Technical methods

A shortened automated procedure for the determination of alkaline phosphatase

JOYCE L. BELL and M. COLLIER with the technical assistance of ANNA MARIE HARTMANN
From the Department of Chemical Pathology,
The Royal Free Hospital, London

The main disadvantage of the standard AutoAnalyzer procedure for determination of alkaline phosphatase is the long time taken for analysis, blanks, and tests having to be run separately. For this reason we have devised an AutoAnalyzer procedure whereby blanks and tests may be read at the same time, thus halving the time of analysis.

REAGENTS

1-AMINOANTIPYRINE Dissolve 1 g. 4-aminoantipyrine in 4 l. distilled water. Filter. Store in a brown bottle.

POTASSIUM FERRICYANIDE Dissolve 5 g. potassium ferricyanide in 1 l. distilled water. Filter. Store in a brown bottle.

BICARBONATE-CARBONATE BUFFER pH 10.0 Dissolve 1.58 g. anhydrous sodium carbonate in 500 ml. distilled water. Add 0.84 g. sodium bicarbonate, dissolve and dilute to 1 litre. Filter. Check the pH before use.

ALKALINE BUFFER SUBSTRATE Dissolve 2 g. phenyl disodium phosphate in 1 l. of the above alkaline buffer. Filter. Check pH. Store in the refrigerator and warm to room temperature before use.

STOCK PHENOL STANDARD Dissolve 1 g. A.R. phenol in 1 l. of 0.1N HCl. The concentration of the stock phenol should be checked before use as follows: Take 25 ml. of stock phenol and 50 ml. of 0.1N sodium hydroxide in a 250 ml. volumetric flask, heat to 65°C. and add 25 ml. of 0.1N iodine to the hot phenol. Stopper and stand at room temperature for 30 to 40 minutes. Add 5 ml. concentrated HCl and titrate with 0.1N sodium thiosulphate. Each millilitre of 0.1N iodine, *i.e.*, each millilitre iodine added—each millilitre thiosulphate used in

FIG. 1. Diagram of the manifold for alkaline phosphatase
(—) manifold for tests
(—) manifold for blanks

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titration, corresponds to 1.567 g. phenol. Adjust so that 1 ml. stock phenol contains 1 mg. phenol.

WORKING PHENOL STANDARDS Working standards containing 10 to 450 μ g. phenol/ml. are prepared by dilutions of the stock.

METHOD

The manifold is constructed as shown in Figure 1. A twin sampling crook is used in order that two samples may be withdrawn simultaneously from the same specimen. Fifteen tube end blocks are used so that only one pump is needed. The 37°C. incubation bath is fitted with two coils so that only one bath is required. The recorder used is fitted with two pens. This is considerably cheaper than two single recorders. The only piece of equipment which it was necessary to duplicate was the colorimeter.

The estimations are run at 40 determinations an hour.

The incubation time is determined as described in the AutoAnalyzer standard procedure. It is important that the incubation time is determined with the tubes which have been used for the test and not the blank runs and that standard curves are run on both the blank and test sides of the manifold.

RESULTS

It was found that the usual manifold for alkaline

phosphatase did not pump well when a 15-tube end block was used and blanks and tests were run at the same time. For this reason a modified manifold was constructed using smaller sized manifold tubing directly scaled down from the standard manifold. This manifold pumped well and also had the advantage that less serum was needed. The standard AutoAnalyzer manifold used 0.64 ml serum whilst the modified manifold only needed 0.32 ml. Using this manifold, however, the incubation time was longer because of the smaller volume of reagents being pumped. This resulted in dilution of the serum being necessary at a relatively low alkaline phosphatase value. For this reason we have increased the size of the buffer-substrate line. The range of the method with our manifold is now 0.65 King-Armstrong units/100 ml. If a wider range is needed the buffer-substrate line may be increased further. Figure 2 shows a typical tracing. The wash out between samples is better than with the standard AutoAnalyzer manifold for alkaline phosphatase. Samples with low phosphatase activity were run directly after samples with high activity then repeated later in the run between water samples; the carry over was found to be negligible. One series of determinations carried out in this way gave the following results:—74, 9, 72, 9, water, 72, water, 7, water, 72, water, 7. Very reproducible results were obtained. One serum analyzed nine times gave the following results:—30, 30, 31, 31, 30, 30, 30, 30, 30.

Table I shows that good correlation was obtained

FIG. 2. Typical recordings of alkaline phosphatase results. Upper tracing is the tests, lower tracing the blanks.

TABLE I

COMPARISON OF RESULTS FOR ALKALINE PHOSPHATASE DETERMINATION BY THE ANALYZER AND BY THE METHOD OF KING AND WOOTTON (1956)

Alkaline Phosphatase (K.-A. units)

No.	Analyzer	Manual
1	47	51
2	52	52
3	7	8
4	22	20
5	104	106
6	26	24
7	13	14
8	31	32
9	13	13
10	14	12
11	9	8
12	11	12

between alkaline phosphatase determinations carried out on the Analyzer, and by hand by the method of King and Wootton (1956).

REFERENCE

King, E. J., and Wootton, I. D. P. (1956). *Micro-analysis in Medical Biochemistry*, p. 85. Churchill, London.

NOTICE

BRITISH COMMITTEE FOR STANDARDS IN HAEMATOLOGY

This Committee has been established by the British Society for Haematology at the invitation of the European Society of Haematology and will work in collaboration with the International (European) Haematology Standardization Committee to encourage the adoption of recommended standards in Britain and to participate in international discussions on standardization. Various professional and trade organizations are represented and the Committee will cooperate with the British Standards Institution. As with the European Standardization Committee expert panels are being convened and will first be concerned with haemoglobinometry, erythrocytometry, haematocrit, normal values, and documentation of haematological data. These topics will be discussed during the Congress of the International Society of Haematology to be held in Stockholm in September 1964.

The Committee's secretariat consists of Dr. S. M. Lewis (Chairman), Dr. A. A. Sharp, and Dr. J. W. Stewart. Further information can be obtained from Dr. S. M. Lewis, Department of Haematology, Post-graduate Medical School, Ducane Road, London W.12.

Book reviews

AN INTRODUCTION TO COMPARATIVE PATHOLOGY By G. A. Gresham and A. R. Jennings. (Pp. xi + 412; 116 figures. 84s.) London and New York: Academic Press Inc. 1962.

The science of pathology originated in the practical study of disease in the human species; this was soon extended to the experimental study of the laboratory animals, but it is only in recent years that the wider interests of disease in animals living in natural surroundings and of disease in phyla other than the vertebrates have received any attention. The theoretical and practical value of this broader concept of pathological studies needs little advocacy, but it is a different problem to bring it within the compass of what pathologists can be expected to learn, when their professional life will be concentrated either on man or on the relatively few species of economic importance. This introduction, written by two pathologists who have done distinguished work in human and animal pathology respectively, is a bold attempt to provide at least a key to what is going on. The format and illustrations are of a high standard and there are few slips.

The authors have perhaps not decided clearly enough on the audience to which they are directing their writing. Some of the material is covered at too elementary a level, too naïvely (is it necessary to point out that fogs are worst for those with pre-existing disease of the respiratory system?) or with too much trivial detail for the senior qualified pathologist, who would prefer a greater emphasis on the comparisons; on the other hand it is doubtful if the elementary student who is not, for example completely familiar with the fundamental genetics of pp. 11-12 or the outline of helminthology of pp. 184-6, is ready to profit much from contemplating the tracheal granulomas of *Filaroides osleri* on the same page as the neoplasma.

Economy by the excision of the elementary matter, and of sentences derived from the lecture ('we shall now consider . . .') would make room for some facets which the reviewer expected to find discussed but are not in fact touched on. Principal among them is the ecological angle of the distribution and kinds of disease found in wild populations—little enough known, but fascinating and important. The wide cover of pathological investigations provided by the zoological gardens in numerous species of animals, notably by Fiennes and his colleagues in London, has not been made use of, even in the discussion of atheroma, which is unexpectedly brief. In view of the present importance of the concept of auto-immune disease, the two brief references indicated in the index are inadequate, and though there is a chapter on 'diseases in which hypersensitivity plays a part', the juxtaposition of tuberculosis and the collagen diseases, without any general survey of the principles, and the closely-written paragraphs on the blood, thyroid, encephalitic, and amyloid auto-immunity, will prove very hard reading for

FIG. 2. Increasing concentrations of FIGLU and glutamic acid plotted against optical densities of eluates from FIGLU and glutamic acid spots.

FIGLU spots were plotted against the concentration. The graph obtained (Fig. 2) shows a straight line except for very low concentration near the limit of sensitivity. Glutamic acid dilutions gave a similar curve.

My thanks are due to Mr. P. H. Motivala for his technical assistance.

REFERENCES

- Kohn, J. (1957a). *Biochem J.*, **65**, 9p.
 — (1957b). *Clin. chim. Acta*, **2**, 297.
 —, Mollin, D. J., and Rosenbach, L. M. (1961). *J. clin. Path.*, **14**, 345.

CORRECTIONS

On page 222 of the paper 'Histology of the aortic media in dissecting aneurysm' by Gerald Manley (*J. clin. Path.*, **17**, 220-224) the two parts of Fig. 2 have been interchanged.

Figure 1 on page 244 of the paper by M. Patricia Jevons and M. T. Parker, 'The evolution of new hospital strains of *Staphylococcus aureus*' (*J. clin. Path.*, **17**, 243-250) has been printed upside down.

On page 301 of the paper 'A shortened automated procedure for the determination of alkaline phosphatase' by Joyce L. Bell and M. Collier (*J. clin. Path.*, **17**, 301-303) under Reagents '1-aminoantipyrine' please read 'Dissolve 1 g. 4-aminoantipyrine in 1 l. distilled water'

A new system for rapid haemoglobin estimations and leucocyte counts

R. E. DAVIS and D. J. NICOL *From the Department of Haematology, Royal Perth Hospital, Western Australia*

Blood samples are usually diluted 1 in 200 for the estimation of haemoglobin and 1 in 500 when counting leucocytes in the Coulter counter. These procedures are performed separately and require two tubes and two diluting pipettes. The purpose of this paper is to describe a system which enables the haemoglobin estimation and leucocyte count to be made from the one diluted sample. The system dispenses with the need for pipettes and diluting tubes and enables both examinations to be completed in less than 40 seconds.

PRINCIPLE

To enable both the haemoglobin estimation and leucocyte count to be made from the one dilution it was necessary to increase the dilution for haemoglobinometry to 1 in 500. Compensation for this was made by increasing the light path in the haemoglobinometer from 10 to 25 mm. The sample of blood is first diluted with N/150 ammonia directly into the haemoglobinometer tube using an automatic dispenser. This lyses the red cells and also causes some swelling of the leucocytes. Without delay the sample is further diluted by adding a saline solution of sufficient strength to make the whole mixture isotonic. This saline diluent also contains saponin to ensure adequate stromatolysis. The leucocytes quickly return to their former size and remain stable. The haemoglobin concentration is read in a photoelectric colorimeter as oxyhaemoglobin and the leucocytes are counted in a Coulter automatic cell counter.

APPARATUS

A standard type of rotary specimen mixer is followed by a Trimatic¹ dispenser fitted with pumps suitable for removing a 40 c.mm. sample of blood and delivering 10 ml. of diluent. Next to the dispenser is a 10 ml. semi-automatic pipette² fitted with a small plastic tube in place of the needle thus reducing frothing. It is set to deliver 10 ml. A Spectronic 20 spectrophotometer, fitted with an adaptor to give a 25 mm. light path, is used to measure haemoglobin. This adaptor is a standard accessory. Leucocytes are counted in a Coulter cell counter model A.

REAGENTS

The diluent used in the Trimatic dispenser is N/150 ammonia and in the semi-automatic pipette is 1.7%

¹Manufactured by Research Specialities Corp. U.S.A. Model No. 2642.

²Manufactured by Becton, Dickinson & Co., Cat. No. 1271.

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