11

CLINICAL LABORATORY INSTRUMENTATION

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The clinical laboratory is responsible for analyzing patient specimens in order to provide information to aid in the diagnosis of disease and evaluate the effectiveness of therapy. The hospital department that performs these functions may also be called the department of clinical pathology or the department of laboratory medicine. The major sections of the clinical laboratory are the chemistry, hematology, and microbiology sections and the blood bank.

The chemistry section performs analyses on blood, urine, cerebrospinal fluid (CSF), and other fluids to determine how much of various clinically important substances they contain. Most applications of electronic instrumentation in the clinical laboratory take place in the chemistry section. The hematology section performs determinations of the numbers and characteristics of the formed elements in the blood (red blood cells, white blood cells, and platelets) as well as tests of the function of physiological systems in the blood (clotting studies are an example). Many of the most frequently ordered of these tests have been automated on the Coulter Counter (see Section 11.5). The microbiology section performs studies on various body tissues and fluids to determine whether pathological microorganisms are present. Until quite recently, there were essentially no applications of electronic instrumentation in microbiology. However, devices that automatically monitor the status of blood cultures (tests for the presence of microorganisms) and tests that semiautomatically measure the sensitivity of microorganisms to antibiotics (susceptibility tests) are now being used in many microbiology laboratories. The application of electronic instrumentation for the blood bank is in its infancy. A few systems that automate the basic classification of the type of the blood product (ABO grouping) are currently being developed.

Because many critical patient-care decisions are based on test results supplied by the clinical laboratory, the accuracy and precision of these results are of great importance. Excellent equipment design and effective quality-control programs are essential. Everyone involved in the design or use of clinical laboratory instruments must be constantly aware that erroneous test results can lead to a tragic outcome.

A second important characteristic of many test procedures is fast response, because in many critical clinical situations, the therapy selected by the physician depends on the test results. The application of electronics in the clinical laboratory has greatly reduced the time required to perform a wide variety of crucial tests.

A major application of electronics in the clinical laboratory is the use of computer systems for information management. Mainframes, minicomputers, and microcomputers are used in commercial systems. Laboratory information systems keep track of patient specimens, organize the flow of work, automatically acquire test results from some types of instruments, maintain test-result databases, report results to on-line devices in patient-care areas, prepare printed reports, assist in quality control, and support a variety of management functions. We shall not discuss laboratory information management systems in detail. The current design trend in laboratory instrumentation, however, is to include data-processing capability in essentially every instrument. Therefore, our discussions of specific laboratory instruments will cover some aspects of laboratory information management.

11.1 SPECTROPHOTOMETRY

Spectrophotometry is the basis for many of the instruments used in clinical chemistry. The primary reasons for this are ease of measurement, satisfactory accuracy and precision, and the suitability of spectrophotometric techniques to use in automated instruments. In this section, *spectrophotometer* is used as a general term for a class of instruments. Photometers and colorimeters are members of this class (Shen *et al.*, 2006).

Spectrophotometry is based on the fact that substances of clinical interest selectively absorb or emit electromagnetic energy at different wavelengths. For most laboratory applications, wavelengths in the range of the ultraviolet (200 to 400 nm), the visible (400 to 700 nm), or the near infrared (700 to 800 nm) are used; the majority of the instruments operate in the visible range.

Figure 11.1 is a general block diagram for a spectrophotometer-type instrument. The source supplies the radiant energy used to analyze the sample. The wavelength selector allows energy in a limited wavelength band to pass through. The cuvette holds the sample to be analyzed in the path of the energy. The detector produces an electric output that is proportional to the amount of energy it receives, and the readout device indicates the received energy or some function of it (such as the concentration, in the sample, of a substance of interest).

The basic principle of a spectrophotometer is that if we examine an appropriately chosen, sufficiently small portion of the electromagnetic spectrum, we can use the energy-absorption properties of a substance of interest to measure the concentration of that substance. In the vast majority of cases, these substances, as they are normally found in a patient's samples (of serum,

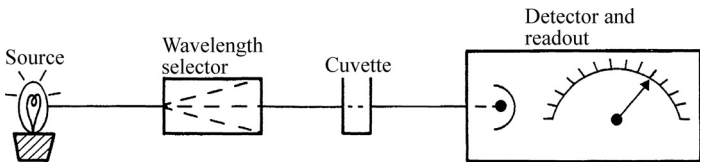

Figure 11.1 Block diagrams of a spectrophotometer (Based on R. J. Henry, D. C. Cannon, and J. W. Winkelman, eds., *Clinical Chemistry,* 2nd ed. Hagerstown, MD: Harper & Row, 1974.)

urine, or CSF, for instance), do *not* exhibit the desired energy-absorption characteristics. In such cases, reagents are added to the sample, causing a reaction to occur. This reaction yields a product that *does* have the desired characteristics. The reaction products are then placed in the cuvette for analysis. The instrument-calibration procedures take into account the possible difference in concentration between the reaction product and the original quantity of interest.

Let us discuss in detail the characteristics of each of the subsystems shown in Figure 11.1.

Power Sources Hydrogen or deuterium discharge lamps are used to provide power in the 200-to-360 nm range, and tungsten filament lamps are used for the 360-to-800 nm range. Hydrogen and deuterium lamps both produce a continuous spectrum; but a problem with these power sources is that they produce about 90% of their power in the infrared range. The output in the ultraviolet and visible ranges can be increased by operating the lamp at voltages above the rated value, but this stratagem significantly reduces the expected life of the lamp. Another problem with tungsten lamps is that, during operation, the tungsten progressively vaporizes from the filaments and condenses on the glass envelope. This coating, which is generally uneven, alters the spectral characteristics of the lamp and can cause errors in determinations.

Wavelength Selectors A variety of devices are used to select those portions of the power spectrum produced by the power source that are to be used to analyze the sample. These devices can be divided into two classes: filters and monochromators. There are two basic types of filters: glass filters and interference filters.

Glass filters function by absorbing power. For example, a blue-colored filter absorbs in the higher-wavelength visible range (red region) and transmits in the lower-wavelength visible range (blue-green region). These filters (consisting of one or more layers of glass plates) are designed to be low-pass, high-pass, or bandpass (a combination of low- and high -pass) filters.

Interference filters are made by spacing reflecting surfaces such that the incident light is reflected back and forth a short distance. The distance is selected such that light in the wavelength band of interest tends to be in phase and to be reinforced; light outside this band is out of phase and is canceled (the

interference effect). Harmonics of the frequencies in this band are also passed and must be eliminated by glass cutoff filters.

Glass filters are used in applications in which only modest accuracy is required. Interference filters are used in many spectrophotometers, including those used in the SMAC (Technicon Instrument Corporation) and the CentrifiChem (Union Carbide). Devices that use filters as their wavelength selectors are called colorimeters or photometers.

Monochromators are devices that utilize prisms and diffraction gratings. They provide very narrow bandwidths and have adjustable nominal wavelengths. The basic principle of operation of these devices is that they disperse the input beam spatially as a function of wavelength. A mechanical device is then used to allow wavelengths in the band of interest to pass through a slit.

Prisms are constructed from glass and quartz. Quartz is required for wavelengths below 350 nm. A convergent lens system is used to direct the light from the source through an entrance slit. The prism bends the light as a function of wavelength. The smaller wavelengths (ultraviolet) are bent the most. This produces an output beam in which the wavelength band of interest can be selectively passed by placing in the light path an opaque substance with a slit in it. The wavelength spectrum of the power passing through the slit is nominally triangle shaped. In prisms, as in filters, the wavelength at which maximal transmittance occurs is the nominal central wavelength. Bandwidths of 0.5 nm can be obtained with this type of device. Prisms have been used over the wavelength range of 220 to 950 nm. The nonlinear spatial distribution of the power emerging from a prism requires relatively complex mechanical devices for control of the slit position to select different nominal wavelengths.

Diffraction gratings are constructed by inscribing a large number of closely spaced parallel lines on glass or metal. A grating exploits the fact that rays of light bend around sharp corners. The degree of bending is a function of wavelength. This results in separation of the light into a spectrum at each line. As these wave fronts move and interact, reinforcement and cancellation occur. The light emerging from a grating is resolved spatially in a linear fashion, unlike the light from a prism, in which the separation of wavelengths is less at longer wavelengths. As in the case of the prism, a slit is used to select the desired bandwidth. The mechanics of the slit-positioning mechanism of a grating are less complicated than those of a prism because of the linearity of the spatial separation of the wavelengths. Gratings can achieve bandwidths down to 0.5 nm and can operate over the range of 200 to 800 nm.

Cuvette The cuvette (Figure 11.1) holds the substance being analyzed. Its optical characteristics must be such that it does not significantly alter the spectral characteristics of the light as that light enters or leaves the cuvette. The degree of care and expense involved in cuvette design is a function of the overall accuracy required of the spectrophotometer.

Sample The sample (actually, in most cases, the substances resulting from the interaction of the patient specimen and appropriate reagents) absorbs light

selectively according to the laws of Lambert, Bouguer, Bunsen, Roscoe, and Beer. The principles stated in these laws are usually grouped together and called Beer's law. The essence of the law was stated by Bouguer: ''Equal thickness of an absorbing material will absorb a constant fraction of the energy incident upon it.'' This relationship can be stated formally as follows:

$$P = P_0 10^{-aLC} \tag{11.1}$$

where

P_0 = radiant power arriving at the cuvette
P = radiant power leaving the cuvette
a = absorptivity of the sample (extinction coefficient)
L = length of the path through the sample
C = concentration of the absorbing substance

Absorptivity is a function of the characteristics of the sample and the wavelength content of the incident light. This relationship is often rewritten in the form

$$\%T = 100P/P_0 = (100)10^{-aLC} \tag{11.2}$$

where $\%T$ is the percent transmittance. The value of a is constant for a particular unknown, and the cuvette and cuvette holder are designed to keep L as nearly constant as possible. Therefore, changes in P should reflect changes in the concentration of the absorbing substance in the sample.

Percent transmittance is often reported as the result of the determination. However, because the relationship between concentration and percent transmittance is logarithmic, it has been found convenient to report absorbance. Absorbance A is defined as $\log (P_0/P)$, so

$$A = \log\left(\frac{P_0}{P}\right) = \log\left(\frac{100}{\%T}\right) = 2 - \log(\%T) \tag{11.3}$$

Note that the relationship

$$A = aLC \tag{11.4}$$

follows from (11.1) and (11.3). As previously stated, the spectrophotometer is designed to keep a and L as nearly constant as possible so that a particular determination A ideally varies only with C. Therefore, the concentration of an unknown can be determined as follows. The absorbance A_s of a standard with known concentration of the substance of interest, C_s, is determined. Next the absorbance of the unknown, A_u, is determined. Finally, the concentration of the unknown, C_u, is computed via the relationship

$$C_u = C_s\left(\frac{A_u}{A_s}\right) \tag{11.5}$$

If this relationship holds over the possible range of concentration of the unknown substance in patient samples, then the determination is said to obey Beer's law. This relationship may not hold, however, because of absorption by the solvent or reflections at the cuvette. Then a relatively large number of standards with concentration values spanning the range of interest must be used to compute a calibration curve of concentration versus absorbance. This curve is then employed to obtain a concentration value for the absorbance value of the unknown.

EXAMPLE 11.1 A filter photometer is being used to determine total concentration of serum protein (grams per deciliter). A technologist runs one standard with a known total protein concentration of 8 g/dl and obtains a $\%T$ reading of 20%, processes a patient sample and gets a $\%T$ reading of 30%, assumes the instrument's operation satisfies Beer's law, and calculates the patient value. What value should be obtained? Do you agree with the methodology? If not, what would you do differently and why?

ANSWER From (11.3), $A_s = 2 - \log \%T = 2 - \log 20 = 2 - 1.30 = 0.70$. $A_u = 2 - \log 30 = 2 - 1.48 = 0.52$. From (11.5)

$$C_u = C_s \frac{A_s}{A_u} = 8 \frac{0.52}{0.70} = 5.9 \text{ g/dl}$$

Do not agree with the methodology. Because of absorption by the solvent or reflections at the cuvette, Beer's law may not hold. Run more than one standard of known concentration to ensure that Beer's law holds.

EXAMPLE 11.2 A spectrophotometer is being calibrated before being used to determine concentration of serum calcium. Four standards (samples of known calcium concentration) are analyzed, and the following values emerge.

Standards	% Transmittance	Calcium Concentration, mg/dl
1	79.4	2
2	39.8	8
3	31.6	10
4	20.0	14

Does this determination follow Beer's law? If a patient sample was processed and a percentage of 35 were obtained, what would the calcium concentration be?

ANSWER

$$A_1 = 2 - \log 79.4 = 2 - 1.90 = 0.10$$
$$A_2 = 2 - \log 39.8 = 2 - 1.60 = 0.40$$
$$A_3 = 2 - \log 31.6 = 2 - 1.50 = 0.50$$
$$A_4 = 2 - \log 20 = 2 - 1.30 = 0.70$$

For all four samples ratio of concentration to Absorbance is given as:

$$\frac{2}{0.1} = \frac{8}{0.4} = \frac{10}{0.5} = \frac{14}{0.7} = 20$$

Therefore, the samples follow Beer's law. For $\%T = 35$,

1. Absorbance $= 2 - \log(\%T) = 2 - \log 35 = 0.46$.
2. Concentration is given as $C_u = C_s(A_u/A_s) = 2(0.46/0.1) = 9.1$ mg/dl.

The amount of light absorbed by a compound is generally a function of wavelength. The chemical reaction used in preparing the sample for spectrophotometry is designed to produce a compound (1) the concentration of which is proportional to that of the compound of interest and (2) the peak of the absorption spectrum of which is separated from the absorption peaks of the other compounds in the sample.

The wavelength band of light allowed to pass through the wavelength selector is generally chosen to cover the peak of the absorption curve symmetrically. There are a number of other factors to consider, however, including the absolute level of absorbance at the peak and its wavelength value. If the absorbance is too great ($A > 1.0$) or too small ($A < 0.11$), the errors of the photometric system become unacceptably large (Henry, 1984). In the case in which the absorbance is very large, the sample can be diluted, but this procedure is time-consuming and can result in errors. The wavelength of the peak must be within the range of the spectrophotometer's capabilities.

Photometric System A spectrophotometer's photometric system includes detectors to measure the amount of power leaving the cuvette (Radiation Sensors, Section 2.16), circuits for amplification of the low currents developed by detectors (Amplifiers and Signal Processing, Chapter 3), and devices to present the results of the determination to the technologist operating the instrument (meters or recorders). Commonly used detectors include barrier layer cells, phototubes, and photoconductive cells.

The design of meters for this application has been somewhat of a problem in the past as a result of the need for precision and the nonlinear relationship between the detected quantity (power) and the quantity of interest (absorption). Now, thanks to the development of low-cost digital electronics, the problems of computation and data presentation have been largely eliminated.

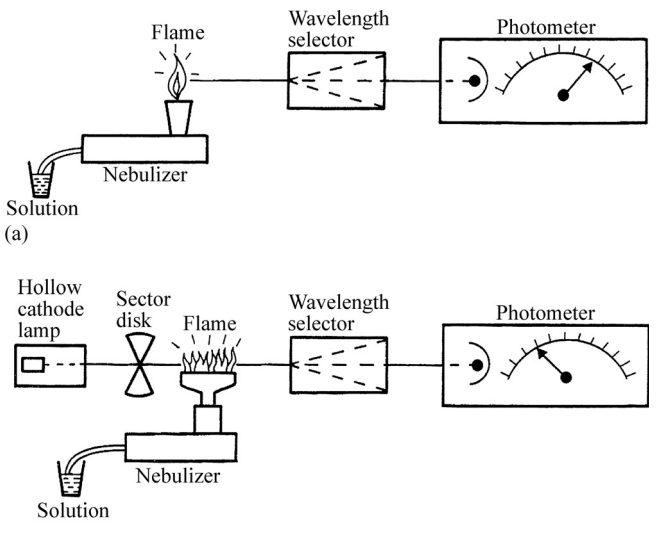

Figure 11.2 Block diagrams of instruments for (a) flame emission and (b) flame absorption. (Based on R. J. Henry, D. C. Cannon, and J. W. Winkelman, eds., *Clinical Chemistry,* 2nd ed. Hagerstown, MD: Harper & Row, 1974.)

FLAME PHOTOMETERS

Flame photometers differ in three important ways from the instruments we have already discussed. First, the power source and the sample-holder function are combined in the flame. Second, in most applications of flame photometry, the objective is measurement of the sample's emission of light rather than its absorption of light, although we shall also discuss atomic absorption-type flame photometers. (Schematic representations of these two types of instruments are shown in Figure 11.2.) Third, flame photometers can determine only the concentrations of pure metals (Lyon and Lyon, 2006).

ATOMIC EMISSION

At the normal levels of power used in flame photometers, only about 1% of the atoms are raised to an excited state. In addition, only a few elements produce enough power at a single wavelength as they move from higher-energy to lower-energy orbits. These two factors have limited the use of atomic-emission flame photometry largely to determinations of Na^+, K^+, and Li^+. Instruments have been developed that can make determinations of other elements, such as Ca^{2+}, but relatively complicated optical systems are required.

As shown in Figure 11.2(a), the sample, combined with a solvent, is drawn into a nebulizer that converts the liquid into a fine aerosol that is injected into the flame. Several types of fuels have been used in flame photometers.

Currently, propane or natural gas mixed with compressed air is used. The solvent evaporates in the flame, leaving microscopic particles of the sample. These particles disintegrate to yield atoms. As we have noted, only a small proportion of these atoms are in the excited state. As the atoms fall to the ground state, they release power at their characteristic wavelength.

A simple optical system—including only a filter and a lens to focus the filtered light on the detector—is normally used for determinations of Na^+ and K^+. More sophisticated optical systems, including a monochromator, are required for other determinations.

Many modern atomic-emission flame photometers are designed to include an internal standard to compensate for variations in the rate of solution uptake, aerosol production, and flame characteristics. Lithium (Li^+) is used for this purpose. It is not normally found in biological samples, it has high emission intensity, and its peak emission wavelength is well separated from those of sodium and potassium. A carefully controlled amount of Li^+ salt is added to the sample. An optical channel is provided to measure the power emitted by the Li^+, and this power, along with the known concentration of Li^+, is used to correct the determination of Na^+ or K^+ for variations in the instrument. Actually, in most applications, the determinations of Na^+, K^+, and Li^+ are done in parallel.

A few problems arise in the use of Li^+ as the internal standard. First, although correction for small variations in the characteristics of the instrument is possible, there is no way to correct for large variations. Second, Li^+ is being used increasingly for treatment of an important psychotic disorder, manic-depressive psychosis. If patients who are receiving Li^+ are not identified to the clinical laboratory, significant errors in determinations of Na^+ and K^+ can occur. It is an unfortunate fact that the clinical laboratory is rarely given any clinical information to use in assessing the accuracy of determinations.

ATOMIC ABSORPTION

This technique has shown great promise for the very accurate determination of the concentration of a variety of elements, including calcium, lead, copper, zinc, iron, and magnesium. It is based on the fact that the vast majority of atoms in a flame absorb energy at a characteristic wavelength. A special power source is used that emits power at the characteristic wavelength of the atom the concentration of which is being determined. This source is a hollow cathode lamp. Such lamps are constructed from the metal to be determined or are lined with a coating of it. In most cases, a separate lamp is needed for each metal determination, but the special characteristics of a few metals make it possible to use one lamp for combinations of two or three of them. The cathode is placed in an atmosphere of an inert gas. When the cathode is heated, the atoms of the cathode leave the surface of the cathode and fill the cathode cavity with an atomic vapor. These atoms become excited as a result of collisions with electrons and ions, and when they return to the ground state, each releases power at its characteristic wavelength, as previously discussed. This power is

directed through the flame [see Figure 11.2(b)], and the amount of absorption is proportional to the amount of the atom present.

Atomic-absorption flame photometers normally require a monochromator and use a photomultiplier as the detector. One additional feature of these devices is that, because the atoms in the flame emit as well as absorb power at the characteristic wavelength, it is necessary to be able to differentiate between the two sources of power reaching the detector. This is accomplished by designing the source to produce pulses of power rather than a steady output. A rotating-sector disk between the source and the flame is generally used for this purpose. The detection electronics incorporate the phase-sensitive demodulator described in Section 3.15 to eliminate the dc component and analyze only the ac signal.

FLUOROMETRY

Fluorometry is based on the fact that a number of molecules emit light in a characteristic spectrum—the emission spectrum—immediately after absorbing radiant energy and being raised to an excited state. The degree to which the molecules are excited depends on the amplitude and wavelength of the radiant power in the excitation spectrum. Small amounts of power are lost in this process, which results in the emission spectrum's being generally higher in wavelength content than the excitation spectrum (Klebe, 2006).

Various power sources, wavelength selectors, and detection circuits are used in fluorometers of varying sensitivity. Mercury arc lamps are commonly used power sources. They produce major line spectra at 365, 405, 436, and 546 nm. Photomultipliers are normally used as detectors. A unique feature of these devices is the need to select operational bandwidths for two spectra—excitation and emission. Figure 11.3 shows a fluorometer block diagram. The

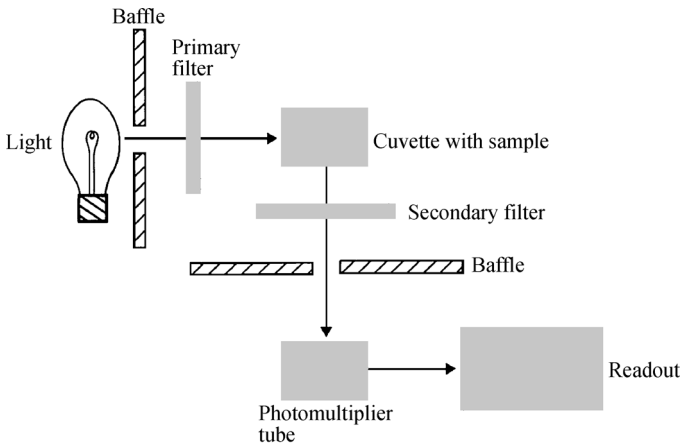

Figure 11.3 Block diagram of a fluorometer (From R. Hicks, J. R. Schenken, and M. A. Steinrauf, *Laboratory Instrumentation.* Hagerstown, MD: Harper & Row, 1974. Used with permission of C. A. McWhorter.)

detector is placed at a right angle to the power source to minimize the chance of direct transmission of light from source to detector. The wavelength characteristics of the wavelength selectors are also chosen such that there is little or no overlap in the wavelengths they pass.

One advantage fluorometry offers is its much greater sensitivity, which may exceed that of spectrophotometric methods by as much as four orders of magnitude. This is because in spectrophotometric methods, the difference between the absorption of a solution assumed to have a zero concentration ($\%T = 100$) of the unknown substance and the absorption of the sample is used as a measure of the concentration of the unknown substance. With highly dilute samples (such as one wherein $\%T = 98$), small errors in the process can cause large percent errors in the determination. In fluorometry, by contrast, a direct measurement of the fluorescence of the sample is used to determine the concentration of the unknown substance.

A special advantage of fluorometry is its great specificity. In spectrophotometric methods, the light absorbed in the wavelength band may be from substances other than the unknown. Only a relatively small number of substances, however, have the property of fluorescence. Thus many substances that might interfere with a spectrophotmetric measurement cannot interfere with a determination of fluorescence. Also, substances that have similar excitation spectra may have different emission spectra, and vice versa. Therefore, appropriate selection of the bandwidths of the two wavelengths selectors can provide additional rejection of noise.

The combination of these characteristics makes fluorometry capable of detecting picogram amounts of unknown substances. Highly dilute samples are used to prevent the light produced by fluorescence from being absorbed by other molecules as it passes through the sample solution.

The principal disadvantage of fluorometry is the sensitivity of its determinations to temperature and pH of the sample (fluorescence in general is pH-sensitive).

11.2 AUTOMATED CHEMICAL ANALYZERS

The Beckman-Coulter Synchron LXi 725 is a state-of-the-art chemistry analyzer. We will review its characteristics as an example of modern clinical laboratory chemistry analyzers. It can perform both immunoassay and chemistry tests. Other important features include integrated closed tube sampling and aliquoting (CTA), the ability to perform a large number of assays, rapid stat testing and a reduction in operator time requirements.

SPECIMEN HANDLING

The LXi 725 utilizes Beckman–Coulter's rack technology to improve workflow by reducing manual processes. Sample tubes are placed in racks and put

directly into a Spinchron DLX centrifuge. The rack is then loaded onto the analyzer. The racks can accommodate multiple tube sizes, sample cups, open or closed tubes. This provides increased efficiency.

Integrated parallel processing of general chemistry and immunoassay testing maximizes the productivity of the system. Both the immunoassay and chemistry analytical units on the LXi 725 can accommodate plasma samples to help improve turnaround time. Specimens that require both the immunoassay and chemistry testing are initially processed in the immunoassay unit. The sample is routed to the chemistry analyzer after an aliquot is obtained for immunoassay testing.

The immunoassay CTA system has the capacity for 100 aliquots per hour. Built-in data management capability tracks the aliquot vessel throughout the testing process. Aliquoting and routing of tests is done automatically without operator intervention. This translates into less labor and lower operating costs with increased output and increased efficiency. Laboratory operating safety is improved by reducing exposure to biohazards and minimizing repetitive motions.

The rack and sample barcodes are read as the rack is queued on the sample-handling carousel. In the CTA system, tubes are positioned for the cap piercing mechanism. The system adapts to a mixed batch of tubes in a single rack.

The CTA includes a piercing probe and a sampling probe. Once a tube is pierced, the sample probe aspirates the proper amount of sample based on the volume required for requested tests and dispenses it into an aliquot vessel.

The CTA reduces the potential for sample carry over by washing both the piercing and sample probe after each pierce and sample aspiration. By reducing the potential for carry over accurate results are obtained even the most sensitive assays such as beta HCG.

A radio sensor in the sample probe automatically determines sample levels within tubes. It detects when there is insufficient sample for the requested tests and the operator is notified when this situation exists.

The LXi 725 also features clot detection at both the immunoassay and the general chemistry sections. When the LXi 725 aspirates a sample the probe is extended into the sample tube. A sample is withdrawn. A pressure transducer detects blockage. If the probe is blocked, a wash solution is flushed through the probe forcing out any obstructions. The probe is now ready to re-aspirate the sample. If the clot is still detected, the operator is alerted, the sample is bypassed, and the system will continue to the next sample.

As soon as the immunoassay aliquots are dispensed, the LXi 725 releases the rack for general chemistry testing. Aliquots for general chemistry tests are prepared using the open tube aliquoting.

After the aliquots have been prepared for the chemistry tests, the rack is sent to the rack output area unless a test result triggers rerun or reflex rules (see below), then the rack is held until all results are delivered.

TEST PERFORMANCE

The LXi 725 has a menu (i.e., list of types of tests) of 145 assays. The system allows 65 test types (24 immunoassays and 41 general chemistries) to be onboard simultaneously. In addition, the general chemistry section accepts programming for over 100 user-defined chemistries. This wide menu enables the laboratory to consolidate workstations. General chemistry, STAT, cardiac, thyroid and other test panels can be performed on the LXi 725.

The immunoassay analysis throughput is up to 100 tests per hour, while general chemistry testing runs at speeds up to 1440 tests per hour. Dual processing enables the LXi 725 to perform a basic metabolic panel in less than 2 min from standby. Troponin results are delivered in less than 14 min. Troponin levels are used in the evaluation of a possible myocardial infarct (MI) so a fast response time is very important. When the troponin value supports the diagnosis of an MI, therapy can be immediately initiated.

Conventional instruments require visual inspection by the technologist to determine the presence of sample interferences from icterus, hemolysis, or lipemia. This determination is highly subjective. The LXi 725 performs an automatic polychromatic analysis on a diluted reagent-free sample using near infrared particle immunoassay (NIPIA) methodology. The system calculates a set of equations that are related to a standard concentration graph. Each index reading can be related to a semiquantitative concentration expressed as a number. This system alerts the operator to a potentially compromised test result.

Paramagnetic particle separation and chemiluminescent detection provide precision, broad dynamic range and excellent sensitivity. Paramagnetic particle separation helps maximize small sample volumes and allows for multiple assay formats for improved performance

Chemiluminescent detector uses alluminometry to take 10 readings of the reaction. The system uses the median response to help eliminate reaction noise and deliver a more accurate result. Chemiluminescence is the generation of light by a chemical reaction, a light source is not used. This eliminates the need to filter out the source light and allows very low concentrations of substances to be detected.

When a stat test is ordered the operator loads the sample into a rack and pushes the priority button. The system will interrupt routine programming to load the new rack, improving the laboratory's delivery of critical test results. It takes approximately 60 s for the CTA to process a sample. Tests results for 11 critical care chemistries are available in less than 2 min from sample introduction. This eliminates the need for a separate instrument to perform stat testing.

SYSTEM CONTROL

The primary system console performs two functions, operation of the analyzer and management of data. The system software offers simple touch screen

operation and single point control of the entire LXi 725 workstation. The DL200 data manager helps improve workflow and results management. It offers a dynamic pending list, add-on tests, review by exception, and automatic reflex testing.

The *dynamic pending list* identifies tests that have not been completed. If an additional test is ordered after the initial specimen submission, this test is called an *add-on test*. The system operator can order the test for a specimen currently in the instrument.

Review by exception is a process that identifies test results that satisfy specified rules (for example a test result that is outside of the reference range for the test) for review by the operator. Often the test is repeated, if the repeat test result agrees with the initial result, the test results may be reviewed by a pathologist. The pathologist may prepare an interpretive report for the patient's clinician.

The review by exception feature is based on user-defined criteria and delta checking, only tests with abnormal results are flagged for operator review. This helps to prevent workflow bottlenecks and improves efficiency. Delta checking is a process in which the current test result is compared with the most recent test result of the same type from the patient (e.g. a potassium level). If the two values differ by a specified amount (the delta value), the test result will be repeated even though the current result is within the reference range. This technique can help identify situations such as specimen identification errors (e.g. the specimen as obtained from the wrong patient).

Automatic reflex testing is a method of computer-based algorithmic test performance. The use of the clinical laboratory to diagnose disease states is often based on an algorithmic approach. An initial set of tests is performed to identify the presence of abnormality in organ function. For example the diagnosis of thyroid disorders. When an initial abnormal value is obtained, the next test(s) in the algorithm is performed. Some algorithms have more than two stages. This approach is more cost effective than if the clinician initially orders all of the tests in the algorithm (so-called shotgun approach) since when the initial test is normal, the performance of the other tests in the algorithm is unnecessary. The advantage of the shotgun approach is that it eliminates the delay resulting from the clinician receiving an initial abnormal result and then ordering the next test in the algorithm.

Automatic reflex testing is implemented by automatically performing the next test(s) in the algorithm when the initial (or subsequent) test in the algorithm yields an abnormal value. This process can decrease the time needed to diagnose a disease condition and eliminate the need for shotgun test ordering. If a result meets the laboratory's criteria for reflex testing, the additional test(s) is ordered automatically.

The LXi 725 holds the primary tube until all testing is final. Controlled through the DL2000 data manager, this feature allows for automatic reflex testing with no operator intervention. If an immunoassay test order contains reflex rules, the CTA system will automatically aliquot the extra volume required for the potential reflux test.

With dynamic download from the LIS to the DL2000, LXi 725 test requests are continuously updated. From this information and the sample barcode, the LXi 725 workstation determines the type of tests required and schedules the system accordingly. After the tests are run, the DL2000 validates the results. Normal results are automatically forwarded to the LIS.

REAGENT AND CALIBRATION

Bar-coded liquid reagents can be loaded at any time during a run maximizing efficiency. Manual tracking of onboard reagent inventory is eliminated. The system tracks identification, lot number expiration date, calibration frequency and precise number of tests remaining in the cartridge or pack.

For around the clock accessibility, the immunoassays stores 24 assays packs at a constant 4 °C to 10 °C. Self resealing immunoassays packs contain enough reagent volume for up to 50 tests. General chemistries cartridges can supply reagents for anywhere from 40 to 400 tests. Easy to use liquid calibrators, for both immunoassay and general chemistry testing, improve productivity by reducing manual processes. Calibration can be performed as needed.

11.3 CHROMATOLOGY

Chromatology is basically a group of methods for separating a mixture of substances into component parts. (Although the use of the term *chromatography* is firmly established, it is really a misnomer: In modern techniques, the colors of the mixture's components are not really used to identify substances.) One phase is fixed—liquid or solid—and the other is mobile—gas or liquid. When a liquid stationary phase is used, the process is called partition. When a solid stationary phase is used, the process is called adsorption.

In all chromatology, differences in the rate of movement of components of the mixture in the mobile phase, caused by interaction of these components with the stationary phase, are used to separate the components. The four possible combinations of stationary and mobile phases have been used in chromatographic methods.

From the viewpoint of the clinical laboratory, these methods are used primarily for the detection of complex substances such as drugs and hormones. For example, gas–liquid chromatographs (GLC) and thin-layer chromatographs (TLC) have been useful in determining what drug or drugs have been taken in overdose cases. The availability of this information is vitally important to the clinician who must select appropriate therapy. The characteristics of the GLC are presented here as an important example of the use of chromatographic methods in the clinical laboratory.

GAS–LIQUID CHROMATOGRAPHS

The basic components of a GLC are shown in Figure 11.4. Prior to being injected into the GLC, the patient sample usually must undergo some initial purification, the extent of which depends on the determination that is being performed. The functions of the major subsystems are as follows:

Injector The injector is used to introduce into the GLC 1 to 5 ml of the patient sample including the solvent in which it is contained (usually a volatile organic solvent). The temperature of the injector is set to flash-evaporate the sample and solvent.

Carrier Gas The inert carrier gas (usually N_2 or He) is the mobile phase of the chromatograph. It sweeps the evaporated sample and solvent gas down the column.

Column The column typically is 1 m long and less than 7 mm in diameter. It is packed with the solid support material (such as diatomaceous earth). The solid support is coated with the liquid phase. The small size of the solid beads produces the separation of the components. The column is enclosed in an oven the temperature of which is carefully controlled. A temperature programmer gradually increases the temperature of the column in a sequence designed for maximal efficiency of separation for the type of substance being analyzed.

Detector The detector is located at the end of the column. Its function is to provide an electric output proportional to the quantity of the compound in the effluent gas. A number of types of detectors are available for use with different types of samples. They include ionization detectors, thermal-conductivity detectors, and electron-capture detectors. Ionization detectors are most commonly used in clinical laboratory applications (Littlewood, 1970).

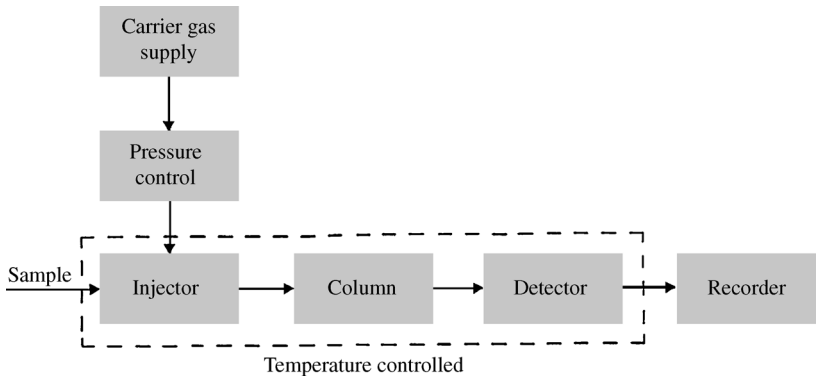

Figure 11.4 Block diagram of a gas–liquid chromatograph (GLC)

All the detectors are sensitive to classes of compounds, not only to some particular component of interest. Therefore, both the concentrations of the detected compounds and the times during the operation of the column when those concentrations occurred (that is, a plot of concentration versus time) are used in determining the types and quantities of components present in the sample. The output of the detector is connected to a recorder.

Recorder In the recorder, the *x* axis represents time, and the *y* axis the output of the detector. The recording thus provides a display of both the quantity of a component that was present (the area under the peak) and the time at which it was eluted off the column. From this information, the components present can be identified by the time they took to leave the column or, preferably, by comparison with recordings obtained by analyzing compounds of known composition with the GLC.

Figure 11.5 shows a recording obtained from the analysis of a blood specimen for the levels of the important anticonvulsant drugs phenobarbital and phenytoin. A measured amount of heptabarbital was added to the specimen to serve as an internal standard. The area under the phenobarbital and phenytoin peaks is compared with the area under the heptabarbital peak to compute the blood levels of these drugs.

Gas–liquid chromatography offers a number of important advantages in the analysis of complex compounds. They include speed, ability to operate with small amounts of sample, and great sensitivity. Most instruments can complete analyses of clinically important substances in less than 1 h, and often in 15 min or less. Only milliliter amounts of the sample are needed. The sensitivity of the device depends on the detector used, but high-quality instruments can detect 1 ng quantities of a compound.

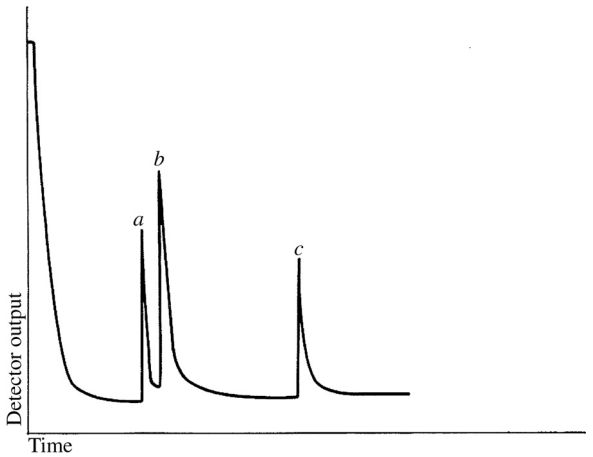

Figure 11.5 Example of a GLC recording for the analysis of blood levels of phenobarbital (peak *a*) and phenytoin (peak *c*). Peak *b* corresponds to the level of heptabarbital (the internal standard).

11.4 ELECTROPHORESIS

Devices based on electrophoretic principles are used in the clinical laboratory to measure quantities of the various types of proteins in plasma, urine, and CSF; to separate enzymes into their component isoenzymes; to identify antibodies; and to serve in a variety of other applications.

BASIC PRINCIPLES

Electrophoresis may in general be defined as the movement of a solid phase with respect to a liquid (the buffer solution). The main functions of the buffer solution are to carry the current and to keep the pH of the solution constant during the migration. The buffer solution is supported by a solid substance called the medium.

Our discussion in this section is limited to zone electrophoresis. In this technique, the sample is applied to the medium; and under the effect of the electric field, groups of particles that are similar in charge, size, and shape migrate at similar rates. This results in separation of the particles into zones. The factors that affect the speed of migration of the particles in the field are discussed in the following paragraphs.

Magnitude of Charge The mobility of a given particle is directly related to the net magnitude of the particle's charge. Mobility is defined as ''the distance in centimeters a particle moves in unit time per unit field strength, expressed as voltage drop per centimeter'' [mobility = $cm^2/(V \cdot s)$] (Henry *et al.,*1974).

Ionic Strength of Buffer The more concentrated the buffer, the slower the rate of migration of the particles. This is because the greater the proportion of buffer ions present, the greater the proportion of the current they carry. It is also due to interaction between the buffer ions and the particles.

Temperature Mobility is directly related to temperature. The flow of current through the resistance of the medium produces heat. This heat has two important effects on the electrophoresis. First, it causes the temperature of the medium to increase, which decreases its resistance and thereby causes the rate of migration to increase. Second, the heat causes water to evaporate from the surface of the medium. This increases the concentration of the particles and further boosts the rate of migration. Because of these effects, either the applied voltage or the current must be held constant in order to maintain acceptable reproducibility of the procedures. For short runs at relatively low voltage levels, either can be held constant. However, when a gel is used as the medium, heating is a significant problem. With this type of medium, constant-current sources are normally used to minimize the production of heat.

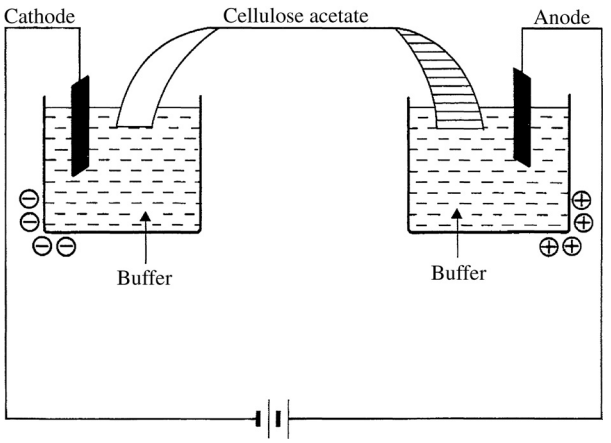

Figure 11.6 Cellulose acetate electrophoresis (From R. Hicks, J. R. Schenken, and M. A. Steinrauf, *Laboratory Instrumentation.* Hagerstown, MD: Harper & Row, 1974. Used with permission of C. A. McWhorter.)

Time The distance of migration is directly related to the time the electrophoresis takes. Other factors that influence migration include electroendosmosis, chromatography, particle shape, ''barrier'' effect, ''wick flow,'' and streaming potential (Henry *et al.,* 1974; Hicks *et al.,*1974).

Types of Support Media A large variety of support media have been used in various electrophoretic applications. They include paper, cellulose acetate, starch gel, agar gel, acrylamide gel, and sucrose. We discuss cellulose acetate electrophoresis here, because it is used extensively in clinical laboratories and because the same general method is used with other media.

Cellulose acetate has a number of desirable properties compared with the paper that was the medium first used in electrophoresis.

Figure 11.6 illustrates the basic process of cellulose acetate electrophoresis. The cellulose acetate strip is saturated with the buffer solution and placed in the membrane holder (the ''bridge''). The bridge is placed in the ''cell'' with both ends of the strip in the buffer wells.

A number of electrophoreses (typically eight) can be done on one strip. The sample for each test is placed on the strip at a marked location. Then the electric potential is applied across the strip. With this type of electrophoresis, constant-voltage-source power supplies are often used. A typical voltage is 250 V, which results in an initial current of 4 to 6 mA. As we have noted, this current increases slightly during the procedure. After 15 to 20 min, depending on the device used, the electric voltage is removed. The next step is to fix the migrated protein bands to the buffer and to stain them so that they can be seen as well as subsequently quantified. This may be done in separate or combined exposures to a fixative and a dye. The membrane is now ''cleared'' to make it transparent. The densities due to the dyed-specimen fractions are not affected. The membrane is dried in preparation for densitometry.

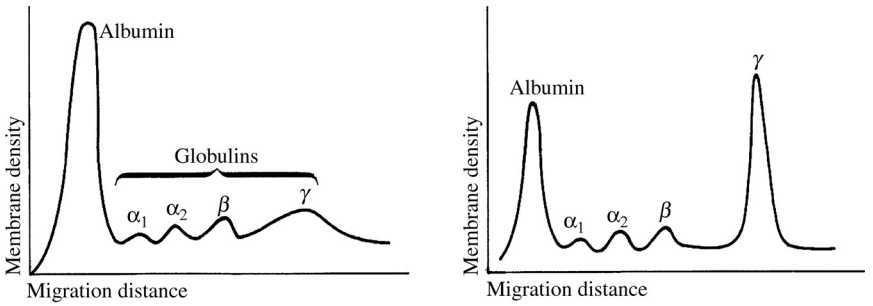

Figure 11.7 Examples of patterns of serum protein electrophoresis The left-hand pattern is normal; the right-hand pattern is seen when there is an overproduction of a single type of gamma globulin.

The densitometer is a device that consists of a light source, filter, and detector (typically a photodiode). The design and operation of this type of device are discussed in Section 11.1.

The membrane is placed in a holder in the densitometer. The path of migration of one of the specimens is then scanned. The low-voltage output of the detector is amplified by a very stable analog preamplifier. The output of the preamplifier is sent to an analog *x*–*y* recorder and to an analog integrator circuit. The *x*–*y* recorder produces a plot whereon the *x* coordinate represents migration distance and the *y* coordinate represents membrane density (which is directly proportional to the amount of specimen component that has moved the corresponding migration distance). The integrator has circuitry that detects the beginning and end of each significant peak and computes the area under the peak. These numbers are printed on the analog recording next to the corresponding peak. This process is repeated for each of the specimens on the membrane.

Figure 11.7 shows examples of the types of plots that are obtained via electrophoresis. These plots are for serum protein electrophoresis.

11.5 HEMATOLOGY

BASIC CONCEPTS

The blood consists of formed elements, substances in solution, and water. This section covers only devices that measure characteristics of the formed elements: red blood cells (RBCs), white blood cells (WBCs), and platelets. The primary functions of the RBCs are to carry oxygen from the lungs to the various organs and to carry carbon dioxide back from these organs to the lungs for excretion. The primary function of the WBCs is to help defend the body against infections. Five types of WBCs are normally found in the peripheral

blood. In order of decreasing numbers in the blood of adults, they are neutrophils, lymphocytes, monocytes, eosinophils, and basophils. In disease, the total number and the relative proportions of these types of WBCs can change; immature and malignant types of WBCs can also appear. Platelets plug small breaks in the walls of the blood vessels and also participate in the clotting mechanism.

The basic attribute of the formed elements in the blood that is measured is the number of elements of each type per microliter (μl). The normal range of the RBC count in an adult male is 4.6 to $6.2 \times 10^6/\mu$l and, in an adult female, 4.2 to $5.4 \times 10^6/\mu$l. The normal ranges of WBCs and platelet counts are the same for men and women. The normal range of the WBC count is $4,500$ to $11,000/\mu$l; that for the platelet count is $150,000$ to $400,000/\mu$l. The hematocrit (HCT) is the ratio of the volume of all the formed elements in a sample of blood to the total volume of the blood sample. It is reported as a percentage, the normal range in adult men being 40 to 54% and, in adult women, 35 to 47%. Hemoglobin (Hb) is a conjugated protein within the RBCs that transports most of the O_2 and a portion of the CO_2 that is carried in the blood. It is reported in grams per deciliter. The normal range in adult men is 13.5 to 18 g/dl, and that in adult women is 12 to 16 g/dl.

A second group of measurements is made to characterize the RBC volume and Hb concentration. These measurements include the mean corpuscular volume (MCV) in cubic micrometers, the mean corpuscular hemoglobin (MCH) content in picograms, and the mean corpuscular hemoglobin concentration (MCHC) in percent. These values are called the RBC indices. Normal ranges for these parameters are as follows:

$$\begin{aligned} \text{MCV}: &\quad 82-98\ \mu\text{m}^3 \\ \text{MCH}: &\quad 27-31\ \text{pg} \\ \text{MCHC}: &\quad 32-36\% \end{aligned}$$

The RBC count (in millions per microliter), HCT (in percent), MCV (in cubic micrometers), Hb (in grams per deciliter), MCH (in picograms), and MCHC (in percent) are related as follows:

$$\text{MCV} = \frac{10\,\text{HCT}}{\text{RBC count}} \tag{11.6}$$

$$\text{MCH} = \frac{10\,\text{Hb}}{\text{RBC count}} \tag{11.7}$$

$$\text{MCHC} = \frac{100\text{Hb}}{\text{HCT}} \tag{11.8}$$

The units for RBC count, Hb, and HCT that are employed in these calculations are such that the units for MCV, MCH, and MCHC are those given above.

EXAMPLE 11.3 Calculate the RBC indices from the following data.

$$\begin{aligned} \text{RBC} &= 5\,\text{million}/\mu\text{l} \\ \text{Hb} &= 15\,\text{g/dl} \\ \text{HCT} &= 45\% \end{aligned}$$

ANSWER

$$\text{MCV} = \frac{10\,\text{HCT}}{\text{RBC count}} = \frac{450}{5} = 90\,\mu\text{m}^3$$

$$\text{MCH} = \frac{10\,\text{Hb}}{\text{RBC count}} = \frac{150}{5} = 30\,\text{pg}$$

$$\text{MCHC} = \frac{100\,\text{Hb}}{\text{HCT}} = \frac{1500}{45} = 33.3\%$$

A new RBC characteristic that is assuming increasing importance in hematology is the volume distribution width [called the *red blood cell distribution width* (RDW)]. Somewhat similar to the standard deviation of a Gaussian distribution, it is a measure of the spread of the RBC volume distribution. In many RBC disorders, RBC production is disordered and a wider range than usual of RBC sizes is produced, leading to an increased RDW value.

Using the RDW with other RBC parameters can aid in the diagnosis of RBC disorders. For example, in iron deficiency anemia (an acquired disorder in which hemoglobin production is reduced due to a lack of iron) the RDW is high and the MCV is low or normal, while in heterozygous thalassemia (an inherited disorder in hemoglobin synthesis is abnormal) the RDW is normal and MCV is low.

ELECTRONIC DEVICES FOR MEASURING BLOOD CHARACTERISTICS

There are two major classes of electronic devices for measuring blood characteristics. One type is based on changes in the electric resistance of a solution when a formed blood element passes through an aperture. Beckman Coulter, Abbott Diagnostics, and others manufacture hematology instruments based on this technique. The other type utilizes deflections of a light beam caused by the passage of formed blood elements to make its measurements. Bayer-Technicon Corporation is a leading manufacturer of hematology instruments that use this approach. Bayer Coulter Corporation has been a leader in blood analyzers for many years and it has developed a large series of instruments. Let us review two of these instruments: the very widely used Coulter STKS and the newest instrument in this series, the Coulter LH 755. The features of the STKS will be presented first, and then the added capabilities of the LH 755 will be discussed.

COULTER STKS

The analyzed sample is blood that has been anticoagulated, with ethylenediaminetetraacetic acid (EDTA). Anticoagulants are substances that interfere with the normal clot-forming mechanism of the blood. They keep the formed elements from clumping together, which would prevent them from being counted accurately. Ethylenediaminetetraacetic acid does this by removing calcium from the blood. The initial step in the analysis procedure is the automatic aspiration of a carefully measured portion of the specimen. Next the specimen is diluted to 1:224 with a solution of approximately the same osmolality as the plasma in Diluter I, Figure 11.8. The diluted specimen is then split, part going to the mixing and lyzing chamber and part to Diluter II.

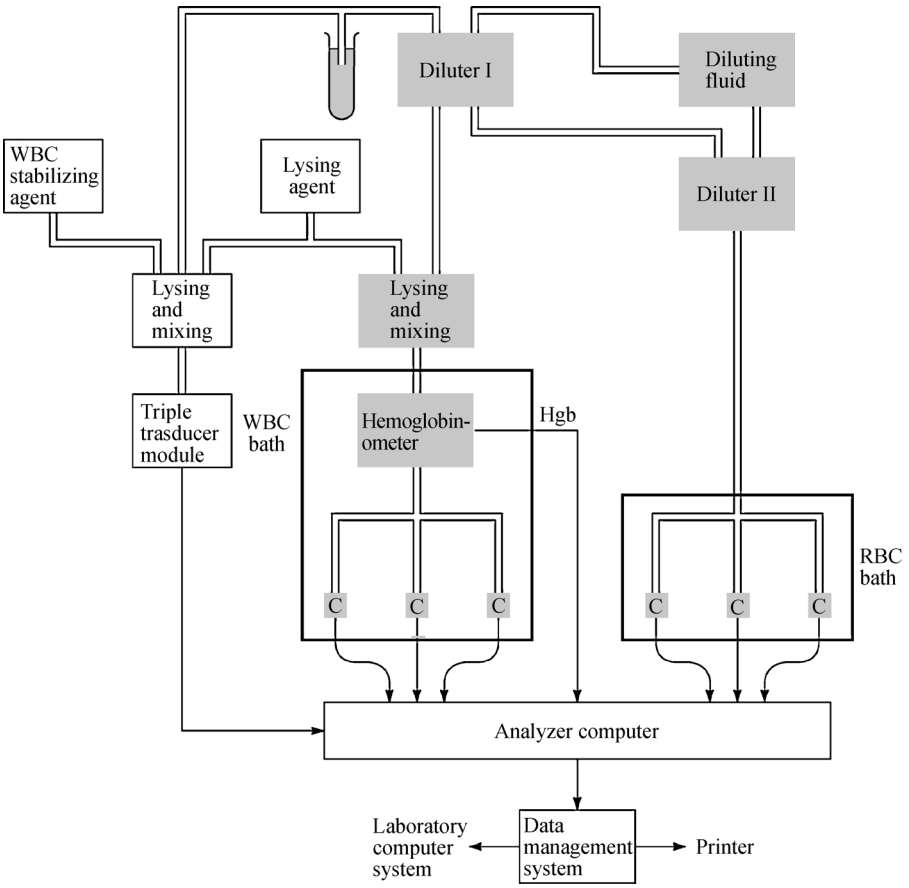

Figure 11.8 A block diagram of a Coulter Model STKS (Modified from J. Davidsohn and J. B. Henry, Todd Sanford Clinical Diagnosis by Laboratory Methods, 15 ed. Philadelphia: W. B. Saunders Co, 1974.)

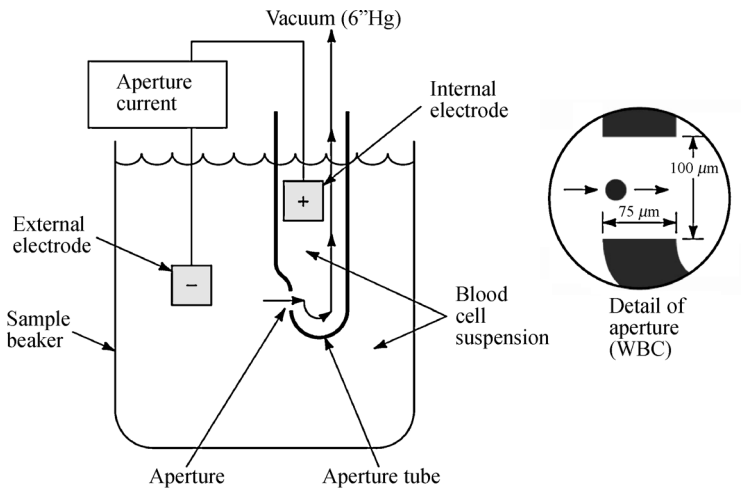

Figure 11.9 Coulter STKS aperture bath.

The function of the diluting and lyzing chamber is to prepare the specimen for the measurement of its hemoglobin content and WBC count. The lyzing agent causes the cell membranes of the RBCs to rupture and release their hemoglobin into the solution. The WBCs are not lyzed by this agent. Adding the volume of lyzing agent increases the dilution to 1:250. A second substance, Drabkin's solution, is present; it converts hemoglobin to cyanmethemoglobin. This is done to conform to the accepted standard method for determining hemoglobin concentration. The advantage of this method is that it includes essentially all forms of hemoglobin found in the blood. The specimen is next passed through the WBC bath, which functions as a cuvette for the spectrophotometric determination (see Section 11.1) of the hemoglobin content. The final step in this process is measurement of the WBC count.

Figure 11.9 outlines the method that is used in making this determination. The same method is used for counting RBCs. A vacuum pump draws a carefully controlled volume of fluid from the WBC-counting bath through the aperture. A constant current passes from the electrode in the WBC-counting bath through the aperture to the second electrode in the aperture tube. As each WBC passes through the aperture, it displaces a volume of the solution equal to its own volume. The resistance of the WBC is much greater than that of the fluid, so a voltage pulse is created in the circuit connecting the two electrodes. The magnitude of that voltage pulse is related to the volume of the WBC (Zhanf, 2006).

EXAMPLE 11.4 Diluted blood with a resistivity of $160\,\Omega\cdot$cm passes through a Coulter Counter cylindrical aperture 100 μm in diameter and 1 mm long. Calculate the resistance of the liquid-filled aperture.

ANSWER

$$R = \rho\frac{l}{a} = 1.60\,\Omega\cdot\mathrm{m}\,\frac{0.001\,\mathrm{m}}{\pi(50\times10^{-6}\,\mathrm{m})^2} = 2.04\times10^5\,\Omega$$

To increase the accuracy of the measurement, the system uses three parallel counting units. They share the common WBC-counting-bath electrode and have individual aperture-tube electrodes. The output of each of these circuits is connected to a preamplifier. The amplified voltage pulses pass through a threshold circuit. The threshold voltage is selected as part of the calibration procedure. Specimens whose WBC count values have been determined by reference methods are processed, and the threshold is set to give counts that agree with the reference values.

Pulses that exceed the threshold enter a pulse-integrator circuit, which produces a dc voltage proportional to the WBC count. The outputs of the three pulse-integrator circuits are sent to a voting circuit. If the three outputs agree within a specified range, they are averaged. If one output disagrees with the other two by more than the specified range, it is not used in computing the average. If all three outputs disagree by more than the specified range, an error indicator is set, and a zero value is produced.

The next step in the signal processing is to correct the average-count signal for coincidence. Coincidence is the passage of two or more WBCs through the aperture at the same time. Statistical analysis is used to estimate the average level of coincidence for the aperture size and any uncorrected count level. An analog circuit makes this conversion. The digital WBC count value is displayed and also recorded on a printer.

We will now examine the right side of Figure 11.8. The first step is the further dilution of the specimen to 1:224 in Diluter II. This second dilution is required because of the much greater concentration of RBC than of WBC in the blood. A system identical to the one described for the WBC count is used to obtain the RBC count.

Cells with volumes greater than 35.9 fl are classified as RBCs. A 256-channel RBC size histogram is prepared. The MCV and RDW are computed from this histogram. The RDW is the coefficient of variation of the RBC volume distribution.

Cells whose volumes are in the 2 to 20 fl range are classified as platelets. The volumes of these cells from each aperture are transformed into a 64-channel histogram. These histograms are statistically processed to yield a platelet count along with a mean platelet volume (MPV) and platelet distribution width (PDW) from each channel. A voting process similar to that described for the WBC count is used to determine the final values for these parameters. The MPV and PDW values are primarily used for quality control functions at this time.

The RBC count, Hb and MCV are input to a special-purpose computer circuit that calculates the values of HCT, MCH, and MCHC by using the relationships given in (11.6) to (11.8).

The Coulter STKS performs a WBC differential count using a flow cytometry approach. At the same time portions of the specimen are being delivered to the WBC and RBC baths, another portion of the specimen is sent to the WBC differential mixing and lyzing chamber. Here the specimen is combined with (1) a lyzing agent to remove the RBCs and (2) a WBC stabilizing agent. The WBC stabilizing agent preserves the characteristics of the WBCs as they are processed in the triple transducer flow cell. Flow cytometry consists of evaluating cells moving in a fluid stream. The triple transducer module includes electronics to create a single line of cells that is passed through a measurement station. In the STKS measurements of low-frequency impedance, high-frequency conductivity and light scatter are made. The cell volume measurement is based on low-frequency impedance (the same approach used to measure RBC and WBC volume), and the internal conductivity is derived from the high-frequency conductivity. A laser illuminates the cells in the measurement station to create the light scatter. The light scatter measurement is made by a forward scatter detector. A measurement of cell internal structure and shape is based on the light scatter measurement. The cell volume, internal conductivity, and cell internal structure and shape measurements are sent to the analyzer computer for processing. The percent lymphocytes, monocytes, neutrophils, basophils, and eosinophils are calculated based on the cell position in a three-dimensional scatter plot. Figure 11.10 is one of the two-dimensional views of this three-dimensional scatter plot. These two-dimensional views can be displayed at the instrument to allow the operator to monitor the functioning of the instrument. The values for basophils and eosinophils are less reliable than those for the other cell types. If the percentage of these cell types exceeds laboratory specified limits (e.g., 10% for

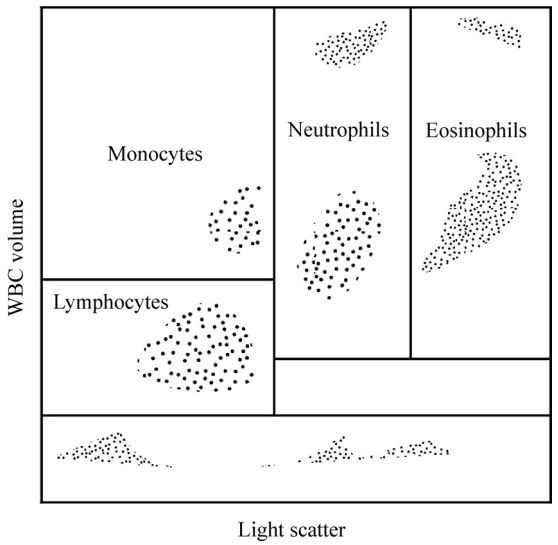

Figure 11.10 Two-dimensional scatterplot

eosinophils or 5% for basophils), a technologist will scan a peripheral blood smear slide to determine if the calculated value is correct. If it is not, a manual WBC differential will be performed. The STKS applies criteria to the scatter plot to determine if the computed WBC differential count appears to be accurate. In the majority of cases, the count is judged accurate and the need to perform a labor-intensive manual count is eliminated. Since the STKS cannot accurately identify immature WBCs (these cells are not normally found in the peripheral blood), the presence of these cells will trigger a message stating that a manual WBC count needs to be performed (Zelmanovic and Kunicka, 2006).

The STKS uses this same approach to measure the number of reticulocytes that are present. The RBCs are dyed before the specimen is placed in the STKS with New Methylene Blue to enhance the differences in characteristics measured in the flow cell between reticulocytes and mature RBCs. The reticulocyte count measurement is made as a separate run of the instrument. This is a very useful capability since a manual reticulocyte count is a time-consuming and relatively inaccurate process.

The blood parameters are printed on a result-report card. The printer includes a patient-identification number that is input to the STKS by the technologist. In computerized clinical laboratory systems, this identifying number, and the blood parameters are directly transmitted to the laboratory computer system.

COULTER LH 755

The LH 755 is a workstation that blends sample handling, advanced testing accuracy and integrated postanalytical slide making and slide staining in real time.

SPECIMEN HANDLING

The LH 755 uses a simple no prep loading method. Operators take a sample tube, place it into a 12-sample cassette and place the cassette in the instrument. Operators do not need to preselect tubes for sample, type them, or sort them before loading since the LH 755 has a random sample testing capability. Samples can be loaded as they come into the laboratory, increasing laboratory productivity.

Before aspiration the LH 755 keeps samples mixed with a gentle motion of the rocker bed. This ensures that cells are properly suspended without the risk of cellular destruction. The aspiration verification system ensures both sample verification and complete sample integrity. It includes a barcode scanner that positively identifies specimens and provides an audit trail to the exact location of the sample tube.

The LH 755 confirms sample integrity using dual optical blood detectors that check every sample for the presence of clots or microbubbles. The LH 755

also features a totally sealed aspiration and dilution system to protect laboratory staff from blood born pathogens. While the LH 755 enclosed needle simultaneously vents and aspirates the sample, it withdraws an aliquot of blood that is sufficient to analyze a sample and make a slide. This eliminates the need for redundant sample processing should a slide be needed.

The aliquot of blood is kept mixed by repetitive gentle inversion in a heated core to ensure proper cellular distribution without damaging the cells. These mechanisms and automated processes minimize operator procedures while improving productivity. Operator safety is increased by minimizing the chance of exposure to patient specimens.

TEST PERFORMANCE

The LH 755 performs complete blood counts, WBC differential counts, and RBC morphology evaluation as well as nucleated RBC (NRBC), platelet and reticulocyte counts. The AccuCount technology delivers expanded linearity and high levels of accuracy, particularly at the clinical decision threshold, and virtually eliminates false-positive flagging. It is a set of mathematical algorithms that is used to process the individual cell volume, high-frequency conductivity, and laser-light scatter measurements made by the instrument.

The AccuCount WBC count has linearity from 0 to $400,000/\mu l$ and eliminates most interference from RBCs, NRBCs, giant platelets, and platelet clumps, which decrease the need for manual intervention. The AccuCount platelet count expands linearity from 0 to $3,000,000/\mu l$. The accuracy of AccuCount has been validated against reference flow cytometry methods.

The LH 755 WBC differential allows laboratories to minimize manual slide reviews. It includes a six-part differential plus the enumeration of NRBCs. The determination of which cells are NRBCs is based on the location of the cell in the NRBC area of the differential plot (cell volume versus rotated light scatter) as well as the presence of cells in the far left (low cell volume) end of the WBC histogram. If cells are present in both locations, the NRBC count is derived from the WBC histogram.

AccuGate technology provides accurate enumeration of populations, blasts identification, and accurate flagging. It is based on using adaptive contouring methods to achieve optimal separation of overlapping clusters of data.

SLIDE PREPARATION

When cells are present that can't be identified by the LH 755 differential method or when user defined decision rules specify that a manual review of the blood specimen is required, a blood smear is automatically prepared. This process is begun by the integrated slide labeling station attaching a label to the slide. The label can contain up to 7 lines of information, or three lines of text and a barcode. It is not affected by fixatives or stains. Once at the smearing station a second slide is released and is used as the spreader blade.

As a drop of blood is applied to the slide, an algorithm makes an intelligent viscosity calculation based on parameters of the CBC data. This calculation determines the amount of time the spreader blade waits for the blood to properly spread out across the slide, as well as the acceleration rate and the final velocity of the spreader blade, creating a slide with a consistent feathered edge. The feathered edge is the portion of the smear where the RBCs are just touching and is the best area in which to make cytologic evaluations.

The prepared smear is dried and stained. The LH 755 gently warms the slide from underneath accelerating the natural evaporation process without introducing drying artifacts. When a slide exits the drying lane it is automatically lowered into a basket. Completed baskets containing 6 to 12 slides are routed to the integrated slide-staining module. The slide stainer's robotic arm then retrieves the basket and moves it into the staining module for automatic fixing, staining and drying.

SYSTEM CONTROL

Age, gender and location specific reference ranges, action and critical limits can be specified. These postanalytical sample review criteria are used so that every sample is assessed the same way every time by every person running the analyzer. Decision rules can be easily defined for parameter values, flags, system messages, location, specific physicians or a combination of these factors. The LH 755 provides fully automated testing that reduces technologist time requirements.

Normal results are auto validated without further technologist intervention. Abnormal results are held for review and follow-up as indicated by the message generator. Samples that require review are sorted and placed in various folders for quick retrieval. A pathologist will review the abnormal slides. He/she will prepare an interpretive report including possible etiologies of the abnormal smear results for the patient's clinician.

PROBLEMS

11.1 Discuss the differences between photometers or colorimeters and monochromators. What are the factors to consider in selecting one or the other for a particular determination?

11.2 Sketch a double-beam spectrophotometer, and explain its operation.

11.3 Explain why fluorometers can be used to detect much smaller quantities of substances than absorption spectrophotometers.

11.4 Assume that you are the biomedical engineer at a 300-bed hospital. The director of the clinical laboratory plans to buy an automated chemical analyzer and wants your advice on which type to buy. What factors would you consider in preparing your response? (This is a broad question, but try to be as specific as possible.)

11.5 The following values are obtained for a specimen of venous blood: MCV = 90 μm^3, HCT = 40%, and MCH = 30 pg. Compute the RBC count, the MCHC, and the Hb concentration.

11.6 Design a circuit to perform the RBC-counting function in a Coulter Counter. Include the voting logic.

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