

**MANUAL OF  
CLINICAL  
IMMUNOLOGY**

# MANUAL OF CLINICAL IMMUNOLOGY

EDITED BY

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## PREFACE

This *Manual* represents a joint effort by members of the American Society for Microbiology (ASM) and the American Association of Immunologists (AAI) to provide medical scientists and physicians interested in clinical immunology with a guide to the rapidly growing field of laboratory immunology. The *Manual of Clinical Immunology* developed as a natural extension of the *Manual of Clinical Microbiology*, now in its second edition as a highly successful publication of the ASM. The first edition of the *Manual of Clinical Microbiology* devoted approximately one hundred pages to various serological tests. The second edition, published in 1974, had very little enlargement of the section on immunological diagnosis, since it was felt that coverage of more than the barest essentials of this rapidly evolving field would require a marked increase in the number of pages. Thus, a companion *Manual of Clinical Immunology* was conceived to deal exclusively with applications of immunology for detection and analysis of a wide variety of diseases, not only those induced by microorganisms.

This *Manual* is directed mainly to laboratory directors and technologists who are responsible for the performance of immunological tests in the clinical laboratory, as well as to graduate students, medical students, post-doctoral trainees, residents, fellows, and clinicians. It is anticipated that the *Manual* will provide authoritative information about the best methods for conducting specific immunological tests. Clinical interpretations of each procedure are also provided, along with a discussion of pitfalls and problems in performing the various analyses.

The organization of the *Manual* is generally similar to that of the *Manual of Clinical Microbiology* in that there are sections dealing with different aspects of immunology with sufficient breadth and background to provide the necessary foundations for clinical studies. The subject matter encompasses laboratory tests designed to measure both humoral and cellular immunological responses of patients but excludes the description of immunological reactions devoted exclusively to identification of microorganisms such as bacteria and viruses. These procedures are covered in the *Manual of Clinical Microbiology*. Thus the emphasis of this *Manual* is on currently used laboratory procedures which aid in the diagnosis not only

of diseases caused by microorganisms, but also other pathological conditions such as autoimmunity, immediate and delayed hypersensitivity states, and malignancy. Analyses using antibodies as specific biochemical reagents are also described. Tests conducted directly on patients are included only if they seem pertinent to laboratory examinations or to the broad area of immunological diseases.

The methodologies described in this *Manual* are presented in a manner to be understandable to laboratory personnel in appropriate positions in academic, community, and general hospitals. Descriptions of laboratory tests are given in sufficient detail that a skilled technologist can perform the procedures without going to other references. Step-by-step methods are given wherever possible. In those areas where a number of different techniques are available, the procedures employed by the authors are described in detail. However, brief discussion is included of other methods, pointing out how and why these methods may differ. All authors have been instructed to keep their descriptions of methods as brief as possible, but to indicate the initial ingredients needed for a test rather than merely describing commercially available reagents or kits. Furthermore, when necessary or available, a number of different vendors or sources for reagents are given. The authors have been requested to point out those vendors or sources of reagents they have used personally.

An early organizational decision taken by the editors and editorial board was that the *Manual* should not provide exhaustive reviews of the literature of clinical immunology. References and citations are used mainly for illustrative purposes. In general, each chapter lists only major reference articles and reviews.

The editors wish to thank the more than one hundred authors for the high quality of their chapters and for accepting the peer review procedure established for the *Manual*. The editors are greatly indebted to the section editors who undertook a very heavy commitment of time and effort. The cooperation of a liaison committee of the AAI, consisting of Dr. Mart Mannik as Chairman, Dr. Robert Schwartz, and Dr. John Fahey, is also appreciated, as are the contributions of the Council of the AAI and the Publications Board of ASM in supporting this endeavor. Mr. Robert A. Day, Managing Edi-

## PREFACE

tor, and the ASM Publications Office staff are to be commended for their patience and cooperation.

It was anticipated by the two sponsoring societies that this *Manual* will fill an important need of various medical specialties and laboratories, including those involved in immunological studies outside the narrower sphere of infec-

tious diseases. Revised editions may well be required every few years, especially since the technology used in immunological laboratories is changing at a very rapid pace.

**Noel R. Rose, M.D., Ph.D.**  
**Herman Friedman, Ph.D.**  
*April, 1976*

# INTRODUCTION

## Clinical Laboratory Immunology: Review and Preview

NOEL R. ROSE AND HERMAN FRIEDMAN

It is the purpose of this *Manual*, as indicated in the Preface, to present information concerning the specific method and rationale for the performance of a wide range of immunological tests that are directly related to patient care. Laboratories devoted to clinical immunology are being established at a very rapid rate in many hospital and medical centers. Historically, many of these laboratories evolved from a serology service as a section of a clinical microbiology laboratory. However, developments in immunology during the last decades have resulted in specialized procedures that are unrelated to microbiology. For example, laboratories devoted to specific clinical applications of immunology, such as tissue typing or tests for autoantibodies, are sometimes administratively part of some other laboratory division or a clinical department outside of the traditional pathology laboratory setting. Many of these specialized laboratories are maintaining their identity and even increasing their scope of operations. It is apparent that clinical immunology is not only a rapidly growing component of laboratory medicine but also an essential ingredient of many areas of clinical medicine. Therefore, some of the traditions associated with other clinical laboratories are either missing or not appropriate. There are very few guidelines at present concerning the establishment of a comprehensive clinical immunology laboratory. Thus, those investigators who wish to do so often have to strike out in new directions.

Historically, laboratory immunology was one of the first clinical laboratory specialties established in medical centers, hospitals, and universities. Long before clinical chemistry became a separate discipline and before anatomical or surgical pathology evolved into important specialties, laboratories were established to carry out the immunological diagnosis of infectious diseases. By the end of the last century, many bacteriologists were offering their services to physicians in providing serological tests for identifying and quantitating bacterial antibodies in patients' sera. They performed Widal tests for typhoid antibodies and a battery of other agglutination tests for monitoring de-

velopment of an immunological response to pathogenic microorganisms. The complement fixation test, initially employed by Wassermann and his colleagues to detect antibody in patients who were infected with *Treponema pallidum*, was widely performed early in this century. Within a short time such complement fixation tests for syphilis became the "work horse" of laboratory immunology. Complement fixation was adapted to the detection of serum antibodies to a wide variety of microorganisms, especially rickettsiae, fungi, and viruses, as well as protozoa and helminths.

Until the modern era of immunology started in the 1960s, most of the work of the serology laboratory continued to deal largely with antimicrobial immunity. Once it was recognized that immunological phenomena are involved in diverse human diseases, as well as in transplantation rejection and tumor progression, clinical immunology laboratories evolved rapidly. Immunofluorescence was introduced in the 1950s, along with other antibody- or antigen-tagging methods using radioisotopes or enzymes. The development of indirect or passive agglutination procedures, including latex fixation and hemagglutination, and a wide variety of techniques designed to measure or quantitate cell-mediated immunity *in vitro* followed in short order. The application of these newer immunological techniques has extended immunology very far indeed from the early days of testing for antibodies to microorganisms. For example, serological and mixed leukocyte reactions for transplantation immunology have provided methods necessary not only for matching prospective donors and recipients for organ grafting, but also for giving important new information concerning immunogenetic relationships and immunological competence. These and similar tests are also being presently exploited for detection of tumor immunity. Precise measurement of immunological capabilities of patients with malignancy as well as patients suspected of having autoimmunity, congenital or acquired immunodeficiency, or even long-standing viral infections is now becoming widely practiced.

A major question often asked by those who wish to establish a clinical immunology laboratory is: "What type of tests should we perform?" There is no general answer to this question until an institution decides what type of patient population must be served in terms of immunological diagnosis.

The scope of a clinical immunology laboratory is generally based not so much on the availability of laboratory tests but more on the type of patient care that a specific institution offers. For example, in a general medical center dealing with a diverse patient population, it is important for a laboratory to have capabilities in the area of antimicrobial immunity as well as many tests dealing with immunopathology, including autoimmune and hypersensitivity diseases. An institution that does not have an organ transplantation program does not need tissue typing or ancillary services considered mandatory for a larger or specialized center. Similarly, a general medical center with no pediatric unit would not need the same type of clinical immunology services offered in a modern children's hospital where congenital immunodeficiency diseases are prevalent. Since many physicians in general hospitals examine and treat patients with malignancy, it seems likely that the newer developments in laboratory immunology related to cancer will have to become widely available. Similarly, many immunological disorders, including immediate and delayed hypersensitivity reactions and diseases with autoantibodies such as rheumatoid arthritis or lupus erythematosus, are not rare in general medical centers, and it is essential to have available not only the techniques for detecting immunoglobulin factors which correlate with such diseases but also personnel who can properly interpret the laboratory results.

The format utilized in this *Manual* can provide a general guide in selecting the most appropriate tests. Equally important, any laboratory that offers immunological services should certainly have available trained supervisory and technical personnel who can utilize the technologies effectively. It is essential for such personnel to understand the nature of tests performed rather than to merely follow "packaged" instructions. This seems to be especially important for those medical centers where some aspects of clinical laboratory immunology have become the province of clinical chemistry or clinical pathology departments. For example, immunoelectrophoresis and gel diffusion tests for serum proteins certainly can be performed by persons with little or no training in immunology. However, when problems arise, do such personnel really understand the basic mecha-

nisms involved? Do they know that a precipitin band occurs only under conditions of optimum molecular ratios of antigen and antibody, and that some classes of antibody molecules are superior to others in producing precipitation but may be less efficient in agglutination or complement fixation reactions? Do they understand that a precipitate does not represent a static one-step reaction but is continuing reaction that is dependent upon temperature? Do they recognize that most of the reagents involved, such as agar and buffers, influence the precipitin reaction? There are many test procedures where interaction between antigen and antibody is not visualized directly but by the binding of a radioisotope. Immunoassays, especially those based on inhibition by small molecules, are commonly performed in radioisotope laboratories; these tests involve very sensitive immunological reactions. Do the people who perform these tests know why it is important to follow certain practices? For example, do they know why a test serum should not be permitted to run down the side of the solid-state immunoassay tube?

It seems apparent from the discussion above that the rapid evolution of a variety of newer immunological techniques and procedures in the clinical laboratory will result in a pluralistic system. Organizations such as the World Health Organization and the International Union of Immunological Societies have directed attention to clinical immunology as a major medical specialty and suggested the different types of laboratory services which should be available in medical schools and hospitals. However, there is no consensus at present as to where such procedures should be carried out. Until there is a "settling out" in this field, it is difficult to state categorically the best arrangement or organization for a clinical immunology laboratory. However, all scientists would agree that only techniques which have been well standardized and are properly controlled should be offered as a service to physicians and their patients. It is likely that within the next few years, and certainly before future editions of this *Manual* appear, there must be further developments in research, education, and standardization so that clinical laboratory immunology will join the ranks of other laboratory specialties in providing improved health services.

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