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ORIGINAL ARTICLE

# Detection of viable and dead *Listeria monocytogenes* by PCR

**L. Herman***

*Non-culturable* Listeria monocytogenes *are detected by polymerase chain reaction (PCR) after treatment with a variety of disinfectants and after different heat conditions. The sensitivity of this PCR detection is strongly dependent on the treatment applied. Dissolving* L. monocytogenes *cells in pure ethanol for 30 days only reduced the PCR sensitivity 100 times compared with untreated cells. Treatment with 1% HCl and sterilization at 124°C for 15 min prevented PCR detection after 1 h. The non-culturable* L. monocytogenes *cells, obtained after a cheese pasteurization of 63°C for 30 min are dead and not in a viable but non-culturable state as was established by reverse transcription (RT) PCR on* L. monocytogenes *RNA.*


## Introduction

*Listeria monocytogenes* is a Gram-positive bacterium which is considered a foodborne pathogen. The classical detection method for *L. monocytogenes* is laborious and time consuming. It involves enrichment for 48 h and subsequent colony forming for 48 h, before identity confirmation (IDF 1990).

Polymerase chain reaction (PCR) offers a very powerful tool to elaborate specific, sensitive and rapid detection methods for bacterial pathogens in food products, clinical and environmental samples. Application of PCR enables the reduction or elimination of the bacterial enrichment procedure so that a 1 day detection method may be obtained, as was reported for the detection of *L. monocytogenes* in raw milk (Herman et al. 1995) and in cheese (Makino et al. 1995).

When no or a very limited culturing of the bacterial cells occur, the question whether such methodology detects dead bacteria has to be examined. It is also not clear whether the classical bacteriological culturing methods are able to detect all viable cells.

Three different states are recognized for bacteria: culturable, viable but non-culturable (VBNC) and dead. As a response to certain environmental stress conditions (e.g., temperature shifts, salt levels, nutrient levels, light), bacteria may loose their ability to grow on media on which they are routinely cultured, while remaining viable (reviewed by Oliver 1993, Oliver 1995). A bacterium in this VBNC state is able to resuscitate to the normal culturable state under proper conditions. The VBNC state is of special concern when considering human pathogens (e.g., *Salmonella*, Roszak et al. 1984) which may become undetectable through routine bacteriological procedures.

Recently, it has been reported that PCR may detect VBNC, as well as culturable cells of *Vibrio vulnificus* in raw oysters. Dead cells

*Correspondence address: Lieve Herman, Government Dairy Research Station, Brusselsesteenweg 370, B-9090 Melle (Belgium).

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*Government Dairy Research Station, Agricultural Research Centre Ghent, B-9090 Melle, Belgium*

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of this pathogen were not detected (Brauns et al. 1991). The same was reported for *Legionella pneumophila* in water (Bej et al. 1991). However, it is known that PCR is capable of detecting DNA in cells from mummies which have been non-viable for centuries (Paabo et al. 1988), typing DNA in hair cells (Higuchi et al. 1988) and detecting formalin preserved cells (Impraim et al. 1987). Masters et al. (1994) have shown that *Escherichia coli* and *L. monocytogenes* were detectable by PCR 20 days after plate counts had declined to zero. This was especially the case for starved and desiccated cells.

An accurate way to test the detection of all viable (culturable and VBNC) bacterial cells is the use of mRNA as the target for PCR amplification. The underlying principle of this approach is that most of the bacterial mRNAs have half lives of <2 min (Belasco and Higgins 1988). Because of short half lives, an RNA extraction procedure has to be used which avoids breakdown of the mRNA by RNase (Bej et al. 1991). Using the extracted RNA as target, cDNA is first synthesized as a target for PCR.

In this article, the influence of different disinfectants and heat treatments on the PCR detectability of *L. monocytogenes* is studied. For pasteurization, it is clearly demonstrated that the cells detected by PCR are dead and not in the VBNC state. The results of this study have practical implications for proper risk assessment of food products and for establishing adequate procedures to prevent PCR contamination.

## Materials and Methods

### *Bacterial culture conditions*

The test organism, *L. monocytogenes* strain Ohio, obtained from INRA (Jouy-en-Josas, France), was grown in brain–heart infusion (Oxoid) at 37°C for 24 h. For determining the direct viable count, decimal dilutions were spread on a tryptone soya agar plate and grown at 37°C for 48 h. The number of colonies per plate was counted and the number of colony forming units per millilitre was calculated. Cells unable to form colonies under these conditions were defined as non-viable (Masters et al. 1994).

### *Stress treatments*

Decimal dilutions of the *L. monocytogenes* culture were made in Ringer solution. To each dilution, a disinfectant was added (Table 1) from a 10-times higher concentrated stock solution. For obtaining 98% ethanol, the bacterial cells of each dilution were collected by centrifugation for 5 min at 13 000 *g* and resuspended. The following disinfectants were used: ethanol (absolute, pro analysis; Merck KGaA, Germany); Dettol$^{R}$ (chloroxylenol as active component; S.A. Reckitt & Colman N.V., Brussels, Belgium); sodium hypochlorite (13% active chlorine, Acros Chimica N.V., Geel, Belgium); RBS35 (R. Borghgraef Solution including anionic and non-ionic surface-active reagentia, phosphates, polyphosphates, hydrates and chlorinated agentia, Chemical Products, Brussels, Belgium); sodium hydroxide pellets (pro analysis, Merck KGaA, Germany), hydrochloric acid (37%, fuming, pro-analysis, Merck KGaA, Germany). The cells were heat stressed in a warm water-bath at different temperatures. Two different treatments at 124°C were performed: heating in an autoclave with temperature control and display, and heating in an oil bath. The samples were cooled to room temperature, kept in the Ringer solution at room temperature and removed at intervals (Table 2) for PCR.

### *DNA extraction*

One millilitre of each dilution was centrifuged for 5 min at 13 000 *g* to collect the bacterial cells. The cells were washed twice with 1 ml water. In order to investigate the inhibitory effects of the disinfectants on the efficiency of the PCR, microcentrifuge tubes were filled with different concentrations of the disinfectants. The tubes were centrifugated for 5 min at 13 000 *g* and washed once with 1 ml water. Different decimal dilutions of *L. monocytogenes* cells were added and concentrated. Crude cell lysates were prepared by resuspending the pellet in 50 µl of 0·05 M NaOH and 0·125% sodium dodecyl sul-

phate and subsequent heating for 17 min at 90°C.

### *PCR amplification*

PCR was performed in a total volume of 50 µl using 1·5 U AmpliTaq DNA Polymerase (Perkin Elmer, Norwalk, Conn.), 50 mM KCl, 1·5 mM $MgCl_2$, 20 mM Tris-HCl (pH 8·3), 0·01% gelatine, 0·5% Tween-20, 50 µM of each deoxyribonucleoside triphosphate, 50 pmol of each primer and 1 µl crude cell lysate. As primers LM1 (5'-CCTAAGACGCCAATCG-AA-3') and LM2 (5'-AAGCGCTTGCAACTG-CTC-3') (Border et al. 1990), derived from the listeriolysin O sequence (LISHLYA; GenBank accession number M24199) (Mengaud et al. 1988) were used. The mixture was subjected to 30 cycles of amplification in a thermal cycler (Cetus 9600; Perkin Elmer). The first cycle was preceeded by an initial denaturation of 1 min at 95°C. Each cycle consisted of a denaturation of 15 s at 95°C, an annealing of 15 s at 50°C and on extension of 10 s at 72°C. The last cycle was followed by a final extension of 8 min at 72°C.

### *Agarose gel electrophoresis*

Ten microlitres of the PCR products was analysed on a 1·5% Seakem ME agarose gel (FMC Bioproducts, Rockland, Maine) in 1× TAE buffer at about 5 V $cm^{-1}$ by a standard protocol (Sambrook et al. 1989). Number VIII (Boehringer, Mannheim GmbH, Germany) was used as a molecular weight marker. Gels were photographed under UV transilluminination (300 nM UV) after staining with 0·001% ethidium bromide.

### *RNA extraction*

Ten minutes before extraction of the RNA, 100 µg $ml^{-1}$ chloramphenicol was added to the bacterial culture in order to destroy all protein synthesis, including production of RNase (Bej et al. 1991). By this way, breakdown of the mRNA by RNases during the extraction is decreased.

*L. monocytogenes* cells from 1 ml culture were lysed as described by Flamm et al. (1984) for the DNA extraction. The RNA was extracted by hot phenol (70°C) at pH 5·5 (Barry et al. 1992). The small quantity of co-extracted DNA was digested by 1 µl (7·5 U $ml^{-1}$) FPLC pure DNase (Pharmacia) in a total volume of 20 µl. The DNase was removed by a phenol–chloroform extraction. For improving the efficiency of RNA precipitation 0·l µl Etachinmate (Eurogentec S.A., Seraing, Belgium) was added. The final pellet was dissolved in 10 µl DEPC treated $H_2O$.

### *Continuous reverse transcription (RT)-PCR*

Continuous RT-PCR was performed on 5 µl RNA by using the EZrTth RNA PCR kit (Perkin Elmer). The rTth polymerase which is used in this system, has a reverse transcriptase and a polymerase activity. The RT-PCR reaction was performed following the suppliers instructions. LM1 and LM2 were used as primers. On 5 µl of the same RNA template a PCR reaction was performed as control.

### *Uncoupled RT-PCR*

RT was performed on 5 µl RNA with 1 µl (12·1 U $ml^{-1}$) of the Molony murine leukemia virus reverse transcriptase (M-MLV-RT) in 0·5×PCR buffer (25 mM KCl, 0·75 mM $MgCl_2$, 10 mM Tris–HCl (pH. 8·3), 0·005% gelatine, 0·25% Tween-20), 250 µM of each deoxyribonucleoside triphosphate, 1 µl RNasin (40 U $µl^{-1}$) (Boehringer Mannheim) and 50 pmol of the reverse primer LM2 in a total reaction volume of 20 µl for 1 h at 37°C. After RT, 4 µl of 10×PCR buffer, 50 µM of each deoxyribonucleoside triphosphate, 1·5 U AmpliTaq DNA polymerase (Perkin Elmer) and 50 pmol of the forward primer LM1 was added in a total reaction volume of 50 µl. The control PCR was performed on 5 µl of the same RNA template.

## Results

### *Effect of different disinfectants on the detection of* L. monocytogenes *by culturing and by PCR*

The effect of different disinfectants (ethanol,

Dettol, NaOH, HCl and NaOCl) on the viability and detectability of *L. monocytogenes* using PCR was tested. The viability was assessed by plate count after 10 min of treatment, the PCR detectability was analysed after 1 h of treatment (Table 1). In a separate experiment, it was confirmed that no significant difference existed between the plate count after 10 min and after 1 h (Table 1 and Table 2). The disinfectants did not inhibit the efficiency to PCR.

After treatment with increasing concentrations of ethanol (0·1 to 98%), a decreasing concentration of culturable cells were counted (100 to 0·03%), whereas by PCR only a 10-fold reduction in detection sensitivity, compared with untreated cells, was established.

Dettol, NaCl and RBS prevented all bacterial growth at concentrations of 0·5, 0·05 and 1%, respectively. Dettol did not reduce PCR detectability when compared with untreated cells, 1% RBS and 0·5% NaOCl resulted in a 100- and 1000-fold reduction, respectively.

NaOH and HCl prevented bacterial growth at a concentration of 1%. PCR detection was reduced 10-fold by 1% NaOH, and was totally absent after a treatment with 1% HCl.

The PCR detectability of the *L. monocytogenes* cells was analysed in function of the duration of the stress treatment (Table 1). Thirty days of treatment with all tested ethanol concentrations and with 0·5% Dettol lowered the PCR detectability 10 times compared with a 1 h treatment. Prolonged treatment for thirty days with 2·5% Dettol resulted in a 1000-fold

**Table 1.** Detection of *L. monocytogenes* by polymerase chain reaction (PCR) after treatment with different disinfectants

| Treatment | DVC[a] (cfu $ml^{-1}$) | PCR sensitivity[b] (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 h | 24 h | 48 h | 7 days | 30 days | 90 days |
| No | $1·1\times10^{9}$ | 100 | 100 | 100 | 100 | 100 | 10 |
| Ethanol 0·1% | $1·1\times10^{9}$ | 100 | 100 | 100 | 10 | 10 | 1 |
| Ethanol 1% | $6·5\times10^{8}$ | 100 | 100 | 100 | 10 | 10 | 1 |
| Ethanol 10% | $5·0\times10^{8}$ | 10 | 10 | 10 | 1 | 1 | 1 |
| Ethanol 98% | $3·2\times10^{5}$ | 10 | 10 | 10 | 1 | 1 | ND |
| Dettol 0·5% | <3 | 100 | 100 | 10 | 10 | 10 | ND |
| Dettol 2·5% | <3 | 100 | 100 | 10 | 1 | 0·1 | 0·1 |
| NaOCl 0·5% | <3 | 0·1 | 0·1 | 0·1 | 0·1 | 0·1 | ND |
| NaOCl 5% | <3 | 0·01 | 0·01 | 0·01 | 0·01 | 0·01 | ND |
| RBS 1% | <3 | 1 | 1 | 1 | 1 | 1 | 0·1 |
| NaOH 0·1% | $2·7\times10^{4}$ | 10 | 1 | 1 | 0·1 | 0·1 | 0·1 |
| NaOH 1% | <3 | 10 | 1 | 1 | 0·01 | <0·001 | ND |
| HCl 0·1% | $1\times10^{2}$ | 100 | 100 | 100 | 10 | ND | ND |
| HCl 1% | <3 | <0·001 | <0·001 | ND | ND | ND | ND |

[a]DVC, direct viable count determined 10 min after heat treatment, determined as described by Masters et al. (1994).

[b]PCR sensitivity, relatively expressed in percent towards the sensitivity obtained for the untreated cells. A PCR sensitivity of 100% is the last decimal dilution of untreated cells which gives a PCR signal after 1 h. This conforms to a dilution of $10^{-5}$ and a detection of $2·2\times10^{2}$ cfu $ml^{-1}$ in this experiment. A PCR sensitivity of 10% means in this experiment that the last decimal dilution, still yielding a positive signal was $10^{-4}$. This means that relatively 10 times more *L. monocytogenes* (cfu $ml^{-1}$) ($2·2\times10^{3}$ cfu $ml^{-1}$) has to be present in order to be detected by PCR. The PCR sensitivity is measured after a stress treatment of different hours or days.

ND, not determined.

reduction, while with NaOCl and RBS no further effect on the PCR detectability was established.

Treatment with 1% NaOH for 30 days abolished all PCR detection.

### *Effect of heat treatments on PCR detectability of* L. monocytogenes

Although no *L. monocytognes* cells could be cultured after a heat treatment of 60°C for 30 min, the PCR detectability stayed the same as for untreated cells 48 h after treatment, and declined only 10-fold after 30 days (Table 3). Ten minutes of 100°C reduced this PCR detection sensitivity from 10-fold, 1 h after treatment, to 100 fold after 30 days. No PCR product was only obtained after heating for 15 min at 124°C.

### *Detection of* L. monocytogenes *cells by PCR*

After a cheese pasteurization of 60°C for 30 min no *L. monocytogenes* cells could be cultured although PCR signals were obtained until at least 30 days after treatment (Table 3). In order to test the detection of all viable

**Table 2.** The influence of different disinfectants on the direct viable count of *L. monocytogenes*

| Treatment | DVC[a] (cfu ml$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 1 h | 24 h | 48 h | 7 days | 30 days |
| Ethanol 0·1% | $1{\cdot}5\times10^{9}$ | $6{\cdot}4\times10^{8}$ | $1{\cdot}4\times10^{8}$ | ND | ND |
| Ethanol 1% | $1{\cdot}4\times10^{9}$ | $6{\cdot}1\times10^{8}$ | $1{\cdot}9\times10^{8}$ | $9{\cdot}8\times10^{7}$ | $3{\cdot}0\times10^{6}$ |
| Ethanol 10% | $3{\cdot}4\times10^{8}$ | ND | $2{\cdot}9\times10^{6}$ | $7{\cdot}5\times10^{5}$ | ND |
| Ethanol 98% | <3 | <3 | ND | ND | ND |
| Dettol 0·5% | <3 | ND | ND | ND | ND |
| Dettol 2·5% | <3 | ND | ND | ND | ND |
| NaOCl 0·5% | <3 | ND | ND | ND | ND |
| NaOCl 5% | <3 | ND | ND | ND | ND |
| RBS 1% | <3 | ND | ND | ND | ND |
| NaOH 0·1% | $2\times10^{4}$ | $2\times10^{3}$ | $6\times10^{2}$ | <3 | ND |
| NaOH 1% | <3 | ND | ND | ND | ND |
| HCl 0·1% | <3 | <3 | ND | ND | ND |
| HCl 1% | <3 | ND | ND | ND | ND |

[a]DVC, direct viable count determined as described by Masters et al. (1994).
ND, not determined.

**Table 3.** Detection of heat killed *L. monocytogenes* by polymerase chain reaction (PCR)

| Heat treatment | DVC* (cfu ml$^{-1}$) | PCR sensitivity | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 h | 24 h | 48 h | 7 days | 30 days | 90 days |
| No | $1{\cdot}6\times10^{9}$ | 100 | 100 | 100 | 100 | 100 | 10 |
| 60° 30' | <3 | 100 | 100 | 100 | 10 | 10 | 1 |
| 100° 10' | <3 | 10 | 1 | 1 | 1 | 1 | 1 |
| 124° 10' | <3 | <0·001 | <0·001 | ND | ND | ND | ND |
| 124° 15' | <3 | <0·001 | <0·001 | ND | ND | ND | ND |

[a]DVC, direct viable count determined as described by Masters et al. (1994).
[b]PCR sensitivity, relatively expressed in percent towards the sensitivity obtained for the untreated cells. A PCR sensitivity of 100% is the last decimal dilution of untreated cells which gives a PCR signal after 1 h. This conforms to a dilution of $10^{-1}$ and a detection of $3{\cdot}2\times10^{2}$ cfu ml$^{-1}$ in this experiment. The PCR sensitivity is measured at different hours or days after heat treatment.
ND, not determined.

*L. monocytogenes* cells (culturable and VBNC), the mRNA was used as target for PCR amplification. The extracted mRNA was applied as a template for a continuous RT-PCR reaction where the rTth polymerase executed a reverse transcriptase and DNA polymerase activity. Continuous RT-PCR on the RNA, extracted from heat treated *L. monocytogenes* cells, gave the same results as untreated cells. Normal PCR on this extracted RNA, which was performed as a control, resulted in a positive signal 10 times less sensitive compared with the one obtained with continuous RT-PCR.

The efficiency of amplification by the AmpliTaq polymerase, applied in the PCR reaction was compared with this of the rTth polymerase applied in the RT-PCR. Amplification was detected until 70 pg DNA by Ampli Taq polymerase and until 7 pg DNA by rTth polymerase as template. Because rTth polymerase used in the continuous RT-PCR was 10 times more efficient than AmpliTaq polymerase, the positive results, obtained with continuous RT-PCR after heating, could be explained by the amplification of small quantities of DNA.

In order to use the PCR on the isolated RNA as a negative control for the RT-PCR, an uncoupled RT-PCR method was applied. The uncoupled RT-PCR produced no signal when RNA extracted from pasteurized *L. monocytogenes* cells was used as a template (Table 4). The signals obtained with RNA, extracted from untreated cells functioned as positive control. As negative control, PCR on the extracted RNA was performed. These results indicated that the non-culturable *L. monocytogenes* cells, obtained after cheese pasteurization, are dead and not in a VBNC state.

**Table 4.** Uncoupled reverse transcription polymerase chain reaction (RT-PCR) with Molony murine leukemia virus reverse transcriptase (M-MLV-RT) on *L. monocytogenes* RNA isolated before and after pasteurisation

| Treatment | DVC[a] (cfu $ml^{-1}$) | M-MLV-RT-PCR[b] | |
|---|---|---|---|
| | | 1 h | 24 h |
| NO | $1{\cdot}25\times10^{9}$ | 100 | 100 |
| 30' 63°C | <3 | <0·1 | <0·1 |

[a]Direct viable count determined as described by Masters et al. (1994).
[b]M-MLV-RT-PCR sensitivity in percent of *L. monocytogenes* cfu $ml^{-1}$ which are still detectable. A M-MLV-RT-PCR sensitivity of 100% is the last decimal dilution of untreated cells which gives a signal after 1 h. This conforms to a detection of $1{\cdot}125\times10^{6}$ cfu $ml^{-1}$ in this experiment.
The M-MLV-RT-PCR sensitivity is measured, 1 and 24 h after heat treatment.

## Discussion

In different stress conditions (treatment with disinfectants and with heat), *L. monocytogenes* cells lost their viability but were still detectable by PCR. Depending on the stress condition, PCR sensitivity measured directly after treatment, varied from 0–100% compared with untreated cells. Also a big variation in the declination of PCR sensitivity during the time was noticed.

The mechanism of DNA destruction by sodium hypochlorite is probably multifactorial. It has been shown that the bases and free nucleotides are destroyed, in some cases with the formation of chlorinated derivatives, e.g., 5-chlorocytidine (Hayatsu et al. 1971). The end result is destabilization and breakdown of the DNA helix. Prince and Andrus (1992) demonstrated that 10% commercial bleach containing 0·5% sodium hypochlorite was sufficient to eliminate DNA templates in the PCR laboratory. However, in our experiments, 0·01% of the *L. monocytogenes* cells stayed detectable by PCR under the same concentration of sodium hypochlorites. This may be explained by a protection of the DNA by some intact bacterial cell-wall structures.

Exposure to acid causes depurination of DNA (Lindahl 1993) and prevent PCR amplification (Masters et al. 1994, Ma et al. 1994) which was confirmed by our experiments. Short exposure to acid, however, may be not sufficient to destroy the DNA template as was reported by Prince and Andrus (1992). Their experiments indicated that treatment of purified DNA with 6·7% HCl did not destroy PCR amplification within 5 min.

Heat can damage DNA by depurination or deamination, and at temperatures above 100°C, causes considerable strand scission and irreversible loss of secondary structure

(Miles et al. 1986). Our results showed that cellular DNA, up to 100°C, could function as a PCR template but this ability is lost at 124°C. Masters et al. (1994) showed that at 121°C the cellular DNA is only partly damaged but still detectable by PCR.

The decimal reduction of PCR detectability as a function of the time, caused by heat and ethanol is probably caused by degradation of DNA by endogenous nucleases. Higher reductions in PCR detectability, obtained after Dettol, sodium hydroxide and acid, could be explained by a prolonged action of the chemical components on bacterial cells and DNA. Treatment with RBS and sodium hypochlorite did not show, in contrast with the other stress conditions, a further damage of DNA by prolonged treatment. This indicated that after the initial cell lysis and DNA damage, further activity of the components was lost and DNA breakdown by endogenous nucleases was avoided.

The effect of different disinfectants on the stability of cellular DNA is also important for establishing disinfection procedures for a PCR laboratory. It is clear that disinfection with ethanol and Dettol is inefficient and less preferable than diluted bleach and acid.

In order to prove that the *L. monocytogenes* cells detected by PCR are dead and not in a VBNC state, the mRNA was used as a template for RT-PCR. Continuous RT-PCR is easy to perform but does not have a decent negative control. It is impossible to make a distinction between a positive signal obtained by amplification of the cDNA or of bacterial cellular DNA. In the uncoupled RT-PCR, two separate reactions have to be performed, first RT and subsequently cDNA amplification. PCR on the extracted RNA can function as a decent negative control.

By means of the uncoupled RT-PCR, our data showed that after heating at 60°C for 30 min the *L. monocytogenes* are dead, and not in the VBNC state. They are, however, detected by PCR as efficiently as the living cells. These results are in contrast to those for *L. pneumophila* where after exposure for 10 min at 70°C the non-viable cells are not detected by PCR (Bej et al. 1991).

In this study, we have shown that *L. monocytogenes*, killed by different treatments, which are of practical significance in the food industry, remain detectable by PCR. It is quite possible that *L. monocytogenes* cells, killed by cheese pasteurization, will remain detectable by PCR. Application of a direct PCR detection method suffers from the drawback that false positive results can be obtained.

Detecting only viable *L. monocytogenes* cells by PCR is possible after RT to cDNA. Because of the instability of many bacterial transcripts, this method is technically difficult because the mRNA molecules can be degraded before extraction is finished. The listeriolysin O transcript, applied in this work, would not be the target of choice for such a RT-PCR detection method because of the lack of sensitivity. It can be assumed that the most important reason for this is its inducible expression. The expression of listeriolysin O is regulated at the transcription level by the pleiotropic activator of virulence, Prf A (Freitg and Portnoy 1994). Although not all information is available yet, it is clear that multiple environmental factors influence the listeriolysin O expression. The most important factors established till now are heat shock and the composition of the growth medium (Park et al. 1992). Because of the inducible expression of a number of bacterial virulence factors (Mekalanos 1992), the extraction efficiency of their transcripts will be variable. To obtain a sufficient sensitivity, more constitutively expressed transcripts have to be chosen as templates.

On the basis of scientific knowledge so far, PCR detection after culturing is advisable for monitoring viable *L. monocytogenes* cells in food products because only the viable cells will multiply. This combination of classical bacteriological and modern PCR technology enables the detection of viable pathogens necessary for a proper risk assessment.

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