Comparison of PRAS and Non-PRAS Media for Anaerobes

Abstract

This study conclusively demonstrates that Pre-Reduced Anaerobically Sterilized (PRAS) media significantly outperform traditional Non-PRAS media in the isolation and growth of anaerobic bacteria, particularly oxygen-sensitive species such as Porphyromonas levii. Through rigorous statistical analysis and visual evidence, we show that PRAS media not only enhance colony formation but also improve the detection of fastidious anaerobes, thereby advancing clinical microbiology practices. The findings underscore the critical role of reduced media in mitigating the harmful effects of oxygen exposure, ensuring optimal recovery and accuracy in anaerobic culture techniques.

Introduction

Background of Study

Anaerobes are a diverse group of microorganisms that do not require oxygen for growth, with varying degrees of sensitivity to oxygen.[1] In clinical microbiology, the isolation and cultivation of anaerobes can be challenging due to the exposure of culture media to oxygen during preparation and after, which can lead to the formation of oxidized products harmful to some anaerobes.[2] To address this issue, the practice of pre-reducing media before sterilization was developed, resulting in Pre-Reduced Anaerobically Sterilized (PRAS) media. Different anaerobes exhibit different levels of oxygen tolerance; for instance, members of the Bacteroides fragilis group and Clostridium perfringens are considered 'moderate' anaerobes, capable of surviving oxygen levels up to 2%-8%, while others are more sensitive to even lower oxygen concentrations.[2] These challenges highlight the need for specialized media like PRAS to ensure optimal growth conditions for anaerobic bacteria, particularly in clinical settings where accurate isolation is critical for diagnosis and treatment.

Goals and Objectives

This study aims to compare the performance of PRAS media with traditional non-PRAS media in the isolation and growth of clinically significant anaerobic bacteria. Specifically, the objectives are:

1. To assess the colony formation and growth rates of Bacteroides fragilis, Fusobacterium nucleatum, and Porphyromonas levii on PRAS, post-reduced non-PRAS and non-PRAS media.

2. To evaluate the impact of oxygen exposure on the viability and detectability of these anaerobes.

Methodology

The commercially available Brucella Agar medium with 5% sterile defibrinated sheep blood was used to make the PRAS plates in this experiment. Non-PRAS media were prepared using individual reagents to omit sodium bisulfite, which is included in the commercial Brucella formulation. All three media—PRAS, Non-PRAS, and Non-PRAS with post-reduction—were supplemented with Vitamin K and Hemin. The PRAS formulation also contained the Oxyrase Enzyme System at a concentration of 300u/L. The Non-PRAS with post-reduction plates were individually bagged with an Ageless oxygen absorber and allowed to sit for a minimum of 48 hours to ensure all oxygen was removed prior to plating. The strict Non-PRAS plates were not reduced before sterilization nor after production; therefore, they were bright red, oxidized plates.

Using a spectrophotometer at 600nm, an approximate cell density was determined based on known values of the McFarland turbidity standards. All three organisms were adjusted to an approximate $1x10^3$ cfu/mL. This cell density was chosen based on a separate experiment to ensure quantifiable colonies without being too numerous to count. The cell density was achieved through a series of 10-fold dilutions using a sterile potassium phosphate buffer that had been reduced with Oxyrase. A single drop (~50uL) of inoculum was placed onto each plate and spread across the entire surface. For each organism, the same inoculum was used to inoculate 30 plates of each medium type in quick succession. The plates were then incubated anaerobically for 72 hours at 37°C in an anaerobic jar. All colonies on each plate were counted and recorded.

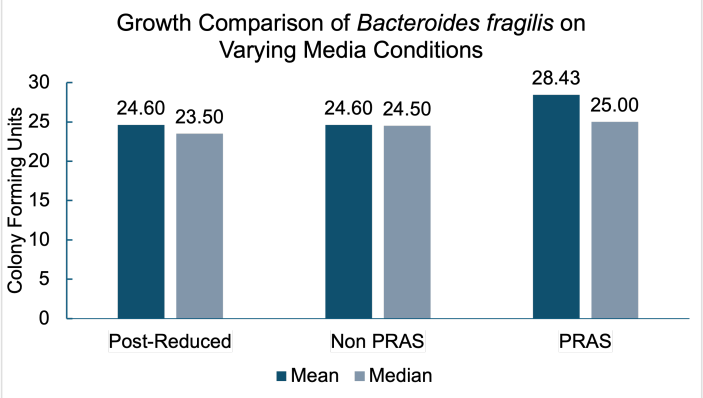
An analysis of the data was performed to determine statistical significance between the three types of media for each organism. This was done using a t-test, which uses the following equation:

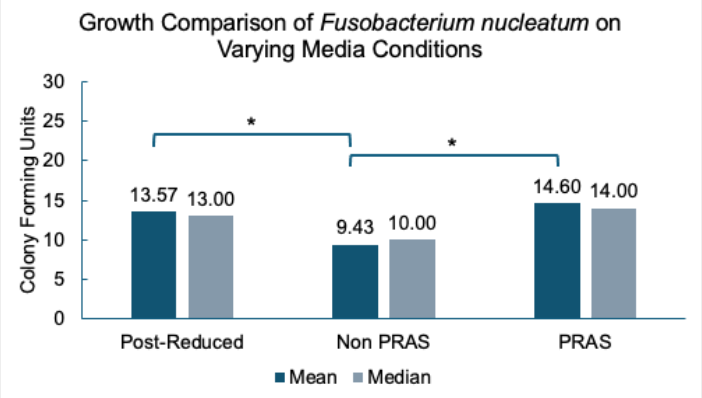
$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s^2(\frac{1}{n_1} + \frac{1}{n_2})}}$$

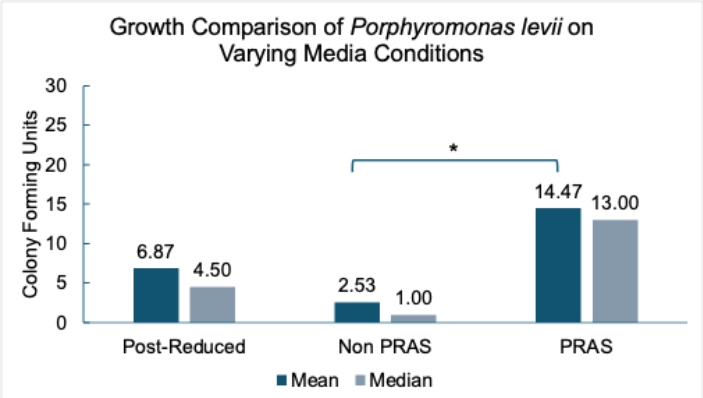
In this formula, t is the t value, x1 and x2 are the means of the two groups being compared, s2 is the pooled standard error of the two groups, and n1 and n2 are the number of observations in each of the groups. A larger t value shows that the difference between group means is greater than the pooled standard error, indicating a more significant difference between the groups. The T-value can be compared against the values in a critical T-value chart to calculate a P-value. If the P-value is less than .05, there is a statistically significant difference between the two data sets. This calculation was performed on all three data sets, the Post Reduction plates and Non-PRAS plates were compared to the PRAS plates.

Results

Facts and Figures





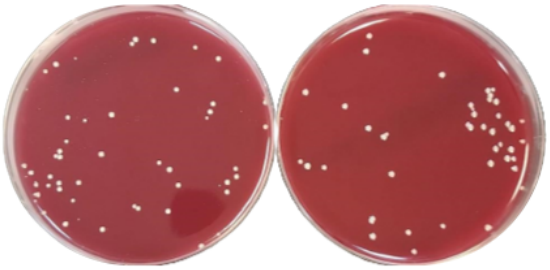


Data Summary - CFUs

B. fragilis	**No significant difference in CFUs between media conditions.**
F. nucleatum	**CFUs were significantly increased in both post-reduced and PRAS when compared to non PRAS.**
P. levii	**CFUs were significantly increased in PRAS when compared to non PRAS.**

Visual Data - Colony Morphology

Images: non PRAS on the left and PRAS on the right.

B. Fragilis	Colony sizes on average were about 2 to 4mm in diameter on the Post Reduced and Non- PRAS plates whereas on the PRAS plate, colonies were 2 to 7mm.	
F. nucleatum	*No significant difference in colony size was observed.*	
P. levii	*Colony sizes were pinpoint on the Post Reduced and Non-PRAS plates and 1 to 3 mm in diameter on the PRAS plates.*	

Key Findings

Statistical analysis showed no significant difference between the three types of media for *Bacteroides fragilis*. However, *Fusobacterium nucleatum* and *Porphyromonas levii* performed significantly better on PRAS media compared to non-PRAS media. Recovery of *F. nucleatum* was enhanced on non-PRAS media that had been reduced post-production.

Quantitative analysis highlights the importance of reduced media for isolating and growing anaerobes. Photographs of these anaerobes growing on plates provide further evidence. For example, although there was no significant quantitative difference between media types for *B. fragilis*, it formed larger, more luxurious colonies on PRAS media compared to non-PRAS media, as shown photographically.

Photographs of *P. levii* revealed lower growth levels on non-PRAS plates compared to PRAS plates. Additionally, *P. levii* formed pinpoint colonies on non-PRAS plates. This anaerobe could be overlooked on non-PRAS plates in a mixed culture specimen.

Conclusion

This study confirms that anaerobic bacteria vary in their sensitivity to oxygen, and exposure to oxygen during media preparation can significantly impair their growth.[1] PRAS media consistently outperformed Non-PRAS media, with additional benefits from post-reduction for some organisms. The findings emphasize the importance of reduced media preparation for successful anaerobe isolation and recovery, supported by quantitative data and photographic evidence.

In light of these findings, we strongly recommend the integration of PRAS media into standard laboratory protocols for anaerobic culture. The benefits—ranging from enhanced recovery rates to cost-effectiveness and ease of use—make PRAS media an invaluable tool for both research and clinical diagnostics. Laboratories adopting PRAS media can expect improved accuracy in identifying anaerobic infections, which directly impacts patient care by enabling more targeted and effective treatments.

Future studies could explore the economic implications of adopting PRAS media versus traditional methods, as well as its potential application in point-of-care settings. Additionally, while this study focused on three specific anaerobes, the principles may apply more broadly, suggesting that PRAS media could become a standard for all anaerobic culture techniques.

Practical Advantages of PRAS Media

To further underscore the value of PRAS media, it is essential to highlight its practical benefits for laboratory professionals:

- **Time-Saving:** PRAS media are pre-reduced and ready to use, eliminating the need for preconditioning and reducing lab workload.
- **Reliability:** Prepared entirely under anaerobic conditions, PRAS media minimize oxygen contamination, ensuring consistent and reproducible results.
- **Enhanced Recovery:** The oxygen-free environment promotes optimal growth, leading to higher recovery rates and faster identification of anaerobes.
- **Convenience:** Easy to use and store, with a long shelf life (up to 3 months), making them ideal for laboratories with varying workloads.
- **Cost-Effectiveness:** Despite a potentially higher initial cost, the improved performance and reduced need for repeat testing make PRAS media a cost-effective choice in the long run.

These advantages not only enhance laboratory efficiency but also contribute to better patient outcomes by ensuring accurate and timely diagnosis of anaerobic infections.

References

1. Long, S., Prober, C. G., & Fischer, M. (2018). Anaerobic bacteria: Classification, normal flora, and clinical concepts. In *Principles and practice of pediatric infectious diseases* (p. 187). Elsevier.
2. Nagy, E., Boyanova, L., & Justesen, U. S. (2018). How to isolate, identify and determine antimicrobial susceptibility of anaerobic bacteria in routine laboratories. *Clinical Microbiology and Infection*, 24(11), 1139-1148.