Journal of Bacteriology and Virology 2010. Vol. 40, No. 2 p.99 – 101
DOI 10.4167/jbv.2010.40.2.99

Communication

A Simple, Effective Method for Bacterial Culture Storage: A Brief Technical Report

Ryan D Winters[1] and Washington C Winn, Jr.[2]

[1]Department of Otolaryngology - Head and Neck Surgery, Tulane University, New Orleans, LA, USA,
[2]Department of Pathology, UVM COM and Fletcher Allen Healthcare, Burlington, VT, USA

Maintenance of large collections of bacterial cultures can be challenging, as individual species may necessitate specific storage techniques, and many important organisms are famously difficult to sustain in culture. We present a simple, cost-effective, reliable method for long-term storage of bacterial strains suitable for fastidious species.

Key Words: Bacterial culture, Long-term storage

It is desirable, oftentimes essential, for laboratories to maintain bacterial strains in culture for extended periods of time for research, teaching and quality control purposes. Common methods employed include lyophilization, water culture storage, repeated sub-culturing, storage at -80°F in defibrinated rabbit blood or freezing in liquid nitrogen. There is a vast amount of literature available detailing different long-term storage methods for microorganisms (1~4), though much of this literature is species-specific (5~15), and several bacterial species are historically difficult to maintain long-term (7, 9, 14). Several of these problematic genera, such as *Haemophilus* and *Neisseria*, include medically important species that are advantageous to possess in culture collections; in addition, much of the published data concerns only aerobic bacteria, with comparatively little published regarding maintenance cultures for anaerobic species (5, 16, 17). In this study, viability of maintenance cultures for a variety of medically important bacteria was followed for one year, with the objective of describing a single simple method for storing a variety of bacterial strains, to include notoriously fastidious aerobes, and a representative anaerobic species.

To this end, one strain of *Neisseria gonorrhoeae*, two strains of *Neisseria meningitides*, two strains of methicillin-resistant *Staphylococcus aureus* (MRSA), one strain of *Haemophilus influenzae*, two strains of *Haemophilus aphrophilus*, two strains of *Clostridium perfringens* and two strains of *Campylobacter jejuni* were obtained from Fletcher Allen Health Care (Burlington, VT, USA) laboratory stock cultures. All were patient isolates and, having been previously identified to species in this department, confirmatory identification was not performed. The *Campylobacter* samples were transferred to chocolate agar plates and incubated in a Bio-Bag (Becton Dickinson, Cockeysville, MD, USA) at 42℃ for 24 h. The *Clostridium* samples were transferred to chocolate agar plates and incubated in a Coy Anaerobic Chamber (Coy Laboratory Products, Grass Lake, MI, USA) for 24 h. All remaining samples were transferred to chocolate agar plates and incubated at 37℃ in room air for 24 h. From these plates, 10 sections of agar measuring approximately 1 × 0.5 cm were cut from areas of dense bacterial growth and placed into individual sterile vials and stored in a Revco Ultralow freezer (Fisher Thermo Scientific, Waltham, MA, USA) at

Received: May 10, 2010/ Revised: May 26, 2010
Accepted: May 27, 2010
*Corresponding author: Ryan D Winters, MD. Department of Otolaryngology - Head and Neck Surgery, Tulane University, 1430 Tulane Ave. SL-59, New Orleans, LA 70112, USA.
Phone: +1 (802) 318-1537, e-mail: rwinters@tulane.edu
**Financial Support: UVM Department of Pathology Resident Research Funds.

a temperature of -40 to -70℃.

Viability of the cultures was assessed at monthly intervals for six months, again at 9 months, and finally at 12 months from initial storage. One vial of each strain was removed from the freezer and allowed to rise to room temperature. The fragment of agar from the vial was placed onto a room temperature chocolate agar plate and rotated so all sides of the fragment contacted the surface of the plate. Each plate was then streaked for isolation and incubated at 37℃ and checked for growth at 24 and 48 h, then again after five days. A culture was considered viable if ≥1 colony forming unit (CFU) was present after incubation, and considered nonviable if no growth was observed after five days incubation. Quantitative analysis was not undertaken in this study, given the samples used were patient isolates and the original number of microorganisms present was not known.

We describe a simple, effective method for long-term storage of a variety of bacterial species including fastidious organisms; it is a method particularly suited for maintenance of large culture collections as it is inexpensive and requires very little labor to implement. Recovery was possible for all species studied at 12 months (exception: *Clostridium perfringens*, which was studied only to six months). While established methods such as commercially available bead storage systems are useful for maintenance of quality control samples that must be repeatedly sampled and re-frozen, a uniform method for culture storage for clinical isolates for possible later use could streamline laboratory procedures for upkeep of large culture collections.

REFERENCES

1) Morgan CA, Herman N, White PA, Vesey G. Preservation of micro-organisms by drying; a review. J Microbiol Methods 2006;66:183-93.
2) Theunissen JJ, Stolz E, Michel MF. The effects of medium and rate of freezing on the survival of chlamydias after lyophilization. J Appl Bacteriol 1993; 75:473-7.
3) Siberry G, Brahmadathan KN, Pandian R, Lalitha MK, Steinhoff MC, John TJ. Comparison and different culture media and storage temperatures for the long-term preservation of *Streptococcus pneumoniae* in the tropics. Bull World Health Organ 2001;79:43-7.
4) Staab JA, Ely JK. Viability of lyophilized anaerobes in two media. Cryobiology 1987;24:174-8.
5) Obara Y, Yamai S, Nikkawa T, Shimoda Y, Miyamoto Y. Preservation and transportation of bacteria by a simple gelatin disk method. J Clin Microbiol 1981;14: 61-6.
6) Alexander GA. Improved lyophilization procedure for storage of reference cultures and clinical isolates. J Clin Microbiol 1981;13:805-6.
7) Murray PR, Baron EJ, Jorgensen JH, Pfallen MA, Yolken RH. 2003. *Manual of Clinical Microbiology*, 8th Ed, Vol I. American Society of Microbiology Press.
8) Hagen JC, Wood WS, Hashimoto T. Effect of chilling on the survival of *Bacteroides fragilis*. J Clin Microbiol 1976;4:432-6.
9) Harbec PS, Turcotte P. Preservation of *Neisseria gonorrhoeae* at -20℃. J Clin Microbiol 1996;34:1143-6.
10) Wasas AD, Huebner RE, Klugman KP. Use of Dorset egg medium for maintenance and transport of *Neisseria meningitides* and *Haemophilus influenzae* type b. J Clin Microbiol 1999;37:2045-6.
11) Charlambous BM, Batt SL, Peek AC, Mwerinde H, Sam N, Gillespie SH. Quantitative validation of media for transportation and storage of *Streptococcus pneumoniae*. J Clin Microbiol 2003;41:5551-6.
12) Votava M, Stritecka M. Preservation of *Haemophilus influenzae* and *Haemophilus parainfluenzae* at -70℃. Cryobiology 2001;43:85-7.
13) Aulet de Saab OC, de Castillo MC, de Ruiz Holgado AP, de Nader OM. A comparative study of preservation and storage of *Haemophilus influenzae*. Mem Inst Oswaldo Cruz 2001;96:583-6.
14) Cody RM. Preservation and storage of pathogenic *Neisseria*. Health Lab Sci 1978;15:206-9.
15) Drumm B, Sherman P. Long-term storage of *Campylobacter pylori*. J Clin Microbiol 1989;27:1655 -6.
16) Aaberge IS, Eng J, Lermark G, Lovik M. Virulence of *Streptococcus pneumoniae* in mice: a standardized method for preparation and frozen storage of the

experimental bacterial inoculum. Microb Pathog 1995; 18:141-52.
17) Cassiday PK, Sanden GN, Kane CT, M'Boup S, Barbaree JM. Viability of *Bordetella pertussis* in four suspending solutions at three temperatures. J Clin Microbiol 1994;32:1550-3.