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Evaluation and Comparison of Two Fully Automated Radioassay Systems with Distinctly Different Modes of Analysis

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Two fully automated radioimmunoassay systems with batch and sequential modes of analysis were used to assay serum thyroxine, triiodothyronine, and digoxin. The results obtained were compared with those obtained by manual methods. The batch system uses antibody coated tubes while the sequential system uses immobilized antibody chambers for the separation of bound from free ligands. In accuracy, both systems compared favorably with the established manual methods, but the sequential system showed better precision than the batch system. There was a statistically significant carryover of thyroxine in the sequential system when there were at least six-fold differences in the concentrations of thyroxine in adjacent samples, but the carryover was not significant in the batch system. Compared with the batch system, the sequential system has a shorter throughtime for individual samples (time from aspiration of the sample to the printout of results) but a longer interval for final overall printout of assay results (lower throughput).

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Manual radioligand assays are time-consuming and labor-intensive. They require a large number of pipetting steps and strict control of assay times and conditions. Therefore, the precision of manually performed radioassays sometimes is relatively poor compared with that of other clinical laboratory assays.

The need to reduce the labor-intensiveness of the technique, to increase the sample throughput, and to improve the precision has made the automation of radioligand assays the subject of extensive studies since 1966 (*1*). However, due to the complexity of these procedures, it was not until 1976 that a fully automated radioligand assay system became commercially available in the United States (*2*). Since then three more fully automated systems have become available. This paper describes our evaluation of performance characteristics of the two most widely used fully automated radioligand assay systems at present.

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MATERIALS AND METHODS

Batch system. This system* consists of a pipetting station, an incubator, an aspirate-and-wash station, a gamma counting station, and a programmable calculator. The system is capable of handling up to 200 tubes per run.

Upon activating the instrument, an aliquot of sample is aspirated from the sample tube to the appropriate antibody-coated tube. The racks of assay tubes move sequentially through the reagent addition and mixing station and then into the incubator loading zone where the tubes are pushed up into an incubation chamber in batches of ten. At the end of the allotted incubation time the tubes are lowered back into the racks and are advanced to the aspirate-and-wash station for repeated aspiration and washing. The racks then proceed to the counting station where the tubes are lifted out of the rack into dual detectors and are counted for various times. On-line data reduction is performed and sample concentrations are printed on a paper tape.

Sequential system. This system† uses a flow-through

system with a reusable antibody chamber containing antibodies covalently bonded to solid support media.

The operator dispenses the standard or sample into cups on a sample carousel, which can accommodate up to 120 cups arranged in two concentric rings of 60 positions each. The instrument then aspirates the standard or sample and mixes it with reagents aspirated by the instrument from reagent reservoirs. A measured volume of prepared sample is pumped through the antibody chamber. The unbound radioactive ligand passes from the antibody chamber to a flow cell in a radioactive detector and is counted. Once the free fraction has been counted, the antibody chamber is flushed with elution buffer, which releases the ligand previously bound to antibody sites. This returns the antibody chamber to its original state, ready for the next sample. The released ligand passes through the same detector system and its radioactivity is counted as the bound fraction. At the same time the system also rinses the sample aspirator.

Two different assay modes are used for assaying thyroxine (T_4), triiodothyronine (T_3), and digoxin. In the T_4 assay, the sample is diluted and discharged into the antibody chamber, followed by injection of radioactive T_4 into the antibody chamber for competitive binding. In the T_3 digoxin assays, the sample is aspirated from an inner ring sample cup on the carousel, and is mixed and incubated in the outer cup with the radioligand and soluble antibody aspirated from reagent reservoirs. After a specific incubation time, the instrument completes the assay by passing the incubation mixture through the antibody chamber. Here the antibody chamber acts as a separating agent by binding the free ligand and allowing the ligand bound to the soluble antibody to pass through the antibody chamber. The process is controlled by a microcomputer program in such a way that several samples are assayed in sequence and the incubation time for each sample is maintained precisely.

Manual methods. Solid-phase radioimmunoassays using antibody chemically immobilized to porous glass particles[‡] were used for manual determinations of T_4 and T_3. The manual digoxin assay[§] used a liquid-phase radioimmunoassay with charcoal separation.

Parameters evaluated. Sensitivity was assessed by the smallest amount of the ligand of interest that can be distinguished from zero, and by the slope of the standard curve plotted on logit-log paper. The smallest amount of detectable ligand was conservatively expressed as the dose necessary to produce $B/B_0 = 90\%$ (*3*). (B_0: percent binding in the absence of added nonradioactive ligand.)

Precision was assessed by determining the within-assay and between-assay coefficients of variation (C.V.), using three commercial control sera[¶] that contained low, medium, and high concentrations of each ligand.

Accuracy was evaluated by comparison of the values obtained by the automated systems with those obtained by the manual method routinely used in our laboratory and also by studying the recovery of added pure ligands.[‖] The method described by Caragher and Grannis (*4*) was used for the recovery study. A pooled serum sample with a low concentration of the ligand of interest was supplemented with a known quantity of pure ligand. Various mixtures of the low-concentration serum pool and the supplemented serum pool were prepared, and the ligand concentrations of each mixture were determined. The determined concentrations of a ligand in a set of such specimens were plotted against the known amount (i.e., percentage) of supplemented pool in the specimens. The apparent analytical recovery of added ligand was ex-

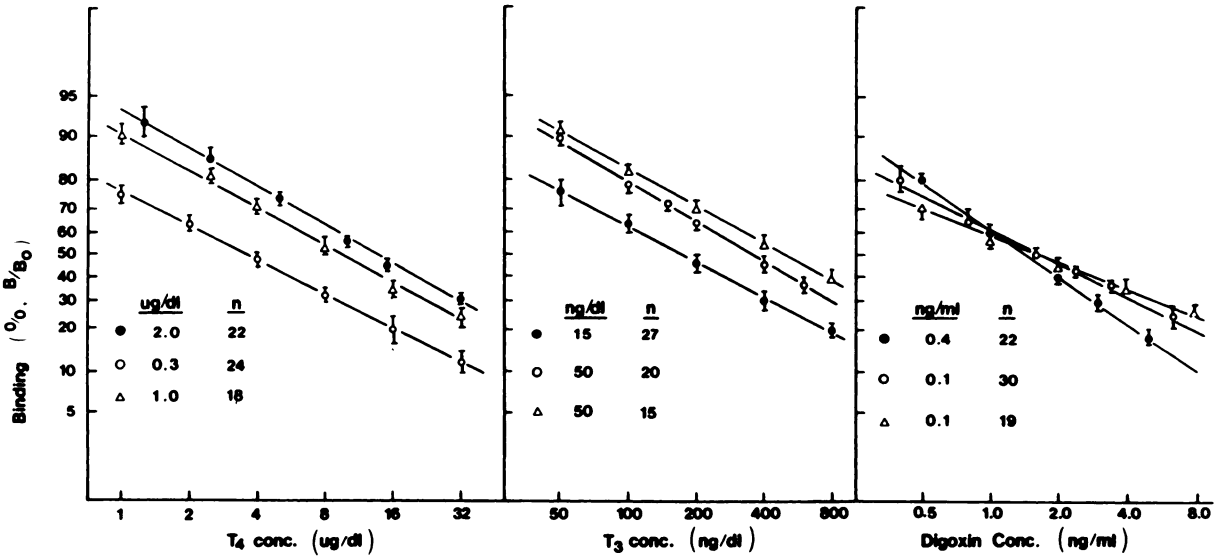

FIG. 1. Composite standard curves (logit-log plot). ● manual, O batch, Δ sequential. Concentrations given in figures indicate smallest detectable concentrations. n denotes number of runs for each composite standard curve. Vertical bars indicate 1 s.d.

TABLE 1. PRECISION EXPRESSED AS WITHIN-ASSAY (INTRA) AND BETWEEN-ASSAY (INTER) COEFFICIENTS OF VARIATION

	T_4			T_3			Digoxin		
	Mean values	CV (%)		Mean values	CV (%)		Mean values	CV (%)	
Methods	(μg/dl)	Intra	Inter	(ng/dl)	Intra	Inter	(ng/dl)	Intra	Inter
	2.5 (n = 19)	10.7	11.7	60 (n = 23)	19.0	15.2	0.7 (n = 29)	14.9	16.3
Batch automated system	7.8 (n = 19)	4.0	6.0	168 (n = 23)	8.8	7.7	1.5 (n = 29)	8.1	7.6
	15.7 (n = 19)	6.9	8.1	388 (n = 23)	5.4	6.0	2.5 (n = 28)	12.1	12.2
	3.1 (n = 12)	7.8	3.8	101 (n = 11)	8.0	5.9	1.0 (n = 19)	4.7	4.1
Automated sequential system	8.5 (n = 18)	4.9	3.6	219 (n = 15)	5.3	8.0	2.1 (n = 19)	3.6	5.2
	16.9 (n = 18)	5.6	3.9	451 (n = 15)	5.3	5.7	3.8 (n = 29)	4.5	5.7
	2.8 (n = 24)	9.3	10.1	62 (n = 28)	11.0	12.9	0.9 (n = 20)	9.6	8.6
Manual assay system	7.6 (n = 23)	5.2	6.0	154 (n = 28)	6.4	9.1	1.8 (n = 24)	8.7	8.5
	16.3 (n = 23)	3.3	3.4	399 (n = 28)	3.9	4.8	3.0 (n = 24)	5.3	5.8

pressed as a percentage calculated by multiplying the ratio of the determined slope to the expected slope by 100.

Total carryover was studied only with the T_4 assay. Alternating groups of three serum specimens with high and low T_4 concentrations were analyzed. The value obtained from the serum sample preceded by two serum samples of equal concentration was assumed to be the true value, and the value of samples preceded by specimens with different concentrations was assumed to be the contaminated value (*5*).

RESULTS

Figure 1 shows composite standard curves of each assay plotted on logit-log paper. The logit-log transformation gave linear standard curves for all assays. The batch system showed the highest sensitivity in terms of the smallest detectable amount for the T_4 and digoxin assays (0.3 μg/dl and 0.1 ng/ml), whereas the manual method showed the highest sensitivity in the T_3 assays (15 ng/dl). The slopes of the standard curves for the T_4 and T_3 assays were about the same for all three systems, but the manual method gave the steepest slope for the digoxin standard.

The sequential system gave the best precision, followed by the manual method and the batch method with overall mean intra- and interassay C.V.s of 5.5 and 5.1%, 7.0 and 7.7%, and 10.0 and 10.1%, respectively (Table 1). The precision of the batch system was especially poor at low ligand concentrations.

Table 2 shows the results of regression analyses of the values obtained by automated systems and by manual methods for each assay. Both automated systems showed good correlation with the manual method, with correlation coefficients ranging from 0.93 to 0.97 for the batch system and from 0.92 to 0.95 for the sequential system.

Results of the recovery study are shown in Fig. 2. The sequential system tended to give higher apparent analytical recovery, ranging from 103.2% for the digoxin assay to 108.5% for the T_4 assay, compared with 96.9% for the digoxin assay and 104.7% for the T_3 assay in the batch system. However, the batch system again showed poor precision in this study, indicated by the wider standard deviations for each concentration point and by the presence of several outliers especially in the T_4 assay. Figure 2 also shows the recovery (in percent) of ligand at each concentration.

Results of the carryover study are summarized in Table 3. There was statistically significant carryover in the sequential system when the sequence of samples was from the "high" to "low" concentrations. For example, the mean true T_4 value of 3.7 μg/dl was about 10% lower than the mean contaminated value of 4.1 μg/dl. When the sequence was from "low" to "high" concentration,

TABLE 2. ACCURACY DETERMINED BY REGRESSION ANALYSIS OF VALUES OBTAINED BY AUTOMATED METHODS AND BY MANUAL METHOD

Methods	Assays (units)	n	Correlation coefficient	Slope	Intercept
Batch automated system	T_4 (μg/dl)	144	0.9696	0.925	0.8
	T_3 (ng/dl)	143	0.9578	0.879	13
	Digoxin (ng/dl)	221	0.9296	1.244	0.1
Sequential automated system	T_4 (μg/dl)	150	0.9482	1.040	0.5
	T_3 (ng/dl)	171	0.9186	0.945	13
	Digoxin (ng/ml)	200	0.9247	1.007	0.2

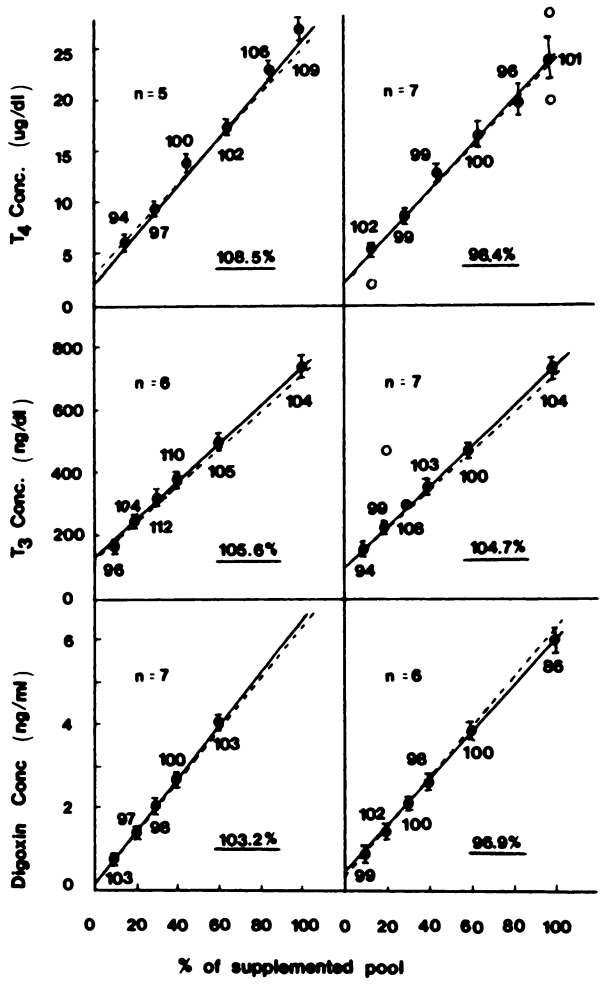

FIG. 2. Recovery of added ligands. - - - expected regression lines, — observed regression lines calculated from measured ligand concentrations. O indicates outliers that were not included in regression analysis. Numbers given at each point indicate percent recovery at each ligand concentration; underlined percentages represent apparent analytical recovery (see Materials and Methods section). Vertical bars indicate ± 1 s.d.

the mean true T_4 value of 20.8 μg/dl was about 10% higher than the contaminated value. The differences between the true values and the contaminated values were not significant in the batch system.

DISCUSSION

The fully automated radioligand assay systems described in this paper are two of four commercially available systems and currently are the most widely used automated systems in clinical laboratories in the United States.

The batch system uses antibody-coated tubes for separation of the bound from free ligands. The samples are processed in a batch of up to 200 tubes. The operation is very similar to the way a technologist performs manual assays on a bench top. The top of the instrument is visible to the operator and the following steps can be observed: the aspiration of sample into the antibody-coated tube, the addition of the radioligand, vortexing, incubation, the separation of bound and free ligands by repeated aspiration and washing of the antibody-coated tube, and the counting of the washed tube. Only the bound fraction is counted in this system.

The sequential system described in this paper uses a flow-through concept, with unique antibody chambers containing antibodies covalently bonded to a solid support medium. Because the antibody chambers have large contact surface areas, the competitive reaction involved in the radioligand assay will occur almost instantaneously when the assay mixture is passed through the chamber at a rate of about 1.1 ml/min in the T_4 assay. In the case of the T_3 and digoxin assays, the assay mixture is preincubated with the soluble antibody for up to 20 min, in order to increase the sensitivity of the assay before the separation of bound and free ligand by the antibody chamber. The bound and free fractions are both counted for each sample in this system. Therefore, the

TABLE 3. TOTAL CARRYOVER IN T_4 ASSAY BY AUTOMATED SYSTEMS

Methods	Sequences	n	T_4 values (mean ± 1 s.e.m., μg/dl) Contaminated	True	p (unpaired t)
Batch	high → low	14	2.6 ±0.1	2.4 ± 0.05	<0.1
	low → high	14	16.5 ± 0.3	15.6 ± 0.2	<0.1
Sequential	high → low	14	4.1 ± 0.03	3.7 ± 0.05	<0.001
	low → high	14	18.7 ± 0.2	20.8 ± 0.3	<0.001

total radioactivity used in each sample, which is an important parameter in the quality control of radioligand assays, can be evaluated.

In the estimation of sensitivity of an assay, the calculated amount of ligand corresponding to two standard deviations from the mean zero binding (B_0) is customarily used as the estimated sensitivity at zero (*3*). In the sequential system, the two standard deviations from the mean zero binding values were in fact less than 3% (C.V.s) in all three assays; that is, the smallest amounts of detectable ligands were the doses necessary to produce B/B_0 = 97%, instead of 90%, and were 0.5 μg/dl, 25 ng/dl, and 0.03 ng/ml, respectively, for T_4, T_3, and digoxin, instead of the 1.0 μg/dl, 50 ng/dl, and 0.1 ng/dl, respectively, shown in Fig. 1. The batch system does not determine the total count, and thus B_0 could not be estimated. Other parameters of sensitivity of the two automated systems are about the same except that the batch system has a higher sensitivity for the T_4 assay. The high sensitivity of the T_4 assay enabled us to adapt the batch automated system for assaying T_4 in 2 μl of serum samples collected in Natelson capillary tubes for neonatal T_4 screening.

The sequential system showed much higher precision than the batch system, with intra- and interassay C.V.s ranging from 3.6 to 8.0%, compared with 4.0 to 19.0% for the batch system and 3.3 to 12.9% for the manual method. The poor precision of the batch system is partly due to the nonuniformity of antibody-coated tubes. The binding of antibody to the assay tube surface is through physical adhesion. Therefore, the binding is relatively nonspecific and is somewhat difficult to control quantitatively. In contrast, the sequential system uses immobilized antibodies bonded covalently to glass beads or fibers, and thus the antibody chambers used in each assay can be more readily controlled quantitatively. In addition, the sequential system uses the same antibody for each sample since the antibody is regenerated in its free form at the end of each analysis. Therefore, qualitative and quantitative variations of antibody used in each sample should be minimal.

Values obtained by both automated systems correlated well with those obtained by the manual methods routinely used in our laboratory (Table 2). The batch system seemed to give slightly better correlation coefficients for all assays, but the proportional errors represented by the slopes of the regression lines (*6*) were higher in the batch system than in the sequential system, ranging from 7.5 to 24.4% for the former and 0.7 to 5.5% for the latter. The constant errors represented by the intercept of the regression lines (*6*) were relatively small for both systems. The constant errors of 13 ng/dl for the

TABLE 4. COMPARISON OF AUTOMATED RADIOLIGAND ASSAY SYSTEMS

	Sequential			Batch		
	T_4	T_3	Digoxin	T_4	T_3	Digoxin
Capacity (cycle* or tubes/run)	120	120	120	200	200	200
Sample volumes (μl)	200	100	100	50	150	150
Incubation time (min)	0	20	18	60	90	30
Throughtime (min)	2.3	22	20	65	95	35
Interval between result printout (min)	2.3	2	2	0.25	0.25	0.25
Assay times for 60 cycles or tubes (min)	138	140	138	80	110	50
Reagent cost/cycle or tube ($)	0.35	0.45	0.40	0.34–0.75	0.60–1.05	0.34–0.75
System cost ($)			68,000			39,000

* Cycle is a term used by the manufacturer to record by an on-line computer the amounts of reagents consumed by a customer. One cycle is equivalent to one tube in a manual radioligand assay. All assays are run in duplicate. See detailed description of each item in this table in Discussion section of text.

T_3 assay are negligibly small because the minimum detectable amounts of T_3 were 50 ng/dl for both automated systems.

Although both systems are a discrete type analyzer, the sequential system uses a flow-through mechanism in which samples are sequentially passed through common tubings, valves, an antibody chamber, and a flow cell for counting radioactivity, whereas the samples in the batch system are handled completely individually in an antibody-coated tube. Therefore, one might encounter a carryover problem in the sequential system even though it was claimed that the entire flow-through system was rinsed thoroughly between each sample. Results of the carryover study in the T_4 assay (Table 3) show that there was a statistically significant carryover of about 10% in the sequential system when the T_4 concentrations in one of two adjacent samples was six or more times the other. Although such a degree of carryover is expected in most automated clinical chemistry instruments, especially in continuous-flow systems, it may cause clinical problems when samples with borderline clinical values are preceded by samples with extremely high or low values. For instance, a sample with T_4 of 4.5 μg/dl could falsely be given a value of 5.0 μg/dl if it is preceded by a sample with T_4 at 27 μg/dl; and a sample with T_4 at 13.8 μg/dl could falsely be called 12.5 μg/dl if it is preceded by one with T_4 at 2.0 μg/dl. The normal range of serum T_4 is 4.5 to 12.5 μg/dl for both automated systems, based on T_4 levels in 257 healthy subjects and 144 patients with thyroidal disease studied in our laboratory. The chance of such sample sequences is small, but it is desirable that the computer of the automated system should have a built-in program to warn an operator of the presence of a greater than five- to six-fold spread between two adjacent values so that the operator would automatically repeat assays for such samples.

Some features of the two automated systems are summarized in Table 4. Capacity denotes the number of cycles or tubes (for standard, control, or patients' sera) that can be assayed in each run without operator intervention after the instrument has been activated. This information is useful for an overnight run. While the batch system has a higher assay capacity, the sequential system can tolerate the addition of more samples to a run that is still in progress. In the batch system, no tube can be added after the last tube of a run has passed through the sampling station.

Sample volumes listed in Table 4 are for a singlicate run. The sequential system requires a relatively large volume (200 μl) of serum for the T_4 assay. This is primarily because the instrument does the dilution and only a small fraction of the diluted sample is consumed in the actual assay. Therefore, if the dilution is done manually, it is possible to perform the T_4 assay with 20 μl of serum.

Throughtime is the time required from aspiration of the sample to the printout of results, and depends largely on the incubation time. The sequential system has shorter throughtime because it requires shorter incubation times, or none. However, the interval between initial throughtime and result printout is shorter in the batch system, mainly because the batch system uses a dual counter for counting two samples at a time, whereas the sequential system uses one and because the sequential system counts both bound and free fractions. It might be possible to shorten the time to printout in the sequential system by installing an additional detector to permit counting of the free and bound fractions at the same time.

Assay times shown in Table 4 indicate the time required to complete 60 cycles or tubes, including about 20 serum specimens plus standards and controls in duplicate. The assay time depends on the number of cycles or tubes assayed per run. The assay time for 60 cycles or tubes is considerably shorter in the batch system due to its shorter time to result printout, but this advantage becomes smaller with fewer cycles or tubes per run due to the longer throughtime and vice versa.

The reagent costs of the sequential system appear to be minimized by its reusable antibody chambers, which the manufacturer claims to be good for at least 3,000 cycles. The antibody is one of the most expensive ingredients in radioligand assay. The reagent costs of the sequential system also include all other consumables including sample and incubation cups and printout tape and are independent of the test volume. The reagent costs (range) for the batch system are dependent primarily on the test volume and do not include sample tubes and printout tape.

We have described the performance characteristics of two fully automated radioligand assay systems and the advantages and disadvantages were discussed. While the accuracy of both systems appears to compare favorably with the established manual methods, the sequential system showed better precision. The high sample throughput and the volume discount of the reagents make the batch system especially appealing to reference laboratories with large test volumes.

FOOTNOTES

* Micromedic Systems, Horsham, PA.

† ARIA II, Becton-Dickinson Automated Immunochemistry System, Salt Lake City, UT.

‡ Corning Medical Diagnostic, Medfield, MA.

§ Becton-Dickinson Immunodiagnostics, Orangeburg, NY.

¶ DADE Division, American Hospital Supply Corp., Miami, FL.

‖ Sigma Chemical Co., St. Louis, MO.

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