Semester – III

16MBU304B MICROBIAL DIAGNOSIS IN HEALTH CLINIC (3H – 3C)

Instruction Hours / week: L: 3 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

Acquire knowledge to identify the common infectious agents with the help of laboratory procedures and use antimicrobial sensitivity tests to select suitable antimicrobial agents.

OBJECTIVES

To provide an understanding of the natural history of infectious diseases in order to deal with the etiology, laboratory diagnosis, treatment and control of infections in the community

Unit I– Importance of Diagnosis of Diseases

Bacterial, Viral, Fungal and Protozoan Diseases of various human body systems, Disease associated clinical samples for diagnosis.

Unit II – Collection of Clinical Samples

How to collect clinical samples (oral cavity, throat, skin, Blood, CSF, urine and faeces) and precautions required. Method of transport of clinical samples to laboratory and storage.

Unit III – Direct Microscopic Examination and Culture

Examination of sample by staining – Gram stain, Ziehl-Neelson staining for tuberculosis, Giemsa-stained thin blood film for malaria. Preparation and use of culture media - Blood agar, Chocolate agar, Lowenstein-Jensen medium, MacConkey agar, distinct colony properties of various bacterial pathogens.

Unit IV – Serological and Molecular Methods

Serological Methods - Agglutination, ELISA, immune fluorescence, Nucleic acid based methods – PCR, Nucleic acid probes, Typhoid, and HIV

Unit V – Testing for Antibiotic Sensitivity in Bacteria

Importance, Determination of resistance/sensitivity of bacteria using disc diffusion method (Kirby Bauer Method) Determination of minimal inhibitory concentration (MIC) of an antibiotic by broth dilution method.

SUGGESTED READINGS

1. Ananthanarayan R and Paniker CKJ (2009). Textbook of Microbiology, 8th edition, Universities Press Private Ltd.
2. Brooks G.F., Carroll K.C., Butel J.S., Morse S.A. and Mietzner, T.A. (2013). Jawetz, Melnick and Adelberg's Medical Microbiology. 26th edition. McGraw Hill Publication.
3. Randhawa, VS, Mehta G and Sharma KB (2009) Practicals and Viva in Medical Microbiology. 2nd edition, Elsevier India Pvt Ltd.
4. Tille P (2013) Bailey's and Scott's Diagnostic Microbiology, 13th edition, Mosby
5. Collee JG, Fraser, AG, Marmion, BP, Simmons A (2007) Mackie and Mccartney Practical Medical Microbiology, 14th edition, Elsevier.

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II B. Sc Microbiology – Microbial diagnosis in health clinic

Objective of the course

In addition to fulfilling the learning objectives provided by individual lecturers, the student should be able to do the following.

- To provide an understanding of the natural history of infectious diseases in order to deal with the etiology, laboratory diagnosis, treatment and control of infections in the community
- Describe the processes involved in the diagnostic tests.
- Integrate experimental strategies learned in the context of individual body systems into the design of experiments involving other systems.
- Describe the human pathogenesis and other diagnostic methods

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II B. Sc Microbiology –Microbial diagnosis in health clinic
Unit I

LECTURE PLAN

S no	Lecture Duration (Hr)	Topics to be covered	Support Materials
1	1hr	Human bacterial diseases	T1 185-240
2	1hr	Human viral diseases	T1 621-666
3	1hr	Human fungal diseases	T1 489-542
4	1hr	Human protozoan diseases	T1 543-620
5	1hr	Clinical samples for diagnosis	R1 660-663
6	1hr	Diagnostic procedures	R1 664-670
		Total	**6 hrs**

Textbooks :T1-Laboratory diagnosis of infectious diseases-paul.G.Engel

References Books: R1- Medical Microbiology – Sherris

Website :

Journal :

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Medical diagnosis (abbreviated **Dx** or $\mathbf{D_S}$) is the process of determining which disease or condition explains a person's symptoms and signs. It is most often referred to as **diagnosis** with the medical context being implicit. The information required for diagnosis is typically collected from a history and physical examination of the person seeking medical care. Often, one or more **diagnostic procedures**, such as diagnostic tests, are also done during the process. Sometimes Posthumous diagnosis is considered a kind of medical diagnosis.

Diagnosis is often challenging, because many signs and symptoms are nonspecific. For example, redness of the skin (erythema), by itself, is a sign of many disorders and thus doesn't tell the healthcare professional what is wrong. Thus differential diagnosis, in which several possible explanations are compared and contrasted, must be performed. This involves the correlation of various pieces of information followed by the recognition and differentiation of patterns. Occasionally the process is made easy by a sign or symptom (or a group of several) that is pathognomonic.

Diagnosis is a major component of the procedure of a doctor's visit. From the point of view of statistics, the diagnostic procedure involves classification tests.

History of medical diagnosis

The **history of medical diagnosis** began in earnest from the days of Imhotep in ancient Egypt and Hippocrates in ancient Greece but is far from perfect despite the enormous bounty of information made available by medical research including the sequencing of the human genome. The practice of diagnosis continues to be dominated by theories set down in the early 20th century.

Ancient Egypt

An Egyptian medical textbook, the Edwin Smith Papyrus written by Imhotep (fl. 2630-2611 BC), was the first to apply the method of diagnosis to the treatment of disease.[

Ancient China

Predated by Babylonian and Egyptian medicine, traditional Chinese medicine (TCM) was described in an ancient Chinese text, the Yellow Emperor's Inner Canon or Huangdi Neijing which dates to the first[6] or second[7] century BCE. The four diagnostic methods of TCM[8] which are still being practiced today are inspection,[9] listening and smelling,[10] inquiry[11] and palpation.[12]

Ancient Greece

Over two thousand years ago, Hippocrates recorded the association between disease and heredity. In similar fashion, Pythagoras noted the association between metabolism and heredity (allergy to Fava beans). The medical community, however, has only recently acknowledged the importance of genetics and its relevance to mainstream medicine.

Islamic world

The Arabic physician, Abu al-Qasim al-Zahrawi (Abulcasis), wrote on hematology in his *Al-Tasrif* (1000). He provided the first description on haemophilia, a hereditary genetic disorder, in which he wrote of an Andalusian family whose males died of bleeding after minor injuries.[13]

The Persian physician, Ibn Sina (Avicenna, 980-1037), in *The Canon of Medicine* (1025), pioneered the idea of a syndrome in the diagnosis of specific diseases.[14]

Middle Ages

The analysis of urine

Physicians used many different techniques to analyze the imbalance of the four humours in the body. Urosocpy was most widely used for diagnosing illness. Physicians would collect patient's urine in a flask called "matula". The matula was specific in shape and had four regions – circulus, superficies, substantia, and fundus – that corresponded to regions of the body. The circulus corresponded to the head; the superficies corresponded to the

chest; the substantia corresponded to the abdomen; the fundus corresponded to the reproductive and urinary organs. Urine was inspected based on four criteria: color, consistency, odor, and presence of precipitate. Physicians analyzed the urine for the four criteria and used that to point out where there was an imbalance of the four humours based on the location in the matula.[17] Physicians also examined blood via phlebotomy, they would observe the viscosity and color of the blood as it was draining from the patient and/or contained in a vial. The color and viscosity denoted whether the patient had an acute, major, or chronic disease; which also assisted the physician with the next course of action.[18] Physicians would also observe a patients pulse by palpation; this technique was performed by carefully noting the rate, power, and tempo of a pulsing artery. By interpreting the pulse of the physician could diagnose the type of fever the patient had.[19] Astrological diagnosis was the least used technique for diagnosing illness. Diagnosis was based on the position of the moon in relation to the constellations, which were associated with different regions of the body (head, arms, chest, etc.).[20] Physicians would diagnose illness with the combined knowledge of zodiac signs and humoral medicine.[21]

19th century

In the first half of the 19th century, the well-known British physiologist Marshall Hall emphasized the necessity of maintaining a close relationship between the theory and practice in medicine.[22] He wrote *On diagnosis* (1817) and *The Principles of Diagnosis* (1834).

The Oslerian ideal

The ideals of William Osler, who transformed the practice of medicine in the early 1900s, were based on the principles of the diagnosis and treatment of disease. According to Osler, the functions of a physician were to be able to identify disease and its manifestations and to understand its mechanisms and how it may be prevented or cured. For his medical students he believed that the best textbook was the patient himself—analysis of morbid anatomy and pathology were the keys. The Oslerian ideal continues today as the basis of the doctor's strategy is, "What disease does this patient have, and what is the best way for treatment?" The emphasis is on the classification of the disease in order to use the remedies available for its effects to be reversed or ameliorated. The human being in question is representative of a class of people with this type of disease; this person's biological individuality is not given any great weight.

Present-day Oslerian practice

Whereas Osler laid the founding principles by which medicine should be practiced, Garrod placed these principles in a greater context of a chemical individuality that is inherited and is subject to the mechanisms of evolutionary selection. The Oslerian ideal of medical practice continues to dominate medical philosophy today. The patient is a collective of symptoms to be characterized and analyzed algorithmically in order to draw a diagnosis and subsequently produce a strategy of treatment. Medicine is about problems based solutions. In keeping with this philosophy, today's pathology reports provide a momentary snapshot of the patient's biochemical profile, highlighting the end result of the disease process.

Influence of DNA technology

Garrod's conception of biological individuality was confirmed with the advent of the sequencing of the human genome. Finally the subtle relationship between inheritance, individuality and environment became apparent via the variations detected in DNA. In each patient's DNA lies a script for how their bodies will change and become ill as well as how they will handle the assaults of the environment from the beginning of their life to its end. It is hoped that by knowing a patient's genes that the biological strengths and weaknesses in respect to these assaults will be revealed and disease processes can be predicted before they have the opportunity to manifest. Although knowledge in this area is far from complete, there are already medical interventions based on this. More importantly, the physician, forewarned with this knowledge can guide the patient towards appropriate lifestyle changes to anticipate and mitigate disease processes.

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Medical uses

A diagnosis, in the sense of diagnostic procedure, can be regarded as an attempt at classification of an individual's condition into separate and distinct categories that allow medical decisions about treatment and prognosis to be made. Subsequently, a diagnostic opinion is often described in terms of a disease or other condition, but in the case of a wrong diagnosis, the individual's actual disease or condition is not the same as the individual's diagnosis.

A diagnostic procedure may be performed by various health care professionals such as a physician, physical therapist, optometrist, healthcare scientist, chiropractor, dentist, podiatrist, nurse practitioner, or physician assistant. This article uses *diagnostician* as any of these person categories.

A diagnostic procedure (as well as the opinion reached thereby) does not necessarily involve elucidation of the etiology of the diseases or conditions of interest, that is, what *caused* the disease or condition. Such elucidation can be useful to optimize treatment, further specify the prognosis or prevent recurrence of the disease or condition in the future.

The initial task is to detect a medical indication to perform a diagnostic procedure. Indications include:

- Detection of any deviation from what is known to be normal, such as can be described in terms of, for example, anatomy (the structure of the human body), physiology (how the body works), pathology (what can go wrong with the anatomy and physiology), psychology (thought and behavior) and human homeostasis (regarding mechanisms to keep body systems in balance). Knowledge of what is normal and measuring of the patient's current condition against those norms can assist in determining the patient's particular departure from homeostasis and the degree of departure, which in turn can assist in quantifying the indication for further diagnostic processing.
- A complaint expressed by a patient.
- The fact that a patient has sought a diagnostician can itself be an indication to perform a diagnostic procedure. For example, in a doctor's visit, the physician may already start performing a diagnostic procedure by watching the gait of the patient from the waiting room to the doctor's office even before she or he has started to present any complaints.

Even during an already ongoing diagnostic procedure, there can be an indication to perform another, separate, diagnostic procedure for another, potentially concomitant, disease or condition. This may occur as a result of an incidental finding of a sign unrelated to the parameter of interest, such as can occur in comprehensive tests such as radiological studies like magnetic resonance imaging or blood test panels that also include blood tests that are not relevant for the ongoing diagnosis.

Procedure

General components which are present in a diagnostic procedure in most of the various available methods include:

- Complementing the already given information with further data gathering, which may include questions of the medical history (potentially from other people close to the patient as well), physical examination and various diagnostic tests.
  A diagnostic test is any kind of medical test performed to aid in the diagnosis or detection of disease. Diagnostic tests can also be used to provide prognostic information on people with established disease.[6]
- Processing of the answers, findings or other results. Consultations with other providers and specialists in the field may be sought.

There are a number of methods or techniques that can be used in a diagnostic procedure, including performing a differential diagnosis or following medical algorithms.[7] In reality, a diagnostic procedure may involve components of multiple methods.[8]

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Differential diagnosis

The method of differential diagnosis is based on finding as many diseases or conditions as possible that can possibly cause the signs or symptoms, followed by a process of elimination or at least of rendering the entries more or less probable by further medical tests and other processing until, aiming to reach the point where only one disease or condition remains as probable. The final result may also remain a list of possible conditions, ranked in order of probability or severity.

The resultant diagnostic opinion by this method can be regarded more or less as a diagnosis of exclusion. Even if it doesn't result in a single probable disease or condition, it can at least rule out any imminently life-threatening conditions.

Unless the provider is certain of the condition present, further medical tests, such as medical imaging, are performed or scheduled in part to confirm or disprove the diagnosis but also to document the patient's status and keep the patient's medical history up to date.

If unexpected findings are made during this process, the initial hypothesis may be ruled out and the provider must then consider other hypotheses.

Pattern recognition

In a pattern recognition method the provider uses experience to recognize a pattern of clinical characteristics.[7] It is mainly based on certain symptoms or signs being associated with certain diseases or conditions, not necessarily involving the more cognitive processing involved in a differential diagnosis.

This may be the primary method used in cases where diseases are "obvious", or the provider's experience may enable him or her to recognize the condition quickly. Theoretically, a certain pattern of signs or symptoms can be directly associated with a certain therapy, even without a definite decision regarding what is the actual disease, but such a compromise carries a substantial risk of missing a diagnosis which actually has a different therapy so it may be limited to cases where no diagnosis can be made.

Diagnostic criteria

The term *diagnostic criteria* designates the specific combination of signs, symptoms, and test results that the clinician uses to attempt to determine the correct diagnosis.

Some examples of diagnostic criteria, also known as clinical case definitions, are:

- Amsterdam criteria for hereditary nonpolyposis colorectal cancer
- McDonald criteria for multiple sclerosis
- ACR criteria for systemic lupus erythematosus
- Centor criteria for strep throat

Clinical decision support system

Clinical decision support systems are interactive computer programs designed to assist health professionals with decision-making tasks. The clinician interacts with the software utilizing both the clinician’s knowledge and the software to make a better analysis of the patients data than either human or software could make on their own. Typically the system makes suggestions for the clinician to look through and the clinician picks useful information and removes erroneous suggestions.[9]

Other diagnostic procedure methods

Other methods that can be used in performing a diagnostic procedure include:

An example of a medical algorithm for assessment and treatment of overweight and obesity.

- Usage of medical algorithms
- An "exhaustive method", in which every possible question is asked and all possible data is collected.[7]This is often referred to as a "diagnostic workup".[10]

Adverse effects

Diagnosis problems are the dominant cause of medical malpractice payments, accounting for 35% of total payments in a study of 25 years of data and 350,000 claims.[11]

Overdiagnosis

Overdiagnosis is the diagnosis of "disease" that will never cause symptoms or death during a patient's lifetime. It is a problem because it turns people into patients unnecessarily and because it can lead to economic waste (overutilization) and treatments that may cause harm. Overdiagnosis occurs when a disease is diagnosed correctly, but the diagnosis is irrelevant. A correct diagnosis may be irrelevant because treatment for the disease is not available, not needed, or not wanted.

Errors

Most people will experience at least one diagnostic error in their lifetime, according to a 2015 report by the National Academies of Sciences, Engineering, and Medicine.[12]

Causes and factors of error in diagnosis are:[13]

- the manifestation of disease are not sufficiently noticeable
- a disease is omitted from consideration
- too much significance is given to some aspect of the diagnosis
- the condition is a rare disease with symptoms suggestive of many other conditions
- the condition has a rare presentation

Lag time

When making a medical diagnosis, a **lag time** is a delay in time until a step towards diagnosis of a disease or condition is made. Types of lag times are mainly:

- *Onset-to-medical encounter lag time*, the time from onset of symptoms until visiting a health care provider[14]
- *Encounter-to-diagnosis lag time*, the time from first medical encounter to diagnosis[14]

Society and culture

Etymology

The plural of diagnosis is *diagnoses*. The verb is *to diagnose*, and a person who diagnoses is called a *diagnostician*. The word *diagnosis* /daɪ.əg□nəʊs□s/is derived through Latin from the Greek word διάγνωσις from διαγιγνώσκειν, meaning "to discern, distinguish".[15]

Medical diagnosis or the actual process of making a diagnosis is a cognitive process. A clinician uses several sources of data and puts the pieces of the puzzle together to make a diagnostic impression. The initial diagnostic impression can be a broad term describing a category of diseases instead of a specific disease or condition. After the initial diagnostic impression, the clinician obtains follow up tests and procedures to get more data to support or reject the original diagnosis and will attempt to narrow it down to a more specific level. Diagnostic procedures are the specific tools that the clinicians use to narrow the diagnostic possibilities.

Social context

Diagnosis can take many forms.[16] It might be a matter of naming the disease, lesion, dysfunction or disability. It might be a management-naming or prognosis-naming exercise. It may indicate either degree of abnormality on a continuum or kind of abnormality in a classification. It's influenced by non-medical factors such as power, ethics and financial incentives for patient or doctor. It can be a brief summation or an extensive formulation, even taking the form of a story or metaphor. It might be a means of communication such as a computer code through which it triggers payment, prescription, notification, information or advice. It might be pathogenic or salutogenic. It's generally uncertain and provisional.

Once a diagnostic opinion has been reached, the provider is able to propose a management plan, which will include treatment as well as plans for follow-up. From this point on, in addition to treating the patient's

condition, the provider can educate the patient about the etiology, progression, prognosis, other outcomes, and possible treatments of her or his ailments, as well as providing advice for maintaining health.

A treatment plan is proposed which may include therapy and follow-up consultations and tests to monitor the condition and the progress of the treatment, if needed, usually according to the medical guidelines provided by the medical field on the treatment of the particular illness.

Relevant information should be added to the medical record of the patient.

A failure to respond to treatments that would normally work may indicate a need for review of the diagnosis.

Concepts related to diagnosis

Sub-types of diagnoses include:

Clinical diagnosis

A diagnosis made on the basis of medical signs and patient-reported symptoms, rather than diagnostic tests

Laboratory diagnosis

A diagnosis based significantly on laboratory reports or test results, rather than the physical examination of the patient. For instance, a proper diagnosis of infectious diseases usually requires both an examination of signs and symptoms, as well as laboratory characteristics of the pathogen involved.

Radiology diagnosis

A diagnosis based primarily on the results from medical imaging studies. Greenstick fractures are common radiological diagnoses.

Principal diagnosis

The single medical diagnosis that is most relevant to the patient's chief complaint or need for treatment. Many patients have additional diagnoses.

Admitting diagnosis

The diagnosis given as the reason why the patient was admitted to the hospital; it may differ from the actual problem or from the *discharge diagnoses*, which are the diagnoses recorded when the patient is discharged from the hospital.

Differential diagnosis

A process of identifying all of the possible diagnoses that could be connected to the signs, symptoms, and lab findings, and then ruling out diagnoses until a final determination can be made.

Diagnostic criteria

Designates the combination of signs, symptoms, and test results that the clinician uses to attempt to determine the correct diagnosis. They are standards, normally published by international committees, and they are designed to offer the best sensitivity and specificity possible, respect the presence of a condition, with the state-of-the-art technology.

Prenatal diagnosis

Diagnosis work done before birth

Diagnosis of exclusion

A medical condition whose presence cannot be established with complete confidence from history, examination or testing. Diagnosis is therefore by elimination of all other reasonable possibilities.

Dual diagnosis

The diagnosis of two related, but separate, medical conditions or co-morbidities; the term almost always refers to a diagnosis of a serious mental illness and a substance addiction.

Self-diagnosis

The diagnosis or identification of a medical conditions in oneself. Self-diagnosis is very common.

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Remote diagnosis

A type of telemedicine that diagnoses a patient without being physically in the same room as the patient.

Nursing diagnosis

Rather than focusing on biological processes, a nursing diagnosis identifies people's responses to situations in their lives, such as a readiness to change or a willingness to accept assistance.

Computer-aided diagnosis

Providing symptoms allows the computer to identify the problem and diagnose the user to the best of its ability. Health screening begins by identifying the part of the body where the symptoms are located; the computer cross-references a database for the corresponding disease and presents a diagnosis.[17]

Overdiagnosis

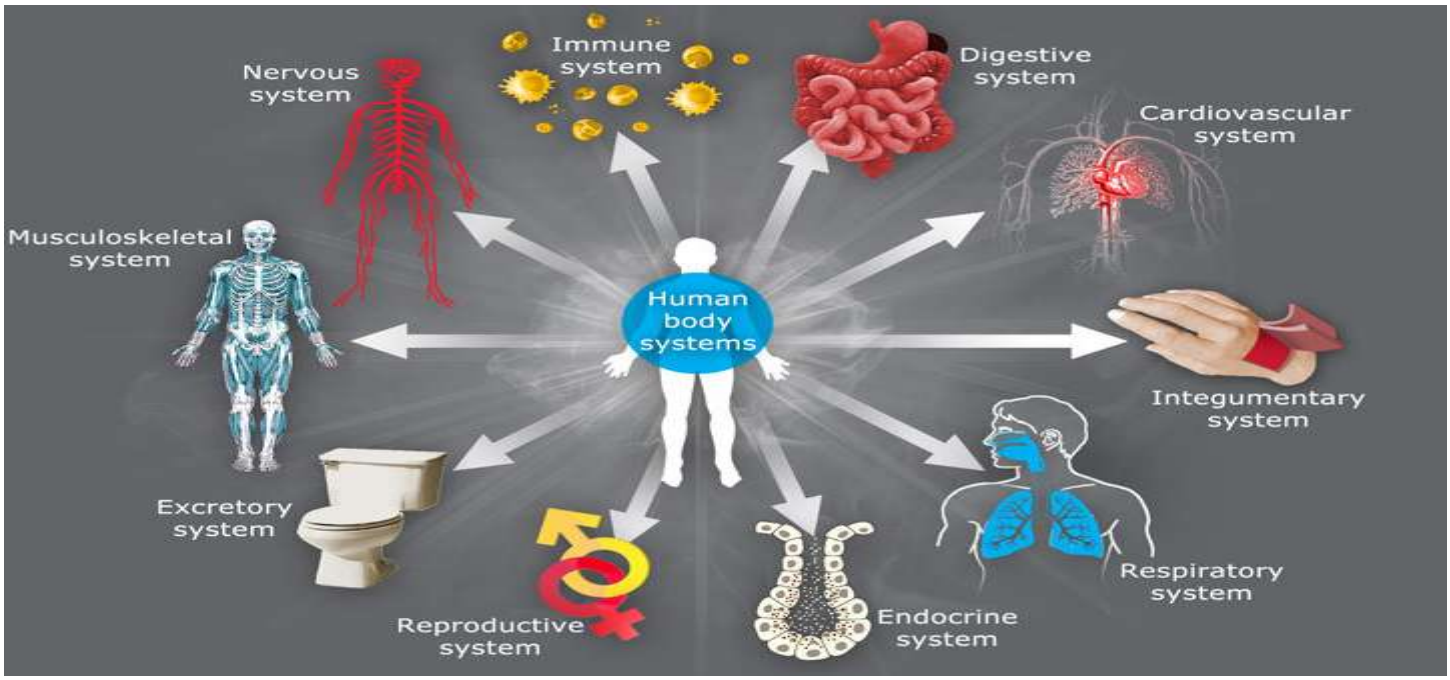
The diagnosis of "disease" that will never cause symptoms, distress, or death during a patient's lifetime

Wastebasket diagnosis

A vague, or even completely fake, medical or psychiatric label given to the patient or to the medical records department for essentially non-medical reasons, such as to reassure the patient by providing an official-sounding label, to make the provider look effective, or to obtain approval for treatment. This term is also used as a derogatory label for disputed, poorly described, overused, or questionably classified diagnoses, such as pouchitis and senility, or to dismiss diagnoses that amount to overmedicalization, such as the labeling of normal responses to physical hunger as reactive hypoglycemia.

Retrospective diagnosis

The labeling of an illness in a historical figure or specific historical event using modern knowledge, methods and disease classifications.



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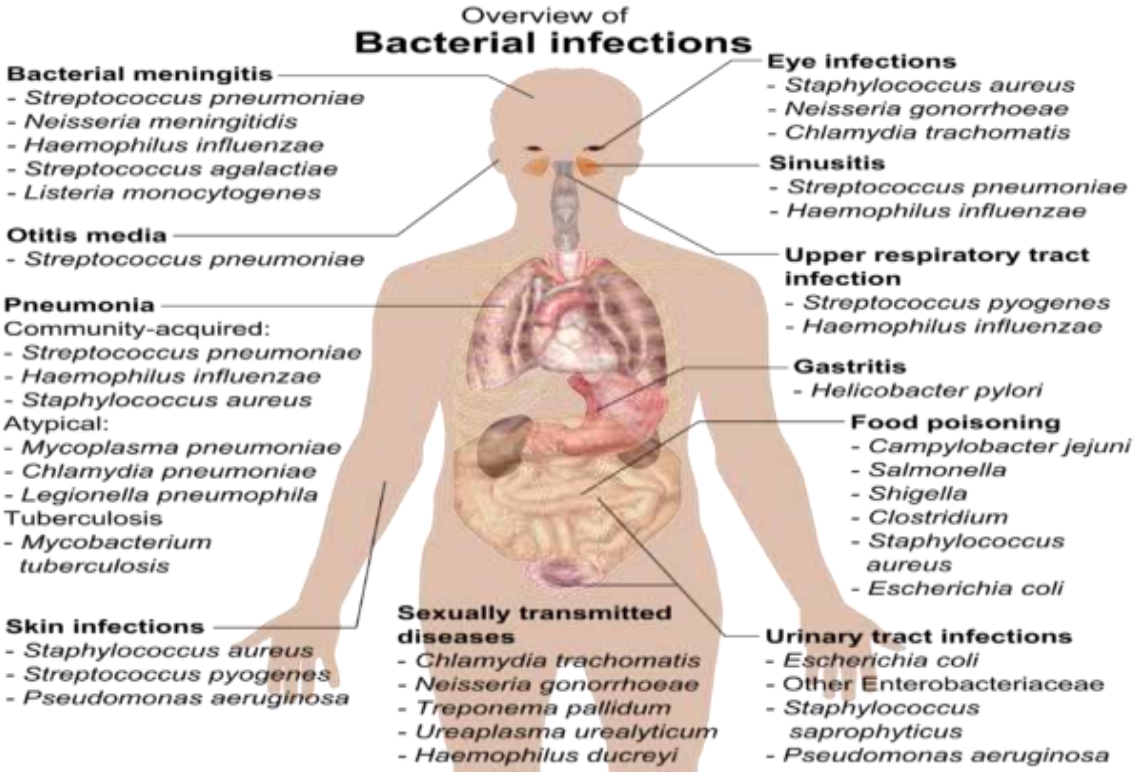

List of systems of the human body

The main systems of the human body are:

1. Cardiovascular / Circulatory system:
    1. Circulates blood around the body via the heart, arteries and veins, delivering oxygen and nutrients to organs and cells and carrying their waste products away.
2. Digestive system / Excretory system:
    1. Mechanical and chemical processes that provide nutrients via the mouth, esophagus, stomach and intestines.
    2. Eliminates waste from the body.
3. Endocrine system:
    1. Provides chemical communications within the body using hormones.
4. Integumentary system/ Exocrine system:
    1. Skin, hair, nails, sweat and other exocrine glands.
5. Lymphatic system / Immune system:
    1. The system comprising a network of lymphatic vessels that carry a clear fluid called lymph.
    2. Defends the body against disease-causing agents.
6. Muscular system/Skeletal system:
    1. Enables the body to move using muscles.
    2. Bones supporting the body and its organs.
7. Nervous system:
    1. Collects and processes information from the senses via nerves and the brain and tells the muscles to contract to cause physical actions.
8. *Renal system / Urinary system*:
    1. The system where the kidneys filter blood.
9. Reproductive system:
    1. The sex organs required for the production of offspring.

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10. Respiratory system
    1. The lungs and the trachea that bring air into the body.

Human Body Organ Systems

The human body is made up of 11 organ systems that work with one another (interdependantly). These systems include the integumentary system, skeletal system, muscular system, lymphatic system, respiratory system, digestive system, nervous system, endocrine system, cardiovascular system, urinary system, and reproductive systems. We will briefly discuss the major functions of each organ system below.

Integumentary system– (skin, hair, nails) Forms the external body covering and protects deeper tissues from injury. Houses cutaneous receptors, sweat glands, oil glands, and synthesizes vitamin D.

Skeletal system– (bones, joints) Supports and protects the body's organs. Provides a framework muscles use (movement). Bones also store minerals and create blood cells.

Muscular system– (skeletal muscles) Maintains posture and produces movement (locomotion). Produces heat.

Lymphatic system– (red bone marrow, thymus, lymphatic vessels, thoracic duct, spleen, lymph nodes) Houses white blood cells (lymphocytes) involved in immunity. Returns leaked fluid from blood vessels to the blood and disposes debris within the lymphatic stream.

Respiratory system– (nasal cavity, pharynx, larynx, trachea, bronchus, lung) Removes carbon dioxide and continually supplies blood with oxygen. Gaseous exchanges occur in the respiratory system (lungs).

Digestive system– (oral cavity, oesophagus, liver, stomach, small intestine, large intestine, rectum, anus) Breaks down food to be absorbed and eliminates indigestible waste.

Nervous system– (brain, spinal cord, nerves) Control system of the body, responds to internal and external changes, activates muscles and glands.

Endocrine system– (pineal gland, pituitary gland, thyroid gland, thymus, adrenal gland, pancreas, ovary, testis) Glands from the endocrine system secrete hormones that regulate many processes like growth, metabolism, and reproduction.

Cardiovascular system– (heart, blood vessels) The heart pumps blood and blood vessels transport it. Blood carries oxygen, carbon dioxide, nutrients, waste and more throughout the body.

Urinary system– (kidney, ureter, urinary bladder, urethra) Eliminates nitrogenous wastes from the body. Regulates acid-base, electrolyte and WATER balance of blood.

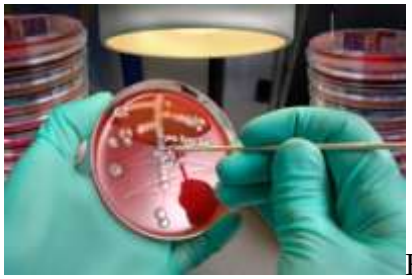
Reproductive systems

MALE (prostate gland, penis, testis, scrotum, ductus deferens)

FEMALE (Mammary glands, ovary, uterus, vagina, uterine tube)

The main function of the reproductive system is to produce offspring. Sex hormone and sperm are produced by the male testes. Male ducts and glands help deliver the sperm. Ovaries produce female sex hormones and eggs. Other female reproductive structures serve as sites of fertilization and development. For instance, the mammary glands produce milk for the newborn.

What are Bacteria?



Bacteria are microscopic, single-cell organisms that live almost everywhere. Bacteria live in every climate and location on earth. Some are airborne while others live in water or soil.

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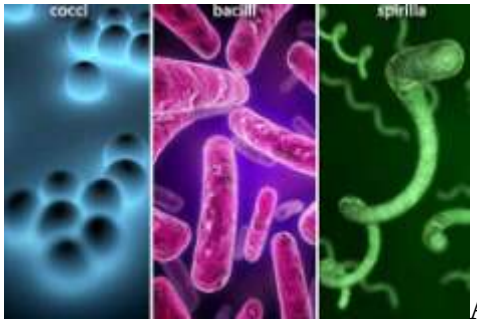
Bacteria live on and inside plants, animals, and people. The word "bacteria" has a negative connotation, but bacteria actually perform many vital functions for organisms and in the environment. For example, plants need bacteria in the soil in order to grow.

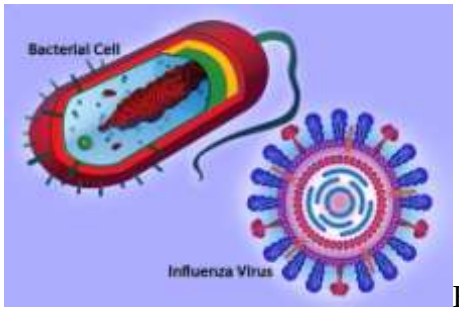
The vast majority of bacteria are harmless to people and some strains are even beneficial. In the human gastrointestinal tract, good bacteria aid in digestion and produce vitamins. They also help with immunity, making the body less hospitable to bad bacteria and other harmful pathogens. When considering all the strains of bacteria that exist, relatively few are capable of making people sick.

What Is a Bacterial Infection?



A bacterial infection is a proliferation of a harmful strain of bacteria on or inside the body. Bacteria can infect any area of the body. Pneumonia, meningitis, and food poisoning are just a few illnesses that may be caused by harmful bacteria. Bacteria come in three basic shapes: rod-shaped (bacilli), spherical (cocci), or helical (spirilla). Bacteria may also be classified as gram-positive or gram-negative. Gram-positive bacteria have a thick cell wall while gram-negative bacteria do not. Gram staining, bacterial culture with antibiotic sensitivity determination, and other tests are used to identify bacterial strains and help determine the appropriate course of treatment.

Bacteria vs. Virus



Bacteria and viruses are different types of pathogens, organisms that can cause disease. Bacteria are larger than viruses and are capable of reproducing on their own. Viruses are much smaller than bacteria and cannot reproduce on their own. Instead, viruses reproduce by infecting a host and using the host's DNA repair and replication systems to make copies of itself.

The symptoms of a bacterial or viral infection depend on the area of the body that is affected. Sometimes the symptoms of the two can be very similar. For example, runny nose, cough, headache, and fatigue can occur with the common cold (virus) and with a sinus infection (bacteria). A doctor may use the presence of other symptoms (such as fever or body aches), the length of the illness, and certain lab tests to determine if an illness is due to a virus, bacteria, or some other pathogen or disease process.

Bacterial Skin Infections

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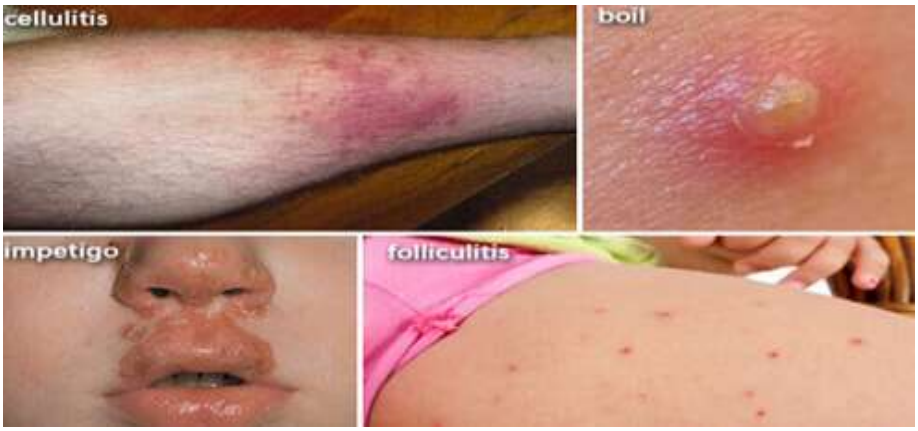

Bacterial skin infections are usually caused by gram-positive strains of Staphylococcus and Streptococcus or other organisms. Common bacterial skin infections include:

- **Cellulitis** causes a painful, red infection that is usually warm to the touch. Cellulitis occurs most often on the legs, but it can appear anywhere on the body.
- **Folliculitis** is an infection of the hair follicles that causes red, swollen bumps that look like pimples. Improperly treated pools or hot tubs can harbor bacteria that cause folliculitis.
- **Impetigo** causes oozing sores, usually in preschool-aged children. The bullous form of impetigo causes large blisters while the non-bullous form has a yellow, crusted appearance.
- **Boils** are deep skin infections that start in hair follicles. Boils are firm, red, tender bumps that progress until pus accumulates underneath the skin.

Bacterial skin infections are treated with oral or topical antibiotics depending on the strain causing the infection.

Foodborne Bacterial Infections

Bacterial infections are one cause of foodborne illness. Nausea, vomiting, diarrhea, fever, chills, and abdominal pain are common symptoms of food poisoning. Raw meat, fish, eggs, poultry, and unpasteurized dairy may harbor harmful bacteria that can cause illness. Unsanitary food preparation and handling can also encourage bacterial growth. Bacteria that cause food poisoning include:

- ***Campylobacter jejuni (C. jejuni)*** is a diarrheal illness often accompanied by cramps and fever.
- ***Clostridium botulinum (C. botulinum)*** is a potentially life-threatening bacterium that produces powerful neurotoxins.
- ***Escherichia coli (E. coli)* O157:H7** is a diarrheal (often bloody) illness that may be accompanied by nausea, vomiting, fever, and abdominal cramps.
- ***Listeria monocytogenes (L. monocytogenes)*** causes fever, muscle aches, and diarrhea. Pregnant women, elderly individuals, infants, and those with weakened immune systems are most at risk for acquiring this infection.
- ***Salmonella*** causes fever, diarrhea, and abdominal cramps. Symptoms typically last between 4 and 7 days.

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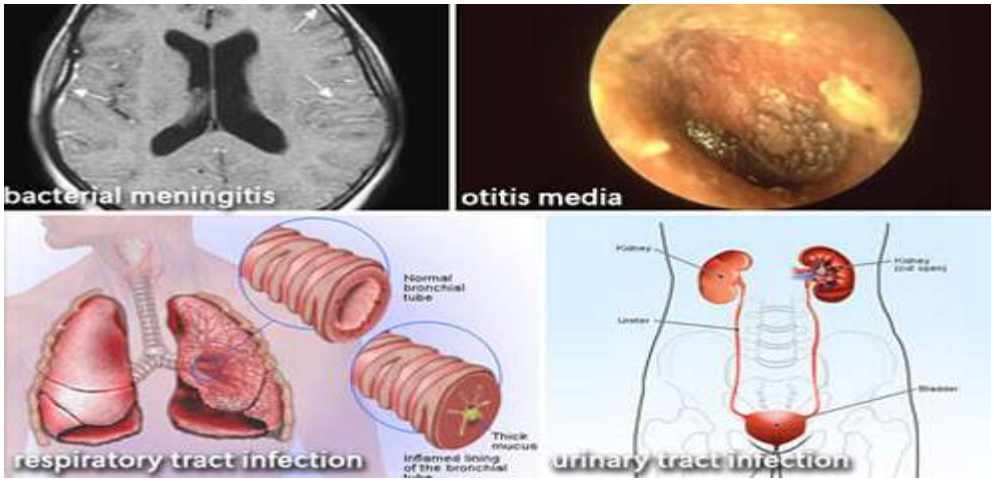
- ***Vibrio*** causes diarrhea when ingested, but it can also cause severe skin infections when it comes in contact with an open wound.

Sexually Transmitted Bacterial Infections

Many sexually transmitted diseases (STDs) are caused by harmful bacteria. Sometimes, these infections aren't associated with any symptoms but can still cause serious damage to the reproductive system. Common STDs caused by bacterial infections include:

- **Chlamydia** is an infection in men and women caused by an organism called Chlamydia trachomatis. Chlamydia increases the risk of pelvic inflammatory disease (PID) in women.
- **Gonorrhea**, also known as "clap" and "the drip," is caused by Neisseria gonorrhoeae. Men and women can be infected. Gonorrhea also increases the risk of pelvic inflammatory disease (PID) in women.
- **Syphilis** can affect men and women and is caused by the bacteria Treponema pallidum. Untreated, syphilis is potentially very dangerous and can even be fatal.
- **Bacterial** vaginosis, which causes an overgrowth of pathogenic bacteria in the vagina (the CDC does not consider this a STD; see second text reference).

Other Bacterial Infections



Harmful bacteria can affect almost any area of the body. Other types of bacterial infections include:

- **Bacterial meningitis** is a severe infection of the meninges, the lining of the brain.
- **Otitis media** is the official name for an infection or inflammation of the middle ear. Both bacteria and viruses can cause ear infections, which commonly occur in babies and small children.
- **Urinary tract infection (UTI)** is a bacterial infection of the bladder, urethra, kidneys, or ureters.
- **Respiratory tract infections** include sore throat, bronchitis, sinusitis, and pneumonia. Bacteria or viruses may be responsible for respiratory tract infections. Tuberculosis is a type of bacterial lower respiratory tract infection.

Antibiotics

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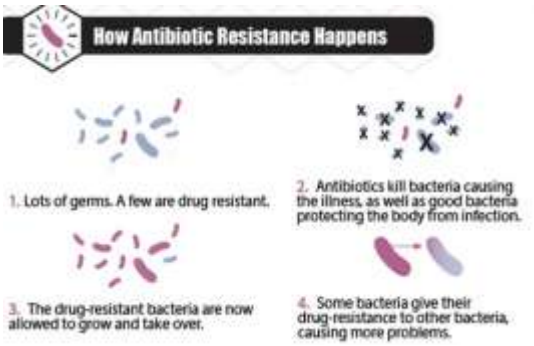
Antibiotics are medications that fight bacterial infections. They work by disrupting the processes necessary for bacterial cell growth and proliferation. It's important to take antibiotics exactly as prescribed. Failure to do so could make a bacterial infection worse. Antibiotics don't treat viruses, but they're sometimes prescribed in viral illnesses to help prevent a "secondary bacterial infection." Secondary infections occur when someone is in a weakened or compromised state due to an existing illness.

Antibiotic Resistance



Overuse and misuse of antibiotics has led to a rise in antibiotic resistance. Antibiotic resistance occurs when bacteria are no longer sensitive to a medication that should eliminate an infection. Antibiotic-resistant bacterial infections are potentially very dangerous and increase the risk of death. About 2 million people in the U.S. suffer from antibiotic resistant infections each year and 23,000 die due to the condition. The CDC estimates 14,000 deaths alone are due to Clostridium difficile (C. difficile) infections that occur because of antibiotic suppression of other bacteria allow C.difficile to proliferate. Most deaths due to antibiotic resistant infections occur in hospitalized patients and those who are in nursing homes.

Good Bacteria and Probiotics

Beneficial bacteria live in the human gastrointestinal (GI) tract and play an important role in digestion and immunity. Most people know it's smart to eat yogurt after completing a course of antibiotics to repopulate the GI tract with helpful bacteria that were wiped out from the antibiotics.

Some studies have shown probiotics can shorten the duration of infectious diarrhea. They may also reduce the risk of developing diarrheal illness due to antibiotic use. Probiotics seem to reduce gas, bloating, and abdominal pain associated with irritable bowel syndrome (IBS). Ongoing research seeks to determine the types and dosages of bacteria that are most beneficial to human health.

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16MBU304A
II BSC Microbiology
Microbial diagnosis in health clinic

Unit I Qu	Opt 1	Opt 2	Opt 3	Opt 4	Answer
The sputun	10C	5C	4C	8C	4C
the blood s	late	early	before diag	after symptom	early
When a pa	pathogen	infection	organism	diagnose	infection
For microb	good	sterile	normal	clean catch mid-stream	clean catch mid-stream
Cary-Blair r	blood	CSF	stool	urine	stool
a parasite c	pathagon	parasite	bacteria	virus	pathagon
the ability c	diagnosis	symptoms	pathogenic	disease	pathogenicity
The most ir	Specimen	Organism	Symptoms	Prophylaxis	Specimen
In case of r	blood	cerebrospi	urine	pus	cerebrospinal fluid
in blood sp	haemoglob	serum	iron	protein	serum
The collect	room temp	4C	2C	5C	room temperature
	agar mediu	peptone w	Cary-Blair r	alkaline medium	Cary-Blair medium
An alternat	alkaline-pe	peptone w	alkaline me	water	alkaline-peptone water
A sterile	container	plastic cont	screw-cap c	metal container	screw-cap container
Specimens	Vaccines	Antimicrob	Symptoms	Culturing	Antimicrobial drugs
To eliminat	Germicide	Soap	Vaseline	Cotton	Germicide
Pus swabs	2	4	6	8	6
During bloc	Isopropyl a	Antiseptic	Soap	Antibiotic	Isopropyl alcohol
About ___	10	20	30	40	20
the blood s	25C	37C	4C	10C	37C
_____ ml o	10	40	30	20	20
In case of _	Arthritis	Gas gangre	Renal failur	Paralysis	Renal failure
If the urine	Amino acid	Boric acid	Sodium chl	Calcium chloride	Boric acid
In case of s	1	2	3	4	3
The CSF sh	Cerebrum	Cerebellum	Ventricle	Arachnoid space	Arachnoid space
In case of i	Ventricle	Arachnoid	Cerebrum	Cerebellum	Ventricle
About ___	10	30	40	20	20
For collecti	Sterile cath	Syringe	Swab	Cotton	Sterile catheter
_____ i	Pus	Mucous	Conjuctival	Conjunctival tissue	Conjuctival scrapings
Respiratory	1	2	3	4	2
The natura	Antibiotic	Toxins	Antibacteri	Amylase	Antibacterial
If not possi	Cotton wol	Syringe	Catheter	Cotton	Cotton woll swab
Salmonella	24	48	32	76	48
Campyloba	2	4	6	8	6
If cholera is	1	3	5	8	8
_____ i	CB medium	Boric acid	Alkaline pe	Phosphate buffered saline	Alkaline peptone water
For suspect	Phosphate	Alkaline pe	CB medium	Boric acid	Phosphate buffered sal
About ___	3	6	9	12	9

_____ is	Trisodium c	Sodium chl	Boric acid	Calcium chloride	Trisodium citrate
About ____	2 to 3	5 to 6	3 to 5	4 to 6	2 to 3
Synovial, p	Antibiotic	Antiseptic	Anticoagula	Antibacterial	Anticoagulant
In case of s	morning	Evening	Mid night	Noon	morning
_____ ba	Rod shaped	Spindle sha	Club shape	Acid-fast	Acid-fast
In suspecte	Lesions	Scrapings	Edema	Necrosis	Lesions
For darkfie	Salt solutio	Saline solut	Anticoagula	Antiseptic	Saline solution
In case of t	Stool	Urine	Blood	Sputum	Sputum
The sputun	Saline solut	Anticoagula	Formalin so	Salt solution	Formalin solution
_____ tec	Ziehl Neels	Gram stain	Endospore	Quellung	Ziehl Neelsen
In bacilliary	Smears	Swabs	Rectal swa	Renal smears	Rectal swabs
In case of s	Urine	Stool	Sputum	Blood	Blood
In case of t	Sputum	Blood	Faeces	CSF	Blood
In order to	Thioglycolla	Sodium citr	Buffered gl	Para amino benzoic acid	Thioglycollate semisoli
A n alterna	Sodium citr	Buffered gl	Para amino	Thioglycollate semisolid medium	Buffered glycerol wate
_____ s	Trisodium c	Formalin so	Sodium citr	Para amino benzoic acid	Para amino benzoic aci
_____ i	Sodium citr	Thioglycolla	Antiseptic	Antibacterial	Sodium citrate
Once the s	Labelled	Opened	Diluted	Defined	Labelled
Samples of	Labelled	Refrigerate	Processed	Incubated	Refrigerated
The sputun	Blood	Pus	Saliva	Tissue	Saliva
when the p	blood	Pus	Sputum	urine	urine
In case of s	blood	Pus	Sputum	urine	blood

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II B. Sc Microbiology – Microbial diagnosis in health clinic

Unit I possible questions

Part B

1. Define microbial diagnosis.
2. Say about bacteria
3. What is disease and infection?
4. What is lab diagnosis?
5. What are MIC and MBC?

Part C

1. give a detailed note on diagnosis and its types
2. Write about the transport media and its importance.
3. Discuss about the investigation by culturing.
4. Describe various sources and methods of infection.
5. Comment on the ELISA
6. Give the laboratory diagnosis of Acid fast bacilli.
7. State the pathogenic role and diagnosis of GI tract pathogenesis.
8. Discuss the properties and UTI caused by *E.Coli*.
9. Explain in detail about Kirby bauer technique of susceptibility testing.
10. Comment on respiratory tract infection and its symptoms.

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II B. Sc Microbiology –Microbial diagnosis in health clinic
Unit II

LECTURE PLAN

S no	Lecture duration	Topics to be covered	Support materials
1	1	Collection of oral cavity, throat, skin	T2 148-150
2	1	Collection of blood &csf	T2 57-59
3	1	Collection of urine & faeces	T2 238-242
4	1	Transportation of samples	T2 4-9
5	1	Revision of unit 1 & 2	
6	1	Unit 1 & 2 test	
	Total no.of hours planned for unit II		6 hours

Textbooks:T1-Lab diagnosis of infectious diseases-paul.G.Engelkirk

References Books:T2 - Manual for medical lab technology. Dr.s.Rajan.

Website:R1 – Medical microbiology – Sherris.

Journal :

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Principles of Diagnosis

General Concepts

Manifestations of Infection

The clinical presentation of an infectious disease reflects the interaction between the host and the microorganism. This interaction is affected by the host immune status and microbial virulence factors. Signs and symptoms vary according to the site and severity of infection. Diagnosis requires a composite of information, including history, physical examination, radiographic findings, and laboratory data.

Microbial Causes of Infection

Infections may be caused by bacteria, viruses, fungi, and parasites. The pathogen may be exogenous (acquired from environmental or animal sources or from other persons) or endogenous (from the normal flora).

Specimen Selection, Collection, and Processing

Specimens are selected on the basis of signs and symptoms, should be representative of the disease process, and should be collected before administration of antimicrobial agents. The specimen amount and the rapidity of transport to the laboratory influence the test results.

Microbiologic Examination

Direct Examination and Techniques: Direct examination of specimens reveals gross pathology. Microscopy may identify microorganisms. Immunofluorescence, immuno-peroxidase staining, and other immunoassays may detect specific microbial antigens. Genetic probes identify genus- or species-specific DNA or RNA sequences.

Culture: Isolation of infectious agents frequently requires specialized media. Nonselective (noninhibitory) media permit the growth of many microorganisms. Selective media contain inhibitory substances that permit the isolation of specific types of microorganisms.

Microbial Identification: Colony and cellular morphology may permit preliminary identification. Growth characteristics under various conditions, utilization of carbohydrates and other substrates, enzymatic activity, immunoassays, and genetic probes are also used.

Serodiagnosis: A high or rising titer of specific IgG antibodies or the presence of specific IgM antibodies may suggest or confirm a diagnosis.

Antimicrobial Susceptibility: Microorganisms, particularly bacteria, are tested in vitro to determine whether they are susceptible to antimicrobial agents.

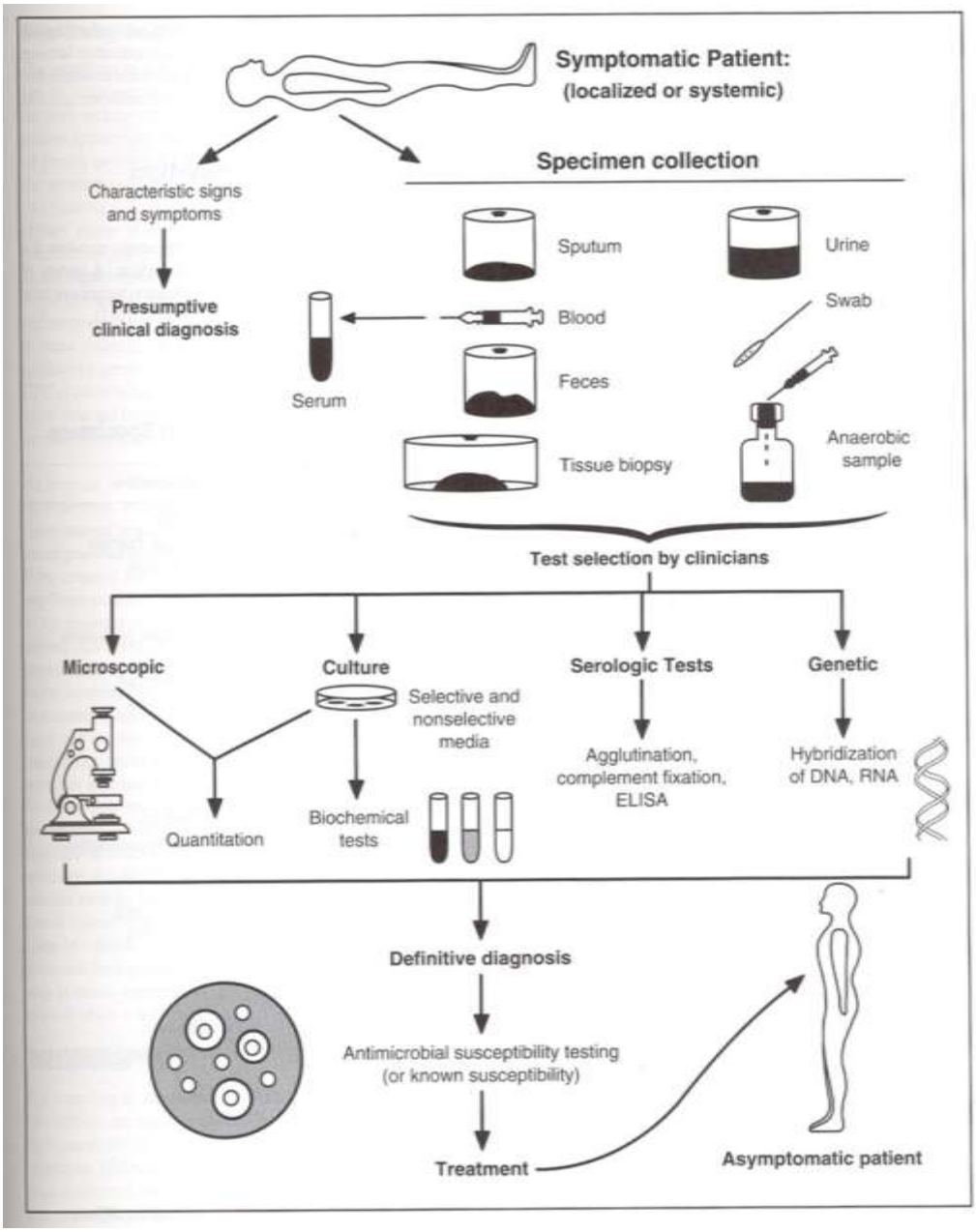
Introduction

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.

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 (Fig. 10-1).

Laboratory procedures used in confirming a clinical diagnosis of infectious disease with a bacterial etiology. The staff of a clinical microbiology laboratory should be qualified to advise the physician as well as process specimens. The physician should supply salient information about the patient, such as age and sex, tentative diagnosis or details of the clinical syndrome, date of onset, significant exposures, prior antibiotic therapy,

immunologic status, and underlying conditions. The clinical microbiologist participates in decisions regarding the microbiologic diagnostic studies to be performed, the type and timing of specimens to be collected, and the conditions for their transportation and storage. Above all, the clinical microbiology laboratory, whenever appropriate, should provide an interpretation of laboratory results.



Manifestations of Infection

The manifestations of an infection depend on many factors, including the site of acquisition or entry of the microorganism; organ or system tropisms of the microorganism; microbial virulence; the age, sex, and immunologic status of the patient; underlying diseases or conditions; and the presence of implanted prosthetic devices or materials. The signs and symptoms of infection may be localized, or they may be systemic, with fever, chills, and hypotension. In some instances the manifestations of an infection are sufficiently characteristic to suggest the diagnosis; however, they are often nonspecific.

Microbial Causes of Infection

Infections may be caused by bacteria (including mycobacteria, chlamydiae, mycoplasmas, and rickettsiae), viruses, fungi, or parasites. Infection may be endogenous or exogenous. In endogenous infections, the microorganism (usually a bacterium) is a component of the patientapos;s indigenous flora. Endogenous infections can occur when the microorganism is aspirated from the upper to the lower respiratory tract or when it penetrates the skin or mucosal barrier as a result of trauma or surgery. In contrast, in exogenous infections, the microorganism is acquired from the environment (e.g., from soil or water) or from another person or an animal. Although it is important to establish the cause of an infection, the differential diagnosis is based on a careful history, physical examination, and appropriate radiographic and laboratory studies, including the selection of appropriate specimens for microbiologic examination. Results of the history, physical examination, and radiographic and laboratory studies allow the physician to request tests for the microorganisms most likely to be the cause of the infection.

Specimen Selection, Collection and Processing

Specimens selected for microbiologic examination should reflect the disease process and be collected in sufficient quantity to allow complete microbiologic examination. The number of microorganisms per milliliter of a body fluid or per gram of tissue is highly variable, ranging from less than 1 to 10^8 or 10^{10} colony-forming units (CFU). Swabs, although popular for specimen collection, frequently yield too small a specimen for accurate microbiologic examination and should be used only to collect material from the skin and mucous membranes.

Because skin and mucous membranes have a large and diverse indigenous flora, every effort must be made to minimize specimen contamination during collection. Contamination may be avoided by various means. The skin can be disinfected before aspirating or incising a lesion. Alternatively, the contaminated area may be bypassed altogether. Examples of such approaches are transtracheal puncture with aspiration of lower respiratory secretions or suprapubic bladder puncture with aspiration of urine. It is often impossible to collect an uncontaminated specimen, and decontamination procedures, cultures on selective media, or quantitative cultures must be used (see above).

Specimens collected by invasive techniques, particularly those obtained intraoperatively, require special attention. Enough tissue must be obtained for both histopathologic and microbiologic examination. Histopathologic examination is used to distinguish neoplastic from inflammatory lesions and acute from chronic inflammations. The type of inflammation present can guide the type of microbiologic examination performed. If, for example, a caseous granuloma is observed histopathologically, microbiologic examination should include cultures for mycobacteria and fungi. The surgeon should obtain several samples for examination from a single large lesion or from each of several smaller lesions. If an abscess is found, the surgeon should collect several milliliters of pus, as well as a portion of the wall of the abscess, for microbiologic examination. Swabs should be kept out of the operating room.

If possible, specimens should be collected before the administration of antibiotics. Above all, close communication between the clinician and the microbiologist is essential to ensure that appropriate specimens are selected and collected and that they are appropriately examined.

Microbiologic Examination

Direct Examination

Direct examination of specimens frequently provides the most rapid indication of microbial infection. A variety of microscopic, immunologic, and hybridization techniques have been developed for rapid diagnosis (Table 10-1).

TABLE 10-1 Rapid Tests Commonly Used to Detect Microorganisms in Specimens

Specimen	Test	Application
Blood	Giemsa	Plasmodia, microfilarie
	EIA	Hepatitis A and B virus, human immunodeficiency virus
Cerebrospinal fluid	Gram stain	Bacteria
	LA; COA	Haemophilus influenzae, Neisseria meningitidis
	India ink wet mount or LA	Streptococcus pneumoniae, Cryptococcus neoformans
Wound exudates, pus	Gram stain	Bacteria
Respiratory secretions	Gram stain	Bacteria
	Acid-fast stain	Mycobacteria, nocardiae
	IFA or genetic probe	*Legionella* species, *Streptococcus pyogenes*
	KOH wet mount	Fungi
	Gomori methenamine silver stain	Fungi, *Pneumocystis carinii*
	FA, EIA	Respiratory syncytial virus
Urine	Gram stain	Bacteria
Urethral or cervical scrapings or exudates	Gram stain, EIA, IFA, EIA, or genetic probe	Neisseria gonorrhoeae *Chlamydia trachomatis*, papillomaviruses
Genital ulcer	FA, EIA, or genetic probe	Herpes simplex virus
Feces	Methylene blue stain	Leukocytes
	Eosin wet mount, trichrome stain	Parasites
	EM, LA, EIA	Rotaviruses
	EIA	Adenoviruses, *Clostridium difficile*

Abbreviations: COA, coagglutination; EIA, enzyme immunoassay; IFA, immunofluorescent antibody; LA, latex agglutination.

Rapid Tests Commonly Used to Detect Microorganisms in Specimens.

Sensitivity and Specificity

The sensitivity of a technique usually depends on the number of microorganisms in the specimen. Its specificity depends on how morphologically unique a specific microorganism appears microscopically or how specific the antibody or genetic probe is for that genus or species. For example, the sensitivity of Cram stains is such that the observation of two bacteria per oil immersion field (X 1,000) of a Gram-stained smear of uncentrifuged urine is equivalent to the presence of $\geq 10^5$ CFU/ml of urine. The sensitivity of the Gram-stained smear for detecting Gram-negative coccobacilli in cerebrospinal fluid from children with *Haemophilus influenzae* meningitis is approximately 75 percent because in some patients the number of colony-forming units per milliliter of cerebrospinal fluid is less than 10^4. At least 10^4 CFU of tubercle bacilli per milliliter of sputum must be present to be detected by an acid-fast smear of decontaminated and concentrated sputum.

An increase in the sensitivity of a test is often accompanied by a decrease in specificity. For example, examination of a Gram-stained smear of sputum from a patient with pneumococcal pneumonia is highly sensitive but also highly nonspecific if the criterion for defining a positive test is the presence of any Gram-positive cocci. If, however, a positive test is defined as the presence of a preponderance of Gram-positive, lancet-shaped diplococci, the test becomes highly specific but has a sensitivity of only about 50 percent. Similar problems related to the number of microorganisms present affect the sensitivity of immunoassays and genetic probes for bacteria, chlamydiae, fungi and viruses. In some instances, the sensitivity of direct examination tests can be improved by collecting a better specimen. For example, the sensitivity of fluorescent antibody stain for *Chlamydia trachomatis* is higher when endocervical cells are obtained with a cytobrush than with a swab. The sensitivity may also be affected by the stage of the disease at which the specimen is collected. For example, the detection of herpes simplex virus by immunofluorescence, immunoassay, or culture is highest when specimens from lesions in the vesicular stage of infection are examined. Finally, sensitivity may be improved through the use of an enrichment or enhancement step in which microbial or genetic replication occurs to the point at which a detection method can be applied.

Techniques

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For microscopic examination it is sufficient to have a compound binocular microscope equipped with low-power (1OX), high-power (40X), and oil immersion (1OOX) achromatic objectives, 10X wide-field oculars, a mechanical stage, a substage condenser, and a good light source. For examination of wet-mount preparations, a darkfield condenser or condenser and objectives for phase contrast increases image contrast. An exciter barrier filter, darkfield condenser, and ultraviolet light source are required for fluorescence microscopy.

For immunologic detection of microbial antigens, latex particle agglutination, coagglutination, and enzyme-linked immunosorbent assay (ELISA) are the most frequently used techniques in the clinical laboratory. Antibody to a specific antigen is bound to latex particles or to a heat-killed and treated protein A-rich strain of *Staphylococcus aureus* to produce agglutination. There are several approaches to ELISA; the one most frequently used for the detection of microbial antigens uses an antigen-specific antibody that is fixed to a solid phase, which may be a latex or metal bead or the inside surface of a well in a plastic tray. Antigen present in the specimen binds to the antibody The test is then completed by adding a second antigen-specific antibody bound to an enzyme that can react with a substrate to produce a colored product. The initial antigen antibody complex forms in a manner similar. When the enzyme-conjugated antibody is added, it binds to previously unbound antigenic sites, and the antigen is, in effect, sandwiched between the solid phase and the enzyme-conjugated antibody. The reaction is completed by adding the enzyme substrate.

Agglutination test in which inert particles (latex beads or heat-killed *S aureus* Cowan 1 strain with protein A) are coated with antibody to any of a variety of antigens and then used to detect the antigen in specimens or in isolated bacteria.

Genetic probes are based on the detection of unique nucleotide sequences with the DNA or RNA of a microorganism. Once such a unique nucleotide sequence, which may represent a portion of a virulence gene or of chromosomal DNA, is found, it is isolated and inserted into a cloning vector (plasmid), which is then transformed into Escherichia coli to produce multiple copies of the probe. The sequence is then reisolated from plasmids and labeled with an isotope or substrate for diagnostic use. Hybridization of the sequence with a complementary sequence of DNA or RNA follows cleavage of the double-stranded DNA of the microorganism in the specimen.

The use of molecular technology in the diagnoses of infectious diseases has been further enhanced by the introduction of gene amplication techniques, such as the polymerase chain reaction (PCR) in which DNA polymerase is able to copy a strand of DNA by elongating complementary strands of DNA that have been initiated from a pair of closely spaced oligonucleotide primers. This approach has had major applications in the detection of infections due to microorganisms that are difficult to culture (e.g. the human immunodeficiency virus) or that have not as yet been successfully cultured (e.g. the Whipple's disease bacillus).

Culture

In many instances, the cause of an infection is confirmed by isolating and culturing microorganism either in artificial media or in a living host. Bacteria (including mycobacteria and mycoplasmas) and fungi are cultured in either liquid (broth) or on solid (agar) artificial media. Liquid media provide greater sensitivity for the isolation of small numbers of microorganisms; however, identification of mixed cultures growing in liquid media requires subculture onto solid media so that isolated colonies can be processed separately for identification. Growth in liquid media also cannot ordinarily be quantitated. Solid media, although somewhat less sensitive than liquid media, provide isolated colonies that can be quantified if necessary and identified. Some genera and species can be recognized on the basis of their colony morphologies.

In some instances one can take advantage of differential carbohydrate fermentation capabilities of microorganisms by incorporating one or more carbohydrates in the medium along with a suitable pH indicator. Such media are called differential media (e.g., eosin methylene blue or MacConkey agar) and are commonly

used to isolate enteric bacilli. Different genera of the Enterobacteriaceae can then be presumptively identified by the color as well as the morphology of colonies.

Culture media can also be made selective by incorporating compounds such as antimicrobial agents that inhibit the indigenous flora while permitting growth of specific microorganisms resistant to these inhibitors. One such example is Thayer-Martin medium, which is used to isolate *Neisseria gonorrhoeae*. This medium contains vancomycin to inhibit Gram-positive bacteria, colistin to inhibit most Gram-negative bacilli, trimethoprim-sulfamethoxazole to inhibit *Proteus*species and other species that are not inhibited by colistin and anisomycin to inhibit fungi. The pathogenic *Neisseria* species, *N gonorrhoeae* and *N meningitidis*, are ordinarily resistant to the concentrations of these antimicrobial agents in the medium.

The number of bacteria in specimens may be used to define the presence of infection. For example, there may be small numbers ($\leq 10^3$ CFU/ml) of bacteria in clean-catch, midstream urine specimens from normal, healthy women; with a few exceptions, these represent bacteria that are indigenous to the urethra and periurethral region. Infection of the bladder (cystitis) or kidney (pyelone-phritis) is usually accompanied by bacteriuria of about $\geq 10^4$ CFU/ml. For this reason, quantitative cultures of urine must always be performed. For most other specimens a semiquantitative streak method over the agar surface is sufficient. For quantitative cultures, a specific volume of specimen is spread over the agar surface and the number of colonies per milliliter is estimated. For semiquantitative cultures, an unquantitated amount of specimen is applied to the agar and diluted by being streaked out from the inoculation site with a sterile bacteriologic loop The amount of growth on the agar is then reported semiquantitatively as many, moderate, or few (or 3+, 2+, or 1+), depending on how far out from the inoculum site colonies appear. An organism that grows in all streaked areas would be reported as 3+.

Chlamydiae and viruses are cultured in cell culture systems, but virus isolation occasionally requires inoculation into animals, such as suckling mice, rabbits, guinea pigs, hamsters, or primates. Rickettsiae may be isolated with some difficulty and at some hazard to laboratory workers in animals or embryonated eggs. For this reason, rickettsial infection is usually diagnosed serologically. Some viruses, such as the hepatitis viruses, cannot be isolated in cell culture systems, so that diagnosis of hepatitis virus infection is based on the detection of hepatitis virus antigens or antibodies.

Cultures are generally incubated at 35 to 37°C in an atmosphere consisting of air, air supplemented with carbon dioxide (3 to 10 percent), reduced oxygen (microaerophilic conditions), or no oxygen (anaerobic conditions), depending upon requirements of the microorganism. Since clinical specimens from bacterial infections often contain aerobic, facultative anaerobic, and anaerobic bacteria, such specimens are usually inoculated into a variety of general purpose, differential, and selective media, which are then incubated under aerobic and anaerobic conditions

General procedure for collecting and processing specimens for aerobic and/or anaerobic bacterial culture.

The duration of incubation of cultures also varies with the growth characteristics of the microorganism. Most aerobic and anaerobic bacteria will grow overnight, whereas some mycobacteria require as many as 6 to 8 weeks.

Microbial Identification

Microbial growth in cultures is demonstrated by the appearance of turbidity, gas formation, or discrete colonies in broth; colonies on agar; cytopathic effects or inclusions in cell cultures; or detection of genus- or species-specific antigens or nucleotide sequences in the specimen, culture medium, or cell culture system.

Identification of bacteria (including mycobacteria) is based on growth characteristics (such as the time required for growth to appear or the atmosphere in which growth occurs), colony and microscopic morphology, and biochemical, physiologic, and, in some instances, antigenic or nucleotide sequence characteristics. The

selection and number of tests for bacterial identification depend upon the category of bacteria present (aerobic versus anaerobic, Gram-positive versus Gram-negative, cocci versus bacilli) and the expertise of the microbiologist examining the culture. Gram-positive cocci that grow in air with or without added CO_2 may be identified by a relatively small number of tests (seeCh.12). The identification of most Gram-negative bacilli is far more complex and often requires panels of 20 tests for determining biochemical and physiologic characteristics. The identification of filamentous fungi is based almost entirely on growth characteristics and colony and microscopic morphology. Identification of viruses is usually based on characteristic cytopathic effects in different cell cultures or on the detection of virus- or species-specific antigens or nucleotide sequences.

Interpretation of Culture Results

Some microorganisms, such as *Shigella dysenteriae*, *Mycobacterium tuberculosis*, *Coccidioides immitis*, and influenza virus, are always considered clinically significant. Others that ordinarily are harmless components of the indigenous flora of the skin and mucous membranes or that are common in the environment may or may not be clinically significant, depending on the specimen source from which they are isolated. For example, coagulase-negative staphylococci are normal inhabitants of the skin, gastrointestinal tract, vagina, urethra, and the upper respiratory tract (i.e., of the nares, oral cavity, and pharynx). Therefore, their isolation from superficial ulcers, wounds, and sputum cannot usually be interpreted as clinically significant. They do, however, commonly cause infections associated with intravascular devices and implanted prosthetic materials. However, because intravascular devices penetrate the skin and since cultures of an implanted prosthetic device can be made only after incision, the role of coagulase-negative staphylococci in causing infection can usually be surmised only when the microorganism is isolated in large numbers from the surface of an intravascular device, from each of several sites surrounding an implanted prosthetic device, or, in the case of prosthetic valve endocarditis, from several separately collected blood samples. Another example, *Aspergillus fumigatus*, is widely distributed in nature, the hospital environment, and upper respiratory tract of healthy people but may cause fatal pulmonary infections in leukemia patients or in those who have undergone bone marrow transplantation. The isolation of *A fumigatus* from respiratory secretions is a nonspecific finding, and a definitive diagnosis of invasive aspergillosis requires histologic evidence of tissue invasion.

Physicians must also consider that the composition of microbial species on the skin and mucous membranes may be altered by disease, administration of antibiotics, endotracheal or gastric intubation, and the hospital environment. For example, potentially pathogenic bacteria can often be cultured from the pharynx of seriously ill, debilitated patients in the intensive care unit, but may not cause infection.

Serodiagnosis

Infection may be diagnosed by an antibody response to the infecting microorganism. This approach is especially useful when the suspected microbial agent either cannot be isolated in culture by any known method or can be isolated in culture only with great difficulty. The diagnosis of hepatitis virus and Epstein-Barr virus infections can be made only serologically, since neither can be isolated in any known cell culture system. Although human immunodeficiency virus type 1 (HIV-1) can be isolated in cell cultures, the technique is demanding and requires special containment facilities. HIV-1 infection is usually diagnosed by detection of antibodies to the virus.

The disadvantage of serology as a diagnostic tool is that there is usually a lag between the onset of infection and the development of antibodies to the infecting microorganism. Although IgM antibodies may appear relatively rapidly, it is usually necessary to obtain acute- and convalescent-phase serum samples to look for a rising titer of IgG antibodies to the suspected pathogen. In some instances the presence of a high antibody titer when the patient is initially seen is diagnostic; often, however, the high titer may reflect a past infection, and the current

infection may have an entirely different cause. Another limitation on the use of serology as a diagnostic tool is that immunosuppressed patients may be unable to mount an antibody response.

Antimicrobial Susceptibility

The responsibility of the microbiology laboratory includes not only microbial detection and isolation but also the determination of microbial susceptibility to antimicrobial agents. Many bacteria, in particular, have unpredictable susceptibilities to antimicrobial agents, and their susceptibilities can be measured in vitro to help guide the selection of the most appropriate antimicrobial agent.

Antimicrobial susceptibility tests are performed by either disk diffusion or a dilution method. In the former, a standardized suspension of a particular microorganism is inoculated onto an agar surface to which paper disks containing various antimicrobial agents are applied. Following overnight incubation, any zone diameters of inhibition about the disks are measured and the results are reported as indicating susceptibility or resistance of the microorganism to each antimicrobial agent tested. An alternative method is to dilute on a $\log_2$ scale each antimicrobial agent in broth to provide a range of concentrations and to inoculate each tube or, if a microplate is used, each well containing the antimicrobial agent in broth with a standardized suspension of the microorganism to be tested. The lowest concentration of antimicrobial agent that inhibits the growth of the microorganism is the minimal inhibitory concentration (MIC). The MIC and the zone diameter of inhibition are inversely correlated. In other words, the more susceptible the microorganism is to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition. Conversely, the more resistant the microorganism, the higher the MIC and the smaller the zone of inhibition.

Two methods for performing antibiotic susceptibility tests. (A) Disk diffusion method. (B) Minimum inhibitory concentration (MIC) method. In the example shown, two different microorganisms are tested by both methods against the same antibiotic. The MIC (A) Disk diffusion method. (B) Minimum inhibitory concentration (MIC) method. In the example shown, two different microorganisms are tested by both methods against the same antibiotic. The MIC of the antibiotic for the susceptible microorganism is 8 μg/ml. The corresponding disk diffusion test shows a zone of inhibition surrounding the disk. In the second sample, a resistant microorganism is not inhibited by the highest antibiotic concentration tested (MIC $\geq$ 16 μg/ml) and there is no zone of inhibition surrounding the disk. The diameter of the zone of inhibition is inversely related to the MIC.

The term susceptible means that the microorganism is inhibited by a concentration of antimicrobial agent that can be attained in blood with the normally recommended dose of the antimicrobial agent and implies that an infection caused by this microorganism may be appropriately treated with the antimicrobial agent. The term resistant indicates that the microorganism is resistant to concentrations of the antimicrobial agent that can be attained with normal doses and implies that an infection caused by this microorganism could not be successfully treated with this antimicrobial agent.

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II BSC Microbiology
Microbial diagnosis in health clinic

Unit II Q	Opt 1	Opt 2	Opt 3	Opt 4	Answer
In Greek '	Suffering	violence	disease	infection	Suffering
The lodge	disease	infection	immunity	parasitism	infection
Initial infe	primary	secondary	re-infectio	nosocomial	primary
Subsequer	Primary	Secondary	Re-infecti	Iatrogenic	Re-infection
When a ne	Primary	Secondary	Re-infecti	Iatrogenic	Secondary
Infection c	Primary	Secondary	Focal	Iatrogenic	Focal
In a patien	Cross	Focal	Re-infecti	Nosocomial	Cross
Cross infe	Cross	Focal	. Re-infection		Nosocomial
Physicians	Iatrogenic	Focal	Nosocomi	Re-infection	Iatrogenic
When clin	Atypical	Inapparent	Subclinica	Clinical	Inapparent
_____	Endogeno	Exogenou	Inapparent	Clinical	Inapparent
Some para	Endogeno	Exogenou	Latent	Atypical	Latent
A person v	Patient	Carrier	Healthy pe	Immunodeficient person	Carrier
A person c	Healthy ca	Convalesc	Contact ca	Paradoxical carrier	Healthy carrier
A person v	Healthy	Convalesc	Contact	Paradoxical	Convalescent
The _____	Acute	Temporary	Chronic	Healthy	Temporary
The term _	Acute	Temporary	Contact	Paradoxical	Contact
The _____	Acute or c	Temporary	Contact	Paradoxical	Acute or chronic
The term _	Healthy	Convalesc	Contact	Paradoxical	Paradoxical
Infectious	Zoonosis	Anoosis	Xanthosis	Phytosis	Zoonosis
When the	Mechanica	Biological	Healthy	Contact	Biological vector
Some path	Congenita	Intracelair	Vertical	Horizontal	Vertical
_____	Infection	Immunity	Pathogeni	Virulence	Pathogenicity
_____	Infection	Immunity	Pathogeni	Virulence	Virulence
_____	Epidemiol	Oncology	Infection	Physiology	Epidemiology
An indivic	Epidemiol	Scientist	Investigat	Environmentalist	Epidemiologist
A _____ i	Health	Disease	Infection	Immunity	Disease
_____ is	Health	Disease	Infection	Immunity	Infection
When a di	Epidemic	Endemic	Sporadic	Pandemic	Endemic
When the	Epidemic	Endemic	Sporadic	Pandemic	Endemic
_____	hyperende	Epidemic	Endemic	Pandemic	hyperendemic
An_____	Epidemic	Endemic	sporadic	Pandemic	Pandemic
The first c	index case	infection	disease	outbreak	index case
A sudden	index case	Infection	disease	outbreak	outbreak
A_____	epidemic	Endemic	Pandemic	Sporadic	Pandemic
The factor	epizootiol	Zootiology	Epidemiol	Entamology	epizootiology
Moderate	epizootic	enzootic	panzootic	zoonoses	enzootic

A sudden	epizootic	enzootic	panzotic	zoonoses	panzotic
A wide di	epizoolic	enzoolic	panzootic	zoonoses	zoonoses
Animal di	epizootic	enzootic	panzootic	zoonoses	zoonoses
A _____	incubation	survival	lag	log	incubation
The _____	incubatio	prodromal	survival p	death	prodromal stage
The _____	infectious	disease	infection	none	infectious disease cyc
_____ i	communic	epidemic	epidemic	none	communicable diseas
The _____	incubation	the period	predormal	none	predormal stage
The water	Quarentur	GTL	Materials	Dispatch	GTL
The Good	Dispatch s	Materials	Quarentur	Stock section	Quarentum section
The water	Catridge	Pad	Sinted gla	Earthern ware	Catridge
The monit	QA	QC	GTL	QB	QC
The micro	Settle plat	Pour plate	Spread pla	Streak plate.	Settle plate
The AHU	Aerosol ha	Air handli	Atmosphe	Auto handling unit	Air handling unit
_____	Air pressu	a. Aeros	Atmosphe	oxygen	Air pressure
The circul	Pad	Candle	HEPA	Catridge	HEPA
The disca	Dissuaded	Dispenced	Decontam	sterilized	Decontaminated
Quality co	QA	QC	GTL	QB	QA
The prope	Epidemic	Endemic	Pandemic	Randamic	Epidemic
There is n	Antibiotic	Antiseptic	Disinfecta	Diluent.	Disinfectants
In the Rid	Phenol	Boron	Acid	Alcohol	Phenol
_____	. HCL	BPL	TNT	SDS	BPL
QA stands	Quality as	Quality as	Quality ac	Quality abolision.	Quality assurance

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II B. Sc Microbiology – Microbial diagnosis in health clinic

Unit II possible questions

Part B

1. Define medical diagnosis
2. Say about human body systems
3. What are clinical samples?
4. What are pathogens and pathogenicity?
5. What is PCR?

Part C

1. Explain in detail about the collection of samples
2. Describe the lab diagnosis of UTI.
3. Illustrate the morphology and pathogenesis of URT pathogens Write about the transport media and its importance.
4. Discuss about the investigation by culturing.
5. Describe various sources and methods of infection.

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II B. Sc Microbiology –Microbial diagnosis in health clinic
Unit III

LECTURE PLAN

S no	Lecture duration (Hr)	Topics to be covered	Support materials
1	1hr	Grams staining &ziehl – Neelson staining	T1 119-127
2	1hr	Giemsa staining	T2 87-88
3	1hr	Culture media – blood, chocolate agar	T1 444-489
4	1hr	Culture media – LJ &macconkey agar	T1 444-489
5	1hr	Colony properties of pathogens	T1667-683
	Total no. of hours planned for unit – 3		6 hours

Textbooks:T1-Lab diagnosis of infectious diseases-Paul.G.Engelkirk

References Books: T2 - Manual for medical lab technology. Dr.S.Rajan.

Website:R1 – Medical microbiology – Sherris.

Journal :

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1. Use universal precautions for collecting and handling all specimens.

2. Whenever possible, collect all culture specimens prior to administration of any antimicrobial agents.

3. Avoid contamination with indigenous flora.

4. Swabs are convenient but inferior to tissue and fluid. Tissue and fluid are essential for fungal and mycobacterial culture.

5. All specimens must be appropriately labeled with two patient identifiers. Identifiers used at University of Iowa Hospitals and Clinics (UI Hospitals and Clinics) include patient name, birthdate and/or hospital number. The requisition will include the patient name, hospital number, hospital service, date and time of collection, specimen type and tests requested. A requisition needs to accompany each different specimen type.

6. Deliver all specimens to the laboratory as soon as possible after collection. Specimens for bacterial culture should be transported at room temperature. If transport is delayed the following specimens should be refrigerated: urines (within 30 min), stool (within 1 h), respiratory specimens. Specimens for viral culture must be transported to the laboratory immediately on ice. See specific specimen and culture type for detailed collection and transport guidelines.

7. Specimens may be hand delivered to the laboratory or transported via the runners from Technical Services if the specimens are not indicated as deliver immediately. Specimens may be transported through the pneumatic tube system if approved by Pneumatic Tube Administration. This includes blood culture bottles (if placed in plastic carrier), Vacutainer® tubes and swabs.

8. Specimens should be in tightly sealed, leak proof containers and transported in sealable, leak-proof plastic bags. Specimens for TB should be double bagged. Specimens should not be externally contaminated. Specimens grossly contaminated or compromised may be rejected.

9. If anaerobic culture is requested, make certain to use proper anaerobic collection containers (fluid: 59546, tissue: 59547, or ESwab, 74541).

10. Further questions may be referred to the Microbiology laboratory (356-2591) or pathology resident (pager 4903 weekdays; pager 3404 evenings and weekends).

Bacterial Cultures: Transport at room temperature unless otherwise specified.

A. Abscess – Tissue or aspirates are always superior to swab specimens. Remove surface exudate by wiping with sterile saline or 70% alcohol. Aspirate with needle and syringe. Cleanse rubber stopper of anaerobic transport vial with alcohol; allow to dry 1 min before inoculating; push needle through septum and inject all abscess material on top of agar. If a swab must be used, pass the swab deep into the base of the lesion to firmly sample the fresh border. Transport time ≤ 2 hours.

B. Anaerobic cultures - Aspirates are preferred rather than swabs. Fluid collections should be aspirated through disinfected tissue or skin. For superficial ulcers, collect material from below the surface (after surface debridement or use a needle and syringe). Submit specimens using anaerobic transport media:
   a. Anaerobic transport vial :Cleanse rubber stopper with alcohol; allow to dry 1 min before inoculation; push needle through septum and inject specimen on top of agar
   b. Anaerobic jar . Place sample on top of agar. Keep jar upright to maintain atmosphere in jar.

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c. A sterile container may be used for tissue if transported to the microbiology lab immediately (add drops of sterile saline to keep small pieces of tissue moist).
d. Copan Liquid Amies Elution Swab – swab specimens are suboptimal, but will be accepted if no other sample can be obtained.
e. Deliver all specimens to the laboratory immediately after collection.
f. Anaerobic flora is prevalent on mucosal surfaces of the oral cavity, upper respiratory, gastrointestinal, and genital tracts; specimens collected from these sites should not ordinarily be cultured for anaerobic bacteria. The following is a list of specimens that are likely to be contaminated with anaerobic normal flora and are NOT routinely accepted for anaerobic culture.
    1. Throat or nasopharyngeal swabs
    2. Gingival or other intraoral surface swabs
    3. Expectorated sputum
    4. Sputum obtained by nasotracheal or endotracheal suction
    5. Bronchial washings
    6. Voided or catheterized urine
    7. Vaginal or cervical swabs
    8. Gastric and small bowel contents (except for "blind loop" or bacterial overgrowth syndrome)
    9. Feces (except for specific etiologic agents such as *C. difficile* and *C. botulinum*)
    10. Rectal swabs - Surface swabs from ulcers and wounds (collect material from below the surface)
    11. Material adjacent to a mucous membrane that has not been adequately decontaminated

C. Blood
a. Adult – Cleanse skin with disinfectant:
    1. Holding the applicator sponge downward, pinch wings on applicator to break ampule and release the antiseptic.
    2. Use a side-to-side motion to scrub the site with the friction pad for a full 30 sec; allow site to dry completely (at least 30 sec) before venipuncture. Do not touch site after prep.
    3. Remove overcaps from bottles (1 aerobic and 1 anaerobic) and cleanse each rubber septum with separate 70% alcohol swabs. Allow septum to dry for 1 min before inoculating.
    4. Draw 20 mL of blood and inoculate each bottle with 10 mL of blood. Do not vent or overfill bottles. Adding low (<8 mL) or high (>10 mL) volumes may adversely affect the recovery of organisms. Transport time <2 h.
    5. For adults with a suspected bloodstream infection (BSI), collect two initial sets of blood cultures sequentially from separate phlebotomy procedures followed by a third and a fourth set at 4-6 hour intervals (will detect >99% of BSIs). Three sets of blood cultures collected within a 24 hour period will detect 96.9 - 98.3% of BSIs. A single set of blood cultures to detect BSIs in adults is inadequate (only 73% sensitivity); two sets of blood cultures will allow detection of 87.7-89.7% of BSI episodes. If patient is allergic to chlorhexidine, prep site with a povidone iodine swab stick applied in concentric circles (start at center). Allow to dry at least 1 min before venipuncture. If patient is allergic to iodine, cleanse site with 70% alcohol for 60 sec.
b. Pediatric – Apart from NICU patients, the minimum volume drawn should be 1 mL per year of age per blood culture set. This volume should be split between an aerobic and anaerobic bottle. See pediatric blood culture order for more detail.

D. Bone marrow aspirate – Prepare puncture site as for surgical incision. Inoculate yellow top tube (104184). Transport time <2 hours.

E. Burn – Clean and debride burn. Place tissue in sterile screw-cap container. Transfer aspirates to a sterile container. These are processed for aerobic culture only. Quantitative culture may or may not be valuable. A 3 to 4 mm punch biopsy specimen is optimum when quantitative cultures are ordered. Cultures of surface samples can be misleading.

F. Catheter Tips – Catheter tips are not routinely accepted for culture. Consult Microbiology laboratory for approval. Foley catheters are not accepted for culture since growth represents distal urethral flora.

G. Cerebrospinal Fluid (CSF) – Obtain CSF for gram stain, cell count, protein, glucose and aerobic culture where able. The kit should contain 4 pre-numbered tubes to be filled in chronological order. Avoid covering tube numbers with stickers to ensure appropriate routing of samples.

- With low volume, one-tube specimens not all testing may be possible and the clinician must determine which tests should be prioritized. If cultures are desired, Microbiology must receive the specimen first to ensure the culturing of a sterile specimen.
- Transport time ≤15 minutes. Do not refrigerate CSF for bacterial culture.

If adequate volume is obtained, orders are placed per tube as follows:

Tube #	**Orders**
#1 – Chemistry/Immunology	Protein and Glucose
#2 – Microbiology	Aerobic culture with gram stain. Select "CSF lumbar puncture", "CSF shunt", or "CSF ventricular trap" as source to ensure appropriate culturing. If cryptococcal meningitis is suspected, fungal culture and cryptococcal antigen should be ordered as well. The Meningitis/Encephalitis PCR Panel should be ordered on all lumbar punctures where infections is being considered and cannot be ordered on non-lumbar puncture samples (order individual tests instead).
#3 – Hematology	CSF Cell Count and Differential. If a manual differential is needed, a pathologist review can be obtained by completing the "Staff Pathologist Slide Review (Information Required)" area of the A-1a Pathology Doctor's Order Form.
#4 – Specimen Control	Specimen storage. Select "Spinal fluid" as source and request to hold for 30 days at -80°C unless 4°C or -20°C storage is needed for a specific test.

Decubitus ulcer – A swab is not the specimen of choice. Cleanse surface with sterile saline. Submit tissue or aspirate inflammatory material from the base of the ulcer in a sterile tube or anaerobic system. Transport time ≤2 hours.

Ear

. Inner ear – Tympanocentesis should be reserved for complicated, recurrent, or chronic persistent otitis media. For intact eardrum, clean ear canal with soap solution and collect fluid via syringe aspiration. Submit in sterile container. For ruptured eardrum, collect fluid on flexible shaft swab via an auditory speculum. Transport time <2 hours.

a. Outer ear – Use moistened swab to remove any debris or crust from ear canal. Obtain sample by firmly rotating swab in outer canal. For otitis externa, vigorous swabbing is required – surface swabbing may miss streptococcal cellulitis.

Eye

. Conjunctiva – Sample each eye with separate swabs (premoistened with sterile saline) by rolling over conjunctiva. When only one eye is infected, sampling both can help distinguish indigenous microflora from true pathogens.

a. Corneal scrapings – Collected by ophthalmologist. Using sterile spatula, scrape ulcers and lesions; inoculate scraping directly onto media (BHI with 10% sheep blood, chocolate, and inhibitory mold agar). Prepare 2 smears by rubbing material onto 1-2 cm area of slide. Transport time ≤15 min.
b. Vitreous fluid – Prepare eye for needle aspiration of fluid. Transfer fluid to sterile tube. Transport time ≤15 min.

Feces - see stool.

Fistula - see abscess.

Fluids - see sterile body fluids.

Genital –Cultures for *Neisseria gonorrhoeae* should be collected using an Copan Liquid Amies Elution Swab (ESwab). Transport to the laboratory immediately.

. Endocervical - Remove cervical mucus with swab and discard. Insert a second swab into endocervical canal and rotate against walls. Allow time for organisms to absorb onto the swab surface.
a. Urethral - Collect urethral specimens at least 1 h after patient has urinated. Insert small swab 2-4 cm into urethral lumen, rotate, leave for 2s to facilitate absorption.

Pilonidal cyst – see abscess.

Respiratory, lower – Transport time ≤2 hours.

. Bronchoalveolar lavage or brush, endotracheal aspirate – Collect fluid in a sputum trap ;transfer to leak-proof container for transport to microbiology labortory; place brush in sterile container with 1 mL sterile saline.
a. Sputum, expectorated - Patient should rinse mouth and gargle with sterile water prior to collection; instruct patient to cough deeply. Collect specimen in sterile transport containers
b. Sputum, induced – Have patient brush gums and teeth, then rinse mouth thoroughly with sterile water. Using a nebulizer, have the patient inhale 20-30 mL of 3 to 10% sterile saline. Collect sputum in sterile container.
c. If Nocardia is suspected, culture for Nocardia should be requested as an add-on test as standard culture is inadequate for its recovery.

Respiratory, upper – Transport time ≤2 hours.

. Oral – remove oral secretions and debris from surface of lesion with a swab. Use a second swab to vigorously sample lesion, avoiding normal tissue. Superficial swab specimens should not be submitted. Tissue or needle aspirates are preferred.
a. Nasal swabs– Insert a sterile swab (use Copan dual swab) into the nose until resistance is met at the level of the turbinates (approximately 1-2 cm into one nostril). Rotate the swab against the nasal mucosa for 3 sec. Apply slight pressure with a finger on the outside of the nose to ensure good contact between swab and inside of nose. Using the same swab, repeat for the other nostril.
b. Sinus aspirates – Aspirate with needle and syringe. Cleanse rubber stopper of anaerobic transport vial with alcohol; push needle through septum and inject specimen on top of agar.
c. Throat - Routine throat cultures will be processed only for growth of ß-hemolytic *Streptococcus* species. Do not obtain throat samples if epiglottis is inflamed, as sampling may cause serious respiratory obstruction. Sample the posterior pharynx, tonsils, and inflamed areas using a Copan Liquid Amies Elution Swab(ESwab).

Sterile body fluids (other than CSF)

. Transport fluid to laboratory in sterile, leak-proof container (BD Vacutainer®, no additive, yellow top,) or anaerobic transport vial (Vial,).

a. Cleanse rubber septum of container with 70% alcohol. Allow septum to dry for 1 min before inoculating.

b. Disinfect overlying skin with iodine or chlorhexidine preparation. Obtain specimen with needle and syringe. Push needle through septum of transport container and inject fluid.

c. Amniotic and culdocentesis fluids should always be transported in an anaerobic transport vial . Agar in anaerobic vial should be clear before inoculation; inject fluid on top of agar.

d. Submit as much fluid as possible. NEVER submit a swab dipped in fluid. NEVER inject fluid into swab container.

e. One aerobic blood culture bottle inoculated at bedside (up to 10 mL) is highly recommended provided adequate sample is available. If blood culture bottle is inoculated, submit separate aliquot inanaerobic transport vial or sterile container for preparation of cytocentrifuged Gram stain and inoculation of solid media (allows quantitation, aids in culture interpretation).

f. Transport time ≤15 min, room temperature.

Stool – Stools submitted on patients admitted for >3 days will be rejected without prior preapproval (pager 4903 weekdays, pager 3404 evenings and weekends).

. Please use Fecal Swabs. 1) Obtain a stool specimen in a clean pan or container. Stool specimens should not contain urine or water. 2) Holding FecalSwab shaft above the red breakpoint mark, insert the entire tip of the FecalSwab into the stool sample and rotate. Do not use FecalSwab as a spoon; rather, coat swab with a visible layer. 3) If visible stool is not coating the FecalSwab tip, reinsert until swab is coated. 4) Using swab and aiming tube away, mash and mix the stool sample against the side of the tube to suspend the sample. 5) Invert the tube several times to homogenize the sample and expose the sample to Cary Blair preservative fluid.

a. The FilmArray Gastrointestinal Panel is a multiplex PCR test capable of qualitatively detecting DNA or RNA of 22 pathogens (bacteria, parasites, and viruses). It requires a FecalSwab. The panel is used to diagnose infection caused by *Campylobacter* species, *Plesiomonas shigelloides*, *Salmonella* species, *Vibrio* species, *V. cholerae*, *Yersinia* species, enteroaggregative *E. coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, Shiga toxin producing *E. coli*, *E. coli* O157, *Shigella*/Enteroinvasive *E. coli*, *Cryptosporidium* species, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia*, Adenovirus F 40/41, Astrovirus, Norovirus, Rotavirus and Sapovirus.

b. Stools for *C. difficile* toxin detection must be transported to the laboratory immediately or refrigerated if transport is delayed. This test requires raw stool, not a FecalSwab.

c. Surveillance cultures may be ordered on Bone Marrow transplant and other immunocompromised patients to detect overgrowth of normal flora by *Staph aureus*, yeast or a gram negative bacillus.

d. Test of Cure Stool Culture (Salmonella, Shigella, EHEC) is only for the listed organisms. For organisms other than these please contact the Microbiology laboratory for approval.

e. Aeromonas Culture – Should be collected in Fecal Swabs. This test may be added onto the FilmArray Enteric Panel.

Tissue – Submit in anaerobic collection jar or sterile screw-cap container ; add drops of sterile saline to keep small pieces of tissue moist. Transport time ≤15 min.

Urine – Collect 4 mL of urine in a sterile specimen container . Transfer urine to a gray top C&S urine container. Tubes must be filled to 3 mL do prevent inhibition of bacterial growth. Transport to the microbiology laboratory. If unable to collect 3 mL of urine, collect in sterile specimen container or yellow top tube and transport urine specimens to the Microbiology Laboratory or refrigerate **within 30 minutes**.

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Refrigerated specimens should be delivered to the lab as soon as possible, and may be rejected if not received within 24 hours of collection.

Gray top C&S urine containers are not acceptable for urinalysis and urine chemistries because the preservative interferes with testing.

Cultures can not be performed as an add-on test to urinalysis. Send separate sample for urinalysis (random urine yellow top, round bottom tube (no additive)) and culture (as above).

. Midstream clean catch method: Patients should be instructed to wash hands prior to collection and offered exam gloves.

1. **Female** patients should be instructed to sit on toilet with legs apart and spread labia with one hand. First void in toilet and then, continuing to void, hold specimen container in "midstream" to collect sample.
2. **Male** patients should be instructed to retract foreskin if uncircumcised. First void in toilet and then, continuing to void, hold specimen container in "midstream" to collect sample.

a. Straight catheter: Clamp catheter below port and allow urine to collect in tubing. Disinfect the catheter collection port with 70% alcohol. Use needle and syringe to aseptically collect 4 mL freshly voided urine though catheter port. Transfer to gray top C&S urine container. Do not collect urine from collection bag.

b. Indwelling catheter: Clamp catheter below port and allow urine to collect in tubing. Disinfect the catheter collection port with 70% alcohol. Use needle and syringe to aseptically collect 20 mL freshly voided urine though catheter port. Transfer to gray top C&S urine container. Do not collect urine from collection bag.

c. Ileal conduit: Remove the external device and discard urine within device. Gently cleanse the stoma with 70% alcohol followed by povidone-iodine swab stick . Using sterile technique, insert a double catheter into the cleansed stoma, to a depth beyond the fascial level, and collect the urine into a sterile container. Transfer to gray top C&S urine container. Use of a double catheter helps to minimize contamination of the specimen with skin flora.

Wound – See abscess.

Fungal Culture

1. Deliver all specimens to the laboratory as soon as possible after collection.
   A. Blood: Cleanse skin with disinfectant. Collect 8-10 mL of blood for adult (1.5 mL for child) and inoculate into an Isolator tube (Adult; Pediatric). Collect in addition to bacterial blood culture bottles. Isolator tubes are for molds, Histoplasma, Blastomyces, and Malassezia spp.; for bloodstream infection by Candida spp., inoculate aerobic blood culture bottles instead.

   B. Skin: Using a scalpel blade, scrape the periphery of the lesion border and transport in a sterile container.

   C. See Bacterial Culture for collection and transport of all other specimen types.

Mycobacterial Culture (AFB Culture)

1. Deliver all specimens to the laboratory as soon as possible after collection. Specimens for mycobacteria should be double bagged and sent sealed in leak-proof containers.
   A. Blood: Media and instructions available upon request from the Microbiology Lab. Test available for limited patient populations only.

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B. Sputum: Collect an early morning specimen on three consecutive days. Collect 5-15 mL in a sterile container.

C. See Bacterial culture for collection and transport of all other specimen types.

D. Swabs are suboptimal for recovery of mycobacteria due to limited material and the hydrophobicity of the mycobacterial cell envelope (often compromises a transfer from swabs onto media). Dry swabs are unacceptable. The lab only accepts Copan Liquid Amies Elution Swab (ESwab) for AFB culture when the ordering physician confirms that the swab is the only possible way to obtain the specimen.

Viral/Molecular Infectious Disease PCR Testing

Collect specimens for PCR testing early in illness when viral shedding is maximal. Place swabs in viral transport medium. Collect bronchoalveolar lavage, tracheal aspirate, or nasopharyngeal wash/aspirate and specimens from normally sterile sites in a sterile, leak-proof container . Transport the specimen to the Microbiology laboratory immediately.

Respiratory Virus PCR
PCR assay includes the detection of respiratory viruses (influenza A including H1N1; influenza B; RSV; parainfluenza 1, 2, 3; adenovirus; human metapneumovirus).

1. Mycoplasma PCR: Collect throat swab in ESwab.

2. Biopsy or tissue: Keep moist with sterile saline or viral transport media [do not use viral transport media (VTM) if bacterial, AFB, or fungal cultures are also requested; VTM is available in kits from Hospital Stores

3. CSF: Collect 0.5 mL in a sterile container. Transport immediately to laboratory.

4. Nasopharyngeal swab: Collect specimen using the flexible minitip flocked swab (Hospital Stores #33595). Measure the distance from the patient's nostril to the nasopharynx (half the distance from nostril to base of the ear) and hold the swab at that location. Do not advance the swab beyond that point. Gently insert the swab along the base of one nostril (straight back, not upwards) and continue along the floor of the nasal passage until reaching the nasopharynx. Rotate swab 2-3 times and hold in place for 5 seconds. Place swab in tube containing viral transport medium. Break off the excess length of swab at the score mark to permit capping of the tube.
5. Nasopharyngeal wash/aspirate:
   A. Assemble equipment:
      • Sterile specimen trap
      • Personal Protective Equipment (gloves, surgical mask, eye protection)
      • Appropriate size suction catheter (8 fr for infants/children, 10/12 fr for adults)
      • Normal saline vial
      • Wall suction
      • Bag or cup of ice for specimen transport to laboratory
   B. Place patient with the head tilted slightly back.

C. With sterile gloved hand, insert suction catheter into the patient's nose to the depth of the nasopharyngeal area (beyond the turbinates). Do not remove catheter until end of procedure (see picture below).

D. With the non-sterile gloved hand, instill approximately 1-2 mL normal saline outside the catheter.

E. Apply suction to aspirate nasopharyngeal secretions.

F. Above steps may need to be repeated to obtain 1 mL sample in specimen trap.

G. Remove catheter from patient. With specimen trap still in-line, rinse catheter with remaining saline to clear secretions.

H. Specimens transported by tube system must be transferred from trap to a leak-proof sterile container (be sure the lid is tightly secured).

6. Respiratory secretions: Collect specimens in a sterile, leak-proof container. Sputum is unacceptable for viral cultures.

PCR Assays are available for the following viruses: HSV1&2, VZV, enterovirus (CSF), EBV (Blood and CSF) and CMV (blood, CSF, or BAL). PCR testing requires a dedicated collection tube and cannot be added onto a previously opened Vacutainer® tube. For CSF from lumbar puncture, most patients should have an order placed for the Meningitis/Encephalitis Panel (LAB8514).

1. HSV 1,2 or VZV PCR: submit CSF in sterile container. Submit vesicle fluid, surface swab, or BAL (sputum and tracheal aspirates are unacceptable) in UTM media. Transport to laboratory immediately.

2. Enterovirus PCR: submit CSF in sterile container. Keep on ice and deliver to laboratory immediately.

3. EBV PCR: Collect one 5 mL pink (EDTA) top tube. For CSF collect a minimum of 1.0 mL in a sterile container. Deliver to laboratory immediately after collection. **EBV PCR is useful only for diagnosis and monitoring of posttransplant lymphoproliferative disorder and similar disorders and is not appropriate for the diagnosis of mononucleosis or meningitis/encephalitis in immunocompetent patients.**

4. CMV Quantitative PCR: Collect one 5 mL pink (EDTA) top tube. For CSF collect a minimum of 0.5 mL in a sterile container. Deliver to laboratory immediately.

5. CMV Qualitative PCR: Submit a minimum of 2.0 mL BAL or 1.0 mL of amniotic fluid in a sterile container. Transport to laboratory immediately.

6. HIV Viral Load by PCR, Hepatitis C Virus RNA by PCR and Hepatitis B Virus DNA by PCR: For each test collect at least 6 mL whole bold in one pink (EDTA) top tube. Deliver immediately to laboratory. Each test requires a dedicated collection tube and cannot be added onto a previously opened Vacutainer® tube. All collection tubes need to be processed within 6 hours of collection.

7. Neisseria gonorrhoeae & Chlamydia trachomatis Detection by PCR: Amplified DNA (PCR) testing is recommended for urine, endocervical, urethral, oral or pharyngeal and rectal swab. Culture is recommended for suspected failure of therapy.

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A. Endocervical, urethral, oral or pharyngeal, rectal swab: Use multicollect specimen kit available from hospital stores (46161). Specimens must be aseptically collected with the orange shaft swab provided with the kit. After collection, specimens may be stored and transported at 2 to 30 ºC for up to 14 days. Do not freeze.

B. Urine: The patient should not have urinated for at least one hour prior to sample collection. Collect urine in a typical collection cup (not provided in multi-collect kit). Using plastic transfer pipette provided in multi-collect specimen kit available from Hospital stores (46161), transfer urine from collection cup into the transport tube until the liquid level in the tube falls within the clear fill window of the transport tube label. Do not overfill. Slightly more than one full squeeze of the transfer pipette bulb may be required to transfer the necessary volume of urine specimen. After collection, specimens may be stored and transported at 2 to 30 ºC for up to 14 days. Do not freeze.

Parasitology

1. Enteric Panel: The Enteric Panel can only be performed from Fecal Swab samples. If test of cure is needed for Salmonella, Shigella or EHEC, Test of Cure Stool Culture instead.
   Please use Fecal Swabs. 1) Obtain a stool specimen in a clean pan or container. Stool specimens should not contain urine or water. 2) Holding Fecal Swab shaft above the red breakpoint mark, insert the entire tip of the Fecal Swab into the stool sample and rotate. Do not use Fecal Swab as a spoon; rather, coat swab with a visible layer. 3) If visible stool is not coating the Fecal Swab tip, reinsert until swab is coated. 4) Using swab and aiming tube away, mash and mix the stool sample against the side of the tube to suspend the sample. 5) Invert the tube several times to homogenize the sample and expose the sample to Cary Blair preservative fluid.

2. Ova and parasite exam: Within 1 hour of collection, transfer a few grams of stool to each vial of SHL collection kit. Order on EPIC as "SHL, Routine O+P with trichrome stain", complete the SHL requisition that is in the box, and deliver to Specimen Control for transport to SHL. A minimum of three stool specimens collected on alternate days is recommended. Onset of diarrhea in patients hospitalized for >3 days is usually not attributed to a parasitic infection. Requests to include Microsporidia or Cyclospora detection must be specified on the SHL requisition.

3. Pinworm exam: Submit scotch tape prep. Touch the perianal folds with clear scotch tape, then attach the tape to a clean glass slide and transport to the laboratory sealed in a ziplock bag. Clear tape must be used, not invisible tape.

4. Parasite exam: For direct examination of parasites (worms), arthropods (insects, spiders), and suspect material passed in stool. This is not a stool ova and parasite (O&P) exam. An O&P can be ordered in EPIC as "SHL, Routine O+P with trichrome stain" and uses SHL collection kit

Instructions:

A. Submit whole worms, worm segments or other objects in 70% alcohol or 10% formalin.

B. Submit arthropods in a clean, dry container.

5. Scabies exam: Sterile mineral oil is available from Pharmacy (item 991565, 10 mL container). Collect skin scrapings as follows:

A. Place a drop of mineral oil on a sterile scalpel blade.

B. Allow some of the oil to flow onto the papule. Scrape vigorously six or seven times to remove the top of the papule. (Tiny flecks of blood should be seen in the oil.)

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C. Transfer the oil and scrapings onto a glass slide (an applicator stick can be used).

D. Add 1-2 extra drops of mineral oil to the slide and mix well. Clumps can be crushed to expose hidden mites.

E. Place a coverslip onto the slide and transport to the Microbiology Lab immediately.

6. Blood Parasite EXAM (R/O Malaria/Blood Parasites): Collect venous blood in EDTA collection tube and deliver immediately to lab. Malaria antigen testing is available 24 hrs/day, 7 days a week. Antigen results will be available within one hour of specimen arrival. Preliminary slide results will be available within 90 minutes if specimen received between 0700-1900 or by 0930 if after 1900. If clinical suspicion for malaria remains after one set of negative smears, additional specimens should be submitted at 12 hour intervals for the subsequent 36 hour period. Note on request if parasite infection other than malaria is suspected.

7. Vaginosis/Vaginitis Panel (Trichomonas, Yeast and Gardnerella): Collect vaginal specimen in Affirm VPIII Collection and Transport System. Deliver to laboratory within 24 hours of collection.

16MBU304A
II BSC Microbiology
Microbial diagnosis in health clinic

Unit III Q	Opt 1	Opt 2	Opt 3	Opt 4	Answer
spe	micrococcu	streptococ	lactococcus	staphylococcus	streptococcus
e	pasteur	emil von be	jenner	alexander ogston	alexander ogston
staphyloco	peptone	beef extrac	NaCl	lactose	NaCl
is the sele	blood agar	PLET	nutrient ag	crystal violet blood agar	crystal violet blood a
local infect	impetigo	pustule	hemorrhag	meningitis	impetigo
The	slide coagu	tube coagu	Grams test	serum test	slide coagulase test
Streptococ	amoxycillin	penicillin	erythromyc	bacitracin	bacitracin
the disease	hide porter	gas gangre	edeme	toxemia	hide porter's disease
_____	micrococcu	streptococ	staphyloco	E.coli	staphylococcus aureu
Vegetative	60C	40C	20C	10C	60C
The spores	20	30	40	60	60
Lepra bacil	100	80	46	20	46
The Lepra l	Common d	Multibacilli	Gas gangre	Symptomatic disease	Multibaciliry disease
was	rifampicin	clofazimine	ethionamic	dapsone	dapsone
_____ v	BCG	Polio vacci	Leprosy va	Rabies vaccine	BCG
The Lepra l	Jenner	Behring	Pasteur	Hansen	Hansen
The diphth	Pasteur	Hansen	Klebs	Loeffler	Klebs
Typing in P	serum typi	Quellung	Bamboo-st	stickland	Quellung
Corynebact	Rod	Cocci	Varied	Spindle shaped	Varied
On repeate	smooth-ro	rough-smo	smooth	rough	smooth-rough
	meningitis	hemorrhag	paralysis	Broncho-pneumonia	Broncho-pneumonia
The strain	Bacillus Ca	Park Willia	Park Willia	Bacillus Calmette 8 strain	Park Williams 8 Strai
The diagno	Bile solubil	serum solu	symptoms	pathogeicity	Bile solubility
The incuba	5 to 6	2 to 5	4 to 9	1 to 3	2 to 5
The BCG va	Fernandez	Pasteur	Jenner	Hansen	Fernandez
Preventatic	prophlaxix	treatment	active imm	antibiotics	active immunization
react	M'Fadyean	Quellung	Nagler	immunization	M'Fadyean's
Staphyloco	Penicillin	streptomyc	amoxycillin	dapsone	Penicillin
is a ty	pus	edema	focal suppu	impetigo	focal suppuration
Streptococ	sore throat	Strep.throa	infection	disease	sore throat
na	Jenner	Behring	Rosenbach	Klebs	Rosenbach
Typical Sta	pus	blood	stool	urine	pus
is the s	PLET	blood agar	Mac-conke	PDA	PLET
In cultures	chain	Bamboo-st	cluster	thread-like	Bamboo-stick
The	exotoxin	antitoxin	size	shape	antitoxin
typ	fever	headache	paralytic	nausea	paralytic
Food poiso	fish	egg	soup	meat	meat

Pneumoco	Nagler	Robert Hoc	Pasteur an	Kleb and Loeffler	Pasteur and Sternberg
The toxin p	exotoxin	endotoxin	antitoxin	toxin	exotoxin
The drug o	Penicillin	streptomyc	metranidaz	Amoxycillin	metranidazole
The irregul	citron bodi	pleomorph	irregular bo	boat bodies	pleomorphs
The arrang	V shaped	L shaped	Chinese	Bamboo- stick	Chinese
The causat	streptococ	micrococcu	Clostridium	Corynebacterium	Clostridium perfringe
Clostridium	PLET	Robertson'	PDA	Mac-conkey	Robertson's cooked n
_____ dyes	Acidic dyes	Basic dyes	Neutral dy	Aniline dyes	Aniline dyes
The most i	Type specif	Type specif	Type specif	Type specific antibody	Type specific capsula
When sput	blood	serum	serum coat	egg	serum coated larynge
is a	lipopolysac	teichoic aci	lactate	iron	teichoic acid
Staphyloc	Tumour	Boils	Lesion	Fever	Lesion
Staphyloc	Vonreckli	Ogsten	Passet	Pasteur	Vonreckling hausen
Staphyloc	Red	Blue	Brown	Yellow	Yellow
Non virule	Water	Hospital	. Skin	Hair	. Skin
Mannitol f	Virulant	Avirulant	Commens	Normal flora	Virulant
Oil paint a	Nutrient b	Nutrient a	Blood aga	Blood broth	Nutrient agar
In intoxica	Toxoid	Tetroid	.Enzyme	Toxin	Toxin
Alpha hae	Complete	Irregular	. Partial	Nolysis	. Partial
Beeta haer	Complete	Irregular	Partial	Nolysis	Complete
Selective r	Manittol s	Mac conk	Blood aga	LJ agar	Manittol salt agar
Streptococ	Alpha	Beta	Gamma	delta	Beta
Pneumoco	Liver	Urinary	Respirator	Bone marrow	Bone marrow

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ar polysaccharide
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2017

II B. Sc Microbiology – Microbial diagnosis in health clinic

Unit III possible questions

Part B

1. Define endemic ad epidemic
2. Say about digestive system
3. What are clinical specimens?
4. What are Infection and immunity?
5. What is IFT?

Part C

1. Give the laboratory diagnosis of Acid fast bacilli.
2. State the pathogenic role and diagnosis of GI tract pathogenesis.
3. Discuss the properties and UTI caused by *E.Coli*.
4. Explain in detail about Kirby bauer technique of susceptibility testing.
5. Comment on respiratory tract infection and its symptoms.

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II B. Sc Microbiology –Microbial diagnosis in health clinic
Unit IV

LECTURE PLAN

S no	Lecture Duration (Hr)	Topics to be covered	Support Materials
1	1hr	Agglutination test	W1
2	1hr	ELISA	W1 & T1
3	1hr	IFT	W1 & T1
4	1hr	PCR	W1
5	1hr	WIDAL	W1 & R1
6	1hr	Typhoid & HIV	W1
7	1hr	Revision of unit 3 & 4	
8	1hr	Unit 3 & 4 test	
Total no. of hours planned for unit-4		**8 hours**	

Textbooks :T1-Laboratory diagnosis of infectious diseases-paul.G.Engel

References Books: R1- Medical Microbiology – Sherris

Website :

Journal :

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Agglutination Reactions

The interaction (immune reaction) between antibody and a particulated antigen resulting a visible clumping called **agglutination.** Antibodies that produce such reactions are called **agglutinins.** Agglutination reactions are similar in principle to precipitation reactions; they depend on the cross linking of polyvalent antigens. The excess of antibody concentration inhibits precipitation reactions, also inhibit agglutination reactions; this inhibition is called the **prozone effect (prozone phenomenon)**. Hence serial dilutions are usually prepared in many similar immunological techniques to avoid this prozone effect by reaching the dilution of the optimum antibody concentration. All type of agglutination reactions are simple to performe (easy), can detect small amounts of antibodies (concentrations as low as nanograms per milliliter) and of low cost.

General feature of antigen antibody reactions

.1 The reaction is **specific**; an antigen combines only with its homologous antibody and vice versa. The specificity however is not absolute and cross reactions may occur due to antigenic similarity or relatedness.

.2 Entire molecules react and not fragment.

.3 There is **no denaturation** of the antigen or the antibody during the reaction.

.4 The combination occurs at the surface, therefore it is the **surface antigens that are immunologically relevant.**

.5 The combination is **firm and irreversible**. The firmness of the union is influenced by the affinity and avidity of the reaction.

.1 **Affinity** refers to the intensity of attraction between the antigen and antibody molecules. It is a function of the closeness of fit between an epitope and the antigen combining region of its antibody.

.2 **Avidity** is the strength of the bond after the formation of the antigen antibody complexes. It reflects the overall combining property of the various antibody molecules in an antiserum, possessing different affinity constants with the multiple epitopes of the antigen.

.6 Antigens and antibodies can combine in **varying proportions**, unlike chemicals with fixed valencies. Both antigens and antibody are multivalent, antibodies are generally bivalent, though **IgM molecules may have five or ten combining sites**. Antigens may have valencies up to hundreds.

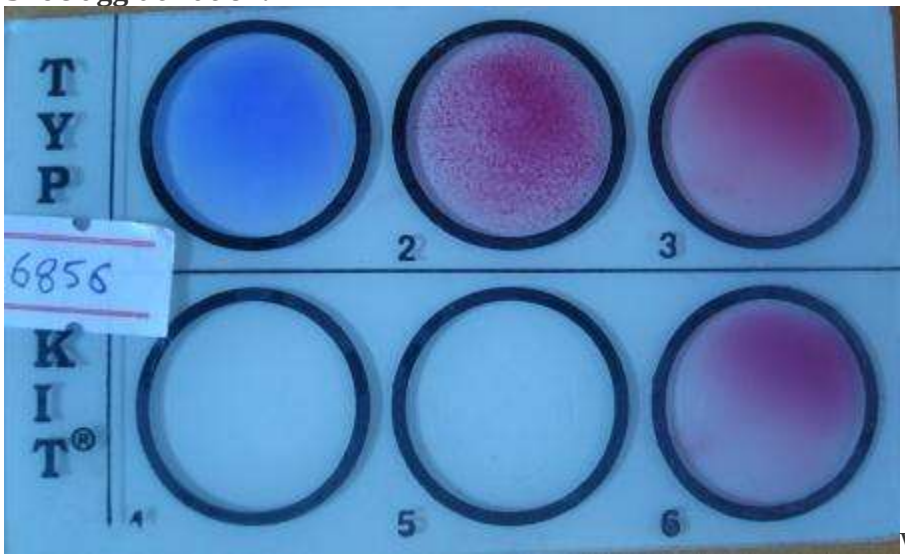
Types of agglutination

.1 Slide agglutination: Serotyping.

.2 Tube agglutination: e.g. **Widal test.**

.3 Indirect (passive agglutination): where soluble antigens are coated on vehicle particle e.g. latex particle, RBCs.

Slide agglutination.



Widal Test: Sample showing H positive in screening test

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- When a drop of the appropriate antiserum is added to a smooth, uniform suspension of a particulate antigen in a drop of saline on a slide or a tile, agglutination takes place.
- A positive result is indicated by the clumping together of the particles and the clearing of the drop. Depending up on the titre of the serum, agglutination may occur instantly or with in seconds.
- Clumping occurring after a minute may be due to drying of the fluid and should be disregarded.
- It is essential to have on the same slide a control consisting of the antigen suspension in saline, without the antiserum, to ensure that the antigen is not autoagglutinable.
- Slide agglutination is a routine procedure for the identification of many bacterial isolates from clinical specimens. It is also the method used for blood grouping and cross matching.

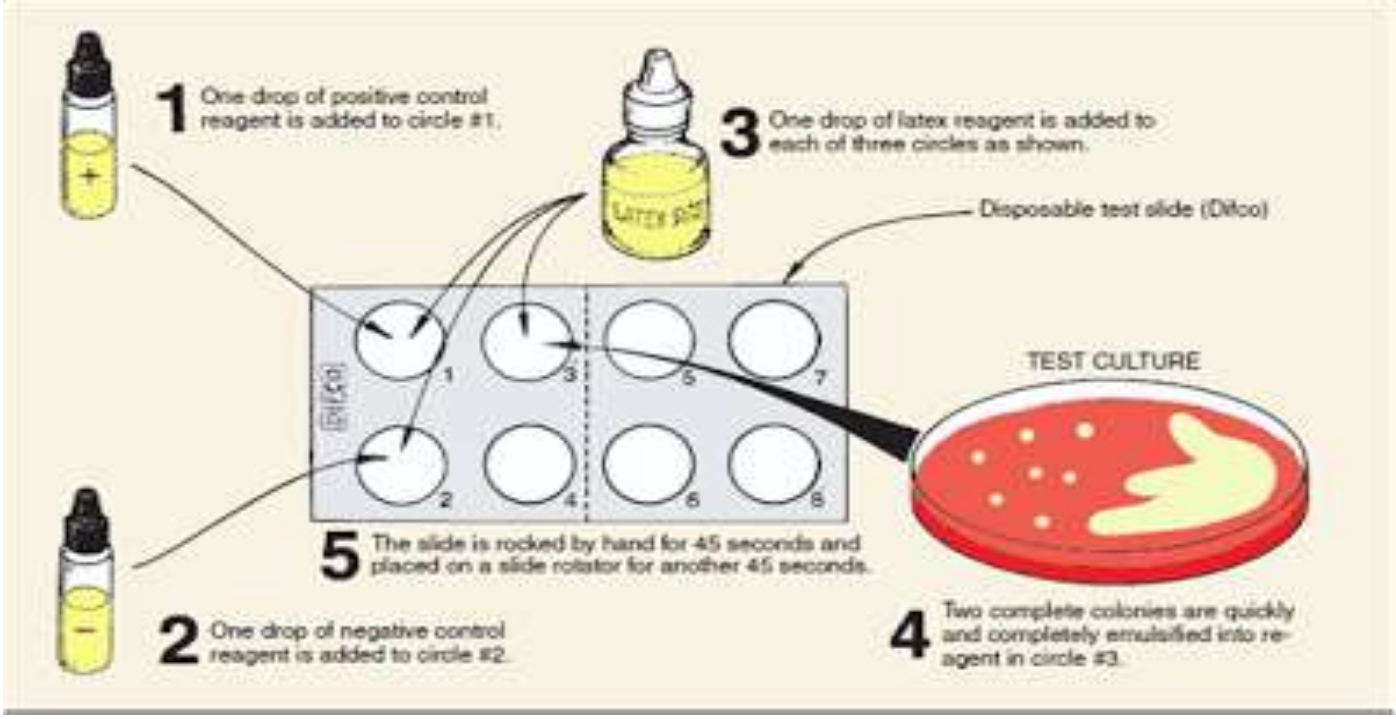

Fig: How to perform slide agglutination

Tube agglutination

This is the standard quantitative method for the measurement of antibodie

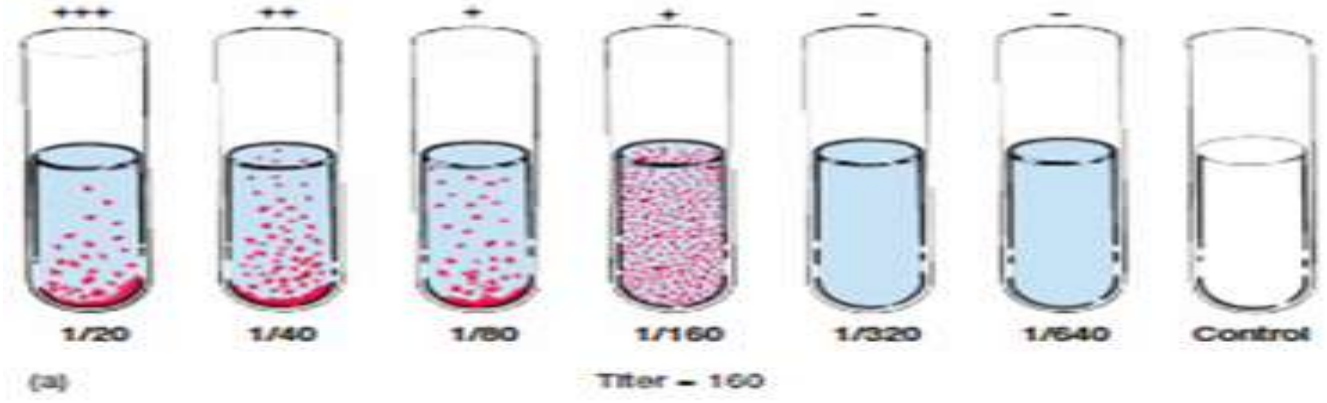

Fig. (a).Tube agglutination test for determining antibody titer.

- When a fixed volume of a particulate antigen suspension is added to an equal volume of serial dilutions of an antiserum in test tubes, the agglutination titre of the serum can be estimated.

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- Tube agglutination is routinely employed for the serological diagnosis of typhoid, brucellosis and typhus fever (weil- felix reaction).

Widal test

The procedure involves adding a suspension of dead typhoid bacterial cells to a series of tubes containing the patient's serum, which has been diluted out to various concentrations. After the tubes have been incubated for 30 minutes at 37° C, they are centrifuged and examined to note the amount of agglutination that has occurred. The reciprocal of the highest dilution at which agglutination is seen is designated as the **antibody titer** of the patient's serum. For example, if the highest dilution at which agglutination occurs is 1:320, the titer is 320 antibody units per milliliter of serum. Naturally, the higher the titer, the greater is the antibody response of the individual to the disease.

Two types of antigens are used, the **H or the flagellar antigen** and the **O or the somatic antigen** of the typhoid bacillus.

Find details information about WIDAL TEST here

Round bottomed felix tubes are used for agglutination. Agglutinated bacilli spread out in a disc like pattern at the bottom of the tubes. The tube agglutination test for brucellosis may be complicated by the prozone phenomenon and the presence of blocking antibodies. Several dilution of the serum should be tested to prevent false negative results due to prozone.

The **weil- felix reaction** for serodiagnosis of typhus fever is a heterophile agglutination test and is based on the sharing of a common antigen between typhus rickettsiae and some strains of proteus bacilli. Another example of the heterophile agglutination test is the streptococcus MG agglutination test for the diagnosis of primary atypical pneumonia.

Examples of agglutination tests using red blood cells as antigens are the Paul Bunnel test and the cold agglutination test. The cold agglutination test is positive in primary atypical pneumonia. The patient's sera agglutinate human O group erythrocytes at 4°C the agglutination being irreversible at 37°C.

Hemagglutination is used in blood typing

Agglutination reactions are routinely performed to type red blood cells. In typing for the ABO antigens, RBCs are mixed on a slide with antisera to the A or B blood group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide. Determination of which antigens are present on donor and recipient blood is the basis for matching blood types for transfusions.

Particle agglutination

Numerous procedures have been developed to detect antigen via the agglutination (clumping) of an artificial carrier particle such as a latex bead with antibody bound to its surface.

Latex agglutination

Antibody molecules can be bound in random alignment to the surface of latex (polystyrene) beads. Antigen present in a specimen being tested binds to the combining sites of the antibody exposed on the surfaces of the latex beads, forming cross- linked aggregates of latex beads and antigen.

- The size of the latex bead (0.8µm or larger) enhances the ease with which the agglutination reaction is recognized.
- Levels of bacterial polysaccharides detected by latex agglutination have been shown to be as low as 1.0 ng /ml because the pH, osmolarity and ionic concentration of the solution influence the amount of binding that occurs, conditions under which latex agglutination procedures are carried out must be carefully standardized.
- Additionally, some constituents of body fluids such as rheumatoid factor, have been found to cause false- positive reactions in the latex agglutination systems available. To counteract this problem. It is recommended that all specimens be treated by boiling or with ethylenediaminetetraacetic acid (EDTA) before testing.

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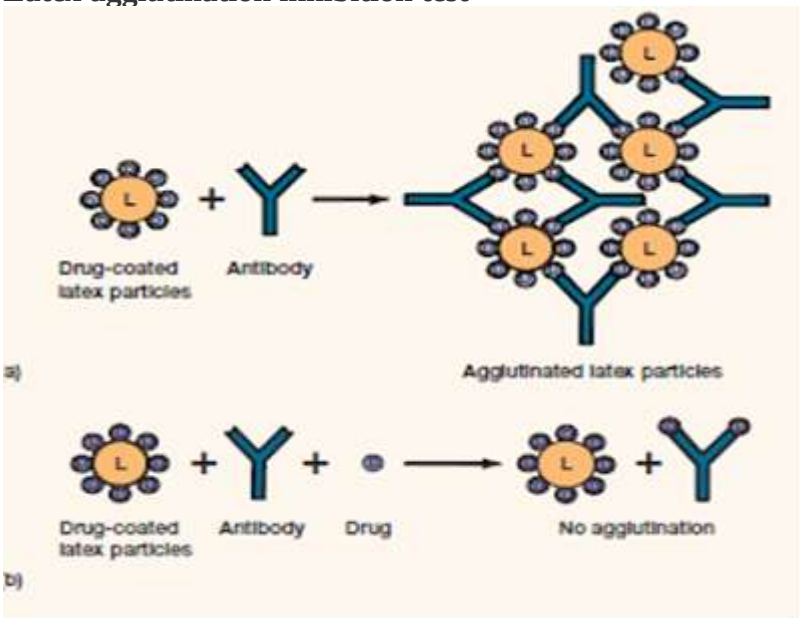
- Commercial test systems are usually performed on cardboard cards or glass slides; manufacturers recommendations should be followed precisely to ensure accurate results.
- Reactions are graded on a 1+ to 4+ scale, with 2+ usually the minimum amount of agglutination seen in a positive sample.
- Control latex (coated with antibody form the same animal species from which the specific antibody was made) is tested alongside the latex. If the patient specimen or the culture isolate reacts with both the test and control latex, the test is considered non specific and therefore uninterpretable.
- Latex tests are very popular in clinical laboratories to detect antigen to *Cryptococcus neoformans* in CSF or serum and to confirm the presence of beta- hemolytic streptococcus form the culture plates. Latex tests are also available to detect *Streptococcus agalactiae*, *Clostridium difficile* toxins A and B and rotavirus.

Coagglutination

In this case the particles are killed and treated ***Staphylococcus aureus*** (cowan I strain), which contain a large amount of an antibody- binding protein, protein A, in their cell walls. In contrast to latex particles, these staphylococci bind only the base of the heavy chain portion (Fc) of the antibody, leaving both (Fab)antigen-binding ends free to form complexes with specific antigen.

Several commercial suppliers have prepared coagglutination reagents for identification of streptococci, including Lancefield groups A, B , C, D, F , G and N; *Streptococcus pneumoniae*; *Neisseria meningitides*; *N gonorrhoeae*; and *Haemophilus influenzae* types A to F grown in culture. The coagglutination reaction is highly specific but may not be as sensitive for detecting small quantities of antigen as latex agglutination. Thus, it is not usually used for direct antigen detection.

Latex agglutination inhibition test



Latex agglutination inhibition test a) negative b) positive

The latex agglutination inhibition test relies on competition for the antibody between a latex- drug conjugate and any drug that may be present in the sample (mostly urine). A urine sample is placed in the mixing well of a slide containing antibody reagent, buffer and latex reagent.

a) If the drug is absent, the latex- drug conjugate binds to the antibody and forms large particles that agglutinate. Therefore agglutination is evidence for the absence of drugs in the urine specimen

b) If a drug is present in the urine sample, it competes with the latex conjugate for the small amount of available antibody. A sufficient quantity of the drug will prevent the formation of particles and agglutination and a positive urine sample does not change the appearance of the test mixture.

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Coombs test

Direct coombs test: Detection of incomplete antibodies on patients RBCs. Antibodies attached on the surface of the RBCs (patient RBCs) + Antihuman globulin = agglutination. .1

Indirect coombs test: Detection of antibodies in patients sera. Rhesus positive RBC + Patient serum (if contains incomplete circulating Abs coats the surface of the RBC)+ Antihuman globulin which makes the bridge = agglutination .2

Hemagglutination of Blood Typing .1

Agglutination reactions (the Figure below), represent the routine laboratories test for determining blood type (blood grouping for ABO system).

Principles:

Recognizing the type red blood cells (RBCs) by typing A and B antigens. (1

RBCs (in the whole blood) are mixed on a slide with antisera (ready from a kit) prepared to react with A and B antigens. (2

If the antigen is present on the cells surface, they will agglutinate RBCs, forming a visible red clump on the slide. (3

Clinical application:

The importance of this test is that it is routinely used in matching blood types (groups) for transfusions by determination of which antigens are present on donor and recipient RBCs.

Bacterial Agglutination: .2

It is used to diagnose bacterial infection, when the immune system starts the production of serum antibodies specific for surface antigens on the bacterial cells. These Abs can be detected by bacterial agglutination reactions.

Principles:

Serial dilutions of serum from a patient suspected to be infected with a given bacterium in tubes. .1

Addition of bacteria to these tubes with constant concentration (same amount and concentration for all tubes). .2

If the person was infected with these bacteria, a visible agglutination will form in the tube of equivalent concentration of serum Abs. This tube is the last tube of positive (with agglutination) among all tubes. It represents the titer of serum antibodies in the patient. For example: If serial twofold dilutions of serum were prepared, and if the dilution of 1/32 shows agglutination but the dilution of 1/64 does not, then the agglutination titer of the patient's serum is 32. .3

Note: In some cases serum can be diluted up to 1/50,000 if it still shows agglutination with bacteria in all tubes (until the appearance of tubes without agglutination). .4

Clinical application:

The agglutinin (specific Abs) titer of an antiserum can be used to diagnose a bacterial infection. Example: Patients with typhoid fever, for example, show a rise in the agglutination titer with *Salmonella typhi bacteria.* Agglutination reactions also provide a way to type bacteria like different species of *Salmonella* can be distinguished by agglutination reactions by typing test using patient antiser

Passive Agglutination: .3

Usually used for soluble Antigens. **.1**

There are two type of this agglutination: **.2**

Agglutination of antigen-coated erythrocytes. It is called Hemagglutination test. .a

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Agglutination of antigen-coated Latex beads. It is called Indirect agglutination test. .b

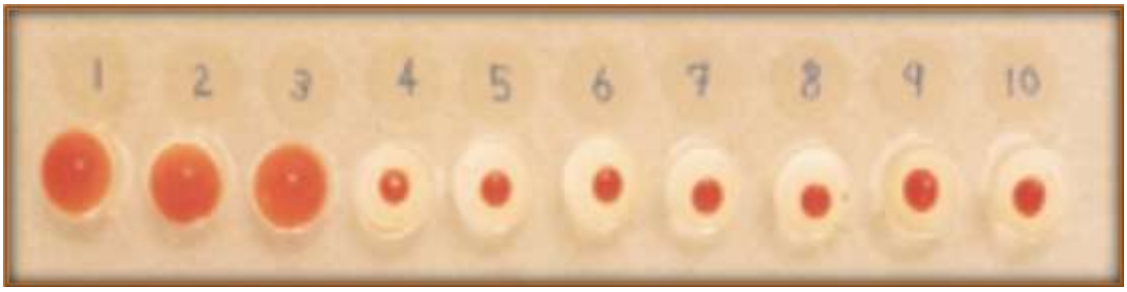
Hemagglutination test (Agglutination of antigen-coated erythrocytes) .A

Principles:

Red blood cells (sheep RBCs are mostly used) in this test are used as carriers of antigens and coloring agents. Antigen-coated red blood cells are prepared by mixing a soluble antigen with tanned red blood cells (that have been treated with tannic acid or chromium chloride to make them sticky), the Ag will be adsorbed of to the surface of the red cells. .1

Diluting serum containing antibody serial dilutions into microtiter plate wells, and the antigen-coated red blood cells are then added to each well. .2

Agglutination can be noticed by the size of the agglutinated red blood cells on the bottom of the well. .3



The formation of a red spot in the well is a marker for no agglutination. .4

There are two type of Hemagglutination tests; direct Hemagglutination and Indirect agglutination. .5

Clinical application:

Determine if a person is using illegal drugs, such as cocaine or heroin using agglutination inhibition assay and the suspected person urine sample.

Latex agglutination (Indirect agglutination test) .B

Principles:

Using synthetic particles called latex beads (polystyrene material); they act as carrier for soluble antigen in agglutination tests. .1

Absorbing Ags on beads surface, many Ags (e.g. proteins) are able to adsorb easly on latex beads surface and change soluble Ag to be a particulated Ag, they are of low cost equipment, do not interfere in Ag-Ab reaction and make the reaction more visible and can be read rapidly (3-5 min) after of mixing the beads with the test sample. .2

Clinical application:

Pregnancy test is of a highly sensitive assay for small quantities of antigen in urine samples. The latex particles coated with prepared chorionic gonadotropin antibodies (anti-HCG) in pregnancy test kits. When urine from a pregnant female, which contained HCG (work here as Ag), mixed with latex beads reagent, if agglutination occurs that will indicate for pregnancy, if absence means no pregnancy. .1

ASOT Anti-streptolysin O (ASO or ASLO) is a test that detects the antibodies formed against streptolysin O bacterial toxin during infection with *Streptococcus pyogens* using Latex agglutination: It **.2**

is important test for the treatment of rheumatic fever and post infectious glomerulonephritis by serial dilution method or slide agglutination method.

Slide ASOT (Antistreptolysin-OTest):

It is a rapid latex particle agglutination test that uses latex reagent, the latex particles coated with streptolysin-O the soluble Ag, the presence of infection means the presence of antistreptolysin-O in the serum leading for agglutination of the latex particles.

Procedure:

Using the micropipette apply the followings on the test card:

One drop application of positive control on the first area. - 1
One drop application of negative control on the second area. - 2
One drop application of serum sample on the third area. - 3
One drop application of latex reagent on all the three areas. - 4
Sample and reagents should be mixed well using wooden sticks or electronic rotator for 2 minutes (100 rpm). - 5
Results: - 6

- Agglutination in the serum sample----------positive.
- No agglutination in the serum sample---------negative.
- Agglutination in negative control or **no** Agglutination in positive control----------error.

Note: the negative ASOT result does not mean there is no infection; second test should be done after 4 weeks. **.3**

ELISA- Principle, Types and Applications

ELISA is an antigen antibody reaction. In 1971, ELISA was introduced by Peter Perlmann and Eva Engvall at Stockholm University in Sweden. It is a common laboratory technique which is usually used to measure the concentration of antibodies or antigens in blood.

ELISA is a plate based assay technique which is used for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. An enzyme conjugated with an antibody reacts with colorless substrate to generate a colored product. Such substrate is called chromogenic substrate. A number of enzymes have been used for ELISA such as alkaline phosphatase, horse radish peroxidase and beta galactosidase. Specific substrate such as ortho-phenyldiamine dihydrochloride (for peroxidase), paranitrophenyl phosphate (for alkaline phosphatase) are used which are hydrolysed by above enzymes to give colored end product.

Principle

ELISAs are typically performed in 96-well polystyrene plates. The serum is incubated in a well, and each well contains a different serum. A positive control serum and a negative control serum would be included among the 96 samples being tested. Antibodies or antigens present in serum are captured by corresponding antigen or antibody coated on to the solid surface. After some time, the plate is washed to remove serum and unbound antibodies or antigens with a series of wash buffer. To detect the bound antibodies or antigens, a secondary antibodies that are attached to an enzyme such as peroxidase or alkaline phosphatase are added to each well. After an incubation period, the unbound secondary antibodies are washed off. When a suitable substrate is added, the enzyme reacts with it to produce a color. This color produced is measurable as a function or quantity of antigens or antibodies present in the given sample. The intensity of color/ optical density is measured at 450nm. The intensity of the color gives an indication of the amount of antigen or antibody.

Types of ELISA

2017

Frequently there are 3 types of ELISA on the basis of binding structure between the Antibody and Antigen.

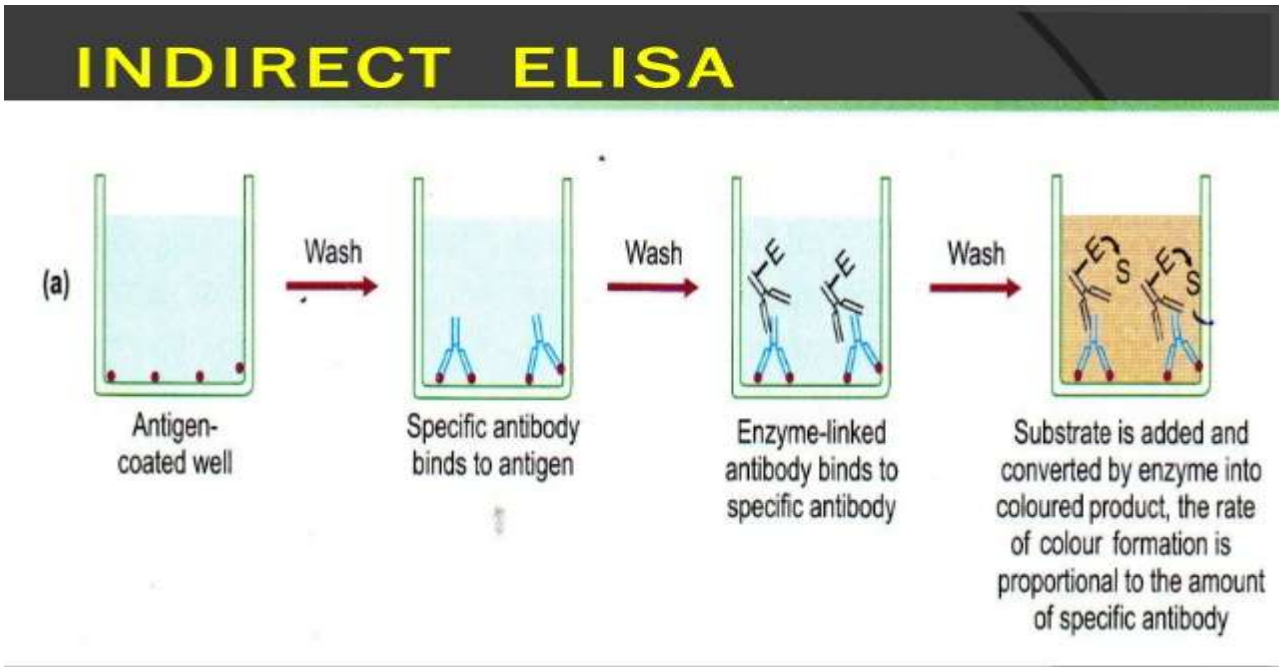
1. Indirect ELISA
2. Sandwich ELISA
3. Competitive

1. Indirect ELISA

Antibody can be detected or quantitatively determined by indirect ELISA. In this technique, antigen is coated on the microtiter well. Serum or some other sample containing primary antibody is added to the microtiter well and allowed to react with the coated antigen. Any free primary antibody is washed away and the bound antibody to the antigen is detected by adding an enzyme conjugated secondary antibody that binds to the primary antibody. Unbound secondary antibody is then washed away and a specific substrate for the enzyme is added. Enzyme hydrolyzes the substrate to form colored products. The amount of colored end product is measured by spectrophotometric plate readers that can measure the absorbance of all the wells of 96-well plate.

Procedure of Indirect ELISA

1. Coat the micro titer plate wells with antigen.
2. Block all unbound sites to prevent false positive results.
3. Add sample containing antibody (e.g. rabbit monoclonal antibody) to the wells and incubate the plate at 37°c.
4. Wash the plate, so that unbound antibody is removed.
5. Add secondary antibody conjugated to an enzyme (e.g. anti- mouse IgG).
6. Wash the plate, so that unbound enzyme-linked antibodies are removed.
7. Add substrate which is converted by the enzyme to produce a colored product.
8. Reaction of a substrate with the enzyme to produce a colored product.



Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

Advantages

- Increased sensitivity, since more than one labeled antibody is bound per primary antibody.
- A wide variety of labeled secondary antibodies are available commercially.
- Maximum immunoreactivity of the primary antibody is retained because it is not labeled.
- Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection.
- Flexibility, since different primary detection antibodies can be used with a single labeled secondary antibody.
- Cost savings, since fewer labeled antibodies are required.
- Different visualization markers can be used with the same primary antibody.

Disadvantages

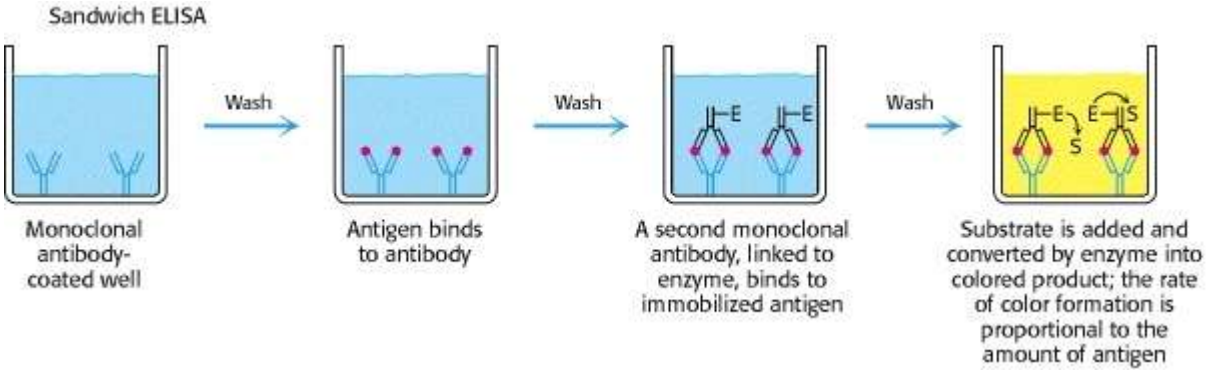
- Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal.
- An extra incubation step is required in the procedure.

2. Sandwich ELISA

Antigen can be detected by sandwich ELISA. In this technique, antibody is coated on the microtiter well. A sample containing antigen is added to the well and allowed to react with the antibody attached to the well, forming antigen-antibody complex. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. Then after unbound secondary antibody is removed by washing. Finally substrate is added to the plate which is hydrolyzed by enzyme to form colored products.

Procedure of sandwich ELISA

1. Prepare a surface to which a known quantity of antibody is bound.
2. Add the antigen-containing sample to the plate and incubate the plate at 37°c.
3. Wash the plate, so that unbound antigen is removed.
4. Add the enzyme-linked antibodies which are also specific to the antigen and then incubate at 37°c.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme to produce a colored product.
7. Reaction of a substrate with the enzyme to produce a colored product.



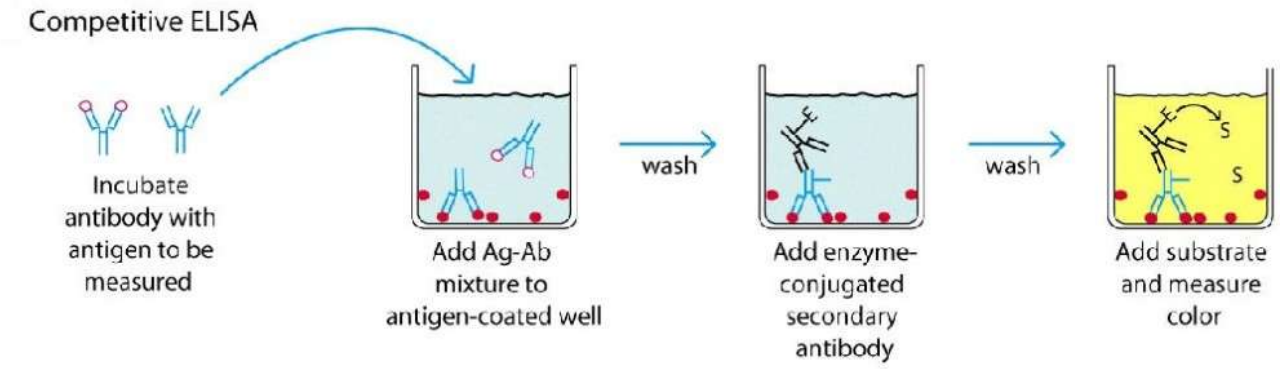
Advantages

- High specificity, since two antibodies are used the antigen is specifically captured and detected.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.
- Flexibility and sensitivity, since both direct and indirect detection methods can be used.

3. Competitive ELISA

This test is used to measure the concentration of an antigen in a sample.

In this test, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to the microtitre well which is coated with antigen. The more the antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. After the well is washed, enzyme conjugated secondary antibody specific for isotype of the primary antibody is added to determine the amount of primary antibody bound to the well. The higher the concentration of antigen in the sample, the lower the absorbance.



Procedure

1. Antibody is incubated with sample containing antigen.
2. Antigen-antibody complex are added to the microtitre well which are pre-coated with the antigen.
3. Wash the plate to remove unbound antibody.
4. Enzyme linked secondary antibody which is specific to the primary antibody is added.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme into a fluorescent signal.

Advantages

- High specificity, since two antibodies are used.
- High sensitivity, since both direct and indirect detection methods can be used.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.

Application of ELISA

1. Presence of antigen or the presence of antibody in a sample can be evaluated.
2. Determination of serum antibody concentrations in a virus test.
3. Used in food industry when detecting potential food allergens.

Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

Applied in disease outbreaks- tracking the spread of disease e.g. HIV, bird flu, common, colds, cholera, STD etc. .4

Antibiotics

I. History of Antibiotics

A. "Magic Bullet" concept (early 1900's)

1. Paul Ehrlich proposed idea of using a drug that would selectively find and kill

pathogen, but not harm human cells (early 1900's)

2. Basis of antimicrobial chemotherapy (drug therapy)

B. Discovery of Penicillin (1928)

1. Alexander Fleming noticed that the growth of *Staphylococcus aureus*was inhibited in the area surrounding a colony of mold that contaminated a Petri plate.

2. The compound was later isolated from the mold and named penicillin

3. 1st Antibiotic

4. Antibiotic – substance produced by one organism that inhibits another organism

C. Sulfanilamide compound in dyes was used (Sulfa Drugs, 1932)

1. Technically an antimicrobial and not an antibiotic, because it is not produced by an

organism, i.e. synthetic drug

2. Resurrected interest in penicillin

D. Florey and Chain (1940, WWII)

1. 1st Successful clinical trials with penicillin

2. Fleming, Chain, and Florey won Nobel Prize

II. Antibiotic – Production and Range

A. Production

1. Naturally occurring – produced entirely by bacteria or fungi (true antibiotic)

2. Semi-synthetic antibiotics – part natural and part designed in lab

3. Synthetic antibiotics – designed in lab

B. Narrow Spectrum Antibiotic

1. Affect narrow range of bacteria, such as only Gram+

C. Broad Spectrum Antibiotic

1. Affect large range of bacteria, such as Gram+ and Gram-

2. Benefit vs. Disadvantage?

3. Superinfection

a) Kill good bacteria and allow opportunistic organism to flourish, such as

Candida albicans in a yeast infection
b) Also, occurs if the strain causing the infection gains antibiotic resistanceto the antibiotic being used to treat the infection

III. Modes of Action, i.e. How do Antimicrobial Drugs Work?
A. Target differences between our cells and pathogens; selective toxicity

B. Modes of Action

1. Inhibit Cell Wall Synthesis

a. Peptidoglycan is only in bacteria cell walls
2. Inhibit Protein Synthesis – Translation at Ribosome

a. Differences in ribosomes allows for selective toxicity

b. Eukaryotes have 80S (60S and 40S) ribosomes, and prokaryotes have 70S (50S & 30S) ribosomes

*NOTE: Mitochondria contain 70S ribosomes; can affect liver and bone marrow cells
*Translation occurs at ribosomes, mRNA to protein

DNA (gene) ——transcription———-mRNA ——translation——- protein
3. Injure Plasma Membrane

4. Inhibit DNA Replication & Transcription

*NOTE: Transcription produces mRNA from gene on DNA
5. Inhibit Synthesis of Essential Metabolites

C. Drugs Used Against

1. Bacteria

2. Fungi

3. Viruses

4. Protozoans

5. Helminths

IV. Antibacterial Antibiotics

A. Inhibit Cell Wall Synthesis

1. Penicillin – prevents peptidoglycan cross-linking

a. 50 chemically related antibiotics

b. Common core of *ß-lactam ring*

c. Natural Penicillins

1) Produced from mold *Penicillium*

2) Penicillin G (injection) and Penicillin V (oral)

3) Against Gram+ (staphylococci, streptococci)

d. Semi-synthetic Penicillins

1) Part produced by mold, part produced in lab

2) Ampicillin – broad spectrum (against Gram+ and Gram-)

3) Amoxicillin and Imipenim – broad spectrum

e. Resistance to Penicillin

1) Penicillinase (a.k.a. ß-lactamase)

a) Breaks ß-lactam ring; inactivates penicillin

2) Staphyloccocus infections resistant to penicillin

a) ß-lactamase gene on plasmid

f. Methicillin (semi-synthetic penicillin) was introduced

1) Initially resistant to penicillinase, but resistance soon appeared

2) Methicillin no longer used

g. Methicillin-Resistant *Staphylococcus aureus* (MRSS)

1) Pronounced “mersa”

2) Now resistant to most penicillins and cephalosporins

2. Cephalosporins

a. Most common prescribed antibiotics

b. ß-lactam ring; similar mechanism to penicillins

c. 4 Generations

1. Each generation more effective against Gram- (broader spectrum)

d. Examples

1) 1st Generation – cephalexin (Keflex®), Cefazolin (Ancef®)
2) 2nd Generation – cephaclor (Ceclor®)
3) 3rd Generation – cephtriaxone (Rocephin®)
4) 4th Generation – cefepime (Maxipime®)

3. Bacitracin

a. Isolated from Bacillus from a girl named Tracy

b. Topical, against Gram+ staphylococci and streptococci

c. Neosporin®

4. Vancomycin

a. Used to treat MRSA

b. It's overuse led to Vancomycin-resistant enterococci (VRE)

c. VRE is a Gram+ pathogen

5. Antimycobacterial Antibiotics – inhibits mycolic acid synthesis

a. Mycolic acid is cell wall component of mycobacteria

a. Isoniazid (INH)

1) Against *Mycobacterium tuberculosis*

B. Inhibitors of Protein Synthesis

1. Aminoglycosides – changes 30S ribsosome, mRNA cannot be read properly

a. Streptomycin – 1st antibiotic agains Gram- bacteria (1944)
b. Neomycin – topical

c. Gentamicin – *Pseudomonas* infections

2. Tetracyclines

a. Broad spectrum antibiotic; produced by *Streptomyces*

b. Interfere with attachment of tRNA at 30S ribosome; interrupts protein synthesis

c. Against Gram+, Gram-, and intracellular rickettsias and chlamydias

d. Treat UTI's

e. Broad spectrum nature leads to suppression of intestinal flora, leading to GI

upsets. Also, superinfections by *Candida albicans*

f. Can discolor teeth in children and cause liver damage in pregnant woman

g. Doxycycline is semi-synthetic tetracycline

3. Macrolides

a. Interferes with growing peptide at 50S ribosome

b. Erythromycin

1) Spectrum of activity similar to penicillin G

2) Cannot penetrate Gram- bacilli

3) Alternate to penicillin

4) Treat streptococcal and staphylococcal infections

c. Azithromycin (Zithromax®) and clarithromycin (Biaxin®)

1) Macrolides with broder spectrum and tissue penetration

2) Used to treat intracellular bacteria such as *Chlamydia*

4. Oxazolidinones (ox-a-zō-lēd-i-nōnes)

a. Interferes with interaction between mRNA and ribosomes

b. Completely synthetic

c. Used in response of VRE and MRSA

d. linezolid (Zyvox®)

C. <u>Injury to Plasma Membrane</u>

1. Triclosan – interrupts fatty acid synthesis

*household antimicrobial

D. Inhibit Nucleic Acid (DNA and RNA) Synthesis

1. Fluoroquinolones

a. Ciprofloxacin – Cipro®

E. Inhibit Synthesis of Essential Metabolites

1. Sulfonamides, a.k.a. sulfa drugs

2. Competitive Inhibition; binds to active site of enzymes

3. Structural analog of PABA; interferes with folic acid synthesis

4. Trimethoprim-sulfamethoxazole (TMP-SMZ)

a. Synergistic effect – much more effective when used in combination

V. Antifungal Drugs

A. Affect fungal sterols in plasma membrane; ergosterol

1. Sterol in our plasma membranes is cholesterol

B. Azoles

1. Clotrimazole and miconazole

a. Treat athlete's foot and vaginal yeast infections

C. Tolnaftate

1. Treat athlete's foot

2. Mechanism unknown

V. Antiviral, Antiprotozoan, and Antihelminthic Drugs

VI. Considerations When Choosing an Antimicrobial Drug

A. Identify Infectious Agent (pathogen causing disease)

1. Physicians direct examination; using experience will prescribe antibiotic

2. Culture and send to lab

3. Antibiotic is prescribed and if lab results show resistance to (or wrong) antibiotic prescribed, then there will be patient follow up to get them on the right antibiotic.

4. Also, even if lab (in vitro) supports that correct antibiotic was prescribed, it may not work in patient (in vivo)

a. Patient may have a few resistant cells that did not show up in lab or there could be one than one pathogen, some of which may be resistant to drug

B. Determining Pathogen Sensitivity to Drug (in lab)

1. Expose pure culture of pathogen to several types of drugs in vitro

2. Kirby-Bauer technique

a. Agar disc diffusion test

b. Look for zone of inhibition

3. Etest®

a. Used to determine Minimum Inhibitory Concentration (MIC)

b. MIC is lowest antibiotic concentration (ug/ml) that inhibits growth

4. Broth Dilution Test

a. Used to determine MIC and Minimal Bactericidal Concentration (MBC)

b. MIC and MBC minimize excessive use to decrease chance of tissue toxicity

c. Microdilution plates used in test

C. Determining Drug Safety

1. Therapeutic Index (TI)

a. Ratio of dose toxic to humans compared to minimum therapeutic dose

b. TI = Toxic Dose / MIC

(10 ug/ml) / (9 ug/ml) = **TI = 1.1**
vs.

(10 ug/ml) / (1ug/ml) = **TI = 10 *much better choice**

D. Patient Factors

1. Take careful history of patient (check for drug contraindication)

a. Preexisting medical conditions

1) Kidney or liver diseases – metabolizing and excreting drug

b. Allergies to medications (penicillins)

c. Elderly – decreased GI absorption

d. Pregnancy – drugs can cross placenta and affect fetus (tetracycline)

e. Other medications currently being used (Drug interaction)

1) Antacids reduce absorption of isoniazid

2) Tetracycline reduces effect of oral contraceptive

16MBU304A
II BSC Microbiology
Microbial diagnosis in health clinic

Unit IVQ	Opt 1	Opt 2	Opt 3	Opt 4	Answer
Vibrio cho	negative	positive	neutral	no reaction	positive
CLED sta	cystine lac	crystalviol	citrate lact	cultural lactose electrolyte defic	cystine lactose electro
Strains of	V.cholera	Hikojima	panama	Ogawa	Ogawa
Widal test	*V.cholera*	*Salmonell*	*Klebsiella*	*Pseudomonas*	*Salmonella*
E.coli is a	Pathogen	predator	Parasite	commensal	Parasite
E.coli exh	.++--	--++	.+-+-	++++	.++--
The K ant	Protein	lipid		carbohydrate	Polysaccharide
._____	EPEC	ETEC	EHEC	EAEC	ETEC
EHEC is a	EPEC	EAEC	VTEC	EXEC	VTEC
SIDS is se	*E.coli*	*Klebsiella*	*Proteus*	*Pseudomonas*	*E.coli*
Klebsiella	Non motil	motile and	Non motil	motile and non capsulated	Non motile and capsu
Klebsiella	Dry	mucoid	Pale	diffuse	mucoid
K. pneumo	Jansen's b	Koch's ba	Friedlande	Escherich's bacilli	Friedlander's bacilli
Klebsiella	++--	--++	+++	----	--++
The tribe p	Fermentor	. non ferm	Late ferme	early fermentors	. non fermentors
The protea	One	two	three	Four	three
Proteus ex	Swarming	no	Fish in str	Darting	Swarming
The predo	Non spori	non acid f	viruses	Gram negative bacilli	Gram negative bacilli
The clinic	*Mycobacte*	*Pseudomo*	*Klebsiella*	*Shigella*	*Shigella*
Shigella i	Flagellate	sporing	capsulated	Non motile	Non motile
The select	Deoxycho	EMB	MSA	Martin Thayer	Deoxycholate citrate
The *Shige*	Hypersens	Lytic	Chemotox	Neurotoxicity	Neurotoxicity
The minin	10-50 bac	10-100 ba	100-1000	1-10 bacilli	10-100 bacilli
Bacillary d	6 hours	1 day	1-7 days	more than 7 days	1-7 days
The main	Rice wate	presence o	Abdomina	Loose scanty feces	Loose scanty feces
The infect	Malaise	gastric ulc	Septicemi	Enteric fever	Enteric fever
Salmonell	Eberth gaf	Shiga	Friedlande	Escherich	Eberth gaffky
Salmonell	Typhoid f	paratypho	Enteric fe	Malaise	Typhoid fever
The incub	6 hours	1 day	1-7 days	7-14 days	7-14 days
The infect	1-10 bacil	100000 ba	10000000	10000 bacilli	100000000 bacilli
Vibrio is _	Helical	Elongated	Twisted	Curved	Curved
Vibrio cho	Pasteur	Koch	Paccini	Boyd	Koch
Vibrio is_	Motile	Non motil	Slime	Capsulated.	Motile
Vibrio cho	Gram posi	Non motil	Spore forn	curved, Cylindrical.	curved, Cylindrical.
._____	Alcohol m	Acid	sea water	V R Medium	V R Medium
Vibrio col	Biochemic	String test	Cultural c	Coombs test	String test
Heiberg cl	2	4	6	8	6

The route	Oral	respiratory	Ingestion	inhalation	Oral
Pseudomo	polar	bipolar	peritrichou	Atrichous	polar
Vibrio is n	Atrichous	peritrichou	polar	Lopotrichous.	polar
Glycocaly	Protein	lipid	Polysacch	carbohydrate	Polysaccharide
Pseudomo	Ruby	lucosin	Pyocynani	Verdin	Pyocynanin
Pyocyanin	Blue	green	. Red	yellow	Blue
Pyocynani	Acid	Base	chloroform	ether	chloroform , Water
Fluorescin	Greenish y	Green	red	blue	Greenish yellow
Pyorubin i	yellow	Green	red	blue	red
Pyomelani	brown	red	Green	yellow	brown
Pseudomo	Pyocyanin	melanin	rubin	verdin	Pyocyanin
The term '	*Proteus*	*Pseudomo*	*E.coli*	*Vibrio*	*Pseudomonas*
._____	Citrimide	EMB	DCA	MSA	Citrimide agar
enteric fev	Salmonell	S. paratyp	S. enterica	Proteus	Salmonella typhii
EHEC stra	verotoxin	exotoxin	endotoxin	exfoliate toxin	verotoxin
._____	Andrews a	Koch	Boyd	Pasteur	Andrews and Laidlaw
The acces	X and Y	X and V	X and Z	Y and Z	X and V
_____	pectinase	elastase	protease	amylase	elastase
._____	Chloramip	Bacitracin	ceftazidim	Streptomycin	Chloramiphenicol
selective r	Mac conk	blood agar	PLET	Dettol agar	Dettol agar
. *T. pallidi*	Syphilis	typhoid	Tuberculo	pertusis	Syphilis
T. pallidu	Acid	Antiseptic	drying	antibiotic	drying
Pseudomo	Primary in	secondary	re- infecti	nosocomial infection	nosocomial infection

ɔlyte deficient

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2017

II B. Sc Microbiology – Microbial diagnosis in health clinic

Unit IV possible questions

Part B

1. Define Serology.
2. Say about antigen
3. ELISA expand and define
4. What is diagnosis?
5. What are PCR?

Part C

1. Explain in detail about the laboratory precautions.
2. Discuss about WIDAL test
3. Comment on the clinical feature and diagnosis of UTI
4. Describe the properties and pathogenicity of LRT pathogens.
5. Give a detail note on opportunistic infection.
6. Write about the types and symptoms of HIV.
7. give a detailed note on diagnosis and its types

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2017

Antimicrobial Susceptibility Testing

Introduction

Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem.

Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately. Inappropriate use results from physicians providing antimicrobial drugs to treat viral infections, using inadequate criteria for diagnosis of infections that potentially have a bacterial aetiology, unnecessarily prescribing expensive, broad-spectrum agents, and not following established recommendations for using chemo prophylaxis. The availability of antibiotics over the counter, despite regulations to the contrary, also fuel inappropriate usage of antimicrobial drugs in India. The easy availability of antimicrobial drugs leads to their incorporation into herbal or "folk" remedies, which also increases inappropriate use of these agents.

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs.

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area.

The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

Principle

The principles of determining the effectivity of a noxious agent to a bacterium were well enumerated by Rideal ,Walker and others at the turn of the century, the discovery of antibiotics made these tests(or their

modification)too cumbersome for the large numbers of tests necessary to be put up as a routine. The ditch plate method of agar diffusion used by Alexander Fleming was the forerunner of a variety of agar diffusion methods devised by workers in this field .The Oxford group used these methods initially to assay the antibiotic contained in blood by allowing the antibiotics to diffuse out of reservoirs in the medium in containers placed on the surface.

With the introduction of a variety of antimicrobials it became necessary to perform the antimicrobial susceptibility test as a routine. For this, the antimicrobial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still the method of choice for the average laboratory. Automation may force the method out of the diagnostic laboratory but in this country as well as in the smaller laboratories of even advanced countries, it will certainly be the most commonly carried out microbiological test for many years to come. It is, therefore, imperative that microbiologists understand the principles of the test well and keep updating the information as and when necessary. All techniques involve either diffusion of antimicrobial agent in agar or dilution of antibiotic in agar or broth. Even automated techniques are variations of the above methods.

3. Factors Influencing Antimicrobial Susceptibility Testing

pH

The pH of each batch of Müeller-Hinton agar should be checked when the medium is prepared. The exact method used will depend largely on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (e.g., aminoglycosides, quinolones, and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected. The pH can be checked by one of the following means:

* Macerate a sufficient amount of agar to submerge the tip of a pH electrode.
* Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup.
* Use a properly calibrated surface electrode.

Moisture

If, just before use, excess surface moisture is present, the plates should be placed in an incubator (35°C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is lost by evaporation (usually 10 to 30 minutes). The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the petri dish covers when the plates are inoculated.

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Effects of Thymidine or Thymine

Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and trimethoprim, thus yielding smaller and less distinct zones, or even no zone at all, which may result in false-resistance reports. Müeller-Hinton agar that is as low in thymidine content as possible should be used. To evaluate a new lot of Müeller-Hinton agar, *Enterococcus faecalis* ATCC 29212, or alternatively, *E. faecalis* ATCC 33186, should be tested with trimethoprim/sulfamethoxazole disks. Satisfactory media will provide essentially clear, distinct zones of inhibition 20 mm or greater in diameter. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

Effects of Variation in Divalent Cations

Variation in divalent cations, principally magnesium and calcium, will affect results of aminoglycoside and tetracycline tests with *P. aeruginosa* strains. Excessive cation content will reduce zone sizes, whereas low cation content may result in unacceptably large zones of inhibition. Excess zinc ions may reduce zone sizes of carbapenems. Performance tests with each lot of Müeller-Hinton agar must conform to the control limits.

Testing strains that fail to grow satisfactorily

Only aerobic or facultative bacteria that grow well on unsupplemented Müeller-Hinton agar should be tested on that medium. Certain fastidious bacteria such as *Haemophilus* spp.,

N. gonorrhoeae, *S. pneumoniae*, and viridans and ß-haemolytic streptococci do not grow sufficiently on unsupplemented Müeller-Hinton agar. These organisms require supplements or different media to grow, and they should be tested on the media described in separate sections.

4. Methods of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include:

Diffusion	**Dilution**	**Diffusion&Dilution**
Stokes method	Minimum Inhibitory Concentration	E-Test method
Kirby-Bauer method	i) Broth dilution	
	ii)Agar	Dilution

4.1 Disk Diffusion

Reagents for the Disk Diffusion Test

1. Müeller-Hinton Agar Medium

Of the many media available, Müeller-Hinton agar is considered to be the best for routine susceptibility testing of nonfastidious bacteria for the following reasons:

* It shows acceptable batch-to-batch reproducibility for susceptibility testing.
* It is low in sulphonamide, trimethoprim, and tetracycline inhibitors.
* It gives satisfactory growth of most nonfastidious pathogens.
* A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Although Müeller-Hinton agar is reliable generally for susceptibility testing, results obtained with some batches may, on occasion, vary significantly. If a batch of medium does not support adequate growth of a test organism, zones obtained in a disk diffusion test will usually be larger than expected and may exceed the acceptable quality control limits. Only Müeller-Hinton medium formulations that have been tested according to, and that meet the acceptance limits described in, NCCLS document M62-A7- Protocols for Evaluating Dehydrated Müeller-Hinton Agar should be used.

Preparation of Müeller-Hinton Agar

Müeller-Hinton agar preparation includes the following steps.

1. Müeller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer's instructions.
2. Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath.
3. Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.
4. The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C).
5. Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.
6. A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

2. Preparation of antibiotic stock solutions

Antibitiotics may be received as powders or tablets. It is recommended to obtain pure antibiotics from commercial sources, and not use injectable solutions. Powders must be accurately weighed and dissolved in the appropriate diluents (Annexure III) to yield the required concentration, using sterile glassware. Standard strains of stock

cultures should be used to evaluate the antibiotic stock solution. If satisfactory, the stock can be aliquoted in 5 ml volumes and frozen at -20ºC or -60ºC.

Stock solutions are prepared using the formula (1000/P) X V X C=W, where P+potency of the anitbiotic base, V=volume in ml required, C=final concentration of solution and W=weight of the antimicrobial to be dissolved in V.

Preparation of dried filter paper discs

Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven.

The loop used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2 mm. This delivers 0.005 ml of antibiotics to each disc.

Storage of commercial antimicrobial discs

Cartridges containing commercially prepared paper disks specifically for susceptibility testing are generally packaged to ensure appropriate anhydrous conditions. Discs should be stored as follows:

* Refrigerate the containers at 8°C or below, or freeze at -14°C or below, in a nonfrost-free freezer until needed. Sealed packages of disks that contain drugs from the ß-lactam class should be stored frozen, except for a small working supply, which may be refrigerated for at most one week. Some labile agents (e.g., imipenem, cefaclor, and clavulanic acid combinations) may retain greater stability if stored frozen until the day of use.
* The unopened disc containers should be removed from the refrigerator or freezer one to two hours before use, so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts cold disks.
* Once a cartridge of discs has been removed from its sealed package, it should be placed in a tightly sealed, desiccated container. When using a disc-dispensing apparatus, it should be fitted with a tight cover and supplied with an adequate desiccant. The dispenser should be allowed to warm to room temperature before opening. Excessive moisture should be avoided by replacing the desiccant when the indicator changes color.
* When not in use, the dispensing apparatus containing the discs should always be refrigerated.
* Only those discs that have not reached the manufacturer's expiration date stated on the label may be used. Discs should be discarded on the expiration date.

Turbidity standard for inoculum preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), should be used. A $BaSO_4$ 0.5 McFarland standard may be prepared as follows:

1. A 0.5-ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2$. $2H_2O$) is added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.
2. The correct density of the turbidity standard should be verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.008 to 0.10 for the 0.5 McFarland standard.
3. The Barium Sulfate suspension should be transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculum.
4. These tubes should be tightly sealed and stored in the dark at room temperature.
5. The barium sulfate turbidity standard should be vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance. If large particles appear, the standard should be replaced. Latex particle suspensions should be mixed by inverting gently, not on a vortex mixer
6. The barium sulfate standards should be replaced or their densities verified monthly.

Disc diffusion methods

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the NCCLS. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here.

NCCLS is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers, educators etc. It promotes accurate antimicrobial susceptibility testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies that are clinically relevant and cost-effective

Interpretative criteria of NCCLS are developed based on international collaborative studies and well correlated with MIC's and the results have corroborated with clinical data. Based on study results NCCLS interpretative criteria are revised frequently. NCCLS is approved by FDA-USA and recommended by WHO.

Procedure for Performing the Disc Diffusion Test

Inoculum Preparation

Growth Method

The growth method is performed as follows

1. At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.
2. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours)

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3. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/ml for *E.coli* ATCC 25922. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

Direct Colony Suspension Method

1. As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from a 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used). The suspension is adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer.
2. This approach is the recommended method for testing the fastidious organisms, *Haemophilus* spp., *N. gonorrhoeae,* and streptococci, and for testing staphylococci for potential methicillin or oxacillin resistance.

Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.
2. The dried surface of a Müeller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.
3. The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

Application of Discs to Inoculated Agar Plates

1. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.
2. The plates are inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied. With the exception of *Haemophilus* spp., streptococci and

N. gonorrhoeae, the plates should not be incubated in an increased CO_2 atmosphere, because the interpretive standards were developed by using ambient air incubation, and CO_2 will significantly alter the size of the inhibitory zones of some agents.

Reading Plates and Interpreting Results

1. After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light. If blood was added to the agar base (as with streptococci), the zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed. If the test organism is a *Staphylococcus* or *Enterococcus* spp., 24 hours of incubation are required for vancomycin and oxacillin, but other agents can be read at 16 to 18 hours. Transmitted light (plate held up to light) is used to examine the oxacillin and vancomycin zones for light growth of methicillin- or vancomycin- resistant colonies, respectively, within apparent zones of inhibition. Any discernable growth within zone of inhibition is indicative of methicillin or vancomycin resistance.
2. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored. When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter.
3. The sizes of the zones of inhibition are interpreted by referring to Tables 2A through 2I (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement, and the organisms are reported as either susceptible, intermediate, or resistant to the agents that have been tested. Some agents may only be reported as susceptible, since only susceptible breakpoints are given.

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4.2 Dilution Methods

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are tested in $\log_2$ serial dilutions (two fold).

Minimum Inhibitory Concentration (MIC)

Diffusion tests widely used to determine the susceptibility of organisms isolated from clinical specimens have their limitations; when equivocal results are obtained or in prolonged serious infection e.g. bacterial endocarditis, the quantitation of antibiotic action vis-a-vis the pathogen needs to be more precise. Also the terms 'Susceptible' and 'Resistant' can have a realistic interpretation. Thus when in doubt, the way to a precise assessment is to determine the MIC of the antibiotic to the organisms concerned.

There are two methods of testing for MIC:

(a) Broth dilution method

(b) Agar dilution method.

Broth Dilution Method

The Broth Dilution method is a simple procedure for testing a small number of isolates, even single isolate. It has the added advantage that the same tubes can be taken for MBC tests also:

Materials

Sterile graduated pipettes of 10ml, 5ml, 2ml and 1ml Sterile capped 7.5 x 1.3 cm tubes / small screw-capped bottles, Pasteur pipettes, overnight broth culture of test and control organisms (same as for disc diffusion tests), required antibiotic in powder form (either from the manufacturer or standard laboratory accompanied by a statement of its activity in mg/unit or per ml. Clinical preparations should not be used for reference technique.), required solvent for the antibiotic, sterile Distilled Water - 500ml and suitable nutrient broth medium.

Trimethoprim and sulphonamide testing requires thymidine free media or addition of 4% lysed horse blood to the media

A suitable rack to hold 22 tubes in two rows i-e 11 tubes in each row.

Stock solution

Stock solution can be prepared using the formula

$$\frac{1000}{P} \times V \times C = W$$

Where P=Potency given by the manufacturer in relation to the base

V= Volume in ml required

C=Final concentration of solution (multiples of 1000)

W= Weight of the antimicrobial to be dissolved in the volume V

Example: For making 10 ml solution of the strength 10,000mg/l from powder base whose potency is 980 mg per gram,the quantities of the antimicrobials required is

$$W = \frac{1000}{980} \times 10 \times 10 = 102.04\text{mg}$$

Note:the stock solutions are made in higher concentrations to maintain their keeping qualities and stored in suitable aliquots at -20^{o}C .Once taken out,they should not be refrozen or reused.

Suggested dilution ranges of some antimicrobials are shown in Annexure II.

Method

Prepare stock dilutions of the antibiotic of concentrations 1000 and 100 µg/L as required from original stock solution (10,000mg/L). Arrange two rows of 12 sterile 7.5 x1.3 cm capped tubes in the rack. In a sterile 30ml (universal) screw capped bottle, prepare 8ml of broth containing the concentration of antibiotic required for the first tube in each row from the appropriate stock solution already made. Mix the contents of the universal bottle using a pipette and transfer 2ml to the first tube in each row. Using a fresh pipette ,add 4 ml of broth to the remaining 4 ml in the universal bottle mix and transfer 2ml to the second tube in each row. Continue preparing dilutions in this way but where as many as 10 or more are required the series should be started again half the way down. Place 2ml of antibiotic free broth to the last tube in each row. Inoculate one row with one drop of an overnight broth culture of the test organism diluted approximately to 1 in 1000 in a suitable broth and the second row with the control organism of known sensitivity similarly diluted. The result of the test is significantly affected by the size of the inoculum.The test mixture should contain 10^{6} organism/ml.If the broth culture used has grown poorly,it may be necessary to use this undiluted. Incubate tubes for 18 hours at 37^{o}C. Inoculate a tube containing 2ml broth with the organism and keep at +4^{o}C in a refrigerator overnight to be used as standard for the determination of complete inhibition.

Calculations for the preparation of the original dilution.

This often presents problems to those unaccustomed to performing these tests. The following method advocated by Pamela M Waterworth is presented. Calculate the total volume required for the first dilution. Two sets of dilution are being prepared (one for the test and one for the control), each in 2ml volumes i-e a total of 4 ml for each concentration as 4ml is required to make the second dilution, the total requirement is 8ml. Now calculate the total amount of the antibiotic required for 8ml. For 64 g/l concentration, 8x64mg/l =512µg in 8 ml. Place a decimal point after the first figure (5.12) and take this volume in ml (i.e 5.12 ml) of the dilution below 512mg/l

and make upto 8ml with broth. In this example given above, the series has to be started again mid way at 2 mg/l which would be obtained in the same way:

8ml of 2mg/l=16µg in 8ml.

1.6 ml of 10 mg/ l + 6.4 ml of broth

Reading of result

MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube.

Because very faint turbidity may be given by the inoculum itself, the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition.

Standard strain of known MIC value run with the test is used as the control to check the reagents and conditions.

Minimum Bactericidal Concentrations(MBC)

The main advantage of the 'Broth dilution' method for the MIC determination lies in the fact that it can readily be converted to determine the MBC as well.

Method

Dilutions and inoculations are prepared in the same manner as described for the determination of MIC. The control tube containing no antibiotic is immediately subcultured (Before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism and incubated at 37^oC overnight. The tubes are also incubated overnight at 37^oC. Read the MIC of the control organism to check that the drug concentrations are correct. Note the lowest concentration inhibiting growth of the organisms and record this as the MIC. Subculture all tubes not showing visible growth in the same manner as the control tube described above and incubate at 37^oC overnight. Compare the amount of growth from the control tube before incubation,which represents the original inoculum. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity .These tubes are not subcultured; the purpose of the control is to confirm by its MIC that the drug level is correct,whether or not this organism is killed is immaterial.

Reading of result

These subcultures may show

- Similar number of colonies- indicating bacteriostasis only
- A reduced number of colonies-indicating a partial or slow bactericidal activity.
- No growth- if the whole inoculum has been killed
- The highest dilution showing at least 99% inhibition is taken as MBC

Micro-broth dilution test

This test uses double-strength Müeller-Hinton broth, 4X strength antibiotic solutions prepared as serial two-fold dilutions and the test organism at a concentration of $2x10^6$/ml. In a 96 well plate, 100 µl of double-strength MHB,

50 µl each of the antibiotic dilutions and the organism suspension are mixed and incubated at 35°C for 18-24 hours. The lowest concentration showing inhibition of growth will be considered the MIC of the organism.

Reading of result

MIC is expressed as the highest dilution which inhibited growth judged by lack of turbidity in the tube. Because very faint turbidity may be given by the inoculum itself,the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition. Standard strain of known MIC, run with the test is used as the control to check the reagents and conditions.

The Agar dilution Method

Agar dilutions are most often prepared in petri dishes and have advantage that it is possible to test several organisms on each plate .If only one organism is to be tested e.g *M.tuberculosis*,the dilutions can be prepared in agar slopes but it will then be necessary to prepare a second identical set to be inoculated with the control organism.The dilutions are made in a small volume of water and added to agar which has been melted and cooled to not more than 60^{o}C.Blood may be added and if 'chocolate agar' is required,the medium must be heated before the antibiotic is added.

It would be convenient to use 90 mm diameter petri dishes and add

one ml of desired drug dilutions to 19 ml of broth.The factor of agar dilution must be allowed for in the first calculation as follows.

final volume of medium in plate = 20 ml

Top antibiotic concentrations = 64mg/l

Total amount of drug = 1280µg to be added to

1 ml of water

2ml of 1280 µg /ml will be required to start the dilution = 2560µg in 2 ml

= 1.28ml of 2000µg /ml

± 0.72 ml of water.

1 ml of this will be added to 19 ml agar.

(Note stock dilution of 2000µg /ml is required for this range of MIC)

The quickest way to prepare a range of dilutions in agar is as follows:

Label a sterile petri dish on the base for each concentration required. Prepare the dilutions in water placing 1 ml of each in the appropriate dish. One ml water is added to a control plate. Pipette 19 ml melted agar, cooled to 55^{o}C to each plate and mix thoroughly. Adequate mixing is essential and if sufficient technical expertise is not available for the skilled manipulation, it is strongly recommended that the agar is first measured into stoppered tubes or universal containers and the drug dilution added to these and mixed by inversion before pouring into petri dishes. After the plates have set they should be well dried at 37^{o}C with their lids tipped for 20 to 30 minutes in an incubator. They are then inoculated either with a multiple inoculator as spots or with a wire loop

or a platinum loop calibrated to deliver 0.001ml spread over a small area. In either case the culture should be diluted to contain 10^5 to 10^6 organisms per ml. With ordinary fast growing organisms, this can be obtained approximately by adding 5 µl of an overnight broth culture to 5ml broth or peptone water.

It is possible to test spreading organism such as *P.mirabilis* by this method either by cutting ditches in the agar between the inocula, or by confining each with small glass or porcelain cylinders pressed into the agar. Although swarming of *P.mirabilis* can be prevented by the use of higher concentration of agar in the medium, this is not recommended for determination of MIC because of the difficulty of ensuring adequate mixing of the drug with this very viscous medium. Selective media should not be used and electrolyte deficient media will give false results because of the effect of variation in the salt content on the action of many antibiotics.

Reading of results

The antibiotic concentration of the first plate showing ≥ 99% inhibition is taken as the MIC for the organism.

4.3 Dilution and Diffusion

E test also known as the epsilometer test is an 'exponential gradient' testing methodology where 'E' in E test refers to the Greek symbol epsilon (ε).The E test(AB Biodisk) which is a quantitative method for antimicrobial susceptibility testing applies both the dilution of antibiotic and diffusion of antibiotic into the medium.. A predefined stable antimicrobial gradient is present on a thin inert carrier strip. When this E test strip is applied onto an inoculated agar plate, there is an immediate release of the drug. Following incubation , a symmetrical inhibition ellipse is produced. The intersection of the inhibitory zone edge and the calibrated carrier strip indicates the MIC value over a wide concentration range (>10 dilutions) with inherent precision and accuracy .

E test can be used to determine MIC for fastidious organisms like *S. pneumoniae*, ß-hemolytic streptococci, *N.gonorrhoeae*, *Haemophilus* sp. and anaerobes. It can also be used for Nonfermenting Gram Negative bacilli (NFGNB) for eg-*Pseudomonas* sp. and *Burkholderia pseudomallei*.

Resistance of major consequence may be detected for e.g., the test is very useful in detecting glycopeptide resistant Enterococci (GRE) and glycopeptide intermediate *S.aureus* (GISA) and slow growing pathogens such as *Mycobacterium tuberculosis.* Further it can be used for detection of extended spectrum beta lactamases (ESBL). In conclusion E test is a simple, accurate and reliable method to determine the MIC for a wide spectrum of infectious agents.

5. Susceptibility of Fastidious Bacteria

DISC DIFFUSION FOR FASTIDIOUS ORGANISMS

Antibiotic susceptibility testing of *S.pneumoniae*

Media for disc diffusion

Müeller -Hinton Sheep blood agar

Standardization of inoculum.

The inocula for seeding the susceptibility media with *S.pneumoniae* is prepared from fresh pure cultures (grown overnight on Chocolate agar). Cell suspensions of the bacteria to be tested are prepared in sterile saline or Müeller-Hinton broth. The cell suspension is prepared by transferring a portion of the fresh growth with a swab or inoculating loop to the suspending medium, using caution when mixing the cells with the suspending medium so as not to form bubbles. The suspension is then compared to the McFarland standard by holding the suspension and McFarland standard in front of a light against a white background with contrasting black lines and comparing the turbidity. If the turbidity is too heavy, the suspension should be diluted with additional suspending medium. If the turbidity is too light additional cells should be added to the suspension.

For *S.pneumoniae* – Direct colony suspension is made in normal saline and turbidity adjusted to 0.5 McFarland standard. Within 15 minutes after adjusting the turbidity of the suspension the plate should be inoculated.

Inoculation of the susceptibility test media

After proper turbidity is achieved, a new sterile swab (cotton or dacron) is submerged in the suspension, lifted out of the broth, and the excess fluid is removed by pressing and rotating the swab against the wall of the tube. The swab is then used to inoculate the entire surface of the supplemented Müeller Hinton agar plate three times, rotating the plate 60 degrees between each inoculation. The inoculum is allowed to dry (usually taking only a few minutes but no longer than 15 minutes) before the discs are placed on the plates. The discs should be placed on the agar with sterile forceps and tapped gently to ensure the adherence to the agar. The plates containing the disks are incubated at $35^{o}C$ for 16 to 18 h in an inverted position in a 5% CO_2 incubator. A candle extinction jar may be used if a CO_2 incubator is not available.

Estimating the susceptibility of the strains

After overnight incubation, the diameter of each zone of inhibition is measured with a ruler or calipers. The zones of inhibition on the media containing blood are measured from the top surface of the plate with the top removed. It is convenient to use a ruler with a handle attached for these measurements, holding the ruler over the surface of the disk when measuring the inhibition zone. Care should be taken not to touch the disk or surface of the agar. Sterilize the ruler occasionally to prevent transmission of bacteria. In all measurements, the zones of inhibition are measured from the edges of the last visible colony-forming growth. The ruler should be positioned across the center of the disc to make these measurements. The results are recorded in millimeters (mm) and interpretation of susceptibility is obtained by comparing the results to the standard zone sizes. For *S.pneumoniae* the zone measurement is from top of plate with the lid removed. Faint growth of tiny colonies that may appear to fade from the more obvious zone should be ignored in the measurement.

Interpretation

Each zone size is interpreted by reference to the Table 2G (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints for *S.pneumoniae*) of the NCCLS M100-S12:

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Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement as susceptible, intermediate and resistant.

Antibiotic susceptibility of *Haemophilus* species

The medium of choice for disc diffusion testing of *Haemophilus* sp. is Haemophilus Test Medium (HTM). Müeller-Hinton chocolate agar is not recommended for routine testing of *Haemophilus* spp.

In its agar form, Haemophilus Test medium consists of the following ingredients.

* Müeller-Hinton agar,
* 15 μg/ml ß-NAD,
* 15 μg/ml bovine hematin, and
* 5-mg/ml yeast extract.

To make HTM, first a fresh hematin stock solution is prepared by dissolving 50 mg of bovine hematin powder in 100 ml of 0.01 mol/L NaOH with heat and stirring until the powder is thoroughly dissolved. Thirty ml of the hematin stock solution are added to 1 L of MHA with 5 g of yeast extract. After autoclaving and cooling to 45 to 50°C, 3 ml of an NAD stock solution (50 mg of NAD dissolved in 10 ml of distilled water and filter sterilized) are also aseptically added. The pH should be 7.2 to 7.4.

Test Procedure

1. The direct colony suspension procedure should be used when testing *Haemophilus* sp. Using colonies taken directly from an overnight (preferably 20 to 24 hour) chocolate agar culture plate, a suspension of the test organism is prepared in Müeller-Hinton broth or 0.9% saline. The suspension should be adjusted to a turbidity equivalent to a 0.5 McFarland standard using a photometric device. This suspension will contain approximately 1 to 4 x 10^8 CFU/ml. Care must be exercised in preparing this suspension, because higher inoculum concentrations may lead to false-resistant results with some ß-lactam antibiotics, particularly when ß-lactamase producing strains of *H. influenzae* are tested. Within 15 minutes after adjusting the turbidity of the inoculum suspension, it should be used for plate inoculation.
2. The procedure for the disc test should be followed as described for nonfastidious bacteria, except that, in general, no more than 9 discs should be applied to the surface of a 150-mm plate or no more than 4 discs on a 100-mm plate.
3. Plates are incubated at 35°C in an atmosphere of 5% CO_2 for 16 to 18 hours before measuring the zones of inhibition.
4. The zone margin should be considered as the area showing no obvious growth visible with the unaided eye. Faint growth of tiny colonies that may appear to fade from the more obvious zone should be ignored in the measurement.

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Zone Diameter Interpretive Criteria

The antimicrobial agents suggested for routine testing of *Haemophilus* sp. are indicated in Annexure I. Each zone size is interpreted by reference to the Table 2E (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints for *Haemophilus* sp.) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement as susceptible, intermediate and diffusion testing of *Haemophilus* spp. with other agents is not recommended.

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Antimicrobial Susceptibility Testing

Introduction

Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem.

Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately. Inappropriate use results from physicians providing antimicrobial drugs to treat viral infections, using inadequate criteria for diagnosis of infections that potentially have a bacterial aetiology, unnecessarily prescribing expensive, broad-spectrum agents, and not following established recommendations for using chemo prophylaxis. The availability of antibiotics over the counter, despite regulations to the contrary, also fuel inappropriate usage of antimicrobial drugs in India. The easy availability of antimicrobial drugs leads to their incorporation into herbal or "folk" remedies, which also increases inappropriate use of these agents.

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs.

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area.

The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

Principle

The principles of determining the effectivity of a noxious agent to a bacterium were well enumerated by Rideal ,Walker and others at the turn of the century, the discovery of antibiotics made these tests(or their

modification)too cumbersome for the large numbers of tests necessary to be put up as a routine. The ditch plate method of agar diffusion used by Alexander Fleming was the forerunner of a variety of agar diffusion methods devised by workers in this field .The Oxford group used these methods initially to assay the antibiotic contained in blood by allowing the antibiotics to diffuse out of reservoirs in the medium in containers placed on the surface.

With the introduction of a variety of antimicrobials it became necessary to perform the antimicrobial susceptibility test as a routine. For this, the antimicrobial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still the method of choice for the average laboratory. Automation may force the method out of the diagnostic laboratory but in this country as well as in the smaller laboratories of even advanced countries, it will certainly be the most commonly carried out microbiological test for many years to come. It is, therefore, imperative that microbiologists understand the principles of the test well and keep updating the information as and when necessary. All techniques involve either diffusion of antimicrobial agent in agar or dilution of antibiotic in agar or broth. Even automated techniques are variations of the above methods.

3. Factors Influencing Antimicrobial Susceptibility Testing

pH

The pH of each batch of Müeller-Hinton agar should be checked when the medium is prepared. The exact method used will depend largely on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (e.g., aminoglycosides, quinolones, and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected. The pH can be checked by one of the following means:

* Macerate a sufficient amount of agar to submerge the tip of a pH electrode.
* Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup.
* Use a properly calibrated surface electrode.

Moisture

If, just before use, excess surface moisture is present, the plates should be placed in an incubator (35°C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is lost by evaporation (usually 10 to 30 minutes). The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the petri dish covers when the plates are inoculated.

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Effects of Thymidine or Thymine

Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and trimethoprim, thus yielding smaller and less distinct zones, or even no zone at all, which may result in false-resistance reports. Müeller-Hinton agar that is as low in thymidine content as possible should be used. To evaluate a new lot of Müeller-Hinton agar, *Enterococcus faecalis* ATCC 29212, or alternatively, *E. faecalis* ATCC 33186, should be tested with trimethoprim/sulfamethoxazole disks. Satisfactory media will provide essentially clear, distinct zones of inhibition 20 mm or greater in diameter. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

Effects of Variation in Divalent Cations

Variation in divalent cations, principally magnesium and calcium, will affect results of aminoglycoside and tetracycline tests with *P. aeruginosa* strains. Excessive cation content will reduce zone sizes, whereas low cation content may result in unacceptably large zones of inhibition. Excess zinc ions may reduce zone sizes of carbapenems. Performance tests with each lot of Müeller-Hinton agar must conform to the control limits.

Testing strains that fail to grow satisfactorily

Only aerobic or facultative bacteria that grow well on unsupplemented Müeller-Hinton agar should be tested on that medium. Certain fastidious bacteria such as *Haemophilus* spp.,

N. gonorrhoeae, *S. pneumoniae*, and viridans and ß-haemolytic streptococci do not grow sufficiently on unsupplemented Müeller-Hinton agar. These organisms require supplements or different media to grow, and they should be tested on the media described in separate sections.

4. Methods of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include:

Diffusion	**Dilution**	**Diffusion&Dilution**
Stokes method	Minimum Inhibitory Concentration	E-Test method
Kirby-Bauer method	i) Broth dilution	
	ii)Agar	Dilution

4.1 Disk Diffusion

Reagents for the Disk Diffusion Test

1. Müeller-Hinton Agar Medium

Of the many media available, Müeller-Hinton agar is considered to be the best for routine susceptibility testing of nonfastidious bacteria for the following reasons:

* It shows acceptable batch-to-batch reproducibility for susceptibility testing.
* It is low in sulphonamide, trimethoprim, and tetracycline inhibitors.
* It gives satisfactory growth of most nonfastidious pathogens.
* A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Although Müeller-Hinton agar is reliable generally for susceptibility testing, results obtained with some batches may, on occasion, vary significantly. If a batch of medium does not support adequate growth of a test organism, zones obtained in a disk diffusion test will usually be larger than expected and may exceed the acceptable quality control limits. Only Müeller-Hinton medium formulations that have been tested according to, and that meet the acceptance limits described in, NCCLS document M62-A7- Protocols for Evaluating Dehydrated Müeller-Hinton Agar should be used.

Preparation of Müeller-Hinton Agar

Müeller-Hinton agar preparation includes the following steps.

1. Müeller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer's instructions.
2. Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath.
3. Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.
4. The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C).
5. Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.
6. A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

2. Preparation of antibiotic stock solutions

Antibitiotics may be received as powders or tablets. It is recommended to obtain pure antibiotics from commercial sources, and not use injectable solutions. Powders must be accurately weighed and dissolved in the appropriate diluents (Annexure III) to yield the required concentration, using sterile glassware. Standard strains of stock

cultures should be used to evaluate the antibiotic stock solution. If satisfactory, the stock can be aliquoted in 5 ml volumes and frozen at -20ºC or -60ºC.

Stock solutions are prepared using the formula (1000/P) X V X C=W, where P+potency of the anitbiotic base, V=volume in ml required, C=final concentration of solution and W=weight of the antimicrobial to be dissolved in V.

Preparation of dried filter paper discs

Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven.

The loop used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2 mm. This delivers 0.005 ml of antibiotics to each disc.

Storage of commercial antimicrobial discs

Cartridges containing commercially prepared paper disks specifically for susceptibility testing are generally packaged to ensure appropriate anhydrous conditions. Discs should be stored as follows:

* Refrigerate the containers at 8°C or below, or freeze at -14°C or below, in a nonfrost-free freezer until needed. Sealed packages of disks that contain drugs from the ß-lactam class should be stored frozen, except for a small working supply, which may be refrigerated for at most one week. Some labile agents (e.g., imipenem, cefaclor, and clavulanic acid combinations) may retain greater stability if stored frozen until the day of use.
* The unopened disc containers should be removed from the refrigerator or freezer one to two hours before use, so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts cold disks.
* Once a cartridge of discs has been removed from its sealed package, it should be placed in a tightly sealed, desiccated container. When using a disc-dispensing apparatus, it should be fitted with a tight cover and supplied with an adequate desiccant. The dispenser should be allowed to warm to room temperature before opening. Excessive moisture should be avoided by replacing the desiccant when the indicator changes color.
* When not in use, the dispensing apparatus containing the discs should always be refrigerated.
* Only those discs that have not reached the manufacturer's expiration date stated on the label may be used. Discs should be discarded on the expiration date.

Turbidity standard for inoculum preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), should be used. A $BaSO_4$ 0.5 McFarland standard may be prepared as follows:

1. A 0.5-ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2$. $2H_2O$) is added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.
2. The correct density of the turbidity standard should be verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.008 to 0.10 for the 0.5 McFarland standard.
3. The Barium Sulfate suspension should be transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculum.
4. These tubes should be tightly sealed and stored in the dark at room temperature.
5. The barium sulfate turbidity standard should be vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance. If large particles appear, the standard should be replaced. Latex particle suspensions should be mixed by inverting gently, not on a vortex mixer
6. The barium sulfate standards should be replaced or their densities verified monthly.

Disc diffusion methods

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the NCCLS. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here.

NCCLS is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers, educators etc. It promotes accurate antimicrobial susceptibility testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies that are clinically relevant and cost-effective

Interpretative criteria of NCCLS are developed based on international collaborative studies and well correlated with MIC's and the results have corroborated with clinical data. Based on study results NCCLS interpretative criteria are revised frequently. NCCLS is approved by FDA-USA and recommended by WHO.

Procedure for Performing the Disc Diffusion Test

Inoculum Preparation

Growth Method

The growth method is performed as follows

1. At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.
2. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours)

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3. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/ml for *E.coli* ATCC 25922. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

Direct Colony Suspension Method

1. As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from a 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used). The suspension is adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer.
2. This approach is the recommended method for testing the fastidious organisms, *Haemophilus* spp., *N. gonorrhoeae,* and streptococci, and for testing staphylococci for potential methicillin or oxacillin resistance.

Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.
2. The dried surface of a Müeller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.
3. The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

Application of Discs to Inoculated Agar Plates

1. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.
2. The plates are inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied. With the exception of *Haemophilus* spp., streptococci and

N. gonorrhoeae, the plates should not be incubated in an increased CO_2 atmosphere, because the interpretive standards were developed by using ambient air incubation, and CO_2 will significantly alter the size of the inhibitory zones of some agents.

Reading Plates and Interpreting Results

1. After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light. If blood was added to the agar base (as with streptococci), the zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed. If the test organism is a *Staphylococcus* or *Enterococcus* spp., 24 hours of incubation are required for vancomycin and oxacillin, but other agents can be read at 16 to 18 hours. Transmitted light (plate held up to light) is used to examine the oxacillin and vancomycin zones for light growth of methicillin- or vancomycin- resistant colonies, respectively, within apparent zones of inhibition. Any discernable growth within zone of inhibition is indicative of methicillin or vancomycin resistance.
2. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored. When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter.
3. The sizes of the zones of inhibition are interpreted by referring to Tables 2A through 2I (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement, and the organisms are reported as either susceptible, intermediate, or resistant to the agents that have been tested. Some agents may only be reported as susceptible, since only susceptible breakpoints are given.

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4.2 Dilution Methods

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are tested in $\log_2$ serial dilutions (two fold).

Minimum Inhibitory Concentration (MIC)

Diffusion tests widely used to determine the susceptibility of organisms isolated from clinical specimens have their limitations; when equivocal results are obtained or in prolonged serious infection e.g. bacterial endocarditis, the quantitation of antibiotic action vis-a-vis the pathogen needs to be more precise. Also the terms 'Susceptible' and 'Resistant' can have a realistic interpretation. Thus when in doubt, the way to a precise assessment is to determine the MIC of the antibiotic to the organisms concerned.

There are two methods of testing for MIC:

(a) Broth dilution method

(b) Agar dilution method.

Broth Dilution Method

The Broth Dilution method is a simple procedure for testing a small number of isolates, even single isolate. It has the added advantage that the same tubes can be taken for MBC tests also:

Materials

Sterile graduated pipettes of 10ml, 5ml, 2ml and 1ml Sterile capped 7.5 x 1.3 cm tubes / small screw-capped bottles, Pasteur pipettes, overnight broth culture of test and control organisms (same as for disc diffusion tests), required antibiotic in powder form (either from the manufacturer or standard laboratory accompanied by a statement of its activity in mg/unit or per ml. Clinical preparations should not be used for reference technique.), required solvent for the antibiotic, sterile Distilled Water - 500ml and suitable nutrient broth medium.

Trimethoprim and sulphonamide testing requires thymidine free media or addition of 4% lysed horse blood to the media

A suitable rack to hold 22 tubes in two rows i-e 11 tubes in each row.

Stock solution

Stock solution can be prepared using the formula

$$\frac{1000}{P} \times V \times C = W$$

Where P=Potency given by the manufacturer in relation to the base

V= Volume in ml required

C=Final concentration of solution (multiples of 1000)

W= Weight of the antimicrobial to be dissolved in the volume V

Example: For making 10 ml solution of the strength 10,000mg/l from powder base whose potency is 980 mg per gram,the quantities of the antimicrobials required is

$$W = \frac{1000}{980} \times 10 \times 10 = 102.04\text{mg}$$

Note:the stock solutions are made in higher concentrations to maintain their keeping qualities and stored in suitable aliquots at -20^{o}C .Once taken out,they should not be refrozen or reused.

Suggested dilution ranges of some antimicrobials are shown in Annexure II.

Method

Prepare stock dilutions of the antibiotic of concentrations 1000 and 100 µg/L as required from original stock solution (10,000mg/L). Arrange two rows of 12 sterile 7.5 x1.3 cm capped tubes in the rack. In a sterile 30ml (universal) screw capped bottle, prepare 8ml of broth containing the concentration of antibiotic required for the first tube in each row from the appropriate stock solution already made. Mix the contents of the universal bottle using a pipette and transfer 2ml to the first tube in each row. Using a fresh pipette ,add 4 ml of broth to the remaining 4 ml in the universal bottle mix and transfer 2ml to the second tube in each row. Continue preparing dilutions in this way but where as many as 10 or more are required the series should be started again half the way down. Place 2ml of antibiotic free broth to the last tube in each row. Inoculate one row with one drop of an overnight broth culture of the test organism diluted approximately to 1 in 1000 in a suitable broth and the second row with the control organism of known sensitivity similarly diluted. The result of the test is significantly affected by the size of the inoculum.The test mixture should contain 10^{6} organism/ml.If the broth culture used has grown poorly,it may be necessary to use this undiluted. Incubate tubes for 18 hours at 37^{o}C. Inoculate a tube containing 2ml broth with the organism and keep at +4^{o}C in a refrigerator overnight to be used as standard for the determination of complete inhibition.

Calculations for the preparation of the original dilution.

This often presents problems to those unaccustomed to performing these tests. The following method advocated by Pamela M Waterworth is presented. Calculate the total volume required for the first dilution. Two sets of dilution are being prepared (one for the test and one for the control), each in 2ml volumes i-e a total of 4 ml for each concentration as 4ml is required to make the second dilution, the total requirement is 8ml. Now calculate the total amount of the antibiotic required for 8ml. For 64 g/l concentration, 8x64mg/l =512µg in 8 ml. Place a decimal point after the first figure (5.12) and take this volume in ml (i.e 5.12 ml) of the dilution below 512mg/l

and make upto 8ml with broth. In this example given above, the series has to be started again mid way at 2 mg/l which would be obtained in the same way:

8ml of 2mg/l=16µg in 8ml.

1.6 ml of 10 mg/ l + 6.4 ml of broth

Reading of result

MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube.

Because very faint turbidity may be given by the inoculum itself, the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition.

Standard strain of known MIC value run with the test is used as the control to check the reagents and conditions.

Minimum Bactericidal Concentrations(MBC)

The main advantage of the 'Broth dilution' method for the MIC determination lies in the fact that it can readily be converted to determine the MBC as well.

Method

Dilutions and inoculations are prepared in the same manner as described for the determination of MIC. The control tube containing no antibiotic is immediately subcultured (Before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism and incubated at 37^oC overnight. The tubes are also incubated overnight at 37^oC. Read the MIC of the control organism to check that the drug concentrations are correct. Note the lowest concentration inhibiting growth of the organisms and record this as the MIC. Subculture all tubes not showing visible growth in the same manner as the control tube described above and incubate at 37^oC overnight. Compare the amount of growth from the control tube before incubation,which represents the original inoculum. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity .These tubes are not subcultured; the purpose of the control is to confirm by its MIC that the drug level is correct,whether or not this organism is killed is immaterial.

Reading of result

These subcultures may show

- Similar number of colonies- indicating bacteriostasis only
- A reduced number of colonies-indicating a partial or slow bactericidal activity.
- No growth- if the whole inoculum has been killed
- The highest dilution showing at least 99% inhibition is taken as MBC

Micro-broth dilution test

This test uses double-strength Müeller-Hinton broth, 4X strength antibiotic solutions prepared as serial two-fold dilutions and the test organism at a concentration of $2x10^6$/ml. In a 96 well plate, 100 µl of double-strength MHB,

50 µl each of the antibiotic dilutions and the organism suspension are mixed and incubated at 35°C for 18-24 hours. The lowest concentration showing inhibition of growth will be considered the MIC of the organism.

Reading of result

MIC is expressed as the highest dilution which inhibited growth judged by lack of turbidity in the tube. Because very faint turbidity may be given by the inoculum itself,the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition. Standard strain of known MIC, run with the test is used as the control to check the reagents and conditions.

The Agar dilution Method

Agar dilutions are most often prepared in petri dishes and have advantage that it is possible to test several organisms on each plate .If only one organism is to be tested e.g *M.tuberculosis*,the dilutions can be prepared in agar slopes but it will then be necessary to prepare a second identical set to be inoculated with the control organism.The dilutions are made in a small volume of water and added to agar which has been melted and cooled to not more than 60^{o}C.Blood may be added and if 'chocolate agar' is required,the medium must be heated before the antibiotic is added.

It would be convenient to use 90 mm diameter petri dishes and add

one ml of desired drug dilutions to 19 ml of broth.The factor of agar dilution must be allowed for in the first calculation as follows.

final volume of medium in plate = 20 ml

Top antibiotic concentrations = 64mg/l

Total amount of drug = 1280µg to be added to

1 ml of water

2ml of 1280 µg /ml will be required to start the dilution = 2560µg in 2 ml

= 1.28ml of 2000µg /ml

± 0.72 ml of water.

1 ml of this will be added to 19 ml agar.

(Note stock dilution of 2000µg /ml is required for this range of MIC)

The quickest way to prepare a range of dilutions in agar is as follows:

Label a sterile petri dish on the base for each concentration required. Prepare the dilutions in water placing 1 ml of each in the appropriate dish. One ml water is added to a control plate. Pipette 19 ml melted agar, cooled to 55^{o}C to each plate and mix thoroughly. Adequate mixing is essential and if sufficient technical expertise is not available for the skilled manipulation, it is strongly recommended that the agar is first measured into stoppered tubes or universal containers and the drug dilution added to these and mixed by inversion before pouring into petri dishes. After the plates have set they should be well dried at 37^{o}C with their lids tipped for 20 to 30 minutes in an incubator. They are then inoculated either with a multiple inoculator as spots or with a wire loop

or a platinum loop calibrated to deliver 0.001ml spread over a small area. In either case the culture should be diluted to contain 10^5 to 10^6 organisms per ml. With ordinary fast growing organisms, this can be obtained approximately by adding 5 µl of an overnight broth culture to 5ml broth or peptone water.

It is possible to test spreading organism such as *P.mirabilis* by this method either by cutting ditches in the agar between the inocula, or by confining each with small glass or porcelain cylinders pressed into the agar. Although swarming of *P.mirabilis* can be prevented by the use of higher concentration of agar in the medium, this is not recommended for determination of MIC because of the difficulty of ensuring adequate mixing of the drug with this very viscous medium. Selective media should not be used and electrolyte deficient media will give false results because of the effect of variation in the salt content on the action of many antibiotics.

Reading of results

The antibiotic concentration of the first plate showing ≥ 99% inhibition is taken as the MIC for the organism.

4.3 Dilution and Diffusion

E test also known as the epsilometer test is an 'exponential gradient' testing methodology where 'E' in E test refers to the Greek symbol epsilon (ε).The E test(AB Biodisk) which is a quantitative method for antimicrobial susceptibility testing applies both the dilution of antibiotic and diffusion of antibiotic into the medium.. A predefined stable antimicrobial gradient is present on a thin inert carrier strip. When this E test strip is applied onto an inoculated agar plate, there is an immediate release of the drug. Following incubation , a symmetrical inhibition ellipse is produced. The intersection of the inhibitory zone edge and the calibrated carrier strip indicates the MIC value over a wide concentration range (>10 dilutions) with inherent precision and accuracy .

E test can be used to determine MIC for fastidious organisms like *S. pneumoniae*, ß-hemolytic streptococci, *N.gonorrhoeae*, *Haemophilus* sp. and anaerobes. It can also be used for Nonfermenting Gram Negative bacilli (NFGNB) for eg-*Pseudomonas* sp. and *Burkholderia pseudomallei*.

Resistance of major consequence may be detected for e.g., the test is very useful in detecting glycopeptide resistant Enterococci (GRE) and glycopeptide intermediate *S.aureus* (GISA) and slow growing pathogens such as *Mycobacterium tuberculosis.* Further it can be used for detection of extended spectrum beta lactamases (ESBL). In conclusion E test is a simple, accurate and reliable method to determine the MIC for a wide spectrum of infectious agents.

5. Susceptibility of Fastidious Bacteria

DISC DIFFUSION FOR FASTIDIOUS ORGANISMS

Antibiotic susceptibility testing of *S.pneumoniae*

Media for disc diffusion

Müeller -Hinton Sheep blood agar

Standardization of inoculum.

The inocula for seeding the susceptibility media with *S.pneumoniae* is prepared from fresh pure cultures (grown overnight on Chocolate agar). Cell suspensions of the bacteria to be tested are prepared in sterile saline or Müeller-Hinton broth. The cell suspension is prepared by transferring a portion of the fresh growth with a swab or inoculating loop to the suspending medium, using caution when mixing the cells with the suspending medium so as not to form bubbles. The suspension is then compared to the McFarland standard by holding the suspension and McFarland standard in front of a light against a white background with contrasting black lines and comparing the turbidity. If the turbidity is too heavy, the suspension should be diluted with additional suspending medium. If the turbidity is too light additional cells should be added to the suspension.

For *S.pneumoniae* – Direct colony suspension is made in normal saline and turbidity adjusted to 0.5 McFarland standard. Within 15 minutes after adjusting the turbidity of the suspension the plate should be inoculated.

Inoculation of the susceptibility test media

After proper turbidity is achieved, a new sterile swab (cotton or dacron) is submerged in the suspension, lifted out of the broth, and the excess fluid is removed by pressing and rotating the swab against the wall of the tube. The swab is then used to inoculate the entire surface of the supplemented Müeller Hinton agar plate three times, rotating the plate 60 degrees between each inoculation. The inoculum is allowed to dry (usually taking only a few minutes but no longer than 15 minutes) before the discs are placed on the plates. The discs should be placed on the agar with sterile forceps and tapped gently to ensure the adherence to the agar. The plates containing the disks are incubated at $35^{o}C$ for 16 to 18 h in an inverted position in a 5% CO_2 incubator. A candle extinction jar may be used if a CO_2 incubator is not available.

Estimating the susceptibility of the strains

After overnight incubation, the diameter of each zone of inhibition is measured with a ruler or calipers. The zones of inhibition on the media containing blood are measured from the top surface of the plate with the top removed. It is convenient to use a ruler with a handle attached for these measurements, holding the ruler over the surface of the disk when measuring the inhibition zone. Care should be taken not to touch the disk or surface of the agar. Sterilize the ruler occasionally to prevent transmission of bacteria. In all measurements, the zones of inhibition are measured from the edges of the last visible colony-forming growth. The ruler should be positioned across the center of the disc to make these measurements. The results are recorded in millimeters (mm) and interpretation of susceptibility is obtained by comparing the results to the standard zone sizes. For *S.pneumoniae* the zone measurement is from top of plate with the lid removed. Faint growth of tiny colonies that may appear to fade from the more obvious zone should be ignored in the measurement.

Interpretation

Each zone size is interpreted by reference to the Table 2G (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints for *S.pneumoniae*) of the NCCLS M100-S12:

2017

Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement as susceptible, intermediate and resistant.

Antibiotic susceptibility of *Haemophilus* species

The medium of choice for disc diffusion testing of *Haemophilus* sp. is Haemophilus Test Medium (HTM). Müeller-Hinton chocolate agar is not recommended for routine testing of *Haemophilus* spp.

In its agar form, Haemophilus Test medium consists of the following ingredients.

* Müeller-Hinton agar,
* 15 μg/ml ß-NAD,
* 15 μg/ml bovine hematin, and
* 5-mg/ml yeast extract.

To make HTM, first a fresh hematin stock solution is prepared by dissolving 50 mg of bovine hematin powder in 100 ml of 0.01 mol/L NaOH with heat and stirring until the powder is thoroughly dissolved. Thirty ml of the hematin stock solution are added to 1 L of MHA with 5 g of yeast extract. After autoclaving and cooling to 45 to 50°C, 3 ml of an NAD stock solution (50 mg of NAD dissolved in 10 ml of distilled water and filter sterilized) are also aseptically added. The pH should be 7.2 to 7.4.

Test Procedure

1. The direct colony suspension procedure should be used when testing *Haemophilus* sp. Using colonies taken directly from an overnight (preferably 20 to 24 hour) chocolate agar culture plate, a suspension of the test organism is prepared in Müeller-Hinton broth or 0.9% saline. The suspension should be adjusted to a turbidity equivalent to a 0.5 McFarland standard using a photometric device. This suspension will contain approximately 1 to 4 x 10^8 CFU/ml. Care must be exercised in preparing this suspension, because higher inoculum concentrations may lead to false-resistant results with some ß-lactam antibiotics, particularly when ß-lactamase producing strains of *H. influenzae* are tested. Within 15 minutes after adjusting the turbidity of the inoculum suspension, it should be used for plate inoculation.
2. The procedure for the disc test should be followed as described for nonfastidious bacteria, except that, in general, no more than 9 discs should be applied to the surface of a 150-mm plate or no more than 4 discs on a 100-mm plate.
3. Plates are incubated at 35°C in an atmosphere of 5% CO_2 for 16 to 18 hours before measuring the zones of inhibition.
4. The zone margin should be considered as the area showing no obvious growth visible with the unaided eye. Faint growth of tiny colonies that may appear to fade from the more obvious zone should be ignored in the measurement.

Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

Zone Diameter Interpretive Criteria

The antimicrobial agents suggested for routine testing of *Haemophilus* sp. are indicated in Annexure I. Each zone size is interpreted by reference to the Table 2E (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints for *Haemophilus* sp.) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement as susceptible, intermediate and diffusion testing of *Haemophilus* spp. with other agents is not recommended.

Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

16MBU304A
II BSC Microbiology
Microbial diagnosis in health clinic

Unit Vqu	Opt 1	Opt 2	Opt 3	Opt 4	Answer
The comm	Fever	gangrene	cold	sore	Fever
Semelweis	puerperal	meningitis	cholera	diarrhoea	puerperal sepsis
Lister ove	acid	base	charcoal	phenol	phenol
The conce	reduce	elevate	. increase	improve	reduce
The incide	10-20%	12-20%	2-12%	10-15%	2-12%
In hospita	Drug resis	common	Nosocomi	saprophyte	Drug resistant
Hospital a	Nominal	Neutral	Normal	Nosocomial	Nosocomial
Hospital a	Exogenou	Endogeno	Epigenous	Eugenous	Exogenous
. _____	Primary in	re infectio	post infect	Iatragenic infection	Iatragenic infection
The oppor	Diet	immune	Invasive	Infection	immune
The hospi	Commens	Contamina	Pathogen	Normal flora	Contaminants
The bliste	Bed sore	Bedbug	Bedding	Bed wet	Bed sore
The sligh	Invention	Infection	Interactior	Innovatoin	Infection
_____	E.coli	Treponem	HIV	Streptococcus pyogenes.	Streptococcus pyoger
Staphyloc	80/81	60/61	10/10/	44/10	80/81
The drug r	Phage	Plasmid	Phasmid	cosmid	Phage
. _____	E.coli	Klebsiella	Pseudomo	Proteus	Pseudomonas
_____	Tetanus	E.coli	Klebsiella	Bacillus	Tetanus
HIV is tra	Sputum	Urine	Blood	Stool	Blood
Viral infe	Pus	Blood	CSF	Swab	Blood
_____	Candida	E.coli	Klebsiella	Cryptococcus	Candida
Stitch abs	Brain	Wound	. CSF	Pus	Wound
Streptoco	Month	Year	Week	Day	Day
Clostridia	Month	Year	Week	Day	Day
Pseudomo	Burns	Wound	Brain	Blood	Burns
Neonatal t	Blood	Body	Umblical c	Brain	Umblical cord
Cathetriza	UTI	RTI	CTI	systemic	UTI
About ___	10 percent	2percent	5percent	7percent	2percent
E.coli and	Mixed	single	. combine	complicated	Mixed
_____	French	Glass	Indwelling	Rubber	Indwelling
Pulmonary	Pneumoni	. Bleeding	Abscesses	Fever	Pneumonia
Multiplica	Bacteria	Bacteremi	Viremia	Septicemia	Bacteremia
Multiplica	Bacteria	Bacteremi	Viremia	Septicemia	Viremia
viral infe	. Fungemi	Bacteremi	Viremia	Septicemia	Viremia
The libera	Fungemia	Bacteremi	Viremia	Toximia	Toximia
Pus filled	Abscesses	Lesion	Necrosis	Fever	Abscesses
Programm	Abscesses	. Lesion	Necrosis	Fever	Necrosis

Stool sam	1 hour	2 hours	3 hours	4 hours	2 hours
Fever indu	Pyrogen	Pyogen	Phelm	Parotid	Pyrogen
Phlebitis s	Bacteria	Bacteremi	Viremia	Septicemia	Bacteremia
Staphyloc	Skin	Catheter	Heart valv	Inhaler	Skin
Many hos	Epidemic	Endemic	Pandemic	Randamic	Endemic
When out	Cultured	Eliminate	Elevated	Intricate	Eliminated
An import	Immuniza	Innovatio	Infection	Intricate	Immunization
At sixth w	OPV	BCG	DPT	TT	BCG
At tenth w	OPV	BCG	DPT	TT	DPT
At ninth m	OPV	BCG	DPT	measles	measles
At 5-6 yea	DT	BCG	DPT	TT	DT
At 10 year	DT	BCG	DPT	TT	TT
At 16 year	DT	TT	DPT	BCG	TT
Pregnant v	DT	BCG	DPT	TT	TT
OPV –O i	At birth	Week	Month	Year	At birth
Booster d	. B	T	Memmory	Plasma	Memmory
Fever of u	Pyrexia	Pyrogen	Pyogen	Phylogenic	Pyrexia
Ulcer on f	Boils	Tuleremia	Wound	Abscesses	Tuleremia
Di dot is a	HIV	Rabies	Meningitis	Carditis	HIV
Inflammat	Meningia	Meningitis	Peritonitis	Carditis	Meningitis
Arthritis is	Immune	Auto imm	Mental	Blood	Auto immune
Allergic m	Hypersens	Hyposensi	. sensitivit	Histeria	Hypersensitivity
Topical oi	Bacteria	Bacteremi	Viremia	Septicemia	Bacteria

ıes.

2017

II B. Sc Microbiology – Microbial diagnosis in health clinic

Unit I possible questions

Part B

1. Define pandemic and how you control it
2. Say about circulatory system
3. What are culture media?
4. What are MIC and MBC?
5. What is Nucleic acid probes?

Part C

1. Describe the morphology, cultural properties and lab diagnosis of GI tract pathogens.
2. Explain in detail about the classification of staining methods.
3. Comment on identification and interpretation of diseases
4. State the application of ELISA
5. Write a detailed account on Kirby bauer method.
6. Elaborate the MIC and MBC procedures.

Prepared by : Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

Reg. No. : ----------
[16MBU312]

KARPAGAM ACADEMY OF HIGHER EDUCATION
KARPAGAM UNIVERSITY
(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
B.Sc. DEGREE EXAMINATION, July 2017
DEPARTMENT OF MICROBIOLOGY

I INTERNAL TEST - THIRD SEMESTER

MICROBIAL DIAGNOSIS IN HEALTH CLINIC

Time: 2 hours **Maximum: 50 marks**
Date / Session:

PART-A

Multiple Choice Questions No.1 to 20 **20 x 1 = 20 marks**

1_____ causes diarrhea, urinary tract infections, bacteremia, and meningitis.
A. *Vibrio cholerae* B. *E.coli*
C. *Salmonella typhi* D. *Serratia*

2. Bacteria are sensitive to _____
A. Interleukins B. Interferons
C. Antibiotics D. Antitumours

3. _____media is used for cultivation of bacteria
A. Nutrient agar B. MacConkey agar
C. EMB agar D. MHA

4. The cocci which forms a bunch and irregular pattern are
A. *Staphylococci* B. diplococci
C. Tetracocci D. *Streptococci*

5. Gram's staining was invented in the year_____
A. 1883 B. 1993
C. 1868 D. 1965

6. _____s is the diagnosis of "disease" that will never cause symptoms or death during a patient's lifetime
A. overdiagnosis B. lab diagnosis
C. self diagnosis D. computer diagnosis

7. When making a medical diagnosis, a _____ is a delay in time until a step towards diagnosis of a disease or condition is made.
A. log time B. lag time
C. death time D. stationary time

8. _____diagnosis is made on the basis of medical signs and patient-reported symptoms, rather than diagnostic tests
A. Clinical B. lab
C. radio D. Principal

9. _____ is a process of identifying all of the possible diagnoses that could be connected to the signs, symptoms, and lab findings, and then ruling out diagnoses until a final determination can be made.
A. Clinical B. lab
C. differential D. Principal

10. _____ is a diagnosis or identification of a medical condition in oneself.
A. Self diagnosis B. Lab diagnosis
C. Clinical diagnosis D. Principal diagnosis

11. Which body system circulates blood around the body via the heart, arteries and veins, delivering oxygen and nutrients to organs and cells and carrying their waste products away
A. Endocrine system B. Excretory system
C. Circulatory system D. Nervous system.

12. This system eliminates nitrogenous wastes from the body. Regulates acid-base, electrolyte and water balance of blood.
    A. Endocrine system B. Excretory system
    C. Circulatory system D. Nervous system.
13. ____forms the external body covering and protects deeper tissues from injury.
    A. Muscular system B. Integumentary system
    C. Lymphatic system D. Cardiovascular system.
14. Which bacteria cause tetanus?
    A. *Vibrio* B. *Salmonella*
    C. *Shigella* D. *Clostridium*
15. Which bacteria cause gonorrhoea?
    A. *Neisseria* B. *Helicobacter*
    C. *Proteus* D. *Salmonell*a
16. MacConkey agar is a _____
    A. Selective media B. Transport media
    C. Differential media D. Basal Media
17. Bacteria cause _____ by secreting or excreting toxins
    A. Disorder B. disease
    C. mutation D. infection
18. _____is an infection of the hair follicles that causes red, swollen bumps that look like pimples.
    A. Cellulitis B. Boils
    C. Folliculitis D. Impetigo
19. Selective media for *Staphylococcus aureus* is _____
    A.MSA B. EMB
    C. TTB D. MHA
20. _____ is a transport media for stool sample
    A. Clary Blair media B. GN broth
    C. TT broth D. Nutrient broth

Part B

Answer all the questions **3x2 = 6 marks**

21. Define Microbial Diagnosis.
22. What are Culture Media?
23. Explain the terms – Infection, Pathogen, Antibiotic, and Prophylaxis

Part C

Answer all the questions **3x8 = 24 marks**

24. A. Explain in short about medical diagnosis.
    Or
    B. Write in detail about processing of samples

25. A. Explain in short about human bacterial diseases.
    Or
    B. Write in detail about the normal flora of human body.

26. A. Give a detailed note on localised and systemic infections
    Or
    B. What are the different types of diagnosis?

Reg. No. : ----------
[16MBU304B]

KARPAGAM ACADEMY OF HIGHER EDUCATION
KARPAGAM UNIVERSITY
(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
B.Sc. DEGREE EXAMINATION, August 2017
DEPARTMENT OF MICROBIOLOGY

II INTERNAL TEST - THIRD SEMESTER

MICROBIAL DIAGNOSIS IN HEALTH CLINIC

Time: 2 hours **Maximum: 50 marks**
Date / Session: **Class : II BSc MB**

PART-A

Multiple Choice Questions **20 x 1 = 20 marks**

1. In hospital environment the normal flora is replaced by _____flora.
   a. Drug resistant b. common
   c. Nosocomial d. saprophyte.
2. Hospital acquired infection are typically_____.
   a. Exogenous b. Endogenous
   c. Epigenous d. Eugenous
3. The opportunity of a microorganism to infect patient is due to _____ impared.
   a. Diet b. Immune
   c. Invasive d. Infection
4. The common post operator consequence in hospital is _____
   a. Fever b. gangrene
   c. cold d. sore
5. CSF is collected from _____
   a. Lymph node b. CNS
   c. Artery d. Brain
6. CSF collection technique called as _____
   a. Phylum b. Swabbing
   c. Lumbar puncture d. Vesicle rupture
7. Leishman stain is used for_____ counting
   a. Colony b. Capsule n
   c. Blood cell d. Gene
8. The apt transport media for stool specimen is _____
   a. Cary-blair b Nutrient broth
   c. Blood agar d. Bile broth
9. An individual who practices epidemiology is an _____.
   a. Epidemiologist b. scientist
   c. investigator d. environmentalist
10. _____ is an illness caused by a pathogen or its products that has been transmitted from an infected person or a reservoir.
   a. communicable disease b. epidemic
   c. endemic d. pandemic
11. Moderate prevalance of a disease in animals is termed _____.
   a. epizootic b. enzootic
   c. panzootic d. zoonoses
12. Quality control checks the quality of the product based on the norms of_____
   a. QA b. QC
   c. GLC d. QB
13. The medium used for antibiotic sensitivity testing is _____
   a. Nutrient agar b. Blood agar
   c. Urea agar d. Muller hinton agar

14. Autoclave employes _____ heat

a. Moist Heat b. Dry heat

c. Electric d. Mechanical

15. Disinfection means _____

a. Demolishing b. Destruction

c. Damaging d. Death

16. The culturing performed for sensitivity testing is _____

a. Lawn b. Single line

c. Quadrant d. Zig zac

17. The _____ is the period in which there is an onset of sign and symptoms.

a. incubation period b. prodromal stage

c. survival period d. death

18. When a disease occurs occasionally and at irregular intervals in a human population it is a _____ disease.

a. epidemic b. endemic

c. sporadic d. pandemic

19. Allergic manifestation is called as_____

a. Hypersensitivity b. Hyposensitivity

c. sensitivity d. Histeria.

20. Inflammation of meninges is called_____

a. Meningia b. Meningitis

c. Peritonitis d. Carditis

Part B

Answer all the questions **3x2 = 6 marks**

21. What are clinical samples
22. What are Culture Media?
23. What are pathogens and pathogenicity?

Part C

Answer all the questions **3x8 = 24 marks**

24. A. Give the importance of safety precaution followed in microbiology laboratory.

Or

B. Write in detail about processing of samples

25. A. Explain about geimsa staining.

Or

B. Write in detail about the normal flora of human body.

26. A. How will you differentiate two groups of bacteria by staining?

Or

B. Give a detailed note on the procedure for staining the tubercle bacilli?

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Reg. No. : ----------

[16MBU304B]

KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act 1956)

Eachanari post, Coimbatore – 641 021, Tamil Nadu, India

III INTERNAL TEST, September 2017

DEPARTMENT OF MICROBIOLOGY

THIRD SEMESTER

MICROBIAL DIAGNOSIS IN HEALTH CLINIC

Time: 2 hours **Maximum Marks : 50 marks**

Date/ Session: **Class: II B.Sc MB**

PART-A **20 x 1 = 20 marks**

1. The medium used for antibiotic sensitivity testing is _____
   a. Nutrient agar b. Blood agar
   c. Urea agar d. Muller hinton agar
2. Autoclave employes _____ heat
   a. Moist Heat b. Dry heat
   c. Electric d. Mechanical
3. Disinfection means _____
   a. Demolishing b. Destruction
   c. Damaging d. Death
4. The culturing performed for sensitivity testing is _____
   a. Lawn b. Single line
   c. Quadrant d. Zig zac
5. The _____ is the period in which ther is an onset of sign and symptoms.
   a. incubation period b. prodromal stage
   c. survival period d. death
6. When a disease occurs occasionally and at irregular intervals in a human population it is a _____ disease.
   a. epidemic b. endemic
   c. sporadic d. pandemic
7. When the disease occurs at a steady low-level frequency at a moderately regular interval it is an ____ disease.
   a. epidemic b. endemic
   c. sporadic d. pandemic
8. Animal disease that can be transmitted to humans are termed _____.
   a. epizootic b. enzootic
   c. panzootic d. zoonoses
9. Mannitol is fermented by _____ *Staphylococcus*
   a. Virulant b. Avirulant
   c. Commensal d. Normal flora
10. *Streptococcus* shows _____ sensitive
    a. Bacitracin b. Ampicillin
    c. Tetracycline d. Kanamycin
11. *Streptococcus* causes_____
    a. Fever b. Malaice
    c. Sore throat d. Dypsia
12. *Streptococcus mutans* is found in _____
    a. Semen b. Dental plaque
    c. Urinary tract d. Respiratory tract
13. *E.coli* exhibits IMViC_____
    a. ++-- b.--++
    c.- +-+ d. ++++

14. *Klebsiella* exhibits_____ colonies.

a. Dry b. mucoid
c. Pale d. diffuse

15. *Shigella* is _____

a. Flagellated. b. sporing
c. capsulated d. Non motile

16 The selective medium used for *Shigella* is_____

a. Deoxycholate citrate agar b. EMB
c. MSA d. Martin Thayer

17. Allergic manifestation is called as_____

a. Hypersensitivity b. Hyposensitivity
c. sensitivity d. Histeria.

18. Inflammation of meninges is called_____

a. Meningia b. Meningitis
c. Peritonitis d. Carditis

19. Fever of unknown origin is called_____

a. Pyrexia b. Pyrogen
c. Pyogen d. Phylogenic

20. Ulcer on finger is called as_____

a. Boils b. Tuleremia
c. Wound d. Abscesses

Part B

Answer all the questions **3x2 = 6 marks**

21. Describe the causes of Typhoid
22. Say about serological methods
23. What are Nosocomial infections?

Part C

Answer all the questions **3x8 = 24 marks**

24. a. Determine the antibiogram using antibiotic sensitivity method.

Or

b. Elaborate the MIC and MBC procedures.

25. a. Explain IFT.

Or

b. Discuss about WIDAL test

26. a. Give a detailed note on ELISA.

Or

b. State the detailed note on PCR.

Reg. No. : ----------

[15MBU304B]

KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act 1956)
Eachanari post, Coimbatore – 641 021, Tamil Nadu, India

B.Sc. DEGREE EXAMINATION, NOVEMBER 2015

DEPARTMENT OF MICROBIOLOGY

THIRD SEMESTER

MICROBIAL DIAGNOSIS IN HEALTH CLINIC

Time: 3 hours **Maximum Marks : 60 marks**

PART-A

Multiple Choice Questions No.1 to 20 (Online Exam) **20 x 1 = 20 marks**

1. The medium used for antibiotic sensitivity testing is _____
   a. Nutrient agar b. Blood agar
   c. Urea agar d. Muller hinton agar
2. Autoclave employes _____ heat
   a. Moist Heat b. Dry heat
   c. Electric d. Mechanical
3. Disinfection means _____
   a. Demolishing b. Destruction
   c. Damaging d. Death
4. The culturing performed for sensitivity testing is _____
   a. Lawn b. Single line
   c. Quadrant d. Zig zac
5. The _____ is the period in which ther is an onset of sign and symptoms.
   a. incubation period b. prodromal stage
   c. survival period d. death
6. When a disease occurs occasionally and at irregular intervals in a human population it is a _____ disease.
   a. epidemic b. endemic
   c. sporadic d. pandemic
7. When the disease occurs at a steady low-level frequency at a moderately regular interval it is an ____ disease.
   a. epidemic b. endemic
   c. sporadic d. pandemic
8. Animal disease that can be transmitted to humans are termed _____.
   a. epizootic b. enzootic
   c. panzootic d. zoonoses
9. Mannitol is fermented by _____ *Staphylococcus*
   a. Virulant b. Avirulant
   c. Commensal d. Normal flora
10. *Streptococcus* shows _____ sensitive
    a. Bacitracin b. Ampicillin
    c. Tetracycline d. Kanamycin
11. *Streptococcus* causes_____
    a. Fever b. Malaice
    c. Sore throat d. Dypsia
12. *Streptococcus mutans* is found in _____
    a. Semen b. Dental plaque
    c. Urinary tract d. Respiratory tract
13. *E.coli* exhibits IMViC_____
    a. ++-- b.--++
    c.- +-+ d. ++++
14. *Klebsiella* exhibits_____ colonies.
    a. Dry b. mucoid
    c. Pale d. diffuse

15. *Shigella* is _____

a. Flagellated. b. sporing

c. capsulated d. Non motile

16 The selective medium used for *Shigella* is_____

a. Deoxycholate citrate agar b. EMB

c. MSA d. Martin Thayer

17. Allergic manifestation is called as_____

a. Hypersensitivity b. Hyposensitivity

c. sensitivity d. Histeria.

18. Inflammation of meninges is called_____

a. Meningia b. Meningitis

c. Peritonitis d. Carditis

19. Fever of unknown origin is called_____

a. Pyrexia b. Pyrogen

c. Pyogen d. Phylogenic

20. Ulcer on finger is called as_____

a. Boils b. Tuleremia

c. Wound d. Abscesses

Part B

Answer all the questions **5x2 = 10 marks**

21. Define medical diagnosis
22. Say about human body systems
23. What are clinical samples?
24. What are pathogens and pathogenicity?
25. What is PCR?

Part C

Answer all the questions **5x6 = 30 marks**

26. a. Determine the antibiogram using antibiotic sensitivity method.

Or

b. Give the importance of safety precaution followed in microbiology laboratory.

72. a. Discuss about the sources and types of infection.

Or

b. Explain about the epidemics and its control.

28. a. Write a detailed account on the diseases affect the human body

Or

b. State the clinical picture of leprosy and its treatment.

29. a. Explain IFT.

Or

b. Discuss the serological diagnosis methods

30. a. Explain the respiratory tract infecting pathogens with reference to lab diagnosis.

Or

b. Comment on Immunoprophylaxis.

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Reg. No. : ----------

[16MBU304B]

KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act 1956)
Eachanari post, Coimbatore – 641 021, Tamil Nadu, India

B.Sc. DEGREE EXAMINATION, NOVEMBER 2017

DEPARTMENT OF MICROBIOLOGY

THIRD SEMESTER

MICROBIAL DIAGNOSIS IN HEALTH CLINIC

Time: 3 hours **Maximum Marks : 60 marks**

PART-A

Multiple Choice Questions No.1 to 20 (Online Exam) **20 x 1 = 20 marks**

1. During vein puncture withdraw approximately _____ mL of blood.
   a. 5 b. 10
   c. 15 d. 20
2. In case of urine collection_____ urine must be collected.
   a. Early b. Midstream.
   c. Late d. Voided
3. During collection of blood, _____ is applied to fix vein.
   a. Blanket b. Belt
   c. Tourniquet d. Catheter
4. Specimen must be collected before administration of _____.
   a. Antiseptic. b. Disinfectant
   c. Antibiotic d. Water.
5. Meningococcal meningitides was formerly known as _____
   a. Meningococcal fever b. cerebrospinal fever
   c. Intestinal fever d. viral fever
6. _____is a selective media for *Niesseria*
   a. PLET b. Blood agar
   c. Mannitol salt agar d. Martin Thayer
7. A person who has recovered from the disease and continous to harbour the pathogen inside the body is called _____ carrier.
   a. healthy b. convalescent
   c. contact d. paradoxical
8. The _____ carrier state lasts for several years.
   a. acute b. temporary
   c. chronic d. healthy
9. *Vibrio* is motile by_____ flagella.
   a. Atrichous b. peritrichous
   c. polar d. Lopotrichous.
10. Fluorescin is_____ colored pigment.
    a. Greenish yellow b. green
    c. Red d. blue
11. MTR protein are found on _____
    a. Flagella b. Cell surface
    c. Cytoplasm d. Pili
12. *Pneumococcus* habitate in the _____ part of human
    a. Liver b. Urinary tract
    c. Respiratory tract d. Bone marrow
13. Pyomelanin is_____ colored pigment.
    a. Brown b. Red
    c. Green d. Yellow
14. Pyocynanin is soluble in_____
    a. Acid b. Base
    c. chloroform , Water d. ether

15. The accessory factors required by *H. influenza* is called as_____

a. X and Y b. X and V
c. X and Z d. Y and Z

16. _____is the drug of choice for *Hemophilus*.

a. Chloramiphenicol b. Bacitracin
c. Ceftazidime, Cefatoxime d. Streptomycin

17. In hospital environment the normal flora is replaced by _____flora.

a. Drug resistant b. common
c. Nosocomial d. saprophyte.

18. Hospital acquired infection are typically_____.

a. Exogenous b. Endogenous
c. Epigenous d. Eugenous

19. The opportunity of a microorganism to infect patient is due to _____ impared.

a. Diet b. Immune
c. Invasive d. Infection

20. The common post operator consequence in hospital is _____

a. Fever b. gangrene
c. cold d. sore

Part B

Answer all the questions **5x2 = 10 marks**

21. Define microbial diagnosis.
22. Say about bacteria
23. What is disease and infection?
24. What is lab diagnosis?
25. What are MIC and MBC?

Part C

Answer all the questions **5x6 = 30 marks**

26. a. give a detailed note on diagnosis and its types

Or

b. Write about the transport media and its importance.

27. a. Discuss about the investigation of epidemics and control.

Or

b. Describe various sources and methods of infection.

28. a. Comment on the ELISA

Or

b. Give the laboratory diagnosis of Acid fast bacilli.

29. a. State the pathogenic role and diagnosis of GI tract pathogenesis.

Or

b. Discuss the properties and UTI caused by *E.Coli*.

30. a. Explain in detail about Kirby bauer technique of susceptibility testing.

Or

b. Comment on respiratory tract infection and its symptoms.

Reg. No. : ----------

[16MBU304B]

KARPAGAM UNIVERSITY

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Eachanari post, Coimbatore – 641 021, Tamil Nadu, India

B.Sc. DEGREE EXAMINATION, NOVEMBER 2016

DEPARTMENT OF MICROBIOLOGY

THIRD SEMESTER

MICROBIAL DIAGNOSIS IN HEALTH CLINIC

Time: 3 hours **Maximum Marks : 60 marks**

PART-A

Multiple Choice Questions No.1 to 20 (Online Exam) 20 x 1 = 20 marks

1. Doors and windows must be _____ during laboratory session.
   a. Open b. Closed
   c. Ventilated d. Aerated.
2. At the beginning and termination of each session wipe the bench tops with _____.
   a. Antiseptic b. Disinfectant
   c. Water d. Antibiotic.
3. Loops and needles should be sterilized by _____.
   a. Autoclave b. Hot air oven
   c. Disinfectants d. Incineration.
4. Wear a paper cap or tie the long hair to minimize the exposure to _____
   a. Air. b. Pathogen.
   c. Flame. d. Water.
5. In a patient already suffering from a disease, a new infection is set up from another host or external source it is termed _____ infection.
   a. Cross b. focal
   c. re-infection d. nosocomial
6. Cross infection occurring in hospitals are called ___ infection
   a. cross b. focal
   c. re-infection d. nosocomial
7. Physicians induced infections are termed _____ infection.
   a. Iatrogenic b. focal
   c. nosocomial d. secondary
8. When clinical effects are not appaent it is called _____ infection.
   a. atypical b. inapparent
   c. subclinical d. clinical
9. A person who has recovered and continous to harbour the pathogen is called _____ carrier.
   a. healthy b. convalescent
   c. contact d. paradoxical
10. In intoxication the disease is caused by _____
    a. Toxoid b. Tetroid
    c. Enzyme d. Toxin
11. Beeta haemolysis means _____
    a. Complete b. Irregular
    c. Partial d. No lysis
12. _____ enzyme brings clotting of plasma
    a. Coagulase b. Lipase
    c. Nuclease d. Ligase
13. *E.coli* is an _____ in humans.
    a. Pathogen b. Predator
    c. Parasite d. Commensal
14. *Klebsiella* is_____
    a. Non motile and non capsulated b. motile and capsulated
    c. Non motile and capsulated d. motile and non capsulated

15. *Proteus* exhibits _____ motility.

a. Swarming b. no

c . Fish in stream d. Darting

16. The clinical picture of dysentery is exhibited by_____

a. Mycobacteria b. Pseudomonas

c. Klebsiella d. Shigella

17. Cathetrization causes _____ infection.

a. UTI b. RTI

c. CTI d. systemic.

18. Multiplication of bacteria in blood called_____

a. Bacteria b. Bacteremia

c. Viremia d. Septicemia

19. Programmed cell death is called as_____

a. Abscesses b. Lesion

c. Necrosis d. Fever

20. Fever inducing agent is called as_____

a. Pyrogen b. Pyogen

c. Phelm d. Parotid

Part B

Answer all the questions **5x2 = 10 marks**

21. Define pandemic and how you control it
22. Say about circulatory system
23. What are culture media?
24. What are MIC and MBC?
25. What is Nucleic acid probes?

Part C

Answer all the questions **5x6 = 30 marks**

26. a. Discuss about the laboratory precaution and guidelines.

Or

b. Explain the collection and processing of urine and pus samples.

27. a. Write about the different types of diagnosis.

Or

b. Outline the protocol of infectious disease cycle?

28. a. Describe the morphology, cultural properties and lab diagnosis of GI tract pathogens.

Or

b. Explain in detail about the classification of staining methods.

29. a. Comment on identification and interpretation of diseases

Or

b. State the application of ELISA

30. a. Write a detailed account on Kirby bauer method.

Or

b. Elaborate the MIC and MBC procedures.

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