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Thermo Scientific™ SureTect™ Listeria species PCR Assay PT0200A

PT0200A
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1. INTENDED USE

The Thermo Scientific™ SureTect™ Listeria species 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 the Applied Biosystems™ 7500 Fast Real-Time PCR System and Applied Biosystems™ RapidFinder Express Software version 2.0 for the detection and identification of *Listeria* species from food or food manufacturing environmental 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 Listeria species PCR Assay targets unique DNA sequences specific to *Listeria* species. The SureTect Listeria species PCR Assay includes all of the necessary reagents for bacterial DNA release and PCR. Enriched samples are pipetted into pre-filled Lysis Tubes, along with Proteinase K and lysis reagent 2, 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 Listeria species 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 Listeria species PCR Assay contains all the necessary reagents for DNA release 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 of Proteinase K (clear colorless liquid).

Lysis Reagent 2 (PT0030B)
1 tube containing Lysis Reagent 2 (clear colourless liquid).

SureTect Listeria species PCR Tubes (PT0210A)
12x8 pre-filled, sealed strips. Each tube contains one SureTect Listeria species 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. Please contact your supplier for further information.
Other culture media formats such as prepared media and Dry-Bags™ are also available;
All items required for sample lysis and PCR set-up are provided as part of the SureTect PCR System Start-Up Package.
Please contact your local supplier for further information.

Enrichment

The use of Oxoid 24 Listeria Enrichment Broth (24 LEB) supplemented with 24 LEB Selective Supplement and 24 LEB Buffer Supplement has been validated by both AOAC-Research Institute (RI) and NF VALIDATION and is required to be used when following the validated enrichment protocols.

Sample Preparation

Homogenizer bags for 25g samples (Unfiltered bag DB4013A, or equivalent), or
Filtered homogenizer bags for 25g samples (DB4011A, or equivalent)
Sample pipette (1-10ml) and extra long filtered pipette tips (1-10ml)
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

Repeater pipettor and filtered tips (for faster, easier addition of Proteinase K and lysis reagent 2 to multiple Lysis tubes)
Multi-channel pipette (and filtered tips (to be used in place of the adjustable single channel pipette for processing multiple samples in parallel)

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)

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

NOTE: When using the PikoReal Real-Time PCR Instrument, the instrument must be connected to a PC loaded with the SureTect Software version 1.2 and assay specific kit file version 1.2.7.34 or higher.

NOTE: When using the Applied Biosystems 7500 Fast Real-Time PCR System, the instrument must be connected to a PC loaded with Windows 7 and RapidFinder Express Software version

2.0 and SureTect Listeria species PCR 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[8].

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 *Listeria monocytogenes* colonies. The use of Oxoid *Brilliance*™ Listeria Agar is recommended and has been validated by both AOAC-RI and NF VALIDATION.

7. TEST PROCEDURE

The SureTect Listeria species PCR assay has been validated for a wide range of sample types using the enrichment protocols detailed below. For preparation of initial suspensions, follow the instructions detailed in ISO 6887 parts 1-5[2] and/or ISO 11290-1[1] standards.

Comply with Good Laboratory Practices (refer to ISO 7218[3]). Additionally, it is advised that users follow the general requirements described in ISO 22174:2005[4] 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.

In the context of NF VALIDATION

Enrichment protocols for use with the PikoReal Real-Time PCR Instrument and SureTect Software

Preparation and enrichment for all human food samples:

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

NOTE: It is essential that both the selective and buffer supplements are used when running the analysis.

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

Weigh 25g of the sample to be tested into a homogenizer bag and add a 1:10 ratio of 24 LEB at room temperature plus 10ml of 24 LEB Buffer Supplement for every 225ml of broth and 1 vial of 24 LEB Selective Supplement for each 500ml of broth.

Homogenize thoroughly for 30 seconds to one minute using a homogenizer (set to 230rpm) or by hand for samples containing hard particles, such as bone, peppercorns, etc. Incubate according

to table below (see section 11, “Performance Validation” for specific NF Validation validated food categories).

NF VALIDATION validated enrichment conditions for use when analysing samples with the SureTect PikoReal and SureTect Software

Category	Incubation temperature	Incubation time
Meat samples	37°C ±1°C	24-28h
Dairy products, seafood, produce and production environmental samples	37°C ±1°C	22-26h

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.

Store at 2-8°C for up to 72 hours, if not processing immediately.
NOTE: The 1.5ml portions of enriched samples can be stored at 2-8^{o}C and retained for confirmation of PCR positive results and in case the PCR analysis needs to be repeated (for up to 72 hours post enrichment).

Preparation and enrichment of food manufacturing environmental samples:

Pre-moisten a sterile sampling swab, cloth or sponge in a suitable diluent (e.g. sterile Peptone water). For sampling of areas that have been cleaned or treated with disinfectants and other cleaning agents, pre-moisten the swab, cloth or sponge with a neutralizing broth, such as Dey-Engley Broth, prior to sampling.
Sample the entire area by rubbing the swab, cloth or sponge in both a horizontal and vertical direction across the entire sampling area.
Place the swab, cloth or sponge into the original packaging (suitable for transport). Samples may be held for up to 2 hours at room temperature or 8 hours in the refrigerator prior to proceeding with the next step.

Add swabs to 10ml, sponges to 100ml, or wipes and 25g of dust or solid production environment samples to 225ml of room temperature prepared 24 LEB supplemented with both buffer and selective supplements. Homogenize thoroughly and incubate as detailed in the table above (see section 11, “Performance Validation” for specific NF VALIDATION validated categories).

Remove the enriched sample from the incubator and remove around 1.5ml and dispense into a new tube and close until ready to process the sample to the next stage.
Store at 2-8°C for up to 72 hours, if not processing immediately.

NOTE: The 1.5ml portions of enriched samples can be stored at 2-8^{o}C and retained for confirmation of PCR positive results or in case the PCR analysis needs to be repeated (for up to 72 hours post enrichment).

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

Preparation and enrichment of meat, dairy (including raw/unpasteurised dairy products), seafood and fish products and vegetable samples:
NOTE: In the context of NF VALIDATION, test portions weighing more than 25g have not been tested.
NOTE: It is essential that both the selective and buffer supplements are used when running the analysis.
NOTE: Filtered homogenizer bags should be used to help with fat and particle separation.

Weigh 25g of the sample to be tested into a homogenizer bag and add a 1:10 ratio of 24 LEB at room temperature plus 10ml of 24 LEB Buffer Supplement for every 225ml of broth and 1 vial of 24 LEB Selective Supplement for each 500ml of broth.

Homogenize thoroughly for 30 seconds to one minute using a homogenizer (set to 230rpm) or by hand for samples containing hard particles, such as bone, peppercorns, etc. Incubate according to table below (see section 11, “Performance Validation” for specific NF Validation validated food categories).

NF VALIDATION validated enrichment conditions for use when analysing samples with the Applied Biosystems 7500 Fast and RapidFinder Express Software 2.0

Category	Incubation temperature	Incubation time
Meat, Dairy, Seafood, Fish, vegetables and environmental samples	37°C ±1°C	24-28h

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.
Store at 2-8°C for up to 72 hours, if not processing immediately.

NOTE: The 1.5ml portions of enriched samples can be stored at 2-8^{o}C and retained for confirmation of PCR positive results and in case the PCR analysis needs to be repeated (for up to 72 hours post enrichment).

Preparation and enrichment of food manufacturing environmental samples:

Pre-moisten a sterile sampling swab, cloth or sponge in a suitable diluent (e.g. sterile Peptone water). For sampling of areas that have been cleaned or treated with disinfectants and other cleaning agents, pre-moisten the swab, cloth or sponge with a neutralizing broth, such as Dey-Engley Broth, prior to sampling.
Sample the entire area by rubbing the swab, cloth or sponge in both a horizontal and vertical direction across the entire sampling area.
Place the swab, cloth or sponge into the original packaging (suitable for transport). Samples may be held for up to 2 hours at room temperature or 8 hours in the refrigerator prior to proceeding with the next step.

Add swabs to 10ml, sponges to 100ml, or wipes and 25g of dust or solid production environment samples to 225ml of room temperature prepared 24 LEB supplemented with both buffer and selective supplements. Homogenize thoroughly incubate as detailed in the table above (see section 11, “Performance Validation” for specific NF VALIDATION validated categories).

Remove the enriched sample from the incubator and remove around 1.5ml and dispense into a new tube and close until ready to process the sample to the next stage. Store at 2-8°C for up to 72 hours, if not processing immediately.

NOTE: The 1.5ml portions of enriched samples can be stored at 2-8°C and retained for confirmation of PCR positive results or in case the PCR analysis needs to be repeated (for up to 72 hours post enrichment).

In the context of AOAC-RI *Performance Tested Methods*℠ certification

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

Preparation and enrichment of all food samples

Weigh 25g of the sample to be tested into a homogenizer bag and add a 1:10 ratio of 24 LEB at room temperature plus 10ml of 24 LEB Buffer Supplement for every 225ml of broth and 1 vial of 24 LEB Selective Supplement for each 500ml of broth.
When testing salami, a 1:20 ratio of the sample should be made into 24 LEB with Buffer and Selective Supplements (e.g. 25g of salami for every 475ml of enrichment broth).
Homogenize thoroughly for 30 seconds to one minute using a homogenizer (set to 230rpm) or by hand for samples containing hard particles, such as bone, peppercorns, etc., then incubate according to table below (see section 11, “Performance Validation” for specific AOAC-RI validated matrices and enrichment conditions).

AOAC-RI PTM validated enrichment conditions

Matrix	Enrichment temperature	Enrichment time
All foods and production environmental samples	37°C ±1°C	22-26h

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

Preparation and enrichment of food manufacturing environmental samples:
Pre-moisten a sterile sampling swab, cloth or sponge in a suitable diluent (e.g. sterile Peptone water). For sampling of areas that have been cleaned or treated with disinfectants and other cleaning agents, pre-moisten the swab, cloth or sponge with a neutralizing broth, such as Dey-Engley Broth, prior to sampling.

Sample the entire area by rubbing the swab, cloth or sponge in both a horizontal and vertical direction across the entire sampling area.

Place the swab, cloth or sponge into the original packaging (suitable for transport). Samples may be held for up to 2 hours at room temperature or 8 hours in the refrigerator prior to proceeding with the next step.

Add the swab, cloth or sponge to 225ml of 24 LEB supplemented with 24 LEB Selective Supplement and 10ml of 24 LEB Buffer Supplement at room temperature. Homogenize thoroughly and incubate as detailed above (see section 11, “Performance Validation” for specific AOAC-RI validated matrices and enrichment conditions).

Remove the enriched sample from the incubator and remove around 1.5ml and dispense into a new microfuge tube and close until ready to process the sample to the next stage.
Store at 2-8°C for up to 72 hours, if not processing immediately.

NOTE: The 1.5ml portions of sample should be stored at 2-8°C and retained for confirmation of PCR positive results and in case the PCR analysis needs to be repeated (up to 72 hours post enrichment).

7.2 Sample lysis

NOTE: When analysing samples with the Applied Biosystems 7500 Fast, 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.

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

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, add 10µl of Lysis Reagent 2 to each of the Lysis Tubes in the same manner.
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 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 heat 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 72h 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

Create the template setup on either the SureTect or RapidFinder Express 2.0 software. Refer to the appropriate software manual[6,7] for directions on how to set a template.

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

Remove the required number of SureTect Listeria species 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 will help to expel the liquid while keeping the tip away from the pellet. Ensure the lysate reaches the bottom of the tube for full rehydration of the pellet

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 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 SureTect or RapidFinder Express Software version 2.0 Manual 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

Positive results can be confirmed by pipetting 10µl of the enriched sample onto *Brilliance* Listeria Agar. Inoculate the plate using a diminishing sweep technique and incubate at 37°C ±1°C for 24-48 hours. Observe the plate for presumptive *Listeria* colonies (blue colonies with or without halos) then confirm as positive by either using the Microbact 12L kit, or the confirmatory tests in the ISO Horizontal method for the detection of *Listeria*[1].
For the AOAC-RI validated method, confirmation should proceed from the secondary enrichment step of the ISO Listeria Horizontal method (e.g. Inoculate 100µl of the 24 LEB enrichment into 10ml of Fraser Broth and incubate at 35 or 37°C for 48 hours, before following the ISO Listeria Horizontal method).
If no typical colonies are present on selective *Listeria* media after 24 hours, re-incubate for a further 24 hours and observe again.
Confirm any presumptive colonies as described above.

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 PikoReal or RapidFinder Express Software version 2.0 Manual for result storage, download and reporting options.

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 Listeria species assay must be confirmed. If confirmation is not started immediately after a positive SureTect test result, store the 24 LEB 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. The conventional tests described in the methods standardized by CEN or ISO from colonies (including the purification step). The confirmation step must start from 24 LEB enrichment

broth. Incubate on a chromogenic agar, PALCAM Agar or Oxford Agar. Incubate the agar according to the recommendations of the package insert. Identify 1 to 5 typical colonies using the conventional tests described.

2. Use the confirmation method described above (7.6), plating the 24 LEB enrichment onto *Brilliance* Listeria Agar and incubating at 37°C ±1°C for 24-48 hours. Observe the plate for presumptive positive colonies of *Listeria* spp. (blue colonies with or without opaque white halos). It is not necessary to confirm colonies which give typical morphology and colour on *Brilliance* Listeria Agar but if required by the user confirm from at least one colony directly from *Brilliance* Listeria Agar or PALCAM Agar (without following a purification step) using the Microbact 12L kit, or the confirmatory methods detailed in the ISO Horizontal method for Listeria.

8. DISPOSAL

Once the PCR run is completed, open the Real-Time PCR Instrument and remove the processed samples. Dispose the PCR tubes as hazardous microbiological waste according to 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 could easily contaminate equipment and the nearby laboratory environment and lead to false positive results in future experiments.

Pregnant women and immune-compromised individuals are advised not to carry out this test as it involves the possible culturing of *Listeria monocytogenes* which can result in harmful infections if not handled correctly. Please refer to your local laboratory procedures and safety requirements. Refer to the relevant Real-Time PCR Instrument manual for guidelines on cleaning equipment and handling possible amplicon contamination.

Dispose of all inoculated culture media, even if shown to be negative for the target organism, as hazardous microbiological waste according to local guidelines.

For disposal of uninoculated culture media or any of the reagents and materials included in the SureTect Listeria species assay and associated tests, refer to the manufacturer's material safety data sheets and apply 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 organism 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 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. 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 that are inhibitory to the growth of the target organism. Therefore, it may be necessary to pre-treat or dilute the sample. Refer to ISO 6887[1] or other appropriate international standards.

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 true 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 Listeria species 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 1ml of the retained enrichment broth into 10ml of 24 LEB (with or without 24 LEB Buffered Supplement) and incubate at 37°C ±1°C for 24 hours. Plate out 10µl of the enrichment onto *Brilliance* Listeria Agar and incubate at 37°C ±1°C for 24-48 hours. Confirm presumptive positive blue colonies (with or without halos) as before. If the result still cannot be confirmed as positive, it is recommended that a second PCR test is run from the new 24 LEB enrichment. If this returns a positive result it is likely to be a true positive result

11. PERFORMANCE VALIDATION

AOAC-RI *Performance Tested Methods*SM Program (Licence Number: 071304)

The SureTect Listeria species assay has been validated by AOAC-RI using:

1. SureTect Software v1.2 and kit file version 1.2.9.53 and the SureTect PikoReal PCR Instrument.
2. RapidFinder Express Software version 2.0 and SureTect Listeria species PCR assay specific kit file version 1 and the 7500 Fast PCR Instrument

according to the *Performance Tested Methods*SM validation scheme for the following food matrices and surface samples:

AOAC-RI *Performance Tested Methods*SM Validated Matrices and Enrichment conditions

Matrix	PCR instrument & software	Validated Enrichment Conditions
Smoked salmon, processed cheese, fresh bagged spinach, Cantaloupe melon, cooked prawns, cooked sliced turkey meat, cooked sliced ham, pork Frankfurters, ground/minced raw beef meat, ice cream, raw ground/minced pork, lettuce, raw ground/minced turkey, pork sausage, raw cod, pasteurized brie, pasteurized 2% milk	PikoReal & SureTect Software or 7500 Fast & RapidFinder Express Software 2.0	1:10 ratio of sample to room temperature 24 LEB with selective and buffered supplement. Incubate for 22-26 hours at 37°C ±1°C
Salami	PikoReal & SureTect Software or 7500 Fast & RapidFinder Express Software 2.0	1:20 ratio of sample to room temperature 24 LEB with selective and buffered supplement. Incubate for 22-26 hours at 37°C ±1°C
Plastic environmental/production surfaces, stainless steel environmental/production surfaces	PikoReal & SureTect Software or 7500 Fast & RapidFinder Express Software 2.0	Add sample to 100ml room temperature 24 LEB with selective and buffered supplement. Incubate for 22-26 hours at 37°C ±1°C

NF VALIDATION™ by AFNOR Certification (Certificate number: UNI 03/09-11/13)

In the context of NF VALIDATION, the SureTect Listeria species assay has been certified as an alternative method for the analysis of all human foods and environmental samples. This validation has been obtained in comparison with the reference method described in the international standard ISO 11290-1/A1[1] according to ISO 16140[5].

1. SureTect Software v1.2 and kit file version 1.2.9.53 and the SureTect PikoReal PCR Instrument.
2. RapidFinder Express Software version 2.0 and SureTect Listeria species PCR assay specific kit file version 1 and the 7500 Fast PCR Instrument.

NF VALIDATION certified categories and enrichment conditions

Matrix	PCR instrument & software	Validated Enrichment Conditions
All human foods (except meat products) and production environment samples	PikoReal Instrument with SureTect Software	Incubate for 22-26 hours in 24 LEB with selective and buffered supplement at 37°C ±1°C
Meat products	PikoReal Instrument with SureTect Software	Incubate for 24-28 hours in 24 LEB with selective and buffered supplement at 37°C ±1°C
All human foods and production environment samples	7500 Fast Instrument with RapidFinder Express Software	Incubate for 24-28 hours in 24 LEB with selective and buffered supplement at 37°C ±1°C

The NF 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 validity of the NF VALIDATION certification, please refer to the certificate UNI 03/09-11/13 available at http://nf-validation.afnor.org/en/ or from our technical support team.

BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140

UNI 03/09 – 11/13

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

http://nf-validation.afnor.org/en/

12. ENRICHMENT MEDIA AND CONFRIMATION PRODUCTS

The following enrichment media and biochemical confirmation kits are available from Thermo Fisher Scientific, Microbiology. Please contact your kit supplier for further information.

Oxoid 24 LEB Complete Base (CM1154B-500g or CM1154R-2.5kg, 500g makes 11.5 litres). 24 LEB Complete Base (CM1154) is pre-supplemented with 24 LEB Selective Supplement and does not therefore require the addition of SR0243E.
24 LEB Base (CM1107B-500g, 500g makes 11.5 litres)
24 LEB Selective Supplement (SR0243E, 10 vials each for 500ml)
24 LEB Buffer Supplement (BO1204E-24x10ml each for 225ml). This product may crystallise during storage. Where crystals are present, the tube should be placed in a 37°C water bath for 5-10 minutes. Ensure that all crystals have dissolved before using the Buffer supplement.

Confirmation of positive results
Brilliance Listeria Agar Base (CM1080B-500g or CM1080R-2.5kg, 500g makes 7.4 litres)
Brilliance Listeria Selective Supplement (SR0227E-10 vials for 500ml)
Brilliance Listeria Differential Supplement (SR0228E-10 vials for 500ml)

Further identification reagents such as a miniaturized biochemical identification panel, e.g. Oxoid Microbact 12L (MB1128A), including Microbact Haemolysin Reagent (MB1249A) or latex agglutination test, e.g. Oxoid Listeria Latex Test (DR1126A).

13. BIBLIOGRAPHY

13.1 References

1. ISO 11290-1:1996/Amd 1:2004; Microbiology of food and animal feeding stuffs-Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1: Detection method.
2. 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.
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.
6. SureTect Software User Manual, Rev 1.2; 2013, Thermo Fisher Scientific.
7. Applied Biosystems RapidFinder Express 2.0 User Manual, 2015, Thermo Fisher Scientific.
8. Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide, Rev A; 2006 Thermo Fisher Scientific.

13.2 Trademark & licensing information

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 or 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.

 Thermo Fisher Scientific, Ratastie 2, FI-01620, Vantaa, Finland.