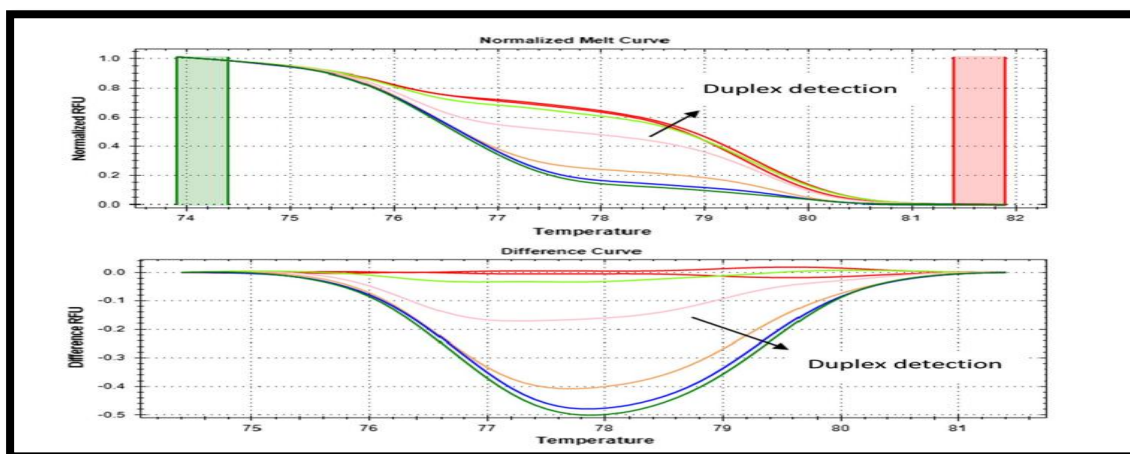




Technology for simultaneous detection of *Listeria monocytogenes* and *Salmonella typhimurium* in meat products using duplex real-time PCR assay with high-resolution melt analysis (qPCR-HRMA).

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Brief description about technology: This study addresses the pressing issue of *Listeria monocytogenes* and *Salmonella typhimurium* contamination in animal-derived foods, highlighting the urgent need for early and accurate detection. The core focus is the development of an innovative duplex real-time PCR assay utilizing SYBR Green chemistry combined with high-resolution melting analysis (HRMA). This duplex qPCR-HRMA approach allows for the simultaneous, rapid, and reliable detection of two major foodborne pathogens in meat products. The assay differentiates amplicons based on distinct melt curve profiles, with a $\sim 4^\circ\text{C}$ difference in melting temperatures— 76°C for *Listeria monocytogenes* and 80°C for *Salmonella typhimurium*. It demonstrated high sensitivity, with a detection limit of 2 pg of DNA, corresponding to approximately 124 genome copies for *L. monocytogenes* and 100 copies for *S. typhimurium*. In spiked meat samples, the method achieved a detection sensitivity of 150 CFU/mL. The assay was rigorously standardized and validated in accordance with ISO 22118:2011, ensuring its reliability for practical food safety applications.



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