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Comparative Analysis and diagnosis on aerobic and anaerobic culturing techniques in Oral Microbiology

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ABSTRACT

Periodontal diseases are associated with microbial infections affecting the periodontal tissues. The microbial challenge is derived mainly from plaque and calculus. Early dedication and identification of the pathogens causing the periodontal diseases is necessary in order to successful treat the infection. Microbial analysis should be used as an diagnostic tool in treating the periodontal infections.

Keywords: aerobic and anaerobic, culturing, periodontal disease.

INTRODUCTION

Infection and diseases in humans are caused by aboard spectrum of bacteria. Aerobic and anaerobic bacteria are both involved in causing infections. Specific virulence factors from these organisms are responsible for the progression of infection resulting in the diseases. Periodontal diseases constituents a group of diseases affecting the periodontal tissues. The most prevalence diseases result due to inflammation process caused due to microbial infections. Plaque and calculus are rich reservoirs of microbes. Certain pathogens such as Porphyromonas gingivalis, Aggregatbacter actinomycetemcomitans and Tannerella forsythia have been shown associated with periodontal diseases. Hence, isolation, identification and diagnosis is necessary for successful treatment. Aerobic and anaerobic culturing techniques are gold standard in identifying the pathogens causing periodontal diseases.

AEROBIC CULTURING[1]

Pathogens have the ability to grow in wider ranges of nutrition and environments strict parasitic, organisms grow under limited nutritional and environmental conditions and thereby require growth factors, optimum temperature and adjustment of pH of media.

Media to supply food and energy

For growth bacteria require,'CO2', 'N', 'O2' mineral salts, vitamins and growth factors

Meat extract

This is used in the medium, as meat infusion

Mineral salts

Inorganic substances **chlorides, phosphates and sulphates** are important for mineral media. Potent **toxins** are derived by **limiting iron supply** .Magnesium activates **phosphatase**.
Copper is required for **Polyphenol Oxidase**.
Zinc for **Carbonic anhydrase**.
Calcium for the action of **Lecithinase.**

Growth factors

H influenza require X and V factors for growth.
'X' factor is heat stable (120˚C), it is Haematin and helps systhesis of Catalase. 'V' factor is Co- enzymes 1, (heat labile) present in all living cells, and essential for bacterial respiration. For routine culture, 5 mg of Paba is used /100 ml broth. Growth factors are studied by adding substances in definite quantities in basal synthetic media.

Standardization of media

Media are standardized for the 'N' content, and pH, (H – ion concentration).

REACTION OF MEDIA

True acidity of any fluid depends on the number of dissociate H – ions present in the solution as is measured by CH (concentration of H – ion) of the fluid. Greater the CH, more acidic is the medium. pH of the Media may be adjusted (to 7.2 usually) with the help of known indicators which give definite ranges of color changes at different pH.

LIQUID MEDIA INCLUDES

1. Meat infusion
2. Nutrient Broth
3. Digest Broth
4. Glucose (Dextrose) Broth
5. Glycerine Broth
6. Bile Broth
7. Sugar Broth
8. Dunham's peptone water
9. Hiss's serum water
10. Litmus milk

SOLID MEDIA

These are obtained by addition of substances like agar – agar or gelatin, to nutrient broth; Nutrient agar forms the basis of most of solid media. These include:-

1. Nutrient Agar
2. Nutrient gelatin

Differential and Enriched Media:

Delicate pathogenic organisms like N. gonorrhoeae and N. meningitides, H. influenza, Bord. pertussis.etc. do not grow on ordinary nutrient agar unless it is enriched by adding blood, serum, ascetic fluid, etc.

Enriched media are:

Blood agar (for Streptococcus, C. diphtheriae)
Chocolate agar (for Neisseria, Haemophilus)
Serum agar (for C. diphtheriae)

ENRICHMENT MEDIA

Materials like stools are inoculated into liquid media for primary culture to enrich pathogens. It allows the growth of desired pathogens in preference to the contaminants.

Differential and Selective Media:

These media contains inhibitory substances and indicators. Growth of the commensal organisms is inhibited and the pathogens get the opportunity to grow and multiply in preference to commensals. In addition certain growth specific substances are incorporated for specific growth of desired organisms:

These are:

MacConkey's medium
Endo's medium
Wilson and Blair's Bismuth Sulphite Agar
S –S (Salmonella – Shigella) agar for Salmonella and Shigella
Tellurite medium selective and differentiating medium for **C. diphtheria.**

ANAEROBIC CULTURING[2]

EPIDERMIOLOGY

Most of the anaerobic bacteria that cause infections in humans are also part of our normal flora. Other pathogenic anaerobes (e.g., Clostridium botacacium and Clostridium tetani) are soil and environmental inhabitants and are not considered part of human flora.

Although person-to-person nosocomial spread of Clostridium difficile among hospitalized patients presents an enormous clinical and infection control dilemma, the majority of anaerobic infections occurs when a patient's normal flora gains access to a sterile site as a result of disruption of some anatomic barrier.

PATHOGENESIS AND SPECTRUM OF DISEASE

Most anaerobic infections involve a mixture of anaerobic and facultatively anaerobic organisms (e.g., Enterobacteriaceae), so that it is problematic to establish the extent to which particular anaerobic species is contributing to infection. In addition, as ubiquitous members of our normal flora, anaerobic organism's free quently contaminate clinical materials. For these reasons, signing clinical significance to anaerobic bacteria isolated in the laboratory is important

SPECIMEN COLLECTION AND TRANSPORT

Material for anaerobic culture is best obtained by tissue biopsy or by aspiration using a needle and syringe. Use of swabs is a poor alternative because of excessive exposure and hence avoided.

A crucial factor in the final success of anaerobic cultures in the transport of the specimen; the lethal effect of atmospheric oxygen must be nullified until the specimen can be processed in the lab oratory. Recapping a syringe and transporting the needle and syringe to the laboratory is no longer acceptable because of safety concerns involving needle stick injuries. Therefore, even aspirates must be injected into some type of oxygen – free transport tube or vial. Three different kinds of anaerobic transport system is a rubber – stopped collection vial containing agar indicator system. The vial is gassed out with oxygen – free carbon dioxide or nitogen. The specimen is injected through the rubber stopper after all air is expelled from the syringe and needle. If only a swab specimen can be obtained, a special collection device with oxygen – free atmosphere is required. When reinserting the swab, care must be taken not to tip the container, which would cause the oxygen – free CO2 or nitrogen to spill out and be displaced by ambient air.

Tissue can be placed in a small amount of liquid to keep it from drying out and then placed in an anaerobic pouch. All specimens should be held at room temperature pending processing in the laboratory, because refrigeration can oxygenate the specimen

Specimen Processing

Specimens for anaerobic culture may be processed on the open bench-top with incubation in anaerobic jars or pouches or in an anaerobic chamber.

Anaerobe Jars or Pouches

The most frequently used system for creating an anaerobic atmosphere is the anaerobe jar. Anaerobe jars are available commercially. A clear, heavy plastic jar with a lid that is clamped down to make it airtight is used. Anaerobic conditions can be set up by two different methods. The easiest method uses a commercially available hydrogen and CO2 generator envelope that is activated by either adding water or by the moisture from the agar plates. Production of heat within a few minutes and subsequent development of moisture on the walls of the jar are indications that the catalyst and generator envelope are functioning properly. Reduced conditions are achieved in 1 to 2 hours, although the methylene blue or resazurin indicators take longer to decolorize. Alternatively the "evacuation-replacement" method can be used. Air is removed from the sealed jar by drawing a vacuum of 25 inches (62.5 cm) of mercury. This process is repeated two times, filling the jar with an oxygen-free gas, such as nitrogen, between evacuations. The heat fill of the jar is made with a gas mixture containing 80% to 90% nitrogen, 5% to 10% hydrogen, and 5% to 10% CO2. Many anaerobes require CO2 for maximal growth. The atmosphere in the jars is monitored by including an indicator to check anaerobiosis. Anaerobe basis of pouches are useful for laboratories processing small numbers of anaerobic specimens. Besides specimen transport, the pouch also can be used to incubate one of two agar plates.

Holding Jars

If anaerobic jars or pouches are used for incubation, the use of holding jars is recommended during specimen processing and examination of cultures. Holding jars are anaerobic jars with loosely fitted lids that are attached by rubber tubing to nitrogen gas. Uninoculated plates are kept in holding jars pending use for culture setup, and inoculated plates are kept in holding jars pending incubation or examination; this minimizes exposure to oxygen.

Anaerobe Chamber

Anaerobic chambers, or glove boxes, are made of molded or flexible clear plastic. Specimens and other materials are placed in the chamber through an air lock. The technologist uses gloves that form airtight seals around the arms to handle items inside the chamber. Media stored in the chamber are kept oxygen-free, and all work on a specimen from inoculation through workup is performed under anaerobic conditions. A gas mixture of 5% CO2, 10% hydrogen, and 85% nitrogen and a palladium catalyst maintain the anaerobic environment inside the chamber.

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