falsely increased concentrations, with far-reaching consequences for the athlete.

We also performed GC/MS analysis on 8 mL of blank urine into which two other reagent strips had been dipped for 1 s: Rapignost® Total Screen (Behring Diagnostics) and Combur10 Test® M (Boehringer Mannheim), respectively. The chromatograms and spectra obtained with these two strips were very comparable but totally different from the Bayer strip. Neither of these two strips contained any caffeine. However, TMB was present in both strips. None of the unknown spectra obtained with these strips has been fully identified.

This report shows that dipping a test strip into urine for as little as 1 s can cause interference in subsequent analyses, such as GC/MS screening. When the Bayer N-Multistix-SG strip is used, this interference can lead to faulty assumptions of caffeine poisoning or caffeine doping. TMB, because it is present in all of the strips tested, can be used as a marker of test strip contamination. We recommend that a urine test strip be dipped in a separate urine aliquot. Unfortunately, current trends to send smaller urine sample volumes to the laboratory (e.g., 8–10 mL in tubes) will make this recommendation increasingly difficult to follow.

Interference of Hemoglobin D in Hemoglobin A₂ Measurement by Cation-Exchange HPLC

To the Editor:

We read the letter from Dr. Dash with interest (1), but we do not agree with her demonstration. We use the same cation-exchange HPLC (Bio-Rad Variant) as Dr. Dash, and we have concluded that hemoglobin (Hb) A₂ cannot be quantified in the presence of Hb D by this method. As shown on Fig. 1, an incomplete return to baseline between the Hb A₂ and Hb D peaks does not allow accurate integration of Hb A₂. To confirm this observation, we compared the Hb A₂ values obtained by HPLC and anion-exchange microchromatography (Quik-Sep; Isolab) in nine samples from Hb D heterozygotes. The latter method separates hemoglobins on the basis of their pI, and the Hb A₂ measurement is not influenced by Hb S or Hb D but is influenced by Hb C, Hb E, or Hb O (2). When anion-exchange chromatography was used, all results (mean, 2.6%; range, 2.4–2.9%) were within the reference interval (2.0–3.5%), whereas those obtained with HPLC were somewhat lower (mean, 1.8%; range, 1.2–2.0%). It has been shown that these methods give similar results in patients without hemoglobin variants (3, 4). In our opinion, the low Hb A₂ values measured by HPLC are most probably attributable to integration problems only. The Hb A₂ values measured by a specific method seem similar in Hb D heterozygotes and Hb A homozygotes.

To detect β-thalassemia carriers, a precise and specific method for Hb A₂ measurement is needed. This is

References

Editor’s Note: The interference described in this Letter is a result of immersing a test strip into a sample. This is not good laboratory practice, for samples or reagents. This Letter should be a reminder to all of us not to place any object other than transfer pipettes into the original specimen or test sample. Although the phrase “dip stick” implies use of this technique, the sample should be transferred to the test strip by another mechanism. When it is acceptable to immerse the strip, a portion of the specimen should be taken to be used only for that purpose. In this age of point-of-care testing, it would seem wise for laboratories to advise non-laboratory staff of this basic principle of testing.
not the case with HPLC in Hb D heterozygotes or in Hb S heterozygotes (3, 4). In these cases, another method is required, such as anion-exchange chromatography or capillary electrophoresis (4), which in turn are not suitable in the presence of Hb C, Hb E, or Hb O. This illustrates that each laboratory should have an alternative method adapted to each case and keep in mind the performances of each method.

References

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Dr. Dash responds:

To the Editor:
I am glad to note that Cotton et al. have corroborated our observation of low hemoglobin (Hb) A₂ values in Hb D cases when measured by HPLC (Bio-Rad Variant).

Our documentation was to emphasize the unreliability of Hb A₂ measurements by this method in the presence of abnormal hemoglobins because the value not only can be spuriously increased, as in Hb S cases, but may also be decreased, as in Hb D cases. Thus, additional methods are required to quantify Hb A₂ in the presence of abnormal hemoglobins.

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