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Streamlining Listeria monocytogenes detection and confirmation in a Brazilian beef producer industrial laboratory: A rapid protocol using qPCR automation and chromogenic media

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Traditional methods such as ISO 11290-1:2017 for the detection of *Listeria monocytogenes* can require more than seven days to results, increasing costs for the industry and compromising effective food safety management. In this context, rapid alternative methods have been increasingly demanded by industrial microbiology laboratories seeking to reduce time to results and operational workload. The aim of this study was to assess the performance of a rapid AFNOR validated protocol for detecting and confirming *L. monocytogenes* in beef samples within the routine of industrial meat producer's laboratories. The three-step alternative tested method comprises enrichment in proprietary media, screening using automated qPCR and confirmation by plating in chromogenic media. For the experiments, sixty raw beef trim samples (25 g) were spiked with 10^3 CFU/sample of *Klebsiella aerogenes* ATCC 13048 to increase background flora. Thirty of these samples were additionally spiked with up to 10 CFU/sample of *L. monocytogenes* strains FDA 806 (n=10), ATCC 19111 (n=10) and ATCC 13932 (n=10). Samples were diluted 10-fold in *Listeria* Special Broth and enriched at 30 ± 1 °C for 24 hr. For screening, DNA extraction and qPCR plate setup were performed using the iQ-Check Prep Automation System, employing the iQ-Check *Listeria monocytogenes* II Kit. PCR runs were conducted using the FAST thermal protocol on a CFX96 Touch Deep Well Real-Time PCR Detection System. For confirmation, ten presumptive and ten negative samples were plated (100 µl) onto the surface of RAPID'*L.mono* chromogenic media. Following incubation at 37°C for 24-48 hr, plates were examined for typical colonies confirming the presence of the pathogen in the samples. Simultaneously, the samples were tested using ISO 11290-1 to compare confirmation methods. Results were analyzed considering sensitivity, specificity, precision, false positive and false negative rates as per DOC-CGCRE-089 (2017) from the INMETRO General Accreditation Coordination. The evaluated rapid protocol successfully detected and confirmed the presence of the pathogen in all spiked samples within 72 hr, achieving a time saving of at least 4 days for positive samples compared to the ISO 11290-1 reference method. The results demonstrated 100% sensitivity, specificity, and precision for both screening and confirmation methodologies, with no false positives or false negatives observed. Additionally, no inhibition was detected in qPCR or bacterial growth assays. The findings from the alternative and the reference method were similar. However, the alternative method required fewer reagents and involves less manipulation steps. The tested rapid AFNOR validated protocol proved to be an efficient and

comprehensive solution for detecting *L. monocytogenes* in industrial microbiology laboratories, contributing to fast and effective food safety management.

Agradecimentos: