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Dept. of *Medical Lab. Technique*

Advance Lab Techniques

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Lec:1

Laboratory Safety

Microbiology laboratory safety practices were first published in 1913. They included admonitions such as the necessity to :-

(1) wear gloves.

(2) wash hands after working with infectious materials,

(3) disinfect all instruments immediately after use.

(4) disinfect all contaminated waste before discarding,

(5) report to appropriate personnel all accidents or exposures to infectious agents.

Sterilization, Disinfection, and Decontamination

Sterilization" is a process in which all living microorganisms, including spore, are destroyed. The organisms may be killed with steam, dry heat, or incineration. If we say an article is sterile, we understand that it is completely free of all living microorganisms

Disinfection" is a process in which vegetative, non sporing microorganisms are destroyed. Agents that cause disinfection are called disinfectants or germicides. Such agents are used only on inanimate objects because they are toxic to human and animal tissues.

There are Many factors limit the success or degree of sterilization, disinfection, or decontamination in a health care setting, such as:-the type of organisms present, the

concentration and exposure time to the germicide, the physical and chemical nature of the surface rough or smooth surfaces), temperature, pH, humidity, and presence of a biofilm.

Methods of Sterilization

The physical methods of sterilization include:

- Incineration
- Moist heat
- Dry heat
- Filtration
- Ionizing (gamma) radiation
- **Chemicals** (ethylene oxide gas, hydrogen peroxide gas

and other liquid chemicals, Alcohols, Aldehydes, Halogens , Per acetic acid, Phenolic).

- **Ethyl or isopropyl alcohol** is nonsporicidal (does not kill endospores) and evaporates quickly. Therefore its use is limited to the skin as an

- Stabilized **hydrogen peroxide** has demonstrated bactericidal, virucidal, sporicidal, and fungicidal activities. Commercially available 3% hydrogen peroxide has been used as a disinfectant on inanimate surfaces.

Who can you sterilize the following materials:-

1- Oil
2- Serum
3- Culture media
4- Loop
5- hood

Methods of sterilization	substances sterilized
Dry heat	
flaming	Slide for fixed bacteria
Hot ear oven	glassware, oils, powder, paraffin
Incineration	biohazardous material- used gloves, needles, etc.
Red hot	inoculating wires, loops
Moist heat	
Pasteurization	serum, milk
Boiling	needles, glass syringes
Autoclave	heat stable media such as nutrient agar

* Filtration :- heat labile media, serum, antibiotics,

Lec:2

Specimen Collection, Transport, and Rejection Criteria

Proper collection of the patient specimen is the single most important step in the diagnosis of an infection, because the results of diagnostic tests for infectious diseases depend on the selection, timing, and method of collection of specimens. Bacteria and fungi grow and die, are susceptible to many chemicals, and can be found at different anatomic sites and in different body fluids and tissues during the course of infection. Because isolation of the agent is so important in the formulation of a diagnosis, the specimen must be obtained from the site most likely to yield the agent at that particular stage of illness and must be handled in such a way as to favor the agent's survival and growth.

Safety Considerations

1. Treat all specimens as potentially hazardous.
2. Use appropriate barrier protection (gloves, gown) when collecting or handling specimens. If splashing is a possibility, protective eyewear, face masks and aprons may be necessary.
3. Do not contaminate the external surface of the collection container.
4. Minimize direct handling of the specimen in transit. Use plastic sealable bags with a separate pouch for paperwork.

General rules apply to all specimens:-

1. The quantity of material must be adequate
2. The specimen should be representative of the infectious process (e.g., sputum not saliva, swab from the depth of wound not from its surface).
3. Contamination of specimens must be avoided by using only sterile equipment and aseptic precautions.
4. The specimens must be taken to the laboratory and examined promptly . special transport media may be helpful.
5. Meaningful specimens to diagnosis bacterial and fungal infections must be secured before antimicrobial drugs are administrated .

Transport of Specimens

Specimens should be transported to the laboratory ideally within 30 minutes of collection, preferably within 2 hours. Adverse environmental changes in oxygen, pH, and temperature can prevent the recovery of certain microorganisms and allow overgrowth of others. If transport to the laboratory is delayed, or if the specimen

will not be processed as it is received in the laboratory, the specimen can be maintained by storage under **certain conditions** or with the use of **preservatives**, **transport or holding medium**.

Transport media are essentially solutions of buffers with carbohydrates, peptones and other nutrients designed to preserve the viability of bacteria during transport without allowing their multiplication. The primary objective of the use of the transport medium is to maintain the specimen as near its original state as possible. Transport medium aims to preserve a specimen and minimize bacterial overgrowth from the time of collection to the processing of the specimen.

However, in general, transport media are classified on the basis of the physical state as semi-solid and liquid and also on the basis of their utility as bacterial or viral transport media. There are many types of transport media:-

.**Amies medium with charcoal**: Charcoal helps eliminate metabolic products of bacterial growth, which may be especially useful in the isolation of fastidious organisms like However it is suggested that, some other pathogens like Campylobacter can also survive in such medium. **Amies medium without charcoal**: Are ideal for the isolation of Mycoplasma and Urea plasma.

Stuarts medium: Commonly used for transporting specimens suspected of having gonococci. Also used for transporting Throat, wound and skin swabs that may contain fastidious organisms.

Preservatives

1. **Boric acid**:- is used in commercial products to maintain accurate urine colony counts.
2. Stool specimens for bacterial culture that are not transported immediately to the laboratory can be refrigerated; if the delay is longer than 2 hours, the specimen can be added to **buffered formalin.**
3. **Anticoagulants** are used to prevent clotting of specimens, including blood, bone marrow, and synovial fluid. **Sodium polyanethol sulfonate (SPS)** is the most common anticoagulant used for microbiology specimens. The concentration of SPS must not exceed 0.025% because some *Neisseria spp*. And certain anaerobes are inhibited by higher concentrations.

4. **charcoal** to absorb fatty acids given off by the swab that can be detrimental to the survival of *Neisseria gonorrhoeae* and *Bordetella pertussis.*

Specimen Storage

Some specimens that will not be transported or processed immediately can be maintained by being stored under certain conditions. The individual responsible for storing the specimen needs to be informed as to the best storage environment for each specimen type. Some specimens, such as urine, stool, sputum, bronchial secretions, swabs (not for anaerobes), foreign devices such as catheters, and viral specimens, can be maintained at refrigerator temperature (4° C) for 24 hours. Pathogens that are cold sensitive may be found in other specimens, and those specimens should be kept at room temperature if culture is to be performed. This includes samples that might contain anaerobic bacteria as well as most other sterile body fluids, genital specimens, and ear and eye swabs.

Unacceptable Specimens and Specimen Rejection

1. The specimen is not submitted in the appropriate transport container or the container is leaking.
2. The quantity of the specimen is inadequate to perform all tests requested.
3. The specimen transport time is more than 2 hours and the specimen has not been preserved.
4. The specimen is received in a fixative such as formalin; stools for O & P examinations are an exception.
5. There is no patient identification on specimen container.

Lec:3

Cultivation

A microbial culture, is a method of multiplying microorganisms by letting them reproduce in predetermined culture media under controlled laboratory conditions. Those media generally provide source of carbon, energy and nitrogen, in the form of available carbohydrates and amino acids. In order to obtain a suitable growth, the artificial medium should provide nutrients and a pH approximating to those of tissues and body fluids. So that culture medium can be defined:- is an artificial environment that provides sources of carbon, energy and nitrogen in the form of available carbohydrates and amino acids for the growth of bacteria.

Purpose of culturing

• Isolation of bacteria.

• Properties of bacteria i.e. culturing bacteria is the initial step in studying its morphology and its identification.

• Estimate viable counts.

• To test for antibiotic sensitivity.

• To create antigens for laboratory use.

• Certain genetic studies and manipulations of the cells also need that bacteria to be cultured in.

culture media can be classified in different ways; based on consistency, based on nutritional component and based on its functional use.

- **Based on consistency**

1- **Liquid media:-** Liquid media are sometimes referred as “broths” (e.g. nutrient broth). These are available for use in test-tubes, bottles or flasks. In liquid medium, bacteria grow uniformly producing a turbidity. Liquid media can be used to propagate large numbers of microorganisms in fermentation studies and for various biochemical tests. Colony morphology of bacteria is not visible in liquid media and presence of more than one type of bacteria cannot be differentiated.

2- **solid media** :- Any liquid medium can be converted to a solid medium by addition of solidifying agents such **as agar-agar, egg yolk** or **serum**. Solid media, such as **nutrient agar** or **blood agar**, are used (1) for the surface growth of microorganisms in order to observe colony appearance, (2) for pure culture isolations, (3) for storage of cultures, and (4) to observe specific biochemical reactions.

3- **Semi-solid media** :-Reducing the concentration of agar to 0.2 - 0.5% renders a medium semi-solid. Semi-solid media can be used in fermentation studies, in determining bacterial motility, and in promoting anaerobic growth. Certain transport media such as **Stuart’s** and **Amies** media are semi-solid in consistency.

- **Based on nutritional component**

Media can be classified as **simple** (Basal media) and **complex** (Enriched media).

- **Based on functional use or application**

These include :-

1- Basal media

Are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar are considered as basal media.

2- Enriched media

Addition of extra nutrients in the form of **blood**, **serum**, **egg yolk** tobasal media makes them enriched media. Enriched media are used to grow nutritionally exacting (**fastidious**) bacteria. **Blood agar**, **chocolate agar**.

3- Selective media

These are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. selective media are generally agar based. Any agar media can be made selective by addition of certain inhibitory agents that do not affect the pathogen. Various approaches to make a medium selective include addition of **antibiotics**, dyes, chemicals, alteration of pH. Examples of selective media are MacConkey agar, Thiosulphate Citrate Bile salt Sucrose agar.

4- Differential media or indicator media

Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Examples: MacConkey agar, Thiosulphate Citrate

Blood agar is also considered differential because it is used to distinguish pathogenic bacteria based on the effect of bacterial enzymes known as hemolysins which lyse red blood cells. Blood agar: contains a basal medium and 5-10% sheep, horse or rabbit

blood.

there are three type of hemolysis:-

1. Beta hemolysis (β- hemolysis): complete lysis or destruction of red blood cells resulting in clear area around colonies. This haemolysis is displayed by *Streptococcus pyogenes* .

2. Alpha hemolysis (α- hemolysis): partial lysis of RBC, resulting in greenish discoloration around colonies this type is demonstrated by *Streptococcus pneumonia.*

3. Gamma – haemolysis (γ-haemolysis): not lyse RBCs this type is typical of *Enterococcus faecalis* .

5- Transport media

Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals, prevent drying of specimen, maintain the pathogen to commensal ratio . Some of these media (Stuart's and Amie's) are semi-solid in consistency.

5- Sensitivity media: a special media used to tested antibiotic sensitivity for given microorganism e.g. Muller Hinton media.

Lec:4

General Urine Examination (Urine Analysis)

Background

- **The urinary system**, also known as the **renal system** or **urinary tract**, consists of the kidneys, ureters, bladder, and the urethra. The purpose of the urinary system is to eliminate waste from the body, regulate blood volume and blood pressure, control levels of electrolytes and metabolites, and regulate blood pH.
- Average urine production in adult humans is about 1–2 liters (L) per day, depending on state of hydration, activity level, environmental factors, weight, and the individual's health. Producing too much or too little urine requires medical attention. **Polyuria** is a condition of excessive urine production (> 2.5 L/day). **Oliguria** when < 400 mL (millilitres) are produced.
- The kidneys take part in several regulatory functions. Through glomerular filtration and tubular secretion, numerous waste products, including nitrogenous products of protein catabolism, and both organic and inorganic acids and bases, are eliminated from the body. Fluid, electrolytes (including sodium, potassium, calcium, and magnesium), and acid-base status are regulated in homeostasis. Furthermore, the kidneys provide important hormonal regulation with erythropoietin and renin production, as well as vitamin D activation. Any derangement of these functions by renal or systemic disease can be reflected as chemically or cytologically altered urine.

- Urine (from Latin Urina,) is a typically sterile liquid by-product of the body secreted by the kidneys through a process called **urination** and excreted through the **urethra.**

Urine Analysis

A urinalysis :- is an array of tests performed on urine. Urinalysis can disclose evidence of diseases, even some that have not caused significant signs or symptoms. Therefore, a urinalysis is commonly a part of routine health screening. Urinalysis is commonly used to diagnose a urinary tract or kidney infection, to evaluate causes of kidney failure, to screen for progression of some chronic conditions such as diabetes mellitus and high blood pressure

Macroscopic Urinalysis

Macroscopic urinalysis is the direct visual observation of the urine, noting its **quantity**, **color**, **cloudiness**, - Normal urine volume **is750 to 2000 ml/24hr**.normal urine is typically light yellow and clear without any cloudiness. Obvious abnormalities in the color, clarity, and cloudiness may suggest possibility of

- an infection, may be caused by excessive cellular material or protein in the urine. (cloudy urine)
- dehydration (dark urine color)
- blood in the urine (hematuria -- visible to the eye may indicate urinary tract infection, stones, tumors, or injuries).
- breakdown of muscle (orange- or tea-colored urine).

Urine Dipstick Chemical Analysis

A urine test strip or dipstick is a basic diagnostic tool used to determine pathological changes in a patient's urine in standard urinalysis.

- **pH**

  The lungs and kidneys are the main regulators of an organism's acid / alkali balance. The balance is maintained through the controlled excretion of acidic hydrogens in the form of ammonia ions, monohydrogenated phosphate, weak organic acids and through the reabsorption of bicarbonate through glomerular filtration in the convoluted tubules of the nephron. The pH of urine normally vary between 4.5 and 8 with the first urine produced in the morning generally being more acidic and the urine produced after meals generally more alkaline.

- **Specific Gravity**

  Specific gravity (which is directly proportional to urine osmolality which measures solute concentration) measures urine density, or the ability of the kidney to concentrate or dilute the urine over that of plasma. Specific gravity between **1.002 and 1.035** on a random sample should be considered normal if kidney function is normal. Any

measurement below this range indicates hydration and any measurement above it indicates relative dehydration.

- **protein**
- A small amount of filtered plasma proteins and protein secreted by the nephron(mucoprotein) (Tamm-Horsfall protein) can be found in normal urine. Normal total protein excretion does not usually exceed **150** mg/24 hours. More than 150 mg/day is defined as **proteinuria.**
- **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 blood glucose level, the rate of glomerular filtration, and 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. A small amount of glucose may be present in the normal urine, but the fasting level in an adult is only about 2–20 mg of glucose per 100 mL of urine.

- **Bilirubin**

Bilirubin is a highly pigmented compound that is a by-product of haemoglobin degradation. The haemoglobin that is released after the mononuclear phagocyte system (located in the liver and spleen) withdraws old red blood cells from circulation is degraded into its components; iron, protoporphyrin and protein. The system's cells convert the protoporphyrin into unconjugated bilirubin that passes through the circulatory system bound to protein, particularly albumin. The kidney is unable to filter out this bilirubin as it is bound to protein, however, it is conjugated with glucuronic acid in the liver to form water-soluble conjugated bilirubin. This conjugated bilirubin does not normally appear in the urine as it is excreted directly from the intestine in bile. Intestinal bacteria reduce the bilirubin to urobilinogen, which is later oxidised and either excreted with the faeces as stercobilin or in the urine as urobilin. Conjugated bilirubin appears in urine when the normal degradation cycle is altered due to the obstruction of the biliary ducts or when the kidney's functional integrity is damaged. This allows the escape of conjugated bilirubin into the circulation as occurs in hepatitis and hepatic cirrhosis).The detection of urinary bilirubin is an early indication of liver disease

and its presence or absence can be used to determine the causes of clinical jaundice.

- **Ketones in Urine**

Whenever a defect in carbohydrate metabolism or absorption or an inadequate amount of carbohydrate is present in the diet, the body compensates by metabolizing increasing amounts of fatty acids. When this increase is large, ketone bodies, the products of incomplete fat metabolism, begin to appear in the blood and are consequently excreted in the urine. In ketonuria, the three ketone bodies present in the urine are acetoacetic (diacetic) acid (20%), acetone (2%), and 3-hydroxybutyrate (about 78%).

- **Nitrite**

Many bacteria that are urinary tract pathogens are able to reduce nitrate to nitrite, and thus will generate a positive urine nitrite test when present in significant numbers (>105–106/mL bladder urine). Common organisms include *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Proteus*, *Staphylococcus*, and *Pseudomonas species*.

False nitrite reactions

A negative reaction will be obtained if an infection is caused by bacteria that do not reduce nitrite. False negatives can occur if the urine has been in the bladder too short a time or the urine is not fresh and the nitrite has decomposed. A false negative reaction may also occur if the urine contains insufficient nitrate.

The microscopic urinalysis

The microscopic urinalysis is the study of the urine sample under a microscope. It requires only a relatively inexpensive light microscope. Cells and cellular debris, bacteria, and crystals in the urine (crystalluria) can be detected by microscopic examination to provide confirmation of the dipstick color change .

1. **Red Blood Cells**

Hematuria is the presence of abnormal numbers of red cells in urine due to: • glomerular damage. • kidney trauma. • urinary tract stones. • upper and lower urinary tract infections. • nephrotoxins. • physical stress. • Red cells may also contaminate the urine from the vagina in menstruating women.

2. **White Blood Cells**

Pyuria refers to the presence of abnormal numbers of leukocytes that may appear with infection in either the upper or lower urinary tract or with acute glomerulonephritis

3- Epithelial Cells

Renal tubular epithelial cells, usually larger than granulocytes, contain a large round or oval nucleus and normally slough into the urine in small numbers. However, with nephrotic syndrome and in conditions leading to tubular degeneration, the number sloughed is increased.

4- Casts

• They are solid and cylindrical structures formed by precipitation of debris in the renal tubules. • Urinary casts are formed only in the distal convoluted tubule (DCT) or the collecting duct (distal nephron). The proximal convoluted tubule (PCT) and loop of Henle are not locations for cast formation. • Hyaline casts are composed primarily of a mucoprotein secreted by tubule cells, hyalin cast are seen in healthy individuals. • RBCs casts are formed when RBCs stick together and in glomerular disease. • WBCs casts are seen in acute pylonephritis and glomerulonephritis. • Granular and waxy casts are seen in nephrotic syndrome.

Lec:5

Gastrointestinal Tract Disease

Digestive System

The digestive system is an entire organs that work together in a process known as **digestion**. It consist of two parts: 1-Gastro Intestinal Tract (GIT) 2-the accessory glands .

Anatomy of the Digestive System

The digestive system consists of two main parts:

1-The gastrointestinal tract (**GIT)**

This tract is divided into the upper and lower gastrointestinal tracts:

Upper Gastrointestinal Tract

The upper gastrointestinal tract includes:

- **Oral cavity**(Mouth)
- **Pharynx**
- **Esophagus:** the fibro muscular tube through which food passes, aided by peristaltic contractions, from the pharynx to the stomach.
- **Stomach:** secretes protein-digesting enzymes called proteases and strong acids to aid in food digestion, before sending partially digested food to the small intestines.
- **Duodenum:** the first section of the small intestine and may be the principal site for iron absorption

Lower Gastrointestinal Tract

The lower gastrointestinal tract includes most of the small intestine (**Duodenum, Jejunum, illium**) and all of the large intestine. According to some sources, it also includes the anus.

2-Accessory glands

The accessory glands consist of the **salivary gland**, **liver**, **pancreas** and **gall bladder.**

Functions Of Digestive System

- The digestive system responsible for **consuming** and **digesting foodstuffs, absorbing nutrients**, and **expelling waste**, and this the ♥main function of digestive system♥.
- **Immune barrier** The gastrointestinal tract is also a prominent part of the immune system. ∗The low pH (ranging from 1 to 4) of the stomach is fatal for many microorganisms that enter it. Similarly, ∗mucus (containing IgA antibodies) neutralizes many of these microorganisms. Other factors in the GI tract help with immune function as well, including ∗ enzymes in saliva and bile .Health-enhancing intestinal bacteria of the ∗gut flora serve to prevent the overgrowth of potentially harmful bacteria in the gut. These two types of bacteria compete for space and "food," as there is limited resources within the intestinal tract. A ratio of 80-85% beneficial to 15-20% potentially harmful bacteria generally is considered normal within the intestines.
- Normal Gastrointestinal **Microbiota (normal flora)** Bacteria make up most of the flora in the colon and up to 60% of the dry mass of feces. Somewhere between 300 and 1000 different species live in the gut. Fungi and protozoa also make up a part of the gut flora, but little is known about their activities. The microorganisms perform a host of useful functions, such as: fermenting unused energy substrates, , preventing growth of harmful, pathogenic bacteria, regulating

the development of the gut, producing vitamins for the host (such as biotin and vitamin K), and producing hormones to direct the host to store fats.

Diseases of digestive system

There are a number of diseases and conditions affecting the gastrointestinal system, including:

Gastroenteritis is an inflammation of the intestines. It occurs more frequently than any other disease of the intestines.

Classification of infection in the digestive system

Infections in the digestive system are classified in two groups:

Exogenous infections –pathogens that come into the body (*Helicobacter pylori* spreads through oral-oral or fecal-oral contact, *C. difficile* and other exogenous infections are frequently acquired in hospital environments)

Endogenous–infections organisms that are part of the normal (*Streptococcus and Enterococcus* are examples.

- **Cancer:** may occur at any point in the gastrointestinal tract, and includes mouth cancer, tongue cancer, esophageal cancer, stomach cancer, and colorectal cancer.
- **Inflammatory conditions: Ileitis** is an inflammation of the ileum; **Colitis** is an inflammation of the intestine, **Appendicitis:** Is inflammation of the vermiform appendix located at the caecum. This is a potentially fatal condition if left untreated; most cases of appendicitis require surgical intervention.
- **Peptic ulcer,** open sore in the lining of the stomach or duodenum
- **Anal fistula,** abnormal tube-like passageway near the anus.
- **Dysphagia**, difficulty in swallowing
- **Jaundice** (icterus), yellow-orange coloration of the skin and whites of the eyes caused by high levels of bilirubin in the blood (hyperbilirubinemia)

- **Dysentery,** is an intestinal inflammation, especially in the colon, that can lead to severe diarrhea with mucus or blood in the feces.
- **Diarrhea** is a condition that involves the frequent passing of loose or watery . Diarrhea is mostly **viral**. *E. coli* can also cause watery diarrhea.
- **Statorrhea,** fat in the feces; frothy, foul-smelling fecal matter, due to malabsorption which result from pancreatic diseases

Symptoms

Several symptoms are used to indicate problems with the gastrointestinal tract:

- ☒ **Nausea,** unpleasant sensation in the stomach associated with a tendency to vomiting.
- ☒ **Vomiting,** which may include regurgitation of food (due to GIT inflammation, acute pain, drugs, pregnancy)or the vomiting of blood (**as** in upper GIT bleeding (Haematomesis)
- ☒ **Melena,** black, tarry stools; feces containing digested blood (which is a sign of upper GIT bleeding)**.**
- ☒ **Diarrhea,** the passage of liquid or more frequent stools **(watery),** more than three times in a day and more than 200g/day.

Lec:6

Stool Analysis

What is the stool analysis test?

A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract.

Stool analysis may be done for these patients:

- Patient with abdominal pain or abdominal discomfort
- Patient with diarrhea
- Patient with anemia
- Patient who is too thin or do not grow well
- Patient with stool color that is changed to abnormal color
- Patient with skin disease as Urticaria which may due to parasitic infection with helminthes.

Composition and characteristics of feces

- Feces consist mainly of cellulose and other undigested foodstuffs, bacteria, and water (as much as 70 percent). Other substances normally found in stools include epithelial cells shed from the gastrointestinal tract,
- small amounts of fats, bile pigments in the form of urobilin, gastrointestinal and pancreatic secretions, and electrolytes.
- The average adult excretes 100 to 200g of fecal material per day. The stool tends to be soft and bulky on a diet high in vegetables and small and dry on a diet high in meat.
- Feces are normally brown because of bacterial degradation of bile pigments to stercobilin.

- The characteristic odor of feces is caused by bacterial action on proteins and other residues that produce substances such as indole, skatole, phenol, hydrogen, sulfide, and ammonia.
- Alterations in color, odor, consistency, or shape may indicate the presence of disease.

Collection Transport of the of Fecal Specimens

- ❖ Collect about 10-15 gm. the stool in a dry, clean, container. Make sure no urine, water, soil or other material gets in the container. If it is not possible to obtain feces collect a specimen by inserting a cotton wool swab into rectum for about 10 sec.
- ❖ The specimen must reach the laboratory within 30 minutes of passing of the stool, since the motile organisms, for example, **Vibrio** and **amoebic trophoziot** are **heat sensitive** and they can die or become unrecognizable after that period.
- ❖ When cholera is suspected, about 1 ml of specimen should be transferred into 10 ml of **alkaline peptone water**, which will act as an enrichment as well as transport medium.
- ❖ When worms or tapeworm segments are present, these should be transferred to a container of **physiological saline** and sent to a laboratory for identification.

General Stool Examination (GST)

Macroscopically or cross examination

1- The color of stool 2- Odor

3-PH (reaction) 4-Consistency of stool

5-Naked eye parasite 7-Gross blood and mucus and pus

1. The color of stool

Why stool color is brown? The characteristic brown color of feces is due to stercobilin and urobinin, both of which are produced by bacterial degradation of bilirubin.

Abnormal color

- **Black color:** indicate iron medication (for treatment of anemia) or upper GIT bleeding (due to peptic ulcer, stomach carcinoma or esophageal varices.(Note: The black color is caused by oxidation of the iron in the blood's hemoglobin.
- **Bright red color (Hematochezia):** indicate lower GIT bleeding (due to piles and anal fissure).
- **Clay color (gray-white):** indicate obstructive jaundice
- **Pale brown color:** with a greasy consistency indicate pancreatic deficiency causing malabsorption of fat (often with offensive odor.
- **Yellow-green color:** occurs in the stool of breast-fed infants who lack normal intestinal flora (low bile. conversion) and may also occurs due to rapid transit of feces through the intestines.
- Red brown color: indicate drugs as Tetracyclines, and Rifambicin antibiotics

- **Odor**

  Normally offensive

Why stool odour is offensive? Fecal odor results from gases produced by bacterial metabolism, including skatole, mercaptans, indoleand hydrogen sulfide formed by bacterial fermentation and putrefaction.

Abnormal:

Very offensive: usually seen in cases of constipation and with certain types of food that produce excessive gases, Bacterial infection and malabsorption. Foul-smelling stool: are characteristic of steatorrhea.

- **PH (reaction)**

  Normally variable and diet dependent and is based on bacterial fermentation in the small intestine. Why stool pH is variable? Because stool pH mainly depends on the type of diet. Abnormal:

  High alkaline stool Physiological cause by using High protein diet

  Pathological : Secretory diarrhea, Colitis or Antibiotic use (impaired colonic fermentation) High acidic stool Physiological :High carbohydrate diet Pathological Poor fat absorption

  • Poor absorption of sugars as in lactose intolerance

- **Consistency:** Normal stools are well formed. In diarrhea and dysentery the stools are semi solid or watery in nature. The cysts have been mostly found in the formed stools, while trophoziot have been most abundantly found in watery stools.
- The presence of blood, mucus or pus. Blood and mucus, it is a case of amoebic dysentery caused by Entameoba histolytica Blood and pus, the case is bacillary dysentery, caused by *Shigella, Compylobacter or E.coli*. Only blood, the diarrhea caused by *Salmonella or E.coli* or *Clostridium difficile*.
- Naked eye parasite Normal: no parasites or larva appear in the stool but in some cause the whole worm or part of its body appear in the stool and can be seen by

naked eye like(segment of tap worm) Two worms can be seen by naked eye in the stool: *Ascaris lumbricoides* and *Enterobius vermicularis.*

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Stool analysis- part-2

Microscopic examination

Microscopic analysis of stool specimens includes examining the sample for , leukocytes, epithelial cells, blood, qualitative fat and parasites (Eggs, larvae cysts).

- Large number of leukocytes clumps of pus cells of > 50 cells per high power field along with macrophages and erythrocytes are typical of shigellosis.
- A smaller number of pus cells of <20 per high power field are found in salmonellosis and in infections which are caused by invasive *E.coli*.

- Few leucocytes (< 5 cells per high power field) are present in cholera, EPEC and ETEC and viral Diarrhoea

Epithelial cells

Normally small to moderate numbers of epithelial cells are present in feces. Large numbers of epithelial cells (or large amounts of mucus), however, indicate that the intestinal mucosa is scratch.

the laboratory diagnosis of most parasitic infections is by demonstrating the ova of the parasite (stool ova and parasites test) in the infected person ‘s stools. The following techniques can be used to examine the stool.

1. Saline wet mount examination and iodine preparation , buffered methylene blue stain
2. Concentration techniques

1- Stool examination (saline wet mount and iodine staining) method

Gastro-intestinal infestations (infections) by parasites (Protozoan/Helminthes) are primarily diagnosed by detecting live motile trophozoites (protozoans); cyst (inactive dormant stage of Protozoa) or eggs and larva (in case of Helminths) in stool. Saline wet mount from the name, it makes uses of Normal saline and is made by mixing a small well mixed quantity (about 2 mg) of the sample(stool) in a drop of saline placed on a clean glass slide and covered with a cover slide. Normal saline is an Isotonic solution that maintains the osmotic pressures in the cells and doesn’t not lyse them. The smear is then examined under the microscope with specific objectives X4,X10,X40. The Saline wet mount is used for the detection of parasitic trophozoites and cysts of protozoa, and eggs and larvae of helminths.

It is particularly useful for detection of live motile trophozoites of protozoans like *E. histolytica*, and helminths larva like *Strongyloides stercolaris* .Iodine Staining Method. It is the same technique as the Saline method but here, Instead of normal saline, odine is used, iodine stains the nuclei of some cyst form of protozoans like *Entamoeba histolystic* making easy to view under the microscope.A common Disadvantage with this method is the ability of iodine to kill the motile (Trophozoites) form of these parasite or protozoans hence not advisable if intended to view motile trophozoites.

2- Buffered methylene blue stain It is used for nuclear stain in *Entamoeba histolytica.*

4- **Concentration methods**

Two types of concentration techniques are used for stool testing

• Sedimentation (Ascaris lumbricoides, shistosoma mansoni,)

- Floatation (enterobius vermicularis, Ankylestoma)

Chemical testing of stool

☒ **PH:** normal stool PH is (6-7.5). The pH of stools is acidic in amoebic dysentery and is alkaline in bacillary dysentery.

☒ **Occult blood (fecal occult blood test or stool occult blood test)**

Occult blood may be present in a variety of conditions, including malignancy of the gastrointestinal(colon, rectum, stomach).The reagent used is benzidine powder. Add a pinch of benzidine powder to a test tube and acidify with 1- 2 drops of glacial acetic acid and mix well.To this is added 1 ml of hydrogen peroxide, which is mixed well again. Place a clean glass slide and put a small amount of stool on it.Add 1- 2 drops of the previously prepared benzidine mixture to the stool sample taken on the slide and observe the colour change. The development of a green to blue colour indicates the presence of occult blood in the stool sample.

☒ **Reducing factors:** mono sugar and di sugar ,there level in stool (6mg/g) any increase in that level indicate disturbance in enzymes that digest sugar (e.g. Lactase, Sucrase).

Stool Culturing

Certain bacteria are normally found in feces (the “normal flora” of the bowel).The presence of pathological types of bacteria may however produce diarrhea and other signs of systemic infection. Thus, most stool cultures are undertaken to evaluate diarrhea of unknown etiology to identify possible causative bacteria.

Bacteria produce diarrhea in three main ways:

(1) The organisms invade the intestinal wall, damaging tissue.

(2) The organisms produce toxins within the intestine that alter gastrointestinal motility.

(3) Toxins produced by bacteria are ingested (e.g., via foods) and produce diarrhea, although the organisms themselves are not detected in feces

Culture media:

1- ***MacConkys Agar***: inhibits most of the gram positive organisms, diffrenciat between lactose fermenters and non lactose fermenters.

2- ***Xylose lysine deoxycholate (XLD) agar***: This selective medium has been recommended for the isolation of Salmonella and particularly Shigella from fecal samples

3- ***Thiosulphate citrate bile salt sucrose (TCBS) agar***: This is an excellent, selective medium for the primary isolation of Cholerae.

4- ***Sorbitol MacConkys agar***: This MacConkys agar contains sorbitol instead of lactose. *E.coli* 0157 produces colorless colonies on this medium because it does not ferment sorbitol so; this medium is useful for screening 0157 *E.coli*.

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Spinal Fluid Analysis

Spinal Fluid is clear, colorless liquid that fills and surrounds the brain and the spinal cord. It contains glucose, electrolytes, amino acids, and other small molecules found in plasma, but it has very little protein and few cells.. Cerebrospinal fluid is made by tissue called the choroid plexus in the ventricles (hollow spaces) in the brain . The cerebrospinal fluid provides a mechanical barrier against shock, supports the brain and provides lubrication between surrounding bones and the brain and spinal cord. When an individual suffers a head injury, the fluid acts as a cushion, dulling the force by distributing its impact. The fluid helps to maintain pressure within the cranium at a constant level. The fluid also transports metabolic waste products, antibodies, chemicals, and pathological products of disease away from the brain and spinal-cord tissue into the bloodstream. CSF is slightly alkaline and is about 99 percent water. The brain produces roughly 500 mL of cerebrospinal fluid per day. This fluid is constantly reabsorbed, so that only 100-160 mL is present at any one time.

Spinal Fluid Analysis:-

Is a set of laboratory tests that examine a sample of the fluid surrounding the brain and spinal cord. ϖ The purpose of a CSF analysis is to diagnose medical disorders that affect the central nervous system. Some of these conditions are as follows :

ϖ Infectious diseases of the brain and spinal cord, including meningitis (inflammation of the thin tissues that cover and protect your brain and spinal cord. This usually is caused by an infection in the cerebrospinal fluid). and encephalitis (inflammation of your brain cells). Myelitis: which may be viral, bacterial, fungal, or parasitic infections.

ϖ CSF tests for infections look at white blood cells, bacteria, and other substances in the cerebrospinal fluid ϖ Autoimmune disorders, such as multiple sclerosis (when your body's immune system attacks your nerves). CSF tests for these disorders look for high levels of certain proteins in the cerebrospinal fluid. These tests are called albumin protein and igG/albumin.

ϖ Bleeding in the brain (hemorrhaging) in the brain and spinal cord., Brain tumors, Leukemia: a kind of blood cancer. 3 Stage rd MSc. Sara. A. Hassan Advanced Lab

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Sample collection Lumbar puncture CSF is withdrawn from the subarachnoid space through a needle by a procedure called a (Lumbar puncture) or (Spinal tap). Lumbar puncture is performed by inserting the needle between the fourth and fifth lumbar vertebrae (L4-L5). This location is used because the spinal cord stops near L2, and a needle introduced below this level will miss the cord Usually three or four tubes are collected. The first tube is used for chemical and/or serological analysis, and the last two tubes are used for hematology and microbiology tests. This method reduces the chances of a falsely elevated white cell count caused by a traumatic tap (bleeding into the subarachnoid space at the puncture site), and contamination of the bacterial culture by skin germs or flora.

Normal result Gross appearance Normal CSF, clear and colorless Opening pressure in children older than six to eight years, 90–180 mm H2O; in infants and younger children, 10–100 mm H2O Specific gravity 1.006–1.009 Total protein 15–45 mg/dL Total glucose 40–80 mg/dL leucocytes 0–6/microL (adults and children). up to 19/microL in infants up to 30/microL (newborns) Gram stain, culture Negative, sterile Syphilis, red blood cell Negative, normally

Routine examination of CSF includes :

1 .Gross examination.

2. Clinical chemistry : Tests for Glucose. Tests for Protein.

3. Hematology :Tests for Red blood cell count. Tests for White blood cell count with differential

4. Serology: (Syphilis)

5. Microbiology: (Bacterial, Viral, Fungal, Parasitic).

1- Gross examination Appearance: Normal:- Clear and colorless . Abnormal: Straw, pink, yellow, or amber pigments (xanthochromia) are abnormal and indicate the presence of bilirubin, hemoglobin, red blood cells, or increased protein. Turbidity (suspended particles) indicates an increased number of cells. Clinical chemistry. 1.GLUCOSE CSF glucose is normally approximately two-thirds of the fasting plasma

glucose. A glucose level below 40 mg/dL is significant and occurs in bacterial and fungal meningitis and in malignancy .

2 .PROTEIN Total protein levels in CSF are normally very low, and albumin makes up approximately two-thirds of the total. High levels are seen in many conditions, including bacterial and fungal meningitis, tumors, subarachnoid hemorrhage, and traumatic tap .

3-Hematology

1. WHITE BLOOD CELL (WBC) COUNT The number of white blood cells in CSF is very low. An increase in WBCs may occur in many conditions, including infection (viral, bacterial, fungal, and parasitic), allergy, leukemia, hemorrhage, traumatic tap, encephalitis, and Guillain-Barré syndrome. The WBC differential helps to distinguish many of these causes.

For example, viral infection is usually associated with an increase in lymphocytes, while bacterial and fungal infections are associated with an increase in polymorphonuclear leukocytes (neutrophils). The differential may also reveal eosinophils associated with allergy and ventricular shunts; macrophages with ingested bacteria (indicating meningitis), RBCs (indicating hemorrhage), or lipids (indicating possible cerebral infarction); blasts (immature cells) that indicate leukemia; and malignant cells characteristic of the tissue of origin .

2.RED BLOOD CELL (RBC) COUNT While not normally found in CSF, RBCs will appear whenever bleeding has occurred. Red cells in CSF signal subarachnoid hemorrhage, stroke, or traumatic tap.

4.Microbiology Gram stain & Culture The Gram stain is performed on a sediment of the CSF and is positive in most cases of bacterial meningitis. Culture is performed for both aerobic and anaerobic bacteria. In addition, other stains (e.g. the acid-fast stain for Mycobacterium tuberculosis, fungal culture, and rapid identification tests (tests for bacterial and fungal antigens) may be performed routinely .

5. Serology Syphilis serology involves testing for antibodies that indicate neurosyphilis. The fluorescent treponemal antibody-absorption test is often used and is positive in persons with active and treated syphilis.