Laboratory Methods in Immunology

Volume II

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Editor

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PREFACE

Some of the most exciting advances in biology, biotechnology, and medicine in the last 10 years have been in immunology or have used immunological techniques. Biologists, from neuroscientists to plant pathologists, from cancer researchers to toxicologists, need to know how to make and use monoclonal antibodies, how to purify proteins on immunoaffinity columns, and how to stain tissue with specific antibody. While biologists from other disciplines have been forced to embrace immunology (often wearing an expression betraying their distaste for the subject), immunologists have needed to acquire at least the language, and often also the technology, of molecular genetics.

These two volumes were planned to contain cookbook-style recipes for immunological techniques, interspersed with reviews on the strategies required, and the choice of techniques. This volume and its companion contain detailed methods and reviews, written by people who are actually using the methods and written to be used at the bench. As the editor, I have had the opportunity of seeing these chapters early, and I have found several of the chapters useful in my own work already.

Volume 1 contains chapters on tissue culture and hybridoma technology, intended to extend and update my book entitled *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc., Boca Raton, Florida, a series of chapters on lymphokines and functional assays *in vitro;* aspects of molecular biological techniques most clearly related to immunology; and immunochemical techniques. Volume 2 starts with a review of techniques used with small laboratory animals and continues with chapters on more specialized procedures with animals. Antigen detection in cells and tissue is covered in the next section, followed by a section containing chapters on the important area of protein purification using monoclonal antibodies. This section includes chapters by several workers with successful experience of affinity purification on an industrial scale.

The authors were all invited to contribute because they were known by me or by members of the Advisory Board to be successful in using their particular technique. Difficulties in setting up published techniques are the bane of many a biologist's life; authors were asked to include *all* important details and even details which just *might* be important, with the aim of providing techniques which do transfer well from one laboratory to another.

THE EDITOR

Heddy Zola, B.Sc., Ph.D., is Chief Hospital Scientist in the Department of Clinical Immunology, Flinders Medical Centre, and Professor of Immunology in the Flinders University of South Australia. He received a Bachelor of Science degree (with Honours) in Chemistry from the University of Birmingham in England in 1962, followed in 1965 by a Ph.D. degree in Biophysics from the University of Leeds in England.

Following his Ph.D. work, Dr. Zola carried out research in the Department of Biophysics at Leeds University and subsequently in the Departments of Biochemistry and Protein Chemistry at the Wellcome Research Laboratories, Beckenham. At that time, his research was principally on the physical properties of large molecules in solution, but he became interested in immunology and transferred to the Department of Experimental Immunobiology at Wellcome in 1975. In 1978 Dr. Zola came to Australia to take up an appointment as Chief Hospital Scientist in the Department of Clinical Immunology at the Flinders Medical Centre, a teaching hospital and research centre in Adelaide, South Australia, and was subsequently appointed Professor at the Flinders University of South Australia.

In the last few years, Dr. Zola's research has concentrated on the use of monoclonal antibodies, flow cytometry, and cell culture to study the differentiation and function of human B-lymphocytes. He is the author of *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc., Boca Raton, Florida, and over 170 research papers.

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ACKNOWLEDGMENTS

This book started from my conviction that there is a need for more books which allow fully detailed descriptions of methods. Books of this type are usually to be found on the laboratory bench rather than on the shelves. Translating this belief into hard copy involved persuading a large number of people to help. They all shared my enthusiasm for this project, at least to a degree, but for all of them to get involved meant giving up a considerable amount of time and putting in a great deal of work. I greatly appreciate the efforts of the authors and the members of the Advisory Board. For the publishers, this book has been developing at a time when their group was undergoing considerable change. The courteous and efficient handling of the manuscript by Janice Morey, Coordinating Editor at CRC Press, and more recently by Carolyn Lea, has made my task easier and more enjoyable than I expected. Mary Brown has as usual provided the efficient secretarial support which is essential to anyone trying to write or edit a book. I would also like to thank two scientists, who, while not members of the Advisory Board, helped me greatly with expert comment on particular subjects — Dr. Keith James of Edinburgh University and Dr. Pam Sykes of Flinders University of South Australia.

LABORATORY METHODS IN IMMUNOLOGY

VOLUME I OUTLINE

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Animal Work, *In Vivo* Studies

Chapter 1

BASIC TECHNIQUES WITH EXPERIMENTAL ANIMALS

John G. Aaskov, David J. Allan, and Judith K. Blackshaw

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I. INTRODUCTION

Animals have been used in medical research at least since Galen (129—199 A.D.) a Greek medical scientist, used a pig for his experiments. Because of the difficulties associated with obtaining human cadavers in the Middle Ages, many of the early anatomic dissections were also carried out on domestic animals. Fundamental physiological studies have employed animals as diverse as frogs (Galvani, 1791) and dogs (Pavlov, 1900+). In more recent years, there has been interest by the community at large in the use of experimental animals in research, teaching, and industrial institutions. This has led to the preparation of "guidelines" and, in some instances, legislation to regulate animal experimentation. While this may have been irksome to some, it has helped scientists design safer, less stressful, and, often, more statistically significant experiments. Before using any animals, investigators should familiarize themselves with all institute, state, county or national guidelines and legislation pertaining to the use of experimental animals. Some sources of such information are

1.	Australia:	National Health and Medical Research Council, Canberra
2.	Canada:	Canadian Association for Laboratory Animal Science
3.	Germany:	Gesellschaft für Versuchstierkunde
4.	Japan:	Japan Experimental Animal Research Association
5.	Scandinavian countries:	Scandinavian Federation for Laboratory Animal Science
6.	Spain:	Sociedad Espanola de Experimentacion Animal
7.	UK:	(1) Laboratory Animal Science Association, the representative national scientific society; (2) Research Defence Society, for legal problems related to the use of experimental animals in the UK; (3) Institute of Animal Technicians
8.	U.S.A.:	American Association for Laboratory Animal Science

It should also be remembered that regulations and guidelines pertaining to the use of toxic or infectious agents in a laboratory also apply in an animal house. This is of particular importance where routine animal care is performed by staff who may not be familiar with the organisms or agents being used. CAGES SHOULD ALWAYS BE CLEARLY AND EXPLICITLY LABELED. Again, it is the responsibility of the scientist to obtain all safety clearances before beginning animal experiments.

Once familiar with the guidelines and regulations pertaining to use of experimental animals and having obtained the necessary licenses, ethics approval, etc., the scientist should develop a working understanding of good husbandry. Although most facilities have trained staff to care for animals, it is in a scientist's own interest to ensure animals are not stressed by overcrowding, poor ventilation, lack of, or incorrect, food, lack of, or stale, water, excessive noise, etc. It is also important (mandatory for some granting bodies) that the genetic backgrounds of animals are checked regularly even if the animals are purchased from a commercial supplier.

II. EQUIPMENT

A. GENERAL

While there are many specialized items of equipment which can be purchased to aid handling of animals, the most common procedures (injections and bleeding) in the most frequently used animals (mice, rats, and rabbits) can be performed with four pieces of equipment which can be constructed in the laboratory.