Intended Use
Diagen Concentrated Neutral Drabkin’s Solution is for use in the measurement of blood haemoglobin concentration.

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 pigment cyanmethaemoglobin. During this process the haem iron (Fe²⁺) is oxidised to give methaem iron (Fe³⁺) and bound to a cyanide radicle. Neutral Drabkin’s is hypotonic and lyses red cells, therefore when blood is added to the solution, cyanmethaemoglobin is formed.

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
Concentrated Neutral Drabkin’s Solution 6 x 25 ml vials
A concentrated solution containing a mixture of: Potassium cyanide, Potassium dihydrogen phosphate and Potassium hexacyanoferrate (III), along with a surface active agent. To prepare, remove the cap and rubber stopper, then dilute the contents of one vial to 1000 ml with distilled water in a volumetric flask.

Procedure
Materials Provided
Materials needed for haemoglobin estimation shown below:
Cat. No.
CNDS620 – Concentrated Neutral Drabkin’s Solution (6 x 25 ml vials).

Materials and equipment required, but not provided:
1. Spectrophotometer (540 nm) and associated cuvettes.
2. A 1000 ml volumetric flask.
3. Pipettes delivering between 20 µl and 4 ml.
4. Distilled water.
5. Diagen Cyanmethaemoglobin Standard:
   CIHS520 (25 x 10 ml).

Technique
1. Mix the blood samples (by gentle inversion) immediately before pipetting from them.
2. Measure exactly 4ml of the diluted Drabkin’s solution into a clean test tube.
3. Pipette 20 µl of blood and wash into the Drabkin’s solution.
4. Mix carefully by inversion.
5. Allow to stand for 4 to 5 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.

Calculation of results
The following equation is used to determine unknown concentrations:

\[
\text{Abs. Unk} = \frac{\text{Abs. Std.}}{\text{Abs. Std. Conc.}} \times \text{Std. Conc.}
\]

For example: 11.5 g/dL Standard had an Abs. = 0.391; the Unknown Abs. = 0.480. The haemoglobin concentration of the unknown is:

\[
0.480 = 0.391 \times 11.5 \text{ g/dL}
\]

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.

Stability and Storage
The solution in the unopened vials may be stored for up to 3 years at 2 - 8°C without deterioration. Once diluted, the Neutral Drabkin’s solution when stored in the dark at 4°C is stable for at least 30 days.

Packaging
Each pack consists of six vials, containing 25 ml of solution. Each vial is sufficient for 1000 ml of Neutral Drabkin’s solution.

References
Concentrated Neutral Drabkin's Solution

Catalogue Number: CNDS620
For in vitro diagnostic use only.

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Warnings and Precautions
Diagen Concentrated Neutral Drabkin’s Solution has the potential to be poisonous, adequate precautions should be taken to minimise risk. Consult the Concentrated Neutral Drabkin’s MSDS (available on request) for further information on any actions that need to be taken prior, during or after use.

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For example: 11.5 g/dL Standard had an Abs. = 0.391; the Unknown Abs. = 0.480. The haemoglobin concentration of the unknown is:

\[
\text{Abs. Unk} = \frac{\text{Abs. Std.} \times \text{Std. Conc.}}{\text{Std. Conc.}} = \frac{0.480 \times 11.5 \text{ g/dL}}{0.391} = 14.1 \text{ g/dL}
\]

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

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Women: 11.5 – 16.5 g/dL

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Limitations
The user must establish the suitability of this material 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 cyanmethaemoglobin solution such as; erythrocyte stroma, lipids and abnormal plasma proteins, the effects of which can be minimized by centrifugation or filtration of the solution. Young et al (4) have reviewed drug effects on haemoglobin assays.

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