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BioMEMS and Medical Microdevices

Steven S. Saliterman

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Chapter 8

Clinical Laboratory Medicine

8.1 Introduction

Of the many possible applications for bioMEMS devices, none have achieved as great a success as LOC devices and μTAS. These devices are used for clinical and research laboratory applications. Many of the biosensors discussed in Chapter 6 have been incorporated into these devices. In addition, DNA and protein microarrays perform studies unimaginable by prior techniques.

Understanding the basic concepts behind laboratory studies and how they are traditionally performed seems prudent at this juncture. Ways to miniaturize these systems with the inherent advantages of higher throughput, greater sensitivity and specificity, lower cost, less reagent and sample requirements, less waste generation, and improved detection schemes are explored later.

Most engineering students, including bioengineering students will not have a strong foundation in laboratory medicine by the time they finish graduate training. If your goal is to become a practicing bioMEMS designer, then it is worth your time to learn laboratory medicine. While basic courses in biology, cell biology, biochemistry, genetics, and physiology are helpful, additional courses in immunology, microbiology, toxicology, and pharmacology can give you the necessary background to communicate with the medical research community.

Laposata's *Laboratory Medicine: Clinical Pathology in the Practice of Medicine* (2002) is an excellent resource and is cited several times throughout this chapter.

8.1.1 Clinical laboratory improvement amendments (CLIA)

Already introduced in Chapter 1, The Center for Medicare and Medicaid Services (CMS) regulates all laboratory testing (except research) performed on humans in the United States through CLIA. Congress passed CLIA in 1988, establishing quality standards for laboratory testing to ensure the accuracy, reliability, and timeliness of patient test results regardless of where the test was performed. A laboratory is defined as any facility that performs laboratory testing on specimens derived from humans for the purpose of providing information for the diagnosis, prevention, treatment of disease, or impairment of, or assessment of health.

Laboratory tests are categorized as *waived*, *moderate complexity*, or *high complexity*. Each laboratory must be either CLIA exempt or possess one of the following CLIA certificates: (1) *registration certificate*; (2) *waiver certificate*; (3) *provider-performed microscopy* (PPM) procedures; (4) *certificate of compliance*; or (5) *certificate of accreditation*.*

*www.phppo.cdc.gov/clia.

Many of the studies described herein and for which μTAS systems will be applied are considered high complexity.

8.1.1.1 Personnel

CLIA defines the following laboratory personnel.

The *director* is responsible for the overall management and direction of the laboratory but does not have to be on-site at all times. A physician with one year of experience directing/supervising a nonwaived laboratory or 20 continuing medical education credits in laboratory practice would qualify, as would a person with a bachelor's degree and two years laboratory training/experience plus 2 years supervisory experience in nonwaived testing. The director could, depending on education and experience, qualify for all other positions.

It is the director's responsibility is to make sure that patient studies are run properly. Procedures must be in place for tracking samples and for reporting results, including critical values. Equipment calibration, standard results, reagent lot numbers, and temperature charts of refrigerators and freezers must be logged and maintained. Safety instruction and inspection, training and periodic retraining must also occur on a continuous basis and be documented.

Testing personnel are responsible for specimen processing, test performance and reporting test results. The minimum requirement is a high school diploma or equivalent and training for the testing performed.

The *technical consultant* is responsible for the technical and scientific oversight of the testing. The minimum requirement is a bachelor's degree with two years laboratory training, or experience in nonwaived testing.

The *clinical consultant* provides clinical consultation. The minimum requirement is a doctoral degree with board certification.

8.1.1.2 Quality management

CLIA certified laboratories must undergo a biennial inspection. Laboratories are required to do the following to maintain quality:

(1) Maintain procedures that ensure proper patient preparation, specimen identification and integrity; and monitor and evaluate the testing process and quality management system.
(2) Maintain a procedure manual (manufacturer's package insert may be acceptable).
(3) Follow manufacturer's instructions for performing the test.
(4) Test a positive and negative control each day that patient samples are tested (package insert may provide flexibility).
(5) Enroll and participate in proficiency testing (if available).

(6) Identify and correct problems, and record remedial actions taken.
(7) Maintain test requests and laboratory records for two years.

8.1.2 Performing laboratory studies

8.1.2.1 Quality assurance issues

The process of performing a laboratory study on a patient begins the moment a study is ordered and ends when the person ordering the study acknowledges (by signature or computer notation) reading the result. All too often the process is viewed as beginning when the specimen reaches the lab and ending when a technician places the result into the computer.

Quality assurance problems may occur in the ordering process, while running the study, reporting results, or if there is a failure to read and respond to abnormal findings. A delay in ordering, misunderstanding an order, or ordering the incorrect study may occur. Once inside the laboratory, problems can occur in labeling the specimen, machines can malfunction, or operators can cause errors. Failure to report results in a timely manner may occur, especially for critical values. Once results are reported, problems can occur with someone actually reading the report and responding to an abnormality. This is more likely to happen when the person who ordered the study is not the same person who is supposed to read it and react to the result. The latter problem can occur when more than one physician assumes primary care over a short period of time, typical of hospitalist run care systems.

Unfortunately, these kinds of problems are often dealt with by a *quality assurance committee* after the fact (if discovered) and not the director. Moreover, these issues are not easily resolved or attributed to just one person, as they include diagnostic imaging and other evaluative procedures.

8.1.2.2 Process steps

Ideally a physician or nurse practitioner orders a study, and either they or an assistant places the request into a *computer order entry* system (COE). Depending on the specified urgency of the study, the laboratory must review the request and dispatch someone to collect the sample (blood, urine, cultures, etc.). The sample must be expeditiously brought to the laboratory for processing.

Typically whole blood, cerebral spinal fluid, urine, and other body fluids or cultures arrive at the lab in vacuum vials, closed plastic containers, or culture medium. These are typically placed in sealed plastic bags labeled “BioHazard” for safety purposes during transportation.

Once inside the laboratory, the least amount of time the specimen is manually handled the better. Figure 8.1 shows the robotic LAB Interlink automated specimen-processor-control station. Figure 8.2 shows the automatic handler and centrifuge (foreground). This system reads specimen bar codes, logs specimens into the

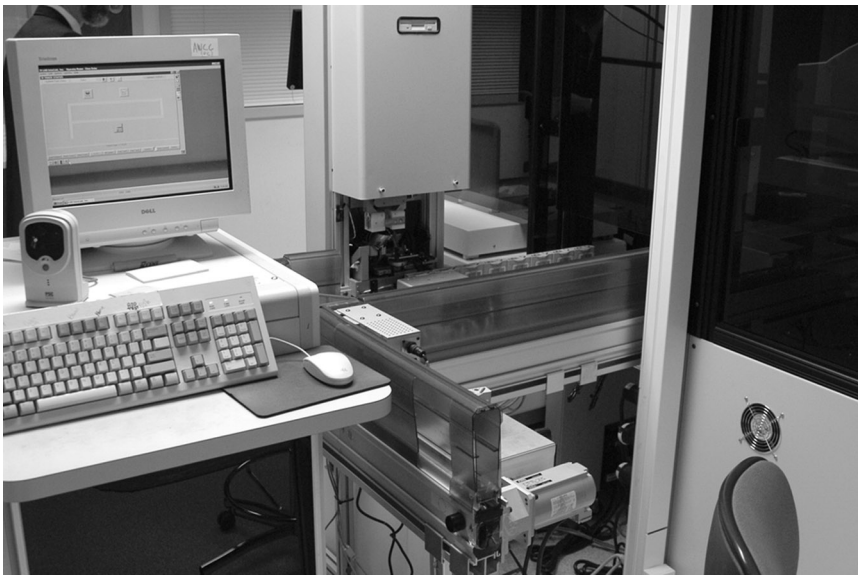

Figure 8.1 The robotic LAB Interlink system places specimens into an automated rail system that includes bringing the specimen for centrifugation and distribution to different instruments throughout the lab. Specimens are identified and bar-coded, and sent along for processing.

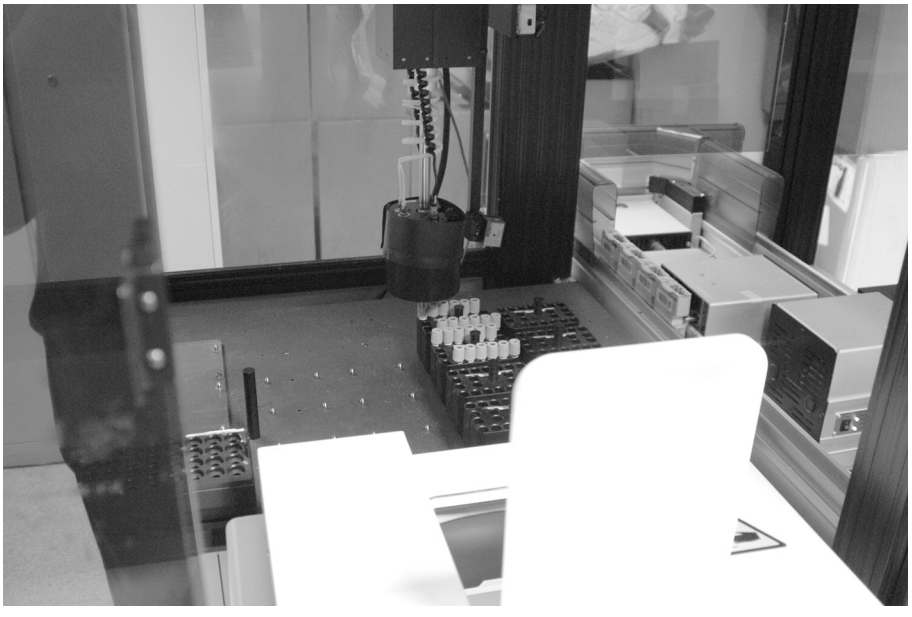

Figure 8.2 Close up of the LAB Interlink system with centrifuge (in the foreground) and robotic manipulator arm.

laboratory information system (for tracking purposes), centrifuges specimens as required, and sorts specimens into racks to go to the individual departments for testing.

Modular in design, the system consists of expandable tracks capable of automated distribution of specimens to instruments and to storage without manual intervention. The LAB-Link can interface to any instrument vendor that complies with the National Committee for Clinical Laboratory Standards (NCCLS). The automation modules can be used to provide both pre- and post-analytical specimen processing as well as to support automated analytical instruments online.[†]

Many samples require centrifugation prior to analysis. Whole blood separates into a straw-colored serum used for chemistries, and concentrated cellular material, which is usually discarded. Hematology vials arrive with a small amount of anticoagulant to prevent clotting, and are not centrifuged. Other fluid samples such as urine require centrifugation as a means of concentrating the analytes.

Each test on the sample provided should be run as quickly as practical, so emergency and urgent studies can move through more quickly. Critical values (studies with life-threatening results) need to be flagged for an immediate personal phone call to the appropriate person. Other studies should automatically be ported to a computer system accessible to the ordering person for review, or promptly delivered as a printout.

Once a study has been reviewed, the responsible person needs to either sign the report or check an appropriate box in the computer noting the result has been reviewed. There needs to be some system in place to recognize that a study has not been reviewed so that corrective action can be taken. The *Electronic Records and Electronic Signatures Rule* (21 CFR Part 11) was established by the FDA to define the requirements for submitting documentation in electronic form and the criteria for approved electronic signatures.

8.1.2.3 Micro-total-analysis-systems (μTAS)

The forthcoming chapters deal extensively with μTAS, and it is important to keep in mind that these systems may need to integrate with established sample transport systems and data reporting. Some μTAS may be part of hand-held units or designed for point-of-care diagnosis with less stringent requirements. Other μTAS may be integrated into high throughput machines for large-scale sample processing, and require control systems, power supplies, plumbing, tubing, connectors, and a user interface.

8.1.3 Reimbursement considerations

Cost savings will ultimately drive the development and implementation of bioMEMS devices in medicine. It is therefore necessary to think about who pays for the new technology and who ultimately benefits from it.

[†]www.labinterlink.com.

Chemistry studies are ordered based on a common nomenclature referred to as the *current procedural terminology* or CPT codes. There are different CPT codes for every preventive medicine, evaluation, management, surgery, laboratory, pathology, and imaging service that can be provided to a patient. These codes are revised annually, and new codes are added while some are removed. Fundamental changes in the way a laboratory test is performed may result in a new CPT code.

The CPT code specifies a laboratory study and is used to code payment. Medicare national limitation amounts are published in the clinical laboratory fee schedule (CLFS). Health maintenance organizations (HMOs) typically reimburse based on the CLFS or a negotiated amount with the ordering facility. *Fee-for-service* or private pay (paying what the laboratory charges), is less commonly seen these days.

Finding a bioMEMS solution to an existing technique that lowers cost may in the short-term benefit the laboratory. However, once the cost of running a particular test is universally reduced, the reimbursement rate is also generally reduced, and it is the payor (Medicare, HMO, or insurance carrier) who benefits in the long run. If overall costs of healthcare come down, then theoretically the insurance premium cost to patients should come down as well. Arguably the latter is rarely observed.

Often studies are matched with an appropriate *IDCM-9 diagnosis code* for reimbursement, assuring the payor that there was a legitimate reason for ordering the study.

8.1.4 Methodologies

Laboratory studies may be broadly categorized as chemistry, hematology, immunology, microbiology, anticoagulation, blood gas, urine, cytology, and pathology studies.

Techniques used include immunoassays such as ELISA, chemiluminescence, nephelometry, direct and indirect antiglobulin detection, immunofixation, immunofluorescence, and latex particle agglutination; protein, immunoglobulin and hemoglobin electrophoresis; lipid ultracentrifugation; spectrophotometry; ion selective, and pH electrodes; impedance measurement; flow cytometry with and without cell markers; slide preparation and staining; and microscopy. Many of these techniques have already been miniaturized for point-of-care systems, and many of these devices incorporate bioMEMS technology.

8.2 Chemistry

8.2.1 Introduction

Technician skills have migrated from bench chemistry to high-throughput machine operation and insuring that there is a steady stream of sample analysis and reporting.

The CPT codes define both *panels* and *individual* tests. For example, the *comprehensive metabolic panel* (CMP) includes an albumin, alkaline phosphatase, ALT, AST, bilirubin, blood urea nitrogen (BUN), calcium, chloride, CO_2, creatinine, globulin, glucose, potassium, sodium, and protein.

There are hundreds of individual tests including electrolytes, proteins, liver and renal function tests, enzymes, tumor markers, various serologies, and medication levels.

8.2.2 Enzyme-linked immunosorbent assay (Indirect ELISA)*

The ELISA test is performed with any body fluid. The sample containing a specific antibody of interest is placed in a *well* that has been coated with a ligand (binding protein) for that antibody. Antihuman antiglobulin antibody that has been conjugated with an enzyme is then added, which binds to the patient's antibodies adherent to the ligand in the well. A substrate specific for the conjugated enzyme is added, imparting color. The amount of color is then measured proportional to the amount of antibody present in the original patient sample. These process steps are shown in Fig. 8.3.

A typical ELISA plate consists of 96 wells on an 8×12 matrix, each well being about 1-cm high and 0.7 cm in diameter.

The Abbott Laboratories (Abbott Park, IL) Commander System is an automated, high-throughput immunoassay testing system for the screening of blood for hepatitis, retrovirus, and other analytes. The Commander System can initiate up to 800 tests per hour and consists of a flexible pipetting center, the parallel processing center, and the dynamic incubator.

False positive results may occur, and some analytes such as HIV require retesting by *Western blot*, where ELISA measures antibodies to whole virus, Western blot is an electrophoretic technique that allows analysis of antibodies directed against several viral proteins such as the envelope, core, or reverse transcriptase.

8.2.3 Chemiluminescence

Chemiluminescence (CL) is the generation of visible, ultraviolet, and infrared light by the release of energy from a chemical reaction. These reactions can be grouped into three types: (1) *chemiluminescent*, which are chemically induced through synthetic compounds and usually involving a highly oxidized species; (2) *bioluminescent* (BL), which arise from a living organism; and (3) *electrochemiluminescent*, which take place by the use of electrical current. Both CL and BL reactions usually involve the cleavage or fragmentation of the O—O bond of an organic peroxidase compound. These bonds are easily cleaved and liberate a lot of energy.

*Alternatively, in the "sandwich" assay, the sample is added to a well coated with an antibody against an analyte of interest, such as a protein, hormone or drug. A second antianalyte antibody conjugated with an enzyme is then added to the well.

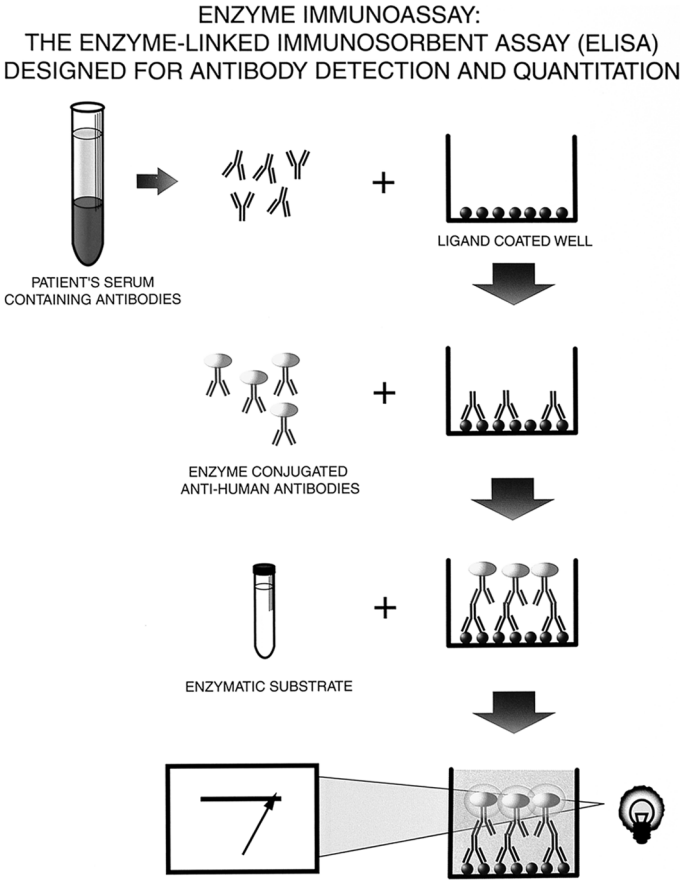

Figure 8.3 The enzyme-linked immunosorbent assay (ELISA). [Reprinted with permission from Laposata (2002), copyright American Society for Clinical Pathology.]

An automated chemiluminescence system is shown in Fig. 8.4. This system performs numerous thyroid, reproductive, oncology, anemia, therapeutic drug monitoring (TDM), cardiac, and endocrine studies.

8.2.4 Nephelometry

Nephelometry is based on the detection of scattered or reflected light. A patient's serum is incubated with a reagent containing specific antibodies. Formation of small precipitates of antigen-antibody complexes increases scattered light, which is proportional to the amount of antigen present. Figure 8.5 shows the process steps for nephelometry [Laposata, 2002].

Figure 8.6 shows a nephelometer. This may be used to detect and quantitate immunoglobulins (IgG, IgM, IgA), complement (C3, C4), rheumatoid factor,

Figure 8.4 This system uses chemiluminescence to tag antibodies, replacing traditional radioimmunoassays. Tests include thyroid (TSH, free T4), tumor markers (CA-125, CEA) and hormones (testosterone, estrogen).

transferrin, prealbumin, haptoglobin, and many other serum proteins and drugs (over 60 plasma assays are available).

One of the earliest books describing nephelometry was written by John Yoe in 1928.

8.2.5 Lipoprotein analysis

Lipoproteins include triglycerides and cholesterol. Cholesterol includes beta lipoproteins (LDL) and the alpha lipoproteins (HDL). Lipoprotein analysis may be performed with electrophoresis or ultracentrifugation. In electrophoresis, plasma is applied to an agarose gel, and four classes of lipoproteins separate. These are the chylomicrons, prebeta lipoproteins (VLDL), LDL, and HDL components.

Ultracentrifugation is performed by adding three specific density solutions to the plasma, centrifuging, and observing the separation into three lipoprotein bands based on density.

8.2.6 Spectrophotometric and ion-selective electrode (ISE)

Spectrophotometric assays measure a change in light absorbance at a specific wavelength, usually caused by dye formation in a series of linked chemical reactions.

The Dade Behring Dimensions RXL (shown in Fig. 8.7) combines spectrophotometric, ELISA, and ion-selective electrodes (ISE) in a single instrument for

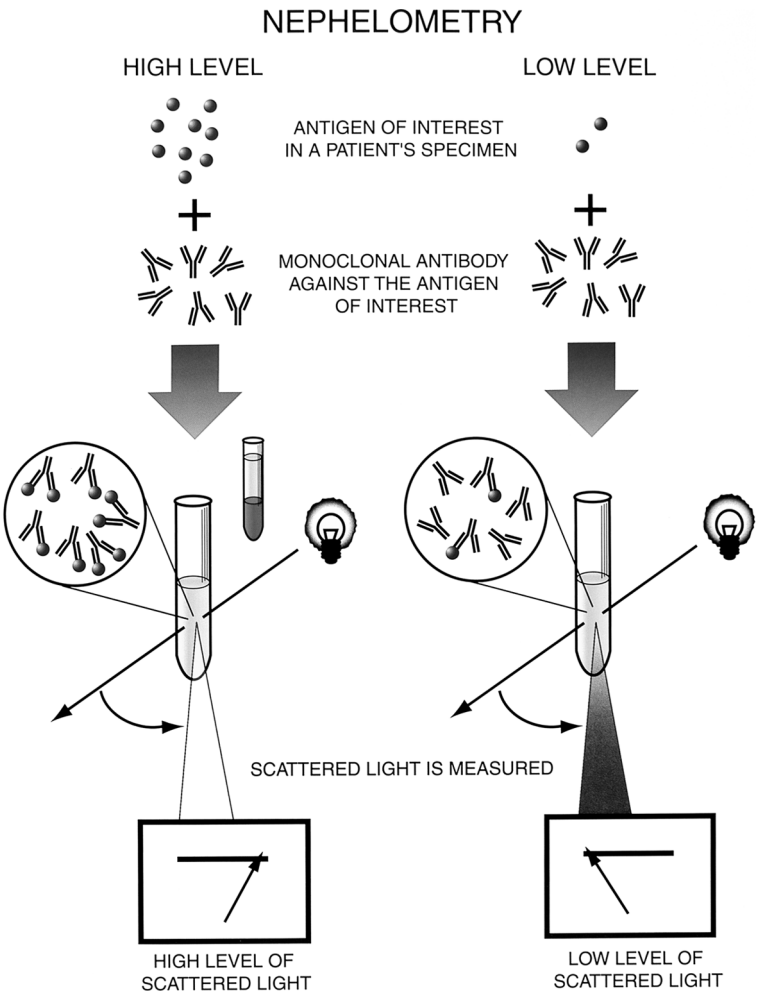

Figure 8.5 Nephelometry. [Reprinted with permission from Laposata (2002), copyright American Society for Clinical Pathology.]

measurement of comprehensive metabolic panels, total cholesterol, TDM, immunoassays for troponin, quantitative HCG, myoglobin, urine chemistries and electrolytes.

8.2.7 Protein electrophoresis

Excessive amounts or deficiencies of a protein may be abnormal. Protein electrophoresis allows differentiation of the different proteins. "Bands" of proteins may be observed, and abnormalities may be seen in various nutritional deficiencies, liver disease, protein disorders, inflammation, multiple sclerosis, cancer, and monoclonal gammopathies (increased production of an immunoglobin).

Figure 8.6 The nephelometer is capable of performing IgG, IgM, rheumatoid factor, transferrin, prealbumin and haptoglobin studies.

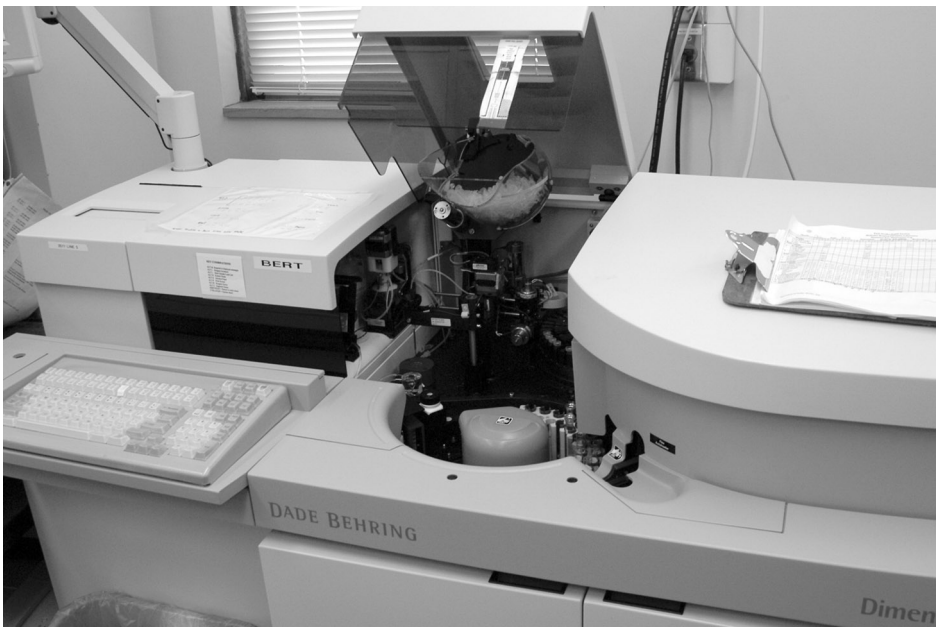

Figure 8.7 The Dade Behring Dimension XL uses spectrophotometric, EIA, and ion-selective electrode methods. It is able to perform general chemistries, liver functions tests, therapeutic drug levels, immunoassays for troponin and quantitative HCG, myoglobin, urine chemistries, and electrolytes.

In protein electrophoresis, a small amount of serum, urine, or concentrated CSF is applied onto an agarose or cellulose gel. Proteins are separated in an electric field and made visible by staining.

8.3 Hematology

8.3.1 Automated blood cell counters

An automated blood cell counter is shown in Fig. 8.8. This performs automated complete blood counts (CBC), white blood cell differentials, and reticulocyte counts. This unit uses a combination of volume, conductivity, and light scatter to achieve sensitivity, specificity, and efficiency in white cell differential and reticulocyte analysis.

The *Coulter principle* of counting and volume determination is based on dc impedance measurement. Particles suspended in a weak electrolyte solution are drawn through a small aperture separating two electrodes, between which an

Figure 8.8 The Coulter Counter GEN S performs complete blood counts (CBC). This includes a built-in slide maker and ability to perform reticulocyte counts.

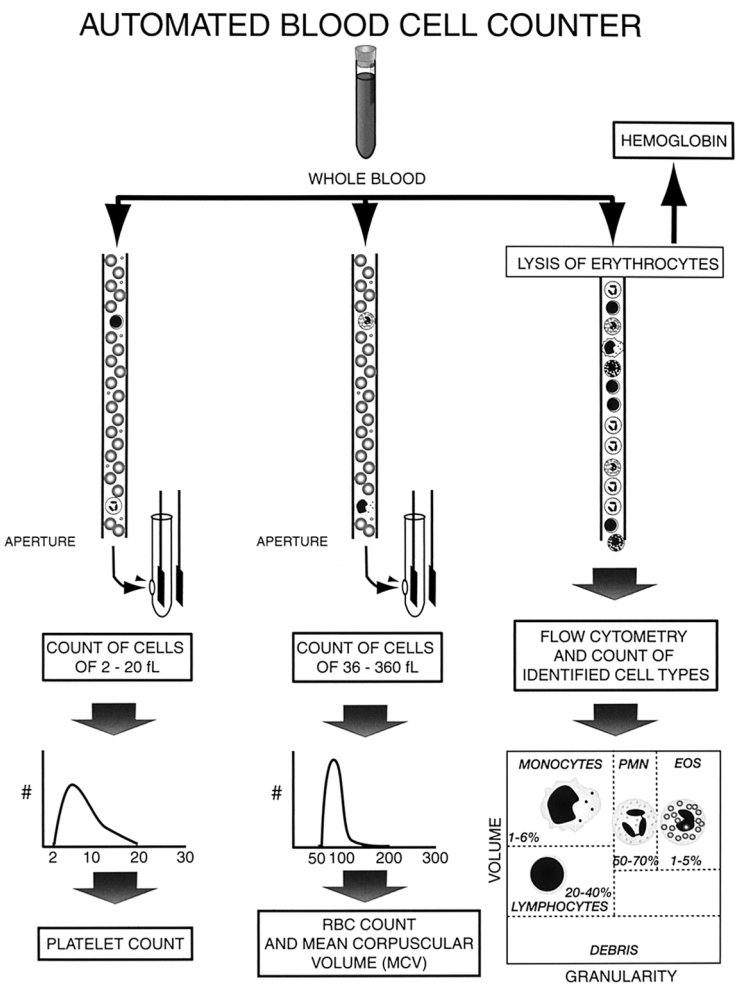

Figure 8.9 Automated blood cell counting. [Reprinted with permission from Laposata (2002), copyright American Society for Clinical Pathology.]

electric current flows. The voltage applied across the aperture creates a "sensing zone." As each particle passes through the sensing zone it displaces its own volume of conducting liquid, momentarily increasing the impedance of the aperture. This is shown in Fig. 8.9.

This change in impedance produces a tiny but proportional current flow into an amplifier that converts the current fluctuation into a voltage pulse large enough to measure accurately. The Coulter principle states that amplitude of this pulse is directly proportional to the volume of the particle that produced it. Scaling these pulse heights in volume units enables a size distribution to be acquired and displayed. In addition, if a metering device is used to draw a known volume of the particle suspension through the aperture, a count of the number of pulses will yield the concentration of particles in the sample. Resolution is the capability to

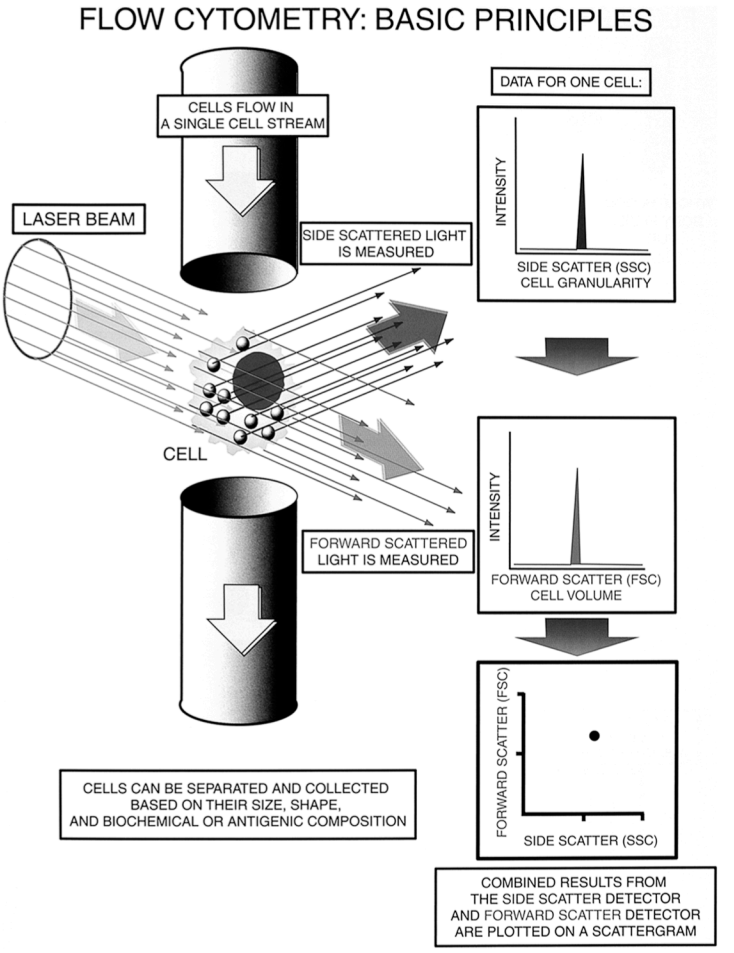

Figure 8.10 Basic flow cytometry. [Reprinted with permission from Laposata (2002), copyright American Society for Clinical Pathology.]

differentiate between different particle sizes. Higher resolution means more detailed size information.[‡]

While this technique is suitable for analyzing red blood cells, analyzing white blood cells requires additional *flow cytometry*. The basic principle of flow cytometry is shown in Fig. 8.10. Here, cells flow in a single cell stream past a laser beam (coherent light source) and detector. Light that is forward scattered and side scattered is measured and plotted together as a scattergram. Forward scatter is the result of diffraction and correlates with cell volume. Side scatter (right angle) is the result of refraction and correlates with internal cellular granularity [Laposata, 2002].

[‡] www.beckman.com.

The Coulter Gen-S improves upon simple flow cytometry by measuring *conductivity, opacity*, and *rotated light scatter* (RLS). Conductivity is measured by applying RF-ac signals across the cell. The RF signal short circuits the bipolar lipid layer of a cell's membrane, allowing the energy to penetrate the cell. This reveals information about cell size and internal structure, including chemical composition and nuclear volume.

Opacity involves compensating for volume-related differences in conductivity characteristics of different kinds of white blood cells. By correcting the conductivity signal so that it is no longer influenced by cell size, measurements are related only to the internal structure of the cell. For example, this allows calculation of the nuclear-to-cytoplasmic ratio, which is useful for distinguishing variant lymphocytes from normal lymphocytes.

Cell-volume-related differences in light scattering can also be accomplished. RLS is the determination of the optimum angle of scatter for each cell type. A scatter detector that covers the range of 10 deg to 70 deg accurately separates different types of white blood cells into distinct clusters without mathematical manipulation. Information is obtained about cellular granularity, nuclear lobularity, and cell surface structure.

Each measured cell is then plotted on a 3D array based on RLS, volume, and opacity. Distinct clusters are observed and may be analyzed to report the percentage of each cell type, and their morphology and density. Deviation of these clusters from their normal quantity, shape, position, or density is an indication of a distributional or morphological abnormality.

8.3.2 Flow cytometry with cell markers

An alternative approach to flow cytometry is to incubate a cell suspension with fluorescent compounds (fluorochromes), each with different emission spectra. The cell suspension is incubated with two or more monoclonal antibodies, each labeled with different fluorochromes. As cells bound with antibodies pass through the detector cuvette, laser light excites the fluorescent compounds causing them to fluoresce at different wavelengths. The light is analyzed for forward and side scatter and the intensity at a given frequency. Data collected from many events is plotted on a histogram. Different antibody markers provide precise identification of cells. This is shown in Fig. 8.11.

The Coulter Cytomics FC 500 flow cytometer conducts 5-color analysis from either single or dual laser excitation. The system plots a 2D display of cell size versus markers. A particular population of cells can be followed through various markers with calculation of percentage of those markers.

8.3.3 Peripheral blood smear

In this test, a drop of whole blood is applied across a glass slide using a *pusher slide*. The slide is stained with Wright stain and reviewed under the microscope for red blood cell morphology and for manual counting of white blood cell types.

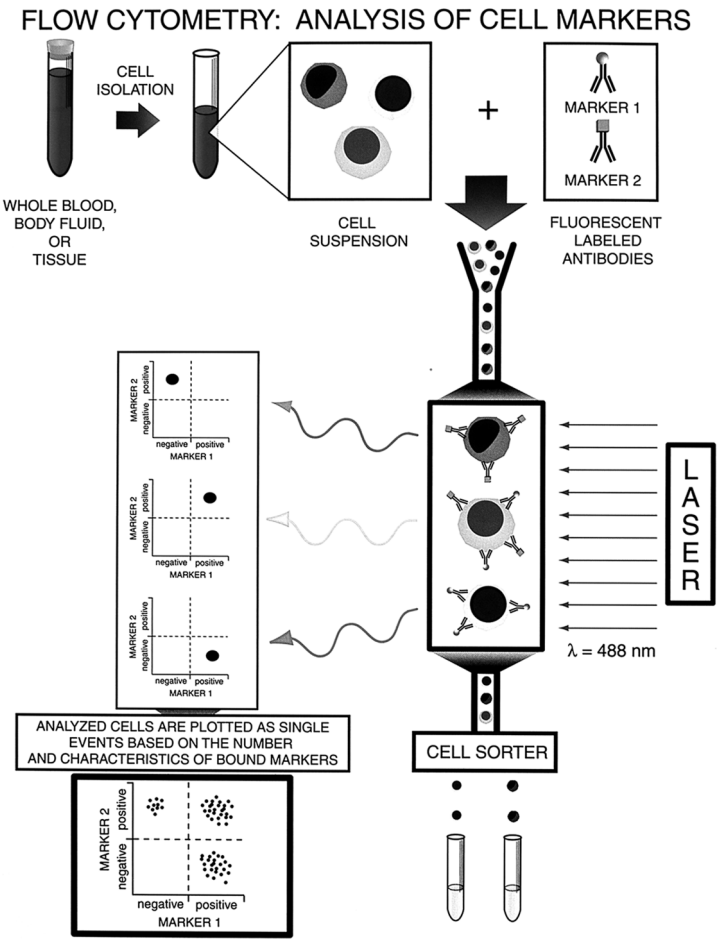

Figure 8.11 Flow cytometry with cell markers. [Reprinted with permission from Laposata (2002), copyright American Society for Clinical Pathology.]

While most slides are read by a technician, abnormal slides may be reviewed by a pathologist for identification of leukemias, verification of high or low counts, identification of abnormal morphology, or intracellular material.

Creating slides can now be done by automated means as well.

8.3.4 Hemoglobin electrophoresis

Hemoglobin electrophoresis begins with centrifugation of whole blood, removal of the serum, and then washing the blood cells with saline. The cells are then lysed to release hemoglobin. Using cellulose acetate for electrophoretic separation, the sample and two controls, one a mixture of hemoglobins and the other normal adult hemoglobin, are applied to different lanes. After separation, the gel is stained and read. Densitometry is used to estimate relative concentrations of different

hemoglobins on the gel. Separation on citrate gel is used to further identify certain abnormal hemoglobins [Laposata, 2002].

8.3.5 Erythrocyte sedimentation rate (ESR)

Elevated ESR may be used as an index of inflammatory states in the body, and although nonspecific, may be elevated due to infections, arthritis, neuromuscular disease, and several other conditions. Anticoagulated blood is placed in an upright tube and allowed to settle over one hour. The amount of settling red blood cells is measured in millimeters, and more than 20 mm/h may be indicative of some underlying disease process.

Measurement of the ESR is a time-honored technique in medicine [Gotzl et al., 1942].

8.4 Immunology

Antibodies are proteins synthesized and secreted by *B-cells* in the body. They bind to *antigens*, most of which are other proteins, polysaccharides, or nucleoproteins. Binding is noncovalent (similar to enzymes and substrates) between the antigen-combining site on the antibody and a portion of the antigen called the *antigenic determinant*, or *epitope.*

Antibodies consist of both light and heavy chains (Fig. 8.12). B-cells are lymphocytes that are produced and mature in the bone marrow. T-cells are lymphocytes that are produced in the bone marrow but mature in the thymus gland. (Lymphocytes are one of the five types of white blood cells.) Both B- and T-cells are specific for a particular antigen, and have specific receptors (BCR, TCR). These receptors are integral membrane proteins, have thousands of copies on the cell surface, are made before the cell ever encounters an antigen, and are encoded by genes assembled by the recombination of segments of DNA.

8.4.1 Antinuclear antibody (ANA)

The ANA test detects autoantibodies directed against a variety of antigens in the body, most located in the nucleus of cells. These occur in *autoimmune diseases*, and include among others rheumatoid arthritis, mixed connective tissue disease, and systemic lupus erythematosus.

Whole blood is centrifuged, and the serum is diluted at a ratio of 1:40 for placement of a monolayer of Hep-2 cells. Anti-IgG fluorescent-labeled antibodies are applied and incubated, then washed and reviewed under a fluorescent microscope. The observed fluorescent pattern is reported as normal, homogeneous, rim, speckled, or nuclear pattern; each suggesting a possible underlying disease. Abnormal results are typically followed up with *extractable nuclear antigen* (ENA) testing for more specificity.

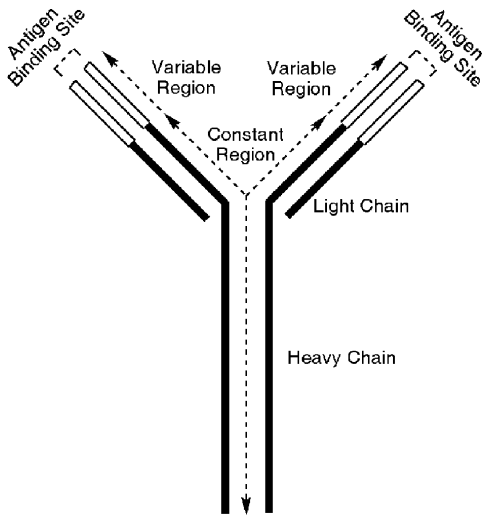

Figure 8.12 Antibodies consist of two heavy and light chains. The *variable region* gives the antibody its specificity for binding an antigen. The *constant region* determines the mechanism used to destroy the antigen. Antibodies are divided into five classes: IgM, IgG, IgA, IgD, and IgE, based on their constant region structure and immune function.

8.4.2 Direct antiglobulin test

The direct anticoagulation test or *direct Coombs test* is used for detecting the presence of immunoglobulin and complement on red blood cells (RBCs). The patient's RBCs are mixed and incubated with a reagent containing polyspecific antibodies against IgG and C3d. The tubes are checked for agglutination and/or hemolysis—both indicators of positivity. If positive, additional tests with anti-IgG antibody and anti-C3d antibody are performed.

8.4.3 Indirect antiglobulin test

The indirect antiglobulin test is used to detect antibodies to RBCs in donor or recipient plasma. This is particularly important in blood transfusion to safeguard against transfusion reactions. The traditional ABO system and Rh-positive blood typing are insufficient for assuring safe transfusions in the nonemergency setting.

Additional antigens from the Rh, MNS, P, Lewis, Kell, Duffy, and Kidd systems may react with a recipient's serum antibodies, and require screening. Cells that express these antigens are used, and these are referred to as *reagent* cells. A patient's plasma is reacted with the various reagents and observed for

agglutination and hemolysis. A positive reaction warrants specific antibody testing to identify the allo-antibody.

8.4.4 Immunoelectrophoresis

Immunoelectrophoresis is usually done to identify a specific monoclonal immunoglobulin when serum, urine, or CSF electrophoresis shows a positive globulin band. A polyacrylamide gel is used for electrophoretic separation of the gamma globulins of the patient's sample and a normal sample. The patient's serum and normal human serum (NHS) are alternately placed on the gel, and then electrophoresis performed to separate the proteins. The troughs are then filled with antisera against light and heavy chains and incubated for 24 hours, during which the antisera diffuses into the gel. The gel is then washed, fixed, and stained. Subsequently seen arcs are expertly interpreted. Comparison with the normal serum provides the determination of IgG kappa M-component, a kappa light chain only M-component, and an IgA lambda M-component [Laposata, 2002].

8.4.5 Immunofixation

Immunofixation has largely replaced immunoelectrophoresis. Serum, urine, or cerebral-spinal fluid can be used to identify specific monoclonal immuno-globulins. Serum is separated on a high-resolution gel in several lanes. Strips of filter paper soaked with specific antisera against light and heavy chains are applied directly onto each lane. The gel is then washed, fixed, and stained. The lanes with stained protein-antibody conjugates are laid beside the patient's protein electrophoreses, to determine if the protein detected by immunofixation is the same protein found in the protein electrophoresis [Laposata, 2002].

8.4.6 Immunofluorescence

Direct and indirect immunofluorescences are used to find a specific antigen in a specimen such as serum, cells, or tissue. Common to both techniques, a smear of the sample is made on a slide. In *direct immunofluorescence*, a fluorescent labeled antibody with affinity for the antigen of interest is incubated with the slide, allowing conjugation of antigen with antibody. The sample is then read using a fluorescent microscope.

Indirect immunofluorescence makes use of an intermediate step. First an *unlabeled antibody* with affinity for the antigen of interest is applied to the test sample and allowed to incubate. Next a *labeled secondary antibody* (an anti-IgG Ab) with specific affinity for the previously formed antigen-antibody complex is applied and fluoroscopically detected. This is illustrated in Fig. 8.13.

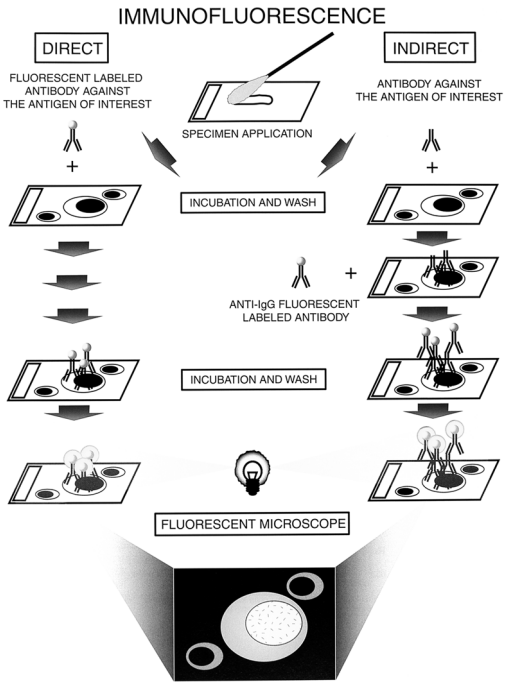

Figure 8.13 Direct and indirect immunofluorescence is when cells or tissues that react positively for the specific antigen fluoresce green. [Reprinted with permission from Laposata (2002), copyright American Society for Clinical Pathology.]

8.4.7 Latex agglutination

Serum, CF, and other body fluids may be tested for specific antibodies or antigens by the agglutination reaction.

Large particles that include red blood cells (hemoagglutination), latex, or gelatin may be used. They are first coated with the specific antibody or antigen of interest. The sample is gently mixed and latex particles cause agglutination to occur if the analyte is present in the sample. When done on a slide, large aggregates can be seen.

8.5 Microbiology

8.5.1 Gram stain

A specimen containing an infectious agent is placed directly on a glass slide and dried. Stains are applied sequentially, including *crystal violet*, *iodine*, and *Safranin dye*, yielding a gram positive or negative result. Morphologic interpretation of the slide is then done, describing cocci or rods, clusters or chains and the location inside or outside of cells. *Gram positive* organisms appear purple, and *gram*

negative organisms appear red. A gram stain can guide immediate antibiotic therapy while cultures are run.

8.5.2 Bacterial culture

Body fluids, swabs, and solid samples can be cultured. A sterile platinum metal *loop* is placed into the sample and then streaked across a *solid culture medium* in a zig-zag pattern. The culture medium is rotated 90 deg and streaked again. After incubation individual colonies may be seen growing on the culture medium. These may be selectively removed by a loop and subcultured for species identification.

8.5.3 Antimicrobial susceptibility

Antimicrobial susceptibility is the process of taking an organism and determining what concentration of different antibiotics will effectively eradicate it. Two methods are commonly used, the *dilution method* and the *disc diffusion method.*

In the dilution method, tubes with increasing concentrations of an antimicrobial agent are prepared, including controls. The suspended organism is added to all but the control tube, incubated, and checked for growth. The first tube that shows no growth identifies the *minimal inhibitory concentration* (MIC). Expressed in milligrams per liter, this defines the minimum concentration of antibiotic in the serum at which the organism is susceptible.

In the disc diffusion technique a suspension of the organism is evenly distributed over the surface of a culture plate. *Discs* embedded with different antimicrobial agents in predetermined amounts are placed onto the surface of the dish. After incubation, a zone of inhibition may be observed around the disc, signifying that the organism growth has been suppressed. The size of the zone determines whether the organism is *susceptible*, *intermediate*, or *resistant* to the antibiotic.

8.6 Urinalysis

8.6.1 Manual urinalysis

When the urine sample arrives at the laboratory it is first inspected for color and turbidity. A urine dipstick with 10 reagent pads performs multiple tests simultaneously. These tests include specific gravity, pH, glucose, protein, ketones, bilirubin, blood, esterase, and nitrite. Microscopic analysis of the urine sediment is performed by placing a small volume of centrifuged urine on a glass slide, and reviewing urinary sediment for abnormalities.

Samples with esterase and/or nitrite positivity may require further study with a urine culture. Proteinuria (protein in the urine) is not normal, and may require quantification or urine protein electrophoresis for further analysis. Simple dipstick techniques are usually inadequate in screening diabetic patients for *microscopic proteinuria*, a subtle finding in diabetic nephropathy (kidney disease). This seems to be a good opportunity for a point-of-care a μTAS-based hand-held unit.

Automated systems also perform automated urinalysis and urine microscopy. Such systems prepare samples, perform chemistries and use image analysis for automated particle recognition.

8.7 Coagulation Assays

Anticoagulation is the process of increasing blood clotting time by administration of heparin, warfarin, or low-molecular-weight heparin injections. Coagulation studies are performed to monitor these drugs and for screening of coagulopathies (abnormal clotting). Anticoagulant drugs are used in patients with heart disease (including coronary artery disease and mural thrombi), atrial fibrillation (an arrhythmia), pulmonary embolism (clot in the lung vasculature), deep venous thrombophlebitis (DVT), artificial heart valves and other prosthetic cardiovascular devices, and other disorders. Coagulopathies include genetic and acquired deficiencies in coagulation factors, abnormal synthesis performance of the liver in hepatic (liver) diseases.

The coagulation cascade is shown in Fig. 8.14. The prothrombin time (PT) evaluates the extrinsic pathway factors VII and common pathway, factors II, V, X. PT is reported as the number of seconds the blood takes to clot when mixed with a thromboplastin reagent.

The International Normalized Ratio (INR) was created by the World Health Organization (WHO) because PT results can vary depending on the thromboplastin reagent used. The INR is a conversion unit that takes into account the different sensitivities of thromboplastins. The INR is widely accepted as the standard unit for reporting PT results.

The partial thromboplastin time (PTT) evaluates the intrinsic pathway; factors VIII, IX, and XII; and the common pathway. Activating agents are added to initiate clotting, and the process is monitored optically.

A coagulation analyzer is shown in Fig. 8.15. It measures the PT (INR) and PTT.

There are significant challenges to treating patients with anticoagulants, including the need for the patient to drive to a facility for measurements (daily to begin with, then every four to six weeks for maintenance), a 45 to 60 minute processing time, and the need to discuss with medication dosing adjustments with a medical professional. There are significant opportunities for μTAS-based point-of-care monitoring. The HemoSense, Inc., INRatio Meter shown in Fig. 8.16 is a hand-held device for point-of-care use.

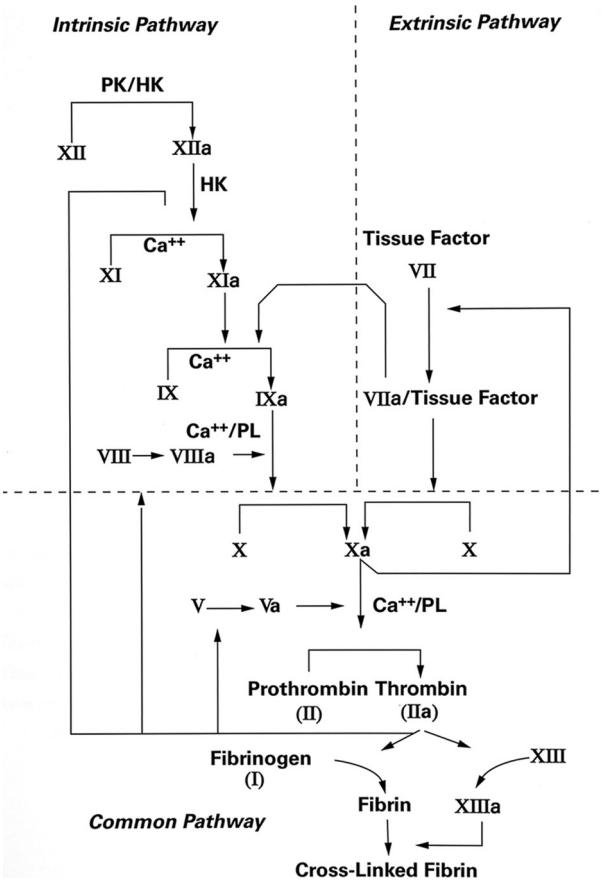

Figure 8.14 The intrinsic and extrinsic pathways of the coagulation cascade. [Reprinted with permission from Laposata (2002), copyright American Society for Clinical Pathology.]

8.8 Arterial Blood Gases

Blood-gas analyzers use ion selective electrodes, pH electrodes, and co-oximetry (spectrophotometric) methods.

Determination of pH, pO_2, and pCO_2 may be performed from whole blood. The pH (hydrogen) electrode consists of an internal Ag/AgCl electrode, reference electrode, and ion buffer solution. The pCO_2 electrode is a pH electrode encased in a plastic sleeve filled with a buffered solution of sodium bicarbonate. Carbon dioxide from the blood diffuses through the membrane causing a drop in pH. The pO_2 electrode is a Clark electrode consisting of a platinum cathode and Ag/AgCl anode. Oxygen migrates through an oxygen-selective membrane and is reduced on the platinum cathode, creating a measurable electrical current. This is illustrated in Fig. 8.17.

Some blood-gas analyzers also measure hematocrit, sodium, potassium, calcium, and chloride.

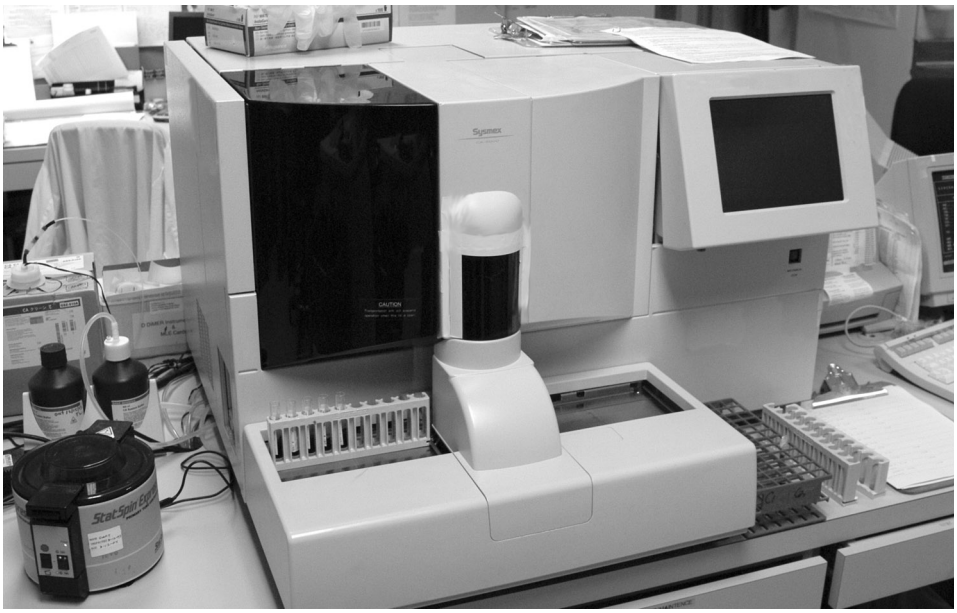

Figure 8.15 The Dade Behring coagulation analyzer is able to detect clot formation for protime (INR) and partial thromboplastin time (PTT).

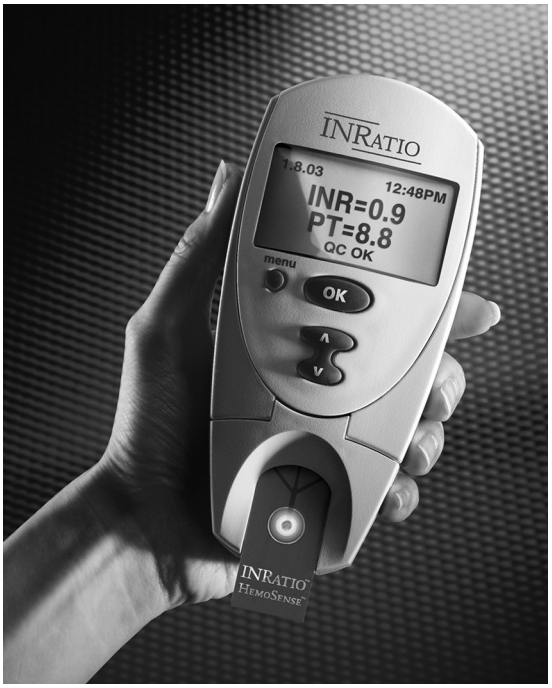

Figure 8.16 The HemoSense INRatio Meter shown is a hand-held device for point-of-care use. (Photo courtesy of Hemosense, Inc.)

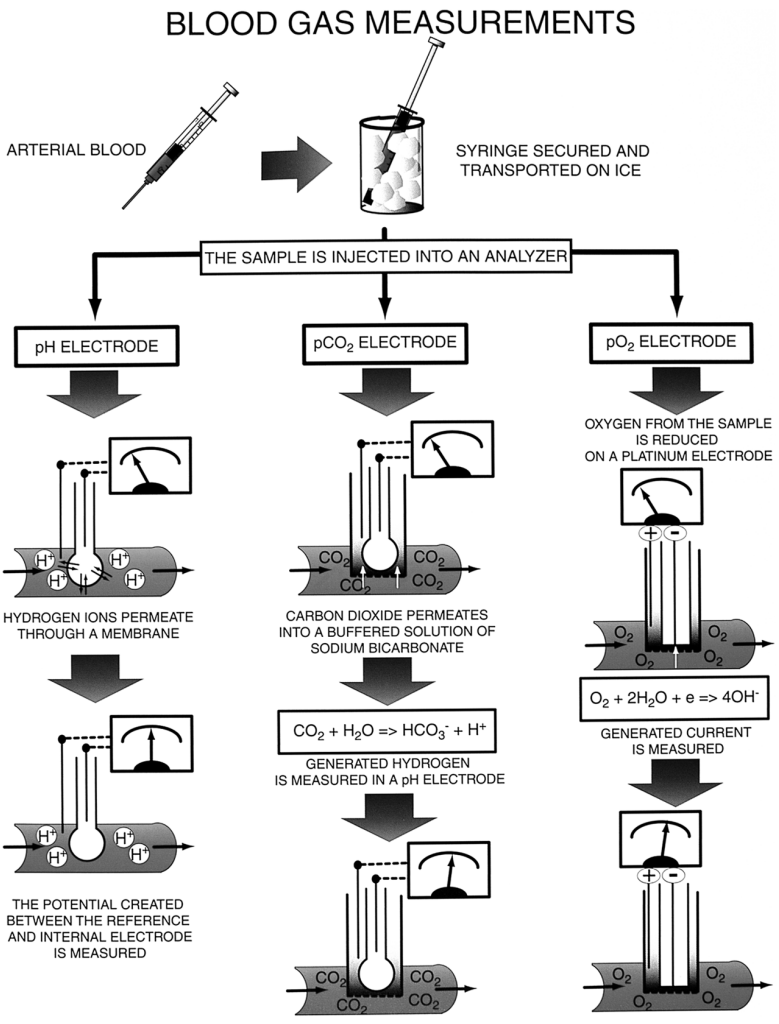

Figure 8.17 Determination of pH, pO_2, and pCO_2 from whole blood. [Reprinted withpermission from Laposata (2002), copyright American Society for Clinical Pathology.]

8.9 Review Questions

1. What categories of certification are clinical laboratories issued from CLIA?
2. What is the responsibility of a laboratory director as defined by CLIA?
3. Describe the process steps required when a laboratory study is ordered.
4. What are the CPT codes and the IDCM-9 diagnostic codes used for?
5. Describe the purpose and methodology for the ELISA test.
6. Describe chemiluminescence and the kinds of reactions that may take place.
7. Describe nephelometry.

8. How are blood-lipids such as cholesterol and triglycerides analyzed?
9. What kinds of studies may be performed with spectrophotometric and ion selective electrode techniques?
10. What samples are typically processed with electrophoresis?
11. Describe the Coulter principle.
12. What is flow cytometry and how is it used?
13. Describe what the Coulter Gen-S does.
14. Describe the use of fluorochromes in flow cytometry.
15. What can be identified by a peripheral blood smear?
16. Describe antinuclear antibodies and how they may be detected in serum.
17. What are the direct Coombs test and the indirect antiglobulin test?
18. Describe the purpose and methodology for immunoelectrophoresis.
19. How is a gram stain performed, and what is its purpose?
20. Describe two techniques for performing antimicrobial susceptibility.
21. Describe the coagulation cascade; and what the protime, INR, and partial thromboplastin time measure.
22. What kinds of electrodes are required to measure serum pH, pO_2, and pCO_2?

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