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A Library Of Serum Drug Assay Error Patterns, And Some Suggestions For Improved Modeling And Simulation Of Pharmacokinetic Behavior

by

Roger W. Jelliffe, M.D. and Babak Tahani

Laboratory of Applied Pharmacokinetics
University of Southern California School of Medicine
2250 Alcazar Stree, CSC 134-B
Los Angeles, CA 90033

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A LIBRARY OF SERUM DRUG ASSAY ERROR PATTERNS, AND SOME SUGGESTIONS FOR IMPROVED MODELING AND SIMULATION OF PHARMACOKINETIC BEHAVIOR.

ROGER W. JELLIFFE, M.D., AND BABAK TAHANI

LABORATORY OF APPLIED PHARMACOKINETICS, UNIVERSITY OF SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, 2250 ALCAZAR STREET, LOS ANGELES CALIFORNIA 90033.

INTRODUCTION

The use of population pharmacokinetic models and of Bayesian methods to make individualized models to describe and simulate drug behavior as precisely as possible in populations and in individual patients has led to improved prediction (and therefore control) of future serum drug concentrations. The Bayesian individualized models utilize a combination of the population pharmacokinetic parameter values and their standard deviations on the one hand, and data of the patient's own measured serum concentrations on the other. The Bayesian posterior parameter values are found by minimizing the Bayesian objective function

$$\left[\mathrm{SUM}\ \frac{(\mathrm{Ppop} - \mathrm{Ppt})^2}{\mathrm{SD}^2\ \mathrm{Ppop}} + \mathrm{SUM}\ \frac{(\mathrm{Cobs} - \mathrm{Cpt})^2}{\mathrm{SD}^2\ \mathrm{Cobs}}\right]$$

where Ppop and Ppt represent the parameter values of the population pharmacokinetic model and of the patient's individualized model respectively, Cobs and Cpt represent the observed (measured) serum drug concentrations and the estimates of those concentrations made with the patient's own individualized pharmacokinetic model respectively, and SD Ppop and SD Cobs are the standard deviations of the various population parameter values and of the various observed serum concentrations respectively.

In this objective function, the credibility of each population pharmacokinetic parameter value is determined by the reciprocal of the variance (the square of the SD) which it has been found to have. Thus the SD of each population parameter value, when squared and its reciprocal is then taken, provides the correct index of credibility (Fisher information) for each population parameter value.

The same is true for the data of the measured serum drug concentrations. However, the usual practice of most clinical laboratories is simply to make sure that the SD's of each assay are within some acceptable limits for that laboratory. Once this is done, the actual error is usually ignored, and is not reported (or even accurately determined) along with the serum concentration itself. The result of this is that the SD's with which serum drug concentrations are measured are usually not precisely considered as a practical matter in the routine fitting of serum drug concentration data.

Practical Determination of Serum Assay Error Patterns

What is needed is a practical means to determine the standard deviation of each serum drug concentration as it is routinely measured by the clinical laboratory. A convenient

and practical way to achieve this goal of computing the probable SD with which a single determination of a serum drug concentration is measured is to do replicate measurements of some representative samples of such concentrations (at least in quadruplicate) and to determine the mean and SD of each sample.

This can be done, for example, on a blank sample, a low sample, an intermediate one, a high one, and a very high one, so that the entire assay range, subtherapeutic, therapeutic, and toxic, is covered. One can then fit this data of the relationship between measured concentration and SD with a polynomial equation, usually of second order. Using this equation, it is then easy to calculate the probable SD with which any subsequent single serum concentration is measured within that range. For example, at the suggestion of Gilman [1] the error pattern of the EMIT gentamicin assay in use at the Los Angeles County - USC Medical Center was determined, and its polynomial equation was found to be

$$SD\ (ug/ml) = 0.570628 - 0.106496C + 0.016852\ C^2$$

This assay thus has an SD of 0.571 ug/ml at 0.0 ug/ml (the blank), yielding a variance of 0.326 and a weight (1/variance) of 3.07. The SD falls to 0.403 ug/ml at 3.0 ug/ml, yielding a variance of 0.162 and a weight of 6.16, double that of the blank. The SD then rises to 0.797ug/ml at 8.0 ug/ml and to 1.71 ug/ml at a concentration of 12.0 ug/ml, when the variance is 2.96 and the weight has fallen to 0.338. Note that the weights range from a high of 6.16 to a low of 0.338, a factor of 18.2 in the credibility given to the serum concentration data points within this range.

The coefficients of the polynomial equation can easily be stored with the Gentamicin population model in the USC*PACK clinical program for adaptive control of Gentamicin dosage regimens so that correct weighting of each measured serum concentration can then be implemented during the Bayesian fitting procedure.

Similarly, the error pattern of the Abbott TDx assay for lidocaine used by the USC Laboratory of Applied Pharmacokinetics was found to be

$$SD\ (ug/ml) = 0.040415 + 0.008604C + 0.004786C^2$$

This assay is more precise than that of the above EMIT assay. Its SD is 0.04 ug/ml at the blank, with a variance of 0.0016 and a weight of 612.2. The SD then rises and the weight progressively falls to 0.077 ug/ml and 169.7 respectively at a concentration of 2.0 ug/ml, to 0.203 and 24.3 at 5.0 ug/ml, and to 0.416 and 5.79 respectively at a concentration of 8.0 ug/ml. With this assay the weights thus vary by a factor of 612.2/5.79 = 111.5 over the range from 0.0 to 8.0 ug/ml.

It is easy for a clinical laboratory to determine its own assay SD over its entire working range in the above manner, thus arriving at a practical mixture of within - run and between - run errors which are useful in the fitting of pharmacokinetic models.

AN EXAMINATION OF THE COLLEGE OF AMERICAN PATHOLOGISTS SURVEY

The College of American Pathologists (CAP) sends out sample specimens containing stated drug concentrations to many clinical laboratories which report their findings back to the College. The College then reports the means and SD's of these findings, and the number of

laboratories reporting. The results are broken down by the drug and by the type of assay used.

We examined the results published by the College in Data Sets 1987 ZM-D, 1988 Z-D, 1989 Z-B, Z-C, and Z-D, and 1990 Z-A, Z-B, and Z-C, for Amikacin, Gentamicin, Digoxin, Lidocaine, Theophylline, and Vancomycin. We then took the calculated the means and SD's of concentrations found for the various specimens and fitted then with a polynomial, usually of second order, occasionally of first order, to provide a library of error patterns for the above assays. These can be used to estimate the SD of individual serum samples for Bayesian fitting of pharmacokinetic models until laboratories can determine their own error patterns in the same way.

RESULTS

Amikacin

Fifteen sample means, ranging from 1.1 to 30.0 ug/ml, and their SD's, obtained from 339 to 725 reporting laboratories, provided the data. Results for the Abbott TDx, The Dupont ACA, and the Syva Emit assays were evaluated. The following polynomial equations for the error patterns were found.

Abbott TDx SD (ug/ml) $= 0.30156 + 0.0053855C + 0.0011184C^2$, $R^2 = 0.983$

Dupont ACA SD (ug/ml) $= 0.46475 + 0.0281310C + 0.0021305C^2$, $R^2 = 0.939$

Syva Emit SD (ug/ml) $= 0.23237 + 0.0470150C + 0.0016876C^2$, $R^2 = 0.965$

All Methods SD (ug/ml) $= 0.32272 + 0.0183650C + 0.0012051C^2$, $R^2 = 0.983$

The Abbott TDx assay was the most precise. The Dupont ACA and Syva Emit assays were less so. As was also the case with all subsequent results, the results found for all methods were heavily dominated by those found with the Abbott TDx assay, as so many laboratories used it.

Gentamicin

Seventeen sample means, ranging from 0.9 to 17.8 ug/ml, and their SD's, obtained from 2512 to 3600 reporting laboratories provided the data. The Abbott TDx, Dupont ACA, and the Syva Emit assays were evaluated. The Baxter Stratus assay was not evaluated as the number and range of data points was much less. The following polynomial equations for the error patterns were found.

Abbott TDx SD (ug/ml) $= 0.02458 + 0.04948C + 0.0020318C^2$, $R^2 = 0.957$

Dupont ACA SD (ug/ml) $= 0.25719 - 0.016215C + 0.0081998C^2$, $R^2 = 0.982$

Syva Emit SD (ug/ml) $= 0.14078 - 0.002263C + 0.0184060C^2$, $R^2 = 0.991$

All Methods SD (ug/ml) $= 0.09114 - 0.043524C + 0.0045964C^2$, $R^2 = 0.992$

It was noteworthy here that two samples were found by all laboratories to have concentrations significantly less than those labeled by the College. Except for this, all other samples had general good agreement between the target values stated by the College and

the means found by the various laboratories. Again, the Abbott TDx assay was the most precise. Its error pattern, and the relationship between it and its polynomial descriptor, is shown in Figure 1.

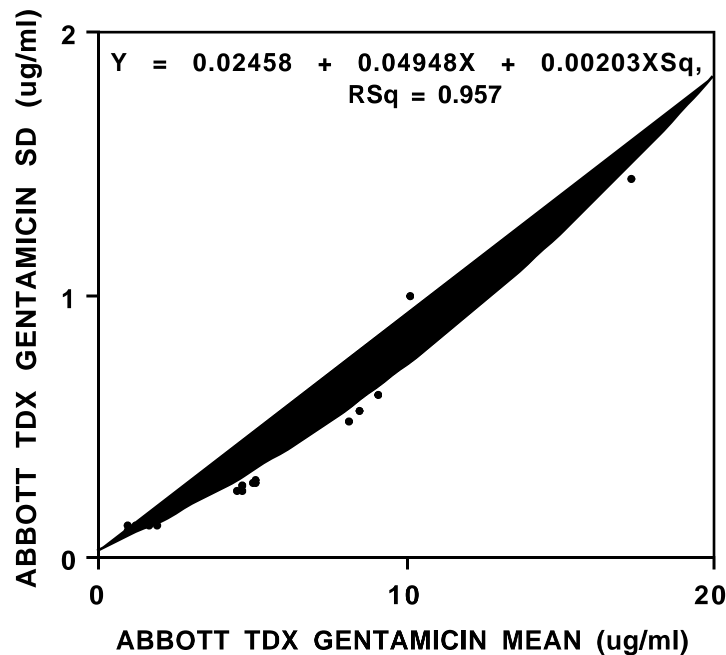

Figure 1. *Relationship between serum gentamicin and the SD of the measurements. Abbott TDX Assay.*

Digoxin

Seventeen sample means ranging from 0.2 to 3.0 ng/ml, and their SD's, obtained from 3160 to 4454 reporting laboratories provided the data. The Abbott TDx, Baxter Stratus, Clinical Assays, Dupont ACA, and Syva Emit assays were evaluated. The following polynomial equations were found.

Abbott TDx SD (ng/ml) $= 0.09211 + 0.0088626C + 0.009941C^2$, $R^2 = 0.948$
Baxter Stratus SD (ng/ml) $= 0.14421 - 0.048708C + 0.022917C^2$, $R^2 = 0.911$
Clinical Assays SD (ng/ml) $= 0.08672 + 0.017052C + 0.011857C^2$, $R^2 = 0.881$
Dupont ACA SD (ng/ml) $= 0.15560 - 0.056293C + 0.035574C^2$, $R^2 = 0.562$
Syva Emit SD (ng/ml) $= 0.16111 + 0.051579C$, $R^2 = 0.451$
All Methods SD (ng/ml) $= 0.12312 - 0.0073104C + 0.020257C^2$, $R^2 = 0.951$

As shown in Figure 2, the Abbott TDx assay was the most precise. It also had the highest R^2 (coefficient of the determination). Because of this, the error pattern of that assay is well characterized by such a polynomial equation. When the Syva Emit assay findings were fitted with a second order polynomial, the curve reached a peak and then began to bend downward. This could yield dangerously low estimates of the SD when extrapolated beyond the range reported here (0.2 to 3.0 ng/ml). Because of this, and because the first order polynomial had essentially the same value of R^2, the first order equation was used here, as shown in Figure 3. The Syva Emit and Dupont ACA assays had the lowest values of R^2,

showing that their error pattern was not at all well characterized by these equations, while the Abbott TDx had a high value of R^2, showing that its error pattern was well captured by its equation. The Clinical Assays error pattern was intermediate. The Abbott TDx assay was the most precise.

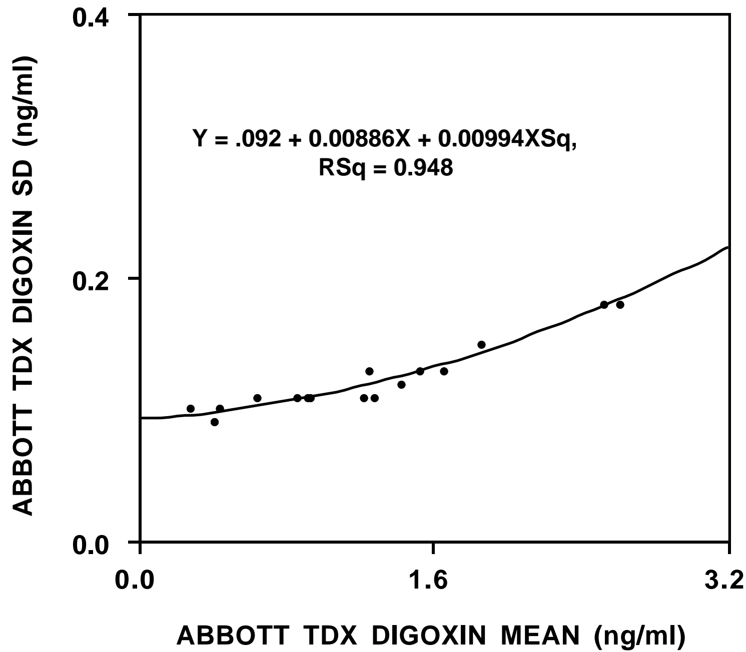

Figure 2. *Relationship between serum digoxin and the SD of the measurements. Abbott TDX Assay.*

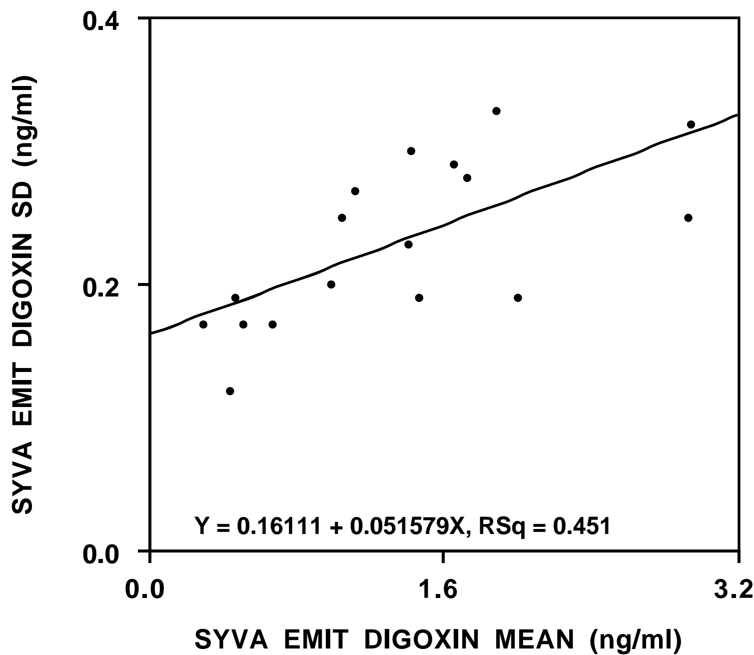

Figure 3. *Relationship between serum digoxin and the SD of the measurements. Syva EMIT Assay.*

Lidocaine

Fifteen sample means ranging from 0.3 to 6.0 ug/ml, and their SD's, obtained from 430 to 799 reporting laboratories, provided data. The Abbott TDx, Dupont ACA, and Syva Emit assays were evaluated. The following polynomial equations were obtained.

Abbott TDx SD (ug/ml) $= 0.053404 + 0.020234C + 0.0036386C^2$, $R^2 = 0.971$
Dupont ACA SD (ug/ml) $= 0.319570 - 0.132040C + 0.0265960C^2$, $R^2 = 0.407$
Syva Emit SD (ug/ml) $= 0.158580 - 0.013422C + 0.0126140C^2$, $R^2 = 0.924$
All Methods SD (ug/ml) $= 0.083569 + 0.008491C + 0.0068741C^2$, $R^2 = 0.985$

The Dupont ACA assay, as shown by its low value of R^2, had a widely varying and inconsistent SD, while the Abbott TDx and Syva Emit assay SD's were well characterized by their equations. The Abbott TDx assay was the most precise.

Theophylline

Seventeen sample means ranging from 3.0 to 30.0 ug/ml, and their SD's, obtained from 3682 to 4696 reporting laboratories, provided data. The Abbott TDx, Baxter Stratus, Dupont ACA, HPLC, and Syva Emit assays were evaluated. The following polynomial equations were obtained.

Abbott TDx SD (ug/ml) $= 0.22605 + 0.023955C + 0.00056926C^2$, $R^2 = 0.978$
Baxter Stratus SD (ug/ml) $= 0.078877 + 0.083394C$, $R^2 = 0.985$
Dupont ACA SD (ug/ml) $= 0.29967 + 0.010201C + 0.1379800C^2$, $R^2 = 0.963$
HPLC assay SD (ug/ml) $= 1.04060 - 0.120450C + 0.0093092C^2$, $R^2 = 0.707$
Syva Emit SD (ug/ml) $= 0.21770 + 0.057018C + 0.07131800C^2$, $R^2 = 0.972$
All Methods SD (ug/ml) $= 0.25463 + 0.039573C + 0.00088179C^2$, $R^2 = 0.976$

The Abbott TDx assay was the most precise, while the HPLC assay was the least. The Dupont ACA assay was next most precise, and the Baxter ACA and Syva Emit assays were intermediate and of about equal precision. The HPLC polynomial had the lowest value of R^2.

Vancomycin

Fifteen sample means ranging from 4.9 to 40.0 ug/ml, and their SD's, obtained from 645 to 862 reporting laboratories, provided data. The Abbott TDx and Syva Emit assays were evaluated. The following polynomial equations were obtained.

Abbott TDx SD (ug/ml) $= 0.57694 + 0.012816C + 0.00058286C^2$, $R^2 = 0.971$
Syva Emit SD (ug/ml) $= 0.93214 + 0.023689C + 0.00177690C^2$, $R^2 = 0.971$
All Methods SD (ug/ml) $= 0.59421 + 0.012291C + 0.00071299C^2$, $R^2 = 0.979$

Both error patterns were well characterized by their equations, with R^2 values over 0.97. The Abbott TDx assay was the more precise.

DISCUSSION

Sources of Error

The errors reported by the College survey are a mixture of within - run and between - run laboratory errors, as well as within - laboratory and between - laboratory errors. They are useful for purposes of therapeutic drug monitoring and Bayesian pharmacokinetic modeling until such time as a clinical laboratory determines its own assay error patterns for its own drugs and develops their own polynomial equations. Fortunately, this is easily done.

The Importance of Models

Recently, a change is taking place in the process by therapeutic drug monitoring and the individualization of drug dosage regimens is performed. Less attention is being paid to the interpretation of the raw data of the individual serum concentration results, and more is being paid to the fitted pharmacokinetic or pharmacodynamic model which is made based on the data of the entire history of the doses given, the patient's renal function or other descriptor of elimination (which may change from dose to dose), the population parameter values and their SD's, and the serum concentrations and their SD's. Such individualized models can usefully cover a span of over a month in some cases, and can reduce the frequency at which serum concentrations need to be obtained. Correlation of the patient's clinical behavior with the behavior of the patient's fitted model is most revealing, especially when the effect of the drug correlates better with concentrations in the peripheral nonserum compartment. For example, a patient may exhibit sinus rhythm at one time and atrial fibrillation at another, with identical serum digoxin concentrations. It is not until the fitted model is made that one can see the good correlation between his or her clinical behavior and that of the peripheral nonserum compartment. Use of models containing nonserum compartments is providing new views of the kinetic behavior of many drugs, including the aminoglycosides, lidocaine, digoxin, digitoxin, and vancomycin. Proper Bayesian fitting, using the correct assay error pattern, is essential. Inaccurate assay error patterns or simple assumptions of a certain coefficient of variation can lead to grossly inaccurate model parameter values, both in individually fitted patient pharmacokinetic models and in population pharmacokinetic modeling.

The Importance of Measuring Blanks

It is interesting that in none of the samples sent out by the College was there a blank sample. Clinical laboratories, however, usually characterize the sensitivity of their assays by choosing a value two SD's above a blank. When concentrations lower than those clearly detectable are encountered, they are often simply reported as being "less than X", where X is two SD's above the blank.

The Importance of Reporting Low Concentrations Below Detectable Limits

While the above practice is useful in toxicological analysis in making a firm decision as to whether a substance is present in the body or not, it is a distinct obstacle to optimal therapeutic drug monitoring. In therapeutic drug monitoring there is no question that the drug has been given. One clearly knows this from the history, the orders, and the nurses' notes, for

example. Indeed, many clinical laboratories will not measure a serum drug concentration unless the time since the last dose is stated on the request slip. Since the patient never excretes the last molecule of the drug, there is no question that the drug is still present in the body. The only question is its concentration. Low trough aminoglycoside concentrations for example, below those clearly detectable, are not only useful but necessary for therapeutic drug monitoring and Bayesian pharmacokinetic modeling. To withhold such results renders that measurement useless for Bayesian modeling, and should not be charged to the patient's bill when done for therapeutic drug monitoring rather than for toxicology. A vital data point is absent.

Rather than reporting a Gentamicin concentration as "less than 0.5ug/ml" for example, the laboratory can easily report the actual value found, and can report it as "0.1 ug/ml, below the secure detectable limits of 0.5 ug/ml", for example. This procedure will answer both the needs of the toxicologists and the pharmacokineticists, and is therefore more generally useful.

The Importance of Collecting High Serum Concentrations

The CAP Survey paid most attention to determining the laboratory errors for concentrations within the therapeutic ranges of the drugs under consideration. However, low trough concentrations, well below the usual detectable limits, are frequently found. Because of this, one might suggest that more such low concentrations, and especially blank concentrations, be included in future surveys.

On the other hand, it is equally important to know know the errors of concentrations found well into the toxic range. Because of this, when high concentrations are encountered, one might suggest that the laboratory run them in replicate as many times as possible, to better characterize the error of the assay at its high end, and to extend the range of the known assay error.

The Importance of Improving Assay Precision at the High End

When doing Bayesian fitting, one can only give equal weight to various serum concentrations when they have the same SD. An assay with a constant SD over its working range is said to be homoschedastic. Such an assay will have a coefficient of variation that decreases by half as the concentration doubles. None of the assays evaluated here are homoschedastic.

In contrast, a heteroschedastic assay error pattern is one in which the assay SD changes over its working range. Even an assay with a constant coefficient of variation is very heteroschedastic. As the concentration doubles, the SD also doubles, the variance quadruples, and the weight given to the assay is reduced to one fourth. If one assumes a constant coefficient of variation, a concentration of 1.0 ug/ml, for example, has a weight 100 times greater than that of a concentration of 10.0 ug/ml, and a concentration of 0.1 ug/ml has a weight 100 times that of the concentration of 1.0 ug/ml, and 1000 times that of the concentration of 10.0 ug/ml! Because of this, when a constant coefficient of variation is assumed for an assay used in Bayesian fitting, high concentrations will be relatively ignored compared to lower ones, and the fitted model will not approach the high concentrations as closely as one might wish. This is also true for the polynomial equations described above.

The difference here is that the polynomial equations are derived from empirically measured SD's over the working range of the assay, and should include blank concentrations as well. Because of this, they are a more correct estimate of the assay error pattern over its working range, and the fit, while often appearing to ignore the high concentrations, is actually being correctly done by current standards. One of two things needs to be improved. Either the current Bayesian fitting procedure based on the Fisher information of the data points is incorrect, or the assays need to have their precision improved at the high end to make them more homoschedastic. To discard the concept of Fisher information would be to overthrow several decades of carefully acquired and searchingly criticized mathematical and statistical knowledge. To improve the precision of assays at their high end is probably the most constructive thing to do. It may even be possible, for example, to alter the ratios of reagents so that the ratio of bound and unbound drug in the assay can be changed, with a resultant change in the error pattern toward homoschedasticity.

CONCLUSION

The results of the survey by the College of American Pathologists have been analyzed to provide a library of polynomial equations which characterize the error patterns of several assays over their working range. These equations can be used to improve the precision of fitting of pharmacokinetic models to optimize the process of model simulation, both for population and for individualized pharmacokinetic models, until each laboratory can determine its own error patterns. The procedure is described, is easy to do, and can be repeated as desired from time to time. Some suggestions are made for improving the quality of therapeutic drug monitoring and for subsequent surveys.

ACKNOWLEDGEMENTS

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1. Gilman T: Personal communication.