Assessment of Ethanol Intoxication and Regulatory Issues

Determination of ethanol concentrations is one of the most common toxicology tests performed in the clinical laboratory that has potential medicolegal ramifications. Several methods can be used to measure ethanol in biologic fluids. Although gas chromatography (GC) and whole blood are considered the reference method and the specimen of choice, this method is too costly and time-consuming for routine use as a primary screening method. Thus, in the clinical setting, enzymatic methods have become the most common approach for measurement of ethanol in serum, plasma, and urine. Devices used for breath-alcohol analysis have been used by law enforcement agencies for the past several years to determine impairment. Recently, the US Department of Transportation (DOT) has approved many of the devices for use in its alcohol-testing program. It also has approved many point-of-care devices for screening of the alcohol concentration in saliva. Although breath-alcohol analysis and saliva testing are performed rarely by the clinical laboratory, they have been used in the emergency department for point-of-care testing.

Measurement of Ethanol in Blood and Urine

Enzymatic Determination of Ethanol

Methods that use the enzymatic oxidation of ethanol with alcohol dehydrogenase (ADH) or alcohol oxidase are sensitive and simple. The principal variants in these methods are the use of ADH or alcohol oxidase, measurement of the change in UV absorbance of the reaction mixture at 260 nm or 340 nm, and visual spectrophotometry of a secondary indicator reaction. The most common enzymatic method is based on the oxidation of alcohol to acetaldehyde by ADH with the concomitant reduction of the oxidized form of nicotinamide adenine dinucleotide (NAD$^+$) to the reduced form of nicotinamide adenine dinucleotide (NADH).

\[
\text{NAD}^+ + \text{Alcohol} \xrightarrow{ADH} \text{NADH} + H^+ + \text{Acetaldehyde}
\]

Acetaldehyde + Tris(hydroxy-methyl) aminomethane (TRIS) → TRIS-Acetaldehyde Complex (Trapped)

For most commercially available assays, the production of NADH is measured spectrophotometrically at 340 nm—the amount of NADH produced is directly proportional to the amount of ethanol present in the sample. Some of these
methods use a second reagent, such as TRIS buffer, to “trap” the acetaldehyde, which drives the reaction to completion.

Ethanol concentrations also can be determined by radiative energy attenuation. A colored dye (chromophore) is generated that inhibits the production of fluorescence from the dye, fluorescein. The light-absorbing properties of the chromophore cause a decrease of measured fluorescent light intensity (attenuated radiative energy). The colored dye, mononitetra (MT)-formazan that attenuates the fluorescence is produced from the unreacted dye, mononitetroazolium (MTT), after it reacts with the NADH produced by the ADH reaction. The decrease in fluorescence intensity is directly proportional to the amount of ethanol present in the sample. The assay can be used with serum, plasma (heparin, EDTA, fluoride, citrate, oxalate), treated whole blood, and urine.

\[
\text{NAD}^+ + \text{Alcohol} \xrightarrow{\text{ADH}} \text{NADH} + \text{H}^+ + \text{Acetaldehyde}
\]

\[
\text{NADH} + \text{H}^+ + \text{MTT} \xrightarrow{\text{Diaphorase}} \text{NAD}^+ + \text{MT-formazan}
\]

All enzymatic assays can be used to analyze serum, plasma (fluoride, oxalate), and urine. Some of the assays, however, are affected by EDTA or heparin; others require special treatment if they are used with whole blood.

**Interference With Alcohol Dehydrogenase Methods**

**Alcohol**
The ADH assay is specific for ethanol and does not cross-react with acetone or acetaldehyde. The enzyme shows slight cross-reactivity with isopropanol of 7%, methanol of 3%, and ethylene glycol of 4%. Some manufacturers claim <1% interference from these alcohols. The amount of interference due to the common toxic alcohols (methanol, isopropanol, ethylene glycol) is rarely of clinical importance. Interference due to n-propanol and n-butanol is greater and depends primarily on the level of alcohol tested and the manufacturer of the method.

**Lactate and Lactate Dehydrogenase**

Pathologic conditions (such as lactic acidosis, diabetes mellitus, chronic liver failure, leukemia) can be associated with elevated lactate and lactate dehydrogenase (LDH) levels. Depending on the method, increased concentrations of lactate in combination with increased LDH levels in a sample can give a false increase in ethanol concentration owing to the formation of NADH, the product measured during the conversion of ethanol to acetaldehyde by ADH. The degree of interference also is assay-dependent. It is the increased production of NADH from the LDH reaction that causes a false-positive ethanol measurement.

\[
\text{NAD}^+ + \uparrow \text{Lactate} \xrightarrow{\text{LDH}} \uparrow \text{NADH} + \text{Pyruvate}
\]

This interference also has been noted in postmortem samples (plasma, urine, and vitreous humor) from infants.

**Ethanol Analysis by Gas Chromatography**

Two approaches (head-space analysis and direct analysis) are used by clinical laboratories to analyze ethanol by GC; each is based on the type of injection method used to introduce the sample into the gas chromatograph. Low-molecular-weight alcohols, such as ethanol, are sufficiently volatile and, thus, occur in measurable concentrations in the air space (head space) above a liquid specimen in a closed container. For head-space analysis, a portion of this air space is sampled and injected into the GC system. Head-space analysis prevents the sample from contaminating the column and injector. Dilutions of the sample are made with a saturated solution of sodium chloride containing the internal standard (usually n-propanol). The sodium chloride increases the vapor pressure of the alcohols and eliminates matrix differences between aqueous calibrators and protein-based blood or serum. The direct method requires the sample dilution with an aqueous solution containing the internal standard before injecting a small volume (<0.5 μL) into the gas chromatograph. The syringe must be rinsed thoroughly between samples.

There are two main types of columns in GC: packed and capillary. Packed columns are filled with particles with an inner diameter of 0.1 to 0.4 cm; the particles can be uncoated or coated with the stationary phase. Capillary columns have particles with an inner diameter of 0.2 to 0.5 mm coated; the inner walls are coated with the stationary phase. Capillary columns have become more popular for ethanol analysis. The type of detector most commonly used with GC is the hydrogen flame ionization detector.
An internal standard should be added to each calibrator, control, and sample for analysis by GC. According to National Committee for Clinical Laboratory Standards guidelines, every single or batch of analyses performed by GC should have at least one and preferably two calibrators, together with an alcohol-free “blank” because changes can occur with the operating parameters and calibration of GC instruments with each startup, and drift often is observed during prolonged operation. At least every 10th specimen should be a control during multiple sequential analyses; use of multilevel controls is recommended. One of the major advantages of GC is the simultaneous determination of ethanol along with other volatile alcohols and some of their metabolites (eg, methanol, isopropanol and its metabolite, acetone).

**Estimation of Ethanol From Osmolality**

An estimation of osmolality or the calculated osmolality often is used by clinicians in an emergency setting; it can be determined by using the following expression:\(^{10}\):

\[
\text{Calculated Osmolality} = 2 \times \text{Na}^+ - \frac{\text{Glucose (mg/dL)}}{20} + \frac{\text{Serum Urea Nitrogen (mg/dL)}}{3}
\]

Unlike the measured osmolality, which is based on the concentration of all particles in the plasma, the calculated osmolality accounts only for the major contributors to plasma osmolality, ie, sodium, glucose, and serum urea nitrogen. The difference between the measured osmolality and the calculated osmolality is called the osmolal gap (reference interval, about 0–6 mOsm/kg H\(_2\)O). The presence of any alcohol in serum or plasma leads to an increase in the osmolality (as measured by freezing-point depression), increasing the osmolal gap. Ethanol contributes about 2.2 mOsm for every 10 mg/dL of ethanol present. The serum concentration of ethanol can be estimated reasonably from the change in the osmolal gap (\(\Delta\) osmolality) by using the following formula:

\[
\text{Alcohol Concentration (mg/dL)} = \Delta \text{ Osmolality} \times \frac{M_e}{10} \times 0.93
\]

The factor 0.93 corrects for the percentage of water in serum or plasma; the molecular weight (\(M_e\)) of ethanol is 49. The osmolal gap correlates well with blood alcohol concentrations (BACs).\(^{11,12}\) However, there are shortcomings when estimating the concentrations.\(^{13,14}\) Cases have been reported in which the concentration of ethanol was overestimated by as much as 30%.\(^{15}\) This discrepancy may be related to the nonideal “osmotic” behavior of ethanol, which can alter the degree of dissociation of solutes within the sample.\(^{15}\)

**Measurement of Ethanol in Breath**

Breath analysis is the most common method used in the United States for medicolegal alcohol determinations. Evidential breath testing (EBT) devices often are used in the emergency department since the devices are not regulated by the Clinical Laboratory Improvement Amendments of 1988 (CLIA ‘88); they also are used routinely in workplace testing and by law enforcement agencies. To be approved by the US National Highway Traffic Safety Administration (NHTSA)—DOT for inclusion in the Conforming Products List, the EBT devices must meet certain specifications (updated periodically in the Federal Register; Table 1).\(^{16}\)

Instruments for breath–ethanol analysis use various methods, including infrared spectrophotometry, GC, electrochemical detection—fuel cell, solid-state sensing, and chemical oxidation with spectrophotometry. Instruments that use infrared spectrophotometry for ethanol breath testing are common. They quantitate ethanol based on the absorption of infrared energy at wavelengths specific for the chemical bonds in the ethanol molecule (such as C—H, O—H, C—O). The greater the concentration of ethanol, the more energy is absorbed; quantitation is based on the Beer-Lambert law. Some of the devices use specialized infrared energy sources in combination with sophisticated optical systems to attain a high specificity. For example, most of the new instruments are designed to identify and compensate for the presence of acetone, because high levels of acetone, which can produce

<table>
<thead>
<tr>
<th>Sample Processing</th>
<th>Prints 3 copies of each test result</th>
<th>Each test numbered sequentially</th>
<th>Processes a test of ambient air with printed record</th>
<th>External calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol concentrations measured (% wt/vol)</td>
<td>0.00, 0.02, 0.04, 0.08, and 0.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compounds measured</td>
<td>Must measure for presence of acetone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscellaneous information</td>
<td>Definition of alcohol to include other low-molecular-weight alcohols including methyl (methanol) and isopropyl (isopropanol)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The Phoenix Portable Breath Analyzer (Lifeloc Technologies, Wheat Ridge, Colo) is a US Department of Transportation-approved, handheld device that utilizes direct and passive alcohol testing. The limits of detection range from 0.000 to 0.400 BAC (blood alcohol concentration) units. The device can store up to 200 test results and has a serial interface for a printer or computer.

The Breathalyzer 7410 (Draeger Safety, Breathalyzer Div, Durango, Colo) is a US Department of Transportation-approved, handheld device that utilizes a temperature-controlled fuel cell allowing the unit to operate efficiently at ambient temperatures that range from 23°F to 104°F (−5°C to +40°C). The limits of detection range from 0.000 to 0.400 gm/210 L of breath. The device is used in conjunction with a printer that can store up to 512 test records via an optical interface. It also can download test records directly to a computer.

Symptoms similar to ethanol-induced intoxication, can occur under certain pathophysiologic conditions (eg, in patients with diabetes, chronic starvation). Other devices can discriminate toluene and methanol, although this is not an NHTSA requirement. However, other instruments, especially older models, do not compensate for acetone, other alcohols, or solvents. Thus, the operator should be aware of the limitations of each instrument. One of the major advantages to this technology is its real-time measurement. One disadvantage of infrared technology is the high cost of achieving specificity and accuracy at low breath alcohol levels. Because the output of the infrared detector is nonlinear for alcohol concentration, the signal must be corrected.

Other popular instruments for breath ethanol testing use an electrochemical oxidation-fuel cell (Fig 1 and 2). Ethanol from alveolar air is converted by oxidation to acetic acid. The oxidation is performed by a fuel cell, which consists of two platinum-coated conduction electrodes separated by an ion-conducting electrolyte layer (Fig 3). The reaction, which generates two electrons per ethanol molecule, produces a measurable current that is directly proportional to the ethanol concentration in the sample. This device generally is used by certified breath alcohol technicians for regulated testing consistent with DOT standards.

Devices that use fuel cells are not subject to interference by acetone, but other alcohols (methanol, isopropanol) and volatile substances (toluene, gasoline, and diethyl ether) can cause false-positive results. Fuel cell technology, used for years for screening or preliminary breath alcohol analysis, also can be used in EBT for ethanol; some devices use the fuel cell in combination with an infrared detector to increase test specificity.

Other breath-ethanol devices use light-emitting diode readouts with solid-state (semiconductor) sensors that are selective for alcohol. Some small portable devices require no specialized
instrumentation and can be used to screen semiquantitatively for alcohol. The method is based on the color change of crystals contained within a disposable plastic tube (Fig 4).

The conversion ratio used by most US manufacturers to calibrate the analyzers (so results can be correlated with BACs) is 2,100:1. This ratio means that 2,100 mL of deep lung air contains the same amount of alcohol as 1 mL of blood. Thus, grams per 210 L of breath and grams per 100 mL of blood are the same numerically. Studies have shown that using the conversion factor of 2,100:1 underestimates the BAC. Thus, several European countries use a ratio of 2,300:1. However, the NHTSA has stated that the 2,100:1 ratio provides the person undergoing testing with a safety factor of 15% to 20% owing to the underestimation of the arterial BAC.

**Measurement of Ethanol in Saliva**

Several devices can measure ethanol in saliva. Some of them can be used for quantitative determination of ethanol; others can be used only qualitatively. Some of the qualitative tests require interpretation of color to semiquantitate alcohol levels.

One qualitative method, the On-Site Alcohol (Roche Diagnostics, Branchburg, NJ) can detect ethanol in the saliva (or urine) in 2 minutes. It is based on two coupled enzymatic reactions involving ADH, the coenzyme nicotinamide adenine dinucleotide (NAD), and diaphorase (Fig 5). The reactions are as follows:

\[
\text{NAD}^+ + \text{Ethanol} \xrightarrow{ADH} \text{NADH} + \text{H}^+ + \text{Acetaldehyde}
\]

\[
\text{NADH} + \text{H}^+ + \text{Tetrazolium Dye} \rightarrow \text{NAD}^+ + \text{Formazan Dye} + 2\text{H}_2\text{O}
\]

In the first step, ADH oxidizes ethanol in the sample to acetaldehyde, and NAD is reduced concomitantly to NADH. The second step uses diaphorase to catalyze the oxidation of NADH to NAD. The resulting hydrogen is transferred to a colorless tetrazolium salt to produce a highly colored formazan dye. In practice, urine or saliva specimens are deposited on a chemically treated pad that volatilizes reducing alcohols. The resulting vapors are concentrated on a membrane saturated with enzyme solution and tetrazolium salt. When the enzyme–tetrazolium salt solution is exposed to ethanol vapor, a formazan dye is formed, producing a purple plus sign, in 2 minutes or less, indicating a positive result. If the result is negative, the detection reagent pad remains pale yellow for 2 minutes. For saliva, the On-Site Alcohol results provide a means of estimating whether the BAC is >0.02% wt/vol (>20 mg/dL). Although the device also can be used with urine, a positive result only indicates recent alcohol ingestion and does not necessarily indicate intoxication, impairment, or BACs >0.02% wt/vol (>20 mg/dL).

The Alco-Screen 02 method (Chematics, North Webster, Ind) is a simple, one-step, saliva screening device for detecting ethanol at levels >0.02% wt/vol (>20 mg/dL). If the result is positive, a distinct colored band appears within 4 minutes. The Alco-Screen 02 method is not specific for ethanol;
Saliva Alcohol test (STC Technologies, Bethlehem, Pa) is used for the quantitative assessment of alcohol in saliva. It is approved for worksite testing by the US Department of Transportation for workplace programs, alcohol treatment centers, and emergency/trauma departments. A, application of the saliva sample using the collector; B, a positive test result of 0.095% wt/vol (95 mg/dL) is shown (note dark bar).

it reacts with methyl and allyl alcohols. It does not react with alcohols that have 5 or more carbons. The reaction is as follows:

\[ \text{Tetramethylbenzidine} + 2\text{H}_2\text{O} \rightarrow \text{Tetramethylbenzidine} + \text{H}_2\text{O} \]

Although the On-Site Alcohol and Alco-Screen 02 meet the required specifications and are listed on the Conforming Products List of the NHTSA-DOT, both devices are sensitive to changes in temperature. Thus, following the manufacturer’s guidelines is absolutely necessary to ensure reliable results.

The Q.E.D. Saliva Alcohol test (STC Technologies, Bethlehem, Pa) measures the concentration of ethanol in saliva (Fig 6). The test is available in two formats, both with scales in milligrams per deciliter and percentage: the Q.E.D. A150 (range, 0.000%-0.145% wt/vol [0-145 mg/dL]; see Fig 6) and the Q.E.D. A350 (range, 0.000%-0.345% wt/vol [0-345 mg/dL]). The device uses ADH reaction to form acetaldehyde and NADH. During the reaction, electron transfer occurs at an alkaline pH in the presence of a trapping agent, a tetrazolium dye, and diaphorase on a solid support, causing the reaction to quantitatively produce a formazan dye (purple). This dye is directly proportional to the ethanol level in the saliva sample. The device has a built-in quality control system (QA spot) that must turn positive for a valid result. Methanol, ethylene glycol, methyl ethyl ketone, and acetone do not interfere with the method at concentrations up to 0.10% wt/vol (100 mg/dL); n-butanol and isopropanol give an apparent increase in ethanol response of 0.06% wt/vol (60 mg/dL) and 0.02% wt/vol (20 mg/dL), respectively, at a similar concentration. Besides being classified by the Health Care Financing Administration as a waived test, it has been approved by the DOT for use in its ethanol testing programs. Although the device is approved by the Food and Drug Administration only for use with saliva, a study has shown it can be used with heparinized or fluorinated plasma.

None of the devices used to determine the presence of ethanol in saliva provide definitive test results. Although they are reliable for screening, confirmation of positive test results should be performed using whole blood with GC in potential medicolegal cases.

**Workplace Testing**

Although the most practical specimen for ethanol testing in the workplace is urine, the predominant opinion is that urine ethanol testing has major limitations that prevent its use for defining impairment with scientific and legal certainty. If an employer believes evidence of impairment exists or that ethanol intake has occurred, eg, a job-related accident or incoherent speech, a blood ethanol test should be used. Urine can be used if the employer’s only interest is whether an employee has used ethanol. The ADH method often is used for the initial screen. The cutoff concentration varies widely, 0.01% wt/vol (10 mg/dL) to 0.06% wt/vol (60 mg/dL) or higher, and is based on the chemistry instrument used for testing; there is no standardized cutoff for urine ethanol testing. Specimen and aliquot tubes should be capped to prevent evaporation of ethanol in urine. False-negative results can occur owing to the volatility of ethanol; for each hour an aliquot remains uncapped, the ethanol concentration can decrease by 10% to 25%.
Department of Transportation Ethanol-Testing Program

The final rules implementing the 1991 Omnibus Transportation Employees Testing Act require routine ethanol testing nationwide for employees engaged in commercial transportation. The final rules became effective March 17, 1994.\textsuperscript{22} Since ethanol consumption is legal during off-duty hours, guidelines require employers to conduct preemployment, postaccident, reasonable-suspicion, random, return-to-duty, and follow-up ethanol testing. The approved method is breath alcohol testing; a list of devices (with output in BAC units) approved by the NHTSA is available.\textsuperscript{16}

The rules define a screening test result of <0.02% wt/vol BAC as negative; a result of \(\geq 0.02\%\) wt/vol BAC requires a confirmation test (performed in duplicate).\textsuperscript{22} Employees cannot resume work for at least 8 hours if the test results are between 0.02% wt/vol and 0.04% wt/vol BAC.\textsuperscript{22} Although the DOT rules specify breath analysis as the approved method, several circumstances are given for which blood testing may be used, eg, a screening or confirmation test for reasonable suspicion or postaccident testing is required when no approved breath-testing device is available or a subject is unable to provide an adequate breath sample.\textsuperscript{22} Guidelines are given in Table 2. The DOT also has established criteria for the use of screening devices that use body fluids, including saliva, to detect the presence of alcohol at \(\geq 0.02\%\) wt/vol BAC.\textsuperscript{23} However, the role of the clinical laboratory in the DOT alcohol-testing program remains unclear.

Americans With Disabilities Act

When the Americans With Disabilities Act (ADA) became law in 1990,\textsuperscript{24} recovering alcoholics were included among the disabled. Under the ADA, alcohol testing is considered a medical examination; drug testing, however, is not. Employers can test applicants for drugs but not for alcohol unless a job offer has been made. Unlike other workplace testing programs, employers cannot deny employment to an applicant or discipline an existing employee solely on the basis of a positive alcohol test result. Although the ADA has no direct effect on the clinical laboratory per se, the laboratory professional should understand its effect on workplace testing. Laboratory personnel may serve as consultants to persons covered by the ADA by describing the use of the test and the specimen collection process.

Clinical Laboratory Improvement Amendments of 1988

After CLIA '88 was implemented, the Department of Health and Human Services announced that any toxicology test performed by a clinical laboratory for forensic purposes was exempt from CLIA '88 regulations.\textsuperscript{25} Thus, alcohol testing for the workplace, for drivers, and other cases involving the legal system are exempt from CLIA '88 regulations. Only ethanol testing performed for medical purposes is regulated; screening procedures for ethanol testing are considered moderate complexity; confirmation testing using GC is classified as high complexity.

<table>
<thead>
<tr>
<th>Situations that require prohibition of employee performance of safety-sensitive functions</th>
<th>Breath alcohol concentration of (\geq 0.04%) wt/vol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of alcohol on the job site</td>
<td>Use of alcohol within the previous 4h</td>
</tr>
<tr>
<td>Involvement in an accident during the past 8h</td>
<td>Physical examination finding or behavior characteristic of alcohol use</td>
</tr>
<tr>
<td>Refusal to take a required alcohol test</td>
<td>Preemployment: during or after hiring but before start of job</td>
</tr>
<tr>
<td>Postaccident: within 8h of the incident; no retrograde extrapolation of alcohol concentration permitted</td>
<td>Supervisor needs to complete 1-h course on manifestations of alcohol abuse.</td>
</tr>
<tr>
<td>Must test 25% of eligible employees</td>
<td>Must test 50% of eligible employees if industry violation rate is 1% or greater</td>
</tr>
<tr>
<td>Can test 10% of eligible employees if violation rate is &lt;0.5% for 2 y</td>
<td>Can test 10% of eligible employees if violation rate is &lt;0.5% for 2 y</td>
</tr>
<tr>
<td>Employee must comply with recommended treatment; alcohol test result &lt;0.02% wt/vol</td>
<td>Employee must be subjected to 6 unannounced tests within 12 mo on return to duty.</td>
</tr>
</tbody>
</table>
Conclusion
Several methods are available to determine ethanol concentrations depending on the type of specimen. Although enzymatic analysis of ethanol in plasma or serum continues to be the most common approach of clinical laboratories, other methods and devices have become popular and more reliable, and many are used by emergency department personnel, private industry, and government and law enforcement agencies for point-of-care testing for ethanol. Many issues related to testing for ethanol consumption in the workplace are highly regulated with clearly defined guidelines. Although the regulations for all clinical laboratories are defined clearly by CLIA '88, the effects of the regulations on some programs remain to be seen, especially with the continued development of novel, sophisticated, and reliable point-of-care testing devices.

References
22. Department of Transportation, Federal Aviation Administration, Federal Aviation Administration, Federal Railroad Administration, Federal Transit Administration, Research and Special Programs: limitation on alcohol use by transportation workers, 59 Federal Register 7301-7611 (1994).