

# Estimation of haemoglobin using a Technicon AutoAnalyzer

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This paper describes a method for the routine estimation of haemoglobin, after conversion to cyanmethaemoglobin, using the AutoAnalyzer. Details are given of the preparation of a stable red cell suspension, and of the method of calibration of the AutoAnalyzer, using this suspension, which allows for the variation in sample dilution due to normal wear and tear of the pump tubes. The manifold (Fig. 1) reduces error due to carry-over and allows full conversion to cyanmethaemoglobin with the potassium ferricyanide solution.

### REAGENTS

**POTASSIUM FERRICYANIDE-CYANIDE SOLUTION** Sodium bicarbonate, 50 g., potassium ferricyanide, 10 g., and potassium cyanide, 2.5 g., are dissolved in distilled water and made up to 1 litre. To 40 ml. of this stock solution are added Brij 35 0.5 ml., ammonia solution 0.880 0.4 ml., and the volume made up to 1 litre with distilled water.

**RED CELL SUSPENSION** Blood is taken into acid-citrate-dextrose solution in the proportion 3:1 and allowed to

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stand at 4°C. until packed. The plasma is aspirated and 15 ml. Streptolin 33 (Glaxo) added to the cells which are run through a fine nylon filter to remove any clots. From this stock suspension 80%, 60%, and 40% dilutions (100 ml. each) are prepared using a sterile saline (100 ml.) and Streptolin 33 (5 ml.) mixture as diluent. The stock suspension and its dilutions are stored at 4°C. in 4 ml. quantities in sterile Bijou bottles and have a usable life in excess of three months. These are used in the day-to-day calibration of the AutoAnalyzer.

### STANDARDIZATION OF SUSPENSIONS

Dilution and conversion of the red cell suspensions to cyanmethaemoglobin followed by comparison with Cyanmethaemoglobin standard (B.D.H.) in a filter photometer proved unsatisfactory as a method of standardization. The haemoglobin values of subsequent blood samples, when diluted manually and read in a filter photometer, differed from those obtained when using the AutoAnalyzer by some 7%. This variation is thought to be due to differences in the spectral characteristics of the two types of filter and, possibly, to the methods of recording, even though Beer's law was obeyed using both instruments. As it is essential that strictly comparable results should be obtained irrespective of the instrument used an indirect method of standardization of the red cell suspensions was devised.

The haemoglobin values of 10 blood samples in the range 10-14 g./100 ml. are determined accurately with

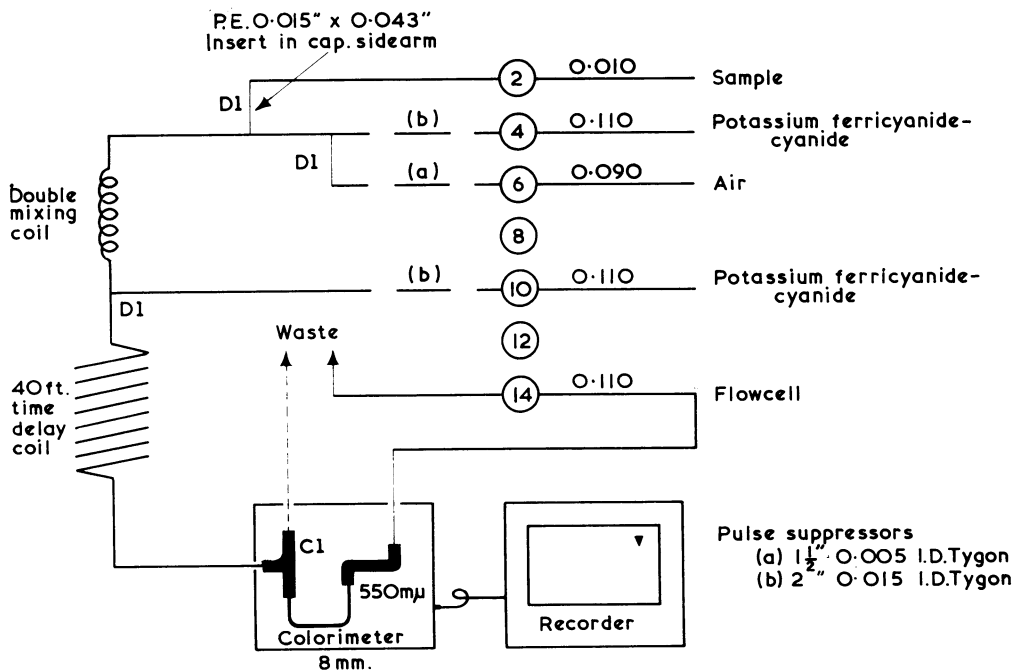


FIG. 1. Flow diagram for haemoglobin estimation.

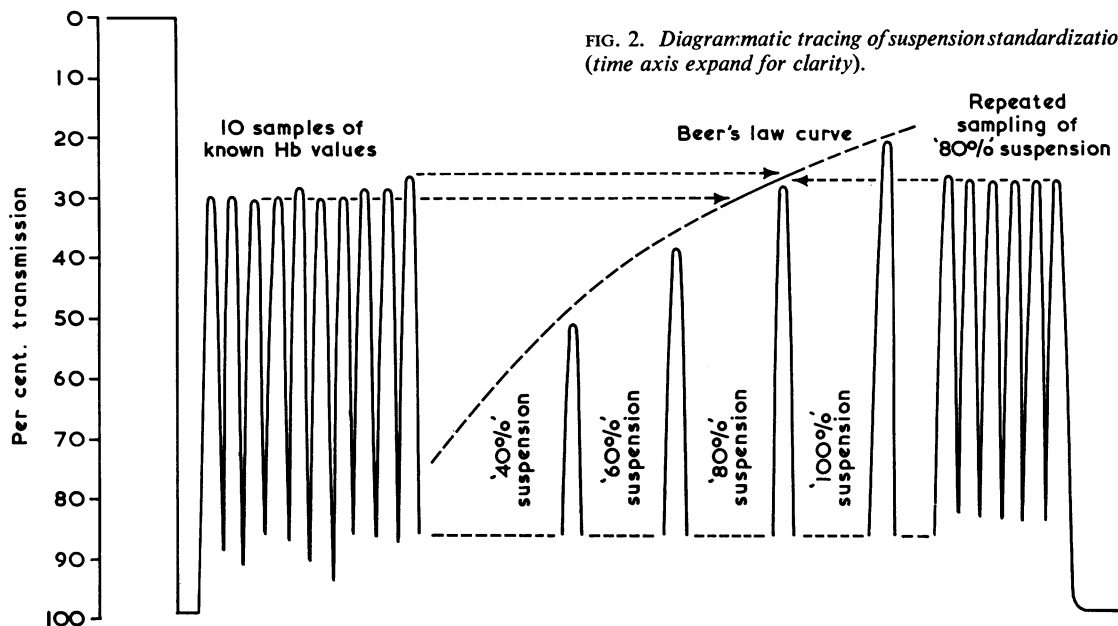


FIG. 2. Diagrammatic tracing of suspension standardization (time axis expand for clarity).

filter photometer. After establishing 0% and 99% transmission lines on the AutoAnalyzer these samples are run at 60/hr. followed by the red cell suspensions in order of increasing concentration, these in turn being followed by repeated sampling of the suspension which comes within the range 10-14 g./100 ml. (Fig. 2). Adequate mixing of the sample is effected by the use of a Technicon Vibra-Mixer accessory attached to the crook of the MK. I sampler. The peak heights of the blood samples are plotted against their determined haemoglobin values using the AutoAnalyzer chart reader, and a line drawn through these points. From this the haemoglobin value of the repeatedly sampled red cell suspension is ascertained. Since the peak height is affected by that preceding it (carry-over error) it is not sufficient to ascribe to the other whole-blood suspensions values calculated from their known dilutions, and the method of determination is as follows:—

The percentage transmission (T) of the repeatedly sampled suspension is converted to the extinction (E) using the formula  $E = 2 - \log T$ . Knowing the dilutions of the suspensions, their extinctions are calculated and reconverted to percentage transmission values. These are plotted on the AutoAnalyzer chart reader against the haemoglobin values obtained by applying the dilution factors of the suspensions to the haemoglobin value ascribed to the repeatedly sampled suspension. These points give a curve obeying Beer's law, independent of the order of sampling, and a template is cut to this curve. The peaks of the red cell suspensions run in ascending order of concentration are offered up to this curve and the values read off. These values are used in the daily preparation of the calibration curve, and since they are dependent upon the order of sampling this must be invariable, *i.e.*, in ascending order.

TEST

Each batch of blood samples for haemoglobin estimation is preceded by the four red cell suspensions. The percentage transmission value for the suspension in the 10-14 g./100 ml. Hb range is plotted against its ascribed haemoglobin value, and the template is used to draw a calibration curve between this point and the 99% transmission reagent baseline. The peaks of the other three suspensions are offered up to check their ascribed values, and if these are satisfactory the tests can then be read off.

COMMENT

Using the method described the results obtained with the AutoAnalyzer are generally within 0.3 g./100 ml. of those obtained photometrically after manual dilution. However, occasional samples will give results which agree to within 0.15 g./100 ml. on duplication by manual and AutoAnalyzer techniques and yet differ by up to 0.75 g./100 ml. when the two methods are compared.

The carry-over error with this manifold is 0.3 g./100 ml. when a haemoglobin value of 14.6 g./100 ml. is followed by a value of 3.65 g./100 ml., this being considerably less than that quoted in the AutoAnalyzer manual.

It is essential that all cups be covered as the fluid loss from these is 1.6%/hr. in still air, and may be as much as 10%/hr. in moving air, in which case the haemoglobin value may be increased by as much as 20%/hr. depending on the packed cell volume.

SUMMARY

Methods for the routine determination of haemoglobin using the AutoAnalyzer and preparation of stable red cell suspensions for the calibration of the instrument are described.