Performance of Solid-State 48 Detector Gamma Counter Using Solid Phase Total Thyroxin and Free Thyroxin Assays

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We evaluated the performance of an automated 48 detector KinetiCount gamma counter using solid phase reagents for total thyroxin (TT_4) and free thyroxin (FT_4). Interassay coefficients of variations (CV) for both total T_4 and free T_4 ranged from 5.4% to 13%. Between-method correlation coefficients were 0.9798 for TT_4 and 0.8844 for FT_4. Detection limits for TT_4 and FT_4 were 3 μg/l and 3 ng/l, respectively. TT_4 was linear up to 200 μg/l. The recovery of TT_4 was between 99 to 101%. A computer-stored standard curve for total T_4 was stable over a 6-day period. The 48 counting chambers demonstrated stability over a 45-day period with no significant changes in counting efficiency ($p > 0.5$). Precision between the 48 chambers gave a CV of 1.6%. Crosstalk between chambers was <0.3% with count rates up to 80,000 cpm. We conclude that the KinetiCount immunoassay system using solid phase separation technology performs well and is suitable for routine clinical use.

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Automation has been widely used in the routine clinical chemistry laboratory. However, automation for radioimmunoassay (RIA) has not been very popular. Completely automated RIA systems were first introduced in the mid 1970s, as manufacturers attempted to design a system which would reduce the labor involved, therefore decreasing cost. The heterogeneous nature of the RIA procedure necessitates a separation step to remove the free fraction from the bound complex. This limits throughput on most automated instruments to data requiring batch processing or continuous flow technology. Also, the traditional automated NaI gamma detector systems are expensive. These expensive systems can only be used with dedicated reagents available from a single manufacture, inhibiting flexibility.

An automated immunoassay processing system has been developed utilizing a kinetic solid phase technology. This system is composed of 48 proportional wire detectors instead of the traditional NaI crystals used in other multiwell counters. This type of detector is more stable and less expensive to manufacture than NaI detectors (*1*). The kinetic solid phase technology allows reactions to occur within their own gamma detector. This speeds up reaction times allowing an entire run to be performed in <1 hr. This computer operated system limits manual time to initial reagent pipettings. Data reduction, quality control graphs, and patient results are then performed by the data management system, freeing the technologist for other tasks. The detector system can also be used as a gamma counter in counting and reducing data of any iodine-125 RIA (^{125}I) procedure, giving flexibility to the user.

We report here an evaluation of this automated immunoassay processing system. Total T_4 and free T_4 assays were chosen to be used in this evaluation. The quality and performance characteristics of the reagents and the instrument were evaluated.

MATERIALS AND METHODS

All instrumentation and reagents were used in compliance with the manufacturer's instructions.

Instrumentation

The KinetiCount 48 gamma counter and immunoassay system was supplied commercially.* This system contained 48 proportional wire detectors. Each proportional wire detector was shielded from the others by an outer brass shell. Xenon

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TABLE 1
Total T_4 Assay Precision

	Interassay		
	Mean		
Sample	(μg/l)	CV%	n
Control 1	27	11.9	18
Control 2	79	5.4	21
Control 3	163	5.4	19

TABLE 3
Analytic Recovery of T_4

Total T_4 μg/l		
Expected	Measured	Recovery, %
51	50.5	99.0
76	76.5	100.7
126	127.5	101.2
176	175	99.4
226	229.9	100.4

gas was filled between this outer brass shell and an inner aluminum tube. Fine wires were located in this gas space and register the ionizing energy from gamma radiation of x-rays. The detector system was connected to a computer which operates the system as well as performs data reduction. The data management system prepared quality control reports and graphs. Reference ^{125}I calibrators were used to perform the stability and precision studies. A Hewlett-Packard HP-87XM microcomputer was used to drive the instrument and analyze the assay data. Comparative methods were counted on a Micro Medic 4-200 gamma counter and data reduced using a Tektronix 4052 computer.

Reagents

Total T_4 was measured by the Phase II Solid Phase Receptacle (SPRS) Method* and compared to the CLASP method.†

Free T_4 was measured directly by the Phase II Solid Phase Receptacle Method and compared with the micro encapsulated method.‡ The Phase II free T_4 method consists of two sequential incubations (2). In the first incubation, the free T_4 in serum or standard, was allowed to bind with the immobilized antibody on the SPRs. After washing with buffer, the remaining free antibody binding sites are measured in the second step by incubation with ^{125}I T_4. The quality control sera used in this evaluation was commercially supplied.§

Methods

Phase II reagents were evaluated on the KinetiCount 48 gamma counter by the following procedure. Standard, control serum, or patient sample was mixed with ^{125}I-labeled T_4 in a disposable reaction tray. The tray was loaded into the heated reaction tray drawer. The antibody-coated solid phase receptacle (SPR) was placed in the well and was sealed by closing the lid. Increased air pressure in the counting chamber pushed liquid from the heated reaction tray well up into the SPR where the reaction occurred. As air pressure was reduced, the liquid is lowered into the tray causing mixing to occur. This cycle was repeated until equilibrium was reached. The bound fraction was then counted in place and data reduction was peformed immediately.

Forty-eight reference ^{125}I calibrators were used to perform the stability and precision studies. Count rates determined by the manufacturer for each standard were supplied on computer disc and initially fed into the computer. Once a week, this set of calibrators were counted and detector counting efficiencies were calculated and stored by the computer. The raw counts for each well were then multiplied by it's computer stored counting efficiency to ensure that each well has the same count. Stability was determined by examining the counting efficiency of each well on Day 1 and comparing it to the counting efficiency on Day 45. A paired Student's t-test was calculated from the change in each chamber's efficiency as a measure of detector stability. Counter precision was evaluated by calculating the coefficient of variation between the raw counters, followed by the CV of the efficiency corrected counts.

TABLE 2
Free T_4 Assay Precision

	Interassay		
	Mean		
Sample	ng/L	CV%	n
Control 1	7.2	13.0	16
Control 2	16.2	9.2	25
Control 3	43.3	6.8	28

RESULTS AND DISCUSSION

Assay Performance

The interassay precision (CV) of the assay studied, determined by quality control samples, are shown in Tables 1 and

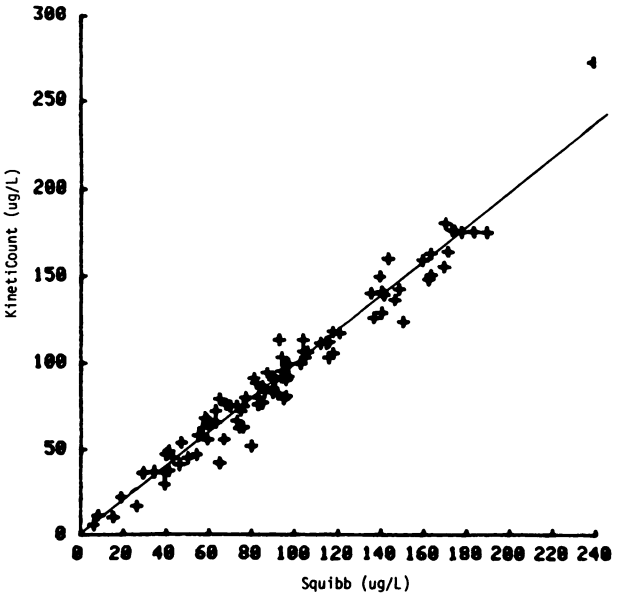

FIGURE 1
Correlation of Squibb CLASP and KinetiCount total T_4 methods. Slope = 0.9801; Intercept = 0.1774; Corr. coef. = 0.9798; No. pairs = 99.0

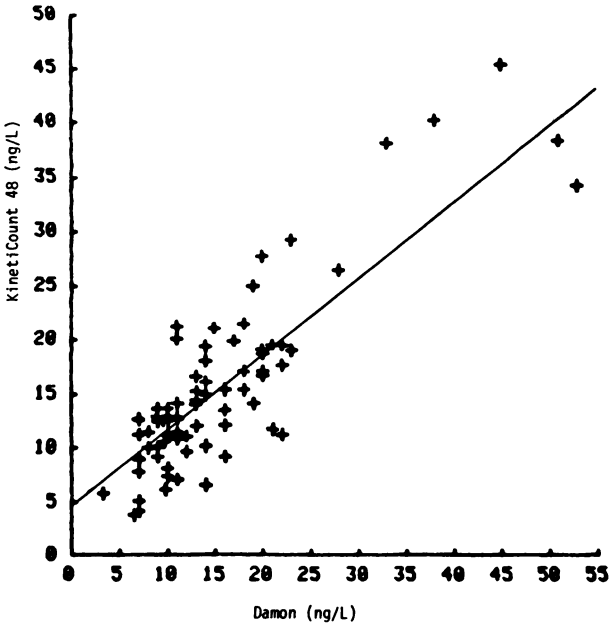

FIGURE 2
Correlation of Damon and KinetiCount free T_4 methods. Slope = 0.7010; Intercept = 4.7831; Corr. coef. = 0.8844; No. pairs = 88.0

2. For total T_4 and free T_4, precision (5% to 13%) was acceptable for routine use. The analytic recovery of the total T_4 assay is shown in Table 3. A known quantity of T_4 was added to a serum pool with a residual T_4 concentration of 26 μg/l. The recovery varied from 99 to 101%.

The correlation of the KinetiCount T_4 and Free T_4 assays with routine methodologies are shown in Figs. 1 and 2. Total T_4 correlated with an r value of 0.9798 and free T_4 with a r value of 0.8844. Sensitivity studies performed for each assay gave a minimum detectable dose for TT_4 of 3 μg/l and for free T_4 of 3.0 ng/l. The minimum detectable dose was calculated from the mean plus 2 s.d.s of the "zero" calibrator.

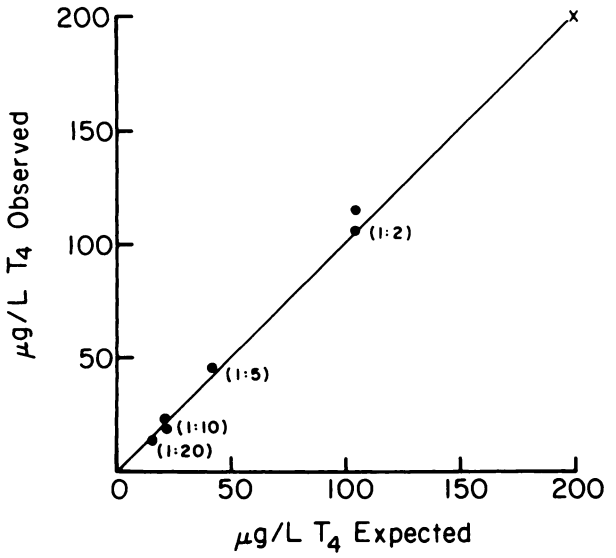

FIGURE 3
KinetiCount total T_4 linearity study

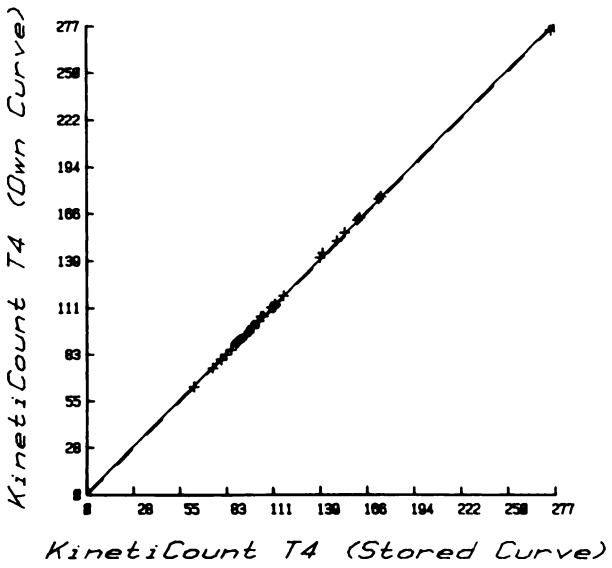

FIGURE 4
Validation study of computer stored curve for total T_4. No. of pairs = 34.0000; Slope = 0.9989; Intercept = 0.9019; Corr. coef. = 0.9998

A parallelism study performed by diluting an elevated T_4 serum sample with the zero standard demonstrated linearity between 0 and 200 μg/l as shown in Fig. 3. A computer-stored standard curve to calculate results was validated for the T_4 assay over a 6-day period. The T_4 results calculated from its own standard curve compared with results calculated from the stored standard curve after 6 days are essentially the same as shown in Fig. 4.

Gamma Counting System

Stability of the counting wells was evaluated by determining whether there was a significant change in the counting efficiency for each of 48 counting wells over a 45-day period of time. The 48 reference ^{125}I calibrators supplied by the manufacturer were counted on Day 1 and Day 45. Counting efficiencies of each well were calculated after corrections for decay were made by the instruments software. A paired t-test calculated from the change in each chamber's efficiency gave a $p > 0.5$, indicating no significant change.

Since it is important that each detector gives the same count for any individual sample, a precision study was performed to determine the variability among the 48 counting wells. The coefficient of variation of the raw counts for the instrument's 48 reference calibrators was calculated. After the software corrected each well for counting efficiency, the CV was again calculated. Raw counts gave a CV of 5.44%, while the corrected counts gave a CV of 1.6%, demonstrating good precision between the 48 wells after correction for counting efficiency.

Within the counter there is an array of 48 proportional wire detectors arranged in eight rows of six detectors. Each detector is shielded from the others by an outer brass shell.

TABLE 4
Crosstalk Evaluation of Counting Chambers*

	1	2	3	4	5	6	7	8
1	80238	87	82936	233	147	452	277	204
2	460	81025	369	222	113	124	136	176
3	81385	356	81426	148	152	127	317	264
4	148	82368	131	349	583	123	160	294
5	81742	131	84483	279	258	337	110	69
6	311	82072	177	360	155	126	72	272

*Radioactive samples were placed in the wells underlined.

The amount of photoelectric absorption of ^{125}I gamma rays from neighboring wells was evaluated and results are given in Table 4. Samples containing ~80,000 cpm were placed in every other chamber for the first three rows. An average count rate of the empty chambers in the last four rows was 210.3 cts/min. This was then subtracted from the backgrounds next to the radioactive samples. The difference was then divided by the average count rate in neighboring wells. Well 2, row 1 showed a 0.3% crosstalk; well 6, row 1 showed a 0.12%; well 3, row 2 showed 0.18%; and well 2, row 3 showed 0.19% crosstalk. These results indicated no significant crosstalk between counting chambers for count rates up to 80,000 counts per minute.

In summary, we have examined the analytic performance of the KinetiCount T_4 and FT_4 assays, and the counting stability, precision, and % crosstalk between wells of the KinetiCount gamma counter. These two assays were found to be acceptable for routine clinical use and gave good correlations with present methods. The use of a computer stored standard curve for total T_4 was stable for up to 6 days. The gamma counter demonstrated counting chamber stability over a period of 6 wk. Precision between the 48 wells was good and crossover of gamma rays from one well to the next was minimal.

FOOTNOTES

* Medical and Scientific Designs, Inc., Rockland, MA 02370.

† A. R. Squibb and Sons, Inc., Princeton, NJ 08540.

‡ Damon Diagnostics, Needham Heights, MA 02194.

§ Bio Rad Laboratories, ECS Division, Annaheim, CA 92806 (Lyphochek Immunoassay Tri Level Control Serum).

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REFERENCES

1. Parsons GH, Rogers CH, Polsky-Cynkin R, et al: Use of a solid state multihead gamma counter in a second generation system for solid phase immunoassay. *Clin Chem* 29:1620–1627, 1983
2. Bayer MF, McDougall IR: Radioimmunoassay of free thyroxine in serum: Comparison with clinical findings and results of conventional thyroid function tests. *Clin Chem* 26:1186–1192, 1980