luted samples is loaded on aromatic sulfonic acid SPE column (500 mg; J. T. Baker Chemical Co., Phillipsburg, NJ) and then sequentially washed with methanol, 0.01 mmol/L HCl, 60 mL aqueous pyridine solution, and deionized water. The glycosides are eluted with 1 mL of a solution of 200 mmol/L NaHCO₃ and 470 mmol/L LiCl (pH 10.00) into a glass tube containing 0.130 mL of 2 mol/L NaOH. After evaporating the eluate, we reconstitute the residue with 1 mL of 200 mmol/L NaHCO₃ buffer (pH 9.20), add 1 mL of 20 mmol/L DABS-Cl solution in acetone, incubate at 70°C for 15 min, and extract with 1 mL of ethanol:water (70:30 by vol); 20 μL of the clear, upper layer is injected into the HPLC column.

We calibrate the assay with GGHYL and GGHYL glycans solutions (0, 25, 50, 100 μmol/L); the calibration curves are linear to 170 μmol/L. The detection limit is 2 μmol/L for both the analytes. Total interassay and intraassay CVs at low (10 μmol/L) and high (60 μmol/L) concentrations were 5% and 7%, respectively, confirming the data previously presented (8). Analytical recovery ranged from 98% to 106% for both analytes. Because the value of interest was a ratio (GGHYL/HHYL) rather than an absolute number, and the recovery of both the analytes was the same, no internal standard was necessary. The comparison between the data obtained for the GGHYL/HHYL ratio in human urines (n = 43) with the modified method (x) and with the method previously reported (y) (9) showed the following results: y = 0.96x - 0.02 (r = 0.93, S_yx = 6.18).

These modifications allow the determination of GGHYL/HHYL ratio in rat urine and can also be applied, with the same advantages, to the analysis of human urine. Using this modified method, we obtained reference intervals for the GGHYL/HHYL ratio in 24-h urine of 0.38–0.48 in normal rats (n = 20) and 1.30–1.60 in normal humans (n = 243). To fully understand the reasons for this difference, we are further studying the glycosylation of collagen in rat connective tissues.

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Increased Detection of Marijuana Use with a 50 µg/L Urine Screening Cutoff

To the Editor:

On September 1, 1994, the U.S. Department of Health and Human Services (DHHS) requirement for the immunoassay screening cutoff concentration of marijuana metabolite was decreased from 100 to 50 µg/L (1). Numerous studies have compared the effectiveness of various immunoassay reagents at 50 and 100 µg/L cutoff values on clinical or controlled specimens (2–9). Here we use historical data to determine the effect of the above reduction on the increased percentage of workers specimens that will screen positive for cannabinoids, the increased percentage of specimens that will be confirmed as positive for 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THCA) by gas chromatography–mass spectrometry (GC-MS), and the effectiveness of the drug detection program.

Specimens (n = 30 402) from unregulated commercial clients were screened on a Varian 5890B gas chromatograph with a Varian 11100 mass spectrometer equipped with an ESI source. After quality control (QC) of the mass spectrometer, the instrument was recalibrated with a standard solution at 50 pg/L. The specimens were assayed by an enzyme immunoassay assay for THC metabolites. The cutoff for THC was 50 pg/L.

Table 1. Distribution of GC-MS values for presumptive positive specimens.

<table>
<thead>
<tr>
<th>GC-MS conc., µg/L</th>
<th>Pos at 50 µg/L, neg at 100 µg/L</th>
<th>Pos at 50 µg/L, pos at 100 µg/L</th>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>0</td>
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<td>50-59</td>
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<td>4</td>
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<tr>
<td>&gt;60</td>
<td>205</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 1. Distribution of GC-MS values for presumptive positive specimens.

No. of specimens screened
92% had THCA concentrations ≥30 μg/L. Of the 97 specimens that screened negative at 100 μg/L, 80% had quantitative values for THCA of ≥30 μg/L. This suggests that reducing the screening cutoff to 50 μg/L will not result in laboratories being flooded with large numbers of specimens for which the THCA concentrations are within 20% of the 15 μg/L confirmation cutoff.

The THCA-positive detection rate for the 30 402 specimens analyzed at the 100 μg/L cutoff was 0.94%. This rate was increased to 1.26% by lowering the cutoff to 50 μg/L. We conclude that the effectiveness of drug detection programs will be enhanced by reducing the screening cutoff for cannabinoids. The cost-effectiveness of a company’s drug testing program is often determined by calculating the cost per positive test. However, the true expense of drug testing is based on the cost of collection, transportation, laboratory analyses, and Medical Review Officer services. The additional specimens associated with positive results will result in an increased laboratory cost for the additional confirmations, but the other costs involved in drug testing will remain the same. Therefore, the cost-effectiveness of a company’s drug testing program based on the cost per positive specimen will actually decrease because of the increased detection rate for cannabinoid use. Any increase in cost due to additional confirmations will be offset by economic savings to employers because of financial liabilities associated with the employment of drug users.

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Lipoprotein(a) Further Invalidates Friedewald Formula

To the Editor:

Li et al. in a recent article (1) concluded that the Friedewald formula should be modified to take into account the contribution of lipoprotein(a) (Lp(a)) cholesterol to total serum cholesterol. Earlier (2), we had shown that the Friedewald formula is invalid for estimating low-density lipoprotein (LDL) cholesterol because it provides cholesterol values that derive from both LDL and Lp(a) cholesterol. This is particularly true for subjects with high concentrations of plasma Lp(a). Until suitable methods for the independent measurement of LDL and Lp(a) cholesterol are developed and generally accepted, it should be recognized that LDL cholesterol values calculated from the Friedewald formula are inaccurate.

References

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Lipoprotein(a) is included in Low-Density Lipoprotein by NCEP Definition

To the Editor:

The recent report by Li et al. (1) appropriately demonstrates the contribution of lipoprotein(a) (Lp(a)) to low-density lipoprotein cholesterol (LDL-C) as estimated by the Friedewald equation (2). Since the Friedewald equation is used almost universally in the routine clinical laboratory to estimate LDL-C, the primary decision quantity in determining the risk of cardiovascular heart disease (CHD) and monitoring treatment, this observation will be of interest to many laboratory managers. The authors’ recommendation that the Friedewald equation be modified to account for the contribution of Lp(a) deserves comment.

The complicated nature of the lipoproteins leads to complex nomenclature and consequent confusion about the terminology. The Friedewald equation (2) [LDL-C = total C – (HDL-C + 0.2 triglycerides)], which has provided a convenient approach to approximating LDL-C, was proposed in 1972. Lp(a), although reported in 1963 (2), was not well characterized during the subsequent decade. The Friedewald triglyceride (TG) factor was selected to approximate the very-low-density lipoprotein cholesterol (VLDL-C) obtained by ultracentrifugation as part of beta-quantitation (BQ), the common research method used in the US Lipid Research Clinics and other major population studies (4). BQ is now generally accepted as the accuracy target for lipoprotein quantification and is the basis for the Reference Method for LDL-C used by the Centers for Disease Control and Prevention (CDC) (5–6). The BQ method separates VLDL-C by ultracentrifugation at d 1.006 kg/L and high-density lipoprotein (HDL-C) after precipitation of the other lipoproteins. LDL-C is calculated as the cholesterol in the d > 1.006 kg/L fraction minus that in the HDL fraction. Since the precipitation methods precipitate Lp(a) together with LDL-C and VLDL-C, the LDL-C fraction determined by BQ includes any Lp(a) present as well as the usually small amounts of intermediate-density lipoprotein (IDL-C) with d 1.006–1.019 kg/L. Thus, BQ (and the CDC Reference Method) obtains a so-called “broad-cut” LDL-C fraction that is approximated reasonably well by the Friedewald equation, avoiding the tedious ultracentrifugation step.

An expert laboratory panel of the US National Cholesterol Education