Comparison of Polymerase Chain Reaction methods and plating for analysis of enriched cultures of *Listeria monocytogenes* when using the ISO11290-1 method

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Abstract
Analysis for *L. monocytogenes* by ISO11290-1 is time-consuming, entailing two enrichment steps and subsequent plating on agar plates, taking five days without isolate confirmation. The aim of this study was to determine if a polymerase chain reaction (PCR) assay could be used for analysis of the first or second enrichment broths, saving four or two days, respectively. In a comprehensive approach involving six European laboratories, PCR and traditional plating of both enrichment broths from the ISO11290-1 method were compared for the detection of *L. monocytogenes* in 872 food, raw material and processing environment samples from 13 different dairy and meat food chains. After the first and second enrichments, total DNA was extracted from the enriched cultures and analysed for the presence of *L. monocytogenes* DNA by PCR. DNA extraction by chaotropic solid-phase extraction (spin column-based silica) combined with real-time PCR (RTi-PCR) was required as it was shown that crude DNA extraction applying sonication lysis and boiling followed by traditional gel-based PCR resulted in fewer positive results than plating. The RTi-PCR results were compared to plating, as defined by the ISO11290-1 method. For first and second enrichments, 90% of the samples gave the same results by RTi-PCR and plating, whatever the RTi-PCR method used. For the samples that gave different results, plating was significantly more accurate for detection of positive samples than RTi-PCR from the first enrichment, but RTi-PCR detected a greater number of positive samples than plating from the second enrichment, regardless of the RTi-PCR method used. RTi-PCR was more accurate for non-food contact surface and food contact surface samples than for food and raw material samples especially from the first enrichment, probably because of sample matrix interference. Even though RTi-PCR analysis of the first enrichment showed less positive results than plating, in outbreak scenarios where a rapid result is required, RTi-PCR could be an efficient way to get a preliminary result to be then confirmed by plating. Using DNA extraction from the second enrichment broth followed by RTi-PCR was reliable and a confirmed result could be obtained in three days, as against seven days by ISO11290-1.

Key-words: *Listeria monocytogenes*, detection, processing environment sample, food sample, RTi-PCR, ISO11290-1 standard.