·Clinical Research·

Evaluation of the new OxyPlate™ Anaerobic System for the isolation of ocular anaerobic bacteria

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Abstract

• AIM: Anaerobic bacteria can cause ocular infections. We tested the OxyPlate™ Anaerobic System (OXY) to isolate pertinent anaerobic bacteria that can cause ocular disease.

• METHODS: OXY, which does not require direct anaerobic conditions (*i.e.* bags, jars), was compared to conventional isolation of incubating culture media in anaerobic bags. Standard colonies counts were performed on anaerobic ocular bacterial isolates under aerobic and anaerobic conditions (anaerobic bags) using agar media: 1) OXY (aerobic only), 2) 5% sheep blood (SB), 3) Chocolate, and 4) Schaedler. The bacteria tested were de-identified ocular isolates cultured from endophthalmitis and dacryocystitis that include 10 *Propionibacterium acnes* and 3 *Actinomyces species.* The colony counts for each bacteria isolate, on each culturing condition, were ranked from largest to smallest, and non-parametrically compared to determine the best culturing condition.

• RESULTS: All anaerobic conditions were positive for all of the anaerobic isolates. SB and Schaedler's agar under aerobic conditions did not support the growth of anaerobic bacteria. Sparse growth was noted on chocolate agar with *Propionibacterium acnes*. As an anaerobic system, SB in an anaerobic bag isolated higher colony counts than OXY (P= 0.0028) and chocolate agar (P=0.0028).

• CONCLUSION: Although OXY did not test to be more efficient than other anaerobic systems, it appears to be a reasonable alternative for isolating anaerobic bacteria from ocular sites. The use of an agar medium in a specially designed plate, without the requirement of an anaerobic bag, rendered OXY as an advantage over other anaerobic systems.

• KEYWORDS: anaerobic bacteria; bacterial isolation; endophthalmitis; dacryocystitis

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INTRODUCTION

Anaerobic bacteria are an important cause of ocular infections including pediatric conjunctivitis, keratitis, and dacryocystitits [1,2], canaliculitis [3], orbital cellulitis [4] and post-operative and post-traumatic endophthalmitis [5]. The Endophthalmitis Vitrectomy Study [6,7] helped to establish a standard protocol for isolating aerobic and anaerobic bacteria.

Currently, the Charles T. Campbell Ophthalmic Microbiology Laboratory uses chocolate agar plates placed within anaerobic bags to isolate ocular anaerobic bacteria. Traditional anaerobic systems require bags, jars, or chambers which can be cumbersome, time consuming and expensive.

Oxyrase Inc. (Mansfield, OH) has introduced a new anaerobic system, OxyPlate™ (OXY), as a commercially available alternative for isolating anaerobes that does not require anaerobic bags, jars, or chambers. OXY is a self-contained anaerobic chamber designed to repeatedly generate and maintain anaerobic conditions (Figure 1). It consists of a dish (OxyDish™) that seals to maintain anaerobic conditions and an enzyme (OxyRase™) that removes oxygen from the Pre-Reduced Anaerobically Sterilized (PRAS) blood agar media and the space above the agar. It is designed to be incubated in a standard incubator aerobically.

OXY needs to be validated for ophthalmic use before one can implement the system for the isolation of anaerobes.

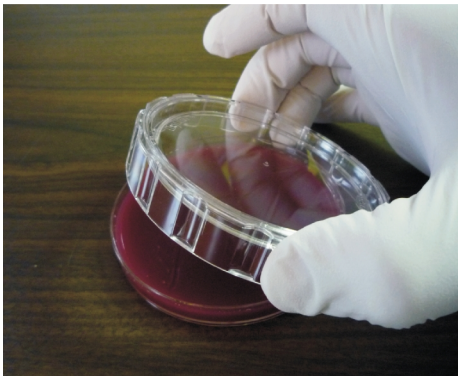

Figure 1 OxyPlate (courtesy of Oxyrase Inc., Mansfield, OH).

Wiggs *et al* [8] demonstrated that the OXY was effective in creating an anaerobic atmosphere and supporting most commonly isolated anaerobic bacteria collected from the Anaerobe Reference Laboratory at the Centers for Disease Control and Prevention. They did find, however, that it was not sufficient for nonpigmented *Bacteroides*, several *Fusobacterium* species and many *Clostridium* strains [8]. It is important to confirm his findings in testing isolates of *Propionibacterium acnes*, which is an important pathogen of chronic endophthalmitis, and Actinomyces species isolates from dacryocystitis.

Our main objective was to determine whether OXY can be used in place of traditional anaerobic systems for the primary isolation and growth of anaerobic bacteria that are pertinent to ocular anaerobic infections.

MATERIALS AND METHODS

Materials We hypothesized that the OXY will be as effective as other anaerobic systems for isolating ocular anaerobic bacteria. The hypothesis will be tested by comparing the isolation of ocular anaerobic bacteria (*Propionibacterium acnes* and *Actinomyces* species) on OXY to the standard anaerobic agar (blood, chocolate) under both aerobic and anaerobic conditions. This approach was chosen because a prospective study with clinical samples would require an extended period and a large volume of intraocular specimen to inoculate multiple media. Testing was undertaken using anaerobic bacteria using the same bacterial concentration and incubation conditions. This study satisfied the requirement of medium verification to replace the existing medium of a current regimen as mandated by laboratory certification (College of American Pathologists, State of Pennsylvania, and Clinical Laboratory Improvement Amendments). It is important to stress that the focus of the study is to test culture media to isolate anaerobic bacteria and not a study to test the ability of media to isolate anaerobic bacteria from ocular clinical samples.

Methods Standard colonies counts were performed on anaerobic ocular bacterial isolates under seven culturing conditions: 1) OXY (Oxyrase Inc., Mansfield, OH); 2) Trypticase soy agar supplemented with 5% Sheep Blood (TSA) (BBL, Sparks, MD); 3) TSA placed in an anaerobic bag; 4) Chocolate II agar (BBL, Sparks, MD); 5) Chocolate II agar placed in an anaerobic bag; 6) Schaedler agar supplemented with 5% Sheep Blood (BBL, Sparks, MD); 7) SCHAED placed in an anaerobic bag.

For anaerobic conditions, the agar media (except OXY) were placed in anaerobic bags that contain a disposable hydrogen generator envelop that generates carbon dioxide with an internal catalyst and also contains an indicator for anaerobic conditions (BD GasPak™, Becton, Dickinson, and Company, Sparks, MD). The envelope is torn open, placed in the bag with the agar medium, and the bag is simply sealed, like a sandwich bag, to produce anaerobic conditions. For anaerobic conditions, an OXY plate is simply opened by squeezing the side of the plate while lifting the lid, inoculating with bacteria, and returning the lid with a twist. All plates (aerobic and anaerobic) were incubated in an air incubator at 37℃. All media were purchased from the respective companies.

The bacteria tested were de-identified ocular isolates cultured from endophthalmitis and dacryocystitis. These represent all anaerobic bacteria isolated between March, 1993 to October, 2009. The anaerobes were stored in a clinical bank as part of a de-identified collection of bacteria used to validate diagnostic testing and to monitor antibacterial susceptibility. These included 10 isolates of *Propionibacterium acnes*, and 3 isolates of *Actinomyces* species. Initially all isolates were grown on chocolate agar plates in anaerobic bags for 72 hours and passed an additional 3 times to maintain anaerobic conditions. [Note: *Propionibacterium acnes* tend to acclimate easily to growth under aerobic conditions. It was important to maintain anaerobic conditions for the anaerobes in this study.] The anaerobic bacterial isolates were suspended in enriched thioglycollate (an anaerobic medium) (BBL, Becton, Dickinson, and Company, Sparks, MD) to a 0.5 McFarland Standard (approximately 1×10^8CFU/mL)[9]. The suspension was diluted by 1/100 in enriched thioglycollate medium for a reduced concentration of 1×10^6CFU/mL, and this was serially diluted 4 times by 1/10 resulting in 1/10, 1/100, 1/1,000, and 1/10,000 dilutions. A volume of 0.1mL of each of the five dilutions was plated in duplicate under each of the seven conditions.

A colony count was determined 6 days after plating by counting the number of distinct colonies on the agar plates. Plates containing 20 to 200 colonies were used to determine

Table 1 Ranks and colony counts (CFU/mL) of culture media for isolating 10 *Propionibacterium acnes* and *Actinomyces israeli*

Media	E596	E91	E47	E136	E566	E150	E301	E297	E264	E385	Median	Actinomyces
TSA	6	6	5	5	5	5	5	6	6	5	5	5
Colony counts	0	0	0	0	0	0	0	0	0	0		0
TSA+Bag	1	1	2	1	2	2	2	2	1	1	1.5	4
Colony counts	2.25t6	2.5t6	5.5t6	2.3t6	8.1t6	1.12t6	3.7t6	1.36t6	2.04t6	1.96t6		4.05t5
Chocolate	5	5	5	5	5	5	5	5	3	5	5	5
Colony counts	1.0t6	1.45t2	0	0	0	0	0	6.0t5	1.85t4	0		0
Chocolate+Bag	4	2	3	2	3	4	3	3	2	2	3	3
Colony counts	1.55t6	2.35t6	5.3t6	2.3t6	8.1t5	1.12t6	3.7t6	1.36t6	2.04t6	1.94t6		4.2t5
Schaedler	6	6	5	5	5	5	5	6	6	5	5	5
Colony counts	0	0	0	0	0	0	0	0	0	0		0
Schaedler+Bag	3	3	1	3	4	1	1	1	5	3	3	2
Colony counts	1.65t6	2.15t6	6.56t6	1.67t6	6.3t5	2.75t6	5.0t6	1.75t6	1.24t6	1.85t6		4.25t5
Oxyrase	2	4	4	4	1	3	4	4	4	4	4	1
Colony counts	1.76t6	1.9t6	6.3t5	1.2t6	1.4t6	1.22t6	2.5t6	1.08t6	1.44t6	8.15e5		5.4t5

Rank of 1 is the medium that had the highest colony count of bacteria; TSA - tryptic soy agar supplemented with 5% sheep blood; Mood Median Analysis for *Propionibacterium acnes* - Oxyrase and Media + bags significantly isolated more anaerobes than media without bags (P=0.0001); Mann-Whitney Analysis for *Propionobacterium acnes* - TSA + bag significantly isolated more anaerobes than Oxyrase and Chocolate + bag (P=0.0028). The isolation of anaerobes was equivalent for Oxyrase and Chocolate + bags (P=0.18).

the colony counts. The average number of colonies for two plates was divided by the dilution and the inoculum (0.1) to obtain the colony count. The colony counts for each bacteria isolate and each culturing condition were rank from largest to smallest (1-7). The ranks for the media from all the bacterial isolates were recorded and non-parametrically compared using Mann-Whitney (2 sample testing) or Mood's Median (multiple sample) testing (Minitab, Sate College, PA) to determine the culturing condition with the highest amount of colony counts. The medium with the highest colony count was determined to be the best for isolating anaerobic bacteria.

RESULTS

The ten *Propionibacterium acnes* and one *Actinomyces* species were deemed obligate anaerobes as defined by the absence of growth aerobically on TSA and SCHAED. All anaerobic conditions were able to isolate all of the anaerobic bacterial isolates. Table 1 summarizes the ranks and colony counts (CFU/mL) of culture media for isolating 10 *Propionibacterium acnes* and *Actinomyces Israeli*. For *Propionibacterium acnes*, Oxyrase and Media+bags significantly isolated more anaerobes than media without bags (P=0.0001, Mood's Median Test). As an anaerobic system, TSA in an anaerobic bag isolated higher colony counts than OXY (P=0.0028, Mann-Whitney) and chocolate agar in an anaerobic bag (P=0.0028, M-W). There was no statistical difference (P =0.18, M-W) between the performance of the OXY and the chocolate agar plate in an anaerobic bag. As expected, TSA and SCHAED agar under aerobic conditions did not support the growth of anaerobic bacteria. Sparse growth was noted on chocolate agar with *Propionibacterium acnes*.

Only one of three *Actinomyces* isolates only grew under anaerobic conditions. For this isolate, OXY isolated the largest number of colonies for *Actinomyces* species among the seven growing conditions.

DISCUSSION

The isolation of anaerobic bacteria from ocular specimens can be a challenge due to prior exposure to toxic concentrations of oxygen. Fortunately, endophthalmitis due to anaerobic bacteria is a rare event, but *Propionibacterium acnes* can be a frequent cause of chronic endophthalmitis and its clinical signs alerts the ophthalmologist to test for its presence[6,7].

Intraocular samples are obtained with a syringe and needle or from the cassette of a vitrectomy specimen. The best culturing technique would be to plant the specimen shortly after collection onto anaerobic agar and liquid culture medium. Placing the agar medium in anaerobic bags immediately increases the chances of isolating anaerobic bacteria. Specimens may also be sent to a laboratory for processing, but any delay in transport may inhibit the growth of obligate anaerobes.

Based on our evaluation, OxyPlate™ is a reasonable alternative to current anaerobic systems. Positive cultures are not based on the quantity of bacteria, but for the presence of bacteria. As noted in the study, all systems were positive for all of the tested isolates, thus all plating conditions would be acceptable for isolating anaerobic bacteria from ocular samples. All anaerobic systems were reasonably priced including anaerobic bags (TSA and chocolate [$2.44], OXY [$2.75), and SCHAED [$3.58 without laboratory discount]).

The advantage of OXY is that it does not require jars or

bags. The disadvantages of OXY included the tendency to accumulate some moisture with storage. The plates also had a tendency to stick and required some finesse to open. The indicator that assures anaerobic conditions would also need to be placed on the plate lid at the laboratory.

In summary, the OXY system is an alternative system for isolating anaerobic bacteria from ocular infections. Laboratories servicing ophthalmic practices will need to evaluate the cost-benefits of OXY over other standard anaerobic systems.

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