The closest instrument, in this study, was the SYNCHRON CX5, which had the least imprecision (CV) of 13.6% [3]. Thus the performances of all of the methods may be satisfactory. However, as Fraser and Peterson have argued, even when biological variation, not analytical variation, is the limiting source of variation in a test, there is still great advantage in using a method with the least possible imprecision and bias [4].

The aco V was the least precise instrument and had the largest mean bias. The overall performances of the other methods were close to each other, although each had its own characteristics. The Ektachem 700 appeared to be the most precise instrument, but its positive bias was significant throughout the lactate reference interval, approaching zero just above the upper limit. In our view, these bias characteristics are less than desirable; others might disagree. The TDx exhibited no bias, but the imprecision was noticeably greater than that of both the Ektachem 700 and the SYNCHRON CX5. Besides having an acceptable imprecision, the SYNCHRON CX5 had only a slightly positive bias.

The performances of all instruments arguably were clinically acceptable and, as is evident, no one method proved to be clearly superior to all others. However, each laboratory must make its selection based on criteria appropriate to its own needs. For our laboratory, we concluded that the SYNCHRON CX5 was the most suitable for measuring lactate in plasma.

References

Influence of Hemoglobin S Adducts on Hemoglobin A₂, Quantification by HPLC, David D. Sub, Jonathan S. Krauss,* and Keith Bures (Med. College of Georgia, Dept. of Pathol., BIH 222B, 1120 15th St., Augusta, GA 30912-3620; *author for correspondence: fax 706-721-7837, e-mail jkrauss@mail.mcg.edu)

Increased hemoglobin A₂ (HbA₂) is an important finding in the diagnosis of β-thalassemia [1]. In the presence of a β-chain variant hemoglobin such as HbC or HbS, HbA₂ is known to be slightly increased [2]; Whitten and Rucknagel [3] suggested that this increase reflected impaired association of β-S with α-chains,
allowing more α-chains to associate with δ-chains. Recently, however, we found that HbA2 measured by HPLC was significantly increased in the presence of HbS—so much so that homozygous HbS samples might be misclassified as HbS-β0-thalassemia.

In 43 healthy subjects the mean HPLC HbA2 was 2.5% (range 2.1–2.9%) and mean microcolumn HbA2 was 2.4% (2.0–2.8%); these results concur with previously reported HbA2 values for normal subjects [4]. In 52 non-HbS-containing samples, HPLC HbA2 and microcolumn HbA2 values agreed well (r = 0.97). In a subgroup of 10 HbAS and 3 HbSS samples, however, mean HbA2 results were 3.9% by HPLC and 2.8% by microcolumn (P <0.001 between methods), confirming previous findings [2,3,5]. Bias plots for HbA2 = HPLC, with and without HbS, differed by −1.14% ± 0.313% and 0.102% ± 0.167%, respectively (P<0.001) (see Fig. 1).

We subjected two samples from untransfused HbSS patients to affinity microchromatography of glycohemoglobin (GHB) (Glyc-Affin® GHB; Isolab), concentrated the GHB eluate (with a B15 concentrator; Amicon, Beverly, MA), diluted this material with HPLC hemolyzing reagent, and subjected it to HPLC. Hemoglobin eluting with HbA2 constituted a mean of 9.6% of the GHB (range 4.8–15.5%; n = 3).

In a retrospective study of HbAA2 (n = 100), HbAC (n = 16), and HbAS (n = 85) subjects, we found that results for HPLC-measured HbA2 varied among groups, with mean HbA2 values of 2.5%, 3.3%, and 3.8%, respectively. This suggests that another factor, in addition to the increased α-δ-chain association in the presence of abnormal β-chains, is responsible for the increased HPLC HbA2 in the presence of HbS. In the HbAC HPLC pattern, two small peaks elute immediately before HbC (probably HbC adducts); in the HbAS pattern, no such peaks appear, with the HbA2 eluting immediately before the HbS. We suspect that coelution of HbS adducts (including glycated HbS) with HbA2 on HPLC is responsible for the increased HbA2.

To illustrate this possibility, we measured HPLC HbA2 in HbS-containing samples after aging for 2 weeks. Previous research indicates that blood samples stored over time show increasing amounts of GHB [6]; therefore, we speculated that if GHB were a contributory factor to the increased HbA2, then HbS-containing samples would show increasing HbA2 over time. Two HbSS specimens and one HbAS specimen measured by HPLC for 14 days showed HbA2 proportions increasing from 4.0% to 5.3%, from 4.4% to 7.4%, and from 3.8% to 5.4%, respectively, consistent with our hypothesis. However, <20% of the affinity GHB eluted with HbA2; hence, the >1% difference between HPLC and microchromatographic HbA2 in the presence of HbS cannot be accounted for by glycated HbS alone but predominantly reflects other HbS adducts.

Recently, Roa et al. [7], using a nonproprietary cation-exchange HPLC method to measure hemoglobins in a population of African American adults, found that mean HbA2 values were respectively 1.2% ± 0.4% and 2.2% ± 1.1% for subjects without and with sickle-cell trait. The differences between their data and ours may reflect the different HPLC methods used.

Another factor that may contribute to the greater accuracy of the microcolumn HbA2 in the presence of HbS is its anionic nature, which results in a reversed elution order for the hemoglobins, causing HbA2 to elute first. For the Variant HPLC system, estimation of HbA2 is effective and efficient in non-HbS-containing samples. In the presence of HbS, however, we conclude that HPLC HbA2 may be falsely increased by the coelution of HbS adducts.

References