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Contamination of Breath Methane Samples in Sterilized Vacutainer Tubes

To the Editor:

In gas-chromatographic measurement of breath methane to investigate its relationship to various carbohydrate malabsorption conditions (1, 2), breath samples often have to be stored for considerable periods before analysis. Sterilized Vacutainer Tubes (Becton Dickinson, Rutherford, NJ 07070), filled with the breath sample under positive pressure and the stoppers secured with adhesive tape, have proved to be a dependable means for storage of breath samples (3, 4).

In investigation of the viability of Vacutainer Tubes for breath methane storage, extremely high methane concentrations for both control and study subjects were discovered and contamination was suspected. I then undertook a study for the presence of methane in Vacutainer Tubes. Methane was detected in sterilized tubes that were silicone coated (Table 1).

Methane was measured with a Model 16 Microlyzer equipped with a molecular-sieve chromatographic column (Quintron Instrument Co., Inc., Milwaukee, WI 53215). Dry air was used as the carrier gas at a flow rate of 35 mL/min. The chromatograph was calibrated with a methane reference mixture in compressed air (Quingas; Quintron Instrument Co.). The smallest detectable concentration of methane was 2 μL/L, with a linear accuracy response range of 2–200 μL/L (5, 6). The retention time for methane elution is 1.2 min. Gas samples for analysis were obtained by injecting a 20-mL sample of room air into a vacuum-intact Vacutainer Tube with a syringe and needle, and then, after 20 min, withdrawing this volume from the Vacutainer Tube into a second syringe. This sample was then injected into the microlyzer (7). A baseline sample of room air was analyzed simultaneously and gave a reading for methane of 0 μL/L.

Jensen et al. (7) noted similar results with hydrogen contamination, and also remarked on the presence of methane, although the amount was not quantified.

Vacutainer Tubes are sterilized by use of ionizing radiation. Ionizing radiation crosslinks silicone polymers; consequently, there is a splitting out of hydrogen, methane, carbon dioxide, carbon monoxide, ethane, and water-soluble silanols (8). Thus, the sterilization process of the silicone-lined Vacutainer Tubes is the cause of the interfering contaminants. In sterilized non-silicone-coated Vacutainer Tubes the presence of methane was negligible.

I recommend that breath methane samples not be stored in irradiated silicone-coated Vacutainer Tubes. Non-silicone-coated tubes may be used without undue contamination.

References

E. Schönau
H. Singer

Table 1. Methane Content of Sterilized Vacutainer Tubes

<table>
<thead>
<tr>
<th>Vacutainer Tube</th>
<th>Methane, μL/L</th>
<th>Range for five tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>6530°</td>
<td>204–248</td>
<td></td>
</tr>
<tr>
<td>8432°</td>
<td>133–143</td>
<td></td>
</tr>
<tr>
<td>VT 100P</td>
<td>0–2</td>
<td></td>
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</tbody>
</table>

* Range for five tubes.
**Siliconized tubes.

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Two Specific Immunoassays of Cyclosporine Compared in Liver Transplant Recipients

To the Editor:

Cyclosporine (Cs A) measurement in blood has been used to guide dosing and reduce the risk of nephro- or hepatotoxicity in patients while maintaining adequate immunosuppression (1, 2). Abbott Laboratories (Abbott Park, IL) has recently developed a nonradioactive monoclonal immunoassay for Cs A in whole blood, based on fluorescent polarization (FPIA). Yatscoff et al. (3) reported a good correlation between the FPIA assay and the Sandimmune selective RIA in blood specimens from renal transplant recipients.

Because the metabolism of Cs A and the excretion of metabolites are strongly influenced by hepatic disease, we compared the Cs A results obtained with two specific assays, FPIA (Abbott) and RIA Cyclo-Trac SP (Incstar, Stillwater, MN), which involve the use of two different monoclonal antibodies: one for one of the metabolites from four liver transplant recipients (average post-transplant time, 89 days; range, 1–335 days). Both assays were performed according to the manufacturers’ instructions (4, 5). A multicytcrystal gamma counter (LB 2104; Berthold, München, F.R.G.) measured 125I activity (counting time per sample, 120 s). The FPIA response was measured with TDX instrumentation (Abbott).

We also analyzed the serum specimens for bilirubin (Hitachi 717; Boehringer, Mannheim, F.R.G.), aspartate aminotransferase (EC 2.6.1.1; AST), and alanine aminotransferase (EC 2.6.1.2; ALT) to determine the extent of liver damage in these patients. The mean (range) for bilirubin, AST, and ALT concentrations were 86.1 (21.7–240.2) mg/L, 91.97 (6–2085) U/L, and 137.8 (10–1495) U/L, respectively.

The average overall CVs were 4.63% and 7.99% for the FPIA and RIA methods, respectively. The regression analysis for Cs A concentrations determined with the Cyclo-Trac (x) and TDX (y) assays was y = 0.972x + 134.13 (r = 0.89, n = 52, S_p = 56.08 µg/L).

To see whether the discrepant Cs A results we found between methods could be attributed to liver damage, we investigated the relationships between bilirubin, AST, and ALT concentrations and the corresponding TDX/Cyclo-Trac ratio. The comparison among all patients showed no significant correlations. The correlations for individual patients are shown in Table 1.

Cs A values measured by the two methods show a weak correlation, the TDX values being higher than those of Cyclo-Trac. This is probably due to the greater cross-reactivity of the antibody used in the TDX assay with the primary Cs A metabolites: M-17 (8.2%), M-1 (15.3%), and M-21 (3.7%) (3). In contrast, the cross-reactivity of the Cyclo-Trac antibody with these metabolites is <2% (6). The metabolites that produce the greatest cross-reactivity in the TDX assay are usually in high concentrations in the blood of transplant patients; moreover, the relative concentrations of Cs A and each metabolite differ considerably among patients (7).

In our group, bilirubin concentrations were always above normal (mean 86 mg/L). Because excretion in the bile is the major pathway for eliminating Cs A metabolites (8), perhaps the higher Cs A results in the TDX assay are explained by the impaired excretion of Cs A metabolites through the biliary system.

In summary, caution seems to be warranted in monitoring Cs A by different specific methods in liver transplant recipients; the differing cross-reactivity of the antibodies may result in substantial disagreement in Cs A values between methods.

References

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Serum Fructosamine and Obesity

To the Editor:

Serum fructosamine is widely used as an indicator of the short-term control of diabetes mellitus. Its value is influenced by the mean blood glucose concentration in the previous two to three weeks as well as by the half-life of serum proteins. Repeatedly observed low concentrations of serum fructosamine, despite poor control in obese Type 2 diabetic patients, prompted us to quantify fructosamine in obese healthy persons.

We measured the serum fructosamine concentrations according to Johnson et al. (1) in 25 healthy non-obese persons (mean age 36, range 20–45 years) and in 25 obese subjects (mean age 33, range 19–50 years). A significant difference in the mean (±SD) body mass index was observed...