Serum/Blood Ethanol

British scientists developed a national scale to assess the harm of drugs for potential misuse. This study was published in *Lancet* (1,2). This study, based on physical, psychological, and social problems caused by misused drugs and alcohol, concluded that alcohol was the most harmful of the drugs that are misused. According to the scale developed by the British scientists, alcohol is three times more harmful than cocaine or tobacco. In this chapter, a brief description of the methods available for the determination of serum/blood ethanol is given. Essentially, these involve serum alcohol determinations for medical management of the patient. In the case of forensic alcohol determinations, a blood sample is required. Serum/blood ethanol levels are determined by GC, which is considered the gold standard. Several automated enzymatic methods are developed, which generally require a serum sample.

4.1 Headspace Gas Chromatograph

A variety of GCs is available in the market. All GCs operate under the same principle. Headspace GC consists of a long column packed with inert material. An aliquot of the sample of blood or serum is put in a vial that is sealed with a rubber septum or stopper and is heated. The vapors from the vial are injected automatically into the column. Vaporized compounds are moved through the column by inert gases. The compounds are separated while moving through the column. At the end of the column, a detector detects the compounds by a signal generated and gives out a peak on a chart that rolls at specified speed. The compounds are identified by the retention times. A known standard compound of known quantity mixed with a sample generates a peak. This standard peak area is compared with the area of the compound in question. The peak areas of the standard are compared with the peak area of the compound in question and are used for quantization.

A technologist using a variety of techniques such as increasing or decreasing the flow rate of inert gases, altering the temperature of the column, or altering the speed of the chart can modify the resolution of the chromatograph. GC analysis is now automated so that a technologist can load the carousel with several sealed vials with blood/serum samples and leave it in
the instrument to be analyzed. With this technique, it is possible to quantify ethanol, acetaldehyde, acetone, methanol, and isopropanol. It is possible to identify volatile hydrocarbons present in serum/blood. The reader may recall that in Chapter 3 this technique was used to identify chloroform in the blood of a patient with chloroform poisoning.

GC is considered the gold standard because false-positives generally do not happen. The method is precise and reliable. However, an experienced technologist is needed to conduct the GC analysis. The machine needs periodic maintenance, and occasionally the column needs to be repacked or replaced with a new column. GC analysis is time-consuming despite some degree of automation. For emergency rooms requiring stat analysis for serum/blood ethanols, other techniques such as automatic enzymatic assays are used. In general, because of its accuracy and absence of false-positive results, GC is the technique used for forensic purposes (3).

4.2 The Vitros Chemistry Analyzer

This instrument is made by Johnson & Johnson. It is a versatile chemistry analyzer and operates on slide technology. In addition to several analytes, serum alcohols can be measured by this instrument. Essentially, a 10-µl sample of serum is pipetted by the instrument on a test slide containing nicotinamide adenine dinucleotide (NAD), alcohol dehydrogenase (ADH), and tris(hydroxymethyl)aminomethane (TRIS). On incubation for 5 min at 37°C, NADH is generated, which is read by the instrument spectro-photometrically at 340 nm. The increase in NADH is converted to milligrams of ethanol per deciliter of serum (4).

4.3 The Axsym Analyzer

This instrument is also a chemistry analyzer capable of determining several analytes including serum alcohols. This instrument is made by Abbott. Ethanols are determined by enzymatic method utilizing ADH and NAD. As in the Vitros instrument, alcohol is converted to acetaldehyde in the presence of NAD. NAD is converted to NADH. However, NADH combines with monotetrazolium dye in the presence of diaphorase. The instrument autopipets an aliquot quantity of serum into a cuvette containing the reagents. The resultant reaction changes the fluorescence of the dye and the changes in fluorescence are read by the machine and converted to milligrams of ethanol per deciliter of serum (3).
4.4 Syva Enzymatic Assay

Syva Company, now called Siemens, also makes reagents for ethanol testing in serum, utilizing conversion of ethanol to acetaldehyde by ADH in the presence of NAD. This coenzyme is converted to NADH by an automated machine. The increase in the absorbance due to NADH is measured at 340 nm and converted to milligrams of ethanol per deciliter of serum. This method was discussed in detail in Chapter 3. As stated in Chapter 3, increase in lactate and lactate dehydrogenase (LDH) is known to give false-positive ethanol readings. Autopsy samples and serum from accident victims with severe injuries and trauma and sera from patients with elevated lactate and LDH are known to give false-positive ethanol levels (4). That is why forensic laboratories measure serum/blood ethanol by GC (5).

4.5 Blood Alcohol Concentration

Blood alcohol concentration (BAC) depends on the number of drinks, the alcoholic content of the drink, the period in which these drinks were consumed, the time of the first drink and the time of the last drink, body weight, sex, age, and food in the stomach. In addition, BAC is influenced by health, medications, and co-abuse of drugs (6).

It is accepted scientifically that a 150-lb man will have a BAC of 0.025% after drinking 1 oz of 100 proof (50%) alcohol. This assumption is accurate under almost all circumstances (7). BAC easily can be calculated as follows.

$$BAC = \frac{150}{\text{Body weight (lb)}} \times \frac{\% \text{ ethanol content}}{50} \times \text{Ounces of alcohol consumed} \times 0.025$$

One drink gives a BAC of 0.02% in a 200-lb man. Thus, after consuming four drinks his BAC would reach 0.08%, the legal limit in most U.S. states. Approximately 0.02% of blood alcohol is dissipated from this man. ADH is low in infants, neonates, and females. For this reason, females generally reach the legal limit with fewer drinks than males do.

For medical management of the patient, serum ethanol concentration is determined. For forensic purposes, blood ethanol concentration is required. For this reason, serum ethanol levels need to be converted to blood ethanol levels and vice versa. The average value for a serum-to-blood ratio was found to be from 1.04 to 1.26 with a mean value of 1.14. Therefore, by dividing serum value by 1.14, blood alcohol concentration is obtained (6). The level of ethanol reaching the brain and its effect on the CNS is very well correlated
with the level of intoxication (6). For this reason, BAC is always correlated with symptoms of intoxication.

<table>
<thead>
<tr>
<th>Blood Ethanol (%)</th>
<th>Intoxication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 0.01–0.05</td>
<td>There is only slight physiological impairment.</td>
</tr>
<tr>
<td>2. 0.05–0.07</td>
<td>Euphoria; increased self-confidence, impairment of reaction responses.</td>
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<tr>
<td>3. 0.07–0.10</td>
<td>Impairment of reaction sponsors, attention, visual acuity, and judgment.</td>
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<tr>
<td></td>
<td>An individual may appear sober.</td>
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<tr>
<td>4. 0.10–0.20</td>
<td>Increased impairment of a sensory motor activity. Reaction times,</td>
</tr>
<tr>
<td></td>
<td>attention, visual acuity, and judgment progress to increase in drowsiness,</td>
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<tr>
<td></td>
<td>disorientation, and emotional liability.</td>
</tr>
<tr>
<td>5. 0.20–0.30</td>
<td>Staggering, drunk, lethargic, sleepy, or hostile and aggressive.</td>
</tr>
<tr>
<td>6. 0.30–0.40</td>
<td>Unconscious and stupor.</td>
</tr>
<tr>
<td>7. +0.4</td>
<td>Coma, and possible death.</td>
</tr>
</tbody>
</table>

### 4.6 Alcohol and Acetaminophen

One should be aware of the danger of taking Tylenol or acetaminophen for a hangover or headache following ethanol consumption. Tylenol is an over-the-counter drug used for pain, flu, headaches, and colds. It is also present in several medications. This drug causes liver failure if more than 10 g are taken in 24 hours. The liver metabolizes both alcohol and acetaminophen. One should be aware that chronic, moderate to heavy alcohol drinking enhances the toxic effects of acetaminophen. This drug is commonly used to commit suicide. Acetaminophen-alcoholic syndrome is a major cause of liver failure in the United States. Approximately 1 to 10% abuse this drug, and 31% of alcoholics use this drug regularly. Fasting also enhances the toxicity of this drug (8).

### 4.7 Could You Be Drunk without Drinking?

Endogenous isopropanol and ethanol could occur in some people. In case of diabetic keto-acidosis, the acetone in the body could be converted to isopropanol. This isopropanol induces intoxication in the individual (9).

Endogenous ethanol can be generated by fungal infections. The following published case from scientific literature (10) illustrates the ethanol intoxication by Candida infection in the GI tract. This fungus causes fermentation of glucose to ethanol in the GI tract by acting on digested food. The ethanol so generated causes intoxication.

A 24-year-old nurse, previously in good health, developed ethanol intoxication without drinking alcohol. Over a 5-month period, she
developed symptoms of faintness, nausea, and sometimes vomiting 1 to 2 hours after eating meals. Occasionally, she reported that she fell asleep during her night duty. She further stated that she fell down occasionally, even during the day while shopping. Her diet consisted of 1800 calories per day with a carbohydrate content of 78%. She complained of general malaise and faintness. What surprised her friends is that she became unconscious two hours after eating an ordinary breakfast. Her colleagues and friends complained that she had a strong smell of alcohol on her breath. The level of consciousness corresponded to stupor and these symptoms lasted for three days. She contends that these episodes became more frequent. She occasionally fell into delirium or coma. She also experienced constipation, lasting a maximum of six days, alternating with diarrhea. Her degree of intoxication decreased after defecation. The ethanol concentration in her breath was measured and shown to be 1208 µ/L. Her blood alcohol concentration was 254 mg/dL. X-ray of the GI tract revealed slight dilation of the duodenum and frequent movement of duodenal contents into the stomach. Serial cultures of the stomach juice, duodenal juice, and fecal specimens showed numerous colonies of Candida, notably in the feces. Maximum live cell count of Candida in her watery stool specimen after an episode was found to be $2.3 \times 10^3$ per gram (10).

References
