Instrumentation in Clinical Chemistry

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Summary. Major advances in instrumentation have revolutionized the clinical chemistry laboratory during the past two decades. This article focuses on some of the more recent developments in instrumentation for clinical chemistry in the areas of general chemistry, immunoassays, urinalysis, electrophoresis, chromatography, and trace metal analyses.

The past 20 years have been an era of dramatic technological development of instrumentation in clinical chemistry. Prior to this time, the tools of the clinical chemist were the test tube, the flask, the burette, the pipette, the cuvette, and the photometer. Laboratory determinations were essentially manual and a significant amount of time was required for each analysis.

At the end of the 1950's, the concept of continuous flow analytical systems that used air-segmented flow streams of reaction mixtures was introduced to the clinical chemistry laboratory (*1*). This marked the beginning of a period of significant advancement in most areas of clinical chemistry. Since that time, major breakthroughs have occurred in instrumentation, the development of new methodologies, automation, data processing, quality control, and, most recently, operational simplification. These developments have transformed the discipline of clinical chemistry from a tedious, repetitive, time-consuming process for each test result to the current state of automatic analyses of many different components done on less than 1 milliliter of sample with the results reported directly to the nursing units by a computer system.

The "instrumentation revolution" in clinical chemistry has not only improved thruput (total handling of the specimens and ensuring a valid result) but also the quality of the final product, the test result. Precision (agreement among replicate analyses of the same material) has improved greatly since the variability inherent in manual procedures has been significantly reduced by instruments (*2*). An instrument, more so than a person, is ideally suited to perform repetitive procedures the same way. Accuracy (closeness of a result to the true value) has also improved, but the user is now more dependent on the instrument manufacturer to provide reliable calibration materials (*3*).

What is the current state of instrumentation in clinical chemistry? Manufacturers are now focusing on operationally simple and reliable instruments that minimize sample and reagent volumes. The use of built-in microprocessors have made possible fully automatic instrumentation (that is, the sample is unattended from the time it enters the instrument until the results are reported). Many systems now include sample identification, operator alerts, performance malfunction detection and correction, autocalibration, data review, and quality control of results as standard components of the instrument. These advancements in instrumentation have changed essentially all the functions of a clinical chemistry laboratory. In this article some of the recent developments and modifications to existing technology for clinical chemistry instrumentation in general clinical chemistry, immunoassays, urinalysis, electrophoresis, chromatography, and trace metal analyses are discussed.

General Clinical Chemistry

General clinical chemistry is the area in which instrumentation has had its most profound effect. Major progress has occurred in accuracy, precision, and turnaround time for the analysis of routine high-volume tests such as electrolytes, urea, and glucose. Many of the major breakthroughs in this area occurred years ago, but some recent innovations should significantly improve this area.

Centrifugal analyzer. The use of centrifugal force to transfer liquids and the simultaneous monitoring of several chemical reactions in a centrifuge marked the first significantly new concept in clinical chemistry instrumentation since the introduction of continuous flow systems (*4*, *5*). With a centrifugal analyzer, chemical reactions take place and are measured in a rotating centrifuge head. The design of the rotor (rotating part of the centrifuge) was the major breakthrough for this analyzer. The center part of the rotator contains cavities in which reagents and the specimen are separated. When the instrument is started, the centrifugal force causes the reagents and sample to combine in an outer compartment (cuvette), which is composed of optically transparent material. As the reaction takes place in this chamber, a light source and photodetector are arranged so that light passes through the cuvettes perpendicularly to the rotor, thereby forming a photometric analysis system (*5*).

Centrifugal analyzers have been available to clinical chemistry laboratories for several years, but recent modifications have improved these analytical systems. The initial systems determined concentration by spectrophotometry. With the use of 90° and 180° optics, it has been possible to expand the detection systems to include fluorometry and nephelometry (*6*, *7*). In addition, a rotor has been designed so that the cuvettes have their longitudinal dimension parallel to the light beam (*8*). This has the advantage of the result being independent of the reagent volume (potential errors in volume with pipetting reaction solutions are minimized). Centrifugal analyzers have become very popular since they can be used for routine chemical and enzymatic tests and therapeutic drug monitoring. The above enhancements will increase the spectrum of tests that can be performed on this instrument.

Multilayered film analyzer. A recent novel development is the multilayered film analyzer (*9*–*12*). In this system no wet chemicals are used; the chemicals are incorporated into a series of thin films on a single-use slide. The sample from the patient is placed on the slide; it permeates the various layers on the slide and the end result is determined colorimetrically. A wide variety of chemical

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and enzymatic assays can be performed with this system.

The several layers (spreading, reagent, semipermeable, indicator, and transparent support) for processing and chemically reacting with the sample are mounted between two slides. The spreading layer is an isotropically porous nonfibrous layer with an 80 percent void volume. The spreading and metering action of this layer compensates for minor differences in the usual sample volume of 10 microliters and serum viscosity. Thus, a constant volume per unit area is naturally applied to subsequent layers of the slide. This obviates the accuracy and precision problems inherent in pipetting small sample volumes. Typically, a chemical or enzymatic reaction occurs in the reagent layer. The semipermeable layer withholds high-molecular-weight substances from the indicator layer where usually a dye combines with a product of the reaction to form a colored compound in proportion to the quantity of the particular component in the specimen. The intensity of the colored complex is measured by reflectance spectrophotometry and, from this measurement, the concentration of the component in the sample is determined. Glucose, urea, calcium, total bilirubin, and uric acid can be determined with this instrument.

This concept has been extended recently for analyses of sodium and potassium by means of potentiometry. Each potentiometric analysis slide contains two identical thin-film, single-use ion-selective electrodes joined by a bridge. The ion-selective electrodes in a flat, film format are made by evaporating thin layers of silver onto a support and then converting some of the silver to silver chloride and overcoating with ion-selective membranes. When 10 μl of a specimen and reference fluid are placed on the slide, a pair of electrochemical half cells is created, and a liquid junction is formed by capillary flow through a small bridge. Depending on the viscosity of the fluid, this junction is obtained within 20 to 30 seconds of drop placement. A stable analytical potential can be reached in about 3 minutes. An electrometer within the analyzer measures the potential difference between the two half cells. From this measurement, the electrolyte concentration of the patient's sample is determined.

A new instrument will be available in the near future that can be programmed to run both colorimetric and potentiometric tests in any combination, on a STAT, discrete or batch basis at a rate of more than 500 tests per hour. Additional tests are being developed. Thus far, reports with this system compare favorably with those of other instruments with respect to precision, accuracy, analytical recovery, and instrument and reagent stability (*11, 13*).

Immobilized enzymes. Techniques for immobilizing enzymes for substrate determination have been used for some time. Recently, Sundaram *et al*. (*14, 15*) have developed a system for the immobilization of enzymes on the inner walls of nylon tubes. This system has been adapted for use with continuous flow systems. It has the obvious advantage of permitting multiple determinations of a given component with the immobilized enzyme. This principle has been applied to the determination of urea with an immobilized urease (*14*) and uric acid with an immobolized uricase (*15*). Results obtained with these two immobilized enzyme systems correlate well with other procedures in common use (*14, 15*).

Flame photometry. The determination of sodium and potassium with the flame photometer has been used for many years. Recently, this instrument has been modified to permit automatic calibration of the flame. In addition, the use of cesium as an internal standard has permitted the analysis of lithium, which was previously used as the internal standard (*16*). Lithium is now used routinely in certain psychiatric disorders, and it has become important for the clinical chemistry laboratory to determine serum concentrations with a short turnaround time.

Electronic pipetting. In the preparation of lyophilized serums for quality-control purposes, it is important to accurately dispense the serum into vials. The use of syringe-type dispensers presents difficulties because of the proteinaceous nature of the liquid and the presence of dissolved gases. Kenney *et al*. (*17*) have described an electronically controlled pipetting device that consists of an inverted (to-deliver) pipette with optical switches that are attached to the pipette and are activated by the presence of liquid in the stem of the pipette. The optical switch can be raised or lowered to permit the system to give the exact volume of liquid required, the latter being confirmed by weighing. The solenoid is the only moving part. The system is described as being more accurate than manual pipetting since draining time is uniform and the closure of the solenoid ensures that no partial drops collect at the tip during delivery. The quoted precision of this system is 0.2 percent or better for volumes ranging between 2 and 50 ml (*17*).

Immunoassay

One of the major developments of the past decade has been the application of immunochemistry to the measurement of chemically important constituents such as hormones, drugs, and enzymes. The ability to prepare antibodies of high specificity has given rise to numerous techniques for labeling antigens and antibodies and to the development of detection systems.

Radioimmunoassay (RIA). The concept of RIA is based on the existence of an equilibrium between an antigen (the component to be measured), an antibody, and a corresponding antigen-antibody complex in a system that includes trace amounts of radioactively labeled antigen. The addition of further antigen (that is, as test or standard) gives rise to a new equilibrium in which there is a decrease in the proportion of radioactive antigen that remains bound to the antibody. A separation step is required in order to determine the amount of labeled antigen that is bound to antibody or free in solution. The concentration of the unknown antigen is determined from a standard curve in which known quantities of the antigen were added to the test system.

The past decade has seen substantial activity in the application of radioisotope-based immunoassays, and in the introduction of radioactivity-counting instruments on a large scale in clinical laboratories. Recently, totally automated RIA systems have become available. These systems are built around different techniques for separating bound from free antigen, such as filtration with glass fiber pads, antibody coated to the reaction tube, and magnetizable particles. The last approach is a recent development and employs magnetizable material consisting of ferric oxide (Fe_3O_4) embedded in a cellulose matrix (*18*). The magnetic particles are covalently linked to the antibody modules. The magnetic separation of free from bound antigen is achieved by activating electromagnets (*19*). The antigen-antibody complex is bound by the magnets, and free antigen goes to a waste bottle. After the free antigen has been removed, the electromagnets are turned off and the antigen-antibody complexes go to the gamma counter. The counts are then translated into the concentration for each standard; the standard curve is constructed, and the unknown concentrations are determined. The results with this automated RIA instrument are similar to those obtained by conventional manual techniques; however, the quoted

precision is improved and operator error is reduced (*18*).

Enzyme immunoassay (EIA). Hazards associated with radioisotope handling, special licensing requirements, and limited shelf life have prompted the development of nonisotopic label assays. Enzymes have proved to be excellent alternative labels. Assay systems involving use of antigens, haptens, or antibodies labeled with an enzyme have been applied to the measurement of substances in biological fluids. One of the most popular techniques is the homogeneous enzyme immunoassay in which the activity of the enzyme when bound to an antigen (hapten) is reduced when the antigen forms complexes with an antibody. Thus, when the enzyme-antigen conjugate is complexed to the antibody, the enzyme substrate is excluded from reaching the active site on the enzyme and the enzyme activity is reduced. This technique precludes the need for the separation of bound from free antigen (thus the name homogeneous EIA) and in effect simplifies the immunoassay making it comparable to an enzyme activity measurement. This has enabled simple colorimetric detection of rate reactions and applicability to a wide variety of instrumentation. This technique has been adapted to continuous flow systems (*20*), bichromatic analyzers (*21*), and centrifugal analyzers (*22*).

Fluorescent immunoassay (FIA). An automated system has been designed in which a fluorescent molecule is substituted for a radioactive label (*23*). The system includes all the necessary reagents and an automated fluorometer. In this competitive-binding fluorescent immunoassay, antigen labeled with fluorescent dye competes with antigens in the sample or standard for a limited amount of antibody immobilized on small polyacrylamide beads. After separating antibody-bound from free antigen, the amount of fluorescence bound to the beads is determined. The separation step removes most of the serum sample components that might interfere with the measurement of fluorescence from the antibody-bound antigen. The polyacrylamide bead is critical to the measurement. The bead has a specific gravity of near 1.0, so that it forms a uniformly distributed suspension in water; and because its refractive index is similar to that of water, it produces minimum light scattering (*23*). This FIA system should be available in the near future.

Nephelometry systems. Nephelometry is a technique in which the light dispersed by particles in a suspension is measured at right angles to the direction of the incident light. The amount of light scattered depends on the number and size of the particles in the light path. This technique has been successfully employed to quantify antigen-antibody complexes. The light source may be an incandescent bulb or a laser. This technique has been applied to the determination of specific proteins in serum such as IgG, IgA, and IgM. A good correlation has been demonstrated between the laser nephelometer and the more conventional radial immunodiffusion method (*24, 25*). Recently, several totally automated systems employing the nephelometric principle have been developed and will be marketed in the near future. These systems should improve thruput and precision for the determination of many specific proteins in body fluids.

Urinalysis

Routine urinalysis includes the qualitative estimation of several components by visual interpretation of colors produced by the reaction of the components with chemicals and enzymes in pads on a stick which is dipped into the urine (dipstick). The time for assessing the color on the pads of the dipstick is important. The perception of the color may vary with personnel, differences in lighting, and differences in the background color against which pads of the dipstick are read. Two instruments have been designed to automatically interpret the color changes on the pads of the dipstick and record the result (*26, 27*). Each of these systems uses reflectance photometry in which the amount of light reflected decreases as the concentration of the substance increases, thus the amount of light reflected is inversely related to the concentration of substance present. There are differing opinions as to whether or not these instruments improve thruput and precision compared to the manual method (*26, 28*).

Electrophoresis

Serum protein electrophoresis, one of the procedures that demands a skillful technologist, has been developed so that all operations can be carried out mechanically by the instrument (*29*). The samples are manually dispensed into depressions of a stainless steel application plate. The application plate is moved into the apparatus and the automatic process commences. The procedures performed automatically include (i) application of samples on cellulose-acetate film; (ii) transport of the strips into the electrophoresis chamber, switching the electrophoresis current on and off; (iii) transport of the strips from the electrophoresis chamber to the staining chamber for staining, destaining, and drying; (iv) transport of the processed membranes into the densitometer for making the membranes transparent; and (v) performing the quantitative evaluation for up to 20 electrophoreses in each strip. The densitometric patterns are recorded and the relative concentration of each fraction is printed on the report.

Chromatography

Faster separations, new detectors, and fully automated operation have been the areas of progress in chromatographic techniques. There has been a growing interest in the use of more powerful separation methods followed by the use of detectors with varying degrees of selectivity. This is an area in which recent developments in instrumentation have enabled the clinical chemistry laboratory to expand its spectrum of tests.

Gas chromatography/mass spectrometry (GC/MS). The techniques of gas chromatography and mass spectrometry have been available to researchers for some time. Recently, the separation process of gas chromatography has been effectively combined with the highly selective detection technique of mass spectrometry in a single instrument. This instrument is useful in toxicology, drug and metabolite identification, and drug level monitoring (*30, 31*). Since mass spectrometry is an extremely sensitive analytical technique and each mass spectra is a unique fingerprint of the compound analyzed, it provides more information per weight of sample than any other analytical technique. An example of the resolving power of GC/MS is its application to the assay of metabolic products in human urine by Gates *et al.* (*32*). These investigators have identified and determined the concentration of 134 organic acids in urine from normal individuals. The application of this highly versatile instrument to clinical chemistry has just begun.

Automated high performance liquid chromatography. High performance liquid chromatography (HPLC) has the potential for becoming one of the most powerful tools in the clinical chemistry laboratory. Excellent resolution of closely related compounds that are polar, ionic, or nonvolatile can be carried out with HPLC. This is most important in clinical work, since most of the drugs and their

metabolites, as well as many biologically active endogenous compounds, fall into one of these categories. Separations are highly reproducible, and very low limits of detection are obtainable. An important feature of HPLC is the ability to detect many compounds in one assay; thus the concentration of a drug and its metabolites or several drugs can be determined at one time.

The first fully automated HPLC system for assay of drugs in serum has recently been described (*33*). The following operations are carried out automatically after the sample has entered the instrument: the compound is extracted from the serum, the extract is evaporated to dryness, the residue is redissolved, the solution is injected onto an HPLC column, the compounds are separated from the HPLC column, and the effluent is analyzed spectrophotometrically. This system requires a sample volume of 150 μl. The quoted coefficient of variation is 4 to 6 percent, and the results correlate well with enzyme immunoassay (*33*).

Trace Metal Analyses

The importance and clinical significance of the concentration of trace metals is just now being understood. It is in this area where future progress in instrumentation will be vital to permit the clinical chemistry laboratory to meet the demands of modern medicine. The introduction of total parenteral nutrition (long-term intravenous feeding as the sole nutritional source) has heightened the interest in this area. Atomic absorption spectroscopy has been the accepted instrument for the determination of heavy metals for several years. The large sample volume required for the determination of multiple trace metals and the lack of sensitivity for some trace metals has prompted evaluation of other techniques.

X-ray fluorescence spectrometry. The x-ray fluorescence spectrometer has been developed over the past decade and recently has become a potential practical analytical technique for the clinical chemistry laboratory. With this technique, an x-ray beam strikes an inner shell electron of an element causing the electron to be ejected from its orbit around the nucleus. The quantum of energy given off when an outer shell electron falls in the space left by the ejected electron is characteristic of a given element and is measured. This system has special appeal since up to 90 elements can be determined rapidly, simultaneously, nondestructively, and quantitatively. This instrument has been used for trace metal analyses in blood, urine, and hair (*34, 35*). If x-ray fluorescence spectrometry is found to be competitive with atomic absorption in terms of sensitivity, it will probably earn a place of its own in the clinical chemistry laboratory.

Anodic stripping voltammetry (ASV). ASV has recently been introduced to the clinical chemistry laboratory. This technique involves reduction of metal ions at an electrode during an electrolysis process which concentrates the metal on the surface of the electrode (*36*). The metal is then oxidized as a voltage scan is made. The curve obtained from plotting current as a function of voltage can be used to quantitate the metal. The instrument is simple to operate and is receiving increasing use for blood lead analyses.

Developments in instrumentation have been a significant factor for enabling the clinical chemistry laboratory to keep pace with modern medicine. In the middle 1960's after the implementation of continuous flow systems, there was a feeling among clinical chemists that the future would probably be limited to refining this analytical system. This proved not to be the case since the 1970's have brought forth several major breakthroughs in instrumentation for clinical chemistry. This ingenuity and productivity will be required in the 1980's to enable clinical chemistry laboratories to meet the diagnostic challenges of modern medicine.

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