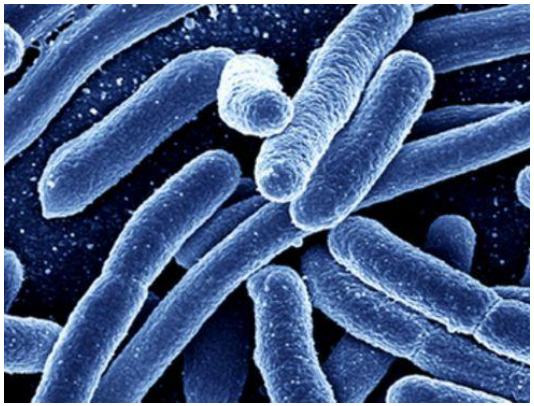
LESSON №1

INTRODUCTION TO SPECIAL BACTERIOLOGY AND VIROLOGY



PURPOSE

- ✔ to study the diagnostic methods of infectious diseases and their use in dentistry

SPECIAL BACTERIOLOGY

- **Special bacteriology** is the branch of microbiology that studies the morphology, ecology, genetics and biochemistry of bacteria and their relation to medicine
- This subdivision of microbiology involves the identification, classification, and characterization of bacterial species

VIROLOGY

- **Virology** is the study of viruses – submicroscopic, parasitic particles of genetic material contained in a protein coat – and virus-like agents
- It focuses on the following aspects of viruses: their structure, classification and evolution, their ways to infect and exploit host cells for reproduction, their interaction with host organism physiology and immunity, the diseases they cause, the techniques to isolate and culture them, and their use in research and therapy
- Virology is considered to be a subfield of microbiology or of medicine

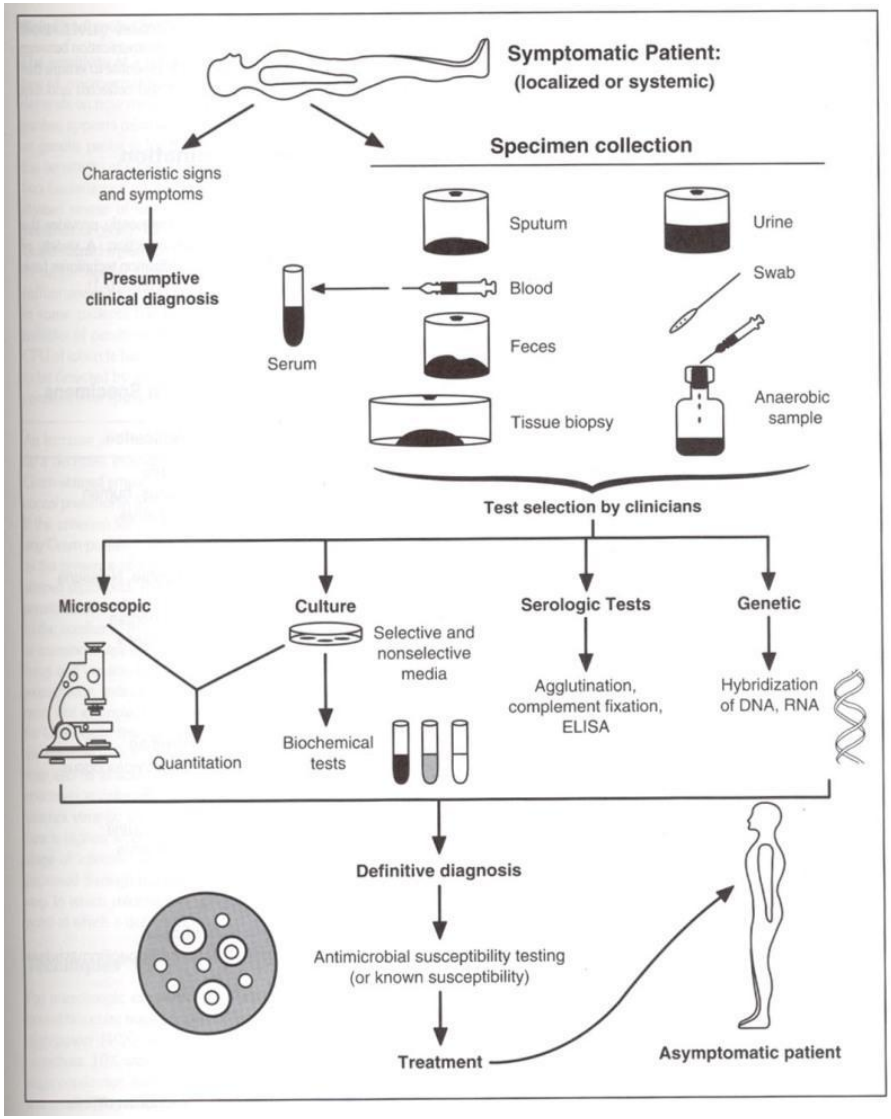
PRINCIPLES OF DIAGNOSIS

Some infectious diseases are distinctive enough to be identified clinically. Most pathogens, however, can cause a wide spectrum of clinical syndromes in humans. Conversely, a single clinical syndrome may result from infection with any one of many pathogens. Influenza virus infection, for example, causes a wide variety of respiratory syndromes that cannot be distinguished clinically from those caused by streptococci, mycoplasmas, or more than 100 other viruses.

PRINCIPLES OF DIAGNOSIS

Most often, therefore, it is necessary to use microbiologic laboratory methods to identify a specific etiologic agent. Diagnostic medical microbiology is the discipline that identifies etiologic agents of disease. The job of the clinical microbiology laboratory is to test specimens from patients for microorganisms that are, or may be, a cause of the illness and to provide information (when appropriate) about the in vitro activity of antimicrobial drugs against the microorganisms identified

Laboratory procedures used in confirming a clinical diagnosis of infectious disease with a bacterial etiology



From: Chapter 10, Principles of Diagnosis
Medical Microbiology. 4th edition.
Baron S, editor.
Galveston (TX): University of Texas Medical Branch at Galveston; 1996.

BACTERIOSCOPIC METHOD

- Bacterioscopic method is detection of microbes in the test material; the study of their morphological and tinctorial properties, the nature of their location in the bacteriological smear in the field of vision
- The study is appointed if there is a suspicion of the patient having an infectious and purulent-inflammatory process or in the obstetric and gynecological area

BACTERIOSCOPIC METHOD

1. Material from the patient is visually studied, a portion is selected in which the causative agent of the disease (lumps of mucus, purulent plugs) can be detected with the greatest probability.
2. It is applied to a slide.
3. The drop is spread over the glass, dried and fixed.
4. The smear stains (by Gram method) and the drug is examined under a microscope.

BACTERIOSCOPIC METHOD

ADVANTAGES

- ✔ Simplicity
- ✔ Ability to quickly obtain results
- ✔ Technical and economic accessibility

DISADVANTAGES

- ✔ To determine the type of microorganisms, it is often insufficient to determine its morphological properties
- ✔ Bacteria with characteristic morphology often undergo changes, especially under the action of antibiotics, and become unrecognizable
- ✔ Concentration of pathogens in the test material can be extremely low, and then they are difficult to detect

BACTERIOLOGICAL METHOD

- The bacteriological method consists in isolating the pure culture of the pathogen (a population containing bacteria of one species) and identifying this pathogen
- A multi-stage bacteriological study lasts 18-24 hours
- Identification is the study of the properties of microorganisms so establishing a particular systematic group of bacteria (species, genus)
- This method is used in to determine such dangerous diseases as tuberculosis, recurrent typhoid or gonorrhea. It is also used to study the bacterial composition of tonsils, cavities of organs

BACTERIOLOGICAL METHOD

1. Isolation of the pathogenic pure culture (inoculation test material on dense nutrient media, elective or differential-diagnostic which is placed in thermostat)
2. The study of bacterial colonies grown on a dense nutrient medium and originating from a single bacterial cell (the colony is a pure culture of the pathogen):
   – cultural properties of the colonies (shape, size, color, edges and surfaces, structure, consistency)
   – tinctorial and morphological properties of the selected culture and verifying at the same time its purity
3. Identification of the isolated pure culture of the pathogen and determination of its sensitivity to antibiotics and other chemotherapeutic drugs

BACTERIOLOGICAL METHOD

ADVANTAGES

- ✔ High specificity (allows to exclude false diagnose)
- ✔ Helps to apply the most effective treatment by accurately determining the reaction of microorganisms to a particular medical device

DISADVANTAGES

- ✔ Lasts a long time
- ✔ Strict requirements to test material
- ✔ Strict requirements for laboratory technicians

SEROLOGIC STUDIES

- Serologic studies are methods of studying certain antibodies or antigens in the blood serum of patients based on the reactions of immunity. With their help, antigens of microbes or tissues are also identified for the purpose of their identification.
- The detection of antibodies to the infectious agent or the corresponding antigen in the serum of the patient allows to establish the cause of the disease.
- Serological studies are also used to determine blood group antigens, tissue antigens and the level of humoral immunity.

SEROLOGICAL METHOD

ADVANTAGES

- ✔ A modern and reliable way to identify such dangerous diseases as HIV, hepatitis, brucellosis, STDs
- ✔ High specificity and sensitivity
- ✔ Most of the reactions of this method are simple in conducting and accounting, available to a wide range of laboratories, usually safe, economical, amenable to standardization

DISADVANTAGES

- ✔ the indirect nature of the result, when the etiology of the disease is judged not by the isolation of the pathogen, but by the immune response to the causative agent
- ✔ the need for parenteral intervention in the patient's body
- ✔ in most cases, late diagnosis, which is due to the natural dynamics of the humoral immune response

ALLERGY DIAGNOSTIC TESTS

- are highly specific and sensitive tests of diagnosing allergic and infectious diseases, in the pathogenesis of which the allergic component predominates
- are based on the local or general reaction of the sensitized organism in response to the introduction of a specific allergen – a delayed type hypersensitivity reaction (HRT)
- are used for detection of an allergen or a group of allergens that have caused a hypersensitivity state
- Skin tests (application, scarification and intradermal tests) provocative tests (nasal, conjunctival, inhalation)

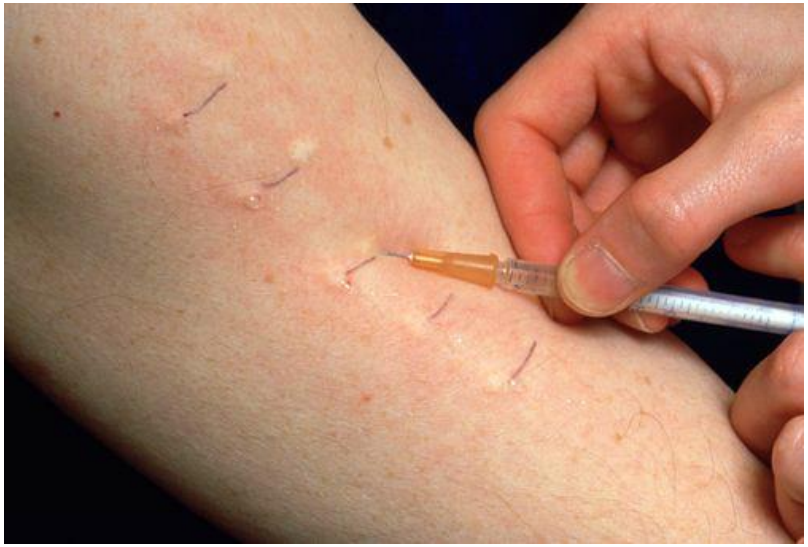
ALLERGY DIAGNOSTIC TESTS

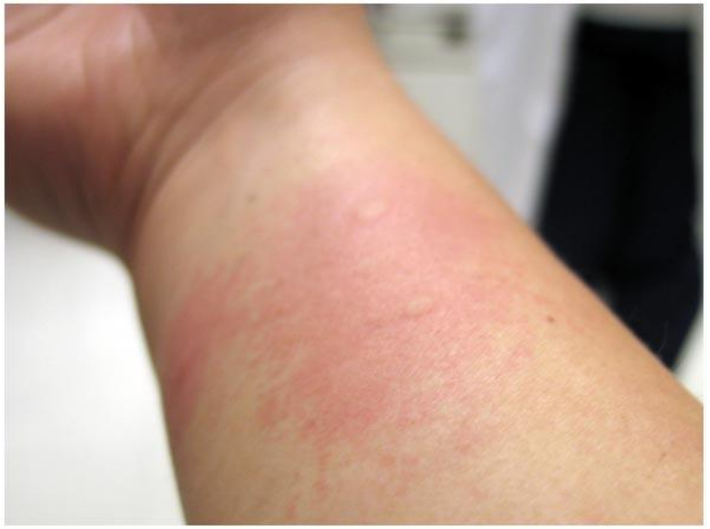
With many infectious diseases, due to activation of cellular immunity, an increased sensitivity of the organism to pathogens and products of their vital activity develops. This is based on allergic tests used to diagnose bacterial, viral, protozoal infections, mycoses and helminthiases. Allergic tests have specificity, but often they are positive for those who have recovered and vaccinated.

ALLERGY DIAGNOSTIC TESTS

Identification of cellular immune response - delayed type hypersensitivity (HRT), scheme

- Introduction intradermally Ag bacteria
- After 48 - 72 hours there is inflammation
- Measure the amount of redness and papules





ALLERGY DIAGNOSTIC TESTS

Allergic diagnostic tests can diagnose

- ✔ tuberculosis (Mantoux reaction)
- ✔ brucellosis (Burne test)
- ✔ tularemia (Tularin test)
- ✔ anthrax (anthraxin test)
- ✔ soft chancre (Duchess reaction)
- ✔ leprosy (Mitsuda reaction)
- ✔ differentiate tuberculoid (lepromin-positive) form from lepromatous (lepromin-negative)

ALLERGY DIAGNOSTIC TESTS

ADVANTAGES

- ✔ Specificity

DISADVANTAGES

- ✔ can be positive not only in infected, but also in vaccinated against these diseases, as well as in people who have recovered many years ago
- ✔ many patients the method of introducing an irritant into the body is contraindicated

BIOLOGICAL RESEARCH METHODS

Biological research methods are aimed at determining the presence of pathogen toxins in the test material and on the detection of the causative agent. Methods include infecting laboratory animals with the test material, followed by isolation of a pure pathogen culture or establishing the presence of a microbial toxin and its nature. The method is highly sensitive, can be used in the early stages of the disease, but is not always available, expensive, long-lasting, unsafe.

MOLECULAR TECHIQUES

- Molecular techniques are based on the analysis of nucleic acids, first of all, the DNA molecule
- The advantage of DNA diagnostics in comparison with biochemical or immunological diagnostics is the use of a unified set of methods that is practically independent of the objectives of the study. These are methods for DNA isolation, PCR, electrophoresis, DNA restriction, hybridization with specific DNA probes, and sequencing. Thus, within the limits of one laboratory it is possible to be engaged in DNA-diagnostics of a wide spectrum of diseases

EXAMPLES OF MOLECULAR TECHNIQUES

- DNA hybridization
- Nucleic acid amplification testing
  - Polymerase chain reaction (PCR)
  - Ligase chain reaction (LCR)
  - Automated DNA amplification
  - Real time PCR

MOLECULAR TECHNIQUES

1. Southern blotting and nucleic acid hybridization

   A labelled DNA probe will bind to the specimen if it contains the specific sequence that is being sought. The captured probe is detected by the activity of it attached label. This technique is specific and rapid, but less sensitive than other methods that involve amplification steps.

MOLECULAR TECHNIQUES

2. Nucleic acid amplification tests

Nucleic acid amplification tests (NAATs) make the diagnosis by amplifying specific regions of the genome from the pathogen. Although different methods are used to amplify pathogen-specific DNA or RNA the aim is the same, to produce sufficient copies for detection. For example, nucleic acid from the pathogen is separated into single strands and primers are designed to bind to targetsequences. A polymerase then catalyses synthesis of new nucleic acid and this process is repeated for multiple cycles.

MOLECULAR TECHNIQUES

2. Nucleic acid amplification tests

Automated systems and commercial kits have made these tests available in many laboratories. Real-time PCR machines measure rising concentrations of target DNA and determine positivity when the concentration passes a set threshold.

NAATs have the advantage that they can detect slow-growing organisms or those that are difficult to grow (e.g. *M. tuberculosis) or make a diagnosis when samples* are rendered falsely negative by antibiotic therapy.

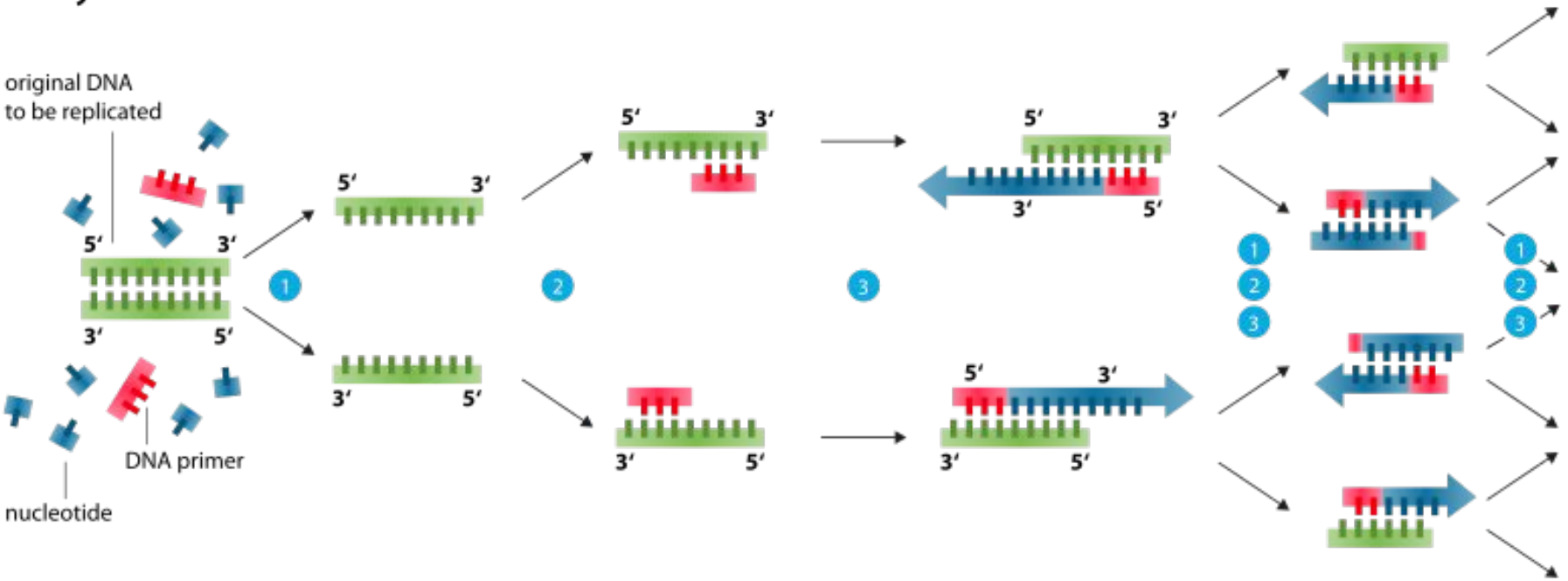
POLYMERASE CHAIN REACTION

- **Polymerase chain reaction** (**PCR**) is a technique used in molecular biology to amplify (accumulate of copies of a certain nucleotide sequence) a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence
- Very sensitive method

POLYMERASE CHAIN REACTION

In the case of molecular diagnostics of infections, a DNA fragment specific for a particular pathogen is amplified, and then, with the help of electrophoresis and staining on DNA, the presence of this fragment, and therefore of the pathogen itself, is tested in the biological sample that was taken for analysis.

Polymerase chain reaction - PCR

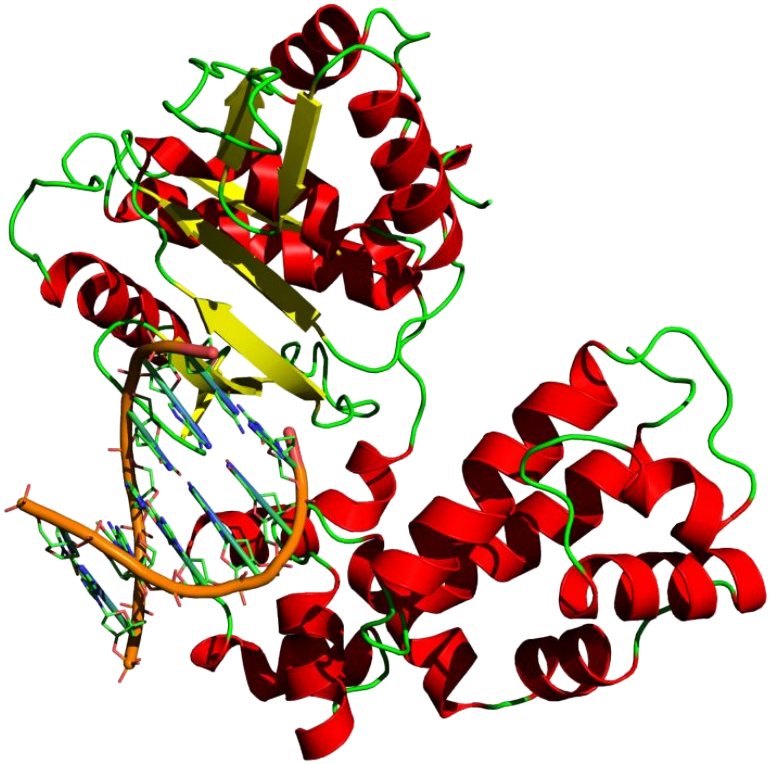


1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

DNA POLYMERASE

DNA polymerases

- are enzymes that synthesize DNA molecules fr om deoxyribonucleotides
- are used DNA polymerase from thermophilic bacteria, because they are thermostable



The three-dimensional structure of DNA-binding helical-hairpin sites in human beta-DNA polymerase

A strip of eight PCR tubes, each containing a 100 µl reaction mixture

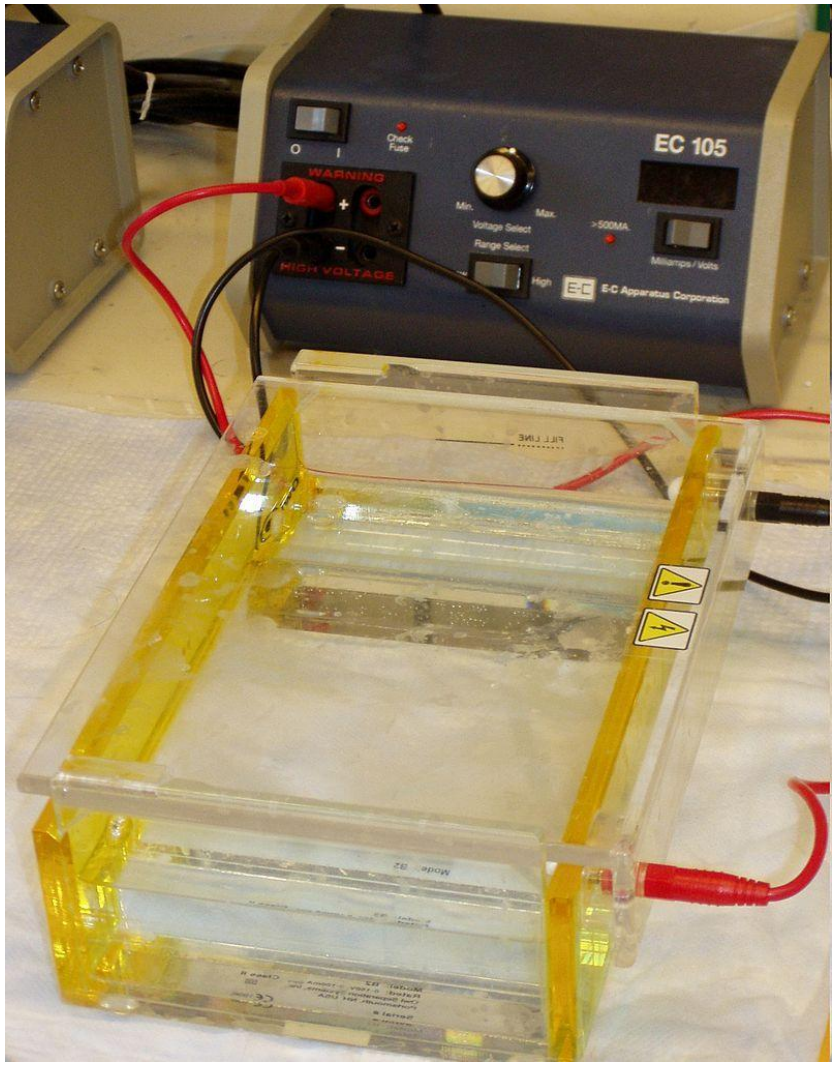
Placing a strip of eight PCR tubes into a thermal cycler

GEL ELECTROPHORESIS

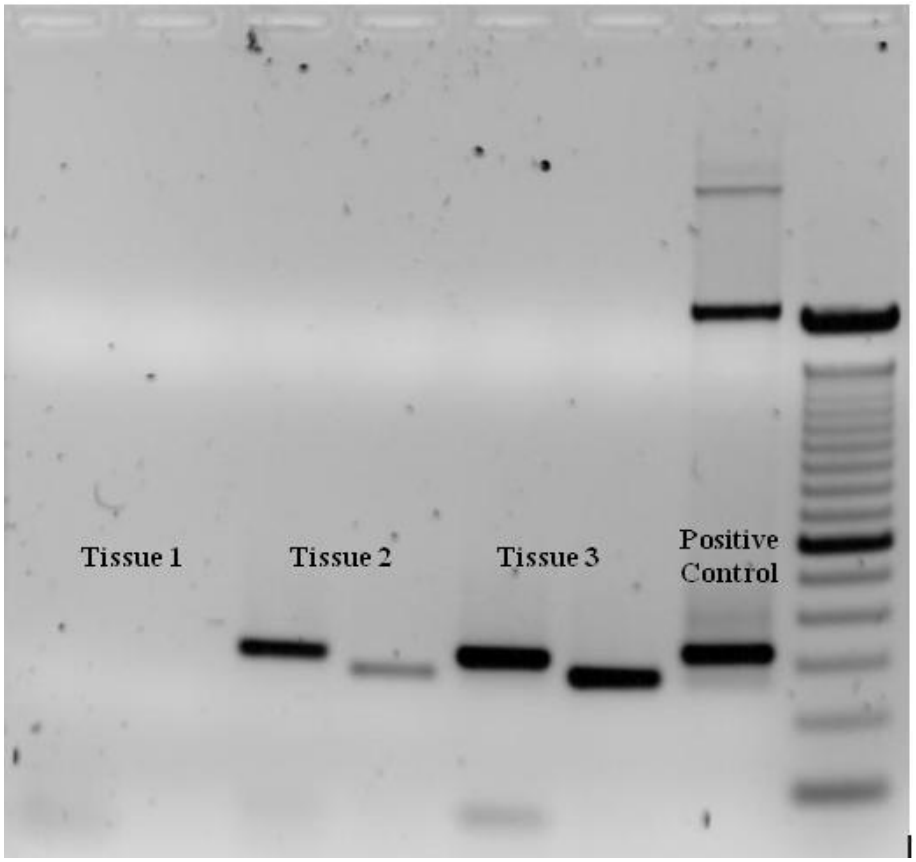
- **Gel electrophoresis** is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge. It is used in clinical chemistry to separate proteins by charge and/or size (IEF agarose, essentially size independent) and in biochemistry and molecular biology to separate a mixed population of DNA and RNA fragments by length, to estimate the size of DNA and RNA fragments or to separate proteins by charge
- Nucleic acid molecules are separated by applying an electric field to move the negatively charged molecules through a matrix of agarose or other substances. Shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel. This phenomenon is called sieving

GEL ELECTROPHORESIS

Gel electrophoresis apparatus – an agarose gel is placed in this buffer-filled box and an electrical field is applied via the power supply to the rear. The negative terminal is at the far end (black wire), so DNA migrates toward the positively charged anode (red wire)



GEL ELECTROPHORESIS

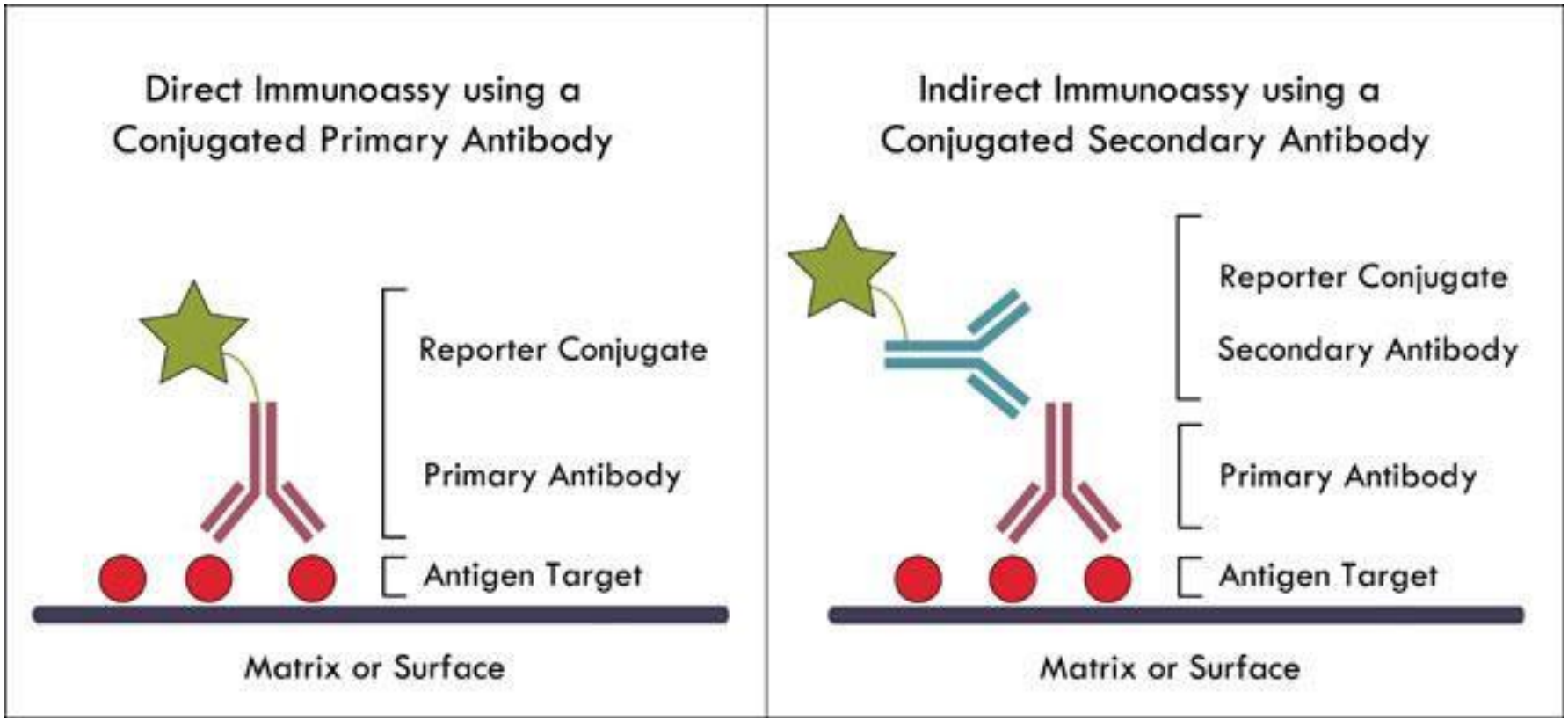


Ethidium bromide-stained PCR products after gel electrophoresis. Two sets of primers were used to amplify a target sequence from three different tissue samples. No amplification is present in sample #1; DNA bands in sample #2 and #3 indicate successful amplification of the target sequence. The gel also shows a positive control, and a DNA ladder containing DNA fragments of defined length for sizing the bands in the experimental PCRs.

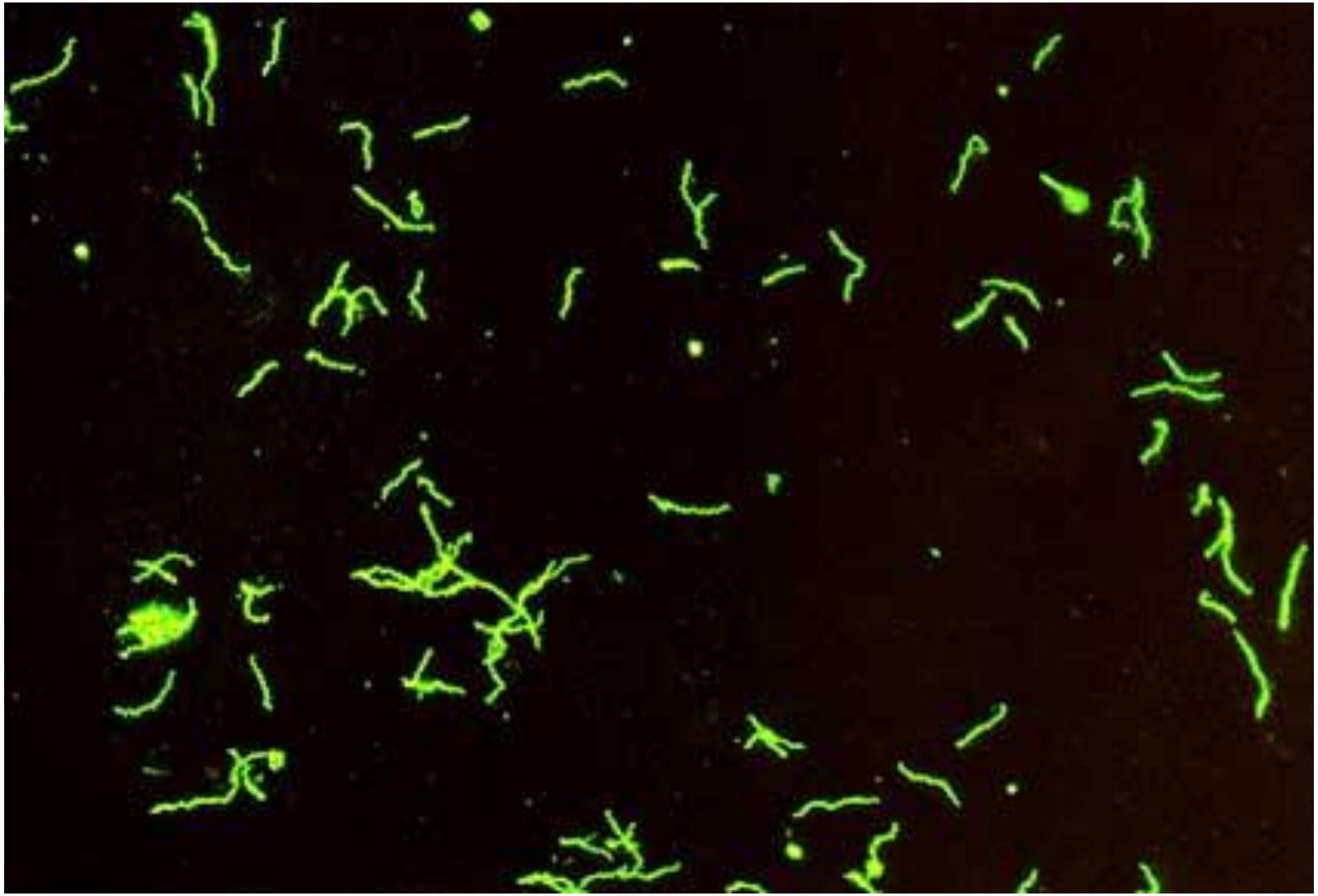
IMMUNOFLUORESCENCE

Immunofluorescence analysis, or the reaction of immunofluorescence, is based on the interaction of antigens with antibodies, but the reagent is then labeled with a dye that glows in the ultraviolet. Luminous antigen-antibody complexes are clearly visible under fluorescence microscopy. It is a rapid and accurate diagnostic method for the detection of antigens of microbes or the detection of antibodies.

IMMUNOFLUORESCENCE



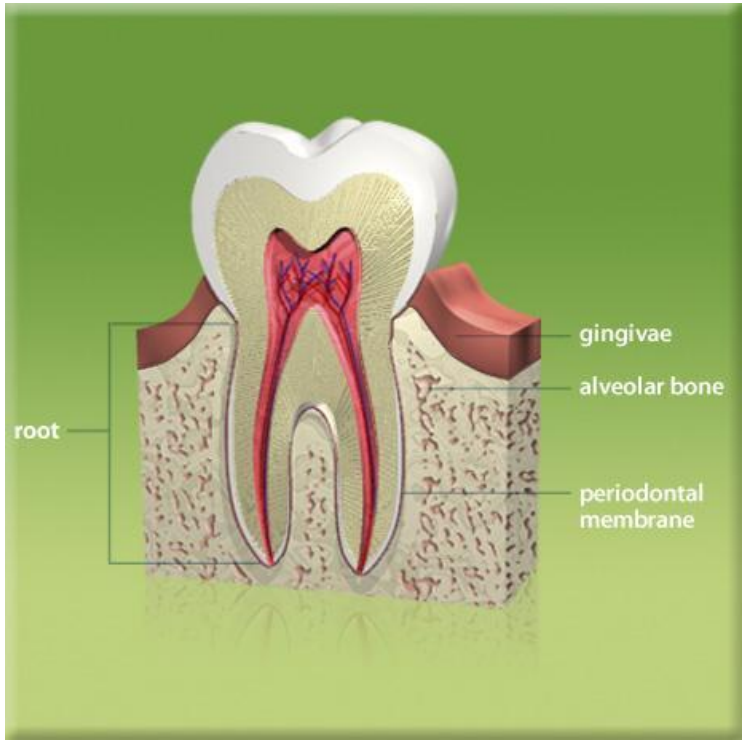
Fluorescent treponemal antibody absorption test (FTA - ABS test)



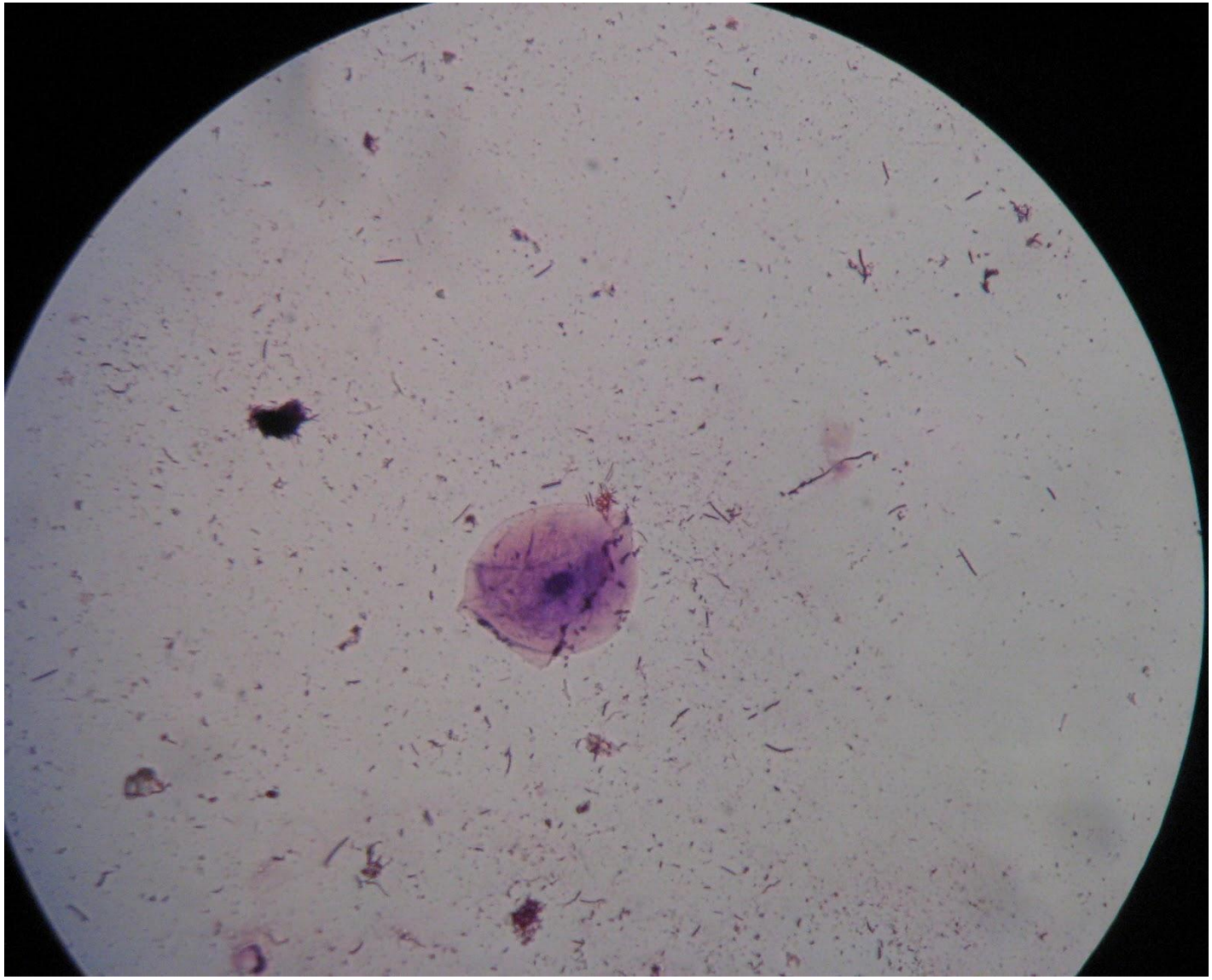
MICROBIOLOGICAL METHODS IN DENTISTRY

- Used to diagnose inflammatory-destructive processes in periodontal tissues
- In the origin and development of the pathological process in the tissues of the periodontal disease, the toxic bacteria of the dental plaque, the disturbance of metabolic mechanisms in tissues, the disturbance of hemodynamics, the regulating role of the nervous and endocrine systems, as well as the immune mechanisms of damage, play a major role

MICROBIOLOGICAL METHODS IN DENTISTRY



Periodontal tissues of a healthy person are filled with microflora. Colonies of microorganisms are concentrated in the surface areas of the gum, as well as on the subgingival dental plaque, where a monolayer with a thickness of up to 20 microbial cells is formed, among which 3/4 are cocci, and 1/4 are rods and spirals.



An example of Gram Stained Oral Microbiota (note presence of cheek cells)

http://microbio146.blogspot.ru/2011/11/lab-35-oral-microbiota.html

MICROBIOLOGICAL METHODS IN DENTISTRY

- When the periodontal lesions are both for diagnosis and for the selection of adequate etiopathogenetic treatment, a microbiological analysis is necessary. Success and failure of treatment often depend on whether it is possible to identify the causative agent of a disease

MICROBIOLOGICAL METHODS IN DENTISTRY

- **Bacterioscopic method** allows to detect causative agents of syphilis, gonorrhea, leprosy, tuberculosis, actinomycosis, fungal diseases
- **Bacteriological examination** is carried out in all cases when it is necessary to clarify the cause of the lesion of the mucous membrane, with specific diseases, purulent processes, to determine the bacilli
- **Molecular techniques** allow detecting periodontal pathogens in the contents of the periodontal pocket is an important information for the dentist when choosing a drug and the method of therapy