Evaluation and utilization of a kinetic enzyme direct measuring photometer

PAUL L. WOLF

From the Division of Clinical Pathology, Department of Pathology, University of California, San Diego, La Jolla, California 92037, USA

SYNOPSIS We have recently utilized a prototype model of the Beckman Enzyme Activity Analyzer System-TR in our laboratory measuring various serum enzyme activities which include: alkaline phosphatase (ALP), E.C.3.1.3.1; creatine kinase (CK), E.C.2.7.3.2; hydroxybutyrate dehydrogenase (HBD), E.C.1.1.1.30; lactate dehydrogenase (LD), E.C.1.1.1.27; aspartate transaminase (AST), E.C.2.6.1.1; and alanine transaminase (ALT), E.C.2.6.1.2. Precision was found to be good. Sample activities could be measured as high as 1000 IU/l. The carryover studies fell within 2 SD of the means of the enzyme control studies. Coefficients of variation for ALP and CK were in the ranges of 0.40-2.14% and 0.52-4.30%, respectively. Correlation studies were done with GemSAEC and Gilford 300 N Spectrophotometer and the results were accurate, precise, and reproducible.

The work load of enzyme determinations in our laboratory has markedly increased in the past few years. Determination of enzyme activity is a most useful diagnostic tool, not only in heart disease but also in diseases of other organs, according to Wolf and Williams (1973). There is an increasing need for reliable, accurate, and precise automated instrumentation for enzyme analyses.

This report describes the capabilities of the Beckman System-TR. Performance studies were done on a prototype model. These studies include precision, linearity, recovery, and correlations with methods existing in the laboratory. To be able to use the Beckman System-TR for routine enzyme analysis we had to evaluate the capability of performance. The evaluation presented here emphasizes the comparison with the GemSAEC and includes studies of precision, range of linearity, and comparison of analytical values between the two instruments.

Materials

APPARATUS

The Beckman System-TR is an automated enzyme instrument designed to perform kinetic rate analyses. It is a double-beam instrument and can measure

absorbances at either 340 nm or 405 nm of wavelength. Reaction is monitored on a strip-chart recorder, and the results are printed in International Units per litre at 37°C.

The instrument was evaluated for the determination of ALP, CK, HBD, LD, AST, and ALT. The rate of analysis was 25 samples per hour for CK to 45 samples per hour for ALP and LD. The throughput for AST and ALT came to 40 samples per hour. The instrument was calibrated at 37°C.

For correlation studies, the same specimens were assayed for the different enzymes using manual methods on the Gilford 300-N Spectrophotometer with thermocuvette (Gilford Instrument Laboratory, Oberlin, Ohio) and the GemSAEC (Electro-Nucleonics, Inc, Fairfield, NJ).

REAGENTS, SAMPLES, AND CONTROLS

We evaluated new enzyme lyophilized controls with three levels of activity—low, medium, and high (Hyland Laboratories, Costa Mesa, Calif). All the reagents needed for an enzyme determination are contained in one bottle (20 determinations per bottle) to be reconstituted with deionized water, except for the ALP which has a specific buffer.

The normal ranges were established from healthy blood bank donors, and correlation studies were done on sera of hospital patients. Sera with visible haemolysis were rejected for the study.

Methods

The ALP activities were estimated, according to the method of Bowers and McComb (1966), utilizing the substrate p-nitrophenyl phosphate. The CK procedure was a composite of methods of Oliver (1955) and Rosalki (1967). The activity of serum HBD was determined as described by Rosalki and Wilkinson (1960). LD activities were assayed according to the methods of Gay, McComb, and Bowers (1968) and that of Amador, Dorfman, and Wacker (1963). Serum AST activity was assayed according to the method of Amador and Wacker (1962). The activity of ALT was determined according to the method of Henry, Chiamori, Golub, and Berkman (1960). The enzyme determinations performed in this study were done at $37 \pm 0.1^\circ\text{C}$.

Procedure

INSTRUMENT OPERATION

The operating instructions provided with the Beckman System-TR were closely followed. A strip-chart recorder was attached to the System-TR for continuous visual monitoring of the reactions.

Results were printed in IU/l at 37°C , preceded by the position number in the turntable (0-19) and the enzyme test being performed. Elevated enzymes were marked with flags to make proper dilutions of specimens.

The changeover from one enzyme test to another was accomplished by priming with air and with the new substrate needed for the next enzyme analysis.

Results

We analysed 10-12 aliquots of three Hyland Control Sera containing enzymes at various levels of activity to determine the analytical precision obtainable with the System-TR. The results from these analyses are listed in table I.

Accuracy and day-to-day precision were determined by analysing aliquots of the same enzyme controls with the three levels of activity. In addition, we also analysed the commercial controls currently used in the laboratory (Monitrol I, Monitrol II, and SMA Ref.). Results of these studies are shown in tables II and III.

All the substrates were stable when observed over a period of 48 hours as determined by assaying

Test ¹	No. of Days of Analysis	Av. No. of tests per Run	IU/l at 37°C				CV%	
			Mean ²		SD			
ALP								
12A	15	12	66-79 ³	72 ⁴	0.70-2.14 ³	5.46 ⁴	1.06-2.71 ³	7.58 ⁴
13A	10	12	129-147	141	0.88-2.09	7.51	0.62-1.45	5.32
14A	11	12	219-249	234	0.98-4.50	14.68	0.40-2.07	6.27
CK								
12A	13	10	145-173	160	2.22-4.70	15.70	1.46-4.43	9.81
13A	16	10	316-375	341	3.65-9.31	27.65	1.13-2.86	8.10
14A	11	10	771-929	866	4.46-21.0	43.35	0.52-2.51	5.00
HBD								
12A	15	10	214-235	228	1.98-6.47	11.61	0.97-2.83	5.09
13A	15	10	424-433	430	3.77-13.23	12.63	0.82-3.05	2.94
LD								
12A	14	10	121-131	126	1.22-2.30	5.46	1.01-1.85	4.33
13A	12	10	216-246	236	2.33-5.40	16.38	0.77-2.82	6.94
14A	11	10	390-434	417	5.21-40.18	43.35	0.37-9.98	10.40
AST								
12A	11	10	81-89	86	1.25-2.37	5.12	1.41-2.80	5.95
13A	13	10	166-179	171	1.09-3.15	8.53	0.61-2.49	4.99
14A	10	10	267-279	275	1.58-3.68	7.51	0.59-1.25	2.73
ALT								
12A	11	10	10-18	15	0.67-2.0	3.75	3.75-13.60	25.0
13A	11	10	55-67	59	0.84-2.89	6.14	1.29-4.80	10.41
14A	10	10	144-195	176	1.21-3.60	19.80	0.68-2.00	11.25

Table I Precision of results for three levels of activity of enzymes in lyophilized sera analysed with the Beckman System-TR

¹Enzyme controls with three levels of activity (low, medium, high) were supplied by Beckman Instruments, manufactured by Hyland Laboratories, Costa Mesa, Calif, USA

²Precision studies were done over a period of three months. Data of mean, SD and CV are from lowest to highest.

³Range of within run mean values, SD, and CV% over three-month period

⁴Absolute mean, SD, and CV% of all values derived during three-month period (day-to-day precision)

Enzyme	Low					Medium					High				
	$\bar{x}$		SD	CV	n	$\bar{x}$		SD	CV	n	$\bar{x}$		SD	CV	n
ALP	71	74	4.25	5.76	31	137	141	5.51	3.90	32	231	239	9.39	3.92	33
CK	151	152	5.81	3.81	15	315	326	10.34	3.17	15	847	845	34.33	4.06	15
HBD	220	229	8.84	3.86	30	418	437	19.25	4.40	30					
LD	120	122	4.87	3.99	31	241	237	7.30	3.08	31	437	424	19.34	4.55	31
AST	76	86	3.60	4.20	32	157	172	5.95	3.46	32	259	281	8.20	2.92	31
ALT	11	16	2.28	14.06	32	58	63	6.00	9.48	32	161	177	19.60	11.09	32

Table II Statistical analysis of lyophilized enzyme control sera determined on the Beckman System-TR

Figures in bold type are the manufacturer's assigned values.

Enzyme	Monitrol I unassayed XLT-307				Monitrol II unassayed XPT-513				Dade SMA Ref. DAS-25 AB			
	$\bar{x}$	<i>SD</i>	<i>CV</i>	<i>n</i>	$\bar{x}$	<i>SD</i>	<i>CV</i>	<i>n</i>	$\bar{x}$	<i>SD</i>	<i>CV</i>	<i>n</i>
ALP	52	5.62	10.88	30	321	14.95	4.65	30	135	14.64	10.81	30
CK	36	4.90	13.78	13	474	27.26	5.74	14	79	8.82	11.12	14
HBD	229	12.80	5.62	30	368	20.50	5.57	30				
LD	122	7.35	4.08	31	369	15.04	4.08	31	246	19.99	8.11	30
AST	28	2.40	8.55	28	106	5.61	5.28	29	93	5.36	5.74	28
ALT	32	4.20	13.13	32	136	12.92	9.53	32				

Table III Summarized results obtained with the Beckman System-TR on three commercial controls¹ during a three month period

¹(Monitrol I, Monitrol II, and Dade SMA Reference; from Division American Hospital Supply Corporation, Miami, Fla 33152)

Enzyme	No. of Dilutions	Maximum Value Observed	Slope	Intercept	<i>r</i>
ALT	21	1222	1.041	-6	0.999
SGOT	26	1483	1.081	-5	0.999
ALP	26	508	1.003	-0.2	0.999
LD	23	1524	0.986	0.70	0.996
CK	20	798	0.7888	3	0.970

Table IV Linearity studies on Beckman System-TR

Test ¹	No.	CV	IU/l					
			Mean	1SD	Min	Max	Range (2SD) 37°C	Range (2SD) ^a Converted to 30°C
ALP	67	28	70	19.5	31	123	34-108	24-86
CK	82	44.4	59	26.4	26	136	7-112	2-66
HBD	64	15.2	190	28.9	113	252	132-248	75-141
LD	75	15.3	120	18.4	76	168	83-157	49-93
AST	92	33.3	16.5	5.5	7	33	6-28	3-18
ALT	93	46.7	15	7.0	3	39	1-29	1-19

Table V Normal values established with the Beckman System-TR, determined at 37°C, and converted to 30°C

¹Data obtained from blood bank donors, male and female, aged 18-65, ostensibly healthy individuals

^a30°C values are derived from calibration curves for each chemistry constructed from 100 patient sera assayed at both 37° and 30°C

control sera with different levels of activity. Average Δ A/min observed for the substrates were from 0.002 OD/min for CK to 0.0013 OD/min for ALT calculated over a 20-minute time period.

Carry-over studies were performed using a

sequence of two water specimens, two controls with high level of enzyme activity, and two controls with low level of activity. Data accumulated were taken from five separate runs using 50 pairs of control sera and water specimens for each method. The

water blanks read 0 ± 3 IU/l and the control values fell within $\pm 2SD$ of the mean assay of the enzymes studied.

Linearity studies were performed utilizing patients' sera with high levels of enzyme activity. Over 20 dilutions were run for all enzymes assayed except HBD. Table IV presents these data.

Recovery studies were performed by analysing

a series of specimens in which a known amount of enzyme had been added. The amounts of enzymes added ranged from 36 IU/l of ALP to 358 IU/l of CK. The average recoveries of the duplicate runs ranged from 95.3 to 106.5%.

The normal values on the six enzyme chemistries were established using healthy blood bank donors. The normal values established with the System-TR

Fig 1 LD correlation studies utilizing the same patient's serum specimen correlating the TR v GemSAEC (iu/l) at 30°C.

Fig 3 AST correlation studies utilizing the same patient's serum specimen correlating the TR v GemSAEC at 30°C.

Fig 2 CK correlation studies utilizing the same patient's serum correlating the TR v GemSAEC at 30°C.

Fig. 4 ALT correlation studies utilizing the same patient's serum specimen correlating the TR v GemSAEC at 30°C.

Fig 5 ALP correlation studies utilizing the same patient's serum specimen correlating the TR v GemSAEC at 30°C.

agreed well with the normal values previously established in our laboratory. Results are shown in table V.

Correlation studies were performed on the same specimens for the different enzymes using manual methods on the Gilford 300-N Spectrophotometer with thermocuvette and a completely automated instrument, the GemSAEC. Results are shown in figures 1 to 5.

Discussion

The throughput of the System-TR is very good for fast enzymes such as ALP and LDH. In addition, the instrument is a versatile one, since the system has a fast and easy test change over. It utilized a one-step pipetting of substrate and specimen, and thus accurate, reproducible, and reliable results are quickly obtained from sample pickup to printout of results.

The results of the precision studies compared well with the GemSAEC. There is no substantial carry-over from samples of high enzyme activities, since there is a rinse cycle between specimen pickup.

The dynamic range and linearity studies demonstrated that dilutions are not necessary up to 1000 IU/l, many times above the normal range. Recoveries are excellent, as evidenced from the data, which would eliminate the possibility of false negative values for enzymes.

Correlation studies with the GemSAEC were very good. Since the results agreed closely between the two instruments, ALP, CK, HBD, LD, AST, and ALT may be measured by either or both instruments in the course of patient treatment.

Our early evaluation of the Beckman-TR found that the loading turntable was easily knocked off with loss of specimen, and a solid base for the turntable was needed. A cover for the turntable was needed to prevent specimen evaporation. The substrate syringe was easily removed from its post. A slight problem with synchronization was also present. All of the deficiencies were corrected after we had made our recommendation.

References

- Amador, E., Dorfman, L. E., and Wacker, W. E. C. (1963). Serum lactate dehydrogenase activity: an analytical assessment of current assays. *Clin. Chem.*, **9**, 391-399.
- Amador, E. and Wacker, W. E. C. (1962). Serum glutamic-oxaloacetic transaminase activity: a new modification and an analytical assessment of current assay techniques. *Clin. Chem.*, **8**, 343-350.
- Bowers, G. N. Jr. and McComb, R. B. (1966). A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. *Clin. Chem.*, **12**, 70-89.
- Gay, R. J., McComb, R. B., and Bowers, G. N. Jr. (1968). Optimum reaction conditions for human lactate dehydrogenase isoenzymes as they affect total lactate dehydrogenase activity. *Clin. Chem.*, **14**, 740-753.
- Henry, R. J., Chiamori, N., Golub, O. J., and Berkman, S. (1960). Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and lactic acid dehydrogenase. *Amer. J. clin. Path.*, **34**, 381-398.
- Oliver, I. T. (1955). A spectrophotometric method for the determination of creatine phosphokinase and myokinase. *Biochem. J.*, **61**, 116-122.
- Rosalki, S. B. (1967). An improved procedure for serum creatine phosphokinase determination. *J. Lab. clin. Med.*, **69**, 696-705.
- Rosalki, S. B., and Wilkinson, J. H. (1960). Reduction of alpha-ketobutyrate by human serum. *Nature (Lond.)*, **188**, 1110-1111.
- Wolf, P. L. and Williams, D. (1973). *Practical Clinical Enzymology and Biochemical Profiling*, pp. 1-2. Wiley-Interscience, New York.