Standard Operating Procedure

Haemoglobin Measurement using DHT Haemoglobinometer

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This Standard Operating Procedure (SOP) has been developed by the Ola During Children's / Princess Christian Maternity Hospitals, Freetown, Sierra Leone – Abertawe BroMorgannwg Health Board, Swansea, UK Link.

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This SOP was developed by:

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This SOP is one of a series of resources developed to support clinical laboratories in low resource settings. These resources are available from the Ibadan-Swansea Partnership website: see http://isp.swanih.org/

We hope that this SOP will be helpful in ensuring the quality of laboratory practice. The information is based on the manufacturer's instructions and, to our knowledge, is correct. However, please note that the responsibility for the use of this SOP rests with individual laboratories and their staff.

We are keen to further develop our resources so that they are as helpful as possible. Please do let us know of any comments, suggestions or feedback from using these resources. Please contact Angela Allen: aallengm@yahoo.co.uk

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Standard Operating Procedure

Haemoglobin Measurement using DHT Haemoglobinometer

Clinical significance: The measurement of haemoglobin concentration is important for

- the detection of anaemia or high haemoglobin levels
- the assessment of changes before and after operations or blood transfusion

Principle of the procedure: Water molecules pass from the alkaline ammonia solution into red blood cells by osmosis. This ruptures the red cell membranes releasing haemoglobin into the solution. The haemoglobin can then be measured by colorimetry.

Specimen: Blood is usually obtained either by finger prick or venepuncture. Blood must be collected into an anticoagulant – either EDTA or heparin.

Equipment, consumables and reagents needed

Equipment and consumables

- DHT Haemoglobinometer
- Glass cuvette provided
- Control cuvette provided (black in colour)
- Pipette 2-20µl (set at 20µl)
- Pipette 100-1000µl (set at 1000µl)
- Pipette tips (2 sizes)
- Glass tubes
- Test tube rack
- Fine marker pen
- Measuring cylinder
- Parafilm
- Tissue
- Gloves

Equipment needed for haemoglobin measurement

Reagents

- Manufacturer's ammonia solution, supplied at a concentration of either 35% or 28%
- Distilled water

Procedure

Step 1: Preparation of ammonia solution

Step 1.1. Make 1000 mls (1L) of a 4% ammonia *stock* solution by diluting the manufacturer's solution. Use a measuring cylinder to measure the volumes as follows:

Concentration of manufacturer's ammonia solution	Volume of manufacturer's ammonia solution (ml)	Volume of distilled water (ml)
28%	143	857
35%	114	886

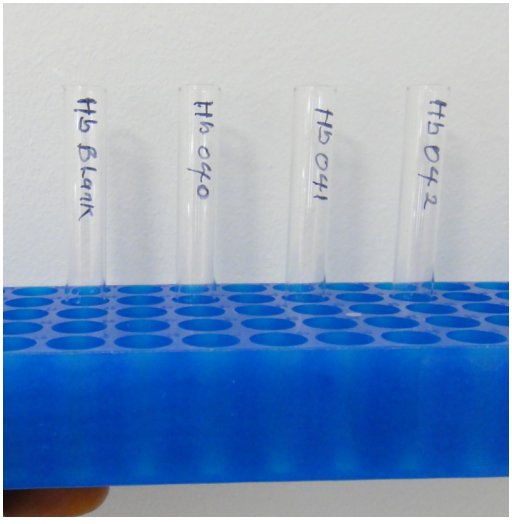
Once prepared, the *stock* solution can be stored in the laboratory at room temperature and is stable for up to one month.

Step 1.2. Make a 0.04% ammonia *working* solution. Decide how much working solution you need according to the number of samples to be analysed. Each test requires 2 mls of *working* solution and then allow an extra 10% for reagent blank and repeat tests.

Number of samples to be tested	**Volume of 4% *stock* solution (mls)**	***Working* solution: make up to this volume with distilled water**
Up to 20	0.5	50
21-40	1.0	100
41-100	2.5	250
101-200	5.0	500

Measure the 4% *stock* solution using a volumetric pipette or a 1ml adjustable pipette and dispense into a clean graduated container. Add distilled water up to the required volume and mix gently.

Step 2: Label tubes



Step 3: Reaction

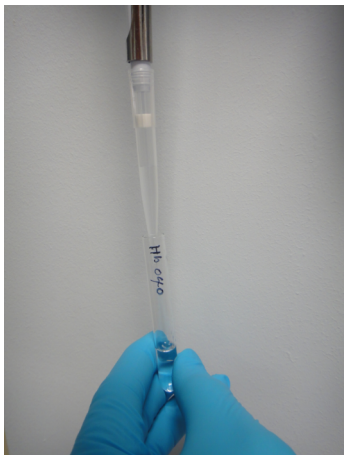
Step 3.1. Pour an aliquot of the 0.04% ammonia *working* solution necessary for the number of tests into a universal container.

To avoid contamination, do not insert a pipette into the working solution bottle.

Step 3.2. Prime a 1000µl pipette

(See Pipetting SOP)

Step 3.3. Pipette 1000µl (1ml) 0.04% ammonia *working* solution twice into each of the blank and sample tubes. Each tube will then contain 2000µl (2ml) of the working solution.



Step 3.4. Prime a 20µl pipette with test blood sample.

(See Pipetting SOP)

20µl pipette

Pipette primed with blood

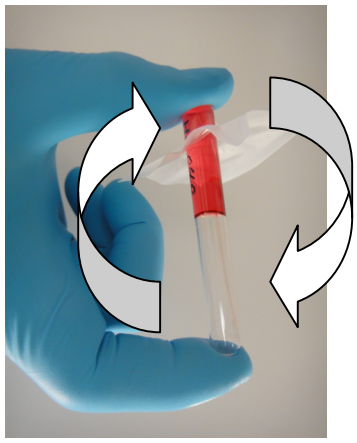
For each patient in turn:

Step 3.5. Pipette 20µl of a well-mixed blood sample and wipe pipette tip to remove excess blood.

Step 3.6. Dispense the 20µl blood sample into the corresponding labelled test tube containing the 0.04% ammonia working solution.

Remember to flush repeatedly to ensure all of blood sample has been dispensed. (See Pipetting SOP)

Step 3.7. Cover with parafilm and then mix by gentle inversion.

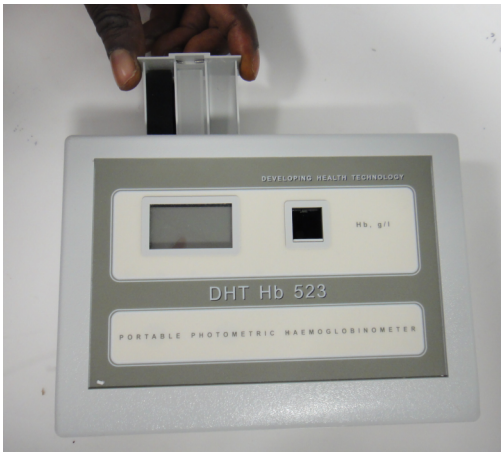


Change pipette tip and repeat for each patient sample.

Step 4: Checking the instrument calibration

Step 4.1. Switch on the DHT haemoglobinometer.

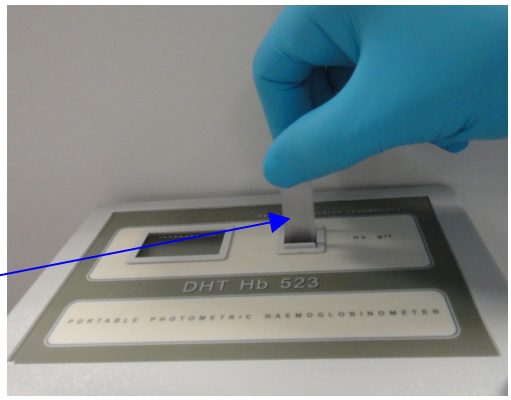
Step 4.2. Take the clear cuvette and the black control cuvette from the drawer at the back of the instrument.



Step 4.3. Insert the empty clear cuvette into the haemoglobinometer.

The cuvette must be inserted so that the transparent side faces the light path (which passes left to right), and the frosted side faces towards you.

Frosted side of cuvette facing towards you



Step 4.4. Take a reading: this should be ± 5 of the manufacturer's setting which can be found on the back page of the manual provided with the instrument.

If the reading is outside this range, check that you have inserted the cuvette correctly.

Reading of 24 which agrees with the manufacturer's setting for this instrument

Step 4.3. Insert black control cuvette into the haemoglobinometer

Note: White numbering should be facing upwards and towards you.

The haemoglobinometer should read within ± 5 of the value written on the cuvette.

Acceptable reading of 163: reading is within ± 5 of the value of 163 written on this cuvette.

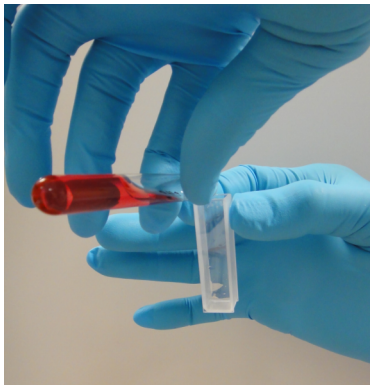
Step 5: Haemoglobin measurement

Step 5.1. Pour all of the contents of the blank tube (2ml of 0.04% ammonia *working* solution) into the clear cuvette and insert into the haemoglobinometer.

The Haemoglobinometer should read ± 0. The haemoglobinometer is now ready to measure a patient sample.

Step 5.2. Pour contents of cuvette back into blank tube. Rinse cuvette with distilled water, invert and pat dry on tissue paper.

Step 5.3.Pour the contents of a sample tube into the clear cuvette.



Step 5.4. Wipe the cuvette clean with a soft tissue (to avoid scratching the cuvette).

Step 5.5. Insert cuvette into haemoglobinometer and take reading.

The display shows haemoglobin concentration measured in g/l. This sample has a haemoglobin concentration of 144 g/l.

Step 5.6. Rinse cuvette with distilled water, invert and pat dry on tissue paper. The cuvette is now ready for the next patient sample – go back to Step 5.3.

Step 6. Cleaning

When all samples have been measured, rinse the clear cuvette and pat dry with tissue paper .

Store the cleaned clear cuvette and the black control cuvette in the drawer in back of haemoglobinometer.

Reporting results / interpretation

The DHT haemoglobinometer reports haemoglobin concentration in grams per litre (g/l). To report the haemoglobin level in grams per decilitre (g/dl) divide by 10. For example, 144 g/l is the same as 14.4 g/dl.

Quality control: checking the accuracy of results

- Repeat any high/low results to ensure that they are correct.
- Measure every 10th sample twice and check that the readings agree
- Measure the packed cell volume on every 10th sample, calculate haemoglobin concentration (PCV in g/dl divided by 3) and check that the results correlate with the haemoglobin values obtained by the haemoglobinometer.
- If using more than 1 clear cuvette, remember to zero and re-calibrate the haemoglobinometer with each empty clear cuvette before testing.