ACTA MEDICA IRANICA
Vol. XV, 1972, p.23-28

Effect of Storage Temperature on the Haemoglobin Content of Stored Blood Samples

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The haemoglobin of a fresh blood sample or one dried on filterpaper, can be measured by micro-method. A fixed amount of blood is mixed with Clorox (Sodium hypochlorite, used in dry-cleaning), then ammonium thiocyanate is added and the amount of released iron as ferric thiocyanate is determined from the resulting colour, by spectrophotometric method.

Introduction

The determination of haemoglobin is important, both in clinical procedures and epidemiological surveys for screening anaemic patients. In some areas of Iran, where laboratory facilities are not available because they are so remote, quantitative determination of haemoglobin is carried out either by methods which are far from being accurate, or blood samples are preserved and then sent to suitably equipped laboratories. This micro-

— These studies were supported by the Institute of Public Health Research, Teheran University and funds of Plan Organization for Project No. 631101.

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method (1) permits both quick and accurate measurement of haemoglobin, with minimal facilities.

The accuracy of this method has been confirmed by the cyan-methaemglobin method. The blood samples can be prepared in various ways including drying on filter paper or in solutions as in the cyan-methaemoglobin method. The transportation of diluted blood samples is difficult but the samples dried on filter paper can be preserved for a long time and are easily transported.

Studies of our methods have shown that the cyan-methaemoglobin method can give accurate results only with blood samples preserved for less than a week, while this new method (1) can be applied to blood samples dried on filter paper and kept for several months, even at 40°C.

The basis of this method is to take 0.02 ml. of whole blood by Sahli pipette and mix it with clorox, then to convert the released iron (III) from haemoglobin to ferric thiocyanate by a solution of ammonium thiocyanate in methylcellosolve (2-methoxyethanol) and measure O.D. or transmittance spectrophotometrically, thus allowing calculation of the haemoglobin content of the blood. The pinkish-red colour of the solution is stable at least for two hours.

Material and Methods

Samplings

The study was carried out in the laboratory and two series of dried samples were prepared and preserved; one at room temperature and the other at 40°C. This temperature was chosen to ascertain the effect of heat (with particular reference to southern regions of Iran where the temperature may reach about 40°C in summer) on the amount of haemoglobin in blood samples.

In this study 50 samples were taken from 50 individuals, both healthy and sick. On the first day the haemoglobin concentration was determined in oxalated samples by this method (1), then six duplicate samples of each were obtained, and dried on filter paper. Three samples were kept at room

temperature, protected from direct light and the remaining three samples were put in screw cap tubes, wrapped in aluminum foil and incubated at 40°C.) The haemoglobin concentration was regarded as the mean value of the three samples. 1500 dried samples were examined in this way. For each sample, 0.02 ml of blood was dried. (Table 1).

Examination Method

The materials and methods are the same as those described by EUGENE W. RICE (J.Lab. and Clin Med. February, 1968, P. 320-321).

But standard solutions are prepared as follows:

The following are put into a 100 ml. beaker:

Iron reduced G.R.	62.5 mg.
Perchloric acid 60 %	3.77 ml.
Pure Hcl	1.25 ml.
Iron-free distilled water	5. 0 ml.

The beaker is then covered with a clean watch glass and mildly heated. The mixture is occasionally stirred with a glass rod, until a greenish-yellow colour is obtained.

After being cooled, the mixture is transferred to a 100 ml. volumetric flask, the cover of the beaker and stirring rod are also rinsed with distilled water into the volumetric flask. The volume is made up to the mark with distilled water.

Calculation

As 0.02 ml of standard solution (62.5 mg per cent corresponded to 18 gr. of haemoglobin) we have therefore.

$$\frac{ODU}{ODS} \times \frac{0.02 \times 18}{100} \times \frac{100}{0.02} \text{ or}$$

$$\frac{ODU}{ODS} \times 18 \text{ gr. Hb per 100 ml of blood.}$$

Table 1. Gm. haemoglobin/100 ml. after storage at 40 C and room temperature at different intervals.

Mean Value of 50 samples*	First day	10		30		60		120		240	
		T	R	T	R	T	R	T	R	T	R
	14.103	14.08	14.06	14.082	14.091	14.094	14.095	14.107	14.09	14.056	14.058

* The mean value of each sample is regarded as the average value of three dried blood samples.
T: 40°C
R: Room temperature

Results

In referring to the results as recorded in Table, no decrease or significant difference in haemoglobin is observed between blood samples immediately analyzed and those dried on filter paper and stored in the dark at 40°C for a period as long as 8 months.

From this experiment, it is concluded that this accurate and valuable method can be reliably used for determination of haemglobin in dried and stored blood specimens.

Haemoglobin measurements of three different standards equivalent to blood haemoglobin concentrations made by this method were reproducible and yielded the same results.

Discussion

It has been confirmed that Clorox used by Rice (1), and by connerty and Briggs (2), causes a faster digestion of haemoglobin than the sulfuric acid potassium persulfate mixture. Also, dissolution of ammonium thiocyanate in methyl cellosolve improves the qualities of the thiocyanate complex.

As is shown in Table 1, the average haemoglobin value of 50 specimens is 14.103 for the first day and 14.056 (40°C) after 8 months.

The difference between the two values of 0.047 is believed to be attributable to errors in calculation and sampling rather than to the effect of time and temperature.

In hot climates, if the specimens are not be analyzed immediately, it is more convenient and practical to dry 20 μ of blood on filter paper and store it for a long period.

Any type of filter paper may be used. In this laboratory Whatman No. 1 filter paper was found quite satisfactory for the storage of blood samples.

(Acknoledgement)

The authors are highly indebted to Prof. M. Faghih, Dean of the School of Public Health and Director of the Institute of Public Health Research and Professor Mohsenin for their advice and suggestions; to S. Vafi, technician, for her assistance.

SUMMARY

In this study 50 specimens of whole blood were dried on filter paper and kept at 40°C. The purpose in choosing this temperature was to determine the effect of the heat on the amount of haemoglobin in blood samples after a few months.

The measurement of haemoglobin was carried out on fresh samples on the first day of sampling and 240 days after storage of dried specimens at 40°C.

This experiment showed no decrease in haemoglobin between blood samples immediately analyzed and those of dried blood maintained at 40°C for periods as long as 8 months.

RÉSUMÉ

Dans ce travail nous avons procédé à la dessication du sang tôtal sur le papier filtre conservé à 40°C. Nous avons choisi cette température pour étudier l'influence de la chaleur sur la quantité d'hémoglobine des spécimens desséchés et conservés pendant plusieurs mois.

Le dosage d'hémoglobine a été effectué le premier jour du prelévèment, sur le sang frais, et 240 jours aprés la conservation des spécimens à 40°C, sur le sang désséché.

Nous n'avons remarqué aucune diminution dans le taux d'hémoglobine des spécimens desséchés; et après huit mois ce taux était comparable au taux du premier jour.

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