Revision -



For in vitro diagnostic use only.

# Intended Use

When added to distilled water, Diagen Drabkin's capsules form a solution that may be used to determine the concentration of haemoglobin in blood.

### 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 haemiglobincyanide. During this process the haem iron ( $Fe^{2+}$ ) is oxidised to give methhaem iron ( $Fe^{3+}$ ) and bound to a cyanide radicle. Alkaline Drabkin's solution made from Diagen Drabkin's capsules is hypotonic and lyses red cells, therefore when blood is added to the solution, haemiglobincyanide 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

# Drabkin's Capsules

6 vials x 6 capsules

A gelatine capsule containing a mixture of: Potassium cyanide, Sodium hydrogen carbonate and Potassium hexacyanoferrate (III). To prepare, remove the cap and rubber stopper, take one capsule and open the contents into a suitable beaker. To this, add 250 mL of distilled water and stir until the Drabkin's powder is completely in solution.

### Warnings and Precautions

Diagen Drabkin's capsules have the potential to be poisonous both in and out of solution. Please take adequate precautions to minimise risk. Consult the Drabkin's capsule 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: <u>Cat. No.</u>

CFDS630 - Drabkin's Capsules (6 x 6 capsule vials).

## Materials and equipment required, but not provided:

- 1. Spectrophotometer (540 nm) and associated cuvettes.
- 2. A 250ml beaker and stirring rod.
- 3. Pipettes delivering between 20 µL and 4 mL.
- 4. Distilled water.
- 5. Diagen Haemiglobincyanide Standard: CIHS520 (25 x 10 mL).

# <u>Technique</u>

- 1. Mix the blood samples (by gentle inversion) immediately before pipetting from them.
- 2. Measure exactly 4 mL of Drabkin's solution into a clean test tube.
- 3. Pipette 20  $\mu L$  of blood and wash into 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.
- $\ensuremath{\mathsf{3}}.$  Insert standard and unknown solutions and note the optical density readings.

#### CFDSIFU-008

The concentrations of the standard solutions will be displayed on the ampoule.

### Note:

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 <sup>(1, 2)</sup>.

### Calculation of results

The following equation is used to determine unknown concentrations:

Unknown (g/dL) =  $\frac{Abs. Unk}{Abs. Std.}$  x Std. Conc. (g/dL)

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 x 11.5 g/dL = 14.1 g/dL

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

### 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.

### **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 haemiglobincyanide solution these include: erythrocyte stroma, lipids and abnormal plasma proteins, the effects of which can be minimized by certrifugation or filtration of the solution. Young et al <sup>(3)</sup> have reviewed drug effects on haemoglobin assays.

# Stability and Storage

Sealed vials may be transported at ambient temperature but should be refrigerated on arrival. Once refrigerated the capsules may be stored for up to 3 years at 2 - 8°C without deterioration. Once reconstituted the Alkaline Drabkin's solution is then stable for up to 30 days when held, excluding light, at 2 - 8°C.

### **Packaging**

Each pack consists of six vials, both containing 6 capsules. Each capsule is sufficient for 250 mL of **Alkaline** Drabkin's solution <sup>(4)</sup>.

### **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.

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