot" test and hold program for raw ground beef components destined for export to the US, and verification testing by AQIS.

# 1. Summary

Establishments will test all raw ground beef components (as defined in this notice) that are eligible for export to the US for the presence of *E. coli* O157:H7, unless the establishment can demonstrate that such products are not destined for the US or not intended for grinding. Samples will be analysed in AQIS approved laboratories using AQIS approved methods and all results will be reported directly to AQIS. All test results will be reported to the AQIS National Microbiological Database. Sampled lots with confirmed positive detections of *E. coli* O157:H7 will be subject to AQIS control and disposition. AQIS will verify *E. coli* O157:H7 testing programs as detailed in this meat notice. AQIS will also undertake its own verification testing for *E. coli* O157:H7 on raw ground beef components destined for the US at a minimum frequency of once per quarter.

All sampled lots will be held or controlled by the establishment until the test result for the lot has been obtained and is negative for *E. coli* O157:H7. If *E. coli* O157:H7 is confirmed as being present in the lot, the affected product will not be exported to the US and disposition will be determined by AQIS.

This Notice describes what is required to be included in the establishment's Approved Arrangement (AA).

# 2. Definitions

# 2.1 Raw Ground Beef Components

Raw ground beef components include beef and veal bulk packed manufacturing trimmings and other beef and veal components such as primal cuts, sub primal cuts, head meat, cheek meat, oesophagus meat, heart, and advanced meat recovery (AMR) product intended for grinding in the USA.

Beef trim may also include product from veal carcases that are packed and labelled as beef under the Australian Meat Classification System Manual 1 (Veal carcase >70kg, Hot Standard Carcase Weight). Irrespective of veal carcase weight, veal must be tested if it is destined for grinding in the US.

# 2.2 Sampled lot

For the purposes of testing raw ground beef components, a sampled lot is defined as all those cartons, packages, or containers of beef components determined by the establishment, based

on the implementation of a robust sampling program (as defined in this Notice) and not exceeding 700 cartons (a container equivalent). All cartons in a sampled lot must be sourced from a single establishment but may contain product across a range of production dates. Cartons must be clearly identifiable with a unique identifier for each sampled lot and the lot must be defined in the establishment's Approved Arrangement.

# 2.3 E. coli O157:H7

For the purpose of this Notice, *E. coli* O157:H7 is an enterohaemorrhagic, Shiga toxin producing strain of *E. coli*.

It is defined as an organism which gives a positive test for detection of *E. coli* serotype O157 from an enrichment broth, and a pure isolate from the enrichment broth is confirmed with biochemical, and serological tests as *E. coli* O157 and the production of Shiga toxin(s) and/or presence of one or more of the Shiga toxin genes is demonstrated by an approved laboratory or by confirming the presence of the H7 antigen or gene using serological and/or molecular test.

**NOTE**: The following terminology is used by FSIS and this document: Presumptive positive = positive on a screening test Confirmed isolate = serologically positive O157 that contains stx gene/s or produces Shiga toxin or is H7 positive.

# 3. Taking samples for testing

# 3.1 Lot identification

Samples collected from cartons need to be clearly and uniquely identifiable to the sampled lots from which they are drawn for purposes of traceability, retention and potential recall. There is no requirement for individual cartons from which samples are collected to be identified. Sampled lots defined at the time of consolidation prior to containerisation should be identified using unique shipping marks prior to the sampling process being undertaken.

# **3.2 Selection of Samples**

- Samples should be collected from a lot based on the establishment's sampling program. The establishment must ensure that the full range of raw ground beef components destined for grinding in the US have an equal opportunity to be sampled in the lot.
  - Samples must not be collected over a small portion of the lot.
- It is acceptable for selected cartons to be removed from production and stored under appropriate conditions for later sampling.
- Samples collected over a number of production days must be stored frozen until pooled for analysis.
- Where samples are collected at independent cold stores each source establishment must be sampled separately and in lots as required under this notice.

**NOTE**: If the lot is less than 12 cartons all cartons must be sampled and the total number of sub-samples collected from these cartons must be at least 60 i.e. N60

# 3.3 Removing a sample

A minimum of 5 small pieces, surface slices or small 'grab' samples, of approximately 5-10g are selected from a minimum of 12 cartons representing the lot. The total number of pieces

sampled per lot must be at least 60 (i.e. N60 method). The total sample weight must be at least 375 g. The sample must be collected using sterile instruments under sanitary conditions.

# **3.4 Lot retention**

The sampled lot must be under the control of the establishment (i.e. able to be recalled) whilst waiting for the results of the screening test or confirmatory test. Product may be able to be stored in another registered establishment or even be containerised as long as it can be shown to still be under the control of the establishment. In the event that a lot is confirmed as being positive for *E. coli* O157:H7, the lot must be retained and placed under AQIS control until disposition (see section 5).

# **3.5 Sample Labelling**

The sample is labelled or bar coded to ensure the following information is available on the laboratory report:

- Establishment number (if samples are to be sent to an external laboratory)
- Date and time of sampling
- Packing line (if applicable)
- Identification of the sampled lot

# **3.6 Sample Storage**

When testing is not going to commence immediately samples must be transferred to active refrigeration ( $0^{\circ}C$  to  $\leq 7^{\circ}C$ ) without delay.

When sample analysis cannot commence on or before the second day following sample collection, samples must be frozen at the establishment and held frozen until transported to the laboratory for testing. Samples collected from frozen cartons must be kept frozen until dispatched to the laboratory for testing. Any questions relating to sample storage should be forwarded to AQIS for clarification.

#### NOTE Samples from frozen cartons cannot be re-frozen once thawed

The temperature of samples on arrival at the laboratory must be measured and the result included on the report to the establishment. The temperature of samples during transport and on arrival at the laboratory must be  $\leq 7^{\circ}C$ . The laboratory can commence analysis of samples that arrive at temperatures  $>7^{\circ}C$ ; however, AQIS and the establishment must be notified immediately so that a decision on the validity of the results can be made. Where samples arrive at the laboratory at temperatures  $>7^{\circ}C$  the establishment must take corrective action to ensure that the temperature of future samples are  $\leq 7^{\circ}C$  on arrival at the laboratory.

# 3.7 Sample dispatch to an AQIS approved laboratory

Where samples are to be transferred to an off-site laboratory for analysis standard procedures for transport must be followed. Samples must be sent to an AQIS approved laboratory for testing using an AQIS approved method.

# 4. Sample Testing

# 4.1 Approved Laboratory

All laboratories used for the analysis of export samples for *E. coli* O157:H7 must be approved by AQIS. Laboratories undertaking screening tests for *E. coli* O157:H7 can be either company owned or commercial laboratories. Only AQIS nominated laboratories can be used for the analysis of verification samples collected by AQIS or for confirmation of presumptive positive samples.

**NOTE**: A list of approved laboratories is maintained at the AQIS export meat program website. Requirements for Approved laboratories can also be found on the AQIS website.

# 4.2 Testing methodology

All testing must be carried out using AQIS approved methods.

All samples with a positive screening test must either undergo further testing for confirmation of *E. coli* O157:H7 by an AQIS approved independent laboratory or the sampled lot treated as a confirmed positive.

**NOTE**: A list of approved test methods can be accessed at the AQIS export meat program website<sup>1</sup>

# 4.3 Sample preparation and testing

Temperature of samples on arrival at the laboratory must be recorded as detailed in 3.6. Samples tested on-site must be analysed as soon as possible after collection, in such cases the temperature of the sample need not be measured prior to testing. If there is a delay in testing samples must be stored at  $\leq$ 7°C. Frozen samples should be thawed in the laboratory at 18-27°C for up to 3 hours before commencing the test (Australian Standard AS 5013.11.2-2006).

Frozen samples must be held frozen until transported to the laboratory. Frozen samples may thaw during transit; this is acceptable as long as they are not re-frozen at the testing laboratory.

Confirmed positive isolates from AQIS verification samples will be typed using pulsed field gel electrophoresis (PFGE), with the elucidated patterns forwarded to FSIS for inclusion in their national database.

# **4.4 Reporting of results**

All presumptive and confirmed positive test results must be reported by the testing laboratory, immediately they become available, by email or fax, directly to the AQIS OPS or ATM and simultaneously to plant management. All results must be recorded by the establishment and the records reviewed by the AQIS OPS or ATM routinely. Laboratories must also ensure that the AQIS approved method used for sample analysis is included on the report (eg AOAC number). It is not acceptable to reference only the laboratory's in-house method number.

# 4.5 Entering data into the National Microbiological database

All test results (negative, presumptive or confirmed positive) must be entered into the National Microbiological Database on a weekly basis, by the AQIS officer as per the AQIS work instruction.

<sup>&</sup>lt;sup>1</sup>http://www.daffa.gov.au/\_\_data/assets/pdf\_file/0020/217721/aqis\_approved\_methods\_microbiological \_testing\_meat.pdf

The unique identifier for the sampled lot must be recorded with the test results. The sampled lot must be able to be traced through the unique identifier.

The name of the testing laboratory and the test method must also be recorded.

# 4.6 Request for Permit (RFP) Validation Rules

The RFP authoriser is required to have knowledge that a lot has been tested and cleared prior to validating the RFP. They are then required to enter a declaration that "the beef intended for grinding in this shipment has been tested negative for *E. coli* O157:H7".

The default endorsement for beef intended for grinding is:

"All lots of raw beef components intended for grinding described by this certificate have tested negative for E. coli O157:H7 in accordance with the AQIS protocol."

Where entire sample lots are confined to one container an optional endorsement can be chosen:

"The raw beef components intended for grinding described by this certificate have tested negative for E. coli O157:H7 in accordance with the AQIS protocol and represent an entire sampled lot which has been subject to N=60 sampling."

The latter statement is only available where the entire sample lot has a unique shipping mark and is confined to a single health certificate.

- It is permissible for multiple entire sample lots each with unique shipping marks to be shipped in one container and covered by a single health certificate.
- The existing rule for a single unique container per RFP still applies.

# 5. Measures in response to a positive result

# **5.1 Product Disposition**

The following diagram shows the decision process for lots that have been tested under the requirements outlined in this Notice.



Disposition of product is to be made in accordance with the provisions specified in the establishment's Approved Arrangement. Disposition of a positive lot must be by heat treatment or other processes that have been validated to achieve a 5D (5-log or 100,000 fold) reduction in *E. coli*. Treatment must be at an establishment with an approved process. Disposition by condemnation must be through rendering or disposal at landfill.

Establishments must make and retain records of screening tests, confirmatory tests and dispositions. Where product has been transferred for treatment or condemnation the records should show that responsibility for the disposition of that product has been transferred to the receiving establishment (e.g. Official Meat Transfer Certificate duplicates).

AQIS authorise the disposition of confirmed product positive lots of raw ground beef components to establishments that can demonstrate they can process product so as to achieve a 5D reduction in *E. coli*. The receiving establishment must have a program approved by the relevant controlling authority detailing how the affected lot will be handled during receival, storage and processing. The process used to eliminate *E. coli* O157:H7 must be validated as being able to deliver a 5D reduction in *E. coli*. Thermal processes must be equivalent to  $\geq 69.4^{\circ}C$  (157°F) for 10 seconds.

If confirmatory testing finds the sample negative for the presence of *E. coli* O157:H7 the lot is released.

## 5.2 Investigations on positive findings

As shown on the flow diagram at 5.1 above, a positive finding and disposition applies to the following lots:

- a) The screening test is positive and the establishment chooses not to proceed with confirmatory testing, or
- b) The confirmatory test is positive.

Establishment management investigations on positive findings should include:

- a) As a guide to assist with the investigation and reporting, the establishment may need to collect, collate and assess appropriate records/information relating to the slaughter (and boning periods) for the positive lot, for example (note this list is not exhaustive):
  - MHA records,
  - process & product monitoring/verification records,
  - pre-shipment reviews,
  - ESAM test results,
  - any other test results (ie. O157:H7 testing),
  - pre-op and operational monitoring,
  - HACCP records,
  - cleanliness, type and source of animals slaughtered,
  - National Establishment Verification System (NEVS) records as appropriate, etc.
- b) A HACCP plan reassessment may or may not be warranted (depending on the outcome of the investigation above).
- c) The investigation must be undertaken in consultation with the On-plant Supervisor (OPS) of the establishment, or the originating establishment where sampling is carried out at cold stores, and Area Technical Manager (ATM) and a written report with any supporting documents must be kept on AQIS file. Any corrective and preventive actions taken must be assessed and verified by the OPS as effective and sustainable.

# 6. Amendment of the Approved Arrangement

Establishments need to amend their Approved Arrangement to reflect their practices in regards to *E. coli* O157:H7 sample selection, testing, transport, and corrective and preventive action.

# 7. AQIS Verification Activities

#### 7.1 Verification of establishment process (Check-the-Checker)

AQIS on plant staff shall verify the establishment process of sample selection and collection in the same way as is currently conducted for the national *E. coli* and *Salmonella* Monitoring (ESAM) program. This verification activity must be conducted once weekly per shift and results entered in NEVS Form 1. The AQIS OPS must also verify that approved laboratories are used for the analysis of samples and that the methods used are also approved by AQIS.

Where an establishment has chosen to identify "sampled lots" at the point of consolidation for loading and is conducting sampling at an independent establishment e.g. a cold store an AQIS authorised officer must verify the establishment process of sample selection and collection. Where an establishment elects to conduct testing <u>only</u> at independent cold stores, testing will be subject to weekly verification by AQIS officers.

Any deficiencies in technique shall be documented and a Corrective Action Request (CAR) raised to ensure that the establishment follows up on the deficiencies with appropriate corrective and preventive action. An inadequate response to the CAR will be followed up as per current instructions to AQIS staff.

All laboratories approved for testing of trim and ground beef components for *E. coli* O157:H7 will be audited annually by AQIS (or an independent third party nominated by AQIS) for compliance to this Notice and for competency in the analysis of samples for *E. coli* O157:H7. All approved laboratories must be enrolled in a recognised proficiency program for *E. coli* O157:H7 and participate in proficiency rounds at least twice a year.

# 7.2 Verification testing

AQIS central office will notify AQIS on-site staff that a verification sample is to be collected and submitted to an independent AQIS approved laboratory for testing for *E. coli* O157:H7. The notification will contain a list of approved laboratories that the establishment can use for the analysis of samples. Samples are to be analysed as per the instructions in the notification. Verification samples cannot be analysed in an on-plant or company owned laboratory.

All establishments with US listing and producing raw ground beef components will be scheduled for verification testing at least quarterly and at most twice monthly. The frequency of testing will be determined by AQIS based on the establishment's compliance history.

To collect verification samples, AQIS staff will notify the establishment that a sample is required. Notification will be given at least one week preceding the collection of the verification samples. The AQIS OPS will directly supervise the establishment employee selecting the test cartons and collecting sufficient samples for analysis (minimum 375g). The establishment may elect to collect a duplicate sample for its own testing, however this is not mandatory. AQIS will take charge of the verification sample. The sample will be identified as detailed in 3.5. The AQIS officer overseeing the process will place the sample into an AQIS tamper evident bag and forward the sample to a nominated AQIS approved laboratory. If storage is required on site samples will be stored as detailed in 3.6 and under conditions of security.

Establishment management should enter into a commercial arrangement with one of the nominated laboratories prior to verification testing to ensure that there is no delay in the analysis of samples and reporting of results. Results of verification testing must be reported to AQIS at the same time that they are reported to the establishment.

**NOTE**: Detailed instructions for handling, storage and transport will be provided with the sample kits distributed to AQIS on-plant staff. As verification sampling is a market access requirement, all transportation and testing costs will be carried by the establishment.

# 7.3 Microbiological independence

In the event the FSIS advise AQIS that there has been a confirmed positive for *E. coli O157:H7* from an establishment at US port of entry, AQIS will immediately request the establishment to provide complete details of the cartons in the sampled lot implicated.

AQIS will then verify that the Establishment has maintained the "Microbiological independence of this sampled lot" from other lots sampled of raw ground beef components which have been exported to the United States based on the following:

a) That other sampled lots of raw ground beef components produced on the same production day(s) have been tested for *E. coli O157:H7* in accordance with this AQIS Meat Notice and on this basis have been certified for export to the United States.

b) Given the legislative requirements for establishments to operate in accordance with their Approved Arrangements, AQIS will confirm there is no evidence of systemic failure that would suggest a higher than usual risk of a similar lot being contaminated on an on-going basis.

AQIS will consider the "microbiological independence" of the sampled lot on the basis of clear separation of the product in the lot from any other lot or lots.

AQIS will provide the outcomes of the investigation to FSIS in accordance with its equivalence agreement.

# 8. Responsibilities

# 8.1 Establishment Management

- Amend their approved arrangements to reflect their *E. coli* O157:H7 testing program as per this notice.
- Ensure that the establishment's Approved Arrangement identifies where sampling will occur and identifies the position/person responsible for sampling.
- Ensure that the persons responsible for collecting and dispatching samples are fully trained.
- Support AQIS check-the-checker and verification activities.
- Endorse Meat Transfer Certificates (MTCs) with a US eligibility statement.
  - Endorse with 'US eligible subject to O157 testing' if testing has not occurred or results not received.
  - Even if the MTCs are endorsed with 'US eligible subject to O157 testing' the establishment must ensure that they maintain control of the product until a definitive result is obtained.
- Maintain control over sampled lots that are positive on a screening test (ie presumptive positives).
- All results must be advised to the AQIS on-plant supervisor. Where testing is done at establishments without permanent AQIS presence the OPS relevant to the source establishment and the cold store's ATM must be advised.
  - Presumptive positives and confirmed positives must be reported immediately.
- Product from a sampled lot that is positive for *E.coli* O157:H7 must be disposed of in accordance with this notice and with the establishment's approved arrangement.
- Enter into an arrangement with a nominated off-site laboratory for testing of verification samples.

**NOTE**: *These responsibilities also apply to independent cold stores where lots are being consolidated and sampled.* 

## 8.2 AQIS OPS

- Consider any amendments made to the Approved Arrangement and make the appropriate recommendation to the ATM.
- Ensure that weekly and other verification activities are conducted as required by this notice. (note: OPS's should verify reporting of results at on-plant laboratories)
- Enter data into the national microbiological database.
- Verify that the establishment retains lots that are confirmed positive or are presumptive positives with the establishment choosing not to conduct confirmatory testing or are implicated by a positive result and inform the ATM.

# 8.3 AQIS ATM

- Consider any amendment to the Approved Arrangement and approve if appropriate.
- Verify, at the frequency dictated by the National Establishment Verification System, that the establishment is complying with the Approved Arrangement.
- Verify that the AQIS OPS is performing the required check-the-checker and verification activities and report the results as appropriate.
- Where there is concern or conjecture on the acceptability of an Establishment's Approved Arrangement, the matter will be referred to AQIS Central Office for resolution.
- Supervise verification activities at independent cold stores as required by this notice.

#### **8.4 AQIS Central Office**

• Liaise with AQIS field staff regarding the implementation of this notice.

#### 8.5 Laboratories

- Ensure compliance with obligations under this notice and the 'General Requirements for Testing Laboratories' document' (posted on the AQIS web site).
- Laboratories are required to report results directly to AQIS at the same time that they are reported to the establishment as a part of their approval. This includes results from AQIS verification testing.

Carol Sheridan National Manager Export Meat

# ANNEX 1 Testing for *E. coli* O157:H7

# A1.1 Sample arrival and storage

The temperature of samples on arrival at off-site laboratories must be measured and the result included on the report to the establishment. The temperature of samples during transport and on arrival at the laboratory must be  $\leq 7^{\circ}C$ . The laboratory can commence analysis of samples that arrive at temperatures  $>7^{\circ}C$ ; however, AQIS and the establishment must be notified immediately so that a decision on the validity of the results can be made. Where samples arrive at the laboratory at temperatures  $>7^{\circ}C$  the establishment must take corrective action to ensure that the temperature of future samples are  $\leq 7^{\circ}C$  on arrival at the laboratory

Samples tested on-site must be analysed as soon as possible after collection, in such cases the temperature of the sample need not be measured prior to testing. If there is a delay in testing samples must be stored at  $\leq$ 7°C.

Frozen samples must be held frozen until transported to the laboratory. Frozen samples may thaw during transit; this is acceptable as long as they are not re-frozen at the testing laboratory. Frozen samples should be thawed in the laboratory at 18-27°C for up to 3 hours before commencing the test. (Australian Standard AS 5013.11.2-2006 Food microbiology Method 11.2: Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Specific rules for the preparation of meat and meat products).

# A1.2 Testing

There are two distinct stages of testing:

- Screening, which generally requires basic skills
- Confirmation, for which specialised tests and skills are required

# A1.2.1 Screening

Screening tests are usually validated for the testing of 25g samples diluted 1:10 with enrichment broth. If compositing samples or using lower dilutions then the test method used must be validated for the sample size and/or the initial dilution used. AQIS must approve any modification to methods prior to their use for the analysis of samples

The following general procedure should be followed for the screening of samples. Weigh out the sample (composite samples can be used if validated for the method). Frozen samples should be thawed as detailed in A1.1. Place the sample into a stomacher bag or blender; add the required amount of enrichment broth for the weight of sample being tested and stomach or blend for one to two minute. If a stomacher or blender is not available then the sample can be palpated by hand for two minutes. Incubate at the temperature and for the time specified in the test method. Analyse the sample as per AQIS requirements (generally as specified in the relevant AOAC publication) or if not specified as per the manufacturer's recommendations. If there is any doubts consult with AQIS before testing samples.

**NOTE**: A list of approved test methods can be accessed at the AQIS export meat program website<sup>2</sup>

<sup>&</sup>lt;sup>2</sup>http://www.daffa.gov.au/\_\_data/assets/pdf\_file/0020/217721/aqis\_approved\_methods\_microbiological \_testing\_meat.pdf

**NOTE**: Some approved methods have variations required by AQIS. The laboratory should check to make sure that the correct methodology is being followed.

All samples with a positive screening test must either undergo further testing for confirmation of *E. coli* O157:H7 by an AQIS approved laboratory or the product treated as a confirmed positive.

**NOTE**: A list of approved laboratories will be maintained at the AQIS export meat program website.<sup>3</sup>

All confirmation of presumptive positive samples must be carried out on a sample of the enrichment broth. When transporting presumptive positive enrichment broths or other presumptive positive samples the laboratory should consult the appropriate regulations for the transportation of infectious substances (see Australian Government, Department of Transport and Regional Services website<sup>4</sup>).

# A1.2.2 Confirmatory testing

Confirmatory tests must be performed in an AQIS approved laboratory.

**NOTE**: AQIS has approved certain methods as suitable for the isolation of E. coli O157 from enrichment broths. Confirmation must include an immunomagnetic separation (IMS) step.

A confirmed positive is reported when at least one colony conforms to the definition of *E. coli* O157:H7.

#### A1.2.3 Typing of *E. coli* O157:H7 isolates

Confirmed positive isolates from AQIS verification samples must be forwarded to an AQIS laboratory approved for the purposes of typing *E. coli* O157:H7 by pulsed field gel electrophoresis (PFGE), with the elucidated patterns forwarded to FSIS.

All confirmed positive test results must be reported by the testing laboratory directly to the AQIS OPS and simultaneously to plant management.

<sup>&</sup>lt;sup>3</sup> http://www.daffa.gov.au/\_\_data/assets/pdf\_file/0008/129662/approved\_lab\_\_list.pdf

<sup>&</sup>lt;sup>4</sup> http://www.dotars.gov.au/transport/australia/dangerous/pdf/guidnote-class62.pdf

Australian Government

Department of Agriculture, Fisheries and Forestry Australian Quarantine and Inspection Service

# ANNEX 2 Published documents relating to *E. coli* O157:H7

The FSIS has published several relevant documents detailing sampling and other requirements for ground beef components (e.g. trim) for *E. coli* O157:H7:

- FSIS Directive 10,010.1 Verification Activities for *Escherichia coli* O157:H7 in Raw Beef Products (Jul 31, 2009)
- FSIS Notice 51-09, Routine Sampling and Testing of Beef Manufacturing Trimmings Derived from Cattle Not Slaughtered in That Establishment (Bench Trim) for *Escherichia coli* O157:H7
- Questions and Answers on FSIS Directives 10,010.1, Revision 1, 5000.2, and 6420.2
- Questions and Answers Regarding Directives 5000.2, 6420.2 and 10,010.1, Revision 1, and the Compliance Guidelines on *E. coli* O157:H7

Compliance Guidelines For Establishments On The FSIS Microbiological Testing Program And Other Verification Activities For *Escherichia coli* O157:H7