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Alcohol Biomarker Testing: Enough Challenges to Drive Laboratories to Drink

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Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are metabolites of ethanol used in clinical and forensic settings to detect recent exposure to alcohol. Although most ethanol is converted to acetaldehyde and then to acetate, minor phase II metabolism mediated by uridine diphosphate glucuronosyltransferases and sulfotransferases conjugates <1% of ethanol to form EtG and EtS, respectively (1). These metabolites reach peak concentrations in plasma approximately 1 to 3 hours after ethanol ingestion, with median half-lives of 2 to 4 hours. EtG and EtS are primarily eliminated in urine but are also detectable in hair and other matrices (2).

Measurement of alcohol biomarkers such as EtG and EtS complements self-reported alcohol consumption, to allow evaluation of drinking behaviors or abstinence. Indications for alcohol biomarker testing include substance use disorder management, pretransplant candidacy, and relicensure, e.g., after intoxicated driving. Although measurement of EtG and EtS is gaining acceptance in clinical and forensic settings, there are some significant limitations that laboratories and end users should recognize, including the inability to distinguish between recent light alcohol consumption vs distant heavy drinking (2).

It is also important to note that ‘ethanol exposure’ is not always synonymous with ‘drinking alcoholic beverages.’ Detectable amounts of EtG and EtS can be present in urine after use of ethanol-containing products such as hand sanitizer and mouthwash; many foods and drinks also contain small amounts of ethanol and/or ethanol metabolites (e.g., soy sauce, nonalcoholic beer and wine, sauerkraut, and mature bananas) (1, 3). These ‘incidental’ exposures can complicate interpretation of low EtG and EtS concentrations.

Furthermore, confounding factors such as urinary tract infections (UTIs) can lead to both false positives and false negatives for EtG (1). EtG can be formed ex vivo in urine when glucose or ethanol are present with specific microorganisms including *Escherichia coli*, a common cause of UTIs. EtG can also be degraded by bacterial hydrolases from *E. coli* and other microbes. The use of fluoride preservatives or frozen storage can reduce the impact of these concerns, although at least 1 study demonstrated ex vivo EtG formation even in the presence of fluoride. In contrast, EtS does not appear to be susceptible to ex vivo formation or degradation in the setting of UTIs, supporting its importance as a complementary biomarker to EtG.

Interpretation and Cutoffs

Given the potentially significant decisions that can be influenced by alcohol biomarker testing, correct result interpretation is extremely important. Unfortunately, there is very little formal guidance available for interpreting EtG and EtS concentrations. The Substance Abuse and Mental Health Services Administration (SAMHSA) released a 2012 advisory on indirect and direct alcohol biomarkers, including EtG and EtS. However, the SAMHSA document only provides interpretive thresholds for EtG in urine (Box 1), without addressing interpretation of EtS (4). Alcohol biomarkers can be measured in other matrices including hair, meconium, and blood. The Society of Hair Testing (SOHT) offers consensus recommendations for EtG testing in hair (Box 1); there are no guidelines for EtG or EtS cutoffs in other matrices.

The SAMHSA described 3 tiers of EtG concentrations based on available research in 2012: very low positive (100–500 ng/mL), low positive

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(500–1000 ng/mL), and high positive (>1000 ng/mL). Interpretation of concentrations in each of these ranges acknowledge that the amount of EtG in a single-timepoint urine sample reflects both the ethanol dose and the time since exposure. Lower concentrations are noted to overlap with “extraneous” exposure, i.e., ethanol from sources other than alcoholic beverages.

Several groups have proposed alternative cutoffs in urine, primarily aimed at maximizing sensitivity for alcohol consumption. Many studies use EtG cutoffs between 100 and 260 ng/mL (0.1–0.26 mg/L) and EtS cutoffs around 40 to 110 ng/mL (0.04–0.11 mg/L) (5, 6). However, literature examining regular hand sanitizer use or ingestion of certain foods demonstrates that some individuals can have urinary EtG and EtS concentrations above these thresholds within a few hours of exposure (3, 7). The SAMHSA advisory suggests EtG concentrations up to 1000 ng/mL can be compatible with recent intense extraneous exposure; however, most studies report typical incidental positive EtG concentrations well below this threshold. In 2 studies attempting to simulate health care worker exposure to hand sanitizer and its vapor, at least 1 subject demonstrated urinary EtG >2000 ng/mL in the absence of ethanol consumption (8, 9). It is difficult to assess how realistically these simulations model true exposures in the health care setting; however, the sometimes strikingly high concentrations demonstrate the challenges of interpreting alcohol biomarker results in authentic specimens.

The difficulty of discerning consumption of alcoholic beverages from other sources of ethanol exposure complicates defining appropriate laboratory test cutoffs. The SAMHSA advisory notes, “The cutoff value selected to distinguish specimens as positive or negative should consider the base rate of problem drinking in the population being evaluated, an individual’s likely exposure to products containing nonbeverage alcohol, and the consequences for the individual and society of the individual being erroneously labeled.” However, no process is outlined for this; given that many laboratories performing alcohol biomarker testing are reference laboratories, such insights into their patient/client population and intended use might be impossible to obtain. In addition, despite observing that ‘further research is needed before firm cutoffs can be established’ (4), the SAMHSA has yet to solidify the EtG cutoffs suggested in the 2012 advisory or update the interpretive thresholds based on more recent studies.

The paucity of formal guidance and the wide range of proposed cutoffs is evident in the variability of analytical cutoffs used by performing laboratories. As of this writing, there are no assays cleared for clinical use by the Food and Drug Administration, requiring clinical laboratories to establish their own performance criteria as laboratory-developed tests. Data from the College of American Pathologists (CAP) Ethanol Biomarkers proficiency testing survey indicate that laboratories using urine immunoassay screens for EtG employ at least 8 different cutoffs from 50 to 1000 ng/mL. Participants using mass spectrometric (MS) assays reported 12 different cutoffs between 10 and 1000 ng/mL for EtG, and 20 different cutoffs from 10 to 1500 ng/mL for EtS. The lowest cutoffs reported were well within the range of EtG and EtS concentrations observed in various studies of incidental exposures; in contrast, high cutoffs bear the risk of false negative results because of the relatively short detection window of these biomarkers.

Detection Windows and Analytical Performance

Numerous studies have demonstrated a correlation between ethanol dose and peak urinary concentrations of EtG and EtS; i.e., heavy or binge drinking will result in higher amounts of these biomarkers than light or intermittent use (1). However, there is substantial inter-individual variability in both the amount of EtG and EtS formed after a given ethanol dose, and in the time required for these biomarkers to decline below the cutoff (5). Unfortunately, there is also quite a bit of variability in study design (e.g., controlled dosing, self-reporting, or acute intoxication) and definitions (e.g., 1 ‘dose’ of ethanol or what constitutes light vs moderate vs heavy drinking), which further complicates the interpretation of the available data.

Several studies administering controlled doses of alcohol indicate that most healthy individuals will have detectable EtG (cutoffs 100–200 ng/mL) and EtS (cutoffs 100–110 ng/mL) for up to 1 day after light consumption, and 1 to 2 days after moderate consumption. Heavy drinking including acute intoxication may extend the detection window to 3 to 5 days (5). However it is important to recognize that the inter-individual variability in EtG and EtS clearance means that, even in healthy subjects, the window for detecting ethanol consumption could be shorter or longer. Higher assay cutoffs result in shorter detection windows and may limit the ability to detect light or moderate drinking (10).

Evaluating detection windows is even more complicated when considering various patient populations rather than healthy volunteers. One study found higher EtG and EtS concentrations with increasing age and marijuana use, and lower concentrations in males and patients with kidney disease (11). In contrast, a study of nonsupervised ethanol consumption in patients with decreased renal function found that EtG and EtS concentrations were higher and detection windows were nearly twice as long compared to a similar study in healthy volunteers (12). Another group that observed higher EtG concentrations in patients with chronic kidney disease also noted that samples from pretransplant patients (liver or kidney) were more prone to

Box 1. Ethyl glucuronide (EtG) interpretive guidelines

SAMHSA (urine EtG)

- A “high” positive (e.g., >1,000 ng/mL) may indicate:
  - Heavy drinking on the same day or previously (e.g., previous day or two).
  - Light drinking the same day.
- A “low” positive (e.g., 500–1,000 ng/mL) may indicate:
  - Previous heavy drinking (previous 1–3 days).
  - Recent light drinking (e.g., past 24 hours).
  - Recent intense “extraneous” exposure (within 24 hours or less).
- A “very low” positive (100–500 ng/mL) may indicate:
  - Previous heavy drinking (1–3 days).
  - Previous light drinking (12–36 hours).
  - Recent “extraneous” exposure.

SOHT (hair EtG)*

- Abstinence Assessment
  - ≤5 pg/mg EtG in the proximal head hair segment with a length of 3 cm up to 6 cm does not contradict self-reported abstinence.
  - >5 pg/mg EtG in the proximal head hair segment with a length of 3 cm up to 6 cm strongly suggests repeated alcohol consumption.
- Chronic Excessive Consumption
  - ≥30 pg/mg EtG in the proximal head hair segment with a length of 3 cm up to 6 cm strongly suggests chronic excessive alcohol consumption.

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*SOHT guidelines provide additional details regarding length and source of hair not included here.

analytical interferences and required use of additional mass transitions to correctly identify EtG (13).

This latter study raises important questions regarding the actual ‘how to’ of performing EtG and EtS testing. Available methodologies include commercial immunoassays and laboratory-developed tests including gas chromatography–mass spectrometry (GC–MS), and liquid chromatography–tandem mass spectrometry (LC-MS/MS). Immunoassays can be prone to false positives, and confirmation of positive results by MS is recommended (4). Reports have also demonstrated underestimation of EtG by immunoassays compared to MS, particularly at concentrations >1000 ng/mL (14). However, since fewer laboratories report semiquantitative immunoassay results than qualitative, the impact of this bias is likely small in actual practice.

Unfortunately, evidence suggests quantitative results from MS-based methods also suffer from challenges including lack of standardization. One study comparing 482 authentic specimens across 4 different LC-MS/MS assays for EtG demonstrated significant differences in high-concentration samples (>1500 ng/mL). Although quantitative agreement was better at lower concentrations, qualitatively up to 18% of samples fell into different diagnostic categories (positive vs negative) at cutpoints ranging from 100 to 1000 ng/mL (15). The authors speculate that direct-injection MS techniques, while convenient and rapid, might not adequately separate analytical interferences from EtG (and EtS) even with the use of isotopically labeled internal standards.

CAP proficiency testing results corroborate lack of comparability between methods, even using a more controlled matrix (synthetic urine). For example, in 6 separate samples targeted between 700 and 800 ng/mL, participating laboratories reported concentrations ranging from <50 to >6000 ng/mL (Fig. 1). Performance in graded testing indicated that on average, 1 in 10 laboratories provided results >35% different from the all-participant mean; this difference is enough to change interpretation between the SAMHSA low-positive, positive, and high-positive ranges (14).

Caveat Tester: Let the Performing Laboratory Beware

Laboratories offering EtG and EtS testing have substantial preanalytical, analytical, and postanalytical concerns they must address to ensure accurate test results and correct interpretation. Ex vivo formation or degradation of EtG in urine can be mitigated through preservatives, frozen storage, and/or concomitant measurement of EtS. The value of EtS as a complementary marker has been debated (16); however, most laboratories participating in CAP proficiency testing for MS-based platforms offer both EtG and EtS (14).

Analytical challenges include the difficult nature of the assays themselves, such as interferences in MS-based testing and limited linear response on

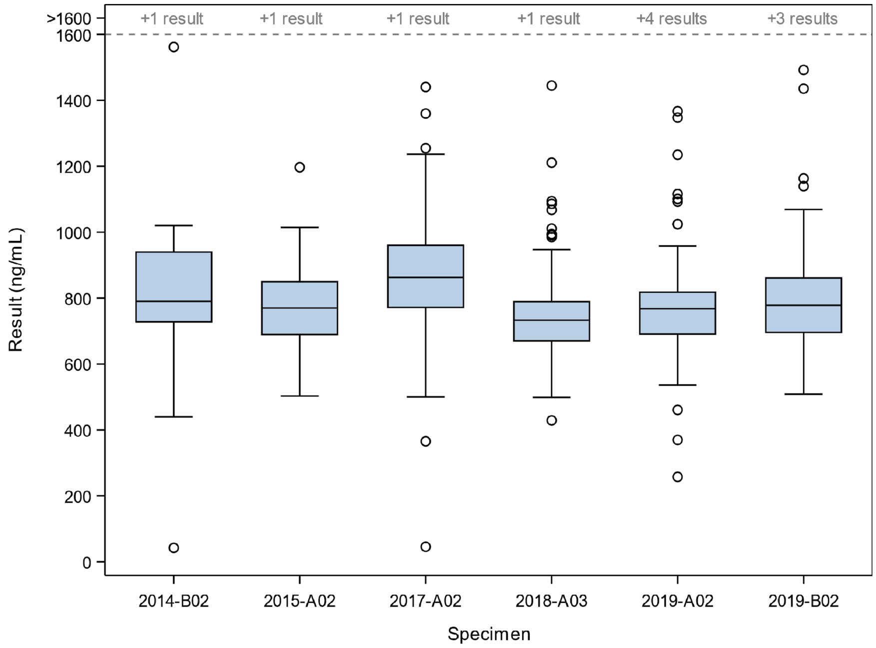

Fig. 1. Result distribution. Box and whisker plots are shown for all challenges targeted between 700 and 800 ng/mL ethyl glucuronide. Whiskers reflect 61.5 times the interquartile range or the extremes of the distribution. Reprinted from Snozek et al. (14) with permission from Archives of Pathology & Laboratory Medicine. Copyright 2021. College of American Pathologists.

immunoassays. More concerning, though, are the lack of standardization and comparability between methods, and the wide variety of interpretive cutoffs used by performing laboratories. No reference methodology exists for EtG or EtS, although initial efforts in this direction have been made for EtG (15). There is therefore no recognized standard for labs to compare against to assess whether their methods are accurate or free from interferences. Proficiency testing provides some objective information regarding accuracy but cannot address the range of potential interferences and unique issues presented by authentic specimens.

The paucity of formal guidance for analytical cutoffs and interpretive thresholds has undoubtedly contributed to the wide disparity between EtG and EtS assays. Ideally, the SAMHSA or other regulatory bodies should systematically evaluate the available literature and provide updated recommendations tailored to clinical and forensic settings. In the meantime, the responsibility for optimizing cutoffs falls to the performing laboratory and should, if possible, consider the intended goals of testing (e.g., monitoring abstinence vs trending during detoxification) and whether results may have negative consequences such as loss of licensure or denial of transplant listing (4, 7). Laboratories should also make efforts to ensure the end users of their test results are aware of the limitations of these biomarkers, e.g., through interpretive comments, outreach education, or other means.

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Urine Drug Testing for Fentanyl and Fentanyl Analogs

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Fentanyl is a potent synthetic opioid prescribed for the management of severe pain. The routes of administration include injection/infusion, transdermal, sublingual, and intranasal routes. The elimination of fentanyl from the human body is mainly accomplished via oxidative N-dealkylation by the liver cytochrome P450 3A4 enzyme into norfentanyl, the major metabolite. Therefore, the capability of the diagnostic assay to detect norfentanyl is critical for successful identification of fentanyl use. Up to 85% of intravenously administered fentanyl is excreted in the urine over a 3- to 4-day period. Within the excreted fractions, 0.4% to 6% is excreted as unchanged drug, 26% to 55% as norfentanyl, and the rest as unknown amounts of hydroxyfentanyl and hydroxynorfentanyl (1). Following the synthesis of fentanyl in 1960, fentanyl analogs such as carfentanyl, sufentanil, alfentanil, and remifentanil were developed for medical or veterinary use. The US Drug Enforcement Administration has classified fentanyl analogs prescribed as analgesics or veterinary medicine as Schedule II narcotics. Many more analogs were synthesized for recreational use. Some common fentanyl analogs causing overdose deaths include acetylfentanyl, acrylfentanyl, butyrfentanyl, carfentanil, cyclopropylfentanyl, fluorofentanyl, 4-fluorobutyrfentanyl, 4-fluoroisobutyrfentanyl, furanylfentanyl, 2-methoxyacetylfentanyl, 3-methylfentanyl, and ocfentanil. Recent studies suggest a surge of novel fentanyl analogs in the global heroin supply has caused unprecedented spikes in opioid overdose deaths (2). The capability of the diagnostic assay to detect common fentanyl analogs is therefore pivotal to the identification of overdoses caused by fentanyl analogs.

According to the US Centers for Disease Control and Prevention, the number of deaths caused by synthetic opioids other than methadone, primarily fentanyl and its analogs, has tripled since 2016 (3). Fentanyl-laced street drugs, such as heroin, cocaine, and methamphetamine, caused outbreaks of fentanyl overdose in multiple metropolitan areas. Although emergency department physicians often treat the presumed opioid overdose patients with naloxone, an opioid antagonist, before drug test results are available, accurate and rapid fentanyl screening still assists with clinical decision-making and management (4). Previous studies suggest fentanyl overdoses require higher naloxone doses and repeated dosing frequencies (5). As the case number of fentanyl overdose rises across the US, some hospital core laboratories either have added fentanyl to their drug screening panels or are considering validating the assay. This article introduces the readers to clinical fentanyl assays currently available and their performance.

Two testing methodologies are available for fentanyl detection: immunoassays and mass spectrometry assays (Table 1). LC-MS/MS is the gold standard for comprehensive drug identification and quantitation. The advantages include high sensitivity, high specificity, and the ability to simultaneously monitor many analytes. However, it also requires highly trained technologists, expensive instrumentation, and batch processing, resulting in high costs and long turnaround times (1–3 days).

Table 1. Comparison of rapid lateral flow assays, immunoassays in central laboratories, and LC-MS/MS for fentanyl.

	Lateral flow assays	Immunoassays in central laboratories	LC-MS/MS
Regulatory status	No FDA[a]-approved or FDA-cleared assays available	FDA-cleared assays available	No FDA-approved or FDA-cleared assays available
Analytical category	Qualitative	Qualitative	Quantitative or qualitative
Principles	Competitive immunoassay	Competitive immunoassay	Chromatography separation and mass spectrometry identification
Turnaround time	5–30 minutes	Hours	1–3 days
Batching	Not required	Not required, random access	Batched
Costs	Low	Medium	High
Sensitivity and specificity	Usually lower sensitivity than central lab immunoassays or LC-MS/MS with some exceptions (10)	Lower specificity than LC-MS/MS	High
Fentanyl analogs	Variable cross-reactivity with fentanyl analogs	Variable cross-reactivity with fentanyl analog	Identify individual fentanyl analogs with high specificity

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[a] FDA, US Food and Drug Administration.

Immunoassays, on the other hand, can be automated, supporting short turnaround times (hours) and relatively low costs. Drawbacks of the immunoassays include lower specificity and sensitivity compared to LC-MS/MS.

At the time of writing, there are 3 US Food and Drug Administration-cleared fentanyl immunoassays for diagnostic use: the ARK Fentanyl II Assay, the Immunalysis SEFRIA Fentanyl Urine Enzyme Immunoassay, and the LZI Fentanyl Enzyme Immunoassay. Both the ARK and the Immunalysis fentanyl assays have a cutoff of 1 ng/mL for fentanyl. The ARK assay has cross-reactivity with norfentanyl at 15 ng/mL (7% cross-reactivity), while the Immunalysis assay has a cross-reactivity with norfentanyl at 20,000 ng/mL (0.005% cross-reactivity). One study found that, compared with Immunalysis, the ARK assay demonstrated higher sensitivity (100% vs 95%) and specificity (96% vs 80%) in an evaluation using 282 patient specimens confirmed by LC-MS/MS (6). Another study revealed that the ARK and SEFRIA assays demonstrated overall good detectability of structural analogs of fentanyl, including acetylfentanyl, acryfentanyl, butyrfentanhyl, 4-chloroisobutylfentanyl, 4-fluorobutylfentanyl, 4-fluorofentanyl, isobutylfentanyl, methoxyacetylfentanyl, and tetrahydrofuranfentanyl (7). On the other hand, the LZI fentanyl assay directly targets norfentanyl with a cutoff of 5 ng/mL, and a cross-reactivity with fentanyl at 3.8 ng/mL (131.58% cross-reactivity). It also has the broadest spectrum of cross-reactivity with fentanyl analogs (Table 2). Further clinical studies are needed to compare the clinical performance of the LZI assay with the others. Nevertheless, results from all immunoassays should be confirmed by LC-MS/MS. The LC-MS/MS methods for fentanyl are laboratory-developed tests that may include fentanyl, norfentanyl, and other analogs in a panel with other opioids. When included in the LC-MS/MS panel, the analogs can be identified with high sensitivity and specificity.

Besides immunoassays used in central laboratories, rapid fentanyl screening strips utilizing immunoassay principles and a lateral flow assay format are also available, mostly in harm reduction settings. Current commercially available strips have relatively high fentanyl cutoffs, ranging from 10 to 200 ng/mL, and therefore may lead to many false negative results when used for testing clinical specimens. They are limited to use in harm reduction settings to provide drug users with the information of possible presence of fentanyl in drug products. A recent study revealed that fentanyl test strips-based interventions could effectively reduce accidental opioid overdose risks (8). The cross-reactivity of these rapid screening strips with different fentanyl analogs varies (9). A rapid lateral flow assay utilizing competitive immunoassay principles was recently developed with a fentanyl cutoff of 1 ng/mL and a

Table 2. FDA-cleared fentanyl immunoassays for central laboratory use.

	ARK fentanyl II assay	Immunalysis SEFRIA fentanyl urine enzyme immunoassay	LZI fentanyl enzyme immunoassay
Manufacturer	ARK Diagnostics, Inc	Immunalysis Corporation	Lin Zhi International
Principle	rG6PDH	β-galactosidase	rG6PDH
Absorbance wavelength	340 nm	570 nm	340 nm
Quantitative or qualitative	Qualitative	Qualitative	Qualitative
Fentanyl cutoff (cross-reactivity)	1 ng/mL	1 ng/mL	3.8 ng/mL (131.58%)
Norfentanyl cutoff (cross-reactivity)	15 ng/mL (7%)	20,000 ng/mL (0.005%)	5 ng/mL
Fentanyl analogs tested at concentration approximately equivalent to the cutoff (cross-reactivity)	Acetyl fentanyl 1.1 ng/mL (90.91%) Isobutyryl fentanyl 1.1 ng/mL (90.91%) ω-1-Hydroxyfentanyl 1.2 ng/mL (83.33%) Acrylfentanyl 1.3 ng/mL (76.90%) Butyryl fentanyl 1.4 ng/mL (71.43%) Furanyl fentanyl 1.5 ng/mL (66.67%) Para-fluoro fentanyl 1.5 ng/mL (66.67%) Ocfentanil 1.6 ng/mL (62.50%) 4-Fluoro-isobutyryl fentanyl 1.9 ng/mL (52.63%) Para-fluorobutyryl fentanyl 1.9 ng/mL (52.63%) Valeryl fentanyl 2.3 ng/mL (43.48%) β-hydroxyfentanyl 9.5 ng/mL (10.53%) Acetyl norfentanyl 12.1 ng/mL (8.26%) (±) β-hydroxythiofentanyl 32.7 ng/mL (3.06%) (±)-3-cis-methyl fentanyl 144.1 ng/mL (0.69%) Carfentanil 448.2 ng/mL (0.22%) Despropionyl fentanyl 471.8 ng/mL (0.21%) Sufentanil 2,362 ng/mL (0.04%) Remifentanil 10,000 ng/mL (ND) Norcarfentanil 38,196 ng/mL (0.003%) Alfentanil 100,000 ng/mL (ND)	Acetyl fentanyl 1 ng/mL (100%) Butyryl fentanyl 1 ng/mL (100%) Despropionyl Fentanyl 40 ng/mL (2.5%) Sufentanil 175 ng/mL (ND)	4-Fluoro-Isobutyryl Fentanyl 20 ng/mL (25%) 9-Hydroxy Risperidone 100,000 ng/mL (ND) Acetyl fentanyl 7 ng/mL (71.43%) Acetyl fentanyl 100 ng/mL (5.00%) Acryl Fentanyl 4 ng/mL (125.00%) Butyryl fentanyl 6 ng/mL (83.33%) Butyryl norfentanyl 40 ng/mL (12.50%) Carfentanil Ocalate 100,000 ng/mL (ND) (±)-3-cis-methyl fentanyl 8 ng/mL (62.50%) Cyclopropyl fentanyl 3.2 ng/mL (156.25%) Cyclopropyl norfentanyl 25 ng/mL (20.00%) Despropionyl fentanyl 100,000 ng/mL (ND) Furanyl fentanyl 5.5 ng/mL (90.91%) Furanyl norfentanyl 180 ng/mL (2.78%) (±) β-hydroxythiofentanyl 100,000 ng/mL (ND) Isobutyryl fentanyl 15 ng/mL (33.33%) Isobutyryl fentanyl 500 ng/mL (1.00%) Methoxyacetyl fentanyl 3.5 ng/mL (142.86%) N-benzyl furanyl norfentayl 11 ng/mL (45.45%) N-benzyl para-furanyl norfentayl 4 ng/mL (125.00%) Norcarfentanil Oxalate 100,000 ng/mL (ND) Ocfentanil 3.8 ng/mL (131.58%) Para-fluorobutyryl fentanyl 4.5 ng/mL (111.11%) Para-fluoro fentanyl 3.2 ng/mL (156.25%) Remifentanil 100,000 ng/mL (ND) Sufentanil 100,000 ng/mL (ND) Thienyl fentanyl 4 ng/mL (125.00%) Thiofentanyl 3.2 ng/mL (156.25%) (±)-trans-3-methyl Fentanyl 6 ng/mL (83.33%) Valeryl fentanyl 70 ng/mL (7.14%) ω-1-Hydroxyfentanyl 300 ng/mL (1.67%)

norfentanyl cutoff of 10 ng/mL (10). The improved analytical sensitivity for fentanyl and rapid turnaround time (5 min) in the point-of-care setting could support useful diagnostic applications if the assay demonstrated wide cross-reactivity with a relatively large number of fentanyl analogs. None of the rapid fentanyl screening strips has been FDA-approved at the time of writing.

In summary, various fentanyl assays have been developed and commercialized to expand our arsenal to fight the opioid epidemic. At the time of writing, the most frequently implemented fentanyl diagnostic assays are automated fentanyl immunoassays in the hospital core laboratories. Positive immunoassay screening results should be confirmed using LC-MS/MS methods. Some clinical laboratories have adopted high-resolution mass spectrometry for direct screening of fentanyl and other opioids without immunoassay testing (11). However, immunoassay screening with reflex to mass spectrometry confirmation remains the mainstream of urine drug testing. The exact identification of fentanyl analogs relies on LC-MS/MS assays that are often only available in specialized reference laboratories. Rapid point-of-care fentanyl testing strips may reduce accidental fentanyl overdose risks, but they need improved sensitivity to be useful for diagnostic applications.

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Toxicology Updates from the 2022 AACC Annual Scientific Meeting

By Alec Saitman, PhD, and Bridgit O. Crews, PhD

This year’s American Association for Clinical Chemistry (AACC) Annual Scientific Meeting held July 2022 in Chicago, Illinois, had a rich and diverse portfolio of toxicology-related sessions. These sessions provided practical and novel information for the novice and the expert toxicologists alike.

On Monday, Drs. Heger and Tacker presented an excellent selection of interactive toxicology case presentations, to help audience members design a “toxicology interpretation toolkit.” This session focused mainly on pain management testing and used actual cases that Drs. Heger and Tacker had to troubleshoot in their own laboratories. This discussion made for an essential session to attend for newer laboratory directors and toxicology staff.

Also scheduled on Monday was the highly anticipated cannabis and driving session. Drs. Fitzgerald and Marcotte described the current state of cannabis exposure testing and how it relates to driving while under its influence. During the session, audience members received unique insight into the complexities of testing different tetrahydrocannabinol (THC) metabolites and biomarkers. These data combined with driving simulation trials of real cannabis users made for a thought-provoking session. Specifically, one of the most interesting details of the study was that study participants were allowed to smoke the amount of cannabis they wanted (self-titration) as opposed to smoking a specific amount prescribed. The speakers argue that this provides better insight into the behavior of cannabis smokers in real world driving settings rather than laboratory-created ones. In the afternoon, Drs. Cotten, Strathmann, and Kuypers discussed implications of psychedelics entering the clinical realm from clinical, laboratory, and business perspectives.

On Wednesday, Drs. Lynch and Roper discussed the nuances of testing for drugs and other toxic substances in pediatric patients. Dr. Lynch began the presentation with multiple cases showing how high-sensitivity mass spectrometry may serve as an important methodology in detecting expected and unexpected drugs and metabolites in these samples. The case studies presented with varying difficulty in the final interpretation, which helped highlight the importance of a robust toxicology clinical department as well as the importance of inter-collaboration with local or state poison control centers and medical examiners offices. In the afternoon, Drs. Morad, Colby, and McMillin discussed the benefits and limitations of meconium versus umbilical cord tissue for neonatal drug testing.

The final scientific session on Thursday, presented by Drs. Saitman and Liao, discussed laboratory approaches for clinical drug testing, including how to address common pitfalls. Their approach centered on leveraging both immunoassay and mass spectrometry to develop comprehensive drug testing programs in laboratories of varying size and complexity.

The Therapeutic Drug Management and Toxicology Division also held the annual division luncheon. Dr. He Sarina Yang received this year's Young Investigator Award and gave an overview of her current research in applying machine learning to drug testing using mass spectrometry. The division also awarded Conner Blair the Best Abstract Award for Outstanding Research for his abstract titled: *Internal Versus External Calibration for Determination of Plasma Methotrexate by LC-MS/MS*. For those individuals who were fortunate to arrive a day early, there was also an informative AACC university session that covered drug test interpretation, presented by Drs. Snozek, Colby, and Delaney.

Overall the meeting was well attended, with attendance more in line with pre-pandemic meetings, not to mention the weather in Chicago was ideal during the meeting week and easy to enjoy with several outdoor networking events overlooking Lake Michigan.

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