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Assessing Enteric Bacterial Viability and DNA Recovery using Fecal Transwab®

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Introduction

United Kingdom National External Quality Assessment Service (UK NEQAS) for Microbiology is an external quality assessment (EQA) provider with participating laboratories worldwide. EQA is an invaluable tool for clinical laboratories monitoring the performance, quality and reliability of their service.

It is estimated that 17 million individuals suffer from an outbreak of gastroenteritis in the UK annually[1]. Known causative agents of gastroenteritis include bacteria, parasites and viruses; with bacterial pathogens accounting for up to 30% of all cases[2]. From the organisms that can cause gastroenteritis in humans, five prevalent faecal pathogens were investigated in this study: *Campylobacter jejuni*, *Clostridium difficile*, *Salmonella* Typhimurium, *Shigella sonnei* and *Yersinia enterocolitica*.

The collection of a stool sample for culture and identification is routine procedure in the investigation of a patient who present with chronic diarrhoea. Culture results from faecal specimens can take several days in order to determine the causative agent, and even then the pathogen may be missed due to being present in low numbers or predominated by normal flora.

Molecular methodologies could provide results in a shorter time frame and with greater sensitivity and specificity. Diagnostic laboratories who have investigated the use of molecular screening in the routine detection of enteric bacterial pathogens have seen the benefits of its implementation; reduction in hands–on time to generate a final result, cost efficiency compared to conventional methods and the possibility of expanding the screening panel[3].

AIMS: To determine the suitability of Fecal Transwab®, manufactured by Medical Wire & Equipment (MWE), as the matrix for specimen delivery. To assess the stability and viability of faecal pathogens for culture and molecular detection over a period of storage.

Keywords

Cary Blair medium, enteric bacteria, External Quality Assessment, Fecal Transwab®, gastroenteritis, multiplex real-time PCR

References

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Materials and Methods

Inoculating Fecal Transwab®

Pure cultures of each of the five organisms were used to prepare 0.5 McFarland suspensions in distilled water. The suspensions of *S.* Typhimurium, *S. sonnei* and *Y. enterocolitica* were further diluted 1:10. A 100µL volume of each of the organism suspensions were then used to inoculate swabs in duplicate.

One set of the inoculated batch of Fecal Transwab®, for each pathogen, were incubated at 4°C and another set at 22°C. The swabs were removed from storage for testing at selected time points throughout the study period. The viability assessment was over a total of 28 days and the DNA recovery over 112 days.

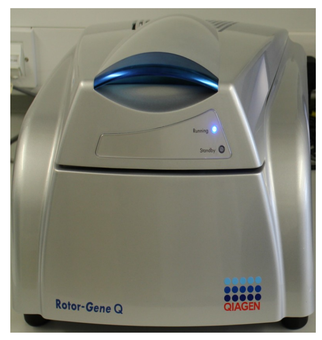


Figure 1: Rotor-Gene Q thermocycler

Molecular detection

To assess DNA recovery all five bacterial pathogens were manually extracted using the QIAGEN QIAamp MiniElute Virus Spin Kit. The detection of the enteric pathogens were achieved using the Fast-Track Diagnostics Bacterial gastroenteritis kit on the QIAGEN Rotor-Gene Q thermocycler platform (Figure 1).

Results

- In total, all 96 test results of the inoculated Fecal Transwab® tested over the period of 112 days and stored at 4°C (Figure 2) and 22°C (Figure 3), for all the respective pathogens, gave a strong PCR positive signal.
- All five enteric bacteria demonstrated good stability during the study period irrespective of the storage temperature, showing no significant increase or decrease in the bacterial load detected.
- Due to the 48 hours incubation period *C. jejuni* and *C. difficile* requires, these inoculated swabs could not be removed from storage at day 2.

Figure 2: DNA recovery of five enteric bacteria stored at 4°C for up to 112 days

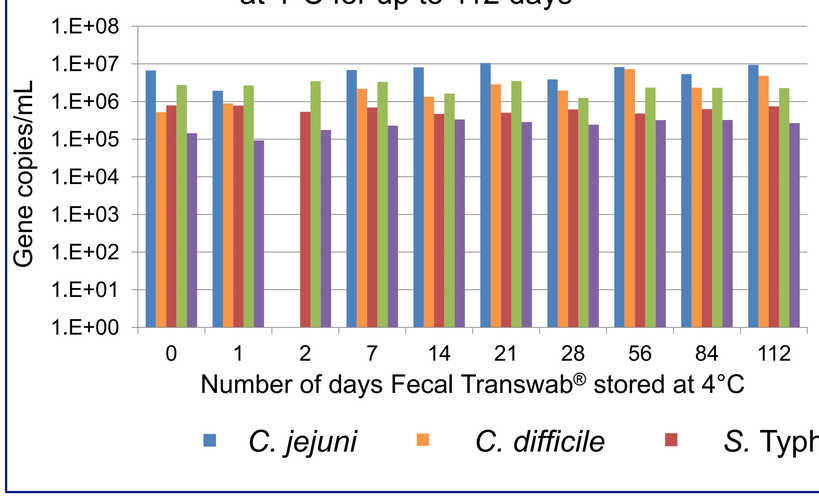
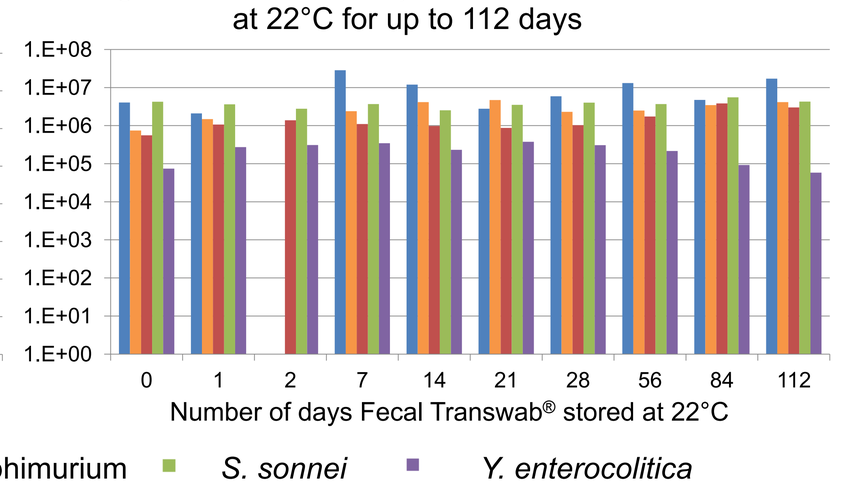


Figure 3: DNA recovery of five enteric bacteria stored at 22°C for up to 112 days



C. jejuni *C. difficile* *S.* Typhimurium *S. sonnei* *Y. enterocolitica*

Discussion

- All bacterial pathogens remained detectable by molecular testing throughout the study period, whether stored at 4°C or 22°C, with no significant impact on the bacterial load detected.
- The results suggests that the swab sample format could be compatible for use with molecular testing, which if used in routine practice can aid in time efficiencies in patient investigation and improve the overall turn around time of patient diagnosis.
- The study results also suggests that the use of swabs in developing an EQA scheme, aimed at the molecular testing of enteric bacteria, could be feasible. However the impacts of large scale production would require consideration and assessment.
- In current literature, DNA recovery and stability studies from transport media and swabs are limited; hence this investigation has introduced new insight into the field.

Acknowledgements

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