APPENDIX 4

Calibration of Klett Summerson Photoelectric Colorimeter with the standard hemoglobin solution.

A factor for the estimation of hemoglobin was obtained by calibrating the colorimeter with standard Hb solution.

Cyanmethemoglobin (HiCN) reference solution was obtained from the CSIR Centre for Biochemicals, New Delhi. The calibration graph was prepared using this solution as follows :

A series of five tubes were set up. Into the tubes the following amounts of HiCN reference solution and the Hb reagent were pipetted out :

Tube No.	HiCN reference solution (ml)	Hemoglobin reagent (Drabkin's) (ml)	Klett reading
1	5.0	none	184
2	3.0	2.0	110
3	2.0	3.0	73
4	1.0	4.0	37
5	none	5.0	-

The hemoglobin concentration in the five tubes were 14.35 g/dl, 8.61 g/dl, 5.74 g/dl and 2.87 g/dl respectively and zero strength (blank).

The readings were taken at a wavelength of 540 nm (green filter) using the Klett Summerson Colorimeter and were plotted on the ordinate against the hemoglobin concentration as described above.

A linear relationship was obtained as shown in the following graph.

A factor of 0.08 g Hb was established from the standardisation and each Klett reading was multiplied by this value to obtain the Hb concentration in g/dl.

FIG 20.
STANDARD CURVE FOR HEMOGLOBIN ESTIMATION BY CYANMETHEMOGLOBIN METHOD