



Intended Use

Diagen Haemiglobincyanide standards are a stabilised and accurately standardised preparation of haemiglobincyanide equivalent to 3.0, 11.5 and 18 g Hb/dL blood at a 1 in 201 dilution. The haemoglobin value of this standard has been accurately assessed by replicate determinations against the WHO International Haemiglobincyanide Standard 98/708 according to BS 3985:2003.

Summary and Principle

To determine the concentration of haemoglobin in blood samples by photometrical method, haemoglobin or oxyhaemoglobin must first be converted into the stable derivative haemiglobincyanide. During this process the haem iron (Fe^{2+}) is oxidised to give methhaem iron (Fe^{3+}) and bound to a cyanide radical. Alkaline Drabkin's solution made from Diagen Drabkin's Capsules (CFDS630) is hypotonic and lyses red cells, therefore when blood is added to the solution, haemiglobincyanide is formed.

Difficulties associated with the estimation of haemoglobin colourmetrically are based mainly on preparation and calibration. It is desirable to have standards which are both able to calibrate a given instrument and also to serve as control and daily check on possible sources of error.

Collection of Blood Samples

Venous blood is collected into tubes containing solid anticoagulants, such as EDTA or heparin.

Capillary Blood is obtained directly from the finger into a clean, dry pipette and tested immediately. If capillary blood is used, exercise care to avoid coagulation.

Reagent

Haemiglobincyanide Standard 6 vials

A haemiglobincyanide solution manufactured from lysed bovine red cells and Drabkin's reagent, diluted to provide a known haemoglobin value. The reagent is provided in sealed vials and is ready for use.

Warnings and Precautions

Diagen haemiglobincyanide Standards have the potential to be poisonous. Please take adequate precautions to minimise risk as the user by following good laboratory practice. Consult the haemiglobincyanide Standard SDS (available on request) for further information on any actions that need to be taken prior, during or after use.

Procedure

Materials Provided

Materials needed for haemoglobin estimation shown below:

Cat. No.

- CMHS510 – Haemiglobincyanide Standard (3.0 g Hb/dL) (6 x 25 mL).
- CMHS511 – Haemiglobincyanide Standard (11.5 g Hb/dL) (6 x 25 mL).
- CMHS512 – Haemiglobincyanide Standard (18.0 g Hb/dL) (6 x 25 mL).

Materials and equipment required, but **not** provided:

1. Spectrophotometer (reading at 540 nm) and associated cuvettes.
2. A 250 mL beaker and stirring rod.
3. Pipettes delivering between 20 μL and 4 mL.
4. Diagen Drabkin's Capsules (CFDS630).
5. Distilled water.

Technique

1. Mix the blood samples (by gentle inversion) immediately before pipetting from them.

2. Measure exactly 4mL of Drabkin's solution into a clean test tube.
3. Pipette 20 μL of blood and expel it into the test tube containing the Drabkin's solution.
4. Mix carefully by inversion.
5. Allow to stand for 15 to 20 minutes, by which time the reaction will be complete.
6. Transfer the solution into a cuvette.

Use of photometer.

1. Set instrument to read at 540 nm.
2. Insert the tube containing reagent blank (Drabkin's solution) and zero the instrument.
3. Insert standard and unknown solutions and note the optical density readings. The concentrations of the standard solutions will be displayed on the vial.

Notes:

1. The colour development time for haemoglobin estimations using the **Alkaline** Drabkin's Solution is 15 to 20 minutes. It is not necessary to add a surface active agent since turbidity due to protein precipitation does not occur at alkaline pH. We also manufacture a pack containing **Neutral** Drabkin's Solution (CNDS620) together with surface active agent in which the development time is 5 minutes^(1,2).
2. Blood specimens should be mixed for at least one to two minutes before removing sample.
3. Blood should be completely removed from the outside of the pipette by wiping with a clean tissue or cotton wool.
4. Contain pipettes (for the blood sample) and graduated delivery pipettes for the diluent should have a tolerance of $\pm 1\%$.
5. Blood should be completely removed from the contain pipette by washing repeatedly in the Drabkin's solution.
6. It is important to seal the vial securely after use and return the standard to the refrigerator.
7. Having read the standard value the solution from the cuvette should be discarded and **not** returned to the vial.
8. The standard should be allowed to reach room temperature in the cuvette or tube before reading the optical density.

Calculation of results

The absorbance values for the three standard solutions should be plotted on a graph. The result obtained for the test solution can then be interpolated from the standard line.

Interpretation

The normal ranges for the concentration of haemoglobin in blood are:

Men: 13.5 – 18.0 g/dL.
Women: 11.5 – 16.5 g/dL.

Quality Control

All laboratories should have in place a quality control system that uses quality control materials (normal and abnormal standard samples). These should be analyzed as test samples to evaluate instrument, reagent and user performance. Controls should be used prior to performing tests on patient blood samples to assess these variables. Diagen haemiglobincyanide standard may be used in this manner. The haemoglobin value of each batch is printed in g/dL of blood on each vial. Using accurate pipettes and with careful attention to technical detail, results should agree within $\pm 0.2\text{g/dL}$ of the true value (for an accurately calibrated instrument).

Limitations

The user must establish the suitability of these materials for their specific application and instrumentation. Sulphaemoglobin is not measured by this procedure. Certain substances can sometimes influence absorbance measurements by causing turbidity within the haemiglobincyanide solution these include; erythrocyte stroma, lipids and abnormal plasma proteins, the effects of which can be minimized by centrifugation or filtration of the solution. Young et al⁽³⁾ have reviewed drug effects on haemoglobin assays.

Stability and Storage

The diluted solutions are stable and may be stored for 2 years at 2 - 8°C. It is advisable to discard any standard which has been removed from the vial after use. Under no circumstances should standard be returned to the vial. The vial should be replaced tightly sealed in the refrigerator after use.

Packaging

6 x 25 mL

Additional Products

CFDS630 - Diagen Alkaline Drabkin's Capsules. The capsules are supplied in packs of 6 vials, each containing 6 capsules, with each capsule making 250 mL of Alkaline Drabkin's Solution. The development time for full conversion of haemoglobin to haemoglobinocyanide is **15 - 20 minutes**. Stored at 4°C the capsules have a shelf life of 3 years.

CNDS620 – Concentrated Neutral Drabkin's Solution. Supplied in packs of 6 x 25 mL vials as a concentrated solution, the liquid has an incorporated surfactant. Each bottle can make 1 litre of Neutral Drabkin's Solution and the development time for full conversion of haemoglobin to haemoglobinocyanide is **4 to 5 minutes**. Stored in the box at 4°C the shelf life of the solution is at least 2 years.

References

1. Van Kampen E.J. and Zijlstra (1961) Clin. Chem. Acta. 6, 538.
2. Munkley, R.M. and Stuart, J. (1970) J. Clin. Path. 23, 190.
3. Young, D.S., Pestaner, L.C. and Gibberman (1975), V., *Clin. Chem.*, Vol. 21, p. 316 D
- 4) Drabkin, D.L. and Austin, J. (1932) J. Biol. Chem. 98, 719.

Key guide to symbols

 Manufacturers catalogue number.  Consult instructions for use.

 Manufacturers batch number.  Requires reconstitution.

 For *in vitro* diagnostic use only.  Product expiry date.

 Biological risks.  Store refrigerated between 2 - 8°C.

 Manufacturer.

 Diagnostic Reagents Ltd.
Thame
Oxon, OX9 3NY
UK
Tel:+44(0)1844 212426
Email: sales@diagen.co.uk
Website: www.diagen.co.uk

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