

## Letters to the Editor

### Standardization of Clinical Enzyme Assays

We were disappointed by the first report of the joint DHSS/ACB working party on standardization of clinical chemistry enzyme assays which dealt with serum alkaline phosphatase (Moss, Baron, Walker, and Wilkinson, 1971). The principal message was that those who use a King-Armstrong method should standardize it by the manual method of King and Wootton (1956). Although most clinical chemists in the United Kingdom still use either a manual or mechanized King-Armstrong method, little help was offered to the increasing numbers of clinical chemists using either  $\beta$ -glycerophosphate or p-nitrophenol phosphate as substrate. The relative merits of the three main substrate systems were regrettably not discussed and the problems associated with the use as secondary standards of commercially prepared dried sera containing added alkaline phosphatase from animal or avian viscera remain unanswered.

The stated aim of the working party is to recommend steps to improve the accuracy and reliability of diagnostic enzyme methods. We would state as general principles that (i) improved precision will be most readily achieved in the United Kingdom by the encouragement of mechanized methods in place of imprecise manual ones; (ii) the accuracy of a mechanized method depends upon it being closely correlated with the initial manual reference method. The reference method should be capable of a kinetic approach and carried out if possible under optimal conditions, eg,  $Mg^{2+}$  concentration.

We conclude that methods using p-nitrophenol phosphate as substrate have certain advantages, such as better precision and kinetic capability over King-Armstrong methods. The modified Bessey-Lowry-Brock method can be easily adapted to ordinary AutoAnalyzer equipment as well as to the newer, more sophisticated analytical systems, and fortunately it appears that the liver and bone isoenzymes have similar affinities for p-nitrophenol phosphate as substrate.

The accuracy and precision of alkaline phosphatase determinations in the United Kingdom will not be significantly im-

proved by the 'official' recommendation of a reference method which, although of great historical importance, still (1) will tacitly encourage the retention in smaller laboratories of existing inferior manual methods which may have a CV of 20 to 30%; (2) uses suboptimal conditions, eg,  $Mg^{2+}$  concentration to measure arbitrary non-SI units; (3) causes difficulties when sera with high activity have to be assayed. Although the 'official' King-Armstrong method now has an incubation period of 0.25h it is suggested that the incubation period can be varied at will for high activity sera. Since it is not usually possible to alter easily the incubation time in mechanized systems, the working party are in effect recommending that most clinical chemists use a manual 'back-up' system for high activity sera.

We hope the working party will reconsider urgently their support for King-Armstrong methods and units.

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#### The King-Armstrong Method

Some of the points raised by Hirst and Howorth (1972) in their criticism of our recommendations (Moss, Baron, Walker, and Wilkinson, 1971) on 'Standardization of alkaline phosphatase assays' have also been made to us by other biochemists and are, we believe, based on a misapprehension of the purpose of our report.

We estimate on the basis of quality-control surveys that rather more than 60% of British clinical laboratories are carrying out alkaline phosphatase estimations by the King-Armstrong procedure or by its later modifications. The use of the AutoAnalyzer adaptation of this method is particularly widespread. Therefore, we felt that some agreement on the meaning of the King-Armstrong unit, and on the way in which automated procedures reporting results in this unit should be calibrated, would have a beneficial effect on the comparability of results reported by the majority of British laboratories. We appreciate that, as Hirst and Howorth

suggest in their letter, kinetic methods with p-nitrophenyl phosphate as a substrate will probably increase in importance until the King-Armstrong unit finally lapses into disuse. However, an IFCC Expert Panel is currently engaged in attempting to define a reference kinetic method for alkaline phosphatase, and we did not wish to duplicate these efforts with the danger of reaching recommendations that might differ from those of the Expert Panel.

We are not able to confirm the estimations of a coefficient of variation of 20 to 30% (at an unstated level of activity) quoted by Hirst and Howorth for the manual phenyl phosphate procedure. Recent estimates of this variation in our own laboratories have given figures of  $\pm 3.9\%$  for the manual method and  $\pm 2.6\%$  for the corresponding AutoAnalyzer procedure, at the 25 King-Armstrong units/100 ml level.

The results of any enzyme estimation could be expressed in SI units, if and when these have been agreed on as far as enzyme activity is concerned, but of course this will in no way affect the accuracy or precision of the methods. The difficulty of dealing with the high activity specimens inherent in all fixed-time methods and, as Hirst and Howorth point out, made worse in the AutoAnalyzer by the difficulty of altering the incubation period. A manual procedure for dealing with high-activity specimens might indeed be preferable to dilution, with its risk of disproportionate changes in activity. However, it must be borne in mind that many automated kinetic procedures are equally inflexible with regard to such factors as their recording intervals and difficulties of interpretation of non-linear progress curves can arise in these methods also (Goldberg, Ellis, and Wilcock, 1971).

We hope that the comments of Hirst and Howorth will not deter those who are using the King-Armstrong method from giving careful consideration to our suggestions.

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[We have given the authors this opportunity to reply to the criticisms of Hirst and Howorth and King et al.—EDITOR]

**Abbreviations for Names of Diagnostic Importance**

A recent communication (Baron, Moss, Walker, and Wilkinson, 1971) is disturbing inasmuch as the group, although representatives of specified organizations, was acting without the authority of these bodies. In addition, the authors state that although the International Union of Biochemistry (1961, 1965) strongly discouraged the use of abbreviations for enzymes, and that this was still upheld, they nevertheless arbitrarily continue, 'we recommend . . .'

The pitfalls of abbreviations have been discussed elsewhere (King, 1969). The system put forward by Baron *et al* differentiates glutamate dehydrogenase and glutathione reductase as GMD and GTD respectively, but what happens to glycerate dehydrogenase and glyoxalate reductase? Alcohol dehydrogenase is AD, and presumably adenosine deaminase would be ADS, trypsin is TPS, and triose phosphate isomerase, TPI. It becomes easier to remember and use the trivial name than the abbreviation and it requires little imagination to see what the computer-controlled data processing systems mentioned would do with some of these abbreviations.

A second communication from this group (Moss, Baron, Walker, and Wilkinson, 1971) deals with the 'standardization' of alkaline phosphatase assay and a further report on the standardization of aspartate and alanine transaminase is promised. This raises the problem of how many standardizations the world can expect, since the German Society of Clinical Chemistry (1970) have already published their standard assays for the transaminases, alkaline phosphatase, lactate dehydrogenase, 2-hydroxybutyrate dehydrogenase, creatine kinase, and 'leucine arylamidase'. The situation is even more complicated because the London group state that an 'expert panel'

of the International Federation of Clinical Chemists has the same subject under discussion.

Doubtless it is natural progression from the first report which discredits recommendations made by an international representative and authorized body to the later reports which disqualify such proposals before they are even made.

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**Correction**

We regret that the list of references, now set out below, was not printed with the Letter to the Editor, 'A red herring in the detection of Bence Jones protein', by R. B. Payne (*J. clin. Path.*, **25**, 183).

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**Book reviews**

**Reference Methods for the Microbiological Examination of Foods.** (Report prepared by the Subcommittee on Food Microbiology of the Food Protection Committee (Pp.39. \$2.25.) Washington, DC: National Academy of Sciences. 1971.

This booklet could be useful although it is incomplete. There is no mention of the surface plate count and its advantages or the importance of anaerobic counts. There is no section on the isolation of *Clostridium welchii (perfringens)* which figures highly in both UK and USA statistics for food poisoning.

All the methods given appear in 'Microorganisms in foods' by the International Committee on Microbiological Specifications for Foods.

Many media are available for the isolation and enumeration of coagulase positive staphylococci so surely a reference method could have been chosen but no information is given.

In the salmonella section there is no guidance on the preparation of samples. The beneficial effect of incubation at 43°C for some liquid enrichment media is not given.

Methods for faecal streptococci, *Vibrio parahaemolyticus*, and *Bacillus cereus* are not given, but there is a clear description of the serological identification of the enteropathogenic *E. coli*.

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**Laboratory Diagnosis of Diseases Caused by Toxic Agents** Edited by F. W. Sunderman, and F. W. Sunderman, Jr. (Pp. xvii + 592; illustrated. £12.60.) St. Louis, Missouri: Warren H. Green, 1970. London: Adam Hilger Ltd. 1971.

This book contains the edited proceedings of an applied seminar held under the auspices of the Association of Clinical Scientists in March 1970. The book is divided into four parts: 'General toxicological considerations', 'General methodological considerations', 'Specific toxic agents', and 'Clinicopathologic considerations'. Fifty-five papers are presented over a very wide toxicological field. Papers range from mode of action of poisons, metabolism, toxicity of food additives