**Principles of diagnosis in the microbiology**

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**ABSTRACT**

**Manifestation of infections:** The clinical presentation of an infectious disease reflects the interactions between the human and the microorganism. These interactions are affected by the human immune status and the microorganism’s virulence factors. Symptoms and signs may vary according to the site involved in the host and progression of the infection. Diagnosis requires a composite of information, physical examination, radiographic findings, laboratory diagnosis data & clinical history. **Microbial Causes of Infection:**Infections caused by bacteria, viruses, fungi, and parasites. The pathogen may be exogenous (acquired from other persons, environmental, or animal sources) or endogenous (from the human floras- present in the body surfaces or inside the body). **Specimen Selection, Collection, and Processing:**Specimens will be selected based on signs and symptoms, and they should represent the disease process and must collect before the administration of antimicrobial agents. Quantity of samples and the rapidity of specimen transportation to the laboratory influence the test results.

**Microbiological Examinations- Direct Examination and Techniques:**Direct examination of the specimens reveals gross pathology. Microscopic examinations identify the microorganisms. Immunofluorescence assay, immuno-peroxidase staining, and other immunoassay detect the specific microbial antigens. Genetic probes identify the genus- or species of the specific DNA or RNA sequences. **Culture:** Isolation of infectious agents are frequently requiring specialized culture media. Non-selective (non-inhibitory) media allows the growth of many microorganisms. Selective media which is contain inhibitory substances that allow the isolation of specific types of microorganisms. **Microbial Identification:** Colony and cellular morphology of the microbial cultures may help in the preliminary identifications of Microorganisms. Microorganisms' growth characteristics may vary in conditions such as the utilization of carbohydrates and other substrates, enzymatic activity, immunoassays, and genetic probes used for the microorganism’s identification. **Serodiagnosis:** A high or rising titer of specific antibodies or antigens, either the presence of specific antibodies or antigens in the clinical samples may suggest or confirm a diagnosis. **Antimicrobial Susceptibility:** Microorganisms (mostly bacteria and fungi) are tested in vitro to determine their susceptibility to antimicrobial agents.

**Keywords:** Microorganisms; Infection; Pathogens; Immunoassays; Virulence factors; Lab diagnosis; symptoms and antimicrobial agents.

1. **INTRODUCTION**

Infectious diseases can be identified clinically. Most pathogenic microorganisms, however, ability to cause a wide spectrum of clinical syndromes in humans. Conversely, a single clinical syndrome may obtain from infection with any one of many pathogens. for example, Influenza virus infection can cause a wide variety of respiratory syndromes that may not be distinguished clinically from those caused by streptococci, mycoplasmas, or more than 80 other viruses.

Therefore, it is necessary to use microbiologic laboratory diagnostic methods to identify the most specific etiological agent of the pathogens. Diagnostic medical microbiology is the discipline that helps to identify the etiologic agents of disease. The role of the clinical microbiology laboratory is to diagnose various specimens from the patients to identify the pathogens that may be a cause of the illness and to provide information about the in vitro activity of antimicrobial drugs against the identified pathogens.

The clinical microbiology laboratory staff should be qualified to advise the physician as well as the processing of the clinical specimens. The physician should provide information about the patient, such as age and sex, tentative diagnosis and the clinical syndrome, date of onset syndrome, significant exposures of the patient, empirical therapy, immunological status, and underlying conditions. The clinical microbiologist should provide an interpretation of the laboratory results.

1. **CLINICAL MANIFESTATIONS**

The clinical manifestations of infection may vary from one to another, depending on various factors, such as the site of entry or acquisition of the pathogens; organ or systemic tropisms of the pathogens; microbial virulence factors; age and sex of the patient, and immunologic status of the patient; diseases conditions; and the presence of implanted prosthetic devices. The signs and symptoms of an infection may be localized or systemic, with fever, chills, hypotension, or hypertension.

1. **MICROBIAL CAUSES OF INFECTION**

Infections are caused by bacteria, viruses, fungi, or parasites. Infection may be endogenous or exogenous in nature. Endogenous infections may occur when the microorganism is collected from the upper to the lower respiratory tract or it penetrates the skin or mucosal barrier in the case of surgery. In exogenous infections, the microorganisms are acquired from the environment (e.g., from soil, food, or water) or from one person to another person or an animal. And the differential diagnosis is based on history, physical examination, radiographic, laboratory studies, and most importantly the selection of correct specimens for the microbiologic examination.

1. **SPECIMEN SELECTION, COLLECTION & PROCESSING**

The Specimens selected for microbiological examinations should be based on the site of the infection involved and be collected in adequate quantity. Swabs for specimen collection, frequently use the method and a small quantity of a specimen for the accurate microbiologic examination and it should be used only for the skin and mucous membrane infections. The skin must be disinfected before taking swab specimens of a lesion.

The type of inflammation present can lead to the appropriate type of microbiologic examination to be performed. For example, if caseous granuloma is observed in the histopathological examinations, then a microbiologic examination should be included for mycobacteria and fungal culture. The surgeon must obtain samples for some examinations from a single large lesion or smaller lesions. If an abscess is found, the surgeon must collect several milliliters of pus and as well as the abscess wall, for microbiologic examinations.

 

**Figure:1 Figure:2**

**Figures: 1 and 2 represent wound swab specimen collection techniques**

1. **MICROBIOLOGIC EXAMINATIONS**
2. **Direct examination Techniques:**

For infinitesimal examination obligatory to have a compound binocular magnifying lens prepared with low-power (1OX), high-power (40X), oil submersion (1OOX), and a great light source. For the immunologic discovery of microbial antigens or antibodies, latex molecule agglutination, agglutination, and enzyme-linked immunosorbent measure (ELISA) are the foremost visit methods within the clinical microbiology research facility.

Hereditary test locations are based on the one-of-a-kind nucleotide arrangements with the DNA or RNA of pathogens. Such nucleotide arrangements are speaking to a harmfulness quality or hereditary fabric of the pathogens. The utilization of atomic innovation within the determination of irresistible maladies has been encouraging and improved by the presentation of quality enhancement strategies, such as the polymerase chain response (PCR). This approach has had major applications within the discovery of contaminations due to microorganisms that are troublesome to culture (e.g. the human immunodeficiency infection) that have not as however been effectively refined.



**Figure: 3 Compound Binocular Microscope**

1. **Culture methods:**

The cause of contamination is affirmed by segregating and refined microorganisms either in fake media or in a living have. Microscopic organisms and parasites are refined in fluid (broth) or strong (agar) fake media. Fluid media give more prominent affectability for the confinement of little numbers of microorganisms. Strong media give disconnected colonies that can be measured on the off chance that fundamental and a few genera and species can be recognized based on their colony morphologies.

Consolidating one or more carbohydrates within the medium together with a reasonable pH marker is utilized to discover differential carbohydrate maturing microorganisms. Such media are called differential media (e.g., eosin methylene blue or MacConkey agar) and are commonly utilized to disconnect Enterobacteriaceae by the color as well as the morphology of colonies.

Culture media can too be made specific by joining compounds such as antimicrobial specialists that repress the inborn greenery while allowing the development of particular microorganisms safe to these inhibitors. One such case is the Thayer-Martin medium, which is utilized to confine Neisseria gonorrhoeae. This medium contains vancomycin to repress Gram-positive microbes, colistin to restrain most Gram-negative bacilli, trimethoprim-sulfamethoxazole to restrain Proteus species and other species that are not hindered by colistin and azinomycin to repress parasites.

Chlamydia and infections are refined in cell culture frameworks, but a few infection confinements require creature vaccination, such as suckling mice, rabbits, guinea pigs, hamsters, or primates. Societies are for the most part hatched at 35 to 37°C within the BOD hatchery depending upon the prerequisites of the microorganism. Clinical examples from bacterial contaminations regularly contain oxygen-consuming, facultatively anaerobic, and anaerobic microbes, such examples are ordinarily immunized into a differential and particular media, which are at that point hatched beneath oxygen-consuming and anaerobic conditions. And The brooding term of societies moreover changes with the development characteristics of the microorganism. Most high-impact and anaerobic microbes will develop overnight, while a few mycobacteria require as numerous as 6 to 8 weeks.



**Figure:4 Different types of solid culture media**



**Figure:5 Liquid culture media (Nutrient Broth)**

1. **Microbial identification:**

Microbial development in societies is illustrated by the appearance of turbidity, and gas arrangement. Recognizable proof of microscopic organisms is based on the development characteristics of the colony and minuscule morphology, biochemical, and physiologic characteristics. The choice and number of tests for bacterial recognizable proof depend upon the category of microbes display and the ability of the microbiologist to analyze the culture. The distinguishing proof of filamentous parasites is based on development characteristics and colony and minuscule morphology. Recognizable proof of infections is based on characteristic cytopathic impacts in cell societies, species-specific antigens, or nucleotide groupings.

 

 **Figure:6 Blood agar beta-hemolytic colony Figure:7 MacConkey agar LF and NLF colony**

1. **Interpretation of culture results:**

Some microorganisms, such as Shigella dysenteriae, Mycobacterium tuberculosis, Coccidioides immitis, and flu disease, are ceaselessly considered clinically vital. Others that commonly are secure components of the intrinsic vegetation of the skin and mucous layers or that are common inside the environment may or may not be clinically critical, depending on the illustration source from which they are detached. Along these lines, their imprisonment from shallow ulcers, wounds, and sputum cannot commonly be deciphered as clinically critical. In any case, they commonly cause contaminations related to intravascular contraptions and inserted prosthetic materials. Specialists must as well consider that the composition of microbial species on the skin and mucous movies may be altered by sickness, organization of anti-microbial, endotracheal or gastric intubation, and the clinic environment.

1. **Serodiagnosis:**

Contamination may be analyzed by a counteracting agent reaction to the contaminating microorganism. The conclusion of hepatitis infection and Epstein-Barr infection contaminations can be made as it were serologically, particularly HIV disease is as a rule analyzed by the location of antibodies to the infection. Even though IgM antibodies may show up generally quickly, getting intense- and convalescent-phase serum tests are as a rule vital to seek for a rising titer of IgG antibodies to the suspected pathogen.



**Figure:8 Serological test**

1. **Antimicrobial susceptibility:**

Defilement may be analyzed by a checking specialist's response to the sullying microorganism. The conclusion of hepatitis disease and Epstein-Barr disease contaminations can be made because it was serologically, especially HIV malady is as a run the show analyzed by the area of antibodies to the disease. Indeed even though IgM antibodies may appear up for the most part rapidly, getting strongly- and convalescent-phase serum tests are as a rule imperative to explore for a rising titer of IgG antibodies to the suspected pathogen.

The obligation of the microbiology research facility incorporates not as it were microbial location and segregation but moreover the assurance of microbial helplessness to antimicrobial operators. Numerous microscopic organisms, in specific, have unpredictable susceptibilities to antimicrobial specialists, and their susceptibilities can be measured in vitro to assist direct the determination of the foremost fitting antimicrobial specialist.

Antimicrobial vulnerability tests are performed by either disk dissemination or a weakening strategy. Standardized suspension of a specific microorganism is immunized onto an agar surface to which paper disks containing different antimicrobial operators are connected. Taking after overnight brooding, any zone breadths of hindrance approximately the disks are measured and the comes about are detailed as demonstrating vulnerability or resistance of the microorganism to each antimicrobial operator tried. The term helpless implies that the microorganism is restrained by a concentration of antimicrobial specialist that can be accomplished in blood with the regularly suggested dosage of the antimicrobial operator and infers that contamination caused by this microorganism may be suitably treated with the antimicrobial specialist. The term safe demonstrates that the microorganism is safe to concentrations of the antimicrobial specialist that can be achieved with ordinary measurements and infers that a disease caused by this microorganism might not be effectively treated with this antimicrobial operator.



**Figure:9 Antibiotic Susceptibility testing**

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