COMPARATIVE STUDY OF COPAN VENTURI TRANSYSTEM & MEDICAL WIRE AND EQUIPMENT (MWE) TRANSWAB MICROBIOLOGICAL SWABS —USING THE ROLL PLATE METHOD

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Abstract

Objectives
To compare/contrast Copan with MWE swabs to determine the differences/similarities in numbers of organisms retrieved from each swab transport system (STS). STS which maintain viability of organisms are preferable over those which do not (avoiding overgrowth). Systems maintaining organisms for extended time, without overgrowth, are beneficial due to potentially prolonged transport times for example. The systems which best maintain organisms, representing the clinical picture will aid the laboratory in the correct determination of results.

Methods
The roll-plate method was used in these viability and overgrowth studies. This is qualitative/semi-quantitative method rather than quantitative (the elution method). Many clinical laboratories use the roll-plate method to directly inoculate media. Organisms tested: *Streptococcus pyogenes* ATCC 19615, *Streptococcus pneumoniae* ATCC 6305, *Pseudomonas aeruginosa* ATCC 27853, *Haemophilus influenzae* ATCC 10211, *Neisseria gonorrhoeae* ATCC 43069, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 60193. This study was performed following the Clinical and Laboratory Standards Institute (CLSI) Quality Control of Microbiological Transport Systems; Approved Standard (Volume 23, Number 34).

Results
A significant variance in the number of colony forming units (CFU) was seen between the systems. In most cases twice as many CFU were released by the MWE Transwab than by the Copan Transystem. Two of the organisms tested showed a greater than a four-fold difference between the release of CFU from the Copan Transystem and the MWE Transwab.

Conclusions
The MWE Transwab performed better in this study. Both systems employed Amies Clear media. A possible reason for the results may involve the amount of media; the MWE Transwab released more transport media from the system onto the agar plates than the Copan Venturi Transystem swabs. The weave of the MWE swab tips tends to open after the swab had been in the transport media aiding in media transfer. In contrast there was very little change in the weave of the Copan Transystem swab tips. This is potentially a factor in the difference between the results obtained in this study.

Introduction

Maintaining the viability of microorganisms from the patient to the laboratory is an important aspect of attaining meaningful and clinically valid results. STS allow maintenance of these microorganisms using different types of media and swab materials to aid organism recovery. Media may be altered to isolate and select for specific organisms, while other media systems are general and maintain many different types of organisms. When STS function correctly, the microorganisms cultured in the laboratory can be considered representative of the clinical state.

The purpose of this study was to compare two STS to determine similarities or differences in number of organisms retrieved after given holding times (5minutes, 24hours, 48hours and 72hours), at refrigerated (4-8ºC) and at room temperature (20-25ºC). STS that maintain organism viability are preferable to those not maintaining viability, although overgrowth needs to be avoided. STS that maintain organisms for an extended length of time (greater than 24hours) are beneficial to the laboratory, as swabs may need to be re-plated for a number of reasons; especially if the direct gram stain indicates the presence of bacteria and no growth is present on plated media. In these cases STS that best maintain viability, and prevent overgrowth, will aid the laboratory in the correct determination of clinically valid results.

Materials and Methods

The two STS used in this study were the Copan Venturi Transystem and the Medical Wire and Equipment Transwab (MWE Transwab). The transport media used in both STS is Amies clear. Both STS employ rayon swab tips and approximately 5ml of transport media. The Copan system has a tube shaped like an hour-glass; designed to keep the media intact

This study was carried out following the NCCLS Quality Control of Microbiological Transport Systems; Approved Standard (Volume 23, Number 34).

The method used for this study was the roll-plate method. This method is a qualitative (or semi-quantitative approximation) means of organism isolation rather than a quantitative method (such as the elution method). In the clinical laboratory the roll-plate method is the primary means to directly inoculate plated media. When counting colonies on agar plates statistical validity is obtained only with counts of 30 – 300 colonies for viability studies and 5 – 50 colonies for overgrowth studies on a 100mm agar plate.

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This method takes into account mechanical variables of direct swabbing action which can influence the release of sample onto culture plates. The roll-plate method involves the agar plates being streaked in three directions each 60° apart to ensure the organism is spread evenly across the agar. However, as the method is only semi-quantitative there is the potential that not all CFU present in the swab tip will be released.

A viability study was carried out on:
- *Streptococcus pyogenes* ATCC 19615 (NZRM 2723 b5)
- *Streptococcus pneumoniae* ATCC 6305 (NZRM 3201 b3)
- *Pseudomonas aeruginosa* ATCC 27853 (NZRM 918 b12)
- *Haemophilus influenzae* ATCC 10211 (NZRM 3245 b4)
- *Neisseria gonorrhoeae* ATCC 43069 (NZRM3246 b5)

An overgrowth study was performed on:
- *Escherichia coli* ATCC 25922 (NZRM 916 b22)
- *Candida albicans* ATCC 60193 (NZRM 3243 b2)

The organisms were plated on media to support the growth of the organism, and to show the growth of any contaminants (non-selective media).
Horse Blood Agar was used for the non-fastidious:
- *Streptococcus pyogenes*
- *Streptococcus pneumoniae*
- *Pseudomonas aeruginosa*
- *Escherichia coli*

Chocolate Supplemented Agar was used to support the growth of the fastidious microorganisms:
- *Haemophilus influenzae*
- *Neisseria gonorrhoeae*

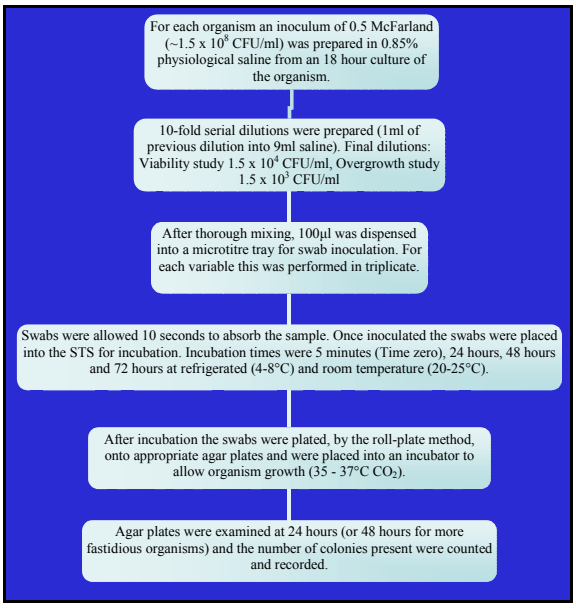
Sabourand Dextrose Agar was used for:
- *Candida albicans*

Results

P. aeruginosa - Viability Study				
Time 0 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^4	32	75	37	84
Time 24 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^4	21	94	TNTC	TNTC
Time 48 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^4	22	79	TNTC	TNTC
Time 72 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^4	24	80	TNTC	TNTC

H. influenzae - Viability Study				
Time 0 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	60	112	73	123
Time 24 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	3	5	1	0
Time 48 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	0	2	0	0
Time 72 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	0	0	0	0

N. gonorrhoeae - Viability Study				
Time 0 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^6	133	TNTC	181	291
Time 24 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^6	10	263	1	17
Time 48 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^6	0	0	0	0
Time 72 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^6	0	0	0	0



S. pneumoniae - Viability Study				
Time 0 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^6	3	213	21	205
Time 24 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^6	0	12	0	10
Time 48 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^6	0	0	0	0
Time 72 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^6	0	0	0	0

S.pyogenes - Viability Study				
Time 0 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	40	180	31	180
Time 24 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	18	62	0	9
Time 48 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	0	1	0	1
Time 72 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	0	0	0	0

E. coli - Overgrowth Study				
Time 0 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^3	5	13	11	14
Time 24 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^3	4	7	TNTC	TNTC
Time 48 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^3	1	7	TNTC	TNTC
Time 72 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^3	0	2	TNTC	TNTC

C. albicans - Overgrowth Study				
Time 0 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	10	17	13	16
Time 24 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	7	13	77	263
Time 48 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	9	13	TNTC	TNTC
Time 72 CFU/ml	Copan 4C	MWE 4C	Copan RT	MWE RT
10^5	7	8	TNTC	TNTC

Acknowledgements

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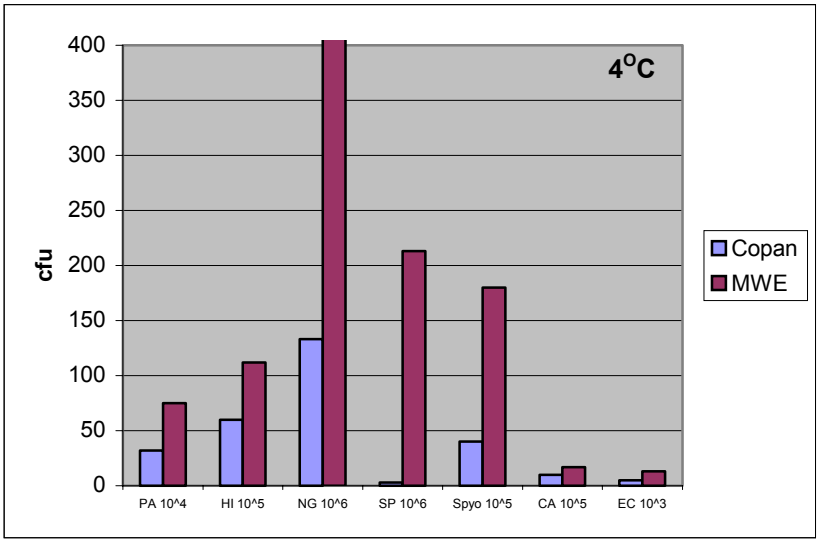

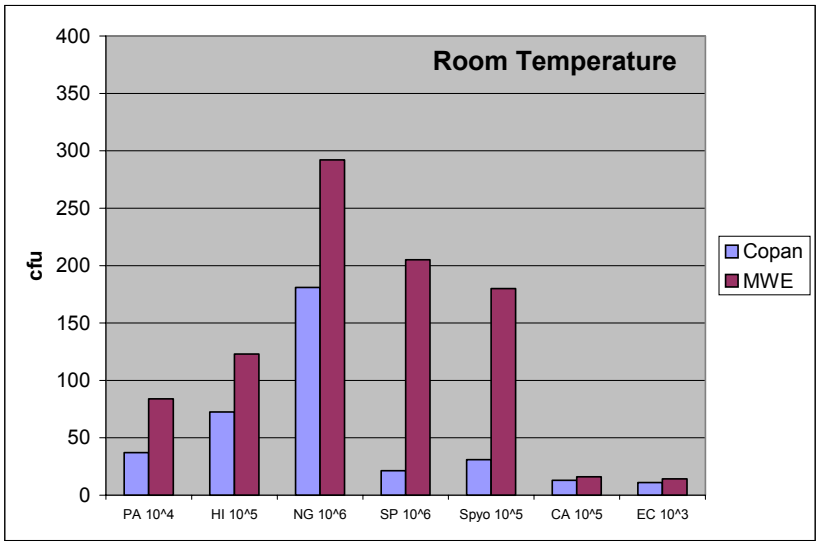

Results

The number of CFU on each dilution of the time zero agar plates was counted and recorded after incubation. The dilutions in the viability studies showing initial counts of 30-300 CFU were used as working dilutions for later incubation times. Only these dilutions were carried further through testing. Plates with counts exceeding 300 colonies were recorded as "too numerous to count" (TNTC). In the overgrowth studies time zero plates with an initial count of 5-50 CFU were used as working dilutions for later incubation times. For results to be statistically valid a decrease of 1 log is allowable in viability studies, and an increase of 1 log allowable for overgrowth studies. This accounts for a ten-fold change in number of CFU.

The results showed distinct differences in the number of CFU between the STS. However, this may not reflect the actual number of CFU present in the swab tip. The roll-plate method does not ensure that all CFU are released during spreading due to the mechanical motion of plating. Viability of all CFU in the swab cannot be guaranteed, some organisms may have become non-viable; this would prevent a colony growing on the agar even after release. In all cases the time zero plates showed a significant difference numbers of CFU present on the plates. The MWE Transwab released more CFU than the Copan Transystem with each organism. Two of the organisms tested showed a greater than a four-fold difference in release of CFU from the Copan Transystem and the MWE Transwab.

Viability study: The organisms in the viability study, showed when STS were held at a refrigerated temperature the number of CFU in the system was maintained when compared to the swabs held at room temperature, where CFU numbers declined significantly over time. The fastidious organisms showed fewer CFU at both 24hours and later holding times. This was the case for both STS, however, the MWE swabs tended to show more CFU on the plates.

Overgrowth study: Refrigeration of the STS helped to prevent overgrowth and tended to sustain the number of CFU. In both *E. coli* and *C. albicans* the number of CFU declined towards 72 hours incubation when refrigerated. While STS held at room temperature showed a significant increase in CFU between the initial time zero plates, and the 24 hour plates; with CFU too numerous to count within 24-48 hours.

Conclusions

There is no difference in the media formulation of both STS. This makes it difficult to ascertain why the MWE Transwab performed better in this study. However, one possible reason could be that the MWE Transwab released more transport media from the system onto the agar plates than the Copan Venturi Transystem swabs. This may have been due to a change in the weave of the swab tip, with the weave of the MWE swab tips tending to become more open after the swab had been in the transport media for a length of time. In contrast there was very little change in the weave of the Copan Transystem swab tips after the same length of time in the transport media. This **could** potentially have been a factor in the difference between the results obtained in this study.

References

1. Arbique, J. C., Forward, K. R., & LeBlanc, J. (1999) Evaluation of four commercial transport media for the survival of *Neisseria gonorrhoeae. Diagnostic Microbiology and Infectious Disease 36;* (2000), 163-168.
2. Gandhi, B., & Mazzulli, T. (2004). Comparative study of a new Copan Amies transport swab M40 Transystem™ with another commercial Amies transport swab. Presented at the 104th General Meeting of the American Society for Microbiology, New Orleans, Louisiana.
3. Gandhi, B., Skulnick, M., & Mazzulli, T. (2005). Survival rate of *Haemophilus influenzae* in two commercial Amies gel swab transport systems at room temperature. Presented at the 105th General Meeting of the American Society for Microbiology, Atlanta, Georgia.
4. Human, R. P., & Jones, G. A. (2004). Evaluation of swab transport systems against a published standard. *Journal of Clinical Pathology, 57;* 762-763.
5. NCCLS Document M40-A. (2003). *Quality control of microbiological transport systems; Approved standard.* (Volume 23, Number 34).