Survival of Fastidious and Non-Fastidious Aerobic Bacteria in Three Bacterial Transport Swab Systems

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Abstract

In the present study we followed the CLSI procedure M40-A to evaluate three specimen transport systems (the new BD CultureSwab™ MaxV(+), the new Remel BactiSwab™ and the Medical Wire & Equipment Transwab® for the survival of fastidious and non-fastidious organisms for 0, 6, 24 and 48 hrs at room temperature. BD CultureSwab™ MaxV(+) outperformed the other two swabs for the recovery of the three fastidious organisms, *H. influenzae, N. gonorrhoeae* and *N. meningitidis* for up to 48 hr. Indeed, BD CultureSwab™ MaxV(+) maintained a constant number of viable *H. influenzae* and *N. meningitidis* for up to 48 hrs, and only 2 log reduction was noted for *N. gonorrhoeae* fulfilling the requirements of M40-A guidelines. However, unlike Remel BactiSwab™ and the Medical Wire & Equipment Transwab® which fulfilled the M40-A requirements for maintaining the viability of *S. pneumoniae*, BD CultureSwab™ MaxV(+) could not maintain the viability of *S. pneumoniae* reference or clinical strains past 6 hours. Excellent overall sensitivity (98%) (95% CI, 89.5-99.7) was observed when the BD CultureSwab™ MaxV(+) rectal swabs were compared to the “gold standard” stool cultures. Thus, the BD CultureSwab™ MaxV(+) rectal swab can be used when investigating gastrointestinal bacterial outbreaks or when health care providers face difficulties in obtaining stool samples particularly from children.
Introduction

One of the crucial steps for accurate laboratory diagnosis of bacterial infections is adequate specimen collection and transport to the laboratory. Aspirates of fluids and exudates from suspected infected sites are superior to samples collected on swabs (2, 13). However, because of the ease of using swabs, clinical microbiology laboratories continue to receive patient samples in swab transport systems (3, 12).

Swab transport systems with semi-gel stabilizing ingredients are effective methods for specimen collection and transport in the event where aspirates cannot be collected. Moreover, these swabs have been shown to protect and maintain the viability of both fastidious aerobic and anaerobic organisms (1, 5, 7). Swab transport systems are easy to use, cost effective, and can maintain the viability of a number of bacterial pathogens. The new BD CultureSwab™ MaxV(+) has the advantage of being flushed with nitrogen gas to stabilize low oxidation reduction ($E_{h}$) potential throughout its shelf life and to prevent media oxidation. Moreover, vegetable protein and amino acids have been included on the swab fiber to maintain the viability of the bacteria (8).

The recent availability of CLSI procedures M40-A (Quality Control of Microbiological Transport Systems) for evaluating swabs systems has tremendously helped in standardizing the methodologies of evaluating the newly manufactured swab systems (4).

The aim of this study was to evaluate the performance of the new Copan M40 Transystem™ Amies without Charcoal (now introduced as Becton Dickinson’s product, BD CultureSwab™ MaxV(+) with the new version of Starplex StarSwab II (now introduced as Remel BactiSwab™ modified Amies Clear (Remel, Eobicoke, Ontario, Canada) and the Medical Wire & Equipment Transwab® in maintaining the viabilities of fastidious and non-fastidious aerobic bacteria. In addition, rectal swabs using the new BD CultureSwab™ MaxV(+) were compared with stool cultures for the recovery of gastrointestinal bacterial pathogens. Maintaining the swabs at 4°C instead of at room temperature has been reported to be superior for recovering viable bacterial pathogens (14). However, because such conditions will most likely not be followed on a day to day basis, we decided to challenge the swabs systems at room temperature.
Materials and Methods

Swab Transport Systems: Three swab transport systems were evaluated according to the CLSI M40-A standard procedures. The bacterial transport systems evaluated were: the new Copan M40 Transystem™ Amies without Charcoal [C-M40] (Copan Innovation Inc., Corona, Calif.) (Now introduced as Becton Dickinson’s product, BD CultureSwab™ MaxV(+) ), the new version of Starplex StarSwab II manufactured under the Remel BactiSwab™ modified Amies Clear [R-BS] (Remel, Eobicoke, Ontario, Canada) and the Medical Wire & Equipment Transwab® [MWE-TS] (Medical Wire & Equipment Co. Ltd., England).

Bacterial Strains: Ten ATCC bacterial strains and three bacterial clinical isolates were used in the study. The ATCC strains were: Streptococcus pneumoniae (ATCC 49619), Haemophilus influenzae (ATCC 49766), Neisseria gonorrhoeae (ATCC 49226), Streptococcus pyogenes (ATCC 19615), Pseudomonas aeruginosa (ATCC 27853), Moraxella catarrhalis (ATCC 25239), Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), Listeria monocytogenes (ATCC 19115), and Neisseria meningitidis (ATCC 13090). The clinical isolates evaluated were Streptococcus agalactiae and two Streptococcus pneumoniae.

Bacterial Culturing: Well-isolated colonies from each of the bacterial strains were streaked for isolation on 5% Sheep Blood Agar (Hy laboratories Ltd. Rohovot, Israel) with the exception to H. influenzae and N. gonorrhoeae which were streaked on Chocolate agar (Hy laboratories Ltd. Rohovot, Israel). The plates were incubated for 18-24 hours in a 5% CO₂ incubator at 37°C. Fresh, well-isolated colonies were utilized for evaluating the three swab systems.

Swabs Evaluation Protocol:

Inoculum preparation: The CLSI M40-A (Quantitative Swab Elution Method) was followed to evaluate the three transport swabs. Briefly, the swab elution method, which allows for quantitative measurement of the ability of a transport system to maintain viable organism, was utilized. Inocula of the isolates were prepared in 0.85% sterile physiological saline (pH 6.8-7.2) to a concentration of approximately 1.5 x 10⁸ CFU/ml (equivalent to 0.5 McFarland standard) from an 18-24 hrs plate culture of each organism. Turbidities were checked with a nephelometer (Biomeurieux, France). Each of the organisms 0.5 McFarland was diluted ten fold in sterile 0.85% physiological saline solution to provide a concentration of approximately 1.5 x 10⁷ CFU/ml. The inoculum was prepared just prior to transferring the organism suspension to the swab
system to be evaluated. The whole procedure did not exceed 20 minutes in order to prevent the loss of the organism’s viability in the inoculum prior to incubation of inoculated swabs.

Inoculation procedure: In triplicate, 100 µl (10^6 organisms) was used to inoculate each of the swabs evaluated. The survival of the organisms on each of the swabs at room temperature was determined at 0, 6, 24, and 48 hrs.

Recovery of viable organisms: At each of these time points, viable organisms on the swabs were recovered in 1 ml of sterile physiological saline after vortexing for 30 sec. This was followed by serially diluting the viable organisms 1:10, 1:100 and 1:1000 in sterile saline. Depending on the organism evaluated, the recovery at time point 0 was between 10^5-10^6 organisms.

Quantitation of viable organisms: In triplicate, 100µl samples were used to quantify the organisms for each of the dilutions on 5% SBA or chocolate agar when required. The organisms were spread over the agar surface with a plate spreader, and the plates were incubated at 37°C in the appropriate incubator. Bacterial recovery was determined by counting the colonies recovered in each of the dilutions. The number of organisms recovered was expressed as an average for triplicate samples evaluated.

Stool and Rectal Cultures

Paired rectal swabs and stool samples were obtained from 198 consecutive children 1 week to 10 years of age presenting with gastroenteritis at Caritas Baby Hospital between August 2004 and October 2004. The samples were collected by well trained health care providers and transported to the laboratory within 1 hr of sample collection. Rectal swabs were obtained within 10 minutes after obtaining the stool specimen by inserting the swab just beyond the rectal sphincter, rotating the swab, and feces should be present on the swab upon removal. Both specimen types were inoculated on MacConkey agar, XLD agar, Preston Campylobacter Selective Agar plate, 5 % Blood agar (all prepared in house from Oxoid LTD, England) and in selenite broth (BioLife, Milano, Italy). Inoculated plates were incubated at 35°C for 24 hours while the Preston Agar was incubated at 42°C in 5% O₂, 10% CO₂, and 85% N₂ conditions for 48 hrs. A subculture at day two from the selenite broth was performed on all samples. Biochemical analyses were performed on all suspected colonies. Conformation and typing of Salmonella isolates were performed with Salmonella O groups antisera (A, B, C, D, and E) using the slide method according to the manufacturer’s guidelines (Israel
National *Salmonella* Center). *Shigella* conformation and typing were performed with antisera against *Shigella sonnei*, *S. flexneri*, *S. boydii* and *S. dysenteriae* using the slide method according to the manufacturer’s guidelines (Denka Seiken Co. LTD Japan).

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Results

Survival of Fastidious Bacteria. Survival of *H. influenzae*, *N. gonorrhoeae*, *N. meningitidis* and *S. pneumoniae* is shown in figure 1. C-M40 outperformed the other two swabs evaluated, R-BS, and MEW-TS, for the recovery of *H. influenzae*, *N. meningitidis*, *H. influenzae* was recovered for up to 48 hrs of incubation at room temperature in C-M40. The number of viable organisms remained stable for 6 hrs followed by gradual increase at 24 hrs (0.5 log) and 48 hrs (1 log) (Fig. 1a). Even though, R-BS maintained the viability of *H. influenzae* for up to 48 hrs, the number of viable organisms started to decrease after 6 hrs, and by 48 hrs a 1.5 log reduction in the number of viable organisms was noted (Fig. 1a). MWE-TS could not maintain the viability of *H. influenzae* since rapid reduction (1 log) in the number of viable organisms was noted after 6 hrs (Fig. 1a). By 24 hrs 1.5 log reduction in the number of viable organisms was detected and no viable organisms were recovered at 48 hrs from MWE-TS.

*N. gonorrhoeae* viability was maintained in C-M40 for 48 hrs, a longer evaluation period than the 24 hrs recommended by M40-A. One log reduction in the number of viable organisms was noted at each of the time points evaluated (Fig. 1b). By 48 hrs 3.5 log reduction in the number of viable organisms was noted. On the other hand, the R-BS and the MWE-TS could not maintain the viability of *N. gonorrhoeae* more than 6 hrs (Fig. 1b). Indeed, there were 2 and 3 log reductions in the number of viable organisms at 6 hrs in R-BS and MWE-TS, respectively.

*N. meningitidis* viability was maintained for up to 48 hrs in C-M40 with no loss of viable organisms, a longer evaluation period than the 24 hrs recommended by M40-A. (Fig. 1c). Unlike C-M40, the two other swab systems, R-BS and MWE-TS could not maintain the viability of *N. meningitidis* beyond 24 hrs, the period recommended by M40-A. By 24 hrs, 1 and 3.5 log reductions in the number of viable *N. meningitidis* was noted in R-BS and MWE-TS, respectively.

While, C-M40 outperformed the other two swabs in maintaining the viability of *H. influenzae*, *N. gonorrhoeae*, and *N. meningitidis*, C-M40 could not maintain the viability of *S. pneumoniae* past 6 hrs. Similar results were obtained after evaluating two *S. pneumoniae* clinical isolates in C-M40 (Data not shown). R-BS and MWE-TS maintained the viability of *S. pneumoniae* for 48 hrs with 1 and 2 log reductions in the number of viable organisms, respectively (Fig. 1d).
Overall, C-M40 satisfied the requirements of M40-A recommendation of no more than 3 log drop in 24 or 48 hrs for the three fastidious organisms *H. influenzae*, *N. gonorrhoeae*, and *N. meningitidis*, while it failed to pass the recommendations for maintaining the viability of *S. pneumoniae*. On the other hand, R-BS satisfied the requirements of M40-A recommendation for the three organisms *H. influenzae*, *N. meningitidis* and *S. pneumoniae*. MWE-TS satisfied the requirements of M40-A recommendation for *S. pneumoniae* only.

**Survival of Non-fastidious Gram-Positive Bacteria.** The survival of *S. aureus*, *S. agalactiae*, *S. pyogenes*, and *L. monocytogenes* in the three transport systems is shown in (Fig 2). Minimal increase in the number of viable *S. aureus*, *S. agalactiae*, *S. pyogenes* was observed in R-BS and MWE-TS transport systems (Fig. 2 a, b, c). On the other hand, there was a 2 log increase in the number of viable organisms in the C-M40 system after 48 hrs (Fig. 2 a, b, c). All three swabs maintained the viability of *L. monocytogenes* at all time points evaluated.

**Survival of Non-fastidious Gram-Negative Bacteria.** The survival of *P. aeruginosa*, *E. coli*, and *M. catarrhalis* in the three transport systems is shown in (Fig. 3). All three swabs maintained a stable number of viable organisms after 6 hrs incubation at room temperature (Fig. 3 a, b, c). Rapid increase in the number of viable organisms (2 logs) was noted for *P. aeruginosa* and *E. coli* after 24 hrs in all three swabs and the number was sustained at 48 hrs (Fig. 2 a, b). All three swabs maintained the number of viable *M. catarrhalis* for 24 hrs (Fig. 2c). This was followed by one and two fold increases in the number of viable organisms at 48 hrs in C-M40 and R-BS, respectively. In contrast, a small reduction in the number of viable *M. catarrhalis* was noted in MWE-TS (Fig. 3c).

**Recovery of gastrointestinal stool pathogens from C-M40 rectal swabs.**

Of the 198 paired stools samples and rectal swabs evaluated for gastrointestinal bacterial pathogens, 51 samples (26%) were positive for one of the common bacterial stool pathogens *Salmonella*, *Shigella*, or *Campylobacter* species. Indeed, of the 51 positive samples 49 were positive in both the stool culture and the rectal swabs, while 2 samples were positive in either the stool specimen or the rectal swab. Thus, the overall sensitivity of the rectal swabs was 98% (95% CI, 89.5-99.7) (Table 1). Stratifying the bacterial pathogens by genus, all 29 *Campylobacter* species isolated from stool cultures were also isolated from rectal swabs. Thus, the sensitivity of the rectal swabs for detecting *Campylobacter* species was 100% (95% CI, 88.3-100) (Table 1). Similarly all
15 *Shigella* species isolated from stool cultures were also isolated from rectal swabs, sensitivity 100% (95% CI, 79.6-100) (Table 1). Six patient samples contained *Salmonella* species. Four were detected in the paired stool samples and rectal swab cultures. For the two discordant pairs, *Salmonella* was detected in the stool culture from one and from the rectal swab culture from the other. For both of these latter samples, the *Salmonella* species in both samples were detected only after subculturing the selenite broth on XLD agar, thus suggesting low numbers of *Salmonella* organisms in the stool sample.
Swab systems are not the best way to collect patient samples for either aerobic or anaerobic specimens (7, 13). However, when swabs are the only choice, C-M40 appears to be an acceptable choice for the maintenance of viable fastidious and non-fastidious organisms for up to 48 hrs. C-M40 outperformed the other swabs for maintaining the viability of the majority of the fastidious and all of the non-fastidious bacteria. Indeed, all the organisms tested in our study were detected for up to 48 hrs post incubation at room temperature satisfying or exceeding the requirements established by the M40-A guidelines, with the exception to the \textit{S. pneumoniae} ATCC 49619 strain and the two \textit{S. pneumoniae} clinical isolates. \textit{S. pneumoniae} survived for only 6 hours in the C-M40 compared to 48 hrs in both the R-BS and MWE-TS. Unlike the observations made in our study, Morosini et al. noted survival of \textit{S. pneumoniae} ATCC 6305 for up to 48 hrs (12). Indeed, Morosini et al noted an increase in the number of viable \textit{S. pneumoniae} at 6 hrs, followed by gradual decline in the number of viable organisms. We cannot explain why there was such dramatic difference between these two studies. We ruled out mishandling \textit{S. pneumoniae} inoculated swabs as a cause for the loss of viable organisms by monitoring the room temperature every 6 hours during the evaluation period.

The survival of the fastidious organisms \textit{H. influenzae}, \textit{N. meningitidis} and \textit{N. gonorrhoeae} in C-M40 was similar to what has been reported by other investigators (8, 12). Melhus et al. noted similar observations with regard to the survival of the two fastidious organisms \textit{H. influenzae} and \textit{N. gonorrhoeae} in C-M40 systems; however, the authors did not evaluate the survival of \textit{S. pneumoniae} in their study (11). Gandhi et al. reported similar survival rates of fastidious organisms (\textit{H. influenzae} and \textit{N. gonorrhoeae}) for up to 24 hours (6). However, unlike what we have repeatedly noted, Gandhi et al reported sudden reduction in the number or viable organisms at 48 hrs of incubation (6). Similar results regarding the survival of non-fastidious organisms, \textit{S. aureus}, \textit{S. pyogenes}, and \textit{P. aeruginosa} have been reported by other investigators (12).

Depending upon which specimen types are cultured, bacterial overgrowth in swab systems is of concern since misinterpretation of bacterial culture results can occur. The CLSI procedure M40-A recommends the performance of over growth studies at 4°C (4). We chose to simulate the actual practice in our environment which is specimen transport at ambient temperatures. Under those conditions, we noted continuous growth of \textit{H. influenzae} in C-M40 up to 48 hrs. This could be beneficial since such
phenomenon will guarantee the recovery of such fastidious organism from some patient specimens’ types (e.g. eye, throat or ear).

Continuous growth of Gram-positive organisms in all swab systems was alarming since they can overgrow some of the fastidious organisms such as *S. pneumoniae* and *N. gonorrhoeae* such as may occur when the infected site is contaminated with normal flora or when two pathogens are the causative agents of the infection. In C-M40, continuous growth of all Gram-positive pathogens evaluated was noted at 24 and 48 hrs (Fig 2), while the majority of the pathogens maintained stable numbers of organisms in the R-BS and the MWE-TS. The only pathogen that continued to grow in all three swabs was *L. monocytogenes*. This is acceptable in all cases since it is not normal flora and its presence should be reported to the physicians. Other investigators noted similar continuous growth of *S. aureus* and *S. pyogenes* in C-M40 while constant numbers of viable organisms were maintained in Starplex StarSwab II and other swab types (8, 12). The stability and continuous growth of *S. agalacticae* in C-M40 could be instrumental in allowing the detection of this pathogen in vaginal swabs from pregnant women. Similar to the Gram-positive organisms evaluated, the non-fastidious Gram-negative organisms evaluated continued to grow in all three swabs with the exception of *M. catarrhalis* which maintained a constant number of viable organisms in MWE-TS. Similar observations were noted for the survival of other *K. pneumoniae* and *P. aeruginosa* (12).

Over all, all three swabs evaluated appear to have similar bacterial release at time point zero (Fig. 1, 2, 3). However, C-M40 was noted to have a better physical design that kept the semi-gel in the bottom of the swab container when compared to that of R-BS and MWE-TS. The addition of specific vegetable protein and amino acids in the C-M40 formula could have in part played a role in the continuous growth of the bacteria evaluated, allowing it to outperform the other swab systems evaluated for the recovery of fastidious and non-fastidious bacterial strains. However, extensive evaluation of the effects of these ingredients on the survival of *S. pneumoniae* should be performed to determine if it played a role in reducing the number of viable organism (log reduction accounts for loss of 90% of viable organisms) as we noted in our study. Inhibitory substances in R-BS and MWE-TS swab materials, and the refinishing chemicals used for bleaching the rayon fibres could have also played a role in allowing C-M40 to outperform these two swabs (15). Our results from this study were consistent with what
the C-M40 manufacture claims with the exception to *S. pneumoniae* which the
manufacturer claim to survive for at least 24 hrs.

Because of the problems that health care providers can face when obtaining stool specimens particularly from children with gastroenteritis, we decided to compare the recovery of bacterial pathogens from rectal swabs and stool samples. Since C-M40 outperformed the other two swabs in the recovery of the majority of the bacterial strains evaluated, we decided to use it for obtaining rectal swabs. Rectal swabs should only be used when health care providers have a difficult time collecting patient specimens and in particular before administration of antibiotics (9). Stool cultures are generally considered the “gold standard” for isolation and identification of gastrointestinal pathogens since rectal swabs are generally considered less sensitive (10). Ours is the first study to evaluate the effectiveness of the newly manufactured C-M40 for collecting stool samples. The sensitivity of the rectal swabs in comparison to the gold standard was 98% (95% CI, 89.5-99.7). C-M40 rectal swabs had 100% sensitivity for the recovery of *Shigella* and *Campylobacter* species when compared to stool cultures. Kaplan et al. reported similar results with Transwab (Medical Wire and Equipment Company, Cleveland, Ohio) when using rectal swabs for the recovery of *Campylobacter jejuni* (9). On the other hand, due to the low *Salmonella* shedding in some of the patients evaluated, the sensitivity of C-M40 for the detection of *Salmonella* were 80% (95% CI, 37.6-96.4) (Table 1). Kotton et al. recently reported 64% sensitivity and 80% specificity for the recovery of *Salmonella* from rectal swabs (10). Overall, rectal swab cultures using C-M40 appear to be as sensitive as stool culture for recovery of these three pathogens. From these results, C-M40 swab systems can be used as rectal swabs when regular stool samples cannot be collected, in particular when looking for *Campylobacter* or *Shigella* species or for investigations of large outbreaks of bacterial gastroenteritis.

The data discussed in this report emphasize the importance of processing specimens collected in swab systems as soon as possible or with a maximum delay period of up to 24 h in order to reduce fastidious organisms death (i.e. *S. pneumoniae, H. influenzae, N. gonorrhoeae, and N. meningitidis*) or non-fastidious organisms overgrowth (i.e. *E. coli* and *P. aeruginosa*). Of the swabs evaluated the C-M40 outperformed the other swabs systems in maintaining the viability of three of the four fastidious organisms evaluated for up to 48 hours. Moreover, C-M40 rectal swabs gave
excellent sensitivity when compared to stool cultures for recovery of *Salmonella*,
*Shigella* and *Campylobacter* from children with gastroenteritis.

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References


Figure Legends

Figure 1: Survival (log recovery) of fastidious bacteria in 3 different transport systems (C-M40; R-BS; and MWE-TS) for 0, 6, 24 and 48 hrs at room temperature.

Figure 2: Survival (log recovery) of non-fastidious Gram-Positive bacteria in 3 different transport systems (C-M40; R-BS; and MWE-TS) for 0, 6, 24 and 48 hrs at room temperature.

Figure 3: Survival (log recovery) of non-fastidious Gram-Negative bacteria in 3 different transport systems (C-M40; R-BS; and MWE-TS) for 0, 6, 24 and 48 hrs at room temperature.

Table 1: Comparison between the “Gold Standard” stool culture and BD CultureSwab™ MaxV(+) rectal swabs for recovery of bacterial stool pathogens.
Fig. 1
Fig. 3
Table 1:

<table>
<thead>
<tr>
<th>Rectal Swab</th>
<th>Overall</th>
<th>Shigella</th>
<th>Campylobacter</th>
<th>Salmonella</th>
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<tr>
<td>Pos.</td>
<td>49</td>
<td>1</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Neg.</td>
<td>1</td>
<td>147</td>
<td>0</td>
<td>183</td>
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</tbody>
</table>

Sens. 98.0% (95% CI, 89.5-99.7)
Sens. 100% (95% CI, 79.6-100)
Sens. 100% (95% CI, 88.3-100)
Sens. 80% (95% CI, 37.6-96.4)

Pos. = Positive; Neg. = Negative; Sens. = Sensitivity; CI = Confidence Interval (95%)