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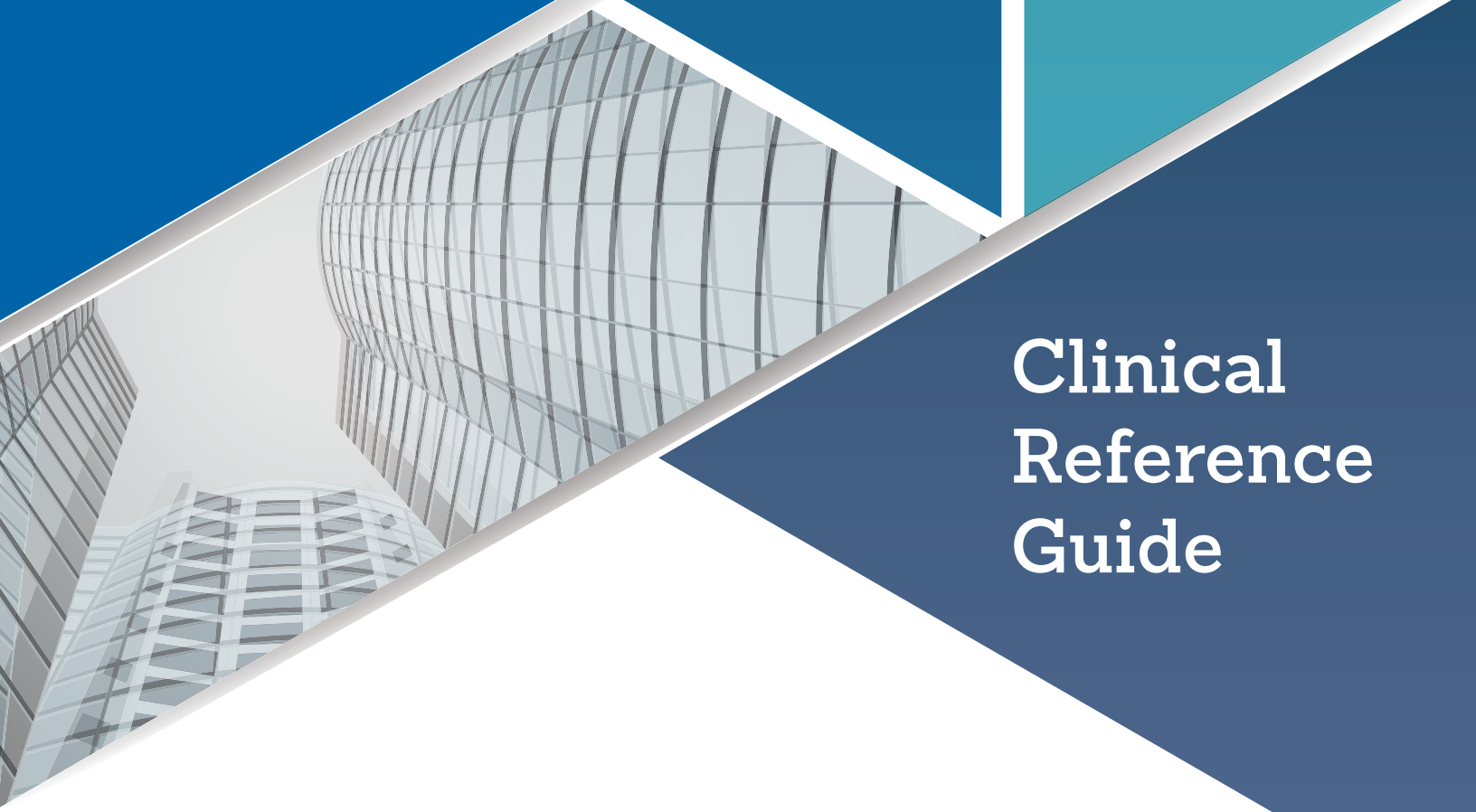

Clinical Reference Guide

Testing for Alcohol Use

Testing for Alcohol Use

Ethyl sulfate (EtS) is a metabolite of alcohol that offers an extended period of detection compared to ethanol. It is detectable for up to three days with typical ingestion but does not provide information regarding degree of intoxication nor the amount of alcohol ingested.

Aegis offers tests for two markers for detection of ethanol use: ethanol and ethyl sulfate (EtS).

A. Ethanol

Aegis offers testing for ethanol in blood, oral fluid, and urine. Ethanol testing determines very recent ingestion with a period of detection less than eight hours.[1-4] Urine ethanol tests may be positive due to post-collection fermentation rather than ethanol ingestion. For example, diabetic patients may excrete glucose in urine; if yeast species such as Candida albicans are present, glucose may then be fermented to ethanol.[1] Yeast is the predominate microorganism implicated in post-collection fermentation to ethanol; however, certain species of bacteria are capable of producing ethanol from glucose as well.[5] A recent study of urine specimens submitted to a laboratory for testing in pain management demonstrated that approximately one-third of ethanol positives were caused by fermentation.[6]

This is concerning, especially when considering that up to 24% of individuals with diabetes are undiagnosed.[7] While not reported in the literature, it is theoretically possible for the presence of ethanol to be detected in oral fluid due to fermentation of sugar by microbial species present in the oral cavity. Candida is found in the mouth of up to 60% of healthy individuals. Excessive amounts of yeast may be present in the oral cavity of patients who are immunocompromised, diabetic, receiving oral corticosteroids and/or broad-spectrum antibiotics, or wearing dentures.[8] Due to the short period of detection and the documented risk of positive results from urine fermentation, ethanol testing is not recommended to evaluate abstinence from alcohol ingestion.

B. Ethyl Sulfate

Healthcare providers may benefit from testing for the direct metabolite of alcohol, EtS. It is excreted as quickly as one hour following alcohol ingestion and has an estimated period of detection of up to three days, detection of up to 3 days, depending on patient-specific factors, amount ingested, and chronicity of ingestion.[9] In rare cases, EtS has been observed up to five days in alcoholic patients undergoing detoxification.[10]

EtS testing tends to be most useful to a provider in cases where a patient's complete abstinence from alcohol is considered part of the treatment agreement. They are generally not useful for identifying cases of alcohol abuse given the lack of correlation between the amount ingested and urine concentration.

Correlating EtS Concentrations to Amount Ingested

EtS concentrations do not correlate to the amount of alcohol ingested. The metabolism and excretion of EtS is highly variable between subjects, and urine concentrations may vary over time in a single subject even when consistent amounts of alcohol are ingested. Therefore, a positive result indicates relatively recent alcohol ingestion, but does not specify the amount ingested.

In addition, EtS presence in urine does not correlate to impairment, as detection of EtS in urine exceeds the time course of ethanol in blood.[11] Consequently, a positive EtS test does not mean the patient was intoxicated at the time of collection.

Incidental Exposures

When interpreting alcohol marker results, it is important to consider exposure to unexpected sources of alcohol/alcohol metabolites outside of alcoholic beverages. Aegis tests for EtS at a conservative threshold of 200 ng/mL. Below is a discussion of the literature regarding exposures to alcohol and/or its metabolites not related to consumption of beverages such as alcoholic beer, liquor, or wine. In the following discussion, concentrations reported as normalized to a creatinine of 100 mg/dL will be indicated with 100 in

subscript (e.g., EtS_{100}).

- Electronic Cigarette Use

Certain liquid formulations used in electronic cigarettes may contain alcohol, and thus, potentially lead to a positive result for an alcohol marker. A retrospective analysis was conducted in 2021 that found ethanol present in nicotine pods, nicotine refill formulations and DOTN (drugs other than nicotine) formulations, which ranges from not present to 217.2 mg/mL. [12] No studies have yet evaluated whether or not EtS is excreted in detectable amounts following the use of electronic cigarettes or vaping.

- Hand Sanitizer Use

Several studies have addressed alcohol metabolite concentrations secondary to hand sanitizer use. A recent study involved pregnant nurses who used hand sanitizer (62% alcohol) as usual during their workday with a reported mean use of 34 times during an eight-hour shift. All five nurses were negative for EtS at a 25 ng/mL threshold. [13]

More intensive application of 62% ethanol hand sanitizer (every five minutes for ten hours on three consecutive days) did not contribute to EtS levels above 100 ng/mL. [14] For individuals working in settings requiring intensive ethanol based hand sanitizer use, a thorough evaluation of the amount of exposure may be required to determine if the exposure exceeds those of documented studies in the literature.

- Yeast and Sugar Ingestion

Ingestion of active baker's yeast (42 g) combined with sugar has resulted in urinary concentrations of EtS up to 1,410 ng/mL; this is thought to be due to in vivo fermentation of sugar into alcohol in the presence of yeast. In the same study, ingestion of 10 g (20 tablets) of brewer's yeast with sugar did not result in measurable levels of EtS, which may indicate a lack of "active" yeast in the product consumed. [15]

Although ingestion of active baker's yeast may lead to unexpected EtS positive results, ingestion of this product in the fashion of this study (e.g., raw consumption) is an uncommon practice. Brewer's yeast is sold as a dietary supplement for health benefits. Although the brewer's yeast product in this study did not result in EtS positives, "active" versions are also available and may potentially lead to unexpected positives; however, unexpected positives are unlikely with "inactive" products.

- Food Ingestions

Certain commonly consumed foods may contain trace amounts of ethanol due to fermentation of glucose; ethanol formation is more likely in unpreserved products or during poor storage conditions. In a recent German study, EtS levels did not exceed Aegis thresholds in patients ingesting up to 2 L apple juice, 1,320 g sauerkraut, or 690 g bananas. However, subjects experienced EtS levels up to 650 ng/mL after ingesting a large amount (1.5-2L) of grape juice. Analysis of grape juices revealed the products contained small amounts of EtS in addition to ethanol.[16] Therefore, it may be advisable for patients requiring abstinence from ethanol to also refrain from drinking excessive amounts of grape juice.

- Nonalcoholic Beverages

Despite the label "nonalcoholic", small amounts of ethanol (e.g., less than 0.5 vol. %) may be present in nonalcoholic beers and wines; [17] therefore, consumption of large amounts may lead to unexpected positive results. Ingestion of 2.5 L of nonalcoholic beer (up to 0.42 vol % ethanol) resulted in levels up to 11,800 ng/mL for EtS100, respectively in one study. Ingestion of outside alcohol by one subject was suspected and may be the cause of the high concentrations observed. The other three subjects in this study had peak concentrations that did not exceed 100 ng/mL for EtS100. [18] In contrast, a separate study involving the ingestion of a similar amount (2-3 L) of nonalcoholic beer (0.36 vol %) showed EtS concentrations up to 169 ng/mL. [16]

Detectable urine concentrations of EtS, up to 2,150 ng/mL EtS100, were observed following consumption of a 7.5 dL bottle of "nonalcoholic" wine in one study. [19] Since a small percentage of alcohol and/or alcohol metabolite may be present, patients requiring complete abstinence from alcohol should avoid consumption of

these beverages.

Kombucha is a popular fermented health beverage made from tea, sugar, yeast, and bacteria. If Kombucha has an alcohol content less than 0.5%, it is considered a nonalcoholic beverage; however, the alcohol content has been reported as high as 3% in some products.[20-22] The likelihood of a positive result for ethanol or alcohol metabolite from ingesting Kombucha will depend on the alcohol content, the quantity ingested, and the individual patient's pharmacokinetics.

- Mouthwash

A couple of studies have addressed frequent use of alcohol-containing mouthwash. The first study involved participants rinsing with 15 mL of 21.6% ethanol mouthwash for a minute every 30s for a total of eight rinses.[19] In the second study, participants rinsed for 30s with 20 mL of 26.9% ethanol mouthwash four times per day for three and one quarter days.[23] In both studies, EtS concentrations did not exceed Aegis thresholds.[19,23]

- Alcohol-Containing Medications

Ethanol is a common inactive ingredient in liquid medications. Over the counter (OTC) medications are limited in the amount of alcohol that may be present in each product. In 1995, the Food and Drug Administration (FDA) issued a final rule that the maximum allowable limit of ethanol as an inactive ingredient in orally ingested OTC products is 10%; the limit is even lower for products intended for children. The alcohol content must be apparent and appear on the front of the product's label. [24] However, certain prescription liquid medications may contain greater amounts of alcohol; for example, the labeling for paregoric liquid reports 45% alcohol content. [25] When considering alcohol-containing medications as a potential source of alcohol metabolite positives, it is important to evaluate the percent of alcohol content in the medication, the dose of the medication, how often and for how long the patient has been taking the medication, and the concentration of EtS in urine. These should be considered together to determine the likelihood that an alcohol metabolite positive is from medication versus ingesting alcoholic beverages. If a patient reports OTC cough and cold medication as a source, it is important to note that several alcohol-free OTC cough and cold formulations are available that may provide an alternative medication for treatment of their symptoms.

- Post-Collection *In Vitro* Production

Post-collection synthesis of EtS was assessed in one study in which EtS failed to form in specimens containing E. *coli*, Klebsiella *pneumoniae*, or Enterobacter *cloacae* in the presence of ethanol or sugar and yeast.[26] However, the limited range of bacterial species studied does not completely rule out the risk of post-collection fermentation. One laboratory has reported concern for potential post-collection synthesis of EtS based on one specimen increasing in EtS concentrations after being stored at room temperature for a year.[27]

EtS is generally considered not to be produced via post-collection fermentation, but more supporting literature is needed. EtS has also been shown to be relatively stable against bacterial degradation. EtS has repeatedly shown stability in the presence of bacteria.[26-28]

- Autobrewery Syndrome

In the literature, case reports of patients with elevated blood ethanol and intoxicated symptoms in the absence of ethanol ingestion have led to the concern that certain individuals may endogenously produce alcohol.[29,30] This is suspected to be due to the combination of bowel stasis, overabundance of yeast in the bowel, and high carbohydrate ingestion.

Autobrewery syndrome is controversial and has not been fully validated in medical literature.[30,31] However, based on case reports, it may be considered as a potential explanation for a positive alcohol marker in patients with combined factors of severe bowel dysfunction, risk of yeast infection (e.g. antibiotics use, immunocompromised), and high carbohydrate diet.[30,31] Autobrewery syndrome should be a rare explanation, and if suspected, patients should be referred for further evaluation and treatment (e.g. antifungal therapy, dietary restrictions).[29-30]

A summary of the potential sources for alcohol metabolite positives can be found in Table 1.

Additional Consideration

Given that EtS is formed by enzymes in the body which are subject to genetic variation, variable excretion patterns for EtS may be observed.[32]

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Table 1: *Potential Sources for Alcohol Metabolite Positives*

POTENTIAL SOURCES
Alcohol-containing beverages (beer, liquor, wine)
Alcohol-containing medications
Autobrewery syndrome[29-30]
Electronic cigarette use[33]
Excessive hand sanitizer use[14,34]
Ingestion of baker's yeast with sugar[15]
Ingestion of large amounts of grape juice[16]
Ingestion of large amounts of nonalcoholic beer or wine[16,18,19]
Kombucha[20-22]

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