Title	Novel PCR-based rapid detection strategies for Escherichia coli O157:H7 and Salmonella in meat products
Author	Luxin Wang and Azlin Mustapha
Citation	Thesis, Doctor of Philosophy, University of Missouri - Columbia. 2009.
Keywords	Escherichia coli O157:H7, Salmonella, Quantum dots; Antibodies

Abstract

Cultural-based detection method takes at least four days to complete. With the use of TaqMan® probes, the real-time PCR technique is a rapid and sensitive way to detect foodborne pathogens. However, DNA-based PCR techniques cannot differentiate between DNA from live and dead cells, while RNA-based PCR can. Ethidium bromide monoazide (EMA) is a dye that can bind to DNA of dead cells and prevent its amplification by PCR. An EMA staining step prior to real-time PCR allows for the effective inhibition of DNA contamination from dead cells. During the EMA treatment process, samples were stained with EMA for 5 min, iced for 1 min and exposed to bright visible light for 10 min prior to DNA extraction, to allow EMA binding of the DNA from dead cells. DNA was then extracted and amplified by TaqMan® real-time PCR to detect only viable *E. coli* O157:H7 and *Salmonella* cells. An internal amplification control (IAC), consisting of 0.25 pg of plasmid pUC19, was added in each reaction to prevent the occurrence of false negative results. The aim of this study was to use this EMA real-time PCR method to detect only viable *Salmonella* and *Escherichia coli* O157:H7cells from poultry and beef products. In addition, the sensitivity of this new designed EMA staining coupled real-time PCR was compared to that of an RNA-based reverse transcription (RT)-real-time PCR. With an optimized EMA staining step, the detection range of a subsequent real-time PCR was 10^{3} to 10^{9} CFU/ml for pure cultures, 10^{5} to 10^{9} CFU/ml for artificially contaminated poultry samples, and 10^{8} to 10^{4} CFU/g for ground beef samples. These detection ranges proved that EMA real-time PCR has better detection efficiency than RT-real-time PCR. After a 12-h enrichment step, EMA combined real-time PCR (EMA real-time PCR) could detect as low as 10 CFU/ml *Salmonella* from chicken rinses and egg broths, as well as 10 CFU/g *E. coli* O157:H7 from ground beef. The use of EMA real time PCR can successfully prevent false positive results from dead cells and represent a simple, yet accurate detection tool for enhancing the safety of food.

Accurate and fast detection methods for foodborne pathogens from various food samples have always been important goals for scientists from many research areas. Quantum dots (QDs) are a family of nanosized particles with a 1 to 10 nm in radius. It has long-term stable photostability, high quantum yield, broad absorption spectra, narrow emission spectra and high signal-to-noise ratio. QD has been used in cell

detection, imaging and DNA hybridization. In this study, EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) and protein A were used to build crosslinkers for making QD antibody conjugates. In order to minimize the interference generated from magnetic beads, a FlowComp(TM) Dynabeads with DSB-X(TM) biotin protein labeling kit (D-20655) was used to isolate the cells from the food matrix and the beads were removed after isolation. Detection signals were dramatically increased with the usage of the bead free isolation method. When bead free QD facilitated detection method was used to detect *Salmonella* and *E. coli* O157:H7 cells from pure cultures, it can detect as low as 10 CFU/ml cells. When it was applied to artificially contaminated ground beef, it can detect 10^6 CFU/g cells. After enrichment, it can detect as low as 10 CFU/g *Salmonella* cells from ground beef. The bead free QD facilitated detection method developed in this study is the first research that combines the bead free isolation method and QD labeling technique together to detect *E. coli* O157:H7 and *Salmonella* from ground beef. Further studies which can improve the detection range and specificity will be worth to try.