# 学会・研究会発表

## 公衆衛生関係（海外）

## Comparison of methods of extracting DNA from human fecal samples contaminated with four bacterial pathogens

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**ABSTRACT**

**INTRODUCTION**: We previously reported our developed screening system using multiplex real-time SYBR Green PCR assays (RFBS24), which were simultaneously evaluated for the detection of 24 target genes of foodborne pathogens in fecal DNA samples. Fecal DNA samples were prepared by the Qkit method (lysing bacterial cells using surfactant). However, the efficiency of DNA extraction from Gram-positive bacteria is low with this method. Therefore, we investigated DNA extraction methods optimized for RFBS24.

**METHODS**: (1) DNA samples were extracted from fecal samples inoculated with *Clostridium perfringens*, *Staphylococcus aureus*, *Salmonella* Typhimurium, and *Campylobacter jejuni* by the Qkit method and Ukit method (disrupting bacterial cells by bead-beating). (2) Real-time SYBR green PCR (SG-PCR) and real-time quantitative PCR (q-PCR) were performed with these samples. (3) Patient fecal DNA samples by the Qkit and Ukit methods in *Salmonella* and *Campylobacter* foodborne outbreaks were tested using RFBS24 and the results were compared with bacterial culture methods.

**RESULTS**: (1) Regarding SG-PCR, the mean Ct value of Ukit DNA samples from the four bacteria were lower than that of the Qkit DNA samples. (2) The copy numbers of Ukit samples from the four bacteria were 8-234 times higher than that of the Qkit samples. (3) The positive rate of RFBS24 using Ukit samples was higher than that using Qkit samples.

**CONCLUSIONS**: The efficiency of DNA extraction was higher with the Ukit method than with the Qkit method. The Ukit method also effectively improved the positive rate of RFBS24 and other PCR tests.