EVALUATION OF ORGANISM RECOVERY AFTER TWELVE MONTHS USING THE ROLL PLATE METHOD DESCRIBED IN CLSI DOCUMENT M40: QUALITY CONTROL OF MICROBIOLOGICAL TRANSPORT DEVICES

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ABSTRACT (REVISED)

Objective: This study was designed to compare two commercial Amies transport systems without charcoal for their ability to support bacterial growth at different temperatures after the swabs were stored at room temperature for 12 months. The swabs compared were the M40 Transystem (Copan Diagnostics Inc.) and the Starswab II (Starplex Scientific Inc.). The roll plate method described in CLSI M40 was used.

Methods: CLSI M40 document recommended ATCC strains of Pseudomonas aeruginosa BAA-427, Streptococcus pneumoniae 6305, Streptococcus pneumoniae 49619, Streptococcus pyogenes 19615, Haemophilus influenzae 10211, Neisseria gonorrhoeae 43069 (GC), Propionibacterium acnes 6919, Prevotella melaninogenica 25845, Bacteroides fragilis 25285, Fusobacterium nucleatum 25586 and Peptostreptococcus anaerobius 27337 were inoculated to Amies without charcoal. Swabs were held at room temperature and 4º C for 0, 24 (GC only) or 48 hours. Bacteria were plated and counted following appropriate incubation conditions. Baseline colony counts were achieved by plating out the zero hour swabs immediately and incubating appropriately.

Results: Acceptable recovery was considered as >5 colonies following the specified holding time from the specific dilution that yielded baseline counts closest to 300 colonies. At 12 months, P. aeruginosa, S. pyogenes, S. pneumoniae 6305, S. pneumoniae 49619, H. influenzae, N. gonorrhoeae, B. fragilis and P. acnes were recovered from both systems at both holding temperatures. P. anaerobius and P. melaninogenica were recovered by the M40 swab at both holding temperatures, but at 4º C only from the Starplex swab. F. nucleatum was not recovered from either system.

Conclusions: Storage temperature was a critical factor for recovery of organisms from both systems. The Starswab II transport system performed well for most organisms at 4º C, but the M40 Transystem swab allowed greater stability of all species at both 4º C and room temperature.

INTRODUCTION

There are several recent studies on optimal temperature requirements for recovery of organisms from transport systems. The 2002 CLSI standard (M40) that describes how these systems should be used does not clearly identify how well different species of bacteria will survive in these transport systems when they are held at different temperatures. Further, many studies have reported percentage recoveries rather than CFU recovered. The CLSI document specifies reporting direct colony count recovery. It is also important to determine if different systems maintain their stability over long periods of time since the transport device may be kept on a shelf in a physician's office for months before use.

For optimal recovery of bacteria collected in these devices, it is critical to understand how well they will survive under different holding conditions. This study was designed to address such issues.

OBJECTIVE

The objective of this study was to use the CLSI M40 document "Quality Control of Microbiological Transport Devices" to determine the ability of two Amies transport media without charcoal to support the growth of organisms after twelve months storage at room temperature. The two Amies transport systems used were the M40 Transystem (Copan Diagnostics Inc.) and the Starswab II (Starplex Scientific Inc.).

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MATERIALS AND METHODS

Strains: Eleven quality control strains were included in this evaluation: Pseudomonas aeruginosa BAA-427, and ATCC cultures of Streptococcus pyogenes 19615, Streptococcus pnuemoniae 6305, Streptococcus pneumoniae 49619, Haemophilus influenzae 10211, Neisseria gonorrhoeae 43069, Bacteroides fragilis 25285, Fusobacterium nucleatum 25586, Peptostreptococcus anaerobius 27337, Prevotella melaninogenica 25845 and Propionibacterium acnes 6919.

Testing Schedule: Both Amies transport systems were tested after 0 and 12 months of storage at room temperature. The data reported here is from the 0 and 12 month testing.

Swabs from freshly manufactured lot numbers were obtained from both companies. The same lot numbers was used for both testing periods.

Methods: Eleven quality control strains were tested. The organisms were suspended in 0.85% saline to a 0.5 McFarland standard (1.5 x 108 cfu/mL) and then log10 dilutions were made in saline. 100 µL of each test dilution (105 to 107 CFU/mL) was inoculated to each transport system by dipping the transport swab in the organism suspension for 20 seconds.The swabs were then placed into the transport device. Baseline counts at each test dilution were performed by plating out the 0 hour swab immediately to appropriate agar media and incubating accordingly for 24 – 48 hours. The swabs were not vortexed before inoculation. The remaining test swabs were held at room temperature and 4º C for 24 hours (N. gonorrhoeae only) and 48 hours and then plated out to appropriate plate media and incubated.

The aerobic agar media was incubated for 24 hours before counting the colonies. The anaerobic media was incubated in an anaerobic chamber for 48 hours. The N. gonorrhoeae plates usually needed 48 hours of incubation in an atmosphere of 5% CO2 before colonies were visible enough to be counted.

RESULTS

Table 1. Colony Counts (CFU's) at 0 and 12 Months

	STARPLEX		COPAN	
Organism / Atmosphere	0 month (CFU)	12 month (CFU)	0 month (CFU)	12 month (CFU)
***P. aeruginosa* BAA-427**	**(10^5)**	**(10^5)**	**(10^5)**	**(10^5)**
Baseline	110	275	47	260
RT	Overgrowth	Overgrowth	Overgrowth	Overgrowth
4º C	6	152	15	105
***S. pyogenes* 19615**	**(10^5)**	**(10^5)**	**(10^5)**	**(10^5)**
Baseline	138	223	54	164
RT	50	128	Overgrowth	Overgrowth
4º C	100	160	38	131
***S. pneumoniae* 6305**	**(10^6)**	**(10^6)**	**(10^5)**	**(10^6)**
Baseline	>400	350	73	283
RT	5	35	12	54
4º C	391	234	11	178
***S. pneumoniae* 49619**	**(10^6)**	**(10^6)**	**(10^6)**	**(10^6)**
Baseline	>400	370	330	260
RT	2	30	0	27
4º C	162	184	0	30
***H. influenzae* 10211**	**(10^7)**	**(10^6)**	**(10^5)**	**(10^6)**
Baseline	>400	433	189	356
RT	0	8	104	381
4º C	206	218	77	229
***N. gonorrhoeae* 43069**	**(10^5)**	**(10^5)**	**(10^5)**	**(10^5)**
Baseline	122	228	66	147
RT	0	7	8	33
4º C	0	55	12	41
***B. fragilis* 25285**	**(10^5)**	**(10^5)**	**(10^5)**	**(10^5)**
Baseline	284	255	69	176
RT	228	186	134	143
4º C	240	166	75	158
***F. nucleatum* 25586**	**(10^7)**	**(10^7)**	**(10^7)**	**(10^7)**
Baseline	0	315	0	260
RT	0	0	0	0
4º C	0	0	0	0
***P. anaerobius* 27337**	**(10^6)**	**(10^6)**	**(10^6)**	**(10^6)**
Baseline	422	328	112	276
RT	0	0	6	77
4º C	0	9	15	182
***P. acnes* 6819**	**(10^5)**	**(10^6)**	**(10^5)**	**(10^6)**
Baseline	160	294	80	266
RT	111	178	101	196
4º C	146	286	36	255
***P. melaninogenica* 25845**	**(10^7)**	**(10^6)**	**(10^6)**	**(10^6)**
Baseline	>400	397	370	380
RT	0	0	5	7
4º C	20	34	22	256

RESULTS (CONT'D)

The CLSI M40 document describes acceptable recovery as >5 colonies (CFU's) recovered following the specified holding time from the dilution that yields baseline counts closest to 300 colonies. The 0 and 12 month test period data is shown in Table 1. The dilution yielding the appropriate baseline colony counts is indicated in Table 1.

0 Months: P. aeruginosa, S. pyogenes, S. pnuemoniae 6305, B. fragilis and P. acnes were recovered from both systems at both holding temperatures. S. pneumoniae 49619 was recovered from the Starplex swab only at 4º C and not by the M40 swab. H. influenzae and P. melaninogenica were recovered by the M40 swab at both holding temperatures, but at 4º C only from the Starplex swab. N. gonorrhoeae and P. anaerobius showed acceptable recovery from the M40 swab only. F. nucleatum was not recovered from either system.

12 Months: P. aeruginosa, S. pyogenes, S. pneumoniae 6305, S. pneumoniae 49619, H. influenzae, N. gonorrhoeae, B. fragilis and P. acnes were recovered from both systems at both holding temperatures. P. anaerobius and P. melaninogenica were recovered by the M40 swab at both holding temperatures, but at 4º C only from the Starplex swab. F. nucleatum was not recovered from either system.

CONCLUSIONS

There was still acceptable recovery for most organisms after 12 months storage with both types of swabs at room temperature for both swab systems. The anaerobes were most difficult to recover; lower colony counts were seen with most of these organisms, and 4º C holding continues to be optimal for these species. There was overgrowth seen with P. aeruginosa in both systems at room temperature and S. pyogenes at room temperature holding with the M40 swab.

With some organisms the dilution yielding the appropriate baseline colony counts differed between the two swab systems. There also appeared to be significant differences between the 0 and the 12 month testing periods for some species. Other studies have shown variation between technologists which may account for the variation seen here. Differences in colony counts at different dilutions and testing periods for the commercial transport systems may indicate that the CLSI M40 document is not a precise measurement tool and care must be taken when evaluating data from swab transport systems.

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