TOWARDS THE DEVELOPMENT OF A RAPID NUCLEIC ACID BASED TECHNIQUE FOR THE SENSITIVE AND SPECIFIC DETECTION OF *SALMONELLA* SPP. ON FRESH MEAT

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Introduction
The safety of food must be assured by a preventative approach based on the application of a food safety management system such as Hazard Analysis Critical Control point (HACCP). The setting of microbiological criteria and testing of food samples against the criteria, can be used to support food safety management systems and provide assurance that process controls and performance criteria or food safety objectives have been achieved (Stewart *et al.*, 2002). To date EU microbiological criteria have not addressed the presence of pathogens such as *Salmonella* on fresh meat carcasses, however this is now being addressed and is included in the EU Commission "Microbiological criteria for foodstuffs". Testing may be carried out by a cultural method (ISO 6579) which takes 3-4 days for positive clearance of samples, or by an equivalent rapid method in EN/ISO 16140:2003. The requirement to test for *Salmonella* means that industry has an immediate need for an alternative rapid method as the delays incurred in waiting for test results will otherwise involve a considerable sized product holding area and delayed product release. The aim of the project is to develop and apply highly specific and user friendly nucleic acid based (DNA and RNA) rapid methods based on real time PCR or fluorescent in situ hybridisation (FISH) approaches for the detection of Salmonella on fresh meat carcasses and chicken skin. The equivalence of the developed method with the traditional method will be carried out according to the procedures outlined in EN/ISO:16140

Materials and Methods
Initial studies included further validation of a 16S rRNA DNA target method (Catarme *et al.*, 2006., Trkov *et al.*, 2003) using a *Salmonella* enterica serovar bank and a range of non-*Salmonella* species by real time and conventional PCR. DNA was extracted from 1 ml of overnight pure cultures of *Salmonella* using the DNAeasy extraction kit (Qiagen), and real time PCR was carried out using 16S rRNA target specific primers and the SYBR Green detection format using the Lightcycler (Roche) platform.
Further studies investigated gene targets identified from the literature in addition to looking for novel gene targets. Identified targets hilA (Pathmanathan *et al.*, 2003), sip b/c (Ellingson *et al.*, 2004), and the ttr (Malorny *et al.*, 2004) were carried out in real time DNA polymerase chain reaction (PCR) protocol using a panel of *Salmonella* serovars and non-*Salmonella* species. When a suitable gene was identified RNA was extracted from twenty three overnight pure cultures of *Salmonella* spp. and twelve non-*Salmonella* strains using the SV total RNA isolation kit (Promega) and RNA was reverse transcribed into cDNA using the ImProm-II reverse transcription system (Promega). Real time PCR was carried out on both the DNA and the cDNA for the twenty three strains and the twelve non-Salmonella strains with the newly designed primer set. The newly developed real time protocol using the SYBR Green detection format was assessed using inocula as low as 10 CFU ml^{-1}.

Results and Discussion
A 16S rRNA DNA target was tested in conventional and real time PCR format using a *Salmonella* enterica serovar bank and a range of non-*Salmonella* strains. It was found that although this method was highly sensitive and specific for *Salmonella* spp. that there was some cross-reactivity with these primers and some of the non-*Salmonella* species. It was concluded that there was a need for a more specific DNA/RNA target for *Salmonella* spp. detection.
From the number of genes tested the target chosen was against the hilA gene. The nucleic acid from twelve typed strains of *Salmonella* were sequenced and aligned and new primers designed against the hilA gene. Using the newly designed primers RNA was successfully reverse transcribed into cDNA and real time PCR was carried out using both DNA and cDNA from all of the twenty three strains. The method was found to be both sensitive and specific for *Salmonella* spp. and there was no cross reactivity with the primers and any of the twelve non-*Salmonella* strains. Further development of the method would be to design a probe towards the PCR product and an internal amplification control (IAC), these design measures would ensure specificity, sensitivity and efficiency of the method.

Conclusions
The requirement for testing for *Salmonella* means that industry has an immediate need for an alternative rapid method as the delays incurred in waiting for test results will otherwise involve a considerable sized product holding area and delayed product release and thus have a significant economic impact. The potential DNA/RNA target for the hilA gene

has been identified for the detection of *Salmonella* spp. and is now being investigated for use in either a real time reverse transcriptase polymerase chain reaction (RT-PCR) protocol or a fluorescent in situ hybridization (FISH) format for the detection of *Salmonella* spp. in meat samples based on specificity, limit of detection and user friendliness.

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