Appendix D. United States of America: *Escherichia coli* O157:H7 Programme

Technical Procedures for Monitoring *Escherichia coli* O157:H7 in Bulk Manufacturing Beef

1 Prelims

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2 Glossary of Terms

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The following words have the following meanings

Approved laboratory – a laboratory approved under the NZFSA Laboratory Approval Scheme.

Adult cattle – bovine species generally greater than 40 kilograms dressed carcass weight, with 3 classes; prime, bull, cow.

Associate trainer – any person approved by the LAS Administrator as competent to

(a) collect samples in accordance with the NMD Programme, and

(b) train restricted samplers.

Beef – meat from adult cattle.

Bovine – red meat species, cattle, with 3 classes; bull, cow, prime.

Bulk manufacturing beef – bulk manufacturing trimmings and ground beef components.

Bulk manufacturing trim – manufacturing trimmings (pieces of meat remaining after intact cuts are removed) intended for use in the production of ground beef.

Certified trainer – any person approved by the LAS Administrator as competent in sample collection and in training associate and restricted samplers.

EHEC – enterohemorrhagic *E. coli*, of which *E. coli* O157 is the most common in New Zealand, are a subset of the STEC (VTEC) group.

E. coli – *Escherichia coli*.

***E. coli* O157:H7 monitoring programme** – monitoring programme for *E. coli* O157:H7 which may include confirmed positive results for other related organisms of the VTEC/STEC group.

Full hygienic cleanup – a wet cleaning and full sanitising of food areas and food support areas of the boning room at least once every 24 hours.

GHP – Good Hygienic Practice.

Ground beef components – Components other than bulk manufacturing trim including two-piece chucks and other primal/sub primal cuts intended for use in raw ground beef or other raw non-intact product; raw oesophagus, head and cheek meat, advanced meat recovery (AMR), low temperature rendered lean free textured beef (LFTB), partially defatted chopped beef, partially defatted beef fatty tissue and heart meat.

HACCP – Hazard Analysis and Critical Control Point.

Heat treatment – application of a 6D listericidal process, or alternative agreed with NZFSA.

LAS – Laboratory Approval Scheme.

Lot – all beef produced and meat from other species concurrently or subsequently processed in a specific boning room between full hygienic cleanups of that boning room.

NMD – National Microbiological Database.

Nontoxigenic isolates – Isolates of confirmed *E. coli* O157 that are negative for all three of the following determinants: Shiga toxin (ST) production, shiga toxin gene(s) (*stx*) and the "H7" gene.

Official assurance verifier- a person recognised under section 103 of the Act to undertake official assurance verification and includes animal products officers employed by NZFSA VA.

Off-site laboratory – a consulting laboratory that is not located within the physical boundaries of any registered slaughterhouse and/or packhouse.

On-site laboratory – a laboratory facility located within the physical boundaries of a registered slaughter operation and/or packhouse.

Operator – an operator of a risk management programme carrying out primary or secondary processing of animal material or product.

Pathogenicity determinants – toxins or other factors (e.g. adherence to intestinal cells, production of haemolysin) involved in the pathogenicity that determine the virulence of any one serotype.

Registered packhouse –packhouse with a registered Risk Management Programme (RMP), including boning operations within an RMP with a broader scope of application than boning alone.

Registered slaughter operation –slaughter operation with a registered Risk Management Programme.

Restricted sampler – a person trained by either an associate trainer or a certified trainer to collect samples in accordance with the NMD Programme.

Sampler – includes certified trainers, associate trainers and/or restricted samplers in accordance with the NMD Programme.

STEC – Shiga toxin producing *E. coli,* also known as VTEC (de facto synonyms).

SSOP – Sanitation Standard Operating Procedures.

VTEC – Verocytotoxigenic *E. coli*, named as such because the toxin these bacteria produce lyses African green monkey kidney (Vero) cells, also known as STEC (de facto synonyms).

3 *Escherichia coli* O157:H7 Monitoring Programme

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3.1 Objectives

To operate a monitoring programme for *E. coli* O157:H7 using the framework of the National Microbiological Database (NMD).

To underpin the Official Assurance issued by the New Zealand government for export of bulk manufacturing beef to the United States.

To provide a specific pathogen-testing component for verification of Good Hygienic Practice (GHP), Sanitation Standard Operating Procedures (SSOPs) and Hazard Analysis and Critical Control Point (HACCP) based risk management plans.

3.2 Application

The *E. coli O157*:H7 testing programme shall be implemented by all US-listed slaughter operations and packhouses exporting to the US manufacturing beef which may be used in the preparation of ground beef.

Results obtained through sampling of boneless manufacturing meat are considered to be representative of other products (e.g. individually wrapped primal cuts) processed concurrently through the cutting/boning room. The boneless meat originates from the same carcasses, represents a large proportion of the total carcass surface, and production maximizes opportunity for re-distribution through a greater level of handling and contact with processing surfaces.

Separate testing programmes are, therefore, not required for other meat products concurrently processed.

Note: Carcasses are not currently exported to the United States and, along with ground beef components not processed in a boning room, head and cheek meats, weasand and heart meat, skirts etc, are not tested under this programme. Any operators wishing to export carcasses or any ground beef components not processed in a boning room to the United

States shall, before commencing export, contact the Programme Manager (Laboratory & Microbiological Programmes) to agree a test programme.

4 Requirements for Physical Containment Level 2 (PC2)

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4.1 Background

E. coli O157:H7 is classified within Risk Group 2 with additional special precautions including the use of gloves and staff with appropriate experience and training (AS/NZS 2243.3 2002, Table 3.2, precautions G and T, respectively).

4.2 Application of AS/NZS 2243.3, 2002, Physical Containment Level 2 (PC2)

All Laboratory Approval Scheme approved laboratories performing *E. coli* O157:H7 analyses must have a copy of *AS/NZS 2243.3 2002, Safety in laboratories, Part 3: Microbiological aspects and containment facilities,* ***Standards New Zealand, Wellington, New Zealand, amended 2003 or later edition*****.** The document can be obtained from Standards New Zealand, 155 The Terrace, Private Bag 2439, Wellington, 6020.

The clauses to be referred to and applied, and any further requirements, are listed in the following sub-sections.

4.2.1 General considerations for both off-site and on-site laboratories

AS/NZS 2243.3 2002 clause numbers 4.3 and 4.7 must be applied.

4.2.2 Considerations for off-site laboratories

AS/NZS 2243.3 2002 Clause Numbers 4.7, 4.8.4, 4.8.4.5.1, 4.8.6 (a), 4.8.6 (b), and 4.8.6 (h) must be applied.

4.2.3 Considerations for on-site laboratories

AS/NZS 2243.3 2002 clause number 4.7 must be applied.

Laboratory location and design

The pathogen laboratory shall be physically separated from other areas of the laboratory (i.e. a sealable room) and shall not be accessible by the general public. Entry to the pathogen laboratory should be via an ante-room with a double-door system. The doors shall open outwards, be self closing and lockable and, where possible, should contain glass panels so that occupants can be seen. Windows in the laboratory shall be closed and sealed.

Where an on-site laboratory has a pathogen laboratory without a double-door system, the laboratory as a whole shall be well separated from the food factory and from general foot-traffic. In addition, the door to the pathogen laboratory shall not be opened when in use.

The laboratory shall be sealable to permit decontamination with gases such as formaldehyde. Means shall be provided to purge the decontaminating gases safely to atmosphere.

A pressure steam steriliser for decontamination of laboratory wastes shall be available, preferably located within the laboratory.

Laboratory ventilation

AS/NZS 2243.3 2002 Clause 4.8.3(f) states that "a directional air flow shall be maintained by extracting room air. Recirculation is permitted but not into areas outside the PC2 facility". In addition, a ventilation system that establishes a negative pressure in the laboratory shall be provided such that there is directional airflow into the working area. The laboratory shall be maintained at an air pressure of at least 50 Pa below the pressure of adjacent rooms. The laboratory shall be structurally designed to take account of operating under negative pressures.

Laboratory access

The pathogen laboratory shall not be accessible by the general public. Specific personnel with access to the pathogen laboratory shall have limited access, and with the following restrictions.

- Sample collectors: Procedures must be in place to deposit samples such that samplers do not enter the laboratory area. If the sampler is also an analyst they shall be subject to all clothing and decontamination requirements. Documented requirements shall be strictly enforced.

- Maintenance staff/trades-persons: Shall have restricted access to the pathogen laboratory only after fumigation, or shall be subject to all clothing and decontamination requirements. Documented requirements shall be strictly enforced.

- Cleaners: Shall have access to the pathogen laboratory only after fumigation. Only laboratory personnel shall perform routine cleaning.

- Administrators/visitors/assessors: Shall have restricted access to the pathogen laboratory only after fumigation, or shall be subject to all clothing and decontamination requirements. Documented requirements shall be strictly enforced.

Authorisation shall be obtained from the Laboratory Manager (or similar authority) prior to entry to the pathogen laboratory.

5 *E. coli* O157 Monitoring Programme

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5.1 Product to be Sampled

Samples shall be collected separately and solely from cartons of non-frozen boneless manufacturing beef (Schedule 1 NMD Programme, section 2.7.1.3).

Operators must ensure that, with time, all product types (e.g. trim, BFH, trunks, skirts) are covered by random sampling (refer section 5.2.1) and represented in the NMD database. An operator may introduce rotational sampling of product types to achieve this end.

Each production day's set of 12 cartons may consist of a number of

(a) product types, and

(b) a mixture of bovine classes (e.g. bull, prime and cow).

Samples must not be collected, or analysed, from thawed product.

Sampling of cartonned frozen meat: Where exceptional and unforeseen circumstances prevent sampling or testing of product core samples may be collected from cartons of frozen meat from the original production date, transported frozen to the laboratory, and analysed directly from the frozen state.

5.2 Collection of Samples

Samplers must be recognised as competent under LAS in NMD bulk meat sampling for bovine species; Certified Trainers, Associate Trainers and/or restricted samplers.

A lot includes all beef produces, and meat from other species concurrently or subsequently processed in a specific boning room, between full hygienic cleanups of that boning room. Twelve (12) cartons of bulk manufacturing beef shall be sampled per lot. A lot cannot include product packed in more than one boning room.

Five pieces of meat representing a specified surface area must be sampled per carton, totalling 60 pieces of meat per lot. More than five pieces per carton may be required where individual pieces are smaller than the specified surface area (refer section 5.2.2).

5.2.1 Carton selection

Samples must be collected before the cartons are closed (Schedule 1 NMD Programme, section 2.7.1.3).

The initial time of sampling must be randomly selected across the period/day of each lot. This time may be brought forward to permit 12 cartons to be selected within the production time of the lot. The operator must record such adjustments to the original randomly selected time.

The first carton to be sampled must be selected randomly from the production line. Subsequent, but not consecutive, cartons must be selected for sampling only after the previous carton has been sampled and returned to the conveyor.

Selection of cartons must be at intervals no less than the time required to undertake the sampling of each carton. (Schedule 1 NMD Programme, section 2.11.1.4, Random selection of run, and section 2.11.1.5 Random selection of time) unless production constraints prevent random selection as described, e.g. only 12 cartons produced in the "lot".

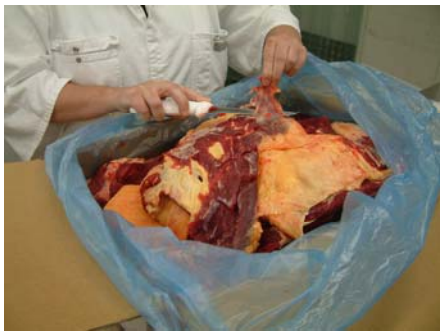
5.2.2 Whole tissue sampling

A total of 60 samples shall be collected from the lot. Five (5) samples shall be collected from each of the 12 cartons selected.

Each sample must be collected using the sampling technique described below.

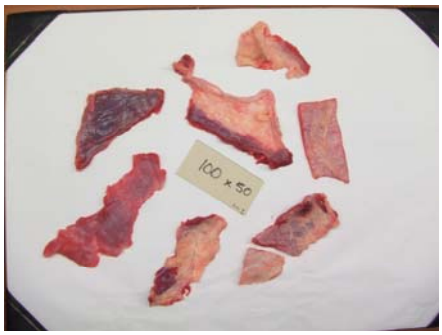
- Select five pieces of meat from separate locations in each carton using Schedule 1 NMD section 3.4.2, Figure 11 as a guideline.
- Where it is not possible to identify an original carcass surface, the sample may be collected from any surface of the meat portion.
- Sample each piece of meat by trimming a thin surface slice using a sharp boners knife, or equivalent (refer photographs). Where possible, a surface from the original outside of the carcass shall be sampled. A surface sample with dimensions of roughly 100mm x 50mm (rectangular) or 50cm^2 (circular 80mm diameter) and <2mm deep will ensure sufficient weight of sample is collected.
- Where the carton contains small pieces of meat (trimmings), the sample may be collected as a grab sample approximating 100mm by 50mm of total surface area from each of the five sampling locations in the carton.
- Collect and bag the five (5) samples from each carton into a labelled sterile sample bag.

Note: Operators must verify weekly that the weight of one set of 60 samples from 12 cartons from the lot, irrespective of the species sampled, is not less than 960g.



Selection of site and commencement of slicing

Surface slice <2mm thick



Approximate surface area

Surface slice of approximately 2mm thick

Frozen storage of fresh samples

In the event that delivery to the laboratory cannot foreseeably be achieved within 24 hours, e.g. courier service not available in the weekend and/or cost-effective alternatives not available, fresh samples may be frozen and delivered frozen to the laboratory at the earliest opportunity. All samples shall be blast frozen immediately after collection of the last sample in the n60 set. Operators shall document procedures to assure the security and identity of samples stored in this manner.

Sampling of cartonned bulk frozen meat

Where frozen meat must be sampled (refer Section 5.1), a minimum of five (5) core samples from each of 12 cartons shall be aseptically collected using a corer, or equivalent (eg. 25mm auger), attached to a drill (reference MIMM 5.5.4) to achieve a total sample weight per carton of not less than 80g. The dimensions of the corer should be approximately 20mm by 140mm length. It may be necessary to collect more than five (5) core samples to achieve the required final sample weight.

Associate Trainers and restricted samplers undertaking frozen core sampling must be trained in frozen core sampling by Certified Trainers.

Less than 12 cartons available for sampling

If less than 12 cartons are available for sampling from the lot because of documented production constraints (refer Schedule 1 NMD Programme section 2.11.4 *Practical constraints and technical failures*), operators may collect more than five (5) samples from each carton to make up approximately 960g of sample (weight equivalent to normal sampling).

Table 1: Proportional sampling for reduced carton numbers

Number of Sample Pieces to Collect Per Carton	
# of cartons available in each specific production lot	# of sample pieces to select from each carton
10	6 pieces
6	10 pieces
5	12 pieces
4	15 pieces
3	20 pieces
2	30 pieces
1	60 pieces

5.2.3 Carton identification

The physically sampled cartons (fresh or frozen) must be marked/labelled, or a unique identifier recorded, in a manner such that they can be verified and readily located as the cartons originally sampled upon notification of a screen test positive result.

Physically sampled cartons shall be traceable to their product type, and to the day and time of production.

5.3 Transportation to the Laboratory

All samples shall be transported to the laboratory as per Schedule 1 NMD Programme, sections 3.5.3 and 3.5.4.

Preparation and analyses of samples from both fresh and frozen meat shall be initiated within 24 hours of collection of the first sample from the lot.

Fresh bulk samples

- Should delivery to the laboratory be delayed, or processing within the laboratory compromised, preparation and analysis of the samples must be initiated no later than 30 hours of collection of the first sample from the lot.
- Should delivery to the laboratory not be achieved within 30 hours of collection of the first sample, and/or temperature requirements are exceeded the samples shall be deemed "void" and shall not be tested.

Fresh samples stored frozen

Blast frozen samples must be stored frozen and transported frozen to the laboratory. The time of commencement of delivery to the laboratory must be recorded and analysis of the samples commenced no later than 24 hours after dispatch from the premises.

NMD records shall indicate that fresh samples were frozen for storage and transport.

Samples from frozen product

Analysis of samples collected from frozen cartons must be initiated within 24 hours, or 30 hours under exceptional circumstances, from the time of collection of the first frozen sample.

All frozen samples shall be stored and transported in a manner that ensures they remain frozen in transit, and be verified as frozen by the laboratory on receipt. Laboratory, operator and NMD records shall indicate that samples have been analysed from frozen.

6 Sample Analysis

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6.1 Sample Analysis

All analyses shall be performed in laboratories LAS approved for analysis for *E. coli* O157:H7, official test 23.1.

Applications by laboratories for LAS approval for official test 23.1 shall include:

- A copy of the documented method from the laboratory's methods manual.
- A resume of competency for personnel eligible to perform the analyses, and for those for whom Signatory status is requested.
- If the laboratory is an on-site laboratory, a written assurance that the on-site laboratory complies with physical containment level two (PC2) and the additional requirements listed in section 4.
- Written evidence that the method has been validated. Validation must consist of five (5) replicates of the following trials:
    - Broth culture: *E. coli* O157:H7 (<10 org/ml) + *E. coli* (10 org/ml) + APC mixture (~1000 org/ml).
    - Spiked meat: *E. coli* O157:H7 (<10 org/ml) + meat flora (indeterminant mixed wild type).

6.1.1 Method

Each analysis shall be performed according to the methods described below. The method for detection of *E. coli* O157:H7 in meat samples is equivalent to that of the USDA/FSIS.

Modification or substitution of methods is not permitted unless approved by LAS, and documented under the Laboratory Approval Scheme (Section 8.5.2).

An ILCP for *E. coli* O157:H7 is required under LAS (Section 6.2.2.2 d iii Pathogens).

6.1.2 Quality Assurance

The quality assurance functions and quality control procedures for methods and media outlined in Chapter 2 of Meat Industry Microbiological Methods,

Edition Four, September 2005 (MIMM) or later edition shall be used, as required under LAS,

Controls: A non-toxigenic *E. coli* O157:H7 positive control (e.g. NZRM 3614) and negative control (*E. coli* NZRM 480 or NZRM 916) shall be run with each batch of samples analysed.

Note: To ensure that visual immuno-assay screen tests provide consistent results, a small quantity of *E. coli* O157 free meat must be added to suspensions of pure bacteria (used in QA control and ILCP procedures) prior to analysis.

- Cooked meat

0.03 - 0.06g per 10ml bacterial suspension,

or

0.75 -1.5g per 250ml enrichment.

- Frozen meat from a carton previously tested O157 negative

1g added to 9 ml bacterial suspension,

or

25g added to 225 ml enrichment.

6.2 Sample Suspension and Enrichment

All samples shall be prepared independently of those for the National Microbiological Database (NMD) Programme.

Preparation

- Aseptically empty the sample bags containing the n60 samples, usually 12 sample bags - one from each of 12 cartons sampled, into a sterile container.
- Thoroughly mix the 60 samples in the container, and then select from the container an analytical unit of 375g.
- Repeat for each set of sixty (60) samples (12 cartons) using re-sterilised, or separate sterile containers.

Suspension and Enrichment Broth

Modified TSB broth + novobiocin + casamino acids (mTSB+n)

Modified Tryptone Soya Broth, Trypticase™ (Tryptic) Soy Broth, or equivalent	33 g
Casaminoacids (casein acid hydrolysate)	10 g
Water	1 L

The use of other manufacturer's modified Tryptone Soya broth or Trypticase™ (Tryptic) Soy Broth base (other than Oxoid) is permitted if the formula is equivalent.

Rehydrate mTSB and casaminoacids in water and stir to dissolve. Autoclave for 20 minutes @ 121ºC. Let media cool to approximately 50ºC. Add 5ml of a filter sterilized, aqueous solution of 4mg/ml sodium novobiocin (adjusted for potency; Sigma N1628) for each litre of medium (20 mg/L). If refrigerated, media must be pre-warmed to an ambient temperature of 18-35ºC prior to use.

Final pH 7.4 ± 0.2 at 25ºC.

Stomaching

- Suspend in a stomacher or filter-stomacher bag the 375g analytical unit in 1000ml of TSB broth + novobiocin + casamino acids (mTSB+n) pre-warmed to 18-35°C, and stomach for 2 minutes, or blend for an equivalent period.

Note: The 375g analytical unit may be separated and suspended in smaller aliquots to facilitate stomaching in standard sized laboratory stomachers; e.g., two subunits of 200g and 175g, each suspended in a portion of the 1000ml of mTSB+n and then combined for incubation.

Note: Tween 80 or Tergitol 7 may be added to samples with high levels of fat (refer Schedule 1 NMD Programme, Section 4.9.1.2).

Enrichment

- Incubate a single suspension for each n60 sample set (total 375g in 1000ml of mTSB+n) at 42±1°C for 15-22 hours.

Note: Ensure a small quantity of *E. coli* O157 free meat is added to suspensions of pure bacteria (used in QA control and ILCP procedures) prior to analysis.

6.3 *E. coli* O157 Screen

Enriched cultures shall be screened for *E. coli* O157 using test procedures currently in use by the FSIS or kits that meet or exceed the following characteristic: sensitivity ≥98%, false negative rate ≤2%, specificity ≥90%, false positive rate ≤10%, and efficiency≥ 94%.

Test kits approved by NZFSA for use for the *E. coli* O157:H7 microbiological programme are:

- BioControl Visual Immuno Precipitate (VIP®) test kit (New Zealand Medical & Scientific). DynaBead immuno-magnetic beads (Dynal Pty, Australia).
- TECRA *E. coli* O157 Visual Immunoassay kit (TECRA Diagnostics). DynaBead immuno-magnetic beads (Dynal Pty, Australia).
- Neogen Reveal® kit, 24h enrichment, immuno-magnetic beads included (InterMed Scientific).
- BAX MP ® Qualicon E. coli O157 PCR screen test.

6.4 Confirmation as *E. coli* O157:H7

Samples returning a positive *E. coli* O157 screen shall be confirmed as containing *E. coli* O157:H7 or equivalent by:

- The Institute of Environmental Science and Research Ltd, Enteric Reference Laboratory (ESR-ERL), Porirua, or
- Any laboratory IANZ accredited for the following procedures.

The laboratory must either:

- forward an aliquot of the *E. coli* O157 screen-positive enrichment culture directly to ESR-ERL or IANZ-accredited laboratory,

or

- isolate *E. coli* 0157 from the enrichment broth in house using immunomagnetic separation (IMS), plating the immunomagnetic beads onto CT-SMAC (preferable) or SMAC agar plates.
- immediately forward sorbitol negative isolates for further confirmation to ESR-ERL or the IANZ-accredited laboratory as subcultures on agar slopes, not agar plates.

Failure to detect a sorbitol negative isolate on CT-SMAC or SMAC agar plates deems the screen test "not confirmed". Further confirmatory testing is not required. The sample result shall be reported as "*E. coli* 0157:H7 not isolated".

The laboratory may also submit to ESR-ERL or the IANZ-accredited laboratory a subculture of their *E. coli* O157:H7 positive control strain to compare with recovered isolates.

Samples must be transported in full compliance with NZS 5433 Code of Practice for Transport of Hazardous Substances on Land.

Further confirmation procedures carried out at ESR-ERL or the IANZ-accredited laboratory, as agreed with NZFSA, include multiplex polymerase chain reaction (PCR) for toxin and pathogenicity genes, and flagella antigen identification techniques (refer Figures 2 and 3).

Figure 2 Method for isolation and confirmation of *E. coli* O157 screen positive enrichment cultures.

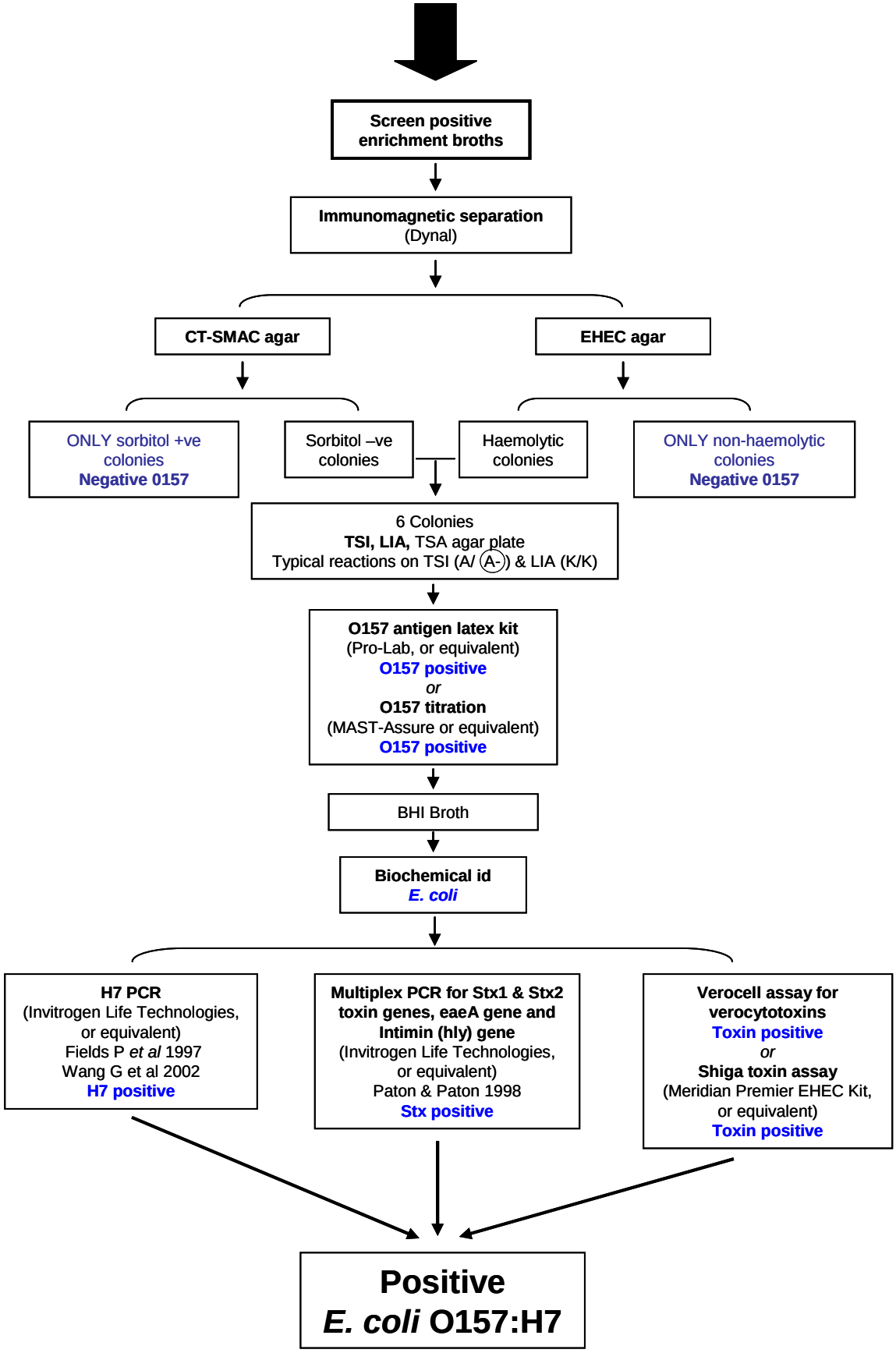
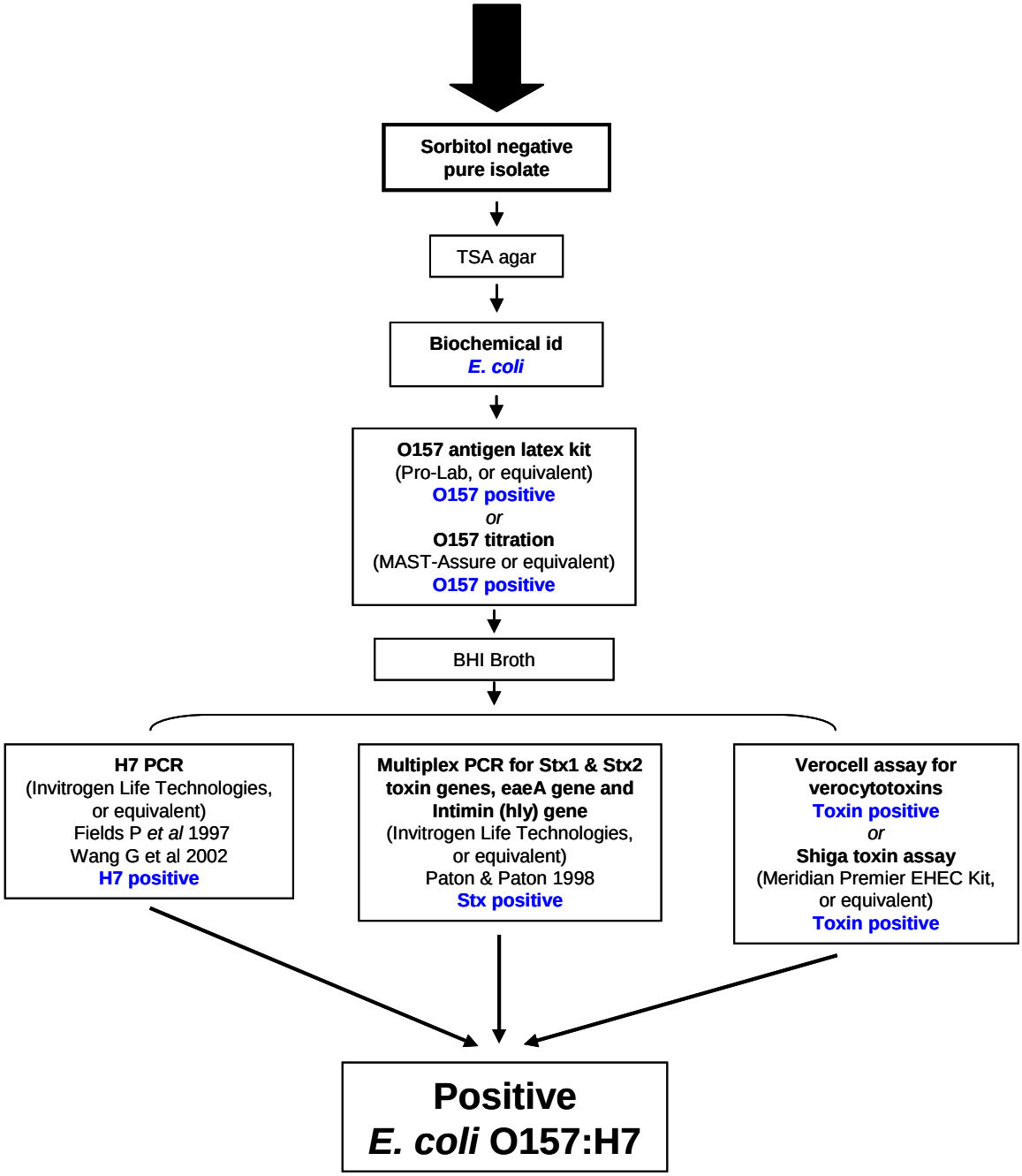


Figure 3 Method for confirmation of pure cultures.



6.5 Classification for Product Disposition

A confirmed positive result for the purposes of positive product disposition is classified as an *E. coli* O157 that has one or more of the following determinants:

1. Positive for Shiga toxin (ST) production.
2. Positive for Stx gene(s) (*stx*).
3. Genetically determined to be H7 (H7 antigen).

E. coli or other cross-reacting organisms, such as *Citrobacter*, that lack all the above pathogenicity determinants will be assigned a negative product disposition.

6.6 Molecular Typing

All isolates from confirmed positive samples shall be molecular typed at ESR by pulse-field gel electrophoresis (PFGE) using the Xba and Bln restriction enzymes, and the PFGE profile submitted to the National Typing Database (NTD).

7 Procedures for Withholding Product until Confirmed Negative

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7.1 Detain Procedure

Operators must establish procedures to ensure that any product is held from further processing or not shipped offshore pending receipt of a negative screen test result or confirmation of a positive screen test result.

7.2 Detain of Product Following Receipt of a Positive Result

Product from a lot as defined in section 2 Glossary of Terms, for which samples return a positive screen for *E. coli* O157 shall be immediately detained according to the procedures described in this section.

Product from a lot as defined in section 2 Glossary of Terms, the samples of which contain an isolate(s) of *E. coli* O157 that is positive for any, but not necessarily all, of the following determinants: Shiga toxin (ST) production, shiga toxin gene(s) (*stx*) and the "H7" gene shall be further detained and reworked according to the procedures described in this section.

Such isolates include *E. coli* O157:H7, and other related VTEC serotype that tests positive for shiga toxin production.

7.3 Application of Result to Other Species Concurrently Processed

In accordance with the definition as applied to USDA spot testing of registered packhouses, a single "lot" consists of all beef (excludes offals), and meat from other species concurrently or subsequently processed, between full hygienic cleanups of the boning room.

When multiple species are concurrently processed in the same boning room between full hygienic cleanups, NZFSA will consider release of product to all markets from a species that has tested negative, or does not require testing. In consideration of the above, NZFSA is likely to request information with regard to likely contamination from the species testing positive to the other species.

Therefore, operators need to consider:

- Physical separation of product, e.g. separate lines, until such time that cartons are packed.
- Control of worker and equipment movement between areas/lines using appropriate sanitary procedures.
- Sanitation between species at common contact points.

7.4 Response to a Positive Screen for *E. coli* O157 (Suspect Lot)

7.4.1 Operator response to positive screen

The operator shall:

- Immediately identify and isolate the whole of the "lot" to which the sample applies, including that already moved off the registered slaughter operation or packhouse.
- Confirm that product from the lot has not been exported nor released for further processing.
- Immediately inform the official assurance verifier.
- Immediately initiate procedures for review of process hygiene with respect to the production date of the lot from which the positive screen result sample was taken, and prepare for escalation of the review process if the screen test is confirmed.

7.4.2 NZFSA VA response to positive screen

Upon notification of a positive screen as per section 7.4.1 above, the official assurance verifier shall:

- Verify that the operator has identified the whole of the "lot" of product;
- Retain the whole of the "lot";
- Confirm there was adequate separation between the affected beef product and product from any other species processed concurrently that is not being included as part of the "lot";
- Notify an NZFSA VA Technical Co-ordinator with details of affected product.

7.5 Response To Final Confirmation Results Of Positive Screen Events

Note that determination of the final product disposition by the NZFSA VA Technical Co-ordinator will be conducted by reviewing the final laboratory report in consultation with the NZFSA Programme Manager (Laboratory & Microbiological Programmes) Export Standards.

7.5.1 Operator response on receipt of final confirmation results

- Immediately inform the official assurance verifier of the final confirmation result, and provide copies of confirmatory laboratory reports.
- Confirm that confirmation result has been entered into the VTEC web database.

Confirmed positive:

Where a sample is confirmed as containing an isolate of *E. coli* O157:H7 or related VTEC, the company shall:

- Immediately implement procedures to withdraw eligibility for sale of the whole “lot” to the US, Canada, and any other markets specifying US requirements.
- Ensure product is heat treated prior to release for human or animal consumption as specified by the NZFSA for the “lot” and maintain records detailing the disposition of the “lot”,
- For processors who elect to undertake heat treatment of product prior to release for human or animal consumption when such is permitted by the NZFSA product disposition, a 6D listericidal process must be applied. Product from a positive lot may be rendered.
- Review the HACCP plan for the process. Document the review, its findings and any corrective actions taken. This may result in changes to the HACCP plan and subsequent revalidation.
- Review records (for example; all pertinent HACCP and SSOP records) and other results from the days before and after the positive result to identify any trends.

Confirmed negative:

Where the screen positive is confirmed as negative for *E. coli* O157:H7 or related VTEC the company shall:

- Release product as per the confirmed negative product disposition notice received from NZFSA relating to the production date of the "lot" concerned.

7.5.2 NZFSA VA response to a confirmed result

NZFSA VA shall ensure that the final laboratory reports received from the operator regarding the production dates with positive screen events are submitted to an NZFSA VA Technical Co-ordinator for review.

Upon notification from the NZFSA VA Technical Co-ordinator of a confirmed presence of *E. coli* O157:H7 or related VTEC, the official assurance verifier shall:

- Ensure product from the "lot" remains under NZFSA VA retain during storage and any transportation until heat treated.
- Verify the operator HACCP review and trend analysis.

Upon notification from the operator of a confirmed absence of *E. coli* O157:H7 or related VTEC the official assurance verifier shall:

- Release product as directed by NZFSA.
- Verify that the operator has carried out a review of process hygiene with respect to the production date of the lot from which the positive screen result sample was taken.

8 Reporting of Results

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8.1 Reporting to the Operator

All test results for the sample shall be reported to the Operator and to the National Microbiological Database (NMD). Results reported shall include:

- Sample format (e.g. fresh meat, fresh meat/frozen sample, frozen meat sample);
- Screen test used;
- Result of screen test;
- Typical isolate(s) obtained by IMS;
- Confirmation, or otherwise, that isolate is *E. coli*;
- Confirmation, or otherwise, that isolate is serotype O157;
- Toxin detection method used (e.g. kit or vero cell culture);
- Result of shiga toxin production test;
- Confirmation, or otherwise, that isolate has an Stx gene;
- Description of Stx gene (e.g. stx1 or stx2);
- Confirmation, or otherwise, that isolate is serotype H7;
- Confirmation, or otherwise, that isolate has the eaeA and HlyA genes;
- Confirmation, or otherwise, that isolate is *E. coli* O157:H7 or related VTEC;

Where a test has not been performed, the report shall state “not tested”.

8.2 Reporting to the National Microbiological Database

Results from the *E. coli* O157:H7 test programme shall be maintained in the NMD in order to:

- Enable the development of a national profile for prevalence of *E. coli* O157:H7 in bulk manufacturing beef;
- Facilitate improvement to the *E. coli* O157:H7 test programme through understanding of the rate of false positive screen and non-confirmed O157 tests.

Premises results shall be entered directly into an electronic database provided by NZFSA.

Operators, or delegated laboratory, must complete data entry for each production date on the same day of receipt of screen test results. Where there are screen positive results the confirmation section of the website data entry screen must be completed immediately on receipt of the final confirmatory results to facilitate product disposition. Final results must be authorised, not left in a provisional state. Product disposition will not be undertake unless a final confirmed and authorised result is available.

8.3 Reporting to the National Typing Database

Molecular typing profiles (PFGE Xba and Bln) for all isolates from confirmed positive samples shall be entered into the ESR administered National Typing Database (NTD). In order to:

- Facilitate epidemiological investigation of, and source attribution for, *E. coli* O157:H7 sporadic cases and outbreaks.

9 References

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