



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

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Author: A. Agersborg
R. Dahl
I. Martinez

Author Affiliation: Norwegian Institute of Fisheries and Aquaculture N-9005 Tromso, Norway.

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Listeria monocytogenes - drug effects - genetics - isolation & purification
Muramidase - pharmacology
Norway - epidemiology
Octoxynol - pharmacology
Polymerase Chain Reaction - methods
Prevalence
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Sensitivity and specificity
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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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