



## Comparison of 3 Amies Gel Transport Systems for the Recovery of 12 Clinically Significant Organisms

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### ABSTRACT

**Objective:** Transport of specimens for bacterial culture is an area of concern to a laboratory servicing rural, community and hospital populations. We assessed the recovery of aerobic and anaerobic organisms using 3 Amies gel transport systems (without charcoal) from 2 manufacturers.

**Method:** A total of 12 ATCC strains, 6 aerobic (*E. coli*, *H. influenzae*, *N. gonorrhoeae*, *N. meningitidis*, *S. pneumoniae*, *S. pyogenes*) and 6 anaerobic (*B. fragilis*, *B. thetaiotaomicron*, *C. perfringens*, *F. nucleatum*, *P. anaerobius*, *P. melaninogenica*) were assessed for recovery after storage at room temperature. Two transport systems from Copan Diagnostics Inc. (Corona, CA, USA), Copan Venturi Transystem (CW), Copan Venturi Transystem (CB) and one transport system from Starplex Scientific (Etobicoke, ON, Canada) STARSwab (SS) were tested in parallel. Swabs were inoculated with 100µL of a 10<sup>7</sup> CFU/ml organism suspension and then incubated for 0, 6, 24 and 48 hours. Following incubation, each swab was placed into 0.9 ml of sterile saline, mixed and three - 10 fold serial dilutions were prepared. A 100 µL aliquot of each dilution was inoculated to blood or chocolate agar and incubated under standard conditions. Colony counts were obtained and the data analyzed as a percent recovery compared to 0 hour growth results.

**Results:** For aerobic organisms, the CW system demonstrated equal or greater recovery at 6, 24, and 48 hours over the CB and SS systems. Both CW and CB systems dramatically increased the recovery of *N. gonorrhoeae* and *N. meningitidis* over the SS system. Anaerobic organism recovery was enhanced with the CW system at 6, 24 and 48 hours over the CB or SS systems for 4 of 6 organisms challenged. The CW system demonstrated greater viability at 48 hours for *P. melaninogenica* and *C. perfringens*.

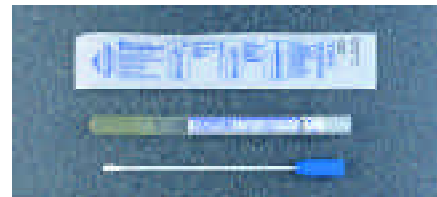
**Conclusion:** The CW system exhibited greater organism recovery over the CB and SS systems for extended incubation times.

### OBJECTIVES

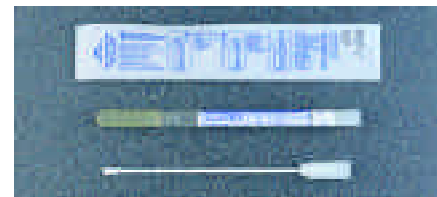
As a large “off site” microbiology laboratory, processing 1700 – 2000 specimens daily from regional, local community and hospital clients, the transport of specimens for bacterial culture is an area of concern. An evaluation of Amies gel (without charcoal) transport systems was undertaken. For our specimen protocols, this formulation of transport media is preferred as it interferes less with the Gram stain interpretation.

Transport media from two manufacturers were selected:  
Copan Diagnostics Inc. of Corona, CA, USA

\* Copan Venturi Transystem with a tight bound fiber swab (CB)



\* Copan Venturi Transystem with a soft , looser bound fiber swab (CW)



Starplex Scientific of Etobicoke, ON, Canada

\* STARSwab system SP130x



ATCC strain isolates were used as challenge organisms. Six aerobic and six anaerobic strains were selected as a cross section representation of isolates obtained in our clinical laboratory.

## METHOD

For each organism, a  $1.5 \times 10^8$  CFU/ml suspension was prepared in 0.85% sterile saline. This suspension was then diluted 1 in 10 for a suspension of  $1.5 \times 10^7$  CFU/ml. A set of 12 swabs from each transport system was prepared and inoculated with 100  $\mu$ l of the  $1.5 \times 10^7$  CFU/ml suspension. The inoculum was placed on the tip of the swab. The swab was inserted into the transport tube. Three swabs were processed immediately to obtain a base line colony count. The remaining swabs were held at room temperature and processed in sets of three at 6, 24 and 48 hours.

At the preset holding times (0, 6, 24 and 48 hours), each of the three swabs was removed from the transport tube and placed into 0.9 ml of 0.85% sterile saline and vortexed for 15 seconds to re-suspend the organism. From this  $1.5 \times 10^6$  CFU/ml suspension, three 10-fold serial dilutions were prepared in 0.9 ml of 0.85% sterile saline to produce suspensions of  $1.5 \times 10^5$  CFU/ml,  $1.5 \times 10^4$  CFU/ml and  $1.5 \times 10^3$  CFU/ml. A 100  $\mu$ l aliquot of each suspension ( $10^6 - 10^3$ ) was inoculated to the appropriate media in triplicate (see Chart 1). The inoculum was distributed across the surface of the media using an automated petri dish streaker (Isoplater 180 - Vista Technology Inc., Edmonton, AB, Canada) (see figure 1). The plates were then incubated at 35°C in the appropriate atmosphere (see Chart 1).

Chart 1

### Challenge Organism Culture Protocol

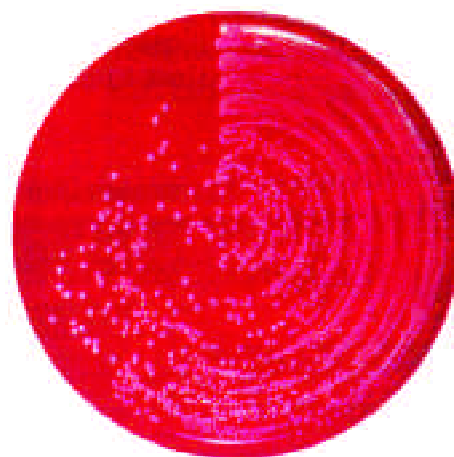
	Culture medium	Atmosphere	Incubation time
<i>B. fragilis</i> ATCC 25285	A	anaerobic	24 hours, 48 hours
<i>B. thetaiotaomicron</i> ATCC 29741	A	anaerobic	24 hours, 48 hours
<i>C. perfringens</i> ATCC 13124	A	anaerobic	24 hours, 48 hours
<i>F. nucleatum</i> ATCC 25586	A	anaerobic	48 hours, 72 hours
<i>P. anaerobius</i> ATCC 27337	A	anaerobic	24 hours, 48 hours
<i>P. melaninogenica</i> ATCC 25845	A	anaerobic	24 hours, 48 hours
<i>E. coli</i> ATCC 25922	D	ambient	24 hours, 48 hours
<i>H. influenzae</i> ATCC 10211	C	7% CO <sub>2</sub>	24 hours, 48 hours
<i>N. gonorrhoeae</i> ATCC 43069	C	7% CO <sub>2</sub>	48 hours, 72 hours
<i>N. meningitidis</i> ATCC 13090	D	7% CO <sub>2</sub>	24 hours, 48 hours
<i>S. pneumoniae</i> ATCC 6305	D	7% CO <sub>2</sub>	24 hours, 48 hours
<i>S. pyogenes</i> ATCC 19615	D	ambient	24 hours, 48 hours

A – pre-reduced Columbia blood agar

C – Chocolate agar

D – Columbia blood agar

\* Media source: PML Microbiologicals, Richmond, BC, Canada\*



(Figure 1)

Plates streaked by Isoplater 180

## RESULTS

Using standardized colony counting methods, counts for each set of three swabs were obtained and averaged. This number was then expressed as a percentage as compared to the 0 hour baseline colony count.

Example:

### ***s. pyogenes* ATCC 19615, 0 hour swabs**

	SC #1	SC #2	SC #3	CB #1	CB #2	CB #3	CW #1	CW #2	CW #3
$1.5 \times 10^7$ CFU/mL	212	242	196	126	147	127	156	141	200
Average	216.6			133.3			165.7		
% Recovery	100			100			100		

### ***s. pyogenes* ATCC 19615, 48 hour swabs**

	SC #1	SC #2	SC #3	CB #1	CB #2	CB #3	CW #1	CW #2	CW #3
$1.5 \times 10^7$ CFU/mL	25	10	22	50	27	46	60	62	54
Average	19			41			58.7		
% Recovery	8.2			30.8			35.4		

### Anaerobic Organism Recovery Percentage

	Starplex (SS)			Copan White (CW)			Copan Blue (CB)		
	6 hours	24 hours	48 hours	6 hours	24 hours	48 hours	6 hours	24 hours	48 hours
<i>B. fragilis</i> ATCC 25285	53.5	29.7	22.8	89.4	93.0	>100	59.8	63.4	54.7
<i>B. thetaiotaomicron</i> ATCC 29741	43.8	10.4	3.8	81.9	13.4	11.1	52.0	8.8	8.9
<i>C. perfringens</i> ATCC 13124	101.4	39.9	0.4	41.6	21.8	4.1	35.4	18.1	2.4
<i>F. nucleatum</i> ATCC 25586	16.0	0	0	56.7	0.1	0	27.6	0.1	0
<i>P. anaerobius</i> ATCC 27337	2.7	0	0	44.4	4.8	0	26.7	2.5	0
<i>P. melaninogenica</i> ATCC 25845	>100	>100	>100	90.5	>100	>100	81.2	>100	>100

### Aerobic Organism Recovery Percentage

	Starplex (SS)			Copan White (CW)			Copan Blue (CB)		
	6 hours	24 hours	48 hours	6 hours	24 hours	48 hours	6 hours	24 hours	48 hours
<i>E. coli</i> ATCC 25922	>100	>100	>100	>100	>100	>100	>100	>100	>100
<i>H. influenzae</i> ATCC 10211	9.8	0.8	<0.01	89.5	35.4	12.0	97.9	31.2	13.5
<i>N. gonorrhoeae</i> ATCC 43069	12.1	0	0	60.7	17.9	9.3	26.4	14.4	9.5
<i>N. meningitidis</i> ATCC 13090	1.1	0	0	80.3	89.5	38.8	105.8	109.9	56.7
<i>S. pneumoniae</i> ATCC 6305	58.9	44.8	14.6	87.3	125.5	47.5	46.4	52.2	46.3
<i>S. pyogenes</i> ATCC 19615	70.4	45.8	8.2	89.9	59.0	35.4	50.5	64.1	30.8

## CONCLUSION

For the six aerobic organisms challenged, the Copan Venturi Transystem (CW) demonstrated equal or greater recovery at 6, 24 and 48 hours over the Copan Venturi Transystem (CB) and the Starplex Scientific STARSwab (SS) transport systems. Both the Copan CB and CW transport systems dramatically increased the recovery of *N. gonorrhoeae* and *N. meningitidis* over the Starplex SS system.

For the anaerobic organisms, the Copan Venturi Transystem (CW) demonstrated greater recovery at 48 hours of *P. melaninogenica* and *C. perfringens*. The remaining 4 anaerobic organisms demonstrated enhanced organism recovery at 6, 24 and 48 hours with the Copan Venturi Transystem (CW). For the challenges in specimen transport encountered in our microbiology laboratory, the Amies gel transport system (CW) from Copan Diagnostics Inc. has been found to best meet our needs.

## ACKNOWLEDGEMENT

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