Adaptation of the EMIT Serum Digoxin Assay to a Mini-Disc Centrifugal Analyzer

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We have modified the EMIT (Syva) serum digoxin assay for use with a mini-disc centrifugal analyzer. A minicomputer is used to control data acquisition, calculations, and readout for 14 cuvettes simultaneously. Thus, it is not necessary to time each individual assay manually or to plot calibration curves on graph paper. We use incubation times and dilutions similar to those used for the manual procedure, but we have changed the buffer solution, decreased the measurement time, and decreased the required serum volume per assay fivefold. We evaluated day-to-day and within-run precision, using Syva calibrators and control sera. EMIT assay values for radioimmunoassay control sera correlate well with the supplier’s stated values.

An enzyme immunoassay technique ("EMIT") for serum digoxin has been recently introduced by the Syva Corp., Palo Alto, Calif. 94304 (1). The assay is similar in principle to the Syva anticonvulsant drug assays, but the procedural steps are significantly different. As in radioimmunoassays for digoxin (2, 3), an antibody that is specific for digoxin is used in the EMIT assay. Unlike radioimmunoassay, an enzyme label is used instead of a radioactive label, and there is no need for radiocounting equipment. Both radioimmunoassay and EMIT are sufficiently sensitive, specific, and accurate to detect digoxin in microgram per liter concentrations (4) without prior extraction of the serum.

An enzyme, glucose-6-phosphate dehydrogenase (EC 1.1.1.49, from Leuconostoc mesenteroides), which is chemically coupled to digoxin, is inactivated when bound to digoxin antibody. When serum containing digoxin is introduced, the competition for antibody binding sites between enzyme-labeled and unlabeled digoxin results in the release of labeled digoxin and an increased enzyme activity, which is monitored spectrophotometrically at 340 nm as NADH is reduced to NAD+. Sera must be deproteinized before analysis, and a serum pretreatment reagent is provided for this purpose.

The manual procedure requires two absorbance measurements per test, at 30-min intervals. Throughput is increased by aspirating samples at 30-s intervals. This sequence is repeated 30 min later and final readings are taken. We have modified the manual procedure substantially by analyzing multiple samples simultaneously rather than sequentially. Other important modifications are described that were needed to ensure compatibility with our centrifugal analyzer system.

Materials and Methods

Reagent A (antibody/substrate), and Reagent B (enzyme-labeled digoxin), and serum calibrators were reconstituted according to Syva's directions. Working reagents are prepared at the beginning of each working day, with use of an automatic pipette (Model 2500; Micromedic Systems, Philadelphia, Pa. 19105). The remaining pipetting operations (Figure 1) are performed with Pipetman P-20 and P-200 pipettes (Cole Scientific, Canoga Park, Calif. 91303). The serum pretreatment reagent (0.5 mol/liter NaOH) and a set of serum calibrators were provided with the assay kit. Under normal refrigeration and proper use, reagents and calibrators are stable for the labeled potency periods. Reconstituted radioimmunoassay controls (Nuclear Medical Systems, Newport Beach, Calif. 92663) and patients' specimens should be stored frozen.

The instrumental system has been described previously (5). A computer program controls start/stop of the sample disc, data acquisition, and data readout. Temperature is controlled at 37 ± 0.1 °C. Solutions, as shown in Figure 1, are mixed by rapid acceleration of the sample disc. Following a 30-s mixing period, 20 absorbance readings are taken per cuvette during 1 min. Then a 20-min delay is entered, after which 20 additional readings are taken during 1 min. Reaction rates are calculated from the average initial and final absorbance readings. Serum digoxin concentrations are then calculated automatically by use of concurrently run Syva calibrators. Up to four patients' specimens may be analyzed, in duplicate, per disc. The manual and modified procedures are compared in Table 1.

Results

Our initial precision and accuracy studies, reported here, were limited to the analysis of Syva calibrators and radioimmunoassay control sera and indicate that our method is satisfactory for use with the EMIT digoxin assay.

Day-to-day precision. Aliquots of a "Level II" radioimmunoassay control serum were analyzed in duplicate once every two weeks for a period of three months. Results are shown in Table 2. For "Level I" radioimmunoassay sera we obtained a mean of 0.62 μg/liter (CV 8.7%). Labeled value was 0.4-0.8 μg/liter (ng/ml).
Within-run precision. For this study 10 replicates of a Level II radioimmunoassay control serum were analyzed per disk. The CV (n = 10) was 4.4%. The mean digoxin concentration found was 1.8 μg/liter, as compared to the labeled value of 1.7–2.7 μg/liter (ng/ml).

Discussion

The EMIIT digoxin assay requires special modifications if a centrifugal analyzer is to be used. Rapid acceleration of the sample disc produces foam in the cuvettes, because of the surfactant in the EMIIT buffer concentrate. We have eliminated this problem by replacing the EMIIT buffer with a surfactant-free 55 mmol/liter Tris-HCl buffer, pH 7.4. The analytical results have been more reliable after this change.

Second, the parallel nature of the centrifugal analyzer allows multiple tests to be performed simultaneously, a significant advantage for reactions with long measurement times. The reliability of the results is enhanced by analyzing serum calibrators and patient specimens on the same disc. Most of the tedious timing steps required for the manual assay have been eliminated, because the analysis is timed by the computer, rather than with a stopwatch.

Third, we have modified the manner in which absorbance readings are obtained. Forty absorbance readings are taken per test, compared to two for the manual procedure. The measurement of the reaction rate is very precise (CV 1%), which reflects both photometric and pipetting precision. Converted to concentrations, the precision is about 5%. The loss in precision in terms of concentration is due to the high serum blank, which we have found to be nearly 90% of the calibrator No. 1 reading. However, no improvement in precision of concentration values is observed by subtracting the serum blank from subsequent readings. Thus, we do not analyze the zero calibrator (serum blank), which results in an additional saving in reagent cost. We do not use the EMIIT logit-log format for plotting calibration curves. Instead, log reaction rate is plotted automatically vs. log concentration, with direct printout of results in micrograms per liter.

Other modifications of the EMIIT digoxin assay have been reported elsewhere (6, 7). We have not found it necessary to employ some of the modifications described by Rosenthal et al. (6), such as filtering and de-gassing the reagents. Turbidity does not interfere with our kinetic procedure, and dissolved gases are removed by the centrifugal force. Incubation temperature is not critical, because samples and calibrators are always incubated and analyzed concurrently. We have obtained satisfactory results when using the preincubation times prescribed by Syva. All reagents and sera should be at room temperature and should be well mixed before use.

We believe the EMIIT digoxin assay is a reliable alternative to radioimmunoassay. With appropriate modifications, good results may be obtained conveniently by using a mini-disc centrifugal analyzer.

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<th>Table 1. Comparison of Modified and Manual EMIIT Digoxin Assay</th>
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References

PLASMA LACTATE DEHYDROGENASE ACTIVITY WILL BE INCREASED IF DETERTENT AND PLATELETS ARE PRESENT

Renzé Bais, Michael P. Prior, and John B. Edwards

Plasma lactate dehydrogenase activity is artfactually increased when analyzed on the SMAC (Technicon) continuous-flow analyzer. The factors responsible are incomplete centrifugation and the presence of detergent in the dilution buffer. The contribution of platelet lactate dehydrogenase in the plasma is demonstrated with a case of thrombocytosis.

Additional Keyphrases: continuous-flow analysis · analytical error · serum/plasma differences

In several reports plasma and serum are compared with regard to lactate dehydrogenase (LD, EC 1.1.1.27) activity. Some investigators find no significant differences (1, 2), others find a higher activity in serum (3–6), others a lower activity in serum (7). Recently, Rothwell et al. (8) re-examined LD activity in serum and plasma and suggested that differences they found in activities were attributable to the presence of platelets in the plasma. They concluded that when plasma is to be used, it must be centrifuged (3000 X g, 15 min) to provide platelet-free plasma.

This report extends that particular investigation and describes some of the conditions under which the platelet LD activity appears in plasma. We were particularly interested in plasma obtained from heparinized blood because this is the only anticoagulant suitable for use with a multi-channel analyzer in which calcium is also being measured.

Materials and Methods

Blood samples were collected and either allowed to clot or added to lithium heparin (12.5 int. units per milliliter). Platelet-rich plasma was prepared by centrifuging at 1000 X g for 10 min, platelet-poor plasma by centrifuging at 3000 x g for 10 min. LD was either assayed on a Beckman Enzyme Activity Analyzer System TR by the method of Wacker et al. (9) or on a Technicon SMAC based on the method of Morganstern et al. (10). Platelet counts were estimated with a Coulter Counter Model F. All enzyme assays were performed at 37 °C, and activity is expressed in IUB units (U/litre).

Results and Discussion

Table 1 shows the differences we observed when the LD activity of heparinized plasma samples was determined with the two instruments. The results shown were selected from one day's routine workload, about 400 biochemical profiles a day. At the time these results were observed, samples were being prepared by centrifuging at 1800 X g for 5 min. There was no correlation between the two activities, and some of the SMAC results were up to three-fold greater than those obtained on the System TR. Statistical analysis of the results by using a paired t-test shows that two sets of results are significantly different at the 0.5% level. However, when serum was used there was no significant difference between results from the two instruments. Because of these discrepancies, we further investigated the problem.

The effect of some different centrifugation conditions on the activity of LD in heparinized plasma is shown in Table 2, the plasma LD activity being determined with both the SMAC and the System TR. The activity with the System TR remains constant whereas in the case of the SMAC the activity was inversely proportional to centrifugation speed. Similar data could be obtained by using platelet-rich plasma that had been diluted with platelet-poor plasma and determining the LD activity on both the SMAC and System TR. In this case the SMAC assays showed a linear relationship between the number of platelets and the activity whereas on the System TR, the