

## VIABILITY OF AEROBIC MICROORGANISMS IN FOUR SWAB SYSTEMS

K. VAN HORN, C.TÓTH\*, and J. WEGIENEK

Westchester County Health Care Corp., Valhalla, N.Y., and College of Mt. St. Vincent, Bronx, N.Y.

### ABSTRACT

Many clinical specimens are collected by a swab transport system. We evaluated and compared the Copan Amies agar swab, Copan Liquid Stuart's swab, BBL Port-a-cul swab, and Starplex Liquid Stuart's swab for maintenance of viability of 18 aerobic microorganisms. Swabs were inoculated with 100- $\mu$ l of a  $10^7$  CFU/ml organism suspension, incubated at room temperature for 0, 4, 24, and 48h, and the swabs used to prepare 10-fold serial dilutions in 0.9-ml saline. A 100- $\mu$ l aliquot of each dilution was inoculated to blood or chocolate agar and incubated for 24-48h in CO<sub>2</sub> or aerobically. Colony counts were obtained and the data analyzed as percent recovery compared to the 0h growth results. After 24h and 48h, all microorganisms were recovered from the Copan Amies agar swab and Copan Liquid Stuart's swab while only 1 *N. gonorrhoeae* was not recovered in BBL Port-a-cul swab (17/18 recovered). 12/18 (67%) microorganisms were recovered in the Starplex Liquid Stuart's swab after 24h and 6/18 (33%) after 48h. Apparent growth of the micro-organism after 48h (>10-fold increased recovery from 0h) was observed for 6/8 (33%) in the Copan Amies agar swab, 4/18 (22%) in the Copan Liquid Stuart's swab, 10/18 (56%) in the BBL Port-a-cul, and 0/18 in the Starplex Liquid Stuart's swab. The Copan Amies agar swab, the Copan Liquid Stuart's swab, and the BBL Port-a-cul swab are acceptable transport systems for recovery of aerobic microorganisms. The Starplex Liquid Stuart's swab is less suitable for transport delays longer than 4 hours based on this data.

### INTRODUCTION

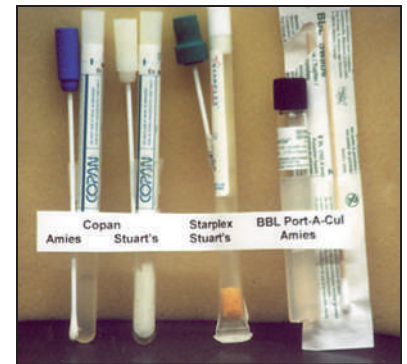
Specimen collection and transport are considered the most important steps in the overall effectiveness of the Microbiology Laboratory to provide clinically relevant results. All medical staff should be aware of the critical nature of selecting the appropriate specimen site, collecting the specimen to avoid commensal flora, and maintaining specimen quality prior to microbiological testing. All appropriately collected specimens should be transported immediately to the laboratory in sterile containers that maintain specimen integrity. Swabs are frequently used to collect specimens, but are the least desirable specimen collection device. The swab and its transport medium should provide an environment for organism survival with minimal organism multiplication or organism death, particularly when transport to a laboratory is prolonged. A variety of swabs are available that are tipped with cotton, calcium alginate, rayon, or dacron. Cotton swabs may contain fatty acids toxic to certain organisms while calcium-alginate should not be used for specimens where extraction reagents may be used. A dacron or rayon tipped swab is considered more ideal. Transport media should prevent specimen desiccation, maintain appropriate pH, and be low in nutrients to prevent overgrowth. Stuart's Liquid medium contains glycerol phosphate which may permit multiplication of some gram-negative bacilli. Amies agar medium contains a buffered balanced salt solution. Both transport media contain the reducing agent sodium thioglycollate. A third medium is Cary-Blair used most often for transport of feces. We tested and compared the Copan Amies agar swab, Copan Liquid Stuart's swab, BBL Port-a-cul swab, and Starplex Liquid Stuart's swab for maintenance of viability of aerobic microorganisms.

### ORGANISMS TESTED

<i>Streptococcus mitis</i>	<i>Moraxella catarrhalis</i>
<i>Streptococcus pyogenes</i>	<i>Neisseria gonorrhoeae</i>
<i>Streptococcus agalactiae</i>	<i>Hamophilus influenzae</i>
<i>Streptococcus pneumoniae</i>	<i>Pasteurella multocida</i>
<i>Enterococcus faecalis</i>	<i>Vibrio parahaemolyticus</i>
<i>Enterococcus faecium</i> (VRE)	<i>Escherichia coli</i>
<i>Staphylococcus aureus</i>	<i>Salmonella</i> group B
<i>Staphylococcus epidermidis</i>	<i>Pseudomonas aeruginosa</i>
<i>Candida albicans</i>	<i>Stenotrophomonas maltophilia</i>

### METHODS

1. The four swab-transport systems tested were the Copan Liquid Stuart's and Amies agar (Copan Diagnostics, Inc., Corona, CA), the Starplex Liquid Stuart's (Starplex Scientific, Etobicoke, Ontario, Can), and the BBL Port-a-cul (Becton-Dickinson {BDMS}, Cockeysville, MD).



2. Each swab type was rolled into 100  $\mu$ l of organism suspension (approximately  $10^6$  CFU/ml) for 5 sec. to completely absorb the inoculum.
3. Four swabs of each type were inoculated and incubated at room temperature for 0, 4, 24, and 48 hours.
4. After appropriate incubation, each swab was removed from the transport tube and placed into 0.9 ml of sterile saline and mixed 10 sec. with a vortex mixer to re-suspend the organisms (approximately  $10^5$  CFU/ml).
5. Two 10-fold serial dilutions in sterile saline were performed to achieve tubes with approximately  $10^4$  and  $10^3$  CFU/ml.
6. 100- $\mu$ l of each of the three organism suspensions ( $10^3$ - $10^5$ ) for each swab were plated to 5% sheep blood agar except *H. influenzae* and *N. gonorrhoeae* (inoculated to chocolate agar). Duplicate plates were inoculated.
7. The inoculum was spread over the entire surface of each plate with a sterile bent plastic rod (Copan Diagnostics).
8. Plates were incubated at 35°C for 24-48 hours in air except for *H. influenzae*, *S. pneumoniae*, and *N. gonorrhoeae* which were incubated with 3-5% CO<sub>2</sub>.
9. Colony counts were obtained by two technologists for each incubation time to minimize bias.
10. Results are expressed as % recovery compared to that swab system's initial inoculum.

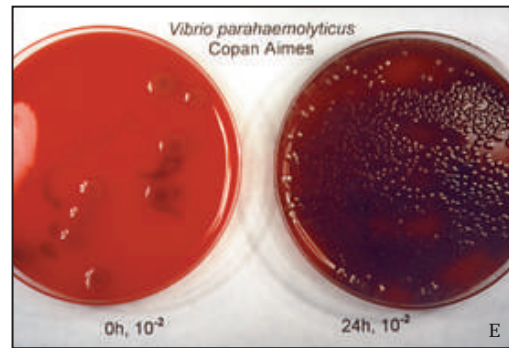
**RESULTS**

1. All 18 aerobic microorganisms tested were recovered after 48h from the Copan Amies and Copan Liquid Stuart's swab-transport systems. (Table 1)

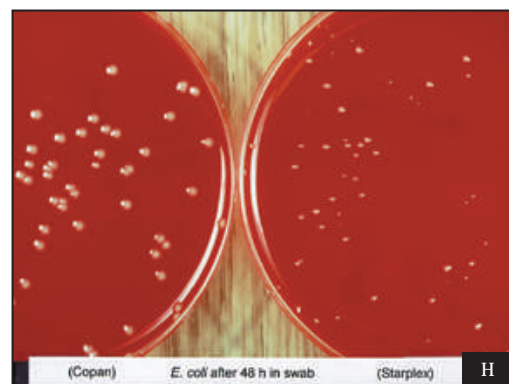
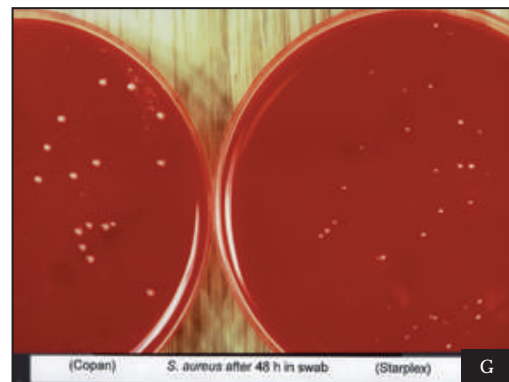
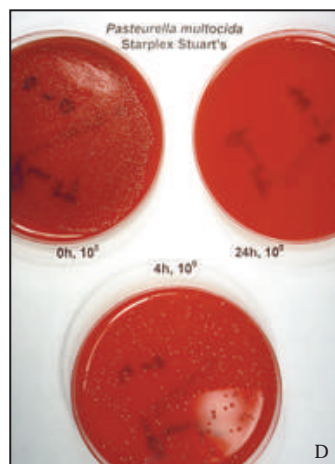
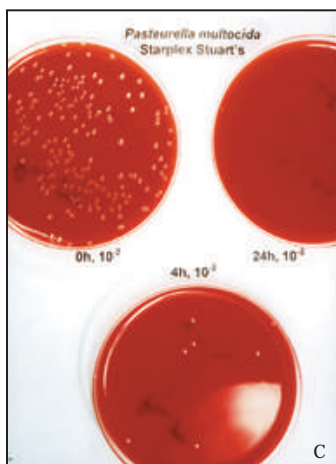
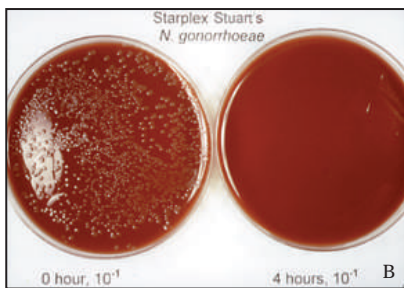
2. Apparent growth in the swab-transport systems was observed for 12/18 (67%) of the organisms in the BBL Port-a-cul, 7/18 (39%) in the Copan Amies, and 5/18 (28%) in the Copan Liquid Stuart's. (Photos E & F {below}, Figures 1 & 2 {next page})

Table 1

Organism	BBL Amies			Copan Amies			Copan Stuart's			Starplex Stuart's		
	4h	24h	48h	4h	24h	48h	4h	24h	48h	4h	24h	48h
<i>S. mitis</i>	23	1.3	3	44	9	5	23	7	0.1	23	0	0
<i>S. pyogenes</i>	65	136	540	76	109	28	86	41	22	21	4	0.1
<i>S. pneumoniae</i>	40	7	3	67	13	10	15	6	0.6	20	3	0
<i>S. agalactiae</i>	100	100	700	100	24	4	32	15	3	32	0.4	0
<i>E. faecalis</i>	100	500	>1000	58	87	140	99	55	35	87	50	8
<i>E. faecium (VRE)</i>	165	175	700	135	17	4	32	15	3	32	1.3	0
<i>S. aureus</i>	100	34	100	87	77	65	40	38	17	26	16	0
<i>S. epidermidis</i>	70	85	82	97	71	77	70	13	15	70	26	1.5
<i>M. catarrhalis</i>	4	0.8	0.4	49	50	17	30	0.8	3	36	0	0
<i>N. gonorrhoeae</i>	4	0	0	17	5	0.2	4	1.2	0.01	0	0	0
<i>H. influenzae</i>	6	1.4	0.03	100	65	12	34	12	0.06	9	0	0
<i>P. multocida</i>	91	193	980	125	290	670	52	10	5	3	0	0
<i>V. parahaemolyticus</i>	52	>1000	>1000	115	>1000	>1000	13	126	500	1	0	0
<i>E. coli</i>	100	530	>1000	150	>1000	>1000	88	800	>1000	70	7	8
<i>Salmonella group B</i>	48	800	>1000	94	>1000	>1000	77	57	82	60	27	15
<i>P. aeruginosa</i>	20	100	>1000	150	460	>1000	42	530	>1000	29	4	0
<i>S. maltophilia</i>	33	130	>1000	77	440	>1000	38	29	400	31	2	0
<i>C. albicans</i>	60	120	1000	51	92	360	83	237	400	70	72	96



- a. All but the *N. gonorrhoeae* (17/18, 84%) were recovered from the BBL Port-a-cul swab-transport system.
- b. Only 6/18 (33%) were recovered after 48h from the Starplex Liquid Stuart's swab-transport system. (Photos A-D)



- a. No organism recovered from Starplex was observed to have colony counts at 48h that were higher than the initial inoculum counts.
- 3. Colony morphology appeared somewhat inhibited for *S. pyogenes*, *E. faecalis*, *E. coli*, and *C. albicans* isolated after 48h, and for *S. aureus* (24h) and *M. catarrhalis* (4h) recovered from Starplex Liquid Stuart's. (Photos G & H)

Fig. 1

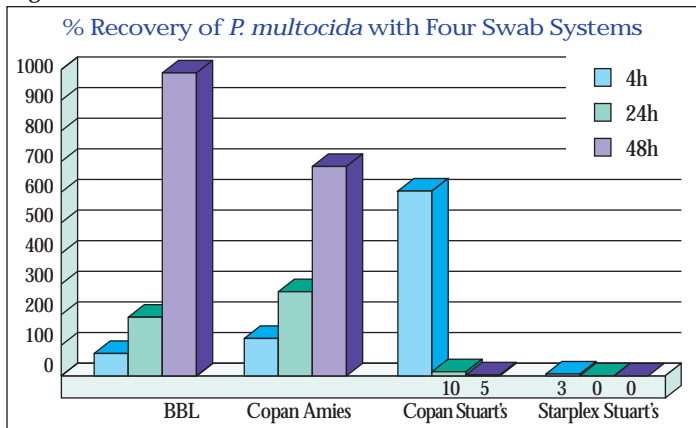


Fig. 5

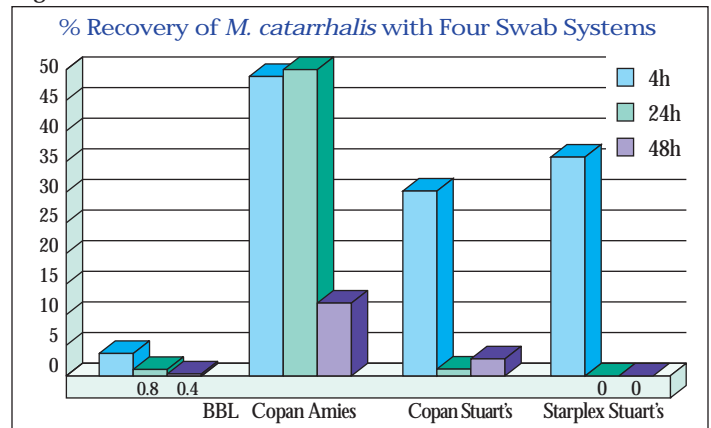


Fig. 2

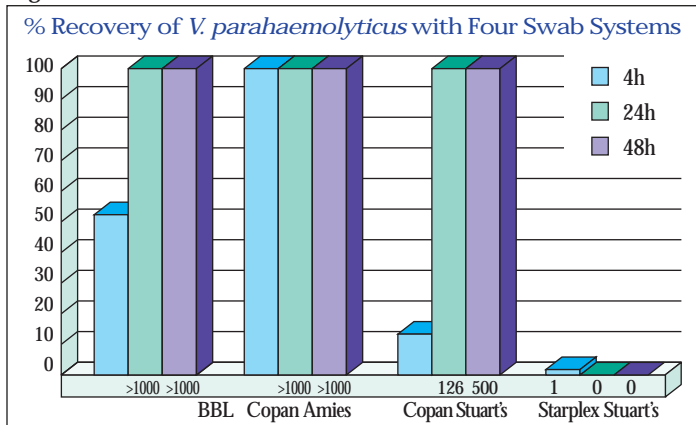


Fig. 6

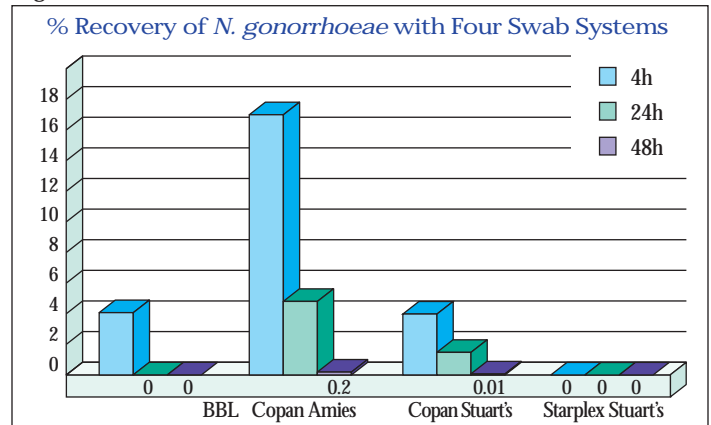


Fig. 3

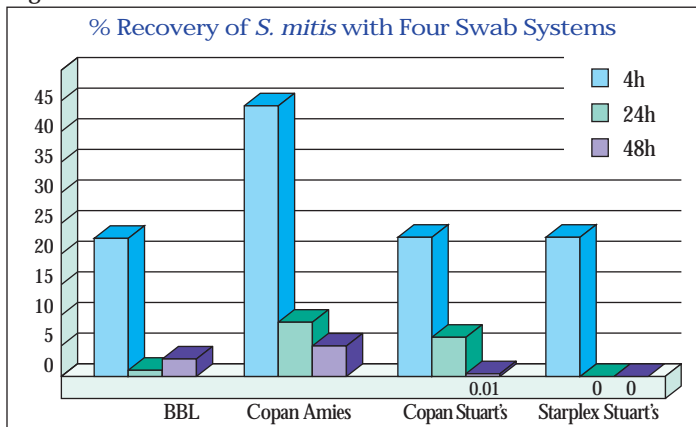
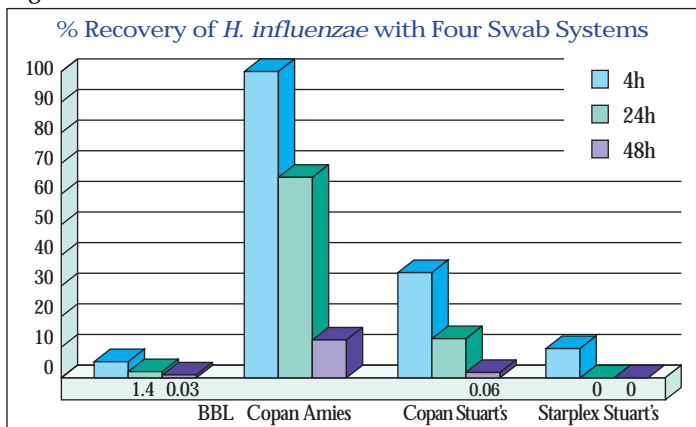


Fig. 4



### SUMMARY AND CONCLUSIONS

- All 18 aerobic organisms tested were recovered after 48h with both Copan Amies agar and Copan Liquid Stuart's swab-transport systems.
  - Apparent growth of the organism in the transport medium after 48h was observed for 7 organisms in Amies and 5 organisms in Stuart's.
- BBL Port-a-cul recovered 17/18 (94%) aerobic organisms after 48h with apparent growth observed for 11 organisms.
- The Starplex Liquid Stuart's swab-transport system recovered 12/18 (67%) after 24h and 6/18 (33%) after 48h.
- The BBL Port-a-cul and both Copan swab-transport systems are adequate for delayed transport of aerobic organisms, although some organisms may tend to grow in the transport medium. The Starplex Liquid Stuart's, based on this data, is adequate only when short transport delays (<4h) are expected.
- Immediate transport of all specimens to the clinical laboratory remains the optimal system.