

Letters to the Editor

EFFECT OF RUST ON HAEMAGGLUTINATION TEST

Microtechniques are now used extensively in diagnostic as well as immunological studies for viruses, protozoa, and bacteria (Sever, 1962; Edwards, 1964; Kessel, Lewis, Pasquel, and Turner, 1965). In microtitrations constant volumes of fluids are picked up and delivered by specially constructed and calibrated metallic microdiluters. According to the Microtiter Instruction Manual of the Cooke Engineering Company (1965), their high titanium, stainless steel, new-pattern microdiluters with a precisely slotted tip are not supposed to rust, but in the hot and humid climate of Calcutta they do rust, though they continue to deliver fluids properly when tested on the special 'go-no-go' delivery tester supplied by the manufacturer. Many of the rust particles become detached from the microdiluters during their agitation in the wells of the dilution plates and produce, both with human group O and goose erythrocytes, an atypical settling pattern at the bottom of disposable plastic V plates. There is a small central button of erythrocytes, showing as well as the peripheral deposit, small black particles rimmed by a clear space. The peripheral concentric erythrocyte deposit closely simulated the typical haemagglutination pattern. Microscopically many black rust particles of various sizes and shapes were observed among discrete erythrocytes, and we have called this atypical pattern pseudo-haemagglutination.

The rusted particles did not alter the typical agglutination pattern or the titre of some of the haemagglutinin-positive enteroviruses and arboviruses. They, however, produced pseudo-haemagglutination with the haemagglutinin-negative enteroviruses as well as the uninoculated tissue culture maintenance medium. It appears that the rusted microdiluters, if used for diluting the antisera or the haemagglutinin-positive antigens, are not likely to modify the positive haemagglutination patterns. But one has to be cautious in using the rusted microdiluters for screening antigens as the detached rust may of itself produce a false-positive haemagglutination pattern.

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REFERENCES

Cooke Engineering Company (1965). *Microtiter Instruction Manual*, 3rd ed. Alexandria, Virginia, U.S.A.

Edwards, E. A. (1964). *J. Bact.*, **87**, 1254.
 Kessel, J. F., Lewis, W. P., Pasquel, C. M., and Turner, J. A. (1965). *Amer. J. trop. Med. Hyg.*, **14**, 540.
 Sever, J. L. (1962). *J. Immunol.*, **88**, 320.

DETERMINATION OF BLOOD GLUCOSE USING 4-AMINO PHENAZONE AS OXYGEN ACCEPTOR

Although the dl adrenaline method has worked very well in this laboratory (Trinder, 1969), the manual version is a little insensitive and the colour development time is rather long. I have now worked out a method of using 4-amino phenazone which is three times as sensitive as the adrenaline method, uses only two relatively stable solutions, and requires only a 10-minute colour development. The two solutions required are:

1 PROTEIN PRECIPITANT Prepared exactly as for the dl adrenaline method but containing 0.1% w/v phen. Keeps indefinitely.

2 COLOUR REAGENT Fermcozyme 653 AM, 5 ml, and 5 ml of 0.1% peroxidase (R.Z. 0.6) are added to 300 ml of a solution containing 1% w/v Na₂HPO₄, 0.1% w/v sodium azide NaN₃, and 0.03% w/v 4-amino phenazone. Keeps at least four weeks at 4°C.

METHOD The proteins from 0.1 ml of blood are precipitated with 2.9 ml of protein precipitant; 3 ml of colour reagent is added to 1 ml of supernatant fluid, and the mixture is incubated at 37°C for 10 minutes with occasional brief shaking to ensure adequate aeration. The optical density of the resulting purple colour is read at 515 mμ or using an Ilford 623 blue-green filter. The optical density of a glucose solution 200 mg/100 ml put through the whole procedure is about 0.47 at 515 mμ. The amounts of blood and protein precipitant used can be varied within wide limits to cover individual requirements.

4-Amino phenazone, when incorporated in cholesterol pellets implanted into the bladders of mice, has been shown to produce more tumours than cholesterol implants alone (Boyland, Busby, Dukes, Grover, and Manson, 1964). However, 4-amino phenazone is not likely to be a dangerous substance in normal laboratory use.

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REFERENCES

Boyland, E., Busby, E. R., Dukes, C. E., Grover, P. L., and Manson, D. (1964). *Brit. J. Cancer*, **18**, 575.
 Trinder, P. (1969). *J. clin. Path.*, **22**, 158.