We have investigated the use and stability of whole blood for the purpose of quality-control material for cholesterol analysis in the Reflotron. In addition, we examined the effect of hemolysis on the cholesterol assay, this having been found to be the limiting factor in the use of whole blood. We obtained blood from the Red Cross Blood Bank, which had been collected in citrate–phosphate–dextrose–adenine within the last 24 h. After portioning the blood into sterile containers in a laminar-flow cabinet, we stored it at 4 °C, removing, at specified times, aliquots to be assayed for cholesterol, in duplicate, in two different Reflotrons. The values obtained with the two instruments were averaged. To determine how much hemolysis had occurred during storage, we centrifuged the sample and determined, in a Coulter Counter, the amount of hemoglobin in the plasma. The Coulter Counter can detect hemoglobin concentrations from 2 g/L.

Figure 1 shows the stability of cholesterol in the whole blood stored at 4 °C. There was no significant change in the cholesterol value for 60 days. In addition, the figure also shows the amount of hemolysis in the sample during the storage period. After 60 days, the cholesterol value decreased, and this decrease corresponded to an increase in the amount of hemolysis in the sample. By adding increasing amounts of hemolysate to a plasma sample and assaying, we demonstrated that the assay is affected by hemoglobin concentrations >4 g/L.

Because many assays can be affected by the nature of the sample, a whole-blood quality-control material is more appropriate for instruments that analyze whole blood.

Whole blood evidently is suitable for use as a quality-control material for cholesterol determinations in the Reflotron, although new material would have to be prepared.
every two months. Also, care must be taken when collecting fingerstick samples, because any hemolysis of the blood droplet—e.g., from excessive pressure—could affect the cholesterol value.

References

Intra-Individual Biological Variation in the N-Acetyl-β-glucosaminidase/Creatininum Ratio for First Morning Urine, J. Huguet, X. Fuentes-Arderius, and C. Ferré (Servei de Bioquímica Clínica, Hospital de Bellvitge “Príncep d’Espanya,” Feixa Llarga s/n, 08907 L’Hospitalet de Llobregat, Barcelona, Spain)

Biochemical quantities within an individual vary randomly about a homeostatic set-point (1). This fluctuation, described as “intra-individual biological variation,” is useful for assessing any change between two consecutive values for a biochemical quantity in a patient (2).

We studied intra-individual biological variation for the ratio between the catalytic concentration of N-acetyl-β-glucosaminidase (EC 3.2.1.30) in first morning urine (Uri-N-acetyl-β-glucosaminidase, b), determined with the 3-cresolsulfonphthalein method in a Hitachi 704 analyzer and the substance concentration of creatininum (the sum of creatinine and the creatininum ion actually measured) in first morning urine (Uri-Creatininum, c, Jaiffe kinetic method, Hitachi 704 analyzer). b is an extremely sensitive index of renal parenchymal damage (3), a reliable quantity for monitoring the progression of kidney diseases (4), and an early indicator of immune rejection in patients with renal allografts (5). Because variations in urine flow may be compensated for by relating b to c, we calculated the ratio between the two.

We studied a group of 12 supposedly healthy individuals (seven women and five men, ages 12-76 y) for 12 weeks. Each week we measured b and c in each specimen and calculated the ratio between the two (Table 1). From this, we estimated the overall intra-individual variance of the ratio (s^2_{ib}). We also studied the relation between the between-days analytical variance and concentration for both biochemical quantities over a wide range of concentrations: b from 38.69 to 650.21 nkat/L, and c from 1.284 to 20.565 mmol/L. These results showed that both quantities were homoscedastic variables (Barlett’s test), although for c this homoscedasticity was observed only after naperian logarithm transformation. Furthermore, we evaluated the between-days analytical variance of the ratio (s^2_{AB}) (see Table 1). The intra-individual biological variance (s^2_{ib}) of the ratio for each subject was calculated from this last value and from s^2_{ib}, as follows:

\[ s^2_{ib} = s^2_{tw} - s^2_{ab} \]

From these, we obtained for each individual the value of the intra-individual biological variation of the ratio, expressed as a CV; the mean of these values was 35.7%. Moreover, we calculated for each individual the CV corresponding to the intra-individual biological variation of b; the mean of these values was 43%.

References


The cloning of the complete dystrophin cDNA has made it possible to identify molecular deletions in Duchenne (DMD) and Becker (BMD) muscular dystrophy patients (1). Utilizing eight cDNA probes, which span the entire message, deletions have been found in 67% of affected patients. Identification of a deletion in the proband permits direct carrier diagnosis of the female relatives by gene dosage (2). If the female exhibits single-copy intensity for the deleted band or bands on the autoradiograph, then she must be carrying the deletion on one of her X chromosomes and would therefore be a carrier. However, if the band or bands are of two-copy intensity, suggesting homozygosity for the fragment, this would indicate noncarrier status.

Variability in the amount of DNA applied onto the gel and differences in the retention of the high-molecular-mass fragments to the filter during Southern transfer can make it difficult to determine visually whether a band is of single- or double-copy intensity. To increase the accuracy of the gene dosage analysis, we have recently started quantifying the autoradiographic bands by scanning with a CS-9000 scanning densitometer (Shimadzu Corp., Kyoto, Japan).

Hybridization of a Hind III digest to cDNA 8 revealed that the DMD proband (A) was deleted for the 10-, 1.6-, and 1.2-kb fragments (Figure 1). To determine whether the relative intensities of these fragments were reduced in the mother (B), the fragments were analyzed densitometrically (Figure 2), and the 10/7-kb absorbance peak ratios for the