GOVT. OF MAHARASHTRA

MULTISKILL TRAINING MODULE FOR LABORATORY TECHNICIANS

FIRST EDITION2018

STATE INSTITUTE OF HEALTH AND FAMILY WELFARE, NAGPUR, MAHARASHTRA

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Acknowledgement

The Multiskills Training Module for Laboratory technicians is a consolidation of the contents of various sections like General Hematological Investigations, Investigations in Diagnosis of Malaria, Falairasis, Tuberculosis, HIV AIDS, Sickle cell diseases etc. It represents the hard work of a large number of faculty members and institutions who were involved in developing this module and provided valuable insights and feedback. This module was compiled by Dr. Harsha Meshram (Wakodkar) & Dr. Purushottam Nandeshwar, Assistant Professor, SIHFW, Nagpur under the guidance of Dr. Sanjay Jaiswal, Director, SIHFW Nagpur with the help of valuable contribution from faculties.

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CHAPTER 1: GENERAL HEMATOLOGY

Standard Operating Protocols for Laboratory Technician

1. Bleeding Time (BT)

a) PROCEDURE

1. Identify the patient from on duty staff nurse.
2. Explain the procedure to the patient.
3. Take patients ring finger.
4. Massage the pulp of finger.
5. Disinfect the area by using alcohol swab and allow it to dry.
6. Take a 3 mm deep bold prick with the help of lancet.
7. Start the stop watch at the same time.
8. The Blood should flow freely without squeezing the finger
9. The blood drop is allowed to drop on a filter paper.
10. The filter paper should be moved so that each drop will flow on placed area.
11. The bleeding of wound should be allowed without squeezing. Each drop should be taken with the interval of 30 sec .
12. When blood stains disappear from the filter paper stop the watch and note the time.
13. This is the bleeding time.

b) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the lab register.

c) NORMAL RANGE

The normal range is 1-5 min.

2. CLOTTING TIME (CT) (Capillary method)

a) PROCEDURE

1. Identify the patient from on duty staff nurse.
2. Explain the procedure to the patient.
3. Take patients ring finger.
4. By using a piece of cotton, apply spirit or 70% alchohol to the patient fingertip.
5. Make a deep (1 mm) incision with a sterile lancet and start the stopwatch.
6. Wipe off the first blood drop and collect blood in the capillary up to 2/3 of its length.
7. After every half minute, break off about 1 cm of the capillary to find out whether fibrin string has formed.
8. When the fibrin string appears , stop the stopwatch and note down the time.

9. This is clotting time.

b) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

c) NORMAL RANGE

The normal range is 4 to 9 mins.

3. DETERMINATION OF HEMOGLOBIN

a) PRINCIPLE

When blood is added to 0.1 N hydrochloric acid , hemoglobin is convered to brown colored acid hematin . The resulting colour after dilution is compared with standard brown glass reference blocks of a sahli hemoglobinometer.

b) REQUIREMENTS

1) Sahli hemoglobinometer : It consists of
   (a) A satandard brown glass mounted on a comparator
   (b)A graduated tube
   (c) Hb – pipette (0.02 ml)
   Refer to the colored plates
2) 0.1N hydrochloric acid
3) Distilled water
4) Pasteur pipettes

Note*: In Sahli's hemoglobinometer the graduations on currently used Hellige tube gives 14.5 g as 100% .These are square tubes with graduations in percent on one side and grams per 100ml (dl) on the other side.*

c) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is clotted if the specimen is clotted notify to the ward and ask for repeat And sufficient specimen
3. By using a pipette add 0.1N hydrochloric acid in the tube up to the lowest mark (20% mark).
4. Draw blood up to 20ul mark in the Hb – pipette. adijust the blood column, carefully without bubbles. wipe excess of the blood on the sides of the pipette by using a dry pipte by using a dry piece of cotton.
5. Transfer blood to the acid in the graduated tube, rinse the pipette well, mix the reaction mixture and allow the tube to stand for at least 10 minutes.
6. Dilute the solution with distilled water by adding few drops at a time carefully and by mixing the reaction mixture, until the color matches with the glass plate in the comparator.

7. The matching should be done only against natural light. the level of the fluid is noted at its lower meniscus and the reading corresponding to this level on the scale is recorded in g/dl.

d) ADDITIONAL INFORMATION

1. Methemoglobin, carboxyhemoglobin and sulfhemoglobin are not converted to acid hematin by 0.1 N hydrochloric acid.
2. This method is useful for places where a photometer is not available.
3. It can give an error up to 1g/dl.

e) PRECAUTION

Immediately after use rinse the HB pipette by using tap water in a beaker. This prevents blocking of the pipette.

f) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

g) NORMAL VALUES

Ctegory	**Hb, g/dl**
* Men	:13.0 - 18.0
* Women	: 12.0 - 16.5
* Children(up to 1 year)	: 11.0 - 13.0
* Children (10-12years)	:11.5 – 14.5
* Infants (full term cord blood)	:13.5 - 19.5

4. DETERMINATION of ERYTHROCYTE COUNT

a) REQUIREMENTS

1. Microscope
2. Improved Neubauer Chamber
3. RBC pipette
4. RBC diluting fluide

It is Prepared as follows

a) Sodium citrate : 3.0g
b) Formalin : 1.0ml
c) Distilled water to : 100ml

This solutions is stable at room temperature (25 C +- 5 C) For at least one year.

NOTE

1. RBC diluting fluid is isotonic with blood hence hemolysis does not take place. Normal saline also can he used but it causes slight creation of red blood cells and allows rouleaux formation.
2. Formalin acts (as a preservative and checks bacterial and fungal growth.
3. Sodium citrate prevents coagulation of blood and provides correct osmotic pressure.

b) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is clotted if the specimen is clotted notify to the ward and ask for repeat And sufficient specimen.
3. Mix the anticoagulated blood carefully by swirling the bulb.
4. In the case of capillary blood the lancet stab should be sufficiently deep to allow free flow of blood. It is drawn quickly in the RBC pipette.
5. Draw blood up to 0.5 mark.
6. Carefully wipe the excess blood outside the pipette by using cotton or a gauze.
7. Draw diluting fluid up to 101 mark.
8. The pipette in rotated rapidly by keeping it horizontal during mixing.
9. After five minutes, by discarding few drops from the pipette and holding it slightly inclined small volume of the fluid is introduced under the cover slip which is placed on the counting chamber.
10. Allow the cells to settle for 2 to 3 minutes.
11. Place the counting chamber on the stage of the microscope.
12. Switch to low power (10x) objective. Adjust light and locate the large square in the center with 25 small squares.
13. Now switch to high power (40x) objective.
14. The red blood cells in the four corner squares and in the center square (marked in the diagram as 'R') are counted.
15. Use following formulae for the calculation of red blood cells.

* Total red blood cells per liter of blood

$$= \text{RBCs / cumm (u1)} \times 10^6$$

or use following formula –

*Red cell count (per litter)

$$= \frac{\text{No.cell counted}}{\text{Volume counted (ul)}} \times \text{Dilution} \times 10^6$$

*Total red blood cell /cu mm (ul)

$$= \frac{\text{No. of red cells counted} \times \text{Dilution}}{\text{Area counted Depth of fluid}}$$

Where (1) dilution =1:200(i.e,200)

(2)Area counted = $\frac{80}{400} = \frac{1}{5}$ sq .mm

Since cells are counted in 5 bigger squares and such square is further divided into 16 small squares.

Each small square = $\frac{1}{400}$ sq. mm

Hence area of (5x16) = 80 such areas = $\frac{80}{400}$

sq. mm = $\frac{1}{}$ sq.mm

10
(3) Depth of fluid =1 mm
10
(4) number of red cells counted =N
Hence Total red blood cells , cu mm
= N x 200 N x 200x50= Nx10,000
1 x 1
5 10

c) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

d) NORMAL VALUES

* Men : 4.5 to 6.0 x 10^6cells /cu mm (ul)
* Female : 4.0 to 4.5 x 10^6cells /cu mm (ul)

5. DETERMINATION OF TOTAL LEUKOCYTE COUNT (TLC)

a) REQUIREMENTS

1. Microscope
2. Improved Neubauer Chamber
3. WBC pipette
4. WBC diluting fluide

It is Prepared as follows

a) Glacial acetic acid : 2.0ml
b) 1%(w/v)gentian violet : 1.0ml
c) Distilled water to : 97ml

This solutions is stable at room temperature (25 C +- 5 C) A pinch of thymol may be added as preservative.

b) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is clotted if the specimen is clotted notify to the ward and ask for repeat And sufficient specimen.
3. Mix the anticoagulated blood carefully by swirling the bulb.
4. In the case of capillary blood the lancet stab should be sufficiently deep to allow free flow of blood. It is drawn quickly in the WBC pipette.
5. Draw blood up to 0.5 mark.
6. Carefully wipe the excess blood outside the pipette by using cotton or a gauze.
7. Draw diluting fluid up to 11 mark.
8. The pipette in rotated rapidly by keeping it horizontal during mixing.
9. After five minutes, by discarding few drops from the pipette and holding it slightly inclined small volume of the fluid is introduced under the cover slip which is placed on the counting chamber.
10. Allow the cells to settle for 2 to 3 minutes.
11. Place the counting chamber on the stage of the microscope.

12. Focous on one of the W marked area (each having 16 small squares) by turning objectives to low power (10x)
13. count cells in all four W marked corner.

c) CALCULATIONS

$$\frac{\text{Number white cells counted x Dillution}}{\text{Area counted x Depth of fluid}}$$

Where : Dillution =20
Area counted =4x1 sq . mm = 4 sq. mm
Depth of fluid =0.1 mm

Hence no. of white cells per cu mm (ul)of whole blood = $\frac{\text{No. of cells counted x 20}}{4 \times 0.1}$

=No. of red cells counted x 50

d) DOCUMENTATION

1. The record is done on the case paper of the patient.
2.Same should be recorded in the indoor lab. register.

e) NORMAL VALUES

* Adult	:	4000 – 10,000/cu mm
* At birth	:	10,000 – 25,000/cu mm
*1 to 3 years	:	6,000 -18,000/ cu mm
* 4 to 7 years	:	6,000 – 15,000/ cu mm
* 8 to 12 years	:	4.500 – 13,000/ cu mm

6. ERYTHROCYTE SEDIMENTATION RATE

a) REQUIREMENT

1) Westergren's ESR tube .
2) Stand for holding the tube .
3) Timer or watch.

b) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is clotted if the specimen is clotted notify to the ward and ask for repeat And sufficient specimen.
3. Mix the anticoagulated blood carefully by swirling the bulb.
4. Fill the Westergren tube exactly upyo zero mark by means of a rubber bulb (avoide air bubbles).
5. Place the tube upright in the stand for exactly one hour.
6. Exactly after one haur , note the level to which the red cells column has fallen.

7. Report the result in terms of mm/after 1 st hour.
8. This is the erythrocyte sedimentation rate of the patients.

c) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

d) NORMAL RANGE

* Male	0-15 mm after 1 st hour.
* Female	0-20 mm after 1 st hour.

7. DETERMINATION OF PLATELATE COUNT

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a) REQUIREMENT

1) Microscope.
2) Improved Neubauer Chamber
3) Platelate diluting fluid

It is prepared as follows

a) Procaine hydrochloride : 3.0 g
b) Sodium chloride : 10 g
c) Distilled water : 100 ml

Filter it trough Whaterman No. 44 filter paper and store in a clean dry plastic container . It is stable at 2-8^0 c.

b) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form received.
2. Check the specimen is clotted if the specimen is clotted notify to the ward and ask for repeat And sufficient specimen.
3. Mix the anticoagulated blood carefully by swirling the bulb.
4. By using RBC pipette draw blood up to 0.5 mark.
5. Wipe excess blood on the outside of the pipette.
6. The diluting fluid is drawn up to mark 101(blood is diluted 1:200).
7. Mix the content in the blub thoroughly.
8. After 5 minutes discard the first drop , then transfer a small drop on one side of the counting chamber.
9. Place the filled mounted counting chamber under a petri dish with a moist filter paper. Let it stay undisturbed for 15 minutes(This permits the platelets to settle and also prevents evaporation of diluting fluid in the chamber
10. Place the counting chamber carefully on the stage of the microscope. Under low power magnification focus red cell counting area and change to high power objective.
11. Keep the condenser down and reduce the light by adjusting the diaphragm. The platelets will apperlike highly refractile particles.
12. Count platelets in all 25 small squres . The area covered by 25 squres is equivalent to 1sq. mm.

c) CALCULATIONS

Platelets per cu mm(ul) = $\frac{\text{Number of platelets counted X Dilution}}{\text{Volume of fluid}}$

where

(a) Dilution= 200

(b)Volume of fluid = 1X0.1 = 0.1 cu mm

(c) Plates per cu mm (ul) = $\frac{\text{Number of platelets X 200}}{0.1}$

= Number of platelets X 2000

d) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

e) NORMAL RANGE

150,000-450,000/ cu

8. DETERMINATION OF BLOOD GLUCOSE LEVEL (GOD-POD)

a) REAGENTS

1) Buffer – enzymes

2) Phenol reagents : ready to use : 100 mg / dl

3) Glucose standard : 100 mg / dl

b) STABILITY OF THE REAGENTS

Reagent 1&2 are stable at 2-8 ^{0}c for six months. Reagents 3 is stable for one year when refrigerated

c) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Take 3 tubes marking as test, std. & blank respectively.
5. Take 1 ml of reagents in each tube respectively .
6. Add 10 ul serum in test-tube marked as test &add 10 ul std reagent in test tube marked as std.
7. After addition incubates the tubes for 10 min at room temp.
8. After 10 minutes measure the the OD values by colorimeter at 530nm(Greeen Filter).

d) Calculations

Plasma (or serum) glucose , mg/dl = $\frac{\text{O. D. Test}}{\text{O. D. Std.}}$ X 100

e) DOCUMENTATION

1. The record is done on the case paper of the patient.
2.Same should be recorded in the indoor lab. register.

f) NORMAL RANGE

1) BSL (R) = 80 – 140 (mg/dl)
2) BSL (F) = 60 -- 100 (mg/dl)
3) BSL (PP) = 100 – 120 (mg/dl)

9. DETERMINATION OF BLOOD UREA LEVEL (DAM)

a) REAGENTS

1) Acid reagent
2) Dam reagents
3) Distlilled water.

b) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Take 3 tubes marking as test, std. & blank respectively.
5. Add. 2 ml of distilled water in each tube respectively .
6. Take 500 ul of acid reagent & DAM reagent in each tube respectively .
7. Add 20 ul serum in test-tube marked as test & add 20 ul std. reagents in test tube marked as std.
8. After addition keep all test tubes in boiling water bath for 20 min .
9. After 20 minutes allow the test tubes to cool or keep under tap water for 3- 5 min measure the the OD values by colorimeter at 530nm(Greeen Filter).

c) Calculations

$$\text{Plasma (or serum) urea , mg/dl} = \frac{\text{O. D. Test}}{\text{O. D. Std.}} \times 40$$

d) DOCUMENTATION

1. The record is done on the case paper of the patient.
2.Same should be recorded in the indoor lab. register.

e) NORMAL RANGE

20- 40 mg/dl

10. DETERMINATION OF BLOOD CREATININE LEVEL (KINETIC)

a) REAGENTS

1) Alkaline picrate reagent (picric acid).
2) Sodium hydroxide 0.75 N.
3) Creatinine strandard.
4) Stock picric acid :0.7 g/dl .
5) Stock sodium hydroxide : 3.0 g/dl .
6) Working alkaline picrate reagent : It is prepared fresh by mixing equal volumes of stock reagents 1 and 2 . Keep at room temaerature for 30 min , before use .

b) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Take 3 tubes marking as test, std. & blank respectively.
5. Add. 1 ml of working reagents in each tube respectively .
6. Add 5 ul of serume in a test tube marked as test .
7. 5 ul Distilled water in a blank marking test tube .
8. Mix it well and stop watch immideately
9. After 20 sec. read OD of test against blank(O.D. T_1)
10. After 80 sec. read OD fo test against blank .(O. D. T_2)
11. Add 5 ul . ml of std in the tube labled as std with 1 ml. of reagent and take 2 readings (one after 20 sec. and next after 80 sec.)Thease readings are OD S_1 and OD S_2.respectively.

c) Calculations

$$\text{Serum Creatinine , mg /dl} = \frac{\text{O.D. } T_2 - \text{O.D. } T_1}{\text{O.D. } S_2 - \text{O.D. } S_1} \times 2$$

d) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

e) NORMAL RANGE

0.7 -1.5 mg/dl

11. DETERMINATION OF SERUM BILIRUBIN BY DMSO METHOD

a) REAGENTS

1. Diazo `A` '' DMSO '' : It contains 5.0 g sulfanilic acid , 12 ml of hydrochloric acid acid & 512 ml of dimethyl sulfoxide in 1 liter of distilled water.

2. Sulfanilic acid : 0.5 g / dl in 1.5 % (v/v) hydrochloride acid .
3. Diazo `B` reagent : 0.5 g/dl , sodium nitrite in distilled water.
4. Billirubin artificial standard (methyl red or cobalt nitrate) : O.D. of this standard corresponds to 10 mg/dl bilirubin.

b) STABILITY OF THE REAGENTS

Reagents 1, 2 and 4 are stable at room temperature (25 ^{0}c + 5 ^{0}c) for one year and reaent 3 is stable at 2-8 ^{0}c for six months.

c) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Take 4 tubes marking as total test, total blank, direct test , direct blank respectively.
5. Add 2.8 ml of reagent (1) in a test tube marked as total test and total blank working reagents in each tube respectively .
6. Add 2.8 ml of reagent (2) in a test tube marked as Direct test and Direct blank working reagents in each tube respectively .
7. Add 100 ul of reagent (3) in a test tube marked as total test and direct test respectively.
8. Add 100 ul of serum in a test tube marked as total blank and direct blank respectively
9. Mix thoroughly , and read intensisities of total tests and direct test against respective blanks at 555nm (green filter530- 580nm).

d) Calculations

$$\text{Total bilirubin , mg /dl} = \frac{\text{O.D. Total test - O.D. Total blank}}{\text{O.D. of std.}} \times 10$$

$$\text{Direct bilirubin , mg /dl} = \frac{\text{O.D. Direct test - O.D. Direct blank}}{\text{O.D. of std.}} \times 10$$

e) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

f) NORMAL RANGE

0.7 -1.5 mg/dl

12. DETERMINATION OF SGPT BY COLORIMETER

a) REAGENTS

Use the reagent s as per company suppliers.

b) STABILITY OF THE REAGENTS

18 months at 2-8 ^{0} C.

Avoid any bacterial contamination . Reagent 4 when diluted and kept in polythelene vials is stable for at least 8 days between 18 and 25^{0} C .

c) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit of SGPT Reagent bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Take reading on colorimeteras per company manual.
7. Calculate the results as per company manual calculation formula.

d) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

e) NORMAL RANGE

13. DETERMINATION OF SGOT BY COLORIMETER

a) REAGENTS

Use the reagent s as per company suppliers.

b) STABILITY OF THE REAGENTS

18 months at 2-8 ^{0} C.

Avoid any bacterial contamination . Reagent 4 when diluted and kept in polythelene vials is stable for at least 8 days between 18 and 25^{0} C .

c) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit of SGOT Reagent bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Take reading on colorimeteras per company manual.
7. Calculate the results as per company manual calculation formula.

d) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

e) NORMAL RANGE

15-36 u/l

14. DETERMINATION OF ALKALINE PHOSPHATE BY COLORIMETER

a) REAGENTS

Use the reagents as per company suppliers.

b) STABILITY OF THE REAGENTS

18 months at 2-8 ^{0} C.

Avoid any bacterial contamination.

c) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit of Alkaline phosphate& Reagent bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Take reading on colorimeteras per company manual.
7. Calculate the results as per company manual calculation formula.

f) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

g) NORMAL RANGE

90-110Iu/l

15. DETERMINATION OF HDL BY COLORIMETER

a) REAGENTS

Use the reagents as per company suppliers.

b) STABILITY OF THE REAGENTS

18 months at 2-8 ^{0} C.

Avoid any bacterial contamination.

c) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit of HDL Cholestrol bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Take reading on colorimeteras per company manual.
7. Calculate the results as per company manual calculation formula.

d) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

e) NORMAL RANGE

30-90 mg/dl

16. DETERMINATION OF SERUM CHOLESTROL BY COLORIMETER

a) REAGENTS

Use the reagents as per company suppliers.

b) STABILITY OF THE REAGENTS

18 months at 2-8 ^{0} C.

Avoid any bacterial contamination.

c) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit of Serum Cholestrol bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Take reading on colorimeteras per company manual.
7. Calculate the results as per company manual calculation formula.

d) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

e) NORMAL RANGE

150-250 mg/dl

17. DETERMINATION OF SERUM TRIGLYCERIDES BY COLORIMETER

a) REAGENTS

Use the reagents as per company suppliers.

b) STABILITY OF THE REAGENTS

18 months at 2-8 ^{0} C.

Avoid any bacterial contamination.

c) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit of Serum Triglycerides bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Take reading on colorimeteras per company manual.
7. Calculate the results as per company manual calculation formula.

d) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

e) NORMAL RANGE

140-175 mg/dl

18. DETERMINATION OF WIDAL (SLIDE AGGLUTINATION) TEST

a) REAGENTS

Use the reagents as per company suppliers.

b) PRINCIPLE

Serum sample of the patient is tested for 'O' and 'H' antibodies by using suspensions. *Salmonella typhi* 'O' and *Salmonella typhi* 'H, respectively . In typhoid fever an increase in the agglutination (titer above 240) is observed . For paratyphoid testing , the antigen suspensions used are S . paratyphoid ' AH ' and S . paratyphoid 'BH'.

c) SPECIMEN

Clear blood serum .

d) REQIREMENTS

1. Glass Plates with ceramic rings
2. Droppers
3. Widal (slide test) kit
4. Timer
5. Applicator sticks (or used match sticks)

e) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit of Widal(Slide Agglutination) Test bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Record the degree of agglutination.And measure the titer and record results .

f) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

19. DETERMINATION OF VDRL (VENERAL DISEASE REASERCH LABORATORY) TEST

a) REAGENTS

Use the reagents as per company suppliers.

b) PRINCIPLE

Since complement present in serum interference with the flocculation reaction , it is inactivated by keeping serum at 56^0C in a water bath . Reagin (antibodies that appear in syphilis) in the serum react with the VDRL antigen (particles of lipid coated with cardiolipin) and forms , floccules.

c) SPECIMEN

Clear blood serum.

d) REQIREMENTS

1. Glass VDRL Plate.
2. Equipments
   (a) Water bath ($56\ ^0$)

(b) Roatating machine
(c) Timer

3. VDRL Reagent kit.

e) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form received.
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit VDRL (VENERAL DISEASE RESEARCH LABORATORY) Test bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Record the degree of agglutination and measure the titer and record results .

f) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

20. DETERMINATION OF RA (RHEUMATIOD) TEST

a) REAGENTS

Use the reagents as per company suppliers.

b) PRINCIPLE

RA factor in patient's serum react with IgG coated latex particles (in the RA tested reagents) and agglutinations are formed.

c) SPECIMEN

Clear blood serum .

d) REQIREMENTS

1. Commercially available RA kit with positive and negative controls.
2. Normal saline.

e) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit RA (RHEUMATIOD ARTHRITIS TEST) ,bring it to room temp.

5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Record the degree of agglutination.And measure the titer and record results .

f) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

21. DETERMINATION OF ANTISTREPTOLYSIN O (ASO) QUANTITATIVE TEST

a) REAGENTS

Use the reagents as per company suppliers.

b) PRINCIPLE

The qualitative slide test reagent contains an aqueous suspension of polystyrene latex particles which are sensitized with Streptolysin O (a purified protein preparation from the cultures of beta hemolytic-C-Streptococci). These particles agglutinate in the presence of ASO present in patient's serum.

c) SPECIMEN

Clear blood serum .

d) REQIREMENTS

1. Latex-ASO reagent Kit.
2. Positive and Negative serum controls.
3. 0.1 and 1.0 ml serological pipettes.
4. Stirring rods.

e) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit ANTISTREPTOLYSIN O (ASO) quantitative test, bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Record the degree of agglutination. And measure the titer and record results .

f) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

22. DETERMINATION OF CRP TEST

a) REAGENTS

Use the reagents as per company suppliers.

b) PRINCIPLE

Latex particles coated with anti-CRP , agglutinate in the presence of CRP.

c) SPECIMEN

Clear blood serum.

d) REQIREMENTS

1. Latex-CRP kit.
2. Normal saline.
3. Serological pipettes 0.1 ml and 1.0 ml.

e) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit of C-Reactive Protein Test,bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Record the degree of agglutination.And measure the titer and record results .

f) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

23. DETERMINATION OF HBsAG TEST

a) REAGENTS

Use the reagents as per company suppliers.

b) PRINCIPLE

Latex particles coated with gammaglobulins from rabbit containing highly purified antibodies. When Latex reagent is mixed with patients serum , distinct agglutination of the latex particles occurs, if the serum contains HBsAg . There is no agglutination if HBsAgis not present in serum.

c) SPECIMEN

Clear blood serum.

d) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit HBsAg Test, bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Read & record the results .

e) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

24. DETERMINATION OF LEPTO TEST

a) REAGENTS

Use the reagents as per company suppliers.

b) PRINCIPLE

Serum antibodies of the IgG & IgM class (when present) , combines with *leptospira* antigen attached to the polystyrene surface of the test strips, buffer substrate (containing chromogen) is added . A pink band appears on the test strip .

c) SPECIMEN

Clear blood serum .

d) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit of LEPTOSPIRASIS bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Read & record the results .

e) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

25. DETERMINATION OF DENGUE TEST

a) REAGENTS

Use the reagents as per company suppliers.

b) PRINCIPLE

Serum antibodies of the IgG & IgM class (when present) , combines with *Dengue* antigen attached to the polystyrene surface of the test strips, buffer substrate (containing chromogen) is added. Then a pink band appears on the test strip .

c) SPECIMEN

Clear blood serum .

d) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit of *DENGUE* bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Read & record the results .

e) DOCUMENTATION

1. The record is done on the case paper of the patient
2. Same should be recorded in the indoor lab. register.

26. DETERMINATION OF CHIKUNGUNIA TEST

a) REAGENTS

Use the reagents as per company suppliers.

b) PRINCIPLE

Serum antibodies of the IgG & IgM class (when present) , combines with *CHIKUNGUNIA* antigen attached to the polystyrene surface of the test strips, buffer substrate (containing chromogen) is added. Then a pink band appears on the test strip .

c) SPECIMEN

Clear blood serum .

d) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Check the specimen is haemolysed if the specimen is haemolysed notify to the ward and ask for repeat And sufficient specimen.
3. Centrifuge the specimen at 3000/rpm for 10 min..
4. Open the kit of CHIKUNGUNIA bring it to room temp.
5. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
6. Read & record the results .

e) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

27. DETERMINATION OF URINE TEST BY STRIP

a) REQUIREMENTS

1. Dry , sterile , wide mouth leak proof bottle
2. First morning discharged (midstream) urine.
3. Uristicks (dipsticks /multisticks)

b) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Take 5 ml of urine in dry and sterile tube .
3. Deep the uristick in a tube cointaining 5 ml urine .
4. After 1 min. Compare the uristicks with the comparator given on the bottle .
5. Read & record the result.

c) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

28. DETERMINATION OF URINE BILE PIGMENTS

a) REQUIREMENTS

1. Dry , sterile , wide mouth leak proof bottle
2. First morning discharged (midstream) urine.
3. Barium Cloride
4. Fouchet Reagent

b) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form recived .
2. Take 5 ml of urine in dry and sterile tube .
3. Take equal quantity of barium chloride & urine sample
4. Centrifuge the specimen at 1000/rpm for 1 min..
5. Decant the supernanat.
6. Add 2 drops of fouchet reagents on the wall of test tube .
7. Shake slightly , if Bile pigment is present then green color ring will be formed emmideatly
8. Read the results on the intensity of rings green color as a trace 1+, 2+ , 3+ & 4+ .

c) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

29. DETERMINATION OF URINE BILE SALTS

a) REQUIREMENTS

1. Dry , sterile , wide mouth leak proof bottle
2. First morning discharged (midstream) urine.
3. Heys Sulpher powder.

b) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form received .
2. Take 10 ml of urine in dry and sterile tube .
3. Sprinkle a little dry sulfur powder on to the surface of urine .
4. Observe the sulfur particles.

c) OBSERVATIONS

1. Sulfer particles sink to bottom : Bile salt present
2. Sulfer particles remain floting : Bile salt absent

d) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

30. DETERMINATION OF STOOL TEST

a) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form received .
2. Observe the sulfur particles.

b) OBSERVATIONS

c) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

31. DETERMINATION OF URINE PREGENCY TEST BY UPT KIT

a) CLINICAL SIGNIFICANDE

HCG (human chorionic gonadotropin) appears in the urine of a pregnant women within 2 weeks , after the first missed menstrual cycle. This can be detected by the UPT test.

b) PRINCIPLE

c) REQUIREMENTS

1. Dry , sterile , wide mouth leak proof bottle
2. First morning discharged (midstream) urine.
3. UPT Kit

d) PROCEDURE

1. Indentify the specimen for correctness in labeling and check it with respect to the requisition form received.
2. Open the kit.
3. Read the company leaflet (operating procedure) and follows the procedure as per company manual.
4. Read & record the results.

e) DOCUMENTATION

1. The record is done on the case paper of the patient.
2. Same should be recorded in the indoor lab. register.

CHAPTER 2: MALARIA

INTRODUCTION

Malaria is one of the most widespread parasitic diseases all over the world. The disease present in 102 countries is responsible for over 100 million reported cases annually and 1-2 million deaths, especially in children. Normally, diagnosis of malaria is based on clinical symptoms such as presence of chills and rigors, intermittent fever, etc. which are non- specific, leading to false diagnosis and over use of anti-malarial drugs, thus increasing the potential of drug resistance, as well as the number of malaria cases.

Early diagnosis, followed by prompt and effective treatment is the key to reducing malaria mortality and morbidity. Consequently, it is essential to recognize the importance of this aspect in the control programme. Laboratory diagnosis of malaria greatly facilitates the management of the disease by confirming the clinical diagnosis and also aids in monitoring drug resistance. Laboratory diagnosis is desirable in all suspected cases of treatment failure and severe forms of the disease, as well as for diagnosis of uncomplicated malaria during low transmission seasons.

Since 1880, when malaria parasites were first detected in the blood of a patient, light microscopy has been the definitive tool for routine malaria diagnosis, especially because clinical diagnosis has low specificity, gives rise to over diagnosis and misuse of antimalarial drugs, resulting in increased cost to the health services. Microscopic examination of blood smears stained with JSB stain (and /or Giemsa, Leishman), continues to be the method of choice-the “Gold Standard”, for confirming the clinical diagnosis of malaria. Microscopy is a reasonably affordable, sensitive and specific technique. It not only allows the differentiation of *Plasmodium* species but also provides an estimate of the parasite load i.e. number of parasites per micro liter of blood. With the advent and spread of antimalarial drug resistance, particularly of multidrug resistant P. falciparum, the need and the importance of accurate microscopic diagnosis has been felt more acutely.

In India, under the National Vector Borne Disease Control Programme (NVBDCP), both microscopy and newer RDTs are being used across the country for diagnosis of malaria. Irrespective of the technique employed, establishment and maintenance of a reliable diagnostic service depends on operational feasibility of the test, availability of adequate trained personnel, equipment and laboratory management systems at all levels. Quality Assurance (QA) and adequate monitoring of laboratory services at the peripheral level have been perceived as one of the important but weak components under NVBDCP which needs to be strengthened. Therefore, it is essential to build and incorporate a Quality Assurance Programme under NVBDCP. As a first step to achieve this goal, the development of Standard Operating Procedures (SOPs) was felt imperative and the SOPs have since been developed. This document describes the Quality Assurance Programme

for malaria microscopy for diagnosis of malaria. There are two more documents which describe the Quality Assurance Programme for malaria Rapid Diagnostic Tests and network of laboratories.

QUALITY ASSURANCE FOR MALARIA MICROSCOPY

Microscopy

It is the most widely used diagnostic test in India, since the inception of a structured malaria control programme in our country. It is till today the “Gold Standard” for laboratory diagnosis, yet it does have some disadvantages, the most important being the subjectivity in interpretation of the result by the examiner.

Strategy of cross-checking of malaria microscopy under NVBDCP

- There has been a well established programme for cross verification of the laboratory results of microscopy under Dte. of NVBDCP, 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.
- Coding: One of the responsibilities of the Zonal Malaria Officers (ZMO) is the coding of the examined slides. The code number (last digit) for cross checking is issued by ZMO (in the first week) of every month, for the negative slides examined in the previous month. If the code for the month is 5, the slide numbers ending with 5 from each section are cross checked.
- Cross checking The PHC/ malaria clinic laboratory technician is supposed to collect all negative slides examined during the previous month with number ending with the code digit and dispatch to the concerned cross-checking laboratory by 10^{th} of every month. All positive blood smears are cross checked in the Regional Office of Health & Family Welfare (ROH&FW), Govt. of India and State Headquarter laboratories. Depending on the workload, it is shared 50:50 between these laboratories. The negative slides are distributed between state/zonal and ROH&FW laboratories, at the ratio of 8.5: 1.5 between former and latter. Instructions are issued to the PHC/malaria clinic laboratory to preserve the rest of the slides, until the cross- checking results are received back.
- Supervision of laboratories for cross checking In 1975, the expert committee on malaria recommended very strongly for supervision of efficiency of laboratories. For this, there was a provision for posting a supervisory laboratory technician at district/zonal/state laboratories. His function was to visit every PHC laboratory to inspect and conduct on the spot corrections in regard to laboratory records, returns, materials and equipment. He was also supposed to cross-check the laboratory procedures and to assist the

technicians to improve their efficiency.

- Results and feed back The results of cross-checking were to be sent to the concerned laboratory by the 10th of the succeeding month. In case of high discrepancy rate i.e., 2% or above, the state programme officer and Regional Director of each ROH & FW was to take the needful remedial action like supervision of the concerned laboratory reporting high discrepancy rate.

Need for strengthening the QA Programme

Over the years, the QA of malaria microscopy in the form of regular cross-checking of examined blood smears could not be sustained upto the desired extent due to various operational and technical reasons. One of the main reasons was/is vacant posts of laboratory technicians at each level that is at PHCs, malaria clinics, at State/Zone and ROH & FW. Besides, the quantity of the negative slides (10%) is too high. In this context, as well as due to increasing trend of P. falciparum cases, emergence of newer foci of drug resistance and high mortality due to malaria, an urgent need has been felt to revitalize the QA component of the laboratory services provided under the Dte. of NVBDCP.

MICROSCOPE

It is one of the most important components of QA on malaria microscopy. It is essential that the application of the different microscopes with specific reference to malaria microscopy should be known by each LT. The purpose of the microscope is to produce an enlarged, well defined image of objects, too small to be observed with the naked eye.

A microscope is an instrument designed to make fine details of the blood film visible.

Types of microscopes

Microscopes vary from an ordinary magnifying lens (magnifies 100 to 1000 times) to that of a sophisticated Electron microscope which magnifies a million times. The simple microscope is nothing but a magnifying lens consisting of two converging lenses fixed at two ends of a brass tube. The lens nearer to the object is called objective lens and the lens through with the final image is observed is called the eye piece or the ocular lens. The objective lens produces a real, inverted, intermediate image of the object which lies within the principal focus of the eye piece, while the eyepiece produces a magnified, virtual and inverted image. The final image is thus inverted, magnified and virtual. A compound light microscope has the capacity to increase an object by 1000 times so that an object of 0.1 micrometer or 100 nanometer is made visible.

Types of light microscopes

- Bright field compound microscope
- Phase contrast microscope

- Dark ground microscope
- Fluorescent microscope

Bright field compound microscope

The common microscope that is suited to see and study the microorganism routinely is the typical compound microscope, either monocular or binocular. Here the microscopic field or area observed is brightly lit and the objects under study appear darker. Generally, these microscopes produce useful magnification of about upto 1000 times than the naked eye.

- Monocular monocular microscopes have single eye piece and are convenient for use by beginners.
- Binocular binocular microscopes have two eye pieces. They are recommended where much work has to be done, as this microscope causes less eye strain and fatigue.

Parts of a microscope

Mechanical

- Stand: It forms the base of the microscope. It consists of a vertical pillar supported on a horse shoe shaped base or foot. It gives stability to the microscope. The stand is attached to the arm or limb by the hinged (inclination) joint, which can be adjusted to any convenient angle. The limb or arm carries the illuminating apparatus, the stage and the observation tube. It also serves as a handle.
- Stage: It is a platform with a circular aperture in the center. Stages are usually of two types:
    - A fixed stage in which the object is fixed by clips as in a monocular microscopy.
    - A mechanical stage in which the object can be moved to the desirable distance, either sideways or forward and backward, as in binocular microscope. This type of stage is preferable for examination of a blood film or to locate a particular point in the object.
- Focussing knobs
  These are located on the side of the microscope; outermost is the course focus and innermost is the fine focus. On the binocular microscopes, these knobs control up/down movement of the stage.
- Magnifying parts
  These are eyepieces and objectives. They are kept separated in a graduated tube.
- Ocular lens or eyepiece
  Usually of 6x or 10x magnification. Only one eyepiece is present in a monocular microscope and two in case of a binocular microscope.
- Objectives
  The objectives are screwed on to the rotating nosepiece which is attached to

the lower end of the tube. They are usually 3-4 in number and designated according to their focal lengths.

- low power dry objective : 16 mm
- high Power dry objective: 4 mm
- oil Immersion objective : 1.6 mm

Their magnifying power and/or their numerical aperture may also be engraved on them. The eyepieces or the oculars are usually designated by their magnification eg. 10x.

- Oil-immersion objective
  This is the most frequently used objective because of the greater magnification and resolution which is required to study the morphology of parasites like malaria parasites. Some of the monocular and all the binocular compound microscopes, have 100x oil immersion lenses. These can be identified by a red band around the lens housing. At magnifications greater than about 500x, light is refracted too much, as it passes through air to yield good resolving power. Thus, optics for these higher magnifications are made to use with a high grade mineral oil as the medium for transmitting light. It is imperative that only immersion oil is used and the lens is cleaned thoroughly with lens paper after each use everyday.
- Body tube
  This contains mirrors and prisms which direct the image to the ocular lens/es.
- Nosepiece
  This holds the objective lenses and rotates with a positive click for each lens.
- Illuminating parts
  This consists of a condenser, an iris diaphragm, a mirror and the light source situated below the stage.
- Condenser
  The condenser is made up of a system of convex lenses. It concentrates the light rays reflected by the mirror to the object plane in the optical axis. The condenser can be raised or lowered. Lowering of the condenser diminishes illumination, whereas, raising it increases the illumination. While using oil-immersion objective, the condenser is completely raised as it requires more light. When the other objectives are used, it is lowered suitably. The condensers move up and down to focus the light beam.
- Iris diaphragm
  This helps to regulate the amount of light. It is opened widely when the oil immersion objective is used, as it requires maximum light and closed partially when the other objectives are in use. The diaphragm is located just below the stage and controls the amount of light which passes to the specimen and can drastically affect the focus of the image.
- Mirror
  This is a plano-concave mirror. It helps to reflect the light into the sub stage condenser. The plane mirror is used, whenever the oil-immersion objective is

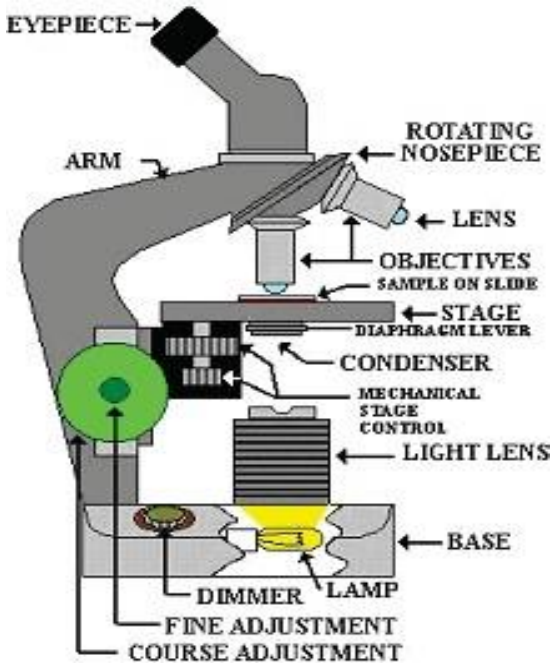
employed. The concave mirror is used with low and dry high power objectives. Under the NVBDCP, both binocular and monocular compound microscopes are being used for malaria microscopy.

- Light source
  The microscope has either built in light sources as in binoculars or external light source as in monocular. The rheostat ON/OFF switch is located either on the microscope or on the external power supply and is used to regulate the intensity of light. Various parts of a compound microscope are shown in Figure 3.

Magnification resolution and working distance

- Magnification is simply a function of making an object appear bigger. Magnification is produced at two stages, by the objective lenses and by the eye piece lenses.

Fig. 3 – Various parts of a compound microscope



The magnifying powers of both objectives and eye pieces are engraved on them and the overall magnification of the given microscope can be calculated by multiplying the magnifying power of the objective by that of the eyepiece.

Total magnification = ocular power x objective power.

The overall magnification achieved by the three objectives, 10x, 40x and 100x, when used with eye piece of 100x, 400 x and 1000 x, respectively.

Resolution:

Merely magnifying an object without a simultaneous increase in the amount of detail seen will not provide the viewer with a good image. The ability of a microscope (or eye) to see the details is a function of its resolving power. Resolving power is defined as the minimum distance between two objects at which the objects can just be distinguished as separate and is a function of the wavelength of light used and the quality of the optics. In general, the shorter the wavelength of the light source, the higher the resolution of the microscope.

Working distance is the distance between the objective lens and the specimen. At low magnification, the working distance is relatively long. As the magnification is increased, the working distance *decreases* dramatically. Oil immersion lenses practically touch the specimen. Be aware of this change in working distance with increasing magnification so as to prevent damage to your specimens.

Focusing procedure

- Low / high power focusing
  - Turn on the light source.
  - Switch to the 10x objective lens.
  - Turn the coarse focus to raise the nose piece.
  - Place the specimen slide on the stage and secure in the proper position. Look at the slide and place it so that the specimen is over the light aperture in the stage.
  - Lower the objective lens to lower limit (close to slide). Raise the lens using the coarse focus knob until you see the image come into focus adjust fine focus similarly.
  - Center the image and adjust the light using the diaphragm.
  - Readjust diaphragm if needed.
  - Now switch objectives to the 40x, if a higher magnification is needed. Readjust fine focus and light (diaphragm), as needed.

  The microscopes should be par focal which means that when you switch from low (100x) to high (400x) power, a focused image at low power will remain more or less in focus at the higher power. Most likely the fine focus and diaphragm have to be readjusted slightly.

- **Procedure for using oil immersion lens**
  - Locate the region of interest of the blood smear and center it with 40x objective.
  - Then the objective lens is raised to its limit (i.e., maximize the distance between stage and objectives) and swing the lens out of the way, about half way to the next position.
  - Place a small drop of immersion oil carefully, placed directly on the bloods smear over the center of the region of interest.

- Rotate the oil immersion objective into position carefully and while looking from the side, lower it using the coarse focus knob until the lens *just* makes contact with the oil drop. The drop leaps up into a column as the contact is made.
- Lower the lens a *smidgen* more and then using the fine focus and looking through the ocular lens, focus on the specimen.
- When done, clean lens with lens paper until no more oil comes off and clean slide if it is to be saved.

- **Handling of the compound microscope**

  The microscope is an instrument of precision and care must be taken to preserve its accuracy, So, precautions have to be taken to keep the microscope and lens system clean. There are only a few ABSOLUTE rules to observe in caring for the microscopes.

  Please report any malfunctions immediately to your supervisor.

  - Always use two hands to carry the scope -one on the arm and one under the base - no exceptions! Never carry the microscope upside down, for the ocular can and will fall out.
  - Use lens paper to clean all the lenses before start of laboratory work and after using the oil immersion lens. Do not ever use anything other than lens paper to clean the lenses. Other papers are too impure and will scratch the optical coating on the lenses.
  - Always remove oil from the oil-immersion objective after its use, with lens paper lightly moistened with alcohol.
  - Always use the proper focussing technique to avoid ramming the objective lens into a slide. This can break the objective lens and/or ruin an precious slide.
  - Always turn off the light when not using the scope.
  - Always carefully place the electric wires out of harm's way. Wires looped in the leg spaces invite a major microscope disaster. Try sliding the wire down through the drawer handles by the side of your bench space.
  - Avoid attack of dust and water to prevent fungal contamination.
  - Never allow the objective lens to touch the cover glass or the slide. Never touch the lenses,
  - Keep the stage of the microscope clean and dry.
  - Do not tilt the microscope when working with oil immersion system.
  - Never lower the body tube with the coarse adjustment while you are looking through the microscope.
  - Never exchange the objective or oculars of different microscopes.

Care of the microscope

Provided normal care and common sense are exercised, the laboratory microscope will be useful for many years.

Removing dust and grease

- When not in use during the day, keep the microscope covered with a clean cloth or plastic cover to protect the lenses from dust that settles out of the air. Overnight, or if it is to remain unused for long periods, place the microscope inside it's box with the door tightly closed.
- To protect the objective lenses, rotate the 10x objective to line up with the ocular. Oil and grease from eyelashes and fingers are easily deposited on lenses and oculars as the microscope is used. Clean these parts with lens tissue or with very soft cotton cloth.
- Clean the oil immersion objective after use. If it is not cleaned, the oil will harden and make the objective useless. A lens tissue or soft cotton cloth is usually sufficient for the purpose. However, never use this tissue or cloth to clean other objectives, the oculars or the mirror, otherwise oil will be transferred to these components.

Preventing the growth of fungus

In warm, humid climates, it is very easy for fungal growths to become established on lenses and prisms. These growths can create problems and may even become so bad that the microscope can no longer be used. The lenses may need to be re-polished by the manufacturer, which is very expensive and may take several months.

Fungus cannot grow on glass when the atmosphere is dry and therefore store the microscope in a dry atmosphere when it is not being used.

To avoid fungal growth, use a desiccant like silica gel, with the ability to absorb water vapour from the air. Self-indicating silica gel is blue when active but becomes pink when it has absorbed all the water. It can then be reactivated by heating.

Transporting the microscope

When the microscope is to be transported from one location to another, ensure that it is properly secured inside its box. The best way to do this is by means of the securing device, which screws into the base of the microscope.

ELECTRONIC BALANCE

Purpose

This SOP describes the process for weighing ingredients of stains using the Analytical Balance.

Specifications

- Microprocessor based single pan
- Weighing capacity upto 100gm
- Weigh upto 3rd decimal place

- Autoself calibration
- Auto zero setting
- Liquid crystal display (lcd)
- High accuracy precision

Procedure

- Switch on the balance by touching the ON/OFF key. The balance undergoes a brief test and is then ready for weighing.
- Open the balance door.
- When using a weigh boat, reset the balance to zero by touching the TARE key.
- Place the sample to be weighed on the weigh boat, and close the balance door.
- As soon as the stability detector symbol (the small ring to the left of the weight display) is seen, the reading is stable and the result can be recorded.

Maintenance

- Clean the balance after every use.
- Maintain the LOG BOOK for balance and record the data after every use.
- Calibrate the balance at regular intervals and maintain the record of calibration in the laboratory.

FORM FOR RECORD OF BALANCE CALIBRATION

CALIBRATION RECORD OF BALANCE

Laboratory Name: Date of Calibration

BALANCE DETAILS

a.) Type of balance:.....

b.) Nominal capacity (Weight in grams): .....

c.) Balance number:.....

RESULTS OF THE TEST

a.) Reference weight used :

b.) Acceptable Range :

REFERENCE WEIGHT (a)	ACTUAL WEIGHT (b)		ACCEPTABLE RANGE	REMARKKS
	(n)	mean		

STATUS OF BALANCE

a.) Difference in actual and reference weight :

b.) Difference in reference and test sample weight :

c.) Balance status :

TEST PERFORMED BY :

1.....

2. .....

CERTIFIED BY

Name:.....

Signature:.....

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	***GENERAL QUALITY ASSURANCE –PH METER***				
SOP No.	**SOP G. 03**	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP G 03 –PH METER

Purpose

This SOP describes the method for using a pH meter required for determination of pH of buffers.

Principle

Before pH is measured, a one- or two-buffer calibration should be performed. The use of two buffers that cover the expected sample pH range is recommended, and calibration must be done every time the pH meter is used.

Specifications

- Ph range 4-12
- Combined ph electrodes
- Liquid crystal display (LCD)
- Temperature control facility

Reagents / Equipment

- Ph meter
- Ph 4.0 or ph 10.0 buffer
- Ph 7.0 buffer
- Distilled H2O
- Beaker

Procedure

This procedure is specific for various makes of pH meter.

Measurement and auto calibration with two buffers

- Select two buffers that cover the range of expected ph. One of the buffers
- Should be near the is potential point (ph 7.0) and the other near the expected sample ph (e.g. Ph 4.0 or ph 10).
- Rinse electrode with distilled water.
- Place electrode on ph 7.0 buffer, then press MODE key. Calibration will be

displayed on screen.

- Press YES. P1 will show on the lower field of the screen.
- When the electrode is stable, Ready will appear on screen and the temperature-corrected ph of the buffer is displayed.
- Press yes if the value shown on screen corresponds to the ph of the buffer. P2 will then appear on the lower field of the screen.
- Rinse the electrode with distilled water, then place on the second buffer.
- When Ready appears, press yes.
- The ph meter automatically advances to the measure mode. Measure is displayed above the main field. Rinse electrode with distilled H2O, then place on sample.
- Once stable, record ph reading from meter display.

Note: Subject to change with the make of pH meter

Maintenance

- Wash the electrode after every use thoroughly with distilled water.
- Maintain the log book for ph meter and record the details after every use with remarks.
- Calibrate the ph Meter at regular intervals and maintain the record for calibration in the laboratory.

CALIBRATION RECORD OF pH METER

CALIBRATION RECORD OF pH METER

Laboratory Name : **Date of Calibration:**

pH METER DETAILS

a.) Type of pH meter:.....

b.) Name of manufacturer: .....

c.) pH Metern number:.....

RESULTS OF THE TEST

a.) Reference buffers used :

i. Name of manufacturer :

ii. Lot No. : pH 4.0

pH 7.0

pH 10.0

b.) Acceptable range :

REFERENCE pH (a)	ACTUAL pH (b)	ACCEPTABLE RANGE	REMARKS
	(n)		

STATUS OF pH METER

TEST PERFORMED BY

1.....

2. .....

CERTIFIED BY

Name:.....

SIGNATURE:.....

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	***GENERAL QUALITY ASSURANCE - CLEANING AND MAINTENANCE OF GLASSWARE***				
SOP No.	**SOP G.04**	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP G 04 - CLEANING AND MAINTENANCE OF GLASSWARE

Purpose

This SOP describes the method for cleaning and maintenance of the glassware used for malaria microscopy and different ways to accomplish it.

Principle

There has to be a minimum level of good laboratory practices which should be maintained at the laboratories under National QA Programme. Cleaning and maintenance of the glassware used for malaria microscopy is one of the basic requirements to achieve the quality laboratory results.

Materials required and procedures for cleaning of micro glass slides for cleaning micro glass slides the following materials are required

- A large plastic basin
- Gauze or cotton wool
- A good quality detergent (powder or liquid)
- 2-4 clean, dry, lint-free cotton cloths
- Clean water.

Micro glass slides

Microscope slides are usually supplied in boxes of 50 or 72. It may be described on the box as "washed" or "pre-cleaned", but the slides will still need to be properly washed, dried and wrapped. It is not possible to make good quality blood films on dirty microscope slides. Blood films made on dirty or greasy slides will wash off easily during staining. It is therefore best to discard slides that have an iridescent bloom or appear white or opaque and are not properly cleaned or slides from old stock with surface scratches or chipped edges.

Process for cleaning

- New slides
  Dip all new slides first in chromic acid overnight and then wash with detergent and clean water:

- after being soaked for a period between 30 minutes and 1 hour, rinse the slides under running tap water or in several changes of clean water.
- wipe each individual slide dry and polish with the clean, dry, lint-free clothes.
- handle cleaned slides by the edges only to avoid finger marks or grease being deposited on the surface.

- **Used slides**
  - Soak used, dirty slides for a day or two in water containing detergent. Use warm water whenever possible.
  - After soaking, clean the slides one by one with a small piece of gauze or cotton wool.
  - Remove all traces of the blood film and oil (used during microscopy) from the slides.
  - Do not leave the slides in the detergent for too long; soaking should be for a few days only, not weeks. If slides are left in the detergent solution for long periods, the water will evaporate, leaving a deposit on exposed slides that is impossible to remove.
  - After cleaning, transfer the slides to a fresh solution of detergent and later rinse under running water or in several changes of clean water.
  - Individually dry with the clean cotton clothes, as described previously.
  - Separate slides that are slightly scratched and considered unsuitable for blood films during cleaning and discard them.

Wrapping cleaned slides

Materials required
Following materials are required to wrap cleaned slides correctly:

- Sheets of thin, clean paper, about 11 cm X 15 cm in size
- Empty cardboard slide boxes (of the type new slides are packed in)
- Rubber bands or adhesive tape.

Method

- Wrap clean slides with thin paper in packs of 10.
- Secure each pack with adhesive tape or a rubber band.
- Place pack in the cardboard slide boxes for later use or dispatch to the field.
- Store slides in a dry place such as a warm-air cupboard. If stored at room temperature with high humidity, the slides will stick together after a few weeks. It will then not be possible to use them unless they are rewashed and dried.

Care of other glassware

Glassware such as measuring cylinders, pipettes and staining troughs must always be cleaned and dried before use. Rinse any glassware that has been used for preparation of stain in clean water immediately after use to remove as much of the stain as possible. It should then be soaked for some time, preferably overnight in a detergent solution.

- washing glassware in detergent gives satisfactory results, provided you rinse it thoroughly in clean water. Deposits of detergent left on glassware can upset the pH of buffered water and spoil the staining so always make sure that glassware is properly rinsed before being dried for future use.
- clean the staining jars atleast once in a week. Any stain deposits that are allowed to dry on glassware will become difficult to remove and may spoil the staining of subsequent blood films. They can be removed by soaking the glassware in methanol and then washing it with detergent in the normal way.
- similarly, wash the beakers used for washing the stained slides once a week.

STANDARD OPERATING PROCEDURES: MALARIA MICROSCOPY

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	*MALARIA MICROSCOPY- PREPARATION OF BLOOD SMEARS*				
SOP No.	SOP: M 01	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 01- PREPARATION OF BLOOD SMEARS

Purpose

This Standard Operating Procedure describes the process for preparing blood smears for malaria microscopy

Principle

To diagnose whether a person is suffering from malaria or not, it is essential to examine the peripheral blood (thik & thin) film for malaria parasite. The thick film is made up of a large number of dehaemoglobinised red blood cells. The thin film consists of a single layer of red cells and is used to assist in the identification of malaria species, after the parasites have been detected in thick film. In a thick film, any parasites present are concentrated in a smaller area than in the thin film and are more quickly seen under the microscope. For QA of microscopy, quality of preparation of the blood smear is of vital importance.

Reagents, equipment required for slide preparation

- Reagents/equipment and other essential items
  - cleaned and wrapped slides
  - spirit swab
  - small bottle with cork for keeping spirit solution
  - cotton
  - clean cotton cloth
  - slide box for 25-50 slides
  - lead pencil
  - register and MF-2 form
  - carbon paper
  - ball point pen

- Specifications of glassware and other items required
  - Glass micro-slides

Glass slides used for blood smear should be clean, grease free, measuring 75mm length x 25mm width x 1.25 mm thickness and having smooth edges without any cuts. The glass should be glazed and not have any visual or chromatic aberrations or scratches.

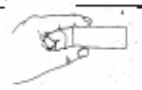
- Lancetl pricking needle
  Auto disposable pricking needles are best suited. However, under the NVBDCP, sterile lancets are being procured and supplied for use in malaria microscopy. Syringe needles or other hollow needles should not be used for collection of capillary blood. After use, the needles/lancets should be disposed/discarded in puncture proof containers after disinfecting them with 1 % hypochlorite solution.

Method of preparation of blood films

Standard precautions for handling and disposal of human blood should be followed *(see* chapter 6 on Bio safety). After the patient information has been recorded on the appropriate form, the blood films are made as under:

- take a clean glass slide free from grease and scratches
- clean the finger of the patient using a spirit swab

Follow the following steps for preparation of the blood smear

	i. select the second or third finger of the left hand
	ii. the site of the puncture is the side of the ball of the finger, not too close to the nail bed
	iii. allow the blood come up automatically. Do not squeeze the finger.
	iv. hold the slide by its edges
	v. the size of the blood drop is controlled better if the finger touches the slides from below
	vi. touch the drop of blood with a clean slide, three drops are collected for preparing the thick smear.
	vii. touch another new drop of blood with the edge of a clean slide for preparing the thin smear.
	viii. spread the drop of blood with the corner of another slide to make a circle or a square about 1 cm
	ix. bring the edge of the slide carrying the second drop of blood to the surface of the first slide, wait until the blood spreads along the whole edge
	x. holding it at an angle of about 45° push it forward with rapid but not too brisk movement
	xi. write with a pencil the slide number on the thin film, Wait until the thick film is dry

While preparing the thick film remember the following points

- using the corner of the spreader, quickly join the drops of blood and spread

than to make an even, thick film.

- do not excessively stir the blood but spread in circular or rectangular form with 3 to 6 movements.
- the circular thick film should be about 1 cm (1/5 inch) in diameter.

The thin film consists of a single layer of red blood cells and under the NVBDCP it is always used as a label to identify the patient.

After preparing the blood smear complete the following process :

- label the dry thin film with a soft lead pencil by writing the blood slide number and date of collection (in the thicker portion of the film).
- do not use a ball point pen for labeling the blood smears.
- allow the thick film to dry with the slide in the flat, level position protected from flies, dust and excessive heat.
- fill up the MF2 form for details of patient as indicated in the columns.
- when the thick film is dried / almost dry, put the slide in the slide box.
- dispatch it to the laboratory as soon as possible.
- second slide used for spreading the blood films may then be used for the next patient and another clean slide from the pack should be used as spreader.
- NEVER WRAP THE SLIDE IN MF2 FORM.

Drying the blood films

- Place the blood films properly in order to allow the thick film to dry evenly.
- Protect from flies and dust. The kind of box, good for both field and laboratory is shown in the Figure 4

Box used in the field to dry blood smears

- Store the slides horizontally, which allows the thick film to dry and be at a level and with even thickness. There is a door to keep out flies and dust and a handle for carrying the box. When the thick film is completely dry, store the slides front to back in the cardboard slide box, previously used for the clean, wrapped slides.

Precautions to be taken while collecting blood (See Chapter 6)

Common faults in making blood films

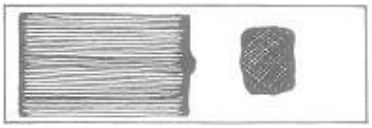
Good quality thick and thin blood smears are the basic requirement for excellent microscopy. A number of faults are observed to be common in making blood films. These can affect the labeling, the staining or the examination, and sometimes more than one of these. Followings are few common faults encountered while making blood films.

- Wrongly positioned blood films

  Care should be taken that the blood films are correctly sited on the slide. If they are not, it may be difficult to examine the thick film. Also, portions of the films may even be rubbed off during the staining or drying process.

- Excess blood

  Thick films made with excess blood, after staining, will appear dark blue in their background. There will be too many white blood cells per thick film field and these could obscure or cover up malaria parasites that are present. If the thin film is too thick, Red Blood Cells (RBC) will be on top of one another and it will be impossible to examine them properly after fixation.



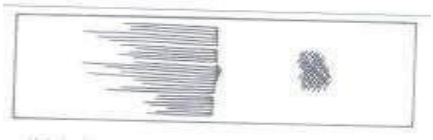
- Too little blood

  If too little blood is used to make the films, there will not be enough RBCs in the thick film and thus, the examiner will not have sufficient fields for standard examination. Besides, the thin films may also be too small for use as a label. If labeled on what so ever little blood is collected, there would not be enough blood film left for examination as well.

- Blood films spread on a greasy slide

  The blood films will spread unevenly on a greasy slide, which makes examination very difficult. Some of the thick film will probably come off the slide during the staining process.

- Edge of spreader slide chipped
  When the edge of the spreader slide is chipped, the thin film spreads unevenly, is streaky and has many “tails”. The spreading of the thick film may also be affected.



- Wrongly preparedl placed blood drops
  If the thin film is too large, the thick film will be out of place and may be so near the edge of the slide that it cannot be seen through the microscope. During staining, some portion of the thick smear on the edge of the slide may wash off.

- Rightly prepared l placed blood drops
  A correctly made combination film should look like as given below note the size and thickness of both films. The patient’s name and other identifying information can be written with an ordinary lead pencil in the thick end of the thin film.

- Other common faults
  Other faults that occur commonly in the preparation of blood films includes the following:
    - flies, cockroaches or ants eat the dry blood and damage the films.
    - blood films made on badly scratched slides (especially when old slides repeatedly reused after washing).
    - the thick film is allowed to dry unevenly.
    - auto fixation of the thick film occurs with the passage of time or

through exposure to heat and staining becomes difficult or unsatisfactory.

- slides are wrapped together before all the thick films are properly dried and the slides stick to one another.
- In warm humid climate, however, auto fixation of unstained slides occurs quite rapidly. Therefore, all the slides should be stained as soon as possible. When long storage is unavoidable, the slides can be kept in a dessicator to delay auto fixation. Besides, it is important to ensure that the slides are packed correctly and that they are not put into strong sunlight or near to any source of heat (e.g. exhaust pipe of a vehicle in the field).

Do not expose the smear to heat, sun light or alcohol as it fixes the thick smear and it would be difficult then to dehaemoglobinise it.

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	***PRESERVATION AND DISPATCH OF SAMPLES***				
SOP No.	**SOP: M 02**	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 02 - PRESERVATION AND DISPATCH OF SAMPLES

Purpose

This SOP describes the correct procedure of collection, preservation and proper transportation of the blood smears to the laboratory under optimum conditions for malaria microscopy.

Principle

Effective diagnosis depends upon the correct procedure and the time of collection as regard to the stage of the disease, preservation and proper transportation of the clinical samples to the laboratory under optimum conditions.

Collection of blood smears for microscopy

See SOP M.1 for preparation of blood smears

Preservation of the blood films at peripheral level

- Place the blood films properly in order to allow the thick film to dry evenly.
- protect from flies and dust. When the thick film is completely dry, store the slides in the cardboard/plastic slide box.
- when long storage is unavoidable, then keep the slides in a dry cool place away from direct sunlight or any source of heat.
- While storing, place the slides horizontally, which allows the thick film to dry with even thickness.

NOTE: The common practice at present is that the slides are wrapped in MF2. It is wrong practice, many a times the MF2 are torn, resulting in loss of patients details in many times. Therefore, under QA it is envisaged that if required, slides along with the MF2 should be wrapped in a separate clean piece of paper.

Dispatch of the blood smear from the periphery

- keep the blood smears collected in the field in the slide box till these are deposited to the malaria laboratory.
- wrap these dry slides and MF2 in a clean paper and in the same packet to avoid mixing up of the slides with those submitted by others and deposit in the laboratory.

- care should be taken while wrapping by avoiding 2 smears keeping together. As both the smears would get attached and both the smears would be lost / damaged.

Receipt of the blood smears in the malaria laboratory

- the LT will receive these slides and the MF2 form.
- after the smears are stained, examined and results handed over to the field/health worker, do not leave the examined slides as such.
- from these slides, remove the oils by gently rubbing with a tissue paper or soft cotton cloth.
- separate the positive and negative slides and pack separately.
- send these slides for cross checking as per instructions, to the competent authority.
- do not discard the batch of slides till the cross checking results are received, as some slides may be required to be re-examined after getting the cross checking results.
- the MO PHC will ensure that all these procedures are observed in malaria laboratory.
- where regular MO is not posted, the malaria inspector should be assigned these supervisory activities.

Note: In warm humid climate, auto fixation of unstained slides occurs quite rapidly. Therefore, stain all the slides as soon as possible.

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	***MALARIA MICROSCOPY - STAINING AND EXAMINATION OF BLOOD SMEARS***				
SOP No.	**SOP: M 03**	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 03 - STAINING AND EXAMINATION OF BLOOD SMEARS

Purpose

This SOP describes the process of staining and microscopic examination of thin and thick smears prepared under SOP-M 01 for malaria parasites.

Principle

For accurate laboratory diagnosis, correct staining and careful examination of the blood smear is of vital importance.
Equipment/glassware/reagents required

Equipment

- compound microscope with 100x oil immersion objective (see SOP :G.01)
- chemical balance (see SOP: G 02 for more details)
- tally counter

Glassware

- flat bottom round flask (1000ml)
- reflux condenser
- pipette
- staining Jar
- beaker

Reagents

- J.S.B. Stain (Jaswant Singh & Bhattacharjee stain)
- Immersion oil

Constituents

The J.S.B. stain comprises of two solutions, J.S.B.I and J.S.B.II. The composition of each staining solution is as follows:

J.S.B. Solution I

- methylene blue (Medicinal) 0.5 gm

- sulphuric acid (H_2SO_4) 1% 3.0 ml
- potassium dichromate ($K_2Cr_2O_7$) 0.5 gm
- disodium hydrogen phosphate dihydrate ($Na_2H\ PO_4\ 2\ H_2O$) 3.5 gm
- distilled water 500 cc

solution II

- eosine 1.0 gm
- distilled water 500 cc

Buffered water

- disodium hydrogen phosphate dihydrate 0.22 gm ($Na_2H\ PO_4\ 2\ H_2O$)
- potassium acid phosphate (KH_2PO_4) 0.74 gm
- distilled water 1000 ml

Others

- enamel tray (for keeping the staining kits)
- heating mantle/stove for boiling stain
- filter paper for filtering the stains for day to day use
- soft cloth

Method of preparation of JSB stain

1) JSB I

- take 500 ml of distilled water in a flat bottom round flask (1000 ml), add 0.5 gm methylene blue and stir the whole solution in order to get it dissolved.
- further, add 3 ml of 1% sulphuric acid in three equal parts with stirring to ensure thorough mixing.
- add potassium dichormate (0.5 gm), which forms a purple precipitate of methylene blue chromate by oxidation of dye in acid medium.
- subsequently, add 3.5 gm of disodium hydrogen phosphate dihydrate and again stir the whole solution thoroughly, ensuring that the precipitate dissolves.
- then boil the solution with reflux condenser for one hour.
- keep the solution thus prepared overnight at room temperature for 24 hours for maturation before use.

2) JSB II

- measure 500 ml of distilled water into a flask and add 1.0 gm of eosin and stir the whole solution.
- leave this solution for 48 hours at room temperature for maturation before use.

3) Buffer water (pH 6.2 - 6.8)

- the ideal pH of water desirable for staining in JSB solution should range between 6.2 to 6.8.
- to prepare buffer wash water, in 1000 ml of distilled water in a flask, add di-sodium hydrogen phosphate (0.22 gm) and potassium acid phosphate (0.74 gm).
- thoroughly stir the solution in order to dissolve the ingredients.

Staining

Preparation for staining

- before staining, filter both the JSB I & II. Keep used filter papers daily till the supervisory visit by a senior officer or supervisory technician. All these filter papers must contain dates.
- change buffer water daily.

Method of staining

- dip the thin smear in methyl alcohol for a second or two for fixation.
- thick smear contains more blood, therefore, if not stained immediately smears are over dried and has to be dehaemoglobinised before staining.
- dry thoroughly in the air.
- immerse the thick & thin smears in solution II for a second or two.
- wash twice or thrice in a jar containing buffer water (pH 6.2-6.8).
- then immerse in solution I for 45 seconds.
- wash 3 or 4 times in buffered water.
- dry in air.

Dehaemoglobinisation of thick smear

- dip dried thick blood smears once into a beaker containing normal water and take out immediately.
- care should be taken during the process as sometimes the thick smear is washed off.
- do not touch or wipe the wet smear.
- however, in freshly prepared slides, dehaemoglobinisation is not required.
- alternatively, keep the dried smears flat and add 2/3 drops of normal water on the dry smears.
- after 30 seconds, the slide is ready for staining.
- care should be taken not to wet the thin smears during the process of dehaemoglobinisation.

Re-staining of old stained blood smears

Sometimes, need arises for re-examination of an old and stained blood smear which is already being examined. Even nicely stained smears in first instance usually fade when kept for a long time. The need of re-examination of old slides also arises due to poor staining in the first instance. In such condition the stained and oiled slide is immersed in xylene for a while, followed by a gentle wipe by a soft cloth which removes the old stains as well as the microscopic oils. The slide is then ready for re-staining following the same procedures as being done in case of fresh smears. In case, the thick smear is too thick or having more blood, the smears should be dehaemoglobinised (*as mentioned at 3.5.2.1 above)* before staining.

Examination of the stained blood smears

- Thick film

  Routinely, thick films are being examined under the NVBDCP. Routine examination of a thick film is based on examination of 100 good fields. In case of any doubt for identification of parasite species, further 100 fields should be examined before a final conclusion is made. This ensures that there is little possibility of a mixed infection (more than one species present in the blood film) being overlooked. A slide should be declared negative only after no parasites have been found in 100 fields of the blood film. A thick blood film consists of many dehaemoglobinized red blood cells packed together in a thick mass.

- Thin film

  Under NVBDCP, routine examination of thin film is not recommended, as it takes almost ten times longer to examine a thin film in comparison to examine a standard thick film. Very low parasitaemia could also be missed in the thin film. Examination of thin film is recommended under NVBDCP in the following circumstances

  - when no thick film has been provided
  - when the thick film is fixed or unreadable
  - when it is necessary to confirm the identification of a species

  When a thin film has to be examined, this should be done in a systematic way as given below:

  - Method
    - place the slide on the mechanical stage of the microscope.
    - position the 100x oil immersion objective over the edge of the film (where the red cells are the thinnest).
    - place a drop of immersion oil on the edge of the middle of the film.
    - lower the oil immersion objective until it touches the immersion oil.
    - examine the blood film by moving along the edge of thin film then moving the slide inwards by one field, returning in a lateral movement and so on
    - examine a minimum of 200 fields in a thin film.

Results

While examining thick and thin smears, along with the malaria parasite, normal components of the blood is also seen as described at 3. 7.4.

- Malaria parasites

  Malaria parasites take up stain in a special way in both thick and thin blood films that enables to distinguish the various parts of the parasite. They pass through a number of developmental stages, However, in all stages, the same parts of the parasite will stain the same colour.

  - Chromatin (part of the parasite nucleus) is usually round in shape and stains deep red.
  - Cytoplasm occurs in a number of forms, from a ring shape to a totally irregular shape. It always stains blue, although the shade of blue may vary between the malaria species.

Stages of the malaria parasite

Stages of the malaria parasite that are seen in peripheral blood films are described below:

Trophozoite stage

This stage is most commonly seen; it is often called the ring stage, as it mostly takes the form of an incomplete ring. The trophozoite stage is a growing stage, the parasite within the red blood cell may vary in size from small to quite large. Pigment appears as the parasite grows. Malaria pigment is a by-product of the growth or metabolism of the parasite. It does not stain but has a colour of its own, which may range from pale yellow to dark brown or black.

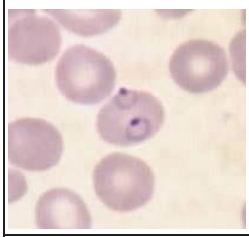
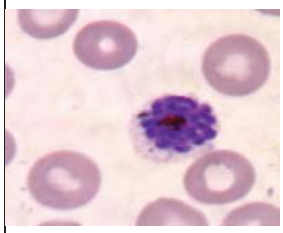
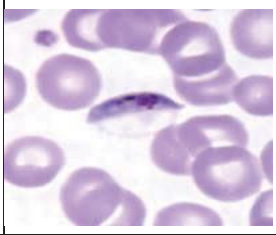
Schizont stage

During the schizont stage the malaria parasite starts to reproduce. This reproduction is referred to as asexual because the parasite is neither male nor female but reproduces itself by simple division. There are several obvious phases in this stage, ranging from parasites with two chromatin pieces to parasites with a number of chromatin dots and definite cytoplasm.

Gametocyte stage

The gametocyte is the sexual stage in which the parasites become either male or female in preparation for the next stage, the extrinsic phase which takes place in the stomach of the female anopheline mosquito. Gametocytes may be either round or banana-shaped, depending on the species. The way in which the parasite takes up the stain also helps to identify male (microgametocyte) or female (macrogametocyte). Some stages of the malaria parasite are shown in Figure 5.

Various stages of malaria parasite

		
Ring stage (Pf)	Schizont stage (Pv)	Gametocyte stage (Pf)

(Photo source: RMRC, ICMR, Diburgarh)

Species of malaria parasite

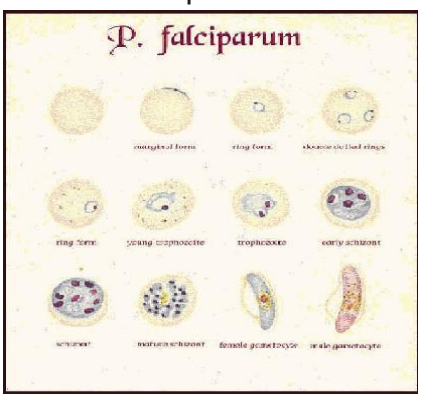
The effect of the parasite on red blood cells is also important because it helps to identify the malaria species. There are four species of malaria parasites that affect humans:

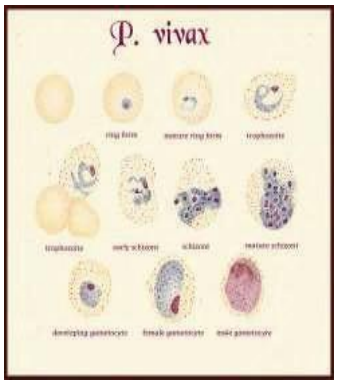
1. P.vivax is the commonest species in India. It is the largest of the malaria parasites found in humans.
2. Plasmodium falciparum, which is also the common species in India and responsible for conditions like cerebral malaria and even death.
3. P.malariae is a less common species in India, prevalant in some parts of Orissa, Madhya Pradesh and Chhattisgarh.
4. P.ovale is not prevalent in India but reported from many countries, especially from Africa.

Appearance of parasite species in thin blood films

The simplest guide to distinguish between the four species of malaria is the effect the parasite has on infected red blood cells. Features to concentrate on include the size of the red blood cell (whether it is enlarged or not) and whether or not staining reveals Schuffner's dots or Maurer's dots (also known as Maurer's clefts) within the cell as shown in the Figure 6.

P. falciparum and *P. vivax* as seen in the thin smears





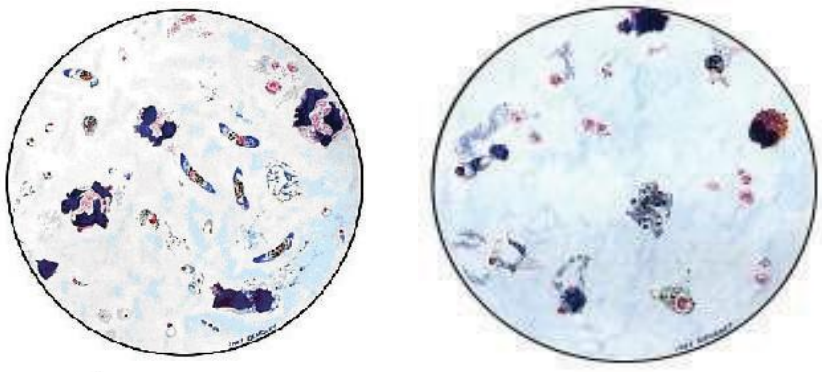
(Photo source WHO)

Appearance of parasite species in thick blood films

In a thick blood film with 100x oil immersion objective and x 7 ocular, no red blood cells are seen. The malaria parasites are seen alongwith the white blood cells as shown in the Figure

However, the parasites appear to be smaller in the thick film than in the thin blood films. The fine rings of cytoplasm of the trophozoites may appear incomplete or broken in thick blood films. Absence of red blood cells may make Schuffner's dots difficult to see. In fact, in the thicker parts of the film it may not be possible to see the stippling at all. Red cells can usually be seen surrounding parasites in the thinner parts of the films often towards the edge.

P. falciparum and *P. vivax* as seen in the thick smears



(Photograph source WHO)

Red blood cells

The shape of red blood cells or erythrocytes is described as a biconcave disc. Erythrocyte is the commonest cell that is seen in thin blood film. There are about 5,000,000 red blood cells in each micro litre of blood. After staining, the RBCs appear pale greyish pink. It measures about 7.5 micrometers in diameter. Red cells do not have a nucleus. Some cells may contain material that has stained differently. Such cells may appear larger than normal cells.

White blood cells

The total number of white blood cells in a microlitre of blood is about 6000-8000, which is much lower than the number of red blood cells. There are several different types of leukocytes as shown in Figure 8 which stain differently.

Typical white blood cells are described below:

Group – 1 Multi lobed leukocytes

Neutrophils

Neutrophils make up about 50-70% of the total white cell count in the blood of healthy persons. They have well defined granules in the cytoplasm and nucleus that stain deep purple.

Eosinophils

Eosinophils make up about 1-4% of the total white cell count in the blood of a healthy person. The granular nature of the cytoplasm is very distinctive, with the granules taking on the pinkish colour of eosin.

Basophils

Basophils are rare leukocytes, usually making up less than 1% of the total was population. Large blue or mauve granules can be seen in the cytoplasm after staining.

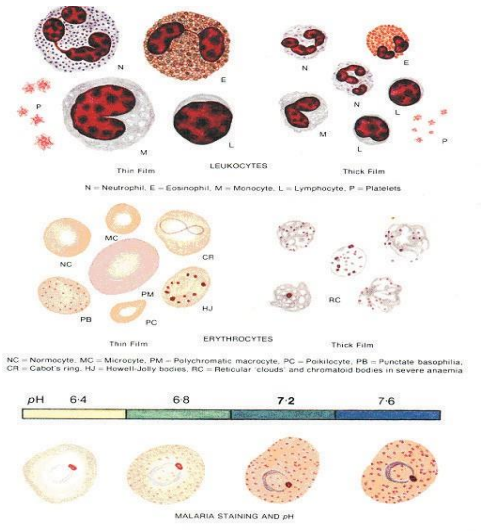
<u>Group – *2* Non-multilobed leukocytes Monocytes</u>

Monocytes usually make up to less than 1% of the total leukocytes. These are the largest of the white blood cells, about 12-18 um in diameter, having a large nucleus, kidney or bean shaped. The cytoplasm may contain a few granules that stain pinkish or red.

Lymphocytes

The two types of lymphocytes: large and small, make up 20-45% of the total white cells. The nucleus of the large lymphocyte is round and appears deep mauve in colour in well stained blood films. The large amount of cytoplasm stains clear water blue and may contain a few mauve staining granules. While the small lymphocyte is slightly larger than a normal red blood cell, it has very little cytoplasm and its nucleus stains a dark blue-black colour.

Components of the Blood



(Photograph source: WHO)

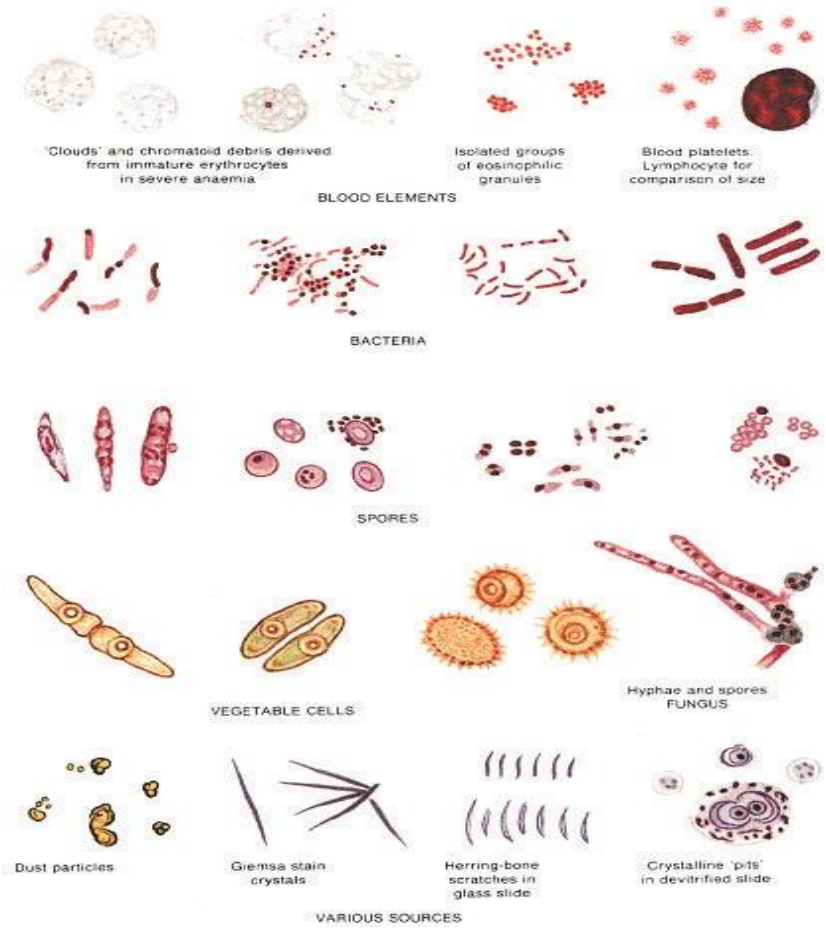
Platelets

Platelets are small, red-staining bodies of irregular shape and without nuclei they number about 1,00,000 per microlitre of blood. They often appear in groups of 5-10 but may clump together in larger numbers, if a blood film has been poorly made. It is important to be able to identify them as they may be confused with malaria parasites by inexperienced microscopists.

Artifacts in blood films

Blood films may contain many features that can cause confusion and problems in diagnosis and problems. Such features are known as artifacts. Some artifacts are depicted in Figure 9.

Artifacts that may cause confusion in diagnosis



(Photograph source: WHO)

Fungus

Will show up as artifacts on blood film. The best way to prevent fungal growths on slides is to stain blood films as soon as possible after preparation and drying them- within 48 hours at most.

Other contaminants

Dust particles floating in the air will settle on blood films while they are drying either before or after staining. Specks of dirt may be transferred from a patient's finger when a blood sample is taken or the original slide may not be perfectly clean. Some artifacts are shown in Figure 9.

Estimation of parasite density

Determination of parasite density in thick film

- using the 40x objective, select a part of the film that is well stained, free of staining debris and well populated with white blood cells. A well made film of even thickness is ideal for determining parasite density.
- place the immersion oil on the thick film.
- swivel the 100x oil immersion objective over the selected portion of blood film.
- confirm that the portion of film selected is acceptable and continue to examine the slide for 100 oil immersion fields. Move the blood film by one oil immersion field each time, following the pattern described by thin film.
- use a tally counter to count the fields as they are examined. For counting of parasite density by following the methods described below:

Counting of the parasite

It is necessary to establish a parasite count for the blood film for the following reasons:

- parasite counts are especially important in *P. falciparum* infections which are potentially fatal:
- to know the severity of malaria.
- for special purposes, such as testing the sensitivity of parasites to antimalarial drugs.
- to know the response of the malaria parasites to the anti malarial treatment being given. This can be monitored over time by plotting the parasite count on the day of treatment and comparing it with the count in a blood film made at some specified time later.
- Besides, parasite count is very essential for preparation of QC/EQA samples to be used for testing malaria RDTs. Prior to preparation of QC/EQA samples, it is recommended to pre-qualify LTs to be utilized in the field to ensure accuracy of subsequent parasite count.
- Parasite count should not start until examination of 100-field is completed and the parasite species and stages present are identified. Two methods are used to establish the parasite count as earlier.

Method 1: Parasites per microlitre of blood

This is a practical method of reasonable and acceptable accuracy. The number of parasites per microlitre of blood in a thick film is counted in relation to a standard number of leukocytes (8000). Although there are variations in the number of leukocytes between healthy individuals and even greater variations between individuals in ill health, this standard allows for reasonable comparisons. For this two tally counters are required, one to count parasites and the other to count leukocytes.

- Step 1
  - **(a)** If 10 or more parasites have been identified and counted, against 200 leukocytes, record the results on the record form in terms of the number of parasites per 200 leukocytes.
  - **(b)** In case, if only 9 or fewer parasites have been counted against 200 leukocytes counted, then continue counting until 500 leukocytes are counted; then record the number of parasites per 500 leukocytes.
- Step 2

  In each case, the number of parasites relative to the leukocyte count can be converted to parasites per microlitre of blood by the simple mathematical formula:

  Number of parasites X 8000
  ---------- = parasites per microlitre
  Number of leukocytes

  In effect, this means that if 200 leukocytes are counted, the number of parasites is multiplied by 40 and if 500 leukocytes are counted the number of parasites is multiplied by 10.

Method 2: the plus system

A simpler method of counting parasites in thick blood film is to use the plus system. This system is less satisfactory and should not be used when parasite count is done for preparation of QC/EQA samples for use with malaria RDTs. However, in the peripheral laboratory under QA/EQA of malaria microscopy for competency/ proficiency testing of the laboratory technicians, this method would be suitable. The system entails using a code between one and four plus signs, as follows:

+	=	1-10 parasites per 100 (thick film) fields
++	=	11-100 parasites per 100 (thick film) fields
+++	=	1-10 parasites per single (thick film) fields
++++	=	more than 10 parasites per single (thick film) field

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	*REPORTING* AND *DOCUMENTATION OF DATA*				
SOP No.	SOP: M 04	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 04- REPORTING AND DOCUMENTATION OF DATA

Purpose

To ensure that the patients can be traced easily for providing radical treatment/follow up, it is important to record all the required information, whether the blood smears are collected during door to door visit (active surveillance) or when the patients attend the clinic (passive surveillance).

Principle

For ensuring uniformity in data across the country and ease in analysis and interpretation, information is usually recorded on specially designed proformae. Under NVBDCP, Malaria Forms (MFs) are used for recording these informations. These MFs are in consonance with the parameters used in the malaria programme.

Reporting format for EQA

Same MFs would be utilized under EQA/QA programme, as a new reporting format may create confusion among the LTs. Besides, it would increase the paper work for the LTs and there would always be a chance for discrepancy while using the same data in separate formats. Therefore, the following MFs that are crucial in providing information regarding malaria laboratory-service would be vital for EQA programme as well, which are as under (Form 01 to 08):

- Form 01 (MF 2) – Normally used by the MPWs for collecting details of the fever cases e.g., patient's name, age, sex and village, etc. A code number is given to each patient in terms of blood smear number. This will help in identification of each fever case screened, for tracing out to provide radical treatment and also for follow up, if necessary.
- Form 02 (MF 4) – Used in the PHC for monthly reporting, it provides details of the agency wise blood smears received and their results.
- Form 03 (MF 5) – Used in the PHC for monthly reporting, it provides species wise details of the positive cases and radical treatment provided.
- Form 04 (MF 7) – Maintained in the PHC, it provides age/sex wise details of each blood smear received (name and address), its result, date of radical treatment, focal spray if conducted in and around the houses reporting *Pf* cases and its detail and also the details of the slides collected from mass and

contact of the *Pf* case including follow ups. It is useful for supervisors to see the time lag between blood smear collection and administering RT and action taken to locate the transmission foci and also to eliminate it.

- Form 05 (MF 8) – Maintained in the PHC, it provides agency wise details of blood smears collected (by individual MPW, FTD, etc), date of collection and submission at the laboratory and date of receipt of result. It is usually referred for cross checking the activities of the MPWs and time taken by the LT to provide the results. It is useful for the supervisors to see the performance of individual field worker.
- Form 06 (MF 9) – Used for village/sub-centre wise monthly analysis of epidemiological situation.
- Form 07 (MF 10) – Provides details of the institutional surveillance
- Form 08 (MF 16) – Provides details of the services provided by community volunteers and by malaria clinics

Data on these forms are considered as the minimum essential under the NVBDCP.

The Medical officer I/C PHC should check all data forms on a daily basis not only for completeness but also to ensure that they are being filled clearly and information collected make sense, specially regarding the services provided to the patients.

Care should also be taken to ensure that samples are correctly labeled and that all laboratory results are reported correctly and properly, as many of these would be used for preparation of test panels for EQA of RDTs.

These MFs would be filled/maintained at PHC level. However in the district level, data entry MUST BE carried out in the web based NAMMIS. As mentioned earlier, all the districts across the country are networked with computerized reporting system (For more details refer **www.nvbdcp.gov.in**).

As the staff associated with malaria control programme is acquainted with these MFs, Dte. of NVBDCP envisages that no change would be made in these forms and these would be used, as such, for EQA programme as well.

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	*CROSS CHECKING OF ROUTINE SLIDES FOR QA*				
SOP No.	SOP: M 05	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 05- CROSS CHECKING OF ROUTINE SLIDES FOR EQA

Purpose

It is one of the important components of EQA, widely used in malaria microscopy, used for evaluation of a LT's performance by some external agency.

Principle

This method of QA in malaria microscopy is the current practice in most countries. All positive and 5% of negative slides are sent to Reference laboratory for cross checking by another superior agency. Feed back is sent promptly to correct the results and also to take remedial action to improve the capacity of an individual LT.

Process

As mentioned in the manual on networking and capacity building, networking of laboratories has been proposed *(*Refer Figure 1, Manual on malaria Networking of laboratory) and the states/UT's are distributed among these Reference laboratories in the network (Table 1, Manual on malaria Networking of laboratory*).*

Primary level laboratory (PHC)

- the respective ROH & FWs or ICMR institutes *(*as mentioned in the chapter 2*,* Manual on malaria networking of laboratory*)*
  would convey a code number (digit) to the states and districts using the NAMMIS (all the districts in the country have access to computerized National Anti Malaria Management Information System or NAMMIS).
- however, if NAMMIS is not working due to some reason, code number would be sent by telegrams/fax etc.
- the code shall be issued on 10th day of the month and the same should be forwarded to the Primary Health Centres on the same day or latest by 11th day of the month.
- on day 12th , the slides would be dispatched from the PHC to their respective District Malaria Offices (DMOs).
- the DMO would send these slides on 13th day to the cross-checking laboratory under intimation to the State Programme Officer. The DMO would

be responsible for ensuring that each PHC sends the slides in time for cross-checking.

Results

- the results of cross-checking by the Reference Laboratories in the Form 09 should be sent to the concerned DMOs by the 15th of the succeeding month with a copy to the state and Dte. of NVDBCP. Dte. of NVBDCP envisages use of NAMMIS for transmission of cross- checking of results as well.
- the district would pass on the results to the PHCs during the monthly review meeting which is held in each district every month.
- the states would compile the data of each district and send to the Dte. of NVBDCP.
- in case of high discrepancy rate i.e., 2% or above, the cross-checking laboratories would take the needful remedial action.
- there will be supervision of the concerned laboratory to find the condition of the microscope and to provide hands on training to the concerned LT (s).
- these remedial actions would be taken in consultation with the state programme officer.

Proforma given at Form No 09 would be used for cross-checking of slides by the designated reference laboratories.

Regional Referral Laboratories

The slides cross checked at Regional Referral Laboratories (ROH&FW/ZMO/ICMR insts) shall be cross checked by National Referral Laboratory to maintain the quality of cross checking.

- This process shall be twice in a year. Once slides examined in the PHCs during transmission period and subsequently cross checked at RRLs. Another during pre/post transmission period.
- Every fouth positive slides and tenth negative slides received from the PHC shall be send by RRLs after cross checking for re-cross checking by NIMR.
- NIMR shall convey the months to the RRLs for sending the slides cross checked by them as the transmission period is not uniform acroos the country.

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	***PREPARATION OF QA PANEL SLIDES FOR EQAS***				
SOP No.	SOP: M 06	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 06- PREPARATION OF QA PANEL SLIDES FOR EQAS

Purpose

This SOP describes the preparation of Malaria positive blood smears of known diagnostic composition, for conducting EQA.

Objectives

The validated and coded blood smears for malaria microscopy will be prepared by the identified external institution for testing the proficiency of the LTs and accuracy in expressing results.

Procedure

One of the important responsibilities of the National Reference Laboratory (National Institute of Malaria Reseach) is to prepare the QA panel slides for conducting EQAS. The slides will be prepared by the following steps:

- prepare blood smears from malaria positive patients, following the method already described in SOP M. 01.
- collect 5ml blood by venepuncture from patients found to be malaria positive (donor's venepuncture: patients found to be malaria positive collect 5 ml blood after obtaining consent).
- on an average, prepare 50 slides from each donor's blood and stain in separate batches to avoid cross contamination during the preparation, following the procedures at SOP M. 03.
- each slide will have of both thick and thin smear.
- stain slides with JSB stain and protect by permanent mounting with cover slip.
- take proper care to mount the slides free from air bubbles.
- label the slides with a code and ID number.
- determine the consensus diagnosis by experienced microscopists from NIMR for each patient/donor and record/report the results as positive or negative for malaria, the species and the parasite density for upper and lower limit.
- fifty (50) sets of twenty (20) slides, i.e. one thousand (1000); each set containing 8 *P. falciparum*, 4 *P. vivax*, 4 *P. falciparum* + *P. vivax* mixed infection and 4 malaria negative slides will be provided for assessing the competence and proficiency of LTs in malaria diagnosis, species identification and density determination.
- to accommodate the anticipated high demand, the distribution of these sets of slides will be on a time-restricted manner twice a year.

NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME (NVBDCP) STANDARD OPERATING PROCEDURE FOR QA					
SOP Title	***EQAS FOR MALARIA MICROSCOPY***				
SOP No.	SOP: M 07	Revision No.	0.0	Effective Date	Dec. 2007
Replacement No		Dated		Page No.	
Next Review on	Maximum 2 years from "effective date"				

SOP: M 07- EQAS FOR MALARIA MICROSCOPY

Purpose

This SOP describes a structural set up for the assessment of the performance of laboratories conducting and participating in malaria microcopy EQAS by cross checking.

Principle and objectives

It is one of the important components of EQA for malaria microscopy for evaluation of a LT's performance and is to be introduced in India. Feed back is sent promptly after receiving the results from PHC for correction, if necessary. The objectives of this SOP are to provide guidelines to every laboratory conducting/participating in EQAS for successful performance of the programme by strictly following all stages described in the SOPM.

Process

Identification of members of the team

As mentioned in the manual on "Networking and Capacity Building" EQA is a team activity involving National, Regional, State, Zonal, District and PHC level members. Therefore, team members have to be identified to carry out the activities as defined for each echelon.

- **Team members at NVBDCP set up**

  PHC level - Medical Officer I/C and LT
  District level - District Malaria Officer
  State level - State Programme Officer
  ZMO - Zonal Programme Officer
  ROH & FW - Medical Officer in charge of laboratory (wherever present), Regional Director and LTs
  National level - QA division Dte. NVBDCP

- **Team members at NIMR set up**

  Field stations - In charge of Parasitology Lab (wherever present) or I/c Field Station and Parasitology

Lab Staff

Head Quarters - Coordinator, QA

- **Team members at Medical College set up**
  Department of Microbiology and/or Pathology - HOD and his/her identified laboratory staff for QA

Setting standards and targets for EQAS

Slide bank and its role in EQAS

- preparation of QC panel slides to conduct EQA as mentioned in SOP : M 6
- maintenance of these slides in the slide bank
- in the current QA programme, the slide bank is located at the NIMR, Delhi
- NIMR would be conducting EQAS of the Regional Reference Laboratories (ROH & FWs/ZMO and NIMR Field Stations)
- NIMR would send the EQA panel slides to the ROH & FWs in the set of 5: 2 Pf, 1Pv, 1 mixed (Pf and Pv) and 1 negative
- each ROH & FW would receive 50 such sets every year

Steps of EQAS

At National Reference Laboratory (NRL) : (NIMR)

Twenty coded positive or negative blood smears along with the proformae properly packed (form 10) will be sent by post to Regional Referral Laboratories (ROH&FWs and NIMR field stations), every 6 months.

At Regional Reference Laboratory (RRL) : (ROH & FWs)

Pack 20 coded positive P. falciparum, P. vivax and mixed infection with different parasite density and negative blood smears (see SOP m6:6.3 for more details) along with proformae (form 10) will be properly packed and sent by post to PHC level laboratory, every 6 months for external quality assessment.

Though both the ROH&FWS and NIMR field stations are involved in cross checking of blood smears, for this activity only ROH & FWs would be given the responsibility. The NRL (NIMR, Delhi) would be sending the EQAS panel slides to the ROH & FWs. The ROH & FWs would be sending these slides to the PHCs either directly in the States under their jurisdiction or through ZMO. The PHCs would send the results to the ROH & FWs in time, who will compile all the reports and send quarterly to the NIMR, Delhi, where the designated official will decode, assess and compare these results and send the report to the Dte. of NVBDCP with a copy endorsed to respective RRL.

At PHC laboratory

Slides are to be examined at the earliest by the Lab. technician, proforma duly filled, counter signed by the medical officer and sent back to respective ROH & FWs for

analysis and feed back.

Analysis of results and problem

At each level, based on the results received in the proforma given at Form 10 following actions have to be taken

Regional Reference Laboratories (ROH & FW) :-

- compare results of malaria microscopy of PHC laboratories with the known key.
- analyse results and identifiy the quality problems.
- analyse problems and provide feedback to respective laboratory.
- send quarterly reports to NIMR.

National Reference Laboratory (NIMR):-

- compare results of EQAS panel of slides received from RRLs.
- analyse EQAS results of RRLs and the PHC level, received quarterly from the ROH & FW and identify the quality problems.
- analysis of problems and feed back.
- quarterly report to Dte. of NVBDCP.

In collaboration with NVBDCP the NRL would undertake the following

- develop solution to the problems for improving quality.
- evaluate the quality improvements after EQAS implementation.
- dispatch the feed back and suggest solutions to PHC laboratory through ROH & FWs and respective SPOs/DMOs.
- The EQAS results would be referred to for accreditation of the LTs.

Confidentiality

Do not, under any circumstances, divulge the results of the tests to unauthorized individuals and results of one laboratory to another.

CHAPTER 3: NATIONAL FILAIASIS CONTROL PROGRAM (Lymphatic Filaiasis)

Filaiasis has been a major public health problem in India next only to malaria. The disease was recorded in India as early as 6th century by the famous Indian physician Sushruta. The discovery of Microfilaria (MF) in the peripheral blood was made first by Lewis in 1872 in Kolkata.

In India, lymphatic filarial cases are reported from 20 states namely Andhra Pradesh,Assam,Bihar,Chhattisgadh,Goa,Gujarat,Jharkhand,Karnataka,Kerala, Madhya Pradesh,Maharastra,Orissa,TamilNadu,UttarPradesh,WestBangal,Pondicherry,Andaman & Nicobar Islands, Div & Daman, Lakshadweep and Dadra Nagar Haveli.

Causative Organisms –

In India, Wuchereria Bancrofti, the causative organisms for filarial transmitted by the vector Culex quinquefasciatus have been the most predominant infection contributing to 99.4% of the problem in the country. Although the vector species breeds preferably in dirty and polluted water.

Brugia malayi infection is mainly restricted to rural areas due to peculiar breeding habits of the vector associated with floating vegetation Mansonia annulifera is principal vector. The breeding of these mosquitoes is associated with aquatic plants such as pistia stratiotes. In the absence of these plants the vector cannot breed.

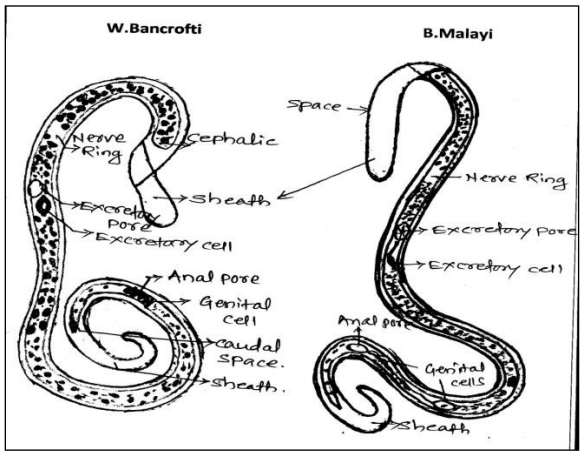
Life cycle of Filaria parasite –

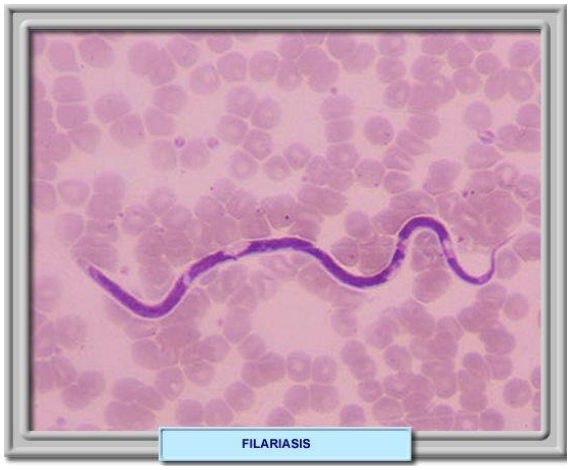
Man is the definitive host i.e. where male & female mature parasite mate and produce Microfilaria, whereas mosquito is the intermediate host. Microfilaria are usually found in the lymphatic system of man.

The parasite cycle in the mosquito begins when the Microfilaria are picked up by the vector mosquito during their feeding on the infected person. The Microfilaria in the mosquito develops into three stages and under optimum conditions of the temperature and humidity. The duration of the cycle in the mosquito is about 10-14 days. When the infective mosquito feeds on other human host, the infective larvae are deposited. Where the infective larvae get into lymphatic system. In the human host the infective larvae develop into adult male & female

MORPHOLOGY OF MICROFILARIAE

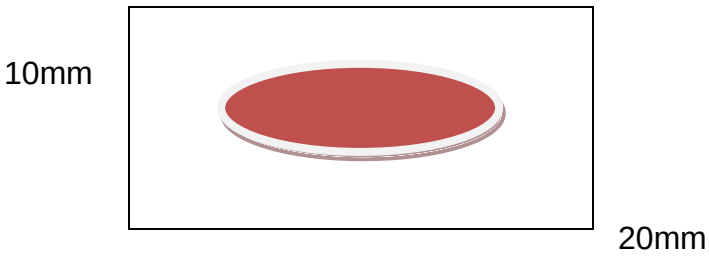
DIGRAMMATIC





Night Blood Smear Collection

- Blood Smears are Collected between 8.30pm to 12pm
- All households in the area covered.
- Blood Smears are made from all individuals, except children under two years of age.
- Record the name, age and address of the person in the register.
- Give the numbering according to register.
- Make sure that the smear is dry before putting the slide in the slide box.
- The blood smear should be at the center of the glass slide and oval shape.



- The thickness of the smears should be the 10 Layers and diameter of the blood smear should be 20mm in length and 10mm in height.
- Blood smear on the glass slide should be spread by using another glass slide as a spreader and spread uniformly only one direction i.e. Clockwise or Anti clockwise
- After drying of blood smear, write the code number and blood smear number at the edge of the slide and put vertically number wise at the slide box.
- Collected blood smear send in laboratory for examination within 24 Hrs.

Blood Smear Examination

Blood Smear staining and Examination for MF

- Collected blood smear from field should be stained within 24 Hrs

- Place the slide horizontally and by using pipette or dropper pour few drops of clean water over the smear to dehaemoglobinise for 10 minutes.
- After that flush water and allow the slide to dry.
- In two wide mouth shallow container, one which contain JSB -1 Stain (Methylene blue) and other contain clean water.
- Immerse the slide first JSB -1 Stain (Methylene blue) for 40 to 60 sec. and then wash it in clean water a couple of times.
- Keep the slide for drying in a suitable cominet so that no dust setties on it.
- After drying, Examine the slide under the microscope under low power Lense I.e.10X /40X. Examination should done one end to last end, total filed should be covered.
- For the conformation of MF oil immersion lenses (100 X) should be used.
- For the reporting, count total MF present in total filed as a result.

Calculation of Parasitological indices -

1. **Microfilaria rate (%)**

$$\frac{\text{No. of slide +ve for MF}}{\text{Total no. of Slide examined}} \times 100$$

2. **Average MF density**

$$\frac{\text{Total no. of Microfilaria}}{\text{Total no. of +ve blood smears}}$$

3. **Disease Rate (%)**

$$\frac{\text{No. of Persons +ve for MF}}{\text{Total no. of Persons examined}} \times 100$$

Entomological data Collection

Entomological data collection should be done from every village as a spot check site, the time in between 6.00 to 10.00 am. By using torch and suction tube mosquito should be collected. Dissection of vector Culex quinquefasciatus mosquito should be done and Entomological survey Larval stage is noted.

The following entomological parameters are to be calculated for each selected village or ward.

1. Ten man-hour vector Density

$$\frac{\text{No. of male \& female C. quinquefasciatus collected}}{\text{No. of man –hours spend for mosquito collection.}} \times 10$$

2. Infection rate

$$\frac{\text{No. of mosquitoes +ve for infection (L1/ L2/ L3 stages)}}{\text{No. of female vector mosquitoes dissected.}} \times 100$$

3. Infectivity rate

$$\frac{\text{No. of mosquitoes +ve for infective L3 stages}}{\text{No. of female vector mosquitoes dissected.}} \times 100$$

4. **Mean number of L3 infective mosquito**

$$\frac{\text{No. of infective larva L3 stages found}}{\text{No. of infective mosquitoes}}$$

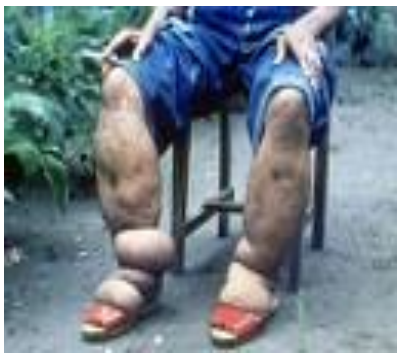
Dosage Schedule

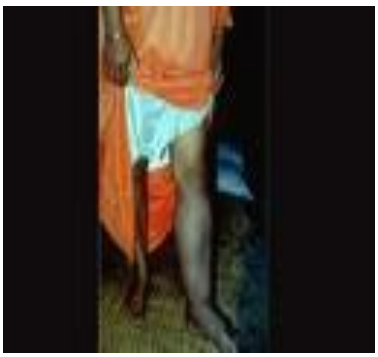
Clinical Manifestations

The Clinical Manifestations of filaiasis depends upon the stage in the course of infection in the human host and the warm load.

The stages in the course of infection as fallows

1. Stage of invasion
2. Stage of asymptomatic or carrier stage.
3. Stage of acute manifestations.
4. Stage of chronic manifestations





Drug Dosage Schedule –

Tab. DEC (Diethyl Carbomazine Citrate)

Dose - 6mg/kg/ 12days

Sr. No.	Age in Years	Tab.DEC	Days
1	Below 2 Yrs.	Nil	---
2	2 to 5 Yrs.	100mg	12 days (one time)
3	6 to 14 Yrs.	200mg	12 days (one time)
4	Above 15 Yrs.	300mg	12 days (one time)

Side effects of Tab.DEC

The drawback with DEC is that it may produce side reactions. These reactions may be of two kinds

1. Those due to drug itself (pharmacological toxicity) Headache, anorexia, nausea, abdominal pain, vomiting, dizziness, weakness or lethargy.
2. Those due to allergic reactions as a result of destruction of microfilaria and adult worms (attributable to filaricidal action) fever, local inflammations around dead worms, pruritus.

CHAPTER 4: RNTCP

AIM OF MODULAR TRAINING

This module contains information on tuberculosis and sputum microscopy. The module includes exercises on the activities and skills which the Laboratory Technician (LT) has to perform to implement the Revised National Tuberculosis Control Programme (RNTCP).

On successful completion of training, including hands-on training in a microscopy laboratory, the LT will be able to understand and perform the laboratory diagnosis of tuberculosis as under RNTCP.

WHAT IS TUBERCULOSIS?

Tuberculosis (TB) is an infectious disease caused by the bacterium, Mycobacterium tuberculosis. Tubercle bacilli mainly affect the lungs, causing lung tuberculosis (pulmonary tuberculosis). However, in some cases, other parts of the body may also be affected, leading to extra-pulmonary tuberculosis.

HOW DOES TUBERCULOSIS SPREAD?

TB germs usually spread through the air. When a patient with pulmonary tuberculosis cough, sneezes, or talks he throws TB germs into the air the form of tiny droplets. These tiny droplets when inhaled by another person may spread TB. When patients with tuberculosis begin taking effective treatment, they stop spreading the germs within a few weeks. But unless they take treatment regularly and complete it, they are likely to develop more dangerous forms of tuberculosis, known as drug-resistant tuberculosis, which they can then spread to others.

MAGITUDE OF TUBERCULOSIS IN INDIA

Tuberculosis remains a major public health problem in the country. Every year, approximately 18 lakh people develop TB disease and about 4 lakhs die of it. India accounts for one-fifth of all new TB cases each year globally and tops the list of 22 high TB burden countries.

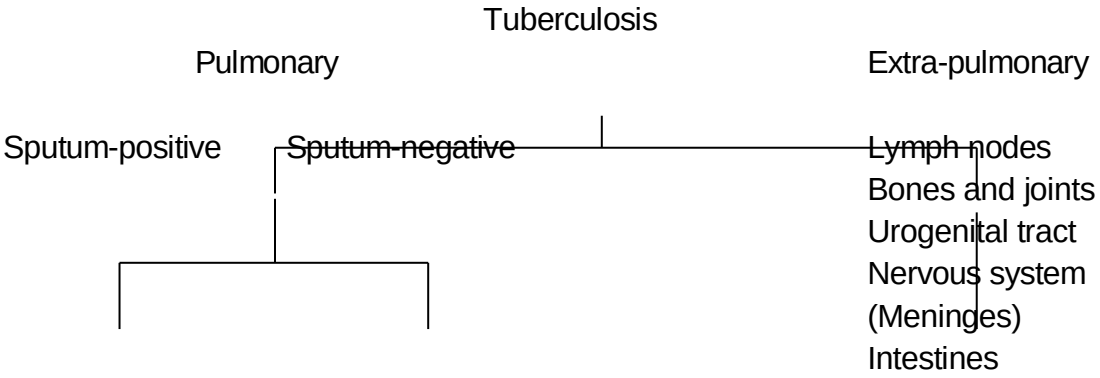
In India, EVERY DAY:

- more than 5,000 develop TB disease
- more than 1,000 people die of TB (i.e. 1 death every $1^1/2$ minutes)

It is estimated that in a year, 216 cases of tuberculosis per 100,000 population will be diagnosed and treated under the RNTCP

CLASSIFICATION OF TUBERCULOSIS

Please try to understand the following chart:



PULMONARY TUBERCULOSIS Sputum Smear-Positive

A patient with at least 1 out of two initial sputum smear examinations (direct smear microscopy) positive for acid-fast bacilli (AFB),

Sputum smear-negative

A patient having symptoms suggestive of TB with at least 2 sputum examinations negative for AFB, and having radiographic abnormalities consistent with active pulmonary TB as determined by the treating MO, followed by a decision to treat the patient with a full course of anti-tuberculosis therapy,

*__Or: A patient whose diagnosis is based on culture positive for *M.tuberculosis* but sputum smear examinations negative for AFB.__

A sputum smear-positive patient with AFB detected by microscopy is much more infectious than a patient who does not show AFB detected by microscopy. Nevertheless, even patients with sputum smear negative for AFB can have pulmonary tuberculosis

EXTRA-PULMONARY TUBERCULOSIS

Extra-pulmonary tuberculosis is TB of organs other than the lungs, such as the pleura (pleurisy), lymph nodes, intestines, genito-urinary tract, skin, joints and bones, meninges of the brain, etc.

Diagnosis should be based on one culture-positive specimen from an extra-pulmonary site, or histological evidence, or strong clinical evidence consistent with active extra-pulmonary TB followed by the MO's decision to treat with a full course of anti-TB therapy.

WHEN SHOULD TUBERCULOSIS BE SUSPECTED

Pulmonary tuberculosis

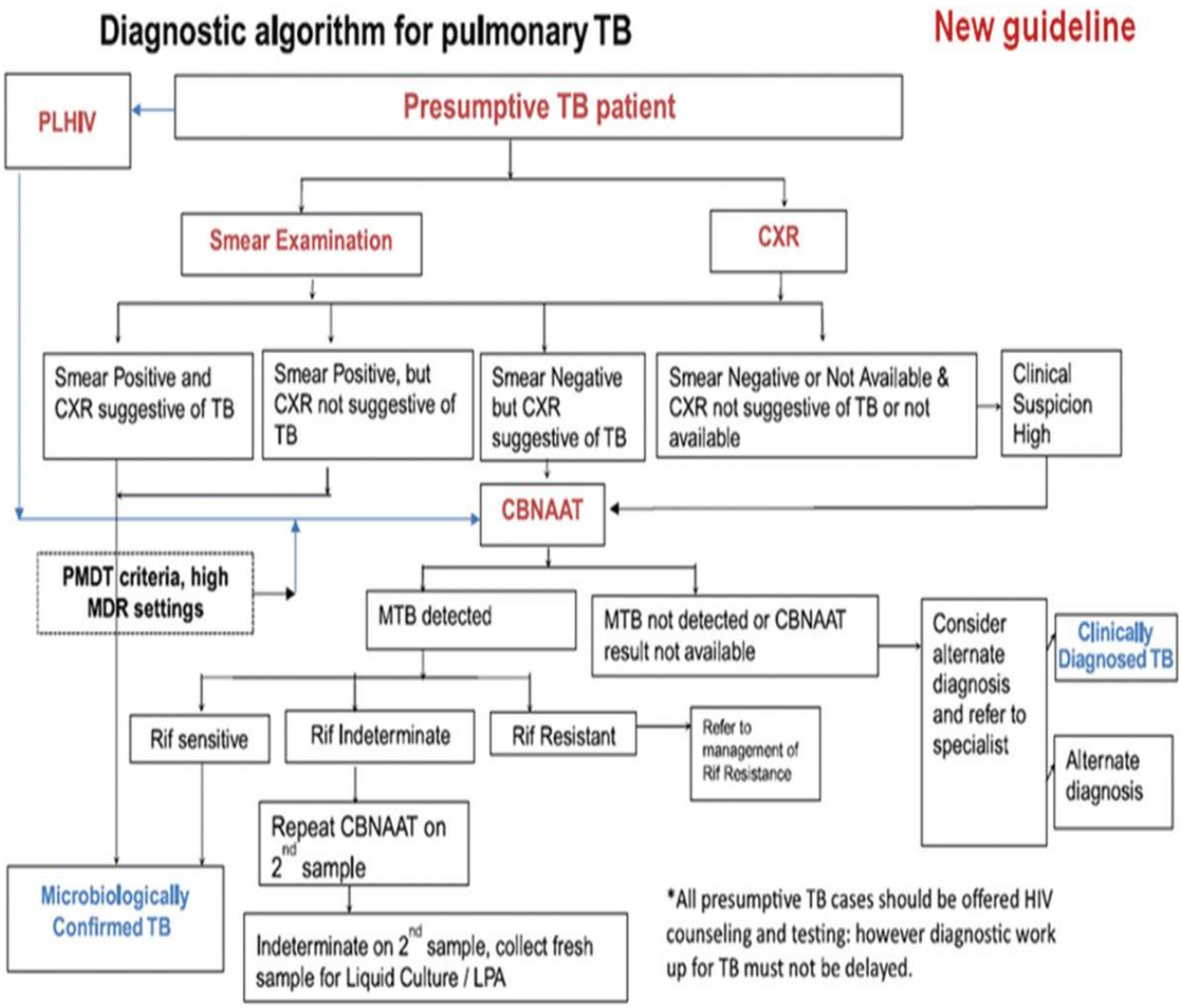
The most common symptom of pulmonary TB is a persistent **cough for 2 weeks or more**, usually with expectoration. It may be accompanied by one or more of the following symptoms:

- Weight loss
- Chest pain
- Tiredness
- Shortness of breath
- Fever, particularly with rise of temperature in the evening
- Blood in sputum in some case
- Loss of appetite
- Night sweats

Extended Criteria of TB Suspects: Cough for any duration in the following patients

- Old Age (Age > 60 Years)
- Any Chronic Disease
- Malnutrition
- HIV (and other immunocompromised patients) *&er>-e*
- Diabetic
- Renal Disease
- Asthma
- TB Contact (Esp. pediatric patients)
- Chronic Alcoholic
- Chain Smoker

The most common symptom of pulmonary tuberculosis is persistent cough for 2 weeks or more (usually with expectoration which is sometimes blood-stained) with or without associated fever and chest pain. Every patient with cough for 2 weeks or more should have sputum sample examined for AFB.



COLLECTING SPUTUM, PREPARING AND STAINING SLIDES, EXAMINING SLIDES, AND RECORDING, REPORTING AND VERIFYING RESULTS

The process of collecting sputum, preparing and staining slides, examining slides, and recording, reporting and verifying results can be divided into six stages.

Stages of sputum smear examination are as follows:

1. Collect the sputum
2. Prepare the slide for examination
3. Examination the slides under the microscope
4. Record the results
5. Report the results
6. Verify the results

STAGE 1: COLLECT THE SPUTUM

Receive patient and laboratory Form. Make sure the form is complete.

The patient should have been referred by a Medical Officer. You should ensure that they have been seen by a Medical Officer.

You must make sure that the Laboratory Form is complete; including the patient's address and reason for examination (Only one fo[r]m needs to be filled out for all 2 sputum specimens collected from a patient.). Confirm the address of the patient again so that the patient is not lost if follow-up is required. If the sputum is for follow-up examination, the patient's TB Number should have been written on the form.

Reason for examination. If the patient has come to the health facility for the first time, the sputum is examined for diagnosis. In this case, 2 sputum samples are examined (SPOT—MORNING). After a patient is diagnosed as a case of tuberculosis, treatment is started. For follow-up examinations, two samples are obtained (MORNING—SPOT).

The schedule for sputum examinations is summarized in the table below.

If the health facility is not an RNTCP designated microcopy centre (DMC), then the patient may be referred to the nearest DMC, or else the patient's sputum is collected and transported to the nearest DMC.

The results of follow-up sputum examinations are important. The treatment a patient receives depends on these results. If the first follow-up sputum examination is positive (that is, after the second month of starting treatment for patients receiving Category I treatment, or after third month of starting treatment for patients receiving Category II treatment), then the treatment is extended for one more month, and the sputum is examined again after that.

Schedule of Sputum Examinations

Category of treatment	Schedule of follow-up sputum examinations
Smear-positive Category 1	At the end of 2, 4 and 6 months of treatment
Smear-positive Category 1 (If sputum-positive at the end of Month 2)	At the end of 2, 3, 5 and 7 months of treatment
Smear-positive Category II	At the end of 3, 5 and 8 months of treatment
Smear-positive Category II (If sputum-positive at the end of month 3)	At the end of 3, 4, 6 and 9 months of treatment

Specimen Identification Number

If specimens are being transported to a DMC from another health facility, a Specimen Identification Number is given at the referring facility, because the Laboratory Serial Number can only be assigned at the DMC. Sputum specimens are assigned specific numbers to keep track of each patient's sputum results. After the Laboratory Form for Sputum Examination is filled up, this number is written on the side of the patient's sputum container. (If a sputum specimen is separated from its Laboratory Form for Sputum Examination, a LT can find out whose specimen it is by using the Specimen Identification No. on the sputum container. The laboratory technician can then locate the form by using the date and the identification number.) Each separate specimen will generally have its own unique Specimen Identification No. For example, 2 specimens from a single patient might have Specimen Identification Nos. A1 and A2. The 2 sputum specimens of the next patient may have Specimen Identification Nos. B1 and B2 1 and 2 correspond to the SPOT-MORNING samples, and this sequence of labeling should be ensured by persons collecting the sputum.

Patient's TB number All patients diagnosed as suffering from tuberculosis are entered in the TB Register maintained by the Tuberculosis Unit. The TB Number is very important. If a patient's sputum is being examined for follow-up, the TB Number should have been written in the space provided on the Laboratory Form. The TB Number should also appear on the patient's Identity Card. If the patient is carrying this card, you can enter the number from this card if it has been omitted from the Laboratory Form.

Demonstrate to the patient how to open and close the sputum container and how to bring up sputum

Give the patient the sputum container with the Laboratory Serial Number written on its side. Show the patient how to open and close the container, and explain the importance of not rubbing off the number you have written on the side of the container.

Explain to the patient that sputum examination is the only sure way to confirm the diagnosis of pulmonary tuberculosis. If it is convenient, you may show AFB-positive slides under the microscope to the patient.

A specimen collected under supervision is likely to yield better results. The person guiding the patient for specimen collection should stand behind and encourage him to cough and produce a good quality specimen. Whenever possible, sputum should be collected in an open place or in a well ventilated room meant for this purpose. Sputum should not be collected in closed rooms, toilets and ill-ventilated rooms. The person collecting the specimen should make sure that no one stands in front of the patient who is trying to cough up sputum. Patients are usually more comfortable if they are separated from other persons at the time of sputum collection.

Demonstrate to the patient by action how s/he should bring up sputum. Patient should preferably rinse his mouth as food particles may give false positive results. The patient is instructed to inhale deeply *(2-3* times), which will initiate the cough reflex in most patients. The sputum is retained in the mouth and spit into the pre labeled container without spilling. Some patients may not be able to expectorate with deep breathing in which case you should demonstrate to them how they should place their palms on the waist, squat or sit and continue deep breathing again. Tapping or thumping of the back may encourage expectoration. (Sitting and placing hands on the waist fixes the shoulder and pelvic muscles and brings the intercostal muscles of ribcage and diaphragm into action).

Most people do not understand the difference between saliva and sputum. Explain to the patient the characteristics of sputum - that it is thick and mucoid, as compared to saliva which is thin and watery. When a patient has only coughed up saliva or has not coughed up at least *2* ml of sputum, the patient should be encouraged to give good specimen. Please review your Laboratory Manual for detailed instructions on collecting sputum samples.

Write information on the Laboratory Form and on the Sputum Containers

Laboratory Serial Number. A new Laboratory Serial Number is assigned to each of the chest symptomatics whose sputum is examined. The Laboratory Serial Number begins with 1 on 1 January each year and increases by one with each patient until 31 December of the same year. Each set of samples *(2* for Diagnosis, *2* for each follow-up examination) is given one Laboratory Serial Number. Diagnosis samples are labelled with a single Laboratory Serial Number with a suffix a-b for the spot-morning samples respectively. For the follow-up examinations, the samples are labelled with a serial number and a suffix a-b with regard to the spot -morning samples respectively. Early morning specimen is always labelled as 'b[1], while the first spot specimen is labelled as 'a'. Remejriber that the/-laboratory seriahiurriber^is given to a set of slides, and not to individual

slides. Enter the Laboratory Serial NumbeTorTthe side of the sputum container and the Laboratory Form.

It is important to label sputum containers properly. Sputum containers should always be labelled on the side, and never on the lid, as the lid from one container may be placed on another container resulting is specimens being labelled incorrectly. If the labelling is incorrect, a patient who should have been treated may not get treatment, whilst a patient who does not have TB may be put on treatment unnecessarily. Label clearly with a marker that will not be easily erased.

Check the sample to see if it is sputum or saliva only

You must make sure that the sputum sample is of good quality for microscopic examination. Please review your Laboratory Manual for information on how to determine whether samples are good quality.

If the sputum sample is good, the chances of finding AFB are greater. If the sputum sample is only saliva, microscopic examination may be falsely negative for AFB. Poor quality sputum samples will result in patients receiving incorrect treatment or no treatment at all. In this case, patients may become seriously ill or die, and also spread tuberculosis to their family and community. For this reason, it is important that you visually examine every sputum sample and record its appearance on the Laboratory Form.

If the sample is poor, ask the patient to cough again until a good sample is obtained. It may take several minutes for the patient to bring out a good specimen.

A good sputum sample is:	**A poor quality sputum sample is:**
• Thick (semi-solid), coughed out deeply from the lungs • Purulent (yellowish mucus) • Sufficient in amount at least 2 ml • Free from food particles	• Contains only saliva (watery) or nasal mucus • Is small in quantity (less than 2ml) • Contain food particle

If you have explained carefully and demonstrated to the patient how to bring out sputum, sample will be of good quality and you will not need to request additional sputum for examination.

Write the visual appearance of the sputum sample on the Laboratory Form

Write the visual appearance of the sputum sample on the Laboratory Form in the space provided. You must make sure that the Laboratory Serial Number on the Laboratory form is the same as the Laboratory Serial Number on the sputum container.

If the patient has provided a sample which is saliva, explain again the importance of a good sputum sample brought out from deep within the lungs. Demonstrate how to bring up sputum, and ask the patient to provide another sample. A patient whose sputum is to be examined for follow-up may only be able to produce saliva, despite their best efforts to produce sputum. These efforts should include having the patient take a series of deep breaths. If this is not successful, try patting the patient gently on the back to help him bring out sputum. If this is not successful, ask the patient to drink something warm and then try to bring out sputum again. If, despite these efforts, the patient is still only able to produce saliva, then the saliva should be examined and the results recorded. While recording, visual appearance is noted as M- for mucopurulent, B- for blood stained, and S- for saliva.

STAGE 2: PREPARE THE SLIDE FOR EXAMINATION

The next step regarding preparing, staining, examining and reporting a sputum smear are summarized below.

Label the slide with the Laboratory Serial Number

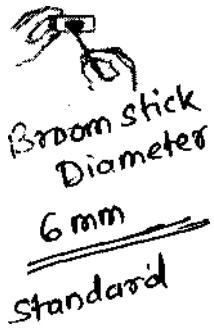
When you are ready prepare the smear, label the slide with the Laboratory Serial Number on the left side. This must be written only with a diamond marker pencil.

Remember that a new Laboratory Serial Number is assigned to each chest symptomatic whose sputum is examined. The Laboratory Serial Number begins with 1 on 1 January each year and increases by one with each patient until 31 December of the same year. Each set of sample (2 for diagnosis, 2 for each follow-up examination) is given the same Laboratory Serial Number. Diagnosis samples are labelled with a single Laboratory Serial Number with a suffix a-b for the spot-morning samples respectively. For the follow up examinations, the samples are labelled with a serial number and a suffix a-b with regard to the spot - morning samples respectively.

Be careful not to leave fingerprints on the slides. Fingerprints can interfere with staining and make accurate examination difficult under the microscope. Only new slides should be used for AFB microscopy, because scratches on old slides can look like AFB, giving a false-positive result.

Spread sputum on the slide using a broomstick and stain the slide

Spreading the sputum correctly on the slide is essential for good staining and accurate microscopic examination.

Explanation of Step in the Preparation and Staining of Slides		
Illustration	**j Step of the staining 1 procedure**	**I Reasons for/Comments on each step**
	Spread sputum on the slide using a broomstick Smear preparation should be done near a flame. This is required as approximately 6 inches around the flame is considered as a sterile zone which coagulates the aerosols raised during smear preparation.	If sample are spread too thickly or too thinly, staining and microscope examination will not be accurate. A different broomstick is used for each smear so that one patient's sputum is not mixed with another patient's sputum mixed with another patient's sputum.
	Allow the slide to air dry for 15-30 minutes	Heating the slide while it is wet could result in bubbling of T8 bacilli into the air.
	Fix the slide by passing it over a flame 3-5 times for 3-4 seconds each time	» Fixation makes the sputum stick to glass slides. • Fixation preserves the shape of the bacilli. • Heating for too long a period could change the shape of bacilli and also cause the slide to break. Heating for too short a period can result in a false-negative result because the TB bacilli will not be well preserved on the slide.
	Pour filtered carbol fuchsin to cover the entire slide	Carbol fuchsin stains the TB bacilli red. The carbol fuchsin solution must be filtered before use. if it is not filtered before use. If it is not filtered, small particles (sediments) get poured onto the slide and can appear red like the TB bacilli under the microscope

	Gently heat the slide with carbol fuchsin on it until vapour rises. DO NOT BOIL.	Carbol fuchsin solution must not be allowed to boil or to dry on the slide, otherwise it will form small particles resulting in a false-positive reading. These particles may look like TB bacilli. When the slide is heated to 80-90°C, the carbol fuchsin on the slide penetrates the wall of the TB bacilli to stain the bacilli red. Allowing the carbot fuchsin to boil, will change the shape of the TB bacilli and may result in a false-negative reading.
	Leave carbol fuchsin on the slide for 5 minutes	The wall of the TB bacillus is thick and waxy. It is essential to give the carbol fuchsin sufficient time to penetrate the wall so that it can stain the bacilli.
	Rinse GENTLY with tap water until all free carbol fuchsin stain is washed away	If water is poured too vigorously, the smear itself will be washed off the slide.
	Tilt the slide to drain off excess water.	If water is not drained, off, it will dilute the next stain/ reagent that is poured, reducing the effectiveness of the next step.
	Pour 25% sulphuric acid onto the slide	Sulphuric acid removes the carbol fuchsin stain from all of the contents of the sputum expect the TB bacilli. For this reason, TB bacilli are known as AFB, or Acid-Fast Bacilli, because the red colour of the AFB from the carbol

		fuchsin remains after they are decolourized with sulphuric acid.
	Let the stide stand for 2-4 minutes	Allowing the slide to stand gives sulphuric acid time to wash out the stain from everything except the TB bacilli. If insufficient time is given, bacteria and sputum contents other than TB bacilli may retain their stain, giving a false-positive result.
	Rinse GENTLY with tap water	• Rinsing too strongly can wash the smear itself off the slide. • Sulphuric acid burns skin. Do not let it splash. • If the slide is still stained red, you may apply sulphuric acid for a second time, letting the slide stand for 1-3 minutes this time. • It is helpful to use sulphuric acid to clean the bottom of the slide, on the opposite side of the smear. This makes it easier to examine the slide under the microscope.
	Pour 0.1% methylene blue onto the slide	• Methylene blue is the counterstain. It colours everything on the smear blue expect the AFB. • The contrast between the AFB which are stained red by the carbol fuchsin and the rest of the smear stained blue by the methylene blue, makes it

		easier to view the TB bacilli.
	Leave methylene blue on the slide for 30 second	• It takes about 30 second for methylene blue to stain the material on the slide.
	Rinse GENTLY with tap water	• Always rinse gently so that the smear is not washed off tie slide.
	Allow the slide to dry and then examine it under the microscope	• Examining a slide when it is still wet may damage the microscope. • Examining a wet slide will also make it difficult to focus the microscope and read the slide correctly. • Do not dry the slides by blotting.

Keep sputum cups and other materials in a safe place until they are discarded

Do not dispose of the sputum containers until you have examined the slides. In this way, if a repeat smear needs to be prepared from the same specimen, you can do so. However, as soon as all slides are examined, you must dispose of all contaminated materials, including sputum containers.

Place the sputum containers, broomsticks, and other contaminated materials in a container with a foot-operated lid. The materials should be submerged in 5% phenol or phenolic compound containing disinfectant phenyl diluted to 5% and should be kept overnight as per Hospital Waste Management guidelines {Annexure 11 and 12).

Infection Control Measures

You are responsible for ensuring that all biological materials are treated as potentially infectious and are handles as per the Standard (Universal) Precautions (Annexure 12). Hand washing is a simple and effective method of minimizing chances of infection for laboratory personnel, (Read Annexure 12 and familiarize yourself with the correct method of hand washing). Cleanliness of the laboratory and good housekeeping is to be ensured by the laboratory technician.

STAGE 3: EXAMINE THE SLIDE UNDER THE MICROSCOPE

Step-by-step examination of the slide, and reasons for/comments on these steps, is summarized in the table below.

Step	Reasons for/Comments on each step
Put one drop of immersion oil on the left edge of the stained smear	• Immersion oil is necessary for observations under the x100 lens. The oil will bridge the gap between the slide and the lens. • Never let the immersion oil applicator touch the slide. Doing so may contaminate the applicator if the applicator touches the slide, you may spread AFB from one slide to the next resulting in false-positive results.
Bring the slide into focus with the x40, then the x100 lens	• The x40 fens aflows you to find a suitable area of the slide to examine. Use the coarse focusing knob for this purpose. • After finding a suitable area, focus the x100 lens with the fine focusing knob. Do not use the coarse focusing knob for final adjustment, as it may break the sfide and damage the microscope. • Never let the lens touch the slide. Doing this will damage the lens and may break the slide. In addition, the lens may pick up pieces of sputum and transfer them onto the next slide examined, giving a false-positive result.
Systematically examine at least 100 fields	• Even the most experienced microscopist needs to examine each slide for at least five full minutes. If you examine each slide for too short a period or not carefully enough, you may miss AFB which are present and report the result as negative when it is actually positive. Examine every slide as if it were from one of your family members. • The appearance of AFB is shown in Annexure III of your Laboratory Manual.
Read results as: negative, scanty, or positive (1+, 2-*-or3+)	• See the table below for grading number of fields to be examined. • Grading of sputum smear results is an indicator of the load of infection and also provides epidemiological information.

Grading of slides in AFB Microscopy

I Examination	Result	Grading	No. of fields to be exarni ned
More than 10 AFB per oil immersion field	Positive	3 +	20
1-10 AFB per oil immersion field	Positive	2 +	50
10-99 AFB per 100 oil immersion fields	Positive	1 +	100
1-9 AFB per 100 oil immersion fields	Positive	Scanty (Record exact number seen)	100
No AFB per 100 oil immersion fields	Negative	—	100

STAGE 4: RECORD THE RESULTS

The "table below summarizes the steps in reporting results, and the reasons for each of these steps.

Step	Reasons for/Comments on each step
Verify the Laboratory Serial Number on the slide and record the result on the Laboratory Form	• Recording results properly is as important as staining and examining a slide correctly. Carelessness can harm patients as well as the programme itself. • Always write the date of the report and sign your name.
Wipe the x 100 lens with lens paper	• The x100 lens is a delicate piece of equipment. • Oil will gradually damage the lens unless it is promptly and carefully wiped off after each session of use. • If you take good care of the microscope, it will last for many years. • Never use spirit or xylene to clean the lens, as this may damage it by dissolving the glue.
Write results from the Laboratory ^orm in the TB Laboratory Register	• For new patients, make sure the correctly in the Laboratory Register. • If the patient is a TB suspect being evaluated for diagnosis, you must tick the "Diagnosis"' column under the

	"Reason for Examination" • For patients who undergo repeat sputum examination for diagnosis, you should write "RE" in the Diagnosis column. • You must enter the TB number in the space provided for all patients whose "Reason for Examination" is follow-up. This number should have been recorded on the Laboratory Form, and allows for cross-checking between your Laboratory Register and the Tuberculosis register. • For patients examined for diagnosis, record the TB Number and category of treatment (when known) in the 'Remarks'column. • Every specimen MUST be entered in the Laboratory Register, regardless of where the patient resides or is treated. • All positive results should be written in the Laboratory registerwith a red **pen.** This allows one to find all positive results quickly.

At the end of each month, the laboratory technician should summarize the sputum smears done that month. The format for the monthly abstract is given in Annexure M, which will be written in the last few pages of the Laboratory Register. Both supervisor and laboratory technician should sign and record the date on this list of findings.

STAGE 5: REPORT THE RESULTS

The completed Laboratory Form back to the treating physician for information and necessary action!) It is important to report these results within one day. The patient's treatment depends on these results, and any delay reduces the value of all the work you have done in examining a slide correctly.

If the patient has been referred from, and will begin treatment at the health unit where the microscopy centre is located, give the results to the treating physician. If the patient was referred from another health unit, ensure that the results of the sputum microscopy are communicated to the treating physician at the referring health facility (a copy of the results/Laboratory form to be sent to the treating physician).

Never give results only to the patient. If the patient falls to bring the results to the Medical Officer or Treatment Centre, s/he may not receive treatment.

CHAPTER 5: HIV AIDS

Tests done for diagnosis of HIV are as follows.

A. COMBAIDS

1. **Introduction:** HIV testing is done by following the Strategy III by NACO. Rapid tests are in vitro qualitative tests for the detection of antibodies to Human Immunodeficiency Virus (HIV) types 1 & 2 in human serum. HIV test kits based on three different principles and/or antigen systems are used for diagnosis of HIV.

2. **Purpose:** The SOP details the test procedures of Comb Aids for HIV testing.

3. **Scope:** It is applicable to the staff of ICTC 1 & 2 and SRL labs

4. **Responsibility:** It is the responsibility of the staff to follow the procedures to the minutest details

5. **Requirements**
   - **a.** Comb Aids test kit.
   - **b.** Distill water
   - **c.** Micro-pipette and tips
   - **d.** Gloves.
   - **e.** Timer.
   - **f.** 1% Hypo chloride solution.

6. **Principle:** Dot immunoassay employs the same principle as Enzyme Immuno Assay whereby the immobilized antigen-antibody complex is visualized by means of color producing (chromomeric) reaction.

7. **Procedure:**
   - **a.** Bring all the reagents to room temperature.
   - **b.** Take out the required number of Combs and label them.
   - **c.** Dilute the Washing Buffer.{Dilute the concentrated Washing Buffer to 1:5 with distilled water or Reagent Grade Water by adding 2ml of concentrated Washing Buffer to 8ml distilled water, taking care to avoid foaming. Fill the wash reservoir with Washing Solution.}
   - **d.** Add 2 drops (0.1ml) of Sample Diluents in micro test wells.
   - **e.** Add 2 drops (0.1ml) of Samples and Controls into each micro test well containing sample diluents.

f. Place the Combs into respective wells. {Gently rock the comb back and forth 2-3 times at the beginning at middle and at the end of incubation in the well.}
g. Incubate for 10 minutes at room temperature.
h. Add 4 drops (0.2ml) of Colloidal Gold Signal Reagent in the required number of micro test wells (Samples and controls)
i. Wash the Combs by moving the Comb forward and backward 10 times in the washing Solution in the wash tray.
j. Place the Combs in micro tests wells containing Colloidal Gold Signal Reagent.
k. Incubate for 10 minutes at room temperature.
l. Wash the combs as explained in step (I).
m. Allow the Combs to air dry and note the color development on the spotted area on the tip of teeth of the Comb for reactivity as well as for control dot appearance.
n. Interpret a pink colored spot/dot, both in the "test" and "control" as positive.
o. Interpret a pink colored spot/dot in the control area as negative.
p. Interpret faint coloration of dot/spot in test area having color intensity between 0.00 and 1.0 color index as negative.
q. Interpret as "Invalid" if no pink colored dot/spot is visible in "control area irrespective of presence of absence of pink colored dot/spot in the test area.
r. Discards the combs as per (MICRO/SOP/GEN-02) Biomedical waste Management

8. Result and interpretation

a. Invalid test: Test will be "invalid" if no pink color spot is visible in "control" area irrespective of presence or absence of pink colored spot in the test area. In such cases the test should be repeated with new comb & fresh specimen.

b. Interpretation:

- **Positive result**: Presence of pink colored spot both in the "test" & "control" area is a positive test. The intensity of spot developed in test area should be equal to or more than 1.0 color index when compared with the reference colour index chart
- **Negative result**: Presence of only one pink colored spot in the control area indicates negative test.
- **Indeterminate result**: faint coloration of the spot in test area having colour intensity between 0.00 & 1.0 colour index is considered as indeterminate. In such a cases repeat the test on same sample. If the result is still "indeterminate", repeat the sample after 4-8 weeks for retesting.

Enter the results in register against respective sample as reactive/non-reactive/indeterminate (checked by in charge technician & supervised by supervisor)

9. **References**:
   Manufacturers insert

10. **Documents:**

11. **Validity:** This document is valid for one year after effective date.

B. CD4 Test

1. **Introduction**
   Flow cytometry: - Flow means Fluid medium, Cyto means Cell, Metry Measurement. It is simultaneous measurement of multiple characteristics of a single cell or particle as they pass through a fluid medium.

2. **Purpose:** To know the progression of HIV infection.

3. **Responsibility:** It is responsibility of the CD4 Laboratory technician to read & follow the SOP.

4. **Procedure**
   4.1 Sample acceptance
   - **a.** Wear Gloves apron & all safety precautions
   - **b.** Check and verify the details mentioned on the samples & in the CD4Laboratory register are same.
   - **c.** Check that the samples are brought to the laboratory in ambient temp/ cold chain when there is a delay beyond four hours.
   - **d.** Check the sample tubes are intact and there is no leakage
   - **e.** See the sample in vaccutainer
   - **f. Reject the samples** if
     - Sample is haemolysed
     - Sample is clotted
     - Sample is frozen
     - Sample tube is broken or cracked
     - Sample not drawn in K2/K3 EDTA vaccutainer
     - Sample not transported at ambient temperature
     - Quantity insufficient
   - **g.** Keep the samples at ambient temperature till they are processed

   4.2 Sample Preparation Procedure:- Perform the procedure using all laboratory safety precautions
   - **a.** Take BD True count tube.
   - **b.** Label it with patients ID
   - **c.** Add 20 ul Tri test antibodies reagent.

d. Then add 50 ul well mixed whole blood sample.
e. Mix by taping the tube
f. Incubate in dark for 15 min at room temperature
g. Add 450 ul of (1x) FACS lysing solution.
h. Vortex & Incubate in dark for 15 min at room temperature
i. Sample is ready to be run in FACS Caliber Machine.

5. **SAMPLE PROCESSING PROCEDURE**
   a. Turn on UPS battery switch.
   b. Start the FACS Caliber Machine (green button)
   c. Turn on the computer, choose user ID and enter password
   d. Check sheath and waste tank in machine
   e. Depressurize system
   f. Fill the sheath tank up to 75% capacity
   g. Empty waste tank and add 400 ml undiluted bleach
   h. Pressurize system
   i. Check the flow cell for air bubble
   j. Press prime button to remove air bubble
   k. Select on loader software on desktop for long cleaning the machine.
   l. Select work list manager software on desktop, enter the patient's details (Name, sample ID) age sex, and case number .Save work list in BD file...
   m. Arrange the ready samples on carasol rack
   n. Put the rack in FACS Caliber Machine
   o. Press **RUN TEST** button on desktop and machine
   p. Collect the printed physician's report
   q. Show results to laboratory in charge/designee, take signature.
   r. Enter the report in CD4 lab register.
   s. Send the reports to collection site and hand over to patient in next visit Take data back up ,keep one copy in laboratory, one in ART centre.

6. **References :** BD BIOSCIENCES guideline manual.

7. **Documents and records**
   Appendix-01

8. **Validity**
   This document is valid till one year after the effective date.

MICRO/CD-4/5/1

DAILY WORK FORMAT FOR CD4

MONTH/YEAR: _____

Date	Temp. Record	Priming / Cleaning of Machine	Calibration of Machine	IQC low/high	Multicheck sample	CD4 testing	Cleaning & priming of Machine	Dialy CD4 pt. Excel Sheet	Sign all Reports

C. AIDSSCAN

1. **Introduction:**
   HIV testing is done by following the Strategy III by NACO. Rapid tests are in vitro qualitative tests for the detection of antibodies to Human Immunodeficiency Virus (HIV) types 1 & 2 in human serum. HIV test kits based on three different principles and/or antigen systems are used for diagnosis of HIV.

2. **Purpose:**
   The SOP details the test procedures for HIV testing.

3. **Scope:**
   It is applicable to the lab staff of ICTC and SRL

4. **Responsibility:**
   It is the responsibility of the staff to follow the procedures to the minutest details

5. **Requirements**
   - Rapid test kit.
   - Distill water
   - Micro-pipette and tips
   - Gloves.
   - Timer.
   - 5% Hypo chloride solution.

6. **Principle**:
   HIV recombinant protein antigens-gp41, c terminal of gp-120 & gp-36 representing the immunodominant regions of HIV1 &2 are immobilized on a Nitrocellulose membrane. As the sample passes through the membrane HIV antibodies if present binds to the above mentioned antigens. These bound

antibodies are visualized by reacting with Protein A Gold conjugates, which binds to the HIV antibodies, giving a distinct red spot against a white background.

7. **Procedure:-**
    - Remove the test device from the pouch just prior to the testing
    - Add 2 drops of buffer solution to the test device
    - Add 2 drops of serum
    - Add 4 drops of buffer solution
    - Add 2 drops of cold conjugate
    - Add 4 drops of buffer solution & read the result within 5 minutes

8. **Validity of the test:**
    1st check the validity of the test. Test will be “invalid” if no spot is visible in “control” area irrespective of presence or absence of spot in the test area. In such cases the test should be repeated on sample.

9. **Result and interpretation**
    - **Positive result**- If three red spots appear (control spot & test spot) appear then sample is reactive for HIV-1 & 2
    - **Negative result**- If only one red spot appear in control area then the sample is negative for HIV-1 & 2

10. **References:**
    Kit Insert

11. **Valididty:**
    This document is valid for one year from the effective date

D. ELISA WASH

1. This test is designed to perform washing operations required in the ELISA technique. The microplate washer performs the washing of the ELISA plate’s wells during the different stages of the technique.

2. **Purpose:**The microplate washer has been designed to supply cleaning buffers required for the ELISA technique in a controlled manner. In the same fashion, the equipment removes from each well, substances in excess from the reaction. Depending on the test performed, the washer can intervene from one to four times, supplying the washing buffer, agitating and removing the unbound reagents 1 until the programmed times and cycles are completed. The washer has of two reservoirs; one for the washing buffer, the other for the waste generated during the washing process.

3. **Scope:** This SOP describes the maintenance and calibration procedures for Photometric micro-plate reader or ELISA washer.

4. **Abbreviations;**
    - **SOP** Standard Operating Procedures

5. **Responsibility**
    - Technical manager and Technical officer is responsible of the yearly maintenances of the instruments
    - It is the Responsibility of the Lab. technician to routine maintenance

6. **Procedure**
    a. **Routine Maintainance**
        - Verify the volume distributed.
        - Test the filling uniformity.
        - Verify the aspiration sub-system's efficiency.
        - Confirm the cleaning of the supply and extraction needles.
        - Clean the washer with distilled water after use, to remove every
        - Vestige of salt in the supply and extraction subsystems channels.
        - The needles may be kept submerged in distilled water.
        - Verify that the body of the washer has been cleaned.
        - If necessary, clean the exterior surfaces with a piece of cloth, moistened with a mild detergent.

    b. **Preventive maintenance Frequency: Quarterly**
        - Disassemble and clean the channels and connectors.
        - Verify their integrity. If leaks or any vestiges of corrosion are detected, adjust and/or replace.
        - Verify the integrity of the mechanical components.
        - Lubricate according to the manufacturer's instructions.
        - Test the adjustment of each one of the subsystems.
        - Calibrate according to the manufacturer's recommendations.
        - Confirm the integrity of the electrical connector and the inter-connection cable.
        - Clean the washer with distilled water after using it in order to remove every vestige of salt in the supply and extraction subsystems' channels.
        - Verify the integrity of the fuse, and that its contact points are clean.

    c. **Calibration:** Calibration of the microplate reader. Do it yearly from authorized agency in annual maintenance contract

    d. **Troubleshooting** : If there is troubleshooting follow trouble shooting table

Problem	Problem Cause	Solution
Upon completion of washing, residual solution remains in the wells.	The washer extraction system demonstrates failure.	Verify if the vacuum system is functioning at the appropriate pressure.
	The conducts/pipes of the vacuum system are of a different diameter than that recommended	Check that the diameter of the channels corresponds to the recommendation by the manufacturer.
	The suction line shows obstructions	Verify that the vacuum lines are clean
	The container for storing the waste is full	Confirm the waste recipient's level.
	The line filter is damp or blocked.	Verify the state and integrity of the suctioning system's fi lter.
	The needles' points are not placed correctly and do not reach the bottom of the wells.	Examine the placement of the needles' points.
	A different microplate is used in the test	Verify the type of plate required for the test.
	The washer has not been purged sufficiently.	Check the purging process.
	The operator has not followed the manufacturer's instructions correctly	Examine the process recommended by the manufacturer. Carry out the required adjustments.
	The plate placed in the washer is incorrectly aligned	Check the placement of the plate in the washer.
	The washer extraction system demonstrates failure	Verify if the vacuum system is functioning at the appropriate pressure.
	The conducts/pipes of the vacuum system are of a diff erent diameter than that recommended	Check that the diameter of the channels corresponds to the recommendation by the manufacturer
The washing cycle is performing inadequately.	The washing solution reserve is exhausted	Examine the cleaning solution storage receptacle. Replace the volume missing.
	The washer was not purged sufficiently at the beginning of the work cycle	Clean adequately in order to homogenize the humidity in each one of its components and to eliminate air bubbles

		Verify the required volume for each type of test and for each plate.
	The plate was placed incorrectly in the washer	Check the correct installation of the plate in the washer.
	The cycle setting was incorrectly selected	Review the cycle setting recommended for each type of plate.
	The plates used are different from those recommended by the manufacturer	Verify that the plates used are completely compatible with the washer
	The fluid level in the wells is inadequate.	Check the manufacturer's specifi cations correct If necessary,
	The washing solution supply tube is not of the diameter or thickness specified by the manufacturer	Check the manufacturer's specifications correct If necessary,
	The pressure is insufficient for delivering the adequate amount of washing solution.	Check the supply system and supply channels, there might be an obstruction in the filling line
The washing container shows fungal and bacterial growths.	The system is not used frequently.	Check the procedures used for preventing fungal and bacterial growth.
	An adequate control procedure (disinfection) is not used.	Check the procedures used for preventing fungal and bacterial growth.
	The tubes and connectors are not changed with the required frequency.	Verify the change frequency suggested by the manufacturer and or the technical department.
	The washing solution has been contaminated	Confirm the procedures used in the preparation and management of the washing solution with the aim of determining the cause of contamination and eliminate it

7. **Documents and records :** Lab equipement manual WHO 2008

8. **Valididty**: This document is valid for one year after its effective date.

E. Elisa reader

1. **Introduction:** The microplate reader also known as “Photometric micro-plate reader or ELISA reader” is a specialize spectrophotometer designed to read results of the ELISA test, a technique used to determine the presence of antibodies or specifi c antigens in samples. The techniqueis based on the detection of an antigen or antibodies captured on a solid surface using direct or secondary, labelled antibodies, producing a reaction whose produce can be read by the spectrophotometer. The word ELISA is the acronym for “Enzyme-Linked Immunosorbent AssayBetter and consistent quality result

2. **Purpose:** The microplate reader is used for reading the results of ELISA tests. This technique has a direct application in immunology and serology. Among other applications it confirms the presence of antibodies or antigens of an infectious agent inan organism, antibodies from a vaccine or auto-antibodies..

3. **Scope:** This SOP describes the maintenance and calibration procedures for Photometric micro-plate reader or ELISA reader**.**

4. **Abbreviations;**
   - **SOP** Standard Operating Procedures
   - **PPE** Personal Protective Equipment

5. **Responsibility**
   - Technical manager and Technical officer is responsible of the yearly maintenances of the instruments
   - It is the Responsibility of the Lab. technician to routine maintenance

6. **Procedure**

   6.1 ROUTUNE MAINTAINANCE
   - Review that optical sensors of each channel are clean.
   - If dirt is detected, clean the surface of the windows of
   - the light emitters and the sensors with a small brush.
   - Confirm that the lighting system is clean.
   - Verify that the reader’s calibration is adequate.
   - When the daily operations begin, let the reader warm up for 30 minutes.
   - Next, do a blank reading and then read a full plate of substrate. The readings must be identical.
   - If not, invert the plate and repeat the reading in order to determine if the deviation originated in the plate or the reader.

- Examine the automatic drawer sliding system. It must be smooth and constant

6.2 Preventive maintenance Frequency: Quarterly

- Verify the stability of the lamp. Use the calibration plate, conducting readings with intervals of 30 minutes with the same plate. Compare readings. There must be no differences.
- Clean the detectors' optical systems and the lighting systems.
- Clean the plate drawer.
- Verify the alignment of each well with the light emission and detection systems.

7 Calibration : Calibration of the microplate reader

- The calibration of a microplate reader is a specialized process which must be executed by a trained engineer following the instructions provided by each manufacturer.

Trouble shooting

Problem	Problem Cause	Solution
The reader gives a reading that does not make sense.	The illumination lamp is out of service.	Replace the lamp with one with the same characteristics as the original.
The reader's readings vary from row to row.	Dirty optical sensors.. The illumination system's lenses or parts are dirty.. Lack of calibration in one or more channels	Clean the sensors Clean the lighting system's lenses. Verify the calibration of each one of the channe
The reader displays high absorbance values.	Reagents expired and/or incorrectly prepared	Check to see if the TMB is colourless and the preparation adequate
	Contamination with other samples	Repeat the test verifying the labelling, the washer and how the pipette was used.
	Incorrect wavelength filter.	Verify the recommended wavelength for the test Adjust if it is incorrect.
	Incorrect wavelength filter.	Verify the recommended wavelength for the test. Adjust if it is incorrect.
	Insufficient washing.	Verify the washing method used. Use an appropriate quality control test
	Very long incubation time or very high temperature	Check incubation times and temperatures.
	Incorrect sample dilution. Check process for sample	Check process for sample dilution

	dilution	
	Some reagent was omitted	Verify that the test has been carried out according to the established procedure
The reader displays low absorbance values.	Very short incubation time and very low temperature,	Check temperatures and incubation times.
	The reagents were not at room temperature.	Check that the reagents are stable at room temperature.
	Excessive washing of the plate.	Adjust the washing process to what the test manufacturers indicate
	Incorrect wavelength fi lter. Verify the wavelength selected.	Use wavelength recommended for the test.
	Expired or incorrectly prepared reagents.	Check the used reagents. Test the dilutions.
	A reagent was omitted.	Verify that the test was done according to the established procedure
	The plate displays scratches at the bottom of the wells.	Prepare a new plate and repeat the test.
The reader displays low absorbance values	Very short incubation time and very low temperature.	Check temperatures and incubation times.
	The reagents were not at room temperature.	Check that the reagents are stable at room temperature.
	Excessive washing of the plate	. Adjust the washing process to what the test manufacturers indicate.
	Incorrect wavelength fi lter. .	Verify the wavelength selected. Use wavelength recommended for the test
	Expired or incorrectly prepared reagents..	Check Test the dilutions the used reagents Verify that the test was done according to the
	A reagent was omitted.	established procedure
	The plate displays scratches at the bottom of the wells.	Prepare a new plate and repeat the test.
	Incorrectly selected or dirty plate	Change the manner in which the plate is washed
	The plate wells have dried	Verify the type of plate used.

	up. The plate is incorrectly placed or is seated unevenly in the reader. Humidity or fingerprints on the outer part of the bottom of the plate. Residual quantities of washing buff er in the wells before adding the substrate. The substrate tablets do not dissolve completely. The substrate tablet has been contaminated by humidity or metal clips or is not complete The position of the blank well could have been changed and an incorrect quantity has been subtracted at each reading	Prepare a new plate and repeat the test. Check the placement of the plate and Repeat he Reading Verify that the plate under the bottom of the wells is clean. Confirm that the washing buffer is completely removed. Verify that the tablets dissolve correctly. Test the integrity and handling of substrate tablets. Verify that the plate set-up is correct.
The reader displays unexpected variation in the optical density readings	The reader's lamp is unstable.	Replace the lamp with one that has similar characteristics as the original.
The reader displays a gradual increase or decrease from column to column	Inappropriate calibration of the plate's advance motor	Calibrate the advance so that at each step the wells remain exactly aligned with the lighting system
The optical density readings are very low compared to the operator's optical evaluation criteria	The reading is being carried out with a different wavelength than required for the test.	Verify the wavelength used when conducting the reading. If this is the problem, adjust the wavelength and repeat the reading. Verify that the recommended wavelength fi lter has been selected
Low reproducibility	Sample homogeneity	Mix the reagents before use. Allow these to equilibrate to room temperature.
Incorrect pipetting	Check the calibration.	Use an appropriate quality

procedure.		Ensure pipette's tips are changed between samples and that excessive liquid inside is removed
	Reader not calibrated	Wait until the reader has warmed up to its operating temaprature
	Expired reagents	Verify the expiry dates of the reagents when washed.

8 **Documents and records : lab equipement manual Who 2008**

9 **Valididty:** This document is valid for one year after its effective date.

F. HIV KIT

1. **Introduction:** This standard operating procedure defines the procedures to be followed to perform rapid tests for the detection of antibodies to HIV-1 and /or HIV-2 in human serum
2. **Purpose:** To confirm the presence of HIV-1 and /or HIV-2 antibodies in human serum
3. **Scope: T**his SOP applicable for the Department of Microbiology, GMC, Aurangabad
4. **Responsibility:** It is the responsibility of ICTC-1, ICTC-2 and SRL technician to follow the SOP for HIV testing
5. **Requirements:**

1. Rapid Test kit 1, 2 and 3	5. Discarding jar with 1% sodium hypochlorite
2. Timer	6. Paper towels / Absorbent papers
3. Marker Pens	7. Micro pipettes and tips
4. Registers	8. Sterile Gloves, masks, goggles
	9. Data sheet

6. **Procedure:**
   a. **Sample preparation:**
      - **Use PPE Refer- MICRO/SOP/GEN- 6**
      - Centrifuge samples at 3000 rpm for 10 min to separate serum to avoid hemolysis
      - The serum is transferred to a sterile screw capped container and the clot s discarded as per **MICRO/SOP/GEN-02**. Biomedical Waste Management.
      - Arrange samples serially as transcribed in the data sheet. (**MICRO/SOP/ICTC/6/1**) Data sheet

- Keep the entire required things ready and align in order before performing the test.

b. HIV testing:

- Use NACO strategy III for HIV testing. **MICRO/SOP/ICTC-12.Reporting of HIV testing results.**
- Use three different tests provided by MSACS/ NACO with different principles of testing for detecting antibodies against HIV**.** Read the kit manufacturer's instructions carefully(**MICRO/SOP/ICTC/7,18,11**)
- Follow the work instructions given for each test.
- Perform the test according to the KIT manufacturer's instructions
- Apply Daily positive and negative external controls of confirmed positive samples from SRL/NARI/Panel sera)
- Apply positive and negative internal controls provided by the kit on every Monday.
- Apply external control on first day of every week and every time a new lot of the kits is used.
- For second and third kit, test with internal controls on opening of kit and test with external controls on further use of the second and third kit.
- Check the internal control band / line / dot for HIV -1 and HIV -2
- Check the positive & negative control results if no results seen with positive control use known positive sample as positive control.
- For interpretation of report follow **MICRO/SOP/ICTC-11**. **Reporting of Results for HIV.**
- Prepare reports in the format given by **MSACS (ICTC/SOP/ICTC/6/2).**
- The test is cross checked by other Technical officer.
- The results are verified by the Technical Manager.
- Samples are stored for 48hrs.at 2-8C Refer **MICOR/SOP/ICTC-13 Storage of samples**
- Samples are discarded after 48 hrs in red bag except those sent for EQAS refers **–(MICRO/SOP/ICTC/14) EQAS for ICTC**
- The test cards are discarded in the yellow bag**.**

c. Interpretation: As per the manufacturer's instruction

7. **Waste disposal:** Refer**: MICRO/SOP/GEN-02**. Biomedical Waste Management and **MICRO/SOP/GEN-07.** Spills Management

8. **Safety precautions:** Follow the universal work precautions. Refer **MICRO/SOP/GEN-06. Biosafety Measures.**

9. **References:** Guidelines on HIV testing, NACO, March 2007

10. Documents and records:

- MICRO/SOP/GEN-02. Biomedical Waste Management
- MICRO/SOP/ICTC-12. Reporting of Results for HIV
- MICRO/SOP/GEN-06**.** Biosafety Measures
- Appendix-1 HIV testing data sheet
- Appendix-2. MSACS Report Format**.**

11. Validity: This document is valid till one year from the effective date.

MICRO/ICTC/6/1: HIV TESTING DATA SHEET

SR NO.	CLIENT OR SPECIMEN	AGE	SEX		QUALITY OF SAMPLE		TEST 1 KIT NAME LOT NO. EXP. DATE			TEST 2 KIT NAME LOT NO. EXP. DATE			TEST 3 KIT NAME LOT NO. EXP. DATE			FINAL RESULT			SENT FOR FURTHER QA TESTING	FINAL QA RESULT			QA REPORTS RECEIVED	ANY
	ID	YRS	M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND	DATE	P	N	IND	DATE	REMARKS
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
	PC		M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
	NC		M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
1			M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
2			M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
3			M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
4			M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
5			M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
6			M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
7			M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
8			M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
9			M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
10			M	F	G	B	R	NR	INV	R	NR	INV	R	NR	INV	P	N	IND		P	N	IND		
TOTAL																								

PC=POSITIVE CONTROL, NC=NEGATIVE CONTROL, M=MALE, F=FEMALE, G=GOOD, B=BAD, R=REACTIVE, NR=NON-REACTIVE, INV=INVALID, QA=QUALITY ASSESSMENT

P=POSITIVE, N=NEGATIVE, IND=INDETERMINATE, GOOD QUALITY OF SAMPLE=NO HAEMOLYSIS, NO LYPEMIC, NO TURBIDITY & SUFFICIENT VOLUME

SAMPLE RECEIVED BY	DATE OF TESTING	TESTED BY	CHECKED BY	INCHARGE	PAGE NO.

MICRO/ICTC/6/2: ICTC HIV TEST REPORTING FORMAT

HIV TEST REPORT FORM

Name and address of ICTC centre: *(Form to be filled in duplicate)*

Name : Surname _____ **First name** _____ **Middle name** _____

Gender: M / F / TG Age: _____ **Years PID #** _____ **Lab ID #**_____

Date and time blood drawn: _____ **(DD/MM/YY)** _____ **(HH:MM)**

Test Details

Specimen type used for testing: Serum / Plasma / Whole Blood

Date and time specimen tested: _____ **(DD/MM/YY)** _____ **(HH:MM)**

Note :

- **Column 2 and 3 to be filled only when HIV 1 & 2 antibody discriminatory test(s) used**
- **No cell has to be left blank; indicate as NA where not applicable.**

Column 1	Column 2	Column 3	Column 4
Name of HIV test kit	Reactive/Nonreactive(R/NR) for HIV-1 antibodies	Reactive/Nonreactive (R/NR) for HIV-2 antibodies	Reactive/Nonreactive (R/NR) for HIV antibodies
Test I :			
Test II :			
Test III :			

Interpretation of the result : Tick(√) relevant

□ **Specimen is negative for HIV antibodies**

□**Specimen is positive for HIV-1antibodies**

□***Specimen is positive for HIV antibodies (HIV 1 and HIV 2; or HIV 2 alone)**

□ **Specimen is indeterminate for HIV antibodies. Collect fresh sample in two weeks.**

**** Confirmation of HIV 2 sero- status at identified referral laboratory through ART centres***

--End of report--

Name & Signature
Laboratory Technician

Name & Signature
Laboratory In-charge

-

G. MERISCAN

1. **Introduction:** HIV testing is done by following the Strategy III by NACO. Rapid tests are in vitro qualitative tests for the detection of antibodies to Human Immunodeficiency Virus (HIV) types 1 & 2 in human serum. HIV test kits based on three different principles and/or antigen systems are used for diagnosis of HIV.

2. **Purpose:** The SOP details the test procedures for HIV testing.

3. **Scope:** It is applicable to the lab staff of ICTC and SRL

4. **Responsibility:** It is the responsibility of the staff to follow the procedures to the minutest details

5. **Requirements**
   - Rapid test kit.
   - Micro-pipette and tips
   - Gloves.
   - Timer.
   - 1% Hypo chloride solution

6. **Principle:**
   MERISCREEN HIV1-2 WB rapid test kits contain a membrane strip, which is pre-coated with HIV-1 & HIV-2 antigen on test region '1' and test region '2' respectively. Recombinant antigen gold conjugate will form a colored band on the test region '1' and the test region '2' of result window. As the test sample flow through the membrane after addition of assay buffer, the antigen gold conjugate complexes with antibodies. The complex moves further on the membrane towards the test region, where HIV antigens are coated and leads to formation of reddish purple band(s). Absence of test band indicates a negative test result.

   The control band is used for procedural control and should always appear if the test procedure is performed correctly .the intensity of control band has nothing to do with intensity of test band(s).

7. **Procedure:-**
   - Wear appropriate PPE
   - Remove the test device from the pouch just prior to the testing
   - Add 10 µl of serum sample.
   - Add three drops of the assay Buffer to the sample well.

- Interpret the test result at the end of 20 minutes .Do not read the result after 30 minutes

8. **Validity of the test:**
   1st check the validity of the test. Test will be "invalid" if no band is visible in "control" area irrespective of presence or absence of band in the test area. In such cases the test should be repeated on sample.

9. **Result and interpretation**
   9.1 Positive result- If three red bands appear (control band & test band) appear then sample is reactive for HIV-1 & 2, the result is positive.
   9.2 Negative result- If only one red spot appear in control area then the sample is negative for HIV-1 & 2

10. **References:** Kit Insert

11. **Valididty:**
    This document is valid for one year from the effective date

H. RAPID SLIDE TEST FOR SYPHILIS

1. **Introduction:** For the quantitative and semi-quantitative detection of regain antibodies for the diagnosis of syphilis by rapid slide method using rapid plasma regain (RPR) antigen.

2. **Purpose:** This SOP outlines the steps taken to perform the Rapid slide method for detection of syphilis.

3. **Scope:** This SOP is applicable to all laboratory staff of Serology Lab, Department of Serology, In Medical Colleges**.**

4. **Abbreviations:**
   - **SOP** - Standard Operating Procedures
   - **PPE** - Personal Protective Equipment
   - **TAT** - Turn Around Time

5. **Responsibility:** It is the responsibility of the
   - Laboratory assistant to receive the samples in the laboratory**.**
   - Laboratory technician to accept or reject the samples by applying acceptance/rejection criteria. to perform the test.
   - Officers : To review reports and sign the report.

6. Materials required:

- Syphstar RPR syphilis Kit
- Slide with reaction circles
- Sample dispensing dropper
- Rubber teat
- Mixing sticks
- Stop watch
- Centrifuge machine
- VDRL rotator

7. Procedure:

7.1 Specimen:

- Centrifuge blood sample at 3000 rpm for 02 minutes.
- Separate serum. In case of delay in testing store serum at 2-8^{0}C
- Use Serum for testing.

7.2 Screening test:

- Use appropriate PPE.
- Bring kits to room temperature.
- Place one drop (0.05ml) of positive control, negative control and specimen in each circle of plastic card, using separate droppers provided in the kit.
- Add one drop of well mixed R.P.R antigen in each of the above circles of plastic card.
- Mix the contents of each circle with separate applicator stick provided in the kit and spread well in the entire circle.
- Rock the plastic card gently for six minutes on VDRL rotator and observe for agglutination.
- External controls (known positive and negative) are applied on first day of the week and/or opening of new kit.
- Discard used glass slides in 1% hypochlorite solution for cleaning.
- Discard disposable cards, dropper and stick in discarding jar.

7.3 Interpretation:

- Black agglutination with positive control within six minutes and no agglutination with negative control validate test results.
- No agglutination with specimen up to six minutes is a negative test and indicates the absence of regain antibodies.
- Black agglutination with specimen within six minutes is a positive test and indicates the presence of regain antibodies.
- Then proceed for semi-quantitative test to determine antibody titre.

7.4 SEMI-QUANTITATIVE TEST:

- Take 5 small test tubes (10X75 mm) for serial dilution of specimen.
- Dilute the serum serially with normal saline from 1:2 up to 1:64 as needed.
- Mix well after each addition.
- Then place one drop (0.05 ml) each of diluted specimen from each of the above test tubes 1-5 to the corresponding circles on plastic card.
- Add one drop (0.05 ml) of normal saline in 6th circle.
- Then add one – one drop of well mixed R.P.R antigen to each of the 6 circles on the plastic card.
- Mix the content of each circle with the separate applicator stick and spread well in entire circle.
- Rock the plastic card gently for six minutes on VDRL rotator and observe the agglutination.

7.5 Interpretation:

- The antibody titre of specimen is the highest dilution showing visible black agglutination.

7.6 Reporting of result:

- Enter the report in report forms for in patients (Ward
- Enter the reports of OPD patients in HMIS system
- TAT for serology reports is within 24 hours.

7.7 Keep positive and negative samples for external control as per requirement

7.8 Discard all negative and positive samples after 48 hours as per SOP for biomedical waste MICRO/SOP/GEN-02.

8. Documents and records:

- SOP for collection and transportation of blood MICRO/SOP/ICTC-04
- Sample receiving acceptance & rejection MICRO/SOP/ICTC-05
- SOP for biomedical waste MICRO/SOP/GEN-02
- Report form MICRO/SOP/SERO/11/3
- Bio-safety SOP No. MICRO/SOP/GEN-06.

9. Validity: This SOP is valid for a period of one year from the date of issue.

10. Reference:

Kit insert

I. SD BIOLINE

1. Introduction: HIV testing is done by following the Strategy III by NACO. Rapid tests are in vitro qualitative tests for the detection of antibodies to Human

Immunodeficiency Virus (HIV) types 1 & 2 in human serum. HIV test kits based on three different principles and/or antigen systems are used for diagnosis of HIV.

2. **Purpose:** The SOP details the test procedures for HIV testing.

3. **Scope:** It is applicable to the lab staff of ICTC and SRL

4. **Responsibility:** It is the responsibility of the staff to follow the procedures to the minutest details

5. **Requirements**
   - Rapid test kit.
   - Micro-pipette and tips
   - Gloves.
   - Timer.
   - 1% Hypo chloride solution.

6. **Principle:** The SD Bioline HIV 1/2 test contains a membrane strip which is percolated with recombinant HIV - 1 capture antigen on test band 1 region and with recombinant HIV - 2 capture antigens on test band 2 regions respectively.
   This stage device has a letter of 1, 2 and C as test line 1; test line 2 and control line on the surface of the device both the test lines and control line in result window are not visible before applying any sample. The control line issued for procedural control.

7. **Procedure**
   - Bring the test kit to room temperature.
   - Lay out the test strip on a white paper towel on a clean flat surface, take test device out of the protective wrapper & label with the patient/Clint detail.
   - Wear gloves & perform fingerpick.
   - Draw up the required amount of whole blood specimen from the fingertip using one of the disposable pipettes supplied and do not re-use. OR add 10 µl of serum sample.
   - Add 4 drops of sample diluents check the result within 20 min.

8. **Result and Interpretation**
   - A color band will appear in the left section of the result window to Show that the test is working properly. This band is control line(C)
   - Color bands will appear in the middle and right section of the result window. These bands are test line 2 and test line 1(2,1).

- **Negative Result**
  The presence of only control line (c) within the result window indicates a negative result.
- **Positive Result**
  - The presence of two lines as control line (C) and test line 1(1) within the result window indicates a positive result for HIV-1.
  - The presence of two lines as control line (c) and test lines 2 (2) within the result window indicates a positive result for HIV-2.
  - The presence of three lines as control lines(c), test line1 (1) and test line 2(2) within the result window indicates a positive result for HIV-1and /orHIV-2.
- **Invalid Result**
  No presence of control line (c) within the result window indicates an indicates an invalid result. The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be re-tested.

9. **Reference:**
   Kit Insert.

10. **Validity:** This document is valid for one year from the effective date

MICRO/SOP/ICTC/ 11/1

HIV -2 Referral Form

Annexure 4: Report Format to be used by HIV-2 Referral Laboratories

To be filled in triplicate. Two copies to be send to referring ART center

(One for Patient and one ART record)

Name of the Referral Laboratory

Name: Surname_____ **Middle name** _____ **First name** _____

Date: _____ (DD/MM/YY) **Gender:** M / F / TG **Age:** _____ Years

ICTC PID # _____ Pre ART Reg. no. _____

Laboratory Sample ID _____

Name of referring ART center:

Date of sample collection (DD/MM/YY)

Date of sample testing (DD/MM/YY) **Test Name**	**Rapid 1**	**Rapid 2**	**Rapid 3**	**HIV1 WB**	**HIV2 WB**
Name of the kit					
Result					

CHAPTER 6: SICKLE CELL EXAMINATION

Introduction

Sickle Cell anaemia and the sickle cell trait are caused by abnormal haemoglobin, HbS. In the presence of HbS, the red cells take on a sickle like shape when oxygen supply to the red cell is decreased. The degree of sickling depends upon the concentration of HbS in the red cell. When the concentration of HbSis 80-100% (as in sickle cell anaemia), sickling of the red cell occurs readily at only slightly reduced oxygen tension. When the concentration of HbS is only 20-40% (as in sickle cell trait), oxygen tension must be much lower for sickling to occur. Sickle shaped red cells, also known as tactoids, are found in the peripheral blood smear of people having sickle cell anaemia. The sickling phenomenon may also be demonstrated in laboratory.

SICKLING TEST

Sodium Metabisulfite Method (Daland & Castle Method)

Principle: Whole blood is mixed with Sodium metabisulphite, a strong reducing agent that deoxygenate the haemoglobin, RBCs containing HbS will take sickle shape.

Sickling phenomenon can be demonstrated in thin wet film of blood sealed between slide & coverslip by petroleum jelly.

Sample: Whole blood, using EDTA, heparin, or ammonium potassium oxalate as the anticoagulant. Capillary blood from finger, toe, or heel may also be used.

Reagent &Equipments

- Freshly prepared Sodium metabisulphite, 2% (w/v)
  Sodium metabisulphite -0.2 gm.
  Distilled water -10 ml.
- Glass slide, glass coverslip.
- Petroleum jelly.
- Glass rod.
- Needle, Syringe.
- Spirit lamp.
- Filter paper.

Procedure :

1. Place one drop of blood to be tested on a glass slide.
2. Add 5 drop of freshly prepared 2% Na metabisulphite.
3. Mix well (with applicator stick or corner of cover glass).

4. Cover the mixture with coverslip and press down lightly to remove any excess mixture and air bubbles.
5. Use the filter paper to soak the excess blood outside coverslip.
6. Using glass rod, seal the coverslip with petroleum jelly.
7. Place the glass slide in a moist chamber (allow the preparation to stand in a petri dish with wet filter paper at room temperature).
8. Apply positive (i.e. AS & not SS) & negative control with each/every batch.

Interpretation :

1. Examine the preparation for the presence of sickle cell after 30 min and after 24 hours.
2. Normal looking red cells or slightly created red cells are reported as negative.
3. Sometimes holly leaf form may be seen and the test is reported positive (often found in sickle cell trait).
4. Positive test should be confirmed by Hb electrophoresis.
5. Positive test is seen in HbSS, HbSA, HbSC, HbSD &HbS-B thalassaemia.

Causes of false negative

- Old and outdated reagents.
- Haemolysed sample.
- Improper sealing of the slide, especially in summer.

Causes of false positive

Inexperienced observer who may interpret created cells as sickled red cell.
Carryover from previous positive sample.

SOLUBILITY TEST FOR SICKLE CELL: (DITHIONITE TUBE TEST METHOD)

Principle: HbS in the RBCs will precipitate in the reduced state when placed in a high molarity phosphate buffer, giving cloudy appearance to the solution.
Other abnormal Hb which show same mobility on electrophoresis can be differentiated as only HbS precipitate in high molarity phosphate buffer.

Sample : capillary / whole blood using EDTA heparin or Na citrate as anticoagulant.

Reagents and Equipments:

- Test tubes 75 X 12 mm
- Lined reader scale (prepared on piece of thick paper containing thin black line)
- Pipettes 20 µl / Hb pipette
- Phosphate buffer, pH 7.1

- Potassium dihydrogen phosphate (KH_2PO_4) - 33.78g
- Dipotassium hydrogen phosphate (K_2HPO_4) – 59.33g
- White saponin - 0.5g
- Distilled water - 250 ml
- Working solution ; Dissolve 0.1 g of Sodium dithionite in 10 ml of buffer just prior to use.

Procedure

1. Place 2ml of working buffer solution in 75 × 12 mm test tube and allow to warm to room temperature.
2. Add 20µl (0.02ml) of whole blood/capillary blood, mix well and allow to stand for 5 min. at room temperature.
3. Place the tube approx. 1 inch in front of lined reader scale.

Interpretation

1. Negative test; Resulting solution will be clear as there in no precipitate formation in the absence of HbS. Black lines on reader scale will be clearly visible.
2. Positive test; Resulting solution will be cloudy due to precipitate formation indicating presence of HbS. Black line on reader scale will not be visible.
3. Positive results should be confirmed by Hb electrophoresis

False Negative test

1. If old, outdated or deteriorated reagent is used. If diathionite/butter mixture is not freshly prepared.
2. Infants under age of 6 months and in heterozygotes when HbS is < 20 %
3. In homozygous state with severe anaemia following recent blood transfusion. In severe anaemia use of packed red cell will overcome this problem

False positive are seen in

1. Severe leucocytosis- A cute leukemia, CML, Leukemoid reaction
2. In hyperproteinemia – multiple myeloma
3. In presence of unstable haemoglobin, especially after splenectomy.
4. Positive results may also be produced by other " sickling"Hb such as HbC-Harlem &HbS-Travis (mobility different from HbS).

Note; Test cannot differentiate between HbSS/HbSA, HbS-βthal, HBS-αthal Examine PS to arrive at diagnosis Normal PS in SA & haemolytic PS in SS (If electrophoresis is not available immediately).

ELECTROPHORESIS

Electrophoresis is the name given to the movement of charged particles through an electrolyte subjected to an electric field. The positively charged particles move through cathode(negative electrode) while negatively charged particles move towards anode

(possisitive electrode). Proteins at alkaline pH have negative charge and hence on electrophoresis move towards anode.
The differential migration depends on the charge on the molecule, pH of the buffer , molecular weight ,temperature, current, voltage and nature of supporting medium. The different positions achieved by the bands help in their identification when compared with the control.

HAEMOGLOBIN ELECTROPHORESIS

Normal healthy red blood cells contain three different types of haemoglobins known as adult haemoglobin (HbA),fetal haemoglobin (HbF) and haemoglobin A_2 ,(HbA_2).Their approximate concentration in adults is as follows –

HbA -96%

HbF – 1%

HbA_2 -3%

In a newborn,the various fractions are HbF(80%),HbA(20%),AND hBA_2 (<0.5%).The percentage of HbF falls progressively and at the end of one year HbF is less than 1%.

All these haemoglobins at alkaline PH have negative charge of different magnitude and in an electrical field run towards anode with different mobilities. HbA with more negative charge moves faster towards anode ,HbA_2 having least negative charge moves very slowly towards anode while other abnormal haemoglobins are in between.

Example –HbS moves in between HbA and HbA_2.

USE OF HAEMOGLOBIN ELECRTOPHORESIS:

1. It is done for the identification and diagnosis of abnormal haemoglobin in haemoglobinopathies.
2. It allows presumptive identification of haemoglobin phenotype i.e. one can differentiate HbS trait (AsSpattern) from sickle cell disease (SS pattern).
3. The band of variant haemoglobin can be eluted and quantitated.
   Example –Quantitation of HbA_2 levels in Thalassemia trait.

Haemoglobin electrophoresis at alkaline pH(8.6) using tris EDTA borate buffer is the principle method of identifying common haemoglobin variants like HbS, HbD, HbE etc. But at alkaline pH, HbS and HbD have similar pattern of migration while HbC trait, HbE trait & HbO trait have similar electrophoretic mobility. Hence at acid pH using citrate buffer (pH 6.2), HbS can be differentiated from HbD.

Sickle cell disease is the most common haemoglobinopathy in Maharashtra. It can occur either in heterozygous state or homozygous state. In the heterozygous state, red cell contain both normal HbA and the variant HbS but HbA is always more than HbS.such patients are said to have sickle cell trait and are known as carriers. They are relatively asymptomatic. In the homozygous state, HbA is totally absent and patients are

said to have sickle cell anaemia and are known as sufferers. A high morbidity and mortality is associated with them.

Sickle cell disease can occur in combination with thallasemia in which there is a quantitative defect in the synthesis of globin chains, either α or β .These patients are known as double heterozygotes. Since there are four genes for a chain synthesis and two genes b chain synthesis, the severity of clinical manifestations and hemoglobin patterns depends on the genotype.

TABLES II:

Type	HbA	HbA_2	HbS	HbF	HbBarts
SS + α-Thal	Absent	N	↑↑	Absent	+
AS + α-Thal	Present	N	< 35%	Absent	Absent
HbS + β^0 Thal	Absent	↑	↑↑	Variable	Absent
HbS + β^0 Thal	↓	↑	↑↑	Variable	Absent

Haemoglobin can be carried out on a variety of supporting media which include –

1.Filter paper electrophoresis-Filter paper as a supporting medium offers advantages as far as cost is concerned but is not sensitive enough to detect small quantities of hemoglobin (<15%).The resolution is poor and long time is required. The A_2 fraction cannot be demonstrated and it is difficult to quantitate the abnormal haemoglobin.But despite the disadvantages, it is useful in field studies.

2. Starch gel electrophoresis ;- Starch gel is a sensitive supporting medium and superior definition of zones can be achieved. It has a sieve like action and so molecules of larger size are retarded. But the process is too cumbersome and migration time is long.

3. Cellulose acetate electrophoresis – Cellulose acetate paper is micro porous and this prevented spreading of bands and hence excellent separation is achieved at low voltages. There is minimal protein adsorption and this eliminates trailing of bands and gives a white background after washing. It is fast (20-30 mts) and elution and quantitation possible, but it is more expensive and requires extra care for satisfactory results.

AGAR GEL ELECTROPHORESIS

EQUIPMENT

1. **Electrophoresis tank**

The electrophoretic tank is a rectangular tank with three separate compartments. The center of the tank is a raised platform where glass slides with the supporting medium are kept. The two side

compartments are for the buffer. The tank is covered with a transparent plastic lid through which the process of electrophoretic migration can be visualized . In front of the tank are two knobs, red and black. As per convention, the red knob is anode and the black is cathode. These are connected through wires to the respective knobs on power supply unit.

2. **Power supply unit**
   This unit is commercially available. It has a meter for current and voltage adjustment. There are two knobs – anode and cathode for the respective knobs of the electrophoretic tank. There is a knob for power (on/off) heat transmission (on/off) and polarity (normal/reverse)
3. Centrifuge machine
4. Refrigerator
5. Incubator
6. Weighing balance
7. Tripod stand
8. Spirit level
9. pH strips
10. Filter paper wick (Whatman No. 1)
11. Needle
12. Template

GLASSWARE

1. Glass pipette
2. Pasteur pipette
3. Beaker (50 ml)
4. Glass syringe (10cc)
5. Petridish (6 diameter)
6. Glass slides (75 mm × 25mm× 0.35mm)
7. Applicator coverslip

REAGENTS

1. **Diffco-bacto agar**
   Agar is dried colloid extracted from seaweed . It contains a mixture of neutral agar, agarose, pyurvated agar with few sulphated groups.
   Agar gel electrophoresis is a sensitive, relatively cheap method with minimal protein adsorption and the results are satisfactory.
   Other supporting media available are-
   - Filter paper electrophoresis- Filter paper as a supporting medium offers advantage as far as cost is concerned but is not sensitive enough to detect small quantities of haemoglobin (<15%) . The resolution is poor and long time is required. The A2 fraction cannot be demonstrated and it is

difficult to quantitate the abnormal haemoglobin. But despite the disadvantages, it is useful in field studies.

- Starch gel electrophoresis- Starch gel is a sensitive supporting medium and superior definition of zones can be achieved . It has a sieve like action and so molecules of larger size are retarded . But the process is too cumbersome and migration time is long-
- Cellulose acetate electrophoresis – Cellulose acetate paper is microporous and this prevents spreading of bands and hence excellent separation is achieved at low voltages. There is minimal protein adsorption and this eliminates trailing of bands and gives a white background after washing. It is fast (20-30 min) and elution and quantitation possible, but it is more expensive and requires extra care for satisfactory results.

2. Buffer

1. Tris EDTA borate buffer (pH 8.6)

Buffer is a solution whose pH does not change on addition or dilution or dilution of a small quantity of acid or base or on storage.

Preparation of stock solution

- Tris powder - 10.2gm
- EDTA - 0.6gm
- Boric Acid - 3.2 gm
- Distilled water - 100ml.

Preparation of working solution:

This is prepared by diluting the stock solution 10 times i.e.100 ml of stock solution in 900 ml of distilled water. The pH of the solution is 8.6 This is confirmed by pH strip. If the pH is more alkaline, it is adjusted to 8.6 by adding 2N HCL drop by drop and checking with pH strip. If it is acidic, the pH is adjusted to 8.6 by adding 1N NaOH (4gms is 100 ml distilled water)

3. Stains:

a) Amidoblack (0.5%)

- Amidoblack – 500mg
- Glacial acetic acid - 3.5 ml
- Distilled water - 97 ml.

Other stains which can be used are-

b) Bromophenol blue-

- Bromophenolblue - 200 mg
- Distilled water – 1000ml

c) Ponceau S-

- Ponceau S powder -500 mg
- Trichloroacetic acid – 100 ml

4. Destaining solution-

7% acetic acid (7ml glacial acetic acid in 100ml distilled water)

Procedure:

Collection of blood sample-

2ml of venous blood sample is collected. The different anticoagulants used are-
Heparin – 0.1 mg/ml or 10-20 IU /ml of blood. Double oxalate- 0.1 ml/ ml of blood

Dipotassium EDTA – 1.5mg/ml

If blood sample is to be transported, acid citrate dextrose is the anticoagulant of choice. (1.5 ml for 10ml of blood)

Preparation ofhaemolysate-

Centrifuge 3 ml of anticoagulated blood at 3000 rpm for 15min. Remove plasma and wash the red cells 3 times in 3 volumes of 9G/L Na Cl (saline) at 3000 rpm for 5 min. After the third wash, remove traces of plasma proteins and as much as WBC layer as possible. Lyse the RBC by adding 2 volumes of distilled water. Shake well for 5 min. in a stoppered centrifuge tube. Centrifuge at 3000 rpm for 30min. Pipette the clear supernatant lysate in to a test tube. Measure the Haemoglobin concentration. Adjust the concentration to 10 g/dL by adding distilled water.

Preparation of agar plates-

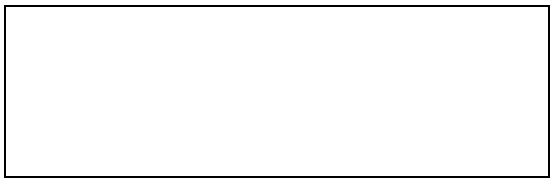
1% purified agar is prepared by dissolving 100 mg of powder in 10 ml of working buffer in a beaker. The beaker is kept on the tripod stand and heated with a spirit lamp. The beaker is shaken intermittently till agar particle dissolve completely and the solution becomes clear.

The reference number of the two test sample is marked on the undersurface of the glass slide on the nodal side in the right upper and lower corner.The glass slide is kept on a flat side, avoiding air bubbles. The slide is kept at room temperature for 10 min so that the agar sets and then kept in the refrigerator for 30-45 min for solidifying.

A template is prepared as shown. The agar slide is removed from the refrigerator and kept on the template. The samples are applied with the help of an applicator like the edge of a coverslip . Apply the sample without cutting the an applicator like the edge of a coverslip. Apply the sample without cutting the surface of the gel, 10-20 µl, allow the applicator in contact with gel for 15 sec. surface of the gel, 10-20 µl, allow the applicator in contact with gel for 15 sec.

TEMPLATE

1) Haemoglobin electrophoresis at alkaline pH
    - Test



- Control
- Test

Controls

- Sickle cell trait (AS)
- Cord Blood (HbF)
- Thalassemia (HbA2)

NOTE :

1) Spirit level should be used to ensure a flat surface.
2) If air bubbles are present while preparing slides, they are removed by applying heated tip of needle
3) If air bubbles are present while preparing slides they are removed by applying heated tip of needle
4) Application of control is essential on each slide for comparison and interpretation of migration patterns of haemoglobin
5) Haemolysates prepared from a known patient of sickle cell trait (SA pattern), cord blood(HBF) and a known patient of sickle – thalassemia (HbA2) should be available as control. Depending on the clinical solution, appropriate control should be applied

Keep the slides on a central platform of the electrophoresis tank. A maximum of three slides can be kept at a time. 100ml of buffer (working solution) is added to each of the side compartments. Filter paper wicks are moistened with the buffer. One end of the wicks is kept on the slide and the other end dips in the buffer. This ensures continuous flow of the buffer onto the slide and prevents agar from drying due to the heat generated during the process.

The anode and cathode of the electrophoretic tank are connected to the respective knobs of the power supply unit. Ensure that voltage and current are both set to zero. Start the power and press the heat transmission knob. Ensure that polarity is normal. The voltage is then adjusted to 300 V while current is gradually adjusted to 7mA per slide.

NOTE:

1) After 5-10 min, water of condensation from the vapours is seen on the undersurface of the lid and this confirms that the machine is working.
2) A check is made from time to time to note if filter paper wicks are drying. If so, the lid is removed and buffer from the side compartment is added on the wicks with the help of a pipette.

After 15 min, the migration is noted. Another check is made after 30 min to note the migration. If discrete bands are noted as compared to the control, electrophoresis can be stopped. The total time required varies between 45-60 min

The slides are removed and kept in a Petridis containing the staining solution i.e. – 5 % amidoblack for 5 min. or bromophenol blue for 15 min. The stained slide is then de-stained for 25 min. to remove excess stain by keeping it in 7% acetic acid. After de-staining, clear bands are visualized. The interpretation is then done by comparing with the control-

Preservation for record keeping-

After de-staining the slide is covered by a moistened filter paper and a nominal weight equivalent to 10 glass slides are kept over the filter paper- This is allowed to dry at room temp- for 2 days or kept overnight in an incubator at 370 C

CELLULOSE ACETATE ELECTROPHORESIS

Equipment;

1. Cellulose Acetate Strips - 4×2 cm.
2. Horizontal Electrophoretic Tank.
3. Power pack.
4. Small forceps.
5. Applicator Coverslip

Reagents

Buffer

Tris EDTA borate buffer (pH 8.6)

Buffer is a solution whose pH does not change on addition or dilution of a small quantity of acid or base or on storage.

Tris powder - 10-2 gm
EDTA _ 0-6gm
Boric acid _ 3-2gm
Distilled water - 1000ml.

Procedure;

(The cellulose acetate strips should be handled by forceps)

1. Fill the compartments of electrophoretic tank with **TEB b**uffer. Soak and position the wicks.
2. Soak the cellulose acetate membranes in TEB buffer for at least 5 minutes. It is important to immerse the membranes slowly so as to avoid trapping air bubbles.
3. Blot the membranes between two pieces of absorbent paper but do not allow them to dry out before applying haemolysates.

4. Apply the haemolysates 10µl with the help of applicator approximately 2-3 cm. from one end of the strip. Allow the applicator tips to remain in contact with the strip for approximately 3 seconds.
5. Place the strip in the tank in such a way that wicks are in contact with the buffer and application line is towards the cathode.
6. Apply power and run at 100 V at 2mA/strip for 2 min or until adequate separation is obtained.
7. Switch off the power, remove the strips from the tank and stain with 0.5% Amidoblack for 5 min.
8. Remove the strips, drain and elute excess stain with 3 consecutive 2 minute wash in 7% acetic acid.
9. Dehydrate in absolute methanol for 2-3 min
10. Clear in 20% (v/v) acetic acid in methanol for 6-8 min
11. Dry in a 65% C oven for 4-6 min
12. Lable and preserve strips in a plastic envelope.
13. If quantitation is to be done, after electrophoretic run, remove the membranes and cut the HbAand HbA2 zones in small pieces and elute into 15ml and 1.5 ml of buffer respectively with occasional shaking.
14. Remove eluted strips and centrifuge the elutes at 3000 rpm for 5 min to sediment debris.
15. Determine the absorbance of each elute at 413 nm using a spectrophotometer. Use TEB buffer as a blank

Calculation -

$$\%HbA2 = \frac{A413HbA2 \times 100}{(10 \times A413HbA) + A413\ HbA2}$$

INTERPRETATION

HbA2 values below 3.5% are normal.

HbA2 values above 3.5% are considered abnormal and are found in beta thalassemia trait (minor) – 3.5-7%

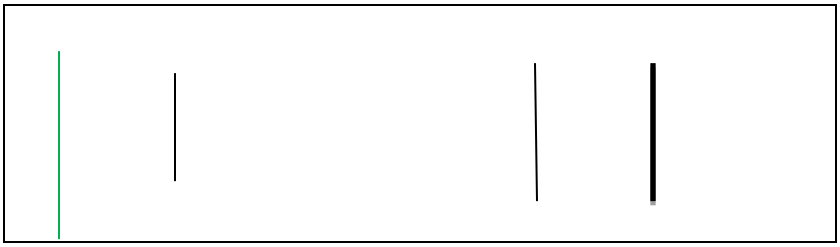
Levels are variable in β – tha major&Intermedia.

Levels are decreased in HbH disease,§ -β-thal, HPFH, Hb-Lepore, Severe Iron deficiencyamneamea

Haemoglobin electrophoresis

- HbEp at Alkaline pH in normal healthy adults

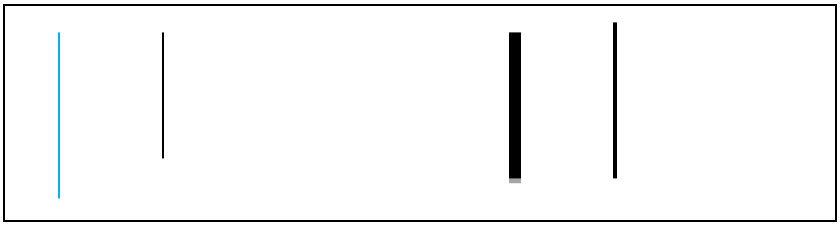
Cathode- **Anode+**



Origin HbA2 HbFHbA

- Hb EP at Alkaline pH in newborn at 6 months

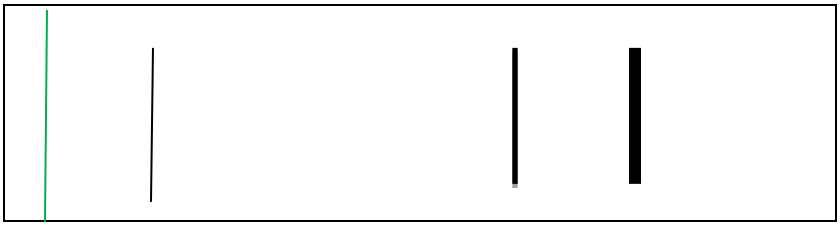
Cathode- Anode



Origin HbA2 HbFHbA

HbEP at Alkaline pH in 6 months -1year

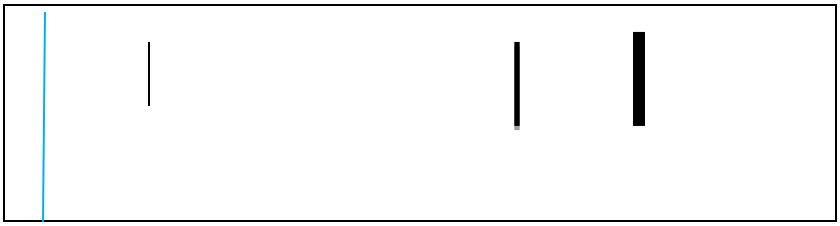
Cathode- Anode+



Origin HbA2 HbFHbA

HbEP at Alkaline pH in Sickle cell/HbD carrier

Cathode- Anode+

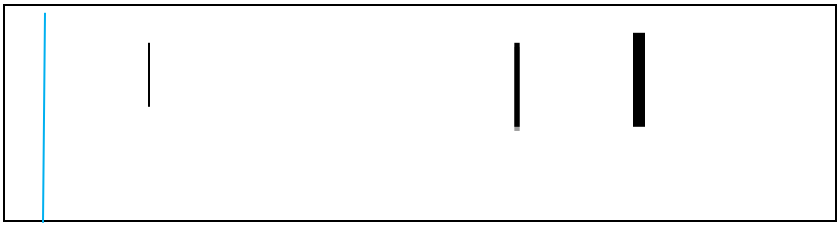


Origin HbA2 HbS/DHbFHbA

T-Test sample

HbEP at Alkaline pH in Sickle cell/ HbD carrier

Cathode- Anode+



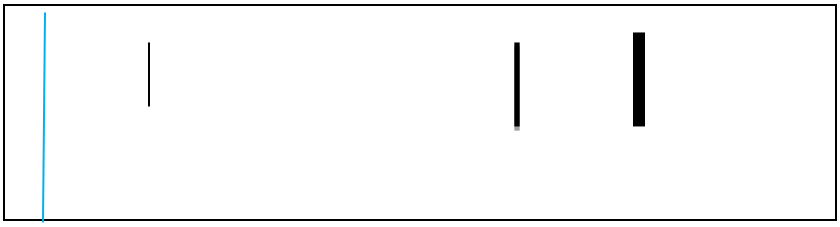
Origin HbA2 HbS/D HbFHbA

T-Test sample

C-Control sample

Origin HbA2HbS/D HbFHbA

Cathode- Anode+



Origin HbA2 HbS/D HbFHbA

Figure II – Hemoglobin Electrophoresis on agar gel at alkaline pH

Table III

Interpretation of results of solubility test &HbEb, & HPLC.

Gp.No	Solubility	HbEp Pattern	HPLC	Privisional diagnosis
1	-ve	F/AF/AA	Hb F>20% Hb A2≥4%	Thalassaemia
2	-ve/+ve	AA2/A2F/SA2	Hb A2>15 %	Hb E cases
3	-ve	AS/SS	HbD+	Hb D cases
4	+ve	AS/SS/SF	Hb S+	SCD
5	+ve	SF	Hb S+/ Hb A2≥4%	S-β
6	-ve	AA	Hb A2 < 4% Hb F < 1%	Normal

Prenatal Diagnosis of Sickle Cell Disease by Polymerase Chain Reaction (PCR)
Sample Collection
Fetal Blood

Fetal blood sampling 39

Initially, prenatal diagnosis of SCD was performed on a small amount of Fetal blood aspirated from the placenta, umbilical cord, or even Fetal heart, either blindly, withultrasound guidance, or through a fetoscope at the 20 th week of pregnancy. 32,33

The procedure was followed by blood analysis on IEF (Isoelectric focusing) and HPLC (high pressure liquid chromatography) which can differentiate from homozygous to heterozygous but was associated with 1-2% Fetal loss. Also, the mid-trimester diagnosis of an affected foetus often led to a painful abortion. The complexity of this approach prevented its large scale implementation in countries such as the U.S. and Britain. When mixture of placental and maternal blood is present, the latter red cells and reticulocytes can be eliminated by selective hemolysis.34 The remaining cells can be used for globin biosynthetic studies. Today, this approach has been replaced by DNA techniques, except in the rare case when the Thalassemic component cannot be identified at the molecular level. The introduction of molecular DNA techniques that allow accurate Fetal diagnosis in the first trimester and termination of pregnancy, if indicated, not later than the 12 th week of gestation changed the picture dramatically.

Amniotic fluid sampling 39

Use of amniotic cells was the easiest approach to DNA analysis, even though they provide reliable results only after the 17 th week of pregnancy. Aspiration of amniotic fluid with a long needle is relatively painless and safe. About 20 ml of fluid contain enough amniotic cells for extraction of up to 20ug of DNA, which is more than enough to identify the defect by PCR

Chorionic villus sampling (CVS)39

Chorionic villus sampling (CVS) can be performed transcervically or transabdominally. The risk of infection is greater with this approach, however. Ultrasound monitoring has made trans abdominal CVS as effective and less risky. Prenatal diagnosis by CVS has a risk-rate for fetal loss or malformations of around 5 per 10,000 cases.

Maternal decidua contamination of trophoblastic tissue must be avoided categorically. Failure to do so can produce catastrophic errors.

Table 1-A Summary of the main advantages of current fetal sampling methods

Methods	Success rate in %	Timing (weeks from LMP)	Time to DNA Diagnosis	Fetal loss rate
CVS	➢ 99	10 on wards	48 hours	1%
Amniocentesis	>99	15 on wards	2-16 days	0.5-1%
Fetal blood sampling	>95	20 on wards	72 hours	2%

Identification of the beta mutation at the DNA level. Early work used a *Hpa I* restriction fragment length polymorphism (RFLP) to distinguish beta ^{A}-globin and beta^{s}- globin genes.[38] The current method of choice employs the polymerase chain reaction (PCR) to amplify the mutated region (CCT, GAG, GAG to CCT, GTG, GAG< codons 5-7) for diagnosis.

B. Molecular Techniques

Polymerase Chain Reaction (PCR)-

Developed in 1984 byKary Mullis,40**PCR** is now a common and often indispensable technique used in medical and biological research labs for a variety of applications 41 including diagnosis of hereditary diseases.

In molecular biology, the polymerase chain reaction (PCR) is a technique to amplify a single or few copies of a piece of DNA, generating thousands to millions of copies of a particular DNA sequence. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA.

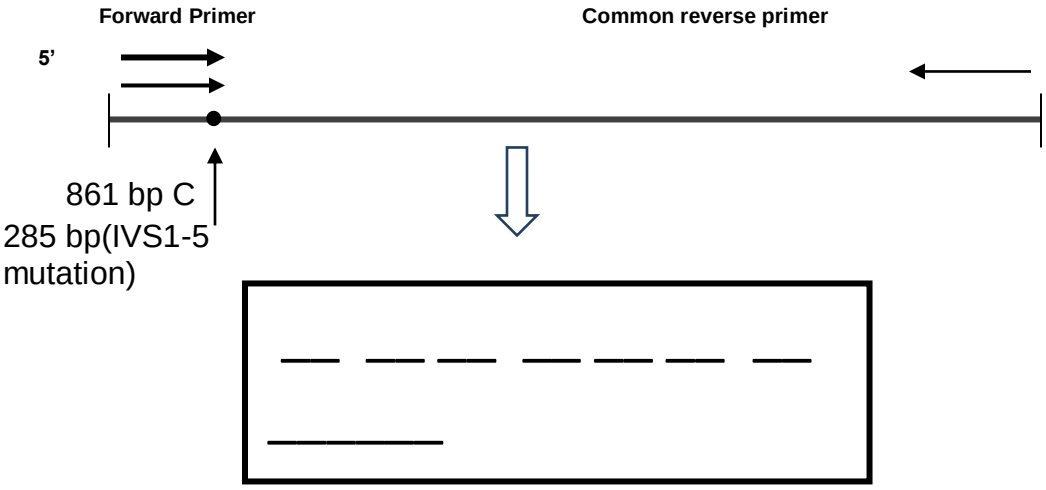
Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the bacterium, Thermusaquaticus. This DNA polymerase enzymatically assembles a new DNA strand from DNA building blocks, the nucleotides, by using single- stranded DNA as a template and DNA oligonucleotides (also called DNA primers), which are required for initiation of DNA synthesis. The vast majority of PCR methods use thermal cycling, i.e., alternately heating and cooling the PCR sample to a defined series of temperature steps. These thermal cycling steps are necessary first to physically separate the two strands in a DNA double helix at a high temperature in a process called DNA melting. At a lower temperature, each strand is then used as the template in DNA synthesis by the DNA polymerase to selectively amplify the target DNA. The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions.

Allele Specific Oligonucleotide Hybridization (ASO)

This technique involves using a synthetic, labeled oligonucleotide (about 20 kilobases long) that binds specifically to the CCT GTG GAG sequence under stringent conditions, but not to the sequence CCT GAG GAG. The reaction is carried out on a membrane using a drop of PCR product. The binding is shown by probes tagged with radioactive isotopes or enzymatic chromogens. The literature contains several variation of this approach. 43 Cross-checks with control probes, e.g. the beta A sequence, are required

Amplification refractory mutation system (ARMS)

Allele specific primers are designed in such in a way that the nucleotide at the ^{3} end of each primer is complementary to the change in DNA sequence caused by the mutation that being looked for. To enhance their specificity, a deliberate additional mismatch is introduced at postion -4 from the^{3} end. Each of these primers is tested with known positive and negative control DNA samples under uniform stringent condition to ensure that successful amplification occurs only in the presence of the mutation that is being looked for



ARMS technique; Schematic diagram

Analysis of restriction endonuclease site (Restriction Enzyme Digestion)

In the past, identification of the mutation by the absence of a restriction endonuclease site was carried out by the Southern blot technique on extracted DNA. The lost cleavage site produces a larger RFLP fragment. Now the technique is used on the PCR amplification product, with the enzyme MstIIwhich cuts the normal CCT-GAG-GAG sequence in two DNA fragments, 228 and 202 bp long. The enzyme cannot cleave the mutant sequence, CCT-GTC-GAG, and yields a single 430 bp DNA fragment. Other

enzymes that recognize the CCT GTG GAAG mutation are DdeIBsu 361 (an isoschizomer of MstII)

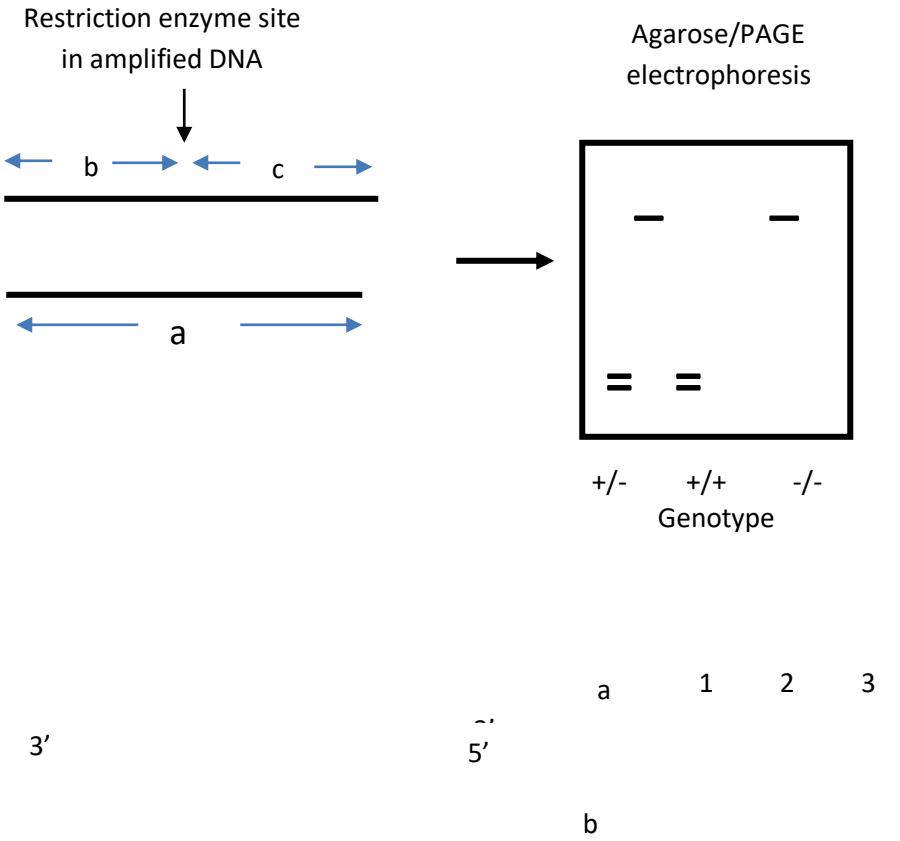


Figure 6- Restriction Enzyme Digestion; Schematic diagram

Variable Number of Tandem Repeats analysis (VNTR)

Sequences of the oligonucleotide primers used for VNTR loci analysis

DIS 80 (MCT-118)	5' GAA ACT GGC CTC CAA ACA CTG CCC GCC G3'
	5' GTC TTG TTG GAG ATG CAC GTG CCC CTT GC3'
IgJH	5' GGG, CCC, TGT, CTC, AGC, TGG, GGA3'
	5' TGG, CCT, GGC, TGC, CCT, GAG, CAG3'
ApoB	5' GAA, ACG, GAG, AAA, TTA. TGG, AGG,GA3'
	5' TCC, TGA, GAT, CAA, TAA, CCT, CG 3?
D4S95	5' GCA TAA AATGGGGATAACAGTAC-3'
	5'- GAC ATT GCT TTA TAG CTG TGC CTC AGT, TT-3'

C- Guidelines for best practice

The guidelines for best practice are-

1. Ensure that fresh parental blood samples are obtained with the Fetal sample in orders to check the parental phenotypes and to provide fresh control DNA.

2. Ensure that the chorionic villous sample has undergone careful microscope dissection to remove contaminating maternal deciduas.
3. Always analyse parental and appropriate control DNAs with the Fetal DNA and always repeat the Fetal DNA analysis to double check the result.
4. Whenever possible, use alternative diagnostic method to confirm the diagnosis.
5. Use a limited number of amplification cycles to minimize any co-amplification of maternal DNA sequences.
6. Check for maternal DNA contamination in every case
7. The Fetal DNA diagnosis report should detail the types of DNA analysis used and clearly state the risk of misdiagnosis due to technical errors based on current data

PCA is highly sensitive to maternal DNA contamination. The diagnostic error rate for prenatal diagnosis by PCR methods including both non laboratory errors and laboratory errors was calculated to be 0.41 % 49

Non laboratory errors occur due to-

"Mis-diagnosis in parents
" Non paternity
" Clerical error
Laboratory errors includes-
" Partial digestion
"Allele dropouts

This can be minimized by using two independent diagnostic methods and by duplicate test.

Two independent diagnostic methods-

1. Direct mutation detection and RFLPs or direct mutation detection and restriction enzyme by PCR on each sample.
2. Polymorphism analysis by PCR using ApoB, IgJH and Ha-RAS variable number of Tandem repeats (VNTR) polymorphism or a number of short tandem repeat polymorphism.

D. New development in Prenatal Diagnosis

Fetal Cells in the Maternal Circulation

This still experimental approach aims to spare mothers at risk from an obstetric procedure. Fetalcells present in the maternal circulation can be tagged with appropriate antibodies and collected by either fluorescence-activated or magnetic sorting. The nuclei are micro dissected, and the DNA is extracted for analysis.

Preconception and Preimplantation Technology

Preimplantation selection is feasible, however, and can be offered to couples who cannot use conventional prenatal diagnosis because selective abortion is prohibited, or who already have had several abortions after diagnoses of affected foetuses. The technique can also be offered to couples for whom multiple attempts at pregnancy are risky.

Pre-conception diagnosis is carried out on the DNA of the first polar body, which is extruded upon maturation of oocytes. The oocytes are obtained after ovarian stimulation, and the polar bodies, which contain identical DNA, are aspirated into micropipettes for analysis. The procedure has been shown to not affect fertilization of the oocytes or viability of the resultant embryos 50.A search for the defect is carried out by PCR methods, and one or more polymorphic markers usually are checked as precautions against errors. Finally, non-affected oocytes are fertilized and transferred into the uterus of the prospective mother to startpregnancy.

Post-conception diagnosis of genetic diseases can be carried out on the second polar body, which is extruded after fertilization. Sequential study of both polar bodies is strongly recommended, because it increases the accuracy of the results when the first polar body reveals an heterozygous state. 50 Then the unaffected , fertilized oocyte(s) is transferred into the uterus of the prospective mother for continuation of the pregnancy.

6. Conclusions

Prenatal diagnosis is considered to be the only solution to prevent sickle cell disease all over the world. The procedure has been simplified by both obstetric and laboratory techniques, and can be carried out with safety in many countries, early enough to allow elective termination of pregnancy.

7. Take home message-

- Prevention is cost effective for SCD.
- Screening , counselling, PND facility to be developed
- Mass education plays a key role before PND acceptability.
- Choice and option a available for PND to rest with the couple.
- Genetic counsellors to help in decision making
- Sampling techniques for PCR based DNA studies are safe for both mother and fetus.
- Newer techniques like preconception and preimplantation technology are less invasive and a new hope for couples.

CHAPTER 7: Space, Equipment and Supplies required for a Blood Bank
(PART XII-B of schedule F)

A. Accommodation for a Blood Bank

Minimum total area shall be 100 square meters having appropriate lighting and ventilation with washable floors and shall consist of following rooms, namely: -1. Registration and Medical Examination room with adequate furniture and facilities for registration and selection of donors.2. Blood Collection Room (This shall be air-conditioned).3. Room for Laboratory for blood group serology. (This shall be air-conditioned).4. Room for Laboratory for Transmissible diseases like Hepatitis, Syphilis, Malaria, HIV antibodies etc.(This shall be air-conditioned).5. Sterilization and washing room.6. Refreshment room.7. Store and Records Room.

Note: The Laboratories of the Blood Bank shall be used exclusively for Blood Bank work.

B. Equipment:

I. For blood collection room, the following would be needed: -

- Donor beds or tables: It shall be suitably and comfortably cushioned and shall be of appropriate size.
- Bed side tables.
- Sphygmomanometer and Stethoscope.
- Recovery beds for donors.
- Refrigerators: Maintaining temperature between 4 to 6 degrees C with recording thermometer and alarm device.
- Weighing machine.

II. Hemoglobin determination:

- Copper sulphate solution (specific gravity 1.053).
- Sterile lancet.
- Capillary tubing (1.3 to 1.4 x 65 mm or Pasteur pipettes).
- Rubber bulbs for capillary tubing.
- Sahli's haemoglobinometer/calorimetric method.

III. Temperature and pulse determination:

- Clinical thermometers.
- Equipment and materials for aseptic cleaning of the thermometer.
- Watch (fitted with a second-hand needle).

IV. Blood containers:

- Disposable plastic packs (closed system) as per the specification of USP.
- Blood collection bottles: 540 ml. with graduated capacity of up to 500 ml graduation mark provided with two rows in opposite direction indicating intervals of 50 ml from 0 to 500 ml.

Anti-coagulants:

Anti-coagulant solution shall be sterile, pyrogen free and of composition that will ensure satisfactory safety and efficacy of the whole human blood and all the separate human blood components.

- Citrate phosphate dextrose solution (CPD) or citrate phosphate dextrose adenine-I (CPDA-I) 14ml. Solution shall be required for 100 ml of blood. In case of double/triple blood collection bags used for blood components preparation, CPDA, blood collection bags may be used.
- Acid Citrate Dextrose Solution (A.C.D. & Formula-A) IP Grade 15 ml. Solution shall be required for 100 ml. of blood. Note: The licensee shall ensure that the anti-coagulant solution bottles/packs conform to the standard laid down in IP/USP. Disposable sterile bleeding sets shall only be used.

V. Disposable sterile bleeding sets shall only be used.

VI. Blood transfusion sets.

Sterile disposable sets with filters and plastic spike shall only be used.

VI. Emergency equipment:

1. Oxygen cylinder (with gauge and pressure regulator).
2. 5 percent glucose or normal saline.
3. Disposable sterile syringes and needles of various sizes.
4. Disposable sterile I.V infusion set.
5. Ampoules of adrenaline, noradrenalin, mephentin, betamethasone or dexamethasone, injectionmetoclopramide.
6. Aspirin and spirit ammonia aromatic.

VII. Accessories:-

1. Such as: Blankets, emesis basins, hemostats, set clamps, sponge, forceps, mouth gauze, dressing jars, solution jars, waste cans.
2. Medium cotton balls, 1.25 cms adhesive tapes.
3. Denatured spirit, tincture Iodine green soap or liquid soap and injection of procaine or xylocaine.

4. Paper napkins or towels.
5. Incinerator
6. Standby generator

C. Refreshment Services:-

Provision for serving refreshments to the donor after phlebotomy shall be made so that he/she may be kept for observation in the Blood Bank for any untoward reactions.

D. Laboratory Equipment: -

1. Refrigerator maintaining a temperature of 4 to 6 degrees with Recording Thermometer. The refrigerator shall have temperature recording and alarm device.
2. Compound Microscope-with low and high power objectives.
3. Centrifuge Table model.
4. Water Baths-one for 37 degree C and another for 56 degree C.
5. Rh viewing box in case of slide temperature.
6. Incubator with thermostatic control.
7. Mechanical shakers for serological tests for Syphilis.
8. Hand lens for observing tests conducted in tubes,
9. Serological graduated pipettes of various sizes.
10. Pipettes (Pasteur).
11. Glass slides.
12. Test tubes of various sizes/micro-titer plates (U or V type).
13. Precipitating tubes 6 mm x 50-mm glass beakers of different sizes.
14. Test tubes racks of different specifications,
15. Interval timer electric or spring wound.
16. Equipment and materials for cleaning glass wares adequately.
17. Shipping containers.
18. RPHA/ELISA Test Kits with Reader for Hepatitis.
19. Wash bottles; filter papers.
20. Ice box for transport of blood units.
21. Hot air oven.
22. Plain and EDT.A Vials.
23. ELISA-Reader, Washer and micropipettes for HIV-antibodies testing (in case HIV-antibodies Testing is done by ELISA Kits).
24. Di-electric tube sealer
25. Chemical balance (wherever necessary)

E. Reagents:-

1. Standard blood grouping sera Anti-A and Ant-B and Anti-AB: All in double quantity and each of different brand or if from the same supplier then each supply should be of different lot numbers.
2. Rh typing sera: All in double quantity and each of different brand or if from the same supplier each supply should be of different lot numbers.
3. Reagents for serological tests for syphilis and positive sera for controls.
4. Anti human globulin serum (Coomb's serum).
5. Albumin 20 per cent to 30 per cent for tests/enzymes.
6. 0.9 per cent saline.
7. Culture media and tubes.
8. Wax pencils and tables.
9. RPHA/ELISA kits for hepatitis.
10. Detergents and other agents for cleaning laboratory glass wares.
11. Elisa Kits/rapid diagnostic kits in case the licensee opts for HIV antibodies testing.

F. General Supplies: -

Autoclave with temperature and pressure recording device.

G. Personnel: -

Every Blood Bank shall have following categories of full time technical staff and their number shall depend upon the quantum of work,

Doctor- Degree in Medicine of a University recognized by the Central Government having experience in Blood Bank for 6 months during regular services. He shall have adequate knowledge and experience in Blood Group serology. Blood Group Methodology and medical principles involved in procurement of blood.

1. Registered nurse.
2. Blood Bank technician with MLT qualification or its equivalent having adequate experience in blood grouping and serology work.
3. Laboratory Assistant with MLT qualifications or its equivalent.
4. Laboratory Attendant.

H. Testing of Whole Human Blood:

1. It shall be the responsibility of the licensee to ensure that the Whole Human Blood supplied conforms to the standards laid down in the current edition of Indian Pharmacopoeia and for all other tests published by the Central Government from time to time.
2. Every licensee shall get samples of every blood unit tested before use for freedom from HIV-antibodies either from such laboratories specified for the

purpose by the Central Government or in his own laboratory. The results of testing shall be recorded on the label of the container also.

Note: -

1. Blood samples of donors in pilot tube and the blood samples of the recipient shall be preserved for 72hours after transfusion.
2. The blood intended for transfusion shall not be frozen at any stage.
3. Blood containers shall not come directly in contact with ice at any stage.

I. Expiry Date:

- The date on which the blood is drawn and the date of expiry which shall be as prescribed under Schedule P to the said Rules.

J. Records & Labels:

The permanent records, which the licensee is required to maintain, are: -

a. Blood Donor Register – Indicating serial number, date of bleeding, name of donor with particulars, age, weight, haemoglobin, blood pressure, medical examination, signature of Medical Officer bleeding the donor, bottle bag number and patient's detail for whom donated in case of recipient donation, remarks on donation (voluntary/replacement /professional). Disposal record.

b. Blood Stock Register – Indicating bottle bag number, date of collection, date of expiry, quantity in ml., ABO/Rh Group, results for testing of HIV antibodies, malaria, VDRL, Hepatitis-B surface antigen, irregular antibodies (if any), name of donor with particulars, utilisation issue number, components prepared or discarded, certified by Medical Officer In-charge). Note: Similar records shall be made for blood components. Group wise stock register shall be maintained.

c. Issue Register – Indicating serial number, date and time of issue, bottle number, ABO/Rh group, total quantity in ml., name of the recipient, group of recipient, unit/institution, details of cross-matching report, indication for transfusion. Particulars of product supplied (whole human blood, red cell/platelet concentrates, cryoprecipitates etc), quantity supplied, compatibility report, signature of issuing persons.

d. Register for ACD/CPD/CPD-A – Bottles/packs giving details of firm, batch number, date of supply and results of testing.(v) Register for Diagnostic Reagents used.

e. Blood Bank must issue the cross matching report of the blood of the patient along with the blood bottle.

f. Transfusion Adverse Reaction Records.

g. Records of Purchase, use and stock in hand of disposable needles, syringes, plastic bags, sets shall be maintained.

K. Labels: -

The label on the blood container shall contain the following particulars namely:

1. The serial number of the bottle.
2. The date on which the blood is drawn and the date of expiry as prescribed under Schedule P to the said Rules.
3. The ABO groups with the corresponding colour; the following colour scheme for labels shall be used for different groups: -

Group	Colour of label
O	Blue
A	Yellow
B	Pink
AB	White

4. The results of the tests for Hepatitis, Syphilis, freedom from HIV antibodies.
5. The Rh group.
6. Total volume of fluid, the preparation of blood, nature and percentage of anticoagulant.
7. Name and address of Blood Bank.
8. License number.
9. Instruction to keep continuously at 4-degree to 6 degree C. The label should also include the following inscriptions –
10. Disposable Transfusion sets with filter must be used in administration equipment.
11. Appropriate compatible cross-matched blood without a typical antibody in recipient should be used.
12. **Caution:** The contents should not be used if there is any visible evidence of deterioration like haemolysis, clotting or discoloration.

Note: The above requirements of Blood bank are subject to modifications at the discretion of the Licensing Authority or the Central License Approving Authority if he is of the opinion that having regard to the extent of manufacturing operations it is necessary to relax or alter them in the circumstances of a particular case.

Part XII C of schedule F deals with minimum requirements for grant of License to process blood components from whole blood.

(Railway Board's No.96/H (FW)/10/19 and 97/IH/7/1,The Gazette of India: Extraordinary (Part IISec.3 (1) - Ministry of Health and Family Welfare - Notification)

FORM 27 C
(Application for grant or renewal of licence)

Application for grant or renewal of license for the Operation of Blood bank processing of Whole Human Blood for components and or manufacture of blood products.1. I/We _____ of _____ hereby apply for the grant/renewal of license to operate a Blood Bank, processing of Whole Human Blood for components and/or manufacture of blood products. Names of the Human Blood Components intended to be processed shall be specified.2. The name, qualification and experience of expert staff: -(a) Name(s) of Medical Officer.(b) Name(s) of Registered nurse.(c) Name(s) of Blood Bank Technician.3. The premises and plan are ready for inspection/will be ready for inspection on _____.4. A fee of Rupees _____ and an inspection fee of Rupees _____ have been credited to the Government under the Head of Account _____.Signature _____ Dated Designation _____

FORM 28-C
(Original Licence)

License to operate a Blood Bank, processing Whole Human Blood for components and/or manufacture of Blood Products. Number of License_____ Date of Issue _____ 1. _____ is hereby licensed to operate a Blood Bank to process Whole Human Blood for components and/or manufacture of blood products as the premises situated at the _____.2. Name of the Product(s) _____.3. Name of approved expert staff _____ 1. _____ 2. _____ 3. _____ 4 The license authorise the distribution and the sale and storage for distribution or for sale by the licensee of Whole Human Blood, Human Blood Components and/or blood product under this Licence subject to the conditions applicable to license for sale.5. The licenseshall be in force from _____ to _____.6. The licenseshall be subject to the conditions stated below and to such other conditions as may.(xi) The licensee shall destroy the stocks of batch unit, which does not comply with Standard tests in such a way that it would not spread any disease/infection by way of proper disinfection method.

FORM 26 G
(Renewal Licence)

Certificate of renewal of license for the operation of Blood Bank and/or for processing of Whole Human Blood for components and/or Manufacture of Blood Products.1. Certified that license N. _____ granted on the _____ for the operation of Blood Bank, Processing of Whole Blood for components and/or manufacture of blood products at the premises situated at _____ has been renewed from _____ to _____. Name of the Product(s) _____.2. Name of the Technical staff _____ 1. _____ 2. _____ 3. _____ Signature _____ Date _____ Designation_____

Source: Notification of Ministry of Health & Family Welfare (Dept. of Health) Dated 22.1.1993

About Blood

Blood is the red colored fluid tissue, that incessantly flows through the different parts of our circulatory system. An average human has 5- 6 liters of blood in the body and it comprises of one twelfth of our body weight.

Blood consists of a straw colored, fluid matrix called plasma, 90% of which comprises of water. The cellular elements namely the Red Blood Cells (RBCs) White Blood Cells (WBC) remain suspended in the plasma. Other substances found in the plasma are glucose, proteins, fats, hormones and enzymes.

The RBCs help to exchange oxygen between the lungs and the tissues. These red cells contain a red pigment called hemoglobin which acts as the carrier vehicle for oxygen. The amount of hemoglobin in a healthy male is 14-16 gms while in a female it is 12-14 gms.

The WBCs are the main components of the immune system that guard the body. They also function as scavengers and cleanse the body of bacteria or any other microorganisms that enter the body.

Platelets are vital in the process of blood **clotting** brought about with the help of coagulation factors.

The **bone marrow** is the prime site of blood cell proliferation. Each day old cells die and are eliminated from the body and each day new blood cells are formed to replenish the stock of blood cells. The life span of RBC is 120 days while the WBC lives for a few days and the platelet for a few hours only.

A person with burn injuries may need only plasma transfusions while a person with anemia may need RBC s alone and a person with clotting problems may need a transfusion with factor VIII or IX.

Plasma can be stored for a year at -80 degrees. Platelets on the other hand can be stored to up to 5 days at 22-24 degrees. Certain coagulation factors such as factor VIII and factor IX can be stored for later use. These are very handy in treating certain disorders, such as Hemophilia. Certain proteins such as albumin and globulin can also be stored.

This is because many patients do not require whole blood. For example, a patient whose **hemoglobin** is low and is therefore anemic, may just require Packed Cells i.e. only red cells; a patient with burns may need more of plasma than cells; a patient with hemophilia may require only Factor VIII.

Blood Groups

For all intents and purposes, Blood in humans is categorized into 4 subgroups – **A, B, O and AB.** Each of these groups may be **Rh positive or Rh negative** depending on the presence or absence of the Rhesus factor, which is believed to have come from the Rhesus monkeys. For example there may be individuals who may be A+ve or A-ve depending on the Rhesus factor. This applies to B, AB and O groups too. Some other categorizations such as the MN blood groups are also recognized. These blood groupings are very handy when it comes to decisions regarding blood transfusions.

O group individuals are called **'Universal Donors'** and **AB group** called **'Universal recipients'**. But this rule is applied only in extremely trying circumstances. It is always wise to transfuse an individual with blood from the same group blood.
For all practical and routine purposes, it is ideal to transfuse to the patient the same group of blood which he belongs to. It is only under very dire emergency that we take O group as universal donor and AB groups as universal recipient. A group individuals have anti-B antibodies while B group people have anti-A antibodies. O, A and B group individuals must always be transfused with the same blood group.
Rh negative patient cannot receive blood from a positive individual. Any change in transfusion will result in antigen-antibody reactions which will lead to serious consequences.

An ideal candidate for blood donation should

- Must be within the age group of 18-60.
- Must weigh a minimum of 45 kgs (90 pounds).

- Have a blood pressure in the range of 160/90 to 110/60
- Have a normal pulse rate between 60 to 100 per minute.
- Hemoglobin content must be a minimum of 12gms%.

Blood Donors-Types

"Donor" means a person who voluntarily donates blood after he has been declared fit after a medical examination, for donating blood, on fulfilling the criteria given hereinafter, without accepting in return any consideration in cash or kind from any source, but does not include a professional or a paid donor.

MINISTRY OF HEALTH AND FAMILY WELFARE (Department of Health)

Voluntary donors -

Here the donor donates blood as a humanitarian act as a result of intense motivation. This is the best kind of blood donation as it is a self-less service. The identity of the donor can be hidden, if he so desires. When a donor gives blood to be stored for later use for an unknown recipient it is called allogenic or homologous blood donation. Camps can be held for this kind of donation and an event where many allogenic donors come to give blood is called a Blood drive or Blood donor session. When blood is donated by a person to be used at a later date by that person it is called autologous blood donation.

a) Replacement donors -

During an emergency, the relatives and friends of an affected individual donate blood, irrespective of blood group, to the blood bank and in turn the bank releases the required group blood for the individual's need. This is quite common is developing countries and rare in the developed world.

b) Professional donors -

These donors exchange their blood for cash or other incentives. They donate blood at frequent intervals and it is very likely that they transmit lethal diseases through the donated blood.

Before donating blood it is ensured that donors are medically fit to donate blood. Their medical history is recorded and they are physically examined to ensure that they don't suffer due to blood donation. Their Hemoglobin or hematocrit level is tested. Being anemic is the primary reason why most donors are turned down. In addition to this the BP, temperature and pulse rate of the patient are evaluated. Pregnant women and elderly folks are usually discouraged from donating blood.

Blood Donation - Process

Blood donation is carried out under the supervision of trained, skilled technicians. The entire procedure, from start to finish, does not take more than 45 minutes.

The blood is usually drawn from the median cubital vein, from the inside of the elbow. An antiseptic such as iodine is used to clean the skin above this vein. This helps to prevent bacterial infection at the site of puncture and also helps to prevent the blood drawn from being infected.

A tourniquet may be used to elevate the blood pressure in the veins of the arm. This helps to ease and speed up the process. Sometimes the donor is given an object to squeeze repeatedly in order to increase blood flow to the targeted vein. Invariably a needle with a larger gauge is used in order to minimize the shearing forces that can cause damage to the RBCs.

A mild sting may be experienced when the needle is inserted, but there should be no pain during the donation.

There are two main procedures that are used to draw blood from a donor.

The first and the most common method is when **whole blood** is drawn from the donor and is collected in a plastic bag that contains anticoagulants and preservatives such as sodium citrate, phosphate, dextrose or adenine. The collected blood separated into its components (mainly the RBCs and the plasma) and stored. It must be noted that most recipients need only certain components and do not require whole blood. Plasma can be used for transfusions and also to make medications. This is a carryover from the World War II times when dried plasma was used to treat wounds.

On an average 450-500 ml of blood is drawn from one individual at a time. This amount of blood, along with the anti coagulants present in the collection bag is referred to as **‘one unit’** of blood.

The second method called **apheresis** consists of drawing blood from the donor, separating the components using a centrifuge, storing the component required for transfusion and returning the remaining components to the donor. Usually the RBCs are transferred back to the donor while the plasma and platelets are put to good use. Apheresis may be carried out using specifically designed instruments.

Whole blood, when kept in CPDA anti coagulant solution at 2 - 4 deg C, can be stored up to 35 days, but the demand is far greater than the supply and the collected blood is used up much before its expiry date.

The frequency by which a donor could donate blood varied from country to country. By general consensus a patient who is donating plasma alone can donate more frequently compared to a donor giving whole blood. This is because RBCs take longer (36 days) to replace compared to plasma(2-3 days).

Donor Recovery

Blood Donors are usually rested at the blood donation site for at least 15-20 minutes. They are encouraged to lie down. Any complication that may develop from donating blood takes place during the donation or immediately afterwards. The rest period may help to monitor this.

After 15 minutes of rest, Blood donation centers usually provide light refreshments such as tea, coffee, milk or fruit juice and biscuits to refresh the donor. The site of needle puncture is covered with a bandage and the donor is recommended to keep it that way for a while. After the initial rest, the donor may resume daily routine. Rigorous physical work is discouraged for a few hours after donation. No special diet is recommended for a donor. A balanced, nutritious daily diet will suffice. The donated blood is replaced within the donor's body within a matter of a day or two.

Complications & Risks to Donors

Studies reveal that there is a minor chance of risk among donors. However, no death has been reported.

Sometimes blood donations can put a person at risks. Donors are usually screened for the presence of risk factors/diseases. Some of the risks faced by a blood donor are listed below:

- A quick change in BP can result in hypovolemic reactions-fainting might ensue.
- Less than 1% suffers from bruising by the needle.
- Some of the donors suffer from bleeding.
- Blood donors may occasionally react adversely to the sodium citrate used to prevent coagulation during apheresis. Sodium citrate, returned to the donor along with blood components that are not required, can bind together the calcium present in the donor's blood. This may cause hypocalcaemia - the symptoms of which include tingling in the lips, convulsion or even graver symptoms. This condition can be prevented by providing calcium supplements to the donor.
- Manual apheresis, without the help of automated machines can lead to transfusion reactions.
- Usage of non-sterilized equipments and needles can increase risk to donors.

Blood Donation - Refrain

Certain category of individuals are strictly forbidden from donating blood.
Do not donate if-

- You have tested positive for **HIV** or hepatitis.
- You are an **intravenous drug abuser**.
- You have **hemophilia or any blood clotting disorder**.
- You had **a heart attack** in the last six to twelve months.
- You had a recent **attack of malaria** recently.
- You had **received blood, plasma or other blood components** during the last year.
- You had **cardiac surgery** in the last year.
- You are taking **cardiovascular medicine**.
- You are **pregnant.**
- You had an **abortion recently**.
- You had **received chemotherapy / radiation** for cancer treatment.
- You are at high **risk of contracting the HIV**.

Recipients

We have seen in length about the donors it is equally important to know about the recipients as well. Very often persons requiring a blood transfusion would be fighting for his life. The following are the individuals who may need blood transfusions.

- A patient who was subjected to a major accident and thereby blood loss.
- A person who is ear marked to undergo a major surgery. For instance, an open-heart surgery may require about 6 units of blood.
- A woman going through childbirth or suffering a miscarriage may require large quantities of life-saving blood.
- Patients with hematological disorders such as hemophilia, Thalassemia, leukemias and anemias
- When a person suffers shock, drug reactions, burns or poisoning, blood transfusion may be the way to save his life.

Faqs / Facts about Blood Donation

Can I donate if I am less than 18 years of age?
If you are younger and wish to donate under special circumstances you should seek permission from your parents and speak to the doctor.

What is the normal safe interval between blood donations?
Normally it is about 56 days for whole blood donation. In men the recommended time period is 3 months and in women 4 months.

Is there any upper age limit to blood donation?
The recommended age limit is 55 years. But a fit and healthy person can donate up to even 60 years or above depending on the requirement.

How much blood is removed during donation and how soon does it get replaced in the body?
The amount of blood withdrawn varies from 350ml- 450ml. It normally takes 24hrs for the blood volume to be replaced. And red cells get replaced in about 6 weeks,.

Will I become physically weak or get any infection after blood donation?
No if you are in normal health there is no cause for concern. The chances of infections are minimum if you donate to a reputed lab as they take all the necessary precautions.

Why should I donate Blood?
Blood is required everyday by hospitals. There are about 80 million units of blood that are donated each year by voluntary and paid donors. However there is still a shortage of blood more so in developing counties. Only 38% of the total blood collected is from the developing countries, where a staggering 82% of the world's population live. Several of these countries are dependent on paid donors.

What happens to recipients who receive incompatible blood?

- Patient complains of shivering, nausea, vomiting and restlessness.
- Pericardial and lumbar pain may occur
- Cold, clammy skin
- Cyanosis
- Pulse rate and respiratory rate increases
- Temperature rises to 38 to 40 deg C
- Blood pressure decreases and the patient enters a state of shock.
- Sometimes complications can lead to death.

What is cord blood donation?
The blood from the umbilical cord of a new-born baby is extracted and stored in a bank. This blood is rich in stem cells which is useful in the treatment of many diseases.

Can I donate blood frequently?
Yes you can donate blood regularly. Many blood banks depend on voluntary regular blood donor. Frequent blood donors have no health ill effects. If you are a frequent blood donor write to Medindia and share your story with us.

Who should I contact if I wish to donate blood?

Contact the nearest blood bank or blood transfusion department of any major hospital in your town. Lion's Blood Bank has branches in many cities. This information is easily available in telephone directories. You can also use Medindia's free utility for this purpose.

Tests we carry out

You may have noticed that each time you give a blood donation we also take blood samples.

Most of these tests are mandatory, in other words we must carry them out on every single blood donation, whether this is your first donation or just one of the many you have given over the years. The tests play a very important role in ensuring that we provide a safe blood supply to patients. We test for your blood group, so that we can select the correct group for the patient.

Any donation that is reactive on any one of the screening tests cannot be used. If your blood is reactive on any one of the screening tests, further tests are carried out to confirm whether the result indicates a true infection.

If this is the case, we will inform you and offer you appropriate advice. If the result is significant to your health you will be asked to discuss the results with one of our clinical staff and, with your permission, we will arrange a referral to your own doctor or a specialist.

We test for the following infections:

- Blood group & Rh factor
- Syphilis
- Human immunodeficiency virus (HIV),
- Hepatitis B virus (HBV)
- Hepatitis C virus (HCV),
- Hepatitis E Virus (HEV)
- Malaria

Treatment of discarded blood units: Disinfection with hypochlorite / formalin versus steam sterilization

Six lakh units of blood are collected annually in India. Possibly 40,000 - 60,000 units need decontamination being positive for HIV, Hepatitis B, Hepatitis C, suspected bacterial contamination or expiry (figures based on 8 - 10 % annual discarding of blood units at our institute). As per Indian Bio medical waste (BMW) rules 1998, these units are required to be chemically disinfected or autoclaved prior to disposal and disinfection is often done by hypochlorite or formalin treatment. Poly vinyl chloride (PVC) content of

blood bags discourages incineration. Scientific data to support autoclaving or chemical disinfection for blood units is lacking. Hence, a study was conducted to compare autoclaving and chemical disinfection by hypochlorite or formalin for PVC blood units that were deliberately contaminated with bacteria / bacterial spores.

CHAPTER 8: BIO-SAFETY IN LABORATORY AND SAFE DISPOSAL OF BIOMEDICAL WASTE IN LABORATORY

Introduction

Bio-safety, especially safety in laboratories is a key component of total quality control programme. There is definitely a potential risk of infection to Health care workers (HCWs), who provide direct or indirect health care to people and thus continuously come in contact with pathogenic organisms, (e.g. nurses, midwives, community health workers, hospital housekeepers and doctors) or handle samples of body fluids/tissues/ morbid specimens (Lab technicians, Microbiologists etc.), handle infected waste and transport potentially infected specimens (laboratory attendants, safai karamcharis etc.). They are exposed to certain infections by nature of their profession. These infections could be bacterial, viral, parasitic or fungal. Some of these are serious like plague, hepatitis, Human Immunodeficiency Virus (HIV) etc. and may even result in death, whereas, others are not serious and only cause morbidity.

Bio-hazards in a laboratory and practice of Bio safety

Laboratories, practicing microbiological work, are exposed to microbiological hazards, besides common hazards like fire, chemical and electrical, etc.

Safety is one aspect of quality, it minimizes the risks of injury, infection or other dangers related to laboratory services delivery. Safety involves the providers as well as the beneficiaries (patients), for example, safety is an important dimension of quality when collecting blood for making blood slides or for using Rapid Diagnostic tests for malaria to prevent transmission of infection such as Hepatitis B and C and HIV.

There are several ways HCWs engaged in malaria diagnosis can acquire blood borne infection from a patient or from his/her specimen either by:

- direct contact with blood/body fluids,
- accidental inoculation of infected blood/body fluids,
- accidental cuts with contaminated sharps,
- indirect contact with contaminated equipment or any other inanimate infected objects.

Before undertaking any QC programme in a microbiology laboratory, all biosafety measures should be ensured and HCWs must take all precautionary measures to protect themselves from accidental injury, while handling the blood (standard work

precautions) and patients must also be protected from infection. The risk of acquiring HIV infection following sharp injuries from a patient or infected blood is extremely low i.e. 0.25 to 0.3 % but that of acquiring hepatitis B or C is higher

Bio safety procedures

Adequate facilities

The laboratory should have adequate facilities, necessary equipment for undertaking the tests and following laboratory safety.

General laboratory specifications

- adequate space should be assigned for a particular laboratory work for the safe functioning.
- laboratory tables should be stable, impervious to water and resistant to disinfectants, chemicals and moderate heat.
- hand-washing basins, with running water, should be provided in each laboratory room. A dependable supply of good quality water is preferable.

Laboratory working place

- All tables must be kept clean, tidy and dry.
- Work surfaces must be decontaminated at the end of the working day.
- All chemicals, solutions and specimens must be properly labelled. Labels must include name, date prepared and expiry date, where applicable.
- Glassware and other materials for reuse must be rinsed properly with water after cleaning with detergent.
- Supplies and materials must be kept in designated drawers and lockers that are labeled with respective contents on the outside.
- Heavy equipment, glassware and chemicals are not to be stored above eye level.
- All equipments must be properly attached to electrical points in a way that prevents overloading and tripping hazards.
- Safety system should preferably have fire safety and electrical back up facilities for emergencies. All laboratory personnel should be trained for required awareness to use the facility in emergency.

Bio-safety practices in a health care setting These include:

Universal work precautions or standard precautions for blood and body fluids

Attention should be paid towards the personal protection during handling of human specimens. e.g., care should be taken to prevent the entry of diseases pathogens

like HIV 1 and 2 and Hepatitis B and C, by the routes mentioned above. Biological and safety hazards inherent in handling human specimen, eg. Contaminated blood and body fluids can be effectively prevented by diligent practice of **standard work precautions** by HCWs by presuming that all the specimens are infected or potentially infections. **Blood** is the single most important source of HIV, HBV, HCV and other blood borne infections to HCWs.

Standard work precautions in a laboratory are:

Hand washing

Hands must always be washed vigorously under running water using a skin disinfectant /antibacterial liquid (i.e. 4% chlorhexidine gluconate with added skin emollients) for at least 10 seconds and 70% alcohol before and after work and at any time before leaving the laboratory.

- **Barrier protection**

  Laboratory gown, preferably wrap around gowns, disposable gloves and protective shoe covers must be worn at all times when working inside the laboratory and especially when handling human blood. Use gloves for all those procedures that may involve accidental, direct contact with blood or infectious materials. A generous supply of good quality gloves is required. Discard gloves whenever they are thought to have been contaminated or perforated, wash hands and put on new gloves. Gloves should be used in addition to hand washing. Laboratory clothings should be removed before leaving the laboratory.

- **Safe laboratory practices**

  Besides the instructions mentioned above

  i. eating, drinking or storing food or drinks is strictly prohibited in the laboratory. Special personal lockers should be provided to the laboratory staff to keep all these items at the entry point of the laboratory area.
  ii. apply strict aseptic techniques throughout the procedure.
  iii. wash hands with soap and water immediately after any contamination and after work is finished. If gloves are worn, wash hands before and after gloves are removed.
  iv. all technical procedures must be performed in a way that minimizes the formation of aerosols and droplets. Work with human blood or serum requires the use of disposable equipment and supplies, whenever possible. Otherwise, all reusable materials must be autoclaved or placed in 1.0% hypochlorite solution for 24 hours before washing.
  v. ensure an effective insect and rodent control programme.

- **Safety procedure for malaria diagnostic tests**

  I. **Collection of blood by finger prick method**

Discard the lancet / pricking needle after the finger prick straight in to a beaker containing 1% freshly prepared solution of sodium hypochlorite or any other appropriate disinfectant.

II. Collection by venepuncture

- wash hands before and after the collection of specimen.
- collect and place the specimen aseptically in an appropriate sterile, leak- proof, airtight container, whenever needed or follow SOP
- tightly close the lid of the container during transportation, if necessary
- completely fill the label on the specimen collection vial.
- collect the specimens by taking precautions to avoid unnecessary contamination of the material but also avoid self-infection, creation of aerosol or gross splashing (especially into eyes) or by injury such as syringe needle or contamination of damaged skin.

Similarly, after venepuncture, the syringe with attached needle may be disposed by different methods. **See under safe handling of sharps**

III. Pipetting

use a rubber teat or automatic suction device properly, as outlined in SOP: G 6 for Pipetting Techniques. Mouth pipetting is strictly forbidden.

Biological safety cabinets, should be used whenever infectious materials are handled and there is an increased risk of aerosol production, which includes centrifugation, blending and mixing, etc.

- ## Safe handling of sharps

Sharps like disposable needles/ hypodermic needles or scalpels and broken glass pose the greatest risk of blood borne pathogen transmission in health care setting through per-cutaneous injury which occurs when needles are recapped, cleaned, improperly discarded or disposed off.

I limit use of hypodermic needles and syringes. They must not be used as substitutes for pipetting.

II never recap, bend, break or remove disposable needles from disposable syringes.

III always destroy needles and syringes by needle cutters, if available or the complete assembly should be placed in the puncture resistant disposal container after decontamination. In case of lancets or other sharps, dispose in the same container

after decontamination. Puncture resistant disposal containers are specially labelled puncture-proof rigid containers fitted with covers. When the container is three-quarters full, it should be placed in an “infectious waste” container and incinerated, with prior autoclaving, if laboratory practice requires it.

IV do not dispose of sharp containers in landfills.

- ## Management of accidental spill of blood

I any spilled biological material on floor/work surface must be covered with paper towel/ blotting paper/news paper/ absorbent cotton

II 1% hypochlorite solution is poured on and around the spill and left for 30 minutes before cleaning.

III all the waste removed with gloved hands and sent for incineration in yellow bags.

- ## Management of accidental injury

I **In the event of a puncture or penetrating injury** noticed during sample collection or any other hazardous procedure:

- wash the affected part thoroughly with water and soap/disinfectant.
- if the eye is splashed, rinse at once either with clean tap water or with irrigating solution held in the laboratory first aid kit or with sterile saline.
- immediately seek medical attention and report to the designated nodal officer or laboratory supervisor.
- document the incident / accident in respective register.

II **Accident reporting**

- date and time of accident.
- sequence of events leading to accident.
- the waste involved in accident.
- assessment of the effects of the accident on human health and the environment.
- emergency measures taken.
- steps taken to alleviate the effects of accidents.
- steps taken to prevent the re-occurrence of such an accident.

Date:_____ Signature: _____

Place:_____ **Designation: _____**

Bio-safety management

It is the responsibility of the laboratory supervisor (the person who has immediate responsibility for the laboratory) to ensure the development and adoption of a bio-safety management plan and a safety operations manual.

The laboratory supervisor should ensure that regular training in laboratory safety is provided. Personnel should be required to read the SOPM on safety and a copy of this manual should be available in the laboratory.

Effective sterilization and disinfection

Definitions

(i) **Sterilization:** Complete destruction of all living microorganisms including spores.

(ii) **Disinfection:** Destruction of vegetative forms of organisms which might cause disease.

(iii) **Disinfectant:** An effective all purpose disinfectant is sodium hypochlorite solution with concentration of at least 1.0%. There are other disinfectants also like lysol,

For purpose of disinfection, disposal and recycling, all the articles may be divided into three categories:

i) **Disposables:** Soak the material overnight in a strong solution of disinfectant before disposing. 1% Sodium hypochlorite / 1% calcium hypochlorite, 10% solution of formalin or 3% lysol may be used as disinfectant.

ii) **Reusable articles contaminated with morbid material:** Discard the material into a jar containing disinfectant solution. Let them remain in this solution overnight. Drain off the disinfectant. Transfer the material to a metal pot or tray with cover. Pour water and boil for 15 min. Cool and drain off the water. Pass on the articles for washing. Current procedures used for sterilisation, ie. Continuous boiling for 20-30 minutes or autoclaving are adequate. Autoclave monitoring is done by using chemical indicator strips. Syringes and needles should never be disinfected by chemical disinfectants.

iii) **Material containing clinical specimen:** Direct on site incineration or autoclaving followed by incineration at a distant site.

Safe disposal of biomedical waste

Definitions

Biomedical Waste is defined as unwanted trash generated during diagnosis, treatment or immunization of human beings, during research activities or testing of biologicals. Laboratories are major source of biomedical waste. These are:

(i) Biologicals l bloodl red cells l body fluids, etc.: Blood samples collected

and stored to use as red cell panel, serum and plasma.

(ii) Expired: contaminated, deteriorated or any condition of infectious biological material generated for disposal.

(iii) Biotechnology waste: Materials generated as waste from the kit like any reagent buffers, diluents, etc.

(iv) Sharp waste: Glass slides, cover slips, needles, glass, Pasteur pipettes, test tubes, scalpels and blades, etc.

(v) Solid waste: other than waste sharps:- Rapid strips, combs, cards, plastic vials, pipette tips, cotton, tissue paper and filter paper contaminated with blood during generated during QC testing.

(vi) Liquid waste: Generated from laboratory during testing, from washing, cleaning and disinfecting activities.

- **Management**

  Management of biomedical waste and disposal in any laboratory that deals with biological testing in terms of quality control and quality assurance applies to all who generate, collect, receive, store, dispose or handle biomedical waste in one or other form. It should be the duty of every person handling the bio-medical waste to ensure that such waste is handled without any adverse effect to human health and environment.

  It is the responsibility of the laboratory personnel to:

  (i) recognize the type of biomedical waste generated in the laboratory, segregation, packing, storage and transportation (category 1-9) and follow treatment and disposal as prescribed in the schedule. *[Ref. The Gazette of India, Extraordinary, Part II-Sec.3 (ii)].*

  (ii) implement waste management in compliance with the prescribed standards.

  (iii) make sure that no waste is left untreated and it should not be kept stored *beyond a period of 48 hours*.

Treatment and disposal recommendations for management of bio-medical waste are

i. place all bio-hazardous waste (apart from sharps) in specially designated colour coded waste containers, separately from non-infectious waste.
ii. all bio-medical waste containers / bags should bear biohazard symbol.
iii. autoclave all infectious solid waste in leak-proof containers e.g. autoclavable, colour- coded plastic bags, before disposal in yellow bags and send to incinerator facility or incinerate within the laboratory, if feasible. Do not dispose infectious material in landfills.
iv. collect all sharps in puncture proof containers and then in blue / white transluscent bags.
v. maintain documentation of waste generated during testing, separately for liquid and solid waste and treatment given and means of their disposal, regularly.
vi. handle waste generated from the kits / during testing in such a way that

infectious and non-infectious materials are discarded separately and treated accordingly before disposal.

vii. decontaminate potentially contaminated liquid waste eg. Blood before discharging to the community sanitary sewer system.

viii. frequently decontaminate the working area with disinfectant.

- Store all biohazardous waste separately from non infectious waste in leak-proof containers (autoclavable, colour- coded plastic bags with biohazard symbol), not more than 2 days and seal tightly when transported. In certain cases double bagging is required to prevent leaking. For disposal of sharps – see 6.3.2.1. The color coding of bags for biomedical waste disposal is shown in Table 2.

Table2: Colour coding of bags for bio medical waste disposal

Colour coding	Type of container	Waste category	Treatment and disposal options
YELLOW	Plastic bag	Human anatomical waste, animal waste, microbiology and biotechnology waste and solid waste.	Incineration / deep burial
RED	Disinfected container / plastic bag	Microbiology and biotechnology waste and solid waste.	Autoclaving / Microwaving / Chemical Treatment i.e. 1% hypochlorite solution
BLUE I WHITE TRANSLUSCENT	Plastic bag / puncture proof container	Sharps waste & solid waste.	Autoclaving / Microwaving / chemical treatment i.e. 1% hypochlorite solution and destruction / shredding
BLACK	Plastic bag	Discarded medicines and cytotoxic Drugs, Incineration ash and chemical waste (solid)	Disposal in secured landfill.

- **Methods of disposal of waste**

The following are the methods of disposal

i. Incineration – it is the best option as it renders the waste noninfectious and changes the form.
ii. Autoclaving and disposal in general waste system at 121° C for 20 minutes.
iii. Needle destroyer /cutter for destroying needle and part of the nozzle of syringe
iv. Chemical – disinfection
v. Deep burial - If incineration is not available, then all Red /Blue / White Translucent bags are collected for final disposal by deep burial. A pit should be dug about 2 mts. deep. It should be half filled with waste then covered with 50 cm of the surface before filling the rest of the pit with soil. It must be ensured that animals do not have any access to burial sites. Covers of galvanized iron wire meshes should be used to cover the waste burial pit.

On each occasion when waste is added to the pit, a layer of 10cm of soil should be added to cover the waste.

Records of all pits for deep burial should be maintained.

Immunization for Hepatitis B –All HCWs should be immunized against HBV.

CHAPTER 9: DUTIES AND RESPONSIBILITIES OF LABORATORY TECHNICIAN

This protocol guides the technicians regarding the work to be done before performing other activities.

(a) Remove shoes or foot ware and wear clean and dry slippers.
(b) Check whether the floor is clean or not.
(c) Check the temperature of all the refrigerators available in laboratory as well as Central Monitoring System Recordings if available.
(d) Also check Temperature of Incubators and Water Bath etc.
(e) Wear cap, mask, gown& gloves.
(f) Do the Quality control of reagents, Distilled water· etc.
(g) Then proceed for routine work.

GENERAL LABORATORY PRECAUTIONS

DO'S	DON'Ts
Minimize splashing or the formation of droplets or aerosols in all procedure while handling each specimen.	Do not eat, drink, smoke or apply cosmetics in the place of work.
Take extraordinary care to avoid accidental wounds from sharp instrument.	Do not do any paper work on potentially contaminated surfaces.
Discard all disposable articles contaminated with blood in Yellow buckets with yellow plastic bags .	Do not wear gloves to examine a patient with intact skin.
Discard needles and other sharp instruments in puncture resistant containers.	Do not touch your eyes, nose, mouth or skin with gloved hands.
Wear gloves when there is to be contact with blood, body fluids, mucous membranes non-intact skin, items or surfaces contaminated with body fluids and for performing all vascular access procedures.	Do not walk around, the workplace wearing gloves.
If you have a breach in skin or hands, then wear gloves in all situations.	Do not try to recap used needles or try to bend or break them with hands.
Use sterile / disposable syringes and needles.	Do not reuse disposable articles.
	Avoid Spillage of Blood.

CLEANING & MOPPING OF FLOOR

This protocol provides the procedure for cleaning of floor to keep the area infection free.

MATERIAL REQUIRED

1. Bucket
2. Tap water
3. Soft Broom
4. Phenyl
5. Duster with handle

PROCEDURE

- The floor cleaning is done daily three times a day
- The floor should be cleaned & mopped in morning, afternoon, & evening time.
- Take 4 Liters of water in a bucket which is already marked .Add 30 ml of phenyl,mix it properly
- The floor is initially swept with a soft broom
- After sweeping mop the floor with the duster soaked in the above prepared solution.
- Change the water in between the procedure. Then it is allowed for drying.
- Bucket should be emptied after each use & washed with detergent & water & dried in sunlight & stored dry.
- The duster is washed with detergent & water& dried in sunlight & stored dry.
- This procedure is used each & every time of cleaning.

WASHING & STERILIZATION

1. NEW TEST TUBES AND SLIDE

Those are soaked in 2% HCL. Then they are washed in running tap water until all the traces of dirt are gone. Then they are boiled in detergent for 30 minutes. Then rinsed thoroughly in running tap water and finally in distilled water.

2. USED GLASSWARE

These are rinsed in Sodium Hypochlorite Solution (5 to 10%) immediately after use. The glass slides, glass pipettes, test tubes and glass receptacles (bulbs etc.) with their teats are washed out with a soft brush in hotsoapy water. Then they are rinsed in running tap water. Finally being placed upside down to drain and sterilized in Hot Air Oven at 80ºC temperature used slides are kept is 5 to 10% Sodium Hypochlorite Solution. Then the slides are kept in boiled soapy water for 30 minutes. Then they

are washed in running tap water, rinsed in distilled water and dried on a clean soft cloth.

3. USED GLASSWARE

The infected glass receptacles (HIV, HBsAg, HCV, VDRL, MP etc.) are kept in 1% Sodium Hypochlorite Solution for 30 minutes and then washed in running tap water. This wastage is then collected at each morning by the attendants, segregated in blue BMW bags and issued to Maharashtra Bio-hygienic Management, Lote (Bio-medical Waste Disposal Project) for final disposal..

4. SHARP WASTAGE

The needles of the cut end of the Blood Bag tube is burnt in electric needle terminator. The cut ends as well as sharps such as lancets are disposed in 1% sodium hypochloride for half an hour, put in punctureproof containers for sharps.The wastage is then collected at each morning by the attendants, segregated in blue BMW bags and issued to Maharashtra Bio-hygienic Management, Lote (Bio-medical Waste Disposal Project) for final disposal.

5. SOILED WASTAGE.

The used gloves, micropipette-tips, cotton swabs and all soiled materials are collected in yellow BMW bags, sent to Maharashtra Bio-hygienic Management Lote (Bio-medical Waste Disposal Project) for final disposal.

6. AUTOCLAGE

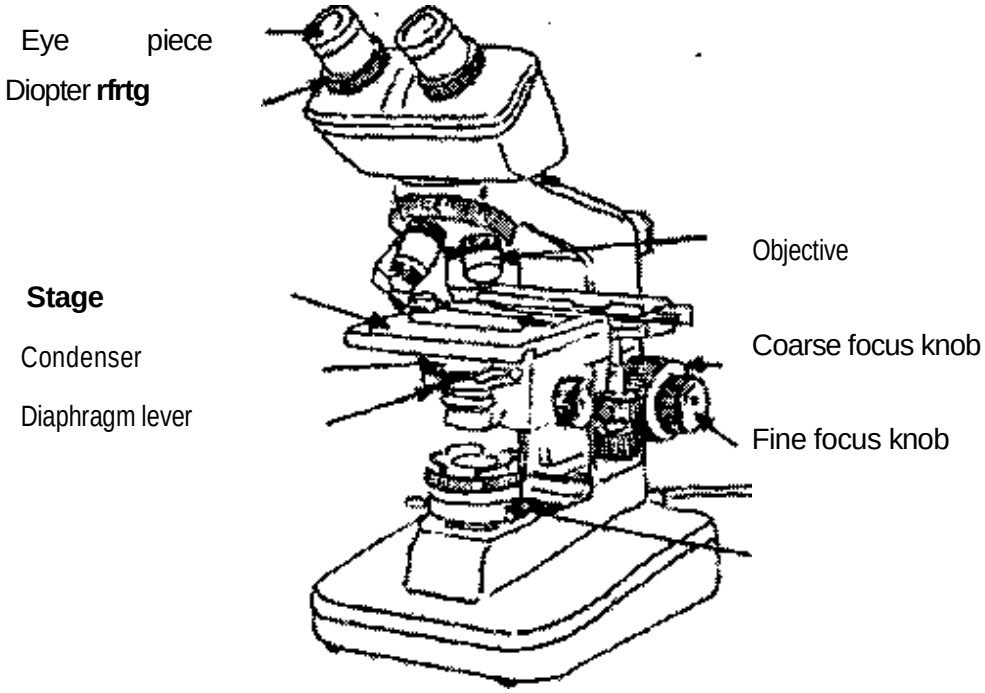
With all the aseptic precautions, the material to be autoclaved is kept in the autoclave drum. Preferably the steam indicator or Autoclave label is affixed to the upper part of the drum after putting the date and time of sterilization on the label. The drum is kept in the autoclave and then switch it on. Allow the autoclave to reach the pressure of 15 to 20 lbs. (10-15 kg.) pressure. After the whistle is heard maintain the pressure for 20 minutes. Switch off the power supply and allow to cool down the sterilizer. Note down the colour change of the autoclave label form green to Grey or black, then the sterilization is complete.

Likewise laboratory precautions are to be taken to maintain the sterility as well the health of the Lab. technicians.

ANNEXURES

ANNEXURE 1

MICROSCOPE AND ITS PARTS



Approximate Time required for routine tests.

Sr. No.	Laboratory Test	Time required approximate
1	Bleeding Time (BT)	2 to 7 mints.
2	CLOTTING TIME (CT) (Capillary method)	4 to 10 mints.
3	DETERMINATION OF HEMOGLOBIN	10 mints.
4	DETERMINATION of ERYTHROCYTE COUNT	25 mints.
5	DETERMINATION OF TOTAL LEUKOCYTE COUNT (TLC)	25 mints.
6	ERYTHROCYTE SEDIMENTATION RATE	1 hours
7	DETERMINATION OF PLATELATE COUNT	25 mints.
8	DETERMINATION OF BLOOD GLUCOSE LEVEL (GOD-POD)	25 mints.
9	DETERMINATION OF BLOOD UREA LEVEL (DAM)	30 mints.
10	DETERMINATION OF BLOOD CREATININE LEVEL (KINETIC)	30 mints.
11	DETERMINATION OF SERUM BILIRUBIN BY DMSO METHOD	30 mints.
12	DETERMINATION OF SGPT BY COLORIMETER	30 mints.
13	DETERMINATION OF SGOT BY COLORIMETER	30 mints.
14	DETERMINATION OF ALKALINE PHOSPHATE BY COLORIMETER	30 mints.
15	DETERMINATION OF HDL BY COLORIMETER	30 mints.
16	DETERMINATION OF SERUM CHOLESTROL BY COLORIMETER	30 mints.
17	DETERMINATION OF SERUM TRIGLYCERIDES BY COLORIMETER	30 mints.
18	DETERMINATION OF WIDAL (SLIDE AGGLUTINATION)TEST	30 mints.
19	DETERMINATION OF VDRL (VENERAL DISEASE REASERCH LABORATORY) TEST	20 mints.
20	DETERMINATION OF RA (RHEUMATIOD)TEST	20 mints.
21	DETERMINATION OF ANTISTREPTOLYSIN O (ASO)QUANTITATIVE TEST	20 mints.
22	DETERMINATION OF CRP TEST	20 mints.
23	DETERMINATION OF HBSAG TEST	20 mints.
24	DETERMINATION OF LEPTO TEST	20 mints.
25	DETERMINATION OF DENGUE TEST	20 mints.
26	DETERMINATION OF CHIKUNGUNIA TEST	40 mints.
27	DETERMINATION OF URINE TEST BY STRIP	20 mints.
28	DETERMINATION OF URINE BILE PIGMENTS	20 mints.
29	DETERMINATION OF URINE BILE SALTS	20 mints.
30	DETERMINATION OF STOOL TEST	20 mints.
31	DETERMINATION OF URINE PREGENCY TEST BY UPT KIT	20 mints.

ANNEXURE 2

CARE OF THE MICROSCOPE

The microscope is the lifeline of the Revised National Tuberculosis Control Programme. Proper handling and maintenance of the microscope, particularly of its ienses, is very important. The following points should be observed:

1. **Place and store the microscope in a dry, dust-free and vibration-free environment, which is specially built in the laboratory, kept warm with a light source to prevent growth of fungus**

- Vibration damages the microscope.
- When the microscope is not being used, cover or keep it in the box so as to keep it free from dust.
- Avoid exposing the microscope to direct sunlight.
- Avoid exposing the microscope to moisture. Humidity may allow fungus to grow on the lens and cause rusting of the metal parts.
- To protect the microscope against the harmful effects of humidity, it is preferable that it be kept overnight in a specially designed storage cupboard. A bulb holder for a 15 watt bulb (candle bulb) will be fixed on the rear wall of the storage cupboard on the right hand side top comer, such that the microscope does not come in contact with the bulb while storing or removing the microscope. The operating switch for the bulb will be located in the storage space on the right side wall towards the front of the storage space (just behind the shutter) for ease of operation. The bulb should remain on when the microscope is stored inside.
- An alternative may be to place plenty of dry bfue silica gel into a shallow plate and place it in the box when the microscope is kept in it. Silica gel is blue in colour when it is dry but when it becomes wet it turns pinkish. As soon as the silica gel becomes pink, change or heat it until it turns blue again and then reuse it.

2. Keep the microscope and lenses clean

- Ail the lenses should be cleaned with dry lens paper or fine silk cloth or lint cloth, immediately after use and at the end of a day's work. Do not wipe the lens with an ordinary cloth.
- Do not leave immersion oil on the surface of the immersion lens.
- Never use spirit or alcohol or xylene to clean the lenses, as these can damage them,
- Never let the oil immersion lens touch the smear.
- Use the fine focusing knob only while using the oil immersion lens.

Reporting form for QA of microscopy

Form 01 (MF 2) : FOR REPORTING OF BLOOD SMEARS BY SURVEILANCE WORKERl MULTIPURPOSE WORKERlPASSIVE AGENCYlHIlMI

Name of Subcentre :_____ Name of PHC: _____ Code Number:_____

Population:_____ Headquarters: _____

Village	No. of House												

Note: This proforma should be in triplicate and three copies forwarded to PHC laboratory Technician who will retain one copy and send the other two to the Surveillance Inspector/Malaria Inspector.

Signature of Laboratory Technician with date of examination Signature of field Worker/MPW/SI/MI/Passive agency

Reporting form for microscopy

Form 02 (MF 4) MONTHLY REPORT OF MALARIA PROGRAMME OF PRIMARY HEALTH CENTRE

Name of the State : _____ Name of the Distt. : _____

Total Population : _____

Sl. No	Name of PHC/Sub-centre	Total population	Active			Mass and contact			Passive			Total			Agewise Positives				RT Done				Death due to mal.	
			Blood Slides			Blood Slides			Blood Slides			Blood Slides												
			Collected	Examined	Positive	Collected	Examined	Positive	Collected	Examined	Positive	Collected	Examined	Positive	Under 1 year	1-4	5-15	15 +	Under 1 year	1-4	5-15	15 +	Pf Microscopically Confirmed	Only Clinically Diagnosed
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25

Signature :.....
Name : MO I/C:.....

Reporting form for microscopy

Form 03 (MF 5) : MONTHLY EPIDEMIOLOGICAL REPORT OF MALARIA PROGRAMME OF PRIMARY HEALTH CENTRE

Name of the State:_____ Name of the Distt:_____

Sl. No.	Name of PHC	No. Positive			Species					RT Given	Total focal spray of rooms	Total fever cases treated with 4 x AQ tabs single dose w/o B.S	Mass therapeutic measures				Balance Tablets			
		Male	Female	Total	PV	PI		others	Total				Single dose 4A.Q.+ 8 A.Q. 600 mg + 45 mg	Single dose Sulpha + Pyri	RT 5 days	Total	4 AQ	8 AQ	Sulpha + Pyri	Qin -ine
						R	RG													
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Total																				

Signature :.....
Name : MO I/C:.....

Reporting form for microscopy

Form 04 (M F 7): DETAILS OF POSITIVES AND REMEDIAL MEASURES

Subcentre: _____ District/PHC: _____

Population: _____ Code No.: _____

Sl. No.	P.C. No.	Source	Group No.	Village	Name of Head of family	Name of Patient	Age	Gender	Code B.S. No.	Date of Collection	Date of examination	Species	Date of receipt of results by MPW	Radical Treatment	If died date of death and species	
														From	To	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17

B.S. Collected				FOCAL SPRAY				TIMELAG BETWEEN		Follow-up smear number and date
Contact		Mass		Date	Targeted Rooms	Sprayed Rooms	% of coverage Rooms	Collection & RT	Collection and focal spray	
No	Result	No.	Result							
18	19	20	21	22	23	24	25	26	27	28

Sign:_____

Name :_____

Designation: _____

Reporting form for microscopy

Form 05 (MF 8) : REGISTER OF BLOOD SMEARS RECEIVED AND EXAMINED (SUBCENTRE-WISE)

Name of Subcentre : _____ Name of PHC : _____

Population : _____ Year : _____ Code No. : _____

Date of receipt	Name of MPW or other agency including FTD etc.	Fever treated w/o B.S. M.S.T. done	Active (A)			Passive (P) FTD			Mass & Contact (M&C)			Period of collection	Date of examination	*Number of B.S. Examined-Positive			POSITIVE SPECIES				Sl.No. of positive cases	Date of despatch of report	
			Sl.No.		Total B.S.	Sl.No.		Total B.S.	Sl.No.		Total B.S.			A	P	M&C	Pv.	Pf.	Pm.	Mixed			
			From	To		From	To		From	To								R	RG				
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Total for the week																							

*In col. No. 15, 16, 17 - the no. of B.S. examined from Active (A), Passive (P) and Mass & Contact (M&C) are posted. Below these the positives among them are posted in a circle.

Sign: _____

Name: _____

Designation: _____

Reporting form for microscopy

Form 06 (MF 9) : EPIDEMIOLOGICAL EVALUATION MASTER REGISTER
(SUBCENTRE-WISE, VILLAGE-WISE and MONTH-WISE)

Name of State : _____ Name of Distt. : _____

Name of PHC : _____ Name of Sub-Centre : _____ Code No : ____

Sl No	Name of village	Population	Target B.S.	Fortnight	B.S. Active	AGENCY-WISE, SEX-WISE POSIVITE								AGE-WISE POSITIVE								Pf. rings only	Total Positive	API
						ACTIVE		PASSIVE		MASS & CONTACT		TOTAL		Pf. & MIXED				Pv. & OTHERS						
						Male	Female	Male	Female	Male	Female	Male	Female	Under 1-Yr.	1-4	5-14	15+	Under 1-Yr.	1-4	5-14	15+			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	A			1																				
				2																				
2	B			1																				
				2																				
Total for the month (Blue ink) (Monthly Report)																								
Total in the next month (Red ink) (supplementary report)																								

*Separate page for each month from columns 5-23 though the list of villages remains common on first page.

Sign: _____
Name : _____
Designation: _____

Reporting form for microscopy

Form 07 (MF 10) : PASSIVE AGENCIES INCLUDING FEVER TREATMENT DEPOTS REPORT
For the Month of _____

Name of PHC : _____ Name of the District : _____

Sl. No.	Name of agency/FTD	OPD-New cases	No. of fever cases	Fever cases treated with 4-AQ without B.S.	B.S. Collected	Number positive	4-AQ consumed	Number R.T. given	8-AQ consumed	Balance of drug	
										4-AQ	8-AQ
1	2	3	4	5	6	7	8	9	10	11	12
2											
3											
4											
5											

Sign:_____

Name :_____

Designation: _____

Reporting form for microscopy

Form 08 (MF 16) : FOR REPORTING DRUG DISTRIBUTIOIN CENTRES, FEVER TREATMENT DEPOTS AND MALARIA CLINICS

Name of the State : _____ Report of the month of : _____

Sl. No.	District	DRUG DISTRIBUTION CENTRES				FEVER TREATMENT DEPOTS				MALARIA CLINICS			
		No.req-uired	No.esta-blished	No. of cases attended during the month	Cumulative No. of cases attended up to date	No.req-uired	No.esta-blished	No. of cases attended during the month	Cumulative No. of cases attended up to date	No. required	No. esta-blished	No. of cases attended during the month	Cumulative number of cases attended up to date
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1													
2													
3													
4													
5													
Total													

Sign: _____

Name & Designation: _____

REPORTING FORM FOR EQA OF MICROSCOPY

Form 09: Proforma for cross-checking by reference laboratory

Name of the Reference laboratory _____

Total No. of blood slides received ----------

Name of PHC _____ Name of District _____

Date of Receipt _____ Name of the State _____

Date of cross checking _____ Month of B.S.C. _____

S.No.	Blood Smear No.	Quality		Result						Remarks
		Smear	Stain	As Examined at PHC			Cross-Checking Organization			
				Neg	Pf	Pv	Neg	Pf	Pv	Quality of slide
1										
2										
3										
4										
5										

Internal Control slide No. used

Quality of slide:- Preparation:-Good(G)/Average(A)/Poor(P)

Staining:- Good(G)/Average(A)/Poor(P)

Diagnosis:-Correct (c) /Incorrect(IC)

Species-Correct©/Incorrect(IC)

Signature of cross checker with date

(COUNTER SIGNED BY I/C REFERENCE LABORATORY)

REPORTING FORM FOR QA OF MICROSCOPY

Form 10: PROFORMA FOR EQAS ON PANEL SLIDES------- EQAS Year.....Round-No.

Name of the Reference laboratory _____

Name of PHC_____ Name of District _____

Date of EQAS performed. _____ Name of the State _____

S.No.	BS No.	Quality		Result							Remarks
		Smear	Stain	species			Stages seen				
				Neg.	PV	PF	Ring	Troph	schizont	gametocyte	
M1.											
M2.											
M3.											
M4.											
M5.											

Internal Control slide No. used

Quality of slide:- Preparation:-Good(G)/Average(A)/Poor(P)

Staining:- Good(G)/Average(A)/Poor(P)

Remark Column:- Signature of Lab. Tech. with date

ANNEXURE 3
ITEMS NEEDED FOR STAINING AND EXAMINING SLIDES FOR AFB

For preparing the sputum smear:

- Wooden broomsticks for spreading sputum
- New microscopy slides
- Carbol fuchsin solution in a plastic squeeze bottle (500 ml capacity)
- 25% sulphuric acid (H2S04) in a plastic squeeze bottle (500 ml capacity)
- 0.1% methyiene blue solution in a plastic squeeze bottle (500 ml capacity)
- Staining rack
- Heat source (spirit lamp or gas burner)
- Tap water

For microscope examination:

- Diamond marker pencil to label slides
- Immersion oil for x100 examination & applicator
- Lens paper or fine silk cloth or tint cfoth to clean the microscope lens
- A notebook to record the number of AFB in each field of the slide

For preservation of slides:

- Soft tissue (toilet tissue) rolls to drain oil
- Slide boxes

ANNEXURE 4

PREVENTION AND CONSEQUENCES OF FALSE-POSITIVE AND FALSE-NEGATIVE SPUTUM RESULTS

HOW TO PREVENT FALSE-POSITIVE SPUTUM RESULTS

- Always use new, unscratched slides
- Use a separate broomsticks for each sample
- Always use filtered carbol fuchsin
- Do not allow the carbol fuchsin to dry during staining
- Decolorize adequately with sulphuric acid
- Make sure there no food particles or fibres in the sputum sample.
- Never allow the oil immersion applicator to touch a slide
- Label sputum containers, slides and Laboratory Forms accurately
- Cross-check the number on the Laboratory Form and sputum container before recording
- Record and report results accurately

Consequences of false-positive sputum results

- Patients are begun on treatment unnecessarily
- Treatment is continued longer than necessary, in the case of follow-up examinations
- Medications will be wasted
- Patients may lose confidence in the programme

HOW TO PREVENT FALSE-NEGATIVE SPUTUM RESULTS

- Make sure the sample contains sputum, not just saliva
- Make sure there is enough sputum (at least 2 ml)
- Select thick, purulent particles to make the smear
- Prepare smears correctly-not too thick, too thin or too little material
- Fix the slide for the correct length of the time, not too short or too long
- Stain with carbol fuchsin for the full 5 minutes
- Do not decolourize with sulphuric acid too intensively
- Examine every smear for at least five minutes before recording it as negative
- Label the sputum containers, slides and Laboratory Forms carefully
- Cross check the number on the Laboratory Forms and sputum container before recording
- Record and report result accurately

Consequences of false-negative sputum results

- Patients with TB may not be treated, resulting in suffering, spread of TB and death
    - Intensive phase treatment may not be extended for the required duration, resulting in inadequate treatment
- Patient may lose confidence in the programme

ANNEXURE 5

JOB RESPONSIBILITIES OF THE LABORATORY TECHNICIAN (LT) IN REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME.

1) Sputum collection

- Instruct and demonstrate to patients the proper methodology on how bring out good quality sputum.
- Label the sputum container properly.
- Before the patient leaves, check the sample to see if it is sputum or only saliva.
- Coordinate with other staff to ensure that patients with productive cough for three weeks or more undergo three initial sputum examinations for diagnosis and those under treatment undergo two sputum examinations for follow up.

2) Sputum processing and examination

- Prepare Smears from the thickest part of sputum, stain read and record results.
- Write the Laboratory No. and visual appearance of the sputum on the Laboratory Form.
- Always use new slides.
- Spread the smear and heat it in order to fix it on the slide.
- Stain the smear by the Ziehl-Neelsen method.
- Examine the stained smear under the microscope.

3) Recording and reporting

- Enter the result of each microscopic examination on the Laboratory form and in the Laboratory Register.
- Maintain the Laboratory Register properly, including the reason for sputum examination.
- Send the Laboratory Form with results recorded to the treating physician promptly.
- Enters the data in monthly lab abstract (Page 28) & signs
- Assist the MO-PHI in completing the monthly PHI report

4) Quality control

- Preserve month-wise in a slide box all the slides according to the entries in the RNTCP TB Laboratory Register.
- Obtain the feedback on the 5 positive and 5 negative slides re-checked by the STLS during their monthly on-site evaluation visit to the respective DMC.
- Collect the slides for random blinded re-checking **(RBRC)** as requested by the STLS and arrange the slides serially in a slide box, complete Annexure B with

ANNEXURE 6

ZIEHL-NEELSEN STAINING PROCEDURE

1) Select a new unscratched slide and label the slide with the Laboratory Serial Number with a diamond marking pencil.
2) Spread sputum on the slide using a broomstick,
3) Allow the slide to air dry for 15-30 minutes.
4) Fix the slide by passing it over a flame 3-5 times for 3-4 seconds each time.
5) Pour filtered carbol fuchsin to cover the entire slide.
6) Gently heat the slide with carbcl fuchsin on it, until vapours rise. Do not boil.
7) Leave carbol fuchsin on the slide for 5 minutes.
8) Gently rinse the slide with tap water until all free carbol fuchsin stain is washed away. At this point, the smear on the slide looks red in colour.
9) Pour 25% sulphuric acid onto the slide.
10) Let the slide stand for 2-4 minutes.
11) Rinse gently with tap water. Tilt the slide to drain off the water.
12) A properly decolourised slide will appear light pink in color .If the slide is still red, reapply sulphuric acid for 1-3 minutes and rinse gently with tap water.
13) Pour 0.1% rnethylene blue onto the slide.
14) Leave rnethylene blue on the slide for 30 seconds.
15) Rinse gently with tap water.
16) Allow the slide to dry.
17) Examine the slide under the microscope using x40 lens to select the suitable area and then examine under xlOO iens using a drop of immersion oil.
18) Record the results in the Laboratory Form and the Laboratory Register.

If the slide has:	**Result**	**Grading**	**No. of fields to**
More than 10 AFB per oil immersion	Pos	3+	20
field 1-10AFB per oil immersion field	Pos	*2*+	50
10-99 AFB per 100 oil immersion	Pos	1+	100
fields 1-9 AFB per 100 oil immersion	Pos	Scanty - record	100
fields		exact number	100
No AFB in 100 oil immersion fields	Neg	seen	

19) Invert the slides on tissue paper til! the immersion oil is completely absorbed' Do not use xylene for cleaning the slides, as it may give false results of repeat examination after storage.
20) Store all positive and negative slides serially in the **same slide-box** until instructed

otherwise by the supervisor.

21) Disinfect all contaminated material before discarding.

ANNEXURE 7

HOW TO DISPOSE OF CONTAMINATED MATERIALS SAFELY

Sputum specimens examined in the laboratory are sotertsatty infectious and after • examination, they must be disinfected and disposed of so fat risk of infection is avoided. All disposable containers are used only once. Slides should never be used again and should be disposed of correctly.

After the smears are examined, remove the lids from all sputum cups and put the cups and removed lids in a bucket containing 5% phenolic compound containing disinfectant solution. The cups and lids should be fully submerged in the solution. Similarly, used broomsticks should also be put in the same bucket containing 5% phenolic compound containing disinfectant solution. The bin/bucket should ha*e a feet-operated lid. Thereafter, the used sputum cups, lids and wooden sticks can deposed off by any of the following methods.

1. Autoclaving in an autoclave or in a pressure cooker. At the end of the laboratory work the sputum cups and the removed lids, *along* with Broomsticks, can be placed in a pressure cooker of approximately 7 liters capacity containing an adequate amount of water to submerge the contents, and boiled for at least 20 minutes using any heating source, electrical or non-electrical. After proper cooling, the material can be discarded along with the other waste.
2. If autoclaving cannot be done, use chemicals such as 5% phenolic compound containing disinfectant solution. Caps of the sputum cups must be removed and the cups, caps and broomsticks submerged in the solution in a secure place overnight. After this the solution, cups, caps and broomsticks can discarded along with the other waste.
3. As a last resort, if none of the above is available, sputum cups, caps and broomsticks can be buried in a pit at a safe distance away from inhabited areas.

Disposal of sputum containers with specimen and wooden sticks

Step 1: After the smears are examined, remove the lids from all the sputum cups. Use gloves whenever you handle infected material.

Step 2: Put the sputum cups, left over specimen, lids and wooden sticks in foot operated plastic bucket/bin with 5% phenol solution. The cups and lids should be fully immersed in the solution,

Step 3: At the end of the day, drain off the 5% phenolic compound'containing disinfectant solution into the drain.

Step 4: Take out the sputum cup/lid/ wooden sticks and put into a reusable metal or autoclave-able plastic container/red bag. The red bag should have a biohazard symbol, and be of adequate strength so that it can withstand the load of waste, and be made off non PVC plastic material.

Step 5: Put this container/bag in to the autoclave with other autoclavable BMW and the contents be autoclaved at 121 °C at 15 psi pressure for 20 minutes. The autoclave shall comply with the standards stipulated in the rules. Under certain circumstances, if autoclaving is not possible, boil such waste in water for at least 20 minutes. However, the District hospital/ CHC/PHC etc. shall ultimately make the necessary arrangements to impart autoclaving treatment on regular basis.

Step 6: After adequate cooling, the material can be safely transported to the common waste treatment facility for mutilation/shredding/ disposal.

If a common waste treatment facility is not available in the area, the sputum cups/ lids/wooden sticks after autoclaving, can be deep buried in a deep burial pit.

Disposal of used syringes/needles/broken vials

Step 1: immediately after administering an injection, cauterize the needle on site using a suitable needle destroyer/cutter, followed by cutting of the plastic hub of the syringe without detaching the needle from the syringe.

Step 2: Put the cauterized needles and broken vials, ampoules in a sturdy puncture proof white translucent plastic/card board container.

Step 3: Segregate and store cut plastic syringes in reusable metal or autoclave-able plastic container/red bag. If a red bag is used, its strength should be such that it can withstand the load of waste inside, and be made of non PVC plastic material.

Step 4: Label both containers with a biohazard symbol as stipulated in the Schedule Ill of the Biomedical Waste (Management & Handling) Rules 1998.

Step 5: Put both the containers into the prescribed bag and transport through a dedicated vehicle to the Common Waste Treatment Facility (CWTF) for autoclaving, mutilation/ shredding/disposal.

Step 6 : If a CWTF does not exist, put both the sharps container (needles) and the metal/plastic container/red bag (syringes) into an autoclave along with the other BMW, and autoclave at 121 °C at 15 psi pressure for 15-20 minutes. Under certain circumstances if autoclaving is not possible, boil such waste in water for at least 20 minutes. However, the District hospital/CHC/PHC etc. should ultimately make necessary arrangements to autoclave the waste on regular basis.

Step 7: Dispose of the autoclaved waste as fellows;

I Dispose the needles and broken vials into the sharps pit,

II Send the syringes for shredding/Mutilation or landfill in deep burial pit.

Disposal of used slides.

Step 1: Place the slides into a puncture proof container or red bag. The red bag should have a biohazard symbol and it should be made of non-PVC plastic material.

Step 2 : Dispose of the slides in the sharps pit.

Under no circumstances the slides should be broken.

ANNEXURE 8
STANDARD (UNIVERSAL) PRECAUTIONS

Standard precautions are used in the care of all patients and apply to blood, all body fluids, secretions and excretions except sweat, regardless of Af-ether tt>ey contain visible blood.
Standard precautions include:

- Hand washing
- Barrier protection
- Safe handling of sharp items
- Safe handling of specimens (blood etc)
- Safe handling of spillage of blood/body fluid
- Use of disposable/sterile items

Hand washing

This is an ideal safety precaution and gloves should not *t<e* regarded as a substitute for hand washing.

For General patient care (hand decontamination)

- Wash hands thoroughly in running water with soap without missing any area. For effective hand washing first wash palms and fingers followed by back hands, knuckles, thumbs, fingertips and wrists. Rinse and dry hand thoroughly.
- Wash hands immediately after accidental contamination with fluid, before eating and drinking and after removing gowns/coat
- Leave soap bars in dry container to prevent contamination

For Surgical care (Surgical Scrub)

- Wash hands up to the elbows,
- Scrub hands for minimum of 2 minutes
- Prevent dripping down of water from unwashed area of arms to washed hands.
- Put on gowns and gloves after drying only.

Barrier Protection

Gloves

- Wear white collecting/handling blood specimens and blood soiled items.
- Wear while disposing waste
- Remove before handling door knobs, telephone, pen, performing office work.
- Discard if cracked, discoloured or punctured.
- Discard if blood spills on them
- Don't reuse disposable gloves.

- Wash hands when gloves are removed or changed,

Masks

- Wear masks and protective glasses if splashing or spraying of blood/body fluids is expected.
- Masks of cotton wool, gauze, or paper mask are ineffective. Paper masks with synthetic material for filtration are an effective barrier against microorganisms.

Caps

Cover hair completely in aseptic units, operating rooms or performing selected invasive procedure.

Gown and aprons

- Wear clean clothes made up of a material easy to clean,
- Change after exposure to blood and body fluids.
- Wear gown or apron of plastic water resistant paper when splashes of blood or other body fluids are likely to occur e.g. during surgery, obstetric procedures, invasive procedures, post mortem and embalming.

Occlusive bandage

- Cover all skin defects e.g. cuts, scratches or other breaks with waterproof dressing before patient care.

Safe Handling of sharps

- Take extra care to avoid autoinoculation.
- Discard all chipped or cracked glassware in appropriate containers
- Never use hands to pick up broken glass. Use a brush and pan.
- Don't manipulate disposable needles. Never bend, break, recap or remove needle from syringe.
- Dispose your own sharps. Don't pass used sharps directly from one person to another.
- Discard of needles into puncture proof rigid containers (Plastic or cardboard boxes)after disinfection in 5% phenol solution. Use needle shredder if available for needles or needles along with syringe nozzle.
- Send sharp disposal containers for disposal when three fourths full.

Safe handling of specimen

- Collect specimens, especially blood and body fluids, in pre sterilized containers properly sealed to prevent leakage or spillage.

- Use autoclaved/pre-sterilized disposable syringes and needles for venupuncture and lancets/cutting needles for finger pricks.
- Cover cuts in hands properly with water proof adhesive bandages.
- Wear disposable gloves while collecting blood/body fluids and maintain proper asepsis.
- Wash hands thoroughly with soap and water, particularly after handling specimens,

Safe handling of blood/body fluids spills

- Cover spills of infected or potentially infected material on the flow with paper towel/blotting paper/ newspaper.
- Pour 5% phenol solution on and around the spill **area** and cover with paper for at least 30 minutes.
- After 30 minutes, remove paper with gloved hands and discard in general waste.

Use of Disposable Sterile Items

- Ensure proper handling of disposable/ sterile item before/ during use. There should be no re-circulation of disposable items.