

The New Era of Automated Immunoassay

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Abstract

Use of immunoassays and other ligand-binding assays in clinical diagnosis has increased dramatically during the last several years. Despite impressive technical advances, “mass production” of these assays in a routine laboratory still presents many difficulties. This review of ligand-binding assay technology highlights some recent developments, emphasizing challenges and possible solutions for cost-effective patient care.

For many years immunoassay (IA) had a narrow role in the clinical laboratory and was performed by tedious manual methods in a specialized section of the laboratory. That situation has changed dramatically. The tremendous versatility of IA together with its increasing automation have thrust it into a leading role in almost all areas of the laboratory (Table 1). It is little exaggeration to say that the future of automated IA is the future of the clinical laboratory.

In contrast with other powerful analytic techniques, such as mass spectroscopy and nuclear magnetic resonance, IA depends on complexity in reagents rather than in instrumentation. This feature has proven highly successful in the clinical laboratory sphere, where the trend has been to abandon generic instruments requiring specialized skills in favor of proprietary clinical analyzers needing little operator involvement. However, the technical simplicity of modern IA formats is deceptive. IA analytes are typically at 3 to 10 orders of magnitude lower concentration than classic chemistry analytes. They may be complex and heterogeneous molecular species. These are demanding, trouble-prone assays, and automation to date has not been fully satisfactory.

The present article is an overview of modern IA and its incorporation into the routine clinical laboratory. This broad subject spans IA technology, automation technology, and method evaluation, so coverage will be selective, emphasizing the interfaces among these areas. In view of the rapid evolution of commercial systems, and detailed descriptions available elsewhere,¹⁻⁵ discussion will be generic, with mention of specific systems only as examples.

Definition and Scope

The term *immunoassay* seems to imply a measurement that pertains to the immune system or depends on an immunologic phenomenon. In practice, the term usually refers to a quantitative measurement that depends on recognition of an

analyte, the antigen (Ag), by a reagent antibody (Ab) directed against it. Similarly constructed assays may use specific binding proteins, such as transport proteins or receptors, that are not Abs. Hence, the term *ligand-binding assay* may be preferable, although it is less familiar and somewhat vague. In other assays, the scheme essentially is reversed; the analyte is an Ab, and its Ag is used as the binding reagent, possibly along with reagent Abs. In still other cases, Ag-Ab complexation is used for qualitative or categoric determinations, for example, in immunocytochemistry or immunohistochemistry. Finally, there is little fundamental difference between assays that depend on recognition of Ag by Ab, and those depend on recognition of a nucleic acid sequence by a complementary probe⁶ (although a practical advantage with nucleic acid “antigens” is that they can be amplified before detection). Many design considerations are common to all of these very diverse “immunoassays,” but the present article emphasizes quantitative chemical determinations in which routine automation has advanced the farthest.

It also may be worth asking what is meant by *automation*. It is logically viewed as any system that minimizes human activity in performing the task. For IA, this can be accomplished not only with mechanical instruments, but also with self-enclosed single-test systems intended for point-of-care or home use.¹

IA Design Options

The range of design possibilities for IA is so multidimensional that classification is difficult, and no scheme is generally agreed on. One useful classification scheme has been proposed by Gosling **Table 2**.⁷ Along with the variety of designs is a bewildering array of acronyms, the more common of which, such as RIA (radioimmunoassay) and ELISA (enzyme-linked immunosorbent assay), often are used indiscriminately and inappropriately. **Table 3** lists several individual characteristics by which IAs can be dichotomized or classified.

Competitive vs Immunometric Detection

This is probably the most fundamental dichotomy in modern IA design. An IA measures its analyte based on a specific binding reaction, and the fundamental requirement is to generate a signal that reflects the extent of binding. The earliest IAs were performed based on detection of Ag-Ab complexation through precipitin bands or immune phenomena such as complement fixation.⁸ A major advance was the introduction of labeling, which initially was applied using the principle of *competitive binding* (competitive-binding assay, CBA). As explained by Ekins,⁹ this amounts to an indirect determination in which the number of occupied sites on the

ligand binder is determined by a signal proportional to the number of *unoccupied* sites. In CBA, a label usually is placed on the Ag to be measured. The original RIA, applied to insulin measurement by Yalow and Berson,¹⁰ was a CBA using insulin labeled with the gamma-emitter iodine 131. A fixed concentration of labeled Ag reagent and a fixed (and comparably low) concentration of Ab reagent are combined with the variable concentration of Ag present in the sample to be analyzed. The higher the concentration of Ag in the sample, the lower the amount of label that will bind to the Ab. It then is essential to separate bound from free label, which in the original RIA was done by a physical separation—protein precipitation followed by centrifugation, decanting of supernatant, and analysis of the pellet in a gamma counter. Now many other types of labels and separation schemes are used for CBA (see “Type of Label”).

An interesting aspect of CBA that was crucial to its early development is that specificity derives from the labeled Ag, and it is not necessary to have a purified Ab. In many cases, the serum of an immunized animal could be used directly as a binding reagent. However, the CBA format also has drawbacks. The Ab concentration cannot be much greater than the lowest amount of Ag to be measured; otherwise the proportional change in unoccupied sites is too small to be measured reliably. Unfortunately, if the Ag and Ab concentrations are both low, the law of mass action dictates a very slow binding reaction, so incubations on the order of 24 hours or longer may be required. This is particularly inconvenient for automation. Another drawback is that the calibration curve is highly nonlinear. Although linearization algorithms usually are applicable,¹¹ reliable calibration typically requires several calibrator levels and frequent adjustment.

Although CBA most naturally uses labeled Ag, variations using labeled Ab are possible if placing the label on the Ag is impractical. In such assays (Gosling’s category 2), the competing ligand reagent typically is immobilized to a solid phase. Labeled Ab then binds competitively to reagent Ag on the solid phase or to Ag from the sample in solution. Label bound to the solid phase is quantified after a wash step. Again, the higher the concentration of Ag in the sample, the less binding of label occurs.

Immunometric assay (IMA), by contrast, measures the amount of binding directly, using label attached to the Ab or other binding reagent.¹² This usually requires that the Ag from the sample to be tested first be immobilized to a solid phase, which may occur by direct adsorption but usually is accomplished via a second, unlabeled binder (the “capture Ab”) attached to the solid phase. Unbound label then is removed by a washing step, and the amount of immobilized label is measured. The amount of bound label thus *increases* with increasing Ag in the test sample, and ideally the increase is linear. The binding kinetics are much more

Table 1
Diagnostic Applications of Ligand-Binding Technologies

Diagnostic Area	Ligand-Binding Methods	Traditional and Alternative Methods
Chemistry		
Low-molecular-weight hormones, metabolites, drugs	RIA, nonisotopic CBA	Standard chemistry; chromatography; GC/MS
High-concentration proteins	Radial immunodiffusion; nephelometry	Electrophoresis; chromatography
Low-concentration proteins, organ markers, tumor markers	RIA, IMA	Enzyme activity measurement; bioassay (sometimes no alternative method)
Hematology, cellular immunology	Immunocytochemistry; flow cytometry	Visual cell recognition; automated pattern recognition
Serology	ELISA; Western blot	Hemagglutination; complement fixation, and others
Microbiology	Organism identification by Abs, nucleic acid probes	Organism identification by biochemical characteristics
Genetics	DNA probes	Classic cytogenetics; protein analysis by enzyme activity, chromatography, electrophoresis
Immunohematology	Cell agglutination; ELISA	—
Surgical pathology	Immunohistochemistry	Morphologic diagnosis using chemical stains, electron microscopy
Diagnostic imaging	Ab-directed nuclear probes	Conventional nuclear probes

Ab, antibody; CBA, competitive binding assay; GC/MS, gas chromatography–mass spectrometry; ELISA, enzyme-linked immunosorbent assay; IMA, immunometric assay; RIA, radioimmunoassay.

Table 2
Gosling’s Scheme for Classifying Immunoassays⁷

Category	Characteristics	Examples
1	Labeled analyte, limited Ab (competitive binding)	Radioimmunoassay (RIA)
2	Labeled Ab, limited reagent (competitive binding)	Some assays using nonisotopic labels having low aqueous solubility, such as acridinium ester
3	Direct detection of immune complexes	Radial immunodiffusion, nephelometry
4	Excess reagent, labeled Ab (immunometric)	Immunoradiometric assay (IRMA); immunoenzymometric assay (IEMA)
5	Assays for quantifying specific Abs	Infectious disease serology
6	Labeled reagent, with signal modulated by immune binding (homogeneous assays)	Enzyme-multiplied IA technique (EMIT); fluorescence polarization IA (FPIA)

Ab, antibody.

Table 3
Dichotomies Useful for Classifying Immunoassays*

Characteristic	Option 1	Option 2
Means of binding detection	Competitive	Immunometric
Type of binder	Biologic	Nonbiologic
	Antibody	Aptamer
	Polyclonal	Imprinted polymer
	Monoclonal	
	Nonantibody	
	Receptor	
	Transport protein	
Binder concentration	Limited	Excess
Use of label	Labeled	Unlabeled
Site of label	Antigen	Antibody
Type of label	Isotopic (radioactive)	Nonisotopic
		Absorbance
		Fluorescence
		Luminescence
		Electrochemical
		Enzyme (used to amplify any of the preceding)
Type of separation	Heterogeneous	Homogeneous
End point	Equilibrium	Kinetic

*The features in each column are not linked, ie, an assay can incorporate selections from both columns. However, the competitive option usually implies limited binder and labeled antigen; the immunometric option usually implies excess antibody and labeled antibody.

favorable because both the capture Ab and labeled detector Ab can be present in great excess, and this, in turn, improves the sensitivity of the assay. Specificity of the assay, in contrast with CBA, depends entirely on the reagent Abs, and IMA development has coincided largely with monoclonal Ab technology.¹³ IMAs for proteins can greatly enhance specificity by using 2 distinct monoclonal Abs for capture and detection directed against spatially distant epitopes on the molecule (the “sandwich” assay). The proportional response of signal to Ag concentration makes calibration easier and more stable and widens the reportable range.

Although IMA has many advantages over CBA and theoretically can be adapted to virtually any analyte (see next section), currently available assays for small molecules are nearly all CBAs. Automated analyzers, to offer a menu including small molecules and proteins, must offer a scheme for IMA and CBA, increasing the complexity of the system.

Small Molecule vs Protein Analyte

This distinction has a major effect on assay design because a small molecule, or hapten, although it may contain several epitopes, may be unable to bind more than 1 Ab molecule at a time because of steric hindrance. Most proteins, in contrast, can bind 2 or more Abs at once. For proteins, it is straightforward to design a CBA or an IMA configuration. The best assay configuration for a specific protein is usually the sandwich-type assay described in the preceding section. Sandwich assays have proven applicable even to peptides as small as 8 to 11 amino acids.¹⁴ For relatively abundant proteins, another option is unlabeled IA (Gosling’s category 3; see “Type of Label”).

Current commercial systems measure small molecules almost exclusively by CBA. It is noteworthy, however, that clever designs allow construction of IMAs for small molecules with their attendant advantages. The immunometric assay for estradiol described by Barnard and Kohen¹⁵ required 3 monoclonal Abs. The first monoclonal Ab was a capture Ab, directed against estradiol and immobilized onto the walls of microtiter wells. The other 2 monoclonal Abs were directed against the antiestradiol idotype. One anti-idiotypic Ab, called *betatype*, recognized an epitope at the unoccupied binding site, and the other (*alphatype*) recognized an epitope near the binding site that was unaffected by occupancy of the site by estradiol but was blocked by presence of the betatype Ab. The microtiter wells were incubated with patient sample, then with betatype Ab to occupy the vacant estradiol sites. Labeled alphatype Ab then was added, and it would bind only to the sites occupied by estradiol. Self et al¹⁶ described a more direct approach using “secondary” monoclonal Abs directed against Ab-hapten complex.

Another possibility is to allow binding of Ag to labeled Ab to take place in solution, then remove uncomplexed Ab

with an immobilized anti-idiotypic capture phase.¹⁷ Yet another approach, termed solid-phase-immobilized epitope IA,^{14,18} uses a bifunctional reagent to covalently attach the hapten after it binds to a solid-phase Ab. A protein-denaturing reagent then releases the hapten from the Ab’s binding site but leaves it tethered to the solid phase. An enzyme-labeled version of the same Ab then serves as an immunometric detector of the attached hapten.

Type of Binding Reagent

The nature of the binding reagent, always a critical feature of an IA, becomes even more important in the context of automation, since the vendor must provide a continuous supply of reagent with consistent performance. Polyclonal Abs are disadvantageous since they are undefined mixtures, “manufactured” in live animals and differing in each production batch. Monoclonal Abs were a great advance, made possible in 1975 by the introduction in the hybridoma method for immortalizing B-cell clones.¹³ Monoclonal Abs still have several disadvantages:

- Immunization of animals is required; difficulties may be encountered with Ags that are nonimmunogenic or highly toxic
- Difficulty selecting a suitable clone
- Difficulty scaling up production to commercial levels
- The need to separate the single Ab produced from other cell proteins
- Practical limitation to rodent species
- Problems related to the Ab product itself, which still is a highly complex molecule, much of it irrelevant to Ag recognition and a potential source of trouble, eg, from binding to heterophilic Abs in patient serum (see “Interfering Abs” under “Specificity”). Simple modifications, such as cleavage to Fab fragments, are useful in some applications but have limited capability.

Many of these problems are addressed by new molecular biologic techniques that have revolutionized the production of Ab-like molecules.¹⁹⁻²² This effort largely has been driven by therapeutic applications, with slight influence until now on in vitro diagnostics, but that situation soon may change. By using recombinant DNA and amplification technologies, binders can be produced from “naïve” DNA libraries, eliminating the need for immunization. Variable regions of the Ab, making up the complementarity determining regions, can be linked with constant regions to mimic a native Ab or can be linked in a much simpler fashion to produce a single chain fragment variable region Ab, which can be joined to produce multivalent binders (one type, the chelating recombinant Ab, achieves higher binding affinity by recognizing adjacent epitopes on a large Ag²³) or modified

in other ways desired for a particular assay. Selection of suitable clones is made easier by phage display and other display technologies, and production can be performed in *Escherichia coli* or in completely acellular systems.

Investigators also have looked toward entirely different classes of molecules as possible binding reagents. Considerable promise has been shown by aptamers, which are polynucleotides with an affinity for a particular ligand.²⁴⁻²⁸ From a mixture of random polynucleotides, the ligand is used to pull out sequences for which it has affinity, then these are amplified using the polymerase chain reaction (PCR). After inducing further variations in the selected sequences, the cycle can be repeated. This process, termed SELEX (systemic evolution of ligands by exponential enrichment), eventually can yield molecules with affinities and specificities comparable to those of monoclonal Abs. The affinity of aptamers can be enhanced further by chemical modification; for example, an aptamer directed against an enzyme can be tethered to an inhibitor that binds tightly to the active site.

Molecular imprinting is another approach to producing polymers with desired binding properties.²⁹⁻³² The polymerization of some type of versatile, monomeric unit is guided by a molecular template made up of the analyte of interest. Polymerization typically is allowed to proceed to formation of a solid mass, from which a particulate binding reagent is produced by grinding. Hence, this technique does not offer the advantage of a pure, defined binder, although it seems that binding properties can be reproduced fairly well. Molecular imprinting may be especially well suited to small molecules with limited aqueous solubility. Competitive assay procedures for several drugs using imprinted binders have been published, but achieving performance in aqueous buffers comparable to that in organic solvents has been difficult.³³⁻³⁵

Synthetic soluble ligand binders ultimately could have the advantages of a completely defined structure, completely defined binding properties, and lower production costs. They also could be tailored to the overall assay design, for example with built-in fluorescence or enzymatic labeling. However, the cost of developing such ideal binders will be high, and the incentive has been diminished by the generally satisfactory performance of Abs in existing assays.

Although pure binding reagents often are expected to yield better assay specificity than polyclonal Abs, this need not always be the case. For example, a monoclonal Ab might be directed against an epitope shared by many proteins, whereas a polyclonal Ab may achieve better specificity by having components directed against several different epitopes on the protein of interest. Ultimately, the best binding performance for difficult assays may require defined "cocktails" containing several pure binding reagents.

Type of Bound-Free Label Separation

This feature of an IA is a major determinant of the mechanics of automation. Most ligand-binding assays are *heterogeneous*, meaning that bound and free label must be separated physically. In the original RIA, this was done by a protein precipitation step followed by centrifugation. That scheme is suited poorly to automation, and modern IAs usually incorporate a solid phase to which Abs or other reagents can be attached. Options regarding the nature of the solid phase are discussed in the next section. The need for a solid phase is eliminated by *homogeneous* IAs, which do not require a physical separation to determine the fraction of bound label.

The first commercially important homogeneous IA was the enzyme-multiplied IA technique (EMIT, Dade Behring, Deerfield, IL) for therapeutic drugs.^{36,37} EMIT is a CBA in which the Ag is labeled with an enzyme; Ag-Ab complexation sterically hinders the enzyme's active site, and so enzyme activity in solution depends on the degree of Ag complexation. Many subsequent types of homogeneous IA also have been based on modulation of enzyme activity, including the cloned enzyme donor IA,^{38,39} the substrate-labeled fluorescent IA,⁴⁰ the prosthetic-group labeled IA,⁴¹ and others.^{37,42} The major alternative has been modulation of fluorescent properties. In the widely used fluorescence polarization IA, binding of a small, fluorescently labeled molecule to Ab is detected via slower decay of fluorescence polarization due to slower molecular tumbling.⁴³ Fluorescence energy transfer also has been applied to homogeneous assays for both haptens and proteins.^{44,45} Another option is microparticle agglutination, which, although using a solid phase, gives a functionally homogeneous assay because no physical separation step is required.⁴⁶

Homogeneous IA is ideal for automation, since it can be directly adapted to existing clinical chemistry analyzers. However, it often is difficult to achieve the sensitivity available from heterogeneous assay, especially for protein analytes.

Type of Solid Phase

Because of the limited power of current homogeneous IA technology, automated IA systems typically require some type of solid phase separation. Use of a solid phase raises several issues such as binding kinetics at the solid-liquid interface, steric hindrance, and immobilization procedures.⁴⁷ With automated IA, there are additional issues of reagent manufacture and instrument engineering. Sandwich-type assays are commonly constructed with a capture monoclonal Ab fixed to a plastic bead or plastic tube. With a multianalyte commercial system, this raises the problem of designing, manufacturing, and dispensing a distinct solid-phase device for each assay. It is generally advantageous to instead use

soluble capture Abs that carry a molecular tag, eg, fluorescein or biotin, and use a generic solid phase with a binder for that tag (antifluorescein Ab or avidin). The mechanics of dispensing beads, tubes, or other solid devices can introduce some awkwardness. An alternative is to use a slurry of microparticles that can be treated somewhat as a liquid. Separation of the microparticles from solution can be accomplished by filtration or by magnetic separation if the particles are paramagnetic. Clogs or other problems may arise due to the nonliquid nature of a particle slurry.

Type of Label

Another feature of the original RIA that made it unfriendly to automation was the radioactive label. Disadvantages of radioactivity include the substantial counting time required, the bulky detector and shielding needed, limited reagent shelf life, continuous changes in calibration, potential health hazards, and the problems associated with radioactive contamination and waste disposal. On the other hand, gamma emitters offer superb sensitivity, are unaffected by solution environment, and are only rarely subject to interference (mainly when patients receive certain isotopes for nuclear medicine studies). Iodine 125 is inexpensive and readily attached to proteins and other molecules. For these reasons, radioactive labels still are used widely in IA and nucleic acid testing, but mainly for research and specialized low-volume clinical application. Since the demise of an early generation of automated RIA instruments, dedicated clinical laboratory analyzers have used only nonisotopic labels.

Nonisotopic IA most commonly uses some type of optical signal as an end point: absorbance, light scatter, fluorescence, or chemiluminescence. Of these, absorbance is the least sensitive because it requires measuring the difference between 2 light intensities. Since concentrations much below 1 $\mu\text{mol/L}$ cannot be measured, chromophores cannot be used directly as labels, but an enzyme label can be detected via a chromogenic substrate. Absorbance measurements are subject to positive interference, eg, with icteric, hyperlipidemic, or hemolyzed samples, but this may be mitigated by washing steps or kinetic measurements. Absorbance is not readily susceptible to negative interference.

Fluorometry is much more sensitive than absorptiometry because the emission light signal, although weak, is measured against a background that is dark, except for scatter from the excitation source. However, fluorescence is very susceptible to negative interference (quenching) and positive interference (from native fluorescence of serum constituents and contaminants). Direct fluorescent labeling has been successfully automated using dissociation-enhanced lanthanide fluorescence IA.⁴⁸ In this system, the label is a chelate of a lanthanide ion, such as europium (Eu^{3+}). The chelate is nonfluorescent, but the Eu^{3+} becomes

fluorescent on addition of a dissociating reagent containing an enhancer. The dissociation allows fluorescence to be measured under optimum solution conditions, and time-resolved fluorometry distinguishes the very long-lived Eu^{3+} fluorescence from that of contaminants. Other adaptations of fluorescence are suited to homogeneous IA, as discussed in "Type of Bound-Free Label Separation." Fluorescence also can be used as a sensitive end point for enzyme detection. For example, alkaline phosphatase cleaves 4-umbelliferyl phosphate to a fluorescent product.

Chemiluminescence (or bioluminescence) is the most intrinsically sensitive optical signal available. Photons of light arise spontaneously in the course of a chemical reaction and, therefore, can be measured against an absolutely dark background. There is little interfering chemiluminescence from serum components and contaminants. However, the label must be made to undergo a chemical reaction for the luminescence to occur. If there is any tendency for this reaction to occur spontaneously, background noise will be produced. The signal is time-dependent, which increases the difficulty of reproducible measurement. Also, like fluorescence, chemiluminescence can be modulated by the solution environment. Luminol derivatives and, more recently, acridinium esters have been used commonly for direct chemiluminescent labeling in IA.⁴⁹ These molecules are relatively stable under ambient conditions, but on addition of an oxidizing reagent, they become luminescent.

Sensitivity can be enhanced even further by using enzyme amplification. Suitable substrates include adamantyl-1,2-dioxetane phosphates that, when hydrolyzed by alkaline phosphatase, become unstable and luminesce with relatively long decay time.⁵⁰ Another interesting approach, now being used commercially, is electrochemiluminescence.⁵¹ Here the label is a ruthenium complex that is much more stable than most chemiluminescent labels, but it is induced to luminesce by an electrical potential applied by an electrode. Molecular access to the electrode can be used as a basis for separation of bound and free label.⁵²

Electrochemical detection has seen little application in commercial IA systems but has some potential: instrumentation is simpler than with optical detection, and operation in an opaque environment is possible. Potentiometric, amperometric, and conductometric detection have been used. Direct labeling with an electrochemically active compound is possible, but enzymatic amplification usually is needed for adequate sensitivity.⁵³⁻⁵⁶ Unlabeled electrochemical detection also has been described (see discussion of surface phenomena in this section).

Although enzymes have been outstandingly useful for signal amplification, they have disadvantages, including their molecular size and complexity, possible instability, and dependence of catalytic rate on temperature and other variables.

Other means of amplification have been devised. Liposomes can be used to package many molecules of a fluorescent or other label.⁵⁷⁻⁵⁹ The liposome is linked to reagent Ab and lysed after binding to a solid phase occurs. The technique of immuno-PCR uses nucleic acid as a label, which is amplified by the PCR and then detected with a complementary probe.⁶⁰⁻⁶³ (Conversely, nucleic acid detection can achieve signal amplification by conjugating multiple copies of a hapten to the probe and adding an enzyme-linked Ab⁶⁴).

Unlabeled IAs (Gosling's group 3) are possible for protein Ags that can bind more than 1 Ab molecule at a time. Ab molecules are likewise multivalent, since a single IgG molecule has 2 binding sites for Ag. When Ab and Ag are mixed in nearly equal concentration (the "equivalence zone"), a macroscopic aggregate occurs that can be detected visually, for example as bands in an agarose gel. This is the basis of classic qualitative immunodiffusion techniques, as well as quantitative manual methods, such as radial immunodiffusion and immunoelectrophoresis.⁸ The major automated adaptation of this principle is nephelometry, which detects the formation of immune aggregates in solution via light scatter.⁶⁵ These assays are especially suitable for proteins at fairly high concentration (>1 mg/L), such as albumin, complement components, immunoglobulin, and transferrin, although much greater sensitivity can be achieved. Nephelometers also can measure haptens, based on inhibition of aggregation of hapten-coated proteins or particles, but this is essentially a type of labeled homogeneous IA.

A more futuristic approach to direct detection of Ag-Ab complexation, without use of a label, is via surface phenomena.^{66,67} Direct immunosensors use a surface coated with a capture Ab. When Ag binds to the surface, it causes some change in a measurable property that can be transduced to an electrical signal. The intrinsic power of this technology has been demonstrated using a myriad of ingenious detection techniques. Surface properties that have been used include refractive index (measured via surface plasmon resonance,^{68,69} the "resonant mirror,"^{70,71} and ellipsometry⁷²⁻⁷⁴), fluorescence,⁷⁵⁻⁷⁷ molecular topography (measured via atomic force microscopy),⁷⁸⁻⁸¹ electrochemical properties,^{56,82} and acoustic properties (measured via the quartz crystal microbalance⁸³⁻⁸⁵ and surface acoustic waves⁸⁶⁻⁹⁰).

Immunosensors offer the utopian promise of a "dip and read" assay with no consumption of specific reagents. This would require regeneration of sensor surfaces, a difficult task, but one on which some progress has been reported using both chemical and electrochemical means.^{75,91,92} Most published immunosensor methods are suitable for protein analytes rather than small molecules. Unfortunately some of the exquisite specificity of current IMAs is lost because there is only a single capture Ab, and no second detector Ab. Nonspecific binding to the sensor can be a major problem for

serum specimens. Direct immunosensors therefore have failed, to date, to be suitable for major use in the clinical laboratory. A possibly useful compromise is to use a sensor design assisted by specific reagents, such as a second Ab or labeled analyte. In this compromise, however, much of the attraction of the sensor is lost, and the technology would have to prove itself advantageous in some way to more conventional current approaches.

Equilibrium vs Kinetic Assay

As with many general chemical assays, the speed of IA often can be improved by measuring the initial rate of binding rather than the final equilibrium. Automated instruments are well suited to rate measurements because they can readily measure the assay signal multiple times and calculate an average slope. Kinetic assay can introduce some imprecision to the assay because rates may be more sensitive to environmental perturbations than the final equilibrium.

Automation Design Options

The broad range of IA design options is made even greater by automation, which can provide capabilities unavailable in a manual format. However, automation also imposes constraints. To achieve inexpensive and efficient operation, mechanical manipulations usually are kept as simple as possible, and a general format is chosen that can be adapted, with only limited modifications, to a multitude of clinical analytes.

Classic Designs

The general approach in designing IA automation to date has been to attempt to perform an established manual format robotically or to adapt an automation design used in general chemistry to IA. Examples of the first approach are early instruments that automated the classic RIA. Owing to the aforementioned difficulties, these were not successful and have become almost extinct. More important examples for today's laboratory are instruments that robotically perform ELISA tests (IAs in microtiter wells). These instruments can be highly advantageous for performing large numbers of ELISA tests but are relatively inflexible for general application.

Most of today's automated IAs are done on instruments that are analogous to general chemistry analyzers. Typical general chemistry analyzers perform simple colorimetric reactions in cuvettes, often with several arranged in a wheel so that they can be rotated into various stations for dispensing, reading, washing, and other steps. With sufficient instrumental complexity, such a design allows for high-throughput performance of a large menu of tests with

random access selection. These analyzers are immediately suitable for homogeneous IA formats, such as EMIT, that are based on absorbance. Homogeneous fluorescence formats can be added by incorporating that detection mechanism. Since many analytes still require a heterogeneous format, however, substantial modification is necessary to offer a full IA menu. Early batch analyzers, such as the Stratus (Dade Behring) and IMx (Abbott Laboratories, Abbott Park, IL), performed heterogeneous assays in batch mode. The next generation of analyzers, including the Immuno 1 (Bayer Diagnostics, Tarrytown, NY), AxSYM (Abbott Laboratories), Access (Beckman Coulter, Fullerton, CA), ACS180 (Bayer), and others, added random access by allowing selection from multiple reagents stored on board. They imitated cuvette-based general chemistry analyzers, generally using microparticles as a solid phase that could be dispensed as a slurry.

Some chemistry and immunochemistry analyzers depart from the typical reaction cuvette design. Continuous flow, the basis of the original AutoAnalyzer and Technicon (now Bayer) SMA instruments, was adapted to homogeneous immunochemistry with the Technicon CHEM-1 analyzer and may be adaptable to heterogeneous assays as well. Dry multilayer reagent films, introduced to general chemistry with the Ektachem analyzers (now Vitros, Johnson & Johnson, New Brunswick, NJ), also are adaptable to immunochemistry.^{93,94} Self-contained testing units, as have been widely used in general chemistry with the ACA analyzer (Dade Behring), are well suited to IA in low volume.

As with general chemistry analyzers, automated IA platforms may be intended for environments ranging from the largest central laboratories to point-of-care environments or even the home. The largest laboratories will be looking for IA modules to be placed on a fully automated track system. For low-volume scenarios, compact batch analyzers, possibly with a restricted menu (eg, cardiac markers) will be useful. At the simplest level, the "automation" may require no instrumentation at all but may be contained entirely within unitized testing devices, as with home pregnancy tests.

Novel Designs

Immunochemistry analyzers may alternatively use technologies that depart in more fundamental ways from general chemistry analyzers. For example, flow cytometers can be adapted to qualitative or quantitative measurement of soluble Ags. This has been done by analyzing, instead of cells, latex microspheres, which serve as the solid phase in a competitive or immunometric IA. Detection uses a fluorescent label on the Ag (competitive format) or Ab (immunometric format). The power of the technique lies in the common ability of flow cytometers to quantify 3 different colors of fluorescence simultaneously. If a green fluorophor (fluorescein) is used as the IA label, varying quantities of red and orange fluo-

rophors can be used to tag microspheres with different Ab coatings, thus allowing 64 or more assays to be performed simultaneously.⁹⁵⁻⁹⁷ A variation, termed *coupled particle light scattering* (Copalis, DiaSorin, Stillwater, MN), performs multiplexed IA using light scattering to distinguish microparticles of different size.^{98,99}

Multiplexed IAs also can be performed via spatial separation of different Abs bonded to a solid surface, with localization accomplished using high-resolution optics.¹⁰⁰⁻¹⁰³ IA analyzers of the future may use microfluidics, which has been applied to competitive IAs with bound-free separation by capillary electrophoresis¹⁰⁴⁻¹⁰⁸ and to latex-agglutination IA.¹⁰⁹ Immunosensor technology, described in the "Type of Label" section, also may be used.

Open vs Closed Systems

The merits of "open" vs "closed" systems have long been discussed with regard to clinical chemistry analyzers. In closed systems, the only source of analytic reagents is the instrument vendor, whereas in open systems, reagents have general availability. For IA, for which laboratories are endeavoring to obtain optimum performance for a wide variety of analyses, the benefits of an open system would seem very attractive. In practice, IA reagents need to be customized for particular platforms and also must provide the major profit incentive to manufacturers, so for the foreseeable future, closed systems will be the norm. When acquiring IA instrumentation, therefore, the laboratory needs to consider carefully not only the characteristics of the hardware, but also the price, availability, and performance of reagents from the same vendor.

Evaluating, Optimizing, and Implementing Automated IA

The technologic advances detailed herein, the increasing clinical application of IA, and the ever-pressing needs to save labor and control costs have made automated IA a compelling technology. The future will bring continuing improvements, but clinical laboratories must be concerned about the imperfections of present systems. Implementing automated IA solutions today requires a thorough skeptical evaluation of all aspects of system performance.

Quality of Results

Although the most obvious features of an automated system are its speed and convenience, the quality of results should be the laboratory's primary consideration. *Quality* seems a more appropriate term than *accuracy*, because the latter implies comparison with an absolute standard of truth,

which may not exist or may not have great clinical relevance. Quality implies overall usefulness of results in patient care, which will relate to different components of accuracy—sensitivity, precision, linearity, specificity, and bias—depending on the application.

Sensitivity

The proper definition of analytic sensitivity and the best means for measuring it have been matters of controversy that are beyond the scope of this article.^{110,111} In this article, and generally in the context of clinical IA, *sensitivity* has referred to the low-end discrimination of an assay. For some important chemical markers, measurement to picomoles-per-liter concentrations may be necessary or desirable. Current assays in which sensitivity has been of particular concern include thyrotropin in diagnosis of hyperthyroidism,^{112,113} prostate-specific antigen in early diagnosis of cancer recurrence,^{114,115} and estradiol in men and postmenopausal women.¹¹⁶ Future expansions to the chemistry menu are likely to increase further the demands on analytic sensitivity. For some microbiologic and nucleic acid testing, detection at the level of a single molecule or organism is desired.

The “lower limit of detection” or “minimal detectable concentration,”¹¹⁷ a frequent descriptor of sensitivity, may be estimated as twice the SD (precision) of a measurement performed on the zero calibrator.⁹ Although useful, this parameter does not directly translate into clinically meaningful sensitivity. Like any precision measurement (see “Precision”), it may be poorer when tested over the long term than, as is commonly done, in a single run. More important, it does not reflect the dependence of usable sensitivity on *specificity*. For example, a thyrotropin assay might be “sensitive” to 0.001 mIU/L of hormone, meaning that such a small amount produces a signal distinguishable from zero. This same assay might, however, be subject to matrix effects causing results to fluctuate by 0.1 mIU/L among different clinical specimens with identical hormone concentrations. Such interference would be utterly insignificant for measuring normal thyrotropin levels but would make measurement of suppressed levels unreliable.

It is also important to recognize that sensitivity depends on signal-to-noise ratio, not just on signal. An intrinsically very sensitive detection signal, such as enzyme-amplified chemiluminescence, will achieve a sensitive assay only if that signal is totally suppressed in the absence of analyte. Systems must be judged on the basis of demonstrated assay performance, not the claimed benefits of a particular technology. A more sensitive signal may have more benefit for assay speed than for analytic sensitivity.

Constraints on sensitivity can be analyte-dependent or assay-dependent. Small amounts of some analytes may, for example, bind nonspecifically to surfaces or macromolecules,

causing irreproducible results at low concentration no matter how the assay is performed.

Precision

Discussions of precision evaluations emphasize its separable components: eg, within-run, within-day, and day-to-day.¹¹⁸ The relative importance of these depends on the clinical application. In some instances, the reason for testing may be to detect a short-term fluctuation, for example, diagnosing ectopic pregnancy based on daily changes in serum choriogonadotropin levels. In this situation, it may be advantageous to run 2 specimens side-by-side, to make use of within-run reproducibility. In many other cases, reproducibility over a much longer term is important. For example, tumor marker results may be monitored over a period of years, and a small increase could be considered clinically significant. To ensure such long-term stability, it is necessary that reagent lot-to-lot variability and long-term instrumental stability be considered part of precision.

An important aspect of precision that is easily overlooked is the vulnerability of a system to sporadic irreproducible results that are entirely erroneous. Classic statistics, based on the gaussian distribution, predict that errors of many SDs have infinitesimal likelihood; in practice, however, they occur, and they may be more likely with new unproven technologies. Although one of the desired features of automation is to remove the possibility of careless human errors, machines can make their own types of blunders; for example, particles or bubbles can cause mispipetting. This is one of the most difficult areas of assay performance to assess rigorously. There have been some efforts to assess the overall frequency of laboratory errors,¹¹⁹⁻¹²¹ but it is difficult to determine how often a particular analytic system may produce a spurious irreproducible result during its routine operation. Most types of quality control fail to detect blunders. The main effective methods are replicate tests and result checks, such as delta checks.^{122,123} Customers can try to subjectively assess the propensity of an automated IA system toward blunders by looking for features such as robust mechanical designs, capability to detect mispipetting, and sophisticated instrumental diagnostics. In addition, during evaluations, they should note carefully the occurrence of statistical outliers and endeavor to find their cause.

Linearity

Linearity is another analytic concept for which rigorous definitions and evaluations are difficult.^{124,125} The term in this context refers to the actual reported result of an assay, not to the signal from which that result is calculated. However, a wide linear range is easier to obtain when the underlying response is intrinsically linear (as with IMA) rather than curvilinear (as with CBA).

In practical terms, assay results need to be verified over their full reportable range. There are 2 components to the reportable range: the directly measurable range of results, and the range that can be measured on dilution. The directly measurable range can depend on a number of factors in the method and instrumental design. Manufacturers often specify a reportable range wider than that spanned by their calibrators. In that case, it is important to have materials that can verify the claim, but these may be difficult to obtain. Dilution can extend the reportable range, but this also must be verified. Proper choice of diluent is essential, and analyte instability and other factors can cause values measured on dilution to be unreliable. A desirable feature of automated platforms is the ability to perform on-board dilutions as needed.

Linearity demands are severe with many clinical IAs because the relevant analyte concentration spans several orders of magnitude. For example, the serum thyrotropin level can be less than 0.001 mU/L in hyperthyroidism and more than 1,000 mU/L in hypothyroidism. With many tumor markers, small elevations can be important early indicators of cancer recurrence, whereas huge elevations can be seen in advanced cancer. Viral Ags also can span a broad concentration range.

Several types of problems can cause IA results to depart from linearity. When the assay response is curvilinear (CBA), drifts in calibration can lead to deterioration of linearity. A well-known situation of severe nonlinearity in IA is the high-dose hook effect, in which assay response eventually decreases as analyte concentration increases.^{126,127} It can lead to dangerous misreporting of exceptionally high results. A readily understood form of the high-dose hook effect occurs with typical IMAs, in which patient sample and detector Ab are incubated simultaneously with the solid-phase capture Ab. If enough analyte is present in the patient sample to saturate the capture Ab, the excess will occupy the binding site of the detector Ab, causing it to fail to bind to the solid phase. Further increase in analyte concentration will cause a further decrease in the observed signal. This type of hook effect can be cured by performing a washing step to remove excess analyte before the addition of signal Ab, but this can add time and complexity to an automated assay. Hook effects are also intrinsic to IAs based on the precipitin reaction, including automated nephelometric IAs, since precipitin formation decreases in the region of Ag excess. Diverse IAs may be subject to hook effects for complex or obscure reasons, so the possibility of hook effect always should be considered.

Specificity

Analytic specificity is one of the most important and complicated characteristics of an IA. At least 2 general types

of nonspecificity, or interference, can affect IA results. First, substances that are chemically similar to the analyte, but whose measurement is not intended, may nevertheless be measured. Such cross-reactivity relates more to binding properties of the reagent Abs than to any particular assay format. Second, entirely spurious interferences can occur, usually more related to the assay format than to binding properties of the Ab.

Cross-reactivity is an inevitable feature of IA, and it is not easy to define precisely what constitutes cross-reactivity as opposed to the desired reactivity of the assay. For example, it generally is not desirable that slight variations in a protein, which do not affect its biologic activity, should affect reactivity in a clinical assay. Protein assays actually can have excessive specificity, as in monoclonal assays for lutropin that were found not to react with a biologically functional variant.¹²⁸ In contrast, choriogonadotropin and lutropin are almost indistinguishable in biologic activity and very similar structurally, yet the small structural difference reflects a difference in tissue source that is crucial in the diagnosis of pregnancy. Some important macromolecular analytes, such as tumor markers, are defined based on their affinity for a certain monoclonal Ab—how can one define cross-reactivity in such a case?

Similar conundrums arise with small molecules. Sometimes, as with drug screening assays, it is desirable that an assay detect a class of molecules (eg, opiates, benzodiazepines, barbiturates) rather than 1 specific molecule. It may be difficult to specify which molecules should belong in the class (opiate agonists only? opiate agonists and antagonists? opiates plus synthetic opioids?), and needs might vary according to circumstances. Requirements for Ab specificity also depend on clinically observed concentrations; for example, interference of triiodothyronine in a thyroxine assay generally would be much less serious than the reverse, since the physiologic concentration of the latter is about 100-fold higher.

In practice, the lack of absolute standards for specificity leads to considerable heterogeneity in the marketplace. Although any particular assay may still be satisfactory, using assay-specific reference ranges, this can lead to harmful confusion. Several important protein analytes can be mentioned as examples. Discrepancies among assays for choriogonadotropin have been recognized for many years.¹²⁹ These can relate not only to gross differences, such as recognition of free subunits or only intact dimers, but also to subtle modifications, such as peptide nicks or alterations in glycation.¹³⁰ Prostate-specific antigen is one of the few tumor markers widely used for cancer screening, with relatively widespread acceptance of a 4.0-ng/mL cutoff. Yet the molecule exists in serum in free and bound forms, and assays may not recognize these equally.^{131,132} A more recently

introduced analyte, cardiac troponin I, exists in serum in a multitude of forms (eg, free, bound to troponin T and/or troponin I, phosphorylated, reduced). Commercial assays for cardiac troponin I vary more than 20-fold and are affected differently, for example, by protein degradation related to sample storage.^{133,134} These problems, although not intrinsically related to automation, can be exacerbated by the restricted choices imposed by selection of a particular automated platform.

Putting aside the dilemmas of defining desirable assay reactivity, it is evident that many IAs have undesirable cross-reactions, especially when making important distinctions among families of molecules such as glycoprotein hormones, steroids, and drug metabolites. The technique of ligand recognition reaches fundamental limitations in such cases, since no binding reagent can recognize every feature of a molecule. Therefore, the most definitive assays use a physical technique, such as gas chromatography–mass spectroscopy, or IA after prepurification. Novel automation designs would seem an attractive way to increase the practicality of IA combined with prepurification, but there has been little progress in this area to date. Prepurification would add considerable cost and complexity, and the perceived need for it in most cases seems slight, but perhaps this needs to be weighed against possible efficiencies from improved diagnosis owing to better IA results.

Spurious interferences are, in contrast with cross-reactions, always undesirable and, in principle, avoidable. Frequent causes are interfering Abs, label interferences, and matrix effects.

Interfering Abs.—Serum contains a high concentration of immunoglobulin with variable and unpredictable binding properties. A patient's serum may contain Abs to the analyte of interest, often related to the disease process that is being investigated (eg, anti-insulin Abs in a diabetic patient). These may cause negative interference, by preventing patient Ag from binding to reagent Abs, or positive interference, by binding the labeled Ag in a competitive format.

It also has long been recognized that native serum Abs can cause problems by reacting with reagent Abs.¹³⁵ With classic RIA, the problem often has been a patient Ab directed against an animal Ab that is used to precipitate the analyte-binding Ab.¹³⁶⁻¹³⁸ This patient Ab would block precipitation, causing decreased radioactivity in the pellet and a spuriously increased assay result. With the more modern double-monoclonal sandwich assays, the culprit usually has been human anti-mouse Ab (HAMA), which can cross-link the capture and detector Abs in the absence of Ag, again causing a spuriously increased measurement.^{135,139-143} Spurious increases seem to be more common (and often more dangerous), but negative interferences due to HAMA and other interfering Abs can also occur.

There are many possible approaches to minimizing effects of interfering Abs.^{135,144-148} In some cases, it is possible to denature the Abs without harming the analyte. For example, acid-heat treatment of serum can remove HAMA interference without denaturing carcinoembryonic antigen¹⁴⁵; alcohol extraction can allow accurate measurement of triiodothyronine when interfering Abs are present.^{149,150} Samples can be tested for heterophilic Abs, which sometimes is done as a control test in single-use devices. Either with all samples or with those that test positive for HAMA, affinity columns can be used to remove immunoglobulin before analysis. In most cases, the effects of HAMA and similar Abs can be blocked by including a large amount of nonspecific immunoglobulin from mouse or other species. Reaction conditions sometimes can be modified to minimize the interference. As discussed in "Type of Binding Reagent," many of the problems may be ameliorated by using modified reagent Abs or nonimmunoglobulin binding reagents. Since Abs can be directed against virtually anything, including a binding idiotype, this approach may never be 100% effective.

Label Interference.—Interference related to the specific type of label used in IA also has been recognized since the early days of RIA. With a radioactive label, if a patient serum happens to contain a radioisotope related to an immediately preceding nuclear medicine procedure, it can be carried over into the final counting tube and affect results. With IAs having colorimetric endpoints, the classic interferents—hyperlipidemia, hemolysis, and icterus—must be considered. With fluorescence labeling, interference can occur due to endogenous fluorescence substances, fluorescent drugs, or fluorescein administration for performance of retinal angiography. Enzyme-labeled IAs usually are not affected by the enzymes present in serum, but they can be in rare cases. An example is a finding of falsely elevated creatine kinase MB results on the Stratus analyzer, which used a filter membrane as a capture device and an alkaline phosphatase label. Washing the filter membrane usually removed all native serum alkaline phosphatase, but the affected patients had a variant macro form of the enzyme that was trapped by the filter.¹⁵¹

Matrix Effects.—A *matrix effect* is an interference arising from substances present at high concentration in clinical specimens, such as protein or lipid, or from a bulk fluid property such as viscosity. The sample matrix may affect, for example, binding kinetics, often in ways that are difficult to pinpoint. Matrix effects are common problems for IAs. They can be an important source of discrepancy between clinical specimens and calibrators, standards, and proficiency materials that are not based on human serum. They also can make certain types of body fluid unsuitable for analysis.

Bias

Bias is a systematic deviation of an assay result from the true value. Bias can have a variety of causes, the most obvious being miscalibration. For reasons discussed in "Specificity," calibration of protein assays can be particularly difficult and even somewhat arbitrary.¹⁵² A system of international standard materials has been established under the auspices of the World Health Organization, but preparations change over time and different ones may be in simultaneous use. A noteworthy example is choriogonadotropin, for which the Second International Standard contained about an equimolar mix of intact choriogonadotropin and its free beta-subunit, whereas in the Third International Standard, the free beta-subunit was removed, and, thus, the total beta-subunit concentration was cut in half. This caused more than 2-fold discrepancies among popular methods for choriogonadotropin measurement, depending on which standard was used and whether the total beta-subunit or intact choriogonadotropin was measured.¹²⁹

For automated IA, clinical laboratories almost always use the calibrators and protocol specified by the vendor. Determining absolute bias requires comparison with a definitive or reference method, which may be unavailable. Relative bias commonly is assessed in clinical laboratories in 2 ways. One is through method comparison studies, which need to be carefully designed and conducted to give a measure of inter-method bias that is itself unbiased.^{153,154} The second is via results of proficiency testing surveys. These can be valuable for revealing the differences among popular commercial methods. When interpreting the survey results, however, it must be remembered that differences may relate not only to calibration differences among methods, but also to properties of the survey material. Survey materials may differ in their biologic matrix from patient specimens and may be spiked with a combination of analytes that would not occur in patient plasma.

As mentioned in "Specificity," bias can have relatively little clinical importance when method-specific decision points are used. Often, however, clinicians base decision points on published literature or personal experience with other methods. Also, especially in this era of managed care, serial monitoring may be performed in different laboratories using different methods.

Analyte Issues

This review has emphasized the variety of challenging assays performed by automated IA. Many analytic difficulties, such as some of the aforementioned cross-reactivity issues, relate more to specific properties of analytes than to any particular IA format or automation design. However, the extent to which they influence results reported by the laboratory may depend greatly on the automated IA design. Aside

from problems of low concentration (sensitivity) and similarity to other molecules (specificity), analyte properties that negatively affect assay results include instability, specific binding, and nonspecific binding.

Instability.—Proteins, including some clinically important peptide hormones such as corticotropin, may degrade spontaneously or from proteases in serum. Small molecules may oxidize, undergo enzymic reactions, or otherwise degrade.

Specific Binding.—If an analyte is bound extensively to a transport protein, the first decision is whether the assay is intended to bind free or total analyte. A total ligand assay usually must use some means to strip the ligand from the transporter. For example, 8-anilino-1-naphthalene-sulfonic acid commonly has been used to dissociate thyroxine from thyroxine-binding globulin, and boiling (not very convenient for automation!) has been used to separate vitamin B₁₂ from its binding proteins. These manipulations can be a source of error. Measurement of free ligand, as with thyroxine, may be more meaningful clinically but raises a number of difficulties, including the impossibility of an absolute standard.¹⁵⁵ Some important proteins, such as prostate-specific antigen and cardiac troponin I, exist in serum in a number of different bound states, and this situation can hamper assay performance and standardization.^{131,156-158}

Nonspecific Binding.—Many molecules tend to bind nonspecifically to proteins, other macromolecules, and to glass and other surfaces. This nonspecific binding usually increases fractionally as total concentration decreases. Some molecules exhibit self-binding (aggregation), a phenomenon that may be more important at high concentrations. Subtle changes in assay design can affect assay results in such cases.

The Pluses and Minuses of Automation

Automation may have beneficial and detrimental effects on the quality of results. Possible benefits include the following:

- Improved test formats. Whereas certain formats, such as classic RIA, are difficult to automate, other formats with especially desirable performance characteristics may be suited intrinsically to automation.
- Improved reproducibility. IAs tend to be less precise than general chemistry assays. Automated platforms may improve the precision of pipetting, mixing, timing, and separation steps, leading to better assay performance.
- Greater within-laboratory uniformity. Results of manual IA may depend on an individual's technique, whereas automated tests presumably will not.
- Greater interlaboratory uniformity. For many IAs there

is considerable disparity in results reported by different laboratories owing to differences in standardization, specificity profiles, and other parameters among assay kits. While automation does not address this problem directly, viable automation platforms ultimately must be adopted by large numbers of laboratories, and it can be anticipated that greater uniformity will result.

Possible adverse effects on result accuracy include the following:

- Compromises in assay design. Manufacturers may compromise assay performance for reasons related to the simplicity, cost, and speed of automation. For example, to minimize wash steps, it is usual with sandwich-type assays to add patient sample and detector Ab simultaneously, without an intervening wash step. This usually causes no problem, but it allows the possibility of a high-dose hook effect. Incubation times and other parameters may be set to increase throughput and harmonize with the other assays on the system rather than to optimize accuracy. Furthermore, the design of automated IAs is necessarily rigid. Individual laboratories generally will be unable to make minor adjustments to improve result quality, and vendors will require relatively lengthy development cycles to improve assay performance.
- Errors related to automation design. These could include, for example, sample-to-sample carryover due to reusable sample probes, sample-to-sample or test-to-test carryover due to reuse of reaction cuvettes, and errors caused by deterioration of reagents in reagent lines.
- Errors related to automation malfunction. A major malfunction may cause systematic errors in 1 assay, a group of related assays, or all assays. More vexing can be sporadic errors (see "Precision").
- Compromises in quality control. While the classic manual RIA was highly susceptible to errors in technique, this was to a great extent compensated by fastidious calibration and quality control. All calibrators, control specimens, and patient specimens typically were run in duplicate (occasionally triplicate). A full set of calibrators, along with nonspecific binding and total count tubes, were run at the beginning of the assay, and control specimens were inserted at the beginning, middle, and end of the patient specimens. Careful examination of the data gave the operator an excellent opportunity to detect a systematic failure of the assay or an error, such as mispipetting affecting a single specimen.

With automated IA, duplicates generally are dispensed with, and standards and controls are run much less frequently. While systematic and sporadic errors may occur less frequently, there is less chance to detect them. Even the final stage of quality assurance, evaluating the reasonableness of patient results, is diminished as these tests become more routine and are reported around the clock by personnel with varied training. This problem can, to some extent, be offset by delta checking and other automated means for checking the reasonableness of results, but these generally are not very satisfactory.

Summary: Evaluating and Maintaining Result Quality

Automated IA combines the power, and the hazards, of sophisticated assay technology, complex instrumentation, and clinical algorithms, often newly introduced, based on measurements of molecules at exceedingly low concentrations. Assurance of dependable results requires the following:

- A thorough initial method evaluation.
- Internal quality control that is customized to the way test results are generated and used. Control materials with concentrations near critical decision points must be included. For example, a highly sensitive thyrotropin assay demands a control material at a suppressed level. Frequency of running controls may need to be considerably higher than minimums recommended by the vendor.
- Participation in external proficiency testing surveys. Results should be examined not only with respect to grading, which may be based on comparison only with the other laboratories using the same method, but also with respect to the overall mean and SD of the laboratory's method in comparison with other methods.
- Continued vigilance for outlier or aberrant results, with attempts to resolve the cause and follow-up with the vendor.
- To the extent possible, clinical correlation of reported results.

Operational Issues

When selecting and implementing a particular platform for automated IA, the laboratory must evaluate carefully its own needs and the capabilities of the analyzer. As usual, the issues are similar to those in general chemistry but tend to be more complex with respect to IA.

Test Selection

Random access—the ability to choose whatever tests are desired, from a large menu, on each individual specimen—is

now as essential in IA as in general chemistry. In both cases, test selection generally is accomplished by the instrument reading a specimen bar code and querying a host computer system. Additional features supporting unattended operation are automatic re-runs, with on-board dilution as needed, and the capability of automated reflex testing based on user-defined criteria.

Reagent and Sample Handling

Operators should be able to place reagents and samples on the instrument with minimal difficulty. Reagent cartridges should be of appropriate size, for the volume of testing, and need infrequent replacement but avoid waste. Different types of sample tubes should be accommodated easily. For many laboratories, the ability to sample from a track system will be important; in this case, a manual insertion mode is useful as well. Sample volume requirements should be minimal, especially for the necessary "dead volume" on top of the test volume requirement.

Ease of Operation and Serviceability

A desire to achieve labor savings is the most important driving force behind laboratory automation. While there is no doubt that automating IA saves manual effort, the post-automation labor needs may be greater than expected. Until the present, many so-called automated systems were limited batch analyzers, requiring frequent operator intervention to change reagents for each assay. With the most modern, random-access analyzers, much greater labor savings can be expected, but one still must consider not only how much operator time is needed, but also the amount of training and the skill level required for the operators, especially in case of subtle instrument malfunction. When there are problems laboratory personnel cannot handle, the laboratory must be assured of rapid service response. This is another vital area requiring major investment by the vendor.

Workstation Consolidation

Besides direct labor savings, additional savings in space, administrative complexity, and operational efficiency may be achieved if 1 automated instrument can consolidate what previously required several workstations. Until recently, most automated platforms offered somewhat incomplete menus, so that the degree of consolidation has been less than may be hoped for in the future. If several different automated analyzers are required, which may differ greatly in their operation, the complexity of the laboratory may be increased. On the other hand, consolidation has hazards of its own, mainly related to lack of redundancy. Considering the urgency of many IA tests, the possibility of instrument breakdown often must be dealt with by having at least 2 instruments of the same model continuously available.

Backup using a different method may be less satisfactory because of intermethod differences. However, a vendor may develop a problem with a particular method or even a group of methods, and it is always useful to have the ability to check a possibly erroneous result with an alternative method.

Nonlabor Costs

Automated instruments often can be designed to consume very low quantities of expensive immunodiagnostic reagents. Balancing this theoretic advantage is the fact that with closed systems, the customer may have to pay inflated reagent charges. Costs of other consumables, such as cups, probes, and tubing, and costs of instrument service also must be considered when evaluating the overall economics of a system.

Turnaround Time and Single-Test Capability

An automated system should be able to perform any test at any time. The actual time to obtain a result once the specimen is on the analyzer is usually of less importance but can be a concern in some cases, eg, for urgent diagnosis of myocardial infarction or ectopic pregnancy. When large numbers of specimens are being processed, overall throughput of the analyzer will also have an important influence on turnaround time, and stat interruption capability is important.

Commercial Issues

The complexities of automated IA put an exceptional demand on system vendors. They first must assemble all of the necessary technology, including a general instrumental approach and individual methods with excellent performance for a broad range of analytes. This generally requires not only a great deal of in-house research and development, but also extensive arrangements with other vendors, such as companies that have developed particular monoclonal Abs. Patents may impede the incorporation of useful technologies developed elsewhere and may encourage development of unique, but not necessarily superior, technologies so that the vendor can obtain its own patents.

The vendor then must establish manufacturing and worldwide service for the hardware platform, while assuring a steady supply of reagents. Complex computer software is also part of the package, and marketing must accommodate a broad range of customers with differing needs. All of this must be accomplished in an era of intense pressure on laboratory spending. Clearly, laboratory customers must evaluate the vendor itself as carefully as it examines the vendor's product. The recent trend has been inevitably toward corporate consolidation, which on balance should be of great benefit to consumers, as entities are created with sufficiently broad scientific and financial resources to meet the demand.

Negotiating the acquisition of a large automated IA platform can be difficult. The laboratory should consider

carefully the different options of purchase, rental, or reagent rental, along with possibly hidden costs of disposables, reagent wastage, nonwarranty service, and other features. It is best to consider in advance, to the extent possible, problems that may arise and how the company may provide restitution.

Conclusion

Progress in automated IA has been impressive, but much remains to be accomplished. Many homogeneous IAs are now almost seamlessly integrated with routine chemistry testing. More demanding heterogeneous IAs can be performed on dedicated systems offering the tantalizing advantages of around-the-clock, random-access testing. However, currently available systems often have been lacking in terms of test menu, convenience, and, in some cases, quality of results. As has been emphasized, the last issue is most critical. As new and powerful diagnostic markers become available, their diagnostic potential can be fully realized only if routine methods are reliable.¹⁵⁹ Better test results come at a price but in the long run probably will help contain health care costs. The emerging mega-corporations in the diagnostics industry have ample resources to meet this challenge, but the laboratory community must be unrelenting in its demand for quality.

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