Cost-effective optimization of real-time PCR based detection of Campylobacter and Salmonella with inhibitor tolerant DNA polymerases

AIMS:
The aim of this study was to cost-effectively improve detection of foodborne pathogens in PCR inhibitory samples through the use of alternative DNA polymerases.

METHODS AND RESULTS:
Commercially available polymerases (n=16) and PCR master mixes (n=4) were screened on DNA purified from bacterial cells in two validated real-time PCR assays for Campylobacter and Salmonella. The five best performing (based on: limit of detection (LOD), maximum fluorescence, shape of amplification curves, and amplification efficiency) were subsequently applied to meat and fecal samples. The VeriQuest qPCR master mix performed best for both meat and fecal samples (LODs of 10^2 and 10^4 CFU ml^-1 in the purest and crudest DNA extractions, respectively) compared with Tth (LOD=10^2 - 10^3 and 10^5 -10^6 CFU ml^-1 ). AmpliTaqGold and HotMasterTaq both performed well (LOD=10^2 -10^4 CFU ml^-1 ) with meat samples and poorly (LOD=10^3 -10^6 CFU ml^-1 /not detected) with fecal samples.

CONCLUSIONS:
Applying the VeriQuest qPCR master mix in the two tested real-time PCR assays could allow for simpler sample preparation and thus a reduction in cost.

SIGNIFICANCE AND IMPACT OF STUDY:
This work exemplifies a cost-effective strategy for optimizing real-time PCR based assays. However, a DNA polymerase suitable for one assay and sample type is not necessarily optimal for other assays or sample types. This article is protected by copyright. All rights reserved.
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