

भा.कृ.अनु.प.-राष्ट्रीय मांस अनुसंधान संस्थान

(पूर्व भा.कृ.अनु.प.-राष्ट्रीय मांस अनुसंधान केंद्र) चेंगिचेर्ला, पोस्ट बॉक्स सं 19, बोडप्पल, हैदराबाद - 500 092, तेलंगाना भारत



ICAR - National Meat Research Institute

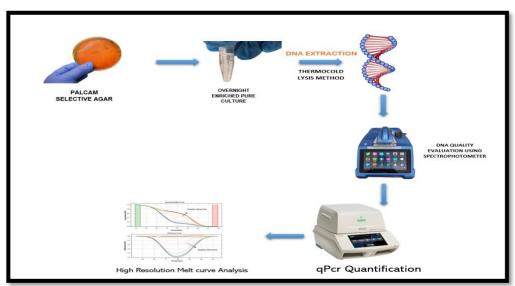
Chengicherla, P.B. No. 19, Boduppal PO, Hyderabad-500 092, Telangana, India (ISO 9001:2015 और FSSC 22000 प्रमाणित, ISO/IEC 17025:2017 NABL मान्यता प्राप्त, FSSAI रेफरल प्रयोगशाला)

<u>Technology for detection of Listeria species and Listeria monocytogenes in meat products</u> <u>using duplex real time PCR assay with high resolution melt analysis.</u>

Inventor: Dr. Vishnuraj M.R.

Brief description about technology

This study tackles the critical challenge of Listeria monocytogenes contamination in animal-derived foods, highlighting the importance of early and accurate detection. The core focus is the development of a duplex real-time PCR assay utilizing SYBR Green chemistry coupled with high-resolution melting analysis (HRMA). To ensure specificity and avoid cross-reactivity with closely related Listeria species, two primer sets were designed—one targeting the Listeria genus and the other specific to L. monocytogenes. Detailed interpretation of HRM curve profiles was carried out to enhance the clarity and reliability of detection. The assay was meticulously optimized through a non-orthogonal approach and rigorously validated in accordance with international standards. It demonstrated high sensitivity, with an absolute limit of detection (LOD $_{\alpha\beta s}$) of 0.78 ng and a limit of quantification (LOQ) of 1.56 ng of target DNA. Additionally, a relative limit of detection (LOD $_{rel}$) of 1% Listeria DNA in a mixed background confirms its effectiveness in complex samples. When applied to artificially spiked samples, the assay achieved a notable detection threshold of 120 CFU/mL, underscoring its potential for practical food safety applications.



Basic process of the developed technology (Duplex real-time PCR assay with high-resolution melt analysis for the detection of *Listeria* species *and Listeria monocytogene*



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DNA Extraction from a Pure culture using Thermocold lysis method

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