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# DATIA focus

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# LONG-TERM ALCOHOL BIOMARKERS

*Emerging Technologies That Allow for the Long-term Detection of Risky Drinking Behavior*

Alcohol abuse continues to be a significant health concern for the United States. In the United States, **15.3 million** people are categorized as having an alcohol use disorder while **1.9 million** have a drug use disorder

**A**dditionally, another 2.3 million have both an alcohol and drug use disorder (Stinson et al, 2005). According to the Judicial Council of California, a survey of family law judges indicated that more than 50% of child custody decisions involved alcohol and drug abuse as a factor (Center for Families, Children, & the Courts, 2007). Reliable and objective measures of alcohol consumption can assist legal, healthcare, and addiction treatment professionals with the evaluation and monitoring of their clients and allow the local substance abuse professional an opportunity to expand their list of available services in their community. Traditional methods to identify and evaluate individuals with alcohol use disorders include a variety of self reporting questionnaires, indirect alcohol biomarkers, and direct short-term alcohol biomarkers. Self-report questionnaires (such as AUDIT, MAST and CAGE) have

limited utility because of participant self-incrimination and recall bias. Indirect alcohol biomarkers (such as CDT, GGT, and MCV) measure the biological effects of abusive alcohol consumption and are not 100% specific to risky alcohol behavior. Many indirect alcohol biomarkers are sensitive to various cancers, infections, and pregnancy. The direct measurement of alcohol in blood, breath, urine and oral fluid has a detection window of approximately 1 hour per drink. These tests are very effective for roadside safety, reasonable suspicion and post accident testing. However, a detection window measured in hours has limited utility in most circumstances. Many situations exist that would benefit from sensitive and specific alcohol biomarkers that detect abusive alcohol consumption. Originally, it was assumed that ethyl glucuronide and ethyl sulfate in urine was a result of beverage alcohol consumption.

Lower costs of more sensitive laboratory instruments have allowed laboratories to develop and offer a new group of tests for direct long-term alcohol biomarkers at a reasonable cost.

However, recent reports in the scientific literature indicate that these compounds can be found in urine due to transdermal absorption from the use of ethanol containing hand sanitizers (Rohrig & Ross, 2006) and innocent ingestion of ethanol containing products such as mouthwash, medicines and certain foods (Costantino *et al*, 2006). Testing urine for ethyl glucuronide and ethyl sulfate satisfies a critical need of ethanol abstinence compliance in our industry. However, the Substance Abuse and Mental Health Services Administration (SAMHSA) warns substance abuse professionals to be very careful with the interpretation of these results (SAMHSA, 2006).

Lower costs of more sensitive laboratory instruments have allowed laboratories to develop and offer a new group of tests for direct long-term alcohol biomarkers at a reasonable cost. These tests are now available to the local substance abuse professional to offer to their community's legal, healthcare, and addiction professionals. Understanding the advantages and disadvantages of these new and exciting tools for the substance abuse profession could be the deciding factor that distinguishes you from other substance abuse testing sources.

### **FAEE Hair**

The first long-term alcohol biomarker that came to this market was testing for Fatty Acid Ethyl Esters (FAEEs) in hair. FAEEs are a group of non-oxidative metabolites that are produced in the presence of ethanol and various fatty acids. FAEEs can be found in a number of specimen types such as hair, fat and a variety of organ tissues. Testing for FAEEs has been used to identify newborns exposed to alcohol in the womb and has been used to evaluate the decedent's alcohol history in post-mortem examinations.

Testing for FAEEs in hair has two main drawbacks. The use of ethanol containing hair care products will produce detectable amounts of FAEEs in the hair and the exposure of clipped hair to ethanol vapor will produce FAEEs in the hair sample (Gareri

*et al*, 2011). The production of FAEEs occurs in the skin cells that surround the hair shaft and in the hair itself. These two facts have limited the usefulness of this test.

### **EtG Hair**

EtG is a minor metabolite of ethanol produced by the conjugation of ethanol with glucuronic acid. Using urine for EtG analysis has been available commercially for almost 10 years, first in Europe and later in North America. Using head hair, which grows at approximately ½ inch per month, to detect EtG was first reported at a scientific conference in 1995 and the data was later published in 2000 (Skopp, 2000). Following the Society for Hair Testing's ([www.soht.org](http://www.soht.org)) release of the "Consensus of the Society of Hair Testing on hair testing for chronic excessive alcohol consumption 2009", several organizations in Europe and North America began offering EtG hair analysis. In summary, the Society of Hair Testing claims that a 1½-inch hair sample containing greater than 30 pg/mg of EtG is a strong indicator of "chronic excessive alcohol consumption" during the previous 3-month period.

One limitation of using hair for EtG analysis is that certain haircare treatments (bleaching, permanent waving, dyeing) negatively affect hair EtG levels (Morini *et al*, 2010). This limitation must be considered when attempting to declare that a donor has been abstinent. This observation was independently confirmed in a report recently released at the Research Society on Alcoholism's (RSA) national conference in June 2011 (Jones *et al*, 2011). This study demonstrated that there may be a gender bias when comparing male and female hair EtG levels to their self-reported drinking histories and more research is needed in this area.

Another limitation (or advantage depending on your point of view) is that consumption of single or small doses of alcohol will not produce a positive hair EtG result. Kronstrand *et al* (2011) reported that when a group of volunteer women consumed 1

drink per day for 3 months (90 drinks) and a group of volunteer men consumed 2 drinks per day for 3 months (180 drinks) that the vast majority of the subjects did not exhibit measurable levels of hair EtG. Those that did have detectable levels of EtG in their hair were significantly below the cutoff of 30 pg/mg. Again, this observation was independently confirmed with data released at the June 2011 RSA national conference (Jones *et al*, 2011).

## EtG nail

Fingernail, which grows approximately 3 mm per month, is very similar in structure to hair in that it is composed of keratinized protein. Originally, it was assumed that analytes were incorporated into the nail where it originates (the matrix). Under this assumption, a clipping of nail would give a two-week history that occurred 6 months ago, severely limiting the usefulness of this specimen type. This assumption was proved to be incorrect in 1991 when analyzing fingernails following the oral administration of a new fungicide (Johnson *et al*, 1991).

Johnson *et al* (2011) found that the drug was appearing in the nail clippings after a couple of days indicating that another mechanism was occurring. The researchers proved that not only was drug incorporated in the matrix (where the nail material originates) but that nail material (and drug) was being incorporated from underneath as the nail grows along the nail bed toward the tip of the finger. The nail gets thicker as it grows in length. A

simple clipping of fingernail provides a history of the entire trip down the nailbed.

The National Institute of Alcohol Abuse and Alcoholism (NIAAA) funded a study (1R44 AA016463-02) that included the collection of head hair, fingernail, and an extensive battery of self-report questionnaires to 606 college-aged students. The hair and fingernail specimens were analyzed for EtG at our laboratory. This group was chosen because in theory they should provide a more accurate self-report because their drinking patterns tend to be more consistent and the stigma and negative effects of alcohol abuse have yet to manifest themselves even though they engage in risky alcohol drinking behavior. In other words, they have not yet lost a wife, kids, or a job because of their drinking pattern; they are just fun loving college kids. The preliminary results of this study were released at the RSA national conference in June 2011 (Jones *et al*, 2011).

The findings of the study were twofold. First, a gender bias may exist when using hair for EtG analysis. When comparing the association between the self-reported number of drinks for the previous 3-months and hair/nail EtG levels, the female hair association was significantly less than female nail, male nail, and male hair (Table 1). One possible explanation for the diminished association for the female hair could be the increased likelihood for female hair care treatments as exemplified in Chart 1, where the female subject self-reported that she had

**Table 1.** Pearson correlation coefficients of EtG in hair and fingernail compared to the number of self-reported drinks for males and females. The association of EtG in female hair is much weaker than female nail, male nail or male hair.

Sample	90-day TLFB	AUDIT
Male nail	0.640	0.505
Male hair	0.556	0.485
Female nail	0.515	0.487
Female hair	0.278	0.245

Abbreviations: TLFB= Time Line Follow Back, AUDIT = Alcohol Use Disorders Identification Test



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bleached her hair, had a negative hair result, but had a positive nail finding. Males may also bleach, perm or dye their hair but in this demographic it is expected that these types of hair care treatments are much more prevalent in the female population. This would be consistent with the diminishing effects of certain hair care treatments previously described by Morini *et al* (2010), but more research is needed in this area.

Secondly, EtG in both hair and nail was not found unless the participant engaged in risky alcohol drinking behavior (binge drinking). Charts 2 and 3 illustrate this observation. Both subjects consumed a similar number of drinks (53 and 58 drinks) during the previous 90-day period however the positive subject had 18 drinking episodes (4.84 drinks/drinking day/100 kg) while the negative subject reported 28 drinking episodes (2.77 drinks/drinking day/100 kg). These two participants demonstrate that the number of drinks is less important than the number of drinks per episode (drinking behavior). A poster presented at the Research Society on Alcoholism's national convention in Atlanta and video concerning this study are available at <http://www.usdtl.com/categories/Presentations/12.html>

### Phosphatidylethanol in blood

A new test that has become available recently is Phosphatidylethanol (PEth) in blood. PEth is an abnormal phospholipid that is formed only in the presence of ethanol and has been reported in a number of tissues and fluids. Once produced in humans, it is incorporated into cell membranes where no enzymatic or metabolic mechanism of elimination is available. PEth decomposes with a very predictable half-life of 4-5 days giving a detection window of 2-4 weeks depending on the starting levels.

Testing blood for the presence of PEth has been used for several years by various medical examiners in Europe to gain insight of the alcohol history of

decedents during post-mortem examinations. Several laboratories in the United States are now offering this assay routinely. Originally, the assay required a venipuncture performed by a licensed phlebotomist, making the collection too expensive or logistically problematic for widespread use. Most recently, the test has been adapted to using dried blood spots which allows for collection of a simple finger stick in a non-clinical setting without the services of an expensive phlebotomist (Jones *et al*, 2011).

### Conclusion

The individuals with an alcohol use disorder outnumber those with a drug use disorder by a factor of over 4 to 1, yet our industry tends to emphasize drug testing. Because of a number of new breakthroughs, a new group of tests for detecting chronic excessive alcohol consumption are now commercially available to assist legal, health care, and addiction professionals assess the drinking behavior of their clients. The results of a combination of tests (EtG/EtS in urine, PEth in blood spot, and EtG in nail) reveal to the substance abuse professional the alcohol history of the donor over the past 3 days, 3 weeks, and 3 months. This level of information using objective measures is new and novel to our industry. These new tests provide the local substance abuse professional powerful additions to the evaluation tool belt, an opportunity to expand their offerings, and the opportunity to distinguish their level of services from their competitors. ■

### Abbreviations

- **AUDIT**—Alcohol Use Disorder Identification Test
- **MAST**—Michigan Alcohol Screening Test
- **CAGE**—an acronym for the 4 questions asked
- **CDT**—Carbohydrate Deficient Transferrin
- **GGT**—Gamma-Glutamyl Transferase
- **MCV**—Mean Corpuscular Volume

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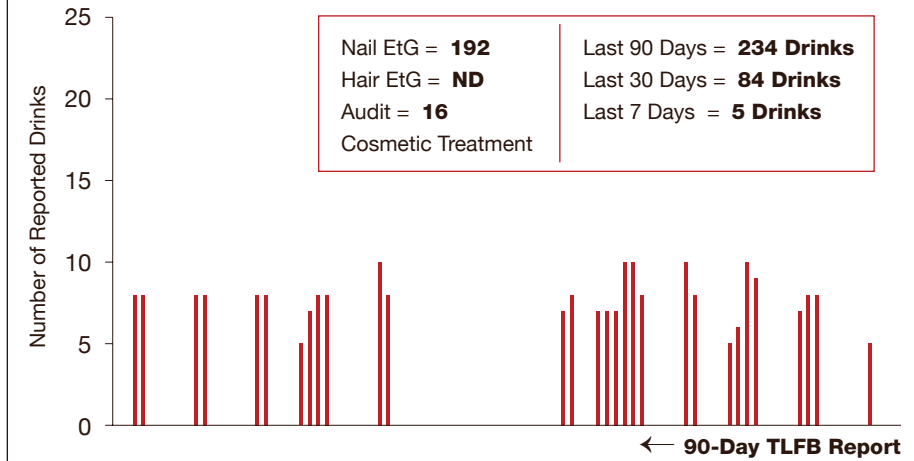
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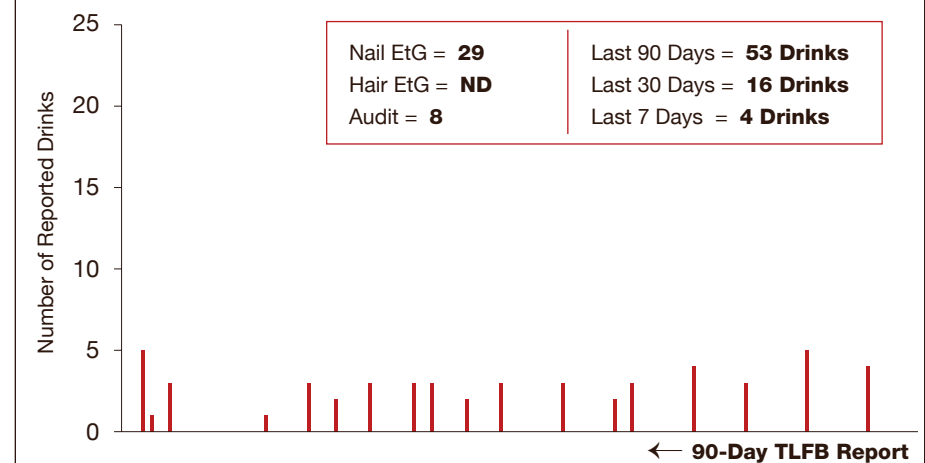
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### Case Study 132 Pound Female



### Case Study 134 Pound Female



### Case Study 168 Pound Male

