

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 with very little additional hands-on time and cost impacts^[4].

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.

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 the 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.

Determining the colony forming units/mL (CFU/mL) for each pathogen (Miles Misra method)^[3]

From the inoculated Fecal Transwab® Cary Blair medium a serial dilution of up to a 10⁻⁶ were performed for *C. jejuni* and *C. difficile*. For *S. Typhimurium*, *S. sonnei* and *Y. enterocolitica* a serial dilution of up to 10⁻⁷ were performed. For each dilution series, spread plates were prepared on Columbia Blood Agar (CBA), using a 20µL volume of each dilution and incubating it under the optimum conditions for the particular pathogen. To determine the bacterial load in each Fecal Transwab®, a colony count was performed on each dilution which yielded a colony count of between 20-100 individual colonies.

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).



Figure 1: Rotor-Gene Q thermocycler

Keywords

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

Results

CULTURE

- The viability of the enteric bacteria were observed for up to 28 days at 4°C in Fecal Transwab® with the exception of *C. jejuni* and *C. difficile* (Figure 2). There was difficulty in counting single colonies for all the enteric bacteria at Day 0, with the exception of *C. difficile*, therefore the readings are not presented on the Figures.
- *C. jejuni* was successfully isolated for up to 4 days at 4°C but failed to grow after just 24 hours of storage at 22°C. There was difficulty in counting single colonies of *C. jejuni* due to the large colony sizes and characteristic spreading across the surface of the culture media, therefore a graphical representation was not produced as accurate data was not obtainable.
- *C. difficile* was viable for up to 21 days at 4°C and remained viable up to 28 days along with the remaining three enteric bacteria (Figure 3).
- The general trend of the culture results showed a decline in the CFU/mL calculated at the end of storage in comparison to the CFU/mL levels seen at the start of the study. There were two exceptions to this trend: *Y. enterocolitica* at 4°C (Figure 2) and *S. Typhimurium* at 22°C (Figure 3). For both pathogens an increase in CFU/mL was seen by the end of 28 days in comparison to the CFU/mL determined at the start of the study.

Figure 2: A graph showing the viability of four enteric bacteria stored at 4°C for up to 28 days

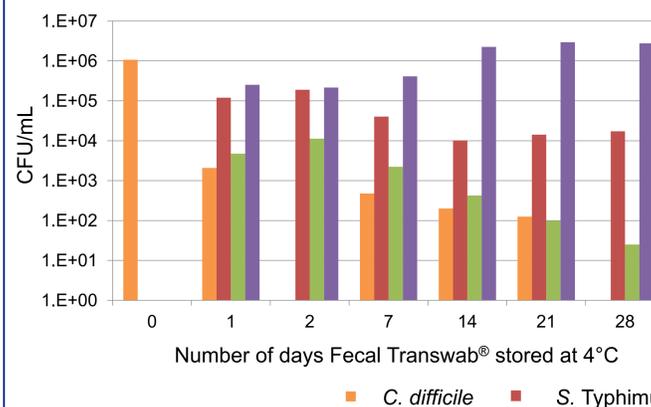
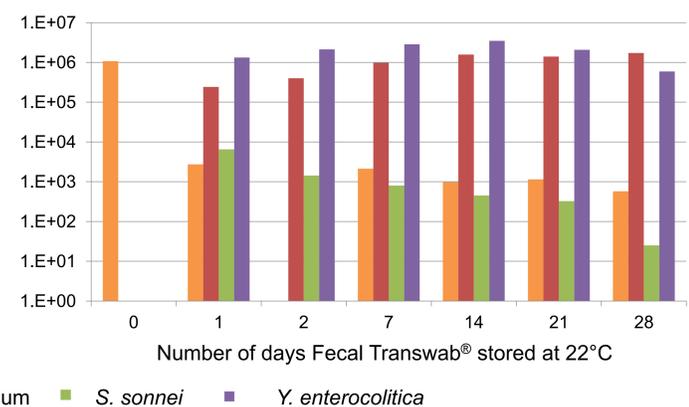


Figure 3: A graph showing the viability of four enteric bacteria stored at 22°C for up to 28 days



MOLECULAR

- In total, all 96 test results of the inoculated Fecal Transwab® tested over the period of 112 days and stored at 4°C and 22°C, for all the respective pathogens, gave a strong PCR positive signal.
- All five enteric bacteria demonstrated good stability irrespective of the storage temperature, showing no significant increase or decrease in the bacterial load detected.

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

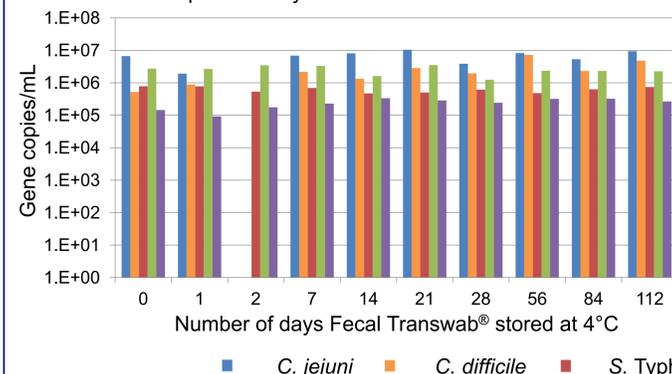
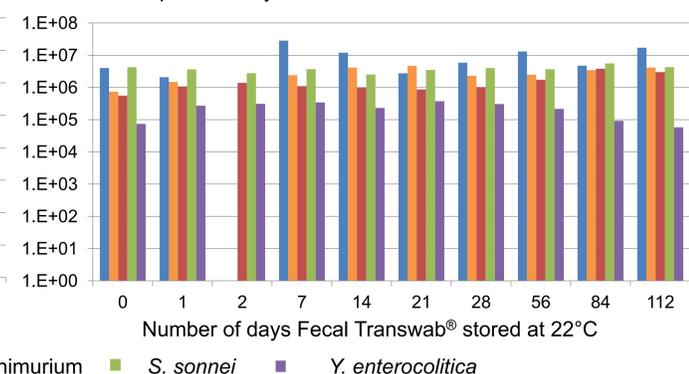


Figure 5: DNA recovery of five enteric bacteria stored at 22°C for up to 112 days



Discussion

Overall, the viability investigation showed good results for *S. Typhimurium*, *S. sonnei* and *Y. enterocolitica*, all showing successful isolation up to 28 days after storage at either 4°C or 22°C. The storage temperatures used in this study reflects those which can be experienced or possibly maintained in the transportation of diagnostic specimens to laboratories for investigation. *C. jejuni* and *C. difficile* are both fastidious pathogens and both these temperature conditions could have an impact on the viability of the pathogens which was seen by a decline in CFU/mL for both these pathogens in the viability assessment. However, the viability achieved at 4°C for *C. jejuni* and at both temperatures for *C. difficile* is considered sufficient to be used for diagnostic purposes. The viability assessment shows that the swab format would not be suitable for the development of an EQA specimen containing *C. jejuni*, *C. difficile* and *S. sonnei* due to the lack of stability. However the viability study would need to be extended past 28 days to assess its suitability for *S. Typhimurium* and *Y. enterocolitica*.

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. In this study 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 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.

References

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