

**HB ESTIMATION FROM DRIED BLOOD SPOT BY
CYANMETHAEMOGLOBIN METHOD**

**Anaemia is the most common nutritional deficiency in India
Anaemia cannot be diagnosed clinically as the symptoms and signs are not sensitive or specific**

Diagnosis of anaemia is made on the basis of Hb estimation

Cyan methaemoglobin method is the gold standard method providing accurate estimation of Hb at affordable cost in primary health care settings

In laboratory or hospital settings 20 µl blood collected from finger prick is directly deposited into 5 ml of Drabkin's solution and the reading taken in colorimeter / spectrophotometer (direct method)

In community settings 20 µl blood collected from finger prick is deposited on a filter paper, dried, put into plastic envelope and transported to the lab for estimation of Hb within the next 30 days (indirect method)

In the following slides collection of blood in community settings and estimation of Hb from the dried blood spot are described

COLLECTING BLOOD FOR HB ESTIMATION



Items required for blood collection on filter paper for Hb estimation





If the blood sample is to be collected on the filter paper, ether should be used for wiping the finger.

Alcohol/spirit, should not be used for wiping the finger.

Alcohol denatures proteins. If alcohol is used for wiping finger and then blood is collected on filter paper, the blood spot will not elute fully.





Dampen the cotton with solvent ether solution



Wipe the left middle finger tip with ether soaked cotton



Gently squeeze the finger tip



Prick the finger tip with the lancet

A drop of blood wells up



Wipe the drop of blood





**Squeeze the finger tip
so that one drop of
blood wells up**



**Pipette out 20 microlitres
of blood from the drop of
blood**



**Pipette containing
20 microlitres of
blood**



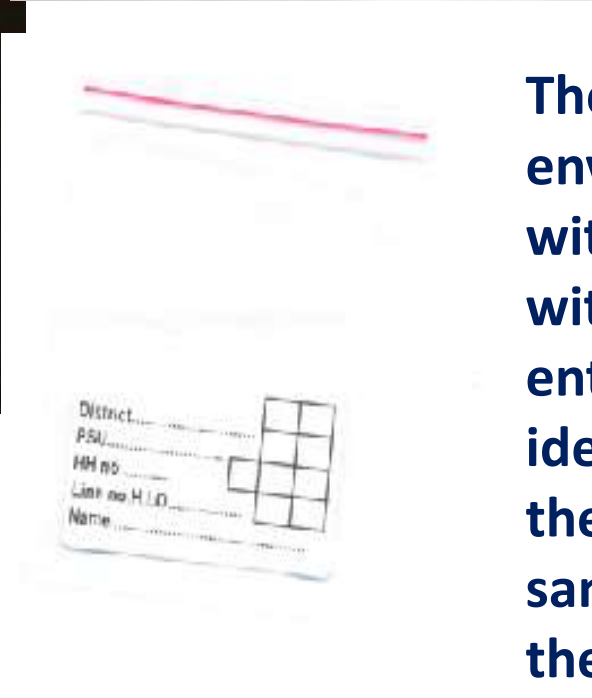
**20 microlitres of
blood deposited on
the filter paper
labeled as number
1 in pencil.**

**Arrows indicate
areas where blood
from the tip has
been deposited.**



Filter paper containing dried blood spot has been inserted into the plastic ziplock envelope and sealed

Insert filter paper containing dried blood spot into the plastic bag and seal it.



The plastic ziplock envelope comes with a sticker with spaces to enter details of identification of the person whose sample is put in the envelope



Blood has been drawn beyond 20 microlitre mark

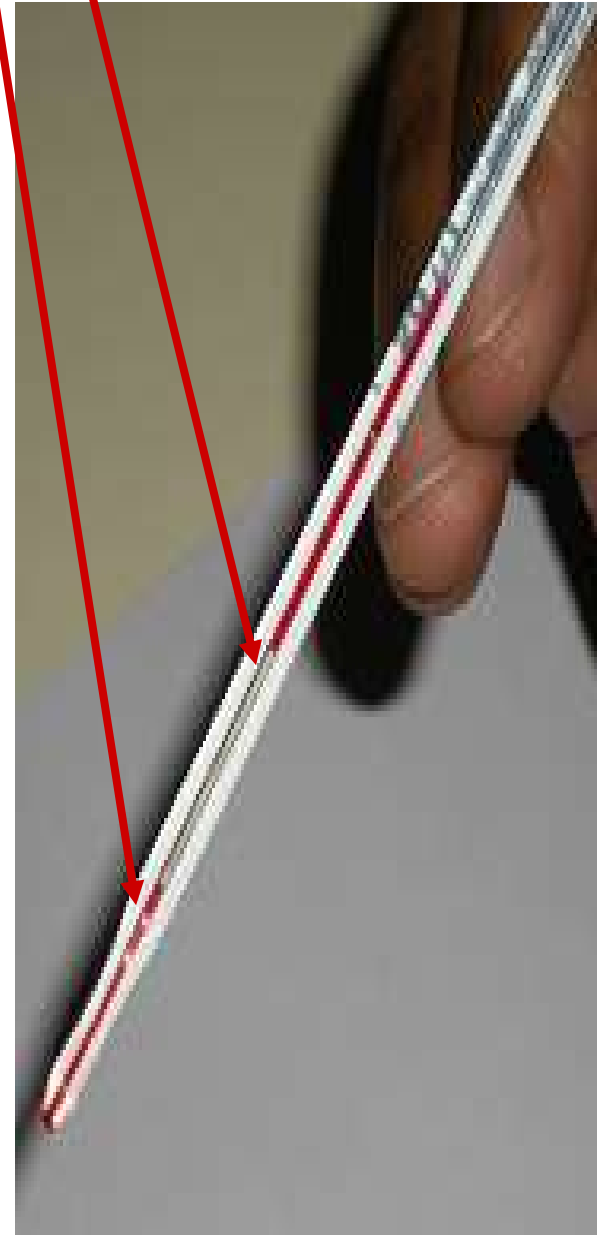
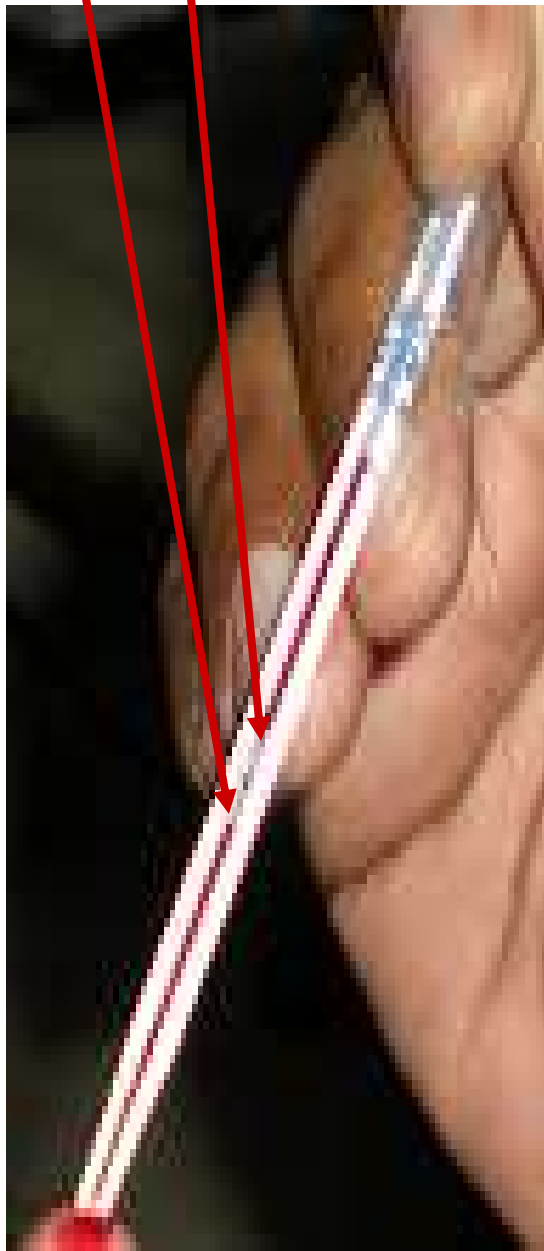


Removing excess blood from the pipette using tissue paper

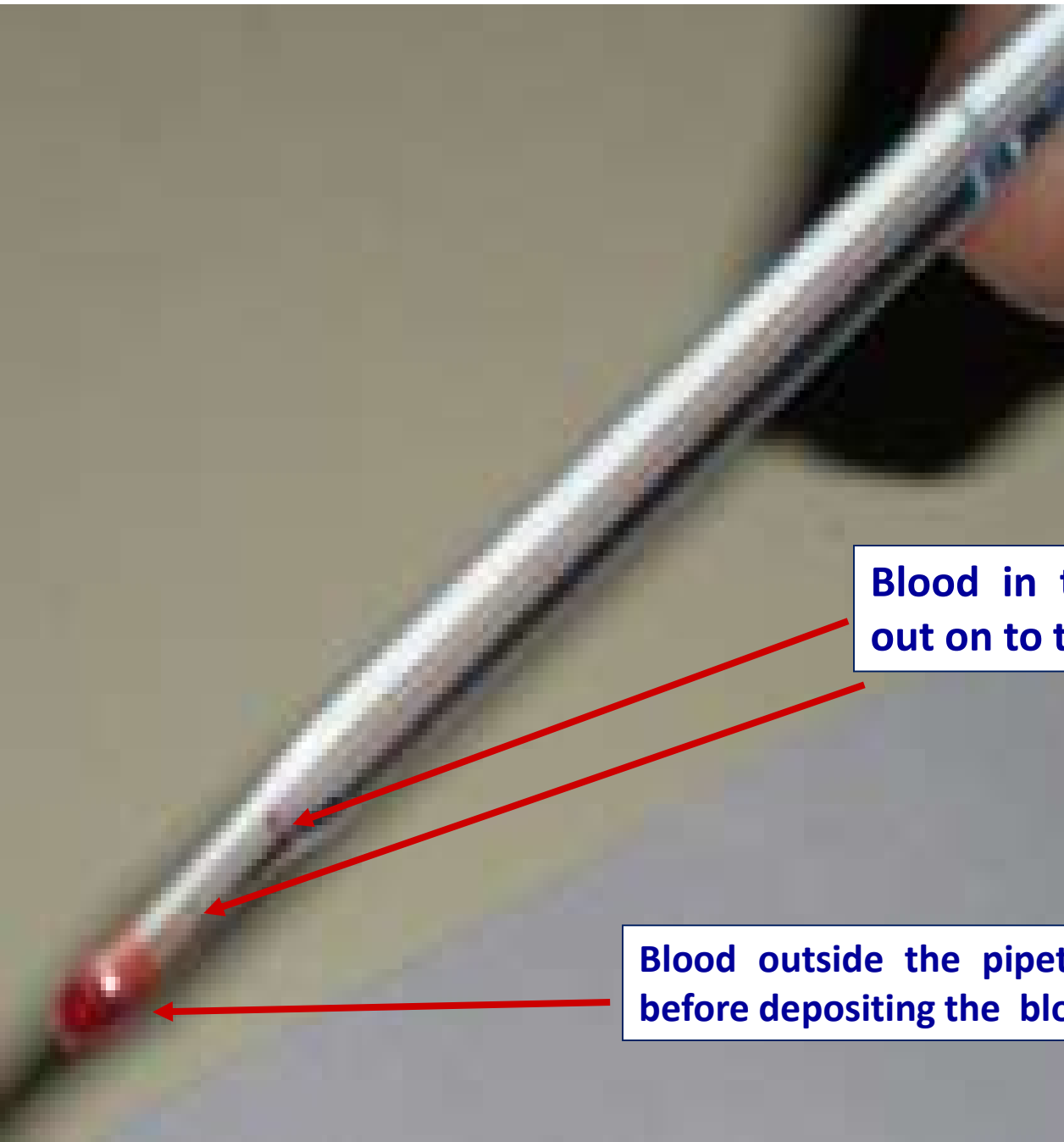


Only 20 microlitre is left in the pipette

ERRORS IN BLOOD COLLECTION – AIR BUBBLES IN THE BLOOD COLUMN IN THE PIPETTE



ERRORS IN BLOOD DEPOSITION ON FILTER PAPER



Blood in the pipette not fully blown out on to the filter paper

Blood outside the pipette has not been wiped out before depositing the blood on the filter paper.

CLEANING AND DRYING THE PIPETTE

As soon as blood is deposited on the filter paper, rinse the pipette twice with Drabkin's solution and blow out into tissue paper /cotton.

Then rinse the pipette twice in distilled water and blow out the water onto tissue paper/cotton.

Dry the pipette by pipetting ether twice and blowing it out. Once it is dry, the pipette is ready for use.



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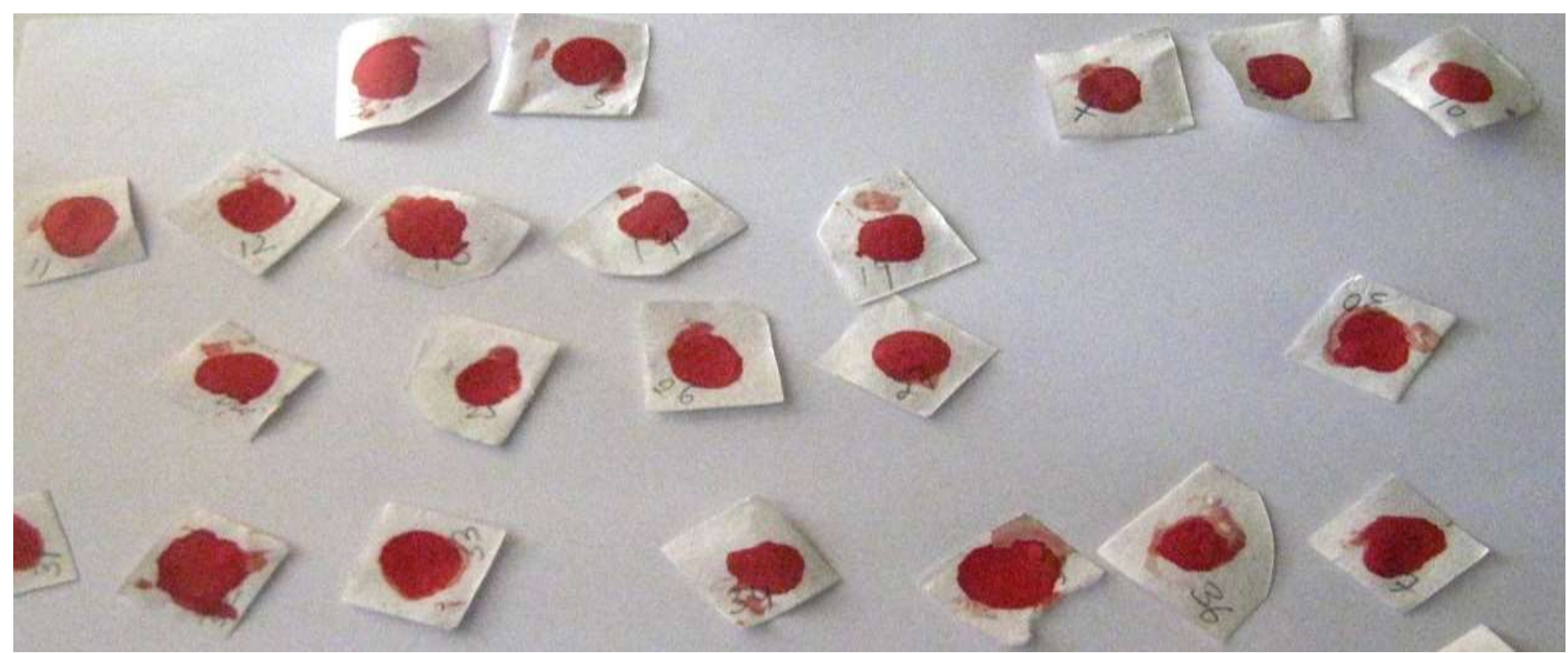
5ml dispenser

Drabkin's solution



Test tubes in test tube rack

**HB ESTIMATION FROM DRIED
BLOOD SPOT BY
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Numbered dried blood spots containing 20micolitres of blood are removed from their plastic covers, placed on a white sheet. Identification data checked with the sticker on the envelope and Schedule II (the lab data sheet).



Test tubes containing filter papers from which blood spot has been fully eluted.

Test tubes containing Drabkin's solution in which blood from filter paper has been fully eluted.





Drabkin's solution in which blood spot has been eluted has been transferred to a cuvette.

Test tube now contain clear filter papers from which blood spot has been completely eluted.



Calorimeter

Cuvette with Drabkin's solution (Blank)

Cuvette with Drabkins solution in which blood spot has been eluted



Hb standard



Calorimeter reading when cuvette containing plain Drabkin's solution is inserted



Calorimeter reading for the cuvette containing Drabkin's solution with eluted blood from blood spot is inserted