1. **INTENDED USE**
   The Thermo Scientific™ SureTect™ Escherichia coli O157:H7 PCR assay is a real-time PCR test intended to be used in conjunction with either
   The Thermo Scientific™ PikoReal™ Real-Time PCR Instrument and Thermo Scientific™ SureTect™ Software
   Or Applied Biosystems™ 7500 Fast Real-Time PCR System and Applied Biosystems™ RapidFinder™ Express Software version 2.0 for the detection and identification of *Escherichia coli* O157:H7 in food samples in laboratories undertaking microbiological analysis.

2. **SUMMARY**
   Real-time Polymerase Chain Reaction (PCR) technology is used for amplifying DNA in a reaction tube which enables detection and analysis of the DNA. SureTect PCR assays are designed for the identification of micro-organisms from foods and food manufacturing environmental samples.

3. **PRINCIPLE OF THE TEST**
   The PCR pellets used in the SureTect assays contain lyophilized (freeze-dried) target-specific primers, dye labelled probes and PCR master mix components. Probes are short oligonucleotides with a quencher molecule at one end that, when not bound to target DNA, greatly reduces fluorescence from the dye at the opposite end. The oligonucleotides target unique DNA sequences found only in the target micro-organism. If present, the target DNA will be amplified and the increasing fluorescent signal generated will be detected by the Real-Time PCR Instrument and interpreted by the software.
   The SureTect assays are based on Solaris™ qPCR technology. The probes have a molecule called Minor Groove Binder (MGB) attached to one end, which enhances the probe-template DNA bond and yields a better signal-to-noise ratio by lowering background fluorescence. Results are achieved in around one hour and twenty minutes of loading the prepared sample in the real-time PCR instrument and are displayed on the PC screen as simple positive or negative symbols with amplification plots also easily accessible for review.
   The SureTect Escherichia coli O157:H7 PCR Assay targets unique DNA sequences specific to *E. coli* O157:H7. The SureTect Escherichia coli O157:H7 PCR Assay includes all of the necessary reagents for bacterial DNA release and PCR. Enriched samples are simply pipetted into pre-filled Lysis Tubes, along with Proteinase K, before incubation to lyse any bacterial cells present in the sample and release their DNA into solution. The lysate is then loaded into the SureTect Escherichia coli O157:H7 PCR Tube to re-hydrate the PCR pellet which contains all of the necessary components and reagents for PCR, including a probe, primers and DNA template for the internal amplification control (IAC). The PCR Tubes are then sealed, loaded into either the PikoReal or 7500 Fast PCR Instrument and the run started using the software relevant to the instrument. On completion of the run the interpreted results will be clearly displayed by the software and can be reported, stored, printed off and downloaded as required.
4. SYMBOL DEFINITIONS

- Catalogue number
- Contains sufficient product for <n> tests
- Consult instructions for use (IFU)
- Temperature limitation (Storage temperature)
- Batch code (Lot number)
- Use by (Expiration date)
- Manufacturer

5. KIT CONTENTS, STORAGE AND PREPARATION FOR USE

The SureTect Escherichia coli O157:H7 PCR Assay contains all the necessary reagents for DNA extraction and real-time PCR for 96 tests.

Contents

Lysis Reagent 1 Tubes (PT0010A)
96 pre-filled, sealed tubes (in 12x8 format). Each tube contains Lysis Reagent 1 (clear, pale blue liquid containing fine white particles). One tube is required for each SureTect PCR assay.

Lysis Tube Caps (PT0020A)
12 strips of 8 caps. The domed cap strips may be cut to enable the required number of caps to be selected for each run.

Proteinase K (PT0050B)
1 capped tube containing Proteinase K (clear colorless liquid).

SureTect Escherichia coli O157:H7 PCR Tubes (PT0410A)
12x8 pre-filled, sealed strips. Each tube contains one SureTect Escherichia coli O157:H7 PCR pellet (pale yellow). Strips can be cut to enable the required number of tubes to be selected for each run.

SureTect PCR Caps (PT0040A)
12 strips of 8 caps. The flat cap strips may be cut to enable the required number of caps to be selected for each run.

Storage

The reagents will remain stable until the expiry date stated on the packaging when stored and handled as directed. The complete box and contents should be stored in the dark at 2-8°C when not in use. Do not use the reagents after the expiration date indicated on the label.

SureTect PCR Strips may be cut, enabling unused materials to be returned to the refrigerator after a short time. The PCR Tubes should be brought to room temperature before opening. This can be facilitated by removing the PCR Tubes required from the packaging and placing on the bench for around 10 minutes before use.
6. MATERIALS REQUIRED BUT NOT PROVIDED
All of the required items listed below are also available from Thermo Fisher Scientific Microbiology. Please contact your kit supplier for further information. Other culture media formats such as prepared media and Dry-Bags™ are available; please contact your local supplier for details. All items required for sample lysis and PCR set-up can be provided as part of the SureTect PCR System Start-Up Package. Please contact your local supplier for further information.

Enrichment
Suitable enrichment medium for growth of *E. coli* O157:H7 from food samples.

**AOAC-RI Performance Tested Method™ validated enrichment protocols:**
The use of pre-warmed (41.5°C) modified Tryptone Soya Broth (mTSB) has been validated during the AOAC-Research Institute (RI) *Performance Tested Methods™* validation study:

**Protocol validated in the scope of NF VALIDATION:**
The use of pre-warmed (41.5°C) Buffered Peptone Water (ISO) has been validated during the NF VALIDATION study done according to the ISO 16140 standard.

**Sample Preparation**
Homogenizer bags for 25g samples
Filtered homogenizer bags for 25g samples
Homogenizer bags for 375g samples
Pipettes and pipette tips
Microfuge tubes (1.5ml)
Sample Tube Rack
CapEase™ tool
Lysis Tube Rack
Adjustable single channel pipette and pipette tips
Two Boekel Scientific™ thermal block heaters
(supplied as part of the SureTect starter pack)

**Optional Lysis Components**
Multi-channel pipette and filtered tips (to be used in place of the adjustable single channel pipette for processing multiple samples in parallel)
Repeater pipettor (and filtered tips (for faster, easier addition of Proteinase K to multiple Lysis tubes)

**PCR set-up**
Rack for PCR Tubes
Multi-channel pipette and filtered tips
Empty PCR Tube strips and caps (required to balance lid pressure if only 1-8 samples are processed)
Computer (supplied as part of the SureTect starter pack)

The SureTect assay can be analysed using either the SureTect PikoReal Real-Time PCR Instrument with the SureTect Software or the Applied Biosystems 7500 Fast Real-Time PCR System with RapidFinder Express Software version 2.0.

**NOTE:** When using the PikoReal Real-Time PCR Instrument it must be connected to a PC loaded with the SureTect Software version 1.2 and assay specific kit file version 1.2.7.55 or higher.
**NOTE:** When using the Applied Biosystems 7500 Fast Real-Time PCR System it must be connected to a PC loaded with the RapidFinder Express Software version 2.0 and SureTect Salmonella species assay specific kit file version 1. The 7500 Fast Instrument must have been calibrated according to the instructions in the Applied Biosystems 7300/7500/7500 Fast Real Time PCR System Installation and Maintenance Guide.

Please review the appropriate software manual or contact our technical support team for details of kit components or PC specifications (Europe/ROW: email: microbiology.techsupport.uk@thermofisher.com telephone: +44 (0)1256 694238/ USA: email: USLEN-TechServices@thermofisher.com telephone 1-800-255-6730) for PC specifications.

**Equipment required but not provided as part of the SureTect System**

The following items are not supplied as part of the SureTect System but can be purchased from Thermo Fisher Scientific Microbiology.

- Homogenizer Laboratory Blender (available from Thermo Fisher Scientific, DB5000A, or similar)
- Homogenizer bag rack (DB5002A, or equivalent)
- Incubators (37°C ±1°C, 41.5°C ±1°C)
- Sterile water, for use when running the SureTect assay on the Applied Biosystems 7500 Fast (available from Thermo Fisher Scientific, BO0209B, or similar)

**Confirmation of positive results**

Suitable culture medium for isolation and identification of *E. coli* O157:H7 colonies. The use of Sorbitol MacConkey Agar with Cefixime and Potassium Tellurite (CT-SMAC Agar) followed by confirmation with either the Oxoid O157 or Wellcolex E. coli O157:H7 latex kits have been validated during the AOAC-RI Performance Tested MethodsSM and NF VALIDATION certification studies.

When using the ISO reference method for the confirmation of samples supplement mTSB with 20mg/l novobiocin.

### 7. TEST PROCEDURE

The SureTect Escherichia coli O157:H7 PCR assay has been validated for a range of sample types using the enrichment protocols details below. For preparation of initial suspensions, follow the instructions detailed in ISO 6887 parts 1-5* and/or ISO 16654 standards. Comply with Good Laboratory Practice (refer to ISO 7218³). Additionally, it is advised that users follow the general requirements described in ISO 22174:2005 when using PCR methods.

#### 7.1 Sampling and enrichment

If preparing culture media for enrichment and confirmation steps in-house, carefully follow the instructions provided by the supplier.

**CAUTION:** Pathogenic enterohaemorrhagic *Escherichia coli* (EHEC) O157 have a low infective dose and can cause severe life threatening illness. Ensure that all procedures for enrichment and handling of enriched cultures take place in an appropriate laboratory (e.g. containment facility) by skilled personnel. Ensure that all relevant national and local regulations are adhered to.

**NOTE:** The short protocols of detection are sensitive to incubation conditions. It is required to scrupulously respect the conditions of temperature indicated in the technical specification. Notably, you must verify that the temperature of pre-warming of the enrichment broth reaches the required temperature. The time of preparation of samples, delay between the end of the pre-
warming step of the enrichment broth and the beginning of the incubation step with the food sample does not exceed 45 minutes. Using a ventilated incubator during the incubation is recommended.

In the context of NF VALIDATION:

Enrichment protocols for use with the PikoReal Real-Time PCR Instrument and SureTect Software or Applied Biosystems 7500 Fast Real-Time PCR System and RapidFinder Express Software version 2.0

NOTE: In the context of NF VALIDATION, test portions weighing more than 25g have not been tested.

NOTE: Filtered homogenizer bags must be used to help with fat and particle separation.

Raw beef meat:
Weigh 25g of the sample to be tested into a homogenizer bag and add 1:10 ratio of pre-warmed (41.5°C) Buffered Peptone Water (ISO). Homogenize thoroughly for 30 seconds to one minute using a homogenizer or by hand for samples containing hard particles, such as bone, etc. Incubate at 41.5°C ±1°C for 8-24 hours (see section 11, “Performance Validation” for specific NF VALIDATION validated matrices and enrichment conditions).

Remove the enriched sample from the incubator and mix the liquid in the homogenizer bag by hand for a few seconds. Allow any food particles to settle. Open the homogenizer bag and using a pipette with an extra long filtered pipette tip remove around 1.5ml and dispense into a new microfuge tube and close until ready to process the sample to the next stage.

NOTE: The 1.5ml portions of samples can be stored at 2-8°C and retained for up to 72 hours following completion of enrichment to allow for confirmation of PCR positive results and in case there is a need to repeat the PCR analysis.

In the context of AOAC-RI Performance Tested MethodsSM certification:

Enrichment protocols for use with the PikoReal Real-Time PCR Instrument and SureTect Software or Applied Biosystems 7500 Fast Real-Time PCR System and RapidFinder Express Software version 2.0

Raw beef trim and ground/minced beef:
Weigh 375g of the sample to be tested into a homogenizer bag and add sufficient pre-warmed (41.5°C) mTSB to give either a 1:4 or 1:5 ratio of sample to broth. The user should choose one of the ratios according to their test requirements as both ratios have been validated during the Performance Tested MethodsSM validation to offer user flexibility.
For 375g samples analysed at a 1:4 ratio add 1.125 litres of pre-warmed (41.5°C) mTSB.
For samples being analysed at a 1:5 ratio add 1.5 litres of pre-warmed (41.5°C) mTSB.
Homogenize thoroughly for 30 seconds to one minute using a homogenizer or by hand for samples containing hard particles such as peppercorns or bone. Incubate at 41.5°C ±1°C for 9-24 hours (see section 11, “Performance Validation” for specific AOAC-RI validated matrices and enrichment conditions).
NOTE: Filtered homogenizer bags should be used to help with fat and particle separation that can make sampling difficult with certain food types.

NOTE: According to the AOAC-RI Performance Tested MethodsSM certification, samples of raw beef with an aerobic plate count of greater than $10^3$ CFU/g are not suitable for analysis with the SureTect PikoReal and SureTect Software v1.2 when following the short enrichment protocol and should be incubated for 24 hours.

NOTE: For short enrichment protocols for beef we would recommend using the Applied Biosystems 7500 Fast with RapidFinder Express Software version 2.0.

**Produce and fruit juices:**
Weigh 25g of the sample to be tested into a homogenizer bag and add a 1:10 ratio (e.g. 225 ml) of pre-warmed (41.5°C) mTSB. Homogenize thoroughly for 30 seconds to one minute using a homogenizer or by hand for samples containing hard particles. Incubate at 41.5°C ±1°C for 8-24 hours (see section 11, “Performance Validation” for specific AOAC-RI validated matrices and enrichment conditions).

Remove the enriched sample from the incubator and mix the liquid in the homogenizer bag by hand for a few seconds. Allow any food particles to settle. Open the homogenizer bag and using a pipette with an extra long filtered pipette tip remove around 1.5ml and dispense into a new microfuge tube and close until ready to process the sample to the next stage.

NOTE: The 1.5ml portions of samples can be stored at 2-8°C and retained for up to 72 hours following completion of enrichment to allow for confirmation of PCR positive results and in case there is a need to repeat the PCR analysis.

**7.2 Sample lysis**
Ensure that the two heating blocks are at the correct temperatures: 37°C ±2°C and 95°C ±2°C.

NOTE: When analysing samples with the Applied Biosystems 7500 Fast PCR Instrument, a negative control sample must be set up for each PCR run. The control must be set up by replacing 10µl of the enriched sample with 10µl of sterile water in the steps detailed below.

Take the required number of Lysis Reagent 1 Tubes and place into a suitable rack. Tap the rack of tubes onto the bench or flick your wrist whilst holding the tubes to remove any liquid from the cap area and to collect the reagents at the bottom of each tube. Bring to room temperature before opening by leaving on the bench for around ten minutes.

Use a suitable pipette to add 10µl of Proteinase K to each Lysis Tube. To avoid contamination, it is important to use a fresh filtered tip every time Proteinase K is withdrawn from the stock tube. Next, using a suitable pipette and filtered tip take 10µl of the aliquoted enriched sample/control from the microfuge tube and add it to one of the opened Lysis Tubes. Ensure when adding the sample that the pipette tip reaches the bottom of the Lysis Tube to facilitate complete mixing of the sample with the Lysis reagent.

Seal the Lysis Tubes with domed SureTect Lysis Caps using the CapEase tool and incubate in the heating block at 37°C ±2°C for 10 minutes. Immediately transfer the tubes to the heating block at 95°C ±2°C and incubate for a further 5 minutes.
Remove the tubes from the block and allow to cool at ambient temperature for 2 minutes before starting the PCR set-up.

The prepared lysates can be stored at 2-8°C for a maximum of 24 hours once the lysis procedure has been completed. This will allow repeat analysis, if required. It is not recommended that lysates are stored for longer than 24 hours.

If required, store an aliquot of the enrichment broth at 2-8°C for up to 72 hours and repeat the lysis procedure on the stored enrichment.

**NOTE:** Care should be taken when cutting strips of caps or tubes to ensure that they are not cut too close to the wall of the tube or cap lid, otherwise the lid will not seal adequately when carrying out the lysis protocol.

**NOTE:** Do not touch the inside of the caps to prevent cross contamination.

**NOTE:** Care should be taken to ensure that incubation temperatures and times are closely adhered to during the sample lysis steps.

### 7.3 PCR set-up

**NOTE:** Ensure that the correct assay is selected from the assay options. In RapidFinder Express Software version 2.0 ensure that “E.coli SureTect” is selected.

Create the template on either the SureTect or RapidFinder Express Software version 2.0. Refer to the appropriate software manual for directions on how to set a template up.

Remove the required number of SureTect Escherichia coli O157:H7 PCR Tubes from the packaging and place into a suitable tube rack. Tap the rack of PCR Tubes onto the bench to ensure the SureTect PCR pellets inside the tubes are located at the bottom. Allow the PCR Tubes to come to room temperature by leaving on the bench for 5 minutes before opening. It is important to return any remaining PCR pellets to the bag, seal and store at 2-8°C as soon as possible.

**NOTE:** PCR pellets should appear as pale yellow pellets. If the pellet is collapsed or not pale yellow in colour, do not use the pellet.

**NOTE:** Care should be taken when cutting strips of caps or tubes to ensure that they are not cut too close to the wall of the tube or cap lid, otherwise the lid will not seal adequately during PCR amplification.

Open the Lysis Tubes with the CapEase tool and using a suitable pipette with filtered tips take 20µl of the lysate. Ensure the lysate is removed from the top half of the liquid, taking care not to disrupt the particles at the bottom of the Lysis Tube. If the particles become disturbed leave the tube for 1-2 minutes to allow the particles to re-settle.

**CAUTION:** Particular care should be taken to ensure that no particles are transferred from the lysate tube into the PCR Tube. The presence of these particles during the PCR process may inhibit PCR from occurring resulting in the internal amplification control and any target DNA failing to amplify.

Transfer the 20µl of lysate into the opened PCR Tube to rehydrate the SureTect PCR pellet. Take care not to touch the pellet when adding the lysate. Touching the pipette tip end to the inside of
the PCR Tube away from the pellet will help to expel the liquid while keeping the tip away from the pellet. Ensure the lysate fully rehydrates the pellet before capping the tube.

**NOTE:** For ease of use a multi-channel pipette can be used to transfer multiple lysates to the PCR Tubes. If on opening the PCR Tube the pellet is not positioned at the bottom of the tube, take a sterile empty pipette tip and gently move the pellet to the bottom of the well. Seal the PCR Tubes with the flat optical caps provided (pre-cut to the number required). Ensure the tubes are properly sealed by pressing down firmly over each opening. Please note that the CapEase tool should not be used for sealing the PCR tubes.

7.4 Starting a run
Open the drawer of the Real-Time PCR Instrument. Load the PCR Tubes according to the plate setup created. Ensure the plate is balanced properly, if running the SureTect PikoReal you must fill at least 2 full rows with tubes. Use blank tubes if necessary. When running the Applied Biosystems 7500 Fast follow the prescribed template.

Close the drawer and start the run. Refer to either the SureTect⁷ or RapidFinder Express 2.0 Software manual⁸ for detailed directions.

**CAUTION:** Once the lysate has been added to the PCR pellets the PCR run should be started within 30 minutes.

7.5 Reviewing results
When the run is completed, remove the processed samples and review the results on the computer screen.

Refer to the SureTect or RapidFinder Express 2.0 Software manual for help with result interpretation.

7.6 Confirming positive results & reporting
**Confirmation of positive results obtained using the methods certified by NF VALIDATION**
In the context of NF VALIDATION, all samples identified as positive by the SureTect Escherichia coli O157:H7 PCR assay must be confirmed. If confirmation is not started immediately after a positive SureTect test result, store the enrichments at 2-8°C. Confirmation must be started within 72 hours following the end of incubation. To confirm the SureTect assay result use one of the following methods:

1. Using the conventional tests described in the methods standardized by CEN or ISO from colonies (including the purification step). The confirmation step must start from the BPW or enrichment broth. Identify 1 to 5 typical colonies using the conventional tests described within the ISO 16654² reference method.

2. a. Directly streak 50µl of the BPW enrichment onto the surface of a plate of CT-SMAC Agar using the diminishing streak technique, so as to obtain isolated colonies. Incubate CT-SMAC Agar at 37°C ±1°C for 18-24 hours. Observe the plate for presumptive *E. coli* O157 colonies (colourless to straw coloured colonies), then confirm as positive using either the Oxoid *E. coli* O157 latex test (DR0620M) or Wellcolex *E. coli* O157:H7 latex test (R30959601). When using the Wellcolex *E. Coli* O157:H7 test kit, to confirm for the presence of the H7 antigen, subculture a presumptive positive colony from CT-SMAC Agar to Blood Agar. Ensure colony purity
before proceeding with the confirmation. For full instructions consult the Wellcolex kit instructions for use.

b. Where direct streaking of the enrichment broth fails to confirm a PCR positive result, perform immuno-concentration using 1ml of the BPW enrichment using anti-\textit{E. coli} O157 magnetic beads. Beads should be resuspended in 100\(\mu\)l of a suitable diluent (e.g. MRD) and 50\(\mu\)l of the beads should be pipette onto a plate of CT-SMAC Agar. Inoculate the plate using a diminishing sweep technique and incubate at 37°C ±1°C for 18-24 hours. Observe the plate for presumptive \textit{E. coli} O157 colonies (colourless to straw coloured colonies), then confirm as positive using either the Oxoid \textit{E. coli} O157 latex test (DR0620M) or Wellcolex \textit{E. coli} O157:H7 latex test (R30959601). When using the Wellcolex \textit{E. coli} O157:H7 test kit, to confirm for the presence of the H7 antigen, sub-culture a presumptive positive colony from CT-SMAC Agar to Blood Agar. Ensure colony purity before proceeding with the confirmation. For full instructions consult the Wellcolex kit instructions for use. Alternatively, SureTect PCR positive results may be confirmed by following the steps in the ISO \textit{E. coli} O157 horizontal method\(^2\).

In the event of discrepant results (positive with the alternative method, non-confirmed by one of the means described above, and in particular for the Latex test) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

\textbf{CAUTION:} In the event of a result that is positive by the SureTect PCR method but which cannot be confirmed using the steps described above, all necessary measures must be taken by the laboratory to establish the true status of the sample before reporting the result. Refer to section 10 - PERFORMANCE LIMITATIONS for further guidance.

Refer to the SureTect or RapidFinder Express 2.0 Software Manual for result storage, download and reporting options.

\textbf{In the context of AOAC-RI Performance Tested Methods\textsuperscript{SM} validation:} Positive results can be confirmed by immuno-concentration of 1ml of the enrichment using anti-\textit{E. coli} O157 magnetic beads. Beads should be resuspended in 100\(\mu\)l of a suitable diluent (e.g. MRD) and pipetting 50\(\mu\)l of the beads onto a plate of CT-SMAC Agar. Inoculate the plate using a diminishing sweep technique and incubate at 37°C ±1°C for 18-24 hours. Observe the plate for presumptive \textit{E. coli} O157 colonies (colourless to straw coloured colonies), then confirm as positive using either the Oxoid \textit{E. coli} O157 latex test (DR0620M) or Wellcolex \textit{E. coli} O157:H7 latex test (R30959601). When confirming for the H7 antigen using the Wellcolex \textit{E. coli} O157:H7 kit, subculture suspect colonies from the CT-SMAC Agar onto blood agar. Alternatively, SureTect PCR positive results may be confirmed by following the steps in either the ISO \textit{E. coli} O157 horizontal method\(^2\) or USDA FSIS MLG reference methods.

\textbf{NOTE:} If following the steps in the ISO \textit{E. coli} O157 horizontal method, a sample of the enrichment medium should be taken after 6 hours and 18-24 hours incubation.

\textbf{CAUTION:} In the event of a result that is positive by the SureTect PCR method but which cannot be confirmed using the steps described above, all necessary measures must be taken by the laboratory to establish the true status of the sample before reporting the result. Refer to section 10 - PERFORMANCE LIMITATIONS for further guidance.

Refer to the SureTect or RapidFinder Express 2.0 Software Manual for result storage, download and reporting options.
8. DISPOSAL
Once the PCR run is completed, open the PCR Instrument and remove the processed samples. Dispose as hazardous microbiological waste according to appropriate local and/or national guidelines.

WARNING: Do not under any circumstances open the PCR Tubes following the completion or partial completion of a PCR run. The amplified DNA (amplicon) in the PCR Tubes can easily contaminate equipment and the nearby laboratory environment leading to false positive results in future experiments.

Refer to the Real-Time PCR Instrument Manual for guidelines on cleaning equipment and handling possible amplicon contamination.

Pathogenic enterohaemorrhagic Escherichia coli (EHEC) O157 have a low infective dose and can cause severe life threatening illness, ensure that all inoculated culture media, even if shown to be negative for the target organism, are disposed of as hazardous microbiological waste according to appropriate local guidelines.

For disposal of uninoculated culture media or any of the reagents and materials included in the SureTect Escherichia coli O157:H7 PCR assay and associated tests, refer to the manufacturer’s material safety data sheets and apply the appropriate local guidelines.

9. QUALITY CONTROL
9.1 Internal Amplification Control
All SureTect PCR pellets contain a probe, primers and DNA template for an internal amplification control (IAC). During PCR the IAC template will be amplified alongside any target DNA present. The probe used for the IAC uses a different coloured fluorescent dye to the probes that are used within the assay to detect target DNA and so can be detected by the Real-Time PCR Instrument through a separate dye channel. The result is that after a successful PCR run the instrument will detect IAC amplification. In the absence of any target DNA being detected by the assay, the presence of IAC amplification curve is confirmation that the PCR process has occurred.

NOTE: Occasionally, the presence of a high level of the target DNA present at the start of the PCR process may cause the IAC to fail to produce an amplification curve. This usually occurs when the target DNA concentration is far more abundant than the IAC control concentration and the target DNA competes more effectively for the components of the PCR reaction. The absence of an IAC amplification curve in the presence of a strong target amplification curve is confirmation that PCR has occurred and a positive call will be made by the PCR analysis software being used (either the SureTect or RapidFinder Express Software version 2.0). If verification of this is required the test may be repeated by first diluting the enriched sample 1:10 – 1:100 and proceeding as before. The failure of both the IAC and the assay target to produce an amplification curve indicates that PCR is invalid and the software will return a “!” result. In this case the test should be repeated.

9.2 Using control organisms
It should not be necessary to incorporate a positive control organisms with routine testing of samples, since it can be shown that the test is functioning through the presence of an amplification curve and result for the Internal Amplification Control (IAC) or presence of a strong amplification curve for the target DNA (in the absence of an amplification curve for the IAC).
However, if for any reason it is required to run control organisms, suitable positive and negative control organisms should be selected. These should then be taken through the complete test procedure (including sample enrichment, lysis and PCR) and processed in parallel to the test samples. Quality control organisms are available from Thermo Fisher Scientific, Microbiology Division. Contact your local supplier for further information.

10. PERFORMANCE LIMITATIONS

10.1 False negative results

The concentration of the target organism must reach around \(10^4\) CFU/ml during the enrichment stage for detection to be possible. Failure of the target organism to reach this level of growth may lead to a false negative result by PCR. Thermo Fisher Scientific can provide detailed information on assay protocols, including enrichment procedures, for the sample matrices that have been externally validated (please contact our Technical Support team (Europe/ROW: email: microbiology.techsupport.uk@thermofisher.com telephone: +44 (0)1256 694238/ USA: email: USLEN-TechServices@thermofisher.com telephone 1-800-255-6730). For other matrices a suitable enrichment protocol must be determined prior to the method being adopted for routine use.

Certain sample types may contain components inhibitory to the growth of the target organism. Therefore, it may be necessary to pre-treat or dilute the sample. Refer to the appropriate local, national or international guidelines, e.g. ISO 68871.

**NOTE:** Some foods may contain compounds that inhibit PCR. Where PCR inhibition is suspected or present during an assay run, the enriched sample should be diluted 1:5 or 1:10 with sterile water before repeating the lysis protocol and analysis.

When testing a sample type or culture medium that has not been validated, it is recommended that a selection of known negative and spiked or known positive samples are tested to ensure that expected results are achieved.

10.2 False positive results

PCR is recognized to be a very sensitive technique for the detection of micro-organisms. It is possible that the SureTect Escherichia coli O157:H7 PCR Assay may produce a positive result that cannot be confirmed by the recommended culture confirmation steps. If this is the case it may be necessary to conduct further steps to confirm the result. The following steps are recommended:

Subculture 0.1ml of the retained enrichment broth into 10ml of mTSB (CM0989) supplemented with 20mg/l novobiocin (e.g. SR0181E). Incubate at 41.5°C ±1°C for 18-24 hours. Plate out 10µl of the incubated mTSB onto CT-SMAC Agar (Sorbital MacConkey Agar supplemented with cefixime and tellurite) (e.g. CM0813 and SR0172) and incubate at 37°C ±1°C for 18-24 hours. Confirm any transparent or almost colourless colonies with a pale yellowish-brown appearance using the confirmation methods detailed in the ISO² or USDA FSIS reference methods. The Oxoid E. coli O157 latex test (DR0620M) or Wellcolex E. coli O157:H7 latex test (R30959601) can be used to confirm the presence of *Escherichia coli* O157 and/or H7 antigens present on presumptive positive isolates on CT-SMAC Agar.
11. PERFORMANCE VALIDATION

AOAC-RI Performance Tested Methods℠ Program (Licence Number: 021501)
The SureTect Escherichia coli O157:H7 PCR Assay has been validated by AOAC-RI using:

according to the Performance Tested Methods℠ validation scheme for the following food matrices: raw ground beef, raw beef trim, spinach and apple juice.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Validated Enrichment Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw ground beef and raw beef trim (1:4 ratio e.g. 375g of sample plus 1,125 ml of mTSB)</td>
<td>Incubate for 9-24 hours in pre-warmed (41.5°C) mTSB at 41.5°C ±1°C†</td>
</tr>
<tr>
<td>Raw ground beef and raw beef trim (1:5 ratio e.g. 375g of sample plus 1,500 ml of mTSB)</td>
<td>Incubate for 9-24 hours in pre-warmed (41.5°C) mTSB at 41.5°C ±1°C†</td>
</tr>
<tr>
<td>Apple Juice and fresh spinach</td>
<td>Incubate 8-24 hours in pre-warmed (41.5°C) mTSB at 41.5°C ±1°C</td>
</tr>
</tbody>
</table>

† Raw beef samples with an aerobic plate count of greater than 10^3 CFU/g are not suitable for the short enrichment protocol when analyzing with the Thermo Scientific PikoReal PCR Instrument and SureTect Software version 1.2 and should be incubated for 24 hours according to the AOAC-RI PTM validation.

NF VALIDATION by AFNOR Certification (Certificate number: UNI 03/10-03/15)
In the context of NF VALIDATION, the SureTect Escherichia coli O157:H7 PCR Assay (along with the SureTect Software and PikoReal Real-Time PCR Instrument) has been certified as an alternative method for the analysis of raw beef meat (using an 8-24 h BPW (ISO) enrichment protocol). The prolongation of the enrichment time to 24 hours allows an improvement in the performance of the method. This validation has been obtained in comparison with the reference method described in the international standard ISO 16654² according to ISO 16140⁵ and the FDIS version of the revision of ISO 16140⁶. The validation included the use of Thermo Scientific SureTect software v1.2 with SureTect Escherichia coli O157:H7 kit file version 1.2.7.55 and the SureTect PikoReal PCR Instrument. For the Applied Biosystems 7500 Fast, the validation included the use of the Applied Biosystems 7500 Fast with Applied Biosystems RapidFinder Express Software version 2.0 and SureTect Escherichia coli kit file version 1. The validation certificate can be obtained from our Technical Support team (Europe: email: microbiology.techsupport.uk@thermofisher.com telephone: +44 (0)1256 694238) or from AFNOR Certification (http://nf-validation.afnor.org/en/). For more information about the end of validity of the NF VALIDATION certification, please refer to the certificate UNI 03/10-03/15 available at http://nf-validation.afnor.org/en/ or from our technical support team (see above).

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Validated Enrichment Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>25g Raw beef meat/products</td>
<td>Incubate for 8-24 hours in pre-warmed (41.5°C) BPW (ISO) at 41.5°C ±1°C</td>
</tr>
</tbody>
</table>
12. ENRICHMENT MEDIA, CONFIRMATION AND OTHER PRODUCTS AVAILABLE FROM THERMO FISHER SCIENTIFIC

- Buffered Peptone Water (ISO) (Oxoid BPW (ISO) product code: CM1049B-500g or CM1049R-2.5kg, 500g makes 25 litres)
- Modified Tryptone Soya Broth (Oxoid, CM0989B-500g, CM0989R-2.5Kg, 500g makes 15.1 litres)
- Sorbitol MacConkey Agar (CM0813B-500g, 500g makes 9.7 litres)
- Cefixime-Tellurite Supplement (SR0172E-10 vials for 500ml)
- Oxoid E. coli O157 latex test (DR0620M)
- Wellcolex™ E. coli O157:H7 latex test (R30959601)
- Homogenizer bags for 25g samples (Unfiltered bag DB4013A, or equivalent)
- Filtered homogenizer bags for 25g samples (DB4011A, or equivalent)
- Homogenizer bags for 375g samples (DB4014A, or equivalent)

13. BIBLIOGRAPHY

13.1 References
1. ISO 6887 Parts 1-5 (most recent edition should be used); Microbiology of food and animal feeding stuffs-Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.
2. ISO 16654:2001; Microbiology of food and animal feeding stuffs-Horizontal method for the detection of Escherichia coli O157.
3. ISO 7218:2007/Amd. 1:2013; Microbiology of food and animal feeding stuffs-General requirements and guidance for microbiological examinations.
4. ISO 22174:2005; Microbiology of food and animal feeding stuffs-Polymerase chain reaction (PCR) for the detection of food pathogens-General requirements and definitions.
5. ISO 16140:2003; Microbiology of food and animal feeding stuffs-Protocol for the validation of alternative methods.

13.2 Trademark & licensing information
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13.3 Field of use
Data obtained using the Thermo Scientific SureTect PCR Assays should not be used for human diagnostic or human treatment purposes. The PikoReal Instrument and 7500 Fast Instrument used in conjunction with the SureTect or RapidFinder Express 2.0 Software and other components of the SureTect PCR System are not approved by the United States Food and Drug Administration or any other US or non-US agency for use in human diagnosis or treatment. The SureTect Assays should not be used as the sole basis for determining the microbiological safety of products for release to consumers. The information generated is only for use in conjunction with the user’s regular quality assurance program. Use for research and development, quality assurance and quality control only, performed by, or under the supervision of a technically qualified person.

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