Comparison of the Starplex II swab with Syringe Collection for the Recovery of Anaerobic Bacteria

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ABSTRACT

Background:
The recovery of anaerobes is challenging and contingent upon their viability in a transport system. We compared syringe and swab methods using the Starswab II S120D (STAR) (Starplex Scientific Inc., Ontario, Canada) anaerobic system, containing pre-reduced Carey Blair medium. Organisms used to challenge the transport system were chosen from the NCCLS M40 document which recommended: *Bacteroides fragilis*, ATCC 25285 *Peptostreptococcus anaerobius* , ATCC 27337, *Propionibacterium acnes*, ATCC 6919 and *Prevotella melaninogenica,* ATCC 25845.
Methods:
A working inoculum of 1.5 X 10 4.5 cfu/ml was prepared for each organism tested. Standard suspensions (100 ul) of the organisms were inoculated into the transport systems. For the Syringe method, 200 ul were aspirated into a syringe and dispensed through the septum of the Starswab to the surface of the medium in the tube. Duplicate swabs were inoculated with the organism suspensions to test swabs held at room temperature (RT) 20-25oC and 2-6oC at 0, 4hr, 24hr and 48hr storage. Following storage, the swabs were removed from the transport device and plated to duplicate CDC anaerobic blood agar and incubated at 35oC in an anaerobic glove box. For the syringe method, 100 ul of inoculum were removed from the transport and placed on the surface of the appropriate plated media at the times mentioned. Culture plates were evaluated following 48 hrs incubation and colony counts were generated.
Results:
The syringe and STAR swab methods performed comparably. The best overall survival was achieved at 2-6oC. *P. acnes* survived throughout all storage times/temperatures. *P. melaninogenica* failed to survive in either system at 24 or 48 hr at RT. *P. anaerobius* was viable up to 24 hr using the syringe method and only 4 hr using the swab method. The swab method maintained viability of *B. fragilis* throughout all storage times and temperatures, while the organism was viable for 24 hr at either temperature with the syringe method.
Conclusions:
The syringe method is equivalent to the swab system for the organisms tested.

INTRODUCTION

The collection and transport of clinical specimens to the microbiology laboratory are essential to dispensing good quality care. The recovery of anaerobic bacteria is challenging due to the protection from exposure to oxygen for survival. Aspirates have been held as preferable to swabs for the recovery of anaerobes. The use of a gassed out collection vial will provide an anaerobic environment during transportation to the laboratory. Most inpatient specimens are received into the laboratory within 4 h after collection. Outpatient specimens, however, may be received in the clinical laboratory 24 h after their collection. These specimens may be stored and transported at a variety of temperatures.The purpose of this study was to compare a syringe system and a swab system for maintaining the viability of several anaerobes at ambient and refrigerated temperatures

METHODS

- Prepare inoculum in sterile soybean casein digest broth to a concentration of approximately 1.5 x 108 CFU/ml from 18 – 24 hour growth of organism.
- Serially dilute to obtain approximate final working concentration of 1.5 x 105 CFU/ml or 1.5 x 104.5 CFU/ml depending on the microorganism.
- Transfer 100 uL (or 200 ul) (use same volume as used for syringe study) of working solution inoculum to sterile vial.
- Insert swab into vial and leave for approximately 10 seconds to absorb inoculum. Transfer swab to tube of Starswab Anaerobic Transport System.
- At defined recovery intervals, retrieve swab from tube of Starswab Anaerobic Transport System and streak swab over the entire agar surface of appropriate agar plate.
- Rotate swab between thumb and index fingers to ensure that all surfaces of the swab equally contact the surface of the agar plate. Repeat by streaking two more times, rotating the plate approximately 60o each time to ensure an even distribution of inoculum.
- Incubate plates in appropriate environmental conditions for 48 hours. Count colonies.

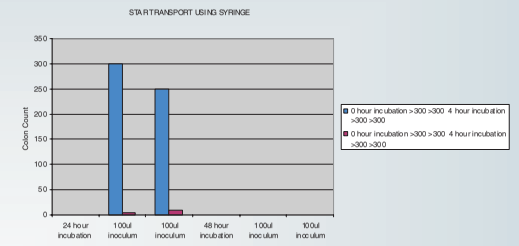
USING SYRINGE

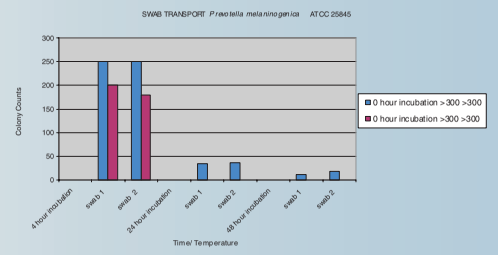
Prevotella melaninogenica ATCC 25845	4 degrees 48 hour growth	room temp 48 hour growth
0 hour incubation		
100ul inoculum	>300	>300
100ul inoculum	>300	>300
4 hour incubation		
100ul inoculum	>300	>300
100ul inoculum	>300	>300
24 hour incubation		
100ul inoculum	300	4
100ul inoculum	250	9
48 hour incubation		
100ul inoculum	0	0
100ul inoculum	0	0
Propionibacterium acnes ATCC 6919		
0 hour incubation		
100ul inoculum	>300	>300
100ul inoculum	>300	>300
4 hour incubation		
100ul inoculum	>300	>300
100ul inoculum	>300	>300
24 hour incubation		
100ul inoculum	>300	>300
100ul inoculum	>300	>300
48 hour incubation		
100ul inoculum	>300	>300
100ul inoculum	>300	>300
Bacteroides fragilis ATCC 25285		
0 hour incubation		
100ul inoculum	>300	>300
100ul inoculum	>300	>300
4 hour incubation		
100ul inoculum	>300	>300
100ul inoculum	>300	>300
24 hour incubation		
100ul inoculum	300	250
100ul inoculum	300	250
48 hour incubation		
100ul inoculum	0	0
100ul inoculum	0	0
Peptostreptococcus anaerobius ATCC 27337		
0 hour incubation		
100ul inoculum	>300	>300
100ul inoculum	>300	>300
4 hour incubation		
100ul inoculum	>300	>300
100ul inoculum	>300	>300
24 hour incubation		
100ul inoculum	>300	210
100ul inoculum	>300	292
48 hour incubation		
100ul inoculum	>300	1
100ul inoculum	>300	3

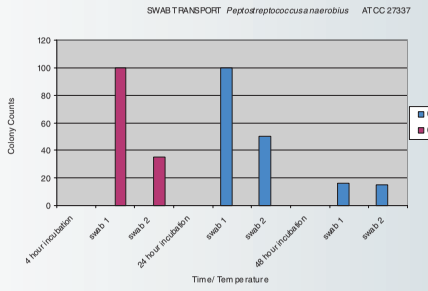
USING SWAB

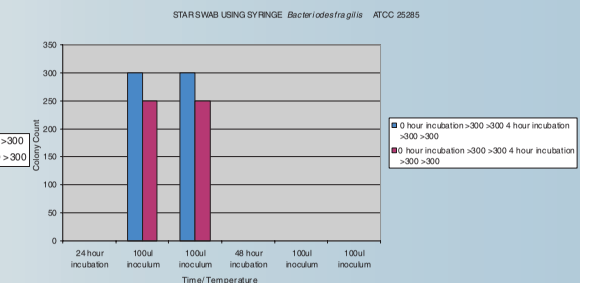
Prevotella melaninogenica ATCC 25845	4 degrees 48 hour growth	room temp 48 hour growth
0 hour incubation		
swab 1	>300	>300
swab 2	>300	>300
4 hour incubation		
swab 1	250	200
swab 2	250	180
24 hour incubation		
swab 1	35	0
swab 2	36	0
48 hour incubation		
swab 1	11	0
swab 2	18	0
Propionibacterium acnes ATCC 6919		
0 hour incubation		
swab 1	>300	>300
swab 2	>300	>300
4 hour incubation		
swab 1	>300	>300
swab 2	>300	>300
24 hour incubation		
swab 1	>300	>300
swab 2	>300	>300
48 hour incubation		
swab 1	>300	>300
swab 2	>300	>300
Bacteroides fragilis ATCC 25285		
0 hour incubation		
swab 1	>300	>300
swab 2	>300	>300
4 hour incubation		
swab 1	>300	>300
swab 2	>300	>300
24 hour incubation		
swab 1	>300	>300
swab 2	>300	>300
48 hour incubation		
swab 1	>300	>300
swab 2	>300	>300
Peptostreptococcus anaerobius ATCC 27337		
0 hour incubation		
swab 1	>300	>300
swab 2	>300	>300
4 hour incubation		
swab 1	>300	100
swab 2	>300	35
24 hour incubation		
swab 1	100	0
swab 2	50	0
48 hour incubation		
swab 1	16	0
swab 2	15	0

RESULTS









CONCLUSIONS

1. All anaerobes survived up to 24 hours
2. Anaerobes can be stored or transported at 4ºC
3. More anaerobes need to be tested at different holding times and dilutions with both syringe and swab
4. A specific inoculum may be needed for each anaerobe
5. Swab and syringe results appear to be similar

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