ENDODONTIC MICROBIOLGY & CULTURING TECHNIQUES

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INTRODUCTION

Preservation of teeth by endodontic therapy has gained lot of popularity because of increased and predictable success rate of our endodontic procedures, the reason for this being the complete understanding of endodontic pathology and our ability to combat the same.

Essentially, endodontic infection is the infection of the dental root canal system and the major etiologic agent of apical periodontitis.
Although various chemical and physical factors can induce periradicular inflammation, scientific evidence clearly indicates that microorganisms are essential for the progression and perpetuation of different forms of apical periodontitis.

INTRODUCTION

- The rationale for endodontic treatment is to eradicate the infection, to prevent microorganisms from infecting or re-infecting the root and/or periradicular tissues.

- Thus, a thorough understanding of the endodontic microbiota associated with different forms of disease is the basis for the success of endodontic treatment.

Pathways of infection

It is proved beyond doubt that presence of microbiota is a major deterrent in endodontic infection by the classical study by Kakehashi *et al*.

Dentinal tubules: After a carious lesion or during dental procedures, microorganisms may use the pathway in a centripetal direction to reach the pulp. Bacteria gain access to the pulp when the dentin distance between the border of carious lesion and the pulp is 0.2 mm.

Open cavity: Direct pulp exposure of traumatic origin such as in coronal fracture, or that of iatrogenic nature due to operative procedures, breaks the physical barrier imposed by dental structures and leaves pulp in contact with the septic oral environment.

Periodontal membrane: Microorganisms from gingival sulcus may reach the pulp chamber through the periodontal membrane, using a lateral channel or the apical foramen as a pathway. This pathway becomes available to microorganisms during a dental prophylaxis, due to dental luxation, and more significantly, as a result of the migration of epithelial insertion to the establishment of periodontal pockets.

Pathways of infection

Blood stream: The bacteria present in the blood would be attracted to the dental pulp following trauma or operative procedure that produced inflammation without causing pulp exposure.

Faulty restoration: temporary seal is broken or if the tooth structure fractures before final restoration, or if the final restoration is inadequate, bacteria may gain access to the periapical tissue and result in infection.

Extent: Microorganisms might reach the principal and/or lateral canals migrating from an infected tooth to a healthy pulp as a consequence of the contiguousness of the tissues

Correlation of Microbes to Infection

- Once the root canal is infected coronally, infection progresses apically until bacterial products or bacteria themselves are in a position to stimulate the periapical tissues, thereby leading to apical periodontitis. Endodontic infections have a polymicrobial nature, with obligate anaerobic bacteria conspicuously dominating the microbiota in primary infections.

- **Intraradicular infections**

1) Black pigmented Gram negative anaerobic rods. These bacteria have been reclassified into two genera: (a) *Prevotella* and (b) *Porphyromonas*.

Prevotella species detected in endodontic infections include

Prevotella intermedia

- *Prevotella nigrescens*
- *Prevotella tannerae*
- *Prevotella multissacharivorax*
- *Prevotella baroniae*

Intraradicular infections

- *Porphyromonas* species detected in endodontic infections include

Porphyromonas endodontalis and

Porphyromonas gingivalis.

2) *Tannerella forsythia*

3) *Dialister* species

4) *Fusobacterium*

5) Spirochetes are highly motile, spiral-shaped, Gram negative bacteria

6) Gram positive anaerobic rods have also been found in endodontic microbiota like

7) Gram positive cocci that are present in endodontic infection:

Intraradicular infections

- Fungi - particularly, *Candida* spp. (e.g.,) *Candida albicans*

Archaea - These are diverse group of prokaryotes which are distinct from bacteria.

Viruses - These viruses require viable host cells to infect and use the cell's machinery to replicate the viral genome. Hence, they cannot survive in a necrotic root canal.

The presence of viruses in the root canal has been reported only for non-inflamed vital pulps of patients infected with human immunodeficiency virus and herpes viruses where living cells are found in abundance.

Among the *Herpes* spp., the human cytomegalovirus and Epstein-Barr virus may be implicated in the pathogenesis of apical periodontitis

Extraradicular infections

The extraradicular infections are dependent on or independent of an intraradicular infection.

The dominant microorganisms present are anaerobic bacteria

- **Bacteria persisting intracanal disinfection procedures and after root canal treatment**

  Some microorganisms are resistant to antimicrobial treatment and can survive in the root canal after biomechanical preparation.

The most common Gram negative anaerobic rods are

Fusobacterium nucleatum

- *Prevotella* spp. and
- *Campylobacter rectus.*

Extraradicular infections

The most common Gram positive bacteria are

Streptococci (*Streptococcus mitis*, *Streptococcus gordonii*, *Streptococcus anginosus*, *Streptococcus oralis*)

- Lactobacilli (*Lactobacillus paracasei* and *Lactobacillus acidophilus*)
- Staphylococci
- *E. faecalis*
- *Olsenella uli*
- *Parvimonas micra*
- *Pseudoramibacter alactolyticus*
- *Propionibacterium* spp.
- *Actinomyces* spp.
- *Bifidobacterium* spp. and
- *Eubacterium* spp.

► Sometimes, yeasts, commonly *C. albicans*, are also found in small amounts.

- ***E. faecalis*** and yeast, mainly *C. albicans*, has been repeatedly identified as the species most commonly recovered from root canals undergoing retreatment, in cases of failed endodontic therapy and canals with persistent infections.

- *E. faecalis* are gram positive cocci and facultative anaerobes. They are normal intestinal organisms and may inhabit the oral cavity and gingival sulcus. When this bacterium is present in small numbers, it is easily eliminated; but if it is in large numbers, it is difficult to eradicate. *E. faecalis* has many distinct features which make it an exceptional survivor in the root canal. These microorganisms can perform the following.

E. faecalis

- Live and persist in poor nutrient environment
- Survive in the presence of several medications (e.g., calcium hydroxide) and irrigants (e.g., sodium hypochlorite)
- Form biofilms in medicated canals
- Invade and metabolize fluids within the dentinal tubules and adhere to collagen
- Convert into a viable but non-cultivable state
- Acquire antibiotic resistance
- Survive in extreme environments with low pH, high salinity and high temperatures
- Endure prolonged periods of starvation and utilize tissue fluid that flows from the periodontal ligament

Pathophysiology

Direct tissue damage can be induced by enzymes, exotoxins and metabolites. Indirect tissue damage can be induced from a host immune reaction capable of causing tissue destruction that is stimulated by bacterial components which include lipopolysaccharide (LPS), peptidoglycan (PG), lipoteichoic acid (LTA), fimbriae, outer membrane proteins, capsular components and extracellular vesicles. The degree of pathogenicity or disease producing ability of a microorganism is known as virulence

Importance of Understanding Microbiology for the Success of Non-Surgical and Surgical Endodontic Treatment

- Chemomechanical preparation of the infected root canal using antimicrobial agents, followed by obturation and coronal restoration, provides a favorable outcome. However, failure of root canal treatment sometimes occurs due to persistent or secondary intraradicular infection

Biofilm

Biofilm is a mode of microbial growth where dynamic communities of interacting sessile cells are irreversibly attached to a solid substratum, as well as to each other, and are embedded in a self-made matrix of extracellular polymeric substances. The microorganisms living in a community must have the following four basic criteria:

1. possess the abilities to self-organize (autopoiesis),
2. resist environmental perturbations (homeostasis),
3. be more effective in association than in isolation (synergy) and
4. respond to environmental changes as a unit rather than single individuals (communality).

- **Development of biofilm**

  Bacteria can form biofilms on any surface that is bathed in a nutrient-containing fluid. The three major components involved in biofilm formation are bacterial cells, a solid surface and a fluid medium.

Biofilm formation occurs in three stages

- Stage 1: Adsorption of inorganic and organic molecules to the solid surface occurs, leading to the formation of conditioning layer.th

  Stage 2: Adhesion of microbial cells to the conditioned layer: There are many factors that affect the bacterial attachment like **pH, temperature, surface energy of the substrate, nutritional availability, time of contact of bacteria, bacterial cell surface charge and surface hydrophobicity.** The bacteria substrate interaction occurs in three phases:

  Stage 3: Development of biofilm and biofilm expansion occurs. In this stage, monolayer of microbes attracts secondary colonizers forming microcolony, and the collection of microcolonies gives rise to the final structure of biofilm.

- **Endodontic biofilms**

  Endodontic microbiota is established to be less diverse compared to oral microbiota. Progression of infection alters the nutritional and environmental status within the root canal, making it more anaerobic with depleted nutritional levels. These changes offer a tough ecological niche for the surviving microorganisms. But complete disinfection of root canal is very difficult to achieve because of persistent microbes in anatomical complexities and apical portion of root canal. Because biofilm is the manner of bacterial growth which survives unfavorable environmental and nutritional conditions, the root canal environment will favor biofilm formation.

  Endodontic bacterial biofilms can be categorized as

intracanal biofilms,

extraradicular biofilms,

periapical biofilms and

biomaterial-centered infections.

Microbial identification

- ✔ Culturing
- ✔ Immunological
- ✔ Molecular based methods.
  - ► DNA-DNA Hybridization.
  - ► PCR techniques.

- Culture is the process of propagating microorganisms in the laboratory by providing them with required nutrients and proper physicochemical conditions, including temperature, moisture, atmosphere, salt concentration and pH.

STEPS IN CULTURE

1. Sample collection and transportation
2. Dispersion
3. Dilution
4. Cultivation
5. Isolation and identification

Advantages:

1. Allow quantification of all major viable microorganism in the samples.
2. Allow determination of antimicrobial susceptibilities of the isolates.
3. Physiological and pathogenicity studies are possible
4. Widely available

Disadvantages:

1. Not all viable bacteria can be recovered
2. Misidentification of strains with ambiguous phenotypic behavior.
3. Once identified, bacteria require identification using number of techniques
4. Strictly depend on mode of sample transport
5. Samples require immediate processing
6. Low sensitivity

Sampling procedure in endodontic retreatment

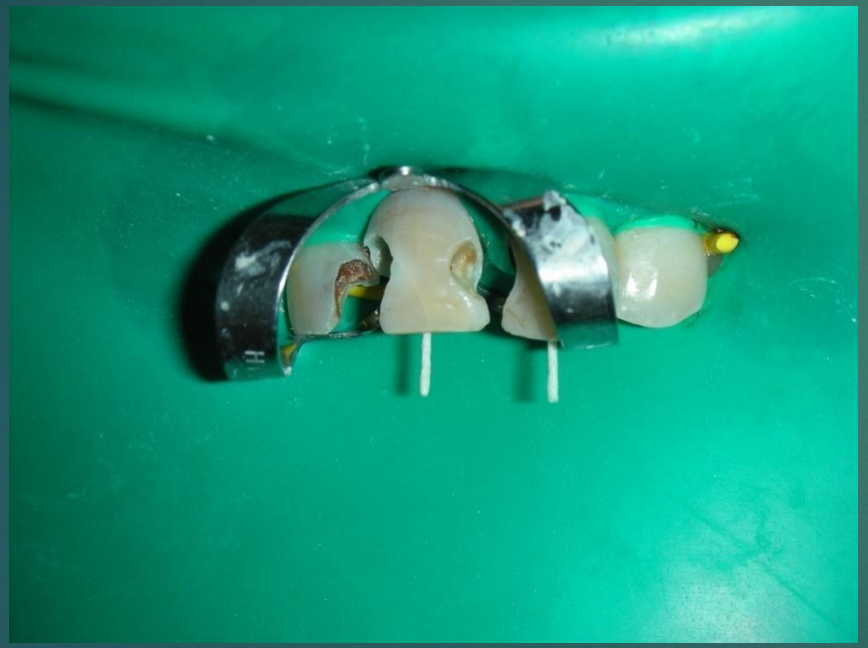
- After supragingival plaque removal by scaling, each tooth was cleansed with pumice & isolated with rubber dam.
- The tooth & surrounding field were cleansed with 3% hydrogen peroxide.
- After isolation & disinfection of the operative field, coronal restorations were removed when present

- Coronal guttapercha was removed by means of sterile gates glidden drill & the apical guttapercha was retrived by H-files.

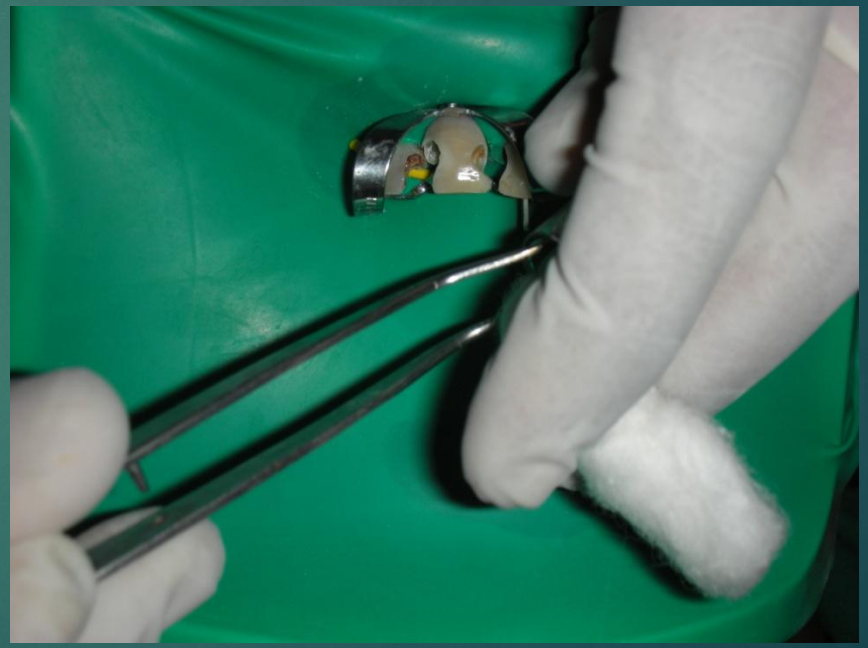
- Removal of root fillings was always performed without the use of chemical solvents.

- Whenever possible, filling material removed from the canals was transferred to cryotubes containing 1 ml of 5% dimethyl sulfoxide in trypticase soy broth.

Absorbent paper point inserted



Transferring into sterile culture tube



- A paper point is inserted to absorb the root canal contents. (minimum 4).
- Each paper point was retained in position for one minute.
- Then the absorbent points are transferred to sterile tubes containing Enterococcus broth (glucose broth) & incubated for 72 hr at 35 degree c.

Turbidity seen after incubation for 72 hrs

ANAEROBIC CULTURE METHODS

SAMPLES FROM ABSCESS

- Fluctuant abscess palpated-most dependent part of swelling determined
- Surface of mucosa cleaned with alcohol or iodoform
- Penetrated with 16-20guage needle, aspiration of exudates
- Aspirates injected in to anaerobic transport medium

False-positive culture:

- Failure in sterilization of the operating field, and instruments.
- Rubber dam leaks
- Use of unsterile paper points and cotton tube plugs
- Air or hand contamination during collection or transport

False negative culture

- Incomplete penetration of a paper point
- Use of a paper point that is too narrow
- Undetectable microbes
- Inadequate amount of specimen
- Presence of antimicrobial materials in the canal
- Insufficient incubation
- Failure to consider culture reversal
- Use of a single culture medium that fails to allow for growth of obligate anaerobic microbes and other hard-to-culture species.

Immunological

- Employs antibodies that recognize specific microbial antigens to detect the target species.
- The reaction can be visualized using a variety of techniques and reactions, including, Direct and indirect immunofluorescence, Flow cytometry & ELISA.

- Advantages:
- ✔ Less time consuming
- ✔ Less expensive analytical method.

- Disadvantages
- ✔ Requires specific antibodies against targeted bacteria.
- ✔ May give false positive because and cross reaction with non-targeted microorganisms.

Molecular diagnostic methods

- In the past two decades, methods based on molecular biology have been introduced for microbial identification.

- These methods rely on the fact that every living being including microorganisms has signature sequences in this genome that can be used as targets for precise identification.

- Even though with the advances in isolation and identification of anaerobic bacteria, Cultivation method have several drawbacks.
- ✔ Slow to provide a diagnostic result
- ✔ Low specificity
- ✔ Low sensitivity (Failure to detect small number of micro organisms) particularly for fastidious anaerobic bacteria
- ✔ Strictly depend upon mode of sample transport
- ✔ Time consuming.

- Several methods based on molecular biology have been used to identify endodontic pathogens, and new techniques may be used in the near future.
- Many of these molecular methodologies are based on the use of ribosomal RNA gene. (rDNA) to identify microorganisms without the need for cultivation. The 16 s rDNA gene is present in all bacteria.

DNA-DNA hybridization methodology

This method allows simultaneous determination of the presence of multitude of bacterial species in single or multiple clinical samples and is particularly useful for large scale epidemiologic research

Polymerase Chain method

- The molecular methods most often used for microbial identification are the PCR methods and its variations.
- The PCR method involves in vitro replication of DNA and has been often referred as genetic Xeroxing approach.
- PCR has unrivaled sensitivity it can defect as few as 1 to 10 bacterial cells in a sample. Making it at least 10 – 100 times more sensitive than any other identification method.

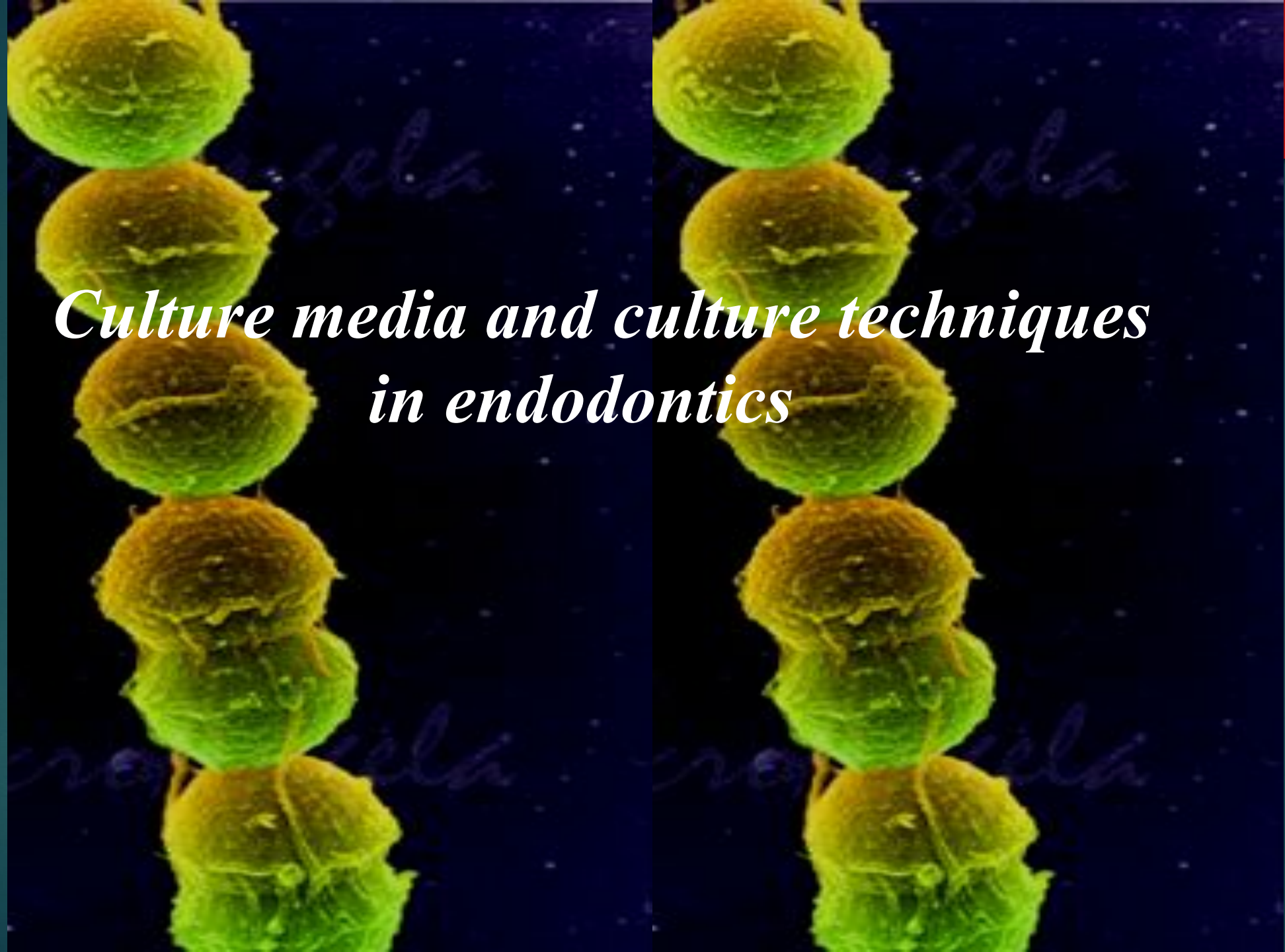
ADVANTAGES OF MOLECULAR BIOLOGY TECHNIQUES

- Detect both cultivable and as-yet uncultivated species or strains.
- High specificity and accurate identification of strains with ambiguous phenotypic behavior.
- Detect species directly in clinical samples.
- High sensitivity and rapid test
- Do not require carefully controlled anaerobic conditions during sampling and transportation.
- Samples can be stored for later analysis.

DISADVANTAGES OF MOLECULAR BIOLOGY TECHNIQUES

- **Most assays are qualitative or semi-quantitative.**
- **Most assays only detect one species or a few different species at a time.**
- **Most assays detect only the target species and fail to detect unexpected species.**
- **Some assays can be laborious and costly**
- **Can be very expensive.**

Thank you



Culture media and culture techniques in endodontics

- Introduction
- Classification of culture media
- Agar in culture media
- Culture media in endodontics
- Culturing techniques
- Antibiotic sensitivity
- Collection of microbial root canal samples(MRS)
- Culture reversal
- Endodontic dilemma
- Conclusion

Introduction

- One of the most important reasons for culturing bacteria in vitro is its utility in diagnosing infectious diseases. Isolating a bacterium from sites in body normally known to be sterile is an indication of its role in the disease process.

- Culturing bacteria is also the initial step in studying its morphology and its identification. Bacteria have to be cultured in order to obtain antigens from developing serological assays or vaccines.

- Certain genetic studies and manipulations of the cells also need that bacteria be cultured in vitro. Culturing bacteria also provide a reliable way of estimating their numbers.

- In Endodontics the vast majority of diseases of the dental pulp and periradicular tissues are associated with microorganisms.

- Therefore the identification of the microbes using culture and carrying out the treatment based on the knowledge of the microflora enhances the success of the treatment.

- Definitions (Stedman)

Culture is a process of propagating microorganisms in the laboratory by providing them with proper environmental conditions.

Culture media : A growth medium or culture medium is a liquid or gel or a solid substance designed to support the growth of microorganisms or cells.

Classification of culture media

Based on consistency:

- liquid,
- semi-solid
- solid and
- biphasic.

Classification based on nutritional component:

- simple,
- complex and
- synthetic (or defined).

- **Classification based on functional use or application**
- basal media,
- enriched media,
- selective/enrichment media,
- indicator/differential media,
- transport media and holding media.

A) **Liquid media:** These are available for use in test-tubes, bottles or flasks. Liquid media are sometimes referred as “broths” (e.g. nutrient broth).

- **Solid media:** Any liquid medium can be rendered solid by the addition of certain solidifying agents.
- **Agar** is the most commonly used solidifying agent.

- **Semi-solid agar(soft agar):**
- Reducing the amount of agar to 0.2-0.5% renders a medium semi-solid.
- Such media are fairly soft and are useful in demonstrating bacterial motility and separating motile from non-motile strains .

- **Biphasic media**:
- Sometimes, a culture system comprises of both liquid and solid medium in the same bottle. This is known as biphasic medium (Castaneda system for blood culture).

- Besides agar, egg yolk and serum too can be used to solidify culture media. While serum and egg yolk are normally liquid, they can be rendered solid by coagulation using heat.

- **Classification based on nutritional component:**
- **Simple media** such as peptone water, nutrient agar can support most non-fastidious bacteria.

- **Complex media** such as blood agar have ingredients whose exact components are difficult to estimate.

- **Synthetic or defined media** are prepared from pure chemical substances and the exact compositions of the medium is known.

- **Classification based on functional use or application:**
- **A) Basal media** are basically simple media that supports most non-fastidious bacteria.
- Peptone water, nutrient broth and nutrient agar considered basal medium.
- **B) Enriched media:** Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes them enriched media.
- Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc are few of the enriched media.

- **Selective and enrichment media** are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria.

- **Enrichment media** also serves to inhibit commensals in the clinical specimen.

Wilson & blair agar

TCBS (Thiosulfate citrate bile salt) agar

- **Differential media or indicator media:** Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour.
- Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies.

- Such media are called differential media or indicator media.
- Examples: MacConkey's agar, CLED(cysteine lactose electolyte deficient) agar, TCBS agar, XLD(Xylose lysine desoxycholate) agar etc.

Mac Conkey's agar LF

Mac Conkey's agar NLF

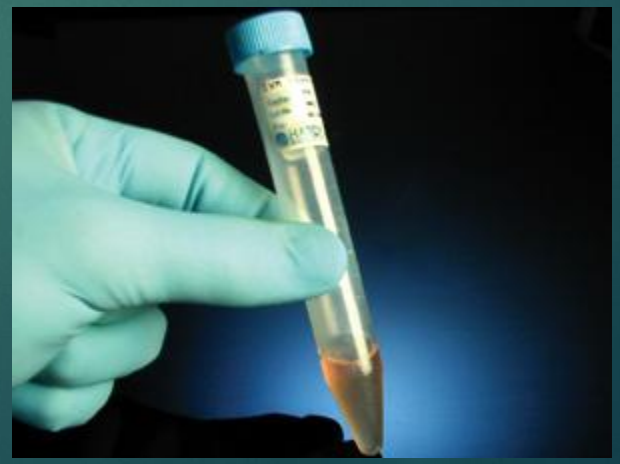
- **Transport media**: Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media.
- Such media prevent

-drying (desiccation) of specimen,

-maintain the pathogen to commensal ratio and

- inhibit overgrowth of unwanted bacteriae.

Ex; Stuart's & Amie's media



- **Anaerobic media:**

- They need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients.

- **Robertson cooked meat** that is commonly used to grow Clostridium sps medium

Culture methods

In the lab the indications for culture are mainly to:

- Isolate bacteria in pure culture
- Demonstrate their properties
- Obtain sufficient growth for preparation of antigens and for other tests
- Type isolates by methods such as bacteriophage and bacteriocin susceptibility
- Determine sensitivity to antibiotics
- Estimate viable counts
- Maintain stock culture

- The culture methods used in the laboratory are
- Streak
- Lawn
- Stroke
- Stab
- Pour plate
- Liquid cultures

- **Streak culture(surface plating)** method is routinely employed for the isolation of bacteria in pure culture
- A platinum loop is charged with the specimen to be cultured.

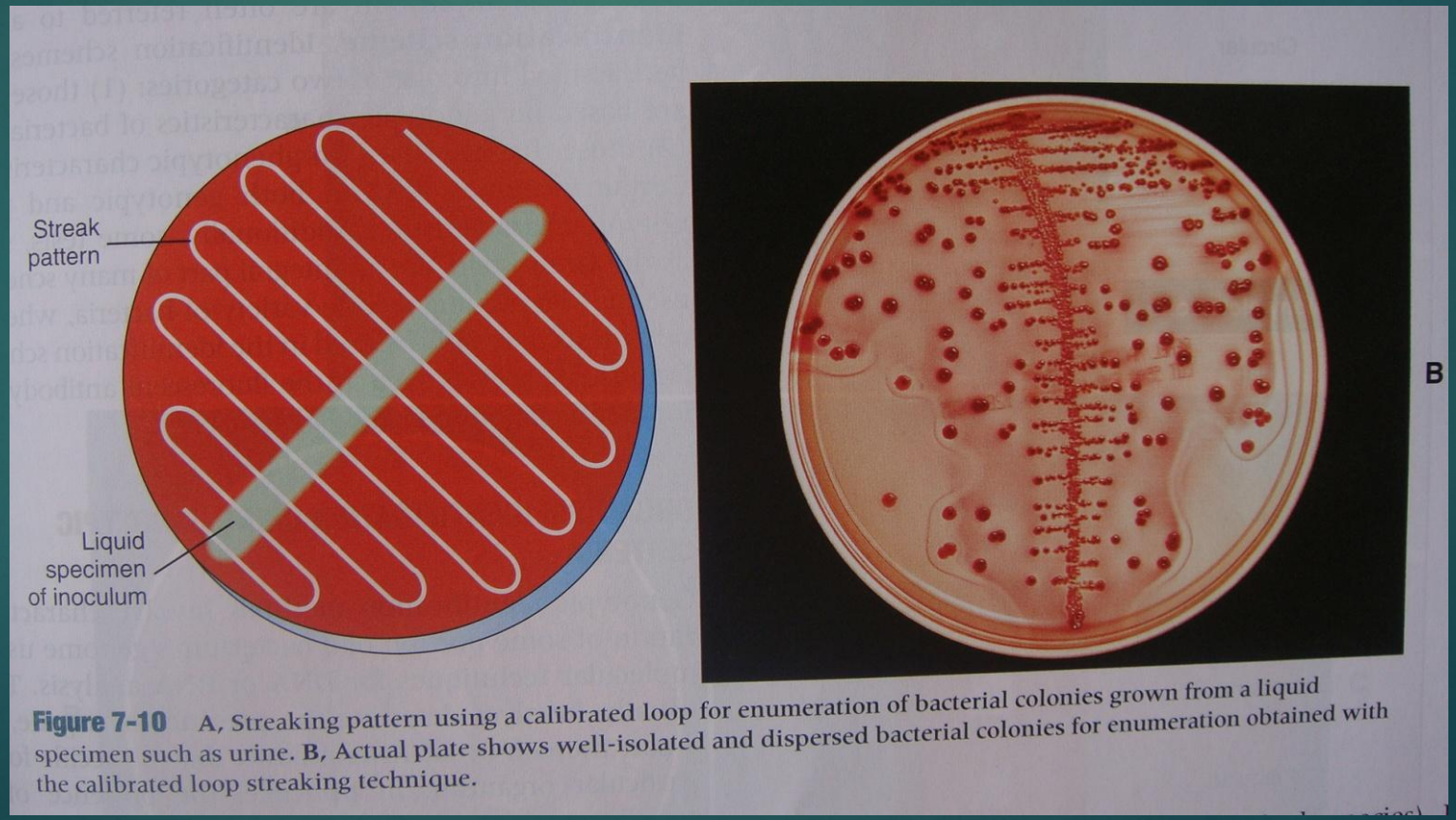
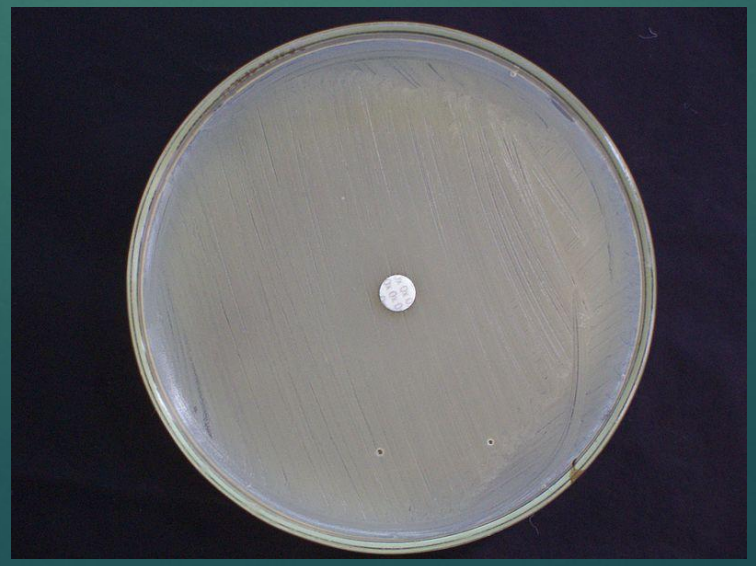


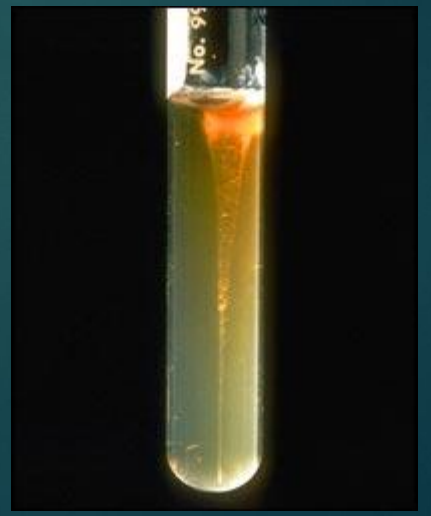
Figure 7-10 A, Streaking pattern using a calibrated loop for enumeration of bacterial colonies grown from a liquid specimen such as urine. B, Actual plate shows well-isolated and dispersed bacterial colonies for enumeration obtained with the calibrated loop streaking technique.

- The **lawn or carpet** culture provides a uniform surface growth of the bacterium and is useful for bacteriophage typing and antibiotic sensitivity testing.



- The **stroke culture** is made in tubes containing agar slope and is employed for providing a pure growth of the bacterium for slide agglutination and other diagnostic tests.

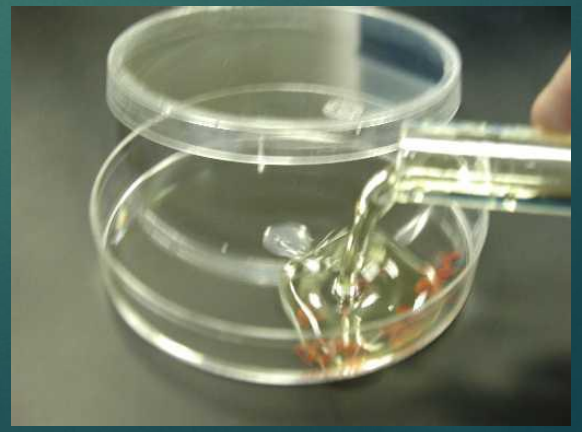
- **Stab culture**
- In a stab culture, the charged needle is passed vertically down the centre of the medium.



- **Pour – plate culture:**

- 15 ml of the agar medium are melted and left to cool in a water bath at 45-50^0 C

- It gives estimate of the viable bacterial count in a suspension and is the recommended method for quantitative urine cultures.



- **Anaerobic culture methods:**

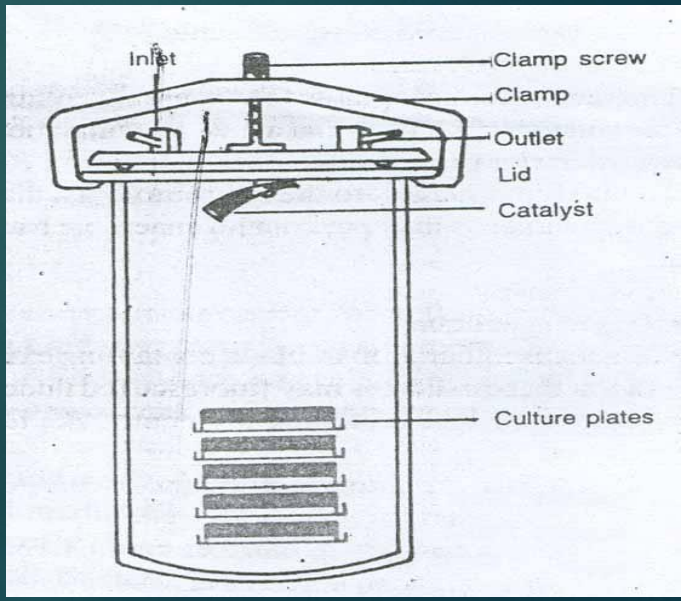
- Exclusion of oxygen from the medium is not that simple, and is effected by growing the organisms within the culture medium such as freshly steamed liquid media and deep nutrient agar with 0.5% glucose and minimal shaking and solidified rapidly by placing the tube in cold water.

- Liquid media soon become aerobic unless a reducing agent such as glucose 0.5-1.0%, ascorbic acid 0.1%, cysteine 0.1%, sodium thioglycollate 0.1%, or particles of meat in cooked meat broth are added.

- **Anaerobic jars:**
- When an oxygen-free or anaerobic atmosphere is required for obtaining surface growths of anaerobes, anaerobic jars provide the method of choice.

- The most reliable and widely used anaerobic jar is the **McIntosh-Fildes**' anaerobic jar. It is a cylindrical vessel made of glass or metal with a metal lid which is held firmly in place by a clamp.

- Inoculated culture plates are placed inside the jar and lid clamped tight. Hydrogen is drawn rapidly.



- The **Gaspak** is now the method of choice for preparing anaerobic jar. The Gaspak is commercially available as a disposable envelope containing chemicals which generate hydrogen and carbon dioxide on the addition of water.
- An indicator should be used for verifying the anaerobic condition in the jar. Methylene blue is generally used for this purpose.

- The major disadvantage of any anaerobic jar system is that the plates have to be removed from the jar to be examined.

- This, of course, exposes the colonies to oxygen, which is especially hazardous to the anaerobes during their first 48 hours of growth.

- Culture media for root canal samples

Media suitable for culturing root canals include:-

- **Brain heart infusion broth with 0.1% agar**
- **Trypticase soy broth with 0.1% agar**
- **Thioglycollate broth.**
- **Glucose ascites broth.**

- **Laboratory testing for antimicrobial sensitivity:**

The action of the antimicrobial agent against an organism can be measured:

1.Agar dilution method

2.Disc dilution method

- Agar dilution method

-each doubling dilution of an antimicrobial agent is incorporated into a single agar plate so that testing 6 doubling dilutions will require 6 plates, plus 1 positive control without antibiotic.

-the surface of each plate is inoculated with $1x10^6$ CFU.

- One or more isolates of bacteria are tested per plate

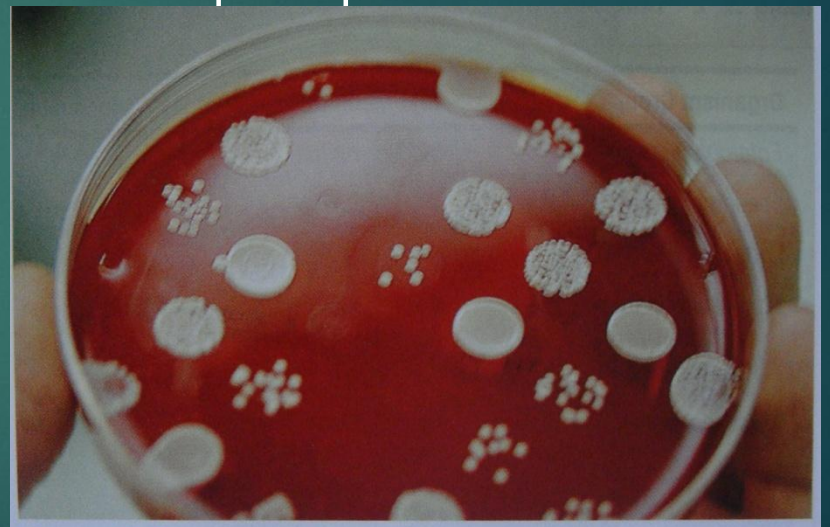


Figure 12-4 Growth pattern on an agar dilution plate. Each plate contains a single concentration of antibiotic, and growth is indicated by a spot on the agar surface. No spot is seen for isolates inhibited by the concentration of antibiotic incorporated into the agar of that particular plate.

- **Disc Diffusion Test:**
- The antibiotic or antimicrobial susceptibility of an organism can be tested by an application of standardized diameter of sterile filter paper discs.
- These filter paper discs are impregnated with different antimicrobial agents on to a lawn of the organism seeded on agar plates.
- After overnight incubation; zones of growth inhibition are measured with a millimeter ruler.

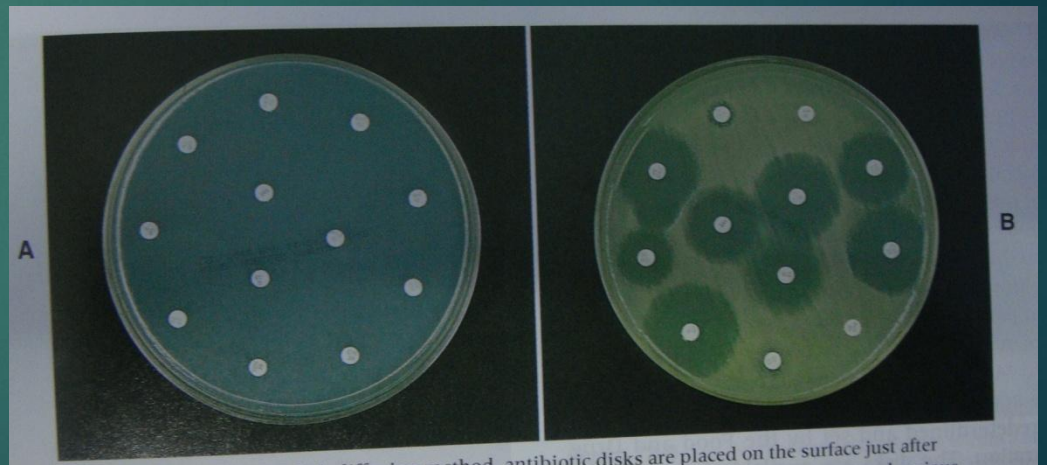
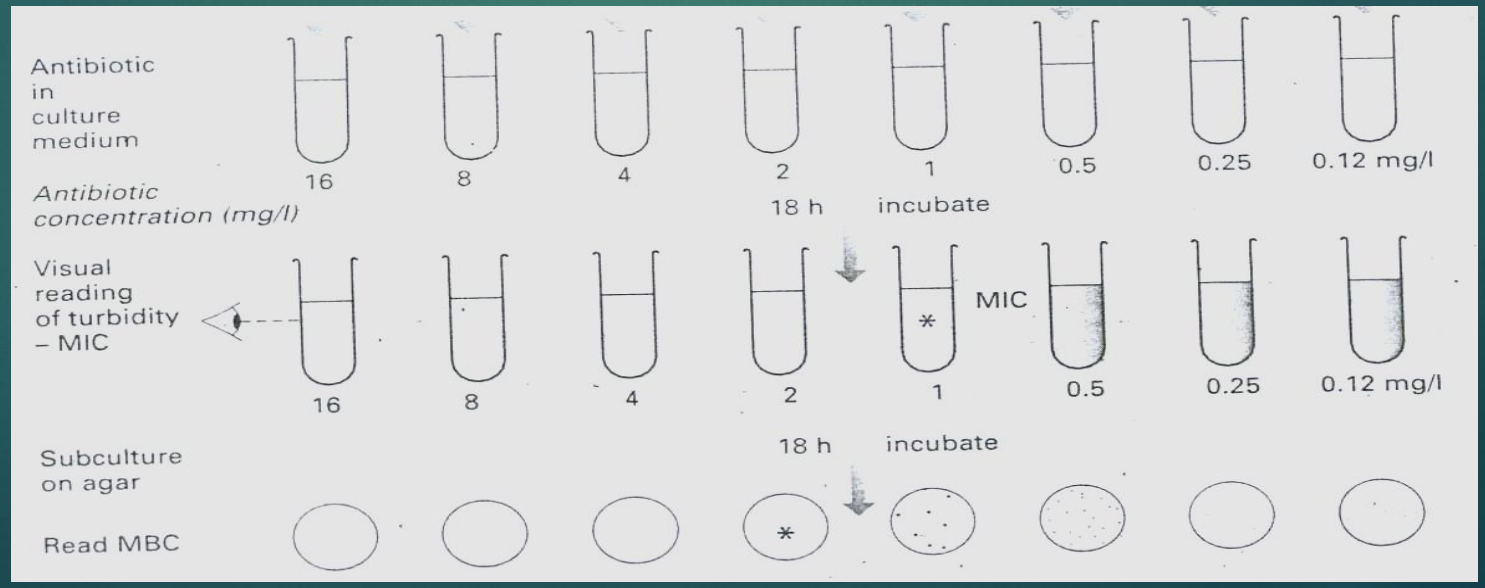


Figure 12-5 A, By the disk diffusion method, antibiotic disks are placed on the surface just after the agar surface was inoculated with the test organism. B, Zones of growth inhibition around various disks are apparent following 16 to 18 hours of incubation.

- **Assessment of minimum inhibitory concentration (MIC):**
- A range of dilutions of antimicrobial agent can be incorporated into a suitable broth in a series of test tubes. The broth is inoculated with a standardized suspension of test organism and for 18-24 hours. The minimum concentration of the drug that inhibits the growth of the test organism in the test tubes is recorded as MIC, i.e., the lowest concentration that will inhibit the visible growth in vitro



- **Minimum Bacterial Concentration (MBC)**

- Subsequently, a standard inoculum from each of the test tubes in which no growth has occurred is sub cultured on blood agar to determine the minimum concentration of the drug required to kill the organism (MBC).

- Minimum Bacterial Concentration (MBC) is defined as the minimum concentration of the drug that kills99.9% of the test organism in the original inoculum.

Endodontic sampling

- That Microbiological Root Canal Sampling (MRS) should form an essential part of endodontic treatment strategy .
- *The protocol for MRS as given by Mueller in the year 1966 is as follows:*
- **Preparation of the operating field**

- scaling of hard and soft deposits, gingivectomy, or temporary restoration and Rubber dam application

- Sterlization of the operating field

-prewashing with 30% hydrogen peroxide and washing with 5-10 % Iodide of tincture iodine

- Sterility control of the operating field

-monitored by sampling the tooth surfaces with charcoal impregnated pellet which will be cultured and if it tests positive to bacteria, any microbial data obtained from that particular sample must be discarded.

- Sampling from the root canal.

- sample is taken with charcoal impregnated points that are transferred to a transport medium or any broth.

- Culturing

- The transport medium is serially diluted and inoculated into an appropriate nutrient broth &/ agar plate.
- These are aerobically or anaerobically incubated for a period of time long enough to allow even slowly growing species to form colonies.

Root canal sampling protocol by Jacinto & Gomes et al

- Access to the root canal is made using sterile burs without water spray or sterile saline solution is used.
- Asepsis is maintained during access, during instrumentation, and removal of the contents from the pulp space, and sample collection.
- Samples are initially collected by means of #15 K-type file with the handle cut off.
- A sterile #15 file is used to agitate canal contents for 60s.
- The file is introduced to a level approximately 1 mm short of the root apex, based on diagnostic radiographs, and a filing motion is used.

- Afterwards, two sequential paper points are placed to the same level and used to soak up the fluid in the canal.
- Each paper point is retained in position for 60 s.
- If the root canal is dry, a small amount of sterile saline solution is introduced into the canal to ensure viable sample acquisition.
- Chemically active irrigants are never used before sampling.

- According to Erkan ,

- The canal orifice is flushed with nitrogen gas during the sampling process.

- According to Elzbieta Zedler,

- Using sterile barbed broach at apical 2-3 mm of the root canal.
- The tip is cut off and transferred to the RTF.

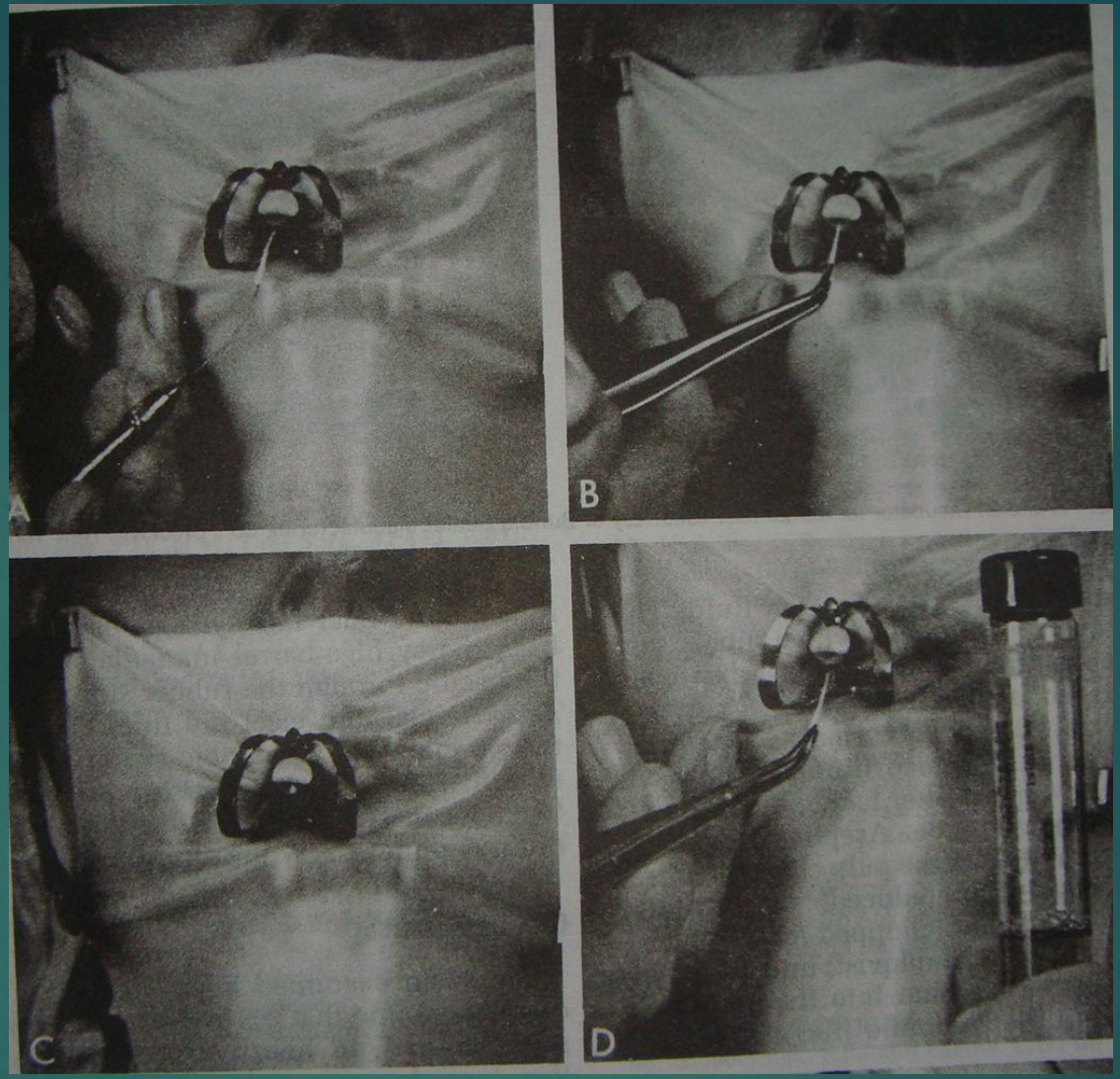
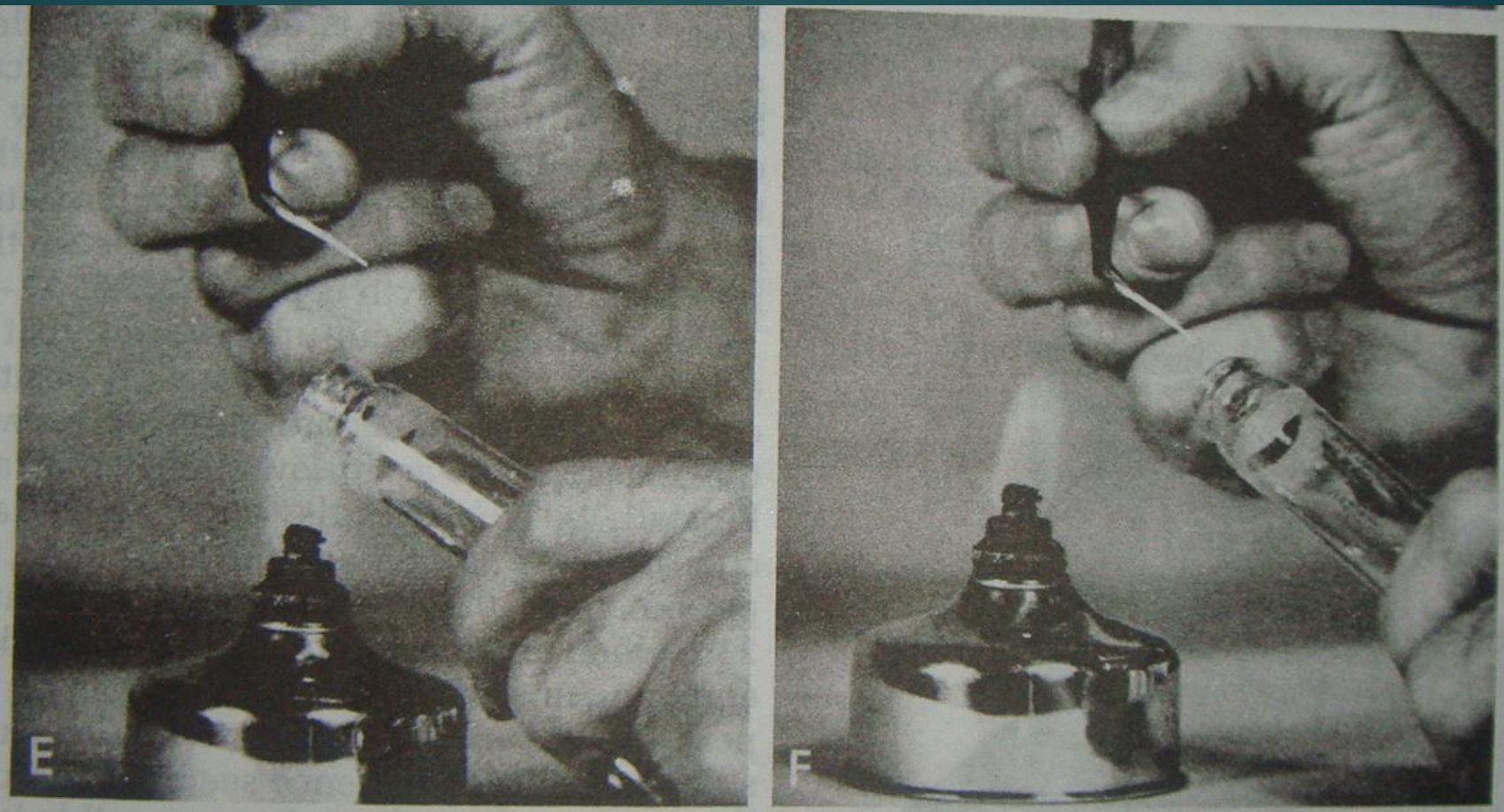


Fig. 13–1. Steps in taking a culture. *A*, Remove the absorbent point from the root canal and discard it. *B*, Wipe away residual antibiotic or antiseptic from the surface of the root canal; repeat twice or three times. *C*, Insert the absorbent point and let remain in the root canal for at least a minute, so the tip of the absorbent point will be moist when it is removed from the root canal. *D*, Remove the absorbent point from the root canal and examine the tip to make sure it is moist. *E*, Unscrew the cap by wrapping the little finger of the right hand around it and turning the culture tube counterclockwise with the left hand; flame the lip of the tube. *F*, Drop the absorbent point into the culture tube and replace the cap.



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- Anaerobic culturing: A clinical concept

Culturing obligate anaerobes is a fastidious process that requires special equipment and media used in temperature controlled oxygen free environment.

- **Mamoru Noda et al (1999)** compared the bacterial detection from intracanal exudate by two different methods:
- (i) *The conventional method:* The samples were put into anaerobic chambers immediately after sampling. The chambers were then moved to an outside laboratory for identification of bacteria.

- (ii) *The preculture method:* As soon as sampled, the samples are directly inoculated into semisolid medium containing menadione, hemin & 0.5% agar and incubated for 24-72hours at 37°C under anaerobic conditions. After incubation, the sample were taken to the laboratory for identification.
- The pre-culture method significantly showed higher frequency of bacterial detection.

- Special medium supplemented with agar is generally required to prevent oxygen diffusion so that toxic intermediate of oxygen do not accumulate & interfere with viability of anaerobic bacteria (Carlson et al 1980).

- **Periradicular sample**

-using an aseptic technique, insert the sterile needle of a Luer Lok syringe into the periradicular space.

-aspirate fluid, eject any air inside the syringe barrel immediately, insert the needle through the rubber septum stopper of an Anaport vial, eject the fluid.

The Anaport vial should be transported to the lab within 4 hrs of sampling.

- **Root canal sample**

-aseptically prepare an access cavity.

-inject a few drops of prereduced, anaerobically sterlized medium(RCM) into the root canal with a sterile endodontic file

-aspirate the fluid with a Luer Lok syringe,

-eject any air from the syringe barrel immediately,

-insert the needle through the rubber stopper of an Anaport vial and eject the fluid,

-transport the sample to the lab within 4 hrs.

- **Sampling through a Swelling**
- Uncontaminated microbial samples from facial swellings of endodontic origin are obtained by needle aspiration following surface disinfection of the mucosa.
- Following aspiration, any air in the syringe should be removed and the sample injected into an anaerobic transport vial for delivery to the laboratory.

- **SAMPLING THROUGH A FISTULA:-**

- The fistula orifice and the surrounding mucous membrane are dried by means of cotton rolls and air syringe.

- The surrounding 2 cm diameter area around the orifice is cleaned with 5-10% H_2O_2 until no bubbling is observed.

- A coarse paper point dipped into the H_2O_2 solution is inserted 2-5mm deep into the fistula orifice. Both the fistula orifice and surrounding mucous membrane area is then cleaned with 10% Iodide of tincture iodide.

- The iodine is then neutralized with 5% thiosulphate solution in a similar manner.
- Then using the blunt end of a sterile paper point obtain sample from the fistula orifice by inserting the paper point upto 2-3mm for 8-10 seconds.
- Sample the innermost part of the fistula by inserting a paper point as deeply as possible into the fistulous tract for 8-10 seconds.
- The sample is then transferred to a suitable media.

- **ADVANTAGES**
- In an average, the incidence of success in endodontics can be raised by approximately 10% by achieving negative culture before canal obturation.
- A negative culture is the most reliable index of root canal sterility available to dentists at their time.
- Root canal cultures are a source of valuable feed back information to the dentist.
- Cultures make it possible to identify the antibiotics sensitivity of canal organisms early in the course of treatment.
- Cultures are one of the several important criteria that may be used to determine when canals are ready to be filled.
- Widely available.

- **DISADVANTAGES**
- Need to purchase a bacteriologic incubator.
- Need to purchase and stock culture media.
- Inconvenience of taking, labeling, reading and recording culture results.
- Not all viable bacteria can be removed
- Low sensitivity
- Strict dependence on the mode of sample transport.
- Costly, time consuming and laborious
- Specificity is dependent on the experience of the operator.
- Specialized equipment needed to isolate strict anaerobe.
- Take several days to weeks to identify most anaerobic bacteria.

- **Culture reversal**

- Grossman examined approximately 1000 cases & found that 2% of the cultures were negative after 48 hrs of incubation, but they turned positive when incubated for 10 days.

- It is advisable to allow more than 48 hrs between taking the culture & filling the root canal, preferably 96 or more hrs & it is recommended that the culture tube be re-examined immediately before obturating a canal to make certain that no evidence of growth is present.

- The incidence of culture reversal, that is negative to positive culture by the time of obturation, varies with the investigator.
- Engstrom & Frostell-14%
- Nicholls-4%
- Seltzer et al-16%
- Serene & McDonald-10%
- Tsatsas & students-23%
- Experienced operators(Winkler)-3%
- Students-9%

- The variation seems to indicate that care in taking the culture, possible leakage between treatments, and the capability of the culture medium to sustain growth of microbes, all play a role in culture reversal.

- The enterococci was inoculated in the CEB which showed growth at the end of 24hrs in spectrometric analysis.

- Root canal sample was inoculated in CEB and the absorption was seen at 3 ½ hrs at 610 & 653 nm compared with previous absorption and peak of absorption was seen at 6 hrs which confirmed E faecalis growth.

Conclusion

- Chromocult with spectroscopy is a rapid chair side kit to identify the E faecalis activity.

- **CONCLUSION**
- Traditionally microbial culture has been the preferred means for examining the vast majority of microbiota.
- Culture is the process of propagating microbes in the laboratory by providing them with proper environmental conditions.
- Various broths, agar & different media are used frequently for routine bacteriology.
- Sufficient knowledge of these media and organisms helps to choose and select the appropriate combination .

- Microbial Root canal Sampling is a definite way of ensuring endodontic success.

- Endodontic success can be reasonably raised by 10% as suggested by literature.

Culturing also serves manifold functions namely, bacterial sensitivity to antibiotics, self assessment of ability to adequately prepare root canals, assessment of temporization measures, successful treatment of refractory cases, selection of proper intracanal medicaments and sterilizing solutions.

CONCLUSION

- Infection of the root canal is not a random event. The type and mix of the microbial flora develop in response to the surrounding environment. Microorganisms that establish in the untreated root canal experience an environment of nutritional diversity. In contrast, well-filled root canal offers the microbial flora a small, dry, nutritionally limited space. Thus, we should obtain a better understanding of the characteristics and properties of bacteria and their biofilms along with the environmental changes, to enhance success.

- ***"On the whole bacterial culturing is not the end in itself-its main purpose clinically is to serve as a predictor of healing".***