Development and application of a novel Peptide Nucleic Acid probe for the specific detection of *Cronobacter* (*Enterobacter sakazakii*) in powdered infant formula

C. Almeida[1,2], N. F. Azevedo[1,2], C. Iversen[3] , S. Fanning[3], C. W. Keevil[2] and M. J. Vieira[1]

[1]IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar 4710-057, Braga, Portugal; [2]Environmental Healthcare Unit, School of Biological Sciences, University of Southampton, Basset Crescent East, Southampton SO16 7PX, United Kingdom; and [3]Centres for Food Safety & Food-borne Zoonomics, UCD Veterinary Sciences Centre, University College Dublin, Belfield, Dublin 4, Ireland.

Cronobacter spp. are causative agents of meningitis, septicemia and necrotizing enterocolitis in neonates and immunocompromised infants. Recently, contaminated powdered infant formula (PIF) has been reported as a source of these infections. In order to minimize the risk of infection, the development of a rapid, sensitive and specific method for the early detection of this bacterium in infant formula is of the utmost importance.

Fluorescence *in situ* hybridization (FISH), a technique that allows direct visualization of whole cells, has been combined with specific peptide nucleic acid (PNA) probes, a new synthetic molecule with a better hybridization performance than DNA probes. In this work, a new FISH method for the detection of *Cronobacter* spp. using a novel PNA probe is reported. This PNA-FISH method was then adapted for the detection of this bacterium in PIF.

The PNA-FISH procedure using the *Cronobacter* probe proved to be a reliable method for the detection of this pathogen in PIF samples and an alternative to existing molecular methods. It presented high specificity and sensitivity, detected less than 1 CFU per 10g of *Cronobacter* in infant formula and provided detection in less than 12 hours. Direct visualization of bacterial cells was possible and the method was simple and easy to use, without any special equipment apart from an epifluorescence microscope. The samples can be also analysed by flow cytometry.