

Compliance of Two Popular Swab Transport Systems with Performance Standards Detailed by the New NCCLS Proposed Standard, M40-P

J.L. PERRY* AND J.S. MATTHEWS. - VA MEDICAL CENTER, WICHITA, KS.

ABSTRACT

Background: Centralization of microbiologic testing with transportation of specimens from outpatient facilities has placed a demand on specimen collection and transport systems. Unlike most medical devices, standards for performance of swab transport devices have essentially been non-existent. The recent release of NCCLS M40-P, *Quality Control of Microbiological Transport Systems: Proposed Standard* details performance standards for this important preanalytical step in clinical microbiology. Two popular swab transport devices were tested in accordance with M40-P procedures to ascertain their levels of compliance. **Methods:** Specified test organisms were used to evaluate Starswab with Amies (STR; Starplex Scientific Inc, Ontario, Can) and Copan EasyFlow with Amies (COP; Copan Diagnostics Inc, Corona, CA). NCCLS quantitative swab elution (vortex) and roll-plate qualitative procedures were used to evaluate inoculated swabs stored at 4°C and room temperature (RT). Compliance was a function of whether organism recovery fell within the proposed standard's acceptance criteria. **Results:** Both swab devices provided NCCLS acceptable performance for *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae* and *Neisseria gonorrhoeae* using the swab elution method. With direct plating, only COP provided acceptable recovery of *Neisseria gonorrhoeae* @ 24h and only STR provided recovery of *Fusobacterium nucleatum* @ 48h. Overgrowth of *Pseudomonas* and Grp A strep occurred in both systems @ RT after 24h. Holding swabs @ 4°C prevented overgrowth and promoted long-term survival of the more fastidious organisms. Swab elution consistently resulted in higher CFU recovery than direct plating and was solely responsible for device compliance when evaluating *Neisseria gonorrhoeae* and the anaerobes. **Conclusions:** These two swab transport systems complied with the NCCLS proposed standard M40-P when results of both testing procedures were combined for each organism tested. Direct plating of swabs held @ RT > 6h provided the poorest recovery of fastidious organisms and promoted overgrowth of others.

INTRODUCTION

Centralization of microbiological testing is an emerging trend. Although the applicability and recovery efficiency of swab systems is debatable, these devices are increasingly being utilized to transport clinical material to a central processing site. Bedside and point-of-care culture procedures, highly desirable and effective, are being replaced with more direct DNA detection/amplification techniques and off-site processing. As new technologies provide alternative methods of organism recovery and detection, establishment of guidelines for the quality control of transport devices becomes essential.

Unlike most medical devices, standards for performance of swab transport devices have essentially been non-existent. Manufacturers have historically not been subjected to guidelines defining minimal performance characteristics. With the release of NCCLS M40-P (*Quality Control of Microbiological Transport Systems: Proposed Standard*), there are now recognized standards for determining the effectiveness of microbiological transport devices.

Two popular swab transport devices were tested in accordance with M40-P procedures to ascertain their levels of compliance with these performance standards.

MATERIALS & METHODS

Inoculum density was standardized using a BaSO₄ turbidity standard and a spectrophotometer for the list of organisms below in accordance with protocols specified by NCCLS M40-P. Inoculum preparation, dilutions, vortexing, plating, incubation, and quantitation followed M40-P guidelines. Inoculated swabs were stored at 20 - 25°C (RT; room temperature) to assess organism recovery and duplicate samples stored at 4-8°C to determine the extent of bacterial overgrowth.

Organism	Strain	Duration of Incubation
<i>Pseudomonas aeruginosa</i>	ATCC BAA-427	48 hours
<i>Streptococcus pyogenes</i>	ATCC 19615	48 hours
<i>Streptococcus pneumoniae</i>	ATCC 6305	48 hours
<i>Haemophilus influenzae</i>	ATCC 10211	48 hours
<i>Peptostreptococcus anaerobius</i>	ATCC 27337	48 hours
<i>Fusobacterium nucleatum</i>	ATCC 25586	48 hours
<i>Prevotella melaninogenica</i>	ATCC 25845	48 hours
<i>Neisseria gonorrhoeae</i>	ATCC 43069	24 hours

Swab Devices Evaluated

Two popular, frequently used swab devices were evaluated for compliance with M40–P criteria. Starswab II consisting of a single swab and clear Amies gel medium (STR; Starplex Scientific Inc., Etobicoke, Ontario, Canada) and Copan Easy-flow, a single swab, clear Amies gel configuration (COP; Copan Diagnostics Inc., Corona, CA).

Vortex Method

After appropriate incubation times and holding temperatures, swabs were vortexed for 15s and serial dilutions made in saline from 10^{-1} - 10^{-5} . Duplicate platings were made in accordance with M40–P^{1,2}. For statistical accuracy, zero time plates had to have colony counts between 30 - 300 for testing to be valid. The final count was an average of the CFU of six plates (3 swabs with duplicate subcultures) from the dilution with 30 - 300 CFU.

Roll Plate Method

Swabs were processed at designated incubation times and temperatures by inoculating to appropriate non-selective media in three planes, rotating the swab between thumb and index finger to insure that all surfaces of the swab equally contacted the surface of the culture media.

Following appropriate incubation, CFU were counted and averaged. The same dilution was counted for zero time and 48h (24h for *N. gonorrhoeae*). For statistical accuracy, the subcultured plates used for enumeration did not exceed 300 CFU and counts of three plates were averaged³.

RESULTS

Chart 1. Results of organism recovery following swab vortexing and room temperature storage (20 - 25°C). M40–P stipulated acceptable recovery or viability to be no more than a 3 log decline in CFU from zero time. Loss of viability was significant only with fastidious and obligate anaerobic organisms. However, organism overgrowth observed at room temperature could result in low numbers of fastidious pathogens being obscured by large increases in more robust microbes.

Chart 2. Vortexed swabs held at 4 - 8°C. Quality control for specimens held at 4 - 8°C includes assessment of bacterial overgrowth (defined as no more than a one log increase in CFU between the zero time CFU count and CFU count after incubation for a specified period). Acceptable limits for 4 - 8°C storage were no more than a log increase in CFU and no more than a 3 log decline in CFU. Only anaerobes in the Starswab II fell out of compliance under these test conditions.

Chart 3. Roll plate data from room temperature storage (20 - 25°C). Room temperature storage assessed viability/recovery and acceptable roll plate performance was defined as ≥ 5 CFU following the specified holding time from the dilution that yielded zero time plate counts closest to 300 CFU. Bacterial overgrowth was evident and similar to vortexed swabs held at room temperature in Chart 1. Significant viability loss was noted with Starswab II inoculated with fastidious organisms and with anaerobic bacteria in both swab devices.

Chart 4. Roll plate data from swabs held at 4 - 8°C. Overgrowth study results were acceptable if there was no more than a 1 log increase in CFU from zero-time counts. Both swabs performed well in preventing overgrowth, but there was a loss of viability for *N. gonorrhoeae* and anaerobes.

Bar Graphs 1 - 4

Acceptable ranges for overgrowth (4 - 8°C) and viability (20 - 25°C), using either the vortex or roll plate method, varied with test combinations as detailed by M40–P. Survival or overgrowth, expressed as a percentage of original swab inocula, was easier to visualize and compare in this format. Graphs 1–4 present data from the various test combinations in bar graph format for two sentinel organisms, *N. gonorrhoeae* and *P. melaninogenica*.

Graph 1. Results from swabs inoculated with *N. gonorrhoeae*, held at both 4°C and RT, then sampled by both roll plate and vortex methods. M40–P stipulated that *N. gonorrhoeae* be recoverable after 24h instead of the usual 48h for other microorganisms. Data for the full 48h are shown for comparison purposes. Significant recovery of *N. gonorrhoeae* at 24h and 48h occurred only after storage at 4°C.

Graph 2. Direct roll plate procedures utilized fewer organisms (30-300 CFU/plate) so a decrease in viability was more dramatic.

Graph 3. Vortex method with *P. melaninogenica* provided recoverable numbers of anaerobes at 24 and 48h only if swabs were stored at 4°C.

Graph 4. *P. melaninogenica* proved to be a much hardier anaerobe than we expected. There was overgrowth with this organism with direct roll plating from swabs stored at 4°C.

DISCUSSION

Both swab devices provided NCCLS M40–P acceptable performance for all challenge organisms using the vortex method to extract swabs held at 4°C (Starswab II provided no recovery of *F. nucleatum* or *P. anaerobius* at 48h).

Using the direct roll plate method, only the Copan Easy-flow swab provided acceptable recovery of *N. gonorrhoeae* at 24h and only Starswab II provided recovery of *F. nucleatum* after 48h. The weakness of the roll plate method is limiting the time zero population density to 300 CFU. There is not as much of a buffer to observe survival trends as with the higher start inoculum of the vortex method.

Overgrowth by *P. aeruginosa* and *S. pyogenes* occurred in both test methods at 24h storage when swabs were held at room temperature. Holding transport devices at 4 - 8°C prevented overgrowth and promoted long-term survival of the more fastidious organisms. Swabs processed by the vortex method consistently resulted in higher CFU recovery than roll plating and was solely responsible for device compliance with evaluating *N. gonorrhoeae* and anaerobes.

RESULTS

Swab Compliance with NCCLS M40-P

Vortex Method: 20-25°C Storage (Recovery Study)

☐ Shaded area is acceptable range of organism viability

Chart 1

Organism Tested	<i>Streptococcus pyogenes</i>			<i>Haemophilus influenzae</i>			<i>Pseudomonas aeruginosa</i>			<i>Streptococcus pneumoniae</i>			<i>Neisseria gonorrhoeae</i>			<i>Fusobacterium nucleatum</i>			<i>Peptostreptococcus anaerobius</i>			<i>Prevotella melaninogenica</i>			
Increase	>1 log ₁₀	★	★	☐					★	☐															
	1 log ₁₀								★	☐															
Decrease	CFU @ 0 Time	★	☐	☐	★	★	★	★		☐	★	★	☐	★		☐	★		☐	★		☐	★		☐
	1 log ₁₀					☐						★			★			★	☐		★	★			
	2 log ₁₀																								
	3 log ₁₀																								
	>3 log ₁₀						☐									★			★			☐		★	★
Incubation Time (h)	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	

★ Copan Easy-Flow ☐ Starplex Starswab II ★☐ Exceeds acceptable range

Vortex Method: 4-8°C Storage (Overgrowth Study)

☐ Shaded area is acceptable range of organism viability/overgrowth

Chart 2

Organism Tested	<i>Streptococcus pyogenes</i>			<i>Haemophilus influenzae</i>			<i>Pseudomonas aeruginosa</i>			<i>Streptococcus pneumoniae</i>			<i>Neisseria gonorrhoeae</i>			<i>Fusobacterium nucleatum</i>			<i>Peptostreptococcus anaerobius</i>			<i>Prevotella melaninogenica</i>			
Increase	>1 log ₁₀																								
	1 log ₁₀																								
Decrease	CFU @ 0 Time	★	★	★	★	★		★	★	★	★	★	★	★	★	★	★	★		★	★	★	★	★	
	1 log ₁₀					★										☐			☐					★	☐
	2 log ₁₀																	★							
	3 log ₁₀																								
	>3 log ₁₀																		☐			☐			
Incubation Time (h)	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	

★ Copan Easy-Flow ☐ Starplex Starswab II ★☐ Exceeds acceptable range

RESULTS

Swab Compliance with NCCLS M40-P

Roll Plate Method: 20-25°C Storage (Recovery Study)

☐ Shaded area is ≥5 CFU following incubation time from ≈300 CFU at 0 time. >1,000 CFU is considered overgrowth

Chart 3

Organism Tested	<i>Streptococcus pyogenes</i>			<i>Haemophilus influenzae</i>			<i>Pseudomonas aeruginosa</i>			<i>Streptococcus pneumoniae</i>			<i>Neisseria gonorrhoeae</i>			<i>Fusobacterium nucleatum</i>			<i>Peptostreptococcus anaerobius</i>			<i>Prevotella melaninogenica</i>		
	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48
>1,000 CFU	★	★					★	★																
≈300 CFU @ 0 Time	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
≤ 5 CFU																								
Incubation Time (h)	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48

★ Copan Easy-Flow ☐ Starplex Starswab II ★☐ Exceeds acceptable range

Roll Plate Method: 4-8°C Storage (Overgrowth Study)

☐ Shaded area is acceptable range of organism viability/overgrowth

Chart 4

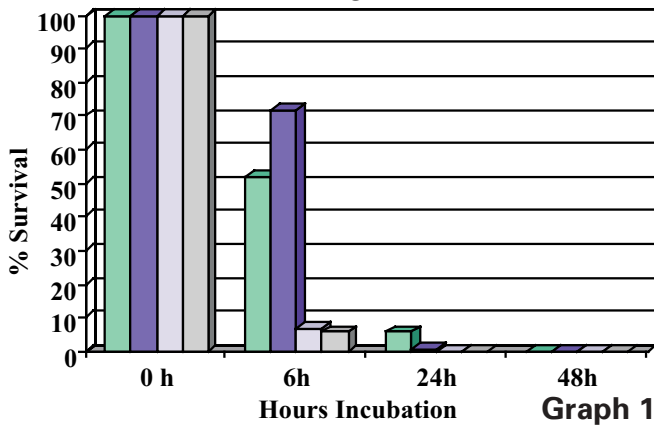
Organism Tested	<i>Streptococcus pyogenes</i>			<i>Haemophilus influenzae</i>			<i>Pseudomonas aeruginosa</i>			<i>Streptococcus pneumoniae</i>			<i>Neisseria gonorrhoeae</i>			<i>Fusobacterium nucleatum</i>			<i>Peptostreptococcus anaerobius</i>			<i>Prevotella melaninogenica</i>		
	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48
>1 log ₁₀																								
1 log ₁₀			★																					
CFU @ 0 Time	★	★	☐	★	★	★	★	★	★	★	★	★	★			★	★		★	★	★	★	★	★
1 log ₁₀															★			☐			☐			
2 log ₁₀															★			★						
Incubation Time (h)	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48

★ Copan Easy-Flow ☐ Starplex Starswab II ★☐ Exceeds acceptable range

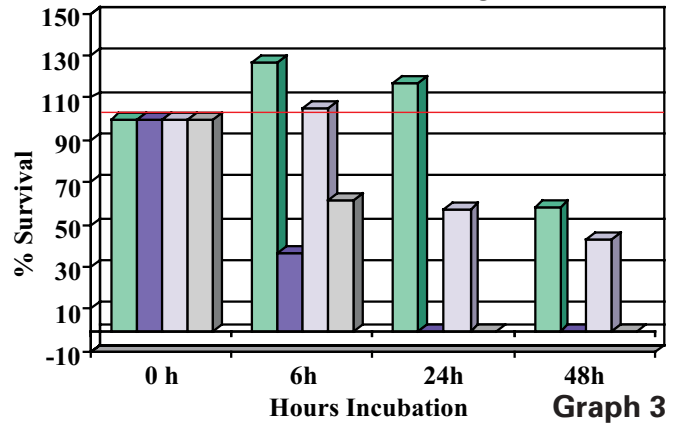
RESULTS

Starswab II vs. Copan Easy-Flow

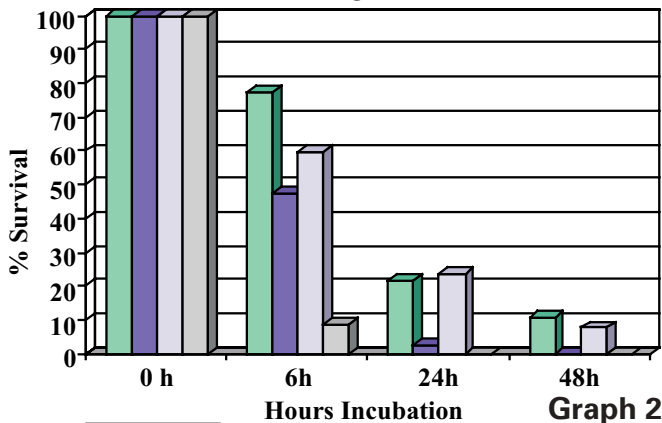
M40-P Roll Plate Method
4°C vs Room Temperature Storage
Neisseria gonorrhoeae



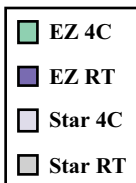
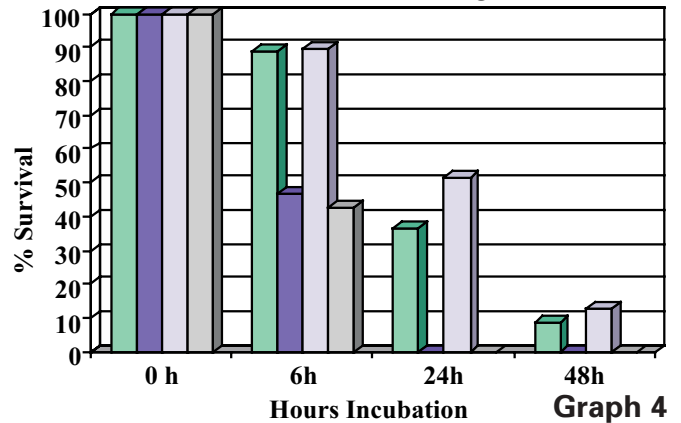
M40-P Roll Plate Method
4°C vs Room Temperature Storage
Prevotella melaninogenica



M40-P Vortex Method
4°C vs Room Temperature Storage
Neisseria gonorrhoeae



M40-P Vortex Method
4°C vs Room Temperature Storage
Prevotella melaninogenica



CONCLUSIONS

Copan Easy-flow and Starplex Starswab II were in compliance with the standards proposed by M40-P, but only when results from both the vortex and roll plate methods were combined. Vortexing swabs held at 4°C provided the best recovery (viability) and the least overgrowth of organisms tested. Direct inoculation using the roll plate method resulted in the poorest recovery of fastidious organisms and promoted overgrowth of others. Findings of this study paralleled those of previous reports and strongly suggest that storage at 4 - 8°C is essential for long term survival of most fastidious organisms^{4,5}.

LITERATURE CITED

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