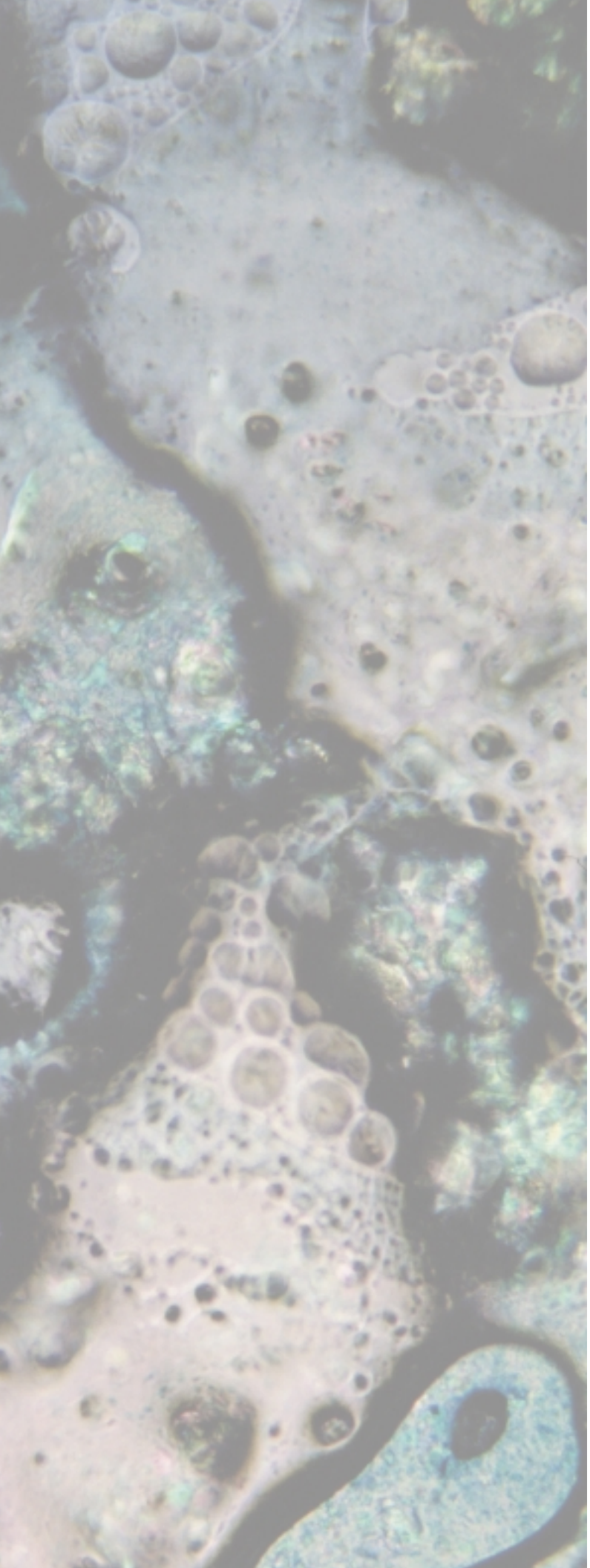

Evaluation of the Starplex Anaerobic Transport Tube for Recovery of Anaerobic Bacteria

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ABSTRACT (C-103)

Specimens collected for the isolation of anaerobic bacteria should be protected from oxygen exposure once collected and until plated to anaerobic media. We tested the Starplex Anaerobic Transport Tube (Etobicoke, Ontario, Canada) for maintenance of viability of 28 anaerobes including 13 gram-negative bacilli, 7 clostridia, 3 cocci, and 5 non-sporeforming gram-positive bacilli. A 10^5-10^6 CFU/ml suspension of each anaerobe was prepared in sterile saline and 100-µl absorbed onto each of 4 Starplex swabs. At 0h, 4h, 24h, and 48h, one swab was placed into 0.9 ml of sterile saline, mixed, and diluted 1:10 and 1:100. From each dilution, 100-µl was plated to reduced blood agar and spread over the surface. Plates were incubated anaerobically at 35°C for at least 48h and colony counts obtained. Good anaerobe viability was maintained for 48h for 22 anaerobes with no growth reduction observed for 2, a 10-fold reduction for 14, and a 100-fold reduction for six. Three additional anaerobes decreased by at least 1000-fold (*F. necrophorum, B. ureolyticus*, and *P. melaninogenica*). One *F. nucleatum* and one *C. innocuum* were not recovered after 24h, and one *C. difficile* was not recovered at 48h. The *F. nucleatum* and *C. innocuum* were part of separate suspensions tested with two mixed organisms. The Starplex Anaerobic Transport Tube appears to be an acceptable method for maintaining the viability of anaerobic bacteria for at least 24h prior to culture.

INTRODUCTION

An essential component for successful cultivation of anaerobic bacteria from clinical specimens is how well that specimen has been protected from the harmful effects of oxygen exposure. A variety of swab and vial systems are commercially available for the collection and transport of specimens for anaerobic culture. Each system is designed to protect the specimen or to minimize specimen exposure to oxygen while in transit to the microbiology laboratory. While aspirated fluid, pus, or tissue is the specimen of choice, many specimens for anaerobic culture are collected with a swab. Therefore, a system that can easily accept and transport fluids, tissue, and swabs might be more effective.

The Starswab Anaerobic Transport System S120D (Starplex Scientific, Etobicoke, Ontario, Canada) is a self-contained, sterile over-wrapped, ready-to-use anaerobic transport system. The system includes two rayon-tipped swabs and a transport vial with modified Cary Blair medium. The medium is balanced, reduced by sodium thioglycolate, non-nutritive, and supplemented with L-cysteine and resazurin to optimize survival of clinical pathogens, including anaerobes, during transport to the microbiology laboratory. The transport vial has a screw cap top with a rubber septum that allows for the introduction of aspirated fluids, small tissue samples, and specimens collected on a swab.

We evaluated the Starswab S120D for its efficiency in recovering anaerobic bacteria from known stock suspensions up to 48 hours incubation at room temperature.

METHODS

Organisms tested (see Table 1)

1. All organisms were recent clinical isolates of the Westchester County Medical Center microbiology laboratory identified by the RapID ANA II system (Innovative Diagnostics Systems, Inc., Norcross, Ga.)

2. All organisms except *A. odontolyticus* were tested as individual suspensions.

3. Mixed inoculum suspensions included:

   a. *F. nucleatum* and *P. magnus*
   b. *C. perfringens* and *B. thetaiotaomicron*
   c. *B. fragilis* and *A. odontolyticus*
   d. *C. innocuum* and *E. coli*

Table 1. Organisms Tested

Bacteroides	Other Gram-Negatives	Clostridia	Cocci / Nonsporers
B. fragilis (2)	*P. intermedia*	*C. perfringens (2)*	*P. anaerobius*
B. thetaiotaomicron[a]	*P. bivia*	*C. difficile*	*P. magnus (2)*
B. caccae	*P. melaninogenica*	*C. sordellii*	*A. meyeri*
B. uniformis	*F. nucleatum*[a]	*C. innocuum*	*A. odontolyticus*
B. ureolyticus	*F. necrophorum*	*C. ramosum*	*E. lentum*
			P. acnes
	E. coli		*Bifidobacterium sp.*

[a]*B. thetaiotaomicron* and *F. nuclearum* tested twice, once as part of a 2-organism mix.

Inoculum

1. Approximately 10^5 CFU/ml of each organism was prepared in sterile saline immediately prior to swab inoculation.

Swab inoculation: Duplicate tests were performed.

1. Four 100-µl aliquots of inoculum were placed into a sterile petri plate.

2. Four Starswab rayon swabs were lightly pressed and rolled into each of the four inoculum aliquots.

3. Each swab was allowed to absorb the inoculum for 15 sec.

4. Each swab was immediately placed according to manufacturer's instructions into one of the 4 Starswab vials.

5. Vials were labeled 0, 4, 24, and 48 hours and incubated at room temperature for the appropriate amount of time.

Swab subculture and results

1. After appropriate incubation, each swab was removed from the Starswab transport vial, placed into 0.9 ml of sterile saline, and mixed 10 sec on a vortex.

2. Ten-fold serial dilutions in sterile saline were prepared to obtain approximate final organism concentrations of 10^2-10^4 CFU/ml.

3. A 100-µl aliquot of each dilution and the original saline with swab was placed onto 2 pre-reduced anaerobic brucella blood agar plates and the inoculum spread evenly with a sterile bent rod.

4. All plates were incubated anaerobically (AnaeroPack System, Carr-Scarborough Microbiologicals, Decatur, Ga.) at 35°C for up to 4 days.

5. Colony counts were obtained from plates with 30-300 colonies.

Table 2. Recovery after 48 hours

No Reduction	10^1 Reduction	10^2 Reduction	10^3 Reduction	No Growth
C. sordellii *P. acnes* *E. coli*	*B. fragilis (2)*[a] *C. perfringens (2)*[a] *P. magnus (2)*[a] *B. caccae* *P. intermedia* *B. thetaiotaomicron*[a] *P. anaerobius* *E. lentum* *C. innocuum* *A. odontolyticus*	*F. nucleatum* *B. thetaiotaomicron* *B. uniformis* *P. bivia* *C. ramosum*	*F. necrophorum* *P. melaninogenica* *B. ureolyticus*	*C. difficile* *F. nucleatum*[a] *C. innocuum*[a]

[a] One strain as part of a 2 - organism mixture

RESULTS

1. A total of 21 anaerobes were tested individually in the Starswab S120D system with 20/21 (95%) recovered after 48 hours. One *C. difficile* was recovered after 24 hours, but not after 48 hours.

2. Seven anaerobes were tested in 2-organism mixtures in the same Starswab vial with 5/7 (71%) recovered after 48 hours.

3. Of the anaerobes recovered after 48 hours, only one *F. necrophorum*, *P. melaninogenica*, and one *B. ureolyticus* had ≥ 10^2 CFU/ml reduction in recovery after 24 hrs.

4. All anaerobes tested as part of 2-organism mixtures were recovered after 24 hrs except one *F. nucleatum* and one *C. innocuum*.
   a. *F. nucleatum* and *P. magnus* (10^1 reduction)
   b. *C. innocuum* and *E. coli* (no reduction)

5. All individually inoculated anaerobes were recovered in equivalent numbers (≤10^1 CFU/ml reduction) after 4 hrs except the one *C. difficile* which had a 10^2 CFU/ml reduction.

6. The Starswab rayon-tipped swabs fully absorbed the 100-µl inoculum for only 49% of the swabs tested. Although not measured, the other swabs appeared to only absorb approximately 50-75% of the inoculum.

SUMMARY

1. The Starplex Starswab Anaerobic Transport system is an acceptable method for maintaining viability of anaerobes for up to 48h during transport.

2. Overall, 90% of the anaerobes tested were recovered after 48 hrs. in the Starswab system.

3. All anaerobes were recovered after 4 hrs. in the Starswab system.

4. The Starswab S120D with two swabs is a simple to use transport system with a sterile over-wrap for use in sterile operating fields.

5. Although the rayon-tipped swabs appeared to be inconsistent in absorbancy, there did not appear to be any discrepancies due to the reduced inoculum on the swab.

6. Future studies need to evaluate the Starswab directly with patient specimens.