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γ -Hydroxybutyrate Concentrations in Pre- and Postmortem Blood and Urine

To the Editor:

With γ -hydroxybutyrate (GHB) becoming popular as a drug of abuse in the US and elsewhere [1], we are receiving increasing requests for the analytical determination of GHB in blood or urine in criminal investigations, especially in sexual assault cases. In a recent report of a fatal poisoning with GHB, the victim had a postmortem blood GHB concentration of 27 mg/L [2], and another three GHB-related fatalities were reported with postmortem blood GHB concentrations of 52–121 mg/L [3].

As a part of a validation study before instituting a GC-MS method described by others [4], we tested for GHB presence in a series of forensic specimens submitted routinely to us by law enforcement agencies and medical examiner offices in cases not known to be GHB-related. No GHB was detected (detection limit, 1 mg/L) in the blood or urine of living persons or in postmortem urine, but very substantial concentrations, ranging from 3.2 to 168 mg/L, were found in 15 of 20 autopsy blood specimens (Table 1). Reanalysis of these 20 blood specimens by gas chromatography with flame-ionization detection on a packed column [5] gave similar qualitative and quantitative results.

These results have great potential significance to the interpretation of postmortem blood GHB concentrations, because the concentration

Table 1. GHB concentrations in blood (n = 20) and urine (n = 8) by GC-MS.

Specimen	GHB concn., mg/L	
	Ave.	Range
<i>Living persons</i>		
Blood	0	0–0
Urine	0	0–0
<i>Deceased persons</i>		
Blood	25	0–168
Urine	0	0–0

range of this apparent “endogenous” GHB overlaps that known to produce clinical effects in patients receiving the drug as an anesthetic agent [6] as well as the concentrations reported to be associated with fatal reactions in medicolegal investigations [2, 3]. The fact that substantial GHB concentrations are found in the blood of deceased persons but not in living persons suggests that GHB is a product of postmortem decomposition.

We are unable to state from our data whether factors such as environmental temperature or storage time contribute to an increase in postmortem blood GHB concentrations. However, we suggest that analysis of GHB in urine, in which the concentrations tend to parallel those in blood but are ~10-fold greater [6], would produce more meaningful results in the investigation of drug-related death.

References

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Joint Limiting Values of N, Mean, and SD

To the Editor:

A graphical Win32 computer program for variance function estimation [1] is currently under development in this department, and design of the data entry module produced an interesting “impossible values” issue. The program incorporates two distinct data entry spreadsheets: (a) a grid for entering or importing sets of raw replicated measurements (e.g., runs of QC or other precision results), and (b) a grid for entering sets of values of N, mean, and SD (e.g., to allow estimation of a variance function from the summarized precision data often found in the literature). Restricting raw measurements to a sensible laboratory oriented range (e.g., 0–10⁷) is a simple matter, but constraining values of N, mean, and SD was more problematic. Suppose, for example, a user enters N = 10, mean = 1.0, and SD = 10 000; each value is within numerical limits, but clearly there is no distribution of 10 values (≥ 0) that can simultaneously have the mean = 1.0 and SD = 10 000. Allowing the manipulation of impossible values is unsatisfying and might also cause numerical instability or even failures if users experiment with highly extreme combinations.

The limiting relationship is surprisingly simple; the largest observable SD for any set of N values (≥ 0) is

$$SD_{\max} = \sqrt{N} \times \text{mean}.$$

Thus, in addition to initial data entry constraints (e.g., $N \geq 2$, mean and SD ≥ 0 , and all values less than a defined upper limit), the following rules can

be usefully applied to whichever of the three values is entered or modified last:

SD: upper limit = $\sqrt{N} \times \text{mean}$

mean: lower limit = SD/\sqrt{N}

N: lower limit = $(\text{SD}/\text{mean})^2$
rounded up to an integer, or 2
(whichever is larger)

Returning to the example above, initial entries of $N = 10$ and mean = 1.0 would restrict SD to a maximum of $\sqrt{10} \times 1.0$, or 3.162. Alternatively, mean = 1.0 and SD = 10 000 would require $N \geq 10^8$ (which would probably be regarded as too large).

These rules are easily incorporated into computer programs that require N, mean, and SD as input data. They cannot prevent all errors (or experimentation), but they do confine data to internally consistent combinations. Laboratory analysts might also be comforted to know that poor reproducibility does have a physical upper limit!

Maximum observable SD. The usual computational formula for the SD of a set of observations, x_i , $i = 1, 2, \dots, N$, is

$$\text{SD} = \left(\left[\frac{\sum_{i=1}^N x_i^2 - \left(\sum_{i=1}^N x_i \right)^2 / N}{N-1} \right] \right)^{1/2} \quad (1)$$

Expanding $\left(\sum_{i=1}^N x_i \right)^2$ yields

$$\begin{aligned} \left(\sum_{i=1}^N x_i \right)^2 &= (x_1 + x_2 + \dots + x_N)(x_1 + x_2 + \dots + x_N) \\ &= \sum_{i=1}^N x_i^2 + C \end{aligned}$$

where $C = 2 \sum_{i=1}^N \sum_{j=1}^N x_i x_j$ ($i \neq j$)

contains the cross-product elements. Substituting

$$\sum_{i=1}^N x_i^2 = \left(\sum_{i=1}^N x_i \right)^2 - C,$$

into Eq. 1 and rearranging yields

$$\begin{aligned} \text{SD} &= \left[\frac{\left(\sum_{i=1}^N x_i \right)^2}{N-1} - C/(N-1) \right]^{1/2} \\ &= [N\bar{x}^2 - C/(N-1)]^{1/2} \end{aligned}$$

where \bar{x} is the mean of the set. The particular case, all $x_i \geq 0$ (typical of virtually all types of medical laboratory results), implies that all cross-product elements in C are ≥ 0 , and therefore the maximum observable SD is $\sqrt{N} \times \bar{x}$ when $C = 0$. There are two cases where $C = 0$: all $x_i = 0$ (trivial case), and all but one $x_i = 0$; i.e., the largest SD of N observations (≥ 0) distributed about a mean \bar{x} , occurs when $(N-1)$ observations are 0 and the single non-0 observation has the value $N\bar{x}$. It also follows that the largest observable CV for any set of N observations (≥ 0) is $100\sqrt{N}$, which could be used to formulate consistency rules for N, mean, and CV.

Reference

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FPIA and EMIT Methods Compared for Cyclosporine Monitoring in Heart Transplant Patients

To the Editor:

Rejection after heart transplantation, especially in the early posttransplant period, is associated with high morbidity and mortality rates. Cyclosporine (CsA) has been widely used in combination with azathioprine and prednisone as immunosuppressants. Besides its narrow therapeutic range, CsA evokes marked inter- and intra-individual differences in bioavailability and pharmacokinetics [1, 2].

Therefore, reliable monitoring assays and adequate CsA concentrations are crucial. The aim of this study was to compare two different immunoassays for CsA monitoring in heart transplant patients. We used both an enzyme-multiplied immunoassay technique (EMIT; Behring) and a monoclonal fluorescence polarization immunoassay (mFPIA; Abbott) to assay in parallel 163 EDTA-anticoagulated blood samples from 87 heart transplant patients.

Our results show a linear correlation between both assays for CsA trough concentrations, with mFPIA values averaging 45% more than EMIT concentrations (mean \pm 2 SD: 173.8 ± 106.1 vs 119.7 ± 78.8 $\mu\text{g/L}$, respectively; Fig. 1). Assay interference from CsA metabolites may be particularly high immediately after heart or liver transplantation or in the presence of renal failure [3].

EMIT reportedly shows no significant interference from the CsA metabolites AM1, AM19, and AM4 N and only a slight cross-reactivity for metabolite AM9 [4]. The mFPIA, however, reportedly shows significant interference from AM1 and AM9 [5]. Our results show an even greater discrepancy between both assays and differ substantially from published data for liver and kidney recipients. LeGatt et al. [6] showed that FPIA-derived CsA concentrations averaged 14.2% more than those obtained with EMIT in kidney recipients. Dias et al. [7] obtained from renal, liver, and heart transplant patients CsA trough FPIA values averaging 32% more than the EMIT results. Dusci et al. [5] reported differences (mFPIA vs EMIT) of 43% and 27% for liver and renal transplant recipients, respectively.

EMIT data on CsA in heart transplantation are sparse. In line with our results are the findings of Seydoux and Goy [8] for a series of 66 CsA measurements in 28 heart transplant patients; the mFPIA values were 50% greater than the EMIT values.

From our results we cannot explain the overt discrepancies between mFPIA and EMIT in renal and cardiac recipients. We speculate that during the immediate posttransplant