

A rapid method for the estimation of serum haptoglobin

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Since the discovery of haptoglobin by Polonovski and Jayle (1939), variations in the serum concentration of this protein have been observed in a variety of diseases; high levels are found in chronic inflammatory, rheumatic, and neoplastic diseases, and in biliary obstruction whereas haemolytic and hepatocellular diseases are associated with a reduction of the concentration of this protein in the serum (Nyman, 1959).

Methods of estimating haptoglobin depend either upon the peroxidase activity of the haptoglobin-haemoglobin complex in acid solution, or upon the difference in electrophoretic mobility between 'free' and haptoglobin-bound haemoglobin. A simple electrophoretic method is described here which can easily be completed in two hours, and requires less than 0.2 ml. of serum.

MATERIALS

AGAR GEL A solution of 0.8% New Zealand agar in 0.05M pH 7.0 phosphate buffer, *i.e.*, Sørensen buffer diluted four-fold.

HAEMOGLOBIN SOLUTIONS For use when normal or low haptoglobin concentrations are anticipated haemoglobin solutions containing 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mg./ml. of carboxyhaemoglobin are prepared by diluting a stock 10 mg./ml. solution with distilled water. Sera with higher haptoglobin contents may be diluted to fall within this range or more concentrated haemoglobin solutions may be used.

BENZIDINE STAIN (Smithies, 1959) Benzidine, 0.2 g., is dissolved in 100 ml. hot distilled water to which 0.5 ml. of glacial acetic acid has been added. This solution is stable for several weeks. Immediately before use 0.2 ml. of 30% w/v H₂O₂ is added.

OR

ORTHO-TOLIDINE STAIN (Kohn and O'Kelly, 1955) Mix equal volumes of 4% o-tolidine in ethanol, glacial acetic acid, and distilled water. Add ¼ volume of 5% w/v H₂O₂ immediately before use.

The benzidine stain is more permanent, but the reagent is carcinogenic.

METHOD

Hot agar solution, 12 ml., is poured onto a level glass plate, 11 × 8 cm. When the gel has set a series of holes of approximately 4 mm. diameter is cut with a cork borer. The holes are then filled with the serum-haemoglobin mixtures. These are prepared by adding

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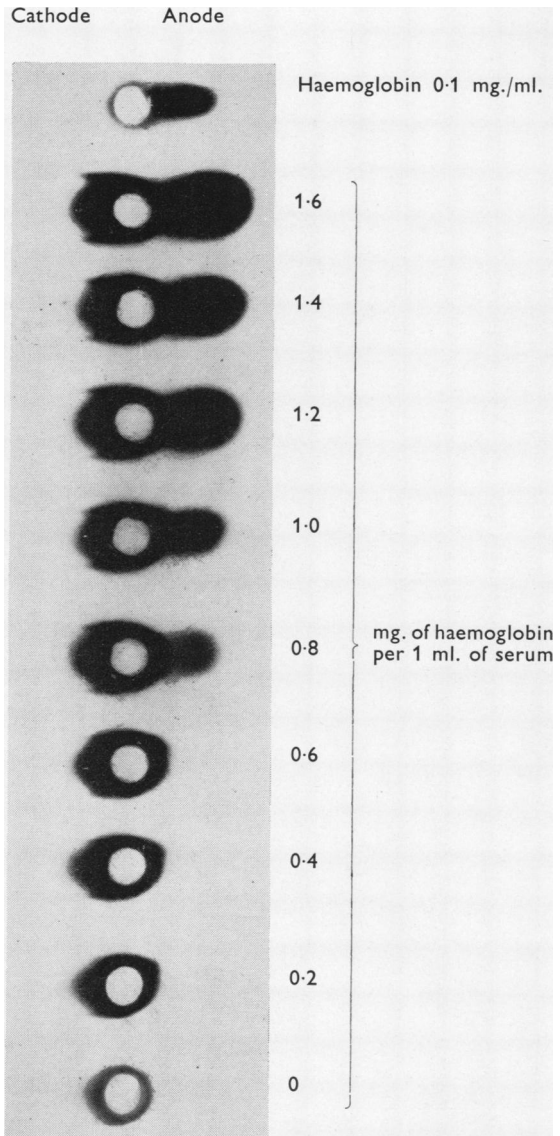


FIG. 1. *Electrophoresis of mixtures of carboxyhaemoglobin with serum in agar gel at pH 7.0 stained with benzidine. The haemoglobin-binding capacity of the serum lies between 0.6 and 0.8 mg./ml.*

haemoglobin solutions to equal volumes of serum, using an 0.02 ml. micropipette, giving mixtures containing from 0.2 to 1.6 mg. haemoglobin per 1 ml. of serum. The longer sides of the agar plate are then connected by Whatman 3 MM filter-paper bridges to reservoirs of 500 ml. of the phosphate buffer. A current of approximately 12 milliamps at 240 volts is passed through the agar for 45 minutes, using platinum wire electrodes in

the buffer reservoirs. The agar plate and the reservoirs are contained within a box to prevent excessive evaporation during electrophoresis.

After electrophoresis the agar plate is immersed in the benzidine stain, which reveals the position of the haemoglobin. Fig. 1, which is a photograph of a portion of a plate, shows that haptoglobin-bound haemoglobin moves slightly towards the anode, and free haemoglobin moves towards the cathode. The binding capacity of the serum haptoglobin in this figure lies between 0.6 and 0.8 mg. of haemoglobin per 1 ml. of serum. Serum to which no haemoglobin had been added shows a small amount of haptoglobin-bound haemoglobin, probably from haemolysis *in vitro*. A solution of 0.01% carboxyhaemoglobin demonstrates that the method is sufficiently sensitive to

detect the haemoglobin concentration increments between adjacent cups.

SUMMARY

A rapid electrophoretic method for the estimation of serum haptoglobin is described. Less than 0.2 ml. of serum is required and results are available in less than two hours.

REFERENCES

- Kohn, J., and O'Kelly, T. (1955). *J. clin. Path.*, **8**, 249.
 Nyman, M., (1959). *Scand. J. clin. Lab. Invest.*, Suppl. 39, p. 15.
 Polonovski, M., and Jayle, M. F. (1939). *Bull. Soc. Chim. biol. (Paris)* **21**, 66.
 Smithies, O. (1959). *Advanc. Protein Chem.*, **14**, 76.

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