

# **Blood Sample Collection, Transport and Analysis Manual:**

*(Source: Dr G.N.V.Brahmam, Deputy Director, National Institute of Nutrition, Hyderabad)*

## ***Equipment***

Whatman No.1 filter paper strip, sterile lancets, micropipette of 20 $\mu$ l capacity, cotton wool.

## ***Procedures***

Label the Whatman No.1 filter paper strip appropriately with identification particulars before collection of blood sample.

Clean the middle finger of the left hand with Spirit and cotton and allow it to dry. Squeeze the finger slightly and hold it firmly with left hand.

Using a lancet, puncture the skin at right angles to the tip of the finger. Discard the lancet after folding it. Wipe off the first drop of the blood with tissue paper and press the finger gently so as to form a drop of blood. Collect the blood into clean Hemoglobin pipette.

Using a clean and dry hemoglobin pipette draw the blood slowly up to a little above the 20 Micro liter mark on the pipette. Care should be taken to avoid any air bubbles in the blood column. Clean the tips of the pipette using a tissue paper. Adjust the volume to 0.02 ml (Up to the mark on the pipette) by touching the tip of pipette with wet tissue paper.

Transfer the blood from the pipette by slowly blowing it out on to the coded filter paper, in the form of a circular spot of about 1 cm diameter, by keeping the pipette perpendicular with its tips touching the filter paper and moving in a circle. Take care not to splash the blood, by blowing very slowly. No trace of blood should remain, either in the pipette or on its tip.

As soon as the sample blood collection is over, wipe the finger with dry cotton. Press the site puncture with spirit swab. Avoid direct contact with blood.

Discard the sample in case the blood sticks to the inner surface of the pipette. Pipette the blood once again with a fresh pipette.

Fold the filter paper diagonally and place it in a breadbox and allow it to dry in shade. Protect the sample from sunlight, flies and dust. Pack the dried samples in polythene cover.

**Transportation and Management:** The dried filter papers will be transferred to a HIS Public

Health laboratory in Hyderabad for the estimation of Hemoglobin by cyanmethemoglobin method (Lewis et.al, 2001). Results will be reported in gram/dl

*Cleaning of Hemoglobin pipettes in the field:* Soon after transferring the blood onto the filter paper, the pipette is rinsed first with Drabkin's solution, followed by dilute Hydrochloric acid ( HCL 1 part + Distilled Water 2 parts) distilled water and acetone in that order. Dry the pipette by blowing air into the pipette. Take care not to pipette the reagents in to the rubber tubing.

*Collection of duplicate samples for quality control:* On every 10<sup>th</sup> subject covered for hemoglobin estimation, duplicate samples will be obtained separately on another coded filter papers (indicating that it is duplicate of a given sample). Every duplicate sample will be analysed to compare the values with the original values. The OD difference between both should not be greater than 0.01.

### ***Precautions***

Since Drabkin's contains small quantity of Potassium Cyanide, care should be exercised while pipetting. Clean the pipette periodically.

Don't touch the blood samples.

Hemoglobin pipettes should be absolutely dry before using it.

Discard used lancet after folding it.

Enter the identification particulars of the samples collected (including duplicate samples), along with their OD & Hb values in a register

Clean the pipette with acid/Darbkin's solutions. Use a thin straight wire to scarp of the blood strains if any, remaining in the pipette. Take care not to damage the tip of the pipette.

### ***Preparation of Drabkin's Solutions***

No. of Samples to analyzed	Drabkin's Concentrate (ml)	+	Distilled Water (ml)	=	Drabkin's solution
2	0.5	+	9.5	=	10

4	1.0	+	19.0	=	20
10	2.5	+	47.5	=	50
20	5.0	+	95.0	=	100
40	10.0	+	190.0	=	200

***Preparation of the samples***

Pipette out exactly 5 ml of the diluted Drabkin’s reagent.

Cut carefully, the portion of filter paper with the blood spot with a pair of scissors and transfer the same in to a pre-coded test tube having 5 ml of Drabkin’s solution.

Allow the filter paper with the blood spot to soak for sufficient length of time (Overnight) in Drabkin’s reagent for complete extraction of the blood. On complete extraction, the filter paper would appear white.

*Calibration of the Colorimeter and Estimation of Hemoglobin:* Instrument has to be calibrated each time, before taking the readings. Calibration has to be repeated after taking readings of every ten samples, or whenever the instrument is used at different time of points on the same day or whenever there is disturbance in the voltage. Adopt the following steps while on the colorimeter.

Make sure that the electric supply is “A.C” and not “D.C” Use voltage stabilizer.

Set the filter in the colorimeter to No.5 position (540nm).

Put on the switch (at the rare of the instrument) and keep the reading switch in % T (Transmittance) mode.

Put mark (transmittance) with permanent link at the top end of all cuvettes (small glass tubes provided with colorimeter) and always place the cuvettes in the instrument such that the arrow exactly coincides with the groove in front of the well.

Put the black tube provided with the instrument, in the well of the instrument and set the reading to “0” by rotating the “Set Zero” adjustment knob clockwise or anti-clockwise.

Take Drabkin’s reagent in a cuvette (up to 3/4<sup>th</sup>) and place it in the “well” of the colorimeter. Adjust the reading to “100” by rotating the set 100 adjustment knob clockwise or anti-clockwise.

Don't disturb this setting until you finish taking for the entire set of samples.

Shift the switch to "OD" (Optical Density) mode before taking readings of standard and samples.

Take the standard solution provided with the kit in the glass cuvette and take the OD reading in the beginning and at the end of each session.

Mix the sample in the test tube well, before transferring into the glass cuvette (up to 3/4<sup>th</sup>).

Transfer the contents of the test tube with the sample into the glass cuvette (with out the filter paper) and take the readings.

Wipe the sides of the cuvette before keeping inside the instrument.

Enter the sample ODs in a register against the correct identification number (Name).

For calculation of the Hb concentration, refer the instructions given along with the kit. Do not use the ready-reckoner (given to you, if any) for deriving the Hb concentration.

$$\text{Hemoglobin (g/dl)} = \frac{\text{OD of Sample} * \text{Concentration of Standard} * 251}{\text{OD of Standard} * 1000}$$

{\*Concentration of Hb Standard = 60 mg/dl, and  
(Dilution factor = 251)}