# HAEMATOLOGY

## Procedure 1. Haemoglobin estimation.

| Performance Checklist | | | | |
|---|---|---|---|---|
| ***Sr. No.*** | ***Steps to be performed sequentially*** | ***Attempt*** | | |
| | | ***F*** | ***R*** | ***Re*** |
| i. | Checks that apparatus is clean and dry. | | | |
| ii. | Takes N/10 HCl in Hb tube up to lowest mark (10% or 2 g%). | | | |
| iii. | Gets an aseptic finger prick and discards the first drop of blood. | | | |
| iv. | Sucks the blood sample into Hb pipette up to 20 µl, without air bubble. | | | |
| v. | Wipes off extra blood on tip of pipette and gently transfers the sample into Hb tube. | | | |
| vi. | Rinses Hb pipette with contents of Hb tube. | | | |
| vii. | Mixes the solution with stirrer and leaves tube undisturbed for 10 mins. | | | |
| viii. | Adds distilled water drop by drop into the tube and mixes the solution with stirrer. | | | |
| ix. | Compares color of solution with standard glass rods, after lifting the stirrer slightly above the level of solution in Hb tube. | | | |
| x. | Notes the reading on Hb tube corresponding to the lower meniscus, when the color matches with standard. | | | |
| ***OVERALL GRADE (B/M/E)*** | | | | |

*Observations and Result:*

**Teacher's feedback:**

*Teacher's signature (with date)*

## Procedure 2. Estimation of Red Blood Cell (RBC) count.

| Performance Checklist | | | | |
|---|---|---|---|---|
| ***Sr. No.*** | ***Steps to be performed sequentially*** | ***Attempt*** | | |
| | | ***F*** | ***R*** | ***Re*** |
| i. | Checks that the apparatus is clean, dry and grease free. | | | |
| ii. | Takes Hayem's fluid in a watch glass. | | | |
| iii. | Gets an aseptic finger prick and discards the first drop of blood. | | | |
| iv. | Sucks the blood exactly up to 0.5 mark of RBC pipette and wipes off any extra blood on the tip of pipette. | | | |
| v. | Sucks Hayem's fluid up to exactly 101 mark of pipette. | | | |
| vi. | Gently mixes the contents by rolling the pipette horizontally between the palms. | | | |
| vii. | Places coverslip on Neubauer's chamber so that it covers counting grids on both sides. | | | |
| viii. | Discards initial 1–2 drops of the fluid from pipette. | | | |
| ix. | Charges the chamber on both sides by ensuring that there is no overflowing of solution into gutters. | | | |
| x. | Counts the cells in 80 smallest squares (1 central and 4 corner blocks of 16 RBC squares each). | | | |
| ***OVERALL GRADE (B/M/E)*** | | | | |

*Observations and Result:*

**Teacher's feedback:**

*Teacher's signature (with date)*

## Procedure 3. Estimation of Total Leucocyte Count (TLC).

| Performance Checklist | | | | |
|---|---|---|---|---|
| ***Sr. No.*** | ***Steps to be performed sequentially*** | ***Attempt*** | | |
| | | ***F*** | ***R*** | ***Re*** |
| i. | Checks that the apparatus is clean and dry. | | | |
| ii. | Takes Turk's fluid in a watch glass. | | | |
| iii. | Gets an aseptic finger prick and discards the first blood drop. | | | |
| iv. | Sucks the blood exactly up to 0.5 mark of WBC pipette and wipes off any extra blood on the tip of pipette. | | | |
| v. | Sucks Turk's fluid exactly up to 11 mark of pipette. | | | |
| vi. | Gently mixes the contents by rolling the pipette horizontally between the palms. | | | |
| vii. | Places coverslip on Neubauer's chamber so that it covers counting grids on both sides. | | | |
| viii. | Discards the initial 1–2 drops of the fluid from pipette. | | | |
| ix. | Charges the chamber on both sides by ensuring that there is no overflowing of solution into gutters. | | | |
| x. | Counts the cells in 64 squares (4 corner blocks of 16 WBC squares each). | | | |
| ***OVERALL GRADE (B/M/E)*** | | | | |

*Observations and Result:*

**Teacher's feedback:**

*Teacher's signature (with date)*

## Procedure 4. Estimation of Differential Leucocyte Count (DLC).

| Performance Checklist | | | | |
|---|---|---|---|---|
| ***Sr. No.*** | ***Steps to be performed sequentially*** | ***Attempt*** | | |
| | | ***F*** | ***R*** | ***Re*** |
| i. | Takes 3–4 clean, dry and grease free glass slides. | | | |
| ii. | Gets an aseptic finger prick and discards the first drop of blood. | | | |
| iii. | Places an adequate-sized blood drop on one end of each slide and holds the spreader in front of blood drop at 45° angle. | | | |
| iv. | Gently moves the spreader backwards while allowing the blood to spread across the edge of glass slide. | | | |
| v. | Moves the spreader forwards, smoothly and swiftly, towards the other end of the slide. | | | |
| vi. | Quickly "air-dries" all smears and selects those with uniform thickness and without striations or vacuoles. | | | |
| vii. | Places the smears on staining rack and after covering them with Leishman's stain, waits for 2 mins. | | | |
| viii. | Adds distilled water drops equal to stain drops, without spilling the contents. | | | |
| ix. | Properly mixes the stain and water mixture by gently blowing intermittently for 8–10 mins. | | | |
| x. | Washes-off stained slides under a gentle stream of water. | | | |
| xi. | Allows the washed slide to dry by keeping them in inclined position. | | | |
| xii. | Selects an ideally stained smear and examines it first under 10X and then under 40X. | | | |
| xiii. | Focuses the smear under oil immersion lens (100X) and counts 100 WBCs using zig-zag method. | | | |
| ***OVERALL GRADE (B/M/E)*** | | | | |

*Observations and Result:*

**Teacher's feedback:**

*Teacher's signature (with date)*

## Procedure 5. Determination of Blood Group.

| Performance Checklist | | | | |
|---|---|---|---|---|
| ***Sr. No.*** | ***Steps to be performed sequentially*** | ***Attempt*** | | |
| | | ***F*** | ***R*** | ***Re*** |
| i. | Takes 4 clean, dry and grease free glass slides and marks them as A, B, C and D. | | | |
| ii. | Places 1 drop of anti-A, anti-B and anti-D sera on slides A, B and D respectively. | | | |
| iii. | Places 1 drop of normal saline on control slide C. | | | |
| iv. | Takes 2–3 ml of normal saline on a watch glass and gets an aseptic finger prick. | | | |
| v. | Adds 1–2 drops of blood to normal saline (in watch glass) to prepare RBC suspension. | | | |
| vi. | Adds 1 drop of blood directly to anti-D serum. | | | |
| vii. | Adds 1–2 drops of RBC suspension to anti-A and anti-B sera. | | | |
| viii. | Mixes all antisera and blood samples using separate toothpicks. | | | |
| ix. | Checks each antisera for agglutination, after 6–8 mins. | | | |
| x. | Confirms findings under microscope for both ABO and Rh blood groups. | | | |
| ***OVERALL GRADE (B/M/E)*** | | | | |

*Observations and Result:*

**Teacher's feedback:**

*Teacher's signature (with date)*

## Procedure 6. Determination of Bleeding Time (BT) and Clotting Time (CT).

| Performance Checklist | | | | |
|---|---|---|---|---|
| ***Sr. No.*** | ***Steps to be performed sequentially*** | ***Attempt*** | | |
| | | ***F*** | ***R*** | ***Re*** |
| **Bleeding Time (Duke's Method)** | | | | |
| i. | Cleans the fingertip with spirit swab and allows it to dry. | | | |
| ii. | Gets a moderately deep finger prick under aseptic conditions and notes the time. | | | |
| iii. | Mops the oozing blood every 30 secs by gently touching the fingertip with filter paper. | | | |
| iv. | Notes the time when blood spots are no longer obtained on filter paper. | | | |
| v. | Counts the number of blood spots and calculates BT. | | | |
| **Clotting Time (Capillary Glass Tube Method)** | | | | |
| i. | Gets a moderately deep finger prick under aseptic conditions and notes the time. | | | |
| ii. | Wipes the first blood drop and allows a large blood drop to form on fingertip (without squeezing). | | | |
| iii. | Fills the blood in a glass capillary by keeping its one end at a lower level than other and notes the time. | | | |
| iv. | Holds the filled capillary tube between his/her palms for 2 mins. | | | |
| v. | Starts breaking off small pieces of tube from one end, every 30 secs. | | | |
| vi. | Stops when the fibrin thread is visible and notes the time. | | | |
| vii. | Counts the number of broken pieces and calculates CT. | | | |
| ***OVERALL GRADE (B/M/E)*** | | | | |

*Observations and Result:*

**Teacher's feedback:**

*Teacher's signature (with date)*

## Procedure 7. Determination of RBC indices.

**Calculate the RBC indices using the following data: Hb: 15 g%, RBC count: 5 million/mm$^3$, PCV: 45%.**

| Performance Checklist | | | | |
|---|---|---|---|---|
| ***Sr. No.*** | ***Steps to be performed sequentially*** | ***Attempt*** | | |
| | | ***F*** | ***R*** | ***Re*** |
| i. | Calculates Mean Corpuscular Volume (MCV) using standard formula. | | | |
| ii. | Calculates Mean Corpuscular Hemoglobin (MCH) using standard formula. | | | |
| iii. | Calculates Mean Corpuscular Hemoglobin Concentration (MCHC) using standard formula. | | | |
| iv. | Compares with normal values and interprets the results. | | | |
| ***OVERALL GRADE (B/M/E)*** | | | | |

***Observations and Result:***

MCV (74–95 µm$^3$) = (PCV per 100 ml of blood × 10)/RBC count in million per mm$^3$

MCH (27–32 pg) = (Hb in g% × 10)/RBC count in million per mm$^3$

MCHC (30–38%) = (Hb in g% × 100)/PCV per 100 ml of blood

**Teacher's feedback:**

*Teacher's signature (with date)*