

# Optimisation of methods for the collection and detection of bacterial pathogens from diarrhoeal human faecal samples using a novel stool collection kit

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## Abstract

Bacterial pathogens such as *Escherichia coli*, *Salmonella*-, *Shigella*- and *Vibrio* species are known to be common causative agents for diarrhoeal disease in humans. This study aimed to develop a culture-independent PCR assay for the detection of bacterial pathogens present in human faecal samples collected using a less intrusive faecal collection technique, the Bio-wipe kit. A multiplex-PCR (m-PCR) was optimised targeting the *E. coli mdh* gene, the *Salmonella IpaB* gene, the *Vibrio sodB* gene, and the *lal* and *IpaH* genes present in entero-invasive *E. coli* and *Shigella* spp. The influence of the DNA extraction method, and sensitivity and specificity of the m-PCR and the Bio-wipe storage conditions on the detection of the bacterial pathogens was investigated. A guanidium thiocyanate DNA extraction method used with laboratory-prepared spin columns could successfully extract DNA from 93% of the samples analysed. The m-PCR could successfully identify and differentiate between the various pathogens tested and was specific for the selected pathogens. Faecal matter was successfully recovered from used Bio-wipes and the bacterial DNA could be detected from these samples at concentrations of 10 cfu. Bacterial DNA could be recovered from the Bio-wipes 5 to 10 d after use when the Bio-wipes were stored at 30°C and 14 d after usage when stored at ambient temperature. Thus the Bio-wipe kit, along with the m-PCR, can be used for collection and detection of bacterial pathogens during outbreaks and in rural settings.

**Keywords:** Bio-wipe kit, bacterial pathogens, faecal matter, PCR, DNA extraction