A Comparison of Commercially Prepared Culture Media for the Isolation of Anaerobic Bacteria

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Revised Abstract

The purpose of this study was to compare the recently launched AnaeroGRO™ pre-reduced anaerobic culture media (Hardy Diagnostics, Santa Maria, CA) to two existing products: BBL™ reducible media (BD, Sparks, MD) and Anaerobe Systems PRAS media (Anaerobe Systems, Morgan Hill, CA).

Several anaerobic ATCC® strains were evaluated on Brucella with Hemin & Vitamin K, BBE, LKV, and PEA agar. ATCC® strains included *B. fragilis* (ATCC® 25285™), *F. nucleatum* (ATCC® 25586™), *P. melaninogenica* (ATCC® 25845™), *C. perfringens* (ATCC® 13124™), *C. difficile* (ATCC® 9689™), and *P. anaerobius* (ATCC® 27337™). Three different lot numbers were parallel tested in triplicate for three consecutive days. A suspension of each strain was prepared in 3.0 ml of buffered gelatin diluent[1] to match a 3.0 McFarland standard. A final 1:100 dilution was prepared from this suspension. Each of the four plates was inoculated with 10µl of the final dilution and incubated anaerobically (48 hours at 37° C). Plates were evaluated at 48 hours for colony size, morphology, relative abundance or luxuriance, and other growth characteristics. Plates were reincubated and read at 72 hours for further pigment production. A total of 1,458 plates were compared in this study.

Colonies on AnaeroGRO™ Brucella, BBE, and LKV were larger for all strains tested. This feature was significant for some strains (*C. perfringens* and *C. difficile*). Anaerobe Systems PEA agar demonstrated slightly better performance in many cases. Double zone hemolysis of *C. perfringens* was easier to visualize on BD and Anaerobe Systems Brucella agar. Package inserts state that pigment is visible at 24 hours on LKV, but that was not evident for any of the three brands in this study. At best, pigment was seen as a tan coloration of the colonies and extended incubation was required before pigment was clearly visible.

AnaeroGRO™ media demonstrated superior performance for all strains tested on Brucella, BBE, and LKV. Colonies were larger & more luxuriant, sometimes appreciably so. Larger, more robust colonies can offer a distinct advantage when working with mixed anaerobic cultures. Pigment production was slightly more intense on Anaerobe Systems LKV. BD and Anaerobe Systems media performed comparably overall.

Introduction

The importance of anaerobes in clinical infections is well documented. However, the ability to recover clinically significant anaerobes from patient samples continues to pose a challenge to routine laboratories. Obviously, proper collection and transportation devices are extremely important, along with the methods employed to maintain viability of the organisms once they are plated. This study focused on various types of primary plating media of three different brands. The media were compared for their ability to recover and support the growth of clinically significant anaerobes. The media were also evaluated for their growth characteristics on each media type.

Materials

The following stock culture strains were used in this study:

Bacteroides fragilis	ATCC® 25285™	*Fusobacterium nucleatum*	ATCC® 25586™
Clostridium difficile	ATCC® 9689™	*Peptostreptococcus anaerobius*	ATCC® 27337™
Clostridium perfringens	ATCC® 13124™	*Prevotella melaninogenica*	ATCC® 25845™

The following commercially prepared anaerobic culture media were evaluated:

- AnaeroGRO™ Pre-reduced anaerobic culture media – Hardy Diagnostics, Santa Maria, CA
- Anaerobe Systems Pre-Reduced Anaerobically Sterilized (PRAS) media – Anaerobe Systems, Morgan Hill, CA
- BBL™ Reducible anaerobic culture media - Becton, Dickinson and Company (BD), Sparks, MD.

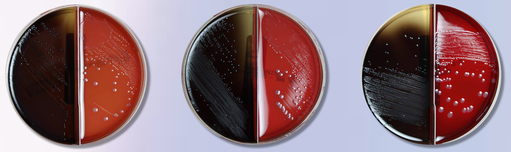
Method

- A suspension of each ATCC strain was prepared in 3.0 ml buffered gelatin diluent[1] to match a 3.0 McFarland standard (9 x 10^8 CFU/mL) from 18-24 hour cultures using a Vitek turbidity meter (bioMérieux).
- A final 1:100 dilution was prepared from this suspension.
- An Eppendorf pipette was used to deliver10µl of the final dilution to each of the four anaerobic plates (Table 1).
- BD plates were reduced by placing under anaerobic conditions for 18-24 hours prior to inoculation as per manufacturer's instructions.
- Plates were streaked to obtain semiquantitative growth and single colonies, and incubated at 37° C in Mitsubishi anaerobic jars for 48-72 hours An anaerobic indicator (resazurin - Oxoid) was included with each jar to ensure anaerobic conditions were met.
- Plates were examined at 48 hours for colony size, morphology, relative abundance or luxuriance, and other growth characteristics.
- Plates were reincubated and read at 72 hours to evaluate further pigment production.
- A total of 1,458 plates were evaluated. Due to supply issues, the BD media was only examined for Lot 3 (in triplicate for three consecutive days).

Table 1 List of Anaerobic Plates Inoculated with each ATCC® Strain

Anaerobic Brucella Agar with Hemin & Vitamin K (BRU)	Monoplate
Anaerobic Bacteroides Bile Esculin Agar (BBE)	Biplate
Anaerobic Laked Blood Agar with Kanamycin & Vancomycin (LKV)	Biplate
Anaerobic Phenylethyl Alcohol Agar (PEA)	Monoplate

B. fragilis on BBE/LKV

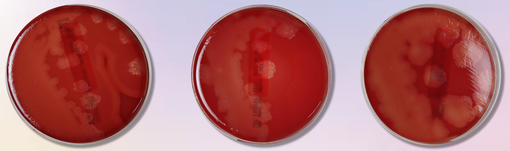


Anaerobe Systems | Becton Dickinson | Hardy Diagnostics

C. difficile on BRU

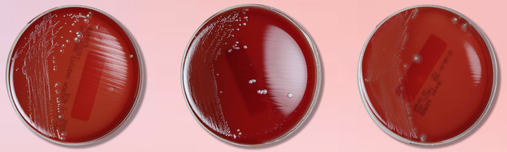
Anaerobe Systems | Becton Dickinson | Hardy Diagnostics

C. perfringens on BRU



Anaerobe Systems | Becton Dickinson | Hardy Diagnostics

P. anaerobius on BRU



Anaerobe Systems | Becton Dickinson | Hardy Diagnostics

Table 2 Chart Comparing Packaging Features for Each Brand

Brand \ Feature	Easy to Open Pre-scored Notch	Oxygen-free Gas Inside Bag	Oxygen-scavenger Sachet	Oxygen-impermeable Foil Pouch	Moisture-absorbing Desiccant
AS		✔		✔	✔
BD					
HD	✔	✔	✔	✔	✔

AS (Anaerobe Systems), BD (Becton Dickinson), HD (Hardy Diagnostics)

AS (Anaerobe Systems)

BD (Becton Dickinson)

HD (Hardy Diagnostics)

Results

- Colonies on AnaeroGRO™ Brucella, BBE, and LKV were larger for all strains tested.
- Colony morphology was typical and as expected for all three brands.
- Anaerobe Systems PEA agar demonstrated slightly better performance in many cases.
- Double zone hemolysis of *C. perfringens* was easier to visualize on BD and Anaerobe Systems Brucella agar.
- Package inserts state that pigment is visible at 24 hours on LKV agar, but that was not evident for any of the three brands in this study. Extended incubation was required before pigment was clearly visible.
- The Certificate of Analysis (Anaerobe Systems) states that *F. nucleatum* ATCC® 25586™ should not grow on PEA agar. However, in this study, it did grow on PEA for all three lot numbers (27 plates total).

Conclusion

- AnaeroGRO™ media demonstrated superior performance for all strains tested on Brucella, BBE, and LKV. Colonies were larger and more luxuriant, sometimes appreciably so. Larger, more robust colonies can offer a distinct advantage when working with mixed anaerobic cultures. Even when isolating colonies using a stereoscopic microscope, there is a distinct advantage to working with larger colonies.
- Pigment production was slightly more intense on Anaerobe Systems LKV.
- BD and Anaerobe Systems media performed comparably well in this study.

Reference

1. Siders, J.A., M.E. Tibbs, L.M. Marler, D.E. Blue-Hnidy, and S.D. Allen. 2005. *IU Anaerobe Laboratory Manual* (Indiana University), Indianapolis, IN 46202.