C-156

Comparison of Five Transport Media for the Extended Maintenance of Viability of Aerobes and Anaerobes

P.P. Patel, **F.O. Wegerhoff**, L.R. Newton, and P.E. Oefinger, Covance Central Laboratory Services, Inc.

8211 SciCor Drive
Indianapolis, IN 46214
Phone: 317-271-4822
Fax: 317-273-7780

Abstract

Maintenance of viability of bacterial pathogens is essential for optimal recovery from clinical samples, and is dependant on transport media when immediate processing of specimens is not feasible. Five transport media were evaluated for the maintenance of viability of pathogens under ambient and refrigerated conditions: A.C.T. I (Remel, Inc.), Port-A-Cul (BBL), Amies with Charcoal (Starplex Scientific, Inc.), Trypticase Soy Broth with 15% glycerol and agar (TSBGA), and Brucella Broth with 15% glycerol and agar (BBGA). Fourteen ATCC strains (*Candida albicans, Enterococcus faecalis, Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Neisseria gonorrhoeae, Neisseria meningitidis, Bacteroides fragilis, Clostridium perfringens, Peptostreptococcus micros)* and 4 clinical strains (*Neisseria gonorrhoeae, Neisseria meningitidis, Escherichia coli, Klebsiella pneumoniae)* were tested. Swabs were immersed in 0.5 McFarland suspensions of each organism, inserted into the transport device, and maintained under ambient and refrigerated conditions. Semi-quantitative culture of swabs occurred on days 1, 3, 5, 7, 10, 21, 30, 60, 90, 120, 150, and 180 using standard media. Aerobes (*E.faecalis, E.coli, K. pneumoniae, S.aureus, S.pneumoniae, S.pyogenes,* and *C.albicans)* maintained viability at both temperatures for 180 days in at least two of the transport media, and *H. influenzae* and *M. catarrhalis* for at least 21 days. ATCC and clinical strains of *N. gonorrhoeae* and *N. meningitidis* maintained viability for 3 - 10 days ambient, and for 10 -180 days refrigerated. Clinical strains of *E. coli* and *K. pneumoniae* remained viable after 180 days at both storage temperatures. Viability of anaerobes at both storage temperatures varied from 30 - 120 days.We conclude that longterm viability of bacterial pathogens may be effectively maintained in a variety of transport devices at either ambient or refrigerated temperatures. Use of selective transport devices for certain organisms may be prudent when immediate processing is not feasible.

Introduction

- Recovery of bacterial pathogens involved in infectious processes is important for the successful treatment and recovery of critically ill patients
- Maintenance of viability of these bacterial pathogens sometimes presents a challenge because of delayed processing, which affects their recovery from clinical samples and correct identification in the laboratory
- The fastidious nature of many bacterial pathogens involved in infectious disease processes requires that they be correctly collected, transported, and processed because their viability rapidly decreases outside the human host
- After collection, immediate processing of specimens containing potential pathogens may not be feasible, and effective transport devices may be required to maintain the viability and integrity of organisms for extended periods of time prior to their recovery in the clinical laboratory
- In this study, we wished to compare the ability of five different transport devices to maintain the viability of selected bacterial pathogens which may be recovered from patients

Materials and Methods

- Five different transport media were evaluated for the maintenance of viability of pathogens under ambient and refrigerated conditions: A.C.T. I (Remel, Inc.), Port-A-Cul (BBL), Amies with Charcoal (Starplex Scientific, Inc.), Trypticase Soy Broth with 15% glycerol and agar (TSBGA), and Brucella Broth with 15% glycerol and agar (BBGA)
- In order to compare these transport systems, ATCC and clinical strains were inoculated into the different transport systems (Tables 1 and 2):
  - 11 ATCC aerobic strains
  - 3 ATCC anaerobic strains
  - 4 aerobic clinical strains
- A suspension of each organism was made in 0.9% saline equivalent to a 0.5 McFarland standard. A cotton swab was immersed in each suspension and then inserted into the different transport media, which were then stored at ambient (20-25°C) and refrigerated (2-8°C) temperatures. Subculture to appropriate media occurred at 1, 3, 5, 7, 10, 21, 30, 60, 90, 120, 150, and 180 days
- *Candida albicans* was cultured on Sabouraud dextrose agar, and *Haemophilus influenzae, Neisseria gonorrhoeae* and *Neisseria meningitidis* on chocolate agar. All other aerobes were cultured on 5% sheep blood agar plates. Anaerobes were cultured on Brucella agar. All plates were inoculated using the four-quadrant method in order to obtain a semi-quantitative enumeration of organism growth
- Aerobes were incubated for up to 48 hours in CO_2 at 34 – 36°C, anaerobes for 48 hours in an anerobic jar at 34 - 36°C, and *C. albicans* for up to 48 hours at 29-31°C. All plates were examined for growth which was recorded as 1+ to 4+

Table 1

Aerobic Organisms	ATCC number
Candida albicans	14053
Enterococcus faecalis	29212
Escherichia coli	25922
Haemophilus influenzae	49247
Klebsiella pneumoniae	13883
Moraxella catarrhalis	25240
Neisseria gonorrhoeae	43069
Neisseria meningitidis	13090
Staphylococcus aureus	29213
Streptococcus pneumoniae	49619
Streptococcus pyogenes	19615
Anaerobic organisms	**ATCC number**
Bacteroides fragilis	25285
Clostridium perfringens	13124
Peptostreptococcus micros	33270

Table 2

Clinical Isolates
Escherichia coli
Klebsiella pneumoniae
Neisseria gonorrhoeae
Neisseria meningitidis

Results

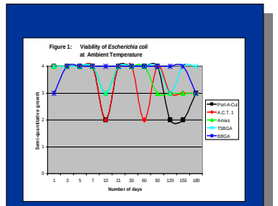
Aerobes

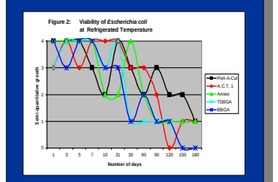
- Among the aerobes, *E. faecalis** was the only organism that fully maintained viability for 180 days in all transport media at both ambient and refrigerated temperatures
- *E.coli* (Figure 1), *K. pneumoniae**, and C. *albicans* (Figure 3) maintained viability in all transport media for 180 days at ambient temperature, whereas *S. pneumoniae* (Figure 5), *S. pyogenes**, and *S. aureus** maintained viability at ambient temperature for 180 days only in Port-A-Cul and A.C.T.1
- *H. influenzae* (Figure 7) maintained viability in all transport media for 5 days at ambient temperature (21 days in BBGA, and 10 days in Port-A-Cul and A.C.T. 1). *M. catarrhalis* (Figure 9) maintained viability in all transport media for 7 days at ambient temperature (30 days in Port-A-Cul and Amies with charcoal)
- *S. pyogenes** and *S. aureus** maintained viability in all transport media for 180 days at refrigerated temperature. *E. coli* (Figure 2) and *C. albicans* (Figure 4) in Port-A-Cul and A.C.T. 1 only, and *S. pneumoniae* (Figure 6) in A.C.T. 1 only. *H. influenzae* (Figure 8) maintained viability in all transport media for 10 days at refrigerated temperature (60 days in Port-A-Cul), and *M. catarrhalis* (Figure 10) for 30 days (120 days in Amies with charcoal)
- Among the clinical strains, *N. gonorrhoeae* (Figures 11 and 12) maintained viability for 3 days at ambient temperature only in Port-A-Cul and TSBGA, but 10 days refrigerated in all transport media. *N. meningitidis* (Figures 13 and 14) maintained viability for 10 days at ambient temperature only in Port-A-Cul, but was fully viable in A.C.T. 1 after 180 days at refrigerated temperature. Clinical strains of *E. coli** and *K. pneumoniae** maintained viability in 3 transport media (Port-A-Cul, A.C.T. I, Amies with charcoal) for 180 days at both storage temperatures

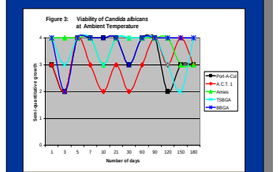
Anaerobes

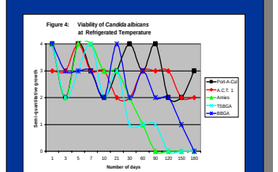
- Among the anaerobes, *B. fragilis* (Figures 15 and 16) maintained viability in all transport media for at least 30 days (180 days in Port-A-Cul and A.C.T. I) at ambient temperature, and up to 60 days refrigerated in all transport media (180 days in A.C.T. I)
- *C. perfringens** maintained viability for at least 7 days in all transport media (30 days in Port-A-Cul, A.C.T. I, and TSBGA) at ambient temperature, and at least 21 days refrigerated in all transport media (90 days in A.C.T. I)
- *P. micros** maintained viability for at least 7 days in all transport media (120 days in Port-A-Cul) at ambient temperature, and at least 21 days refrigerated in all transport media (180 days in A.C.T. I)

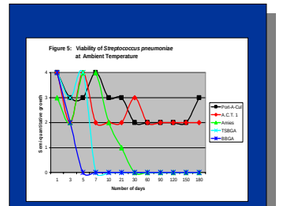
* = Figures not shown

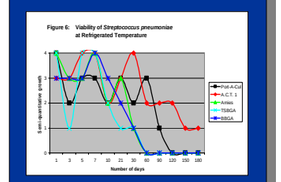


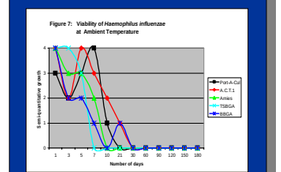


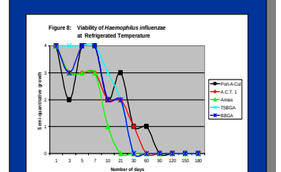


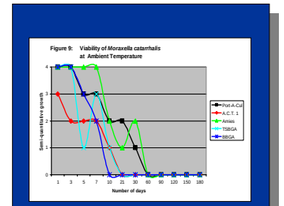


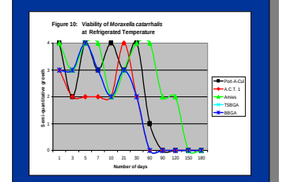


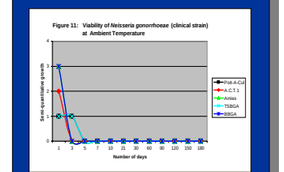


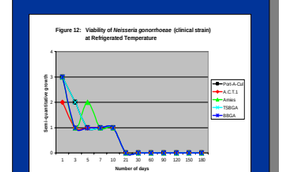


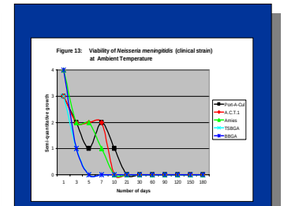


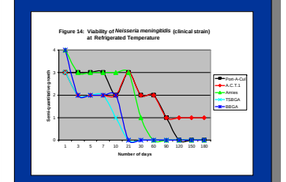


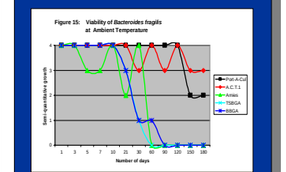


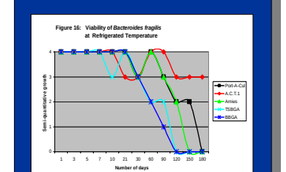












Conclusions

- The new transport media which we devised (TSBGA and BBGA) are as least as good as Amies with charcoal, and in some instances are notably better
- Port-A-Cul and A.C.T. I are comparable in their ability to maintain the viability of a wide variety of organisms for extended periods of time
- A.C.T. I appears to better maintain the viability of fastidious organisms such as *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* than other transport media, especially at refrigerated temperature
- We conclude that longterm viability of bacterial pathogens may be effectively maintained in a variety of transport devices at either ambient or refrigerated temperatures
- Use of selective transport devices for certain organisms may be prudent when immediate processing is not feasible.