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Clinical (Non-forensic) Application Of Ethylglucuronide Measurement: Are We Ready?

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Abstract

Ethyl glucuronide (Etg) and ethyl sulfate (EtS) are minor metabolites of ethanol. Multiple studies have documented that, depending upon the amount of alcohol consumed, they can be measured in biological fluids for hours to days after the parent compound can no longer be detected. Testing for the presence of EtG, in a manner analogous to urinary drug abuse screening, has largely been restricted to forensic and law enforcement situations. Despite a real need for an objective and possibly quantitative marker of ethanol exposure for use in conjunction with outpatient clinical trials and treatment programs, measurement of these metabolites has seen only limited clinical application. The barriers to more extensive clinical use of EtG/EtS testing, particularly misleading assay results that can occur as a consequence of inadvertent exposure to non-beverage ethanol containing substances, are reviewed and put into perspective. Additional information needed to develop guidelines for optimal clinical utilization of EtG/EtS measurements is discussed.

Keywords

Ethyl glucuronide; ethyl sulfate; ethanol metabolite; laboratory test; alcohol screening

Introduction

Gauging the success of clinical trials that address new strategies or therapies for the management of substance use disorders requires an objective and accurate indicator of abstinence or, in some instances, a quantitative measure of recent drug usage. Treatment programs similarly rely on such information for optimal patient management. Urine assays for the parent drug and/or its major metabolite(s) that provide a detection window of at least several days are available for most commonly abused substances. For example, the major metabolites of most opiates, cocaine, nicotine, amphetamine related drugs, and cannabinoids can be detected in the urine in excess of 48 hours after customary use. Alcohol has been an exception. Blood, plasma or breadth ethanol concentrations of ethanol can be measured for less than 10-12 hours following alcohol ingestion, even with fairly heavy drinking. Thus, even programs with frequent monitoring of blood or breadth alcohol concentrations may miss recent alcohol consumption. Self-reports, while very useful, are not always reliable.

The major pathways for disposition of ethanol involve oxidation by the cytosolic enzymes alcohol dehydrogenase and aldehyde dehydrogenase and to a much lesser extent microsomal CYP2E1. Within approximately the last decade, ethyl glucuronide (EtG), and more recently

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ethyl sulfate (EtS), minor metabolites of ethanol produced by conjugation pathways, have been investigated as markers of ethanol exposure and their pharmacokinetics characterized (Droenner et al., 2002; Hoiseth et al., 2007), They have been the subject of multiple studies and excellent reviews (Dahl et al., 2002; Helander et al., 2009; Hoiseth et al., 2008; Neumann et al., 2008; Palmer, 2009; Schmitt et al., 2007 Wurst et al., 2002,2003a,2005). The purpose of this communication is not to duplicate these excellent contributions, but rather to specifically consider and put into perspective issues and gaps in our knowledge that may have constrained the wider application of ethanol metabolite measurements in the clinical milieu, especially in conjunction with outpatient clinical trials and treatment programs.

Although comprising only about 0.1% or less of ethanol's total disposition, by employing sensitive technology EtG and EtS, produced by minor conjugation pathways, can usually be detected for 24 or more hours after one or two “drinks”, and for as long as two to four days after heavier consumption. Their presence, at concentrations above accepted thresholds, is specific for ethanol exposure, although not invariably ingestion of alcohol containing beverages. EtG concentrations seen after alcohol consumption may vary by several orders of magnitude depending upon the amount consumed and the time elapsed since ingestion. Twenty-four to thirty-six hours after the ingestion of the equivalent of one or two drinks, concentrations may barely exceed 100 ng/mL. On the other hand, subjects with recent heavy drinking, or admitted for detoxification may demonstrate peak urinary EtG concentrations well in excess of 100,000 ng/mL (Helander et al., 2009).

EtS appears to follow the same pattern of urinary excretion as EtG following ethanol exposure although absolute concentrations are lower (Halter et al., 2008; Helander et al., 2004,2005; Hoiseth et al., 2008; Wurst et al., 2006). Rarely, low concentrations of EtS have been observed in the absence of measurable EtG (Helander et al.,2005; Wurst et al., 2006). This has led to the recommendation that both compounds be measured concurrently for more definitive evaluation of alcohol consumption. However, insufficient information and experience with EtS is currently available to make a definitive recommendation at this time, especially with respect to clinical utilization. It may be that measurement of EtS will be most useful for confirmation when there is a credible contradiction between clinical impression or self-report and EtG test result. Although recognizing that there may well be an important role for EtS measurements, this communication will focus on EtG with reference to EtS where particularly appropriate.

An excellent review has the provocative title “Ethylglucuronide-The Direct Ethanol Metabolite on the Threshold from Science to Routine Use” (Wurst et al., 2003). This still appears to be its status, especially with respect to non forensic and non medical-legal applications such as outpatient treatment programs and clinical trials, despite the need for an objective marker of alcohol use in those contexts. Several recent studies, performed in the setting of treatment programs, which have documented significant discrepancies between patient self-report and the results of ethanol metabolite analysis, highlight the potential clinical value of such measurements (Junghanns et al, 2009; Skipper et a.l, 2004; Wurst et al., 2003b). However, gaps in our knowledge and/or perceived limitations in validity of EtG assays as a definitive marker of alcohol ingestion has limited its more extensive use, especially in the clinical milieu. These concerns are as follows: 1. Specificity for alcohol consumption; 2. Assays cost, complexity and logistics; 3. Selection of appropriate “cut off” concentrations; 4 Interpretation in conjunction with self-reports and other markers; and 5. Possible impact of genetic polymorphisms.

Although these issues are each considered separately, they are, as must be emphasized, interdependent. Since the concept of “cut off” concentration is relevant to each of the topics

discussed below, we define it here, at the outset. Cut off, as used herein, refers to the concentration above and below which assay results are considered positive or negative respectively for recent alcohol consumption. In order to establish appropriate cut-off levels for biomarker assays several issues need to be completely understood, including assay performance (variability, lower limit of detection, and analytic interference), as well those factors that effect clinical sensitivity and specificity. These issues will be discussed briefly in subsequent sections

1. Specificity for Alcohol Consumption

One problem that has inhibited wider acceptance of EtG measurements is that a positive test may occasionally occur without consumption of ethanol containing beverages. Exposure to ethanol containing mouthwashes and hand sanitizers have been most often implicated as a cause of spurious positives (Constantino et al., 2006; Jones et a.l, 2006; Rosano and Lin., 2008) and have led to a cautionary warning from SAHMSA (SAHMSA, 2006). Following repeated use of ethanol containing mouth washes and hand washes, urinary concentrations of EtG can exceed 100 ng/mL, a commonly used cut off for tandem mass spectrometry (LC/MS/MS) assays of EtG. The unquestioned importance of ethanol exposure other than through alcohol consumption must be considered in the context of intensity of exposure to the contaminating source, concentration cut offs employed and the intended use of the assay results. A brief review of the several published studies that have addressed this issue may help to put the problem into perspective.

Following atypically intense exposure to commercial mouth washes containing approximately 20% ethanol, sufficient alcohol is either swallowed or else absorbed through the bucal mucosa, to result in measurable urinary EtG concentrations. Repeated oral rinses over a period of 15 minutes, sufficient to exhaust a 4 oz bottle (Constantino et al, 2006), or hourly rinsing for eight hours (Jones et al, 2006) resulted in peak EtG concentrations exceeding 300 ng/mL in some subjects, but not more than 400 ng/mL. Based on reports of EtG's pharmacokinetics (Hoiseth et al, 2007; Droenner et al, 2002), all would have been undetectable at 24 hours following exposure. Following more modest exposure, concentrations above 100 ng/mL appear to be unusual but may occur. Employing a dosing regimen somewhat more applicable to real life, subjects rinsed three times a day for five days, and spot urine collections were obtained at approximately 12 hrs after the last exposure. Fifty three of 55 samples were negative with concentrations less than 100 ng/mL, while two barely exceeded this threshold (Constantino et al, 2006). Fortunately, alcohol free mouthwashes are available and can be recommended to obviate this challenge to the interpretation of EtG.

False positive EtG reports following use of ethanol containing hand sanitizers have received more publicity. Compared to commercially available mouth washes, hand sanitizers have about three times the ethanol concentration, and, as used, are in contact with a much larger absorptive service area. Thus, they can be expected to be more problematic. Anecdotal reports in the newspapers (Helliker, 2006) and on the internet have described instances wherein health care workers exposed to hand sanitizers, but who denied consumption of alcoholic beverages, have lost their jobs on the basis of positive EtG tests, and a level as high as 770 ng/mL has been described following repeated exposure throughout the day. The possibility of concentrations as high as 1500 ng/mL following incidental exposure to hand sanitizers has been postulated (Skipper in Helliker, 2006).

Data resulting from careful experimental studies are somewhat less striking but do not contradict anecdotal reports. Urinary EtG concentrations from nine adults who used a commercial hand sanitizer (61% w/w ethanol) twenty times a day for five days, varied from

less than 10 ng/mL to 114 ng/mL the next morning, exceeding 100 ng/mL in only three samples (Rosano and Lin, 2008). In an earlier communication, (Rohrig et al., 2006), one of two subjects demonstrated a level of only 62 ng/mL following application of a hand sanitizer every 15 minutes through out the day, but levels were undetectable following less frequent use. Similarly, hourly use for eight hours resulted in undetectable EtG levels in one subject, but a maximal level of 103 ng/mL in a second immediately following the final exposure. However, following hourly exposure up to the elbow, concentrations as high as 799 ng/mL occurred (Jones et al 2006). Although incidental exposure is unlikely to lead to positive EtG levels in most cases, SAMHSA has, with good reason, recommended caution in utilizing a positive EtG tests, in vacuo, as definitive and incontrovertible evidence of alcohol beverage ingestion (SAMHSA,2006). Such reports have elicited skepticism regarding the reliability of the test as a regulatory or forensic tool, and have likely impeded its acceptance into the clinical milieu as well.

Exposure to ethanol containing medications, of which there are many, is another potential source of “false” positives. Forbidding patients/subjects access to such medications may be unrealistic, and even unwise. However, we are not aware of any studies regarding the impact of the small amounts of ethanol derived from appropriate dosing with such agents on EtG levels. Such experiments could be easily and definitively performed, through precise dosing with the small amounts of ethanol found in various medications, according to recommended schedules, and following urinary EtG concentrations over time. Another potential artifact that should be mentioned for completeness derives from the possible in-vitro conversion of glucose to alcohol and subsequently EtG through the sequential action of yeast and some strains of bacteria in urine samples, particularly those from diabetics (Helander et al, 2007). Conversely, degradation of EtG but not EtS has been observed in samples stored at room temperature (Helander and Dahl, 2005). Both artifacts should be avoidable through freezing of samples immediately after collection (Schloegl et al, 2006). EtG concentrations are reported to be stable for 24 hours, and to show negligible degradation after 48 hours with ordinary refrigeration at 4°. Fluoride (10/mg/mL) also appears to be protective (Helander and Dahl, 2005) although additional definitive studies with various preservatives are still needed. Thus, other options are available for use in environments wherein prompt freezing of samples may not be feasible. Alternatively, concurrent measurement of EtS, which should not be produced in vitro, has been recommended as a safe guard.

In summary, occasional use of ethanol containing mouth and hand washes appear to produce spurious positive EtG tests uncommonly, although studies are needed employing more conventional and casual exposure such as one or two applications per day. More intense and frequent use of hand washes, as occurs amongst some categories of health care workers, is of more concern. Thus higher cut off's might be prudent for this group, for whom avoidance of such exposure may not be feasible. The trade off might be reduced sensitivity for light drinking and a shorter time window of positivity. However, these well documented “interferences” ought not to impede the application of ethanol metabolite measurements in the clinical milieu, especially since, in the absence of detectable blood alcohol concentrations, it is the only available source of objective information regarding recent alcohol consumption. This issue can be considered analogous to the problem of the impact of poppy seed exposure on assays for opiates, which may be addressed by instructing subjects or patients to avoid such exposures. Subjects in alcohol use treatment programs or clinical trials, could be instructed to avoid ethanol containing mouth washes or hand washes or if possible, ethanol containing medications. Special guidelines might be needed for health care workers in treatment programs where frequent repeated exposure to ethanol containing hand washes may be unavoidable. However, the problem is not insurmountable. One potential solution would be to establish individualized cut-offs for health care workers by monitoring EtG levels under controlled conditions in which the person would repeatedly use

a hand sanitizer to determine a level that would be expected through occupational exposure. While this would be time intensive, the effort would be justified for a health care worker in recovery whose employment is at risk. Just about all clinical laboratory tests, even the best, are subject to occasional confounding factors, and require knowledge and experience for proper interpretation.

2. Assay Cost, Complexity and Logistics

Most published studies of EtG have utilized LC/MS/MS, which is considered to be state of the art . This elegant technology is highly sensitive, accurate and reasonably efficient, but costly and requires highly skilled personnel. The cost of a single instrument is in excess of $250,000. Assays must usually be sent off site to reference laboratories, and turn around time is measured in days rather than minutes or hours. Thus, it is not suitable for on site or point of care testing, and does not allow for real time feed back to patients as might be optimal for contingency management interventions.

The usual urinary drug abuse testing paradigm consists of an initial screening immunoassay which provides rapid results, followed by confirmation of positives by a more specific mass spectrometry based procedure. The confirmatory step, considered de rigueur for forensic, regulatory or occupational applications, is sometimes discretionary for treatment programs depending on the structure of the program and consequences of a positive test result. Immunoassays, even the best ones, are subject to occasional false positives, but are fast, economical and are technically suitable for point of care or on site testing in the clinic. Recently, an enzyme immunoassay (EIA) for EtG has been marketed, although at the time of this writing, is not yet FDA approved. A published evaluation, in which this new immunoassay was compared with a LC/MS/MS showed an excellent correlation and comparable sensitivity (Bottcher et al., 2008). However, a field test of this procedure showed a high incidence of discrepancies with LC/MS/MS when a 100ng/mL cut off was used. (Dekalb County Drug Court, 2008) The correlation improved and was considered acceptable employing a higher cut off of 500 ng/ml for the immunoassay and that is the value recommended by the manufacturer. Prior to routine implementation of this immunoassay for EtG in the clinical milieu, several questions to be answered include: 1.Can a cut off as low as 100 ng/mL be employed with a screening immunoassay if positives are confirmed by LC/MS/MS? 2. Is such a low cut off needed or is 500 ng/mL adequate? Actual field experience with systematic clinical evaluation of various cut off's may be needed to answer this question. 3. What are the explanations for the false immunoassay positives? Note that extraneous sources of ethanol, such as hand sanitizers would not explain discrepancies with LC/MS/MS since the source of the EtG is not distinguished by either procedure. Ingestion of chloral hydrate has been reported to yield a metabolite that cross reacts in the immunoassay (Arndt et al., 2009), but this is an uncommonly used medication. 4. Are false negatives likely in those uncommon circumstances when EtS but not EtG may be present? 5. Can a point of care procedure suitable for use on site to allow immediate feedback to the subject/patient be developed. The availability of an antibody to EtG should make this possible, as has been done with immunoassays for other drugs and compounds of clinical importance.

3. Selection of appropriate “cut off” concentrations

Recommendations for cut off values do not always adequately consider that “one size may not fit all.” The following considerations are relevant to the choice of cut off values: Assay performance; Need to exclude extraneous sources of ethanol exposure; and Structure and goals of the clinical trial or treatment program.

Assay performance

Generally, the closer the cut off value is to the detection limit of the assay (the concentration that can be confidently differentiated from the background signal in the absence of EtG), the poorer the reproducibility of the assay. Many studies have employed a 100 ng/mL cut off based upon documented LC/MS/MS assay performance at a concentration which will usually detect light drinking for at least 24 hours. One study which optimized an LC/MS/MS assay to achieve a cut off of as low as 10 ng/mL, detected a median concentrations of EtG of 19ng/mL (range <10-62ng/mL) in nine adults with no known exposure to ethanol (Rosano et al, 2008). The source of ethanol and thus EtG was assumed to be endogenous, but if correct this finding suggests that a cut off of less than 100 ng/mL might yield misleading information. The currently available immunoassay (see 2 above) assay, appeared, in practice, to show a significant reduction in accuracy with a 100 ng/mL cut off, but performed acceptably at 500 ng/mL.

Need to exclude extraneous exposure

This topic has been discussed at length (see 1. above). For programs wherein the goal is regulatory, and the outcome may be punitive, 100 ng/mL may be too low. Published data suggest 500 ng/mL would adequately exclude most instances of extraneous exposure, although, as noted, anecdotal reports suggest higher cut off's may be needed. However, for clinically oriented programs wherein subjects can be specifically instructed to avoid extraneous sources of ethanol exposure, 100ng/mL should be acceptable, although further confirmation of this recommendation is needed. For some categories of health care workers different strategies may be needed with respect to cutoffs, and/or scheduling of sample collections.

Structure and goals of the clinical program

Relatively high cutoffs, sufficient to evaluate a 12 to 24 hour window, may be suitable for confirmation or evaluation of recent heavy drinking. Such instances might include detoxification programs, and auto accident investigations. With clinical trials and treatment programs, especially those that target abstinence, the lowest practical cut off that is still specific for exogenous ethanol exposure, might be preferred. Outpatient programs that focus on use reduction may be more concerned with changes in EtG concentration and quantitative correlation of EtG concentrations with drinking history. Such programs might be comfortable with higher cut offs, especially if testing is frequent. In our experience, subjects may show entry level EtG concentrations in the thousands or even tens of thousands of ng/mL which, in some, may drop by orders of magnitude during follow up

4. Interpretation: Use in conjunction with self-reports and other markers

This is perhaps the most complicated issue, and more information is needed for a definitive recommendation. The following comments relate to clinical programs and not forensic applications. Attempts to define sensitivity and specificity of EtG testing in the clinical milieu require criteria for defining true positives and negatives. If self-reports were a true gold standard this entire discussion would be moot. However, the potential for bias in self-reports as a component of treatment studies or clinical trials, whether intentional or inadvertent, is well recognized. The most widely accepted approach to assessing drinking behavior is retrospective or prospective daily estimation methods in which subjects are asked to recall the quantity of alcohol consumed on each day during the reporting period (Del Boca and Darkes, 2003) In clinical trials, researchers obtain these reports under conditions intended to enhance the reliability and validity of these reports. For example, a research assistant rather than the treating clinician obtains the information, the confidentiality of the reports is emphasized, and the potential consequences associated with

truthful reporting are minimized (e.g., by the exclusion of participants with legal problems that require reporting to probation officers). Nonetheless, these results are clearly estimates of drinking and underreporting undoubtedly occurs among some patients. For example, between 12.2% and 28% of those followed for several weeks after completing an inpatient study denied drinking but were positive for EtG (Junghanns et al., 2009). Moreover, bias in self-reports probably increases as researchers move from efficacy studies in which very select groups of patients are studied to effectiveness trials in which a broader group of patients are included or when self-reports are obtained in clinical practice (Langenbucher and Merrill, 2001).

Information derived from definitive studies of EtG concentration vs. time relationships, and their inter-individual variation, over a range of ethanol dosages should enable a reliable estimation of the accuracy of self-reports and the rational integration of self- reports with EtG testing. Such information, used in conjunction with self-reports might, in some contexts also allow a quantitative approximation of ethanol consumption rather than simply a yes or no answer. One very large epidemiological type study (Wurst et al., 2004) which compared Receiver-Operating Characteristic plots (ROC'S) for various cut off concentrations of EtG, focused on risk of developing or having an alcohol use disorder. Although providing important epidemiological data, this was not entirely applicable to the needs of clinical trials. Regardless, advertisements touting the sensitivity of EtG measurements to the effect that it is reliably detectable three or more days following consumption (e.g. “80 hour test”) can be misleading. As with other drug and/or drug metabolites, the windows for detection for EtG and/or EtS in biological fluids are a function of their respective pharmacokinetics and the dosage of the parent compound, in this case alcohol, Rosano et al (Rosano et al, 2008) observed that the fractional clearance of ethanol as EtG seemed to increase with increasing ethanol dosage which suggests saturation of the major metabolic pathways with greater diversion to conjugation routes. Ethanol may also induce some glucuronidation pathways, including UGT1A1 which is involved in the glucuronidation of ethanol and thus might impact on the interpretation of metabolite concentrations (Monaghan et al., 1996).

Clinical laboratory tests are often best interpreted in conjunction with other relevant information. Various other markers of alcohol use have been described (SAMSHA, 2006). Phosphatidylethanol and fatty acid ethyl esters (Borucki et al., 2005) are, like EtG, minor ethanol metabolites, but less well characterized. Phosphatidylethanol appears to reflect sustained heavy alcohol use and is relatively insensitive to recent consumption (Hartman et al.,2006). GGT and CDT, on the other hand, reflect the metabolic consequences of alcohol use and are not actual ethanol derivatives. GGT is a very sensitive but relatively non-specific liver function test, which can be useful for the evaluation ethanol use disorders if other causes of liver dysfunction can be excluded. CDT has perhaps received the most attention and best documentation (Fleming et al., 2004; Anton et al., 2002). Studies indicate that it is a sensitive indicator of heavy ethanol use, and may even respond within days of relapse (Anton and Youngblood, 2006). Concerns regarding its specificity appear to have been addressed by more advanced assays that target the alcohol specific disialo isoform (Jeppson et al., 2007). EtG and CDT considered together might provide a more comprehensive evaluation for the study and management of patients with alcohol use disorders. For example, a CDT measurement might provide information as to whether a high EtG was a sporadic finding or instead reflected sustained behavior. Strategies for optimal integration of the results of these two tests need to be developed. More definitive information is needed to confirm and expand the following preliminary conclusions:

a. Interpretation of a negative EtG assay

Despite generic statements indicating a several day window for detecting alcohol use, a negative test only has significance in the context of alcohol dose and time elapsed since

consumption. The literature indicates that a single drink usually, but not invariably, results in an ETG concentration exceeding 100ng/mL cut off for 24-36 hours, Thus a negative result can usually be considered confirmatory of a self report denying alcohol use for about the most recent 24 hours *and/or* "heavier" drinking for two-to four days.

b. Interpretation of a positive result

A positive EtG test refutes a self report of abstinence, but not necessarily one of recent (last 24 hours) abstinence. A positive test does not differentiate between recent light drinking or less recent heavier drinking. More precise information regarding urinary EtG concentration vs. time relationships for various levels of consumption, ranging from light to heavy, may allow more precise use of quantitative EtG results in conjunction with self-reports regarding the time and quantity of drinking. With respect to programs that focus on moderation, we do not yet have sufficient information to determine how accurately changes in an individual's urinary EtG concentration reflect changes in alcohol intake.

5. Impact of Genetic polymorphisms

Uridine-diphosphate-glucuronosyltransferases (UGT's) show considerable genetic polymorphism, and are responsible for conjugation of multiple endogenous and exogenous substrates. Gilbert's disease, which involves reduced expression of the gene coding UGT1A1, the enzyme responsible for conjugation of bilirubin (Bosma et al., 1995) may affect in excess of ten percent of the population (Beutler et al, 1998). The clinical importance of the same allele, with respect to disposition of the chemotherapeutic agent, irinotecan, is well established (Innocenti et al.,2004).

There is only scant data in the literature regarding the pharmacogenetics of ethanol glucuronidation. In-vitro studies employing human liver microsomes (Folti and Fisher, 2005), with ethanol as the substrate, demonstrated that most ethanol UGT activity was associated UGT1A1 and UGT2B7, although multiple isoforms contributed to the formation of EtG. The authors concluded that reduced activity of any one UGT isoform would likely be compensated by the remaining glucuronyl transfersase isoforms and thus that genetic polymorphisms in glucouronyltransferase activity ought not to constrain the validity of EtG as a marker of ethanol exposure. Rarely, low concentrations of EtS have been found when EtG was undetectable, although studies especially designed to identify individuals with reduced ethanol glucucuronidation activity have not been performed. Aside from false negatives, the possibility that inter-individual differences in UGT activity, regardless of the mechanism, may be responsible for variations in excretion of EtG cannot be ruled out. This topic deserves further investigation. Alternatively, variations in the capacity of the major pathways for ethanol's disposition might influence the amount that is channeled through the minor glucuronidation and sulfation pathways.

Conclusions

EtG is a reliable and relatively long term marker of ethanol exposure. Yet measurement of this minor ethanol metabolite has yet to fully realize its potential as a valuable asset to clinical trials or treatment programs concerned with the management of alcohol use disorders. The current state of the the art with respect to clinical application of EtG measurements is summarized in Table 1. With possible very rare exceptions, the presence of EtG in the urine at a concentration above 100 ng/mL is indicative of exposure to exogenous ethanol, whether though intentional consumption or through use of extraneous ethanol containing substances. An awareness of the effect of such exogenous exposure on EtG measurements, particularly to hand sanitizers is important. However, in the context of treatment programs and clinical trials it should be possible to preclude exposure from these

sources in most individuals with the possible exception of some health care workers. The significance of any negative or positive urinary EtG result and EtG concentration will vary with the frequency of sampling, and the amount and the likely time interval between alcohol consumption and sampling and inter-individual differences in pharmacokinetic. Therefore, interpretation in conjunction with self-reports is essential. Studies are needed to more precisely define the relationship between urinary EtG concentrations over time with the quantity of ethanol consumed. Careful studies of EtG in the laboratory and in clinical trials in combination with self-report may allow rational selection of cut off values and guidelines for optimal clinical use of this test. Knowledge regarding the extent of concordance between EtG values and self-reports in various treatment populations may also help develop guidelines regarding the need for EtG testing and their frequency in various programs. EtG (and EtS) assays are an objective source of information regarding alcohol consumption, and when used in conjunction with self-reports, potentially provide an important tool for evaluating outcomes in clinical trials and for patient management in treatment programs.

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Table 1

Current Status and Future Research Required to Inform the Clinical Use of EtG/EtS

Issue	Current status	Information needed
Cut Off's	100ng/ml-500 ng/mL range has been employed in various studies.	May depend upon application: abstinence testing; use reduction evaluation; testing health care workers.
Assay sensitivity	LC/MS/MS more than adequate for clinical use (<100ng/mL) Immunoassay: Good	Practical sensitivity of immunoassay limited by apparent false positives. Further studies are needed to better define sensitivity in practice.
Assay specificity	LC/MS/MS-Highly specific for ethanol exposure. Immunoassay: Specificity probably as good or better than most drug of abuse immunoassays;	More experience with the clinical application of immunoassays for EtG testing and their limitations is needed.
Assay cost	LC/MS/MS: High cost of instrumentation and cost per assay. Immunoassays: Cost should be consistent with other drug immunoassays; Suitable for large scale screening.	Guidelines for immunoassay screening and indications for LC/MS/MS confirmation, analogous to that in use for other drugs of abuse need to be developed. Need for confirmation will depend upon the structure and goals of the program: may be required for forensic use but be discretionary for clinical applications. More immunoassay options are needed. Currently available immunoassay * is not yet FDA approved.
On site (clinical) testing in real time.	LC/MS/MS: not suitable. Current Immunoassays are not optimized for on site testing.	Immunoassay should be adaptable to on site testing using existing immunoassay technologies, but developmental work needed.
Clinical sensitivity	Urinary EtG detectable for ~24hrs (one drink) to 72 hrs and possibly longer after heavy consumption.	More studies needed to better define inter and intra subject variability in dose response relationships
Clinical specificity	EtG specific for ethanol exposure. Interferences may occur from recent non-beverage ethanol exposure (hand and mouth washes, medications, possibly)	Instructions to avoid nonbeverage alcohol should usually be suitable. Strategies for monitoring health care workers subject to frequent exposure to ethanol containing hand sanitizers need to be developed and evaluated.
UGT activity	Hepatic UGT 1A1 and to a lesser extent other UGT's catalyze glucuronidation of ethanol	Effects of genetic polymorphisms of glucuronyl transferases, especially 1A1 on urinary EtG concentrations have not been studied. Similarly, the effect of advanced (alcoholic) liver disease on formation of EtG is unknown.
EtS	Parallels EtG excretion in most instances. May rarely be present in absence of EtG. No immunoassays for EtS are available	Studies to establish if and when measurement of EtS is necessary
Correlation with other biomarkers	Widely used biomarkers that reflect the consequences of alcohol use include GGT and CDT	Guidelines for optimal integration of EtG testing with other biomarkers for clinical management need to be developed.

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