To validate the method, we used this novel approach to genotype 155 DNA samples from Caucasians with known mutations (9) and found complete agreement. The allele frequencies for NAT2*4 (wild type), *5A/B/F, *5C/D, *6A/B/C/D, and *7A/B were similar to published frequencies (5). A G191A mutation was not detected. The concordance rate was 100% for each polymorphic site with DNA from whole blood, whereas the sensitivity was 70% and the specificity was 100% for a second set of DNA samples (n = 400) extracted from plasma or serum. Overall, we noticed some lower absolute fluorescence for C481T, but intensities were still high enough to allow unambiguous results. However, it must be noted that >30 different NAT2 alleles are currently known (http://www.louisville.edu/medschool/pharmacology/NAT2.html). Several single-nucleotide polymorphisms have been reported in the 5'- and 3'-flanking regions of the gene, which also might affect phenotypes through endogenous or exogenous regulation or other interactions. In addition, striking ethnic differences in the frequencies of the slow acetylator alleles and phenotypes (10, 11) must be considered. Therefore, analysis of additional mutations (C282T and A803G) should be performed when mixed Hispanic or Orientals individuals are studied to minimize chances for misclassification (12).

In conclusion, the procedure is very simple, results are robust, and repeats on 20% of the samples were in complete agreement. This approach allows the detection of five major NAT2 mutations (G191A, T341C, C481T, G590A, and G857A) and prediction of the resulting phenotype in just 1 h, making it suitable for clinical applications (13).

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References

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Imprecision of Cerebrospinal Fluid Net Bilirubin Absorbance, Adie Viljoen,” Simon W. Walker, Kay S. Walker, and Patrick J. Twomey (Department of Clinical Biochemistry, Royal Infirmary of Edinburgh, Edinburgh, Scotland, UK; * address correspondence to this author at: Department of Clinical Biochemistry, Room 6114 Level 2, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh, EH16 4SA Scotland, United Kingdom; fax 44-131-242-6812, e-mail adie@mailbox.co.za)

Analysis of cerebrospinal fluid (CSF) has an important role to play in the diagnosis of subarachnoid hemorrhage (SAH) (1). Computed tomography, the first line of investigation for suspected SAH, shows a decrease in diagnostic accuracy as the time interval between the suspected hemorrhage and the time of investigation increases. A computed tomography scan would be positive in 98% of patients presenting within 12 h after an event, but positivity decreases to ~50% in patients presenting after 1 week, 30% after 2 weeks, and 0% after 3 weeks (2).

An increase in bilirubin in the CSF is the key finding supporting the occurrence of SAH (3). Guidelines for the analysis of CSF for bilirubin in suspected SAH have recently been published in the United Kingdom (3). The presence of bilirubin is assessed by calculating the net bilirubin absorbance (NBA) according to Chalmers’ modification (4) to the original method of Chalmers and Kiley (5). A single NBA cut point of 0.007 absorbance units (AU) is recommended in the decision tree for interpretation and reporting of results (3). The interpretative comment “No evidence to support SAH” is advised if the NBA value is ≤0.007 AU and no oxyhemoglobin is detected. In contrast, if the corrected NBA is >0.007 AU and no oxyhemoglobin is detected, the interpretative comment “Consistent with SAH” is recommended (3).

Laboratories and clinicians need to define and be aware of the quality of the tests they provide when these tests are the basis for clinical decisions. To the best of our knowledge, the analytical imprecision profile for NBA has not been published to date. Our laboratory has performed 92 analyses of CSF for suspected SAH in the last year. Of these, 84 were patient samples and 8 were analyzed as part of the UK National External Quality Assessment Scheme. We therefore felt it important to ascertain the imprecision of the NBA at the medical decision cutoff (0.007 AU) and other absorbance values.

The spectrophotometer used for the investigation undergoes a yearly service check during which both the wavelength accuracy and the absorbance accuracy are assessed. The most recent was conducted within 1 month.
of this investigation. The absorbance inaccuracy was assessed by measuring the absorbance of four reference standards. The mean bias was 1.3%. External quality-assurance (proficiency testing) samples were used to assess the absorbance at the desired values. Samples were analyzed in 200-μL quartz microcuvettes with a 1-cm light path. Samples were scanned with a Uvikon 922 dual-beam spectrophotometer (Kontron Instruments) from 600 to 350 nm at a scan speed of 50 nm/min and a 2-nm bandwidth with data collection points set at 1-nm intervals. Blanking was performed across the entire wavelength range by use of deionized water in the reference cell. Six samples with mean NBA in the range 0.177–0.006 AU were scanned 20 times. A predicted baseline, which forms a tangent to the scan between 350 and 400 nm and again between 430 and 530 nm, was constructed as recommended (3, 4). Baselines were drawn manually, and the NBA was measured with use of a ruler in each case. To derive the NBA, the same individual measured the absorbance of the scan above the predicted baseline at 476 nm for each of the 20 scans. The within-operator imprecision for deriving the NBA was calculated, and a precision profile was constructed (Fig. 1). Increasing NBA values corresponded with decreasing CVs (Table 1).

To calculate the analytical imprecision of the spectrophotometer, 20 repeat measurements were made of the absolute absorbance at 476 nm, using the sample that had a derived NBA of 0.007 AU. The calculated CV was 1.4%. The imprecision contributed by the spectrophotometer to the derived NBA is therefore minimal.

In view of the relatively large CV around the medical decision cutoff (17%), two other individuals derived the NBA, using photocopies made from the original scans of the sample with the lowest absorbance (Table 1). The individuals derived the NBA by drawing a baseline and measuring the NBA with a ruler for each of 20 scans, as outlined in the guidelines (3). Each analyst was blinded to the NBA calculated by the other individuals. The mean value calculated by the three individuals was 0.0062 AU (0.0062, 0.0062, and 0.0063 AU, respectively), and the mean CV was 19% (22%, 18%, and 17%, respectively). Calculated measurements ranged from 0.0047 to 0.0084 AU, with 17 (7, 5, and 5, respectively) of the 60 results having a calculated NBA greater than the cut point of 0.007 AU.

We subsequently reexamined the 84 sample scans that were processed by our laboratory in the past year and calculated the NBA (3). Five of the 84 patient samples had a calculated NBA between 0.006 and 0.007 AU. These samples were reported as negative for the presence of bilirubin but could potentially have a significant NBA considering the amount of variation apparent in this region. Bilirubin was present in 8 of the 84 samples (NBA range, 0.0136–0.636 AU). In the remaining 71 samples, the NBA measurements were <0.0036 AU.

It is important to be aware of the imprecision of the NBA calculation at the recommended medical decision limit and the impact of this imprecision on clinical decision-making. This is especially the case if a single NBA cut point is used. If no internal quality control is performed, as we believe is the case in the majority of laboratories, knowledge of the imprecision will often remain unknown. Our data represent a best-case scenario because the intraassay variation, and not the interassay variation, was assessed. We did not evaluate the between-day analytical variation because of possible sample deterioration caused by progressive light exposure and temperature variations. Day-to-day variation and interlaboratory variation are likely to be higher in practice when the impact of interassay and instrument-related factors is considered. External quality-assurance data showed interlaboratory CVs of 15% and 29% at respective mean values of 0.045 and 0.015 AU (unpublished data, UK National External Quality Assurance Scheme).

Catheter angiography, which is used to locate the source of the hemorrhage, is an invasive, resource-intensive, and costly procedure that has a low but recognized risk of morbidity and mortality (6). Patients suffering from a severe benign headache mimicking SAH may be exposed unnecessarily to this risk if a false-positive bilirubin result is obtained. On the other hand, it is important to diagnose SAH quickly because a "warning leak" may be followed by another, often catastrophic, event (7). Because the UK guidelines recommend that a single NBA cut point is used for reporting (3), it is important that clinicians and laboratory personnel are aware of the uncertainty of the measurement at this critical clinical decision cutoff. Where appropriate, laboratories must supply information about the uncertainty of their measurement results (8). We would suggest that laboratories that analyze CSF for the presence of bilirubin determine the imprecision of the NBA at the medical decision cutoff.

Table 1. Mean NBAs, CVs, and the associated 95% confidence intervals.

<table>
<thead>
<tr>
<th>Mean NBA, AU</th>
<th>CV, %</th>
<th>95% confidence interval, AU</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0062</td>
<td>22</td>
<td>0.0035–0.0088</td>
</tr>
<tr>
<td>0.007</td>
<td>17</td>
<td>0.0047–0.0093</td>
</tr>
<tr>
<td>0.014</td>
<td>11</td>
<td>0.011–0.017</td>
</tr>
<tr>
<td>0.034</td>
<td>9.0</td>
<td>0.028–0.04</td>
</tr>
<tr>
<td>0.086</td>
<td>6.0</td>
<td>0.075–0.095</td>
</tr>
<tr>
<td>0.177</td>
<td>3.5</td>
<td>0.16–0.18</td>
</tr>
</tbody>
</table>
From these data, results within a multiple of the standard deviation at the medical decision cutoff could be reported as equivocal for the presence of bilirubin (8). The 95% confidence interval for the medical decision cutoff in this laboratory was calculated to be 0.0047–0.0093 (Table 1). Thus, 5 of the 84 (6%) patient specimens previously reported as negative for the presence of bilirubin would fall into this equivocal zone. Our data also emphasize the importance of clinical liaison in suspected cases of SAH because this should form part of the service offered to clinicians. In particular, issues such as “rule in” and “rule out” or sensitivity and specificity need to be discussed with the investigating clinician. It has previously been reported that the UK recommendations (3) have a sensitivity and a specificity of 80% and 100%, respectively, compared with cerebral angiography (9). Should investigating clinicians want to rule in a diagnosis of SAH where the NBA is in the equivocal range, CSF ferritin may add additional sensitivity before referral for the invasive procedure of cerebral angiography (9). The clinical consequences of missing a positive NBA may be catastrophic. Because the manual calculation of very small NBAs at the medical decision cutoff produces large CVs, we would recommend the use of built-in software programs that are able to objectively calculate the NBA.

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References


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