



## **Validation of the Roll Plate Method Described in NCCLS Document M40: Quality Control of Microbiological Transport Devices.**

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**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 room temperature storage for 12 months. The swabs were the M40 Transystem (Copan Diagnostics Inc.) and the Starswab II (Starplex Scientific Inc.). The roll plate method described in NCCLS M40-A was used. This paper will report on the initial recovery.

**Methods:** Suggested ATCC strains of *P. aeruginosa*, *S. pneumoniae*, *S. pyogenes*, *H. influenzae*, *N. gonorrhoeae*, *P. acnes*, *P. melaninogenica*, *B. fragilis*, *F. nucleatum* and *P. anerobius* were inoculated to Amies without charcoal. Swabs were held at room temperature at 4°C for 0, 24 or 48 hours. The test swabs were plated and counted following appropriate incubation.

**Results:** The M40-A document indicates acceptable recovery as  $\geq 5$  colonies recovered following the specified holding time from the specific dilution that yielded baseline counts closest to 300 colonies. *P. aeruginosa*, *S. pyogenes*, *B. fragilis* and *P. acnes* were recovered from both systems. *S. pneumoniae* and *H. influenzae* were recovered from both systems at 4°C, but only from the M40 swab at room temperature. *N. gonorrhoeae*, *P. anaerobius* and *P. melaninogenica* showed acceptable recovery from the M40 swab only.

**Conclusions:** Unacceptable recovery was observed with *N. gonorrhoeae*, *P. anaerobius* and *P. melaninogenica* with Starplex Amies swab. *S. pneumoniae* and *H. influenzae* were not recovered at room temperature from the Starplex swab. Acceptable recovery was observed for all organisms from the M40 swab.

### **Introduction:**

There are several recent studies on optimal temperature requirements for recovery of organisms from transport systems. The 2002 NCCLS standard (M40-P) 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 NCCLS 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 answer such issues.

### **Objective:**

The objective of the study was to use the NCCLS M40 document "Quality Control of Microbiological Transport Devices" to determine the ability of two Amies transport media without charcoal to support the growth of organism after 12 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.).

### **Materials and Methods:**

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

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

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

**Methods:** Ten quality control strains were tested. The organisms were suspended in 0.85% saline to a 0.5 McFarland standard ( $1.5 \times 10^8$  cfu/ml) and then  $\log_{10}$  dilutions were made in saline. 100ul of each test dilution ( $10^5$  to  $10^7$  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 4°C for 24 hours (*N. gonorrhoeae* only) and 48 hours and then plated out to appropriate plated 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 CO<sub>2</sub> before colonies were visible enough to be counted.

## Results

Table 1. Colony Counts at 0 Months

	Starplex Starswab II	Copan M40 Transystem
Organism	CFU	CFU
<i>P. aeruginosa</i>	(10 <sup>5</sup> )	(10 <sup>5</sup> )
Baseline	110	47
RT	overgrown	overgrown
4°C	6	15
<i>S. pyogenes</i>	(10 <sup>5</sup> )	(10 <sup>5</sup> )
Baseline	138	54
RT	50	Overgrown
4°C	100	38
<i>S. pneumoniae</i>	(10 <sup>5</sup> )	(10 <sup>5</sup> )
Baseline	144	73
RT	0	12
4°C	48	11
<i>H. influenzae</i>	(10 <sup>5</sup> )	(10 <sup>5</sup> )
Baseline	396	189
RT	0	104
4°C	50	77
<i>N. gonorrhoeae</i>	(10 <sup>5</sup> )	(10 <sup>5</sup> )
Baseline	122	66
RT	0	8
4°C	0	12
<i>B. fragilis</i>	(10 <sup>5</sup> )	(10 <sup>5</sup> )
Baseline	284	69
RT	228	134
4°C	240	75
<i>P. anaerobius</i>	(10 <sup>5</sup> )	(10 <sup>5</sup> )
Baseline	33	112
RT	0	6
4°C	0	15
<i>P. acnes</i>	(10 <sup>5</sup> )	(10 <sup>5</sup> )
Baseline	160	80
RT	111	101
4°C	146	36
<i>P. melaninogenica</i>	(10 <sup>5</sup> )	(10 <sup>6</sup> )
Baseline	130	370
RT	0	5
4°C	0	22

<i>F. nucleatum</i>	(10 <sup>5</sup> )	(10 <sup>5</sup> )
Baseline	No growth	No growth
RT	No growth	No growth
4°C	No growth	No growth

### Results:

The NCCLS M40 document indicates acceptable recovery as  $\geq 5$  colonies recovered following the specified holding time from the dilution that yields baseline counts closest to 300 colonies. The 0 month test period data is shown in Table 1. The dilution yielding the appropriate baseline colony counts is indicated in Table 1.

*P. aeruginosa*, *S. pyogenes*, *B. fragilis* and *P. acnes* were recovered from both systems. *S. pneumoniae* and *H. influenzae* were recovered from both systems at 4°C but only from the M40 Transystem swab at room temperature. *N. gonorrhoeae*, *P. anaerobius* and *P. melaninogenica* showed acceptable recovery from M40 Transystem swab only.

### Conclusions:

Unacceptable recovery was observed with *N. gonorrhoeae*, *P. anaerobius* and *P. melaninogenica* with the Starplex Amies swab. *S. pneumoniae* and *H. influenzae* were not recovered at room temperature from the Starplex swab.

With some organisms the dilution yielding the appropriate baseline colony counts differed between the two swab systems. Differences in colony counts at different dilutions from the commercial transport systems may indicate that the NCCLS M40 document is not precise.

This project will continue with testing at 12 months.

### References:

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