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An Evaluation of Faecal Transport Swabs Using the Swab Elution Method in Accordance With Approved Standard CLSI M40-A2.

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INTRODUCTION

Specimen collection using medical devices such as swabs is an integral part of patient infection diagnosis. Faecal swabs can be used for bacterial cultures in place of traditional stool samples. Efficient absorption, transportation and release of specimens without overgrowth, are essential features that faecal transport swabs must possess. The Clinical and Laboratory Standards Institute's standard M40-A2 includes testing for faecal transport systems using Enterobacteriaceae associated with gastrointestinal diseases. The testing encompasses conditions reflecting actual use with storage conditions, including cold (2 to 8°C) and controlled room temperature (20 to 25°C), and testing time intervals up to 72 hours. For recovery of samples to be considered acceptable, plate counts should remain stable and be within 2 log10 of the initial counts for each microorganism.

The aim of this study was to evaluate and compare the recovery efficacy of three faecal transport swabs using the quantitative elution method of CLSI M40-A2 adapted for enteric bacteria.

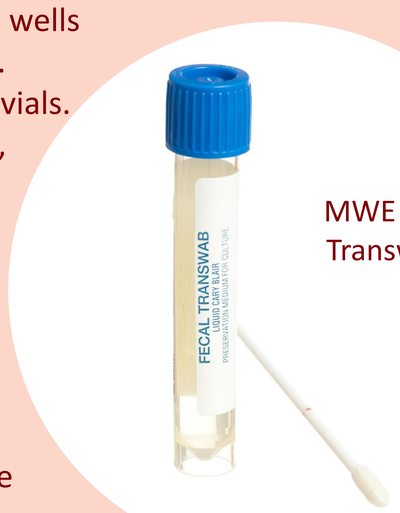
METHODS

Methods were adapted from the CLSI M40-A2 quantitative elution method for faecal transport swabs and enteric bacteria. The performance of three medical devices was evaluated and compared: MWE Fecal Transwab®, a polyurethane foam tip with 2 mL vial of Cary-Blair medium; MWE Fecal Transwab® Purflock, a multifilament polyester flocked fibre tip with 2 mL Cary-Blair medium; and Copan FecalSwab™ , a nylon flocked swab with 2 mL of Cary-Blair medium.

- Organisms used in this study included *Escherichia coli* ATCC 11775, *Salmonella typhimurium* ATCC 14028, and *Shigella flexneri* ATCC 12022.
- Culture suspensions were prepared in phosphate buffered saline (PBS) via direct colony inoculation from an 18-24 hour agar plate for each organism to achieve approximately 1.5 x 108 CFU/ml (0.08-1.2 OD at 600nm) and then further diluted 1/5 in PBS to give approximately $3.0x10^7$ CFU/ml.
- For each swab tested, 4 time points (0, 24, 48 & 72h) and two temperatures (RT & 4°C) were used as holding conditions.
- For each swab to be tested, and each organism/holding temperature/holding time, 50µl aliquots of the suspension were dispensed into wells of a microtitre plate and used to inoculate the swab.
- Inoculated swabs were placed in their respective vials.

For time zero, devices were vortexed for 15 seconds, swabs removed and discarded, and the transport medium was used to prepare serial dilutions. Dilutions were then spiral plated (100µl) on tryptone soya agar using an Neutec™ Eddy Jet™ 2 Spiral Plating System.

- For each swab/organism/temperature, at each holding time, the sampling procedure outlined above was used. Plate counts were used to calculate CFU/ml of recovered bacteria.



MWE Fecal Transwab®

DISCUSSION

The number of viable organisms remained stable for devices stored at 4°C and there was less than 1 log change over the 72 hour period for all test organisms and swab types.

At RT, the MWE Fecal Transwab® also maintained viable counts of all three organisms with less than 1 log change in CFU/ml. For the MWE Fecal Transwab® PF, at RT, there was a >2 log increase in CFU/ml increase for *E. coli*, approximately 2 log reduction for *S. typhimurium*, whilst no change in log CFU/ml was observed for *S. flexneri*. At the same storage condition i.e. RT, there was an increase in viable count for all three organisms with the Copan FecalSwab™ with 3.02 log CFU/ml increase in recovery for *E. coli*, and an increase of 2.51 log and 2.61 log respectively for *S. flexneri* and *S.typhimurium*.

At 4°C, the recovery of microorganisms was maintained for 72 h for all three swabs, compared to swabs stored at RT, thus all three swabs were compliant with the M40-A2 protocol at the cold temperature.

At RT, M40-A2 compliance was observed with MWE Fecal Transwab® (for all organisms) and MWE Fecal Transwab PF with *S. typhimurium* and *S. flexneri* with less than 2 log change in CFU/mL between 0 and 72 hours. The Copan FecalSwab™ at RT was not M40-A2 compliant for any of the tested organisms.

CONCLUSION

In this study all three devices recovered the target organisms acceptably, and in accordance with the M40-A2 standard. The Copan device did seem to promote growth at room temperature which is not desirable for a transport device as this can interfere with diagnostic testing in real situations.

RESULTS

Table 1. Recovery CFU/ml of *E.coli* ATCC 11775, *S. typhimurium* ATCC 14028, and *S. flexneri* ATCC 12202 at time 0, 24, 48 and 72 h with all swabs and temperatures.

Organism	Time (h)	FTPF 4°C	FTPF RT	FT 4°C	FT RT	CF 4°C	CF RT
***E. coli* ATCC 11775**	0	N/A	2.08E+05	N/A	2.07E+05	N/A	1.79E+05
	24	1.66E+05	1.08E+06	1.10E+05	9.17E+05	1.91E+05	TNTC
	48	1.30E+05	1.88E+07	8.53E+04	2.77E+06	1.66E+05	2.35E+08
	72	8.13E+04	5.86E+07	5.94E+04	8.82E+06	5.81E+04	1.88E+08
***S. typhimurium* ATCC 14208**	0	N/A	8.56E+05	N/A	6.93E+05	N/A	6.95E+05
	24	7.62E+05	1.92E+05	6.33E+05	5.13E+05	3.87E+05	TNTC
	48	5.87E+05	1.49E+05	3.84E+05	1.71E+06	4.99E+05	TNTC
	72	3.13E+05	1.30E+04	3.06E+05	TNTC	5.27E+05	2.74E+08
***S. flexneri* ATCC 12022**	0	N/A	2.20E+05	N/A	2.30E+05	N/A	2.50E+05
	24	2.23E+05	1.65E+05	1.34E+05	1.27E+05	1.88E+05	1.45E+07
	48	1.51E+05	7.52E+04	1.17E+05	7.23E+04	1.82E+05	5.99E+07
	72	4.55E+04	2.14E+05	8.51E+04	3.17E+04	2.30E+05	8.05E+07

RT- room temperature TNTC – too numerous to count. FTPF - MWE Fecal Transwab PF. FT - MWE Fecal Transwab®. CF – Copan FecalSwab™

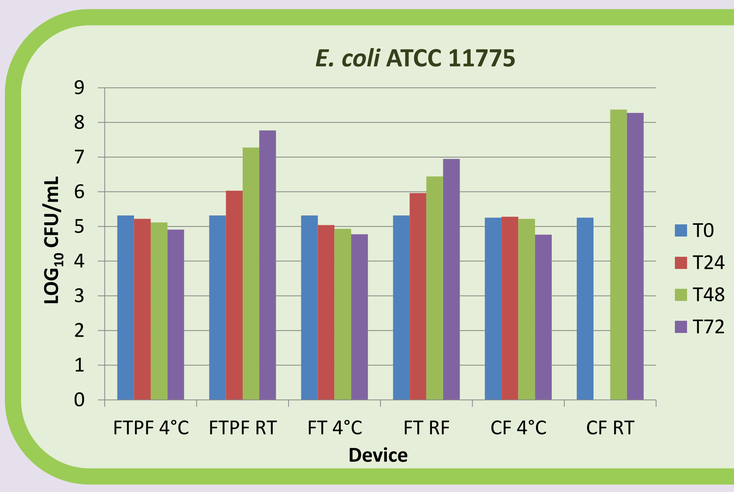


Figure 1. Log_{10} CFU/mL of *E.coli* ATCC 11775 at time points 0, 24, 48 and 72 hours for all swab devices and temperatures tested.

FTPF - MWE Fecal Transwab PF. FT - MWE Fecal Transwab®. CF – Copan FecalSwab™

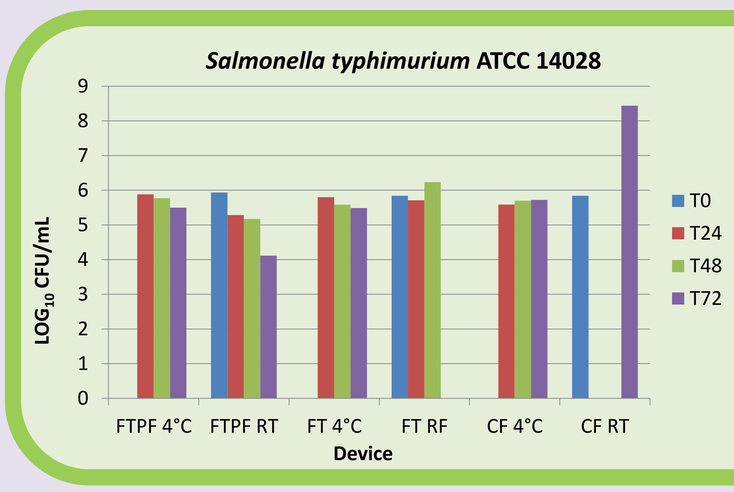


Figure 2. Log_{10} CFU/mL of *S. typhimurium* ATCC 14028 at time points 0, 24, 48 and 72 hours for all swab devices and temperatures tested.

FTPF - MWE Fecal Transwab PF. FT - MWE Fecal Transwab®. CF – Copan FecalSwabTM

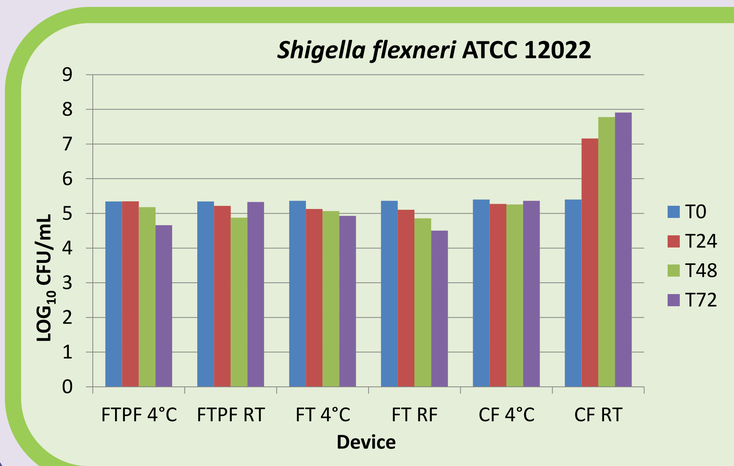


Figure 3. Log_{10} CFU/mL of *S. flexneri* ATCC 12022 at time points 0, 24, 48 and 72 hours for all swab devices and temperatures tested.

FTPF - MWE Fecal Transwab PF. FT - MWE Fecal Transwab®. CF – Copan FecalSwab™

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REFERENCES

Clinical and Laboratory Standards Institute (CLSI). *Quality Control of Microbiological Transport Systems; Approved Standard-Second Edition*. CLSI document M40-A2. Wayne, PA: CLSI; 2014.

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