EDUCATIONAL COMMENTARY – URINE TESTING FOR ALCOHOL AND ETHYL GLUCURONIDE

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Learning Outcomes

On completion of this exercise, the participant should be able to

- recognize settings in which alcohol detection would be useful;
- explain what ethyl glucuronide (EtG) is and how it relates to alcohol;
- understand causes of false-positive or false-negative results in detection of alcohol in urine;
- determine when testing for EtG would be more informative than testing for alcohol itself; and
- know under what circumstances EtG is used as proof of alcohol use in legal proceedings.

Introduction

Alcohol, one of the most frequently abused substances, is also difficult to monitor, as it is metabolized quickly in the human body. Although consumption of alcohol is legal in most situations, alcohol can cause significant mental and physical impairment when consumed in excess. In the United States and other countries the consumption of alcohol is regulated by age, retail sale is regulated by time of day or seller type, and use is restricted in public spaces and in relation to operation of motor vehicles. Most employers prohibit employees from consuming alcohol during working hours and have disciplinary policies to deal with employees whose consumption of alcohol at any time affects their ability to perform job duties.

Alcohol concentrations are most frequently tested in law-enforcement situations such as traffic stops or other situations that could be linked to the use of alcohol, in addiction recovery programs, and in pre-employment drug screening. Alcohol can commonly be detected in urine, breath, saliva, and/or blood. Although laboratory testing for alcohol is highly sensitive and specific, alcohol can be a difficult substance to monitor, as it metabolizes within hours of use.

Ethanol, the chemical name for alcohol, is assayed most commonly in the blood or urine. Typically, testing is used to quantify the concentration of alcohol in the body, which is related to the amount consumed by the individual in the form of food or drink (beer, wine, or liquor). Incidental amounts of alcohol can also be identified from the use of mouthwashes, medication that contains alcohol, and some hand sanitizers.¹
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(cont.)

Chemically, alcohol is defined as any organic compound whose molecule contains hydroxyl functional group(s) bound to a saturated carbon atom. Fermentation is an anaerobic chemical process where yeast, either naturally occurring or intentionally added, interacts with carbohydrates, particularly sugars. When fruit or grains ferment, the organic compound ethyl alcohol is generated as a byproduct. This compound, when consumed in excess, can be responsible for the mental and physical impairment associated with alcohol abuse.

The human body metabolizes alcohol via an oxidative pathway and a nonoxidative pathway. During oxidative breakdown, ethanol is converted to acetate (Figure) and is excreted from the body, mostly in the urine, although small amounts may be excreted in sweat or exchange of breath.

<table>
<thead>
<tr>
<th>CH₃CH₂OH</th>
<th>ADH</th>
<th>CH₃CHO (ACETALDEHYDE)</th>
<th>ALDH</th>
<th>CH₃COO⁻ (ACETATE)</th>
</tr>
</thead>
</table>

Figure. The Chemical Breakdown of Alcohol.²

On ingestion of any substance containing alcohol, about 20% of the ethanol is absorbed in the stomach and 80% in the intestines. The ethanol is then distributed into the total body water, where it cycles through the liver. Ninety percent to 95% of the ethanol is broken down in the liver by aldehyde dehydrogenase and microsomal ethanol-oxidizing enzymes before it is eliminated in the urine. This oxidative pathway is responsible for alcohol’s pathologic effect, physical and mental impairment, on the human body. The remaining 5% to 10% of ethanol is expelled in sweat, urine, and air exchange. However, a small amount, less than 1%, follows the nonoxidative pathway, which produces the byproducts ethyl glucuronide (EtG) and ethyl sulfate.³

**Ethyl Glucuronide**

Ethyl glucuronide is a nonvolatile, water-soluble metabolite of ethanol. Literature about EtG dates back to the 1950s when it was isolated from rabbit urine.¹ It is described as “... a minor non-oxidative metabolite of alcohol formed by the in-vivo conjugation of ethanol with activated glucuronic acid in the presence of membrane bound mitochondrial UDP glucuronyl transferase in the liver. Only .02-.04 percent of alcohol is metabolized by this pathway, however, EtG can be detected in urine for up to three to five days following consumption of alcohol. EtG is not detectable unless alcohol has been consumed.”⁴ Commonly, EtG testing is used to document recent alcohol consumption and to confirm abstinence in treatment programs or in the workplace. In legal proceedings, results of EtG testing are accepted as
proof of alcohol consumption. The presence of EtG in urine can be used to demonstrate ingestion of alcohol after ethanol is no longer measurable. Although EtG can be detected in the urine, the concentration does not indicate the amount of alcohol consumed. At this time no documented studies exist that correlate EtG concentrations with alcohol concentrations in blood or breath. Testing for EtG is common in the United States and is commercially available.

Laboratory Testing

Alcohol can be identified in a variety of specimens in a variety of ways. Most common are immunoassays for ethanol in the urine and in the blood. Immunoassays are highly specific as they measure the precise binding between an antibody and antigen in the presence of alcohol. However, the sensitivity of the assay depends on the timing of the assay; as alcohol is metabolized quickly, there is a very short window of time when it can be detected in the blood, breath, saliva, and urine. A more sensitive test is the immunoassay for EtG, as the metabolite remains present longer. This byproduct of the nonoxidative pathway can be detectible and indicative of alcohol consumption up to 5 days. If confirmatory testing is required, mass spectrometry for EtG is recommended. Mass spectrometry is a highly sensitive and specific testing methodology that ionizes a substance and sorts the ions based on their charge to mass ratio to identify its exact chemical makeup.

Because the sensitivity of EtG is high, any consumption of alcohol or application of products containing alcohol can be detected. It is important that cutoff values have been established to differentiate between true alcohol consumption and the use of mouthwash, for example. Because at low levels it is not possible to distinguish consumption of alcohol from exposure to certain personal products, punitive measures are discouraged when low levels are detected, and combination testing of other biomarkers is recommended.

False-Negative Results

Although EtG is stable for up to 5 days after consumption of alcohol, there is one notable instance in which false-negative results can occur. When a patient has a urinary tract infection caused by the bacteria *Escherichia coli*, hydrolysis by bacterial glucuronides can cause EtG to degrade at a faster rate. Because of this, EtG testing could result in a false negative.

Specimen handling can also affect the detection of EtG in urine. If the urine sample is not preserved and is left at room temperature, overgrowth of bacteria can occur, resulting in a degraded sample.
False-Positive Results

False-positive results are seen more frequently than false-negatives; however, they are more difficult to differentiate since many topical hand-hygiene products, medications, and mouthwashes contain ethanol that can be absorbed by the body. Because EtG testing is highly specific, use of such products or consumption of even small amounts of certain foods can lead to a positive result as they, too, would produce EtG in the body.

Legal Considerations

According to the National Institute on Alcohol Abuse and Alcoholism (NIAAA), a 2015 survey documented that 26.9% of individuals 18 years of age and older reported binge drinking in the past month, and 7% reported engaging in heavy alcohol use in the past month. NIAAA defines binge drinking as “a pattern of drinking that brings blood alcohol concentration (BAC) levels to 0.08 g/dL. This typically occurs after four drinks for women and five drinks for men—in about 2 hours.” Heavy use was defined as “binge drinking on five or more days in the past month.” Additionally, nearly 20% of patients treated in general medical practice report drinking at levels considered risky or hazardous.

Legal reasons for testing include investigation of underage drinking, drunk driving, or alcohol consumption that has contributed to an accident or a crime; verification of whether a parolee has complied with conditions of parole; and employee drug testing, pre-employment screening, or on-the-job accident investigations. For legal reasons, the chain of custody of the sample must be unbroken; this means that each handoff must be documented, from collection to test results. Ethyl glucuronide is used to detect alcohol levels in the urine by the military, employers, courts, and the penal system. Because false-positive results can occur, EtG testing with legal ramifications has been scrutinized, as it can be difficult to differentiate incidental exposure to ethanol from alcohol consumption.

Conclusion

Alcohol consumption can be tested for in a variety of settings and results of this testing can be used as legal documentation in a court of law. Testing for ethyl glucuronide, a byproduct of the nonoxidative pathway of alcohol breakdown, allows alcohol to be detected up to 5 days following consumption in comparison to testing for ethanol, which cannot. Immunoassays and mass spectrometry assays can detect even trace amounts of EtG, so false-positive results are seen more commonly than false-negatives. False-positive results are more difficult to detect, as the EtG molecule is identical in the regulated and nonregulated substance.
References


