A System for Computer Analysis of Kinetic Enzyme Data from a Modified Beckman DSA-560

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We define the following criteria for enzyme assays as being beyond compromise: narrow bandpass double-beam spectrophotometer, multiple readings on the sample (kinetic procedure), temperature control of assay reaction mixture to ±0.1°C, wash-out between samples less than 1% of the preceding sample, and sample size of 100 μl or less. In addition, the following specifications for a system were considered desirable: automatic handling of the sample from initial pipetting through readout, adequate computer compatibility, versatility so that new procedures and different methods could be used, relatively short set-up time, and hard-copy backup in the event the computer failed. We have developed a system for enzyme assays that meets all of the above criteria. It consists of an analyzer (Beckman DSA-560) coupled to two modified Eskalab Spectrophotometers Alpha. The system is controlled via a special-purpose hybrid computer designed and built in our laboratory. Data from the system is analyzed on-line by an IBM 1800 computer. Details of the operation and reliability of the system in a routine clinical chemistry laboratory are discussed.

Additional Keyphrases Eskalab colorimeters • GOT and GPT assay • IBM 1800 computer

In this paper, we describe a system designed to produce accurate enzyme assays in a high-volume clinical laboratory that already has most of its assays on-line to an IBM 1800 computer.

System Design

Criteria. In defining the problem of enzyme assays, we selected those criteria listed in the abstract, above.

Instrumentation. We selected the Discrete Sample Analyzer (DSA-560; Beckman Instruments, Fullerton, Calif. 92634) because it seemed most nearly to meet these criteria and specifications. We discovered that the DSA colorimeter has a half-width bandpass of 30 nm at a wavelength of 340 nm, a violation of one of the criteria. Consequently, we investigated the use of a Beckman DB-GT, which has an adjustable bandpass and an Eskalab Spectrophotometer Alpha (Smith Kline Instruments, Inc., Palo Alto, Calif. 94306) which has a 4-nm bandpass. Computer analysis of the signal from these instruments showed that each had about the same amount of “fast noise” (high-frequency voltage fluctuation); however, the amount of “slow noise” (low-frequency voltage fluctuation) was significantly greater in the Beckman DB-GT than in the Eskalab Alpha (1). The slow noise had a timing and magnitude such that the variance observed on replicated samples was about 10 times greater with the DB-GT than with the Alpha. Accordingly, we decided to install two Eskalab Spectrophotometers on the DSA-560 (1).

The Eskalab spectrophotometers have unique sample compartments but are readily converted to standard 1-cm square cuvets by inserting the square-cell adapter supplied with the instrument. A second square-cell adapter can be inserted on the reference side. We have found that Eskalab flowcells with the 1-cm light-path can be filled by the DSA colorimeter pump and that they have acceptable wash-out characteristics. Temperature is controlled in the flowcell by pumping water from a closely-controlled thermostirator through the water jacket that is an integral part of the cell.

The Alpha displays only 0.5 absorbance (A) units at full scale, and a manual selector is used to choose the proper range from zero to 1.5 A. If the system is to function unattended, a method is needed for automatically selecting the proper range. In addition, an autoranging amplifier is required so that the signal from the spectrophotometer can be displayed on a chart recorder at an expansion sufficient for enzyme assays. We found that 0.1 A full scale is adequate expansion. Finally, we noted that when the flow cells supplied by Eskalab were used in the reference beam and the sample beam, erratic signals were recorded for darkly colored sera.

We solved these problems as follows. There are three absorbance ranges on the Eskalab Alpha: 0-0.5 A, 0.5-1.0 A and 1.0-1.5 A. Operating the standard range selector inserts a neutral density screen in the reference light path and operates an electrical switch. To permit range changing under remote control by the IBM 1800 computer, we modified the Eskalab spectrophotometers by re-

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1 See also the Acknowledgments section.
placing the manual range-change actuator with a ratchet that can be operated by either of two pneumatic cylinders, one to change the range upward, the second to change it downward. The electrical switch gives a digital signal to the IBM 1800 to indicate which range is in use.

The need for an autoranger was met by building a small hybrid computer. It expands the signal from both spectrophotometers and automatically adjusts it by means of motor-driven potentiometers so that, regardless of the absorbance of the sample, $0.1$A represents a full-scale deflection on the chart recorders and a $5$-V deflection for the IBM 1800 computer. The hybrid computer also provides the unexpanded signal and "interrupts" to the main computer.

The occasional erratic signals from the spectrophotometer were traced to the fact that darkly colored samples were so dense that the high-voltage power supply for the photomultiplier tube was unable to compensate for them. To solve this problem, we replaced the slit in the spectrophotometer with a round aperture having about three times the area of the slit. Thus, more light reaches the detector and the requirements on the high-voltage power supply are eased. This modification does not affect the bandpass of the instrument at 340 nm, where the enzyme assays of current interest are read, because a separate interference filter is used at this wavelength. However, the bandpass at other wavelengths will be affected.

Methodology. We chose to measure L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2. (GPT) and L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1. (GOT) activities in serum because they seemed to present the greatest challenge analytically. The reagents are from commercial reagent kits (Boehringer Mannheim Corp., New York, N.Y. 10017; GPT optimized and GOT optimized). The kit is reconstituted into a single solution, taking into account the dilution by the wash water added during sample pipetting. Sixty assays are performed per hour. Figure 1 shows the set-up diagram for the DSA. A preincubation period of 10 min is allowed to eliminate any lag-phase reactions (2). The reagent pipettor-diluters were modified as recommended by the manufacturer so that the reagent is pumped through the syringe with no water washout. This modification was made after repeated problems were experienced with the check valves. The spectrophotometer flowcells are filled by the DSA colorimeter pump with the usual air-segmented stream.

The assay is performed by measuring the rate of change of absorbance with time—the greater the rate, the greater the enzyme activity. However, a sample with very high enzyme activity shows very slow absorbance changes because the substrate has been consumed during the preincubation period. In order to automatically differentiate between a sample with very high activity and one with low activity, it is necessary to define conditions of substrate exhaustion in a way that can be recognized by the computer. Substrate exhaustion can be defined in terms of the absorbance seen when the sample is first in the flowcell; however, this absorbance varies with the density of the sample under assay. Substrate exhaustion can be accurately determined only if a serum blank is used in the reference flowcell to correct for the density of the sample.

Data acquisition and handling. With each sample advance, an interrupt is given to the IBM 1800 to signal the start of a new assay (Figure 2). Simultaneously, the hybrid computer grounds the recorder outputs to eliminate signals generated by the passage of air segments through the flow cells. After the flow cells have filled, the IBM 1800 monitors the unexpanded output and actuates the pneumatic cylinders to select the proper absorbance range. When this has been done, the hybrid computer adjusts the signals and sends the expanded signal to the recorder and the IBM 1800. Subsequently, the IBM 1800 reads and stores the expanded analog signals at 1-s intervals. This sequence of events occurs at standard timed intervals after the sample-advance interrupt (Figure 2).

The enzyme activity in each sample is calculated from the last 30 data points. The slope obtained from a least-squares linear regression analysis on the 30 data points is multiplied by a factor to correct for sample size, total reaction volume, and the molar absorptivity of the substrate consumed (or produced). Enzyme activity is expressed in International Units per liter. An analysis of variance performed on each regression analysis yields a measure of the spread or scatter of the data points around the regression line. The calculated results, the scatter, and messages concerning the status of controls are printed on a typewriter in the laboratory. Experience with the system has shown that the computer can call attention to substrate exhaustion and noisy tracings. Thus, while the run is in progress, the results are printed as they are calculated and error conditions are noted automatically. For off-line operation, the range is changed manually and the results calculated with the slope obtained from the chart recorder tracings.

Results and Discussion

Our system has been in routine operation with one channel for nine months and with two channels for five months. Two methodologic problems have been resolved easily. The first problem, that of plugging of the sample-pump probe by clots, is avoided by carefully removing all fibrin before loading the sample on the tray. The second prob-
lem, that of inadvertent use of insufficient sample volume, is avoided by extending the Teflon sample probe so that it reaches the bottom of the sample cup. The sample cups are filled with sample and inserted into the sampler tray to the same depth each time; thus, unless the analyst sees an empty cup, he can safely assume that enough sample was taken for analysis.

Three quality-control pools of frozen serum are assayed with each group of determinations. The results of these assays over the past three months are summarized in Table 1. With the sample volume and reagent volume described in Figure 1, 0.001 A/min equates with 2.5 U/liter. Because the sample is read for 30 s, a serum with an activity of 15 U/liter has an observed absorbance change of 0.003 A. The reproducibility of this small an absorbance change is reflected in the rather small standard deviation of 2.6 U/liter found for GOT and GPT in quality-control pool 1 (see Table 1). This datum includes the variability contributed by different operators, different lots of reagents, and different days.

In summary, the system has been found to meet all of the criteria and specifications detailed earlier. In addition, the system has proven to require little servicing besides routine preventive maintenance. Finally, the system is capable of performing accurate enzyme assays with a high degree of reproducibility in an automated clinical laboratory with a production rate of two results per minute.

The medical technologists in chemistry rotate through a research assignment as well as routine assignments. They contributed materially to the work described in this paper.

### Table 1. Enzyme Activity Measurements for Three Quality-Control Pools of Serum during Three-Months

<table>
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<th>Pool no.</th>
<th>GOTa</th>
<th>GPTa</th>
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<tbody>
<tr>
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<td></td>
<td>x 14</td>
<td>17</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td>x 112</td>
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<td></td>
<td>SD 6.3</td>
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</table>

*a Results in U/liter.

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**Fig. 1.** Set-up diagram for the simultaneous determination of GOT and GPT on modified DSA-560

**Fig. 2.** Timing of events during a single enzyme analysis

Note the relationship, in time, of the air segments passing through the flow cell, the range-change impulse, the display of the expanded signal and finally, the next sample-advance interrupt.

### References
