STUDY REPORT

# SureTect Salmonella species PCR Assay Workflow NF VALIDATION ISO 16140 – Extension Study: Method Comparison

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## Summary

As part of the NF VALIDATION™ ISO 16140 extension study of the Thermo Scientific™ SureTect™ Salmonella species PCR Assay workflow (alternative method), a method comparison study was conducted by ADRIA Développement, Quimper, France. The extension study was designed to validate use of the SureTect Salmonella species PCR Assay with two real-time PCR systems: the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument and SureTect Software v1.2 for dairy samples and the Applied Biosystems™ 7500 Fast Real-Time PCR System and Applied Biosystems™ RapidFinder™ Express version 2.0 Software for meat products, dairy products, infant formula, seafood and vegetables and production environment samples. This report presents the results from the expert laboratory study conducted to validate the alternative method against the ISO reference method detailed in ISO 6579:2002, following the rules of ISO 16140-2:2016.

## Methodology

Expert Laboratory Study

A total of four hundred and thirty-eight samples covering meat and dairy products, dried infant formula, seafood and vegetables and production environment samples were analyzed during the expert laboratory study, which was designed to validate the performance of the SureTect Salmonella species PCR Assay on the SureTect PikoReal Instrument with SureTect Software v1.2 for dairy samples and 391 samples were analysed for the use of the SureTect Salmonella species PCR Assay with the Applied Biosystems 7500 Fast System and RapidFinder Express v2.0 Software with SureTect Salmonella Kit File for meat, dairy, infant formula, seafood and vegetables and production environment samples.

A total of 149 samples were artificially contaminated by a spiking protocol and 21 samples by a seeding protocol, when samples were analysed with the SureTect PikoReal Instrument. When samples were analysed using the Applied Biosystems 7500 Fast System, 25 samples were artificially contaminated by a spiking protocol and 136 by a seeding protocol.

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## Protocol

Alternative Method

For the SureTect PikoReal Instrument, twenty-five gram dairy samples were homogenised with 225 ml of room temperature Thermo Scientific™ Oxoid™ ONE Broth Salmonella Base supplemented with 12 mg/l novobiocin and enriched by incubating for 20 to 24 hours at 37±1 °C. Additionally 25 gram samples were homogenised with 225 ml of room temperature Buffered Peptone Water (ISO) (BPW (ISO)) supplemented with 12 mg/lnovobiocin and enriched by incubating for 20 to 24 hours at 37±1 °C.

For the Applied Biosystems 7500 Fast System, twenty-five gram samples were enriched as follows:

- Meat (excluding raw beef meats) and seafood and vegetables samples were homogenised with 225 ml of room temperature BPW (ISO) supplemented with 12 mg/l novobiocin and enriched by incubating for 20 to 24 hours at 37±1 °C.
- Raw beef meat samples were homogenised with 225 ml of pre-warmed (41.5°C) BPW (ISO) and enriched by incubating for 9 to 24 hours at 37±1 °C.
- Infant formula samples were homogenised with 225 ml of room temperature BPW (ISO) and enriched by incubating for 16 to 20 hours at 37±1 °C.
- Dairy samples were homogenised with 225 ml of room temperature Oxoid ONE Broth Salmonella Base supplemented with 12 mg/l novobiocin and enriched by incubating for 20 to 24 hours at 37±1 °C. Additionally, samples were homogenised with 225 ml of room temperature BPW (ISO) supplemented with 12 mg/l novobiocin and enriched by incubating for 20 to 24 hours at 37±1 °C.
- Production environment samples were homogenised with room temperature BPW (ISO) and enriched by incubating for 20 to 24 hours at 37±1 °C, following the below dilution ratios:
  - Twenty-five gram production environment samples and wipes were homogenised with 225 ml of BPW (ISO)
  - Production environment swabs were homogenised with 10 ml of BPW (ISO)
  - Production environment sponges were homogenised with 100 ml of BPW (ISO)

For the complete list of validated protocols, please see flow diagrams on the next page.

Ten microlitres of SureTect Proteinase K Reagent were added to each of the required SureTect Lysis Tubes (supplied prefilled with Lysis Reagent 1). Next 10 µl of the enriched samples were added to the Lysis Tubes, which were then heated at 37±1 °C for 10 minutes, followed by 95±1 °C for 5 minutes. When performing PCR using the Applied Biosystems 7500 Fast Instrument, a negative control sample was prepared by adding 10 µl sterile water to a SureTect Lysis Tube. The tubes were cooled at room temperature prior to transferring 20 µl aliquots of the lysates to SureTect Salmonella species PCR Tubes containing SureTect Salmonella species PCR tablets.

The PCR Tubes were then immediately sealed and transferred to the SureTect PikoReal Instrument or the Applied Biosystems 7500 Fast System for processing.

Positive PCR results were confirmed by streaking a 10 µl loopful of the enrichment broth onto a plate of Thermo Scientific™ Oxoid™ *Brilliance*™ Salmonella Agar and confirming typical purple-coloured colonies with either the Thermo Scientific™ Oxoid™ Microbact™ GNB 24E Kit or the Thermo Scientific™ Oxoid™ Salmonella Latex Test.

ISO Method

Twenty-five gram samples were analyzed according to ISO 6579:2002. Each sample was enriched by incubating at 37±1 °C for 16 to 20 hours in 225 ml of BPW (ISO). After primary enrichment, 0.1 ml of the BPW enrichment was inoculated in 10 ml of Rapapport-Vassiliadis Soya Peptone (RVS) Broth and incubated for 21 to 27 hours at 41.5±1 °C. After incubation, plates of Xylose-Lysine-Deoxycholate (XLD) Agar and another chromogenic agar were streaked and incubated for 21 to 27 hours at 37±1 °C. A further 1 ml of the BPW enrichment was inoculated in 10 ml of Muller-Kauffman Tetrathionate Broth supplemented with novobiocin (MKTTn) and incubated at 37±1 °C for 21 to 27 hours. After incubation plates of XLD Agar and another chromogenic agar were streaked and incubated for 21 to 27 hours at 37±1 °C. Up to five characteristic colonies were streaked onto Nutrient Agar to produce pure cultures and growth was confirmed by biochemical and serological identification tests, as detailed in the reference method.

## Alternative method flow diagram: Protocol for all samples using the SureTect PikoReal Instrument

**Day: 0**

- Add 25 g raw beef meats to 225 ml of pre-warmed BPW (at 41.5±1 °C).
  *Incubate at 41.5±1°C for 8-24 hours.*
- Add 25 g meat samples to 225 ml of BPW.
  *Incubate at 37±1 °C for 20-24 hours.*
- Add 25 g seafood or vegetable samples to 225 ml of BPW.

- Add 25 g egg samples to 225 ml of BPW.
  *Incubate at 37±1 °C for 20-24 hours.*
- Add 25 g pet food samples to 225 ml of ONE Broth Salmonella supplemented with ONE Broth Salmonella Supplement.
  *Incubate at 37±1 °C for 20-24 hours.*

- Add 25 g dairy to 225 ml of BPW supplemented with 12 mg/l novobiocin.
  *Incubate at 37±1°C for 20-24 hours.*

  OR
- Add 25 g dairy to 225 ml of ONE Broth Salmonella supplemented with 12 mg/l novobiocin.
  *Incubate at 37±1 °C for 20-24 hours.*

**Day: 1**

Add 10 µl of SureTect Proteinase K to each required SureTect Lysis Tube.

Add 10 µl enriched sample to the SureTect Lysis Tube.

Incubate SureTect Lysis Tubes at 37±1 °C for 10 minutes followed by 95±1 °C for 5 minutes.

Allow lysates to cool at room temperature for at least 2 minutes, then transfer 20 µl to SureTect Salmonella PCR Tubes.

Load SureTect PCR Tubes to SureTect PikoReal Instrument
Start PCR and review results at end of run

Report negative results

**Day: 2**

Confirm positive results by inoculating 10 µl of enrichment onto *Brilliance* Salmonella Agar and confirming presumptive positive colonies with the Microbact GNB 24E Kit, the Salmonella Latex Test or the ISO reference method confirmatory tests.

## Alternative method flow diagram: Protocol for all samples using the Applied Biosystems 7500 Fast Instrument

**Day: 0**

- Add 25 g raw beef meats to 225 ml of pre-warmed BPW (at 41.5±1°C).
  *Incubate at 41.5±1 °C for 9-24 hours.*
- Add 25 g meat samples to 225 ml of BPW supplemented with 12 mg/l novobiocin. *Incubate at 37±1 °C for 20-24 hours.*
- Add 25 g powdered infant formula to 225 ml of BPW.
  *Incubate at 37±1 °C for 20-24 hours.*

- Add 25 g of production environment sample to 225 ml of BPW. Add 1 swab to 10 ml of BPW. Add 1 sponge to 100 ml of BPW. Add 1 wipe to 225 ml of BPW.
  *Incubate at 37±1 °C for 20-24 hours.*
- Add 25 g seafood or vegetable samples to 225 ml of BPW supplemented with 12 mg/l novobiocin.
  *Incubate at 37±1 °C for 20-24 hours.*

- Add 25 g dairy to 225 ml of BPW supplemented with 12 mg/l novobiocin.
  *Incubate at 37±1 °C for 20-24 hours.*

  OR
- Add 25 g dairy to 225 ml of ONE Broth Salmonella supplemented with 12 mg/l novobiocin.
  *Incubate at 37±1 °C for 20-24 hours.*

**Day: 1**

Add 10 µl of SureTect Proteinase K to each required SureTect Lysis Tube.

Add 10 µl enriched sample to the SureTect Lysis Tube. Prepare a negative control sample by adding 10 µl sterile water to a SureTect Lysis Tube.

Incubate SureTect Lysis Tubes at 37±1 °C for 10 minutes followed by 95±1 °C for 5 minutes.

Allow lysates to cool at room temperature for at least 2 minutes, then transfer 20 µl to SureTect Salmonella PCR Tubes.

Load SureTect PCR Tubes to Applied Biosystems 7500 Fast Instrument. Start PCR and review results at end of run

Report negative results

**Day: 2**

Confirm positive results by inoculating 10 µl of enrichment onto *Brilliance* Salmonella Agar and confirming presumptive positive colonies with the Microbact GNB 24E Kit, the Salmonella Latex Test or the ISO reference method confirmatory tests.

## ISO reference method: Protocol for all food and production environment samples

**Day: 0**

Add 25 g of sample to 225 ml Buffered Peptone Water (BPW)

*Incubate at 37±1 °C for 16 to 20 hours*

**Day: 1**

Add 0.1 ml of BPW enrichment to 10 ml of Rapapport-Vassiliadis Soya Peptone Broth.

*Incubate at 41.5±1 °C for 21 to 27 hours.*

Add 0.1 ml of BPW enrichment to 10 ml Muller-Kauffman Tetrathionate Broth supplemented with novobiocin.

*Incubate at 37±1 °C for 21 to 27 hours.*

**Day: 2**

Streak 10 µl of enrichment onto Xylose-Lysine-Deoxycholate (XLD) Agar and onto a second *Salmonella* selective agar.

*Incubate at 37±1 °C for 21 to 27 hours.*

**Day: 3**

Streak up to five presumptive colonies onto Nutrient Agar.

*Incubate at 37±1 °C for 21 to 27 hours.*

**Day: 4**

Confirm pure cultures as *Salmonella* spp. by performing biochemical and serological identification tests.

## Results

Expert Laboratory Study

The alternative method was shown to be a reliable alternative to the ISO reference method for the detection of *Salmonella* species from the food and production environment samples analyzed during the expert laboratory study.

Table 1 shows the confirmed results for the alternative method using the SureTect PikoReal Instrument and the ISO reference method and table 2 shows the confirmed results for the alternative method using the Applied Biosystems 7500 Fast System and the ISO reference method results.

**Table 1: NF VALIDATION ISO 16140 extension study confirmed results for the ISO reference method and the alternative method when using the SureTect PikoReal Instrument for all samples**

| Protocols | Methods | ISO Reference method positive results | ISO Reference method negative results |
|---|---|---|---|
| Dairy products BPW + novobiocin & Raw beef 8 hour incubation | Alternative method positive results | 183 | 11 |
| | Alternative method negative results | 9 | 230 |
| Dairy products BPW + novobiocin & Raw beef 24 hour incubation | Alternative method positive results | 185 | 9 |
| | Alternative method negative results | 10 | 229 |
| Dairy products Oxoid ONE Broth Salmonella Base + novobiocin & Raw beef 8 hour incubation | Alternative method positive results | 183 | 11 |
| | Alternative method negative results | 11 | 228 |
| Dairy products Oxoid ONE Broth Salmonella Base + novobiocin & Raw beef 24 hour incubation | Alternative method positive results | 185 | 9 |
| | Alternative method negative results | 12 | 227 |

**Table 2: NF VALIDATION ISO 16140 extension study confirmed results for the ISO reference method and the alternative method when using the Applied Biosystems 7500 Fast System for all samples**

| Protocols | Methods | ISO method positive results | ISO method negative results |
|---|---|---|---|
| Dairy products BPW + novobiocin & Raw beef 9 hour incubation | Alternative method positive results | 154 | 20 |
| | Alternative method negative results | 20 | 197 |
| Dairy products BPW + novobiocin & Raw beef 24 hour incubation | Alternative method positive results | 155 | 19 |
| | Alternative method negative results | 20 | 197 |
| Dairy products Oxoid ONE Broth Salmonella Base + novobiocin & Raw beef 9 hour incubation | Alternative method positive results | 154 | 20 |
| | Alternative method negative results | 21 | 196 |
| Dairy products Oxoid ONE Broth Salmonella Base + novobiocin & Raw beef 24 hour incubation | Alternative method positive results | 155 | 19 |
| | Alternative method negative results | 21 | 196 |

Thirty-three positive deviation results were recorded during the expert laboratory study.

Thirty-one negative deviation results were recorded during the expert laboratory study. The presence of *Salmonella* was detected in 9 of these samples (by subculture of the BPW enrichment into RVS Broth before streaking onto *Brilliance* Salmonella Agar).

The remaining 22 negative and the 33 positive discordant results were most likely due to the unpaired study design and the related sampling heterogeneity as *Salmonella* could not be isolated from the samples by the culture confirmation method meaning that it is likely that no target cells were present in the portion of matrix used for the alternative method.

The relative trueness, sensitivity for the reference and alternative methods and false positive ratios are listed in Tables 3 and 4.

The observed data and results confirmed that the alternative method and the ISO reference method show equivalent performance.

**Table 3: NF VALIDATION ISO 16140 extension study: relative trueness, sensitivity for the reference and alternative methods and false positive ratio results when using the SureTect PikoReal Instrument for all samples**

| | SureTect Assay methods comparative study | | | |
|---|---|---|---|---|
| | **Dairy products BPW + novobiocin & Raw beef 8 hour incubation** | **Dairy products BPW + novobiocin & Raw beef 24 hour incubation** | **Dairy products Oxoid ONE Broth Salmonella Base + novobiocin & Raw beef 8 hour incubation** | **Dairy products Oxoid ONE Broth Salmonella Base + novobiocin & Raw beef 24 hour incubation** |
| Relative Trueness | 95.4% | 95.7% | 95.0% | 95.2% |
| Relative Sensitivity for the reference method | 95.6% | 95.1% | 94.6% | 94.2% |
| Relative Sensitivity for the alternative method | 94.6% | 95.6% | 94.6% | 95.6% |
| False positive ratio | 2.6% | 2.6% | 2.1% | 2.2% |

**Table 4: NF VALIDATION ISO 16140 extension study: relative trueness, sensitivity for the reference and alternative methods and false positive ratio results when using the Applied Biosystems 7500 Fast System for all samples**

| | SureTect Assay methods comparative study | | | |
|---|---|---|---|---|
| | **Dairy products BPW + novobiocin & Raw beef 9 hour incubation** | **Dairy products BPW + novobiocin & Raw beef 24 hour incubation** | **Dairy products Oxoid ONE Broth Salmonella Base + novobiocin & Raw beef 9 hour incubation** | **Dairy products Oxoid ONE Broth Salmonella Base + novobiocin & Raw beef 24 hour incubation** |
| Relative Trueness | 89.8% | 90.0% | 89.5% | 89.8% |
| Relative Sensitivity for the reference method | 89.7% | 89.2% | 89.7% | 89.2% |
| Relative Sensitivity for the alternative method | 89.7% | 90.2% | 89.7% | 90.3% |
| False positive ratio | 0.5% | 0.5% | 0.0% | 0.0% |

## Conclusion

The method comparison study conducted as part of this NF VALIDATION extension study demonstrated that the alternative method is equivalent in performance for the food and production environment samples analysed to the ISO reference method detailed in ISO 6579:2002, when using either the SureTect PikoReal Instrument with SureTect Software v1.2 or the Applied Biosystems 7500 Fast System and RapidFinder Express v2.0 Software. The NF VALIDATION certificate and a summary of the expert laboratory report for this study are available from **http://nf-validation.afnor.org/en/.**

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