[D&PA 29]

Validation of a Peptide Nucleic Acid Fluorescence *in situ* Hybridization for the specific detection of *Salmonella* species in food matrices

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Salmonella is a Gram-negative flagellated rod-shaped bacterium that is one of the most important etiological agents in bacterial foodborne diseases [1,2]. Despite human salmonellosis generally presenting as a self-limiting episode of enterocolitis, the infection can degenerate into chronic and debilitating conditions [2]. To diagnose a *Salmonella* infection, standard cultural methods are routinely used, which implies bacterial identification by biochemical and serological tests, to confirm the suspectcolonies grown on the selective agar [3]. However, this methodology is time-consuming and takes too long to deliver the results [4]. Due to these limitations, more rapid techniques for detection have been developed [5-7]. For that, in this study, we developed a novel Peptide Nucleic Acid Fluorescence *in situ* Hybridization (PNA FISH) method for the specific detection of *Salmonella* spp.

The method was based on a new PNA probe, SalPNA1692, coupled with a novel blocker probe ina 1:1 ratio. The method was optimized for the detection of *Salmonell*a in food samples through an evaluation of several rich and selective enrichment broths. The best outcome was achieved using Buffered Peptone water as a pre-enrichment for 24 h followed by 16 h of selective enrichment in RambaQuick broth. For validation in food samples, fresh ground beef was artificially contaminated with two ranges of inoculum: a low level (0.2–2 CFU/25 g) and a high level (2–10 CFU/25 g). For both levelsof contamination, the confirmed positives were the same comparing the PNA-FISH method and the reference method (ISO 6579-1: 2017). The new PNA-FISH method presented a specificity of 100 % and is a faster time-to-result method, making it a good candidate for routine application in food safety laboratories.

Acknowledgments: This work was financially supported by: LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC). This study was also financed by Project NORTE-01-0247-FEDER-046970.

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