Republic of Sudan

Federal Ministry of Health

Communicable & non Communicable disease control Directorate

Laboratory Diagnosis of Malaria

(Microscopy & Rapid diagnostic tests)

Training Manual

April 2017

Acknowledgment

This manual is an effort of the technical working group for Laboratory Diagnosis of Malaria with the guidance of WHO of both Basic Malaria microscopy (tutor and learner guide).

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Contents

Introduction

Rational of the manual

This manual is developed to help trainees to perform accurate laboratory diagnosis of Malaria and also give the trainers a glance on how to conduct the training efficiently**.**

Who are the manual intended for?

The Malaria laboratory diagnosis manual constitutes a framework in which trainers construct their course. It provides the minimum information required to train people in laboratory diagnosis of Malaria. It mainly targets people with a relatively moderate to high educational level on entry to the course; trainers can adjust the course for participants with higher levels of education. Workers can consider this course as refreshment**.**

Selection of participants

The selection of participants for the training will be representing from public,private,and NGOs health centers and hospitals When state Malaria reference laboratory conduct their routine supervision, they select the target of training according to it, beside some health facilities report high false positive results which enable them to be eligible for training.

Awarding of certificates

It is important to award a certificate that records the final achievement of each graduate. The certificate of achievement will award to successful graduates that they have reached the recommended level of competence. The certificate should be reevaluated regularly, such as every third year. a copy of certificate in the annexes (1).

Course methodology

Lectures and presentations should be kept to a minimum.

Practical sessions having the majority in the course in addition to demonstrations, small discussion groups and role-play involving the trainees are the most effective ways to teach.

Requirements for the training

Depending on the situation, the following logistics should be available:

- A multimedia projector,
- A personal computer (PC)
- Colored marker pens;
- Teaching microscope', fitted with two to five viewing heads.
- Flip charts
- Binocular Microscope(at least 2 participants in one microscope)
- Set of teaching slides
- Hall
- Laboratory equipped with water basins and running water

Reference slide sets for demonstration during training

A set of reference slides of Giemsa-stained thick and thin blood films containing the species that the trainees are likely to see locally should be available. It should consist of 25–30 slides of Plasmodium falciparum and P. vivax specimens, with, if possible, examples of the less common P. malariae. P. ovale

Opening and closing ceremonies,

These activities are an important part of the course as it will break the ice between participants and facilitators.

Time table

Suggested time table is provided so as to unify the sequence of learning and to ensure the smooth flow of learning units.

Suggested time table is in annex (2)

Facilitators

The facilitators who run the course will be from a core of experienced trainers, who may therefore be familiar with the objectives of the national program, also tutors who classified as grade 1 (External competency assessment - WHO) are highly recommended to be involve in the training.

In addition to full-time facilitators, other teachers may be requested to address a particular subject, such as 'Policies of the national malaria control program and epidemiology of Malaria.

Supplies, equipment and other materials

Supplies and equipment should be ordered well in advance of the course, as many items can be difficult to obtain at short notice. The basic equipment and reagents required for the training course are listed in Annex (3)

Instructional methods:

1-lectures

2-Laboratory training.

3-Group discussions and presentations .

Evaluation methods:

- Attendance.
- Reports on individual participation.
- Pre and post-tests.

Pre and post test

Pre and post test are very important tools as they are measuring the skill and knowledge gained before and after the training course.

Written questions in addition to known slides have to be examined under microscopes will be suitable for the Pre and post test. (Scores given more to microscopic examination than theoretical part)(10 slides with 70 scores and 10 questions with 30 scores)

Suggested questions are listed in annexes (4).

Overall Objectives

By the end of training, participants should be able to:

- Emphasize the situation of Malaria in Sudan, signs and symptoms, and correct diagnosis and treatment
- Recognize the importance of following Standard Operating Procedures (SOPs) in Malaria laboratory diagnosis
- Apply biosafety measures
- Demonstrate the safety measures when handling human blood
- Prepare thick and thin blood film in one slide according SOPs
- Recognize the importance of Romanowsky Giemsa stain and usage in Malaria Microscopy
- identify the microscope parts ,usage , care and maintenance
- recognize the components of blood
- detect malaria parasites correctly on blood films, and confirm parasite-negative films
- Accurately count malaria parasite;
- identify the Malaria parasite components
- distinguish P.f/P.m/P.v& P.o in thin and thick blood film
- demonstrate artefacts seen in blood films that are often mistaken for blood component or Malaria parasites
- identify other blood parasites
- identify the correct way of recording patients information, how to report Malaria results and how to calculate parasite count accurately
- Apply proper QA and QC measures in Malaria diagnosis
- Use and maintain the microscope
- explain the importance of quality assurance in Malaria diagnosis

- recognize to diagnose Malaria by RDTs

Unit 1 : Current situation of Malaria in Sudan

Interactive session (30 minutes)

Unit objectives are:

1. Explain the importance of Malaria as disease
2. Identify the magnitude of the Malaria in Sudan
3. Recognize the need for laboratory diagnosis
4. Highlighting about updated Malaria treatment protocol

Current situation of Malaria in Sudan

Malaria constitutes a major public health problem in Sudan. Almost, 75% of population is at risk of developing malaria. Malaria transmission is unstable putting the whole country under the risk of malaria epidemic. The possibility of epidemic increased with heavy rains, floods and in case of interruption of control activities.

Results of the Sudan Malaria Indicators Survey in 2016 (Sudan MIS 2016), showed a parasite prevalence ranging from <1 in Red Sea, Northern, River Nile and Khartoum States to >20% in Central Darfur State with an overall national prevalence of 5.9%. In South Darfur, West Darfur, Blue Nile and South Kordofan states the prevalence approached or exceeded 10%. The prevalence correlates with age, as children are 3 times more likely to get malaria than adults.

The main species is the P. falciparum (pf) representing 87.6% of cases. However, the P. vivax (pv) unexpectedly reaches 8.1% and mixed infection (pf & pv) approached 5%. P. vivax alone plus mixed infection exceeded 15% in North Darfur, West Darfur, South Darfur, River Nile and Khartoum states.

Suspected malaria:

Malaria is suspected when a patient presents with fever (or history of fever within 48 hours) with or without other symptoms and signs suggestive of malaria (e.g. headache, vomiting, sweating). The health care worker should consider other common causes of fever in the area as a cause of the current presentation and as co-infection with malaria. In Sudan, these could be pneumonia, influenza ,tonsillitis, chest infection, brucellosis, typhoid ,measles, abscess, urinary tract infection, etc….

Confirmed malaria:

Malaria is confirmed by demonstration of asexual stages (eg ring stage) of the parasite by microscopy or by detection of the parasite antigens using rapid diagnostic tests (RDTs) in suspected cases

Life cycle:

Containing 2 hosts occurring in human as an intermediate host and female anopheline mosquitoes as definitive host

Although there are different species of the malaria parasite, the basic life cycle of each follows the same basic pathway. Malaria disease is caused by a single-celled protozoan parasites of the genus Plasmodium which belongs to the order haemosporidia. Plasmodium is a heteroxenous parasite having two phases, extrinsic phase in the female of Anopheles mosquito (definitive host) which is called sporogony and the intrinsic phase in the man (intermediate host) which is called schizogony.

Types of plasmodium in Sudan

Basically there are 4 types of plasmodium that infect human and available in Sudan**;**

- Plasmodim falciparum
- Plasmodim vivax
- Plasmodium malariae
- Plasmodium ovale
- *Plasmodium knowlesi* (this is the fifth type which is the parasite of monkeys and can infect humans through the bite of anopheles mosquitoes in south east Asia)

Laboratory Diagnosis of malaria

Malaria can be diagnose microscopically, immunological methods and molecular biological techniques.

The microscopical method depends on the identification of parasites on human blood as it is the gold standard method because it is informative (parasite species, stage and density)

Immunological method includes demonstration of parasite antigens in blood by RDTs which is an easy, simple and rapid method but prohibited in patient who received treatment during the last 21 days.

Unit Two: Cleaning of slides

Unit objectives are:

1. To identify the effect of using new slides without cleaning
2. To recognize the standard methods for cleaning and storage of slides

interactive and practical session (90 minutes)(20 min interactive +70 min practical)

It is an important step towards accurate diagnosis because new slide containing waxy materials that interfere with blood components and thus affect the quality of stain.

This will be obtained by Leaving slides in a detergent solution for 1-2 hours in a detergent solution Then wash by clean tap-water, and wipe with a dry clean linen cloth, or wipe the surface with ethylated spirits (95% ethanol) and dry with a clean cloth .

Used slides can be cleaned with prolonged soaking in detergent for 24 hours and then wash with running tap water with a clean linen clothes and put it in paper, Then wipe dry with a clean linen cloth and keep in box of 15- 20 slides and keep together.

Select a slide with at least one smooth end to serve as a spreader, each spreader can be used for many times if the spreader edge remains smooth.

The edge must be wiped carefully and dried before and after each use and the slide must be discarded if the spreading edge becomes chipped or the slides are scratched

Safety in Malaria laboratory

Unit objectives are:

1. To explain the safety precautions when dealing with blood
2. To identify the risk of chemicals in the lab

Interactive session (30 minutes)

Following safety measure is crucial in Malaria laboratory to avoid direct and indirect contamination with human blood which stated clear in Malaria laboratory diagnosis SOPs.

A list of precautions can minimize the risk of contaminations such as :

1. wearing labcoat ,gloves
2. not allowed to eat,drink and smoke inside the laboratory
3. using of saftey designed box for disposable needles and lancets
4. cover the exposed wounds and injuries
5. dispose the waste materials as recommended

Quality assurance of Malaria laboratory diagnosis

Unit objectives are:

1. To emphasize the concept of quality assurance
2. To scale up the participation in quality assurance system
3. To ensure the accuracy of malaria laboratory diagnosis.

Interactive session (45 minutes)

A QA program deals with the dynamic ongoing process of monitoring the diagnostic laboratory's testing system for reproducibility in order to permit corrective action when established criteria are not met. This includes sampling specifications, testing methods, reporting and documentation for procedures ensuring that the necessary and relevant steps have been taken for quality services.

The components of a QA program:

- adhering to Standard Operating Procedures
- ensuring correct methods of specimen/sample collection
- ensuring quality of reagents used and calibration of equipment
- performing the tests with proper precision and accuracy
- interpreting of the results correctly
- monitoring and evaluation
- coordinating and supervising
- adequate training and re-training (experienced personnel)
- giving timely feedback

- detecting errors in the techniques and taking corrective steps
- Documenting procedures, results, etc.

Standard operating procedures in malaria diagnosis

The Standard Operating Procedure (SOP) is the most important document in a laboratory. It describes in detail the complete procedure for performing tests and ensures that consistent and reproducible results are generated. The instructions given in a SOP must be strictly adhered to by all those who are related with the functioning of the laboratory.

Strategy of cross-checking of malaria microscopy

There has been a well-established program for cross verification of the laboratory results of microscopy, wherein all the blood smears found positive at the Primary Health Centres (PHC) or other peripheral laboratories are supposed to be cross-checked for parasite species and stage by the designated centers. The negative slides are also cross checked as well. It was envisaged that all positives and 10% of all negative blood smears examined at PHC/ Malaria Clinic would be cross-checked.

Unit three: The Microscope

Unit objectives are:

1. demonstrate the correct set-up and use of the microscope
2. describe the correct way to maintain the microscope in good working condition

Small group discussion session (30 minutes)

is an instrument used to see and investigating small objects that are very small to be seen by naked eye.

There are many types of microscopes. Binocular is the most common which uses light to magnify the sample. Other major types of microscopes are the electron and ultra-microscope

Use and care of microscope

in order to obtain long duration for the microscope , the correct use and care are important elements thus to avoid malfunctioning.

Unit Four: Preparation of thick and thin film

Interactive and practical sessions (120 minutes)

Unit objectives are:

1. To recognize correct way of making thick and thin blood films
2. Demonstrate the correct ways of labelling blood films;
3. Demonstrate how to separate thick and thin blood films of acceptable quality from unacceptable ones, giving reasons for their rejection;

The thick film is more sensitive in detecting parasite and also minimizes time in examination while the thin film may cause very little distortion of the parasite while spreading of the smear and permits species identification which may not be possible in thick films.

Comparison between thick and thin blood film

Differences	***Thick film***	***Thin film***
Blood amount and distribution	Large amount of blood distributed in small area	Small amount of blood distributed in large area
Thickness	20-25 layers of RBCs	One layer
Fixation	Not fixed	Fixed with methanol
Examination time	Short time	Long time
purpose	Detection and parasite count	Identification

Unit Five: Stains and staining methods

Interactive and practical sessions (120 minutes)(30 min interactive session +90 min practical)

Unit objectives are:

1. Demonstrate the correct method for preparing and storage concentrated giemsa stain
2. To recognize the staining methods and optimum PH for staining Malaria parasites

There are many stains that stain blood components , but the recommended stain for Malaria Microscopy is Giemsa ,as it can stain both thick and thin film at the same time , group staining and result in good colouration of blood component and different parts of Malaria parasite.

Basically it is alcohol-based Romanowsky stain, which is a mixture of eosin that stains parasite chromatin and stippling red or pink, and methylene blue that stains parasite cytoplasm and white cells blue.

Components of Giemsa stain

Giemsa (powder)	3.8 g
Methanol absolute	250 ml
Glycerol pure	250 ml

The concentrated Giemsa stain should be stored in dark bottle in cool and dry place avoiding direct sun light and evaporation .

Staining methods :-

There are Two methods of Staining with geimsa:

1. The rapid method 10% for 10 minutes

   It is fast which used for routine laboratory work.

   Method:

   - Fix thin film with absolute methanol.
   - Make 10% working solution of Geimsa stain with buffer PH 7.2 .
   - Each slide need 3ml to3.5ml to cover it.
   - Use the dropper to drop the stain on to slide.
   - Stain blood film for10 mines.
   - Pour clean water gently to float off the scum of stain and solution of stain become feasible and clear
   - Drying in drying rack

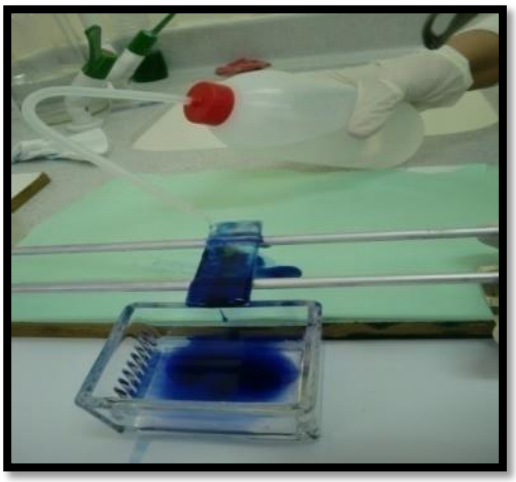
2. The regular (slow) method 3% for 30 minutes.

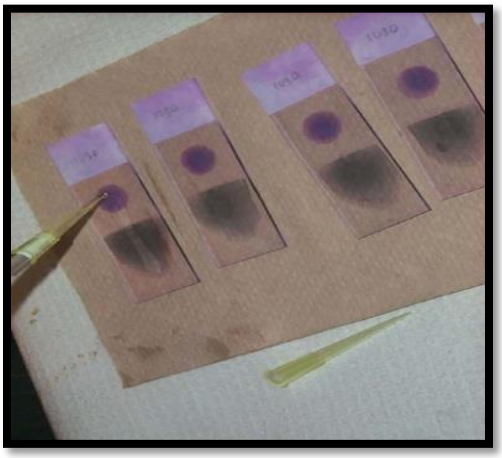
Preferred to use in surveys, research and training purposes **.**

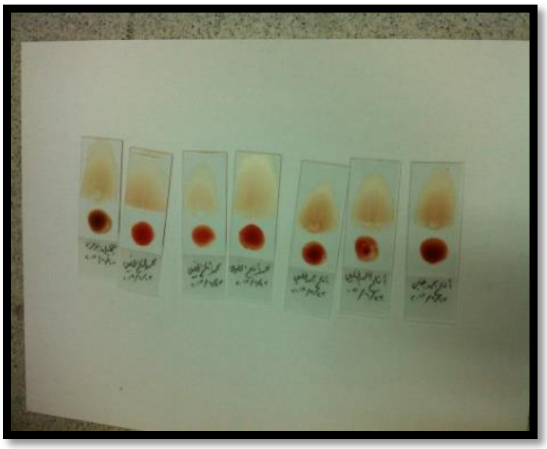
Method:

- Fix each thin film with methanol.
- Place the slides back to back in staining trough.
- Prepare 3% Giemsa stain with buffer solution 7.2 PH
- Pour the stain in the trough avoiding pouring it direct to the thick film.
- Stain for 30 mines.
- Pour clean water gently to float off the scum of stain and solution of stain become feasible and clear

- Drying in drying rack







❖ **Microscopic examination of malaria depends on a proper preparation of a thick & thin blood film stained with Giemsa stain.**

Unit Six: Blood components

Interactive and practical sessions (60 minutes)(20 min interactive session +40 min practical)

Unit objectives are:

1. list the three major components of normal blood;
2. recognize and classify the normal components of blood
3. recognize correctly the main parts of a white blood cell

Blood component is important to be recognized as it is first step to differentiate them with Malaria parasite

The main components of blood include;

Red blood cells

The shape of the red blood cell, or erythrocyte, is described as a biconcave disc. It is the commonest cell in thin blood films. There are about 5 000 000 in each microlitre (µl) of blood. With Giemsa staining, the red cell appears as a pale-greyish to light-pink disc measuring about 7.5 µm in diameter.

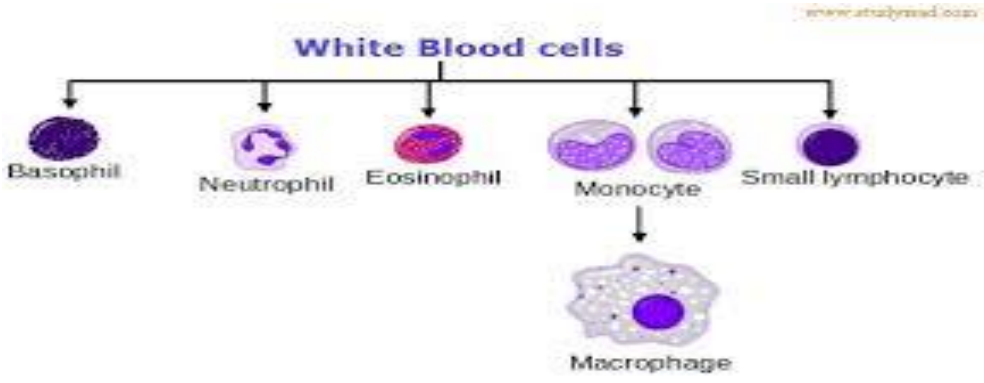
White blood cells

The number of white blood cells, or leukocytes, per microlitre of blood is normally 6000–8000, much fewer than red cells. The number can vary widely under certain conditions and in some individuals. There are several types of leukocyte. As each stains differently, they are easy to distinguish with practice. The parts of a typical white blood cell are shown in the illustration.

Each leukocyte has a nucleus surrounded by cytoplasm; sometimes, the cytoplasm is granular. Some leukocytes have a multilobed nucleus, as shown in the illustration above. Leukocytes are divided into two groups, polymorphonuclear and mononuclear leukocytes.

Platelets

Platelets are small, irregularly shaped bodies, without a nucleus but with fine red granules on a blueish background. Like eosinophils, platelets can be used as sensitive indicators of the quality of staining. Numbering about 100 000 per microlitre of blood, they usually occur in groups of 5–10 but form larger clumps when a blood film is poorly made. Inexperienced microscopists may confuse them with malaria parasites.



Unit Seven: Malaria parasite components

Unit objectives are:

1. list the main parts of Malaria parasite component with an aid of diagram
2. detect and identification of Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale and Plasmodium vivax

Interactive sessions (15 minutes)

Malaria parasite components **Interactive session** 15 mins

Plasmodium falciparum& Plasmodium malariae **Interactive session** 60 mins

Plasmodium falciparum& Plasmodium malariae **practical session** 360 mins

Plasmodium ovale and Plasmodium vivax **Interactive session** 60 mins

Plasmodium ovale and Plasmodium vivax **practical session** 360 mins

The participants must identify the diagnostic points of the plasmodia and to differentiate between various stages and species.

Unit Eight: artefacts and other blood parasites

Unit objectives are:

1. Demonstrate common contaminants and artefacts seen in blood that may misdiagnose

Artefacts and other blood parasites Interactive sessions (30 minutes)

Some objects in blood films may cause confusion and uncertainty. There may be other blood parasites in your area, which are routinely collected in surveys or from patients. These non-malaria parasites should have been demonstrated to trainers. Although they are not artefacts, you must be familiar with them, as this is very important to the malaria control program. This locally important subject will be explained in detail by your trainer.

Recording patient information, Malaria Result and parasite count

Interactive session's small group discussion +practical session (120 minutes)

Unit objectives are:

1. Demonstrate the correct method of recording patient information
2. Unify the way of Malaria result writing
3. Recognize how to calculate parasite density

It is important to ensure that patients' details can be traced easily by recording all appropriate information when they attend the health center. Most information is stored in a daily register database and requires specially designed forms, which usually cover:

- State ,locality and administrative unit where the patient live
- Serial number ,Name ,sex and age

A patient's details are confidential. It is unethical to discuss information in a patient's records with unauthorized persons. Patient records should be stored securely, safe from unauthorized access.

The way of writing the Malaria result is very important as it is indicates the result of diagnosis to be easily read by the physician.

Parasite count is crucial step in malaria microscopy as it help in patient follow-up of parasitemia to monitor the drug efficiently .

Unit Nine: Malaria rapid diagnostic tests (RDTs)

Interactive session + small group discussion +practical session (90 minutes)

Unit objectives are:

1. Demonstrate the importance of using RDTs
2. Demonstrate the types of RDTs
3. Recognize the principles & method to perform
4. Identify the way of interpret the result and how to write it

Early diagnosis and prompt effective treatment is a main strategy of Malaria control to reduce Malaria morbidity and mortality, in area where microcopy services is abscent,care providers have to use RDTs as a step in expansion in Malaria Microscopy services.

RDTs are a simple and fast way for health workers to test for malaria parasites (antigens)in a patient's blood. They are more accurate than presumptive diagnosis .RDTs can also help identify patients who do not have malaria so that these patients can receive correct treatment.

To work properly, RDTs need blood and a chemical called 'buffer'. Adding too much or too little blood or buffer can cause the test to give an invalid result or be difficult to read. Adding blood and buffer in the wrong place can also cause an invalid result.

Annex 1: time table

Annex 2: evaluating of the course questionnaire

Annex 3: list of the supply requirements for the training

Annex 4: example of awarded certificate

Annex 5: suggested pre test

Annex 3 : List of supplies needed for the training

- **Consumables**

1. Giemsa powder
2. Methanol
3. Glycerol
4. Glass beads
5. Forested ends slides
6. Lancets
7. Cotton wool
8. Alcohol swabs or 70% ethanol
9. Gauze
10. Buffer tabs
11. Immersion oil
12. Detergent

- **Glassware**

1. Measuring cylinder
2. Coplin jars
3. Drying rack
4. Stop watch
5. Talley counter
6. Staining rack

Annex 5 :Theoretical pre-test

Time: 30 mins

Scoring :10 marks per each question

1) With an aid of a simple diagram mention the components of malaria parasite.

2) What is the purpose of thick & thin blood film?

3) What are the ingredients of Giemsa stain?

4) What is the optimum PH for staining malaria parasites with Giemsa stain?

5) What is the name of dots present in P. vivax?

6) How can you count the malaria parasites?

7) Define relapse & in which species it occurs?

8) Mention 2 formats of malaria RDTs

9) Mention 2 antigens that released by malaria parasites.

10) What is the principle of malaria RDTs & what do you do if there is no line appears in your control line ?

Annex 2 : Questionnaire for evaluating training

Instructions for completing the questionnaire

Use the following code to indicate the extent to which you agree or disagree with each of the statements made in the questionnaire:

1) Disagree strongly :2.5 scores
2) Disagree 5 scores

4) Agree :7.5 scores

5) Strongly agree :10 scores

No.	Question	1	2	4	5
1	Overall, the organization of the training programme was satisfactory				
2	The training programme covered all the subject matter in adequate detail. (If you disagree with this, state which subjects should have been given greater coverage.)				
comments					
3	The tutors and facilitators for this training course had sufficient knowledge and teaching ability to give you the necessary skills and competence.				
Comments					
4	The time allocated to each part of the training was adequate relative to the total time available. (If you disagree with this, state which topic should have been allotted more or less time				
Comments					
5	Overall, the teaching methods used in this training course were effective.				
6	The use of the various teaching methods listed below was quite appropriate:				
7	Large group presentations				
8	Practical demonstrations (laboratory)				
9	Small group discussion				

10	Quizzes, tests and other evaluation exercises				
11	The teaching materials provided were satisfactory in all respects				
Suggestion for improvements					
12	The general atmosphere of the training course made this a good learning experience				
Comments					
13	Every effort was made to help me achieve the learning objectives				
Comments					
14	I was able to achieve all the learning objectives of the training programme.				
Comments					

What overall rating would you give to this training programme?

Circle your response

Lowest 1 2 4 5 highest

Annex 1 : Suggested Time Table

Period	Day1	Day 2	Day 3	Day 4	Day 5	Day 6
08:00-08:50	Opening ceremony	Preparation of thick & thin blood films T/P	Malaria parasite components	Examining blood films (pv+po) A	Artefact and other blood parasite and	Species revision T/P
09:00-09:50	Introduction to the course	Stains and staining methods **T/P**	Examining blood films (pf+pm) B	Examining blood films (pv+po) B	Keeping accurate result & parasite count B	Rapid Diagnostic tests A
10:10-11:00	**Breakfast**	**Breakfast**	**Breakfast**	**Breakfast**	**Breakfast**	**Breakfast**
11:10-12:00	Pre test **T/P**	Microscope Small g d	Examining blood films (pf+pm) B	Examining blood films (pv+po) B	Malaria result	Rapid Diagnostic test B
13:00-13:50	**prayer**	**prayer**	**prayer**	**prayer**	**prayer**	**prayer**
14:00-14:50	Malaria ,the disease Malaria Life Cycle A/DA/**D**	Blood components **T**	Examining blood films (pf+pm) B	Four species examination A	Recording and malaria reports	Post test
15:00-15:50	Cleaning OF Slides and safety & QA A/D	Blood components **P**	**Comparison** (pf+pm) B	Comparison between malaria species	Quiz	Closing ceremony

T: Theory P: Practical SGD:Small group discussion