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MOLECULAR BASED METHOD FOR THE DETECTION OF Salmonella IN MEAT PRODUCTS

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Fresh meat and meat products are often contaminated by the zoonotic pathogen Salmonella resulting in gastroenteritis in humans, one of the major causes of food borne outbreaks and sporadic cases around the world. Although culture based standard methods are reliable and produce accurate results, these are time consuming. Hence, efforts have been made to develop novel methods for Salmonella detection. The Real-Time Polymerase Chain Reaction (RT-PCR) based methods are the most promising due to its ability to quantify. rapidity and excellent detection limit. However, the detection sensitivity often has constraints as the presence of Polymerase Chain Reaction (PCR) inhibitors inherent to food matrices. The objective of this study was to develop a rapid and sensitive diagnostic test to detect Salmonella in meat products using commercially available kits and to evaluate kits' ability to produce accurate results by overcoming PCR inhibitors. The method included an 18 h non-selective enrichment in buffered peptone water, followed by DNA extraction using Oiagen DNeasy[®] mericon[®] food kit. The method was applied to raw and processed meat samples inoculated with a Salmonella reference strain. The RT-PCR was performed using Oiagenmericon[®]Salmonella kit which also included an internal amplification control (IAC) in duplex PCR format. Further, DNA was extracted from a tenfold dilution series of the reference strain and subjected to RT-PCR in order to determine the sensitivity of the RT-PCR assay. Due to the technical errors occurred in the RT-PCR machine, analysis was performed by visualization of PCR products, on a 1 % agarose gel. Successful amplification in PCR inferred that the extraction method effectively removed PCR inhibitors. The developed method has shown a detection limit of 15-20 CFU g⁻¹ of food with a possibility of a further increased sensitivity. The results of the RT-PCR sensitivity assay suggest that the sensitivity could go beyond the 10⁻¹⁰ dilution. Amplified products of DNA that were extracted from artificially inoculated meat samples showed a band intensity which is similar to that of DNA from 10⁻⁴ dilution of sensitivity assay, indicating the efficacy of the enrichment step to increase the detection sensitivity. The overall analysis time was 26 h which is far less than the conventional method. In conclusion, the developed molecular based method is a rapid method with an increased sensitivity and with further validations, this method can be successfully used to test commercial meat products.

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Keywords: DNA extraction, Meat, PCR inhibitors, Real-Time PCR, Salmonella