Hemoglo-

(Cyanmethemoglobin Method)

Code : 10013 / 14 (1000 ml / 5 Ltr.)

(For the analyser/Colorimetric estimation of Hemoglobin in whole blood)

In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST:

Hemoglobin (Hb) has the major function of supplying oxygen to the tissue cells. Hb estimation is one of the commonest screening test for the diagnosis of anemia. Decreased levels of HEMOGLOBIN are observed in all varieties of anemia, resulting from hemorrhage or from deficiency of Iron, Vitamin B_{12} or Folic acid. Red cells hemolysis resulting from autoimmune process or due to enzyme abnormality (G-6-PD) may result in anemia. The defective globin chain synthesis as in thalasaemias or structural abnormalities of the hemoglobin molecule as in abnormal hemoglobins may result in severe anemia. Increased levels of Hb concentrations are observed in polycythemia vera, congenital cyanotic heart disease and in hemoconcentration due to various clinical causes e.g., heatstroke and dehydration. Exercise has been reported to lead to a reversible increase of about 3-10%.

Techniques used to determine HEMOGLOBIN have measured it as oxyhemoglobin, carboxyhemoglobin, cyanmethemoglobin, acid and alkaline haemin, by its oxygen capacity and by iron content. The International Committee for Standardisation in Hematology(ICSH) recommended the cyanmethemoglobin method as a Standard method. This method is simple, rapid and reliable. It measures all types of HEMOGLOBINS except Sulfhemoglobin (Hbs). HEMOGLOBIN KIT incorporates the recommendations of ICSH. Cyanmethemoglobin Standard complies to the specifications defined by ICSH which are based on the molecular weight of Hb 64, 458 and a millimolar extinction coefficient of 44. The Drabkin's solution & Cyanmethemoglobin Standard are specially stabilized.

PRINCIPLE:

The HEMOGLOBIN is treated with a reagent containing Potassium Ferricyanide, potassium cyanide and potassium dihydrogen phosphate. The ferricyanide forms methemoglobin which is converted to cyanmethemoglobin by the cyanide. The cyanmethemoglobin has an absorbance which is proportional to the Hemoglobin concentration.

REAGENTS:

1.	Drabkin's Solution (Ready to use)	1000ml	5000 ml
2.	Cyanmethemoglobin Standard	10 ml	10 ml

The reagents are ready to use and usable to the expiration date when stored at 2-8^{o}C, if contamination is avoided.

SAMPLE :

Whole blood, E D T A, Heparin or Oxalate blood.

EXPECTED RANGE:

* Males	13.5 – 18.0 gm%
* Females	11.5 – 16.4 gm%

LINEARITY:

Hemoglobin kit is linear upto 20 gm%

Samples exceeding 20 gm% should be diluted and reassayed. The result has to be multiplied by the dilution factor.

DIRECTIONS FOR USE ON ANALYSERS:

Reaction Type	:	End point with std
Wave Length	:	540nm (green filter)
Incubation Temp	:	Room Temperature
Incubation Time	:	5 mins.
Linearity	:	20 gm%
Unit	:	gm%

Cyanmethemoglobin Standard is used for direct comparision with blood, which when treated with Drabkin's Solution is also converted to Cyanmethemoglobin.

PROCEDURE:

Pipette in a clean dry test tube labelled (T)

	Test (T)
Drabkin's solution	5.0 ml
Blood	0.02 ml

Rinse the pipette well with the reaction mixture. Mix well and keep at R.T. for 5 minutes. Measure the absorbance of Test (T) and Cyanmethemoglobin Standard (S) against distilled water on photocolorimeter using green filter or on a spectrophotometer at 540 nm (Hg 546 nm).

CALCULATIONS:

$$\text{Hemoglobin in gm\%} = \frac{\text{A of T}}{\text{A of S}} \times \frac{251}{1000} \times$$

251 = Blood dilution factor

1000 = Factor to convert milligrams to grams

NOTES:

★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology. Value of Std. stamped on label in mg%

★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

BIBLIOGRAPHY:

1. ICSH Committee (1978) J. Clin. Path. 31,139.
2. Dace, J.V. (1985) Practical Hematology, 6th edition, P 28.
3. Drabkin, DL. (1932) J. Biol. Chem 98,719.

Manufactured in India by :

M/s Excel Diagnostics Pvt. Ltd.

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OD of Sample																			
0.68																		19.04	18.96
0.67																	19.10	18.76	18.68
0.66																19.19	18.81	18.47	18.39
0.65															19.28	18.90	18.52	18.19	18.11
0.64														19.36	18.98	18.60	18.23	17.90	17.82
0.63													19.45	19.05	18.68	18.31	17.94	17.62	17.54
0.62												19.55	19.14	18.75	18.38	18.01	17.65	17.34	17.26
0.61											19.64	19.23	18.82	18.44	18.08	17.72	17.36	17.05	16.97
0.60										19.74	19.31	18.91	18.51	18.13	17.78	17.42	17.07	16.77	16.69
0.59									19.84	19.41	18.99	18.59	18.19	17.83	17.47	17.13	16.78	16.48	16.40
0.58								19.96	19.50	19.08	18.66	18.27	17.88	17.52	17.17	16.83	16.49	16.20	16.12
0.57							20.08	19.61	19.16	18.75	18.33	17.95	17.57	17.21	16.87	16.54	16.20	15.92	15.84
0.56						20.20	19.72	19.26	18.82	18.42	18.01	17.63	17.25	16.90	16.57	16.24	15.91	15.63	15.55
0.55					20.36	19.89	19.36	18.91	18.48	18.09	17.68	17.31	16.94	16.60	16.27	15.95	15.62	15.35	15.27
0.54				20.46	19.95	19.47	19.00	18.56	18.13	17.76	17.35	16.99	16.62	16.29	15.97	15.65	15.33	15.06	14.98
0.53			20.61	20.08	19.57	19.10	18.65	18.21	17.79	17.43	17.02	16.67	16.31	15.98	15.67	15.36	15.04	14.78	14.70
0.52		20.76	20.21	19.69	19.20	18.73	18.29	17.86	17.45	17.10	16.70	16.35	16.00	15.68	15.37	15.06	14.75	14.50	14.42
0.51	20.92	20.35	19.81	19.31	18.82	18.36	17.93	17.51	17.11	16.77	16.37	16.03	15.68	15.37	15.07	14.77	14.46	14.21	14.13
0.50	20.50	19.95	19.42	18.92	18.44	18.00	17.57	17.16	16.77	16.44	16.04	15.71	15.37	15.06	14.77	14.47	14.17	13.93	13.85
0.49	20.08	19.54	19.02	18.53	18.07	17.63	17.21	16.81	16.43	16.11	15.72	15.39	15.05	14.76	14.46	14.18	13.88	13.64	13.56
0.48	19.66	19.13	18.63	18.15	17.69	17.26	16.85	16.46	16.08	15.78	15.39	15.07	14.74	14.45	14.16	13.88	13.59	13.36	13.28
0.47	19.24	18.73	18.23	17.76	1731	16.89	16.49	16.11	15.74	15.45	15.06	14.75	14.43	14.14	13.86	13.59	13.30	13.08	13.00
0.46	18.82	18.32	17.83	17.38	16.94	16.52	16.14	15.76	15.40	15.12	14.74	14.43	14.11	13.83	13.58	13.29	13.01	12.79	12.71
0.45	18.41	17.91	17.44	16.99	16.56	16.16	15.78	15.41	15.06	14.79	14.41	14.11	13.80	13.53	13.26	13.00	12.72	12.51	12.43
0.44	17.99	17.51	17.04	16.60	16.18	15.79	15.42	15.06	14.72	14.46	14.08	13.79	13.48	13.22	12.96	12.70	12.43	12.22	12.14
0.43	17.57	17.10	16.64	16.22	15.81	15.42	15.06	14.71	14.37	14.13	13.75	13.47	13.17	12.91	12.66	12.41	12.14	11.94	11.86
0.42	17.15	16.69	16.25	15.83	15.43	15.05	14.70	14.36	14.03	13.80	13.43	13.15	12.86	12.61	12.36	12.11	11.85	11.66	11.58
0.41	16.73	16.29	15.85	15.45	15.05	14.69	14.34	14.01	13.69	13.47	13.10	12.83	12.54	12.30	12.06	11.82	11.56	11.37	11.29
0.40	16.31	15.88	15.46	15.06	14.68	14.32	13.98	13.66	13.35	13.14	12.77	12.51	12.23	11.99	11.76	11.52	11.27	11.09	11.01
0.39	15.90	15.47	15.06	14.68	14.30	13.95	13.63	13.31	13.01	12.81	12.45	12.19	11.91	11.69	11.45	11.23	10.98	10.80	10.72
0.38	15.48	15.07	14.66	14.29	13.92	13.58	13.27	12.96	12.66	12.48	12.12	11.87	11.60	11.38	11.15	10.93	10.69	10.52	10.44
0.37	15.06	14.66	14.27	13.90	13.55	13.21	12.91	12.61	12.32	12.15	11.79	11.55	11.29	11.07	10.85	10.64	10.40	10.24	10.16
0.36	14.64	14.26	13.87	13.52	13.17	12.85	12.55	12.26	11.98	11.82	11.47	11.23	10.97	10.76	10.55	10.34	10.11	9.95	9.87
0.35	14.22	13.85	13.48	13.13	12.79	12.48	12.19	11.91	11.64	11.49	11.14	10.91	10.66	10.46	10.25	10.05	9.82	9.67	9.59
0.34	13.80	13.44	13.08	12.75	12.42	12.11	11.83	11.56	11.30	11.16	10.81	10.59	10.34	10.15	9.95	9.75	9.53	9.38	9.30
0.33	13.39	13.04	12.68	12.36	12.04	11.74	11.47	11.21	10.96	10.83	10.48	10.27	10.03	9.84	9.65	9.46	9.24	9.10	9.02
0.32	12.97	12.63	12.29	11.97	11.66	11.37	11.12	10.86	10.61	10.50	10.16	9.95	9.72	9.54	9.35	9.16	8.95	8.82	8.74
0.31	12.55	12.22	11.89	11.59	11.29	11.01	10.76	10.51	10.27	10.17	9.83	9.63	9.40	9.23	9.05	8.87	8.66	8.53	8.45
0.30	12.13	11.82	11.50	11.20	10.91	10.64	10.40	10.16	9.93	9.84	9.50	9.31	9.09	8.92	8.75	8.57	8.37	8.25	8.17
0.29	11.71	11.41	11.10	10.82	10.54	10.27	10.04	9.81	9.59	9.51	9.18	8.99	8.77	8.62	8.44	8.28	8.08	7.96	7.88
0.28	11.29	11.00	10.70	10.43	10.16	9.90	9.68	9.46	9.25	9.18	8.85	8.67	8.46	8.31	8.14	7.98	7.79	7.68	7.60
0.27	10.88	10.60	10.31	10.05	9.78	9.54	9.32	9.11	8.90	8.85	8.52	8.35	8.15	8.00	7.84	7.69	7.50	7.40	7.32
0.26	10.46	10.19	9.91	9.66	9.41	9.17	8.96	8.76	8.56	8.52	8.20	8.03	7.83	7.69	7.54	7.39	7.21	7.11	7.03
0.25	10.04	9.78	9.51	9.27	9.03	8.80	8.61	8.41	8.22	8.19	7.87	7.71	7.52	7.39	7.24	7.10	6.92	6.83	6.75
0.24	9.62	9.38	9.12	8.89	8.65	8.43	8.25	8.08	7.88	7.86	7.54	7.39	7.20	7.08	6.94	6.80	6.63	6.54	6.46
0.23	9.20	8.97	8.72	8.50	8.28	8.06	7.89	7.71	7.54	7.53	7.21	7.07	6.89	6.77	6.64	6.51	6.34	6.26	6.18
0.22	8.78	8.57	8.33	8.12	7.90	7.70	7.53	7.36	7.19	7.20	6.89	6.75	6.58	6.47	6.34	6.21	6.05	5.98	5.90
0.21	8.37	8.16	7.93	7.73	7.52	7.33	7.17	7.01	6.87	6.85	6.56	6.43	6.26	6.16	6.04	5.92	5.76	5.69	5.61
0.20	7.95	7.75	7.53	7.35	7.15	6.96	6.81	6.66	6.51	6.54	6.23	6.11	5.95	5.85	5.74	5.62	5.47	5.41	5.33
0.19	7.53	7.35	7.14	6.96	6.77	6.59	6.45	6.31	6.17	6.21	5.91	5.79	5.63	5.55	5.43	5.33	5.18	5.12	5.04
0.18	7.11	6.94	6.74	6.57	6.39	6.23	6.10	5.96	5.83	5.88	5.58	5.47	5.32	5.24	5.13	5.03	4.89	4.84	4.76
0.17	6.69	6.53	6.35	6.19	6.02	5.86	5.74	5.61	5.49	5.55	5.25	5.15	5.01	4.93	4.83	4.74	4.60	4.56	4.48
0.16	6.27	6.13	5.95	5.80	5.64	5.49	5.38	5.26	5.14	5.22	4.93	4.83	4.69	4.62	4.53	4.44	4.31	4.27	4.19
0.15	5.86	5.72	5.55	5.42	5.26	5.12	5.02	4.91	4.80	4.89	4.60	4.51	4.38	4.32	4.23	4.15	4.02	3.99	3.91
0.14	5.44	5.31	5.16	5.03	4.89	4.75	4.66	4.56	4.46	4.38	4.27	4.19	4.06	4.01	3.93	3.85	3.73	3.70	3.66
0.13	5.02	4.91	4.76	4.64	4.51	4.39	4.30	4.21	4.12	4.23	3.94	3.87	3.75	3.70	3.63	3.56	3.44	3.42	3.34
0.12	4.60	4.50	4.37	4.26	4.13	4.02	3.94	3.86	3.78	3.90	3.62	3.55	3.44	3.40	3.33	3.26	3.15	3.14	3.06
0.11	4.16	4.09	3.97	3.87	3.76	3.65	3.59	3.51	3.43	3.57	3.29	3.23	3.12	3.09	3.03	2.97	2.86	2.85	2.77
0.10	3.76	3.96	3.57	3.49	3.38	3.28	3.23	3.16	3.09	3.24	2.96	2.91	2.81	2.78	2.73	2.67	2.57	2.57	2.20
0.09	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54

OD (Standard) ➔

The Concentration of Hemoglobin in the sample can be read directly from above chart by corresponding the absorbance of sample with the absorbance of standard.