Detection of Vibrio from Seafood using the new SureTect Vibrio Multiplex PCR Assay

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INTRODUCTION

The new Thermo Scientific™ SureTect™ Vibrio cholerae, V. parahaemolyticus and V. vulnificus PCR Assay has been developed to simultaneously detect and differentiate Vibrio cholerae, V. parahaemolyticus, and V. vulnificus from molluscs and crustaceans. This study evaluated a rapid time to result for the detection of V. parahaemolyticus and V. vulnificus from molluscs and crustaceans using the SureTect Vibrio PCR Assay and comparing performance to the ISO 21872-2 method.

RESULTS

The ISO method routinely failed to isolate Vibrio species on TCBS medium due to overgrowth of competing microflora including closely related organisms such as V. alginolyticus. To compare the performance of the SureTect Vibrio PCR solution to the ISO reference method, the SureTect Vibrio PCR Assay was used to test ISO reference method enriched samples and the two sets of PCR data were compared (Figure 4). The SureTect Vibrio PCR method demonstrated superior performance to the ISO reference method with 63 positive deviations versus six negative deviations.

Unpaired Study Key

Positive Agreement:
SureTect Method Positive, Reference Method Positive

Negative Agreement:
SureTect Method Negative, Reference Method Negative

Positive Deviation:
SureTect Method Positive, Reference Method Negative

Negative Deviation:
SureTect Method Negative, Reference Method Positive

MATERIALS AND METHODS

The study was conducted by testing naturally and artificially contaminated seafood, including molluscs and crustaceans, for both the candidate (Figure 2) and ISO reference methods. Artificial contamination with V. cholerae, V. parahaemolyticus and V. vulnificus was conducted at a level 1-5 CFU/25 g. Reference method samples were tested according to ISO21872-2 methodology and compared to the SureTect Vibrio PCR method (unpaired study).

RESULTS

The ISO method routinely failed to isolate Vibrio species on TCBS medium due to overgrowth of competing microflora including closely related organisms such as V. alginolyticus. To compare the performance of the SureTect Vibrio PCR solution to the ISO reference method, the SureTect Vibrio PCR Assay was used to test ISO reference method enriched samples and the two sets of PCR data were compared (Figure 4). The SureTect Vibrio PCR method demonstrated superior performance to the ISO reference method with 63 positive deviations versus six negative deviations.

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CONCLUSIONS

- The SureTect Vibrio PCR method utilises an eight hour enrichment.
- Total time to result for the SureTect Vibrio PCR method is 10 hours compared to the ISO method of 40 hours.

TRADEMARKS/LICENSES

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Figure 1: Instrumentation for use with the SureTect range

Figure 2: Process flow for the SureTect Vibrio PCR Assay

Figure 3: Example amplification plot from the Thermo Scientific Analysis Software

Figure 4: PCR results agreement between the SureTect method and ISO reference method

Figure 2: Process flow for the SureTect Vibrio PCR Assay

Sample prep

25 g oyster meat and liquor diluted in 225 ml alkaline saline peptone water (prewarmed, 35 °C)

4–20 hours enrichment at 35 °C

PCR

Lysis conducted with the SimpliAmp Thermal Cycler

PCR conducted with the SureTect assay and QuantStudio 5 Food Safety PCR instrument

Confirmation

10 µL streaked onto TCBS medium

Inoculate at 35 °C for 18-24 hours

Purify suspect colonies and confirm species

Figure 3: Example amplification plot from the Thermo Scientific Analysis Software

Figure 4: PCR results agreement between the SureTect method and ISO reference method