Radioimmunoassay in Clinical Biochemistry

Edited by C. A. Pasternak

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Department of Biochemistry
University of Oxford

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FOREWORD

This volume contains a selection of articles written by leading workers in the field of clinical radioimmunoassay, an assay technique which is rapidly becoming one of the most important and most widely applicable methods of estimation in medicine. Consequently the book has a broad coverage as well as much technical detail and it should prove a most useful guide for clinical biochemists and immunologists wishing to use these methods in hospital and research laboratories.

June 1975

R. R. PORTER, F.R.S.
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PREFACE

This collection of articles is based on the papers presented at a Symposium on Radioimmunoassay and Related Topics in Clinical Biochemistry, which was organized by LKB Instruments Ltd., and which was held in Oxford from 24–26 September 1974. The success of the meeting indicated the need of clinical research workers for an up-to-date summary of the various techniques currently in use. This volume has been prepared to meet that demand. New applications and variations are presented, together with some of the pit-falls that may occur; the topics range from automation and costing of assay procedures, through mechanisms of hormone action, to the problem of drug abuse. The book is therefore likely to appeal to a wide audience of students and research workers unfamiliar with the field, as well as to clinical biochemists engaged in the day-to-day use of radioimmunoassay. The articles appear virtually as submitted, with minimal editorial changes. It is hoped that what they therefore lack in uniformity of style and presentation is compensated for by a freshness and individuality of approach.

To Dr. P. Knox thanks are due for preparing the Index at exceptionally short notice.

August 1975
Oxford

C. A. PASTERNAK

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PART I

General Methodology

RADIOIMMUNOASSAY DESIGN

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ABSTRACT

The fundamental principles of radioimmunoassay design have for many years been controversial. Accepted wisdom in the field dictates that assay should be set up using an amount of antibody to bind roughly 50 per cent of a vanishingly small amount of tracer.

The fallacious grounds for this frequently misleading belief is briefly discussed, and its replacement by a more rationally based assay design concept described. The latter entails numerical computer optimization techniques for which simple programs, intended for wide distribution and easy implementation, are currently being written.

Preliminary results derived by the use of these techniques reveal that, under certain conditions, assay optimization requires the use of assay protocols differing fundamentally from those which are now conventional. For example, in certain methods characterized by low 'misclassification error', greatest sensitivity is achieved by delaying addition of a *large amount* of tracer ligand until shortly before termination of the reaction.

In general, one of the most important determinants governing the design of an optimal assay system is the form of the relationship between the error in the response metameter and the magnitude of the response. Although the importance of the relationship has seldom been stressed, and is rarely reported in RIA literature, information regarding this parameter is vital, not only to optimal assay design but also to the evaluation of the effect of different experimental stratagems, and in the comparison of different assay methods.

INTRODUCTION

Since the inception and earliest use of saturation or radioligand assay techniques (Yalow and Berson, 1959, 1960; Ekins, 1960), there has been continuing disagreement between the two groups regarding the theoretical principles governing assay design (Ekins, 1969; Ekins *et al.*, 1970). This controversy may have bewildered many in the field since both laboratories have shown themselves capable of setting up assays of high sensitivity, frequently using assay protocols which have not been grossly dissimilar.

It is hardly surprising that this should be so, since the basic approach to the choice of reagent concentrations, times of incubation etc. to yield working assays of high sensitivity is reasonably obvious. It appears self-evident for example, that, in setting up a conventional radioimmunoassay, one should use an amount of the radioactively

labelled ligand which is small in relation to the amount of the unlabelled material that one wishes to measure. Moreover it also appears obvious that, since the assay response depends on the final distribution of radioactivity between two fractions, the amounts of activity in each should be roughly equally balanced, to obviate the large errors that might arise in the measured distribution if activity were predominantly in either the free or the bound fraction. Clearly such precepts constitute a good, commonsense, approach to assay design, and it would be notable if assay protocols in conformity with these principles were not to yield assays of acceptable sensitivity and precision.

We have tried to show that the theoretical work of Yalow and Berson (1959, 1960) was based on false premises, employed fallacious arguments, and yielded assay protocols which showed differences in detail from those yielded by a more rigorous and conceptually valid approach. More importantly, it is now evident that in certain circumstances, optimal assay protocols may in fact differ very significantly from those which appear at first sight to be self-evident, and for which the theoretical analysis of Yalow and Berson (1959, 1960) has provided an apparent validity.

OPTIMAL ASSAY DESIGN

The purpose of this presentation is to reveal the inadequacy of the conventional approach to the choice of assay reagent concentrations and other design parameters, and to illustrate how assays differing markedly in design from the norm may, in certain circumstances, be superior.

What is an optimal assay system? Since the aim of any assay technique is the measurement of an unknown amount of some substance with maximal precision, an optimal assay design is clearly one which achieves this end in minimum time.

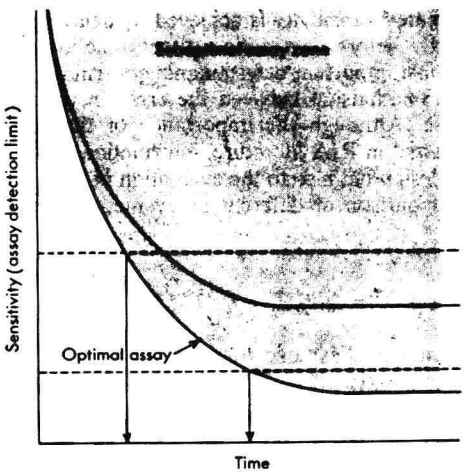

Fig. 1. Definition of optimized assay systems, optimized with respect to assay sensitivity. The optimal assay curve shown defines the maximal sensitivity achievable within a given overall assay time. Note that the composition of the optimal assay system will change for different overall assay times. An individual, sub-optimal assay system will yield a sensitivity/incubation time curve falling to the right of the curve linking optimal assay systems. A given level of sensitivity is achieved in a shorter time in an optimized system and levels of sensitivity are attainable which cannot be achieved using a sub-optimal design.