Use of a Solid-State Multihead Gamma Counter in a Second-Generation System for Solid-Phase Immunoassay

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Simultaneous advances in detector technology and solid-phase separation systems, as well as the availability of powerful desktop computers, have made possible the development of "second-generation" solid-phase immunoassays. These retain the advantages of classical solid phase while significantly accelerating reaction kinetics. Hapten assays—such as for digoxin, thyroxin, and triiodothyronine uptake—in batches of 48 are processed in about 20 min from reagent introduction until hard-copy printout, with minimal operator involvement. The system also functions as a 48-detector gamma counter, capable of counting and reducing data for any 125I-based RIA that can be run in a 12 × 75 mm test tube. System control, data management, and computer screen displays of kinetic data are provided by an unmodified Hewlett Packard HP-87XM computer. User-friendly disc-based software facilitates the creation and storage of counting and data reduction protocols for as many as 30 RIAs from various manufacturers as well as up to 30 of our own assays.

Additional Keyphrases: kinetic assays · digoxin · thyroxin · triiodothyronine uptake · data processing · proportional wire counter

RIA is the last and probably the most challenging area of clinical chemistry to be automated. The necessity of separating bound from free fractions and the expense of traditional gamma detectors mean that systems in current use have limited throughput. Often, expensive equipment can only be used with dedicated reagents available from a single supplier (1-4). Because of these limitations, some analysts have turned to nonisotopic systems, only to find that sensitivity limitations (5-7) and single-source reagent supplies (5-8) inhibit the flexibility necessary for the full-service immunoassay laboratory.

We describe an immunoassay-processing system that expedites the workflow in the RIA laboratory. The reagents are based on the well-known principles of coating antibody onto plastic surfaces (9). Low air pressure under microprocessor control initiates the reactions and separates the bound and free fractions. Each reaction takes place within its own gamma detector. The equipment can also function as a stand-alone multiwell gamma counter capable of counting and performing data reduction on any 125I-based RIA.

The system takes advantage of proportional wire detectors instead of the NaI crystals used in other multiwell counters. Proportional wire detectors have been used rou-

tinely since the 1940s (10, 11) in high-energy physics. Initially they resembled Geiger tubes, gas-filled cylinders with an anode wire down the axis. Such devices were limited to giving positional information, telling only that a particle had or had not traversed the counter's volume. The work of Charpak et al. (12) in 1968 led to multwire chambers. Many wires were placed side by side in a common gas, but each wire was connected to a separate electronic circuit. Thus it became possible to locate tracks to the nearest millimeter or better, simply by recording which wire each particle "hit" (i.e., deposited the most energy on). Such detectors are both more stable and less expensive to build than NaI detectors and therefore offer distinct advantages in a multiwell system.

With the equipment we discuss, a flexible data-management system is provided for manipulation and analysis of the assay results. This can include an optional "history" system, for preparing quality-control reports and graphs. Results from more than 250 48-tube assay runs may be stored on each floppy disc, and this data can be collected during use of the system as a gamma counter or as an assay processor, or entered manually. Nine different control values as well as parameters such as assay sensitivity, curve midpoint, and slope can be tracked. Histograms and reports of patients' results allow each laboratory to establish its own reference ranges for its population of patients.

Materials and Methods

Instrumentation

The KinetiCount 48 Immunoassay System (Medical & Scientific Designs, Inc., Rockland, MA 02370) is driven by an unmodified HP-87XM microcomputer (Hewlett-Packard, Corvallis, OR 97330). Several simple moving parts in the instrument allow it to function as an assay processor and as a gamma counter. An electric motor lowers and raises the antibody-coated solid-phase receptacles into and out of the reaction tray drawer. Another motor allows the solid-phase receptacle in each gamma counter position to access four different reagent wells in the reaction tray drawer. The drawer and lid are manually operated and are fitted with switches monitored by the computer to ensure that they have been properly closed during certain critical reaction steps. The low air pressure and the heated tray drawer used to accelerate the reactions are monitored and controlled electronically.

The heart of the instrument is the array of 48 proportional wire counters arranged in eight rows of six detectors. Figure 1 is a schematic diagram of one detector. Each detector is shielded from the others by an outer brass shell. A 17-mm (i.d.) inner aluminum tube allows gamma radiation from 125I into the body of the detector. Sealed between the concentric brass outer housing and inner aluminum tube is xenon gas under a pressure of about 5 atm (about 500 kPa). Fine wires located in this gas space are maintained at a positive potential of about 3500 V relative to the grounded...
brass outer housing. These wires are ganged on the same detection circuit and surround a region of defined geometry. Any x-rays or gamma rays originating inside that region have a certain probability of causing ionization in the surrounding gas container, which will register on the wires. The amplitude of the signal from the wires is proportional to the x-ray or gamma ray energy, a feature that can be used to distinguish the decay of one nuclide from another or from background processes.

*Theory of Operation*. The sequence of steps in detecting the decay of an $^{125}$I nucleus is as follows: The decay of $^{125}$I produces gamma rays with 35.5 or 27.5 keV of energy. For each decay, there is a 53% probability that two such gamma rays will emerge, and a 40% probability that one will emerge (13). The inner wall of the chamber is thin enough to allow approximately 90% of the emerging gamma rays to pass into the gas–wire envelope.

Because xenon is a neighbor element to iodine, the energy levels of its electron shells are ideally suited for photoelectric absorption of the $^{125}$I gamma rays. At a pressure of 500 kPa, and with the relatively small chambers in the present system, the probability of detecting a single $^{125}$I gamma ray is 23%. Given that more than half of the time the decay involves two gamma rays, the probability of detecting a decay is closer to approximately 30%. Each detected decay corresponds to a deposition of 20 keV or more of ionization energy in the gas and is roughly equivalent to the release of 1000 electrons. By operating with a gas gain (multiplication of electrons in the cascade by the anode wire) of $10^8$ to $10^9$, the wires will register several picocoulombs of collected charge per decay (Figure 2). The signal generated by this gain factor can easily be amplified by inexpensive preamplifiers to a level suitable for storage in a computer memory without venturing too close to the boundaries of electrical breakdown. Moreover, the signal is easily distinguished from most sources of background noise.

*Operation of the gamma counter*. To use the gamma counter, one loads plastic 12 × 75 mm tubes into the plastic well liners (which also serve as reaction chambers when the system is used as an assay processor). After the operator selects a previously created and stored counting and data-reduction protocol, the system makes a rapid estimate of the tube with the highest counts in the array and displays on the computer screen an appropriately scaled bar graph display of counts vs well number. During the 3-min counting period the cumulative display is updated at 10-s intervals, allowing a rapid overview of the entire batch of tubes being counted. Data reduction proceeds similarly to that for the proprietary solid-phase reagents and is described in the Discussion.

*Operation of the assay processor*. Liquid reagents such as tracer buffer and samples are pipetted into the disposable plastic reaction trays, which are loaded into the reaction tray drawer in the lower front part of the instrument.

The solid-phase receptacle of the KinetiCount 48 resembles a pipette tip that is closed on the top and has a small opening at the tip; this allows liquid to enter and leave the receptacle (Figure 3). Antibody is coated onto the inner surface of the receptacles by a method described in the *Reagents* section. The antibody-coated receptacles are dropped into each gamma counter, positioned to correspond to an occupied reaction tray well in the drawer below. After the operator closes the reaction tray drawer and the lid and selects the assay to run, the upper stage of the instrument moves down, simultaneously lowering all of the antibody-coated solid-phase receptacles into their respective reaction tray wells, creating a sealed system enclosing all of the liquid reagents and the receptacles in the wells (Figure 3). The tip of each solid-phase receptacle fits through a brass washer, which shields the detectors from 99% of the radioactivity in the wells below.

Low air pressure (5–7 p.s.i.g., 35–50 kPa) forces liquid into the receptacles until the air trapped in the receptacle is at the same pressure as the air outside the receptacle (Figure 3c). The liquid entering the receptacles initiates the immunoreaction in all of the wells simultaneously, obviating trends and "end-of-run" effects in the assay. Because each solid-phase receptacle is enclosed in its own individual

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**Fig. 1.** Cutaway schematic of proportional wire detector

Outer brass housing and inner aluminum tube trap xenon gas within the detector. Gamma rays passing from the gamma-detector well through the aluminum inner liner ionize xenon atoms. Electrons ejected from the xenon are accelerated toward the positively charged wires, causing additional ionizations by collision along the way. The cloud of electrons that eventually collects on the wires is processed as an electrical signal. The positively charged xenon ions are eventually neutralized at the grounded brass outer shell.
gamma detector, the total amount of radioactivity in each receptacle can be counted. Any receptacle that appears to have significantly less radioactivity than the others in the array is pointed out.

A typical pressure cycle lasts for 45 s, after which the pressure in the reagent chamber is released. The liquid reactants drain cleanly from the solid-phase receptacles back into their original wells, leaving only antibody-bound tracer behind (Figure 3d). A brief count of the radioactivity bound to each receptacle is taken at this point and the results are displayed graphically. Typical reactions involving small molecules go through 15 of the pressure/release cycles described above. The brief counts of bound radioactivity made in every cycle are noted in a cumulative bar graph, as illustrated in Figure 4. Replicates of samples are averaged, and individual values that are significantly different from the average are displayed. At the end of the specified number of cycles, a final count of the bound radioactivity is made, and the system proceeds automatically to data reduction.

Data-Management System

All of the software is provided on four discs (Figure 5), only two of which are used at any one time. The System disc contains the main control and data management programs, which enable the system to function as a processor of solid-phase receptacle assays, as a gamma counter, and as a data-management station for manually entered data. It also provides access to the two other program discs, the Utility disc and the History disc. The Data disc provides storage for information such as assay data, calibration data, and data-reduction protocols. Each time an assay is processed or the radiation is counted, or whenever data are entered manually, all of the count data as well as several of the assay variables are automatically stored on the Data disc.

The Utility disc contains programs by which the user creates and updates assay protocols. User-defined variables include the number of replicates of standards, controls, and patients' samples, the dose units desired on the printout, normal range limits, replicate reproducibility "flag" limits, and the response format desired. Data may be calculated as
counts, as % B/B₀, or as % B/T vs dose. The values, ranges, and the preferred location of as many as nine controls in the array may also be specified and will be stored. In addition, the user may define the fit desired (four-parameter logistic, log-logit, cubic spline, point-to-point, and ratio). Simple instructions on the display screen allow rapid recovery from errors.

The History disc enables the user to catalog assays stored on the Data disc and to retrieve data on individual assays. Additional features include the ability to create trend charts or tabular reports of as many as nine different controls or various assay variables from the assays stored on the Data disc. Tabular reports of these stored values may also be created. Histograms of patients' results can be used to define each laboratory's reference interval for a particular analyte.

Reagents

Tracers for digoxin, triiodothyronine (T₃), and thyroxin (T₄) were obtained as concentrates (Cambridge Medical Diagnostics, Billerica, MA 01865) and diluted into appropriate buffers with preservatives and release agents.

Antisera were obtained from a variety of commercial sources. Pools capable of supporting projected needs for each assay for several years have been secured and backup pools have been evaluated. Coating and wash buffers have been described elsewhere (9). The plastic solid-phase receptacles are molded from large lots of virgin polypropylene by a subcontractor, then coated in large, pressurized chambers in batches of up to 20,000 at a time. Batch coating means that no time trends can develop in the coating process. The coated receptacles are dried overnight before being packaged in plastic with desiccant pellets, humidity control in drying solid-phase-bound antibody being critical for product quality (14).

The human serum base for the standards is purchased from AMF, Seguin, TX 78155, and from Boston Biomedical Laboratories, Boston, MA 02120. Digoxin standards are made by adding USP digoxin to the base materials, essentially according to the method outlined by Butler and Chen (15). Standards for T₃ and T₄ are prepared in charcoal-stripped serum, according to a procedure described by Perlstein (16). The euthyroid calibrator for the T₃ uptake test is made from pooled normal human serum and calibrated by an inorganic adsorbent method (17). The hyperthyroid control is made by adding T₄ to the euthyroid base. For a standard having a very low T₃ uptake value, we add to charcoal-stripped serum some sera from donors with a familial excess of thyroxin-binding globulin (18). Standards are preserved by adding sodium azide (1 g/L).

These reagents are marketed under the name "Phase II" (Medical and Scientific Designs).

Immunoassay Procedure

Aliquots of standards, controls, and patients' samples are pipetted into disposable plastic trays. Sample sizes are 10 μL for T₄, 25 μL for T₃ uptake, and 100 μL for digoxin and T₃. Tracer buffer aliquots are 500 μL for all four assays. Solid-phase antibody is added by dropping a solid-phase receptacle into each detector well as described above. Trays are loaded into the heated tray drawer. Timing, temperature, pressure, and volume are controlled highly accurately by the instrument. Digoxin, T₄, and T₃ uptake reactions are incubated for 15 cycles, whereas T₃ reactions are incubated for 30 cycles.

Comparison Studies

To compare the performance of this system with other methods, we used the following commercially available reagents and instruments according to the manufacturer's instructions. GammaCoat Digoxin and Free/Total T₄ kits (CA 527 and CA 533) were obtained from Travenol Laboratories, Inc., Clinical Assays Division, Cambridge, MA 02139. Tri-Tab resin T₃ uptake kits were obtained from Nuclear Medical Laboratories, Irving, TX 75061. The RIA-BEAD Total T₃ RIA kit was from Abbott Laboratories, North Chicago, IL 60064.

Reference-range samples were obtained from Boston Biomedical Laboratories; patients' samples were obtained from a local reference laboratory.

We also compared results with those obtained with two NaI crystal counters—an Autologic counter (Abbott Laboratories), and a Packard 5110 (Packard Instruments, Downers Grove, IL 60515)—and with an NE 1600 multiwell gamma counter (Nuclear Enterprises, Edinburgh, Scotland).

For comparative data reduction we used a Hewlett-Packard HP-85-based software package (RIAssist™) obtained from Ventrex Laboratories, Inc., Portland, ME 04103. Statistical data analysis was done with an HP-87-based Basic Statistics and Data Manipulation Package from Hewlett Packard, Inc.

Results

Counter Stability

To study short-term stability, we placed 48 gamma-radiation sources in the 48 wells and counted for 3 min/h, over a 10-h period. The counts detected were typically in the range of 40,000 to 70,000 in the 3-min interval. Given the well-understood statistical nature of radioactive decay, the error in counting any radioactive sample is equal to the square root of the number of counts detected, so that the expected CV was around 0.4%. For the 10 sets of 48 readings, the average theoretical CV was calculated to be 0.42%; the average observed CV was 0.46%.

Longer-term stability has been measured by repeated comparisons with calibrated NaI counters over a period of six weeks. In these comparisons we used a variety of test tubes with various concentrations of 125I. Reproducibility of count rate included all the normal handling and positioning of tubes, as well as long-term changes in the proportional chambers, their electronics, and the power supplies. The average disagreement in count rates was less than 1%.
It is important that a given sample count is the same, regardless of which detector cell is counting. To check this, we counted a specimen generating 20,000 counts per minute in each of the 48 wells. The inter-well CV measured was 0.84%, compared with the theoretical CV of 0.71%.

Gamma Counter Results

 Tubes from a previously run coated-tube assay for thyroxin were counted and evaluated with the KinetiCount 48 System. The results were compared with data obtained with a conventional multihead gamma counter and commercially available software. Excellent agreement was obtained between the two counters, as shown by the regression analysis data accompanying Figure 6 (top). When these counts are converted into doses, both by the commercially available software for the conventional multiwell counts and by our data-management system for the counts obtained on our system, correlation is again excellent (Figure 6, bottom).

 Other studies, involving a wide variety of reagents, have shown similar agreement when the system is used as a gamma counter and (or) as a data management system.

Assay Performance

Reproducibility studies, as measured by a random sampling of coated receptacles assayed at the midpoint of the standard curves, consistently show CVs of counts of 1.5–2.5%.

Results of inter- and intra-assay precision studies for digoxin are shown in Table 1. The analytical recovery of the digoxin assay is reported in Table 2. The specificity of the digoxin assay, tested in cross-reactivity studies, is shown in Table 3. Precision and accuracy are comparable for T4, T3 uptake, and T3. In addition, we determined reference intervals for these analytes with sera from apparently healthy blood donors, selected without conscious bias (Figure 7).

[Table 1: Digoxin Assay Precision]

<table>
<thead>
<tr>
<th>Sample</th>
<th>Digoxin, ng/mL</th>
<th>CV, %</th>
<th>Digoxin, ng/mL</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool A</td>
<td>0.77 (0.05)</td>
<td>6.5</td>
<td>1.05 (0.07)</td>
<td>6.3</td>
</tr>
<tr>
<td>Pool B</td>
<td>1.79 (0.13)</td>
<td>7.2</td>
<td>1.97 (0.10)</td>
<td>4.9</td>
</tr>
<tr>
<td>Pool C</td>
<td>3.09 (0.20)</td>
<td>6.3</td>
<td>3.08 (0.20)</td>
<td>6.5</td>
</tr>
</tbody>
</table>

*The pools used for the intra-assay precision studies were not necessarily those used for the interassay precision studies.

*Mean (and SD), n = 10 each.

[Table 2: Analytical Recovery of Digoxin]

<table>
<thead>
<tr>
<th>Added</th>
<th>Measured</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.90</td>
<td>0.89</td>
<td>99</td>
</tr>
<tr>
<td>1.20</td>
<td>1.26</td>
<td>107</td>
</tr>
<tr>
<td>1.80</td>
<td>1.86</td>
<td>103</td>
</tr>
<tr>
<td>3.60</td>
<td>3.71</td>
<td>103</td>
</tr>
</tbody>
</table>

Fig. 6. Gamma counter correlations between the KinetiCount 48 (y) and an NE1600 Multiwell gamma counter (x)

Top linear regression of counts per minute: slope = 1.045, intercept = -112, r = 0.999, n = 48. Bottom, regression analysis of thyroxin concentrations from antibody-coated tubes counted and data reduced by the KinetiCount 48 vs data obtained with an NE1600 multiwell gamma counter with data reduction by commercially available software: slope = 1.022, intercept = 0.067 µg/dL, r = 0.991, n = 24

Fig. 7. Reference interval studies for Phase II thyroid-function kits

Bars indicate the number of patients' samples that fall into a particular range; while the superimposed smooth curve represents the idealized normal distribution. For T4, n = 200, x = 8.02 µg/dL, SD = 1.79 µg/dL, x ± 2 SD = 4.4–11.6 µg/dL. For T3 uptake, n = 200, x = 38.6%, SD = 2.72%, x ± 2 SD = 33.3–44.2%. For total T3, n = 200, x = 1.50 ng/mL, SD = 0.26 ng/mL, x ± 2 SD = 0.98–2.02 ng/mL.
Results of correlation studies between these reagents and conventional reagents are shown in Figure 8. All three thyroid-function tests show good agreement with published reference intervals for normal subjects (19-21). Nonetheless, every laboratory using these reagents should establish its own reference intervals for its own patient population.

Discussion

Detectors

Proportional wire detectors have several intrinsic advantages over conventional NaI crystal detectors. The simplicity of design and manufacture assure low cost and reliability. There are no halide crystals to hydrate, corrode, or age and no vacuum tubes to cause baseline drift with time. It is difficult to imagine a device chemically and mechanically more stable than a hermetically sealed metal can filled with a noble gas. Efficiencies are essentially locked in at manufacture, where critical variables such as geometry and xenon pressure are tightly controlled.

A traditional NaI counter has a cup-shaped crystal shielded against external radiation, and is joined at the bottom to a photomultiplier tube in such a way that light pulses originating in the NaI crystal are detected by the photomultiplier tube. In comparison with these “well-counters,” the present device is simpler, more compact, and less expensive. It requires virtually no shielding, because it is nearly insensitive to all radiation but that from 129I, as discussed below. The stability of operation is superior to that of NaI crystal–photomultiplier combinations. Moreover, it is well suited to an application in which a tube extends through the sensitive region, allowing access immediately below the counter. Finally, because of the small size and low cost per cell, we have been able to combine 48 detectors of this type to develop a sensitive gamma counter.

Specificity of Gamma Detection

For a detector dedicated to a certain operation (e.g., RIA counting of 125I radioactivity), rejection of other signals makes the device less sensitive to backgrounds and decreases contamination of the operating environment by other radionuclides.

Because the detector is “tuned” to 129I, it has reduced efficiency for counting disintegrations of 125I, the long-lived isotope often used for gamma-counter calibration. In fact, any counter will have a different response for 129I and 125I, but this is often overlooked because the NaI well-counters are sufficiently similar to one another that the suppliers of 129I can quote a fixed scale factor for 125I simulation. For an equal number of 129I and 125I decays, an idealized counter, which would surround the sample and register 100% of all energetic gamma rays (energy greater than 5 keV), would count 129I with 82.5% of the efficiency of 125I. For conventional NaI well-counters, the scale factor = 0.76, perhaps varying by several percent depending on the particular design. The present proportional counter device, with com-

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Table 3. Digoxin Assay Cross Reactivity in the KinetiCount 48 System

<table>
<thead>
<tr>
<th>Compound</th>
<th>% cross reactivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lanatoside</td>
<td>64.1</td>
</tr>
<tr>
<td>Deslanoside</td>
<td>70.2</td>
</tr>
<tr>
<td>Digitoxin</td>
<td>1.7</td>
</tr>
<tr>
<td>Dihydroroxygen</td>
<td>3.0</td>
</tr>
<tr>
<td>Gitalin</td>
<td>1.6</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>&lt; 0.015</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>&lt; 0.015</td>
</tr>
<tr>
<td>Furosemide</td>
<td>&lt; 0.015</td>
</tr>
<tr>
<td>Quinidine</td>
<td>&lt; 0.015</td>
</tr>
</tbody>
</table>

*Results reported as the amount of material necessary to produce 50% inhibition, divided by the amount of digoxin necessary to produce the same inhibition.

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Fig. 8. Correlation studies comparing Phase II reagent kit results with values obtained with conventional reagents

Digoxin: $n = 102$, $y = 1.11x + 0.17$ ng/mL, $r = 0.9503$. Thyroxin: $n = 88$, $y = 0.92x + 0.34$ µg/dL, $r = 0.9591$. T3 uptake: $n = 102$, $y = 1.069x - 4.58$, $r = 0.9586$. Total T3: $n = 100$, $y = 0.87x + 0.26$ ng/mL, $r = 0.9726$.
pact geometry and filled with pressurized (500 kPa) xenon, has a scale factor = 0.53.

The scale factor is a constant of the device and is invariant with time or use. Hence $^{125}\text{I}$ can be used to calibrate with the present device as readily as any other, so long as the appropriate scale factor is applied. However, cost considerations argue for gamma counter calibrators made from $^{125}\text{I}$ and corrected for decay by software. The specificity of the detectors for $^{125}\text{I}$ is even more evident when one uses them to detect isotopes that emit at substantially different energy levels than $^{125}\text{I}$. For example, $^{57}\text{Co}$ emissions are well above the absorption range for xenon and, therefore, $^{57}\text{Co}$ is detected only 10% as well as $^{125}\text{I}$.

Gamma Counter

Solid-state electronics throughout the instrument reduce instrument size, heat load, and component failure rates. The instrument measures $45 \times 68 \times 68$ cm and fits on a laboratory bench. A dual floppy disc drive provides a total of 540 kilobytes of mass storage. Hard copy is provided in a 20 $\times$ 28 cm format (8 1/2 $\times$ 11 in.) by a dot matrix printer. The KinetiCount 48 requires only 5 A of 120 V current and an air-pressure line capable of delivering about 2.8 L (0.1 cu. ft.) of air per minute at 138 kPa (20 p.s.i.g.).

The removable plastic well liners may be cleaned or replaced if they become contaminated. When the system is closed and is at rest, it automatically reviews the background counts in each well and alerts the operator of a possible contamination when a background count is significantly higher than usual for that particular well.

Each time the radioactivity in an assay is counted on the system, the raw count data, the counting and data-reduction protocol information, and the curve-fit parameters are stored automatically on a floppy disc. The output on the display screen continually informs the operator as to how full the History system is. The ability to group a large, stable array of counters economically provides a system having a potential throughput of 1000 tubes per hour.

Reaction Kinetics

The geometry of the solid-phase receptors and the mixing of the liquid reactants substantially accelerate the reactions (Figure 9). Relative to similar reactions in a coated test tube, the four- to fivefold increased rate of binding of tracer to the solid phase is due in part to coated antibody surface area and reaction volumes that lead to an effective solid-phase radius ($R_e$) of 0.19 cm (22) vs 0.44 cm for a typical coated test tube. Further application of Nayak’s analysis of solid-phase reaction kinetics (22) suggests that some reaction acceleration could be expected relative to other, more conventional, solid-phase geometries, but the extent of overall increase indicates that the mixing at the solid-phase–liquid-phase interface also plays an important role.

During each cycle, as the liquid drains back into the wells, the reactants mix thoroughly. Because solid-phase immunoassays are subject to liquid-phase reactant depletion conditions at the liquid–solid-phase–antibody boundary layer, reactants not initially located adjacent to the solid-phase–antibody must diffuse to the well from bulk solution, thus slowing the reaction rate. When liquid reactants are reintroduced into the receptors by repressurizing the reaction chamber, the reaction can proceed at nearly its maximal rate because the layer of depleted reagent has been replenished by remixing the solution. For example, a digoxin reaction carried out in solid-phase receptors that were in constant contact with the reaction solution for one cycle of 11 min (a duration equivalent to the fifteen 45-s incubations in a digoxin assay) showed only 56% of the binding exhibited in our usual protocol.

The kinetics of coating the antibody onto the plastic receptors shows an acceleration similar to that found for antigen–antibody reactions.

Assay Processor

The KinetiCount 48 assay processor and data-management system is flexible and allows the operator to monitor the reactions in process. The bar graph (Figure 4) that appears on the computer screen serves as an immediate quality-control check, the scale of the relative response on the y-axis being proportional to the average total counts for each occupied well in the array. A response scale less than 20 000 counts may indicate expired tracer or operator error. Empty wells are indicated by an asterisk below the well number on the display screen. Receptors that contain more radioactivity than the average of all occupied sites in the array are also noted on the screen. This condition may reflect endogenous radioactivity in the patient’s sample, perhaps from (e.g.) previous in-vivo nuclear medicine treatment (23). Such wells are not included in the data-reduction procedure but the apparent counts are shown.

The bar graph is updated with each cycle. A qualitative standard curve can be constructed from the bar-graph display within 7–8 min of the start of the assay. During this phase of the assay, only a brief count of the bound phase can be accommodated, so only qualitative information may be derived from the display. Nevertheless, the immediacy of feedback on replication, curve shape, empty wells, and relative binding of patients’ and control samples can be very useful. Because the operator can identify these situations within minutes of initiating the reaction rather than hours later, corrective action may be taken quickly.

At the completion of the last cycle, the pressure is released for the last time and a final count of the antibody-bound radioactivity is taken for each of the solid-phase receptors. These data are first subjected to a least-squares log-logit fit, which is displayed on the computer screen as a plot of counts (or $B/B_0$ or $B/T$) vs log standard dose. The system then starts a four-parameter logit (4 PL) fit of the same data, using the derived logit-log parameters as the starting points (24). When two successive iterations improve the squared residuals by less than 0.1% for the calculated backfit and actual standard curve points, the 4 PL fit is considered to have converged. The standard curve from this fit is superimposed on the log-logit fit, which is still on the
display screen. With most solid-phase assays the logit fit does an adequate job in the low- and medium-dose standards but often flattens prematurely in the high-dose region. The 4 PL fit consistently does a better job of fitting all of the standards to a standard curve.

When the 4 PL fit is complete, the system signals the operator, who may at this time edit the data for the standard curve. The graphics capabilities of the system allow the operator to alternately view a standard curve data plot and the numerical standard count values during this process. Edited data points are notated and omitted from data reduction, but are never lost and may easily be restored.

When the operator accepts the data plot, the system automatically stores the raw count data, the assay protocol information, and some curve-fit parameters on disc. Hard-copy print-out is also provided.

Besides the assays already mentioned, assays for free T4, cortisol, phenytoin, theophylline, beta-chlorogonadotropin, and thyrotropin are being developed. All require substantially shorter incubation times than comparable assays currently available commercially, and their performance is comparable with the results described here, as will be discussed in future communications.

References