Clinical Analysis

Clinical analysis are performed in the laboratory, which is a part of the healthcare organization. healthcare organizations offer a variety of services to physicians and patients with the goal of providing the best possible patient care.

Microorganisms populate the healthy human body by the billions as benign passengers (normal flora) and even as participants in bodily functions. Those relatively few species of microorganisms that are harmful to humans, either by production of toxic compounds or by direct infection, are characterized as pathogens.
Most infectious disease is initiated by colonization (the establishment of proliferating microorganisms on the skin or mucous membranes). The major exceptions are diseases caused by introduction of organisms directly into the bloodstream or internal organs.

Microbial colonization may result in:

1- elimination of the microorganism without affecting the host
2- infection in which the organisms multiply and cause the host to react by making an immune or other type of response
3- Transient or prolonged carrier state.

Infectious disease occurs when the organism causes tissue damage and impairment of body function.

The human microbiome is the total number and diversity of microbes found in and on the human body. In the past, the ability to cultivate organisms from tissues and clinical samples was the gold standard for identification of normal flora and bacterial pathogens.
However, the recent application of culture-independent molecular detection methods based on DNA sequencing indicates that the human body contains a far greater bacterial diversity than previously recognized.

Molecular methods are capable of detecting fastidious and nonculturable species. Even using advanced molecular techniques, it is difficult to define the human microbiome because microbial species present vary from individual to individual as a result of physiologic differences, diet, age, and geographic habitat.

Identifying the organism causing an infectious process is usually essential for effective antimicrobial and supportive therapy.

Initial treatment may be empiric, based on the microbiologic epidemiology of the infection and the patient's symptoms. Definitive microbiologic diagnosis of an infectious disease usually involves one or more of the following ***five basic laboratory techniques***:

1- Direct microscopic visualization of the organism
2- Cultivation and identification of the organism
3- Detection of microbial antigens
4- Detection of microbial DNA or RNA
5- Detection of an inflammatory or host immune response to the microorganism

A clinical history is the most important part of patient evaluation. For example, a history of cough points to the possibility of respiratory tract infection, whereas dysuria (painful or difficult urination) suggests urinary tract infection.
A history of travel to developing countries may implicate exotic organisms. For example, a patient who recently swam in the Nile has an increased risk of ***schistosomiasis***.
Patient occupations may suggest exposure to certain pathogens, such as ***brucellosis*** in a butcher or ***anthrax*** in farmers.
The age of the patient can sometimes guide the clinician in predicting the identity of pathogens. For example, a gram positive coccus in the spinal fluid of a newborn infant is unlikely to be ***Streptococcus pneumoniae*** (***Pneumococcus***) but most likely to be ***Streptococcus agalactiae*** (group B) which is sensitive to penicillin G. By contrast, a gram-positive coccus in the spinal fluid of a 40year-old patient is most likely to be ***S. pneumoniae***. This organism is frequently resistant to penicillin G and requires treatment with a third generation cephalosporin (such as Cefotaxime or ceftriaxone) or vancomycin.

In many infectious diseases, pathogenic organisms (excluding viruses) can often be directly visualized by microscopic examination of patient specimens, such as sputum, urine, and CSF.

The health-care setting provides abundant sources of potentially harmful microorganisms. These MOs are frequently present in the specimens received in the clinical laboratory. Understanding how microorganisms are transmitted ***(chain of infection)*** is essential to preventing infection (as represented by the biohazard symbol in fig (1).

Fig. 1: the symbol of biohazard

MOs from the source are transmitted to the host. This may occur by 1) direct contact (e.g., the host touches the patient, specimen, or a contaminated object), 2) inhalation of infected material, 3) ingestion of a contaminated substance (e.g., food, water, specimen), or 4) from an animal or insect vector bite.

Preventing completion of the chain of infection is a primary objective of biological safety. Figure (2) uses the universal symbol for *biohazardous* material to demonstrate how following prescribed safety practices can break the chain of infection.

Proper handwashing and wearing *personal protective equipment* (PPE) are of major importance in the laboratory. Concern over exposure to blood-borne pathogens, primarily hepatitis B virus (HBV) and human immunodeficiency virus (HIV), resulted in the drafting of guidelines and regulations by the Centers for Disease Control and Prevention (CDC). Figure (2) demonstrates the Chain of infection and safety practices related to the biohazard symbol.

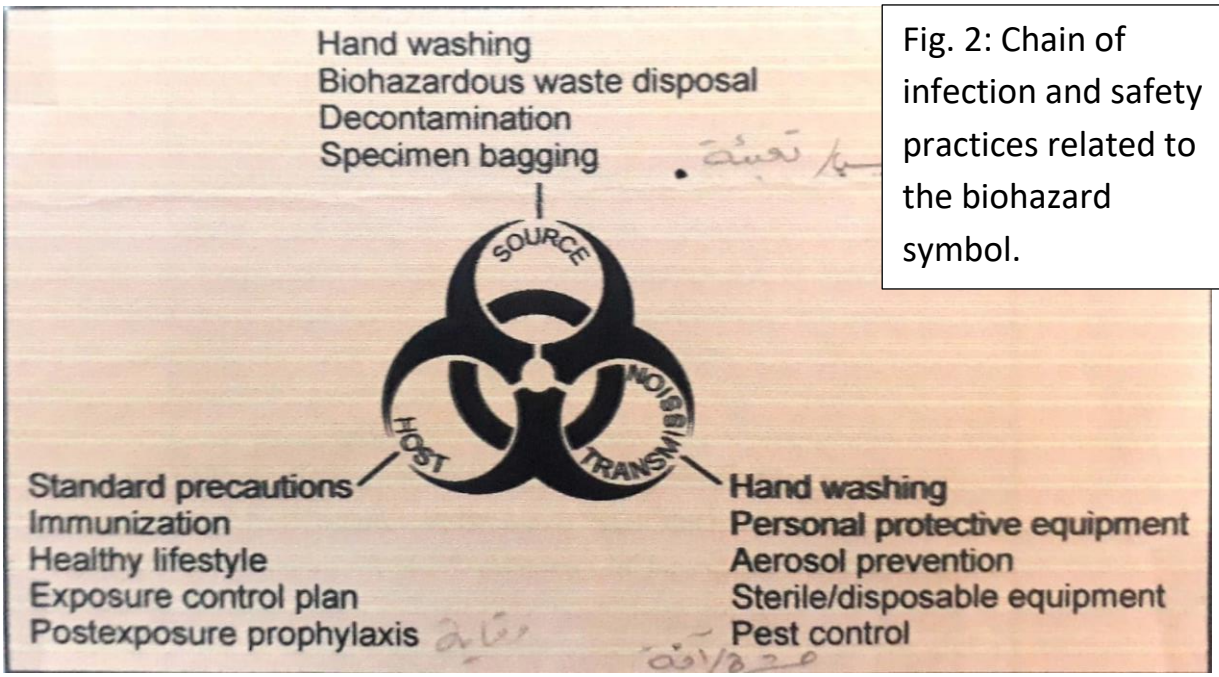


Fig. 2: Chain of infection and safety practices related to the biohazard symbol.

Safety in the Clinical Laboratory: The clinical laboratory contains a variety of safety hazards, many of which are capable of producing serious injury or lifethreatening disease. To work safely in this environment, laboratory personnel must learn: * what hazards exist, *the basic safety precautions associated with them, and *how to apply the basic rules of common sense required for everyday safety.

QUALITY ASSESSMENT

In order to make gains from the assessment work done and to reduce medical errors, results from quality assessment activities must be evaluated and communicated.

Variables Affecting the Quality of Laboratory Testing: Errors can occur throughout the testing process and include errors from **preanalytical, analytical, and postanalytical sources**. Quality control is used to monitor the analytical (or testing) process. This is critical to ensure the accuracy of laboratory test results. As testing procedures are now very sensitive and specific, these preanalytical and postanalytical errors are more prevalent than **analytical errors**. Table 1-1 provides examples of behaviors that can lead to preanalytical, analytical, and postanalytical errors.

Table 1-1 Examples of Laboratory Testing Errors

PREANALYTICAL ERRORS	ANALYTICAL ERRORS	POSTANALYTICAL ERRORS
Patient identification errors	Technologist error	Computer result entry error
Improper patient preparation	Instrument Calibration error	Test interpretation errors
Inappropriate test orders	Reagent deterioration	Illegible report
Incorrect container/additives	Pipetting errors	Failure to deliver report
Specimen labeling errors	Instrument bias or failure	Incorrect patient information
Improper specimen collection or handling	Test procedure steps not followed	Transcription errors
Improper timing of collection	Timing errors while running test	Delayed report
Hemolyzed or contaminated specimen	Instrument not operated correctly	Failure to phone critical results

Quality Control: is a set of procedures and practices that monitor the testing process and those procedures that verify the reliability, accuracy of testing. **Standards** and **controls** are used in this process. Standards contain a known amount of the analyte being tested and are used to calibrate the test. Controls are materials of the same matrix as the sample (composed of serum for serum tests and composed of urine for urine tests) that have an established acceptable range for the analyte being tested. The controls are always run with the test, and control values are monitored statistically to assess the validity of the test results. If the controls do not fall in acceptable range, the test results may be invalidated.

By monitoring the control values daily or with each shift, the accuracy of the test method can be observed. Controls are usually in the normal patient level and in the clinically significant abnormal level(s) (usually high and possibly also low levels). Quality control must be recorded and analyzed to be of any benefit.

Collection and Physical Examination of Urine

Urine is the most obtainable specimen used in laboratory testing. Test results often depend on the collection and handling of specimens. Several techniques and preservatives are used in the collection of urine, which should be used appropriately to allow for the most accurate results. The physical examination of urine includes the observation of urine appearance and concentration and to a lesser extent urine odor and the presence of foam. These observations, along with chemical testing of urine, aid in the screening and diagnosis of disease.

SPECIMEN COLLECTION METHODS

The performance of an accurate urinalysis begins with the proper collection technique. There are several methods available, depending on the type of specimen needed. The first important step is the use of a clean, dry container. Disposable containers are preferred by most laboratories, since they avoid the possibility of contamination from improperly washed glass urine bottles. Samples that are to be cultured must be collected in sterile containers.

Clean-catch or clean-voided midstream specimen is usually the method of choice for obtaining non-contaminated specimens. During the collection the initial portion of the urine stream is allowed to escape while the midstream portion is collected into a sterile container. The final portion of the urine flow is also discarded.

Three-glass collections are similar to the clean-catch collection and are used to determine prostate infection. here, all portions, beginning, middle, and final portion of the void, are collected in three separate containers. The prostate is massaged prior to collection in the third container. Urinary tract infections will show increased WBCs counts and bacteria in the second and third containers, while prostate infections will demonstrate WBCs counts and bacteria higher in the third container than in the second.

Catheterization of the bladder is sometimes necessary to obtain a suitable specimen. This method may be used if the patient is having difficulty voiding. It can also be used in a female patient to avoid vaginal contamination, especially during menstruation. However, since this procedure carries with it the possibility of introducing

organisms into the bladder which may, in turn, cause infection, it should not be routinely used for the collection of culture specimens.

Suprapubic aspiration of the bladder is sometimes used in place of catheterization for obtaining a single urine sample. It involves the insertion of a needle directly into the distended bladder. This technique avoids vaginal and urethral contamination and can also be useful in getting urine from infants and small children. The specimen obtained by this method can also be used for cytology studies.

To obtain suitable specimens from infants and small children, pediatric **urine collection bags**, which are attached to the genitalia, are available. These collection bags are soft and pliable and cause little discomfort to the patient. As in all urine collections, however, care must be taken to avoid fecal contamination.

Unacceptable urine collection techniques include collecting the sample into a container that may still have detergent residue or bleach, or one that has not been adequately cleaned. Urine collected in a bedpan that also contains feces is not an acceptable specimen, nor is urine that has been squeezed out of a diaper.

SPECIMEN PRESERVATION

Ideally, the specimen for routine urinalysis should be examined while fresh. If this is not possible, then it should be refrigerated until examined. Specimens left at room temperature will soon begin to decompose, mainly due to the presence of bacteria in the sample. **Urea-splitting bacteria** produce ammonia, which then combines with hydrogen ions to produce ammonium, thereby causing an increase in the **pH** of the urine. This increase in pH will result in the decomposition of any casts which may be present, because casts tend to dissolve in alkaline urine. If glucose is present, the bacteria may use it as a source of energy which could then result in a false-negative test for glycosuria.

There are times when a urine specimen must be saved for a longer period of time than is recommended. This is a common occurrence when specimens are sent to commercial laboratories for analysis. There are several chemical preservatives that can be added to the specimen but most of them interfere in some way with the testing procedure. For this reason, the routine use of preservatives is not recommended.

PRESERVATIVES

Preservatives that can be used to preserve random screening specimens include toluene, formalin, thymol, formaldehyde- generating preservative tablets, and chloroform, boric acid, and chlorhexidine.

- **Formalin** (1 drop/30 mL urine) is a good preservative for urinary sediment but if used in too large a concentration it will precipitate protein and will give a false-positive test for reducing substances.
- **Toluene** (2 mL/100 mL urine) preserves **ketones**, proteins, and reducing substances, but it is not effective against bacteria already present in the urine. Because toluene floats on the surface of the urine, it may be difficult trying to separate the preservative from the specimen for testing. In addition, toluene is flammable.

TIMING OF COLLECTION

A **random** sample is usually sufficient for the performance of most urinary screening tests; but, since the first specimen voided in the morning **(first-morning)** is more concentrated, it is usually the specimen of choice.

Because urinary substances are excreted in varying concentrations throughout the day, it is necessary to collect timed specimens to accurately quantitate some substances such as creatinine, glucose, total protein, electrolytes, hormones, and urea. The most commonly used sample is the 24-hour specimen. In this procedure, the patient empties the bladder and discards the urine. This is usually done about 8 AM. All urine is collected for 24 hours thereafter, including the sample at 8 AM the next day. The container that is used for the 24-hour specimen should be kept in the refrigerator during the entire collection period. Various chemical preservatives may need to be added to the collection container depending on the substance to be tested. For some tests, such as creatinine and protein, refrigeration alone is sufficient.

EXAMINATION OF PHYSICAL CHARACTERISTICS

The routine urinalysis includes the examination of (a) physical characteristics, such as color, appearance, and specific gravity; (b) chemical characteristics, including pH, protein, glucose, ketones, blood, bilirubin, nitrite, leukocyte esterase, and urobilinogen; and (c) microscopic structures in the sediment.

Samples collected for routine urinalysis should be at least 15 mL in volume. If only one specimen has been sent to the laboratory for both microbiology and urinalysis studies, the sample must be cultured first or separated into a sterile container for microbiology, before routine testing is performed.

- **COLOR:**

Normal urine has a wide range of color, which is mainly determined by its concentration. This color may vary from a pale yellow to a dark amber, depending on the concentration of the pigments **urochrome** and, to a lesser extent, **urobilin** and **uroerythrin**. The more pigment there is, the deeper the color will be. There are, however, many factors and constituents that can alter the normal urine color. These include medications and diet as well as various chemicals that can be present in disease. Table (1) lists some of the substances that may influence the color of urine. This table should not be considered as an all-inclusive list, for there are numerous drugs that are capable of changing the color of urine. It should be noted that the pH of the urine influences the color that many chemicals produce. In addition, there may be several coloring factors present in the same urine, which may result in a different color than that expected.

Very pale or colorless urine is very dilute and can result from high fluid consumption, diuretic medication, natural diuretics such as coffee and alcohol, and in such disease states as diabetes mellitus and diabetes insipidus.

The most common cause of red urine is the presence of red blood cells (RBCs) **(hematuria)**. Red urine may also be due to the presence of free hemoglobin **(hemoglobinuria)**, myoglobin **(myoglobinuria)**, or large amounts of uroerythrin which can occur in acute febrile disease.

In addition, some individuals have an inherited metabolic sensitivity which results in the excretion of red urine after eating beets. This color is due to the presence of complex pigments called anthocyanins.

Patients with jaundice will excrete bile pigments such as bilirubin, and the urine will be yellow–brown to yellow–green in color. The green pigment is due to biliverdin,

the oxidized product of bilirubin, and if the specimen is left standing, the green color will intensify.

Multivitamins and riboflavin can give a bright yellow color to urine. Even food dyes such as those used in candies can be excreted in the urine, thus affecting its color.

Although some laboratories have eliminated the routine reporting of urinary color, one must not overlook the clues given by this physical characteristic. For example, if bilirubin is not included in the routine urinalysis because of the type of dipstick that is used, but the color of the urine strongly suggests its presence, then a test for bilirubin should be performed and the results reported. This may be the first indication to the physician of the patient's problem. Any abnormal color such as black or brown should always be reported.

- **CLARITY:**

Normal urine is usually clear but it may become cloudy due to the precipitation of amorphous phosphates in alkaline urine, or amorphous urates in an acid urine. Amorphous phosphates are a white precipitate which will dissolve when acid is added. Amorphous urates frequently have a pink color from urinary pigments, and they will dissolve if the specimen is heated.

Urine can be cloudy from the presence of leukocytes or epithelial cells. The presence of these cells can be confirmed by microscopic examination of the sediment. Bacteria can also cause cloudiness, especially if the specimen has been sitting at room temperature. Mucus can give the urine a hazy appearance, and RBCs can result in a smoky or turbid urine. Fat give urine a milky appearance.

- **FOAM:**

Although not routinely reported, foam may be a significant finding. A white stable foam that is formed upon agitating the specimen can be seen in urine containing a moderate or large amount of protein. Foam that is present in agitated urine specimens may appear yellow to yellow–green if sufficient amount of bilirubin is present.

- **ODOR:**

Although not routinely reported urine odor may be a significant observation. Ketones smell sweet or fruity. A specimen contaminated with bacteria may have a pungent smell from the ammonia that is produced. A "musty or mousy" odor of an infant's urine may indicate phenylketonuria. A "sweaty feet" odor is found in individuals who have excessive amounts of butyric or hexanoic acid.

- **CONCENTRATION:**

The specific gravity is the ratio of the weight of a volume of urine to the weight of the same volume of distilled water at a constant temperature. It is an indicator of the concentration of dissolved material in the urine; however, it is dependent not only upon the number and weight of the particles in the solution. The specific gravity is used to measure the concentrating and diluting ability of the kidney in its effort to maintain homeostasis in the body. The concentrating ability of the kidney is one of the first functions to be lost as a result of tubular damage.

The normal range of specific gravity for a random specimen is 1.003–1.035, although in cases of excess hydration the reading may be as low as 1.001 (water is 1.000). The specific gravity value varies greatly depending on the state of hydration and the urinary volume. Because the specific gravity varies throughout the day, a single random reading may not give the physician sufficient information, so a 24-hour collection may be ordered. The range for a 24-hour specimen is 1.015–1.025.

Hyposthenuria is a term that is used to describe a urine with a consistently low specific gravity (<1.007). The excretion of urine of unusually high specific gravity is called **hypersthenuria**, and this can result from deprivation of water. **Isosthenuria** refers to a fixed specific gravity of 1.010, which indicates poor tubular reabsorption.
Some of the causes of increased specific gravity include dehydration, proteinuria, glycosuria, heart failure, renal stenosis, , lipid nephrosis, and water restriction.

Table **Causes for Urine Color and Clarity**

APPEARANCE	PATHOLOGIC CAUSES	NONPATHOLOGIC CAUSES
White	Chyle	Phosphates
	Lipids	Vaginal creams
	Pyuria (many WBCs)	
Yellow to amber to orange	Bilirubin	Acriflavine
	Urobilin (excessive)	Azo Gantrisin
		Carrots
		Concentrated urine
		Food color
		Nitrofurantoin
		Pyridium
		Quinacrine
		Riboflavin
		Rhubarb
		Senna
		Serotonin
		Sulfasalazine
		Vitamin B complex
Yellow to green	Bilirubin-biliverdin	
Pink to red	Hemoglobin	Aminopyrine
	Myoglobin	Antipyrine
	Porphobilin	Beets (anthocyanin)
	Porphyrins	Bromosulfophthalein
	Red blood cells	Cascara
		Diphenylhydantoin
		Food color
		Methyldopa
		Phenacetin Phenolphthalein
		Phenolsulfonphthalein
		Phenothiazine
		Pyridium
		Senna
Red to purple	Porphyrins	
Red to brown	Methemoglobin	
	Myoglobin	
Brown to black	Bilirubin	Chloroquine
	Homogentisic acid	Hydroquinone
	Indican	Iron compounds
	Melanin	Levodopa
	Methemoglobin	Methyldopa
	Myoglobin	Metronidazole
	Phenol	Nitrofurantoin
	p-Hydroxyphenylpyruvate	Quinine
	Porphyrins	Resorcinol
Blue to green	Biliverdin	Acriflavine
	Indicans	Amitriptyline
	Pseudomonas infection	Azure A
		Chlorophyll
		Creosote
		Evans blue
		Methylene blue
		Phenyl salicylate
		Thymol
		Tolonium
		Triamterene
Clear	Very dilute as in diabetes insipidus	Polyuria
Hazy to cloudy to turbid	Varying degrees of casts	Varying degrees of creams, lotions, and salves
	Cells	Crystals
	Crystals and calculi	Fecal contamination
	Fat (lipid, chyle)	Microorganisms
	Microorganisms	Mucus
		Radiographic dyes
		Powders
		Spermatozoa

Bloodstream Infections

Invasion of the bloodstream by MOs constitutes one of the most serious situations in infectious disease. MOs present in the circulating blood—whether continuously, intermittently, or transiently—are a threat to every organ in the body. The suffix *emia* is derived from the Greek word meaning "blood" and refers to the presence of a substance in the blood; *bacteremia* refers to the presence of bacteria in the blood, *fungemia* refers to the presence of fungi in the bloodstream, and *septicemia* indicates bacteria are present in the blood, producing an infection and reproducing within the bloodstream.

Bacteria:

The organisms most commonly isolated from blood are:
Staphylococcus aureus, *Escherichia coli*, Coagulase-negative staphylococci, *Enterococcus* spp. , *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, streptococci species, *Enterobacter cloacae*, *Proteus* spp. ,
Anaerobic bacteria: *Bacteroides* and *Clostridium* spp.

Of importance, the laboratory isolation of certain bacterial species from blood can indicate the presence of an underlying, occult, or undiagnosed neoplasm. Alterations in local conditions at the site of the neoplasm allowing bacteria to proliferate and seed the bloodstream have been suggested as a potential mechanism for the association between bacteremia and cancer. Another possible mechanism is reduced killing of bacterial cells by the host phagocytes. Organisms associated with neoplastic disease include *Clostridium septicum* and other uncommonly isolated clostridial species, *Streptococcus galldyticus*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, and *Campylobacter* spp.

Fungi:

Fungemia is usually a serious condition, occurring in immunosuppressed patients and in those with serious illness. *Candida albicans* is by far the most common species, but *Malassezia furfur* can often be isolated in patients, particularly neonates.

Except for *Histoplasma*, which multiply in leukocytes, fungi do not invade blood cells, but their presence in the blood usually indicates a focus of infection elsewhere in the body.

Fungi in the bloodstream can disseminate (be carried) to all organs of the host, where they may grow, invade normal tissue, and produce toxic products.
Fungi gain entrance to the circulatory system via:

1- Loss of integrity of the gastrointestinal or other mucosa
2- Through damaged skin
3- From primary sites of infection, such as the lung or other organs
4- By means of intravascular catheters.

Systemic fungal infections begin as pneumonia and may disseminate from the lungs, which serve as the portal of entry.

Parasites:

Parasites may be found transiently in the bloodstream as they migrate to other tissues or organs. Their presence, however, cannot be considered consistent with a state of good health. For example, tachyzoites of the parasite *Toxoplasma gondii* may be found in circulating blood. They invade cells within lymph nodes and other organs, including the lungs, liver, heart, brain, and eyes. The resulting cellular destruction accounts for the manifestations of toxoplasmosis.

Malarial parasites invade host erythrocytes and hepatic parenchymal cells. The significant anemia and subsequent tissue hypoxia (reduction in oxygen levels) may result from destruction of RBCs by the parasite.

Parasites in the bloodstream are usually **detected by**:

A= Direct visualization. Those parasites for which traditional diagnosis is dependent on observation of the organism in peripheral blood smears include *Plasmodium, and Trypanosoma*.

B= Rapid serological methods

C= Molecular methods are currently used.

Viruses:

Although many viruses do circulate in the peripheral blood at some stage of disease, the primary pathology relates to infection of the target organ or cells. Those viruses that preferentially infect blood cells are:

1- Epstein- Barr virus (invades lymphocytes).
2- Cytomegalovirus (invades monocytes, polymorphonuclear cells, and lymphocytes).
3- Human immunodeficiency virus (HIV) (involves only certain T lymphocytes and perhaps macrophages).
4- Other human retroviruses that attack lymphocytes.

The **pathogenesis** of viral diseases of the blood is the same as that for viral diseases of any organ; by:

** Diverting the cellular machinery to create new viral components
** The virus may prevent the host cell from performing its normal function.
** The cell may be destroyed or damaged by viral replication
** Immunologic responses of the host may also contribute to the pathogenesis.

Types of bacteremia:

Bacteremia may be **transient**, **continuous**, or **intermittent**. Most people have transient bacteremia when having dental procedures have had oral flora gain entry to the bloodstream through breaks in the gums, devices or instrumentation inserted through contaminated mucosal surfaces, and surgery involving non-sterile sites. These circumstances may also lead to septicemia, although normally the bacteria are cleared

from the blood by scavenging leukocytes, resulting in no infection, Septicemia can occur when the bacteria multiply more rapidly than the immune system is capable of killing and removing the organism.

During the early stages of specific infections, including typhoid fever, brucellosis, and leptospirosis, bacteria are continuously present in the bloodstream.
In most other infections, such as in patients with undrained abscesses, bacteria can be found intermittently in the bloodstream.

Of note, the causative agents of meningitis, pneumonia, pyogenic arthritis, and osteomyelitis are often recovered from blood during the early course of these diseases. In the case of transient infection, such as an abscess, bacteria are released into the blood approximately 45 minutes before a febrile episode.

Signs and symptoms of septicemia may include fever or hypothermia (low body temperature), chills, hyperventilation (abnormally increased breathing leading to excess loss of carbon dioxide from the body), skin lesions, change in mental status, and diarrhea. More serious manifestations include hypotension or shock, and major organ system failure.

Types of blood stream infections:

The two major categories of bloodstream infections are:

- Intravascular (those that originate within the cardiovascular system) .
- Extravascular (those that result from bacteria entering the blood circulation through the lymphatic system from another site of infection).

Of note, other organisms, such as fungi, may also cause intravascular or extravascular infections.

Factors contributing to the initiation of bloodstream infections are:

- Immunosuppressive agents.
- widespread use of antibiotics that suppress the normal flora and allow the emergence of resistant strains of bacteria.
- Invasive procedures allowing bacteria access to the interior of the host.
- More extensive surgical procedures.

Gram-negative bacteria contain lipopolysaccharide (LPS) in their cell walls, called endotoxin. This LPS may be released during the normal growth cycles of bacteria or after the destruction of bacteria by host defenses. Endotoxin (or the core of the LPS, lipid A) has been shown to mediate numerous systemic reactions. Although gram-positive bacteria do not contain the lipid A endotoxin, many produce exotoxins, and the effects of their presence in the bloodstream may be equally devastating to the patient.

Drawing Blood for Culture: MOs found in blood can be enriched in culture for isolation and further studies. Blood for culture must be obtained aseptically. Once removed from the circulation, unclotted blood must be diluted in growth media.

Specimen Volume:

- **Adults.** There is a direct relationship between the volume of blood and an increased probability that the laboratory will isolate the infecting the organism. Therefore, collection of two sets of cultures using10 to 20 mL of blood per culture is strongly recommended for adults.
- **Children.** For infants and small children, only 1 to 5 mL of blood should be drawn for bacterial culture.

Blood Culture Media: The diversity of bacteria recovered from blood requires a diverse of media to enhance the growth of these bacteria. Basic blood culture media contain a **nutrient broth** and an **anticoagulant**. Most blood culture bottles available commercially contain **trypticase soy broth**, **brain-heart infusion broth**, supplemented peptone, or **thioglycolate broth**. More specialized broth bases include **Columbia or Brucella broth**.

Handling Positive Blood Cultures:

✓ **Gram stained smear** of an air-dried drop of medium should be performed to identify the cellular morphology, which may be especially valuable for detecting gram-negative bacteria among red cell.

Determining the clinical significance of an isolate is the physician's responsibility. If no organisms are seen on microscopic examination of a bottle that appears positive, subcultures should be performed anyway.

✓ Subcultures from blood cultures suspected of being positive, whether proved by microscopic visualization or not, should be made to various media that would support the growth of most bacteria, including anaerobes. Initial subculture may include chocolate agar, 5% sheep blood agar, MacConkey agar, and supplemented anaerobic blood agar.

✓ Numerous rapid tests for identification and antimicrobial susceptibilities can be performed if a monomicrobic infection is suspected (based on microscopic evaluation). A suspension of the organism that approximates the turbidity of a 0.5 McFarland standard, can be used to perform either disk diffusion (qualitative) or broth dilution (quantitative) antimicrobial susceptibility tests.

✓ Molecular methods, including conventional and real-time polymerase chain reaction (PCR) assays and microarrays have been used to directly identify MOs. in blood culture bottles.

Guidelines that can assist in distinguishing probable pathogens from contaminants are as follows:

Probable pathogen

- Growth of the same organism in repeated cultures obtained at different times.
- Growth of certain organisms in cultures obtained from patients suspected of endocarditis, such as enterococci, or gram-negative rods in patients with clinical gram-negative sepsis
- Growth of certain organisms such as members of Enterobacteriaceae, *Streptococcus pneumoniae*, gramnegative anaerobes, and *Streptococcus pyogenes*
- Isolation of commensal microbial flora from blood cultures obtained from patients suspected to be bacteremic (e.g., immunosuppressed patients or those having prosthetic devices).

Probable contaminant

- Growth of *Bacillus* spp., *Corynebacterium* spp., *Propionibacterium acnes*, or coagulase-negative staphylococci in one of several cultures
- Growth of multiple organisms from one of several cultures (polymicrobial bacteremia is uncommon)
- The clinical presentation or course is not consistent with sepsis (physician-based, not laboratory-based criteria)
- The organism causing the infection at a primary site of infection is not the same as that isolated from the blood culture.

Chemical Analysis of Urine

The routine urinalysis includes chemical testing for pH, protein, glucose, ketones, occult blood, bilirubin, urobilinogen, nitrite, and leukocyte esterase. The procedures are either qualitative (positive or negative) or semiquantitative (e.g., trace through 4+) measurements. Technological advances have provided for the development of reagent test strips (dipsticks) that allow for the rapid, simultaneous determination of these substances. Abnormal urine chemistry results not only aid in the assessment of renal disorders but can also disclose many systemic disorders.

A **reagent strip** is a narrow strip of plastic with small pads attached to it. Each pad contains reagents for a different reaction, thus allowing for the simultaneous determination of several tests. The colors generated on each reagent pad vary according to the concentration of the analyte present. Colors generated by each pad are visually compared against a range of colors on specific color charts. Figure (1) illustrates a typical urine chemistry reagent strip.

Fig. 1

The procedure for using the dipstick is as follows:

the test areas of the strip must be dipped in well mixed urine and removed immediately. then after particular times, the test areas should be compared with the color charts on the container.

URINARY pH

One of the functions of the kidney is to help maintain acid–base balance in the body. To maintain a constant **pH** in the blood about 7.4, the kidney must vary the pH of the urine to compensate for diet and products of metabolism. This regulation occurs in kidney with the secretion of both hydrogen and ammonia ions into the filtrate, and the reabsorption of bicarbonate.

The secretion of H^+ in the tubule is regulated by the amount present in the body. If there is an excess of acid in the body (acidosis), more H^+ will be excreted and the urine will be acid. When there is an excess of base in the body (alkalosis), less H^+ will be excreted and the urine will be alkaline.

The pH of the urine may range from 4.6 to 8.0 but averages around 6.0, so it is usually slightly acidic. There is no abnormal range as such, since the urine can normally vary from acid to alkaline. For this reason, it is important for the physician to correlate the urine pH with other information to determine whether there is a problem.

If pH is the only test needed to be done on a urine specimen, litmus paper or Nitrazine paper can also be used to obtain an approximate reading.

PROTEIN

The presence of increased amounts of **protein** in the urine can be an important indicator of renal disease. It may be the first sign of a serious problem. There are, however, physiologic conditions such as exercise and fever that can lead to increased protein excretion in the urine in the absence of renal disease. There are also some renal disorders in which **proteinuria** is absent.

In the normal kidney, only a small amount of low– molecular weight protein is filtered at the glomerulus. The structure of the glomerular membrane prevents the passage of high–molecular weight proteins including albumin (mol wt = 69,000). After filtration, most of the protein is reabsorbed in the tubules.

Notes:

- The dipsticks are more sensitive to albumin than to other proteins, whereas the heat and acid tests are sensitive to all proteins.
- Agitation of the urine will cause a white foam to develop on the surface of the urine. Observing foam may be helpful as an indicator of proteinuria.

REAGENT TEST STRIPS

Because proteins act as hydrogen ion acceptors at a constant pH. Usually, the indicator (Tetrabromphenol blue) changes from yellow to blue (or green) between pH 3 and pH 4, but in the presence of protein, this color change will occur between pH 2 and pH 3.

when in the absence of urine protein, the reagent produces a yellow color. The development of any green to blue color indicates the presence of protein. The intensity of the color is proportional to the amount of protein that is present.

The dipstick procedure is very sensitive to albumin, the protein that is primarily excreted as the result of glomerular damage or disease. Other urine proteins such as gamma globulin, glycoprotein, ribonuclease, lysozyme, hemoglobin, and Bence-Jones protein are much less readily detected than albumin. Therefore, a negative urinary dipstick result does not necessarily rule out the presence of these proteins.

GLUCOSE

The presence of significant amounts of glucose in the urine is called **glycosuria** (or glucosuria). The quantity of glucose that appears in the urine is dependent upon the 1) blood glucose level, 2) the rate of glomerular filtration, and 3) the degree of tubular reabsorption. Usually, glucose will not be present in the urine until the blood level exceeds 160–180 mg/dL, which is the normal renal threshold for glucose. When the blood glucose exceeds the renal threshold, the tubules cannot reabsorb all of the filtered glucose, and so glycosuria occurs. Normally, this level is not exceeded even after the ingestion of a large quantity of carbohydrate.

To screen for or monitor glycosuria, The procedures depend on use the enzyme glucose oxidase which is specific for glucose. importantly, a correlation must be made with the blood glucose level as well as the case history, family history, and clinical picture. A previously undiagnosed glycosuria should be followed up by such studies as * a glucose tolerance test, * 2-hour postprandial glucose, and *fasting blood sugar.

REAGENT STRIP GLUCOSE OXIDASE TEST

Reagent strips that are contain the enzyme glucose oxidase detect only glucose. These strips use the following double sequential enzyme reaction:

Reaction A:

$$\text{Glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

Reaction B:

$$H_2O_2 + \text{chromogen} \xrightarrow{\text{peroxidase}} \text{oxidized chromogen}$$

The chromogen that is used varies among the different reagent strips. They are many chromogens used by main manufacturers but the most common used are: Potassium iodide, Glucose oxidase and Peroxidase. Glucose results are read at 30 or 60 seconds.

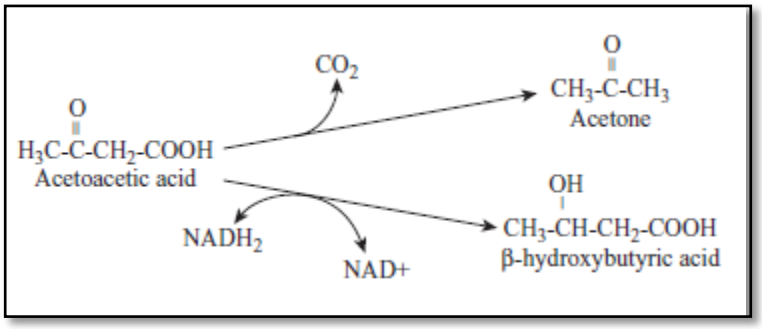
The results are reported as negative to 4+ (The results are semiquantitative values).

KETONES

Ketones, or ketone bodies are formed during the catabolism of fatty acids. One of the intermediate products of fatty acid breakdown is acetyl CoA. Acetyl CoA enters the citric acid cycle (Krebs cycle) in the body if fat and carbohydrate degradation are appropriately balanced.

The first step in the Krebs cycle is the reaction of acetyl CoA with oxaloacetate to yield citrate. When carbohydrate is not available or is not being properly utilized, all available oxaloacetate will be used to form glucose, and so there will be none available for condensation with acetyl CoA. CoA cannot enter the Krebs cycle; therefore, it is diverted to the formation of ketone bodies.

The ketone bodies are acetoacetic acid (diacetic acid), β -hydroxybutyric acid, and acetone. Acetoacetic acid is the first ketone that is formed from acetyl CoA, and the other ketones are formed from acetoacetic acid as shown in the reaction:



Acetoacetic acid and β- hydroxybutyric acid are normal fuels of respiration and are important sources of energy. In fact, the heart muscle and the renal cortex prefer to use acetoacetate instead of glucose. But glucose is the major fuel of the brain in well-nourished individuals, even though the brain can adapt to utilize acetoacetate in the absence of glucose.

Acetone is lost into the air if a sample is left standing at room temperature. Therefore, urines should be tested immediately or refrigerated in a closed container until testing.

REAGENT TEST STRIPS : Multistix contains the reagents sodium nitroprusside and an alkaline buffer, which react with diacetic acid in urine to form a maroon color, as in the following reaction:

Color change is from buff-pink to maroon and the reaction is reported as either negative, trace, moderate, or large or negative to 160 mg/dL. fig ( ) displays a keton color chart.

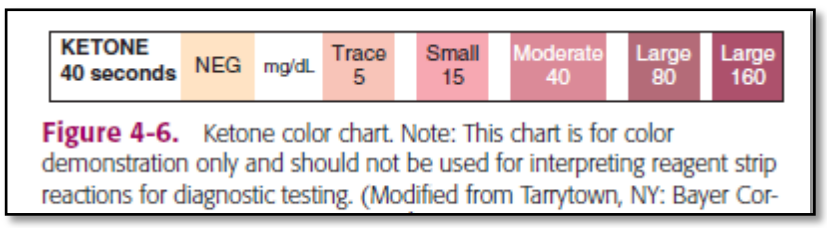


Figure 4-6. Ketone color chart. Note: This chart is for color demonstration only and should not be used for interpreting reagent strip reactions for diagnostic testing. (Modified from Tarrytown, NY: Bayer Cor-

BILIRUBIN AND UROBILINOGEN

Bilirubin is formed from the breakdown of hemoglobin . It is then bound to albumin and transported through the blood to the liver. This free or unconjugated bilirubin is insoluble in water and cannot be filtered through the glomerulus. In the liver, bilirubin is removed by the parenchymal cells and is conjugated with glucuronic acid to form bilirubin diglucuronide. This conjugated bilirubin, which is also called direct bilirubin, is water soluble and is excreted by the liver through the bile duct and into the duodenum.

Normally, very small amounts of conjugated bilirubin regurgitate back from the bile duct and into the blood system. Therefore, very small amounts of conjugated bilirubin can be found in the plasma. Because conjugated bilirubin is not bound to protein, it is easily filtered through the glomerulus and excreted in the urine. Normally, no detectable amount of bilirubin (sometimes referred to as “bile”) can be found in the urine.

In the intestines, bacterial enzymes convert bilirubin, through a group of intermediate compounds, to several related compounds which are collectively referred to as **urobilinogen**. Most of the urobilinogen (a colorless pigment) and its oxidized variant, urobilin (a brown pigment), are lost in the feces. About 10–15% of the urobilinogen is reabsorbed into the bloodstream, returns to the liver, and is reexcreted into the intestines.
A small amount of this urobilinogen is also excreted by the kidneys into the urine. The normal level of total bilirubin in the serum is about 1.0 mg/dL or less. This consists mainly of indirect or unconjugated bilirubin, but there is also a very small amount of direct or conjugated bilirubin present.

When the level of total bilirubin exceeds approximately 2.5 mg/dL, the tissues of the body take on the yellow color of bilirubin, and this is called jaundice. If the jaundice is due to an increase in unconjugated bilirubin, no bilirubin will be excreted in the urine because unconjugated bilirubin cannot be filtered at the glomerulus. But if jaundice is due to an increase in the water-soluble conjugated bilirubin, then bilirubin will be present in the urine.
REAGENT TEST STRIPS: Most dipsticks are based on the coupling reaction of a diazonium salt with bilirubin in an acid medium. Bilirubin results range of colors correspond to levels of bilirubin from negative to large (3+)

False-Negative Results

1- Large amounts of ascorbic acid decrease the sensitivity of this test. Repeating the test at least 10 hours after the last dose of vitamin C will produce more accurate results.
2- Elevated levels of nitrite will lower the bilirubin result.
3- A false negative result will be obtained if the bilirubin has been oxidized to biliverdin, as occurs when specimens are exposed to room temp. and light.

NITRITE

The **nitrite** test is a rapid, indirect method for the early detection of significant and asymptomatic bacteriuria.

Common organisms that can cause urinary tract infections, such as *Escherichia coli*, Enterobacter, Citrobacter, Klebsiella, and Proteus species, produce enzymes that reduce urinary nitrate to nitrite. For this to occur, the urine must have incubated in the bladder for a minimum of 4 hours. Hence, the first morning urine is the specimen of choice.

Reagent strips for the detection of nitrite in the urine commonly use *p*-arsanilic acid and a quinoline compound.

Nitrite reacts with *p*-arsanilic acid to form a diazonium compound. This compound then couples with the quinolone compound to produce a pink color.

Nitrite results are read at 30 or 60 seconds, depending on the manufacturer. Any degree of uniform pink color should be interpreted as a positive nitrite test suggesting the presence of 10^5 or more organisms per milliliter. The color development is not proportional to the number of bacteria present. The test is reported as positive or negative.

False-Negative Results:

a/ high specific gravity or b/ elevated level of ascorbic acid. c/ high levels of urobilinogen

A negative test should never be interpreted as indicating the absence of bacterial infection. There are several reasons for this:

1. There may be pathogens present in the urine that do not form nitrite. **2.** The urine may not have remained in the bladder long enough for the nitrate to be converted to nitrite. **3.** There are cases in which the urine does not contain any nitrate, so bacteria may be present but the dipstick will be negative. **4.** Under certain circumstances, the bacterial enzymes may have reduced nitrate to nitrite and then converted nitrite to nitrogen, which will give a negative nitrite result.

IntechOpen

CHAPTER REVIEW REPORT

CHAPTER:	The aflatoxin producing fungi Aspergillus flavus
AUTHORS:	Nadeem Hasani
ACADEMIC EDITOR:	Lukman Abdulra'Uf
DATE:	09.02.2021, London

TITLE

Chapter title:

It suits the manuscript's content

ORIGINALITY

iThenticate Report Findings: Similarity index: 82%

Is the chapter acceptable in its current form?

No

How severe is the plagiarism?

Significant

The revision required is a major revision to reduce the percentage plagiarism to less than 20%

METHODS USED

Are the research methods and analysis well explained?

No

Suggestion:

No research methods and analysis involved

KEY RESULTS

Is the discussion carried out in a satisfactory manner?

Yes

Does the conclusion support the presented research ?

Yes

REFERENCES

Is the reference list adequate?

Yes

EDITOR'S COMMENTS

Aflatoxins are a type of toxins produced by Aspergillus species, including A. flavus Link, A. parasiticus Speare, and A. nomius Kurtzman , Horn and Hesseltine . These toxins are responsible for damage to 25% of the world's food crops. The fungi produce the contaminating toxins both pre- and postharvest. Aflatoxin is responsible for large economic losses to agriculture in the United States and other developed countries, but in developing countries, where the use of contaminated

grain cannot always be avoided, aflatoxins also cause human and animal disease. Aflatoxin exposure contributes to the development of liver cancer in parts of the world where it is endemic , making it a significant contributor to a major public health problem. The presence of other mycotoxins, particularly fumonisins, along with aflatoxin in field samples brings additional concerns for the safety of food and feed supplies. (Reference required)

Until the 1980s, numerous reports and reviews were available on the impact of aflatoxins on livestock . From the 1990s to today, numerous works have appeared reporting studies on toxicological problems caused by aflatoxins, focusing mainly on the molecular biology of aflatoxin in both the fungi and host, aflatoxin management through conventional breeding, and genetic engineering to produce resistant lines of the susceptible crops and their release to general use. Biological control of aflatoxin using nontoxigenic strains of Aspergillus flavus in corn, peanut, and cotton made substantial progress during this period. Commercial use of this technology in the field is now showing promise for controlling aflatoxin contamination. (Reference required)

EDITOR DECISION

MAJOR REVISION

Lec:5 **Dr.Enas AL-Layla**

UPPER RESPIRATORY TRACT INFECTIONS

Introduction

The upper respiratory tract extends from the larynx to the nostrils and comprises the oropharynx and the nasopharynx together with the communicating cavities, the sinuses and the middle ear(Fig1).

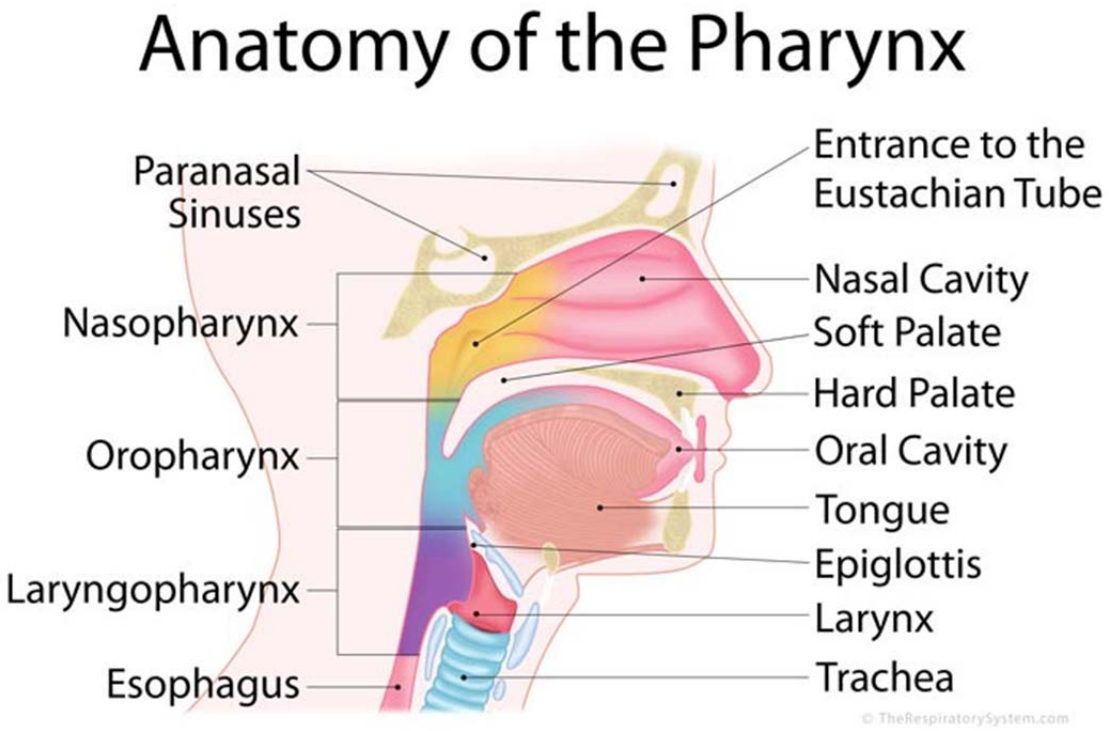


Fig 1:The Pharynx structure

The upper respiratory tract can be the site of several types of infection:

1- pharyngitis sometimes involving tonsillitis, and giving rise to a “sore throat”
2- nasopharyngitis
3- otitis media
4- sinusitis
5- epiglottitis.

Of all those infections, pharyngitis is by far the most frequent; in addition, the untreated infection may have serious sequelae. Only pharyngitis will be considered here.

Most cases of pharyngitis have a viral etiology and follow a self-limiting course. However, approximately 20% are caused by bacteria and usually require treatment with appropriate antibiotics.

As the physician is rarely able to make a distinction between viral and bacterial pharyngitis on clinical grounds alone, treatment should ideally be based on the result of bacteriological examination.

Bacteriological diagnosis of pharyngitis is complicated by the fact that the oropharynx contains a heavy, mixed, normal flora of aerobic and anaerobic bacteria. The normal flora generally outnumbers the pathogens and the role of the bacteriologist is to distinguish between the commensals and the pathogens. Where possible only the latter should be reported to the physician.

Normal flora of the pharynx

The normal flora of the pharynx includes a large number of species that should be neither fully identified nor reported when observed in throat cultures:

• viridans (a-haemolytic) streptococci and pneumococci
• nonpathogenic *Neisseria* spp.
• *Moraxella* (formerly *Branhamella*) *catarrhalis* (this can also be a respiratory pathogen)
• staphylococci (*S. aureus*, *S. epidermidis*)
• diphtheroids (with the exception of *C. diphtheriae*)
• *Haemophilus* spp.
• yeasts (*Candida* spp.) in limited quantity
• various strictly anaerobic Gram-positive cocci and Gram-negative rods, spirochaetes and filamentous forms.

Note:-

The throats of elderly, immunodeficient, or malnourished patients, particularly when they have received antibiotics, may be colonized by:

1- Enterobacteriaceae (*Escherichia coli*, *Klebsiella* spp., etc.)
2- nonfermentative Gram-negative groups (*Acinetobacter* spp. and *Pseudomonas* spp.).
3- Such patients may also have in their pharynx a proliferation of *S. aureus* or of *Candida* spp., or other yeast-like fungi.

Although these microorganisms do not cause pharyngitis, except in association with granulocytopenia, it is advisable to report such isolates to the clinician, as they occasionally indicate the existence of (or may sometimes give rise to) a lower respiratory tract infection (e.g. pneumonia) or bacteraemia. However, an antibiogram should not be performed routinely on these colonizing microorganisms.

Bacterial agents of pharyngitis

1-*Streptococcus pyogenes* (Lancefield group A) is by far the most frequent cause of bacterial pharyngitis and tonsillitis.

2-Non-group-A, b-haemolytic streptococci (e.g. groups B, C and G) are uncommon causes of bacterial pharyngitis and if detected should be reported.Pharyngeal infections due to *S. pyogenes*, if not properly treated, may give rise to sequelae such as rheumatic fever, and, less often, glomerulonephritis. Specific identification of, and antibacterial treatment directed against, *S. pyogenes* are primarily intended to prevent the occurrence of rheumatic fever.

3- *Corynebacterium diphtheriae* causes a typical form of infection, characterized by a greyish-white membrane at the site of infection (pharynx, tonsils, nose, or larynx). Diphtheria is a serious disease and the diagnosis is made on the basis of clinical findings. The physician would then generally make a specific request to culture for diphtheria bacilli.

4-*Neisseria gonorrhoeae* (Gonococcal pharyngitis).Culture of throat swabs for gonococci should be done on specific request from the clinician, using the appropriate selective medium (modified Thayer–Martin medium).

5-Necrotizing ulcerative pharyngitis (Vincent angina) is a rare condition characterized by a necrotic ulceration of the pharynx with or without formation of a pseudomembrane. It is associated, at the site of infection, with a heavy mixed flora of strict anaerobes dominated by Gram-negative fusiform rods and spirochaetes, generally referred to as *Fusobacterium* spp. and *Treponema vincentii*, and possibly others. Although both species belong to the normal mouth flora, their presence in large numbers in a Gram-stained smear of ulcerated lesions should be reported as a "fusospirochaetal complex". This microscopic diagnosis need not be confirmed by anaerobic culture, which is difficult and time-consuming. However, the presence of this complex does not exclude the need to search for other pathogens, particularly *S. pyogenes.*

6-Although small numbers of *C. albicans* or other *Candida* species may be part of the normal oral flora, oral candidiasis results when the number of organisms increases considerably in certain pathological conditions, e.g. in malnourished premature babies, in immunodeficient adults (e.g. patients with HIV/AIDS), or in patients who have received broad-spectrum antimicrobials or cancer therapy. The affected area—tongue, tonsils, throat or buccal mucosa —may be extremely red, or covered with white patches or a confluent grey.

Fig 2:pharyngitis

white membrane (thrush): The diagnosis of candidiasis is best made by:
1- finding numerous yeast cells, some of them forming long mycelium-like filaments, in a Gram-stained smear of the exudate.

2-Swabs from the upper respiratory tract may be submitted to the laboratory, not for the diagnosis of a clinical infection, but to detect a potential pathogen in a healthy subject, a pharyngeal or a nasal "carrier". This should only be done as part of well-defined epidemiological surveys.

The following pathogens can give rise to a carrier state in the upper respiratory tract:
• *Staphylococcus aureus*. Sampling of patients and staff for nasal carriers is sometimes performed as part of an investigation of hospital outbreaks of Meticillin-resistant *S. aureus* (MRSA).

• *Neisseria meningitidis*. Carriage of meningococci may be very prevalent (20% or more) even at non-epidemic times. Identification of pharyngeal carriers of meningococci is rarely needed, and need not be performed prior to the administration of prophylactic antibiotics to family or other close contacts of patients with meningococcal disease.

• *Streptococcus pyogenes*. Carriage of this organism in low numbers may be prevalent, especially among schoolchildren (20–30%).

• *Corynebacterium diphtheriae*. The carrier rate of the diphtheria bacillus is high in non-vaccinated populations. In such communities, it may be justified to identify and treat carriers among the close contacts of a patient with proven diphtheria. Carriers are rare when an immunization programme is correctly implemented.

Collection and dispatch of specimens
1-Ideally, specimens should be collected by a physician or other trained personnel.
2-The patient should sit facing a light source. While the tongue is kept down with a tongue depressor, a sterile cotton-wool swab is rubbed vigorously over each tonsil, over the back wall of the pharynx, and over any other inflamed area.
3-Care should be taken not to touch the tongue or buccal surfaces.
4-It is preferable to take two swabs from the same areas. One can be used to prepare a smear, while the other is placed into a glass or plastic container and sent to the laboratory. Alternatively, both swabs may be

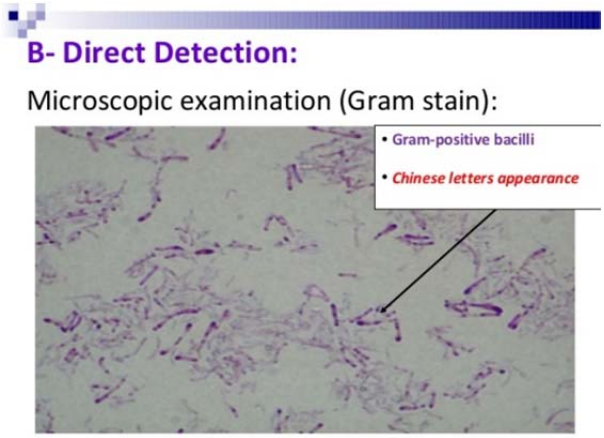
placed in the container and dispatched to the laboratory. If the specimen cannot be processed within 4 hours, the swab should be placed in a transport medium (e.g. Amies or Stuart).

Direct microscopy

1-The fusospirochaetal complex of necrotizing ulcerative pharyngitis (Vincent angina) and *Candida* are best recognized on a Gram-stained smear, which should be prepared if the physician makes a special request.

2-The Gram stained smear is not useful for the detection of streptococci or *Neisseria* spp.

3-The direct smear has poor sensitivity and specificity for the detection of the diphtheria bacillus, unless the specimen has been collected with care and is examined by an experienced microbiologist. In the absence of a physician's request or of clinical information, a Gram-stained smear should not be made for throat swabs.



Culture and identification

Culture for *Streptococcus pyogenes*

1-Immediately upon receipt in the laboratory, the swab should be rubbed over one-quarter of a blood agar plate, and the rest of the plate streaked with a sterile wire loop.

2-The blood agar should be prepared from a basal agar medium without glucose (or with a low glucose content), e.g. tryptic soy agar (TSA). Acidification of glucose by *S. pyogenes* inhibits the production of haemolysin. Blood from any species, even human blood (fresh donor blood), can be used at a concentration of 5%. The plates should be filled to a depth of 4–5 mm. Sheep blood is preferred because it does indicate haemolysis of some commensal *Haemophilus* spp. and it gives no haemolysis with the *zymogenes* variant of *Enterococcus faecalis.*

The recognition of b-haemolytic colonies can be improved, and their presumptive identification hastened, by placing a co-trimoxazole disc (as used for the susceptibility test) and a special low-concentration bacitracin disc over the initial streaked area. Because *S. pyogenes* is resistant and many other bacteria are susceptible to co-trimoxazole, this disc improves the visibility of b-haemolysis. Incubation in a candle-jar will detect most b-haemolytic streptococci. After 18 hours and again after 48 hours of incubation at 35–37^{o}C, the blood plates should be examined for the presence of small (0.5–2 mm) colonies surrounded by a relatively wide zone of clear haemolysis.

After Gram-staining to verify that they are Gram-positive cocci, the colonies should be submitted to specific identification tests for *S. pyogenes.* For clinical purposes, presumptive identification of *S. pyogenes* is based on its susceptibility to a low concentration of bacitracin. For this purpose, a special differential disc is used containing 0.02–0.05 IU of bacitracin. The ordinary discs used in the susceptibility test, with a content of 10 units, are not suitable for identification. A b-haemolytic streptococcus showing any zone of inhibition around the disc should be reported as *S. pyogenes*. If the haemolytic colonies are sufficiently numerous, the presence or absence of an inhibition zone may be read directly from the primary blood agar plate.

. In reporting the presence of *S. pyogenes* in a throat culture, a semiquantitative answer should be given (rare, +, + +, or + + +) . Patients with streptococcal pharyngitis generally show massive growth of *S. pyogenes*, with colonies over the entire surface of the plate. Plates of carriers generally show fewer than 20 colonies per plate. Even rare colonies of b-haemolytic streptococci should be confirmed and reported.

Culture for *Corynebacterium diphtheriae*

Although the diphtheria bacillus grows well on ordinary blood agar, growth is improved by inoculating one or two special media:

• *Löffler coagulated serum or Dorset egg medium.* Although not selective, both of these media give abundant growth of the diphtheria bacillus after overnight incubation. Moreover, the cellular morphology of the bacilli is more "typical": irregularly stained, short to long, slightly curved rods, showing metachromatic granules, and arranged in a V form or in parallel palisades. Metachromatic granules are more apparent after staining with methylene blue or Albert stain than with the Gram stain.

• *A selective tellurite blood agar.* This medium facilitates isolation when the bacilli are few in number, as is the case for healthy carriers. On this medium, colonies of the diphtheria bacillus are greyish to black and are fully developed only after 48 hours. Suspicious colonies, consisting of bacilli with a coryneform morphology on the Gram-stained smear, should be subcultured to a blood agar plate to check for purity and for "typical" morphology. It should also be remembered that colonies of the *mitis* biotype of *C. diphtheriae*, which is the most prevalent, show a marked zone of b-haemolysis on blood agar.

A presumptive report on the presence of *C. diphtheriae* can often be given at this stage. However, this should be confirmed or ruled out by some simple biochemical tests and by demonstration of the toxigenicity. As the latter requires inoculation of guinea-pigs or an in vitro toxigenic *C. diphtheriae* is catalase- and nitrate-positive. Urea is not hydrolysed. Acid without gas is produced from glucose and maltose, generally not from saccharose. The fermentation of glucose can be tested on Kligler medium. Urease activity can be demonstrated on MIU and nitrate reduction in nitrate broth in the same way as for Enterobacteriaceae. For the fermentation of maltose and saccharose, Andrade peptone water can be used as a base with a 1% final concentration of each carbohydrate. Results can usually be read after 24 hours, although it may be necessary to reincubate for one night.

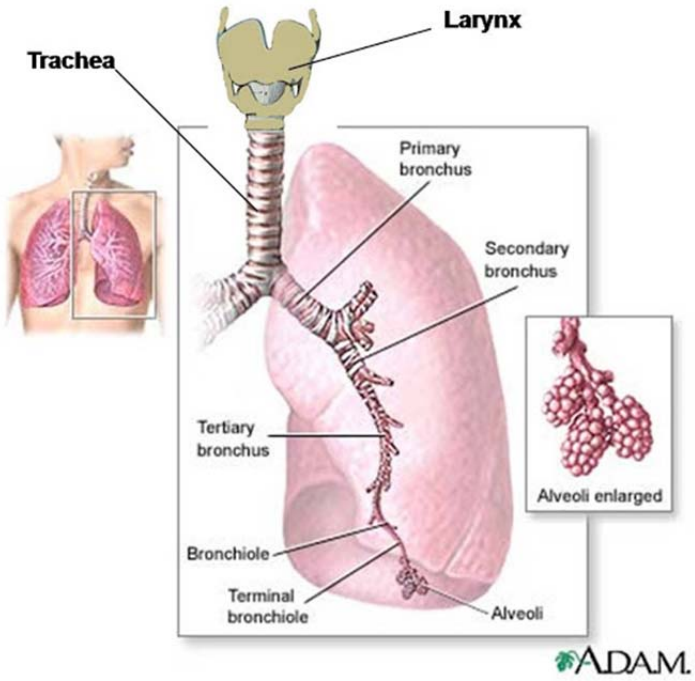
the clinical diagnosis of diphtheria. Therapy should not be with held pending receipt of laboratory reports.

Lec:7&8 **Dr.Enas AL-Layla**

Lower respiratory tract

The lower respiratory tract or lower airway is consists of the trachea, bronchi (primary, secondary and tertiary), bronchioles (includ ing terminal and respiratory), and lungs (including alveoli). It also sometimes includes the larynx.

The lower respiratory tract is also called the **respiratory tree** or **tracheobronchial tree**, to describe the branching structure of airways supplying air to the lungs, and includes the trachea, bronchi and bronchioles.



Normal flora of the lung:-

Investigation of the lung microbiome is a relatively new field, and may lead to new ways of thinking about respiratory disease . The lungs of healthy humans were previously believed to be sterile, based on results of classical, culture-based studies . In fact, the National Institutes of Health's initial Human Microbiome Project did not include the lung as a site of investigation. However, recent culture-independent methods demonstrate that the lungs of healthy never-smokers are inhabited by communities of bacteria that are very few in number but composed of diverse types of bacteria .

The **lung microbiota**, is the pulmonary microbial community consisting of a complex variety of microorganisms found in the lower respiratory tract particularly on the mucous layer and the epithelial surfaces. These microorganisms include bacteria, fungi, viruses and bacteriophages. The bacterial part of the microbiota has been more closely studied. It consists of a core of nine genera:
Prevotella, *Sphingomonas*, *Pseudomonas*, *Acinetobacter*, *Fusobacterium*, *Megasphaera*, *Veillonella*, *Staphylococcus*, and *Streptococcus*.

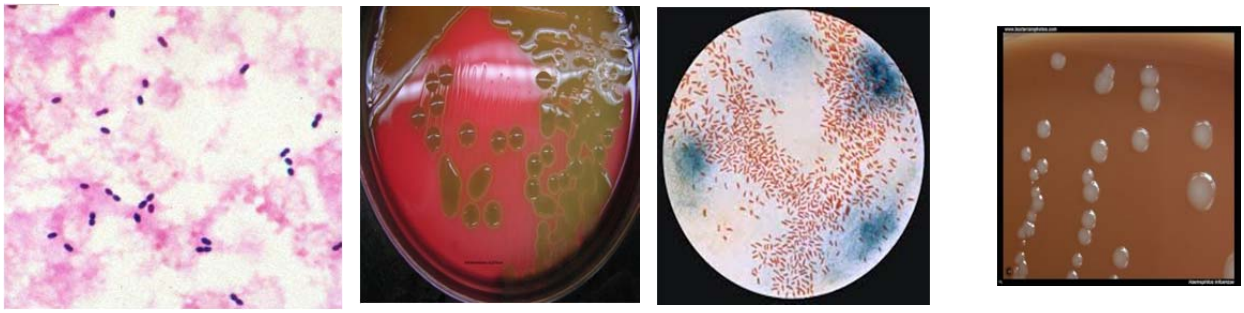
Lower Respiratory Infections

Infections of the lower respiratory tract include bronchitis, bronchiolitis and pneumonia. These syndromes, especially pneumonia, can be severe or fatal. Although viruses, mycoplasma, rickettsiae and fungi can all cause lower respiratory tract infections, bacteria are the dominant pathogens; accounting for a much higher percentage of lower than of upper respiratory tract infections.

1-Bronchitis and Bronchiolitis

Etiology

Bronchitis and bronchiolitis involve inflammation of the bronchial tree. Bronchitis is usually preceded by an upper respiratory tract infection or forms part of a clinical syndrome in diseases such as influenza, rubella, pertussis, scarlet fever and typhoid fever. Chronic bronchitis with a persistent cough and sputum production appears to be caused by a combination of environmental factors, such as smoking, and bacterial infection with pathogens such as *H. influenzae* and *S. pneumoniae*. Bronchiolitis is a viral respiratory disease of infants and is caused primarily by respiratory syncytial virus. Other viruses, including parainfluenza viruses, influenza viruses and adenoviruses (as well as occasionally *M. pneumoniae*) are also known to cause bronchiolitis.



From the left:*Str.pneumoniae* gram stain,it's on blood agar,Haemophilus influenzae gram stain,it's on chocolate agar

Clinical Manifestations

Symptoms of an upper respiratory tract infection with a cough is the typical initial presentation in acute bronchitis. Mucopurulent sputum may be present, and moderate temperature elevations occur. Typical findings in chronic bronchitis are an incessant cough and production of large amounts of sputum, particularly in the morning. Development of respiratory infections can lead to acute exacerbations of symptoms with possibly severe respiratory distress.

Coryza and cough usually precede the onset of bronchiolitis. Fever is common. A deepening cough, increased respiratory rate, and restlessness follow. Retractions of the chest wall, nasal flaring, and grunting are prominent findings. Wheezing or an actual lack of breath sounds may be

noted. Respiratory failure and death may result.

Microbiologic Diagnosis

Bacteriologic examination and culture of purulent respiratory secretions should always be performed for cases of acute bronchitis not associated with a common cold. Patients with chronic bronchitis should have their sputum cultured for bacteria initially and during exacerbations. Aspirations of nasopharyngeal secretions or swabs are sufficient to obtain specimens for viral culture in infants with bronchiolitis. Serologic tests demonstrating a rise in antibody titer to specific viruses can also be performed. Rapid diagnostic tests for antibody or viral antigens may be performed on nasopharyngeal secretions by using fluorescent-antibody staining, ELISA or DNA probe procedures.

2-Pneumonia

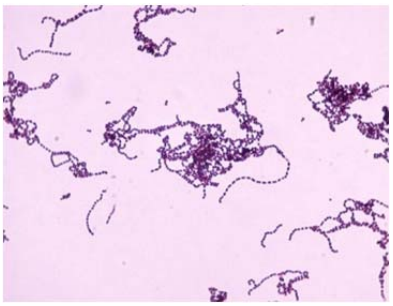
Pneumonia is an inflammation of the lung parenchyma Consolidation of the lung tissue may be identified by physical examination and chest x-ray.

Etiology

Bacterial pneumonias

2-*Streptococcus pneumoniae* is the most common agent of community-acquired acute bacterial pneumonia. More than 80 serotypes, as determined by capsular polysaccharides, are known, but 23 serotypes account for over 90% of all pneumococcal pneumonias in the United States. Pneumonias caused by other streptococci are uncommon.

2-*Streptococcus pyogenes* pneumonia is often associated with a hemorrhagic pneumonitis and empyema.




From the left:*Str.pyogenes* gram stain,it's on blood agar

3-Community-acquired pneumonias caused by *Staphylococcus aureus* are also uncommon and usually occur after influenza or from staphylococcal bacteremia.

4-Infections due to *Haemophilus influenzae* (usually nontypable)

5-*Klebsiella pneumoniae* are more common among patients over 50 years old who have chronic obstructive lung disease or alcoholism.

6-The most common agents of nosocomial pneumonias are aerobic gram-negative bacilli that rarely cause pneumonia in healthy individuals. *Pseudomonas aeruginosa, Escherichia coli, Enterobacter, Proteus*, and *Klebsiella* species are often identified.

7-Mycobacterium tuberculosis can cause pneumonia. Although the incidence of tuberculosis is low in industrialized countries, *M tuberculosis* infections still continue to be a significant public health problem in the United States, particularly among immigrants from developing countries, intravenous drug abusers, patients infected with human immunodeficiency virus (HIV), and the institutionalized elderly. Atypical *Mycobacterium* species can cause lung disease indistinguishable from tuberculosis.

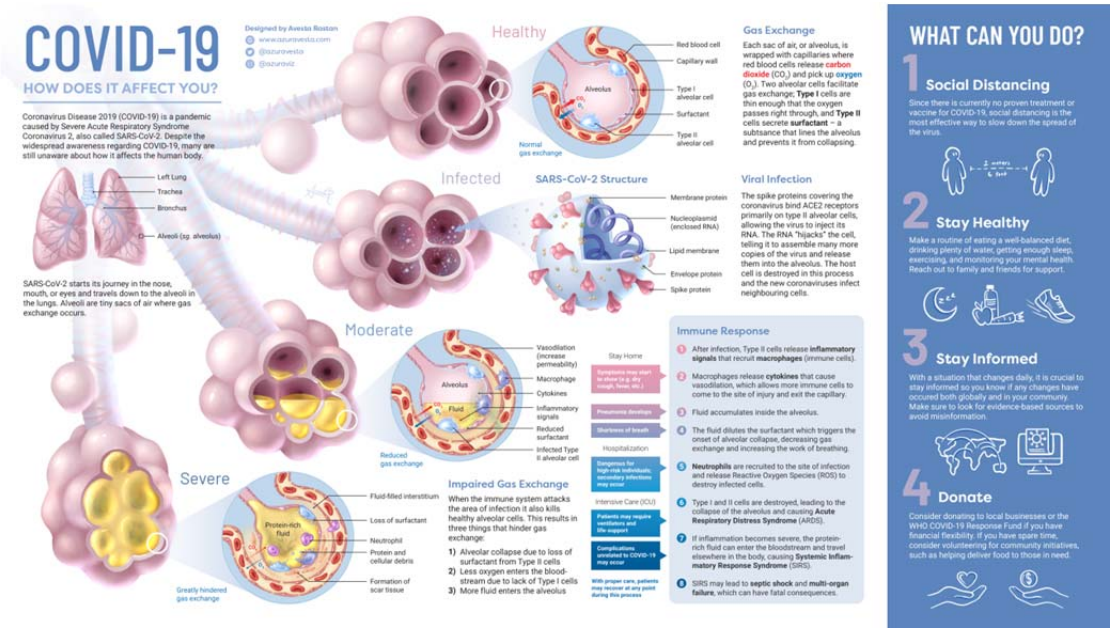
8-*Chlamydia* spp noted to cause pneumonitis are *C. trachomatis, C .psittaci* and *C. pneumoniae*. *Chlamydia trachomatis* causes pneumonia in neonates and young infants. *C. psittaci* is a known cause for occupational pneumonitis in bird handlers such as turkey farmers. *Chlamydia pneumoniae* has been associated with outbreaks of pneumonia in military recruits and on college campuses.

9-Viral pneumonias are rare in healthy civilian adults. An exception is the viral pneumonia caused by influenza viruses,SARS-CoV-2 which can

have a high mortality in the elderly and in patients with underlying disease..

Pathogenesis and Clinical Manifestations

Infectious agents gain access to the lower respiratory tract by the inhalation of aerosolized material, by aspiration of upper airway flora, or by hematogenous seeding. Pneumonia occurs when lung defense mechanisms are diminished or overwhelmed. The major symptoms or pneumonia are cough, chest pain, fever, shortness of breath and sputum production. Patients are tachycardic. Headache, confusion, abdominal pain, nausea, vomiting and diarrhea may be present, depending on the age of the patient and the organisms involved.



Clinical Presentation of COVID-19

In general, common cold CoVs tend to cause mild URT symptoms and occasional gastrointestinal involvement. By contrast, infection with highly pathogenic CoVs, including SARS-CoV-2, causes severe 'flu'-like symptoms that can progress to acute respiratory distress (ARDS), pneumonia, renal failure, and death. The most common symptoms are fever, cough, and dyspnea. The incubation period in COVID-19 is rapid: ~5–6 days versus 2–11 days in SARS-CoV infections. As the pandemic is progressing, it has become increasingly clear that COVID-19 encompasses not only rapid respiratory/ gastrointestinal illnesses, but can also have long-term ramifications, such as myocardial inflammation. Furthermore, severe COVID-19 is not restricted to the aged population as initially reported; children and young adults are also at risk. From a

diagnostic perspective, COVID-19 presents with certain hallmark (CRP,D-Dimer,Ferittin test) laboratory and radiological indices, which can be helpful in assessing disease progression.

Microbiologic Diagnosis

Etiologic diagnosis of pneumonia on clinical grounds alone is almost impossible. Sputum should be examined for a predominant organism in any patient suspected to have a bacterial pneumonia; blood and pleural fluid (if present) should be cultured. A sputum specimen with fewer than 10 white cells per high-power field under a microscope is considered to be contaminated with oral secretions and is unsatisfactory for diagnosis. Acid-fast stains and cultures are used to identify *Mycobacterium* and *Nocardia* spp. Most fungal pneumonias are diagnosed on the basis of culture of sputum or lung tissue. Viral infection may be diagnosed by demonstration of antigen in secretions or cultures or by an antibody response. Serologic studies can be used to identify viruses, *M. pneumoniae, C. burnetii, Chlamydia species, Legionella, Francisella*, and *Yersinia*. A rise in serum cold agglutinins may be associated with *M. pneumoniae* infection, but the test is positive in only about 60% of patients with this pathogen.

Rapid diagnostic tests, are available to identify respiratory viruses: the fluorescent-antibody test is used for *Legionella*. A sputum quellung test can specify *S. pneumoniae* by serotype. Enzyme-linked immunoassay, DNA probe and polymerase chain reaction methods are available for many agents causing respiratory infections.

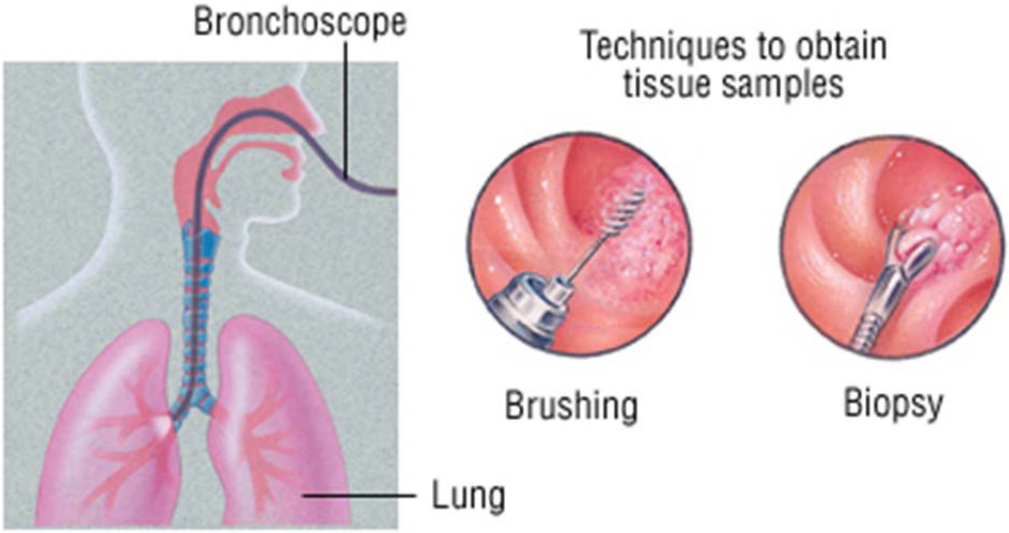
Some organisms that may colonize the respiratory tract are considered to be pathogens only when they are shown to be invading the parenchyma. Diagnosis of pneumonia due to cytomegalovirus, herpes simplex virus, *Aspergillus* spp. or *Candida* spp require specimens obtained by transbronchial or open-lung biopsy. *Pneumocystis carinii* can be found by silver stain of expectorated sputum. However, if the sputum is negative, deeper specimens from the lower respiratory tract obtained by bronchoscopy or by lung biopsy are needed for confirmatory diagnosis.

Bronchoscopy.

The diagnosis of pneumonia, particularly in HIV- infected and other immunocompromised patients, often necessitates the use of more invasive procedures. Fiberoptic bronchoscopy has dramatically affected the evaluation and management of these infections. With this method, the bronchial mucosa can be directly visualized and collected for biopsy, and the lung tissue can be sent for transbronchial biopsy to evaluate lung

cancer and other lung diseases. Although transbronchial biopsy is important, the procedure is often associated with significant complications such as bleeding.

During bronchoscopy, physicians can obtain bronchial washings or aspirates, bronchoalveolar lavage (BAL) samples, protected bronchial brush samples, or specimens for transbronchial biopsy. Bronchial washings or aspirates are obtained by instilling a small amount of sterile physiologic saline into the bronchial tree and withdrawing the fluid when purulent secretions are not visualized. Such specimens will still be contaminated with upper respiratory tract flora such as viridans streptococci and Neisseria spp. Recovery of potentially pathogenic organisms from bronchial washings should be attempted, because such specimens may be more diagnostically relevant than sputa.



Microscopic Examination of Urinary Sediment

The examination of urinary sediment is an important component of routine urinalysis, which aids in the detection and evaluation of renal and urinary tract disorders as well as other systemic diseases. This microscopic procedure is used to confirm chemical findings. The microscopic examination should be performed on a centrifuged sample.

CELLS

ERYTHROCYTES: RBCs in the urine may have originated in any part of the urinary tract. They can appear in a variety of forms depending upon the environment of the urine. When the urine specimen is fresh, the red cells have a normal, pale, or yellowish appearance and are smooth, biconcave disks (Fig. 1).

Normally, RBCs do not appear in the urine, although the presence of 1–2 RBC/HPF is usually not considered abnormal.

Injury or rupture of the blood vessels of the kidney or urinary tract releases RBCs into the urine.

Hematuria is the presence of an increased number of RBCs in the urine. In addition, the protein test will be positive if large amounts of blood are present.

LEUKOCYTES: WBCs can enter the urinary tract anywhere from the glomerulus to the urethra. Normal urine can contain 2 to 8 WBCs/HPF. WBCs are larger than RBCs.

WBC are usually spherical and can appear gray in color, and may appear singly or in clumps (Fig. 2). The WBCs that are seen in urine are mostly neutrophils, which can be identified by their characteristic granules and nuclear lobulations (Fig. 3).

Sometimes **pyuria** (pus in the urine) is seen in conditions such as appendicitis and pancreatitis. Pyuria is also found in noninfectious conditions such as acute glomerulonephritis, dehydration, stress, fever, and in noninfectious irritation to the ureter, bladder, or urethra. The presence of many WBCs in the urine, especially when they are in clumps, is strongly suggestive of acute infection such as cystitis, or urethritis.

EPITHELIAL CELLS: The epithelial cells in the urine may originate from any site in the genitourinary tract. Normally, a few cells from these sites can be found in the urine as a

result of the normal sloughing off of old epithelial cells. A marked increase indicates inflammation of that portion of the urinary tract from which the cells are derived.

There are three main types of epithelial cells:

1) **Renal tubular epithelial cells** are slightly larger than leukocytes and contain a large round nucleus. Fig. (4)
2) **Transitional epithelial cells** are two to four times as large as white cells Fig. (4). They may be round, pear-shaped, or may have taillike projections. Occasionally, these cells may contain two nuclei.
3) **Squamous epithelial cells** are easily recognized as large, flat, irregularly shaped cells. They contain small central nuclei and abundant cytoplasm (Fig. 5). The cell edge is often folded over and the cell may be rolled up into a cylinder.

Squamous epithelial cells have little diagnostic significance.

CRYSTALS

Crystals are compounds, its formation occurs in the kidney or urinary tract and can result in the formation of urinary calculi (stones).

Microscopic evaluation of urine is important for detection of crystals, because no chemical test detects the presence of crystals.

ACIDIC URINE

- **Uric acid:** crystals can occur in many different shapes, but the most characteristic forms are the diamond or rhombic prism (Fig. 6) and the rosette (fig. 7), Uric acid crystals are usually stained with urinary pigments and are, therefore, yellow or red–brown in color.

The presence of uric acid crystals in the urine does not necessarily indicate a pathologic condition, nor does it mean that the uric acid content of the urine is definitely increased. Pathologic conditions in which uric acid crystals are found in the urine include gout.

- **Calcium oxalate** crystals are colorless octahedral or “envelope”- shaped crystals which look like small squares crossed by intersecting diagonal lines (Fig. 8). Rarely, Calcium oxalate crystals can be present:

1- normally in the urine especially after the ingestion of oxalate-rich food such as tomatoes, spinach, garlic, and oranges. Increased amounts of calcium oxalates, suggest the possibility of oxalate calculi.

2- pathologic conditions in which calcium oxalates can be present in increased numbers include ethylene glycol poisoning, diabetes mellitus, liver disease, and severe chronic renal disease.

3- following the intake of large doses of vitamin C. Oxalic acid is one of the breakdown products of ascorbic acid, and oxalic acid precipitates ionized calcium.

- **Amorphous Urates:** Urate salts of sodium, potassium, magnesium, and calcium are frequently present in the urine in a noncrystalline, amorphous form. These amorphous urates have a yellow–red granular appearance (Fig. 9). they have no clinical significance.

- **Cystine** crystals are colorless, refractile, hexagonal plates with equal or unequal sides (Fig. 10). The presence of cystine crystals in the urine is always important, also Cystine crystals can form calculi.

- **Tyrosine** crystals are very fine, highly retractile needles occurring in sheaves (Fig.11). Tyrosine crystals can be seen in tyrosinosis.

- **Cholesterol** crystals are large, flat, transparent plates (Fig. 12).presence of cholesterol plates in the urine indicates excessive tissue breakdown, and they are seen in nephritis.

ALKALINE URINE

- **Triple phosphate** (ammonium magnesium phosphate) crystals can be present in neutral and alkaline urines. they are colorless prisms with from three to six sides that frequently have oblique ends (Fig. 13). They are frequently found in normal urine but can also form urinary calculi. Pathologic conditions in which they may be found include chronic pyelitis, chronic cystitis, enlarged prostate, and when the urine is retained in the bladder.

- **Amorphous Phosphates:** are granular particles have no definite shape and they are usually visibly indistinguishable from amorphous urates. The pH of the urine helps distinguish them and have no clinical significance.

- **Calcium phosphate** crystals are long, thin, colorless prisms and can have one pointed end, be arranged as rosettes or stars, or appear as needles (Fig 14). These crystals may be present in normal urine, but they may also form calculi.

- **Ammonium biurate** (ammonium urates). they may occasionally be found in acidic urine. Ammonium biurates are yellow–brown spherical bodies with long, irregular spicules (Fig.15). Their appearance is often described with the term "thorn apple." they may also occur as yellow–brown spheroids without spicules (Fig. 16). Ammonium biurates are abnormal only if found in freshly voided urine.

CASTS

Urinary casts are formed in the lumen of the tubules of the kidney. They are so named because they are molded in the tubules. The renal tubules secrete a mucoprotein called Tamm–Horsfall protein which is believed to form the basic matrix of all casts.

The presence of casts in the urine is frequently accompanied by proteinuria. However, casts can be seen in the absence of protein, making microscopic examination of urine an important tool in the diction of casts. Casts are always renal in origin.

1- **Hyaline casts :** the most frequently occurring casts in the urine. They are composed of only protein, They are colorless, transparent, and usually have rounded ends (Fig. 17). Hyaline casts can be seen in even the mildest kind of renal disease. A few hyaline casts may be found in the normal urine, and increased amounts are frequently present following physical exercise and physiologic dehydration.

2- **Red blood cell casts** mean renal hematuria and they are always pathologic. They are diagnostic of glomerular disease, and subacute bacterial endocarditis, also can be present in right-sided congestive heart failure, and renal vein thrombosis. RBCs casts may appear brown to almost colorless (Fig. 18). The cast may contain only a few RBCs in a protein matrix, or there may be many cells packed close together with no visible matrix.

3- **White blood cell casts** are present in renal infection and in noninfectious inflammation. The majority of white cells that appear in casts are polymorphonuclear neutrophils. The WBCs in the cast may be few in number, or there may be many cells tightly packed together (Fig. 19).

4- **Granular casts** (fig. 20) may be the result of the degeneration of cellular casts or they may represent the direct aggregation of serum proteins into a matrix of Tamm–Horsfall mucoprotein. Granular casts almost always indicate significant renal disease; however, granular casts may be present in the urine for a short time following strenuous exercise.

5- **Epithelial cell casts** may be present in urine after exposure to nephrotoxic agents or viruses (e.g., CMV, hepatitis virus), which cause damage that accompanies glomerular injury.

The epithelial cells may either be arranged in parallel rows in the cast or may be arranged haphazardly and vary in size, shape, and stage of degeneration (Fig. 21).

6- **Waxy casts** are yellow, gray, or colorless, and have a smooth homogeneous appearance (Figs. 22). They frequently occur as short broad casts with blunt or broken ends, and they often have cracked or serrated edges. Waxy casts are found in patients with severe chronic renal failure. They may also be found in acute renal disease.

7- **Fatty casts**: have incorporated either free fat droplets or oval fat bodies Fig. (23). Fatty casts are seen when there is fatty degeneration of the tubular epithelium, as in degenerative tubular disease. They are frequently seen in the nephrotic syndrome and toxic renal poisoning.

BACTERIA

The urine is normally free of bacteria while in the kidney and bladder, but contamination may occur from bacteria present in the urethra or vagina, or from other external sources. When a properly collected, freshly voided specimen contains large numbers of bacteria, especially when accompanied by many white cells, it is usually indicative of a UTI.

YEAST

Yeast cells are smooth, colorless, usually ovoid cells with doubly refractile walls. They can vary in size and often show budding (Fig. 24). They may sometimes be mistaken for red cells. Yeast may be found in UTIs, especially in patients with diabetes. also be present in the urine as a result of skin or vaginal contamination. *Candida albicans* is the most common yeast to appear in the urine.

Mucous threads

Mucous threads are long, thin, wavy threads of ribbonlike structures which may show faint longitudinal striations (Fig. 25). Mucous threads are present in normal urine in small numbers, but they may be very abundant in the presence of inflammation or irritation of the urinary tract. Some of the wider threads may be confused with cylindroids or hyaline casts.

CONTAMINANTS

A variety of foreign objects can find their way into the urine specimen during collection, transportation, while being tested, or while on the microscope slide.

1- **Starch crystals** are frequently found in the urine. They are round or oval, are highly refractive, and vary in size. Cornstarch crystals are almost hexagonal in shape, and they contain an irregular indentation in the center (Fig. 26).

2- **Cloth fibers** may come from clothing, diapers, toilet paper. Fibers which are long and flat are easily recognizable (Fig. 27). However, fibers those are short and are approximately the same size as casts can be mistaken for casts.

PARASITES

Parasites may occasionally be found in the urine, either because they are indigenous to the urinary tract or as the result of vaginal or fecal contamination. Chemical analysis does not detect parasites in urine. Microscopic evaluation of urinary sediment is important if parasitic infections are suspected.

1- ***Trichomonas vaginalis*** is flagellate organism that is about the same size as a large white cell (Fig. 28). In the unstained wet mount, the organism should not be reported unless it is motile. Sometimes when bacteria are next to a white cell, the cell may be mistaken for Trichomonas, which is why motility is the diagnostic feature. This organism may be found in males, although it is more common in females. *T. vaginalis* is frequently accompanied by WBCs and epithelial cells.

2- ***Enterobius vermicularis*** **(pinworm):** ova and occasionally also the female adult may be found in the urine. The ova are very characteristic in shape, having one flat and one rounded side (Fig. 29). If the urine is found to contain many ova, examination of the original urine container may reveal the adult worm.

3- ***Schistosoma haematobium*** is a blood fluke that inhabits the veins in the wall of the urinary bladder. The adult deposits eggs in the capillaries of the mucosa. Abscesses develop around the eggs, and the eggs can be found in the urine accompanied by RBCs and WBCs. This type of schistosomiasis is endemic in Africa, especially around the Nile Valley, in the Middle East, and around the Mediterranean. The *S. haematobium* ovum has a characteristic terminal spine and measures about 50 microns by 150 microns (Fig. 30).

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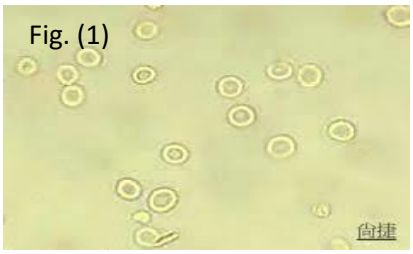

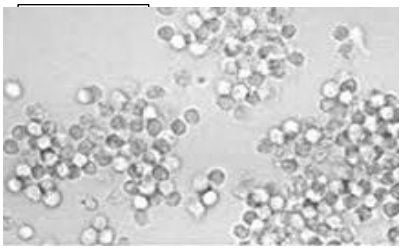

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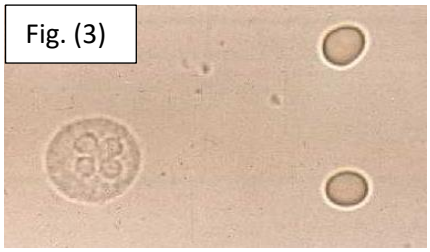

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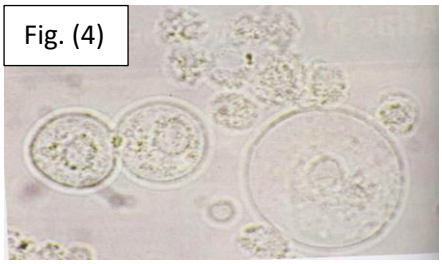

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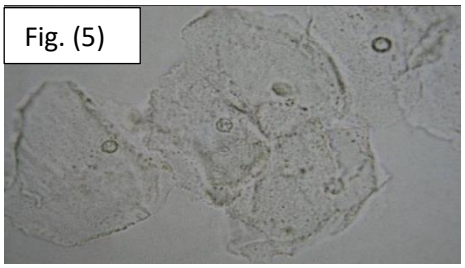

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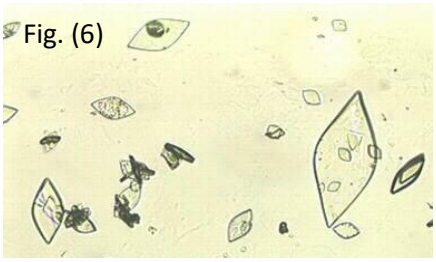

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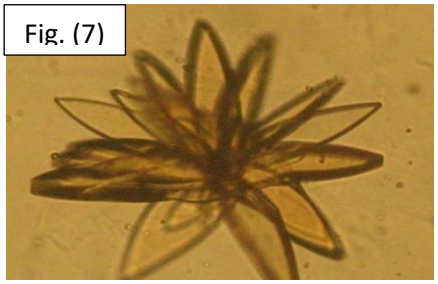

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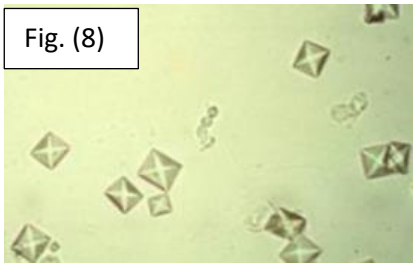

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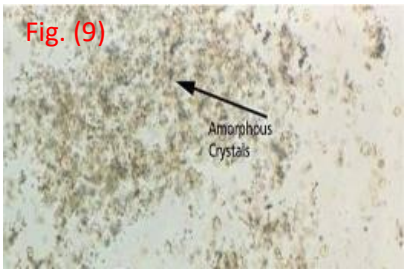

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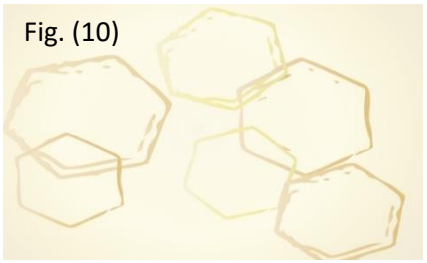

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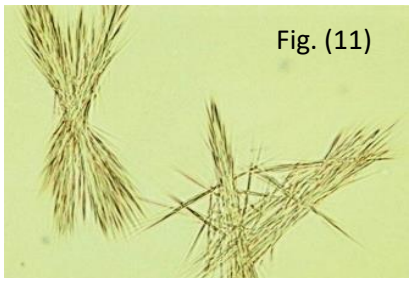

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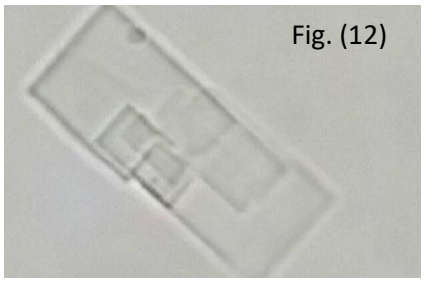

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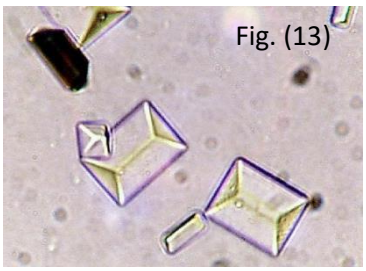

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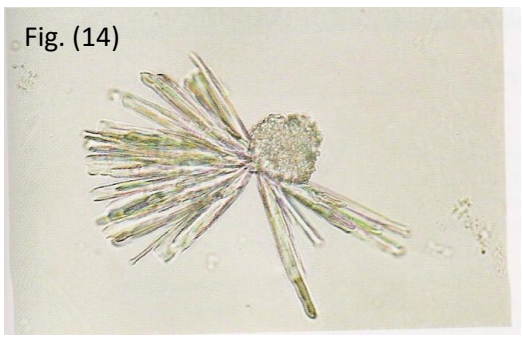

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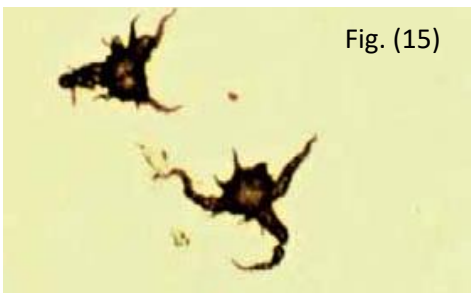

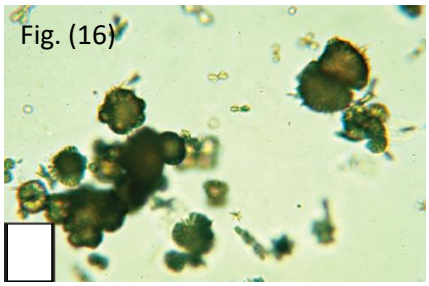
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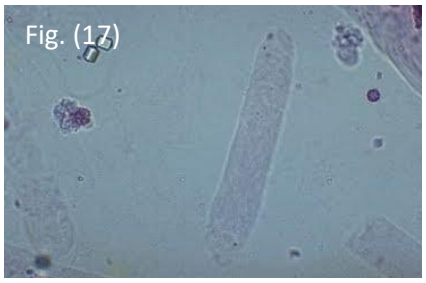
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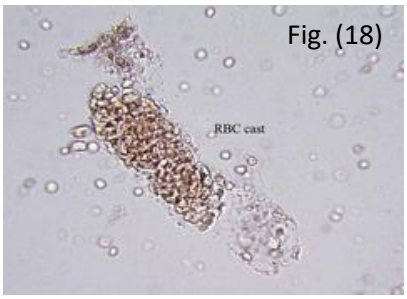

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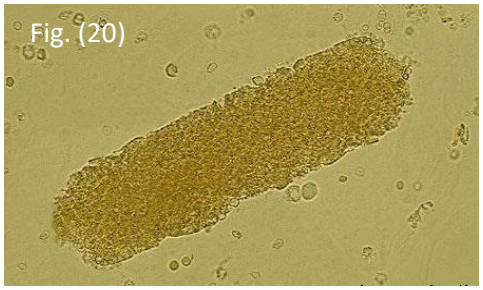
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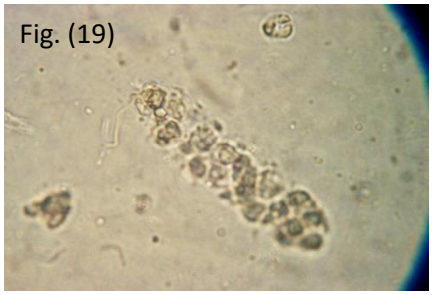
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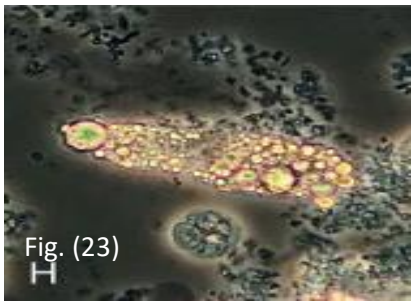
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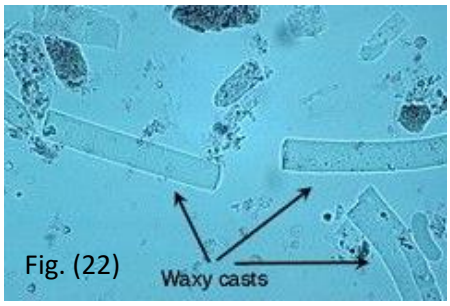

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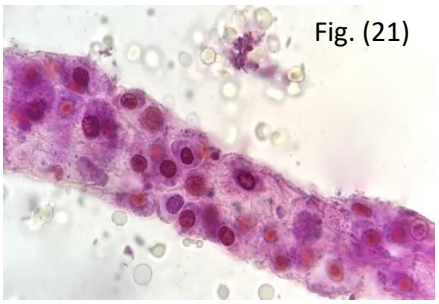
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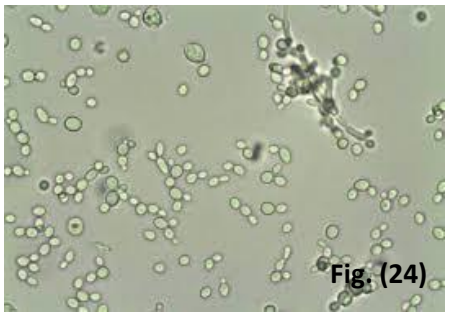


Fig. (24)

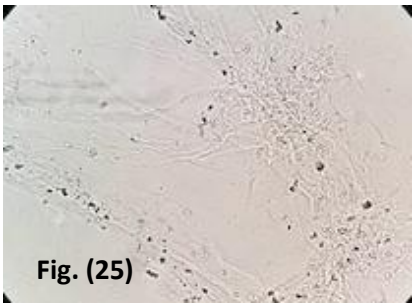
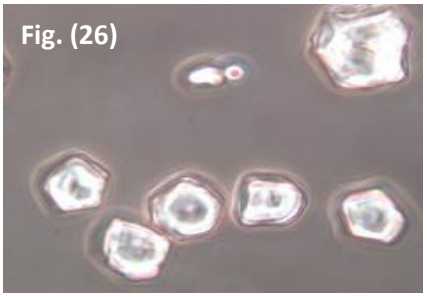


Fig. (25)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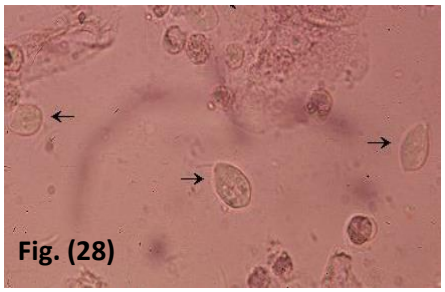


Fig. (28)


Fig. (29)

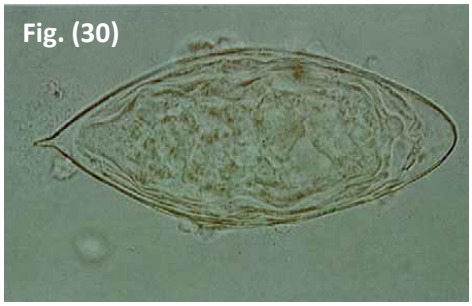


Fig. (30)

Bacterial Analysis

Lec:11,12 **Dr.Enas AL-Layla**

Bacterial Culture

In nature, bacteria exist as mixed populations. In the laboratory these populations must be separated so that characteristics of individual species may be observed. A number of basictechniques are used in microbiology with this end in mind.

First, microorganisms must be removed from natural environments and cultured in the laboratory. This requires artificial media and surfaces on which bacteria may grow. This also requires knowledge of nutritional requirements and environmental requirements (such as temperature of incubation and the requirement of oxygen).

Second, bacteria of interest must be separated from all other bacteria in the environmental sample. This requires separation techniques that allow isolation of a pure culture of one type of bacteria.

Third, once a pure culture is achieved, no contaminating bacteria can be introduced from the environment. This requires that all media and lab supplies be sterile (that is contain no bacteria that may contaminate the culture of interest).

Fourth, techniques are needed that facilitate working with pure cultures. This requires aseptic technique and techniques of storage for pure cultures.

bacteria culture test

Bacteria are a large group of one-celled organisms. They can live on different places in the body. Some types of bacteria are harmless or even beneficial. Others can cause infections and disease. A bacteria culture test can help find harmful bacteria in your body. During a bacteria culture test, a sample will be taken from your blood, urine, skin, or other part of your body. The type of sample depends on the location of the suspected infection. The cells in your sample will be taken to a lab and put in a special environment in a lab to encourage cell growth. Results are often available within a few days. But some types of bacteria grow slowly, and it may take several days or longer.

The importance of bacteria culture test

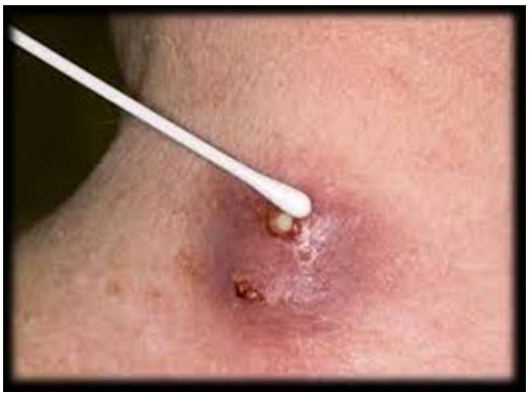
Your health care provider may order a bacteria culture test if you have symptoms of a bacterial infection. The symptoms vary depending on the type of infection.

1-Urine Culture

- Used to diagnose a urinary tract infection and identify the bacteria causing the infection
- Test procedure:
- You will provide a sterile sample of urine in a cup, as instructed by your health care provider.
- Culture media is MacConkey Agar,Blood Agar

2-Wound Culture

- Used to detect infections on open wounds or on burn injuries
- Test procedure:
- Your health care provider will use a special swab to collect a sample from the site of your wound.
- Culture media is MacConkey Agar,Blood Agar and Mannitol salt agarMSA.



3-Skin culture:-

A skin culture is used to test for germs that affect your skin, fingernails, or toenails. It can be called a mucosal culture if it involves your mucosa membranes. These are the moist linings inside certain areas of your body, such as your mouth and nose.

Some conditions that may require a skin culture include:

- rash that seems to be infected
- open sore that isn't healing correctly
- fungal infection
- impetigo, a common skin infection that's caused by strep or staph bacteria
- athlete's foot

How a Skin Culture Is Performed

A skin culture is a quick, simple process that can be done in a hospital or your doctor's office.

1-Take a cotton swab and gently run it over the affected area. If you have an abscess or blister, your doctor may decide to lance, or cut, it. This will allow them to gather a sample of the pus or fluid inside.

2-If doctor wants a sample of fingernails or toenails, they may simply snip off a portion of nail. This is done in the same way that trim nails at home.

3- Samples will be packaged and sent to a laboratory, where the majority of the work is done. There, technicians will run tests to learn if any bacteria, fungi, or virus may be causing symptoms. These tests may take longer if they involve samples of nails.

3-Usually, the test results are accurate enough to determine the specific strand of virus, fungi, or bacteria causing your symptoms. This will help your doctor choose the best course of treatment.

4- Culture media is MacConkey Agar,Blood Agar ,Mannitol salt agar(MSA) and Saborad dextrose agar (SDA).

4-Ear culture:-

Why the Test is Performed
The test may be done if you or your child has:

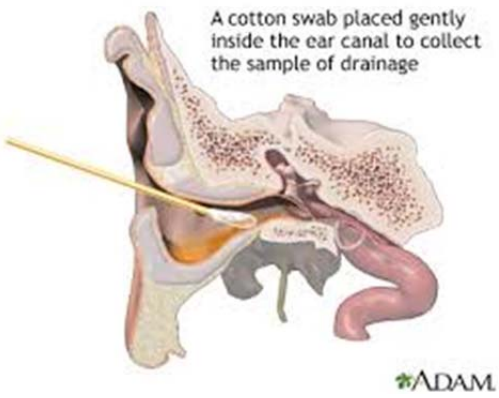
- An ear infection that is not getting better with treatment
- An infection of the outer ear (otitis externa)
- An ear infection with a ruptured eardrum and draining fluid

1-A sample of ear drainage is needed. Your health care provider will use a cotton swab to collect the sample from inside the outer ear canal. In some cases, a sample is collected from the middle ear during ear surgery.

2-The sample is sent to a lab and placed on a special dish (culture media).

3-The lab team checks the dish every day to see if bacteria, fungi, or viruses have grown. More tests may be done to look for specific germs and determine the best treatment.

4- Culture media is MacConkey Agar,Blood Agar ,Mannitol salt agar(MSA) and Saborad dextrose agar (SDA).



5-Eye swab culture

Onsite collections: Conjunctiva:

1. Swab: Pass moistened swab two times over lower conjunctiva. Avoid eyelid border and lashes. Place in swab transport medium.

2. Contact Microbiology for media for direct inoculation.

3. Scrapings: Instill one or two drops of topical anesthetic and scrape the lower tarsal conjunctiva. Inoculate plates directly (BAP, CHOC).

Corneal scraping:

1. Procedure performed by ophthalmologist.

2. Contact Microbiology for media for direct inoculation.

3. Direct culture inoculation: BHI with 10% sheep blood, Chocolate agar and MSA.

Why do I have to wait so long for my results?

Your test sample doesn't contain enough cells for your health care provider to detect an infection. So your sample will be sent to a lab to allow the cells to grow. If there is an infection, the infected cells will multiply. Most disease-causing bacteria will grow enough to be seen within one to two days, but it can take some organisms five days or longer.

Imprtant Note:-

If your results show you don't have a bacterial infection, you **should not** take antibiotics. Antibiotics only treat bacterial infections. Taking antibiotics when you don't need them won't help you feel better and may lead a serious problem known as antibiotic resistance. Antibiotic resistance allows harmful bacteria to change in a way makes antibiotics less effective or not effective at all. This can be dangerous to you and to the community at large, as this bacteria can be spread to others.

Organisms can be grown in liquid media (broth) or on solid media. For example, Nutrient media is referred to as *Nutrient Broth* when in the liquid form, and *Nutrient Agar* when in the solid form. Agar, a galactan obtained from marine algae is used as the hardening agent in solid media. Broth, with added agar powder, is heated to 121oC in an autoclave, dissolving the agar and sterilizing the medium. The molten medium may be poured into plates or tubes. The agar-media will remain liquid at temperatures above 45oC. Below 45oC the agar will harden, and supply a solid surface for the growth of bacteria. Agar plates and slants may be inoculated after they have solidified. To avoid any condensation-droplets

from falling onto agar surfaces and smearing the inoculum, agar plates are inverted during incubation.

Precisely the opposite problem arises with anaerobes: all 02must be excluded. This is accomplished in several ways.
(1) Anaerobic media containing reducing agents such as thioglycollate or cysteine may be used. The medium is boiled during preparation to dissolve its components and drive off oxygen. The reducing agents eliminate any residual dissolved 02 in the medium so that anaerobes can grow beneath its surface.
(2) Oxygen also may be eliminated from an enclosed work area, often called an anaerobic chamber or anaerobic workstation. Most of the air is removed with a vacuum pump followed by purges with nitrogen gas . gas mix containing hydrogen is then introduced into the workstation. In the presence of a palladium catalyst, the hydrogen and last remaining molecules of 02 react to form water, creating an anoxic environment. Often C02 is added to the chamber because many anaerobes require a small amount of C02 for best growth.
(3) One of the most popular ways of culturing small numbers of anaerobes is by use of a GasPak jar, which Catalyst chamber Contains palladium pellets Gas generator envelope Water is added to chemicals Lid in envelope to generate H2 and C02. Carbon dioxide promotes more rapid growth of microorganisms.
out of the chamber
(4) A similar approach uses plastic bags or pouches containing calcium carbonate and a catalyst, which produce an anoxic, carbon dioxide-rich atmosphere.