# Methods of Immunological Analysis

Edited by R. F. Masseyeff,
W. H. Albert, N. A. Staines

Volume 3

## Cells and Tissues

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# Preface to the Series

Analysis based on the principle of the specific reaction between an antigen and its complementary antibody is now a major technique in all fields of biological and chemical science; in human and veterinary medicine, in environmental science, and even in plant physiology and pathology. The inherent advantage of immunological analysis, its specificity for the target analyte with its consequences of minimum sample preparation and sensitivity, is so great that it is now almost impossible to ignore the possible use of this technique in any application requiring the detection or measurement of organic molecules.

Immunological analysis has its roots in the 19th century, in the agglutination reactions of red blood cells or bacteria, and in immune precipitation and complement fixation reactions. While these principles are still in use, their further development was limited by the low sensitivity of immune precipitation and the insufficient reproducibility of agglutination and complement fixation. The explosive growth of immunological analysis in the second half of the present century is the result of advances in fundamental understanding of the immune response and of the molecular nature of the antigen-antibody reaction, coupled with practical developments such as the ability selectively to introduce a signal-generating label into antigens or antibodies without affecting their immunological reactivity. Of particular relevance to immunological analysis, fundamental research has revealed the fine structure of the binding sites of antibodies, progressively disclosing the molecular basis of their astonishing variability and providing a clearer understanding of the determinants of specificity. Research has also demonstrated the importance of correct presentation of a potential antigen to the antibody-producing cell, while developments in organic synthesis, especially of peptides, have greatly expanded the range of putative antigens. The development of monoclonal antibodies has provided an almost limitless range of reagents of clearly defined specificities, capable of further refinement through genetic engineering. Great technical ingenuity has been expended on the practical aspects of immunological analysis, in developing methods of increased sensitivity and reliability for measuring the analytical signal, and in increasing the convenience and speed of analysis through mechanization and automation.

The result of these advances has been to present the analyst, whose background may not be in immunology, with an embarrassment of riches when faced with the selection of an immunoassay to meet his particular requirements. The need for a comprehensive source of reliable, practical information on immunological analysis is apparent, considering, for example, Bergmeyer's "Methods of Enzymatic Analysis". The importance of enzyme labels in immunoassay required four volumes for its treatment in that series: nevertheless, it was clear that the much wider scope of immunoassay in

general demands its own series. Thus, "Methods in Immunological Analysis" was conceived. Its aim is to provide the analyst with a guidebook with which to set out confidently into the rich territory of immunological analysis.

The Editors retained some of the organizational features that made the success of "Methods of Enzymatic Analysis". A survey of the fundamental observations on which immunological analysis is based, namely, the nature of the immune response and physicochemical aspects of the structures of antigens and antibodies and their interaction, is followed by discussion of the strategies available in immunoassay. Tested and reliable methods for a wide range of analytes are described in a detailed and rigorously specified format that is intended to provide working protocols that obviate the need for reference to other sources. Furthermore, informed by the principles of analysis set out in the series, the analyst should be able to modify the protocols where necessary to meet his individual needs. In addition to this "cook-book" approach, the series contains papers on the various applications of immunoassays in different fields, with emphasis on medical aspects. The place of immunological assays in the context of other methods is discussed for each group of assays. Chapters on method comparison provide additional guidance. Special problems in standardization, disclosed by results of quality assessment surveys, are covered in specific papers. The editors' aim is not only to provide recipes for the assay of a variety of analytes, but also to assist the reader in the selection of the most suitable method for his needs, and thus to provide him with the knowledge needed for an optimal use of immunological methods and their interpretation.

Immunological analysis exploits the specificity of the reaction between differently-specific antibody molecules (possibly as many as ten million) and their ligands. The results of immunological analysis are method-dependent. This, however, is not well appreciated, even by experienced immunoassayists. Such problems in immunological analysis derive from the incomplete characterization of the reagent antigens and antibodies as well as from matrix effects, and lead to problems of standardization and comparability of results. The introduction of monoclonal antibodies in many applications has improved the reproducibility of immunological reagents, but formidable problems of standardization remain, and are fully discussed in this series.

As well as striking an appropriate balance between fundamental principles and practical descriptions, the Editors have endeavoured to make a systematic selection from the vast array of methodological principles and variations that are now represented in immunological analysis. This situation is rendered even more complex by the changing balance of method development, from the universities and research institutes towards industry, often with consequent patent protection and restricted availability of certain reagents. Fortunately, however, many procedural variations are of a minor nature, dictated in some cases only by the need to establish commercial novelty. By emphasizing the modular nature of assays and their classification in terms of the analytical principles involved, and by avoiding confusing abbreviations and acronyms, the Editors have attempted to bring some order into the potentially bewildering array of available methods.

The analytes for which assays are described have been chosen for their importance, e.g., the frequency with which their measurement is required, and also for their

suitability for the demonstration of particular analytical approaches. Several types of methods are available for certain analytes with a long history of biomedical importance, such as a variety of drugs or hormones. In such cases alternative, reliable methods have been described so that an intending user should be able to select the one that is most appropriate to his available resources. However, less well-known methods and those which demonstrate original analytical principles have not been neglected. Methods subject to patent protection, or offered in the form of commercial reagent kits, have not been excluded, provided that the nature and composition of their reagents is disclosed in sufficient detail for them to be repeated with readily available substances. Authors of methods which depend on specific immunological reagents have been asked to state whether these reagents can be provided to intending users.

Throughout, the Editors' aim has been to provide a comprehensive handbook of value to the specialist and non-specialist alike.

All contributions are written in English. However, since the authors, as well as their potential readers, come from all over the world, a uniform style of grammar and spelling has been adopted to provide readers with a consistent and unambiguous text. Modern English spelling has been chosen which minimizes the differences between English and American usage, in the hope of making the volumes readily accessible to the widest possible readership.

The Editors' grateful thanks are due to the authors who placed their specialist knowledge so freely at the service of their colleagues, and who patiently accepted the editorial requirements of the series. The ultimate success of the volumes is entirely due to their willing cooperation. The Editors owe a special tribute to Professor H. U. Bergmeyer and to Professor D. Moss for contributing their immense experience. They are also grateful for the expert support of VCH, especially of Mrs. N. Banerjea-Schultz, while the technical assistance of L & J Publikations-Service has greatly facilitated the rapid production of the volumes.

Nice, July 1992 René F. Masseyeff

# Preface to Volume 3

The detection and quantification of the component molecules of cells require an approach subtly different from that used to deal with analytes already present in solution. This volume, the Third in the "Methods of Immunological Analysis" series, is concerned with the approach to using immunological methods for the analysis of cells and tissues. The constituents of cells naturally do not exist or function in isolation of each other, and it is the very intimate relations these molecules have that makes their analysis such a challenge. Many, probably most, are inaccessible in normal circumstances, hidden by lipid membranes or inside organelles; others are relatively insoluble or buried beneath other molecules. So, in order to exploit the exquisite specificity of antibodies to analyse cells, the component analytes, for some purposes, have to be revealed or separated from their neighbours for accurate assessment. This is the starting point of this Volume.

In the first sections, the methods necessary for the isolation of cells from many different tissues are described. The emphasis is upon human cells, but many references are made to animal cells in acknowledgement of their importance in experimentation. Throughout, the common principles essential to handling cells from many varied sources are stressed.

The culture of cells, over both long and short periods, is an essential component of many experiments. Indeed, the availability of well-characterized and standardized cell lines has been central to the elucidation of cell structure and function. Thus, methods of tissue culture are dealt with here, not only with regard to mammalian cells but also those from arthropods and plants which both enjoy a new prominence in contemporary research. The propagation and characterization of bacterial cells are covered too. The importance of eukaryotic cell clones is exemplified by a section on the T-lymphocytes of the immune system which, because of their great heterogeneity of specificity and function, are ideal candidates for cloning. Because B-lymphocyte clones have been used extensively to make monoclonal antibodies, these were dealt with in Volume 2 of the series.

Aside from cell cloning, there are many ways in which cells can be physically separated from each other, and the systems involving gradient centrifugation, panning and magnetic beads are discussed critically. In a related way, the separation of sub-cellular fractions and components is necessary in many studies, and this too is discussed.

Using standardized immunoassay methods it is possible to quantify the amount of particular analytes in cell extracts, assuming of course a quantitative recovery in the extraction process. However, there are many situations where it is desirable to quantify the analyte *in situ* and to define precisely its subcellular location. For reasons mentioned

earlier, there may be considerable problems in this but there has been much encouraging progress through the development and application of methods of, for example, image analysis, confocal microscopy, flow cytometry and immunocytochemistry. The importance and versatility of these technologies are dealt with in critical detail.

An important aspect of immunological analysis of cells is the use of antibody-based methods in immunocytochemistry. In a a major section, the various approaches to this most central of cell biological methods are described and their comparative uses and performance discussed. Precise protocols are given in the style established in Volume 2 of this series: the common features of immunocytochemical methods and their modular nature is emphasized and exploited in a step-by-step approach to setting up and applying them. These now can be used to localize with some precision the target antigens in cells and tissues when examined under light or electron microscopy, but the methods do not yet approach the quantitative accuracy of conventional immunoassays for soluble analytes. The reasons for this are discussed and the limitations of the cytochemical approach dealt with critically.

Immunocytochemical methods have found wide application in many areas of cell biology, and an authoritative commentary is provided on this by several different authors. The ways in which immunological methods have greatly facilitated the analysis of cells and tissue organization are exemplified by their use in unravelling the development of lymphocytes. Immunological methods have, however, been central to dissecting cell organelles and structures as well, and the ways in which immunochemical and immunocytochemical methods have been used to investigate nuclear and cytoplasmic structures are described so that they will enable the interested reader to undertake analysis of any tissue, cell or organelle by similar approaches.

Much of the value in the application of immunological methods has been in the study of cell phenotype, which in essence reflects the structural organization of the cell. From this can be inferred, for example, the means and circumstances of the regulation of gene expression. It is only now, however, that immune methods are applied successfully to analyse cell function. Two major approaches that are described critically here have been developed for primarily immunological purposes, but they clearly have applications in many other areas. The first of these is the ELISPOT method that allows the enumeration of cells secreting any particular molecule – an antibody, hormone or cytokine for example. This has great potential to be applied in many circumstances. The second is the use of immunocytochemical methods to identify cells making soluble secretable molecules such as cytokines. Here as elsewhere comparisons are made with other methods, in this case the use of nucleotide probes and PCR methods, in order that the value of immunological methods can be fairly appreciated. Judging from progress in developing these methods in recent years, there is justified optimism that they will be refined to the point where they will provide accurate quantitative data. Without doubt, other methods will be derived from them.

The title of this particular volume of this series might imply that it is devoted to the analysis of immune cells. As this account makes clear, this is not the case. Certainly, immune cells are mentioned in many places, but largely because they are the object of study by cell biologists in general, and not immunologists in particular. The criterion for inclusion of a particular approach or method here has been that it uses an immunological

probe, almost always an antibody, to examine a cell. The last part of the volume nonetheless considers the ways in which immune cells can be analysed by methods conventionally understood by immunologists, but not necessarily using antibodies. The ways in which immune cells are examined nowadays would provide material for many volumes beyond the size and scope of this: here we confine ourselves to our brief to explain how immunological methods can be put to work in cell biology. Discussion and commentary form vital parts of the volume, but they are only the support for its main purpose which is to give precise laboratory protocols that can be established by the non-expert and applied without reference beyond this volume.

Many authors have contributed to this volume and to them, the editors express their sincere thanks. As in the other volumes of the series, the excellent help and cooperation of Professor H. U. Bergmeyer and Professor D. Moss have been especially valuable and appreciated.

March 1993 The Editors

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