

Evaluation of probe chemistries and platforms to improve the detection limit of real-time PCR

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Abstract

A validated qPCR-based *Salmonella* method targeting a 94-bp sequence of the *ttr* gene was used as a model to compare six different combinations of reporter and quencher dyes of a TaqMan probe, on three different instruments, to improve the detection limit in a realtime PCR assay with the aim of a same-day analysis. The use of locked nucleic acids (LNA) and Scorpion probes were also tested. The combination FAM–BHQ1 or Cy5–BHQ3, both dark quenchers, gave the best results (Cycle threshold (Ct) of 25.42 ± 0.65 and 24.47 ± 0.18 at 10^3 DNA copies). When comparing different probe technologies, the LNA probe (FAM–BHQ1) was the most sensitive with the strongest fluorescence signal (dR last 48066), resulting in 0.6 to 1.1 lower Ct values than TaqMan probe, and 1.9 to 4.0 lower Ct than the Scorpion system (FAM–BHQ1). The RotorGene real-time PCR instrument gave 0.4–1.0 lower Ct values (more sensitive) than the Mx3005p, and 1.5–3.0 lower than the ABI 7700. Using the LNA in a RotorGene instrument, we detected the following *Salmonella* DNA copies in 1-ml pre-enriched samples: fishmeal (100 copies), chicken rinse (100 copies) and pig feces (10 copies). The detection probability of the final assay on inoculated fecal samples was 100% at 2×10^4 copies per ml. In conclusion, the LNA probe with annealing temperature of 65 °C could be useful for more sensitive detection limits.

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