

## Introduction

For the extensive control of the microbiological risks in the food production industry, the EU-Commission introduced regulation No. 2073/2005 (from 15. November 2005). This regulation dictates the investigation of following focal points: *Listeria monocytogenes* and *Salmonella spp.* as safety criteria in foodstuffs and *E. coli* as a process hygiene criterion. For *L. monocytogenes* the detection limit for consumable products is ND\*/g at the production level, and <math>10^2</math> cfu/25 g at retail.

The official method for the detection of *L. monocytogenes* in foodstuffs (§64 LFGB 00.00-22) requires the enrichment and culturing of the bacteria in selective media. Processing samples in this way may take up to five days for a positive result. Through the implementation of polymerase chain reaction (PCR) or RNA-based methods in foodstuff analyses it will be possible to dramatically reduce the time required to process samples with a similar sensitivity and specificity as the plate-culture method.

\*not detectable

## Materials and methods

In the scope of this investigation five samples (negative for *L. monocytogenes* by plate-culture) were tested prior to their expiration date. The samples were subsequently spiked with 0, 10, 100, 1000, 10000 and 100000 cfu *L. monocytogenes*/g and subsequently enriched in Pepton Water (Oxoid, Wesel, Germany) for 24 hours (Fig. 1). The detection of *L. monocytogenes* was performed by culture method on PALCAM-Agar (Oxoid) (Fig. 2), PCR (Sirs-Lab, Jena, Germany) and RNA-hybridisation (RNA-FastScan®, ScanBec, Halle, Germany).

The PCR utilizes a set of primers which target a *L. monocytogenes*-specific invasion protein whereas the RNA-hybridization occurs with probes directed against 16S rRNA. Additionally 78 random samples were taken out of our routine investigation process and compared by the aforementioned methods.



Fig.1: Foodstuff probe (Fish) in enrichment broth during the incubation



Fig.2: Typically colony form from a *Listeria spp.* mix-culture after 24 hours incubation directly from the enrichment broth

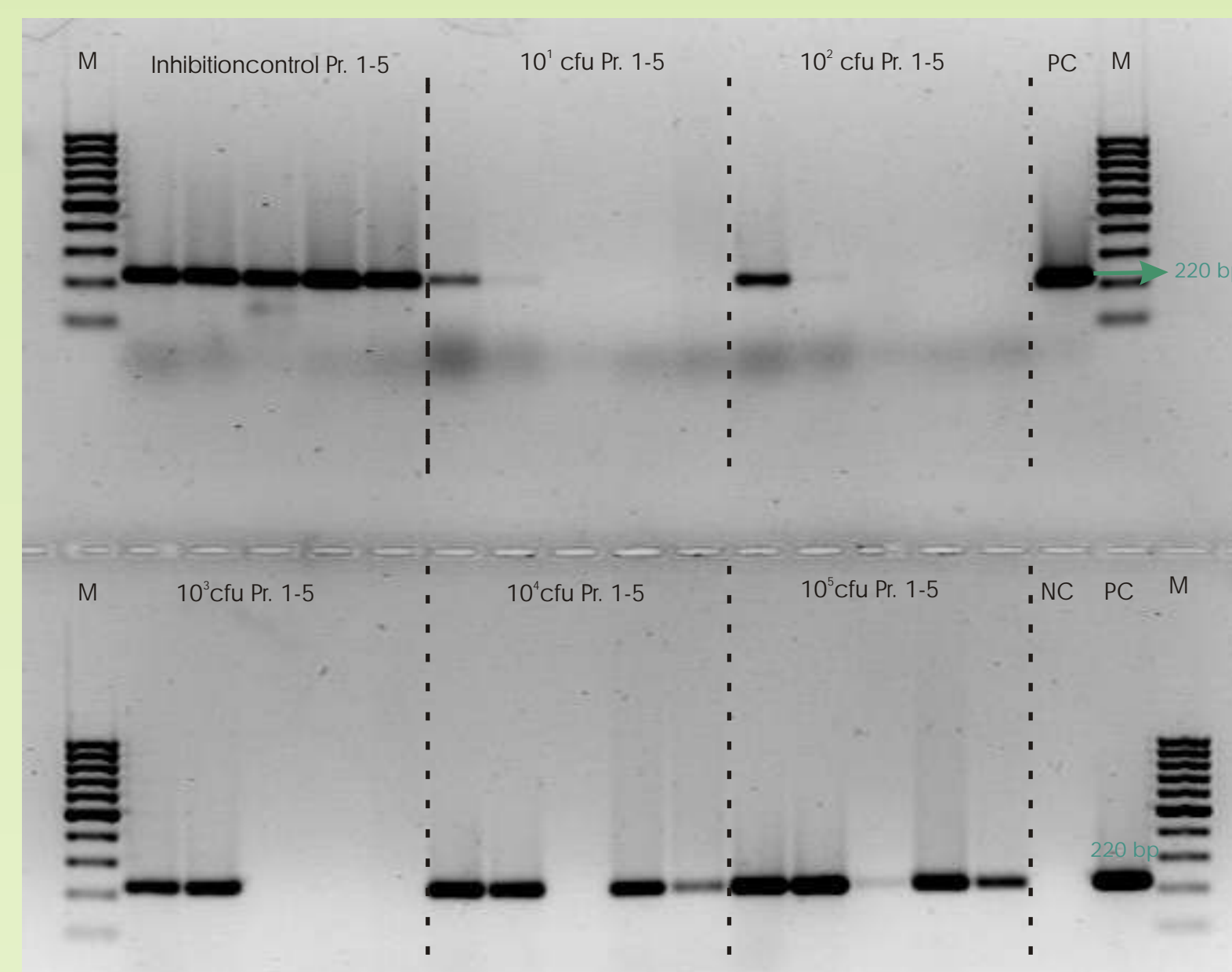


Fig 3: PCR-Gel from spiked foodstuffs

	Culture method	PCR	RNA-Hybridization
Sensitivity	1,0	0,4 - 1,0	0,0 - 0,6
Specificity	(1,0)	1,0	1,0
Detection limit	1 cfu/g	10 ng/ml	1000 cfu/ml

Tab.1: Comparison of sensitivity, specificity and detection limit of the reference method (culture), PCR and RNA-Hybridization of spiked foodstuffs

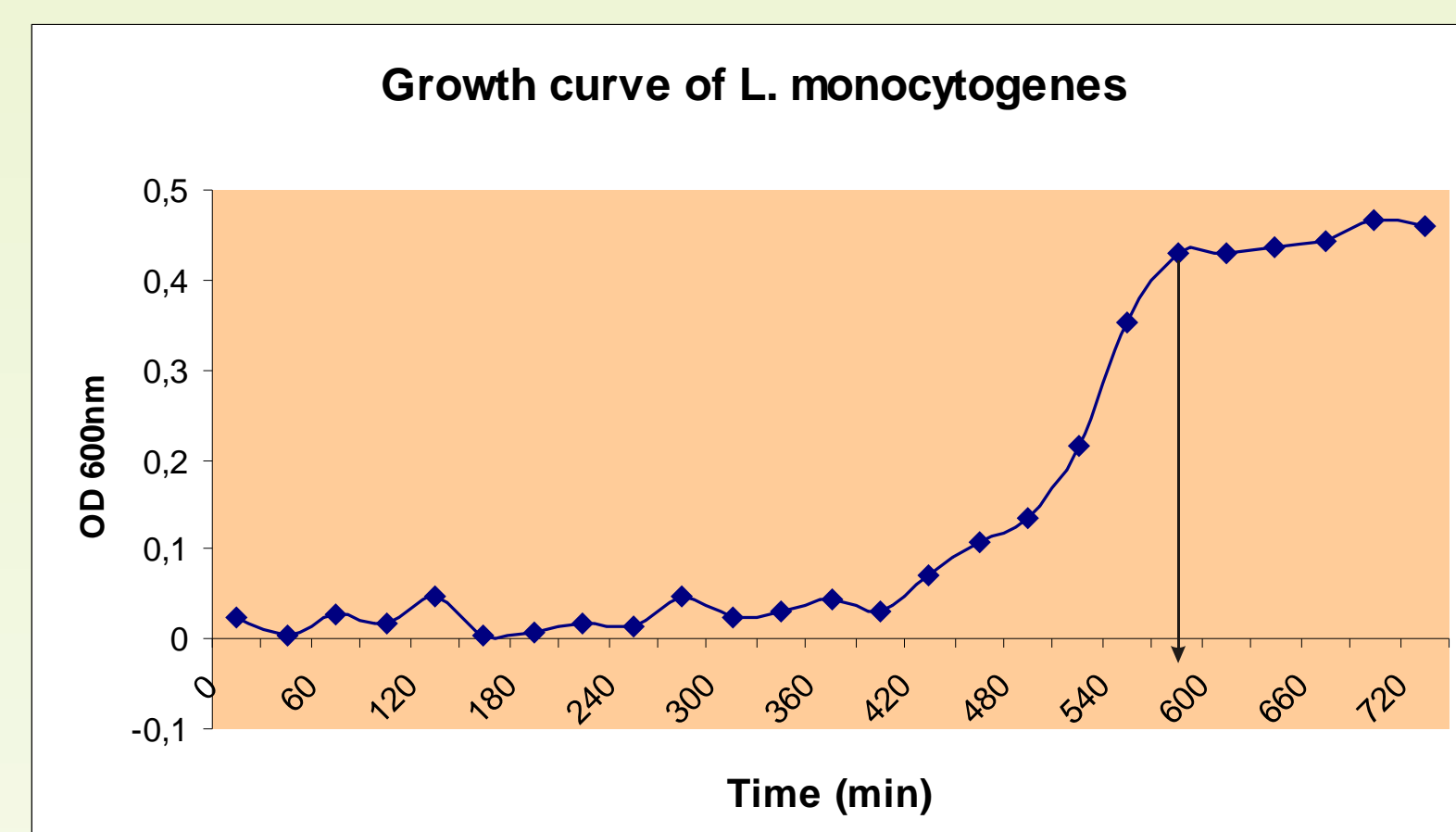


Fig 4: growth curve from *L.monocytogenes* in peptone water

## Results and Discussion

All samples (excluding 0 cfu/g) inoculated with *L. monocytogenes* tested positive by culture methods, with >math>10^6</math> cfu/g after 24-hour incubation. Table 1 shows a comparison between the three methods utilized in this study, based on sensitivity and specificity. For the PCR the sensitivity\* achieved at the detection limit of 10ng DNA/ml was: 0,4 for samples inoculated with  $10^1$ - $10^3$  cfu/g; 0,8 for  $10^4$  cfu/g and 1,0 for  $10^5$  cfu/g respectively (Fig. 3). The specificity#, calculated from the 0 cfu/g inoculation and cross reactivities, of the PCR method is 1,0. Inhibition of the PCR-reaction due to sample-matrix effects was not seen.

The RNA-hybridization assay achieved a sensitivity of 0,0 at  $10^1$ - $10^2$  cfu/g, 0,4 at  $10^3$  cfu/g and 0,6 at  $10^4$ - $10^5$  cfu/g *L. monocytogenes*, with a detection limit of  $10^3$  cfu/ml. The specificity of this reaction is 1,0. Two of the five inoculated samples (Gouda/Mozarella, ham-and-onion Mettwurst) showed no results at all inoculation concentrations utilized. This effect in addition to the generally low sensitivity, especially at high inoculation levels, and a very heterogeneous background signal ( $OD_{450nm}$  0,189-0,011) suggest interference due to non-specific sample-matrix effects.

Inhibitory effects on both PCR and RNA-hybridization were observed when *L. monocytogenes* was pre-enriched in selective medium (Fraser, 1/2-Fraser, ONE-Bouillon) (results not shown). Figure 4 shows a growth curve of *L. monocytogenes* in buffered peptone water. Logarithmic growth occurs approximately between six and ten hours after inoculation, after which the culture enters the stationary phase. Extension of the pre-enrichment cycle to >24-hours therefore does not increase the sensitivity of the detection systems.

*L. monocytogenes* was present in three of the 78 random samples tested by the reference method (plate culture/biochemical). Two of these three positive samples were detected as positive by PCR, the remaining sample consequently resulted in a false negative result. The RNA-hybridization assay detected one of the three samples as positive and the remaining two as false negative.

\* Sensitivity = Number of Positive Results / Number of True Positives + Number of False Negatives  
# Specificity = Number of True Negatives / Number of True Negatives + Number of False Positives

## Conclusion

The PCR system used in this study is a suitable method for the detection of *L. monocytogenes* in foodstuffs. For broad spectrum application the quantification system must achieve a reproducible detection limit of  $10^2$  cfu/g. In our opinion this goal can be achieved by optimisation of the sample preparation protocol.

In comparison, the RNA-hybridization assay showed several flaws, especially with regards to sample-matrix effects. As with PCR this problem may be overcome by honing the sample preparation strategy. A further shortcoming of the RNA-hybridization assay is the relatively high detection limit ( $10^3$  cfu/ml). The advantages of this system are the obligatory detection of living cells (PCR may detect non-viable cells), and since the method is based on an ELISA-platform it is easily automatable and allows the simultaneous detection of many samples (screening).