

This document is downloaded from DR-NTU, Nanyang Technological University Library, Singapore.

Title	Detection of <i>Listeria monocytogenes</i> by using the polymerase chain reaction(Main article)
Author(s)	Bessesen, Mary T.; Luo, Qian; Rotbart, Harley A.; Blaser, Martin J.; Ellison III, Richard T.
Citation	Bessesen, M. T., Luo, Q., Rotbart, H. A., Blaser, M. J., & Ellison III, R. T. (1990). Detection of <i>Listeria monocytogenes</i> by using the polymerase chain reaction. <i>Applied and environmental microbiology</i> , 56(9), 2930-2932.
Date	1990
URL	http://hdl.handle.net/10220/18733
Rights	© 1990 American Society for Microbiology. This is the author created version of a work that has been peer reviewed and accepted for publication by <i>Applied And Environmental Microbiology</i> , American Society for Microbiology. It incorporates referee's comments but changes resulting from the publishing process, such as copyediting, structural formatting, may not be reflected in this document. The published version is available at: [http://aem.asm.org/content/56/9/2930.full.pdf+html].

Detection of *Listeria monocytogenes* by Using the Polymerase Chain Reaction

MARY T. BESSESEN,^{1,2} QIAN LUO,^{1,2} HARLEY A. ROTBART,³ MARTIN J. BLASER,^{1,2†}
AND RICHARD T. ELLISON III^{1,2*}

Medical Service, Department of Veterans Affairs Medical Center,¹ and Division of Infectious Diseases,
Department of Medicine,² and Division of Infectious Diseases, Department of Pediatrics,³
University of Colorado School of Medicine, Denver, Colorado 80220

Received 15 March 1990/Accepted 2 July 1990

A method was developed for detection of *Listeria monocytogenes* by polymerase chain reaction amplification followed by agarose gel electrophoresis or dot blot analysis with a ³²P-labeled internal probe. The technique identified 95 of 95 *L. monocytogenes* strains, 0 of 12 *Listeria* strains of other species, and 0 of 12 non-*Listeria* strains.

Listeria monocytogenes has long been acknowledged as a cause of sporadic human infection in immunocompromised patients, newborns, and pregnant women (4). However, in recent years, outbreaks of listeriosis which have been associated with the consumption of contaminated food have been recognized (2, 6, 13). It has therefore become increasingly important to identify contamination of food with *L. monocytogenes* to prevent new outbreaks. Isolation and identification of the organism by standard microbiological techniques are slow and laborious (4). Thus, we have sought to develop a method for the rapid detection of the organism using genetic techniques.

All pathogenic strains of *L. monocytogenes* are known to be hemolytic, with two hemolysins identified (11). Transposon mutagenesis studies have indicated that the sulfhydryl-activated hemolysin, listeriolysin O, is an important virulence factor for the organism (3, 8). The gene for this protein has been identified and sequenced from a serotype 1/2a *L. monocytogenes* strain by Mengaud and colleagues (9). We have used a portion of this gene sequence to develop a polymerase chain reaction (PCR) assay for detection of *L. monocytogenes* (12).

Initial studies were performed with the Maritime *L. monocytogenes* strain (13). Chromosomal DNA was purified by standard techniques (7), and PCR amplification was performed by the methods of Saiki et al. (12) with a pair of 24-mer oligonucleotide primers that define a 606-base-pair segment of the listeriolysin gene (9). Thirty amplification cycles were performed, each consisting of denaturation at 94°C for 2 min, primer annealing at 51°C for 2 min, and extension at 74°C for 2.5 min. The reaction products were analyzed by agarose gel electrophoresis followed by ethidium bromide staining. When 10-fold dilutions of the purified chromosomal DNA were tested, the predicted 606-bp segment was successfully amplified in samples containing 18 ng to 1.8 µg of chromosomal DNA (Fig. 1A). Studies performed with purified chromosomal DNA from *Listeria ivanovii* ATCC 19119 and *Listeria seeligeri* SLCC 3954 at concentrations of 10 ng and 1 µg resulted in no detectable amplified DNA, as expected. To confirm the identity of the 606-bp

amplification product from the *L. monocytogenes* DNA, slot blot hybridization was performed with a ³²P-labeled 24-mer oligonucleotide probe that is internal to the primer pairs on the listeriolysin O gene. The probe hybridized with the PCR products of samples containing as little as 5 ng of template *L. monocytogenes* DNA (Fig. 1B), but no hybridization signal was observed with the primers or unamplified *L. monocytogenes* DNA (data not shown).

We next determined whether the PCR technique could be used with crude bacterial cell lysates. *L. monocytogenes* cells were suspended in sterile distilled water, and lysis was attempted with sonication for 60 s, boiling for 2 min, or treatment with 1% sodium dodecyl sulfate and 1 mg of lysozyme per ml. By using hybridization with the ³²P-labeled oligomeric probe described above, it was possible to detect amplification products ranging from approximately 4 to 400 boiled *L. monocytogenes* CFU (Fig. 2A); results were less favorable with the other lysis techniques. Using agarose gel electrophoresis (without a probe) to detect the amplification product, we were able to consistently identify as few as 10⁴ *L. monocytogenes* CFU suspended in water (Fig. 2B).

To define the sensitivity and specificity of the assay, the PCR technique was applied to other bacterial strains by using crude cell lysates of 10⁷ CFU as the template and agarose gel electrophoresis for product detection. A 606-bp PCR product was obtained from 95 of 95 human, food, environmental, animal, and research laboratory *L. monocytogenes* isolates (Table 1). Some strain-to-strain variability in the relative efficiency of the cell lysis by sonication or boiling was observed (data not shown). In contrast, with the same cell concentration and lysis techniques, a PCR product was not obtained from 12 strains representing non-*L. monocytogenes* *Listeria* species and 12 strains of other genera (Table 1). Each of these strains was tested at least in duplicate, by both the boiling and sonication cell lysis methods.

The utility of the PCR assay for detection of *L. monocytogenes* in commercial whole homogenized milk or human cerebrospinal fluid (CSF) was then studied. Fluid samples were inoculated with the Maritime strain, and the cells were pelleted by centrifugation at 5,000 × g, washed in saline and then in water, and then lysed by microwaving. Successful amplification of listerial DNA occurred in each fluid at cell concentrations of 10⁵ CFU/ml (Fig. 3).

* Corresponding author.

† Present address: Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232.

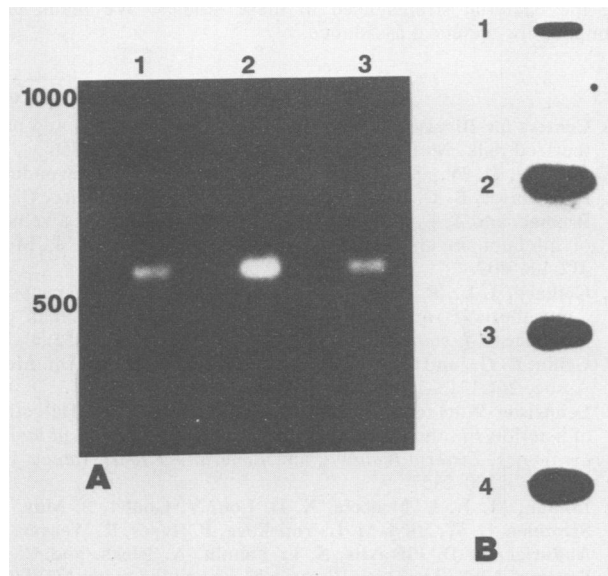


FIG. 1. Products of PCR amplification of purified listerial DNA. (A) Agarose gel electrophoresis. Demonstration of a 606-bp amplification product with 18 ng (lane 1), 180 ng (lane 2), and 1.8 µg (lane 3) of template DNA obtained by electrophoresis on a 1.2% agarose gel with 0.125 µg of ethidium bromide per ml. (B) Slot blot. PCR products with 5 ng (slot 1), 50 ng (slot 2), 500 ng (slot 3), and 5 µg (slot 4) of template were fixed to a nylon membrane and hybridized with a ³²P-labeled internal oligonucleotide probe, and the autoradiograph was exposed for 3 h.

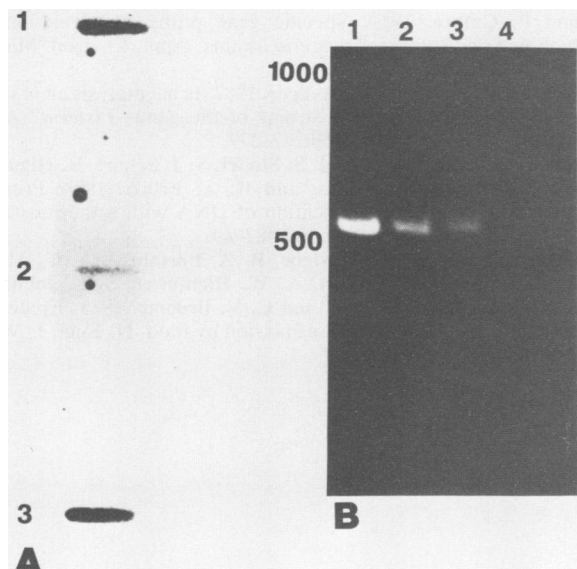


FIG. 2. Products of PCR amplification of crude listerial cell lysates. (A) Slot blot. Products of PCR obtained with 4 (slot 1), 40 (slot 2), and 400 (slot 3) boiled *L. monocytogenes* cells per reaction as template were fixed to a nylon membrane and hybridized with a ³²P-labeled internal oligonucleotide probe. (B) Agarose gel electrophoresis. Products of PCR with 10⁶ (lane 1), 10⁵ (lane 2), 10⁴ (lane 3), and 10³ (lane 4) cells were electrophoresed on a 1.2% agarose gel with 0.125 µg of ethidium bromide per ml.

TABLE 1. Ability of PCR assay for *L. monocytogenes* to distinguish between *L. monocytogenes*, other *Listeria* species, other beta-hemolytic organisms, and other human pathogens

Bacterium and source	No. of strains		
	Studied	Beta-hemolytic	PCR positive
<i>Listeria</i> species			
<i>L. monocytogenes</i>			
Sporadic human cases	57	57	57
Human outbreaks	2	2	2
Foods	13	13	13
Environment	9	9	9
Animal isolates	4	4	4
Laboratory isolates	10	9	10
<i>L. innocua</i>	2	0	0
<i>L. grayii</i>	2	0	0
<i>L. ivanovii</i>	3	3	0
<i>L. seeligeri</i>	3	2	0
<i>L. murrayi</i>	1	0	0
<i>L. welshimeri</i>	1	0	0
Other			
<i>Streptococcus pyogenes</i>	1	1	0
<i>Streptococcus agalactiae</i>	1	1	0
<i>Streptococcus pneumoniae</i>	1	1	0
Viridans streptococci	1	1	0
<i>Staphylococcus aureus</i>	1	1	0
<i>Escherichia coli</i>	2	1	0
<i>Haemophilus influenzae</i>	1	0	0
<i>Neisseria meningitidis</i>	1	0	0
<i>Pseudomonas aeruginosa</i>	1	1	0
<i>Vibrio cholerae</i>	1	1	0
<i>Salmonella typhimurium</i>	1	1	0

The results of this study demonstrate that PCR amplification of the listeriolysin O gene may provide a rapid alternative to standard techniques for the detection of *L. monocytogenes* in biological fluids and food products. All *L. monocytogenes* strains tested to date have been successfully detected by this assay regardless of isolate source, serotype, or electrophoretic type. This compares favorably with a recently reported probe which detects all serotypes except 4a by conventional nucleic acid hybridization (10).

Recent studies using DNA hybridization techniques and affinity-purified antibodies have suggested that *L. ivanovii* ATCC 19119 and *L. seeligeri* SLCC 3954 contain a gene homologous to the *L. monocytogenes* listeriolysin gene and produce a protein recognized by antisera to the *L. monocytogenes* protein (5). We did not detect a PCR amplification product from whole-cell lysates or purified DNA from these strains. This discordance is likely due to the high degree of stringency of the PCR procedure which we employed.

This assay may prove to be useful for rapid detection of *L. monocytogenes* in CSF and blood samples in the clinical laboratory. In the food industry, where contamination of surfaces, equipment, and food with nonpathogenic *Listeria* spp. is common, the ability to rapidly detect pathogenic *Listeria* strains without cross-reaction with nonpathogenic strains can potentially improve upon current approaches. However, because the concentration of bacteria in contaminated foods is frequently less than 10³ cells per ml (1), the sensitivity of the assay must be improved before it can be used directly on clinical or food samples.

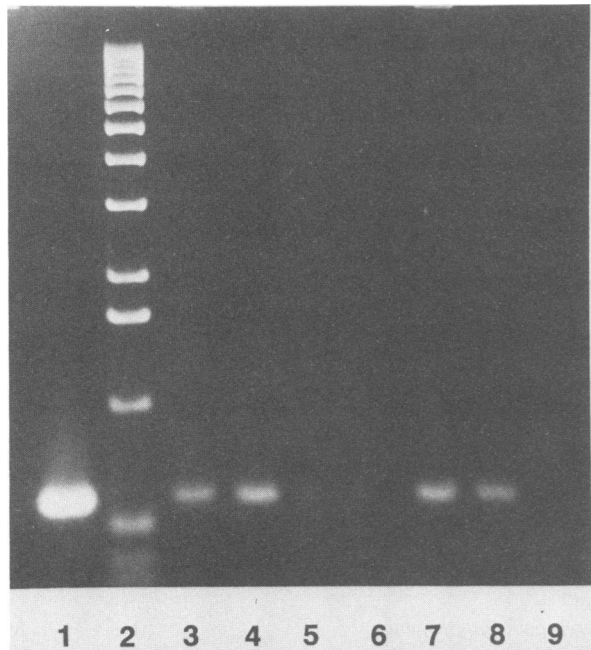


FIG. 3. Agarose gel electrophoresis (1.2% agarose gel with 0.125 μg of ethidium bromide per ml) of products of PCR amplification of *L. monocytogenes* cells in milk or CSF. Lane 1, 10^7 CFU in saline; lane 2, 1-kilobase ladder; lane 3, 10^7 CFU in milk; lane 4, 10^6 CFU in milk; lane 5, 10^5 CFU in milk; lane 6, 10^4 CFU in milk; lane 7, 10^7 CFU in CSF; lane 8, 10^5 CFU in CSF; lane 9, 10^4 CFU in CSF. The amplified segments all appeared at a size of approximately 606 bp.

This research was supported by the Committee on Food Microbiology of the International Life Sciences Institute Nutrition Foundation. R. T. Ellison is the recipient of a Department of Veterans Affairs Research Associate career development award, and M. J. Blaser is the recipient of a Department of Veterans Affairs Clinical Investigator career development award.

We thank Walter Schlech (Dalhousie University, Halifax, Nova Scotia, Canada), L. Barth Reller (Duke University, Durham, N.C.), L. Reimer (Veterans Affairs Medical Center, Salt Lake City, Utah), Donald E. Conner (National Food Processors' Association, Washington, D.C.), David W. Francis (Food and Drug Administration, Cincinnati, Ohio), Trinad Chakraborty (Universität Würzburg, Würzburg, Federal Republic of Germany), Michael Vasil (University of Colorado Health Sciences Center, Denver, Colo.), Bruce Dunn (Department of Veterans Affairs Medical Center Denver Clinical Microbiology Laboratory), Richard Finkelstein (University of Missouri, Columbia, Mo.), Ilkka Helander (National Public Health Institute, Helsinki, Finland), and Jean-Claude Piffaretti (Istituto Cantonale Bacteriologico, Lugano, Switzerland) for provid-

ing the bacterial strains used in these studies. We thank Gail Campbell for technical assistance.

LITERATURE CITED

- Centers for Disease Control. 1988. Update—listeriosis and pasteurized milk. *Morbidity and Mortality Weekly Report* 37:764-766.
- Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N. Engl. J. Med.* 312:404-407.
- Gaillard, J. L., P. Berche, and P. Sansonetti. 1986. Transposon mutagenesis as a tool to study the role of hemolysin in the virulence of *Listeria monocytogenes*. *Infect. Immun.* 52:50-55.
- Gellin, B. G., and C. V. Broome. 1989. Listeriosis. *J. Am. Med. Assoc.* 261:1313-1320.
- Leimeister-Wächter, M., and T. Chakraborty. 1989. Detection of listeriolysin, the thiol-dependent hemolysin in *Listeria monocytogenes*, *Listeria ivanovii*, and *Listeria seeligeri*. *Infect. Immun.* 57:2350-2357.
- Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekura, P. Hayes, R. Weaver, A. Audurier, B. D. Plikaytis, S. L. Fannin, A. Kleks, and C. V. Broome. 1988. Epidemic listeriosis associated with Mexican-style cheese. *N. Engl. J. Med.* 319:823-828.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Mengaud, J., J. Chenevert, C. Geoffroy, J.-L. Gaillard, and P. Cossart. 1987. Identification of the structural gene encoding the SH-activated hemolysin of *Listeria monocytogenes*: listeriolysin O is homologous to streptolysin O and pneumolysin. *Infect. Immun.* 55:3225-3227.
- Mengaud, J., M.-F. Vicente, J. Chenevert, J. M. Pereira, C. Geoffroy, B. Gicquel-Sanzey, F. Baquero, J.-C. Perez-Diaz, and P. Cossart. 1988. Expression in *Escherichia coli* and sequence analysis of the listeriolysin O determinant of *Listeria monocytogenes*. *Infect. Immun.* 56:766-772.
- Notermans, S., T. Chakraborty, M. Leimeister-Wächter, J. Dufrenne, K. J. Heuvelman, H. Maas, W. Jansen, K. Wernars, and P. Guinee. 1989. Specific gene probe for detection of biotyped and serotyped *Listeria* strains. *Appl. Environ. Microbiol.* 55:902-906.
- Rocourt, J., and H. P. R. Seeliger. 1987. Is haemolysis an *in vitro* marker of the pathogenic strains of the genus *Listeria*? *Ann. Inst. Pasteur/Microbiol.* 138:277-279.
- Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487-491.
- Schlech, W. F., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome. 1983. Epidemic listeriosis—evidence for transmission by food. *N. Engl. J. Med.* 308:203-206.