Evaluation of DNA Extraction Kits for the Detection and Differentiation of *Salmonella* Enteritidis and Typhimurium in Chicken Feces by Multiplex PCR

Ruchuda Kanjanapen[1,*] and Nanticha Rattanamangkalanon[1]

Abstract

Background: *Salmonella* spp. are important causative agents of human salmonellosis, particularly *S.* Enteritidis and *S.* Typhimurium. Human can become infected through consumption the contaminated food, especially chicken meat, where contamination may originate from environmental sources. Currently, the multiplex PCR technique has been applied for the detection of *Salmonella* spp. However, it may yield false-negative results due to the presence of PCR inhibitor commonly found in chicken feces, such as bilirubin, phytic acid, and bile salts. Therefore, selecting an appropriate DNA extraction method is crucial to reduce false-negative results.

Objectives: This study aim to evaluate three DNA extraction methods from chicken fecal samples for the detection of *Salmonella* spp. and differentiation of the serovars Enteritidis and Typhimurium using the multiplex PCR technique.

Methods: Chicken fecal samples confirmed negative for *Salmonella* spp. by the standard culture method (ISO 6579-1: 2017/Amd 1: 2020) were spiked with reference strains *S.* Enteritidis ATCC 13076 and *S.* Typhimurium ATCC 14028 at concentrations ranging from 10^1 to 10^7 cfu/mL, with one sample prepared for each concentration. DNA extraction was performed using three methods: Taco™, MagMax™, and QIAamp® Fast DNA Stool Mini Kit. The extracted DNA was subsequently analyzed by multiplex PCR. The most suitable extraction method was selected based on DNA yield and purity, and differences among extraction methods were statistically analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level ($\alpha = 0.05$). The selected method was then applied to field chicken fecal samples submitted to the laboratory, and multiplex PCR results were compared with those obtained using the standard culture method.

Results: Significant differences in DNA yield and purity were observed among the three extraction methods. The MagMax™ method produced the highest DNA yield and purity (average DNA concentration: 110.27 ng/µL; DNA purity: 2.19), followed by the QIAamp® Fast DNA Stool Mini Kit, whereas the Taco™ method yielded the lowest DNA concentration. Consequently, MagMax™ was identified as the most appropriate method for this study. Multiplex PCR detection of *Salmonella* spp. detection and differentiation of *S.* Enteritidis and *S.* Typhimurium in field chicken fecal samples demonstrated complete agreement with the standard culture method. Among the evaluated methods, MagMax™ and Taco™ exhibited advantages in terms of reduced processing time, lower labor requirements, simplified procedures, and lower reagent costs; however, both methods require an automated nucleic acid extraction system. In contrast, the QIAamp® Fast DNA Stool Mini Kit was more suitable for laboratories without automated extraction instruments, although it required more labor-intensive, time-consuming procedures and incurred higher costs.

Conclusion: DNA extraction using the MagMax™ method demonstrated high efficiency and suitability for extracting DNA from chicken fecal samples for the detection of *Salmonella* spp. and differentiation of *S.* Enteritidis and *S.* Typhimurium using multiplex PCR. Therefore, the three evaluated DNA extraction methods may serve as alternative approaches for researchers to adapt according to laboratory conditions.

Keywords: DNA extraction method, chicken feces, multiplex PCR, *S.* Enteritidis, *S.* Typhimurium

[1] Veterinary Research and Development Center (Eastern Region), Klong Kio, Ban Bueng, Chon Buri 20220, Thailand

[*] Corresponding author; E-mail: ruchuda277@gmail.com