GCC

Haemoglobin determination by Cyanmethaemoglobin procedure

B160

Introduction

The basis of the method is the dilution of blood with a modified Drabkins Reagent. Hb, Hbi & HbCo but not SHb are converted to HiCN. The absorbance of the mixture is then measured with a spectrophotometer or photoelectric cell at 540nm.

| | | K3Fe[CN6] | | KCN | |
|---------------------|----------|-----------|----------------|-----|--------------------|
| The reaction being: | Hb(Fe2+) | → | Hi (Fe3+) | → | HiCN |
| | | | Methaemoglobin | | Cyanmethaemoglobir |

This reaction is linear up to 20 g/dl (20g/100ml) haemoglobin therefore the absorbance of the solution at 540nm is directly proportional to the amount of haemoglobin present. All forms of Hb except Sulphaemoglobin are measured.

Measurements of haemoglobin from venous or capillary aids in the diagnosis of various conditions that affect the normal concentration of haemoglobin in blood eg: anemia, polycythemia. Of the various methods available for the determination of haemoglobin in blood, the cyanmethaemoglobin method is the internationally adopted method of choice.

Contents

Each pack contains 6 vials. Each vial is a concentrate and when diluted with deionised water will make 1 litre of completed Drabkins Reagents. Therefore each set of 6 vials will make a total of 6 litres finished reagent.

Health and Safety Information

The modified Drabkins reagent employed in this procedure contains cyanide. Use appropriate precautions in use.

Wear full body / breathing protection when using. Use only in an efficient ventilated fume-hood.

Contact with acids will produce HIGHLY TOXIC hydrogen cyanide. Dispose of by diluting with large quantities of water to drain (see below). TOXIC - POISON contains Potassium Cyanide.

H300 Fatal if swallowed. H331+311 Very toxic if inhaled or in contact with skin. P301+310 IF SWALLOWED - Wash mouth with plenty of water. Immediately call a POSION CENTRE or physician. P303 IF ON SKIN (or hair) - Wash with plenty of soap and water. EUH031 contact with acids liberates toxic gas. H411 Toxic to aquatic life with long lasting effects. P405 Store locked up. Call a POSION CENTRE or physician if you feel unwell or symptoms persist.

Disposal

Local and National Environmental / Waste-disposal regulations should be strictly adhered to. Reconstituted reagent should be greatly diluted with water after use and run to drain. Dilution of the reconstituted reagent with dilute aqueous Sodium thiosulphate will assist conversion of cyanide ion to the less harmful thiocyanate ion before disposal to drain.

Storage and stability

Store new product in a closed/locked cupboard away from light at room temperature. Store reconstituted reagent at room temperature 16 - 20 °C away from light. Reconstituted product should be used within 4 months. Reconstituted reagent should be a clear yellow solution. Discard if any sign of turbidity develops.

Specimen collection and storage

Preferably use venous blood collected in EDTA. Other anticoagulants may be used - citrate, heparin or oxalate. Capillary blood may also be used.

Sources of Technical Error

Include – pipetting error, dirty, scratched or unmatched cuvettes, deteriorated reagents.

Reagent Deterioration

Cyanmethaemoglobin reagent is unstable exposed to bright light. After preparation, keep in brown borosilicate glass bottle in a closed or locked cupboard or wrap bottle in aluminium foil. Keep reagent tightly capped.

Interferences

Several medical conditions can cause erroneous Hb values, such as gross lipemia, leukocytosis and macroglobulinemia. For further technical advice on this subject, please contact us directly.

Method

For each test prepare a control, reagent blank and samples.

Controls - use a certified known standard eg. 16g/dl (16g/100ml) or 20g/dl (20g/100ml).

If using a 20g/dl stock standard, prepare in a clean tube or matching cuvettes the standard and reagent blank and samples as follows:

| Standard | Reagent Blank | Patient Sample 1 etc. |
|-------------------------------|--------------------------|-------------------------|
| 5.0 ml Drabkins reagent | 5.0 ml Drabkins reagent | 5.0 ml Drabkins reagent |
| 0.02 ml 20g/dl known standard | 0.02 ml De-ionised water | 0.02 ml whole blood |

Prepare any other controls as required.

Mix all test reagents well. Use matching cuvettes. Allow all test reagents to stand at room temperature 15 - 26°C for at least 3 minutes to allow full conversion to HiCN.

Adjust spectrophotometer to zero absorbance at 540nm using the Reagent Blank.

At 540nm read absorbance result for standard, any other controls and unknown Patient Sample. Accurately record results.

Reaction Product Stability

Cyanmethaemoglobin is accepted to be stable. However, recommendation should be that samples are read within one hour of preparation.

Calculation of Results

Abs. = Spectrophotometer absorbance

Example: If a 20g/dl Standard had an absorbance of 0.562, and the patient sample absorbance was 0.400 then the patient sample haemoglobin concentration will be:

Quality Control

It is recommended that controls are included in each set of assays (normal and abnormal), analysed by the same procedure.

Expected Values

| Adult males | 13.0 - 18.0 g/dl | Adult females | 11.0 - 16.00 g/dl |
|-------------|------------------|---------------|-------------------|
| Children | 10.0 - 14.0 g/dl | Newborn | 14.0 - 23.0 g/dl |

The above values should serve only as a guide. Each laboratory should establish its own range.

GCC Diagnostics guarantees that the highest quality reagents are supplied with this product to give reliable results time and again and that this product conforms to the information contained in this leaflet.

The user should, however, determine the suitability of this product for their particular use. If you wish to report any findings to us or if you require any help or further information on the use of this product please contact us.

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