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Evaluation of the New Sigma-Transwab® for Maintaining Viability of Aerobic and Anaerobic bacteria.

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Abstract

Background:. Appropriate specimen collection and transportation are crucial for accurate laboratory diagnosis of bacterial infections. Different swab systems are used for a variety of specimen types and they must maintain the organisms viability during the transport process. The Medical Wire & Equipment Σ-Transwab® is a new device with a liquid Amies transport medium that can be used for traditional culture, Gram strains, automated processing systems, and molecular assays. The swab is foam tipped, which allows the flow through of the liquid medium, reagents and microorganisms. In this study, Σ-Transwab® was evaluated for maintaining of viability of all recommended (10 strains) bacteria according to the CLSI M40-A swab elution (quantitative) method.

Methods. Aerobic, facultative anaerobic, anaerobic and fastidious bacteria selected for this study were 10 control strains recommended by CLSI M40-A method. Quantitative viability studies were performed in triplicate at either controlled room temperature (RT), refrigerator temperature (4ºC), or both of these for three different batches of Σ-Transwab® at the same time. For each organism viable counts were performed at zero (0) time, 24 h and 48 h according to the CLSI-M40A quantitative method**.**

Results:. The Σ-Transwab® met CLSI acceptance criteria for all aerobic and faculatative isolates stored at both temperatures and for all anaerobic isolates stored at refrigerated temperature. The Σ-Transwab® also met CLSI criteria for three of five anaerobic strains at room temperature. *Neisseria gonorrhoeae* was recovered after both 24h or 48h at 4C with only 1log reduction in recovery after 48h. *Pseudomonas aeruginosa* is included in M40-A as an indicator for overgrowth and is normally only tested at 4C. However Σ-Transwab® was also acceptably close to the standard at room temperature.

Conclusion: The Σ-Transwab® system is an acceptable swab transport system for both aerobes and anaerobes. This system, across the 3 batches, consistently met CLSI acceptance criteria for most aerobic and anaerobic isolates when it was tested under refrigerated and room temperature storage conditions, while *Neisseria gonorrhoeae* survived very well for 48 hours at 4C.

Introduction

In clinical diagnostics, successful sampling and transport of bacteria to the laboratory is crucial for an accurate diagnosis and treatment of the patient. Swabs are a very common method of specimen collection and the swab material and transport medium play a major, but often overlooked role in sampling. The ideal swab system must absorb organisms from the infection site, maintain viability during transport and allow release of organisms from the swab to the appropriate media for culture.

In this study the performance of Σ-Transwab® was evaluated using the standard M40-A (CLSI) method. The Σ-Transwab® is a liquid Amies medium transport system containing a soft polyurethane foam bud, which is highly absorbent and has an open cell structure which allows complete flow through of medium and reagents with a maximum release of microorganisms into the liquid medium. Liquid Amies medium is suitable for both automated and conventional processing. Also, the medium provides a suspension for quick Gram-stains and a multiple cultures. This swab system can be used for bacterial and fungal culture and additionally can be used in modern molecular testing methods, such as PCR

Methods

1. **A suspension from a freshly grown isolate of each strain was prepared in sterile saline diluted 1:10. Serial 10-fold dilutions were prepared from the suspension and plated onto appropriate agar. The plates were incubated at 37°C for 24h, and colony forming units counted to confirm inoculum concentration.**
2. **Swabs were inoculated with 50µl of inoculum suspension for 10 sec allowing the fluid to absorb and then inserted back into the transport device**
3. **Swabs were incubated at room temperature and at 4°C for 0h, 24h and 48h (as required for M40-A**
4. **After the appropriate incubation period each swab was vortexed and serial dilutions were prepared from the liquid transport medium.**
5. **Serial dilutions were inoculated onto the appropriate agar using spiral plater (Don Whitley Scientific, BS5687).**
6. **All plates were incubated at 37°C for 48h in the appropriate conditions. After incubation, a quantitative count was performed using Acolyte counter (Don Whitley Scientific) All experiments were carried out in triplicate**.

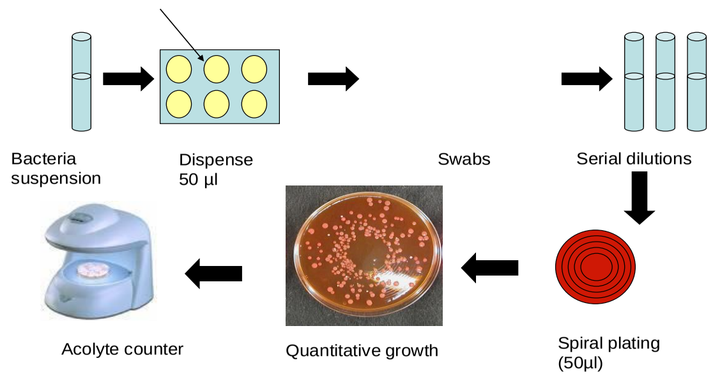


Figure 1. Method of processing the swab for evaluation of bacteria viability. Strains: Ten quality control strains were included in the evaluation.

Pseudomonas aeruginosa ATCC 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
Propionibacterium acnes. ATCC 6919

Table 4. Recovery of *Streptococcus pneumoniae* and *Haemophilus influenzea* from three different batches of Σ-Transwab® after holding at 4ºC and RT* .

Bacteria	Time	Batch number					
		Batch 10J20		Batch 10J21		Batch10J22	
		4C	RT	4C	RT	4C	RT
RT							
Streptococcus	Time Zero	1.57×10^6	1.57×10^6	1.23×10^6	1.23×10^6	1.42×10^6	1.42×10^6
pneumoniae							
(cfu/ml)	24h	1.12×10^6	8.56×10^6	1.12×10^6	9.27×10^6	1.12×10^6	1.24×10^6
	48h	8.65×10^5	1.02×10^7	1.01×10^6	1.12×10^7	1.12×10^6	9.11×10^6
Haemophilus influenzae	Time Zero	3.54×10^6	3.54×10^6	3.52×10^6	3.52×10^6	3.28×10^6	3.28×10^6
(cfu/ml)	24h	4.25×10^6	8.64×10^6	3.48×10^6	8.76×10^6	3.42×10^6	4.12×10^6
	48h	6.26×10^6	1.36×10^7	3.52×10^6	9.96×10^6	3.37×10^6	4.33×10^6

Other findings

The 3 batches of Σ-Transwab® recovered all the aerobic and facultative strains for 48 hours at both 4C and room temperature

The 3 batches of Σ-Transwab® recovered ***Bacteroides fragilis, Peptostreptococcus anaerobius***, ***Propionibacterium acnes***, and ***Prevotella melaninogenica*** for 48 hours at both 4C and room temperature, and ***Fusobacterium nucleatum*** for 24 hours at both 4C and room temperature

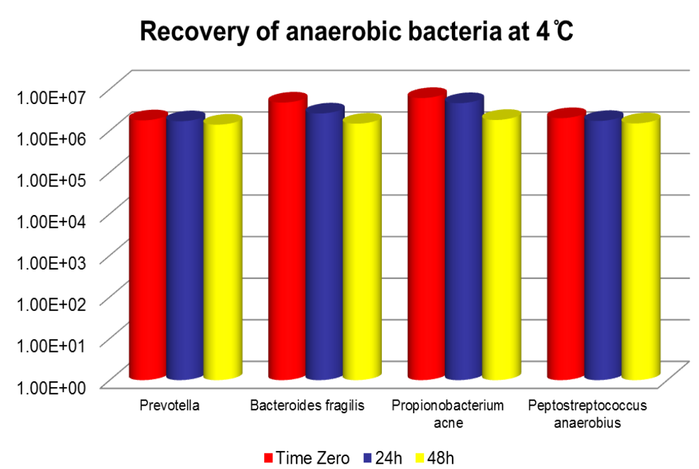
Neisseria gonorrhoeae was recovered from all 3 batches after both 24h or 48h at 4C with only 1log reduction in recovery after 48h.

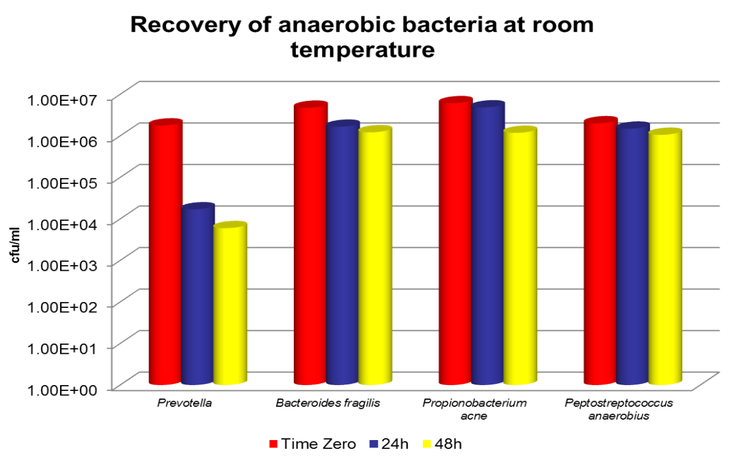
Pseudomonas aeruginosa is included in M40-A as an indicator for overgrowth and is normally only tested at 4C. However Σ-Transwab® was also acceptably close in all 3 batches to the standard at room temperature.

**** Further results are available on the handout***

Results

Tables 2 & 3. Results of the recovery of anaerobic bacteria held at 4ºC and at Room Temperature (Batch 10J20*)





Discussion/ Conclusion

The Σ-Transwab® met CLSI acceptance criteria for all aerobic and facultative anaerobic isolates stored at both temperatures, for all anaerobic isolates at 24 hours at both temperatures, for all anaerobic isolates at 48 hours stored at refrigerated temperature, and for all but one anaerobic isolates at 48 hours stored at room temperature . *Neisseria gonorrhoeae* was recovered after both 24h or 48h at 4ºC with only 1log reduction in recovery after 48h. *Pseudomonas aeruginosa* is included in M40-A as an indicator for overgrowth and is normally only tested at 4C. However Σ-Transwab® was also acceptably close to the standard at room temperature. This system, across the 3 batches, consistently met CLSI acceptance criteria for most aerobic and anaerobic isolates when it was tested under refrigerated and room temperature storage conditions, while *Neisseria gonorrhoeae* survived very well for 48 hours at 4C.

References

Quality Control of Microbiology Transprt Systems. Approved Standard. CLSI Document M40-A, 2003

The Σ-Transwabs® were provided by Medical Wire & Equipment

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Σ-Transwab® with Liquid Amies Medium

RESULTS:

	Holding Temp	Holding Time (hours)			
Lot No.			**10J20**	**10J21**	**10J22**
Pseudomonas aeruginosa (ATCC® BAA-427)		0	1.36×10^6	1.33×10^6	1.16×10^6
Pseudomonas aeruginosa (ATCC® BAA-427)	4C	48	1.23×10^6	1.26×10^6	1.09×10^6
Pseudomonas aeruginosa (ATCC® BAA-427)	RT	48	3.51×10^7	2.97×10^7	3.03×10^7
Streptococcus pyogenes (ATCC® 19615)		0	1.47×10^5	4.13×10^5	3.90 x 10^5
Streptococcus pyogenes (ATCC® 19615)	4C	48	2.01 x 10^5	2.24 x 10^5	1.99×10^5
Streptococcus pyogenes (ATCC® 19615)	RT	48	1.96×10^5	1.49×10^5	1.66×10^5
Streptococcus pneumoniae (ATCC® 6305)		0	1.57×10^6	1.23×10^6	1.42×10^6
Streptococcus pneumoniae (ATCC® 6305)	4C	48	8.65×10^5	1.01×10^6	1.12×10^6
Streptococcus pneumoniae (ATCC® 6305)	RT	48	1.02×10^7	1.12×10^7	9.11×10^6
Haemophilus influenzae (ATCC® 10211)		0	3.54×10^6	3.52×10^6	3.28×10^6
Haemophilus influenzae (ATCC® 10211)	4C	48	6.26×10^6	3.52×10^6	3.37×10^6
Haemophilus influenzae (ATCC® 10211)	RT	48	1.36×10^7	9.96×10^6	4.33×10^6
Bacteroides fragilis (ATCC® 25285)		0	4.76×10^6	4.87×10^6	4.67×10^6
Bacteroides fragilis (ATCC® 25285)	4C	48	1.48×10^6	1.08×10^6	1.84×10^6
Bacteroides fragilis (ATCC® 25285)	RT	48	1.22×10^6	9.24×10^5	2.29×10^6
Peptostreptococcus anaerobius (ATCC® 27337)		0	2.01×10^6	1.52×10^6	1.92×10^6
Peptostreptococcus anaerobius (ATCC® 27337)	4C	48	1.50×10^6	1.55×10^6	2.31×10^6
Peptostreptococcus anaerobius (ATCC® 27337)	RT	48	1.06×10^6	7.73×10^5	6.74×10^5
Fusobacterium nucleatum (ATCC® 25586)		0	4.03×10^6	3.49×10^6	2.69×10^6
Fusobacterium nucleatum (ATCC® 25586)	4C	24	3.38×10^5	3.48×10^5	6.64×10^5
Fusobacterium nucleatum (ATCC® 25586)	RT	24	5.84×10^5	2.63×10^5	2.11×10^5
Propionibacterium acnes (ATCC® 6919)		0	6.12×10^6	4.12×10^6	3.16×10^6
Propionibacterium acnes (ATCC® 6919)	4C	48	1.82×10^6	9.66×10^5	3.04×10^5
Propionibacterium acnes (ATCC® 6919)	RT	48	1.18×10^6	2.40×10^5	6.33×10^5
Prevotella melaninogenica (ATCC® 25845)		0	1.78×10^6	1.53×10^6	1.38×10^6
Prevotella melaninogenica (ATCC® 25845)	4C	48	1.4×10^6	1.26×10^6	1.03×10^6
Prevotella melaninogenica (ATCC® 25845)	RT	48	6.00×10^3	1.21×10^4	2.00×10^3
Neisseria gonorrhoeae (ATCC® 43069)		0	8.05×10^5	7.10×10^5	6.53×10^5
Neisseria gonorrhoeae (ATCC® 43069)	4C	24	8.81×10^4	1.51×10^5	9.55×10^4
Neisseria gonorrhoeae (ATCC® 43069)	4C	48	4.63×10^4	1.08×10^5	5.25×10^4