Medical Laboratory NTQF Level III

Learning Guide #44

Unit of competence: performing parasitological tests

Module Title: performing parasitological tests

LG Code: HLT MLT3 07M07 LO2-LG44

TTLM Code: HLT MLT3 TTLM 0919v1

LO2: **Process samples and associated request details**

Instruction Sheet	Learning Guide #2

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics –

Process samples for microscopic identification of parasites

2. Parasitological Sample Collection and Processing
    - 2.1. Checking of request papers and samples
    - 2.2. Sorting of specimens according to its urgency
    - 2.3. Acceptance and rejection criteria
    - 2.4. Sample log and labeling
    - 2.5. Processing of samples
    - 2.6. Storage of sample and its components

This guide will also assist you to attain the learning outcome stated in the cover page. Specifically, upon completion of this Learning Guide, you will be able to –

- Check details of request form and sample before they accepted
- Sort specimens according to tests requested
- Comply reasons to rejected samples and request forms to their sources with reasons for rejection
- Log accepted samples and request forms to easily tracking mechanisms
- Process samples for testing
- Store samples appropriately for testing.

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described in number 3 to 19.
3. Read the information written in the “Information Sheets 1”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-check 1” in page 7.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 1).
6. If you earned a satisfactory evaluation proceed to “Information Sheet 2”. However, if your rating is unsatisfactory, see your trainer for further instructions..
7. Submit your accomplished Self-check. This will form part of your training portfolio.
8. Read the information written in the “Information Sheet 2”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
9. Accomplish the “Self-check 2” in page 9.
10. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 2).
11. Read the information written in the “Information Sheets 3. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
12. Accomplish the “Self-check 3” in page 12.
13. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 3).
14. Read the information written in the “Information Sheets 4. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
15. Accomplish the “Self-check 4” in page 14.
16. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 4).
17. Read the information written in the “Information Sheets 5. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
18. Accomplish the “Self-check 5” in page 17.

19. Read the information written in the “Information Sheets 6. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
20. Accomplish the “Self-check 6” in page 30.
21. Read the information written in the “Information Sheets 7. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
22. Accomplish the “Self-check 7” in page 32.
23. If you earned a satisfactory evaluation proceed to “Operation Sheet 1” in page 34, “Operation Sheet 2” in page 35 and “Operation Sheet 3” in page 36, Operation Sheet 4” in page 37, “Operation Sheet 5” in page 38 and “Operation Sheet 6” in page 39, Operation Sheet 7” in page 39, “Operation Sheet 8” in page 41, “Operation Sheet 9” in page 42, operation sheet 10 page 42 and “Operation Sheet 11” in page 343. However, if your rating is unsatisfactory, see your trainer for further instructions.
24. Read the “Operation Sheet 1 - 11 and try to understand the procedures discussed.
25. Go to your trainer if you need clarification or you want answers to your questions or you need assistance in understanding a particular step or procedure.
26. Do the “LAP test” in page 44 (if you are ready). Request your trainer to evaluate your performance and outputs. Your trainer will give you feedback and the evaluation will be either satisfactory or unsatisfactory. If unsatisfactory, your trainer shall advice you on additional work. But if satisfactory you can proceed to Learning Guide # 45.

Information Sheet-1	Parasitological Sample Collection and Processing

2. Parasitological Sample Collection and Processing

Introduction

Parasite disease continue to be a significant threat throughout the word. These disease are usually brought about by climate conditions desirable for parasitic survival as well as poor sanitation and personal hygiene practices of the inhabitants. Certain population are more at risk of contracting parasitic infections, including forging visitors and those traveling and emigrating to other countries. Successful laboratory identification of parasite requires the knowledge and practice of laboratory testing in the pre-analytic, analytic and post analytic steps. For example, in pre-analytic phase, a specimen received in the laboratory that is compromised because of improper collection, labeling or transport should be rejected and anew specimen requested. Similarly laboratory techniques performed in the analytic phase of testing of these samples should be completed with care to ensure that the accurate results are obtained. Interpretation and reporting of the results obtained, completed in the post analytic phase of the testing, should be accurately reported in the timely manner.

- **Definitions Specimen:** A piece or portion of a sample selected for examination. The specimen may, or may not be representative, whereas the sample may have been selected to be representative.

- **Stool specimen**

  Adult healthy human defecate three times a day. The common pattern is once a day. Stool tends to be soft and bulky on diet high in vegetables and small and dry on a diet high in meat. 2/3 of stool is water.

- **Stool sample is composed of**
  - Waste residue of indigestible material in food
  - Bile pigment and salts

- Intestinal secretions, including mucous
- Leucocytes that migrate from the blood stream.
- shed epithelial cells
- Large number of bacteria (1/3 of total solid)
- Inorganic material chiefly calcium and phosphate
- Digested food present in very small quantities.

- **Blood specimen**

  Systemic or blood-borne parasitic infections are diagnosed by demonstrating the diagnostic stage(s) of the responsible parasite(s) in a blood specimen. The proper collection and handling of blood specimens is essential to obtain adequate smears for examination. There are some parasites that can be detected by observing motility in a wet preparation of a fresh blood sample under low- and high-power magnification. Blood smears can be prepared from fresh whole blood without anticoagulant (fingertip or earlobe) or from venipuncture collection with anticoagulant.

- **Cerebrospinal Fluid and Other Sterile Fluids**

  Cerebrospinal fluid (CSF) specimens may be collected for the diagnosis of amebic conditions as well as African sleeping sickness. The CSF must be examined promptly to detect the motility of these parasites. Special stains can also be performed on CSF including Giemsa, trichrome, and modified trichrome stains. The specimen can be cultured on non-nutrient agar seeded with Escherichia coli. The CSF sediment is inoculated to the medium, sealed, and incubated at 35' C. The plate is then examined for evidence of the amebae feeding on the bacteria. Other pathogens that might be recovered from the central nervous system include *Toxoplasma gondii*. Sterile fluids other than CSF include several specimen types, such as fluid present in cysts, aspirates, peritoneal fluid, pleural fluid, and bronchial washings. All these samples may be examined using wet preps and/or permanent stains.

- **Tissue and Biopsy Specimens**

  Tissue and biopsy specimens are recommended for the recovery of a number of parasites, including intracellular organisms such as Leishmania spp. and T.

gondii. Surgical removal of the specimen followed by the preparation of histologic tissue sections and impression smears is the preferred method for handling these Samples. Hepatic abscess material is the specimen of choice for patients suspected of liver abscesses caused by E. histolytica.

- **Sputum**

Sputum is typically collected and tested from patients suspected of being infected by the lung fluke. Other parasitic infections that may be found in sputum samples include E. histolytica, Entdmoeba gingiualis, Ascaris lumbricoides, and hookworm. An early-morning specimen is best and should be collected into a wide mouthed container with a screw cap lid. Saliva should not be mixed with the specimen. The sample may then be examined directly via wet preps. Microscopic examination of the sediment can include wet preps and permanent stains.

- **Urine and Genital Secretions**

Urine is the specimen of choice for the detection of Schistosoma haematobium eggs and may also yield *Trichomonas vaginalis*. The specimen should be collected into a clean container with a watertight Lid. The sample should be centrifuged on arrival at the laboratory. Microscopic examination of the sediment should reveal the parasites' if they are present. Vaginal and urethral specimens, as well as prostatic secretions, are typically collected and examined for the presence of T. vaginals trophozoites. These specimens may be collected on a swab or in a collection cup equipped with a lid. Saline wet preparations are the method of choice for demonstrating the motile trophozoites. Culture methods are available, including a commercial product that uses a culture pouch. All these methods are highly successful for diagnosing this sexually transmitted parasite.

- **Skin Snips**

Skin snips may be made using one of two collection techniques. The objective of both procedures is to obtain skin fluid without bleeding. One of

the methods involves making a firm (scleral) punch into skin with a specially designed tool. The other technique uses a razor blade with which a small cut into the skin is made. The resulting material obtained by both techniques may then be placed in approximately 0.2 mL of saline. After a 30-minute incubation period, the sample may be microscopically examined.

Self-check 1	Written test

Write True if the statement is correct and False if it is incorrect

1. Specimen is the representative of the whole, but sometimes it may not represent the whole. (2 points)
2. Skin snip is a specimen that has to be taken without fluid. (2 points)
3. *Trichomonas vaginalis* is a protozoa that diagnosed by taking stool specimen.(2 points)
4. Pre analytical phase the most important in clinical laboratory.(2 points)
5. Blood specimen is collected in the lab for diagnosing tissue parasites. (2 points)

Note: satisfactory rating is 5 points, unsatisfactory <5 points. You can ask your instructor for copy of correct answer.

Answer Sheet

1._____

2. _____

3. _____

Score = _____

Rating: _____

Name: _____ Date: _____

2.1. Checking of request papers and samples

Pre-analytical variables refers to any and all procedures that occur during sample collection, prior to sample analysis. This involves patient identification, physical sample collection, sample transportation to the testing site and sample preparation. Patient samples are sometimes collected by the patient themselves, for example, faecal parasitology samples. It is important that the laboratories have set protocols to ensure that appropriate collection kits with instructions for collection, safety precautions, and labeling are available for their patients. It is suggested that instructions for the patients be in the languages for the community the laboratory is serving or presented as simple easy-to-understand graphics.

Proper patient identification is mandatory to produce quality test result in the laboratory. Some of the pre-analytical activities in the lab are the following.

- **Patient preparation:** Some tests require that the patient be fasting. There may also be special timing issues for tests such as blood glucose, drug levels, and hormone tests. The client from whom sample is to be taken has right to know the type of the sample to be collected, the reasons why we collect the sample and the procedure applied to collect the sample.
- **Patient identification:** we have to properly check whether we have collected a sample from appropriate patient and the request paper and sample must be labeled correctly with some informations such as; name of the patient, age, sex, ward address of the patient, required test. The person collecting the sample must accurately identify the patient. This might be done by questioning the patient, by questioning an accompanying family member, or by the use of an identifying wrist band or other device.
- **Sample collection:** appropriate procedures must be applied to collect the sample. Some of the important informations to be considered here are:
    - Specimen container
    - Volume of the specimen
    - Time of collection
    - Type of anticoagulants for blood specimens
    - Preservatives to be considered etc….

- **Sample transportation:** specimens can be transported to reference laboratories for more specialized tests or for quality control purpose. Here proper labeling, packing, and correct preservative selection are mandatory. Generally the pre-analytical phase is the phase where the laboratory has no direct control on the process. Pre-analytical factors that can affect results include: sample type, sampling time, sample handling, patient's preparation and the nutritional status of the patient.

Self-check 2	Written test

Choose the best answer from give alternatives

1. One of the following is **Not** pre analytical activity in the laboratory?
   A. Patient identification
   B. Patient preparation
   C. Sample collection
   D. Sample processing
2. One of the following is **Not** informations to be considered during specimen collection
   A. Specimen container
   B. Volume of the specimen
   C. Time of collection
   D. Reporting result

Note: satisfactory rating is 2 points, unsatisfactory 1 points. You can ask your instructor for copy of correct answer.

Answer Sheet

1._____

2. _____

Score = _____

Rating: _____

Name: _____ Date: _____

2.2. Sorting of specimens according to its urgency

Once a sample enters the laboratory, there are a number of steps needed prior to testing. These pre-examination steps include:

- Verifying the sample is properly labelled, adequate in quantity, in good condition, and appropriate for the test requested. The test request must be complete and include all necessary information;
- Recording sample information into a register or log;
- Enforcing procedures for handling sub-optimum samples, including sample rejection, when necessary.

The objectives of the sorting concepts are to monitor and control the sample flow regarding the whole laboratory cycle.

The advantages of sorting sample are:

- Control and monitor sample material from delivery to disposal
- Documentation of know-how and regulations in the system
- Flexible assignment of the expert staff and quick integration of new employees.
- Defined & structured process
- Continuous sample cycle time
- Continuity regarding sample flow and capacity utilization by recursive sample sorting and defined buffer zones (to smooth peaks)
- Preparation of sample material for automation
- Automated handling of standard samples
- Sorting of special material on manual work places
- Programming of special rules by the maintenance personnel
- Special workflows can be configured
- Daily analysis of the order data

Collection of sufficient quantity is important to permit detection of organisms and to prevent rapid drying. The stool specimen should contain at least 4 ml.

Process & examine stool specimen immediately after collection, if not, preserve stool specimen.

- Liquid stool: < 30 minutes of passage at Room Temperature.
- Semi-formed stool: < 1 hour of passage at Room Temperature.
- Formed stool: < 24 hours of passage, 4 at ^{0}C.

Collect approximately 100g of faeces in a clean, dry container without preservatives for parasitological examination. A screw-top container labeled with full information identifies the patient is most suitable. Make sure that any adult worms or segments passed are included. For collection of stool specimens for bacteriological examination (e.g. for culture of cholera and other bacteria that cause dysentery).

Self-check 3	Written test

Answer the following question

1. List advantages of sorting laboratory specimen. (7 points)

Note: satisfactory rating is 3.5 points, unsatisfactory <3.5 points. You can ask your instructor for copy of correct answer.

Score = _____
Rating: _____

Answer Sheet

1._____
_____
_____
_____
_____
_____

Name: _____ Date: _____

2.3. Acceptance and rejection criteria

The laboratory should establish rejection criteria and follow them closely. It is sometimes difficult to reject a sample, but remember that a poor sample will not allow for accurate results. It is the responsibility of the laboratory to enforce its policies on sample rejection so that patient care is not compromised. Management should regularly review the number of rejected samples and reasons for rejections, conduct training on sample collection, and revise written procedures for sample management as needed.

The following are examples of samples that should be rejected:

- unlabeled sample;
- stool contaminated with urine during collection
- broken or leaking tube/container;
- insufficient patient information;
- sample label and patient name on the test request form do not match;
- If it is transported sample collected in wrong tube/container; for example, using the wrong preservative
- Inadequate volume for the quantity of preservative;
- insufficient quantity for the test requested;
- Prolonged transport time, or other poor handling during transport.

N.B. Record the reason for rejection in the log book and include all pertinent information.

Self-check 4	Written test

Answer the following questions

1. List the characteristics of laboratory specimen that should be rejected. (9 points)

Note: satisfactory rating is 4.5 points, unsatisfactory <4.5 points. You can ask your instructor for copy of correct answer.

Score = _____

Rating: _____

Answer Sheet

1._____

_____

_____

_____

_____

_____

_____

_____

_____

Name: _____ Date: _____

2.4. Sample log and labeling

The label must contain the following legible information**:**

- Patient name.
- Patient medical record number,
- Patient location.
- Collection date and time.
- Specimen type and/or source.
- The initials of the person collecting the sample.
- Test required (note any special handling required)
- Ordering physician.

Potential outcomes of collection and labeling errors:

- delays in reporting test results
- unnecessary re-draws/re-tests
- decreased customer satisfaction
- increased costs
- incorrect diagnosis / treatment
- injury
- Death.

During Labeling:

- Make sure that container label & the requisition match.
- Label should be on the container not on the lid, since the lid can be mistakenly placed on a different container.
- Ensure the labels on the containers are adherent under refrigerated conditions.

Fig, 2.1. Labeling specimen

The laboratory should keep a register (log) of all incoming samples. A master register may be kept, or each specialty laboratory may keep its own sample register.

Assign the sample a laboratory identification number – write the number on the sample and the requisition form. If computers are used for reports, enter the information into the computer.

The register should include:

- date and time of collection;
- date and time the sample was received in laboratory;
- sample type;
- patient name and demographics, as required;
- laboratory assigned identification (e.g., number 276_01_06_2009);
- tests to be performed

The laboratory needs a system to allow for tracking a sample throughout the laboratory from the time it is received until results are reported.

This can be done manually by careful keeping of records.

- confirm receipt of samples, include date and time;
- label samples appropriately; keep with the test requisition until laboratory ID is assigned;
- Track aliquots–traceable to the original sample**.**

If computers are available, maintain a database for tracking. The following information about each sample should be entered into the database:

- identification number;
- patient information;
- collection date and time;
- type of sample: for example, urine, throat, cerebrospinal fluid for culture;
- tests to be performed;
- name of ordering physician (or other health care provider);
- location of patient, such as ward, clinic, outpatient;
- diagnostic test results;
- Time and date results are reported.

Table 2.1. Sample parasitology log book

Date	Specimen no.	Patient	Sent by	Specimen provided	Examination requested	Results	Results sent (date)
2.1.01	1	Mr F	Dr A	Stool	Intestinal parasites	Direct microscopy: moderate no. of *Ascaris lumbricoides* ova seen	2.1.01
2.1.01	2	Ms M	Dr C	Stool	Intestinal parasites	Direct microscopy: no ova or parasites seen Concentration technique: no ova or parasites seen	2.1.01
2.1.01	3	Mrs L	Medical ward 1	Skin snips	Onchocerciasis	No parasites seen	3.1.01
3.1.01	4	Mr S	Dr R	Stool	Parasites	Occult blood: positive Direct microscopy: many trophozoites of *Entamoeba histolytica* and a few hookworm ova seen	3.1.01

Self-check 5	Written test

Answer the following questions

1. What are the informations that should be included during specimen labeling? (8 points)

Note: satisfactory rating is 4 points, unsatisfactory <4 points. You can ask your instructor for copy of correct answer.

Score = _____

Rating: _____

Answer Sheet

1._____

_____

_____

_____

_____

_____

_____

_____

_____

Name: _____ Date: _____

2.5. Processing of samples

2.5.1. **Stool sample processing :** Collect about 4 gm of fresh faces uncontaminated by urine or soil using wooden applicator stick or spatula to a container

A. The Container should be

- Wide necked
- Screw top
- Free from antiseptics
- Preferably, this is incinerated after use.

B. Label all the specimen with

- Patients name
- Date of collection
- Time of collection
- Ward number

C. Sample from patient with a confirmed or suspected diagnosis of certain infections disease such as cholera, shigella should be labeled “Risk of infection biohazard”

D. If delay is unavoidable use preservative. Note that stool sample should be

- Faces should be urine free
- Collect before antimicrobial agents are administered
- Examine diarrhea stool soon.
- Do not refrigerate stool specimen

❖ **Macroscopic examination stool specimen**

Is the primary step in the diagnosis of fresh stool specimen for parasitic infection and includes:

A. Consistency
B. Composition
C. Color and presence of adult parasites should be reported

A. *Consistency*

The presence of protozoan trophozoites in the stools will depend on the consistency and passage rate of the faces.

i. Formed **(**normal shape)

- Water content is absorbed
- Few trophozoites principally the cyst stage protozoa is common

ii. Semi formed or unformed soft stool with no regular shape as in diarrheal

iii. Watery /liquid – contain and any flakes of mucous and blood

B. *Composition*

The stool may contain blood mucus or pus, which is the evidence of ulceration due to pathogenic parasites.

C. Color

Pale yellowish stools are passed in statorrhoeaic conditions such as giardiasis.

D. Adult parasites

Faces may contain adult helminthes or segments present such as *A. lumbricoides*, *Entrobium vermicularis* or gravid segments of *Tenia species*.

Dysenteric and watery specimens must reach the laboratory as soon as possible after being passed (within 15 minute) otherwise motile parasites such as E.*histollytica* & *G.lambalia* may not be detected. Other specimen should reach the laboratory within 1 hour of being collected.

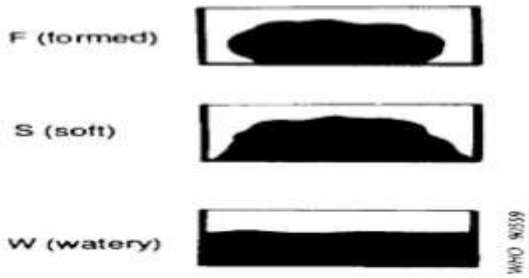


Fig 2.2 consistency of stool sample

❖ **Microscopic examination of faces**

- Necessary materials and reagents
- Gloves
- Normal saline
- Applicator stick
- Cover glass
- Slide

Microscopic examination of stool is used to observe cellular exudates and motile protozoan trophozoites, which are not easily seen by our naked eye.

❖ **Concentration technique for fecal parasite**

The two concentration techniques are

- ✓ Floatation concentration technique
- ✓ Sedimentation concentration technique

➢ ***Floatation Concentration Technique***

Uses high specific gravity of a solution to float the lighter ova and cyst

▪ **Zinc sulphate technique /Znso$_4$/**

- Is recommended for concentrating cysts of *Gardia lamblia*, *E. histolytica* and egg of *Trichuris trichuria*.
- Other nematode eggs are concentrated less well, while operculated trematodes egg are not concentrated because they are ruptured in the zinc sulphate solution.
- The technique is not suitable for concentrating egg or cysts in fatty faces.
- A zinc sulphate solution is used which has a specific gravity of 1.180 – 1.200: faces are emulsified in the solution and the suspension is left undisturbed for the eggs and cysts to float to the surface where they are collected on a cover glass.

Required materials and reagents

- Zinc sulphate solution 33% W/V specific gravity (1.80 – 1.200)
- Test tube of about 15 ml capacities, which has a completely smooth rim.

2.5.2. Blood sample processing

- Blood is the only fluid tissue that constitutes 6 – 8% of the total body weight and consists of cells suspended in fluid plasma the three types of blood cells are
  - Platelets (thrombocytes)
  - White blood cells (leucocytes)
  - Red blood cells (erythrocytes)
- The fluid portion (plasma) forms 45 – 60% of the total volume of blood.
  - Red cell possess (occupy) most of the remaining volume.

- Platelate and WBC occupy relatively small proportion of the blood mass.

- Obtaining blood from a finger (capillary blood)

  Some tests may be performed with a few drops of blood other test require several cubic centimeters of blood. When test call for a few drops of blood. The blood is obtained from a finger or lobe of the ear for blood film preparations.

- There are two types of blood films
  - Thick blood film
  - Thin blood film
- **Thick blood film preparation:** First step is identifying the patient and label the slide with request paper, prepare the blood film fig

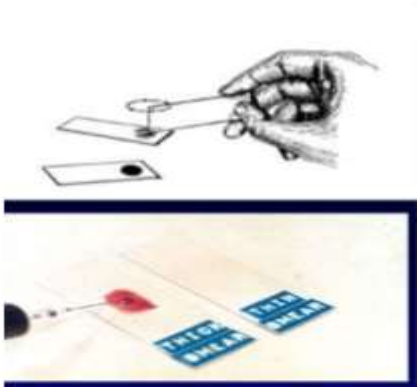


Fig 2.3. ***Preparation of thick blood film***

- **Preparation of Thin Films:** First step is identifying the patient and label the slide with request paper, prepare thin film as fig (2.4).

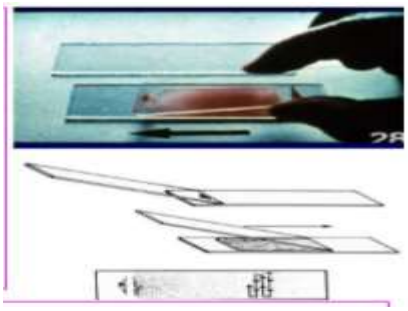


Fig. 2.4. ***Preparation of thin films***

- **Spreading a thin blood film:** This takes practice. A well-made thin blood film should have a smooth tail end (not ragged) and be free of vertical lines and 'holes'. A poorly spread film is extremely difficult to report because the red

cells, parasites, and white cells will appear distorted. It is essential to use a spreader which has a smooth ground glass polished edge.

- **Drying thick blood films:** It is good practice to keep a separate box or deep tray for drying malaria blood films. Cover it with a lid made from netting to protect the films from insects and dust (flies will rapidly 'clean' blood from a slide). If the box or tray is placed in a warm sunny place, the thick film will dry quickly (do not allow the blood to remain in the sun after it has dried). In humid climates, it may be necessary to use a hand dryer or an incubator to dry thick blood films.
- **Fixing thin blood films**: Use absolute methanol (methyl alcohol) or when not available, ethanol (ethyl alcohol) to fix thin blood films. The alcohol must be free from water otherwise it will not fix the cells properly. Always make sure the stock bottle of alcohol is kept tightly stoppered. For routine use, keep a small amount of alcohol in a dispensing bottle, which can be closed in between use.

Advantage of thin blood film	Advantage of thick blood film
➢ Required to confirm the *plasmodium* species	➢ Good for rapid detection malaria parasites , particularly when they are few *P. malariae* parasitaemia is normally low
➢ Enabling the parasites to be seen in the red cells	➢ About 30 times more sensitive (detecting about 20 parasites/µl)
➢ Greatly assists in the identification of mixed infection	➢ In a thick film the blood is not fixed
➢ Value in assessing whether a patient with falciparum malaria is responding to treatment	
➢ Gives the opportunity to investigate **anemia** and **white cell abnormalities**	

- **Staining malaria parasites:** Malaria parasites in thick and thin blood films require staining at pH 7.1–7.2 using a Romanowsky stain (contains azure dyes and eosin).
    - The stains most frequently used in district laboratories are:
        - **Field's stain**: This water-based Romanowsky stain is composed of two solutions, Field's stain A and Field's stain B. It is buffered to the correct pH and neither solution requires dilution when staining thick films. When staining thin films, Field's stain B requires dilution. Compared with Giemsa working stain, Field's

stains are more stable. They stain fresh blood films well, particularly thick films. The rapid technique is ideally suited for staining blood films from waiting outpatients and when reports are required urgently.

- **Giemsa stain:** This is an alcohol-based Romanowsky stain that requires dilution in pH 7.1–7.2 buffered water before use. It gives the best staining of malaria parasites in thin films. It also stains thick films well providing they are completely dry (overnight drying is recommended), the concentration of stain is low, and the staining time is sufficiently long. Less satisfactory results are obtained when the concentration of Giemsa stain is greatly increased to reduce the staining time. Care must be taken to prevent water from entering the stock stain. Giemsa stain is commonly used in malaria survey work because many films can be stained at one time and differentiation of the different species in thin films is excellent.

Notes: the mature gametocytes of Pv, Po and Pm are hard to distinguish from mature troph of these species.

Artefacts

Potential source:

Vegetable spores, yeast, pollen, algae and bacteria in the stain or on the slide

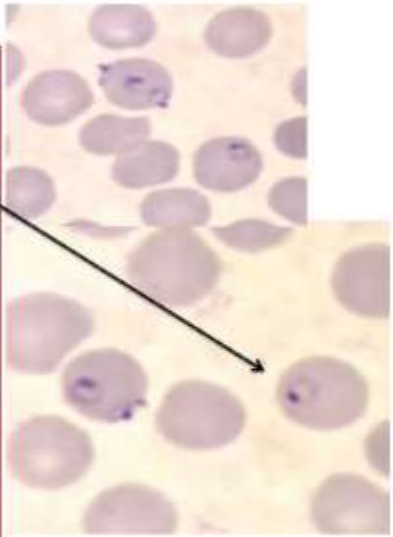
- Platelets
- Howell-jolly bodies in anaemic patients
- Ghosts of immature red cells mimicking schuffner's stippling

Examination

P. falciparem

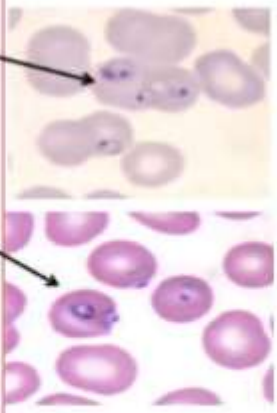
Young Trophozoite (Ring forms)

- Stage frequently found in blood film
- **Size**: Very delicate, 0.15-0.5 diameters of RBCs which is unaltered in size.
- **Shape**: small fine pale blue ring
- **Chromatin:** 1 or 2 small red dots.
- Often with double chromatin dot
- May lie on red cell membrane **(accole forms)**
- **Pigment**: absent



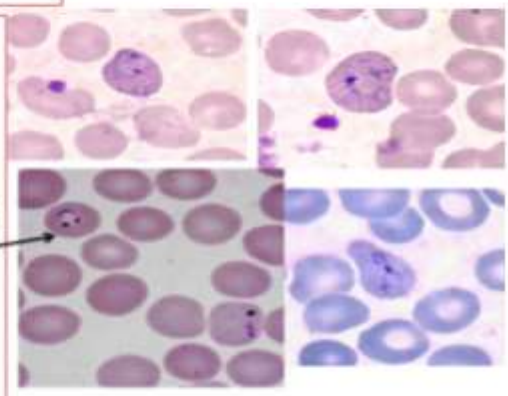
Mature Trophozoite

- Stage rarely seen in peripheral blood
- RBC unaltered in size, sometimes stippled, pale
- **Size**: small
- **Shape**: compact thin blue ring , comma or exclamation mark
- **Chromatin**: 1 or 2 red dots
- **Pigment**: black or dense brown mass
- Stippling Maurer's cleft



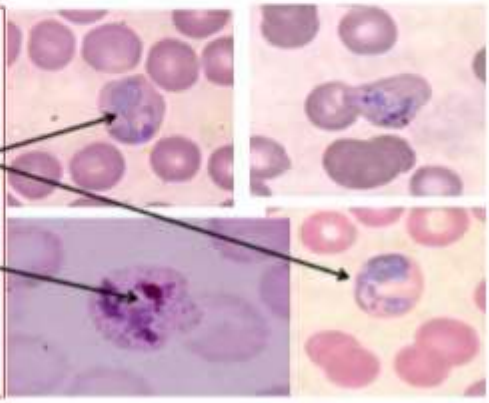
Plasmodium vivax

- **Young Trophozoites**
- Stage frequently seen
- Size : 0.3-0.5 diameter of RBC which is unaltered in size.
- Cytoplasm-irregular blue quite thick ring
- Chromatin : one rather large red dotted/ some times two
- Pigment: absent.
- RBCs are enlarged and distorted.
- Schüffner's dots may be seen



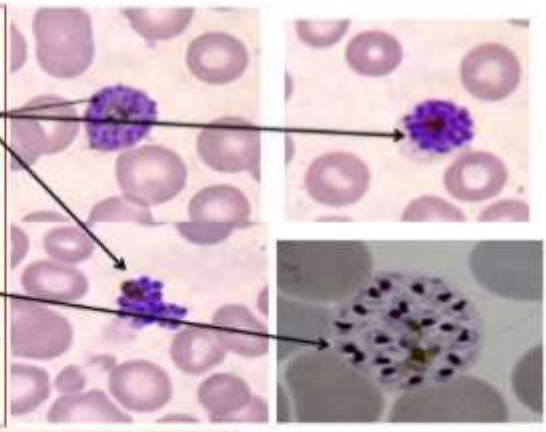
Mature Trophozoite

- Not frequently seen
- Size : large (RBC enlarged, stippled)
- Parasite- large blue irregular/ amoeboid
- Chromatin: Dots or threads of red colour
- Pigment: Golden brown and scattered



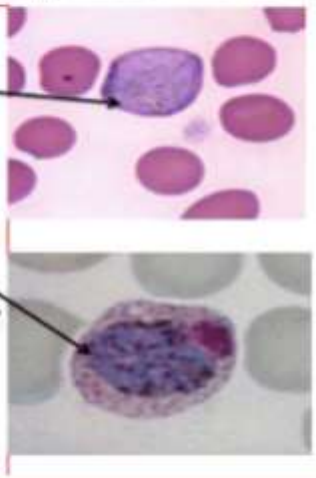
Schizonts

- quite frequently seen
- RBC much enlarged
- Size: Almost fills red blood cells
- Shape: amoeboid or segmented, parasite large, filling enlaged RBC
- Cytoplasm: pale blue
- Merozoites: 14-24; average 16
- Pigment: Golden brown central loose mass



Gametocytes

- Are **round to oval** with blue stain
- Scattered brown pigment
- Dense **red triangular nucleus** often at one end
- May almost **fill the red blood** cell (RBC).
- RBCs are enlarged and may be distorted.
- Under optimal conditions, **schüffner's dots** may appear more fine than those seen in *p. Ovale*.
- Difficult to differentiate from late trophozoites



Plasmodium malariae

Young Trophozoite

Size: Up to 1/3 Red blood cell.

Cytoplasm:thicker & dense (compact) blue ring

Chromatin: one large red dot

Pigment: *absent*

Mature Trophozoite

RBC unaltered

Parasite- compact often band or rounded s

Chromatin: round do or red band

Pigment: dark brown or black pigment, often concentrated along one edge of the band

Band forms can be seen in thin films

Occasionally "birds-eye" ring form may be seen 8-10 merozoites in mature schizont

- 'rosette'

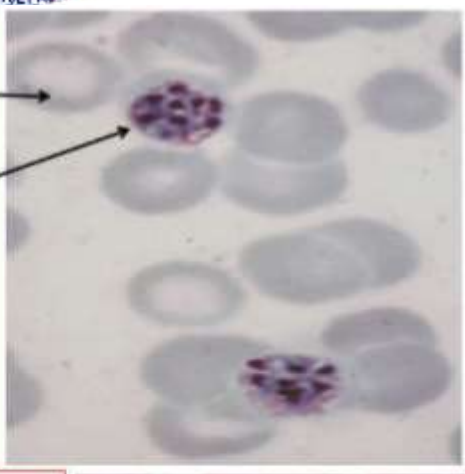
Schizont

RBC unaltered

Size: small compact nearly fills red cells

Merozoites 6-12; average 8; sometimes forming rosette

Pigment: Brown aggregated



Gametocytes

Shape- large, oval/round

Nucleus-1 round red chromatin at one edge

Pigment-black brown course

RBC unaltered, parasite small round filling RBC

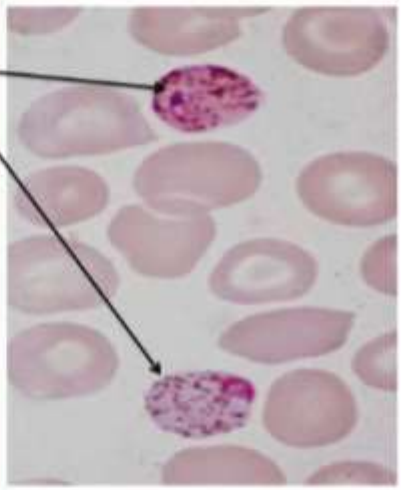
Infected Red Blood Cells

Oldest erythrocytes are infected

Stippling- None (**Ziemanns dots** often after prolonged leishmania staining

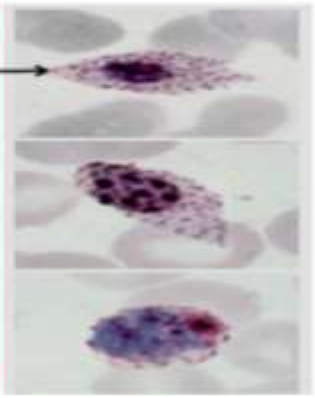
Parasite Density: low density,

Rarely more than 1% of cells infected (easily missed in Laboratory diagnosis



P. ovale

- Red cells enlarged
- Comet forms common (top right
  - Schüffner's dots
  - Rings large and coarse.
- similar to *P. vivax*
- subtle differences
  - 'compact' trophozoite
  - fewer merozoites
  - elongated erythrocyte
- Mature schizonts similar to those of P. *malariae* but larger and more coarse



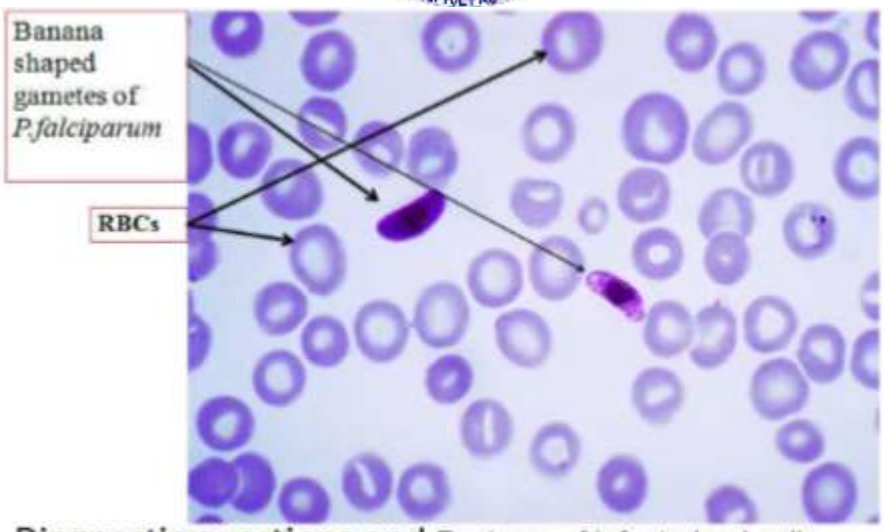
Microscopic differentiation

- Microscopic differentiation of species depend on Host cell and Parasite characteristics

1. Feature of infected red cell and ghosts
   - change in size, shape and colour
   - Presence of dots, maurer's clefts (not on ghost cell) on infected red cell
   - Single or multiple infection of each cell
2. Parasite morphology at specific stages
   - Number and size of chromatin beads
   - Shape and size of cytoplasm
   - Degree of pigmentation within cytoplas:
   - Stages of parasite seen together

Microscopic features of three blood stages

- trophozoites, schizonts, gametocytes
  - All these three stages have Red nuclear chromatin, blue cytoplasm and pigment (except young troph)
- What are the key features of:
- trophozoites?
  - single (Sometimes double) chromatin bead
  - Cytoplasm as a ring uniform or fragmented mass
  - Pigment absent from young (ring) form



Diagnostic questions and Features of infected red cells

- Are some infected RED CELLS enlarged?

  YES…..
- Marked enlargement….PV
- Moderate enlargement…PO

  NO……
- Infected red cell occasionally smaller than normal…..PM
- Infected red cell show no change in size…PF

- Are some infected RED CELLS changed in shape?
- YES…
- Angular distortion is observed…..PV
- Enlarged or oval with a ragged edge (fimbriation)…..PO
- Sometimes crenation ……Pf

- No change of shape in infected cell ….Pm
- Is there marked loss of colour?
- YES……
- Decolourization is characteristics of Pv and PO
- NO……
- Retention of colour is characteristics of Pf and Pm

- Multiplicity of infection and stippling or cleft
- Are some red cells infected with more than a single parasite?
- YES: Pf
- NO: Pm and Po, Pv typically does
- Is there evidence of red cell dots?
- Yes: schuffner's ……PV and Po

➢Maurer's cleft……Pf

➢Notes: The red cell ghosts in thick films can show schuffner's dots but do not show maurer's clefts

➢NO:….Pm

➢Thus Microscopy for Diagnosis of malaria:

➢An established method for the laboratory confirmation of malaria.

➢The careful examination of well prepared and stianed blood film by expert microscopist remaines currently the "GOLD STANDARD" for detecting and identifying malaria parasites

Advantages of using a microscope are:

➢It is senstive, when used by skilled and careful microscopist

➢Can detect densities as low as 5-10 parasites per µl of blood(WHO,1990)

➢It is informative, when parasites are found they can be identified in terms of their

➢Parasite densities can be quantified(from ratio of the parasites per number of leukocytes or erythrocyte)

➢Relatively inexpensive

Reporting blood films for malaria parasites

Reporting thick blood films: plus sign scheme

Parasites

1 – 10 per 100 high power fields ..... +

11 -100 per 100 high power field..... ++

1 – 10 in every high power field..... +++

More than 10 in every high power field ..... ++++

Example: *P. falciparum* trophozoites +++, gametocytes +, with malaria pigment in white cells

If no parasites are found after examining 100 fields (or if indicated 200 fields), report the film as: Malaria thick film: NPF (No parasites found

2. Immunologic/Biochemical techniques-Rapid Diagnostic Tests-RDTs

Immuno-chromatographic tests for malaria antigens

- Are based on the capture of the parasite antigens from the peripheral blood
- Uses either monoclonal or polyclonal antibodies against the parasite anti targets.
- RDTs do not require a laboratory, electricity, or any special equipment.
- **Targets**
  1. Histidine-rich protein 2 of *P. Falciparum*,
  2. Pan-malarial *plasmodium* aldolase, and
  3. Parasite specific lactate dehydrogenase (pldh)

Self-check 6	Written test

Answer the following questions

1. Macroscopic examination of stool specimen includes _____, _____and _____. (3 points)
2. Discuss stool wet mount technique with necessary reagents & materials needed. (3 points)
3. Discuss concentration technique with necessary reagents & materials needed. (6 points).

Note: satisfactory rating is 6 points, unsatisfactory <6 points. You can ask your instructor for copy of correct answer.

Score = _____

Rating: _____

Answer Sheet

1._____

_____

_____

2._____

_____

_____

3._____

_____

_____

_____

_____

_____

Name: _____ Date: _____

2.6. Storage of sample and its components

Sample storage:

Written policies should be developed that include:

- description of what samples should be stored;
- retention time;
- location–consider ease of access;
- conditions for storage, such as atmospheric and temperature requirements;
- System for storage organization, one method being to store samples by day of receipt or accession number.

Sample retention:

Set a laboratory policy for retention of each type of sample. Some samples can be quickly discarded, and others may need to be retained for longer periods. Monitor stored samples, and do not keep for longer than necessary, as refrigerator and freezer space may be limited. Sample freeze/thaw cycles must be monitored, as samples may deteriorate with these conditions.

Planning is required for samples that may need long-term storage. An organized, accessible system using computer tracking would be useful for these samples. The inventory of stored samples should be reviewed at specified intervals to determine when they should be discarded.

Sample referral:

When referring samples to other laboratories for testing:

- obtain a laboratory handbook with detailed procedures from each laboratory;
- ensure the sample is labelled correctly, in the correct container, accompanied by a requisition form that specifies the required test(s), and includes the sending laboratory's contact information;
- carefully monitor samples that are referred:
  - keep a record of all tests / samples referred, date of referral, name of person referring the test;
  - record and report results received for each referred sample;
  - Monitor turnaround times and record any problems encountered.

Sample disposal:

The laboratory is responsible for ensuring that disposal of all laboratory waste is handled in a safe manner. To ensure proper disposal of patient samples:

- develop a policy for sample disposal; apply local, as well as country regulations for disposal of medical waste;
- Establish and follow procedures to disinfect samples prior to disposa

Self-Check 7	Written Test

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Discuss sample storage. (3 points).

Note: **Satisfactory rating 1.5 points** **Unsatisfactory - below 1.5 points**
You can ask you trainer for the copy of the correct answers.

Answer Sheet

Score = _____
Rating: _____

1. _____
   _____
   _____
   _____

Name: _____ Date: _____

Operation Sheet 1	Procedures preparing stool wet mount Direct saline and Iodine mounts:

1. With a wax pencil write the patient's name or number and the date at the left – hand end of the slide
2. Place a drop of saline in the center of the left half of the slide and place a drop of Iodine solution in the center of the right half of the slide.

   NB: use warm saline (37^0) if the presence of amoebic trophozoite is suspected
3. With an applicator stick, pick up a small portion of the specimen (size of a match head) and mix with the drop of saline
4. Similarly, pick up a small amount of the stool and mix with the drop of iodine, to prepare an iodine mount
5. Cover the drop of saline and the drop of iodine with a cover slip. Hold the cover-slip at an angle, touch the edge of the drop, and lower gently on to the slide. This will reduce the chance of including air bubbles in the mount
6. Observe the preparation under the microscope with the condenser lowered and the light intensity adjusted, So that the opaque structure of the trophozoit adjusted and cysts can be seen.
7. Scan the total area of the cover slip using the l0x objective initially, and 4ox objective as required. Take note of any trophozoite and their motility and any red blood cells ingested by amoebae. *Note also the presence of cysts, ova, red blood cells polymorph nuclear cells and macrophages.*

2. Using a rod or stick, emulsify an estimated 1g (pea-size) of faeces in about 4ml of 10% formol water contained in a screw-cap bottle or tube. ***Note: Include in the sample, faeces from the surface and several places in the specimen.***

3. Add a further 3–4ml of 10% v/v formol water, cap the bottle, and mix well by shaking.
4. sieve the emulsified faeces, collecting the sieved suspension in a beaker.
5. Transfer the suspension to a conical (centrifuge) tube made of strong glass, copolymer, or polypropylene. Add 3–4ml of diethyl ether or ethyl acetate. ***Caution: Ether is highly flammable and ethyl acetate is flammable, therefore use well away from an open flame, e.g. flame from the burner of a gas refrigerator, Bunsen burner, or spirit lamp. Ether vapour is anaesthetic, therefore make sure the laboratory is well-ventilated.***

6. Stopper* the tube and mix for 1 minute. If using a Vortex mixer, leave the tube unstoppered and mix for about 15 seconds (it is best to use a boiling tube). ****Do not use a rubber bung or a cap with a rubber liner because ether attacks rubber.***

7. With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube).
8. Centrifuge immediately at 750–1000g (approx. 3000 rpm) for 1 minute.
9. Using a stick or the stem of a plastic bulb pipette, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, faecal debris, and formol water. The sediment will remain.
10. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to resuspend and mix the sediment. Transfer the sediment to a slide, and cover with a cover glass.
11. Examine the preparation microscopically using the 10X objective with the condenser iris closed sufficiently to give good contrast. Use the 40X objective to examine small cysts and eggs. To assist in the identification of cysts, run a small drop of iodine under the cover glass . Although the motility of

Strongyloides larvae will not be seen, the non-motile larvae can be easily recognized.

12. If required, count the number of each species of egg in the entire preparation. This will give the approximate number per gram of faeces.

Operation Sheet 3	Procedures preparing stool concentration technique with zinc sulphate flotation technique

1. Fill the tube about one quarter full with the zinc sulphate solution. Add an estimated 1 gram of faeces (or 2ml if a fluid specimen). Using a rod or stick, emulsify the specimen in the solution.
2. Fill the tube with the zinc sulphate solution, and mix well. Strain the faecal suspension to remove large faecal particles.
3. Return the suspension to the tube. Stand the tube in a completely vertical position in a rack.
4. Using a plastic bulb pipette or Pasteur pipette, add further solution to ensure the tube is filled to the brim.
5. Carefully place a completely clean (grease-free) cover glass on top of the tube. Avoid trapping any air bubbles.
6. Leave undisturbed for 30–45 minutes to give time for the cysts and eggs to float. *Note: Do not leave longer because the cysts can become distorted and the eggs will begin to sink.*
7. Carefully lift the cover glass from the tube by a straight pull upwards. Place the cover glass face downwards on a slide. *Caution: Avoid contaminating the fingers. Mature E. histolytica and G. lamblia cysts are infective when passed in the faeces.*
8. Examine microscopically the entire preparation using the 10X objective with the condenser iris closed sufficiently to give good contrast. Use the 40X objective, and run a drop of iodine under the cover glass, to identify the cysts.
9. Count the number of T. trichiura eggs to give the approximate number per gram of faeces. *Note: Parasites can also be recovered from the surface of the floatation fluid after centrifuging. If however, a centrifuge is available, the safer formol ether technique is recommended for concentrating eggs and infective cysts from faecal specimens.*

Operation Sheet 4	Procedures for preparing Capillary blood method (thick and thin films on same slide)

1. Cleanse the lobe of the finger (or heel if an infant) using a swab moistened with 70% v/v alcohol. Allow the area to dry.
2. Using a sterile lancet, prick the finger or heel. Squeeze gently to obtain a large drop of blood. Collect the blood preferably in a small plastic bulb pipette.
3. Using a completely clean grease-free microscope slide and preferably a malaria slide card, add a small drop of blood to the center of the slide and a larger drop about 15mm to the right
4. Immediately spread the thin film using a smooth edged slide spreader (see fig 2.3 &2.4). Blood from anaemic patients needs spreading more quickly with the spreader held at a steeper angle.
5. Without delay, spread the large drop of blood to make the thick smear. Cover evenly an area about 15 x15mm (see Fig. 2.3). It should just be possible to see (but not read) newsprint through the film. When spreading the blood, mix it as little as possible to avoid the red cells forming marked rouleaux which can cause the blood to be easily washed from the slide during staining.
6. Using a black lead pencil, label the slide with the date and the patient's name and number. If a slide having a frosted end is not used, write the information neatly on the top of the thin film (after it has dried).
7. Allow the blood to air-dry with the slide in a horizontal position and placed in a safe place.

Operation Sheet 5	Procedures for staining thin films with Field's staining technique

1. Place the slide on a staining rack and cover the methanol-fixed thin film with approximately 0.5ml of diluted Field's stain B.
2. Add immediately an equal volume of Field's stain A and mix with the diluted Field's stain B. Leave to stain for 1 minute. Note: The stains can be easily applied and mixed on the slide by using 1ml graduated plastic bulb pipettes.
3. Wash off the stain with clean water. Wipe the back of the slide clean and place it in a draining rack for the film to air-dry.

   Results for malaria thin film

   Chromatin of parasite . . . . . Dark red
   Cytoplasm of parasite . . . . . Blue
   Schuffner's dots . . . . . Red
   Maurer's dots (clefts) . . . . . Red-mauve
   Malaria pigment in white cells. . . . . Brown-black
   Red cells. . . . . Grey to pale mauve-pink
   Reticulocytes. . . . . Grey-blue
   Nuclei of neutrophils . . . . . Dark purple
   Cytoplasm of mononuclear cells . . . . . Blue-grey
   Granules of eosinophils . . . . . Red

Operation Sheet 6	Procedures for staining thick films with Field's staining technique

1. Holding the slide with the dried thick film facing downwards, dip the slide into Field's stain A for 5 seconds. Drain off the excess stain by touching a corner of the slide against the side of the container. ***Caution: Thick blood films are not fixed and the stains do not kill parasites, viruses, or other pathogens which may be present in the blood.***
2. Wash gently for about 5 seconds in clean water. Drain off the excess water.
3. Dip the slide into Field's stain B for 3 seconds. Drain off the excess stain.
4. Wash gently in clean water. Wipe the back of the slide clean and place it upright in a draining rack for the film to air-dry.

   Results for malaria thick film

   Chromatin of parasite . . . . . .Dark red

   Cytoplasm of parasite . . . . . .Blue-mauve

   Schuffner's dots around . . . . . .Pale red

   P. vivax and P. ovale parasites

   Malaria pigment . . . . . .Yellow-brown or brown-black

   Nuclei of small lymphocytes . . . . . .Dark purple

   Nuclei of neutrophils . . . . . .Dark purple

   Granules of eosinophils . . . . . .Red

   Cytoplasm of mononuclear cells . . . . . .Blue-grey

   Reticulum of reticulocytes . . . . . .Blue-grey stippling in background

Operation Sheet 7	Procedures for staining thick and thin films with Geimsa staining technique

1. Immediately before use, dilute the Giemsa stain as required: 3% solution for 30 minute staining

   Measure 50ml of buffered water (or saline) pH 7.1–7.2. Add 1.5ml of Giemsa stain and mix gently. The stain can be measured using a dry graduated plastic bulb pipette or a small volume (2ml) plastic syringe. 10% solution for 10 minute staining Measure 45ml of buffered water, pH 7.1–7.2 in a 50ml cylinder. Add 5ml of Giemsa stain (to 50 ml mark) and mix gently.

2. Place the slides face downwards (This is necessary to prevent fine particles of stain being deposited on the films). in a shallow tray supported on two rods, in a Coplin jar, or in a staining rack for immersion in a staining trough.

   Thick blood films must be thoroughly dried and thin blood films must be fixed (methanol for 2 minutes).

3. Pour the diluted stain into the shallow tray, Coplin jar, or staining trough. Stain as follows: 30 minutes if using a 3% stain solution 10 minutes if using a 10% stain solution

4. Wash the stain from the staining container using clean water (need not be distilled or buffered). Important: Flushing the stain from the slides and staining container is necessary to avoid the films being covered with a fine deposit of stain.

5. Wipe the back of each slide clean and place it in a draining rack for the preparation to air-dry.

   Results

   Chromatin of parasite . . . . . Dark red

   Cytoplasm of parasite . . . . . Blue

   Schuffner's dots . . . . . Red

   Maurer's dots (clefts) . . . . . Red-mauve

   Red cells . . . . . Grey to pale mauve

Reticulocytes . . . . . Grey-blue

Operation Sheet 8	Procedures for reporting thick blood films

1. When the thick film is completely dry, apply a drop of immersion oil to an area of the film which appears mauve coloured (usually around the edges).
2. Spread the oil to cover an area about 10mm in diameter (there is no need to add a cover glass). This is to enable the film to be examined first at a lower magnification.
3. Select an area that is well stained and not too thick. Change to the 100X objective (if required add a further small drop of oil).
4. Examine for malaria parasites and malaria pigment. Confirm the Plasmodium species by examining the thin blood film.
5. Report the approximate numbers of parasites (trophozoites, schizonts, and gametocytes) and also whether malaria pigment is present in white cells.
6. If no parasites are found after examining 100 fields (or if indicated 200 fields), report the film as: **Malaria thick film: NPF (No parasites found).**

Operation Sheet 9	Procedures for reporting thin blood films

1. When the stained thin film is completely dry, apply a drop of immersion oil to the lower third of the film and spread the oil to cover most of this part of the film.
2. Examine the film first with 40x objective to check the staining, morphology, and distribution of the cells and to detect malaria schizonts, gametocytes, and trophozoites.
3. Change to the 100x objective to examine the parasites. Identify the different Plasmodium species.
4. If no parasites are found after examining 100 fields (or if indicated 200 fields), report the film as: **Malaria thick film: NPF (No parasites found).**

Operation Sheet 10	Procedures for diagnosing urine for *S. hematobium eggs*

1. Collect 10–15ml of urine (between 10.00h and 14.00h) in a clean dry container.
2. Report the appearance of the urine. In moderate to heavy infections, the urine will usually contain blood and appear red or red-brown and cloudy. When visible blood is present, add 2 drops of saponin solution
3. Transfer 10ml of well mixed urine to a conical tube and centrifuge at RCF 500–1000g to sediment the schistotome eggs (avoid centrifuging at greater force because this can cause the eggs to hatch).
4. Discard the supernatant fluid. Transfer all the sediment to a slide, cover with a cover glass, and examine the entire sediment microscopically using

the 10x objective with the condenser iris closed sufficiently to give good contrast.

5. Count the number of eggs in the preparation and report the number/10ml of urine

Operation Sheet 11	Procedures for diagnosing skin snip for *O.Volvulus microfilariae*

1. Cleanse the skin using a spirit swab. Allow the area to dry.
2. Insert a sterile fine needle almost horizontally into the skin. Raise the point of the needle, lifting with it a small piece of skin measuring about 2mm in diameter.
3. Cut off the piece of skin with a sterile razor blade (or scalpel). Immerse the skin snip in a conical centrifuge tube containing about 1ml of fresh physiological saline and leave it at room temperature for up to 4 hours. Do not tease (pull apart) the skin because this is not necessary and can damage the microfilariae.
4. Using forceps, remove the skin snip, place it on a slide, and cover with a cover glass. Centrifuge the contents of the tube at medium to high speed, i.e. RCF 500–1000, for 5 minutes. Remove and discard the supernatant fluid. Transfer the entire sediment to a slide.
5. Examine both the skin snip and sediment microscopically for microfilariae using the 10x objective with the condenser iris closed sufficiently to give good contrast. Report the number of microfilariae as scanty, few, moderate numbers, or many.

LAP Test	Practical Demonstration

Name: _____ Date: _____

Time started: _____ Time finished: _____

Instructions: Given necessary materials, reagents and color plates you are required to perform the following tasks within --- hours.

Task 1: perform Stool wet mount procedure directly with saline and Iodine.

Task 2: perform stool sedimentation concentration technique with formol ether concentration technique.

Task 3: perform stool concentration technique with zinc sulphate flotation technique.

Task 4: prepare thick and thin blood films with capillary blood

Task 5: perform staining of thin films with Field's staining technique

Task 6: perform staining of thick films with Field's staining technique

Task 7: perform staining thick and thin films with Geimsa staining technique

Task 8: report thick blood films

Task 9: report thin blood films

Task 10: perform urinalysis for *S. hematobium eggs*

Task 11: perform skin snip for *O.Volvulus microfilariae*

List of Reference Materials

- Cheesbrough M, 1998. District Laboratory Practice In tropical countries.
- Jeffrey H.C and Leach R.M. 1975. Atlas of Medical Helmintology and Protozoology
- Parasitology Handbook, 1998. London School of Hygiene and Tropical Medicine.
- Smith JW,et al.Diagnostic Medical Parasitology. Chicago: American Society of Clinical Pathologoy,1976.
- Strickland GT.Hunter's tropical medicine. 7th ed. Philadeliphia: WB Saunders, 1991.
- Sullivan J.T, 1997. A color Atlas of Parasitology. Taticheeff , Seyoum, Yahya A,

Prepared By

No	Name	Educational Background	LEVEL	Region	College	Email	Phone Number
1	Kalicha Boru	Laboratory	B	oromia	Nagelle HSC	boru9683@gmail.com	0912493885
2	Furo Beshir	Laboratory	A	Harari	Harar HSC	nebi.furo@gmail.com	0911739970
3	Motuma Chali	Laboratory	B	oromia	Nekemte HSC	lammiifcaalii@gmail.com	0938456753
4	Abdirahman Mahad	Laboratory	A	Somali	Jigjiga HSC	abdirahman7584@gmail.com	0911044715
5	Adisu Tesfaye	Laboratory	B	Somali	Jigjiga HSC	adistesfaye21@gmail.om	0931747320
6	Kebebe Tadesse	Laboratory	B	BGRS	Pawi HSC	no	0926841290
7	Tagel Getachew	Laboratory	A	Harari	Harar HSC	tagegetachew@gmail.com	0915746748