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Oxyrase Cell-Membrane Preparations Simplify Cultivation of Anaerobic Bacteria

ABSTRACT Clinical microbiologists have sought to isolate, identify, and characterize anaerobic bacteria since the organisms and their role in wound infections were discovered. Methods to create an environment for anaerobic growth have not changed for 100 years. A new approach—the Oxyrase enzymatic method—promotes anaerobiosis in the solid culture medium itself, allowing colonies to form without barriers associated with jars, bags, and chambers. In response to space restraints, increased workload, and cost-cutting directives, we evaluated the Oxyrase method for the primary isolation of anaerobic bacteria from clinical wound specimens. By using plated media (containing the oxygen-reducing substance) to isolate and identify anaerobic bacteria, a single technologist could report culture results more rapidly because all cultures—aerobic and anaerobic—for a patient could be processed simultaneously.

Anaerobic bacteria are part of the normal flora of healthy skin and mucous membranes. Ironically, these organisms can cause serious and even life-threatening infections in persons and animals. They play an important role in the most commonly encountered categories of infection: skin and soft tissue, osteomyelitic, pleuropulmonary, intra-abdominal, and female genital tract. Downsizing, cost-cutting, capitation, and HMO economics may eliminate or at least minimize anaerobic culture workups. This may seem justified because antimicrobial drugs with excellent activity against anaerobic bacteria are available. Using these drugs as the first option, however, increases both the cost of treatment and the risk of bacterial resistance to these agents.[1]

Conventional methods for achieving anaero-biosis rely on (1) barriers created by jars, bags, or chambers in tandem with vacuum and purge, or (2) catalytic reactions that provide a limited and contained environment suitable for growing the microorganisms. Many scientists favor the anaerobic chamber.[2] Although well-designed for growing anaerobic bacteria, the chamber is not practical for today's multifunctional, resource-strained, clinical microbiology laboratory. Costly to maintain and awkward to use, the chamber also occupies valuable space and disrupts work flow.

Adler and colleagues made a discovery that led to the first new means to grow anaerobic bacteria without extraneous paraphernalia. They found that cell membrane fractions derived from *Escherichia coli* and related microorganisms contained respiratory enzymes that reduced oxygen directly to water. These researchers were quick to recognize that these enzymes could produce anaerobic environments when added to any liquid or solid growth media (Fig 1).[3-6]

We have extensive experience with traditional methods for isolation and identification of anaerobic bacteria. Because we were unfamiliar with anaerobiosis generated by adding cell membrane fractions to culture media, we did a study to compare the results obtained by the traditional method with the results of the cell membrane fraction method.

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Materials and Methods

We inoculated Brucella blood agar (with and without oxygen-reducing substance) and kanamycin-vancomycin-laked blood agar (with and without oxygen-reducing substance) with 212 infected wound specimens.The plates enriched with oxygen-reducing substance (OxyPlates, Oxyrase, Mansfield, OH) came ready-to-use from the manufacturer. Due to their wealth of anaerobic flora, specimens not routinely recommended[7] for anaerobic workup (perirectal abscess, decubitus ulcer, and appendiceal abscess) were included in the study. OxyPlates were incubated at 35°C in 6% carbon dioxide, and plates lacking oxygen-reducing substance were incubated at 35°C in an anaerobic chamber. We examined all anaerobic plates after 48 hours and again at 18- to 24-hour intervals. Their total incubation time was 5 days.

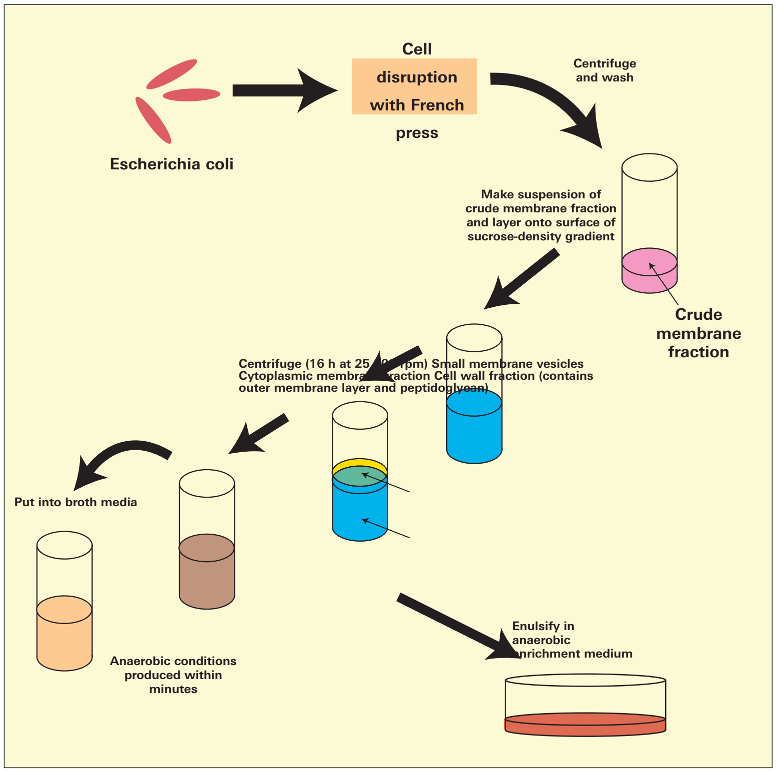

Fig 1. Steps in preparing Escherichia coli membranes that create anaerobic conditions in bacteriologic media. Courtesy of James Copeland.

Results

Eighty-seven cultures showed growth of anaerobic bacteria (180 strains, Table 1) with OxyPlate and anaerobic chamber methods. Forty-one strains—predominantly *Peptostreptococcus* species (28%), *Eubacterium* species (20%), and *Propionibac-terium* species (20%)—failed to grow in the anaerobic chamber plates but grew on the OxyPlates (Table 2). Fourteen strains did not grow on the OxyPlates (Table 3). (The latter conclusion was somewhat subjective because the same organisms recovered from the chamber had been isolated from OxyPlates associated with other specimens.) We noticed no colonial variation between the OxyPlate and anaerobic chamber isolates. The growth rates were similar with both methods.[8,9]

Discussion

During the comparison study we noted that the total patient culture workup could be done by 1 technologist simultaneously at a single workbench (Fig 2). Side-by-side comparison of the aerobic and anaerobic plates allowed for earlier recognition of anaerobic colonies and quicker preliminary reporting (Fig 3). Removal of the anaerobic chamber freed valuable space and allowed us to create a paper-free work log in which we entered all results electronically instead of on work cards. Brucella blood agar results obtained with the Oxy-Plate method for 2 species are shown in Figure 4.

Table 1. Anaerobic Isolates Recovered* By Oxyplate and Anaerobic Chamber Methods

Isolate (No. Recovered)
Actinomyces israelii (1)
Actinomyces meyeri (2)
Actinomyces viscosus (1)
Bacteroides caccae (2)
Bacteroides capillosus (4)
Bacteroides fragilis (5)
Bacteroides fragilis group (4)
Bacteroides ovatus (4)
Bacteroides ruminicola (1)
Bacteroides thetaiotaomicron (8)
Bacteroides uniformis (5)
Bacteroides urealyticus (6)
Bifidobacterium species (1)
Campylobacter gracilis (1)
Clostridium clostridioforme (4)
Clostridium innocuum (1)
Clostridium perfringens (1)
Clostridium ramosum (3)
Eubacterium lentum (4)
Eubacterium species (4)
Fusobacterium species (1)
Fusobacterium symbiosum (1)
Fusobacterium varium (2)
Mobiluncus species (2)
Peptostreptococcus anaerobius (4)
Peptostreptococcus asaccharolyticus (7)
Peptostreptococcus magnus (8)
Peptostreptococcus prevotii (8)
Peptostreptococcus species (21)
Peptostreptococcus tetradius (1)
Porphyromonas asaccharolytica (3)
Porphyromonas endodontalis (1)
Porphyromonas gingivalis (1)
Prevotella bivia (8)
Prevotella corporis (1)
Prevotella disiens (1)
Prevotella loescheii (1)
Prevotella melaninogenica (4)
Prevotella nonpigmented group (2)
Prevotella oralis group (3)
Prevotella pigmented group (1)
Prevotella species (6)
Propionibacterium acnes (15)
Propionibacterium avidum (4)
Propionibacterium granulosum (1)
Propionibacterium species (2)
Streptococcus intermedius (7)
Veillonella species (2)

*Identified by RapidANA (Remel, Lenexa, KS) and antibiotic disk schematics, which are special potency disk patterns. The disk method is a standard practice for identifying anaerobes (eg, vancomycin, colistin, kanamycin, nitrate species).

Table 2. Isolates Recovered From Media Conatining Oxygen-Reducing Substance

Isolate (No. Recovered)
Actinomyces meyeri (1)
Bacteroides fragilis (1)
Bacteroides ovatus (1)
Bacteroides thetaiotaomicron (1)
Bacteroides urealyticus (3)
Bifidobacterium species (1)
Clostridium clostridiforme (2)
Eubacterium species (8)
Peptostreptococcus species (11)
Porphyromonas species (1)
Prevotella species (2)
Propionibacterium species (8)
Streptococcus intermedius (1)

Table 3. Isolates Recovered in Anaerobic Chamber Media Only

Isolate (No. Recovered)
Bacteroides fragilis (2)
Bacteroides urealyticus (1)
Clostridium perfringens (1)
Eubacterium species (1)
Fusobacterium species (2)
Peptostreptococcus species (2)
Prevotella species (2)
Propionibacterium species (3)

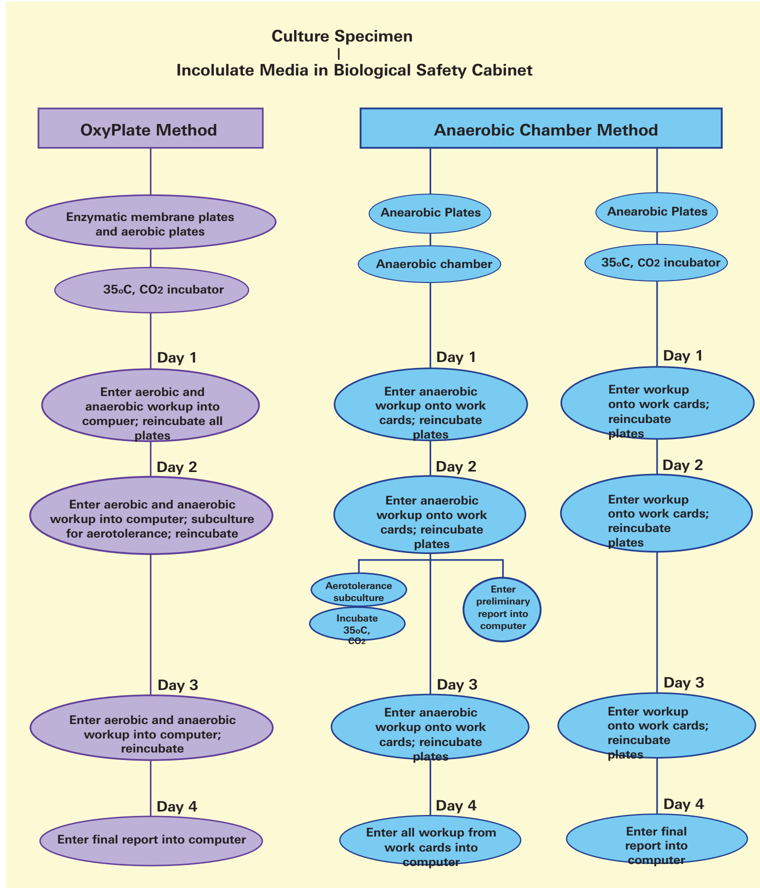

Fig 2. Steps in OxyPlate (Oxyrase, Mansfield, OH) and anaerobic chamber methods for culturing anaerobic bacteria.

We could stack and store all media plates (aerobic and anaerobic) in 1 incubator and with the aerotolerance subcultures. We could open and close OxyPlates several times during the 5-day incubation period and the enzyme continued to reduce oxygen in the media and in the space between the lid and the agar surface after each exposure.

By averaging initial and replacement costs, gas tank replenishment, and maintenance fees, we discovered that the price of using 2 conventional pre-reduced anaerobic agar plates in tandem with the anaerobic chamber was comparable to that of using 2 OxyPlates for the primary plating of each wound specimen.

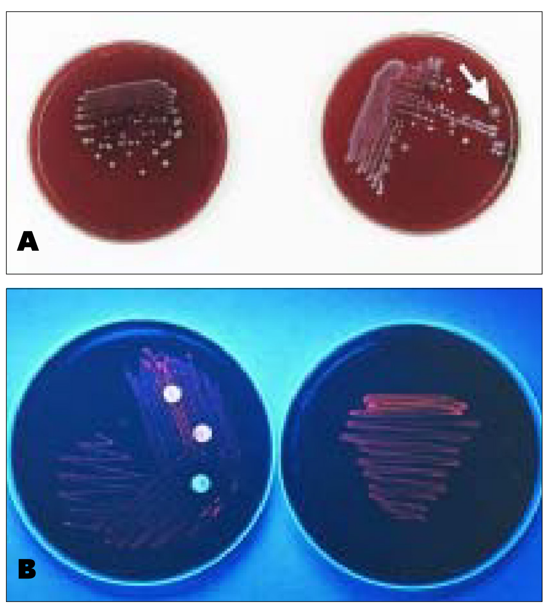

Fig 3. Aerobic and nonaerobic growth patterns for side-by-side comparison.

Conclusion

Cultivating and identifying anaerobic bacteria is a high-complexity procedure that requires extensive education and proficiency review of our technologists. By eliminating the cumbersome barrier apparatus, we gained time that allowed technologists to focus on recovering and characterizing these microorganisms. Using OxyPlates reduced the workup workload to the level required to identify aerobic bacteria.l

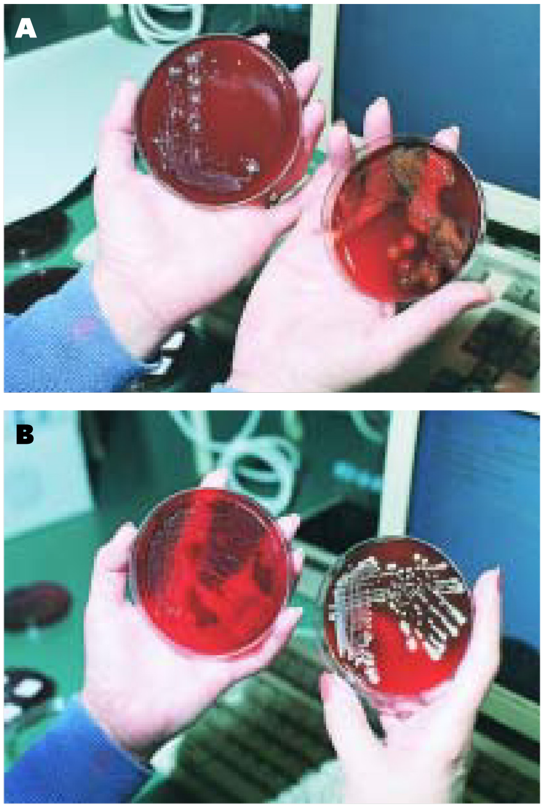

Fig 4. Plates cultured for anaerobic bacteria by the OxyPlate (Brucella blood agar). A, Clostridium sporogenes colonies in pure culture (left) and in mixed culture (right). B, Pigmented Porphyromonas species colonies with brick-red fluorescence under UV light.

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