Prospective evaluation of different faecal preservation media for subsequent molecular diagnostics

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Background and aims

Travellers' diarrhoea (TD) is a constant threat to international travellers, affecting ≥50% of individuals visiting high-risk destinations [1]. There is more widespread use of rapid molecular diagnostic tests, and an increased recognition of the importance of mixed infections as causes of TD [2]. We compared multiplex PCR test results of faeces preserved in different storage media.

Methods

The BioFire® FilmArray® multiplex PCR gastrointestinal panel (bioMérieux) was used to test freshly passed faecal samples (the reference standard), and samples stored in three different storage media (Figure 1).

A B C

Figure 1. Storage media evaluated for sample preservation. A- DNA/RNA shield DX™ (Zymo Research); B- OMNIgene® 200 (DNAgenotek®); C-Whatman FTA™ Elute cards (GE Healthcare).

Fresh faecal samples obtained during a previously reported diarrhoea outbreak amongst British military personnel deployed to Kenya in Feb-Apr 2022 were analysed [3]. Figure 2 shows the processing steps.

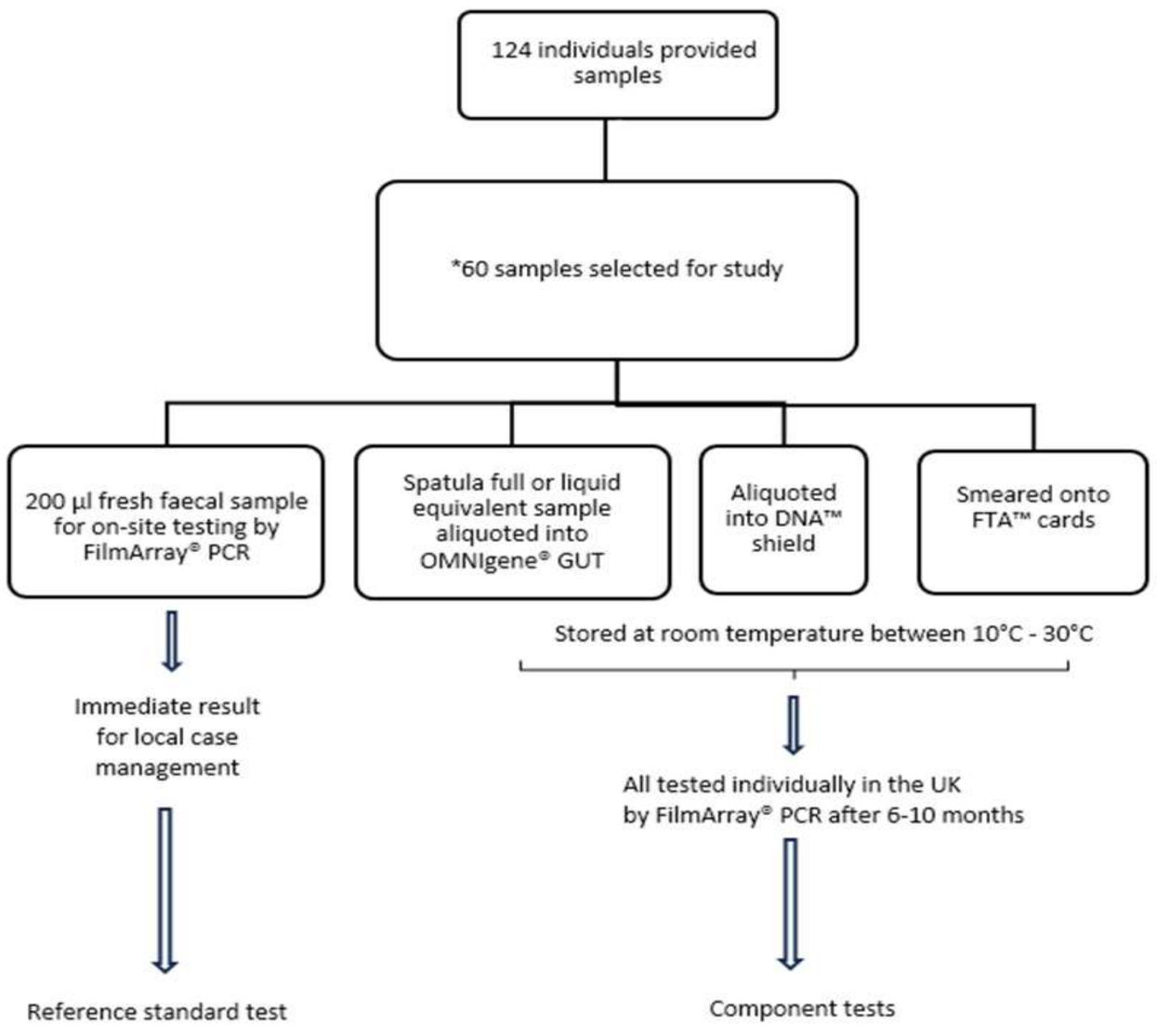

Figure 2. On-site and repatriated faecal sample processing steps. Stepwise approach from onsite sample collection and analysis, to repatriation using different storage media, followed by UK laboratory testing at different timepoints as shown above. *60/124 (48.4%) corresponding samples were selected to match the initial fresh faecal sample test (reference standard).

Results

- Sample size (n=60) with 80% male; median ([IQR] age 24 [22-28] years)
- Most common pathogens evaluated: *Cryptosporidium* spp., Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Shiga toxin producing *E. coli* (STEC), *Campylobacter* spp.
- *Campylobacter* spp., EAEC: test sensitivity was high across all three tests (86.4-100%)
- Good concordance of OMNIgene®200, DNA/RNA shield™ with fresh faecal sample test
- FTA™ Elute card tests had low sensitivity for STEC and poor specificity for *Campylobacter* spp.
- Agreement between FTA™ Elute cards when compared with the fresh faecal sample test was low-moderate (kappa coefficient ≤ 0 - 0.49) for all enteropathogens.

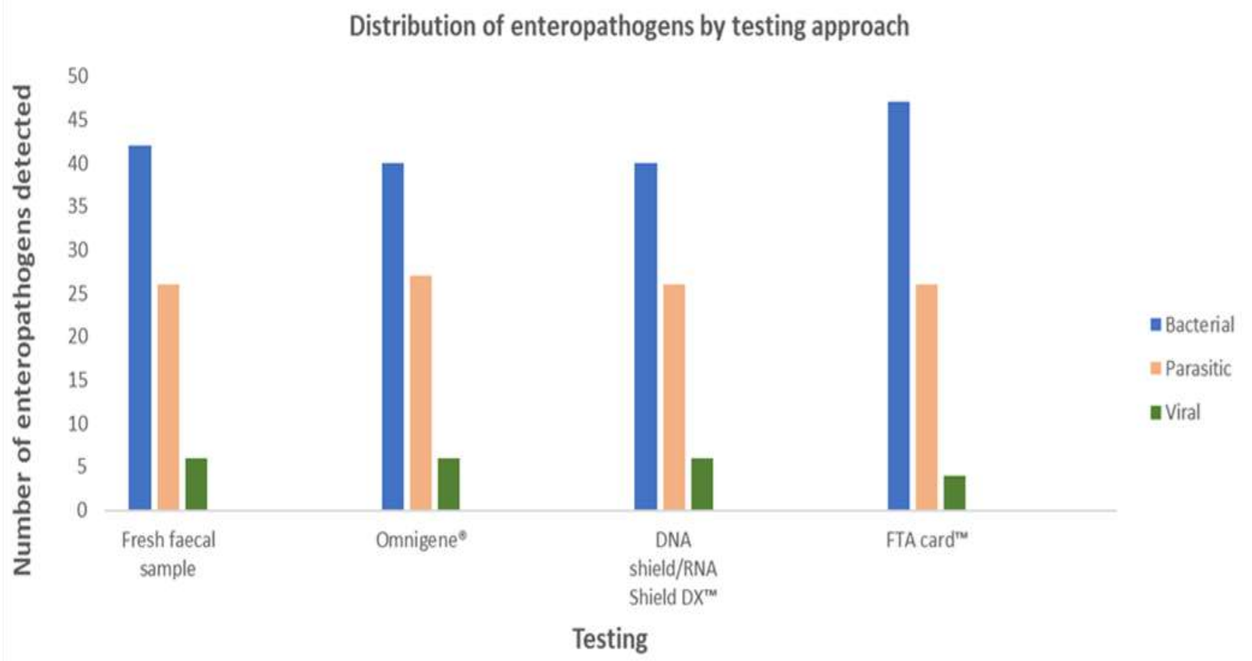

Figure 3. Pathogen group distribution by testing modality. Different coloured bars represent the number of samples in which bacterial, viral, and parasitic targets were detected.

Enteropathogen	Percentage observed agreement (POA) between different individual component tests versus Fresh sample reference standard test		
	OMNIgene®	DNA shield™	FTA™ card
Cryptosporidium spp.	98.3%	96.7%	71.9%
Enteroaggregative *E. coli* (EAEC)	93.2%	93.3%	72.4%
Enteropathogenic *E. coli* (EPEC)	91.5%	91.7%	67.2%
Shiga-like toxin-producing *E. coli* (STEC)	91.5%	89.8%	77.6%
Campylobacter spp.	100%	100%	69%

Colour Key
Substantial 81-100%
Moderate 61-80%

Figure 4. Heatmap summarising differences in percentage agreements between individual tests versus the fresh faecal sample test (reference standard) test.

Conclusions

- Successful field use of FilmArray® with comparable detection rates across storage methods in first simultaneous comparison of these three media with fresh faecal sample clinical test results
- Stored samples tested up to 18 months later with good concordance observed in OMNIgene®200 and DNA/RNA shield™ when compared with fresh faecal sample test
- Distorted performance of FTA™ Elute card testing requires further optimisation
- Testing of samples stored in these media is suitable for research studies, but applicability with other molecular diagnostic platforms, or clinical diagnostics requires confirmation.

References

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3. Toriro R, Pallett SJC, Woolley SD, Bennett CJ, Hale I, Heylings J, et al. Outbreak of diarrhoea caused by a novel Cryptosporidium hominis subtype during British military training in Kenya. OFID. 2024:ofae001