Lab.N0-4

STOOL EXAMINATION

General Stool examination

General Stool Examination (GSE) is carried out in laboratories for various diagnostic purposes. Examination of stool is very helpful in the diagnosis of disease of the gastrointestinal tract. Mostly a clean container which does not contain any detergent or disinfectant is sufficient for all types of stool examinations including stool culture. It consist the following tests:-

A.PHYSICAL EXAMINATION OF STOOL:

Sample should be examined immediately after collection. Samples left standing prolonged will deteriorate helminthes, Ovum, other parasites and increase the numbers of monilia and bacteria which gives wrong results, however the following aspects of stool should be examined:

- (1) Quantity: the adult person excretions about 150-250 gm. /day of feces, about (1/3-1/2) of feces dry weight is bacteria.
- (2) Consistency and form: Normal stool is <u>well formed</u>. But in constipation (Dehydration) the stool is <u>solid (Hard)</u> and the <u>semi-solid (soft or loose)</u> seen when taking certain medications and laxatives. In abnormal cases such as diarrhea and dysentery the stool appear <u>liquid</u>, or watery in nature. In cholera the stools have a <u>rice water appearance</u>. In cases of malabsorption of fats the stools are <u>pale bulky</u> and <u>semi-solid</u>.

(3) Colour:

- 1- Normal colours of stools are <u>light to dark brown</u> due to the Presence of bile pigments.
- 2- <u>Dark black:</u> In cases with bleeding into the intestinal tract the stools become dark tarry in nature due to the formation of acid hematin ميغة تستخرج من الهيموجلوبين المؤكسد. if the bleeding is in the small intestines. In case of bleeding in large intestines or rectum stool color may be <u>bright red</u> due to fresh blood.
- 3- Red color: Resulted from eating certain colorful foods such as red beets.
- 4- <u>Clay colour:</u> The stool may be clay coloured due to absence of stercobilinogen in biliary tract obstruction.

(4) Odor: The normal fecal odor of stool resulted from Indole and Skatol. Odor of stools may become offensive in conditions like, Intestinal amoebiasis. In cases of bacillary dysentery and cholera the stools are not foul smelling due to the absence of fecal matter.

(5) Blood:

- 1- The blood is present on the outer surface of the feces and this caused either by contamination from menstrual cycle blood in women or bleeding hemorrhoids from the blood vessels.
- 2- Blood should be noted in stools if present as it is indicative of Ulceration or presence of any other pathology like malignancy.
- (6) Mucus: Is present in certain conditions like amoebic or bacillary dysentery.
- (7) **Parasite:** Stools may contain adult helminthes. Nematodes like Ascaris are easily visible as their size is large. Hook worms and Proglottids of cetodes may also present. These may be visible to the naked eye.

B. MICROSCOPIC EXAMINATION OF STOOL:

The laboratory diagnosis of most parasitic infections is by the demonstration of ova of the parasite in the stools of the infected person. The stool is collected in a clean container. The stool can be examined by the following techniques.

(a) Wet mounts examination.(b) Iodine examination.

- (a) Saline wet mount examination: The stool is emulsified in normal saline and a large drop is placed on a glass slide and is then covered with a cover slide. Then examined under a light microscope, it is important to examine specimen under 10X objective lens at first to observe large molecules, cells, ova and helminthes, then to the 40X objective to complete the test. It is preferable to keep the condenser down and the intensity of the light low for proper visualization of the ova and cysts. The thickness of the film should be such that one is able to see the printed letters of the newspaper through it.
- (b) Iodine examination: Iodine preparation leads to better visualization of morphological details of ova and cysts as it stains the glycogen in them. However it has the disadvantage that the live trophozoites of *Entamoeba histolytica* and other live parasites cannot be seen as the iodine kills them. The examination instructions in normal saline must be followed the same in iodine test.



Microscopic examination include the following:

- (1) Pus cells: Observed in stool the same procedure as in urine.
- (2) **RBCs:** Observed in stool the same procedure as in urine.
- (3) Monilia: Observed in stool the same procedure as in urine.
- (4) Protozoa:
 - (a) *Entamoeba histolytica*: To investigate the vegetative phase (trophozoite) and cyst, causing amoebic dysentery disease.
 - (b) *Entamoeba_coli*: trophozoite + cyst <u>Note: -</u> most of children diarrhea less than 2 years cause by *Entamoeba_coli*.
 - (c) <u>*Giardia lamblia*</u>, trophozoite + cyst, Cause watery diarrhea disease in children, especially.
 - (d) <u>Balantidium coli</u>, trophozoite + cyst, causing Balantidiasis in colon.

(5) Worms :

- (a) <u>Enterobius vermicularis (pinworm)</u>: investigating the eggs that are of convex and flat surface and a pointed end.
- (b) <u>Ascaris lumbricoides</u>: investigating for eggs which characterized by the content of granular yellow to Brown irregular albumin membrane.
- (c) Hookworm (*Ancylostoma duodenale*): investigating the eggs where the egg yolk is divided and surrounded by a thin membrane.
- (d) Tapeworms, (Taenia *solium*): investigating the worm pieces called (gravid segments or Proglottids) that comes out with the feces.
- (e) <u>Schistosoma mansoni</u>: Investigating the eggs distinct by lateral spin.

C. CHEMICAL EXAMINATION OF STOOL

- (a) **pH:** The pH of stools is acidic in amoebic dysentery and is alkaline in bacillary dysentery.
- (b) Occult blood: Presence of blood in feces which is not apparent on gross inspection and which can be detected only by chemical tests is called as occult blood. Causes of occult blood present in a number of diseases including malignancy of the gastrointestinal tract.

The reagents used are:

- 1-Benzidine reagent: Development of blue colour is indicative of presence of occult blood in the stool specimen.
- 2- Orthotolidine: Development of green colour

Benzidine test is also highly sensitive and <u>false-positive</u> reactions are common. Since bleeding from the lesion may be intermittent, repeated testing may be required.

Causes of False-positive Tests

- 1. Ingestion of peroxidase-containing foods like red meat, fish, poultry, turnips, horseradish, cauliflower, spinach, or cucumber. Diet should be free from peroxidase-containing foods for at least 3 days prior to testing.
- 2. Drugs like aspirin and other anti-inflammatory drugs, which increase blood loss from gastrointestinal tract in normal persons.

Causes of False-negative Tests

- 1. Foods containing large amounts of vitamin C.
- 2. Conversion of all hemoglobin to <u>acid</u> hematin (which has no peroxidase-like activity) during passage through the gastrointestinal tract.

D. STOOL CULTURE

How to take stool samples:

- 1. Specimen of stool (at least 4 ml or 4 cm³) is collected in a clean, dry, container with a tightly fitting lid.
- 2. Do not let sample stand for a long period of time (so as not to die eccentric parasitic and inspection preferably within an hour of taking the sample).
- **3.** Early morning is the best sample because the stool here all night and the chances of the emergence of complex parasites and eggs are the largest.
- **4.** For children prefer to urinate first before taking a stool sample so as not to mix urine with stool sample.
- 5. Stool should not be contaminated with urine, water, soil, or menstrual blood. Urine and water destroy trophozoites; soil will introduce extraneous organisms and also hinder proper examination.
- 6. Patient's name, date, time & number must be labeled on the sample container.
- 7. Stools should be examined as early as possible after receipt in the laboratory (preferably within 1 hour of collection). If delay in examination is anticipated, sample may be refrigerated because <u>Parasites</u> are best detected in warm, freshly passed stools.
- 8. Patient must not use laxatives prior collecting stool sample.
- 9. Patient must refrain الامتناع عن from taking certain medicines before the test such as: pH medications, diarrhea medications, anti parasite drugs, antibiotics.
- 10. The patient must wear gloves before handling a stool sample in order to avoid transmission or use the tool to move the sample in the container. And don't take a stool sample base of the bathroom (toilet) floor. لابد من ارتداء قفازات قبل الإمساك بعينة المراز حتى تتجنب نقل العدوى أو استخدم أداه لنقل العينة في الوعاء
- **11.** The patient must wash his hands thoroughly after taking the sample. And the sample water or soap does not mix.

Procedure:

Stool is cultured by taking a sample by loop and cultured on different types of culture media according to the type of bacteria or diagnosis of case investigated as follows:

- 1- It is cultured on <u>Thiosulfate citrate bile salts sucrose agar media</u> if the patient is suspected of cholera infection.
- 2- It is cultured on <u>Lowenstein–Jensen medium</u> for Mycobacterium tuberculosis if the person suspected of gastrointestinal tuberculosis.
- 3- It is cultured on blood agar if suspected of infection with Staphylococcus aureus which it is blood hemolytic.
- 4- It is cultured on MacConkey agar medium to detect lactose fermentation bacteria in pink color colonies include (*E .coli, Klebsiella* and *Enterobacter*). But if it's not lactose fermenter, it is either *Proteus* which is identified by (diffusion phenomenon), or *pseudomonas* which is identified by (pyocynin test). And to distinguish between *Shigella* and *Salmonella*, by using serological and biochemical test.

