

## TECHNICAL NOTE

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# Improper Sealing Caused by the Styrofoam Integrity Seals in Leakproof Plastic Bottles Lead to Significant Loss of Ethanol in Frozen Evidentiary Urine Samples\*

**ABSTRACT:** Evidentiary urine samples ( $n = 345$ ) stored frozen at  $-20^{\circ}\text{C}$  in their original containers (leakproof 100 mL plastic bottles) upon re-testing for ethanol resulted in concentrations that were significantly lower (average loss =  $-30\%$ ) than those prior to their storage at  $-20^{\circ}\text{C}$  ( $p \leq 0.0001$ ). The observed loss of ethanol was independent of the method of thawing or the concentration of ethanol in the samples, but was dependent on the sample volume in the container, i.e., the larger the volume of sample the larger the magnitude of ethanol loss. The loss of ethanol was determined to be due to improper sealing by a Styrofoam integrity seal attached to the mouth of the container. Accordingly, adopting leakproof plastic containers that do not contain Styrofoam integrity seals, but rather an outside and across the cap tape integrity seal for evidence collection and long-term storage, will prevent loss of ethanol due to evaporation.

**KEYWORDS:** forensic science, ethanol, urine, head-space gas chromatography

Social use of ethanol and its abuse continues to be important from the viewpoint of clinical and forensic interest (1). Accordingly, almost every state in the United States and many foreign countries around the world have per se laws governing the measurement of ethanol concentrations for forensic purposes (1). The goal of measuring ethanol concentrations is to establish the degree of intoxication either at the time of sample collection or another time. For this purpose, one of three principal media, viz., blood, breath and/or urine, are used to measure ethanol concentrations. Often the sample choice is single and ethanol concentrations in the other samples may need to be derived. If the sample of choice were urine, then the interpretation of the degree of intoxication would be difficult but not impossible, because urine is not a dynamic body fluid like blood. Accordingly, the accuracy of measurement of ethanol concentrations in evidentiary samples and their interpretation, especially in the case of urine, are extremely important.

The accuracy of measurement and interpretation of ethanol results may be compromised for many reasons. One such scenario is the presence of high concentration of sugar, contamination of such samples by ethanol producing/metabolizing bacteria and/or yeast,

e.g., *E. coli* and *C. albicans*, and storage of contaminated samples at room temperature that promotes in vitro ethanol production/consumption (2-8). To correct such a situation NaF (10 mg/mL) is added to samples at the time of collection and the samples are stored at  $0-4^{\circ}\text{C}$  prior to analysis (9-11). The latter corrective step is made possible by collection of samples in containers having a fixed amount of sodium fluoride. This in turn will lead to the presence of elevated levels of NaF in samples because small volumes of samples are collected in bottles containing a fixed concentration of NaF, which has been shown to result in salting out of ethanol during storage and analysis (9,10,13).

Improper storage methods and procedures may also result in compromising the accuracy of measurement and interpretation of ethanol results. Described herein is one such scenario, which we have discovered and corrected. We, like many other laboratories, routinely treat urine samples with NaF (10 mg/mL) and store them at  $4^{\circ}\text{C}$  prior to any toxicological analysis. After the completion of ethanol analysis, the urine samples are treated in one or more of the following ways. First, urine samples are stored frozen at  $-20^{\circ}\text{C}$  until they are tested for drugs of abuse (a preventive measure to preserve drugs of abuse and their metabolites). Second, urine samples are stored frozen at  $-20^{\circ}\text{C}$  until they are returned to the agencies that collected them for safekeeping and/or to use them as items of physical evidence in court proceedings. Third, urine samples are stored frozen at  $-20^{\circ}\text{C}$  until they are transferred to defense counsel for re-testing of ethanol and drugs of abuse when the state laboratory results are disputed. Finally, urine samples are stored frozen at  $-20^{\circ}\text{C}$  until they are destroyed when they are no longer required. Although this practice has been discontinued lately, the

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