

# **Resource Manual for Laboratory Technician:**

## **Introduction**

Jan Swasthya Sahyog, Ganiyari in collaboration with National Health Mission, Madhya Pradesh is working in 6 eastern districts of Madhya Pradesh namely Anuppur, Umaria, Mandla, Sidhi, Shahdol and Dindori. In these districts JSS is doing interventions to improve the quality of health through 3 projects iGUNATMAC, Pushprajgarh Health and Nutrition Intervention(PHNI) and Sickle Cell Anaemia Control Mission. Alongwith NHM Sickle Cell Project is being implemented with the support of SRIJAN (Self-Reliant Initiative Through Joint Action). The objective of the work in these districts involves improving quality of care for maternal and newborn health in public health facility, sub centre strengthening, improving nutritional status of children below 3 years of age and ensuring proper and timely diagnosis, treatment and quality care for sickle cell patients in public health facility in the above mentioned 6 districts.

In our attempt to improve quality of care given to patients from different departments, we found laboratory, which can be compared to lungs of a human body to very essential and critical. Maternal deaths due to malaria in pregnancy, deaths due to sickle cell crisis, complications due to hyperbilirubinemia in newborns, etc all such deaths could be prevented with right report at the right time from lab. Medical laboratory services in public health settings are essential but often neglected . Their role is poorly recognised by governments and donors. We found reliability on test results for proper diagnosis and treatment to be insignificant . Furthermore there is increased dependency on private labs irrespective of quality of result, this increases out of pocket expenditure for patients. Insufficient HR, insufficient supply of consumables, lack of trainings, lack of continuum of education for technicians, lack of exposure to good laboratories and its practices, lack of appreciation on reporting critical life saving results, etc are few reasons why the quality of our labs and results are not as per required standard.

It is high time now to imbibe in our laboratory technicians the importance of Standard Operating Procedure and following each step to the last detail in order to get good quality results. We should understand the value of Internal Quality Assurance, External Quality Assurance and incorporate them in our laboratories.

This booklet aims to cover essential investigations that are critical to provide good quality care by performing tests till the last details. We hope this effort will help to identify high risk patients at the right time and save many precious lives. We also hope with more continuum of education for technicians and improvement in labs , trust on lab reports and making decisions based on them will be regained.

# Tests to Diagnose Anaemia

## 1) Haemoglobin Estimation by Flotation in Copper sulphate Solution

### INTRODUCTION & SCIENTIFIC PRINCIPLE:

Most of the weight of blood is contributed by haemoglobin and water. Since the water content of blood is constant except in case of dehydration, the weight of blood is practically determined by its haemoglobin content. Consequently, blood that has more haemoglobin is heavier than blood that has less haemoglobin in it. We can find out how heavy someone's blood is by putting a drop of it in a copper sulphate ( $\text{CuSO}_4$ ) solution of known density. If the sample of blood is heavier than the  $\text{CuSO}_4$  solution, the drop will sink. Conversely, if the blood sample is lighter than the  $\text{CuSO}_4$  solution, the drop will float. The density of the  $\text{CuSO}_4$  solution is chosen by prior experiment to correspond to any particular haemoglobin concentration, such as 9 grams of haemoglobin per dl (dl = 100 ml) of blood.

Any solute can be used to obtain solutions of different densities but  $\text{CuSO}_4$  is preferred since the copper in copper sulphate reacts with protein in the blood to form copper proteinate, which holds the drop together, giving it shape and preventing it from diffusing throughout the solution.

### MATERIALS:

- $\text{CuSO}_4$  solutions of different strengths:
  - 6.25% (*pathlaa ghol*) to correspond to 6 g / dl of haemoglobin
  - 7.3% (*madhyam ghol*) to correspond to 9 g / dl of haemoglobin
  - 8.0% (*gadhaa ghol*) to correspond to 11 g / dl of haemoglobin.
- (To reduce bulk, preferable not to buy readymade  $\text{CuSO}_4$  solutions. Instead, use  $\text{CuSO}_4$  in the solid form in measured quantities in small, labelled paper wrappers. The labels indicate the kind of solution that will be obtained when the contents are dissolved in 40 ml of clean water.)
  1. Graduated measuring cylinder for measuring 40 ml of water.
  2. Plastic containers for holding the different kinds of  $\text{CuSO}_4$  solutions
  3. 70% iso-propanol and cotton swabs for cleaning the skin before drawing blood
  4. Sterile lancets for drawing blood
  5. Latex gloves
  6. Notebook and pencil for recording patient's name and test result (not included in the kit)

### METHOD:

- Transfer the  $\text{CuSO}_4$  given in the paper wrapper into a plastic container. Measure 40 ml of clean water using the measuring cylinder. Transfer the measured amount of water to the plastic container and stir to dissolve the copper sulphate.
- Make all three kinds of solutions and keep the wrappers of  $\text{CuSO}_4$  under the respective plastic containers to indicate the strength of the solution.
- Clean the patient's left ring finger (or the right one for left-handed patients) with a cotton swab soaked in 70% iso-propanol. Let the isopropanol dry off completely. Prick the side of the tip of the finger with the sterile lancet. The prick should be deep enough to allow blood to flow freely. If the prick is not deep enough, please do not squeeze the fingertip to bring out blood. Instead, make a fresh and deeper prick. Wear latex gloves for personal protection from blood-borne diseases.
- Bring the tip of the finger very close to the surface of the  $\text{CuSO}_4$  solution and let a drop of blood fall on it. Afterwards, remove the finger and observe the drop carefully. Note whether it sinks ( ↓ ) or floats ( ↑ ) in the  $\text{CuSO}_4$  solution and record accordingly.

- Sometimes a drop will sink to a short depth and then come up. Such a drop should be recorded as floating ( ↑ ). Sometimes a drop will sink slowly to a short depth and remain there without either sinking further or floating and should be recorded as such ( ↔ ). If in doubt, repeat the test using another drop of blood.
- At least 20 drops of blood can be tested in 40 ml of solution before it becomes too turbid to use.

#### INTERPRETATION:

Depending on whether a sample of blood floats or sinks in the different  $\text{CuSO}_4$  solutions, we can find out whether its haemoglobin level is less than or more than certain cut-off values.

Movement of the drop in 6.25% $\text{CuSO}_4$ ( <i>patlaa ghol</i> )	Movement of the drop in 7.3% $\text{CuSO}_4$ ( <i>madhyam ghol</i> )	Movement of the drop in 8% $\text{CuSO}_4$ ( <i>gadhaa ghol</i> )	Haemoglobin content of blood	Implication
↑	↑	↑	Less than 6 g / dl	Needs urgent medical attention, especially if pregnant
↔	↑	↑	6 g / dl	Needs urgent medical attention, especially if pregnant
↓	↑	↑	More than 6 g / dl but less than 9 g / dl	Needs medical attention but not urgently unless severely symptomatic
↓	↔	↑	9 g / dl	Can be treated by VHW. Seek medical attention in case of no relief
↓	↓	↑	More than 9 g / dl but less than 11 g / dl	Can be treated by VHW. Seek medical attention in case of no relief
↓	↓	↔	11 g / dl	Give iron and folic acid if pregnant. Otherwise give dietary advice only
↓	↓	↓	More than 11 g / dl	

## **2) Digital Hb using Colorimetric Method( Handy Span):**

### **Procedure for measuring Hb in Handy Span:**

1. Switch on the machine and set date, when done press F4 button.
2. Check factor it should be 34.67.
3. Take 1.5 ml reagent only as blank in Cuvette.
4. Press F4 button to get reading of blank, once reading is done, do blanking done.
5. Remove Cuvette.
6. Machine will show read sample, start taking reading of samples one after another.

### **Test Procedure:**

Add 10 $\mu$ l whole blood sample /EDTA sample in 1.5ml Haematin Reagent, keep for 10 minutes and then take reading.

### **Normal Range:**

Hb: Female-12 to 16gm

Male- 12 to 18 gm.

**Note: Detail description of the machine is available in Operator's manual, which will be provided along with the machine.**

### 3) Cell Counter

#### Procedure:

1. Before starting cell counter, make sure there is sufficient reagent i.e diluent, rinse and lyse solutions. Printer should have sufficient paper in it.
2. It takes about 10 minutes for the machine to start, it is advisable not to use any button during this start process.
3. Flush the interior of machine once before running any sample.
4. Before running sample, run the controls provided by the company and make sure all the results are within the acceptable range.
5. Only EDTA sample should be used for estimation of CBC. Mix the sample well before inserting probe of the machine into it.
6. There should be a sample ID for each sample , which is to be enlisted in the machine before testing the particular sample.
7. Before shutting down, run cleanser , test sample as described in the operator's manual of the machine and then turn off the machine.

#### Quality Control:

Usually the company of the machines provides a set of controls (which is stabilised lysable human erythrocytes, simulated platelet components, simulated WBC's and constituents of animal origin in a medium containing stabilizers and preservatives). This product should be treated and analyzed the same as patient specimens and run in accordance with the instructions accompanying the instrument, kit or reagent being used.

Once the control sample is analysed in cell counter, the mean value of the results obtained by running the control should fall within the corresponding acceptable range of mean values provided by the company. It is always recommended that each laboratory should establish its own means and acceptable ranges and use those values provided with the controls as a guide.

#### Interpretation of Few Parameters:

Parameters	Normal Range	Interpretation
Hematocrit (HCT)	Male- 45-50% Female-37-45%	Ratio of volume of red blood cells to the volume of whole blood.
Mean Corpuscular Volume(MCV)	80-100 femtoliters(fL)	Average volume of a red blood cell.
Mean Corpuscular Hemoglobin(MCH)	27-32 picograms	Average amount of hemoglobin in average red cell.
Mean Corpuscular Hemoglobin Concentration(MCHC)	32% to 36%	Average concentration of hemoglobin in a given volume of red cells.
Red Cell Distribution Width(RDW)	11 to 15	Measurement of the variability of red cell size and shape. Higher numbers indicate greater variability in size.
Mean Platelet Volume(MPV)		The average size of platelets in a volume of blood.

#### 4) Sahli's/acid hematin Method

##### Principle:

Blood is mixed with N/10 HCl resulting in the conversion of Hb to acid hematin which is brown in color. The solution is diluted till it's color matches with the brown colored glass of the comparator box. The concentration of Hb is read directly.

##### Equipment required

- Hemocytometer which consists of comparator box which has brown colored glass on either side
- Hb pipette which is marked upto 20mm
- Tube with markings of Hb on one side
- Glass rod
- Dropper

##### Reagents required

- N/10 HCl
- Distilled water

**Sample:** Venous blood collected in EDTA as described earlier

##### Procedure

1. Add N/10 HCl into the tube upto mark 2g%.
2. Mix the EDTA sample by gentle inversion and fill the pipette with 0.02ml blood. Wipe the external surface of the pipette to remove any excess blood.
3. Add the blood into the tube containing HCl. Wash out the contents of the pipette by drawing in and blowing out the acid two to three times. Mix the blood with the acid thoroughly.
4. Allow to stand undisturbed for 10min.
5. Place the hemoglobinometer tube in the comparator and add distilled water to the solution drop by drop stirring with the glass rod till it's color matches with that of the comparator glass. While matching the color, the glass rod must be removed from the solution and held vertically in the tube.
6. Remove the stirrer and take the reading directly by noting the height of the diluted acid hematin and express in g%.

##### Advantages

- Easy to perform
- Quick
- Inexpensive
- Can be used as a bedside procedure
- Does not require technical expertise

##### Disadvantages

- Less accurate.
- All hemoglobins (oxyhemoglobin, sulphemoglobin) are not converted to acid hematin and hence the value of Hb obtained is less than the actual value.
- The color of acid hematin develops slowly.
- Color of acid hematin fades with time and dilution must be done exactly after 10 min when the color development is maximum
- Individual variation in matching of color is seen.
- If the matching point is passed, the whole procedure has to be repeated.
- Color of glass in the comparator box tends to fade with time .
- Lack of a true standard.

## Tests to detect Malaria

### **What is Malaria?**

Malaria is a vector borne infectious disease caused by Plasmodium parasites. These parasites are primarily spread by bite of infected female anopheles mosquito. There are 4 main types of plasmodium species that infect humans:

- Plasmodium falciparum
- Plasmodium vivax
- Plasmodium malariae
- Plasmodium ovale

### **How does it happen?**

Once bitten, the parasite enters the bloodstream and travels to the liver. The infection develops in the liver before re-entering the bloodstream and invading the red blood cells. The parasites grow and multiply in the red blood cells. At regular intervals, the infected blood cells burst, releasing more parasites into blood.

### **When Malaria tests should be done:**

- a. If a patient has fever with or without chills
- b. For all pregnant women at least once during ANC check up and ideally for each ANC visit in endemic area.
- c. Patients presenting with altered sensorium
- d. Patients presenting with any of the following complications
  - i. Jaundice
  - ii. Shock
  - iii. Pulmonary edema
  - iv. Acute respiratory distress syndrome
  - v. Hypoglycemia
  - vi. Kidney failure
  - vii. Splenomegaly
  - viii. Dehydration

### **Tests to detect malaria:**

- a) Thick Smear Test
- b) Thin Smear Test
- c) Rapid Diagnostic Kit (RDK)

#### **a) Thick and Thin Smear Test:**

To confirm if someone is infected with malaria or not, it is important to test their peripheral smear (Thick and Thin smear). Number of RBC's present in thick smear is more; hence thick smear is seen to confirm presence of malaria parasite in RBC's. There is only one layer of RBC's in thin smear hence it is used to identify species of malaria parasite and stage of their life cycle. Thick and thin smears are considered as "gold standard" test for malaria.

### **How to make thick and thin blood smear:**

- i. Whenever possible use separate slides for thick and thin smear.

- ii. **Thin Smear-** Place a drop of blood at the end of a specimen slide. Bring a clean spreader slide, held at a 45 degree angle, toward the drop of blood on the specimen slide, wait until the blood spreads along the entire width of the spreader slide. While holding the spreader slide at the same angle, push it forward rapidly and smoothly. The smear should appear tongue shape.
- iii. **Thick Smear-** Using the corner of a slide, spread the drop of blood in a circle the size of a dime (diameter 1-2cm). Do not make the smear too thick or else it will fall off the slide. One should be able to read newsprint through the smear.
- iv. Wait until the thick and thin films are completely dry before staining. Thin film can be fixed in methanol (100% or absolute) and let it dry completely before staining. Thick film should not be fixed.

### Staining of thick and thin smear:

#### Field Stain method:

##### Principle:

Red blood cells are lysed during this procedure. Diagnosis is based on the appearance of the parasite. The parasites appear more concentrated in thick blood films as compared to thin blood films.

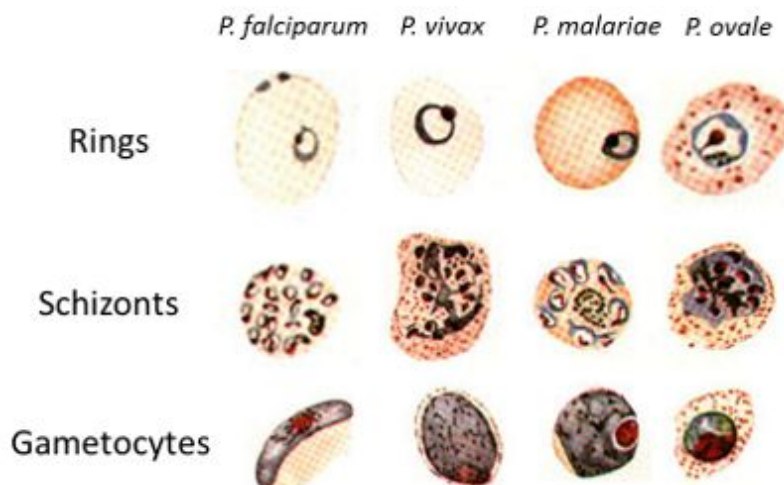
##### Procedure for Thick Smear

- i. Air dry thick smear film, do not fix the smear.
- ii. 1-2 dip in Field stain A
- iii. Wash in tap water; keep water in a tray/basin to wash slide
- iv. 1-2 dip in Field Stain B
- v. Wash in tap water; keep water in a tray/basin to wash slide
- vi. Air dry slide before examining in microscope

##### Procedure for Thin Smear

- i. Air dry smear and fix in Methanol
- ii. Air dry smear again after fixing in Methanol
- iii. 1-2 dip in **Field Stain B**
- iv. Wash in tap water; keep water in a tray/basin to wash slide
- v. 8-10 dips in Field Stain A
- vi. Wash in tap water; keep water in a tray/basin to wash slide
- vii. Air dry slide before examining in microscope.

### Interpretation of results: Differentiation of Malaria Parasites





Finding	<i>P.falciparum</i>	<i>P.vivax</i>	<i>P.malariae</i>	<i>P.ovale</i>
<b>RBC Size</b>	Not enlarged	Enlarged	Not enlarged	Enlarged
<b>RBC Shape</b>	Round, sometimes crenated	Round or ovale frequently bizarre	Round	Round or oval, often fimbriated
<b>RBC Colour</b>	Normal but may become darker; may have a purple rim	Normal to pale	Normal	Normal
<b>Stippling</b>	Maurer's spots appear as large red spots, loops and clefts; up to 20 or fewer	Schuffners dots, appear as small red dots. Numerous.	Ziemann's dots, few tiny dots, rarely detected	Scchuffner's dots (James's dots) Numerous small red dots
<b>Pigment</b>	Black or dark brown ; in asexual forms as one or two masses; in gametocytes as about 12 rods	Seen as a haze of fine golden brown granules scattered through the cytoplasm	Black or brown coarse granules scattered	Intermediate between <i>P.vivax</i> and <i>P.malariae</i>
<b>Early Trophozoite (ring)</b>	Smallest, delicate; sometimes two chromatin dots; multiple rings commonly found	Relatively large; one chromatin dot, sometimes too often two rings in one cell	Compact ; one chromatin dot; single	Compact; one chromatin dot; dot single
<b>Schizont</b>	Medium size; compact; numerous chromatin masses; coarse pigments; rarely seen in peripheral blood	Large; amoeboid; numerous chromatin masses; fine pigments	Small; compact; few chromatin masses; coarse pigments	Medium size; compact; few chromatin masses; coarse pigments
<b>Gametocyte</b>	Crescent shaped, larger and slender; central chromatin	Spherical; Compact	Similar to <i>P.vivax</i> but smaller and less numerous	Like <i>P.vivax</i> but smaller

#### Quality Check of Stain:

- Stain 3 to 4 slides with different timings in Field stain A and B, look at the smear in microscope, see if RBCs have normal red orange appearance, nucleus lobes in WBC should appear purple and granules in pink colour.

#### Rapid Diagnostic Test for Malaria:

Malaria Pf/ Pv Ag test kit is an in-vitro diagnostic immuno chromatographic assay for the qualitative detection of infection with plasmodium parasites causing malaria it does not assess parasite densities.

#### PRINCIPLE:

The following plasmodium antigens are detected in this test:

- Histidine rich protein 2 specific for p falciparum (Pf-HRp2)
- Plasmodium lactate dehydrogenase to plasmodium vivax (Pv-pLDH)

**REAGENTS AND MATERIALS REQUIRED:**

Each kit Contains:

1. Test strip in test device packed
2. Sample Loop
3. Assay buffer : Protein stabilizers, detergent and preservatives.
4. Sterile lancets, Alcohol swabs, Disposable gloves, Pen and Timer, Sharp box, Non- Sharps disposal container, Venipuncture blood collection materials and precision pipette plus tips (if whole blood is collected by venipuncture)

**STORAGE AND STABILITY:**

- Store the kit between 1-40°C.
- Do not store the kit in the freezer .
- Protect the kit from humidity.
- The kit has a shelf life of 24 months from the date of manufacture. The test kit is stable until the expiration date marked on the kit box and/or the packaging of individual components when stored as specified. In case, the desiccant pouch changes colour from blue to light pink or colourless, the test device should not be used.

**WARNING AND PRECAUTIONS:**

- Apply standard biosafety precautions for handling and disposal of potentially infectious material.
- The buffer contains sodium azide as a preservative which may be toxic if ingested. When disposed of through a skin. Flush with large quantities of water.
- Do not use any other buffer than the buffer supplied within this kit.
- Do not use the kit beyond the expiration date.
- Do not use if the packaging is damaged.
- Do not use if the product has been exposed to excessive heat or humidity
- Perform the test immediately after opening the cassette packaging
- Allow all reagents and Sample (s) to attain room temperature (18°C to 30°C) before use.
- Do not touch the nitrocellulose part of the device. Fingerprint or scratch on nitrocellulose membrane may give erroneous results.
- Do not use haemolysed specimen for testing.

**SPECIMEN COLLECTION AND PREPARATION:**

Capillary blood or venous blood with the following anticoagulant: EDTA, heparin, Oxalate or Citrate.

Time Between collection and analysis:

- Capillary: Immediately
- Venous: immediately. If immediate testing is not possible, store the whole blood specimen at 2-8°C for maximum 72 hours.

## TEST PROCEDURE:

### Before Testing :

1. Prepare all necessary materials:
  - a. When stored in the refrigerator, bring the kit components to room temperature minimum 30 minutes before use.
  - b. Prepare all the materials ready for use.
2. Check the expiration date of the test. If expired, do not use it but take another test from an unexpired kit.
3. Check that the cassette packaging is not damaged. If damaged, discard the cassette packaging and use another test.
4. Open the cassette packaging and check the desiccant. If there is a humidity indicator and it shows saturation (colour changed from blue to pink/white), throw away the cassette and take another cassette packaging if the color of desiccant in the non- sharps disposal container.
5. Take the cassette and place it on a horizontal surface.

You see :


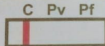
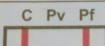
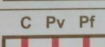
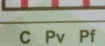
- A. A result window (marked with C,Pv, Pf)
  - B. A circle well marked "S" (for specimen)
  - C. A square well "B" (for buffer)
  - D. Write the patient name or patient identifier on the cassette.
6. Put on gloves.

### Sample:

- In case of capillary whole blood , take small loop and collect 5 µl of blood by dipping the circular end of the loop into the whole blood drop. In case of venous sample, collect sample into a tube containing the correct anticoagulant.
- Place the circular end of the loop in the circle well(marked"S") so that it touches the strip. Press down lightly to transfer the whole blood to the strip.
- Take the buffer bottle . Hold the open buffer bottle vertically above the square well. Squeeze the buffer bottle gently and apply exactly 4 drops into the square well.
- Write the time on the cassette or set a countdown timer to the required reading time.
- Read test results after a minimum of 20 minutes but no later than 30 minutes. Use a good light source when reading the test results.

### INTERPRETATION OF RESULTS:

- After 20 but no later than 30 minutes: compare the test lines with the presentation in the table below.
- Where possible have the results confirmed by a second reader within this time frame.
- Line intensities may vary from faint to strong intensity. Consider also faint test line as a positive result.
- Record the test results as noted in the table below. Consult the national guidelines for malarial case management to complement the table below.

Lines that you see	Picture/Drawing	Record the following result, take the following action
NO line at 'C' (=control)		Invalid. Take a new cassette packaging and repeat the test.
Line at 'C' and NO other line		Negative
Line at 'C' AND at 'Pf'		Positive for <i>P. falciparum</i>
Line at 'C', at 'Pf' AND at 'Pv'		Positive for as a mixed infection with of <i>P. falciparum</i> and <i>P. vivax</i>
Line at 'C' AND at 'Pv'		Positive for <i>P. vivax</i>

### LIMITATIONS OF THE TEST:

1. As will all diagnostic test, the test result must always be correlated with clinical findings.
2. False positive result can occur:
  - Presence of heterophile antibodies in patient's sample with Rheumatic diseases and autoimmune disorder may lead to false results.
  - Some viral infection (such as hepatitis B or hepatitis C, dengue)
  - Parasitic infection (eg. Schistosomiasis and Trypanosomiasis)
3. False negative result can occur:
  - If antigen concentration/parasite densities present in the specimen is below the detection limits of the assay or the analyte of interest that are detected are not present during the stage of disease in which a sample is collected.
4. A negative result at any time does not preclude the possibility of exposure or infection.
5. Repeat the test in case of very faint band or if have any doubt for test band.
6. This kit is designed for primary screening of malaria infection.
7. Although the test is accurate in detecting HRP- 2 Specific to *P. falciparum* or pLDH specific to *P. Vivax* in blood samples, low incidence of false results may occur. Other clinically available tests should be used if questionable results are obtained.
8. "Pv" band may turn negative after successful anti – malarial therapy.
9. In few cases, HRP-2 band appears in certain post malaria, however, such observation are also observed in certain untreated malaria. In such cases, re- testing after 2 days is recommended.

### Reference:

Literature of Meril Diagnostics for One Step test for Malaria Pf/Pv Ag.

## Chapter - 2: Diagnosis of Sickle Cell Disease

### 1) Sickle Cell Preparation (Slide Method)

#### Principle:

RBCs become sickle shape in Sickle cell disease. Due to its oxygen carrying nature, sickle shape RBCs also appear in normal RBC shape on routine microscopy. The sickle shape appears only in deoxygenated sickle RBCs. In Sickle prep method, sodium dithionate helps in deoxygenate the RBCs. Wax sealing prevents further air/oxygen contact of RBCs, thus sickle RBCs remain in sickle shape. These can be easily identified on microscopy. De-oxygenation with sodium dithionate takes about an hour. In sickle prep investigation, blood sample is subjected to de-oxygenation after sealing the sample with wax followed by microscopy to identify sickle RBCs.

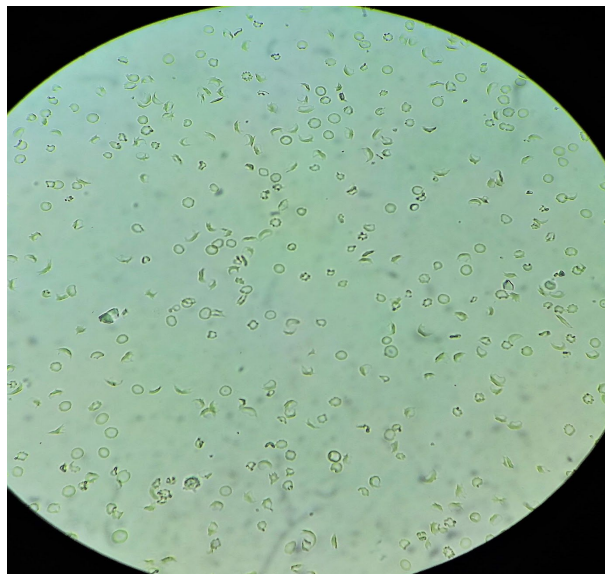
#### Requirements for the test:

- Slide
- Sickle Buffer
- Cover Slip
- Wax
- Match Box
- Dropper
- Lancet
- Needle
- Spirit
- Bunsen Burner/Spirit Lamp
- Electronic Balance
- Chemicals Required- Disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) and Sodium dithionate ( $\text{Na}_2\text{S}_2\text{O}_4$ ).

#### Process of making Buffer and Working Solution

**Buffer:** Mix well -Disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) – 8.1 grams and distilled water 833 ml. This mixed solution can be kept in dark brown bottle for 4-5 months.

**Working Solution:** Mix well a solution of 12.5 ml of buffer and 110 mg sodium dithionate ( $\text{Na}_2\text{S}_2\text{O}_4$ ) in a screw cap test tube. This working solution can be stored in refrigerator upto a week.



### Procedure for Sickle Cell Preparation:

1. Place a small drop of blood(5 $\mu$ l), EDTA sample/finger prick in the middle of a microscopy slide and add a drop of working solution on the sample. Mix Well.
2. Cover this mixture with a cover slip.Be careful in placing the cover slip, no air bubbles should be present in the mixture after cover slip is placed on it.
3. Seal the coverslip border with liquified wax.
4. Observe the slide under 40X after 1 hour.
5. If Sickle Cell Preparation test is positive , we would find sickle shaped RBC's. In case of negative sickle test, there would be no change in RBC shape.

### Precautions:

1. Heat the wax only until it liquefies. Do not heat it in high temperature. Using very hot wax for sealing will affect results.
2. For making working solution, be very particular of using 110mg strictly. Higher quantity can crenate RBC's resulting in false positive results.
3. Avoid using more than 5 $\mu$ l blood for slide preparation, this will cause overlapping of RBC's and affect reporting of result.

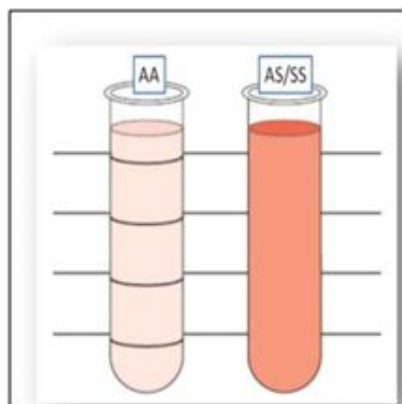
### Quality Control:

To check if the working solution is giving correct result, make a sickle cell preparation for known sickle positive sample with freshly made working solution. After one hour if the RBC's in the preparation appear sickle shaped then we can go ahead with using the solution for testing other samples. IF, NOT then discard the working solution and make a fresh one again and repeat quality check.Every time working solution is made, **quality control should be done** before proceeding with sickle preparation for other samples. **Once working solution is made it is usable for maximum 12 hours.**

## 2) Solubility Test:

### Principle:

Solubility test for detection of Hemoglobin S is based on the solubility difference between HbS and HbA in concentrated phosphate buffer solution. In this test RBC'S are lysed by a hemolytic reagent and the released hemoglobin is then reduced by sodium dithionate in a concentrated phosphate buffer. In the presence of sodium dithionite, HbS precipitates causing turbidity of the reaction mixture. HbA and other hemoglobins are soluble and hence a transparent solution is seen. A positive result is indicated by a turbid suspension through which the ruled lines are not visible. A negative result is indicated by a transparent suspension through which the ruled lines are visible.



**Fig - The Sickle cell solubility test**

**Preparation of working reagent:**

1. To prepare 20ml working reagent take one vial of solubility buffer(R1) and one vial solubility reagent powder(R2).
2. Add one ml of solubility buffer in one vial of solubility reagent powder. Mix well and let it stand for 10 minutes.
3. Mix the reagent thoroughly before use

**Sample:**

EDTA whole blood sample

**Procedure:**

1. Label test tube appropriately, add 2 ml of working reagent to each tube
2. Add 1 drop (20 $\mu$ l) of whole blood sample.
3. Mix well and allow to stand for 10 minutes.
4. Read the turbidity in the test tubes by holding the tubes against a dim illumination and viewing the black lines printed on a paper.

**Interpretation of Results:**

1. A turbid solution(black lines on background of result reading stand are barely visible and cannot be seen) indicates a positive result for sickle cell.
2. A clear solution (black lines on background are clearly visible) indicates a negative result for sickle cell.

**Quality Control:**

After preparation of working reagent do solubility test on known positive and negative sample for sickle cell before proceeding with other samples.

**Note:**

If hemoglobin concentration is 8g/dl or less , the sample volume for testing should be doubled to 40 $\mu$ l in screening method. False negative results may occur in infants under 6 months of age due to high levels of hemoglobin F.

### **3) Hemoglobin Electrophoresis**

**Principle:**

Haemoglobin molecules dissolved in water are electrically charged. The magnitude of the charge and its polarity (positive or negative) are determined by the kind of haemoglobin and by the acidity or alkalinity of the solution. In an electrical field, charged haemoglobin molecules move towards the cathode or anode depending on the polarity of the charge and for the molecules having the same polarity of charge, the speed of movement depends on the magnitude of the charge. Because of differences in speed, different kinds of haemoglobin molecules in a mixture migrate different distances in a given span of time and get separated in discreet migration fronts that appear as bands on the electrophoresis medium (agarose gel or cellulose acetate paper). For example, in a mildly alkaline medium (pH 8.2 or 8.4), normal adult haemoglobin (HbA) and sickle haemoglobin (HbS) both move towards the anode but HbA moves faster leaving HbS behind. Ultimately all the HbA molecules accumulate in a distinct band ahead of the HbS molecules, which also move together in a trailing

band. The different bands can be identified by comparing their positions with those obtained from control samples of known varieties of haemoglobin. These can also be clearly visualized and identified on an agarose gel stained with Amido black 10B Stain.

#### **Materials Required:**

- Electrophoresis unit
- Electrophoresis power supply
- Spatula (for detaching the gel from the casting tray for staining purpose)
- Petri dish for staining and de-staining
- Centrifuge machine
- Pipettes(1000 and 10 micro lt)
- 50ml beaker
- Bunsen Burner, Tripod Stand, Wire Mesh
- Conical Flask

#### **Specimen Handling and Collection**

Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future). Ensure that the blood sample is at room temperature before beginning the protocol.

#### **Procedure**

##### **I. Sample preparation**

The quantity of sample required for the electrophoresis testing is 250  $\mu$ l of blood, if patient's hemoglobin is less than 7 gm% then take 500  $\mu$ l sample. For testing purposes red blood cells are to be separated from the fresh blood sample to isolate hemoglobin from them. For doing that, Centrifuge the fresh whole blood sample at approximately 3000 rpm for 10 minutes at room temperature. Discard the supernatant plasma carefully such that the red blood cell pellet obtained, is not disturbed. Wash the red blood cell pellet with 2 ml of Normal Saline. Centrifuge the resuspended pellet at approximately 3000 rpm for 10 minutes. Again discard the supernatant carefully and wash the pellet with another 2ml of Normal Saline. Perform this wash step 5 times with Normal Saline to obtain a red blood cell pellet. Discard the supernatant carefully at the end of the washing steps such that the red blood cell pellet remains undisturbed. Make sure that there is no normal saline remaining in the tube.

##### **II. Lyse**

1. Add 200-500  $\mu$ l of Distil Water, mix the suspension well and let it stand for 10 minutes.
2. Add 200 $\mu$ l of CCL<sub>4</sub> (Carbon Tetra Chloride), Mix well and centrifuge at approximately 3000rpm for 10 minutes.
3. Take the tubes carefully out of the centrifuge without disturbing the supernatant and pellet.

##### **III. Preparation of Working Buffer**

1. Mix 8 gm Tris buffer pre-weighed and 3.6 gm Glycine to 500ml of distilled water in a conical flask.
2. Check pH of the buffer, it should be 7.4.
3. This working buffer can be stored in fridge and reused 3-4 times, provided its pH is maintained at 7.4.

##### **IV. Agarose gel preparation**

1. Prepare agarose gel by adding pre-weighed 320mg of Agarose in 40ml working buffer. Dissolve it completely by boiling in the working buffer.  
**NOTE:** Prepare fresh diluted Gel Running Buffer as indicated in general preparation instructions  
**NOTE:** The agarose powder should be dissolved in diluted working buffer by boiling and swirling intermittently such that the agarose dissolves completely. Do not over boil the agarose so as to minimize water loss due to evaporation.



- Cool the melted agarose for about 10 minutes, cover the open sides of the boat with cello tape. Pour the melted agarose while hot in the casting unit of electrophoresis unit with the two combs placed in their respective notches. Ensure that the gel poured spreads evenly on the surface of the casting tray to form a thin gel. Allow the gel to set. The gel will solidify completely in 15 minutes.

**NOTE:** Do not pour the gel when it is boiling hot as it leads to water loss due to evaporation which will alter the concentration of agarose in the gel. The formation of a thin uniform gel is essential to minimize resistance produced, which leads to generation of heat due to high voltage and high current required for the electrophoretic run.

- Position the casting tray after the gel has set such that the wells are oriented towards the cathode.
- Pour 450 ml of working buffer into the electrophoretic tank. Ensure that the agarose gel is submerged completely in the working buffer.
- Load 5  $\mu$ l of supernatant from each sample into each well.
- Connect the electrodes of the Electrophoresis unit to Electrophoresis power supply unit and run the gel at 150 to 200 V and 90 mA for 1 hour. To ensure that the run has started the user can observe bubbles in the buffer from the sides of the electrophoresis unit. Usually migration of protein band can be read after 1 hour without staining but if the bands are lightly coloured and for clear reading we can go ahead with staining.

## V. Staining of gel for visualization of hemoglobin protein bands

- Slice the gel carefully along the edges of the casting tray using a spatula, such that gel can slide down easily into the staining tray. Avoid breakage of gel during handling.
- Pour Amido Stain onto the gel such that the gel is completely submerged in the staining liquid. Allow staining by shaking the gel in the staining solution for 10 minutes.
- Decant the stain used in a container. This stain can be reused 8-10 times. It should be stored in brown glass bottle.
- Rinse the gel with 100 ml distilled water by pouring it in the tray and shaking the gel intermittently.
- Decant tap water and pour 5% acetic acid into the tray for 30 minutes. Shake the gel intermittently.
- Discard the acetic acid after 30 minutes.
- Discard the acetic acid and take reading of the bands.

### Preparation of Amido Black 10B stain:

- Add 10mg Amido black 10B in 100ml of 5% Glacial Acetic acid

### Interpretation Of Results:

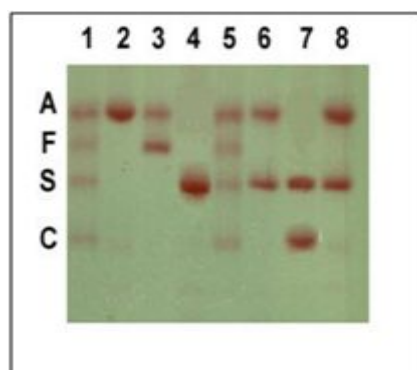


Fig: Hb Electrophoresis slide after staining

## Troubleshooting Guide

SN.	Problem	Possible Cause	Solution
1.	Poor separation of hemoglobin protein bands	Blood sample used is not freshly collected	Freshly collected blood sample should be used. Do not use old and stored blood samples
		Hemolysate obtained is not freshly used	Do not store hemolysate obtained in . Prepare hemolysate freshly every time before each electrophoretic run
2.	Smearing of hemoglobin protein bands observed	Incomplete removal of Normal Saline in Sample preparation	Ensure that Normal Saline is completely removed from the red blood cell pellet in the Sample preparation step carefully without disturbing the red blood cell pellet
		Insufficient washing with Normal Saline	Wash the red blood cell pellet sufficiently with Normal Saline.
		Collection of red blood cell debris	Ensure that while collecting the supernatant hemolysate, the intermediate layer of red blood debris should not get collected along with upper layer of the supernatant
3.	Melting of Agarose gel	Use working buffer	Use freshly prepared working buffer
4.	Poor resolution of the hemoglobin bands	The band might have not migrated to 4-4.5 cm	Please run the gel for another 15 minutes to obtain proper migration of bands

### Safety Information

Alkaline Hemoglobin Electrophoresis is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach.

### 4) Reticulocyte Count

Reticulocytes are immature but already anucleated erythroid cells with residual amount of RNA. In healthy individuals reticulocytes are in the bone marrow for about 3 days and spend 1 day in circulation before they mature into an RBC. Under steady state conditions, RBC production equals RBC losses. With worsening anemia and increase in erythropoietin stimulation, the bone marrow releases reticulocytes at an earlier stage in their maturation and reticulocytes are in the peripheral circulation for a longer time. The reticulocyte count is therefore useful as a marker to estimate the degree of erythropoiesis and the appropriateness of the bone marrow response to anaemia. Since sickle cell anaemia is a chronic anaemia, the

reticulocyte count will be elevated at baseline and it is important to measure when there is a drop in the haemoglobin to make sure the bone marrow is responding adequately.

### Staining of reticulocytes:

#### Principle:

Brilliant cresyl blue is an isotonic medium which selectively stains nucleic materials of reticulocytes which can be seen under a microscope.

#### Equipments Required:

- Slide
- Test tube
- Retic Reagent
- Microscope

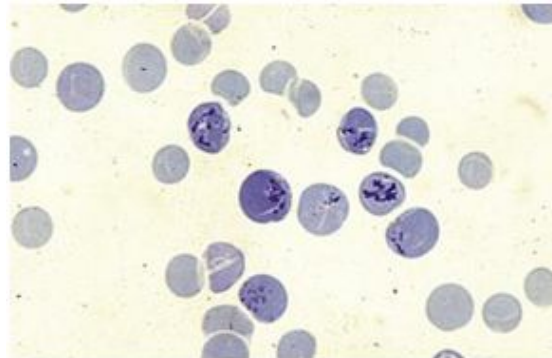
#### Sample:

EDTA Sample/Whole Blood

#### Procedure:

1. Take 200µl blood sample in 5 ml glass test tube.
2. Add 100µl retic stain (2:1), mix and incubate at 37°C for 20 minutes .
3. Prepare few smears in microscopy glass slides.
4. Report in 100x, oil immersion lens.

#### Reporting:



Reticulocytes in Brilliant Cresyl Blue Staining

In Brilliant Cresyl Blue Stain under oil immersion lens nuclear material in reticulocytes appear in dark blue colour and cytoplasm in blue colour.

#### Calculation:

$$\text{Retic Count} = \frac{\text{Total no. of retic seen} \times 100}{\text{Total no. of RBC counted}}$$

**Normal Value:** 0.2-2%

#### Reagent Preparation:

- A.  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  (Concentration = 23.4gm / litre)
- B.  $\text{Na}_2\text{HPO}_4$  (21.3gm / lit)

Quantity of reagents: (A = 64ml) + (B = 36ml) = 100ml (pH 6.5)

## **25ml+ 250mg Brilliant Cresyl Blue Or New Methylene Blue**

### **Quality Control:**

The performance of stain must be periodically checked by known samples. Accuracy of reporting reticulocyte count is subject to the professional experience of each person as well as the use of a good optical system that could make clear magnification of the cells from smear.

## Chapter -4 : BIOCHEMISTRY for Semi Automatic Analyze

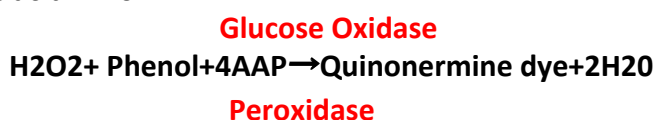
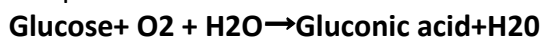
### 1) Random Blood Sugar (RBS)- Trinder's Method, End Point

#### What is the clinical significance of measuring glucose in serum?

Glucose measurements are used in the diagnosis and treatment of carbohydrate metabolism disorders including diabetes mellitus, hypoglycaemia and various other conditions.

#### Principle

Glucose in the sample is oxidised to yield gluconic acid and hydrogen peroxide in the presence of Glucose oxidase. The enzyme peroxidase catalyses the reaction of 4- aminoantipyrine with phenol, which gives a coloured compound, with absorbance proportional to the concentration of glucose in sample.



#### Materials Required:

- Reagent- Glucose reagent: Phosphate Buffer 100 mmol/l, Glucose oxidase > 7 U/ml, Composition

- Reagent 1. Glucose Reagent: Phosphate Buffer 100 MMol/l, Glucose Oxidase > 7 U/ml, Peroxidase > 0.14 U/ml, Phenol 5 mmol/l, 4-aminoantipyrine 0.5 mmol/l, stabilizers
- Glucose Standard: Concentration as stated on the label.

Semi auto or fully automated analyzer, Calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/ rack, heating bath, controls, saline.

#### REAGENT PREPARATION, STORAGE & STABILITY

Keep away from direct light sources.

Stability: up to expiration date on labels at 2-8°C.

Stability since first opening of bottle: preferable within 60 days at 2-8 °C

#### REAGENT DETERIORATION

- Discard the reagent if absorbance exceeds 0.3 against distilled water.
  - Keep the Standard vial plugged after use, in order to avoid deterioration
- Use fresh unhaemolysed serum. Serum or plasma should be separated from the cells, as soon as possible, to prevent glycoly. The addition of sodium fluoride is recommended to inhibit glycolysis. serum / plasma is stable for 3 days at 2-8 °C

#### TEST PROCEDURE

Dispense	Blank	Standard	Sample
Reagent 1	1ml	1ml	1ml
Distilled water	10µl	-	-
Standard	-	10µl	10µl
Mix, incubate for 10 min at 37°C Read absorbance of standard (As) and Samples (Ax) against reagent black.			

**Assay Protocol 2:**

Dispense	Standard	Sample
Reagent 1	1000 µl	1000µl
Standard	10µl	-
Sample	-	10µl

Mix. Incubate 30 seconds at 37°C then record absorbance as A, After exactly 60 Seconds, record again absorbance as A2

**RESULT CALCULATION****Serum/plasma:**

Glucose mg/dl =  $A_x/A_s \times \text{Concentration of Standard}$

or

Glucose mg/dl =  $A_2 - A_1(\text{Sample}) / A_2 - A_1(\text{Standard}) \times \text{Concentration of Standard}$ .

SI Conversion factor: 1 mg/dl x 0.0555 = 1 mmol/l

**EXPECTED VALUES**

Glucose Fasting : 70 - 110 mg/dl                      OR                      3.8 - 6.1 mmol/l

Post Prandial: 90 - 140 mg/dl                      OR                      5.0 - 7.8 mmol/l

It is recommended that each laboratory verifies this range or derives reference interval for the population it serves.

**QUALITY CONTROL AND CALIBRATION**

It is recommended to perform internal quality control with assayed normal and assayed abnormal to confirm the validity of the test and assure the accuracy of patient result.

Using the recommended calibrator or the standard included, calibrate the assay :

- a. when using a new reagent or lot
- b. when QC values are out of range

## (2) Creatinine Kit- Jaffe's Method, Initial Rate

### Clinical Significance:

Creatinine is a waste product formed from creatine phosphate. It is removed from plasma by glomerular filtration and then excreted in urine. Creatinine is a useful indicator of renal function. Elevated levels of creatinine are associated with abnormal renal function.

### Principle of the Method:

Creatinine reacts with picric acid in alkaline environment to form an orange - red color complex. Developing of this orange-red color may be followed photometrically at 500-520nm.

### Materials Required:

- Reagent 1: Picric acid 11mmol/l
- Reagent 2: NaOH 0.3 mmol/l
- Creatinine Standard: Concentration 2.0mg/dl
- Semi Auto Analyzer
- Cuvette holder
- Glass or high quality polystyrene cuvette
- Test tube /rack
- Heating bath
- Controls
- Saline

### Reagent Preparation, Storage and Stability

Reagent 1 and Reagent 2 are ready to use. Upon opening of kit, store reagents R1 and R2 at 15-30°C and standard at 2-8°C.

### Specimen:

Preferably Serum/ Plasma (Heparinated). Avoid severely hemolysed specimen collection. Samples are stable for 7 days at 2-8°C.

### Test Procedure

Dispense	Standard	Sample
Reagent 1	500µl	500µl
Reagent 2	500ul	500µl
Standard	100ul	-
Sample	-	100µl

Mix, incubate for 60 seconds at 37°C, then record absorbance as A1- After exactly 60 seconds, record agani absorbance as A2.

### Result Calculation

**Serum/Plasma:** Creatinine mg/dl=  $A2-A1(\text{sample})/A2-A1(\text{standard}) \times \text{Concentration of Standard}$

### Expected Values

Men: 0.6-1.4mg/dl

Women: 0.6-1.2mg/dl

### Quality Control and Calibration

It is suggested to perform internal quality control with normal and abnormal controls, to confirm the validity of the test and assure the accuracy of patient result.

### Reference:

Literature taken from Meril Diagnostics Creatinine Kit

### 3) Serum Glutamic Oxaloacetic Transaminase(SGOT)

#### IFCC Method, Kinetic

#### **Clinical Significance:**

Transaminases are widely distributed in animal tissues. Both AST(SGOT) and ALT(SGPT) are normally present in human plasma, bile, CSF and saliva. Elevated AST levels are observed in viral hepatitis and other liver disease , cirrhosis, myocardial infarction.

#### **Principle:**

L-Aspartate+2-Oxoglutarate→Oxaloacetate+L-Glutamate

AST

Oxaloacetate+NADH→Malate+NAD

MDH

Pyruvate + NADH→L-Lactate+NAD

LDH

AST: Aspartate aminotransferase

MDH: Malate dehydrogenase

LDH: Lactate dehydrogenase

#### **Materials Required:**

- SGOT-Reagent 1 and Reagent 2
- Glass or high quality polystyrene cuvettes
- Semi automated analyzer
- Calibrated Micropipettes
- Test tube/ rack
- Controls Saline

#### **Reagent Preparation, storage and stability:**

Mix reagent 1 and reagent 2 in ratio 4:1. Keep away from direct light sources. Stability of reagent up to expiration date on labels at 2-8°C. Stability of working reagent , 30 days at 2-8°C. Discard the working reagent if absorbance < 1.0 at 340 nm against distilled water.

#### **SPECIMEN**

Use serum, plasma. SGOT is stable for 4 days at 2-8 °C or 1 month at -20 °C

#### **TEST PROCEDURE**

Dispense in tube : working reagent	500 µl
Add Sample	50 µl
Assay Protocol 1: Mix and incubate 60 seconds at 37°C, then record first reading of absorbance. Perform other 2 reading at 60 seconds intervals. Calculate the Δ A/Min.	

Assay Protocol 2: Mix and incubate 30 seconds at 37°C, then record first reading of absorbance. Perform other 2 reading at 30 seconds intervals. Calculate the Δ A/Min.
----------------------------------------------------------------------------------------------------------------------------------------------------------------------------

#### **RESULT CALCULATION**

Perform calculations in units per litre, Multiplying the Δ A/Min by the factor.



Activity in U/l =  $\Delta$  A/Min x 1768

**EXPECTED VALUES** <45 U/l at 37°C OR 0.8  $\mu$ kat/l

it is recommended that each laboratory verifies this range or derives reference interval for the population it serves.

**QUALITY CONTROL AND CALIBRATION**

It is suggested to perform internal quality control with assayed normal and assayed abnormal to confirm the validity of the test and assure the accuracy of patient result.

when using the calibrator calibrate the assay:

- a. When using a new reagent or lot
- b. When QC values are out of range

## 4) Test for Bilirubin

### DCA Method

#### CLINICAL SIGNIFICANCE

Bilirubin a breakdown Product of hemoglobin is transported to the liver in association with albumin, this bilirubin is water insoluble known as indirect unconjugated bilirubin. in liver bilirubin is conjugated to glucuronic acid to form direct bilirubin or conjugated bilirubin excreted into intestine via biliary system

$$\text{TOTAL BILIRUBIN} = \text{INDIRECT BILIRUBIN} + \text{DIRECT BILIRUBIN}$$

Total Bilirubin is elevated in obstructive conditions of bile duct, hepatitis, cirrhosis of liver and hemolytic disorders. indirect bilirubin is elevated by pre-hepatic causes such as hemolytic disorders or liver diseases. monitoring of indirect bilirubin in neonates is of special importance as it is the indirect (free) bilirubin bound to albumin that is able to cross the blood brain barrier more easily increasing the danger of cerebral damage.

#### PRINCIPLE OF THE METHOD

Total (Conjugated and unconjugated) Bilirubin couples with a DCA reagent in the presence of a surfactant to form azobilirubin. the diazo reaction is accelerated by the addition of surfactant as a solubilizing agent. the increase in absorbance at 546 nm due to azobilirubin is directly proportional to the total bilirubin concentration.

#### KIT COMPONENTS

1. R1 - Total Bilirubin Reagent
2. R2 - Total Bilirubin sodium Nitrite Reagent

Composition in the test : 2-4 dichloroaniline 0.5 mmol/l, cetrimide 30 mmol/l, hydrochloric acid 100 mmol/l, and sodium nitrite 150 mmol/l.

#### MATERIALS REQUIRED

- Laboratory instrumentation
- Spectrophotometer
- thermostatic
- cuvette holder
- semi automated
- calibrated micropipettes
- glass or high quality polystyrene cuvettes
- test tube
- heating bath
- controls
- saline

#### REAGENT PREPARATION STORAGE & STABILITY

Reagents are ready to use keep away from direct light sources

Stability: Unopened bottle up to expiration date on labels + 15 to +30°C

#### SPECIMEN

Serum, plasma (Heparinate only) Bilirubin in serum is stable for 7 days at 2-8 °C

#### TEST PROCEDURE

Dispense	Blank	Test
Reagent 1	1 ml	1 ml

Reagent 2	25 µl	25 µl
Distilled water	100 µl	-
Sample	-	100 µl
Mix, incubate for 5 min at 37 °C. Read absorbance at 546/670 nm against reagent blank. Reading should be taken immediately after incubation is over (2 minutes maximum).		

### **RESULT CALCULATION**

Serum

Total Bilirubin mg/dl=abs. of Test - Abs. of Blank x12

SI conversion factor:1 mg/dl x 17.1 = 1 µmol/l

### **EXPECTED VALUES**

Total Bilirubin:

Adults: 0.1 - 1.2 mg/dl      OR      1.7 - 20.5 µmol/l

Infants: 1.2 - 12 mg/dl      OR      20.5 µmol/l

### **QUALITY CONTROL AND CALIBRATION**

It is recommended to perform internal quality control with assayed normal and assayed abnormal confirm the validity of the test and assure the accuracy of patient result.

When using Calibrator calibrate the assay:

- a. When using a new reagent or lot
- b. When QC values are out of range

## **5) SGPT(Serum Glutamic Pyruvic Transaminase)**

### **IFCC Method, Kinetic**

#### **Clinical Significance:**

The aminotransferases (transaminases) are widely distributed in animal tissues. Both AST and ALT are normally present in human plasma, bile, CSF and saliva. Elevated ALT levels are observed in viral hepatitis, cirrhosis, obstructive jaundice. Decreased levels found in B6 vitamin deficiency and renal dialysis patients.

#### **Principle:**

**ALT**

L-alanine+2-Oxoglutarate $\rightarrow$  Pyruvate+ L-Glutamate

Pyruvate+NADH $\rightarrow$ L-Lactate+NAD

**LDH**

ALT: Alanine aminotransferase

LDH: Lactate dehydrogenase

#### **Materials Required:**

- Reagents- R1, R2- Composition in test: Tris buffer 80 mmol/l pH 7.5, L-alanine > 500 mmol/l, 2-Oxoglutarate 12 mmol/l, NADH 0.18mmol/l, LDH $\geq$ 2000U/l and stabilizers.
- Semi automated analyser.
- Calibrated micropipettes.
- Glass or high quality polystyrene cuvettes,
- Test tube rack
- Heating bath
- Controls
- Saline

#### **Reagent Preparation, Storage and Stability:**

Mix reagent 1 and reagent 2 in ratio 4:1. Keep away from direct sunlight sources. Stability of reagent up to expiration date on labels at 2-8°C. Stability of working reagent: 30 days at 2-8°C. Discard the working reagent if absorbance < 1.0 at 340 nm against distilled water.

#### **Specimen:**

Use Serum. SGPT is stable for 7 days at 2-8°C.

#### **Test Procedure:**

Dispense in tube : working reagent	500 $\mu$ l
Add Sample	50 $\mu$ l

#### **Assay Protocol 1:**

Mix and incubate 60 seconds at 37°C, then record first reading of absorbance. Perform other 2 reading at 60 seconds intervals. Calculate the  $\Delta A/\text{Min}$ .

#### **Assay Protocol 2:**

Mix and incubate 30 seconds at 37°C, then record first reading of absorbance. Perform other 2 reading at 30 seconds intervals. Calculate the  $\Delta A/\text{Min}$ .

#### **Result:**

Perform calculations in units per litre, Multiplying the  $\Delta A/\text{Min}$  by the factor.

Activity in U/l =  $\Delta$  A/Min x 1768

**Expected Values:** <45 U/l at 37°C

It is recommended that each laboratory verifies this range or derives reference interval for the population it serves.

**Quality Control and Calibration:**

It is suggested to perform internal quality control with assayed normal and assayed abnormal, to confirm the validity of the test and assure the accuracy of patient result. When using recommended Calibrator, calibrate the assay:

1. When using a new reagent or lot
2. When QC values are out of range.

**Reference:**

Literature taken from Meril Diagnostics SGP

## Chapter-5: Test for TB Diagnostics

### 1.) AFB Staining method for Sputum(Ziehl Neelsen Stain)

#### **Principle:**

Heating the smear allows greater penetration of carbol fuchsin into the cell wall. Mycolic acids and waxes complex the basic dye, which then fails to wash out with mild acid decolorization.

**Sample Quality:-** Good Quality - Yellowish, Semi solid part of sample.

#### **Required Materials:-**

- ZN Stain kit
- Mask, gloves, spirit, lamps, slides
- Staining rack

#### **Process & Directions:-**

1. Prepare a smear on clean, dry glass side.(Preferably New)
2. Allow it to air dry and fix with gentle heat.
3. Flood smear with carbolfuchsin stain, steam the slides gently for 1 minute. This can be accomplished by flaming from below the rack with a gas burner or spirit lamp. Do not permit the slides to boil or dry out.
4. Allow it to stand for 5 minutes without further heating
5. Rinse with water and tilt slides to drain
6. Decolorize with acid alcohol(95% ethanol and 3% HCl) for 3 minutes .
7. Rinse with water and tilt slides to drain
8. Flood slides with methylene blue reagent for 1 minute.
9. Rinse with water and tilt slides to drain. Air dry.
10. Examine under oil immersion for presence of acid fast bacilli.

#### **Reporting :-**

**RNTCP. Grading of the sputum smears is done according to the following table:**

Microscopy Field	Result	Grading	No. of fields examined
No. AFB in 100 oil immersion fields	Negative	0	100
1-9 AFB in 100 oil immersion fields	Scanty	Record actual number	200
10-99 AFB in 100 oil immersion fields	Positive	1+	100
1-10 AFB per oil immersion field	Positive	2+	50
More than 10 AFB per oil immersion field	Positive	3+	20

## 2.) Ammonium sulphate – Sodium hydroxide Concentration Technique for the Diagnosis of Tuberculosis

### PRINCIPLE

- Sodium hydroxide turns sputum samples liquid
- Ammonium sulphate acts like alum to precipitate tuberculosis bacteria to the bottom of the tube

### ADVANTAGE

- 89% increase in the detection rate of tuberculosis bacteria in sputum and other samples
- Inexpensive
- Very easy to learn and perform
- Minimum hands-on time compared to other concentration techniques
- No need for a centrifuge

### SPECIAL UTILITY

- Classifies a larger number of patients as sputum-positive and avoids the expense / logistic difficulties of a chest X-ray, particularly in rural areas.
- Facilitates the follow-up of sputum-positive patients during treatment.

### METHOD

<b>Reagent:</b>	Ammonium sulphate	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	30 g
	Sodium hydroxide	NaOH	10 g
	Distilled Water		1 litre

(Please use freshly made distilled water since some rapidly growing mycobacterial species can grow in distilled water stored for a long time, particularly in plastic containers, and give rise to false-positive results on microscopy. For the same reason, use clean glassware heated at 150°C for 2 hours while making and using this solution to avoid the possibility of introducing mycobacteria that may be non-viable but stainable)

Mix to dissolve. Autoclave at 121°C for 15 minutes. The solution keeps indefinitely in screw-capped glass bottles at room temperature.

### **Glassware and other supplies:**

- Clean test tubes made of borosilicate glass; size 15 x 150 mm; heated at 150°C for two hours in a hot-air oven (you may take the help of a local bakery if you do not have a hot-air oven in your lab),
- New microscope slides, 25 x 75 mm,
- Glass Pasteur pipettes, cotton plugged, heated as described above,
- Rubber bulb,
- Rubber caps, freshly taken out of used Penicillin vials or similar vials,
- Test tube rack to hold 15 x 150 mm test tubes,
- Bacteriological loop,
- Spirit lamp and Spirit or Bunsen burner and LPG cylinder,
- Match box or lighter,

- Wax pencils *or* Adhesive stickers and pen *or* Glass etching pencil for labeling

**Procedure:**

1. Enter the patient’s name, registration number, age, sex, address, type of sample, and date in the register.
2. Label the test tube with the patient’s registration number and date using a marker pen or sticker.
3. Pour the sputum sample into the tube taking care not to contaminate the outside surface. Add double the volume of ammonium sulphate – sodium hydroxide solution using a glass pipette and a rubber bulb. There is no need to measure the volume precisely; visual estimation works fine.
4. Cap the test tube with a new rubber cap taken out from a penicillin vial (avoid washing and reusing rubber caps to avoid false positive results due to carry-over of AFB from previous samples).
5. Invert the tube several times while pressing on the rubber cap firmly with the thumb to avoid spillage.
6. Let the tube stand overnight in a test tube rack without further movement.
7. Next morning, remove the rubber cap and decant the supernatant solution in one smooth movement into a discard jar containing 3% phenol or 6% lysol.
8. Bring the tube back to the upright position.
9. Resuspend the deposit in the few drops of solution that remain on the walls of the test tube by gently tapping the bottom of the tube several times.
10. Remove the resuspended deposit with a Pasteur pipette and smear it on a new microscope slide using a bacteriological loop that has been flamed and cooled. Label the slide with the patient’s registration number and date using an etching pencil. Let the smear dry completely. Fix the smear by dropping just enough methanol to cover the smear completely and then letting it evaporate. If you do not have methanol, you can fix the smear by passing the slide twice over a spirit lamp or Bunsen burner flame until the bottom of the slide is uncomfortably hot to the touch. Do not to heat the slide too much.
11. Let the smear cool down. Gently pour about 1 ml of tap water on the smear to dissolve the ammonium sulphate and sodium hydroxide. Tilt the slide gently to let the water run off along with the solutes. Let the smear dry at room temperature.
12. Stain by the Ziehl-Neelsen method and examine for acid-fast bacilli (AFB) as usual. Please remember that sometimes all the AFB in the entire smear are concentrated in a small area. Therefore scan at least 200 fields to avoid missing small numbers of AFB.
13. Enter report in register and on the patient’s file.

**Experience of Jan Swasthya Sahyog with the Ammonium Sulphate – Sodium Hydroxide Concentration Technique**

<b>Direct smear positive Concentration smear positive</b>	<b>Direct smear positive Concentration smear Negative</b>
119 Samples	10 Samples
<b>Direct smear positive Concentration smear Negative</b>	<b>Direct smear Negative Concentration smear Negative</b>
115 Samples	1722 Samples



<b>Total Samples tested</b>	<b>1966</b>
Sensitivity of Direct Smears for Diagnosis of pulmonary TB	<b>53%</b>
Sensitivity of Conc. Smears for Diagnosis of pulmonary TB	<b>96%</b>
Additional Yield because of Concentration Technique	<b>89%</b>

#### **REFERENCE**

- Vasantha Kumari R.; Concentrated Sputum Smear Microscopy: A Simple Approach to Better Case Detection in Pulmonary Tuberculosis
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### **3.) Urine Analysis**

A urinalysis is a group of physical, chemical, and microscopic tests. The tests detect and/or measure several substances in the urine, such as byproducts of normal and abnormal metabolism, cells, cellular fragments, and bacteria.

Urine is produced by the kidneys, two fist-sized organs located on either side of the spine at the bottom of the ribcage. The kidneys filter wastes out of the blood, help regulate the amount of water in the body, and conserve proteins, electrolytes, and other compounds that the body can reuse. Urine is generally yellow and relatively clear, but each time a person urinates, the color, quantity, concentration, and content of the urine will be slightly different because of varying constituents.

Many disorders may be detected in their early stages by identifying substances that are not normally present in the urine and/or by measuring abnormal levels of certain substances. Some examples include glucose, protein, bilirubin, red blood cells, white blood cells, crystals, and bacteria. They may be present because:

1. There is an elevated level of the substance in the blood and the body responds by trying to eliminate the excess in urine.
2. Kidney ailment is present.
3. There is a urinary tract infection present, as in the case of bacteria and white blood cells.

A complete urinalysis consists of three distinct testing phases:

1. Visual examination, which evaluates the urine's color and clarity
2. Chemical examination, which tests chemically for about 9 substances that provide valuable information about health and
3. in disease and determines the concentration of the urine
4. Microscopic examination, which identifies and counts the type of cells, casts, crystals, and other components such as bacteria and mucus that can be present in urine

#### **Urine Microscopy:**

The microscopic exam is performed on urine sediment – urine that has been centrifuged to concentrate the substances in it at the bottom of a tube. The fluid at the top of the tube is then discarded and the drops of fluid remaining are examined under a microscope. Cells, crystals, and other substances are counted and reported either as the number observed "per low power field" (LPF) or "per high power field" (HPF). In addition, some entities, if present, are estimated as "few," "moderate," or "many," such as epithelial cells, bacteria, and crystals. Cells and other substances that may be seen are listed below.

Following elements are usually found in urine:

- Erythrocytes (RBC's)
- Leukocytes (WBC's)
- Epithelial Cells
- Casts
- Crystals
- Parasites eggs and larvae
- Trichomonas vaginalis
- Sperm

#### **Red Blood Cells (Erythrocytes)**

Normally, a few RBCs are present in urine sediment (0-2 RBCs per high power field, HPF). More than 3 cells/hpf is considered abnormal. Presence of increased amount of erythrocytes in the urine may indicate a variety of urinary tract and systemic conditions.

- Renal Disease
- Lower urinary tract disease
- Extra Renal Disease
- Toxic reaction due to drugs

It is important to collect a urine specimen correctly and for women to tell their healthcare provider that they are menstruating when asked to collect a urine specimen.

Blood in urine is not a normal finding. RBC's can be intact, crenated and swollen.

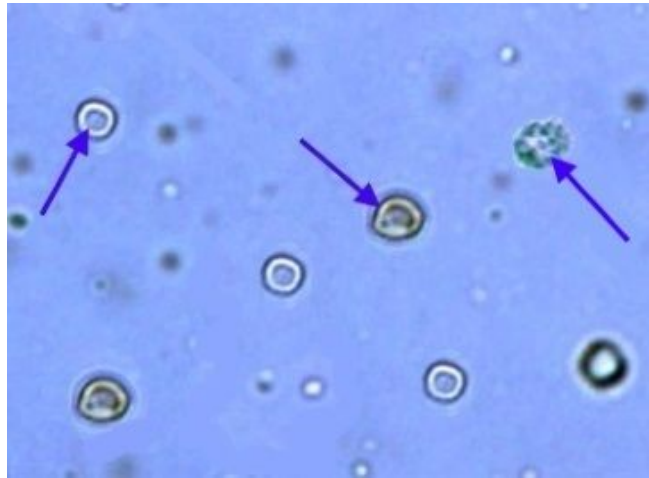
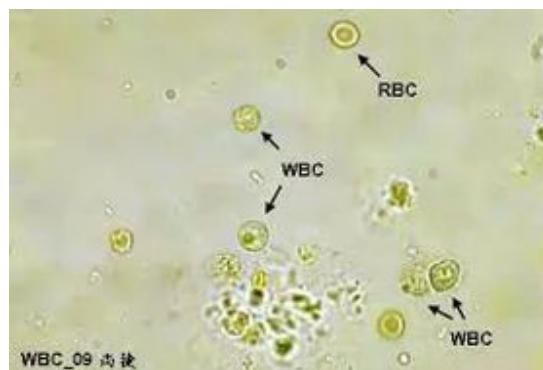


Fig- RBC's in Urine- ring shaped

### White Blood Cells (Leucocytes)

The number of WBCs in urine sediment is normally low (0-5 WBCs per high power field, HPF). An increased number of WBCs seen in the urine under a microscope indicate an infection or inflammation somewhere in the urinary tract. If also seen with bacteria, they indicate a likely urinary tract infection.



### Epithelial Cells:

Epithelial cells are usually reported as "few," "moderate," or "many" present per low power field (LPF). Normally, in men and women, a few epithelial cells can be found in the urine sediment. In urinary tract conditions such as infections, inflammation, and malignancies, an increased number of epithelial cells are present.

### Casts:

Casts are the only formed elements of urine that have their kidney as the sole site of origin. They can be of different shape, size and stability. Different types of casts are as follows:

#### a) Hyaline Casts



Fig- Hyaline Cast

Zero to 2 hyaline casts per lower power field(lpf) is considered normal. Hyaline casts are translucent. Increased numbers are seen with renal diseases, heat exposure, dehydration, fever, etc.

**b) Waxy Casts:**

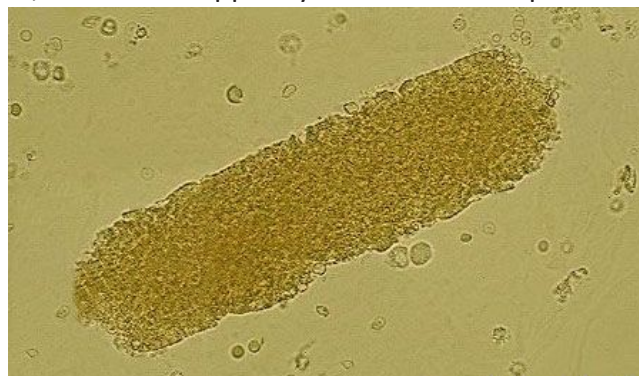
Some cast become denser in appearance and are known as waxy. Waxy casts can be easily seen because of high refractive index. Mostly seen in patients with chronic renal failure.



**Fig: Waxy Cast**

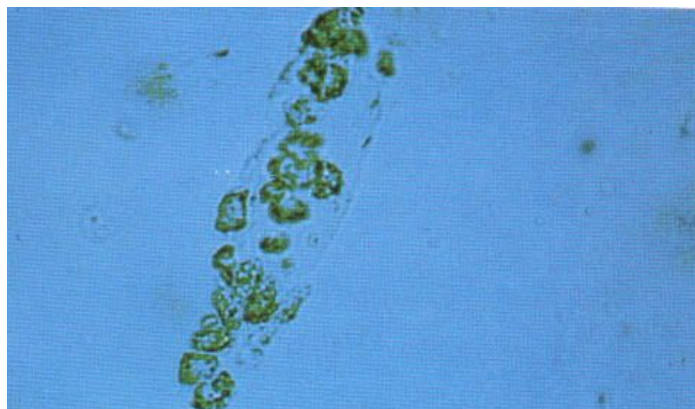
**c) Cellular Casts:**

- **Erythrocyte(RBC) Casts-** Presence of this cast in urine indicates bleeding within nephron. In urine, these casts appear yellow under low power objective.



**Fig: Erythrocyte(Red Blood Cells ) Cast**

- **Leukocyte(WBC) Casts** - WBC casts are refractile, have granules and multilobulated nuclei are frequently visible.



**Fig- Leukocyte Cast**

- **Renal Tubular Epithelial Cell Casts-** It might be difficult to distinguish renal tubular epithelial cell casts from leukocyte casts. The only way to distinguish between the two is that renal tubular epithelial cells have singular round nuclei. Renal tubular epithelial casts are seen in

urine in case of acute tubular necrosis, viral disease, heavy metal poisoning and exposure to a variety of drugs.

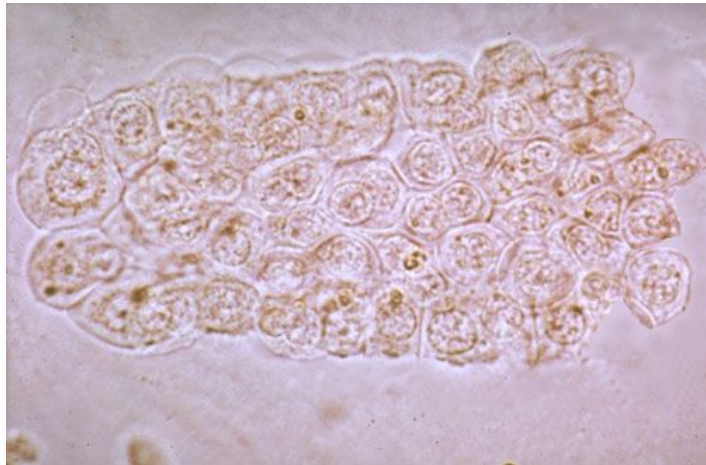


Fig- Epithelial Cell Cast

- **Mixed Cellular Cast-** Two types of cells in single cast is referred to as mixed cellular cast. Example- Leukocyte/ Epithelial cell cast, Erythrocyte/ Leukocyte cast. When cell type cannot be recognised accurately it is referred to as cellular cast. To confirm as to which cell to report mention the type of cell that is dominant.
- **Granular Cast-** Granular cast are commonly seen. Granules may be large or small and may originate from plasma protein aggregates, cellular remains of RBC's, WBC's, etc. Granular cast are seen in glomerulus and tubular disease, viral infections, chronic lead poisoning and renal allograft rejection.

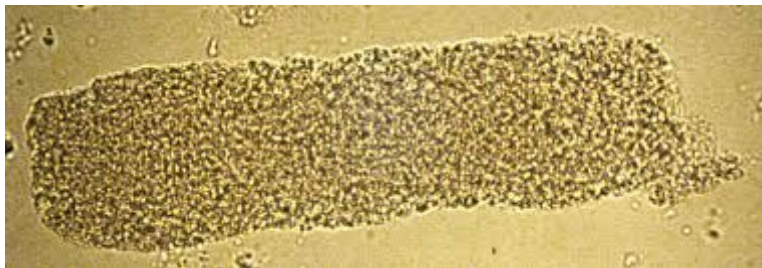


Fig- Granular Cast

- **Fatty Cast-** Fatty cast usually seen when heavy proteinuria is present and are a feature of nephrotic syndrome.



- **Crystal Cast-** Casts containing urates, calcium oxalate and sulfonamides are referred to as crystal cast. Presence of crystal cast indicates deposition of crystals in tubules or collecting duct. These cast should be carefully distinguished from clumps of crystals forming at room or refrigerator temperature.

## CRYSTALS

Crystals are formed when urinary salts precipitate due to alterations of multiple factors which affect urinary salts solubility. Few factors are changes in pH, temperature and concentration. Although most crystals in urine are of limited clinical significance, proper identification is important so that one does not miss important crystals associated with pathologic condition. Knowledge of urine pH is important in crystal identification, because it is the pH of urine that determines which crystal will precipitate.

### **Crystals found in normal acid urine:**

- **Amorphous urates (calcium, magnesium, sodium and potassium urates)**- Amorphous urates precipitate upon standing in concentrated urine of slightly acid pH. When large quantities are present, the urine sediment may appear pink- orange to reddish brown on macroscopic examination, this appearance has been referred to as brick dust.
- **Crystalline urates (sodium, potassium and ammonium)**- These urates form small brown circles or colourless needles in slightly acid urine.
- **Crystalline uric acid**- Uric acid crystals occur at low pH (5 to 5.5) and are seen in many shapes such as prism, oval forms with pointed ends, four sided flat plates and irregular plates. Mostly they are coloured, typically yellow or reddish brown. Rarely, they are colourless and hexagonal. Large no. of uric acid crystals reflect increased nucleoprotein turnover, especially during chemotherapy of leukemia or lymphoma. increased giant toes, may be seen with Lesch-Nyhan syndrome and may provide circumstantial evidence of the nature of small stones lodged in the ureters,
- Conjunction with raised serum uric acid levels. Indicate urate nephropathy of gout.
- **Calcium oxalates.** may appear at pH 6 or in neutral urine.

Their classic form is that of a small, colorless octahedron that resembles an envelope. Dumbbell shapes and ovoid forms may occur. Longer forms occur in calcium oxalate monohydrate. Oxalate crystals are insoluble in acetic acid.

Oxalate crystals in large numbers may reflect severe chronic renal disease. Oxaluria increased absorption of oxalates from food following small bowel disease notably for Crohn's disease.

### **Crystals Found in Normal Alkaline Urine**

**Amorphous phosphates (calcium and magnesium).** amorphous phosphates have a granular appearance microscopically, they tend to be colorless and will produce a fine or lacy white precipitate macroscopically. Clumps or masses can often be seen by light microscopy. Phosphates will dissolve in acids.

They do not dissolve in dilute sodium hydroxide solutions or alcohol.

**Crystalline phosphates.** Triple phosphate most easily identified urine crystals, They are colorless, three to six-sided prisms with oblique ends referred to as **coffin lids** may form colorless sheets or flakes. Overall, phosphate crystals have little if any clinical significance. often seen in infected urine of alkaline pH.

**Calcium carbonate** small and colorless, with dumbbell or spherical shapes. They may form pairs, fours, or clumps.

Ammonium biurate. They have a yellow-brown color appear as spheres with radial concentric striations and irregular projections or thorns referred to as **thorn apples**.

### **Crystals Found in Abnormal Urine**

**Cystine.** Cystine crystals are colorless, refractile, hexagonal plates which appear in acid urine. They are soluble in water at pH less than 2 or greater than 8, and they may be confused with hexagonal forms of uric acid. Both cystine and uric acid are soluble in ammonia water, but cystine will also dissolve in dilute hydrochloric acid, and uric acid will not.

Cystine crystals occur in patients with cystinuria and may be associated with cystine calculi. Confirmatory testing consists of the cyanide-nitroprusside reaction (see the Cystinuria section later in the chapter).

**Tyrosine.** In acidic urine, tyrosine forms fine silky needles that may be arranged in sheaves or clumps, especially after refrigeration. These may be colorless or yellow, appearing black as the microscope is focused

Tyrosine and leucine crystals are occasionally seen in the urine of patients with severe liver disease

**Leucine.** These crystals are also rare, occurring as yellow, oily-appearing spheres with radial and concentric striations. Leucine and tyrosine crystals may occur together; leucine may be precipitated with tyrosine crystals if alcohol is added to the urine.

**Sulfonamide (sulfadiazine) crystals.** These crystals may take on various morphologies, depending on the form of drug involved. They may be seen as yellow-brown sheaves of wheat with central bindings, striated sheaves, eccentric bindings, petals, needles, and round forms with radial striations.

**Tumor Cells.** Malignant tumor cells exfoliated from the renal pelvis, ureter, bladder wall, and urethra are best identified using cytologic techniques.

Myeloma cells have also been noted in urine, both with and without apparent renal involvement.

**Bacteria.** Finding bacteria in urine may or may not be significant, depending on the method of urine collection and how soon after collection of the specimen the examination takes place. If bacteria are identified with Gram stain in an uncentrifuged urine specimen under an oil-immersion lens, this suggests that more than 100,000 organisms/mL are present (i.e., significant bacteriuria). Most commonly, rod-shaped bacteria are seen because the enteric organisms are the causative agents in the majority of urinary tract infections (Fig. 28-45). Leukocytes will usually be seen in the sediment as well.

Acid-fast bacilli may be seen in urine sediment, but because the urethral flora may contain non-pathogenic acid-fast organisms, the presence of tuberculosis in urine must be substantiated by culture and/or polymerase chain reaction methods.

**Fungi.** Yeasts (most commonly *Candida* species) may be causative agents in urinary tract infection (e.g., in diabetes mellitus), but yeasts are also common contaminants from the skin, the female genital tract, and the air. On microscopic examination, they may be confused with erythrocytes; the presence of budding helps to identify them as yeast cells. Pseudohyphae of *Candida* are occasionally found.

**Parasites.** Parasites and parasitic ova may be seen in urine sediments as a result of fecal or vaginal contamination. When noted, repeat examination should be performed on a fresh, clean-voided urine specimen. Although *Trichomonas vaginalis* may be present in urine as a result of vaginal contamination, urethral or bladder infection can occur; when suspected, the protozoa should be searched for immediately in a wet preparation of the sediment. Motility of the organism is helpful in making the appropriate identification. In patients with schistosomiasis due to *Schistosoma haematobium*,

typical ova are shed directly into the urine accompanied by erythrocytes from the urinary bladder. Amebas are rarely seen in the urine; they may reach the bladder from lymphatics or more likely from fecal contamination of the urethra. The pathogenic *Entamoeba histolytica* is usually accompanied by erythrocytes and leukocytes.

### **Contaminants and Artifacts**

Partially digested muscle fibers or vegetable cells may be found when fecal contamination occurs Spermatozoa are occasionally present,

### **METHODS FOR URINALYSIS**

#### **BASIC (ROUTINE) URINALYSIS PROCEDURE**

- Take about 5 ml urine in a test tube and centrifuge it at 200 rpm for 10 minutes.
- Decant the supernatant.
- Gently resuspend the sediment in the remaining supernatant.

#### **Examine with low- and high-power objectives**

The fine focus should be used continuously while scanning. Systematically progress around the entire examination chamber, being careful to examine along the edges for casts.

Count the number of casts in at least 10 lpf, average, and report the number of casts per lpf. A reasonable range may be used in reporting (e.g., 0 to 2, 2 to 5, 5 to 10). Use high power to identify casts by type Identify and count erythrocytes, leukocytes, and renal epithelial cells using the high-power objective. Count at least 10 hpf, average, and report as cells.

#### **Comment on the following:**

- a. Squamous and transitional cells if present in large numbers or as fragments (transitional cells).
- b. Bacteria, yeast, and microorganisms. Bacteriuria detectable on low power should be reported as at least 2+.
- c. Crystals (quantitated under low power). The presence of abnormal crystals should be confirmed chemically and correlated with the patient history.
- d. Large amounts of mucus.



## Chapter- 6) Maintenance of equipment:

### a) Balance:

An instrument which measure the mass of a body or a substance using gravity force which acts on that body. The balance has other names such as scale and weight. Electronic balance and Analytical balance are commonly used in laboratory.



1) Electronic Balance



2) Analytical Balance

### Installation Requirements:

- An environment with no air currents or sudden changes in temperature and free from dust.
- Place the balance in perfectly levelled table/counter.
- Avoid installing equipment which produces elevated magnetic fields or vibrations like centrifuges, electrical motors, compressors and generators in its vicinity.
- Avoid locating it directly under the air-conditioning system (air currents) and sunlight.
- An electrical outlet which complies with the current electrical standards in the country or the laboratory. It must be in good condition and equipped with a ground pole and switches.

### Routine Maintenance of Balance:

- Clean the weighing plate so that it is kept free of dust. Cleaning is done by using a piece of clean cloth which may be dampened with distilled water. If there is a stain, a mild detergent can be applied.
- Clean the weighing chamber, externally and internally, eg analytical balance.
- Always use a clean, pre-weighed container for weighing (glass container or weighing paper if possible). Note that plastic can become electromagnetically charged and is not recommended for weighing powdered or granulated chemicals.
- Any spill must be cleaned immediately to avoid corrosion or contamination. Use 70% ethanol to disinfect the pan of the balance.

### b) Centrifuge

#### Purpose of Centrifuge:

The centrifuge uses centrifugal force (the force generated when an object rotates around a single point), for separating solids suspended in a liquid by sedimentation, or liquids of diverse density. The rotational movements allow forces much greater than gravity to be generated in controlled periods of time. In the laboratory, centrifuges are generally used in processes such as the separation of solid components from biological liquids through sedimentation and in particular of blood components: red cells, white cells, platelets among others and for conducting multiple tests and treatments. There are several kinds of centrifuges. The most widely used in public health, surveillance and clinical laboratories are the table-top centrifuge, the ultracentrifuge, the haematocrit centrifuge and the standing centrifuge.

#### Routine Maintenance of Centrifuge:

- Personnel trained and familiar with the use, care, risks and handling of the centrifuge should operate it.

- Verify that the centrifuge external components are free of dust and stains. Avoid affecting the rotor with spills. Clean the rotor compartment using a mild detergent.
- Verify the locking /safety mechanism of the centrifuge's cover. This is fundamental in guaranteeing operators' safety as this mechanism keeps the cover of the centrifuge closed while the rotor is turning.
- Verify the state of gaskets and watertight joints.
- Annually-Test operation controls needed for selection of the different parameters of the centrifuge: speed, time, temperature, alarms selectors and analogous or digital instruments.
- Annually- Verify the actual rotation speed against the selected one using a normal load. The testing is done with a tachometer or a photo tachometer. If the hatch is not transparent, the procedure indicated by the manufacturer must be followed.
- Confirm the functioning of the brake system.
- Avoid spilling liquids on control keys. The keys must be operated with the fingertips: The operator should avoid using fingernails, as this can result in the perforation of their protective membrane.

### 3)Microscope:

#### Parts of the Microscope



- Ocular-it's the eyepiece; it's where you look through to see the image of your specimen.
- Body tube-the long tube that holds the eyepiece and connects it to the objectives.
- Nosepiece-the rotating part of the microscope at the bottom of the body tube; it holds the objectives.
- Objective lenses- low (4x), medium (10x), high (40x), oil immersion(100x), the lens in the microscope that first receives light rays from the object and forms the image. The

microscope may have 2, 3 or more objectives attached to the nosepiece; they vary in length (the shortest is the lowest power or magnification; the longest is the highest power or magnification). You will probably not be using the oil immersion lens, but if you do, you will need to place a drop of oil on the cover slip and slide the oil immersion objective into place. After use, you will also need to clean the objective to get the oil off.

- Arm-part of the microscope that you carry the microscope with.
- Coarse adjustment knob -large, round knob on the side of the microscope used for focusing the specimen; it may move either the stage or the upper part of the microscope.
- Fine adjustment knob-small, round knob on the side of the microscope used to fine-tune the focus of your specimen after using the coarse adjustment knob.
- Stage-large, flat area under the objectives; it has a hole in it (see aperture) that allows light through; the specimen/slide is placed on the stage for viewing.
- Stage clips- a metallic clip on top of the stage that holds the slide in place.
- Aperture-the hole in the stage that allows light through for better viewing of the specimen.
- Diaphragm-controls the amount of light going through the aperture.
- Light -source of light usually found near the base of the microscope; the light source makes the specimen easier to see.
- Base – Supports the microscope

### **Using the Microscope**

- Always observe the specimen or object using the LOWEST POWER object first.
- Focus using the COARSE ADJUSTMENT KNOB to bring the object into focus. Bring the object into sharp focus by using the fine adjustment knob.
- Focus, and then move to a higher power objective, if needed.
- Use only the FINE ADJUSTMENT KNOB when using the HIGHEST (longest) power objective. Keep both eyes open to reduce eyestrain. Keep eye slightly above the eyepiece to reduce eyelash interference.

### **Care & Use**

#### **Handling The Microscope**

- Always use two hands to move the microscope. Place one hand around the arm, lift the scope, and then put your other hand under the base of the scope for support.
- Be gentle. Setting the microscope down on the table roughly could jar lenses and other parts loose. The microscope seems like a simple instrument, but each lens (eyepiece and objective) is actually made up of a number of other lenses; banging your microscope around, shakes about 15 to 20 lenses. Always have clean hands when handling your microscope.

#### **Storing The Microscope**

- Return the lowest power objective in place
- Wrap the cord around the base
- Dust is an enemy to microscope lenses; always keep the microscope covered when not in use.

#### **Cleaning the Microscope**

- Don't let the microscope get too dirty – always use the dust cover when not in use.
- To clean the eyepiece – use a high quality lens paper. First brush any visible dust from the lens, and then wipe the lens. Do not use facial tissues, they are made from ground up wood fibers and could damage the lenses.
- To clean the objective lenses – use a fresh piece of the lens paper each time so that you don't transfer dust from one lens to another.
- Use lens paper on all glass parts of the microscope.
- Clean oil immersion lens with chemicals provided by your instructor

#### 4) Pipettes

Pipettes are devices used for measuring or transferring small volumes of liquid from one container to another with great precision. There are many pipette models. Fixed volume and variable volume pipettes with mechanical controls are highlighted herein.

##### **Purpose of pipettes:**

Pipettes are devices widely used in clinical and research laboratories to supply very exact quantities of fluids.

##### **Using the pipette:**

In order to obtain precise, exact and reliable results, it is necessary for pipette operators to know in detail correct pipetting procedures. This is achieved by training and detailed follow-up regarding the use of pipettes. The general outlines for the appropriate use of pipettes are as follows. Before using a pipette, verify that it is correctly calibrated and suitable for the transfer of liquid volume to be performed.

##### **Recommendations:**

- Verify that the pipette is in a vertical position to aspirate a liquid. The vertical position guarantees that there is no uncertainty due to minimal variation at the surface of the liquid.
- Use the recommendation outlined by the manufacturer for the minimum immersion depth of the pipette's tip to aspirate liquids. The depths vary according to the pipette type and capacity. A general guide is shown in the following

**Table of tip immersion depth according to the pipette volume range:**

Volume range of the pipette( $\mu$ l)	Depth of the immersion(mm)
1-100	2-3
100-1000	2-4
1000-5000	2-5

- Dispense the liquid drawn by letting the tip touch the wall of the receiving tube. The pipette's tip must form an angle ranging between 30 and 45° with the tube at 8 to 10 mm above the surface of liquid.

##### **Routine Maintenance:**

##### **Inspection:**

##### **Frequency: Daily**

Pipettes require frequent inspection in order to detect abnormal wear and tear or damage and/or to verify that they are in good working condition. Inspection must cover the following aspects:

- Verify the integrity and adjustment of the mechanisms. These must move smoothly. The piston must move smoothly.
- Confirm that the tip holder is not displaying distortions or signs of being worn out, as it is essential for the exactitude of measurements.
- Put on a tip and fill it with distilled water. The pipette must not show any leak.

##### **Cleaning and Decontamination:**

- Every day, verify that the pipette is clean. If dirt is detected, it must be cleaned using a suitable solvent or a mild detergent solution. Check the manufacturer's recommendation regarding the compatibility of the pipette with solvents to select the appropriate one.
- If a pipette has been used with harmful substances, it is the responsibility of the user to ensure that it is completely decontaminated before it is used in other procedures or removed from the laboratory.

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