Detection of *Kudoa septempunctata* 18S Ribosomal DNA in Patient Fecal Samples from Novel Food-Borne Outbreaks Caused by Consumption of Raw Olive Flounder

Feces

CASE STUDY

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Introduction

A method to detect *K. septempunctata* 18S ribosomal DNA in fecal samples of outbreak patients using real-time PCR. A spiking experiment was performed to assess whether a previously developed real-time PCR assay was applicable to detect *K. septempunctata* in feces. Simultaneously, three commercially available kits were compared to determine relative extraction efficacy of *K. septempunctata* DNA.

Overview

- **Keywords:** Food-borne disease, Parasite identification, Human feces, qPCR, *K. septempunctata*
- **Aim of the study:** Identification of a standard method for DNA extraction from fecal parasites
- **Application:** Quantitative PCR
- **Sample name:** Human fecal sample
- **Sample type:** Feces
- **Material:** FastDNA™ SPIN Kit for Soil containing Lysing Matrix E, QIAamp® DNA Stool Mini Kit, UltraClean™ Fecal DNA Kit
- **Buffer:** Provided with each of the three commercial DNA extraction kits

Protocol and Parameters

To compare the amount of *K. septempunctata* (parasites) DNA extracted using the three kits.

1. 200 mg of each sample and 200 µL of DNA elution buffer were used during the extraction procedure for each kit.
2. Extracted DNA was stored at -20°C until use.

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Results

Real-time PCR was performed to compare parasite DNA extraction from human fecal samples using three commercial DNA extraction Kits: QIAamp® DNA Stool Mini Kit (Qiagen); FastDNA™ SPIN Kit for Soil (MP Biomedicals); UltraClean™ Fecal DNA Kit (MOBIO Laboratories). Superscript letters A to C indicate the DNA extraction efficiency in each experiment, from high (A) to low (C), as determined using the Turkey-Kramer test with a significance level (P) of 0.05.

Mean C_T ± SD$^{\alpha}$ at low and high concentrations of spiked *K. septempunctata* spores in fecal samples. The FastDNA™ SPIN Kit for Soil resulted in the lowest average C_T values compared to other kits for the majority of the samples tested.

	Low (1.6 x 10^4 spores/g)			High (1.6 x 10^6 spores/g)		
Sample	QIAamp	FastDNA	UltraClean	QIAamp	FastDNA	UltraClean
A	37.65 ± 0.747^{C}	27.55 ± 0.286^{A}	35.54 ± 0.751^{B}	30.70 ± 0.125^{C}	24.25 ± 0.547^{A}	29.48 ± 0.596^{B}
B	37.15 ± 0.435^{C}	29.40 ± 2.264^{A}	32.94 ± 0.330^{B}	31.83 ± 0.366^{C}	27.03 ± 0.323^{B}	25.31 ± 0.212^{A}
C	36.96 ± 0.422^{C}	27.75 ± 0.108^{A}	32.85 ± 0.193^{B}	29.52 ± 0.166^{C}	20.64 ± 0.215^{A}	25.65 ± 0.853^{B}

Conclusion

The FastDNA™ SPIN Kit for Soil proved to be the best DNA extraction method providing the highest PCR amplification.

FastPrep technology gives higher yields and increases detection limit threshold of PCR. FastDNA SPIN Kit for Soil is the most efficient method for extracting parasite DNA from fecal samples.

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