The Direct Detection of Microorganisms in Clinical Samples

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Foreword

When this writer began his association with the field of clinical microbiology more than 40 years ago, test tubes, petri dishes, and pipettes together with a microscope, an autoclave, and an oven for dry sterilization constituted the major laboratory equipment. Although commercial dehydrated media were available, many laboratories still prepared basic infusion media from fresh ground beef or horse meat. Color comparison with indicator solutions or papers was the usual way of adjusting pH. Electronic pH meters and spectrophotometers were available but uncommon. Anaerobic cultures were done only in relation to trauma and suspected gas-gangrene, and the techniques used were frequently inappropriate for the isolation of anaerobic bacteria. Cultures for fungi were rarely done. Viral examinations, except for rabies, existed in only a few special centers because tissue culture techniques were just being developed for virus diagnosis. Antibiotics had not been discovered and sulfonamides had been in use for only a few years. Bacteremic patients had a high mortality rate, and osteomyelitis, meningitis, pleurisy, acute glomerular nephritis, rheumatic heart disease, and other afflictions were commonplace.

Complement fixation tests for syphilis and febrile agglutination tests for typhoid and paratyphoid fever, brucellosis, tularemia, and infectious mononucleosis were performed in many laboratories. Stools were cultured for *Salmonella* and *Shigella* and examined for ova and parasites. Definitive serotyping of *Salmonella* by agglutination tests and grouping and typing of group A streptococci by precipitin tests were sophisticated procedures available in relatively few laboratories.

The advent of antimicrobic agents stimulated a need for more rapid and precise identification of pathogenic microorganisms to serve as a guide to appropriate therapy. The "cold war" years of the 1950s stimulated interest in civil defense

against potential agents of biological warfare. As a result, the fluorescent antibody techniques developed by Albert Coons were extended and applied to the detection or identification of a variety of microbiological disease agents, the goal being to obtain rapid and specific information earlier than by cultural procedures. Bacterial genetics was a new frontier just beginning to be explored by Lederberg and Tatum. In the late 1940s when a graduate student concluded her seminar by stating that "it appears that bacteria have a sexual apparatus but they don't use it all the time," many of the distinguished faculty of her department were left in shock. It was difficult for many microbiologists to accept the fact that bacteria are subject to the same mechanisms of heredity that apply to higher forms of life. Genetic analyses of microbial cells including recombinant DNA, DNA base composition, and plasmid determinations are now indispensable tools of the taxonomist. Led by W. E. C. Moore and his group at Virginia Polytechnic Institute, anaerobic bacteriology has, during the past 20 years, added an important new dimension to the services of the clinical laboratory.

The 1970s and 1980s have provided a virtual explosion of techniques for detection, identification, and quantitation of microbial agents, both in pure culture and in clinical specimens by both direct and indirect means. Rapid progress has been possible for two important reasons: (1) commercial companies have applied the technology of computers and microprocessors to the design and manufacture of instruments that mechanize or automate clinical laboratory analyses, and (2) cooperation in the design, development, and evaluation of useful equipment has existed between industrial and clinical laboratory scientists. Notable examples are gas–liquid chromatography (GLC), enzyme-linked immunosorbent assays (ELISA), radioimmunoassays (RIA), and instruments for testing blood, urine, and antimicrobic susceptibility.

Certain problems that tend to limit the application of direct detection techniques for microorganisms are discussed in this book. These problems usually involve the specificity, sensitivity, and predictive value of a given test and the amount of tolerance that is acceptable. In cost effectiveness, the most successful tests are those that can be applied in a mechanized or automated mode to large-volume clinical specimens such as blood, urine, and serum. Specimens containing mixed flora usually require more processing time because of difficult interpretation, need for isolation of individual components, or possible nonspecific reactions. The weakness of some systems of direct detection is economic, in that manufacturers of diagnostic products do not provide reagents for performing important but low-volume tests. A second problem is the poor quality of some commercial products.

The basic requirements for progress are that laboratory scientists communicate their needs to industry, that the expected volume of tests to be performed are sufficient to support the development of new equipment and reagents, and that the applications are relatively rapid and cost effective. Continued cooperation

between clinical microbiologists and the manufacturers is essential for improvement in diagnostic methodology and, indirectly, for better patient care.

The contributors to the present volume—all highly experienced laboratory scientists—have critically reviewed the most practical and widely used procedures for direct detection of microorganisms in clinical specimens. This is, however, a dynamic field that will continue to expand and evolve under the influence of the "information revolution."

William B. Cherry

Preface

Recent years have seen a steady growth in efforts in the clinical laboratory to improve the diagnosis of infectious diseases. There has been a particularly striking renewal of interest in the possibility of detecting microorganisms directly in clinical samples by identifying their soluble antigens or metabolites. Improved diagnostic methods are clearly needed, and numerous investigators have now entered the field of so-called rapid diagnosis in infectious diseases. The word rapid is not a precise one in this context, however, because many of the methods being studied are not rapid, and it is doubtful if any method could be more rapid than the time-honored gram stain. What is sought in infectious diseases is perhaps better labeled "timely" diagnosis, a diagnosis made sufficiently early that the clinician can intercede effectively with therapy or prophylaxis. Whether a test can provide timely diagnosis in a particular situation depends on many variables, including methodologic requirements of the test, the type and stage of the infection, and the nature of the therapeutic possibilities.

As older diagnostic tests are expanded or new ones are developed, it is vital that there be continual assessment of the value of these tests and that a critical attitude be maintained. This book was written to consider for the first time in a critical fashion the multiple methods being used to detect microorganisms directly in clinical samples. Applications to virology and mycology are considered as well as the generally more familiar applications to bacteriology. Interesting work is going on in all these areas, and lessons learned in one prove useful in all. Although certain of the methods discussed here may be applicable to the detection of organisms in pure culture, this subject represents a burgeoning field of research in its own right, and no attempt has been made to include it.

The book is organized into three sections. Established techniques for visualization of intact organisms in clinical samples are considered first. Chapters in

the second section deal with immunologic techniques for detecting soluble microbial antigens. Precipitin and agglutination techniques are discussed sequentially, followed by enzymatic and radioimmunoassays. In the third section, diverse nonimmunologic methods for detecting soluble constituents of organisms and their metabolites are discussed. The contents of individual chapters range from purely methodologic discussions to general critiques of the diagnostic process itself. Contributors have been selected because of their extensive personal experience with their subjects. A pragmatic and critical attitude has been encouraged, not only in the area of methodologic "nitty-gritty" but also in terms of the overall diagnostic usefulness of the methods. T attempt at a comprehensive and critically oriented review of methods for detection of microorganisms in clinical samples appears overdue. It is particularly directed at clinical microbiologists and infectious disease clinicians and researchers. Individuals working in analogous areas, such as detection of tumor-specific antigens, detection of unique cell markers, and related fields, may also find sections of interest in this book. Controversial material is presented here as such, and the reader is forewarned that different authors in this text have not always come to the same conclusions. It has not been our goal to attempt a consensus where none currently exists but, rather, to stimulate needed additional research in these areas of controversy.

We especially thank our colleagues whose interests in diagnostic methods in infectious diseases have provided the data that are the basis of this book. We thank also the staff of Academic Press for their patience and kind assistance.

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