The Development of a Membrane Surface Adhesion- Polymerase Chain Reaction technique for the rapid detection of *Listeria monocytogenes*.

O. M. Cloak, G. Duffy, J.J. Sheridan, T. Mooney
The National Food Centre, Teagasc, Dunsinea, Castleknock, Dublin 15, Ireland.

Background : *Listeria monocytogenes* is a pathogen which has been linked to several outbreaks of food poisoning involving a wide range of foods including coleslaw, milk, cheese, chicken and pate (McLauchlin *et al.*, 1996). Currently available cultural methods for the detection of *L. monocytogenes* are labourious and time consuming taking 4 days to obtain a presumptive result and a further 2-3 days to confirm and speciate (Farber and Peterkin, 1991) . Approaches to rapid methods for the detection of the pathogen have largely been directed towards the use of antibody and nucleic acid based tests. In general, these techniques require lengthy sample enrichment (48 h) to increase the numbers of target cells to a level of 10^5 - 10^6 cfu ml^{-1} prior to detection so these rapid tests can take up to three days to carry out. Thus, there is a real need for rapid methods of pathogen detection.

Objectives : To develop a rapid method for the detection of *L. monocytogenes* in food samples by combining a membrane surface adhesion isolation method with detection by the polymerase chain reaction.

Methods : Food samples (25g) were enriched overnight (18 h) at 30°C in buffered peptone water (225ml) and tested for the presence of *L. monocytogenes* using the procedure described in figure 1. A polycarbonate membrane was attached to a glass slide using 1% bacteriological agar and then immersed in the enriched culture for 15 minutes. This allowed the bacteria in the enrichment broth to attach to the surface of the membrane. After immersion the membrane was detached from the slide and placed in an eppendorf tube. The DNA was extracted from the bacteria by a chemical procedure (Hoffman and Winston, 1987) which also degraded the polycarbonate membrane. The extracted DNA was amplified using a PCR assay and primers (Thomas *et al.*, 1991). The primers employed were specific for the genetic sequence of listeriolysin O, a virulence factor in *L. monocytogenes*. The template DNA was initially denatured at 95°C for 5 min and then 35 cycles of PCR amplification were run under the following conditions : denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min and DNA extension at 72°C for 2 min. The amplified PCR products were detected using gel electrophoresis. The method was compared with a standard cultural procedure for the detection of *Listeria monocytogenes* (Duffy *et al.*, 1994). This method was applied to inoculated broth culture, inoculated meat culture and retail meat samples.

Results and discussion: Surface adhesion onto a polycarbonate membrane was successfully used to isolate bacteria from the enriched food sample. This simple and fast extraction procedure has previously been employed in conjunction with immunofluorescence (Sheridan *et al*, 1997). It is considerably faster and gives similar yields to immunomagnetc separation (Duffy *et al.*, 1997). The DNA was extracted from cells chemically. The advantage of this procedure was that it also degraded the polycarbonate membrane. This study showed that the listeryolysin O primer yielded a 520 bp product. This primer proved to be highly specific for *L. monocytogenes* only. In inoculated broths the surface adhesion PCR technique had a detection level of approximately $\log_{10}$ 3-4 cfu ml^{-1} . For inoculated meat samples a minimum level of $\log_{10}$ 4.0 cfu ml^{-1} *Listeria* in the culture was necessary to produce a visible PCR product band (520bp). For the 120 retail samples analysed using standard cultural technique and the Surface adhesion - PCR method, a total of 23 samples tested positive for *L. monocytogenes* using the standard method while the Surface adhesion - PCR method detected 20 positive samples. The results showed that in these undetected samples the level of *Listeria* was approximately $\log_{10}$ 2 - 3 cfu ml^{-1} which is below the detection limit of the PCR test. A longer enrichment period may address this problem. This method took approximately 24h to carry out and is considerably faster than previously described PCR methods (Bessesen *et al.*, 1990) . It has considerable potential to be developed for use with a wide range of foods and a range of food pathogens.

Conclusions : The membrane surface adhesion assay is a rapid, sensitive and specific method for the detection of *L. monocytogenes* in food samples and can be completed within a 24 hour time period.

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Figure 1 : A surface adhesion polymerase chain reaction technique for the isolation and detection of *L. monocytogenes* from an enriched food sample.