A METHOD FOR THE ESTIMATION OF SERUM HAPTOGLOBINS

Corporal W. E. G. HOWARD, A.I.M.L.T., S.R.M.L.T., R.A.M.C.

Department of Pathology, Royal Army Medical College

Introduction

An attempt has been made to devise a simple and reproducible technique for the estimation of the haemoglobin-combining capacity of serum which could be easily carried out in laboratories where the test is performed only infrequently.

The technique utilises the well-documented property of some serum components, (mainly the alpha-2 fractions) of combining both in vivo and vitro with free haemoglobin (Hall, 1964, Row, 1961, and Smith 1965). This effect may be useful in the investigation of the nature and time of intravascular haemolysis.

When known concentrations of haemoglobin are added to serum and the resultant mixture electrophoresed at pH 7 the haptoglobin/haemoglobin complex is attracted towards the anode, while free haemoglobin migrates to the cathode by endosmosis. The dilution at which free haemoglobin separates from the mixture may thus be taken as the haemoglobin-combining power of the serum and expressed as mg per 100 ml of serum.

Materials

Electrophoresis bath

No suitable bath was available; consequently one was constructed in the laboratory at a cost of less than ten shillings (Fig 1). It was made from a plastic partitioned

Fig. 1—Diagram of electrophoresis bath.

nail/screw container, 18 x 12 x 4 cm. This was originally divided internally into ten compartments and fitted with a lid. The internal partitions were utilised by removing the longitudinal former and reducing the transverse formers in height by a half an inch. Curved supports of perspex were bonded to the inner formers using chloroform as a

solvent. The electrodes were constructed from platinum wire connected to terminals which projected through the lid.

Power source

Any power source capable of delivering a constant 6-8 mA was found suitable.

Supporting media

Oxid cellulose acetate strips 20 x 5 cm, reduced to 10 x 5 cm were used.

Buffer

Of the several buffers tried, "Sorenson's" phosphate buffer (Varley, 1962) pH 7 and molarity of M/15 was found most satisfactory. Approximately 200 ml were required to fill the bath and soak the supporting media. The working life of the buffer was extended by reversing the current after each run.

Stock haemolysate solution

Cells from a freshly collected blood sample were well washed with physiological saline and adjusted to a haemoglobin content of 10 g per 100 ml. From this a standard haemolysate equivalent to 1,000 mg per 100 ml was made. When converted to carboxy-haemoglobin with coal gas this haemolysate was found to be stable for several months at minus 20°C.

Working haemolysate solution

Using distilled water as a diluent, working solutions were prepared from the stock solution at the following concentrations:—20, 40, 60, 80, 100, 120, 140, 160, and 180 mg of haemoglobin per 100 ml.

Stain

Orthotolidine was found to be most suitable for demonstrating the small concentrations of haemoglobin on the strip. The stain was prepared immediately before use as follows:—4 per cent orthotolidine in ethanol 10 ml: glacial acetic acid (AR) 10 ml: distilled water 10 ml: hydrogen peroxide (20 vols) 3 ml. (When higher concentrations of hydrogen peroxide were used it was found the stained areas faded much more rapidly.

Sample

A fresh sample of serum was obtained. This may be stored for two days at 4°C. It is important that no haemolysis is caused during the taking or separation of the serum.

Method

Preparation of bath

The bath was charged with buffer after the filter paper wicks and beds had been damped and put in place. The beds must be evenly moistened and the buffer level in all compartments equal.

Serum/haemolysate mixture

To prepare the serum haemolysate mixture, equal volumes of serum and working

haemolysate were mixed. The volumes used are immaterial but 0.1 ml amounts delivered by a "Sanz" pipette were found satisfactory.

Application of serum/haemolysate mixtures

A cellulose acetate strip was marked down the mid-line with pencil, soaked in buffer, blotted, and placed with its width across the supports. The mixtures were applied without delay (Fig 2) using a fine capillary pipette capable of delivering about 0.001 ml.

Fig. 2—Diagram indicating the position of haemoglobin/serum mixtures on strip

A serum blank and free haemoglobin marker (10 mg per 100 ml) were included. The lid was firmly placed in position and a current of 6-8 mA applied for 20 minutes. The strip was removed and immediately placed in the stain; areas of haemoglobin appeared dark blue.

Discussion

By the method described the point at which the serum haptoglobins became saturated with haemoglobin was shown when free haemoglobin separated from the haemoglobin/haptoglobin complex. Haemolytic episodes were clearly demonstrated in a patient suffering from intravascular haemolysis and normal sera showed a drop in binding capacity equivalent to amounts of added haemoglobin. The normal range by this method was found to be between 40 and 180 mg of haemoglobin bound per 100 ml of serum and the method gave reproducible results, even in the hands of different operators.

Summary

A simple electrophoretic technique for the estimation of haemoglobin-combining capacity is described.

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