Polymerase study: Improved detection of Salmonella and Campylobacter through the optimized use of DNA polymerases in diagnostic real-time PCR

Søndergaard, Mette Sofie Rousing; Löfström, Charlotta; Al-Habib, Zahra Fares Sayer; Nielsen, M.T.; Hoorfar, Jeffrey; Josefsen, Mathilde Hasseldam

Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

Improved detection of *Salmonella* and *Campylobacter* through the optimized use of DNA polymerases in diagnostic real-time PCR

Mette S. R. Søndergaard, Charlotta Löfström, Zahra F. S. Al-Habib, Mette T. Nielsen, Jeffrey Hoorfar and Mathilde H. Josefsen.

National Food Institute, Technical University of Denmark, Søborg, Denmark. Email: msrso@food.dtu.dk

Diagnostic analyses of foodborne pathogens are increasingly based on molecular methods such as PCR, which can improve the sensitivity and reduce the analysis time. The core of PCR is the enzyme performing the reaction: the DNA polymerase. Changing the polymerase can influence the sensitivity and robustness of a PCR assay, as some polymerases are more resistant to inhibitors, and thus be a simple strategy for assay optimization. Identifying an optimal polymerase can even render costly and time-consuming sample preparation unnecessary.

The aim of this study was to evaluate the performance of 16 commercially available polymerases and four master mixes in two validated PCR assays, for *Campylobacter* and *Salmonella*, respectively, to develop more sensitive, robust and cost effective assays. The polymerases were screened on purified DNA and the five best performing, for each PCR assay, were then applied on sample matrices known to contain PCR inhibitors (i.e. minced meat samples for *Salmonella* and chicken fecal samples for *Campylobacter*).

The samples were prepared for PCR by three methods: No DNA extraction, lysis by boiling and semi-automated DNA extraction for *Salmonella* and lysis by boiling and two different DNA extraction methods for *Campylobacter*.

Results show that VeriQuest qPCR master mix have the best general performance, while the AmpliTaq Gold and HotMasterTaq DNA polymerases performed well with meat samples and poorly with fecal samples. Tth DNA polymerase performed well only with the purest DNA extractions and intermediate or bad with the crude extractions, while TaKaRa ExTaq HS only performed well with the purest extractions of fecal samples and intermediate with semi-automated magnetic beads based extracted fecal samples.

In conclusion, our data shows that exchanging the DNA polymerase from e.g. Tth to VeriQuest qPCR Master Mix could allow for a less thorough pre-PCR sample preparation with maintained sensitivity, resulting in savings in time and costs.