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AUTOMATION AND MECHANIZATION IN CLINICAL MICROBIOLOGY

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Abstract - It is widely recognized that the diagnostic clinical laboratory has been revolutionized during recent years by extraordinary progress and instrumentation in automation. The microbiology and immunology laboratories have not developed until quite recently a sophisticated instrumentation and automation methodology. However, such advances are dependent on both modification and expansion of conventional techniques and on development of refined equipment to perform many of the time consuming but necessary manual operations. A variety of newer automated technologies are briefly described and discussed.

It is quite apparent that a clinical microbiology and immunology laboratory depends not only upon application of modern technology but also interpretive ability and rational decision making as to what are clinically relevant determinations. Like many of the other clinical laboratory specialties, the clinical microbiology laboratory provides a diagnostic function by evaluating results obtained with clinical specimens as well as comparing these results with expectations and knowledge about infectious diseases. Microbiology per se has been one of the most difficult of the clinical laboratory specialties to adjust to automation and/or instrumentation. There are many reasons for this. Firstly, microorganisms responsible for human diseases often require multiple testing for identification; these necessary tests are often difficult to mechanize and/or automate. In addition, the microbiologist must obtain information concerning morphology, clonal characteristics, and other features of the organism. Also, one must know about the source of the specimen, the patient's medical history and any other pertinent information which will assist diagnosis. Additionally, many specimens obtained from tissues or fluids may contain normal microbial flora necessitating the separation of these microorganisms from those which may be pathogenic or opportunistic. The identification of pathogens also usually demands considerably more information than provided by a single or even a few tests. Different methodologies are typically required to completely characterize any given bacterial isolate, including growth requirements, chemical reactivity and serologic typing.

Instruments designed to detect the growth of a microorganism or for identification cannot be made universally applicable, since growth requirements of such microbial groups as aerobic versus anaerobic bacteria as well as nonfastidious versus fastidious organisms are often quite different. In addition, among the problems complicating instrumental isolation and identification of microorganisms are that disease producing microbes in patient specimens are often present in insufficient number to insure their identification through the usual instruments without resorting to enhancement or propagation of their growth through culture. Besides this, many microorganisms replicate so slowly that they require days or even weeks rather than hours for colony formation, delaying the completion time of a given test. Because of this, much of the effort in developing instrumentation for microbiology has focused on methods for detecting organisms in a specimen that should only be sterile, such as blood, cerebrospinal fluid or urine, rather than identifying these organisms. Other instrumentation, for determining whether organisms in a positive isolate are susceptible to antibiotics has been developed, and automated equipment providing relatively rapid antibiotic susceptibility tests are now available. Finally, instruments designed to identify microorganisms by various biochemical and physico-chemical tests are now being developed and there is substantial hope that they may be found useful in the clinical microbiology laboratory.

An additional approach to identification of microorganisms or microbial infections involves the use of specific antibodies. Thus, in terms of infectious diseases, serology and clinical immunology have developed parallel to classic microbiology. In recent years, it has become quite clear that a wide variety of immunologic tests are available not only for infectious diseases, but also for many immunopathologic conditions, including autoimmune and immunodeficiency diseases and even for cancer. Yet, although the earliest immunologic tests were based on assays that could be detected by instrumentation or automation, it has

only been in the last decade or so that a significant effort has been made to automate techniques for detecting diagnostically useful antibodies or even antigens in patient sera. Thus, it is clear that recent advances in areas of microbiology and immunology have depended upon the development of instruments and automated equipment which permit the determination of many of the parameters determined conventionally by manual methods. Some of these automated procedures are listed below.

TABLE 1. Automated systems for bacteriology

Bacterial Identification	Antibiotic Susceptibility
API System	Autobac
Autobac	FIAX - Automated Immunofluorescence
Automicrobic System	MIC - 2000
Bactec - Radioimmunoassay	MS - 2
Counterimmunoelectrophoresis	Radioimmunoassay
Electrical Impedance (Bactobridge, Bactometer)	Sceptor
Gas - Liquid Chromatography	Sensititer
Minitek	
Phadebact	
Sceptor System	

BACTERIAL GROWTH DETECTION

Various newer modalities have been designed to help detect the presence of microbes in patient specimens. A variety of different technologies are available and can be categorized as follows:

1. Radiolabeling techniques - Radioisotopic detection apparatuses have been developed which permit quantitation of radioisotopically labeled gases evolving from culture media in which microorganisms are growing (1). There are several models of the basic instruments first developed and marketed by Johnston Laboratories in Maryland. A series of instruments, labeled Bactec, include completely manual models as well as automated or semi-automated models. In brief, culture bottles containing radioisotopically labeled nutrients such as carbohydrates and amino acids are inoculated with the patient specimen which normally should be sterile. If microorganisms are present in the specimen, even in small numbers, their replication results in the metabolism of the nutrients, releasing radiolabeled CO_2 which is detected by an automatic isotope counter. The apparatus is capable of more rapid detection of bacterial growth than by visual examination and/or by overnight culturing. For example, in many instances blood cultures become positive within 12 to 18 hours after initial inoculation with a specimen.

2. Impedance Monitoring - Several apparatuses have been developed which permit detection of microbial growth based on alteration in electrical impedance (2). Such instruments, which essentially use a modified Wheatstone bridge, are useful for detecting bacteria in blood, urine or spinal fluid, usually producing a positive reaction within a few hours after inoculation of a sample well with a patient specimen. The impedance monitoring apparatuses currently on the market are not fully automated but can provide significant savings in time and effort, as well as in media, for screening specimens for the presence or absence of bacteria. There are some difficulties in interpreting results and/or diminishing "background noise". Nevertheless, the simplicity of the apparatus suggests that impedance monitoring devices in the future will be valuable for clinical microbiology.

3. Automated Detection Systems - A completely automated detection and bacterial identification system has recently been introduced by Vitek Corporation (Hazelwood, Missouri) (3). This apparatus is based on a system developed initially for the Aerospace program for detecting life on other planets. In brief, microchambers in plastic identification cards contain various media and are inoculated by an automated vacuum system. Up to 150 cards may be inoculated at one time in the apparatus. Each of the microchamber wells in a card is then examined microscopically by a laser beam system for evidence of turbidity, gas formation, pH alterations, etc. The information so obtained is computed and compared to information on the characteristics of common bacterial pathogens contained in a computer storage bank. In general, the detection of an organism can often be made within 6-8 hours after inoculation of the cards.

4. Antibiotics Susceptibility Testing - Once an organism has been isolated from a patient specimen and shown to be pathogenic, antibiotic susceptibility tests are generally performed. Classic methods in most laboratories consist of the paper disc impregnation tests. This has been the conventional procedure for detecting antibody susceptibility. A number of instruments have recently been developed for automated or semi-automated

(a) Autobac System (Pfizer, Inc., New York City, New York) (4). This instrument detects the minimal inhibitory concentration (MIC) of an antibiotic through the use of specialized cuvettes. The susceptibility of an isolated organism can be determined usually within three to five hours.

(b) The MS-2 System (Abbott Laboratories, Houston, Texas) uses kinetic/turbidimetic procedures to permit sequential detection of bacterial growth and assessment of effective and antimicrobial agent activity against this growth (5). This instrument uses a computer system to determine the growth rate of isolated organisms. Results may be obtained within two to six hours, both qualitatively and as related to MIC assays.

(c) Automicrobic System. This system is utilized not only for detecting bacteria and identifying the organism, but also for antibiotic susceptibility testing (6). Pure cultures of an organism are transferred from culture positive cuvettes to sensitivity microplates. The automatic processing of the plates permits determination of antibiotic susceptibility results within several hours.

(d) Microtitration Instruments. Antibiotic microtitration can also be performed using several mechanized instruments permitting serial dilutions of antibiotics (7). Such instruments obviate the need for tedious manual-fold serial dilutions. A variety of semi-automated microtitrations permit serial dilution of the antibiotics and addition of standardized cultures of microorganism to each well, followed by determination of turbidity using microspectrophotometry.

TABLE 2. Serologic assays: automation and rapid methods

Enzyme-linked assays: Automatic spectrophometric readers
FIAX
Microdiluters
Nephelometric assays
Radioassays

5. Serology. Serologic procedures have been available for many decades for detecting antibodies to organisms, permitting serodiagnosis of infectious diseases. Similar serologic tests have been developed for a wide variety of diseases unrelated to infections including immunodeficiency diseases, autoimmune diseases, and even newer techniques for detecting tumor associated antigens such as carcinoembryonic antigen and alpha fetal protein. Instruments for serology have been available for nearly a decade and include microtitration instruments for serial dilution of patient serum as well as performing some of the steps usually done manually for addition of various reagents such as antigen, complement, target erythrocytes, etc., to test plates. In addition, there is a wide variety of instruments available for radioimmunoassay (8), as well as more recently for enzyme immunoassay, (9), and fluorometric (10) detection procedures. These instruments have been developed so that they can be utilized for detecting either antigen or antibody. Solid phase immunoassays using plastic wells or surfaces coated with antigen or antibody are performed with appropriate incubation procedures, either directly, or by indirect testing. In addition, newer fluorometric apparatuses based on comparative assessment of the fluorescence detected on solid phase material following incubation with the tested serum or specimens permit quantitative analysis of antigen-antibody reactions, eliminating the difficulty of potentially dangerous isotopes (11). Finally, nephelometric assays can be performed with automated apparatuses yielding very sensitive assessment of antigen-antibody reactions (12). Newer apparatuses available for serology permit rapid, accurate detection and quantitation of antigens, antibodies, or immune complexes not only for infectious disease diagnosis but also for autoimmunity diagnosis, transplantation immunology, cancer immunology, and a wide variety of other clinical, chemical and physiological analyses.

CONCLUSIONS AND DISCUSSION

The advent of mechanized and/or automated equipment in clinical microbiology and immunology is markedly changing these clinical laboratory areas, as has already occurred in clinical chemistry. It is important to note that the same advantages have occurred in other areas of the laboratories and are obvious for clinical microbiology and immunology. For example, physicians in health care facilities often need information concerning laboratory results at the earliest possible times. Thus, many time consuming microbiologic and immunologic procedures appear to be appropriate for mechanization or automation. Development of completely automated equipment which assists both detection and quantitation of microorganisms as well as the testing for antibiotic susceptibility is occurring at a rapid pace. Such instruments, once in wide use, may be linked to computer systems and used to disseminate information in the way that chemistry results are joined to computer banks. Acceleration in test procedures that does not compromise quality and which can more rapidly

disseminate results and store information will obviously contribute to improvement of health care.

It is evident that microbiologic and immunologic laboratories are moving very rapidly into the areas of mechanization and automation. Thus, it can be expected that developments in the coming decade for these laboratory services will bring them into the mainstream of modern bio-medical science.

REFERENCES

1. E.L. Randall, In: Microbiology. Washington, D.C.: American Society for Microbiology (1975).
2. S. Specter, R. Throm, R. Strauss and H. Friedman, J. Clin. Microbiol. 489 (1977).
3. C. Aldridge, P.W. Jones, S. Gibson, J. Lanhan, M. Meyer, R. Vannest and R. Charles, J. Clin. Microbiol. 6, 406 (1977).
4. R.D. Jenkins, D.C. Hale and J.M. Matsen, J. Clin. Microbiol. 11, 220 (1980).
5. C. Thornsberry, J.P. Anhalt, J.A. Washington II, L.R. McCarthy, F.D. Schoenknecht, J.C. Sherris and H.J. Spencer, J. Clin. Microbiol. 12, 375 (1980).
6. R.E. Tilton and R.C. Tilton, J. Clin. Microbiol. 11, 157 (1980).
7. R.N. Jones, T.L. Gavan and A.L. Barry, J. Clin. Microbiol. 11, 426 (1980).
8. W.D. Odell and D. Heber, In: Manual of Clinical Microbiology, 2nd Ed. Rose, N. and Friedman, H. (eds.). Washington, D.C.: American Society for Microbiology, 329 (1980).
9. A. Voller, D. Bidwell and A. Bartlett, In: Manual of Clinical Immunology, 2nd Ed. Rose N. and Friedman, H. (eds.). Washington, D.C.: American Society for Microbiology, 359 (1980).
10. A. Kawamura, Jr., Fluorescent Antibody Techniques and Their Applications. 2nd Ed. Baltimore: University Park Press (1977).
11. R. A. Harte, Clin. Immunol. Newsletter 1(2), 2 (1980).
12. G. Virella, W.A. Hipp, J.F. John, B. Kahaleh, M. Ford and H.H. Fudenberg, Int. Arch. Allergy Appl. Immunol. 58, 402 (1979).