COMPARISON OF FOUR SWAB SYSTEMS FOR RECOVERY OF ANAEROBIC BACTERIA


ABSTRACT
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Specimens for anaerobic culture require specialized collection and transport devices to protect the specimen from oxygen during transport. We compared the Copan Venturi Transystem swab, the BBL Port-A-Cul swab, the BBL Culturette swab, and the BBL Culturette EZ swab transport systems for recovery of 47 anaerobic bacteria. Swabs were inoculated with 100-µL of a freshly prepared 10^7 CFU/µL suspension of the organism and incubated at room temperature for 0, 4, 24, and 48 h. The swabs were then used to prepare three 10-fold serial dilutions in 0.9-mL sterile saline. A 100-µL aliquot of each dilution was inoculated to reduced anaerobic blood agar and incubated in an anaerobic atmosphere for 48-96 h. Colony counts were obtained for each organism dilution and the data analyzed as percent recovery compared to the 0 h (initial inoculum) growth results. After 24 h room temperature incubation of each swab system, both the Port-A-Cul and Copan Venturi Transystem recovered 44/47 (94%) of the anaerobes tested. The Culturette recovered 39/47 (83%) and the Culturette EZ recovered 37/47 (79%) after 24 h. After 48 h, the Copan Venturi Transystem recovered 41/47 (87%), the Port-A-Cul recovered 39/47 (83%), the Culturette recovered 32/47 (68%), and the Culturette EZ recovered 28/47 (60%). Five Bacteroides sp., 5 Clostridium sp., 4 Fusobacterium sp., 3 Peptostreptococcus sp., and 1 each of Prevotella and Bifidobacterium were not recovered in the Culturette EZ after 48 h. Five Clostridium sp., 3 each of Fusobacterium sp. and Peptostreptococcus sp., 2 Bacteroides sp., and 1 each of Prevotella and Veillonella were not recovered in the Culturette system after 48 h. The Port-A-Cul failed to recover 3 Peptostreptococcus sp., 3 Clostridium sp., 1 Fusobacterium sp. and 1 Prevotella sp. while the Copan Venturi Transystem failed to recover 2 each of Clostridium sp. and Fusobacterium sp., and 1 each of Prevotella sp. and Peptostreptococcus sp. after 48 h. The Copan Venturi Transystem and BBL Port-A-Cul are satisfactory transport systems to protect the specimen from oxygen during transport for recovery of anaerobic bacteria after 24 and 48 h. The Culturette and Culturette EZ are less satisfactory after 24 h and should not be used for long term (48 h) transport of specimens for anaerobic culture.

METHODS

1. Four collection/transport swab devices were tested for their ability to maintain viability of 47 anaerobic bacteria (identified by the RapID ANA II, Remel-Atlanta, Atlanta, Ga.).
   a. BBL Port-A-Cul (Becton-Dickinson Microbiology Systems, BD M S, Cockeysville, Md.)
   b. BBL Culturette (BD M S)
   c. BBL Culturette EZ (BD M S)
   d. Copan Venturi Transystem (Copan Diagnostics, Corona, Ca.)

2. Four swabs of each type were rolled for 10 sec. into 100-µL of an organism suspension of approximately 10^7 CFU/mL to completely absorb the inoculum.

3. Swabs were incubated at room temperature for 0, 4, 24, and 48 h.

4. After the appropriate incubation period, each swab was removed and placed into 0.9-mL of sterile saline and mixed 10 sec. on a vortex to re-suspend the organisms.

5. Two 10-fold serial dilutions in sterile saline were performed to achieve tubes with approximately 10^1-10^8 CFU/mL.

6. 100-µL of each of the 3 serial dilutions of each organism for each swab were plated to anaerobic brucella blood agar (BD M S) reduced 24 h prior to use.
   a. Duplicate plates were inoculated for each organism inoculum.
   b. At 48 h, the swab itself, after dilutions were prepared, was rolled over the surface of an anaerobic plate to determine if organisms may have been trapped in the swab itself.

7. The inoculum was spread over the entire agar surface with a sterile bent plastic rod (Copan).

8. All plates were incubated at 35°C in an anaerobic atmosphere (AnaeroPack, Mitsubishi Gas Chemical America, New York, N.Y.) for 48-96 h.

9. Two microbiologists obtained colony counts for each incubation time.

10. Results were expressed as % recovery compared to the 0 h initial inoculum for each swab system.

RESULTS (Tables 1-4)

Recovery of anaerobes after 24 hours room temperature incubation in four swab systems.

1. The BBL Port-A-Cul swab system recovered 44 of 47 (94%) with one strain of C. perfringens, P. anaerobius, and P. asaccharolyticus not recovered.

2. The Copan Venturi Transystem recovered 44 of 47 (94%) with one strain of F. nucleatum, P. intermedia, and C. cadaveris not recovered.

3. The BBL Culturette recovered 39 of 47 (83%) with one strain of B. ureolyticus, P. intermedia, F. nucleatum, F. mortiferum, F. necrophorum, C. cadaveris, and both P. anaerobius strains not recovered.

4. The BBL Culturette EZ recovered 37 of 47 (79%) with one strain of F. mortiferum, F. necrophorum, P. intermedia, C. cadaveris, C. perfringens, P. asaccharolyticus, and both strains of F. nucleatum and P. intermedia not recovered.

INTRODUCTION

The most common indigenous flora of the human body are anaerobic bacteria, therefore, special care needs to be taken to ensure their recovery from sites of infection. Proper collection and transport of specimens for anaerobic culture is mandatory for the appropriate recovery and identification of any infecting anaerobic bacteria. Anaerobic transport of specimens has been suggested as the ideal method of transport for aerobic, anaerobic, and facultative organisms. Unexpected transport delays may disrupt the timely culture of these specimens, therefore, the transport system needs to be adequate to maintain the viability of organisms over extended periods of time.

The following transport systems were compared for their ability to maintain the viability of 47 anaerobic bacteria over a period of 48 hours of room temperature incubation: Copan Venturi Transystem with Amies agar; BBL Port-A-Cul which contains a "balanced formula of reducing agents and resazurin in a buffered isotonic base"; BBL Culturette for aerobes and anaerobes which contains a modified Stuart's liquid medium; and the BBL Culturette EZ for aerobic specimens which contains a non-toxic and unique polyurethane-tipped swab with no transport medium to cause dilution of the specimen.
OBSERVATIONS

1. The BBL Port-A-Cul swab system recovered 39 of 47 (83%) with the addition of one strain of F. necrophorum, P. intermedius, P. anaerobius, C. perfringens, and C. difficile not recovered.

2. The Copan Venturi Transystem recovered 41 of 47 (87%) with the addition of one strain of F. necrophorum, P. anaerobius, and C. difficile not recovered.

3. The BBL Culturette recovered 32 of 47 (68%) with the addition of one strain of B. uniformis, P. asaccharolyticus, Veillonella sp., C. perfringens, C. innocuum, and two C. difficile not recovered.

4. The BBL Culturette EZ recovered 28 of 47 (60%) with the addition of one strain of B. caceae, B. ovatus, B. uniformis, B. ureolyticus, B. vulgatus, C. difficile, C. innocuum, C. perfringens, and Bifidobacterium sp. not recovered.

SUMMARY/CONCLUSIONS

1. After 24 hours incubation, the BBL Port-A-Cul and Copan Venturi Transystem recovered 94%, the BBL Culturette recovered 83%, and the BBL Culturette EZ recovered 79% of the anaerobes tested.

2. After 48 hours incubation, the Copan Venturi Transystem recovered 87%, the BBL Port-A-Cul recovered 83%, the BBL Culturette recovered 68%, and the BBL Culturette EZ recovered 60% of the anaerobes tested.

3. All four swab systems are acceptable for maintenance of viability of anaerobes up to 24 hours post-inoculation, although the BBL Port-A-Cul and Copan Venturi Transystem are somewhat better than the BBL Culturette and BBL Culturette EZ.

4. The BBL Port-A-Cul and Copan Venturi Transystem are acceptable for maintenance of anaerobe viability when transport delays exceed 24 hours up to 48 hours. The BBL Culturette and BBL Culturette EZ cannot be recommended when transport delays will exceed 24 hours.

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