



## HEMOGLOBIN PROCEDURE

Intended for the Quantitative Determination of Hemoglobin in the blood

### SUMMARY AND EXPLANATION

Hemoglobin is a red pigmented protein which serves to transport oxygen from the lungs to body tissues. It gives blood the characteristic red color. In anemia, hemoglobin levels are abnormally low and this condition suggests an underlying disease. Miale (1) has described the various anemias. Polycythemia and erythrocytosis increase hemoglobin levels.

ATLAS HEMOGLOBIN PROCEDURE is based on the determination of cyanmethemoglobin, which has become the internationally adopted method (2).

### PRINCIPLE

Ferricyanide oxidizes oxyhemoglobin to methemoglobin, and cyanide converts methemoglobin to cyanmethemoglobin (3). Absorbance measurements are made at 540 nm. The CYANMETHEMOGLOBIN REAGENT contains a surfactant to promote rapid hemolysis and to accelerate formation of cyanmethemoglobin. The reaction is completed in 3 minutes.

### REAGENTS: FOR IN-VITRO DIAGNOSTIC USE

#### DRABKINS REAGENT

##### Concentrated 40x

##### Preparation of Working Solution:

The working solution is prepared by diluting 1 part of Drabkin's reagent (40x) with 39 parts of distilled water (e.g. 5 ml of Drabkin's reagent (40x) is to be diluted with 195 ml distilled water).

Protect from light and heat.

Ready to use solution has a shelf life of 7 days.

### INSTRUMENTS

Use a spectrophotometer or colorimeter calibrated at 540 nm.

### SPECIMEN COLLECTION

#### PRECAUTION:

1. Whole blood with EDTA as an anticoagulant is recommended.
2. The specimens may be collected also with heparin, citrate or oxalate as anticoagulants.
3. If **capillary** blood is used, exercise care to avoid coagulation.
4. Young et al (4) have reviewed drug effects on hemoglobin assay.

#### SAMPLE STORAGE:

Hemoglobin in whole blood collected with EDTA appears stable for one week at room temperature (15 - 30°C).

#### ADDITIVES:

No special additives or preservatives other than anticoagulants are needed.

#### INTERFERING SUBSTANCES:

Gross lipemia, leukocytosis and macroglobulinemia may falsely elevate the hemoglobin value.

### PROCEDURE

#### MATERIALS PROVIDED:

CYANTHEMETHEMOGLOBIN REAGENT.

#### MATERIALS REQUIRED BUT NOT PROVIDED:

1. CYANMETHEMOGLOBIN STANDARD.
2. 0.02 mL micropipettor
3. 5.0 mL pipette or dispenser
4. Test tubes, rack and timer

#### REACTION CONDITIONS:

Wavelength 540 nm

Reaction Type	Endpoint
Incubation Temperature	15 - 30°C
Incubation Time	3 minutes

Sample Volume	0.02 mL
Reagent Volume	5.0 mL
Total Volume	5.02 mL

PERFORMANCE OF TEST - AUTOMATED PROCEDURE:  
This is a manual test procedure only.

#### PERFORMANCE OF TEST - MANUAL PROCEDURE:

1. Place 2 mL of HEMOGLOBIN STANDARD in tube labeled Standard.
2. Dispense 5.0 mL DRABKIN REAGENT into tubes labeled Reagent Blank, Control, Sample 1, etc.
3. Place 0.02 mL specimen into the appropriately labeled tube. Use deionized water as specimen for Reagent Blank. Mix well.
4. Allow the test samples to stand at room temperature (15 - 30°C) for at least 3 minutes.
5. Adjust instrument to zero absorbance at 540 nm using Reagent Blank.
6. Read and record absorbance values for Standard, Controls and Unknowns.

NOTE: For a direct read-out instrument, set read-out concentration value of the Standard and read the unknown concentrations directly.

#### STABILITY OF FINAL REACTION:

Cyanmethemoglobin appears quite stable. However, the test samples should be read within an hour before evaporation of the reaction solutions becomes significant.

#### QUALITY CONTROL:

The reliability of test results should be monitored routinely using quality control materials (normal and abnormal) analyzed in the same manner employed.

#### CALIBRATION:

CYANMETHEMOGLOBIN STANDARD, which is available in a ready to use form and is recommended. The assigned value of this standard is 20 g/dL hemoglobin, and a calibration curve may be prepared.

### CALCULATION OF RESULTS

The following equation is used to determine unknown concentrations:

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

#### EXAMPLE

A 20 g/dL Standard had an Abs. = 0.550: the Unknown Abs. = **0.395**. The hemoglobin concentration of the unknown is:

$$\frac{0.395}{0.550} \times 20 \text{ g/dL} = \mathbf{14.4 \text{ g/dL}}$$

#### QUALITY CONTROL:

the reliability of test results should be monitored routinely using suitable quality control materials (normal and abnormal) analyzed in the same manner as the Unknowns. Failure to achieve assayed values of freshly prepared control sera should be thoroughly investigated before patient values are reported.

### LIMITATIONS

Sulfhemoglobin is not measured by this procedure.

### EXPECTED VALUES

**ADULT MALE:** 12.7 - 18.3 g/dL

**ADULT FEMALE:** 11.0 - 15.8 g/dL

This range represents the 95% confidence interval from a clinically normal population. Each laboratory should establish its own range of expected values.

## PERFORMANCE CHARACTERISTICS

### LINEARITY:

This method is linear to 20 g/dL hemoglobin.

### PRECISION:

Hemoglobin controls at three levels were assayed 20 times **each** for within run precision, and for 10 working days to establish run to run precision.

#### WITHIN RUN

LEVEL	MEAN	ST. DEV.	%CV
I	8.1	0.13	1.6
II	11.1	0.17	1.5
III	16.6	0.12	0.7

#### RUN TO RUN

LEVEL	MEAN	ST. DEV.	%CV
I	8.2	0.20	2.4
II	11.1	0.18	1.6
III	16.5	0.19	1.2

### SPECIFICITY:

The cyanmethemoglobin procedure is the most widely used and preferred method for hemoglobin assay (2). A comparative study of the HEMOGLOBIN PROCEDURE and another widely used commercial method showed a 99% correlation.

### SENSITIVITY:

This procedure has a sensitivity of 0.04g/dL per 0.001 absorbance unit.

### REFERENCES:

1. Miale, J.B., *Laboratory Medicine: Hematology*, C. V Mosby Co., St. Louis, 1972, p. 494.
2. Van Kampen, E.J., and Zijlstra, W.G., *Clin. Chem. Acta* **6**: 538 (1961).
3. Tiez, N.W., *Fundamentals of Clinical Chemistry*, W.B. Saunders Co., Philadelphia, 1976, p. 411.
4. Young, O.S., Pestaner, L.C. and Gibberman, V., *Clin. Chem.*, Vol. 21, p. 316 D (1975).

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