Methods of Enzymatic Analysis

Third Edition

Editor-in-Chief: Hans Ulrich Bergmeyer
Editors: Jürgen Bergmeyer and Marianne Graßl

Volume X
Antigens and Antibodies 1

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VCH

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Volume VII	Metabolites 2: Tri- and Dicarboxylic Acids, Purines, Pyrimidines and Derivatives, Coenzymes, Inorganic Compounds
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Volume IX	Proteins and Peptides
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Volume XI	Antigens and Antibodies 2
Volume XII	Drugs, Pesticides

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Preface to the Series

"Methods of Enzymatic Analysis" appeared for the first time in 1962 as a one-volume treatise in German. Several updated and improved editions in English and German have been published since then. The latest English edition appeared in 1974.

In the meantime, enzymatic analysis has continued to find new applications, refinements and extensions at a pace that justifies – indeed, demands – the preparation of a new and completely revised edition. However, the field has grown so enormously that it can no longer be surveyed adequately by one person. Fortunately, therefore, I am supported in this new enterprise by Dr. M. Graßl, who is highly experienced in biochemical analysis, and Dr. J. Bergmeyer, who represents the younger generation of biochemists.

With the 1974 edition of "Methods of Enzymatic Analysis" as a starting point for our work towards the new edition, it soon became obvious that many chapters had to be eliminated, re-written or added. Moreover, the increased number of analytes that can now be determined enzymatically and of enzymes regularly requiring analysis, especially in the clinical laboratory, together with the emergence of an entirely new field of application through the technique of the enzyme-immunoassay, demanded a new arrangement and subdivision of the contents, if the vast range of material was to be dealt with properly and lucidly.

The result is the plan of the work printed on the page opposite the title page of this volume. Of course, it would be impossible to publish a whole series such as this at one moment and still maintain an equal degree of topicality for all contributions. Therefore, we decided to produce the series at a pace of several volumes per year. The volumes will not necessarily appear in their numerical order, but will be made available as they can be planned and completed.

As before, the purpose of the work is to provide reliable descriptions of well-developed procedures of enzymatic analysis in the broadest sense of the term. Special efforts are being made to arrange every chapter, and to co-ordinate the contents of all chapters, in such a way that the volumes are useful as laboratory manuals for daily work.

Internationally-agreed enzyme nomenclature as well as quantities and units correlating with the "Système International d'Unités" are used wherever possible in order to make statements and data unambiguous and comparable over time and space.

All contributions are and will be written in English: however, contributors come from all over the world and their manuscripts naturally show various versions of English. These have to be harmonized in style and spelling in order to achieve uniformity throughout the series without, we hope, entirely eliminating each author's personal approach. Professor Donald W. Moss has kindly agreed to undertake this task. We agreed with him to use modern English spelling, but to try to minimize differences between British and American practice. We hope that this will be considered

as a fair solution and one which will make the series accessible to as wide a readership as possible.

Thanks are due to the authors in the first place for responding so readily to our invitations, for writing their chapters so diligently within a short time and for communicating their experience and expertise. We are also indebted to all colleagues who gave their advice and to Professor Moss for accepting the task of language editor. Finally I wish to record my gratitude to Verlag Chemie for the fruitful and excellent co-operation during all stages from planning to production.

Tutzing, February 1983 Hans Ulrich Bergmeyer

Preface to Volume X

This volume is the first of two which provide working instructions for the determination of antigens (proteins, viruses and micro-organisms with antigenic properties) and antibodies. There is a great demand in clinical, virological, bacteriological, veterinary and other laboratories for reliable and reproducible methods for the detection and quantitation of these complex biological entities. The rapid development of enzyme-immunoassay (EIA) techniques has made available specific and convenient procedures for the detection and measurement of these analytes many of which, until recently, could only be traced by elaborate biological assays.

Enzyme-immunoassays already cover an immense field, and one which is still rapidly expanding. Therefore, contrary to the goal of most volumes of this series, it was impossible to describe the assays of every possible antigen and antibody. Rather, our ambition has been to provide model systems from different areas of biology, or from human or veterinary medicine. In this way, the reader who does not find instructions for the solution of a specific problem will nevertheless find related assays offering relevant information.

The selection was guided by several criteria: the scientific importance of the antigen or the corresponding antibody, its practical importance, special methodological features of the assay and, last but not least, availability of experienced authors.

Again, the sequence of chapters follows the chemical nature of the analytes or their function. In some cases the placement may be debated: as an example, we decided that the chapter Thyroglobulin and Antibodies is more appropriately placed with "Transport Proteins" in Volume IX than under "Allergic and Autoimmune Diseases" in Volume X. As a result, all parameters the determination of which may be of interest in thyroid diseases are described in one volume. The same is true for Collagen, Complement Component C5a Antigen and Sex-Associated Antigen: they are less related to Allergic and Autoimmune Diseases (this volume) than to Structural and Regulatory Proteins (Volume IX).

To our knowledge, this is the first collection of methods of this kind that has ever been presented to facilitate diagnosis in allergic and autoimmune diseases, as well as in viral diseases (Parts 3 and 4). The chapters in Part 2 are particularly useful for investigating the immune status of a patient.

Most of the assays described in this volume are clinically orientated and are also applicable in epidemiology. However, clinicians and epidemiologists use the terms "sensitivity" and "specificity" with meanings that differ considerably from those implied by analytical chemists. In analytical chemistry, "sensitivity" refers to the ability of an assay to distinguish different levels of the analyte, while "specificity" is the ability to measure the analyte in the presence of possibly interfering substances. In clinical usage, e.g. when screening a population, sensitivity is the ratio of the number of people suffering from a disease and found to do so by the assay, to the total

number of people suffering from the disease (as determined by other methods). Specificity in the clinical sense is the ratio of the number of people who are in fact free from the disease and giving a negative test, to the total number of people who are in fact free from the disease.

When using immunological techniques, the analyst is confronted with the problem of how to characterize the reagents, which include antibodies and enzyme-antibody conjugates. This topic is discussed in Part 1 ("General"), which also contains chapters on Antigen- and Antibody-Secreting Cells, and on the advantages and drawbacks of using polyclonal and monoclonal antibodies. We have made every attempt to get as much information as possible from authors and manufacturers about the composition of immunological reagents and the concentrations of their constituents. Unfortunately, we have not been successful in every case.

We assume that in the foreseeable future the number of enzyme-immunoassays of antigens and antibodies will continue to increase. This volume is intended to stimulate the application and improvement of these methods for routine use, and the development of similar methods for other analytes important in the life sciences.

Thanks are due to the authors for their fine co-operation in the preparation of manuscripts, and again to the editorial consultant, Professor R. F. Masseyeff.

Tutzing, January 1986 Hans Ulrich Bergmeyer

Contents of Volumes I – XII

(Chapter Headings only)

Volume I
Fundamentals

Volume II
Samples, Reagents, Assessment of Results

Volume III
Enzymes 1: Oxidoreductases, Transferases

Volume IV
Enzymes 2: Esterases, Glycosidases, Lyases, Ligases

Volume V
Enzymes 3: Peptidases, Proteinases and Their Inhibitors

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Metabolites 3: Lipids, Amino Acids and Related Compounds

Volume IX
Proteins and Peptides

Volume X
Antigens and Antibodies 1

Volume XI
Antigens and Antibodies 2

Volume XII
Drugs, Pesticides

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p. 175