Optimization of Enrichment Medium and DNA Extraction Method for Detecting 
Salmonella spp. in Food Using Real-time PCR

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Introduction: Real-time polymerase chain reaction (PCR) analysis has been widely employed for detecting pathogens contaminating food products. In this study, we evaluated the inhibitory effect on PCR assays by enrichment broth culture of Salmonella spp. and compared the ability of several DNA extraction methods to eliminate PCR inhibitory effects, allowing for efficient detection of Salmonella using a real-time PCR-based method.

Materials and Methods: Overnight cultures of Salmonella serotype Enteritidis (SE) were diluted 1: 10 in buffered peptone water (BPW), Rappaport Vassiliadis (RV), Muller-Kauffmann tetraionate with novobiocin (MKTTn), or phosphate-buffered saline (PBS) as a control, followed by real-time PCR analysis. Three DNA extraction schemes, PrepMan™ Ultra Reagent, PrepMan™ Ultra Reagent with an additional washing step, and the DNeasy® Tissue Kit, were compared for their effectiveness in removing potential PCR inhibitors from the enrichment media. In addition, an optimum enrichment medium and an optimum DNA extraction method to detect Salmonella spp. in food (steamed pork) using real-time PCR assays were evaluated.

Results: The inhibition of PCR reactions was statistically significant (P<0.05) in RV and MKTTn broths, as compared with BPW or PBS. The sensitivity of the real-time PCR analysis was improved by using the PrepMan™ Ultra Reagent with an additional washing step rather than the DNeasy® Tissue Kit. When applied to detection of SE in steamed pork, the real-time PCR method coupled with a single 24-h enrichment with BPW and the PrepMan™ Ultra Reagent with an additional washing step for DNA extraction detected an equivalent number or more positive samples when compared with results from a conventional culture method.

Conclusion: In conclusion, our study has demonstrated that enrichment media used for Salmonella detection have inhibitory effects on real-time PCR assays that decrease the sensitivity of the assay. The inhibitory effects of the enrichment broths were readily removed by modification of the DNA extraction method. Further study is necessary to identify the components of enrichment broths responsible for these inhibitory effects. Prudent selection of an enrichment medium, combined with an optimum DNA extraction method, will result in improved detection of foodborne pathogens in various food products by real-time PCR.

Reference