Classification: N/A

Background: Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS) are direct ethanol metabolites produced by a minor pathway of the liver. EtG and EtS are considered highly sensitive, water soluble, non-volatile metabolites that can be detected at quantifiable levels in hair, urine, saliva, plasma and body tissues up to 80 hours after use whereas parent alcohol testing is limited to 8-12 hours for detection of consumption. The primary mode of metabolism of ethanol is through the alcohol dehydrogenase pathway (ADH). However, the microsomal ethanol oxidizing system (MEOS) has become very important to the forensic and clinical communities for producing metabolites that can be tested for an extended period of time over their parent compound. This enzymatic process is a minor pathway that is specifically selective to ethanol and not to any other common alcohols such as methanol and isopropyl alcohol (common household rubbing alcohol). If EtG and EtS (or only EtS), are present in the urine it is from the direct exposure to or ingestion of ethanol, or alcoholic beverages.

Metabolism and Detection in Urine: Elimination of ethanol through the body is primarily through the liver (>90%) via enzymatic oxidation with 5-10 % eliminated unchanged through the kidneys, skin, sweat, and lungs. To date, the metabolism of ethanol occurs by three pathways: alcohol dehydrogenase pathway (ADH), microsomal ethanol oxidizing system (MEOS) (cytochrome P450 [CYP] 2E1), and the peroxidase-catalase system. The MEOS pathway produces the phase-II metabolites of ethanol formed by a conjugation reaction of glucuronic acid via uridine diphosphate (UDP)-glucurolyosyltranferase (UGT) catalysis creating Ethyl Glucuronide (EtG) and by sulfotransferase (SULT) creating Ethyl Sulfate (EtS). Approximately 0.2-0.6% of a dose of ethanol is recovered as EtG in urine. EtS is generally present in the urine at 1:5 of the EtG. This can vary depending on individual donor genetic polymorphisms. EtG and EtS testing can detect very small acute doses to chronic alcohol consumption from as soon as 1.5 hours up to 80 hours post consumption. EtG and EtS are subject to dilution affects of the urine, and can be artificially lowered by consuming fluids in excess.
**EtS Testing in Conjunction with EtG:** A common phenomenon of alcohol testing in urine, as well as postmortem samples, is the post collection creation of ethanol due to fermentation processes naturally occurring in the sample. This fermentation can cause positive confirmed results for alcohol, requiring further testing to eliminate other contributing factors. These same factors can not only cause post collection creation of the EtG biomarker but also degradation, causing positive and negative results. This will typically occur in the rare group of events where a sugar source is present as well as active bacteria. An example of this would be the diabetic patient with a urinary tract infection. If their diabetes is uncontrolled, they can create and excrete excess glucose into the urine that can then ferment to alcohol by microbial growth. Then, *E.coli*, the predominant strain of bacteria found in urinary tract infections, the human bowel, and food borne illnesses, metabolizes the ethanol into EtG. Conversely, the *E.coli* can consume the delicate glucuronide bond of the EtG as a food source causing depletion of the EtG.

The inclusion of the EtS biomarker, in conjunction with EtG, eliminates the potential for false positive or negative results as it is not vulnerable to the *E.coli* bacteria by creation or degradation. EtS has proven to be a more robust and reliable biomarker of ethanol exposure that is quickly replacing EtG as the gold standard for alcohol consumption. When EtS is present, regardless of whether EtG or alcohol levels are present in the urine, it is consistent with direct exposure to or ingestion of ethanol, or alcoholic beverages.

**Appropriate Cut Offs:** EtG/EtS testing is sensitive down to the lowest levels of exposure and results should always be interpreted with this in mind. Many testing laboratories choose to set low cut off levels for EtG and EtS and allow the customer to interpret results according to the individual program requirements. Testing agencies should take caution and evaluate the needs of their programs to set realistic expectations for ethanol exposure due to incidental opportunities in the environment. Food products, Nyquil, mouthwashes, hand sanitizers, some medications and even non-alcoholic beverages contain concentrations of alcohol that will cause results for EtG and EtS. Presently, EtG levels above 500 ng/mL and/or EtS levels above 100 ng/mL are popularly accepted in the drug testing industry as the threshold values where direct alcohol consumption must have occurred.

**Screen Test:** Performed by Enzyme Multiplied Immunoassay Technique (EMIT) for the EtG metabolite at a cut off of 500 ng/mL.

**Confirmation Test:** Performed by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)

**Cut Off Levels:**
- EtG: 500 ng/mL
- EtS: 100 ng/mL