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Heatherwood and Wexham Park Hospitals NHS
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An investigation of the suitability of liquid transport medium for recovery of enteric pathogens from faecal specimens

Kamran Khan & Jamie Laughlin

Objective

In recent years a number of automated processing systems have been introduced into clinical microbiology laboratories. These systems require a liquid specimen such as blood or urine as the matrix for processing. Transport swabs are also available with liquid medium for respiratory, urogenital, and wound specimens. Faecal specimens, however, which account for a considerable proportion of specimens in a routine clinical laboratory, could not be processed unless first emulsified and suspended in a broth. Recently a transport swab for faecal specimens (Fecal Transwab® by Medical Wire) has been developed which at the time of collection converts faeces samples into liquid specimens; suitable for direct processing on automated platforms. The present study was devised to investigate the performance; over typical transport periods, of this new device with a range of important enteric pathogens.

Methods

The Clinical and Laboratory Standards Institute (CLSI) standard M40-A; describes methods for assessing the ability of transport devices, to maintain various microorganisms in a viable condition for up to 48 hours, during transport at ambient (room temperature, roughly 18°C) or refrigerated temperatures (4°C). This standard, however, does not include any enteric pathogens. The present study used the principles and methods of CLSI M40-A to evaluate the new transport medium, adapted for the enteric microorganisms which are the target for faecal swabs. A 0.5 McFarland stock solution was prepared for Escherichia coli 0157, Campylobacter spp, Shigella dysenteriae, Shigella flexneri, Shigella boydii, Shigella sonnei, Vibrio cholerae, Salmonella typhi, Salmonella typhimurium, and Salmonella enteritidis. The initial stock solutions were diluted to produce serial dilutions from 10-1 to 10-4. These initial dilutions were required to calculate a workable recovery rate. 50µl was plated on to the appropriate media and following 24 hour incubation the number of colonies was counted. The 10-2 dilution was selected as it yielded between 30 and 300 colonies which were ideal for counting the number of colonies produced. All swabs were tested in triplicate to insure no random anomalies occurred, such as, failure to grow or human error. The swabs were inoculated using the target organism at the selected dilution, to comply with the M40-A standard the swabs were held at both ambient and refrigerated temperatures. Three swabs were tested at time 0 hours by plating out 50µl on to non selective agar; six swabs were tested after 24 hours holding period, three from ambient and three from the refrigerated. After a 48 hour holding time a further six swabs were tested for each target organism from both temperature ranges. The numbers of colonies recovered were recorded. All target organisms were tested with this method with exception of Campylobacter spp, this delicate organism was incubated on to non selective media which was incubated for 48 hours to allow growth to occur.

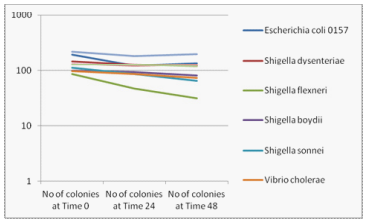

Fig 1 Recovery at refrigerated temperature (4-8°C)

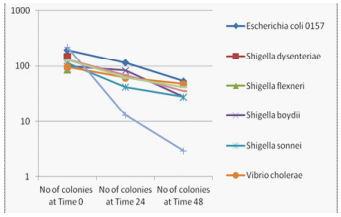

Fig 2 Recovery at ambient temperature (20-25°C)

Results

CLSI M40-A requires that organism numbers should not fall by more than 3 logs (log 10) over the stated holding period. Acceptable numbers were recovered for all the organisms tested for the refrigerated swabs, while acceptable recovery was also seen at ambient temperatures for many of the enteric organisms including Vibrio, Escherichia coli O157, and Salmonella. A previous study had shown good recoveries of Clostridium difficile under such conditions. However Campylobacter and Shigella dysenterae were not recovered at ambient temperatures.

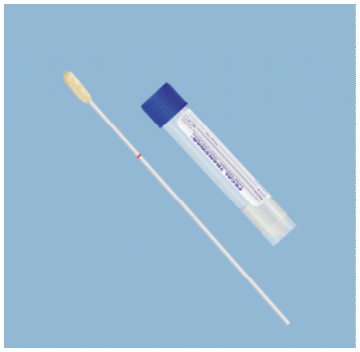

Table 1 shows the average numbers of colonies from the refrigerated swabs at 0 hours, 24 hours and 48 hours

Organism	No of colonies at Time 0	No of colonies at Time 24	No of colonies at Time 48
Escherichia coli 0157	197	126	140
Campylobacter spp	3+	3+	2+
Shigella dysenteriae	151	130	120
Shigella flexneri	86	48	32
Shigella boydii	100	93	81
Shigella sonnei	112	86	65
Vibrio cholerae	97	87	74
Salmonella typhi	222	185	201
Salmonella typhimurium	133	121	128
Salmonella enteritidis	129	129	118

Table 2 shows the average number of colonies from the swabs kept at ambient temperature at 0 hours, 24 hours and 48 hours

Organism	No of colonies at Time 0	No of colonies at Time 24	No of colonies at Time 48
Escherichia coli 0157	197	115	54
Campylobacter spp	3+	No growth	No growth
Shigella dysenteriae	151	No growth	No growth
Shigella flexneri	86	1	No growth
Shigella boydii	100	83	28
Shigella sonnei	112	42	28
Vibrio cholerae	97	62	48
Salmonella typhi	222	13	3
Salmonella typhimurium	133	69	36
Salmonella enteritidis	129	62	42

Conclusion

This investigation has shown that the Fecal Transwab® device can efficiently recover a range of enteric pathogens at ambient and refrigerated temperatures during simulated transport conditions over a 48 hour period, in compliance with the principles of CLSI standard M40-A. Campylobacter did not survive well at ambient temperatures, reflecting its inherent instability, even in stool samples.These results demonstrate that MWE's Fecal Transwab® is suitable for the transport of faecal specimens at ambient or refrigerated temperatures. If Campylobacter is suspected, the specimen should be refrigerated and transferred as rapidly as possible to the laboratory. Fecal Transwabs® can be used can be used either for manual inoculation or with the newer automated methods such as Kiestra's InoqulA.

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