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Simultaneous Detection and Differentiation of Campylobacter from Poultry in less than 24 Hours

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INTRODUCTION

Campylobacter jejuni, C. coli and C. lari from contaminated poultry are causative agents of invasive infections resulting in 1.3 million cases in the United States annually. It is challenging to differentiate these species due to their similar 16s rRNA sequences and phenotypic traits.

This study evaluated performance of the Thermo Scientific™ SureTect™ Campylobacter jejuni, C. coli and C. lari PCR assay in detecting and differentiating three *Campylobacter* targets in poultry samples vs Hygiena™ BAX™ System Real-Time PCR Assay for Campylobacter.

MATERIALS AND METHODS

Pure Isolate Study

Fifty-eight *Campylobacter* isolates and 58 closely-related isolates used to test inclusivity (Figure 1) and exclusivity (Figure 2) respectively.

Matrix Study

Twenty-eight poultry samples including carcass rinse, raw meat with skin and ready to re-heat meat were spiked with *Campylobacter* isolates and tested via PCR using the SureTect (Figure 3) and BAX (Figure 4) methods.

Figure 1. Inclusivity

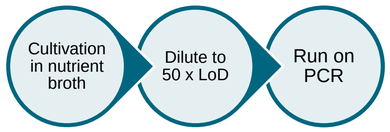


Figure 2. Exclusivity

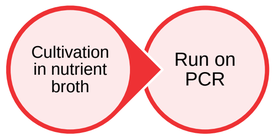


Figure 3. SureTect Workflow

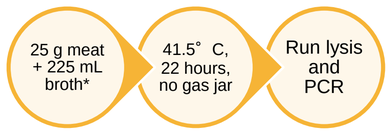
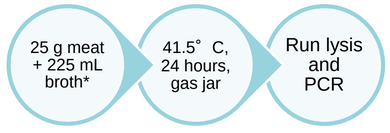


Figure 4. BAX Workflow



^LoD: Limit of Detection, 10^4 CFU/mL
*prewarmed Bolton Broth with selective supplement, without blood

RESULTS

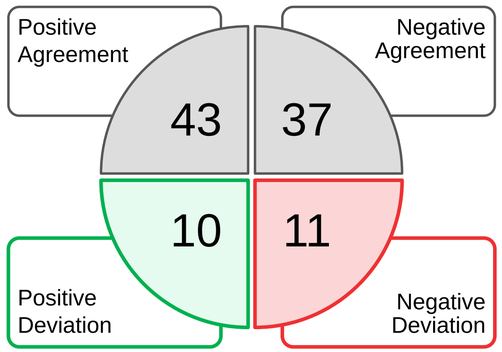
Figure 5. Inclusivity Results (SureTect vs. BAX)

	C. jejuni	*C. coli*	*C. lari*
SureTect	**100%**	**100%**	**100%**
BAX	**100%**	**85%**	**46%**

The SureTect PCR assay demonstrated superior inclusivity compared to the BAX PCR assay for *C. coli* and *C. lari* (Figure 5).

Exclusivity of both assays was 100%.

Figure 6. Method Agreement for *Campylobacter* species



Unpaired Study Key:
Positive Agreement = SureTect Positive, BAX Positive
Negative Agreement = SureTect Negative, BAX Negative
Positive Deviation = SureTect Positive, BAX Negative
Negative Deviation = SureTect Negative, BAX Positive

Table 1. Rate of PCR Positive Detection per Target

Method	*C. jejuni*	*C. coli*	*C. lari*
SureTect	47	30	5
BAX	45	9	3

The method comparison demonstrated that the 22 hour SureTect workflow and 24 hours BAX workflow were comparable when detecting *Campylobacter* species (Figure 6). Co-infection scenarios were frequently missed with the BAX assay compared to SureTect (Table 1); *C. coli* was detected in 21 fewer samples.

CONCLUSIONS

The SureTect PCR Assay demonstrated superior inclusivity performance to the BAX PCR assay.

The SureTect workflow enables users to reliably detect and differentiate *Campylobacter* from poultry samples, without using gas jars, within 24 hours.

TRADEMARKS/LICENSING

LT 2494A
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ThermoFisher SCIENTIFIC

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Thermo Scientific SureTect Cronobacter Species PCR Assay: NF VALIDATION using the Applied Biosystems QuantStudio 5 Food Safety PCR Instrument

Amanda Manolis[1], Jessica Williams[1], Ana-Maria Leonte[1], David Crabtree[1], Katharine Evans[1], Maryse Rannou[2], Muriel Bernard[2]. [1]Thermo Fisher Scientific, Microbiology Basingstoke, UK, [2]ADRIA Développment, Quimper, France

INTRODUCTION

The aim of this study was to extend the NF VALIDATION claims of the Thermo Scientific™ SureTect™ Cronobacter species PCR Assay (SureTect Cronobacter method) for the detection of *Cronobacter* species from powdered infant formula (PIF) and production environment samples to include use with the Applied Biosystems™ QuantStudio™ 5 Food Safety System (Figure 1).

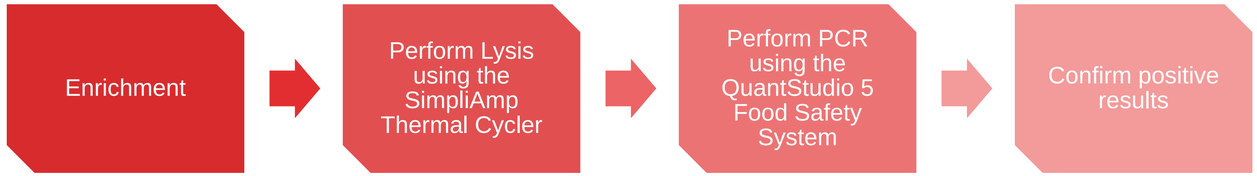
Figure 1. Thermo Scientific™ SureTect™ Food Safety System

Left to right:
Applied Biosystems™ SimpliAmp™ Thermal Cycler
Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR Instrument
Laptop with Applied Biosystems™ RapidFinder™ Analysis Software
Thermo Scientific SureTect PCR Assays

MATERIALS AND METHODS

The SureTect Cronobacter method (Figure 2) was compared to ISO 22964:2017[1], in accordance with ISO 16140-2:2016[2], and the previously validated workflow including the Applied Biosystems™ 7500 Fast Food Safety PCR Instrument with Applied Biosystems™ RapidFinder™ Express Software.

Figure 2: Workflow for the SureTect Cronobacter PCR Assay



RESULTS

During the sensitivity study, a total of eight negative deviation results occurred (six negative deviations and two positive presumptive negative deviations), these are likely due to the low spike levels and the natural variation of an unpaired study design.

A total of 14 positive deviations occurred, showing that the SureTect Cronobacter method has a superior performance for detection in comparison to the reference method. The SureTect Cronobacter method showed considerable improvement from the reference method when testing 300 g PIF samples, which gave nine out of the 14 positive deviations.

The sensitivity study results (Table 1) show that the SureTect Cronobacter method achieved a superior combined sensitivity (91.7%) compared to the reference method (85.4%).

Table 1. Sensitivity study result summary

Category	Sensitivity of the SureTect Cronobacter method (%)	Sensitivity of the Reference method (%)	Relative trueness (%)	False positive ratio (%)
PIF 10 g	100.0	96.7	98.4	5.9
PIF 300 g	86.5	75.7	81.8	10.5
Production environment samples	89.7	86.2	89.6	2.6
Combined result	91.7	85.4	89.4	6.3

The relative level of detection (RLOD) study was performed by analyzing PIF 300 g using the Quantstudio 5 Food Safety PCR System. The RLOD result met the acceptability limit (Table 2).

Table 2. RLOD study result summary

Category	RLOD	Acceptability limit
PIF 300 g	1.482	≤2.5

CONCLUSION

- The SureTect Cronobacter species PCR Assay workflow using the Applied Biosystems QuantStudio 5 Food Safety System has equivalent or improved performance compared to ISO 22964:2017.
- A total of 14 positive deviations show the SureTect Cronobacter method detected more positives than the reference method, particularly for 300 g PIF.
- The SureTect Cronobacter species PCR Assay is an accurate and sensitive method for the detection of *Cronobacter* species from powdered infant formula (10 g and 300 g) and production environment samples.

REFERENCES

1. ISO 22964:2017 Microbiology of the food chain -- Horizontal method for the detection of Cronobacter spp.
2. ISO 16140-2:2016 Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

TRADEMARKS/ LICENSING

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ThermoFisher SCIENTIFIC

thermoscientific

AOAC-RI PTM and NF VALIDATION of the Thermo Scientific SureTect Salmonella species PCR Assay using the QuantStudio 5 Food Safety PCR Instrument

Jessica Williams[1], Ana-Maria Leonte[1], Katharine Evans[1], Annette Hughes[1], Charlotte Cooper[1], Maryse Rannou[2], Muriel Bernard[2] Ben Bastin[3]. [1]Thermo Fisher Scientific, Microbiology Basingstoke, UK, [2]ADRIA Développment, Quimper, France, [3]Q Laboratories Inc., Ohio, US.

INTRODUCTION

Studies were performed to extend the current AOAC-RI Performance tested method℠ (PTM) and NF VALIDATION™ by AFNOR Certification claims for the Thermo Scientific™ SureTect™ Salmonella species PCR Assay (candidate method) to include the use of the Applied Biosystems™ QuantStudio™ 5 Real-Time Food Safety PCR Instrument with associated Applied Biosystems™ RapidFinder™ Analysis software (figure 1).

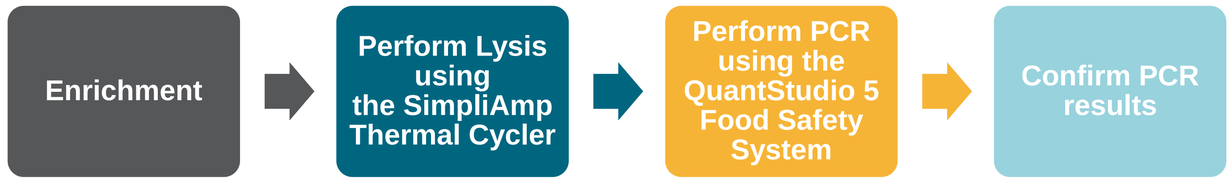
Figure 1. Thermo Scientific™ SureTect™ Real-Time PCR System

Left to right:
Applied Biosystems™ SimpliAmp™ Thermal Cycler
Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR Instrument
Laptop with Applied Biosystems™ RapidFinder™ Analysis Software
Thermo Scientific™ SureTect™ PCR Assays

MATERIALS AND METHODS

- The AOAC-RI PTM method modification study was conducted in comparison to ISO 6579-1:2017[1].
- The NF VALIDATION extension study was conducted in comparison to ISO 6579-1:2017 in accordance with ISO 16140-2:2016.
- The candidate method workflow is summarized in figure 2.

Figure 2. Thermo Scientific SureTect Salmonella species PCR Assay Workflow Summary



RESULTS

NF VALIDATION

Table 1: Sensitivity, Relative Trueness and False Positive Ratio of the Candidate method

Category	Sensitivity of the alternative method %	Sensitivity of the reference method %	Relative trueness %	False positive ratio %
A:	88.4	88.8	88.8	2.6
B:	88.5	88.1	88.4	3.5
C:	88.4	88.4	88.6	2.2
D:	88.5	87.7	88.2	3.1

A = Total of Dairy enriched with Thermo Scientific™ Buffered Peptone Water (BPW) and novobiocin, Raw beef 9hr and all other products
B = Total of Dairy enriched with Thermo Scientific™ One Broth Salmonella (OBS), Raw beef 9hr and all other products
C = Total of Dairy enriched with BPW and novobiocin, Raw beef 24hr and all other products
D = Total of Dairy enriched with OBS, Raw beef 24hr and all other products

The results in table 1 show that the alternative method is better or equivalent in performance to the ISO 6579-1:2017 reference method. The studies performed as part of the NF VALIDATION met the requirements of ISO 16140-2:2016.

AOAC PTM Validation

Table 2: POD Analysis Summary of the Candidate Method

Matrix	Spike level	N	Reference method positives	Alternative method positives	dPOD[a]	95% Confidence interval[b]
All food matrices[c]	n/a	20	0	0	0	-0.16, 0.16
	Low	80	46	38	-0.1	-0.25, 0.25
	High	20	16	15	-0.05	-0.30, 0.21
All surface matrices[d]	n/a	15	0	0	0	-0.20, 0.20
	Low	40	10	10	0	-0.19, 0.19
	High	10	8	8	0	-0.34, 0.34

[a]Difference in POD between the alternative and reference methods
[b]If the 95% CI does not contain a zero the results are statistically significant at the 5% level
[c]Raw ground beef (9 hr and 24 hr protocols), Skimmed milk powder, Lettuce
[d]Plastic surface swabs (1x1") and sponges (4x4")

The results in table 2 show no statistically significant differences between the candidate method and the reference method. Inclusivity and exclusivity testing demonstrated that the candidate method successfully detected all target *Salmonella* species isolates and correctly excluded all non-target isolates

CONCLUSION

Superior Salmonella detection

- Detects Salmonella species in a broad range of food and environmental surfaces
- Superior or equivalent performance to the reference method.

AOAC and AFNOR Validated

- Data satisfied the acceptability criteria of AOAC PTM and NF VALIDATION by AFNOR Certification

Improved workflow using the QuantStudio 5 Food Safety System

- The QuantStudio 5 Food Safety System uses a 6-channel, 96-well cloud-enabled platform
- Suitable for running PCR solutions for food pathogen and authenticity testing
- Instrumentation offers touch screen technology along with intuitive software

REFERENCES

1. ISO 6579-1:2017 Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella spp
2. ISO 16140-2:2016 Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

TRADEMARK STATEMENT

LT2486A
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ThermoFisher SCIENTIFIC

thermoscientific

Thermo Scientific SureTect E. coli O157:H7 PCR Assay: AOAC-RI PTM Validation using the Applied Biosystems QuantStudio 5 PCR Instrument

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INTRODUCTION

The Thermo Scientific™ SureTect™ E.coli PCR Assay (candidate method) has been certified by AOAC-RI *Performance tested method*^{SM} (PTM 021501) for the detection of *Escherichia coli* O157:H7 from 375 g ground beef, 375 g raw beef trim, 25 g spinach and 25 mL apple juice.

The aim of the study was to extend the current claims for the candidate method to include the use of the Applied Biosystems™ SimpliAmp Thermal Cycler for sample lysis and the Applied Biosystems™ QuantStudio™ 5 Real-Time Food Safety PCR Instrument with associated Applied Biosystems™ RapidFinder™ Analysis software (QuantStudio 5 Food Safety System) as shown in figure 1.

Figure 1. Thermo Scientific SureTect Real-Time PCR System

MATERIALS AND METHODS

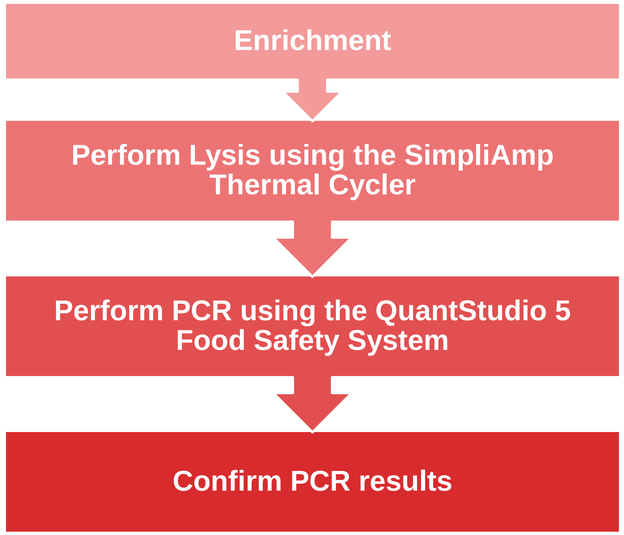
The QuantStudio 5 Food Safety System uses a 6-channel, 96-well cloud-enabled platform suitable for running a wide range of PCR solutions for food pathogen and authenticity testing.

The candidate method was analysed with raw ground beef, raw beef trim and spinach according to the protocols detailed in figures 2 and 3.

Figure 2. Sample Preparation and Reference Method Summary

Raw ground beef and beef trim	• 1-in-4 and 1-in-5 ratio in prewarmed Oxoid™ Modified Tryptone Soya Broth (mTSB) • Sampled at 9 and 24 hours • Compared to USDA/FSIS MLG 5.09[1]
Spinach and apple juice	• 1-in-10 ratio in prewarmed mTSB • Sampled at 8 and 24 hours • Compared to ISO 16654:2001[2]

Figure 3: Summarized Workflow for the SureTect E. coli O157:H7 PCR Assay Extension Study



RESULTS

Inclusivity

All 52 *E. coli* O157:H7 isolates were correctly identified.

Exclusivity

All 30 exclusivity isolates were correctly excluded.

Probability of Detection (POD) Analysis

Statistical analysis using Probability of Detection (POD) at 95% confidence levels demonstrated no statistical difference between the candidate and reference method during any of the validation studies (table 1).

Table 1. Summary of POD Results

Spike level	N	Candidate method positives	Reference method positives	dPOD	95% CI
n/a	15	0	0	0.00	-0.20, 0.20
Low	60	23	20	0.05	-0.12, 0.22
High	15	15	15	0.00	-0.20, 0.20

There were no statistically significant differences for any matrix individually.
N = Number of samples
dPOD= difference in POD between methods
95% CI= if the 95% confidence interval does not contain a zero the results are statistically significant at the 5% level

CONCLUSION

The SureTect E. coli O157:H7 PCR method using the QuantStudio 5 PCR Instrument for PCR and RapidFinder Analysis software for data analysis proved to be a suitable substitute to the reference methods for *E. coli* O157:H7 detection in raw ground beef, raw beef trim, spinach and apple juice.

REFERENCES

1. USDA FSIS MLG5.09: Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges. Effective date 01/15/15.
2. ISO 16654:2001 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of Escherichia coli O157

TRADEMARKS/ LICENSING

LT2466A
July 2019

ThermoFisher SCIENTIFIC

thermoscientific

Improved Confirmation of STEC Contaminants Using the Thermo Scientific SureTect E. coli O157:H7 and STEC Screening and Identification PCR Assay

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INTRODUCTION

Microflora from food samples pose a challenge for shiga-toxin producing *Escherichia coli* (STEC) culture confirmation testing.

These studies assessed performance of the Thermo Scientific™ SureTect™ E. coli O157:H7 and STEC Screening and Identification PCR Workflow confirmation method using Thermo Scientific™ Ocoid™ Tryptone Bile X-Glucuronide Medium (TBX), Thermo Scientific™ Chromogenic Coliform Agar (CCA) and CHROMagar™ STEC.

MATERIALS AND METHODS

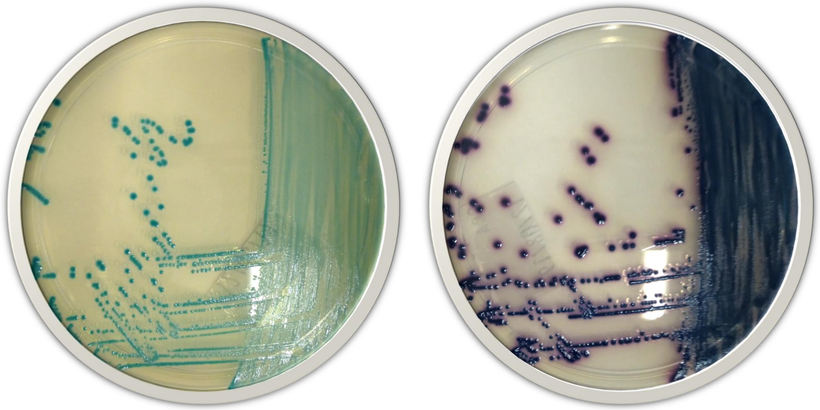
Pure Isolate Study

"Big Six" STEC pure isolates (n=38) were streaked onto CCA (Figure 1) and CHROMagar STEC and the inclusivity of both plating media compared.

Matrix Study

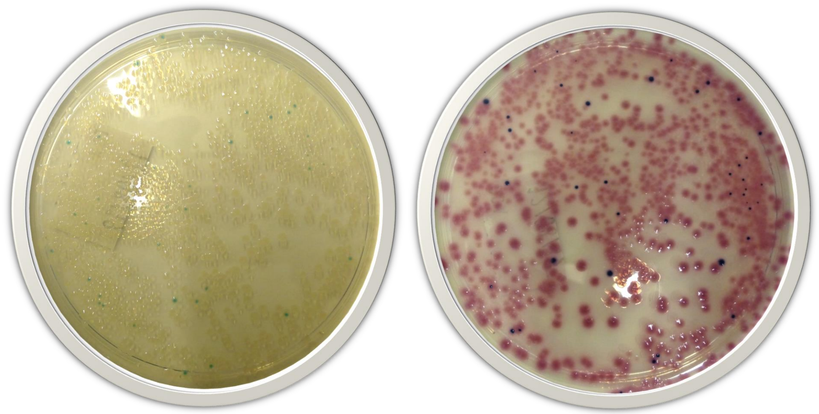
Ten vegetable samples, including sprouted seeds, were artificially contaminated with STEC at 0.67-1.79 CFU/25 g and enriched alongside unspiked samples with the same microflora. These were tested with the SureTect PCR kit and confirmed via the SureTect confirmation plating protocol (Figure 2).

Figure 1. Typical *E. coli* growth on TBX (left) and CCA (right)



TBX differentiates *E. coli* (green) from background (colourless). CCA uses chromogenic compounds to differentiate *E. coli* (dark blue) from background (pink).

Figure 2. Presumptive STEC with high levels of background flora from vegetables on TBX (left) and CCA (right)

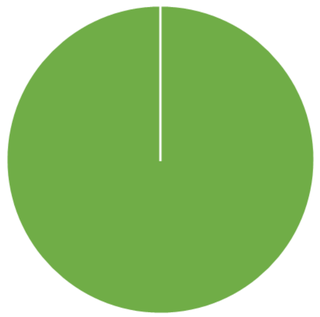


Isolating *E. coli* from CCA is simpler compared to TBX when background flora is present.

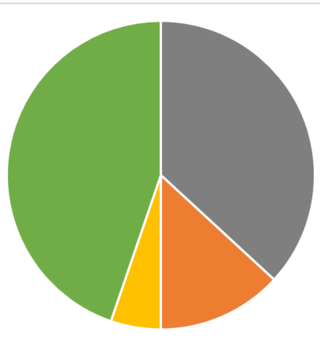
RESULTS

Figure 3. Comparative growth levels of STEC on:

a.) CCA – 38/38 isolates grew well



b.) CHROMagar –19/38 isolates had inhibited growth



Key:

- Growth to tertiary bed
- Growth to secondary bed
- Growth in primary bed only
- No growth observed

Pure Isolate Study

CCA facilitated typical morphology for 36 (94.7%) pure isolates, while CHROMagar exhibited this for 22 (57.9%). Two isolates grew strongly on CCA but had pink morphology rather than the expected blue.

CHROMagar failed to recover 14 STEC isolates and exhibited reduced growth levels for a further five (Figure 3).

Matrix Study

The SureTect method confirmed six STEC isolates despite high levels of background flora. Five positive samples were confirmed using a direct streak method on CCA while the final positive was confirmed using immunomagnetic separation and CCA. CHROMagar STEC had comparable performance to CCA.

TBX failed to confirm presence of STEC for three out of six confirmed positive samples.

CONCLUSIONS

Effective isolation of STEC relies upon the considered selection of confirmation plating media. These studies demonstrate the effectiveness of the SureTect confirmation plating method using CCA, enabling highly contaminated STEC samples to be confirmed rapidly and reliably.

TRADEMARKS/LICENSING

LT 2493A, September 2019

ThermoFisher SCIENTIFIC

thermoscientific

Evaluation of a New Multiplex PCR Assay for Detection of STEC from Beef Meat Samples

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INTRODUCTION

Shiga toxin-producing *Eschericia coli* (STEC) are a group of pathogenic organisms that may cause severe disease including hemolytic uremic syndrome (HUS). STEC outbreaks have been linked to a number of food sources including beef and vegetables.

The Thermo Scientific™ SureTect™ STEC PCR Assay (candidate method) detects multiplex genetic targets for O157:H7 and other STEC from food and environmental samples. The SureTect STEC PCR Assay kit comprises two multiplex reactions for the simultaneous detection of the following targets:

- **Screening Assay:** O157:H7, *stx*, *eae*
- **Identification Assay:** O26, O103, O111, O145, O45, O121

This study evaluated the performance of the SureTect STEC PCR Assay (candidate method) for the detection of STEC from beef meats vs. the ISO 13136:2012 reference method[1].

METHODS

Three categories of beef meat samples (raw, seasoned and frozen) were divided into 25 g portions and artificially contaminated with a range STEC isolates from different serogroups (Table 1). The samples were then tested using the candidate method workflow (Figure 1) and associated instrumentation (Figure 2). A replicate set of samples was tested according to the ISO reference method.

Beef Matrix Type	Spiked (N)	Spike Level (CFU)	Unspiked (N)
Raw	7	0.4 – 3.6	7
Seasoned	7	0.4 – 2.2	4
Frozen	7	1.8 – 3.0	7

Post enrichment, all candidate method samples were tested and streaked onto isolation agars for confirmation including; Thermo Scientific™ Oxoid™ Chromogenic Coliform Agar and Thermo Scientific™ Oxoid™ TBX Medium.

In cases where plating direct from enrichment broth was unsuccessful, a purification step using serogroup-specific Dynabeads and Immunomagnetic separation (IMS) was used before plating.

MATERIALS

Figure 1. SureTect STEC PCR Assay Process Flow

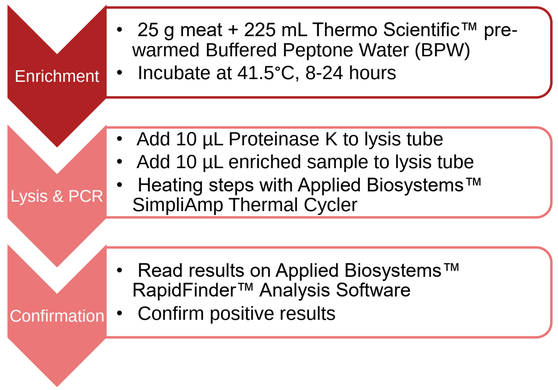


Figure 2: Applied Biosystems™ QuantStudio 5™ Food Safety System

*Applied Biosystems™ QuantStudio 5 Food Safety Real-Time PCR Instrument and laptop with Applied Biosystems™ RapidFinder™ Analysis software

RESULTS

Table 2. Candidate Method Performance by Beef Meats Category

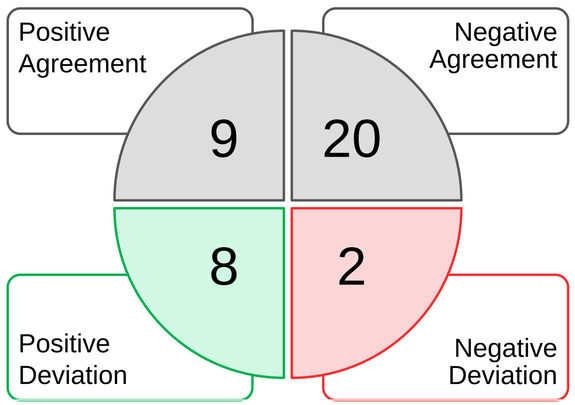
Method Performance	Raw Beef	Seasoned Beef	Frozen Beef
Positive Agreement	2	5	2
Negative Agreement	8	4	7
Positive Deviation	4	2	2
Negative Deviation	0	0	2

Unpaired Study Key:

Positive Agreement = Candidate Method Positive, Reference Method Positive

Positive Deviation = Candidate Method Positive, Reference Method Negative

Figure 3. Overall Candidate Method Performance with Beef Meats



Negative Agreement = Candidate Method Negative, Reference Method Negative

Negative Deviation = Candidate Method Negative, Reference Method Positive

RESULTS SUMMARY

The reference method obtained fewer positive results from raw and seasoned meat than the candidate method. The methods performed comparably with frozen beef meat (Table 2).

The difference between the negative deviations and positive deviations was -6 (Figure 3), indicating that the candidate method performed better than the reference method.

CONCLUSIONS

Improved Performance

The SureTect STEC PCR workflow demonstrates improved performance over the ISO reference method

Rapid Time to Result

Samples are enriched for only 8 hours with the SureTect method compared to the ISO reference method of 18 hours

Simple Enrichment

The SureTect workflow utilises enrichment in BPW without the need for antibiotics or proprietary media

ACKNOWLEDGEMENTS

The authors would like to thank ADRIA Développement, Quimper, France, for conducting data generation for this study.

REFERENCES

1. ISO/TS 13136:2012 Microbiology of food and animal feed -- Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens -- Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups

TRADEMARKS/LICENSING

LT 2477A
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ThermoFisher SCIENTIFIC

thermoscientific

AOAC-RI PTM and NF Validation of the Thermo Scientific Listeria Species PCR Assay using the QuantStudio 5 Food Safety PCR Instrument

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[1]Thermo Fisher Scientific, Basingstoke, UK, [2]ADRIA Développement, Quimper, France, [3]Q Laboratories Inc., Ohio, US.

INTRODUCTION

Studies were performed to extend the current AOAC-RI *Performance tested method*℠ (PTM) and NF VALIDATION™ by AFNOR Certification for the Thermo Scientific™ SureTect™ Listeria species PCR Assay (candidate method) to include the use of the Applied Biosystems™ QuantStudio™ 5 Real-Time Food Safety PCR Instrument with associated Applied Biosystems™ RapidFinder™ Analysis software.

Figure 1. Thermo Scientific™ SureTect™ Real-Time PCR System

Left to right:
Applied Biosystems™ SimpliAmp™ Thermal Cycler
Applied Biosystems QuantStudio 5 Real-Time PCR Instrument
Applied Biosystems RapidFinder Analysis software
Thermo Scientific™ SureTect™ Kits

The QuantStudio 5 Food Safety System uses a 6-channel, 96-well (Figure 2) cloud-enabled platform suitable for running a wide range of PCR solutions for food pathogen and authenticity testing. Instrumentation offers easy to use touch screen technology along with intuitive software to streamline the workflow.

Figure 2. Thermo Scientific SureTect PCR Tubes being loaded in the QuantStudio 5 Food Safety PCR Instrument

RESULTS

AFNOR Validation

The NF VALIDATION by AFNOR Certification extension studies were conducted in comparison to ISO 11290-1:1996 in accordance with ISO 16140-2:2016[2].

Table 1: Sensitivity, relative trueness and false positive ratio of the candidate methods

Number tested	Sensitivity of the Candidate method (%)	Sensitivity of the Reference method (%)	Relative trueness (%)	False positive ratio (%)
378	78.9	77.9	78.3	4.8

The candidate method gave equivalent or improved performance to the reference method (shown in table 1) and satisfied the requirements of ISO 16140-2:2016.

RESULTS

AOAC PTM Validation

The AOAC PTM method modification study were conducted in comparison to ISO 11290-1:1996[1] in accordance with AOAC Appendix J[3].

Table 2. POD analysis of the SureTect Listeria species PCR Assay and the ISO 11290-1:1996 Reference Method

Matrix type	Spike level	No. tested	Reference method positives	Candidate method positives[a]	dPOD[b]	95% CI[c]
All food matrices[d]	n/a	20	0	0	0.00	-0.16, 0.16
	Low	80	46	38	-0.10	-0.25, 0.05
	High	20	16	15	-0.05	-0.30, 0.21
All surface matrices[e]	n/a	15	0	0	0.00	-0.20, 0.20
	Low	40	10	10	0.00	-0.19, 0.19
	High	10	8	8	0.00	-0.34, 0.34

[b]Difference in POD between the candidate and reference methods
[c]If the 95% confidence interval does not contain a zero the results are statistically significant at the 5% level
[d]Raw ground beef (9 hr and 24 hr protocols), skimmed milk powder, lettuce
[e]Plastic surface swabs (1x1") and sponges (4x4")

The Probability of Detection (POD) analysis (table 2) demonstrated no statistically significant differences between the candidate methods and the reference method.

Inclusivity and exclusivity testing demonstrated that the candidate methods successfully detected all target *Listeria* spp. isolates and excluded all non-target isolates.

CONCLUSION

Superior Listeria detection

- Detects Listeria species in a broad range of foods
- Superior or equivalent performance to the ISO 11290-1:1996 reference method.

AOAC and AFNOR Validated

- Data satisfied the acceptability criteria of AOAC PTM and NF VALIDATION by AFNOR Certification

Improved workflow using the QuantStudio 5 Food Safety System

- The QuantStudio 5 Food Safety System uses a 6-channel, 96-well cloud-enabled platform
- Suitable for running PCR solutions for food pathogen and authenticity testing
- Instrumentation offers touch screen technology along with intuitive software

REFERENCES

1. ISO 11290-1:1996, including Amendment 1:2004 'Microbiology of the food chain -- Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. -- Part 1: Detection method'
2. ISO 16140-2:2016 Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
3. AOAC Appendix J:

TRADEMARKS/ LICENSING

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September 2019

ThermoFisher SCIENTIFIC

thermoscientific

AOAC-RI PTM and NF Validation of the Thermo Scientific Listeria monocytogenes PCR Assay using the QuantStudio 5 PCR Food Safety Instrument

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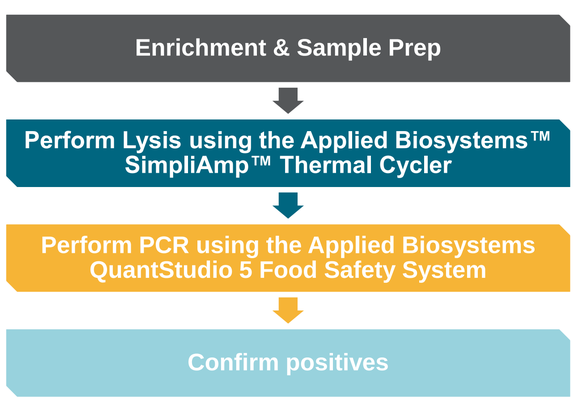
INTRODUCTION

Studies were performed to extend the current AOAC-RI *Performance Tested Method*℠ (PTM) and NF VALIDATION™ by AFNOR Certification claims for the Thermo Scientific™ SureTect™ Listeria monocytogenes PCR (candidate method) to include the use of the Applied Biosystems™ QuantStudio™ 5 Real-Time Food Safety PCR Instrument with associated Applied Biosystems™ RapidFinder™ Analysis software.

MATERIALS AND METHODS

For the extension of the AOAC-RI PTM validation method modification studies were conducted in comparison to ISO 11290-1:1996[1]. For the NF VALIDATION by AFNOR Certification extension studies were conducted in comparison to ISO 11290-1:1996 in accordance with ISO 16140-2:2016[2].

Figure 1: Workflow for the SureTect Listeria monocytogenes PCR Assay



RESULTS

AOAC PTM Validation

- The Probability of Detection (POD) analysis (table 1) demonstrated no statistically significant differences between the candidate methods and the reference method.
- Inclusivity and exclusivity testing demonstrated that the candidate methods successfully detected all target *L. monocytogenes* isolates and excluded all non-target isolates.

Table 1. POD analysis of the SureTect Listeria monocytogenes PCR assay and the reference method

Matrix type	Spike level	Number tested	Reference method positives	Candidate method positives[a]	dPOD[b]	95% CI[c]
All food matrices[d]	n/a	20	0	0	0.00	-0.16, 0.16
	Low	80	46	38	-0.10	-0.25, 0.05
	High	20	16	15	-0.05	-0.30, 0.21
All surface matrices[e]	n/a	15	0	0	0.00	-0.20, 0.20
	Low	40	10	10	0.00	-0.19, 0.19
	High	10	8	8	0.00	-0.34, 0.34

[b]Difference in POD between the candidate and reference methods
[c]If the 95% confidence interval does not contain a zero the results are statistically significant at the 5% level
[d]Raw ground beef (9 hr and 24 hr protocols), skimmed milk powder, lettuce
[e]Plastic surface swabs (1x1") and sponges (4x4")

AFNOR Validation

- Data was analyzed in accordance with ISO 16410-2:2016 (table 2).
- The candidate methods gave equivalent or improved performance to the reference method.

Table 2: Sensitivity, relative trueness and false positive ratio of the candidate methods

Number of samples	Sensitivity of the Candidate method	Sensitivity of the Reference method	Relative trueness	False positive ratio
387	80.2 %	77.8 %	81.9 %	2.7 %

Figure 1. Applied Biosystems QuantStudio 5 Food Safety Real-Time PCR Instrument

CONCLUSION

The AOAC-RI PTM and NF VALIDATION studies demonstrated that the SureTect Listeria monocytogenes assay is a suitable method for the detection of *Listeria monocytogenes* from a variety of food and environmental surface samples when using the QuantStudio 5 Food Safety System (Figure 1).

REFERENCES

1. ISO 11290-1:1996, including Amendment 1:2004 'Microbiology of the food chain -- Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. -- Part 1: Detection method'
2. ISO 16140-2:2016 Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

TRADEMARKS/ LICENSING

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ThermoFisher SCIENTIFIC

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Thermo Scientific SureTect Listeria monocytogenes PCR Assay and SureTect Listeria species PCR Assay: AOAC-RI PTM and NF VALIDATION using the QuantStudio 5 PCR Instrument

Amanda Manolis[1], Jessica Williams[2], Ana-Maria Leonte[2], David Crabtree[2], Katharine Evans[2], Maryse Rannou[3], Muriel Bernard[3], Ben Bastin[4]. [1]Thermo Fisher Scientific, Austin, US, [2]Thermo Fisher Scientific, Basingstoke, UK, [3]ADRIA Développement, Quimper, France, [4]Q Laboratories Inc., Ohio, US.

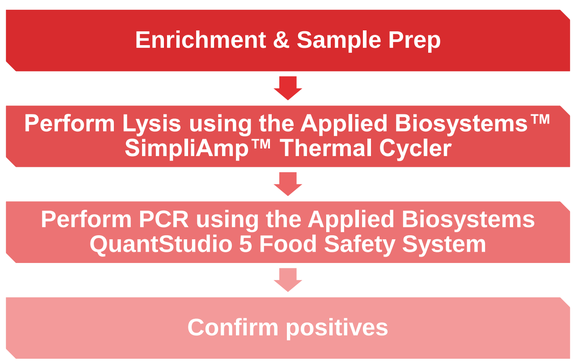
INTRODUCTION

Studies were performed to extend the current AOAC-RI Performance tested method^{SM} (PTM) and NF VALIDATION by AFNOR Certification claims for the Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay and the Thermo Scientific™ SureTect™ Listeria species PCR Assay (candidate methods) to include the use of the Applied Biosystems™ QuantStudio™ 5 Real-Time Food Safety PCR Instrument with associated Applied Biosystems™ RapidFinder™ Analysis software.

MATERIALS AND METHODS

For the extension of the AOAC-RI PTM validation method modification studies were conducted in comparison to ISO 11290-1:1996[1]. For the NF VALIDATION by AFNOR Certification extension studies were conducted in comparison to ISO 11290-1:1996 in accordance with ISO 16140-2:2016[2].

Figure 1: Workflow for the SureTect Listeria monocytogenes and SureTect Listeria species PCR Assays



RESULTS

AOAC PTM Validation

- The SureTect L. monocytogenes and SureTect L. species Assays returned identical results.
- The Probability of Detection (POD) analysis (table 1) demonstrated no statistically significant differences between the candidate methods and the reference method.
- Inclusivity and exclusivity testing demonstrated that the candidate methods successfully detected all target *L. monocytogenes* and *Listeria* spp. isolates and excluded all non-target isolates.

Table 1. POD analysis of the SureTect Listeria monocytogenes and the SureTect Listeria species PCR assay and the reference method

Matrix type	Spike level	Number tested	Reference method positives	Candidate method positives[a]	dPOD[b]	95% CI[c]
All food matrices[d]	n/a	20	0	0	0.00	-0.16, 0.16
	Low	80	46	38	-0.10	-0.25, 0.05
	High	20	16	15	-0.05	-0.30, 0.21
All surface matrices[e]	n/a	15	0	0	0.00	-0.20, 0.20
	Low	40	10	10	0.00	-0.19, 0.19
	High	10	8	8	0.00	-0.34, 0.34

[a]Represents both SureTect Listeria monocytogenes and SureTect Listeria species PCR Assay results (results were identical)
[b]Difference in POD between the candidate and reference methods
[c]If the 95% confidence interval does not contain a zero the results are statistically significant at the 5% level
[d]Raw ground beef (9 hr and 24 hr protocols), skimmed milk powder, lettuce
[e]Plastic surface swabs (1x1") and sponges (4x4")

AFNOR Validation

- Data was analyzed in accordance with ISO 16410-2:2016 (table 2).
- The candidate methods gave equivalent or improved performance to the reference method.

Table 2: Sensitivity, relative trueness and false positive ratio of the candidate methods

SureTect PCR Assay	Number tested	Sensitivity of the Candidate method (%)	Sensitivity of the Reference method (%)	Relative trueness (%)	False positive ratio (%)
Listeria monocytogenes	387	80.2	77.8	81.9	2.7
Listeria species	378	78.9	77.9	78.3	4.8

Figure 2. Thermo Scientific SureTect Real-Time PCR System (Thermo Scientific SureTect PCR Assays, Applied Biosystems QuantStudio 5 Food Safety System and Applied Biosystems SimpliAmp Thermal Cycler)

CONCLUSION

The AOAC-RI PTM and NF VALIDATION studies demonstrated that the SureTect PCR Assays are suitable methods for the detection of *Listeria monocytogenes* and *Listeria* spp. from a variety of food and environmental surface samples when using the QuantStudio 5 Food Safety System (Figure 2).

REFERENCES

1. ISO 11290-1:1996, including Amendment 1:2004 'Microbiology of the food chain -- Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. -- Part 1: Detection method'
2. ISO 16140-2:2016 Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

TRADEMARKS/ LICENSING

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