Reason for Limitations of Heterophilic Blocking Tube Use on Certain Beckman Coulter Access Assays

To the Editor:
A recent Technical Brief by Ellis et al. (1) showed the usefulness of heterophilic blocking tubes (HBTs) in identifying false-positive specimens, thereby providing an estimate of the incidence of false-positive test results on several analyzers, including the Roche Elecsys and Abbott Architect. The authors of the Technical Brief went on to suggest that HBT treatment led to particular overrecoveries in several assays on the Beckman Coulter Access analyzer. It is important to know that the reason for this observed limitation is the unique design of those particular Access assays, which according to the Technical Brief (1) “contain solid-phase goat–anti-mouse [monoclonal antibody complexes].” This design (which can be identified by the Access package inserts) prohibits the use of any blocker containing murine or mouse components (e.g., HBTs). For those assays that contain an anti-mouse component, it is advisable to check for false positives by determining whether results are linear on dilution.

Reference

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Hypertriglyceridemia: Interaction between APOE and APOAV Variants

To the Editor:
Schaefer et al. (1) recently reported an association between a distinct combination of variants in the apolipoprotein E (APOE) and APOAV genes and hypertriglyceridemia. Among 170 hypertriglyceridemic (HTG) patients, all carriers of APOE22 (n = 7) had at least 1 APOAV Trp19 allele, but this combination was not found in controls with triglyceride (TG) concentrations within the reference interval.

APOE is a structural component of TG-rich lipoproteins; it serves as a ligand for lipoprotein receptors and plays an important role in the catabolism of remnant particles (2, 3). Of the 3 common apoE isoforms, apoE4 (Cys112→Arg) and apoE2 (Arg158→Cys) differ from the commonest isoform, apoE3, by a single amino acid substitution. The APOE4 allele has been shown to be associated with increased plasma cholesterol and with an increased risk of coronary heart disease. In contrast, the APOE2 allele is associated with low plasma concentrations of cholesterol and is believed to be protective against coronary heart disease [reviewed in Refs. (2, 3)].

APOAV variants (e.g., T→1131>C and Ser19→Trp) play an important role in modulating plasma TG concentrations in humans (4). An association between the APOAV Ser19→Trp polymorphism and TG concentrations has been found in many population samples, but the total impact of this variant is not the same in different ethnic groups (4–7). The Trp19 allele was found to be associated with extremely high concentrations of plasma TG (8), and Trp/Trp homozygotes have a higher risk of myocardial infarction (6). Recently, it was reported that apoAV interacts physically with lipoprotein lipase and significantly increases its activity (9). Computational analysis of the apoAV protein suggests that the change of Ser19 to Trp could lead to impaired export of apoAV from the liver (10).

Using a previously described method, we have analyzed (by PCR and restriction analysis) APOE and APOAV variants (T→1131>C, Ser19→Trp, and Val153>Met) (6, 11) in 2559 unrelated Caucasians. This population sample included 1191 males [mean (SD) age, 49.2 (10.8) years; TGs, 2.0 (1.3) mmol/L; total cholesterol, 5.8 (1.0) mmol/L; body mass index, 28.2 (4.0) kg/m²] and 1368 females [age, 48.8 (10.6) years; TGs, 1.5 (0.8) mmol/L; total cholesterol, 5.8 (1.2) mmol/L; body mass index, 27.6 (5.5) kg/m²] recruited as a representative 1% population sample in 9 Czech districts according the WHO protocol (12). Additionally, 111 HTG individuals [TGs >10 mmol/L; mean (SD), 22.4 (24.1) mmol/L; age, 51.4 (9.6) years] and 8 individuals with type III hyperlipidemia [all APOE22 genotype; TGs, 6.7 (6.7) mmol/L; age, 56.2 (13.8) years].

As described before, we have found an association between increased concentrations of plasma TG and the presence of the Trp19 allele (6). The same allele was also found to be more frequent in HTG patients (8). In contrast to Schaefer et al. (1), we found no significant interaction between the APOAV Trp19 variant, APOE2, and hypertriglyceridemia: of 111 HTG patients, 4 were carriers of the APOE22 genotype and 1 of these had the APOAV Trp19 allele. Of the
13 APOE32 heterozygotes, 5 had Trp at position 19. Among the HTG patients were 39 other Trp19 carriers, and but only 5 of them had at least 1 APOE2 allele (for more details, see Table 1).

In the population sample of 2559 individuals, we detected 20 APOE2 homozygotes, and 4 of them also had the APOAV Trp19 allele. Moreover, none of these 4 individuals had high TGs (1.37, 1.67, 2.32, and 4.52 mmol/L, respectively).

Finally, of 8 APOE22 homozygotes with type III hyperlipidemia, only 3 were carriers of the APOAV Trp19 allele.

On the other hand, it is noteworthy to mention that the proportion of carriers of the Trp19 allele was high among the HTG patients with APOE34 or APOE24 genotypes. In the HTG group, >50% of the patients with the Trp19 allele also had APOE42 or APOE43 genotypes, in contrast to only 13% of such individuals in the population (P < 0.001; for details, see Table 1).

The other APOAV variants (T−1131C and Val153Met) exhibit no interaction with APOE in the genetic determination of different forms of hypertriglyceridemia (details not shown).

In summary, our results exclude the possibility that there is an exclusive interaction between the APOE22 genotype and APOAV Ser19>Trp variant and hypertriglyceridemia in Caucasians, as suggested in the study by Schaefer et al. (1). In contrast, we detected a strong association between hypertriglyceridemia and a combination of the genotypes APOE34 (APOE22) and APOAV +Trp19. Whether the interaction between the APOE polymorphism and the APOAV Ser19>Trp variant can lead to hypertriglyceridemia in other ethnic groups needs to be analyzed further.

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References

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Table 1. APOE and APOAV variants in the population sample and in the HTG patients.a

<table>
<thead>
<tr>
<th>APOE genotype</th>
<th>Total study population</th>
<th>HTG patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APOE, n (%)</td>
<td>APOE, n (%)</td>
</tr>
<tr>
<td></td>
<td>Ser/Ser</td>
<td>Ser/Trp</td>
</tr>
<tr>
<td>Total</td>
<td>2198 (85.9)</td>
<td>352 (13.8)</td>
</tr>
<tr>
<td>e2/e2</td>
<td>20 (0.8)</td>
<td></td>
</tr>
<tr>
<td>e2/e3</td>
<td>318 (12.4)</td>
<td>4 (20.0)</td>
</tr>
<tr>
<td>e3/e3</td>
<td>1754 (68.5)</td>
<td>238 (13.6)</td>
</tr>
<tr>
<td>e4/e3</td>
<td>398 (15.6)</td>
<td>54 (13.6)</td>
</tr>
<tr>
<td>e4/e2</td>
<td>36 (1.4)</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>e4/e4</td>
<td>33 (1.3)</td>
<td>6 (18.2)</td>
</tr>
</tbody>
</table>

a Numbers and percentages (in parentheses) of individuals in either the total analyzed groups or (in bold) just for each row so that frequency of the Ser/Trp genotype could be compared among the carriers of different APOE genotypes.

b Significant differences (P < 0.005) between the total population and patients.
Mutations in K-ras Codon 12
Detected in Plasma DNA Are Not an Indicator of Disease in Patients with Non-Small Cell Lung Cancer

To the Editor:
The demonstration that cell-free circulating DNA detected in the plasma of cancer patients is genetically identical to that of the primary tumor has generated substantial interest, leading to >200 publications (http://www.ncbi.nlm.nih.gov). Recently, Wang et al. (1) reported in this journal that the method chosen for DNA isolation might contribute significantly to mutation detection (in their case, K-ras mutations in the plasma of patients with colorectal cancer). Briefly, they recommended the use of a modified guanidine/Promega resin method (G/R) to isolate DNA, affirming that this method enhances assay sensitivity. We used the same approach to detect K-ras mutations in the plasma of patients with non-small cell lung cancer (NSCLC) and compared the Qiagen vs the G/R method for isolation of circulating DNA. We purified DNA from plasma samples and cancer tissues from 12 patients. The DNA in 2 aliquots of the plasma from each patient was isolated by the Qiagen method (1,2) and by the G/R method according to Wang et al. (1). Additionally, DNA was isolated by the Qiagen method from matched plasma and tissue samples (n = 10 for each) and from 76 plasma-only samples (36 from cancer patients and 40 from cancer-free volunteers) according to Kopreski et al. (2). There was no difference in the number of K-ras mutations detected in the plasma samples collected from patients (n = 15; 41.7%) and from volunteers (n = 12; 30.0%; odds ratio = 1.6; P = 0.21). In addition, when we evaluated the presence of K-ras mutations in the matched plasma and neoplastic tissue samples, we observed no correlation. Finally, when we compared the results (K-ras status) for DNA samples isolated from plasma by the 2 different methods with the results obtained for the DNA isolated from tissue samples (12 patients), we observed K-ras codon 12 mutations in 2 different tissue samples, whereas we detected no mutations in plasma DNA isolated with the Qiagen method and 2 mutations different from those identified in the corresponding tumor tissue in the plasma DNA isolated with the G/R method. The correlation between controls and cases was not significant (χ² = 0.7; P = 0.5). The correlations between results obtained for tissue DNA and for plasma DNA isolated by the Qiagen or G/R method also were not significant (P = 0.4 and P = 1.0, respectively, nonparametric test for cases vs samples), nor was the correlation between the G/R and Qiagen isolation methods (P = 0.2).

These data show no relationship between K-ras mutations found in DNA from plasma and tumor tissue from NSCLC patients. Our results do not support the suggestion that K-ras mutations detected in plasma DNA are markers for tumor detection.

Ramirez et al. (3) analyzed K-ras mutations (codon 12) in tumor and paired serum DNA of 51 NSCLC patients undergoing surgery and detected mutations in 9 tumors and 12 serum samples. As suggested by Gautschi and Ziegler (4), serum may contain not only DNA of tumor origin, but also a variable fraction of DNA derived from in vivo- and in vitro-damaged hematopoietic cells. Indeed, lymphocytes stimulated with phytohemagglutinin or antigen may release DNA (5). Furthermore, human leukocytes stimulated by neutrophil-derived hydroxyl radicals may cause activation of K-ras codon 12 (6). In addition, inhaled particles in exposed individuals can generate reactive oxygen species that can activate K-ras (7). Moreover, K-ras mutations are detected in neogenetic lesions of subpleural fibrinous lesions, including ciliated bronchial epithelium and metaplastic epithelium (8). Because chronic inflammation may be present in patients without cancer as well as those with cancer (9), the analysis of K-ras mutations in plasma may be influenced by such factors. Finally, as reported recently by Keohavong et al. (10), K-ras mutations are frequently found in histologically normal tissues near tumors, suggesting that such mutations may represent an early event in the development of lung cancer. K-ras mutations thus may be present before clinically detectable tumors. As a final point, it is important to note that K-ras mutations have been detected in patients with ulcerative colitis, Crohn disease (11,12), and Helicobacter pylori-associated chronic gastritis (13). We observed K-ras mutation in 2 volunteers diagnosed with chronic gastritis, in 1 patient with kidney failure, and in 2 who were heavy smokers.

In light of these observations, plasma DNA assays for the detection of mutations in codon 12 of K-ras do not provide a reliable method to screen populations for the somatic mutations frequently found in neoplasms. Further confirmatory studies are required.

References
5. Rogers JC, Bolt D, Kornfeld S, Skinner A,