Technical methods

Simple method for estimating plasma haemoglobin during open heart surgery

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Standard methods to measure plasma haemoglobin use orthotolidine or benzidine. These have disadvantages of (a) being time consuming, (b) depending for accuracy on critical timing of colour development, and (c) both compounds are considered to be carcinogenic.

Some laboratories give an approximate result, e.g., a one, two, or three plus degree of plasma haemoglobin.

In estimating plasma haemoglobin directly, errors occur associated with varying degrees of turbidity of the plasma. It was found that Lissapol NDB (I.C.I. Ltd.) will clear plasma turbidity with no significant effect on the haemoglobin content.

The following method was found to be rapid, simple, and sufficiently accurate by comparison with standard techniques.

**METHOD**

Primed pump blood, 9 ml., is taken into 1 ml. of 3·8 g% sodium citrate and 9 ml. of blood after the patient has been heparinized is taken into 1 ml. of 3·8 g% sodium citrate. After centrifugation the supernatant plasma is taken off from each and mixed together in equal amounts.

Mixed plasma, 3 ml., is used for the haemoglobin standard curve and 3 ml. for the blank. To clear the plasma, 7 drops of Lissapol are added to each sample and mixed by inversion several times.

A haemoglobin standard is previously prepared from haemolysed blood with a concentration of 1,000 mg. Hb%.

A calibration curve is prepared by repeatedly adding 0·03 ml. of the above haemoglobin standard and reading after each individual addition in a photoelectric colorimeter using an Ilford green filter no. 625.

Each addition is equivalent to 10mg.% of plasma haemoglobin.

If the optical density of the plasma blank is too high to 'zero' the colorimeter dilute the blank and sample 1 in 2 with ammoniated water and add only 0·015 ml. of the haemoglobin standard for each reading.

To obtain plasma haemoglobin levels during and after the operation use 3 ml. of citrated plasma, add 7 drops of

Automated screening for sheep erythrocyte agglutination

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The routine examination of serum from patients with rheumatoid arthritis using the differential agglutination of sheep erythrocytes is a standard procedure.

With only minor modification the techniques in use are basically those of Rose, Ragan, Pearce, and Lipman (1948) in which sheep cells are sensitized by a potent rabbit anti-sheep serum to a minimum agglutination dose, and used as indicators in a two-fold dilution system of complement-inactivated serum. The differential titre is achieved by a further titration using unsensitized sheep cells. These techniques are time consuming and in routine testing some 60% of sera prove to have a titre of less than 32. The following automated screening technique reduced the number of titrations by detecting sera with titres greater than 16.

The serum under test is inactivated at 56°C. for 30 minutes and samples then placed in autoanalyser standard sample cups. Tests may be run at 40 to 60 tests per hour. Washed sheep cells are sensitized using a rabbit anti-

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Lissapol and mix, read against the blank as above; the result is obtained from the calibration curve (Fig. 1).

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sheep serum as for the manual test with a final concentration of 1%. The cells are kept in suspension by means of a magnetic stirrer. Serum samples are diluted with 0.85% sodium chloride, mixed in a single mixing coil and sensitized sheep erythrocytes added, mixing again in a single coil, then into a 40-foot glass coil at 37°C.

From the delay coil, the agglutinates settle in a straight horizontal piece of glass tubing. Removal of agglutinates is accomplished by a standard T piece. The remaining cell suspensions are re-mixed in a single glass mixing coil and passed into a 15 mm. flow cell in an N colorimeter using 550 mμ filters.

Initially a base line of 98% transmission is set with all lines pumping saline. Sheep cells are pumped through the appropriate line until a 5% transmission is obtained. Any sample giving a rise of more than 2% transmission from this base line is regarded as a potential positive and titrated manually. To facilitate the identification of samples a known positive is placed at the beginning of the batch and at every tenth cup as a marker.

Some 500 tests have so far been performed and the results compared with those from the manual technique. No titre below 1/16 as shown by the manual method has been recorded by the automated method nor has any automated titre of less than 1/16 been subsequently found to be greater after manual titration.

This screening technique represents a saving in manual titration of approximately 60% of samples.

REFERENCES


CORRECTION

This is figure 2 of the paper by V. P. Pugh and R. W. T. Gaze, entitled 'The Reiter protein complement-fixation test using the AutoAnalyzer' (J. Clin. Path., 19, 595), which was wrongly printed.