

EVALUATION OF SPECIMEN TRANSPORT MEDIA IN THE VIABILITY PRESERVATION OF FASTIDIOUS AND ANAEROBIC BACTERIA

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ABSTRACT

The viability of both aerobic and anaerobic organisms from specimens stored in transport media over a long period of time is essential for the correct identification of the pathogen, diagnosis, and appropriate treatment. We evaluated two brands of commonly employed transport media: Copan Transystem – culture swab amies w/o charcoal (CP) and Cral transport media – (CR) in order to assess the ability of preserving fastidious and clinically important bacteria over time. A total of five microorganisms were tested, *Haemophilus influenzae* (HI) ATCC 10211, *Neisseria gonorrhoeae* (GC) ATCC 43069, *Streptococcus pneumoniae* (SP) ATCC 19615, *Peptostreptococcus anaerobius* (PA) ATCC 27337 and *Fusobacterium nucleatum* (FN) ATCC 25586. A 0,5 McFarland suspension of each strain was prepared in saline from an 18-24 hrs growth of the organism and then three ten fold dilutions were prepared. For each dilution, swabs were inoculated in triplicate with 100 µl of each organism suspension. Swabs were held at room temperature (RT) and 4°C for 0, 6, 24 and 48 hours. Survival was evaluated and colony counts were obtained for each incubation period and compared to the 0 h colony count to determine % recovery of each organism. For HI, the recovery rate for CP at RT was 64% after 24 hr period, and 34,8% after 48 hrs, whilst the recovery rate was zero for CR after 24 hrs. For SP at RT, 75% was recovered after 24 hrs and 18.7% after 48hrs in CP media. No recovery was observed for SP inoculated in CR swabs after 24 hrs. Concerning GC, CP recovered 24.7% after 24hrs and none after 48hrs at RT. No recovery was observed in the CR swabs after 6 hrs at RT. For PA, 7% and 2.6% recovered after 24 and 48 hrs, respectively in CP at RT. Recovery in CR media was low (7.6%) at 6 hr period, and none thereafter. An excellent recovery rate of 100% was observed in CP media up to 24 hrs, dropping to 7.4% after 48hr for FN. For CR, survival rate was of 70% after 6hrs, and none thereafter. Recovery rates were greater for all the swabs held at 4°C, even for anaerobes, when compared to those held at ambient temperature. Most specimens are still transported under RT nowadays, often remaining in the transport media for long period of time. Based on the presented data, we emphasize the importance of good quality transport media in order to preserve the viability of the microorganisms, mainly the fastidious and the anaerobes.

INTRODUCTION

The successful isolation of anaerobes and fastidious organisms depends extensively on adequate collection and transport to the clinical microbiology laboratory. Swab transport systems with solid media have been developed for the transport of patient samples for anaerobic cultures, apparently protecting both anaerobic and fastidious aerobic organisms (1). Since swabs are an easy-to-use method of collection and transport, laboratories continue to receive clinical specimens on them. However, it should be noted that a number of materials are superior to samples collected on swabs (2). Certainly, a transport system which maintains organisms viability for 24 to 48 h is of extreme importance. On a routine basis, our laboratory receives samples submitted for aerobic and anaerobic cultures from different regions of São Paulo State and other states from Brazil. Thus, this study aimed at comparing two specimen transport media, Venturi Transystem Transport Swabs (Copan Diagnostic Inc.) **and Cral Transport Media (Lab Service srl, formerly EuroMed, Lecce, Italy)**, in the viability preservation of fastidious and anaerobic bacteria.

MATERIAL AND METHODS

ORGANISMS

Five organisms were used in the evaluation:
Haemophilus influenzae ATCC 10211
Neisseria gonorrhoeae ATCC 43069
Streptococcus pneumoniae ATCC 49619
Peptostreptococcus anaerobius ATCC27337
Fusobacterium nucleatum ATCC 25586

TRANSPORT SYSTEMS

Venturi Transystem Transport Swabs (Copan – Diagnostic Inc.) – Amies w/o charcoal, lot #2183, expiry: 2003/10

Cral transport medium, lot # 782, expiry: 2003/08 (blind testing)

STUDY PROTOCOL:

Directed Swabbing Technique – For each organism, a 0.5 McFarland standard representing a 1.5×10^8 CFU/ml suspension was prepared in 0.9% physiological saline from 18-24h cultures (48h for anaerobes), using a Vitek turbidity meter (bioMérieux). >From this working suspension, four 1:10 serial dilutions were prepared: 1:10, 1:100, 1:1000 and 1:10,000 representing 1.5×10^7 , 1.5×10^6 , 1.5×10^5 , and 1.5×10^4 CFU/ml respectively. For all organisms, the 10^{-2} , 10^{-3} and 10^{-4} dilutions were used to inoculate swabs.

Using an Eppendorf repeater pipette, 100µl volumes of each organism suspension was transferred into wells of a microtiter plate. Each swab type was rolled into 100µl of the organism suspension for ten seconds to completely absorb the inoculum, and then inserted into the transport device. For baseline counts (zero time), three swabs of

each organism/dilution were removed from the transport device after 15 minutes and spread over the entire agar surface using the roll plate technique. The remaining swabs were held at room temperature and 4°C for 6, 24 and 48hrs. Swabs were plated out at the end of each time period and incubated at 35°C for 48 hours. Counts were then performed.

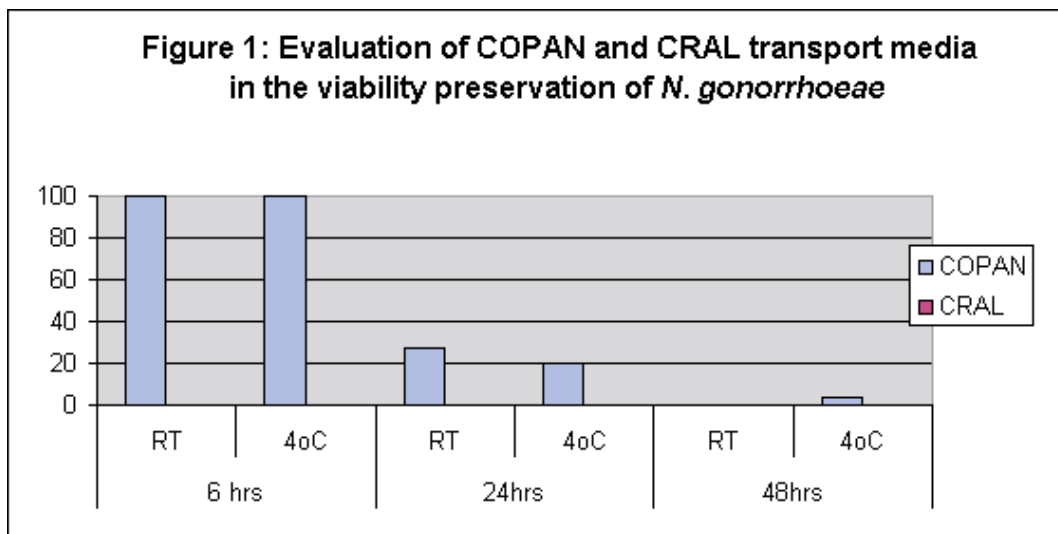
Colony counts of >300 CFU were approximated and averaged for each of the three swabs for each time point and dilution. Average counts of 6, 24 and 48 hours stored swabs for a specific dilution and organism were compared to the zero hour inoculated swab, for the same dilution and organism.

RESULTS

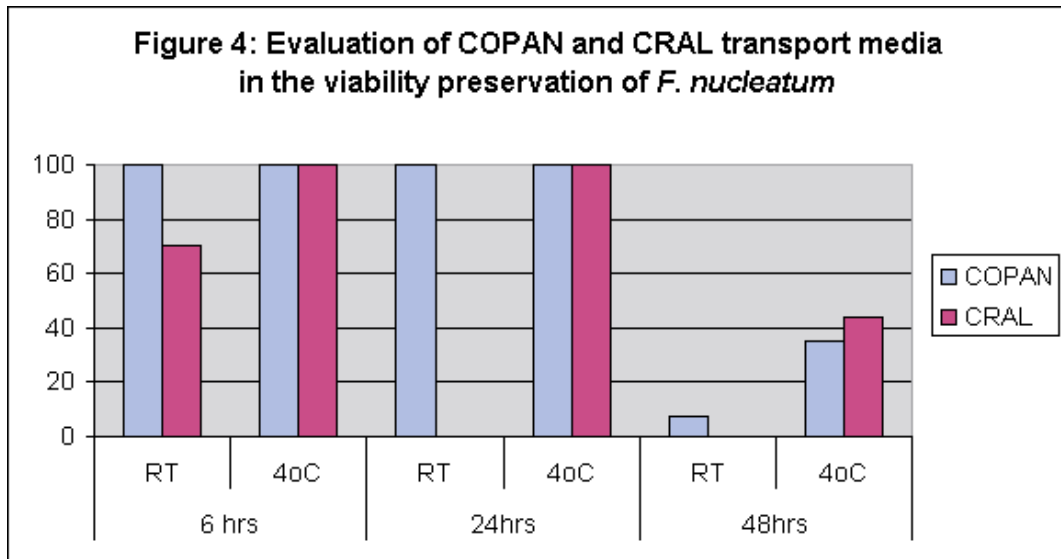
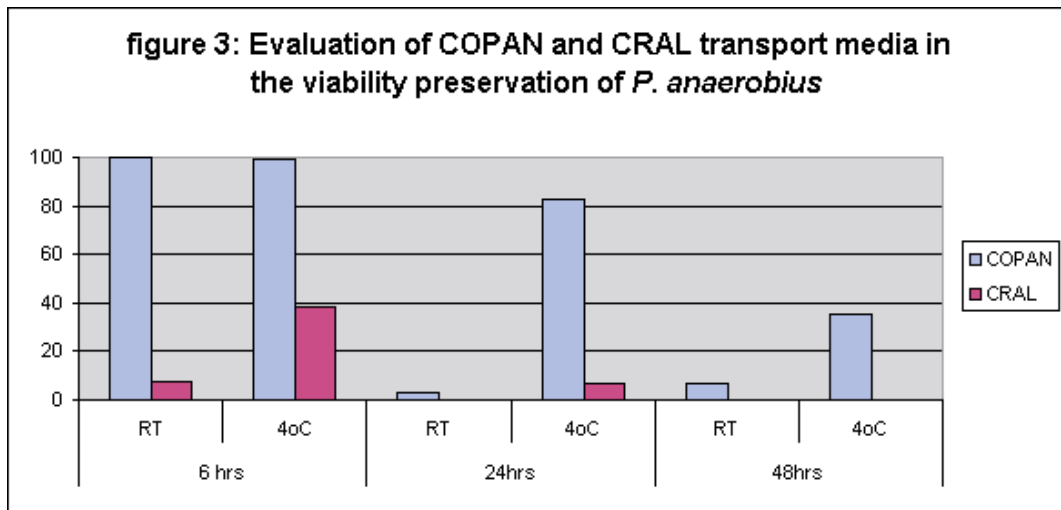
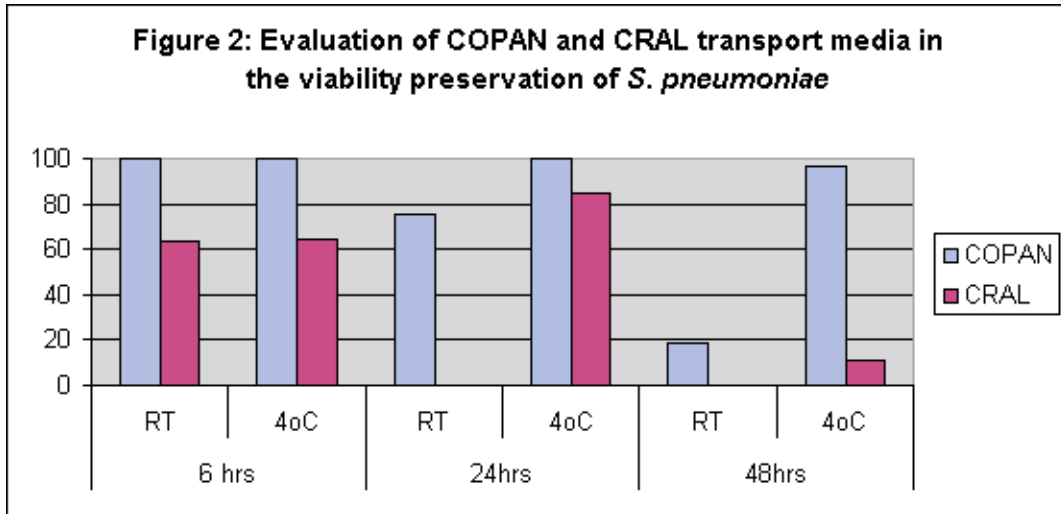
Table 1 - Comparison of organism recovery rates from Copan and Cral swabs (direct plating method). Colony count (% survival)^a at room temperature and 4°C/ 10^{-3} dilution

Organism	Swab	0 hr	6 hrs		24hrs		48hrs	
		(100%)	RT (%)	4°C (%)	RT (%)	4°C (%)	RT (%)	4°C (%)
<i>N. gonorrhoeae</i>	Copan	>300	>300 (100)	>300 (100)	82 (27,3)	59 (19,7)	0 (0)	11 (3,6)
	Cral	72	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>H. influenzae</i>	Copan	>300	>300 (100)	>300 (100)	192 (64)	188 (62,6)	104 (34,6)	194 (64,6)
	Cral	253	49 (19,4)	230 (90,9)	0 (0)	59 (23,3)	0 (0)	11 (4,3)
<i>S. pneumoniae</i>	Copan	>300	>300 (100)	>300 (100)	227 (75,6)	>300 (100)	56 (18,6)	293 (96,6)
	Cral	>300	191 (63,6)	194 (64,6)	1 (0,3)	255 (85)	0 (0)	33 (11)
<i>P. anaerobius</i>	Copan	>300	>300 (100)	297 (99)	8 (2,7)	248 (82,7)	21 (7)	106 (35,3)
	Cral	168	13 (7,7)	65 (38,7)	0 (0)	11 (6,5)	0 (0)	0 (0)
<i>F. nucleatum</i>	Copan	>300	>300 (100)	>300 (100)	>300 (100)	>300 (100)	22 (7,3)	105 (35)
	Cral	>300	210 (70)	>300 (100)	0 (0)	>300 (100)	0 (0)	132 (44)

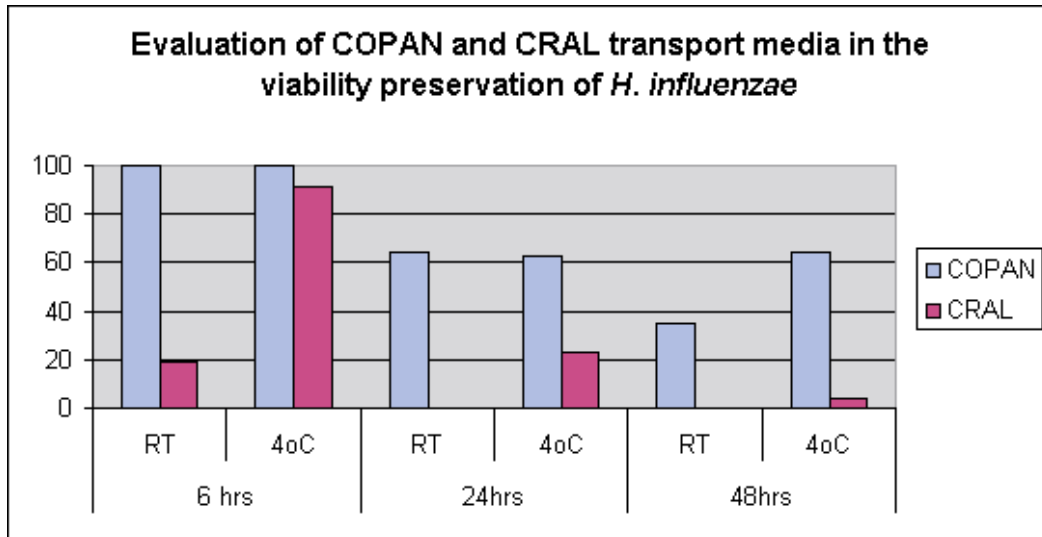
a: % survival is calculated relative to the zero-time count (100%)



RESULTS



RESULTS



DISCUSSION/CONCLUSION

The Copan and Cral swab transport systems were evaluated for their ability in order to preserve the viability of the microorganisms after incubation at room temperature and at 4°C for 6, 24 and 48 h.

Copan swab showed higher recovery rates (100%) at room and 4°C temperatures for all aerobes and anaerobes evaluated after 6 h.

Higher survival rates (100%) were observed for *F. nucleatum* in the Copan swab even after 24 h at room temperature.

N. gonorrhoeae was the most fastidious organism tested in this study. This organism's survival was better up to 6 hours, independent of storage temperature, in Copan swab.

For *S. pneumoniae*, *H. influenzae* and *P. anaerobius*, Copan swab showed the best recovery rates when held at 4°C temperature.

Swab transport system can vary in their ability to provide recovery of fastidious aerobes and anaerobes. In the present study, the Copan Venturi Transystem device demonstrated better viability and recovery for all microorganisms tested, at all time points and at both temperature when compared to the Cral Transport Media.

REFERENCES

1. Roelofsen E, Van Leuween M, Meijers-Severs M, Wilkinson H, Degener JE. 1999. Evaluation of the effects of storages in two different swab fabrics and under three different transport conditions on recovery of aerobic and anaerobic bacteria. *J Clin Microbiol*, 37:3041-3043.
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