3,4-Dihydroxybenzylamine (DHBA) is used as the internal standard.

We observed an interference with the internal standard in patients taking part in a clinical study in our intensive care unit. Evaluation of the case histories revealed that these patients have received dopamine intravenously before blood sampling. An example of this interference in a patient receiving dopamine is shown in Fig. 1A. The sample from this patient showed an approximately 1.5-fold spurious increase in the response of the internal standard compared to other samples in the same run. Because calculations of catecholamine concentrations were performed relative to the internal standard, falsely low results (approximately 67% of the actual concentration) would be reported if the falsely increased internal standard response was not manually corrected.

Testing dopamine (Carinopharm) in vitro, we found the same interference (Fig. 1B). Because dopamine has a retention time of approximately 20 min with this method, a dopamine impurity might lead to the spuriously increased internal standard readings. Therefore, we asked the manufacturer about potential contaminants and learned that minor amounts of 3-O-methyl dopamine, 4-O-methyl dopamine, and homoveratrylamine are detected via thin-layer chromatography within the QC procedure. Therefore, we analyzed the retention time of these substances. We found that 3-O-methyl dopamine (Fisher Scientific) interfered with the HPLC analysis, leading to an increase of the internal standard only with substantial amounts, e.g., 100 mg/L. The other tested substances showed no interference with the internal standard (data not shown). To analyze whether the disturbance is specific for the applied dopamine, we tested dopamine standards from 2 other sources, Sigma-Aldrich and Fresenius-Kabi, and also found an artificial increase of the internal standard (data not shown). In vivo, 3-O-methyl dopamine is a degradation product of dopamine. Dopamine is converted to 3-O-methyldopamine via catechol-O-methyltransferase (3). The concentration of 3-O-methyldopamine in plasma is usually <1 μg/L, but may increase markedly in pheochromocytoma patients. We tested whether 3-O-methyldopamine also interfered with catecholamine determination in urine. To retain catecholamines we applied a urine pretreatment process that immobilizes phenylboronic acid via formation of cyclic esters. This specific interaction is possible only for cis-diol compounds. Indeed, this pretreatment eliminated the disturbance caused by 3-O-methyldopamine (data not shown).

Because we knew that the chromatographic separation of 3-O-methyldopamine, the peak with the retention time of 13 min, should contain 2 different substances, DHBA and 3-O-methyldopamine, we repeated the analysis of 3-O-methyldopamine with increased resolution of our method and a new column. The columns not able to resolve the peak, however, were maintained according to the manufacturer’s instructions. Alternatively, more specific detection methods, such as mass spectrometry, might be applied, but such methods are not as widely used for catecholamine analysis. Interference with the internal standard is a major pitfall in HPLC analysis. Numerous reports are published that describe such interferences, which are often found coincidentally (4, 5), and they probably go unrecognized even more frequently.

Our findings show that the possibility for spurious increases in the internal standard must be considered because they may lead to falsely low results. DHBA, a widely used internal standard for the determination of catecholamines, would not be easy to replace, but minor modifications of the method might be beneficial and must be developed. As this report exemplifies, manufacturers and analysts are permanently challenged to improve their methods.

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References

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The Vietnamese Khin Population Harbors Particular N-Acetyltransferase 2 Allele Frequencies

To the Editor:

Vietnam ranks 13th among the 23 countries that must deal with 80% of global deaths from tuberculosis (1). The National Tuberculosis Control Program of Vietnam implemented the WHO DOTS (directly observed therapy, short course) strategy in 1989. The standard treatment regi-
men for previously untreated patients consists of 2 months of streptomycin, isoniazid (INH), rifampin, and pyrazinamide, followed by 6 months of INH and ethambutol (the 2SHRZ/6HE regimen).

INH, a central component of this chemotherapy, is extensively metabolized by the polymorphic N-acetyltransferase 2 (NAT2) enzyme. N-acetyltransferase 2 (arylamine N-acetyltransferase) (NAT2) gene polymorphisms are associated with individual phenotypes generally classified as INH rapid (homozygous or heterozygous) and slow acetylators. Among the described NAT2 alleles, NAT2*5, *6, *7, 12*, *14, *17, and *19 have been associated with the slow acetylator phenotype, and NAT2*4 and *13 are associated with fast acetylation. Under treatment with standard dosages, patients who are slow metabolizers may be at risk for INH side effects, which include peripheral neuropathy, hepatic toxicity, and psychiatric symptoms. On the other hand, insufficient drug concentrations may be more frequent among fast metabolizers. The determination of the NAT2 gene allele pattern of a population can supply valuable baseline information for determining drug dosage and expected efficacy for specific groups.

Although Vietnam has exceptional DOTS coverage, with extensive use of INH, information on population-related NAT2 pharmacogenetics has been unavailable. Accordingly, we sequenced the full NAT2 gene in 72 unrelated adult Vietnamese volunteers [51 male and 21 female, average age: 29 (18–45) years], sampled at the National Institute of Malaria, Parasitology and Entomology, Hanoi, Vietnam. With the exception of 2 Thai individuals, all volunteers were self-identified as belonging to the Khin ethnic group, the prevalent population in Vietnam. All study participants gave written informed consent. All protocols were approved by the Ministry of Health Hanoi, Vietnam; the Swedish Medical Products Agency, Uppsala, Sweden; and the Ethics Committee of Göteborg University.

We used an ABI 6100 Nucleic Acid PrepStation (Applied Biosystems) to extract genomic DNA from peripheral blood samples. The NAT2 gene was analyzed through full sequencing with PCR amplification in ABI 7420 thermocyclers. The primers used were as follows: 5’-TCACAGAGGAAATCAAATG-3’ (NAT2f) and 5’-GTGCTATCAGATATCCCTCTC-3’ (NAT2r).

Our results show that 90% (65 of 72) of the investigated individuals harbored at least 1 NAT2 fast allele (Table 1), suggesting a particularly low frequency of slow acetylators, even in an Asian population. This high prevalence of fast acetylator–associated alleles is essentially attributable to the exceptionally high frequency of the NAT2*13. Although this allele has been observed at frequencies ≤5% in other populations, in our study we detected it at a prevalence of 31%, significantly different from previous studies on a sample of 264 Thai individuals (*13 <5.1%; P <0.0001) (2) (Table 1). Notably, the NAT2*7 allele, consistently found in Asian populations, was not observed in our study. This finding also contrasts with the 20% prevalence in the Northeast Thai population, and the 6.3% prevalence among 64 individuals from the Cambodian Khmer population (6.3%) (2) (Table 1).

Table 1. Genotype frequencies and deduced phenotype among Vietnamese individuals predominantly from the Khin ethnic group.

<table>
<thead>
<tr>
<th>Genotypes and deduced phenotypes</th>
<th>Allele frequencies vs other Southeast Asian populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotype</td>
<td>NAT2 genotype</td>
</tr>
<tr>
<td>Rapid (homozygous)</td>
<td>*4/*4</td>
</tr>
<tr>
<td></td>
<td>*4/*13</td>
</tr>
<tr>
<td></td>
<td>*13/*13</td>
</tr>
<tr>
<td>(heterozygous)</td>
<td>*13/*6A</td>
</tr>
<tr>
<td></td>
<td>*6B/*13</td>
</tr>
<tr>
<td></td>
<td>*7</td>
</tr>
<tr>
<td>Slow</td>
<td>*4/*12B or *12A/*13</td>
</tr>
<tr>
<td></td>
<td>*6A/*6A</td>
</tr>
</tbody>
</table>

All alleles were found to be in Hardy-Weinberg equilibrium.

b The ambiguous *4/*12B or *12A/*13 haplotype found in 1 individual was not included in the allele frequency calculations.

c 95% CI.

d Ref. (2).

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ND, not detected, although some alleles might be included in the NAT*4 group, due to allele differentiation limitations of the performed essays.

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During analysis we found cases in which it was not possible to distinguish between haplotypes (Table 1) because the presence of multiple heterozygous single-nucleotide polymorphisms prevented us from determining which alleles were in cis (same chromosome) or trans (different chromosomes). For example, when we have both the 282 C>T and 803 A>G in the heterozygote form, 2 possible haplotypes appear: NAT2*4/*12B (one chromosome 282C/803A + the other 282T/803G) and NAT2*12A/*13 (282C/803G + 282T/803A).

The observed 9.7% frequency of slow acetylators among the Vietnamese Khin predicts a low incidence of adverse effects caused by overexposure to INH, and potential drug-drug interactions associated with this drug (3). For this minority group of slow acetylators, a decrease in drug dosage would likely reduce the risk of adverse events without compromising the efficacy of the drug, because a dose of INH as low as 3 mg/kg has recently been shown to be sufficient for a successful therapy (4). On the other hand, a dose reduction below 6 mg/kg in fast acetylators can compromise INH efficacy. The observed high frequency of fast alleles may hypothetically affect the success of the standard INH dosing in the overall Vietnamese population, particularly in terms of early bactericidal activity (5).

Limited pharmacogenetic data are available to account for the diversity of the Southeast Asian populations. Future studies should be performed to identify particular population characteristics that might influence pivotal pharmacotherapy in these geographic regions.

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References

C-Reactive Protein (CRP) in Neonates: Comparing VITROS Slide and High-Sensitivity CRP Methods

To the Editor:
C-reactive protein (CRP) is measured on VITROS® Chemistry Systems (Ortho-Clinical Diagnostics) using a “MicroSlide” method. At our hospitals plasma CRP concentrations <10 mg/L are used as an indicator that it is safe to discontinue antibiotic therapy in neonates with known or suspected sepsis (1). After implementing the neonatal sepsis protocol, we found that the VITROS CRP slide gave results up to 33 mg/L higher in neonatal specimens (Fig. 1, open triangles) than the Behring Nephelometer II (BNII, Dade Behring) highsensitivity CRP (hsCRP) assay, a well-characterized method for CRP measurements (2).

According to the manufacturer’s instructions, hemolysis falsely increases VITROS CRP slide results, but the increases we observed were not limited to hemolyzed specimens. It has been reported (3) that collecting specimens in serum separator tubes increases CRP values measured by the VITROS CRP slide; however, the differences we saw were much greater than those attributed to serum separator tubes. Because the falsely increased results in neonates were at or near our decision point of 10 mg/L, we concluded that the VITROS CRP slide method was not suitable for use with our neonatal

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