Sample preparation and DNA extraction procedures for polymerase chain reaction identification of Listeria monocytogenes in seafoods.

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Abstract: Five grams of seafood products were inoculated with one to 500 viable or 10(9) heat-killed cells of Listeria monocytogenes. The presence of the pathogen was detected by the polymerase chain reaction (PCR) with primers specific for fragments of the listeriolysin O (hly) gene (two sets) and for the invasion-associated protein (iap) gene (one set). For DNA preparation, boiling, either alone or in combination with lysozyme and proteinase K treatment, was not always sufficient to lyse L. monocytogenes, while treatment with Triton X-100 produced consistently good DNA suitable for amplification. To avoid false-negative and false-positive results, 48 h incubations were necessary and a subculturing step after an initial 24 h incubation greatly improved the results. The primers that amplified regions of the listeriolysin O gene gave clearer and stronger products than primers for the invasion-associated protein gene. Using this method we were able to detect one to five L. monocytogenes cells in 5 g of product in a total of 55 h.

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