In this chapter, a brief description of unusual and unexpected results is given. These do happen in busy clinical toxicology laboratories and are not unique to any one laboratory. Most of these results were published in peer-reviewed scientific journals. Unusual results include:

1. False-positive serum ethanol levels
2. Attempted suicide by chloroform
3. Acute ethanol intoxication in a 7-month-old infant
4. Carry-over cocaine
5. Nonalcoholic beer and blood alcohol levels
6. Attempted suicide by phenylbutazone
7. Other unusual requests

### 3.1 False-Positive Serum Ethanol Levels

In the clinical toxicology laboratory, approximately 40% of the workload is usually serum alcohols and the remaining workload deals with drug screens, volatiles, other therapeutic drugs, over-the-counter drugs, and plant and animal toxins. GC is the gold standard for serum/blood alcohol determination. However, to determine serum alcohols in a busy medical center by GC is slow, costly, and requires professional time of an experienced technologist (1). With the discovery of enzymatic methods of serum alcohol determination, the tests became rapid and inexpensive. Once the Food and Drug Administration (FDA) approved this method, several manufacturers came up with reagents and instruments to measure serum alcohol levels. The enzymatic method follows the reaction principle as given in the following.

\[
\text{ADH} \quad \text{Ethylalcohol} + \text{NAD} \rightarrow \text{Acetaldehyde} + \text{NADH}
\]

\[
\text{ADH} = \text{alcohol dehydrogenase}; \quad \text{NAD} = \text{nicotinamide adenine dinucleotide}
\]

The machine measures absorbance of NADH generated at wavelengths of 340 nm. An increase in absorbance due to NADH is proportional to ethyl alcohol concentration in the sample. Using the enzymatic method developed
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by Syva Company, Palo Alto, California on Cobas Mira S instrument, ethyl alcohol determinations were carried out (2).

Problems began to appear in pediatric patients from a children’s hospital. The first patient was a four-month-old boy with postnatal respiratory problems. He developed cardiopulmonary arrest due to upper respiratory infections. A serum sample obtained in the emergency department gave a serum alcohol of 105 mg/dL by the enzymatic method. Headspace GC did not detect any alcohol.

The second patient was a two-month-old girl born to a mother with a history of alcohol and cocaine abuse. The infant died due to cardiopulmonary arrest. Enzymatic serum ethanol determination gave an alcohol level of 60 mg/dL. GC determination did not detect any alcohol.

When these sera samples were retested, the same results were obtained—positive by enzymatic method and no alcohol by GC. The cause for a false-positive alcohol reading by the enzymatic method and no alcohol by GC was investigated. The same sera samples were retested by other enzymatic methods with a different instrument. Identical results were obtained—positive serum ethanol by enzymatic method and no serum ethanol by GC. The possibility that another endogenous enzymatic reaction that converts NAD to NADH might generate falsely elevated alcohol readings was investigated. One such possible interfering reaction appeared to be

\[
\text{LDH} \\
\text{Lactate + NAD} \rightarrow \text{Pyruvate + NADH} \\
\text{LDH} = \text{Lactate dehydrogenase}
\]

To confirm this possibility, a series of experiments was performed by obtaining serum samples from a number of autopsy subjects. Lactate and LDH enzyme activity levels and alcohol levels were determined in these sera samples. Alcohol levels were determined by four different methods. They are Abbot, Roche, Syva, and GC. Serum samples from healthy volunteers were obtained and their LDH and lactate levels were determined. Samples were spiked with increasing concentrations of LDH and lactate and tested for alcohol levels by GC, Abbot, Roche, and Syva methodologies. These experiments proved that lactate and LDH present in the serum give falsely elevated ethanol levels but not by GC (3).

Published reports in the scientific literature show that elevation of lactate and LDH occurs in postmortem serum samples and in sera of trauma and injuries, immune-compromised patients, and patients with systemic fungal infections. In these cases, it is useful to check ethanol levels by headspace GC. In case of automobile accidents involving severe traumas, both lactate and LDH increase and the elevated lactate does not clear rapidly (1,3–5). If the victim is transported by an ambulance to the hospital, the paramedics
may give IV Lactate–Ringer solution. This is another factor in the elevation of lactate on top of already elevated LDH. Under these circumstances, false-positive ethanol occurs if the laboratory measures serum alcohol levels by an enzymatic method utilizing ADH and NAD. Even if the controls and calibrators work, the enzymatic method still can give falsely elevated alcohol levels due to the presence of LDH and lactate.

### 3.2 Chloroform Poisoning

The next interesting case is a suicide attempt using chloroform. The effects of chloroform (CHCl$_3$), carbon tetrachloride (CCl$_4$), and other hydrocarbons on small experimental animals like mice and rats are well studied. In the earlier medical literature, the use of CCl$_4$ to treat intestinal helminthic infestations was indicated (6). CCl$_4$ is no longer used in human medicine because of its toxicity. A suicide attempt by chloroform provided a rare opportunity to study its effects in humans.

A 33-year-old Caucasian female who worked in a dentist’s office injected herself intravenously with 0.5 ml of chloroform. She became unconscious, but woke up the next morning and realized that she was not dead. She then drank about half a cup of chloroform and became unconscious but woke up several hours later. She vomited and became unconscious again. She was found unresponsive on the kitchen floor. She was brought to the emergency room, and was found to be alert and in no apparent distress.

She was treated with hyperbaric oxygen, cimetadine, and N-acetylcysteine. Analysis of chloroform in her blood was done by headspace GC (Figure 3.1). Her sequential serum samples were analyzed daily for up to 11 days to monitor her liver necrosis, liver function, and liver regeneration. Markers for liver necrosis are alanine aminotransferase, units/dL (ALT), aspartate aminotransferase, units/dL (AST), lactate dehydrogenase, units/dL (LDH), and alkaline phosphatase, units/dL (ALP). Biomarkers for liver function are total bilirubin (TBIL), direct bilirubin (DBIL), prothrombin time (PT), and activated partial prothrombin time (APTT). The markers for liver regeneration including γ-glutamyl transferase (GGT), α-fetoprotein (AFP), des γ-carboxy prothrombin (DCP), and retinol-binding protein (RBP) were quantitated (7). The values were divided into three groups:

1. Markers for liver necrosis
2. Markers for liver function
3. Markers for liver regeneration

The values were normalized and the averages of these values were plotted for each day, up to 11 days (Figure 3.2). From the graph, it is obvious that liver
necrosis peaked by day four and declined rapidly, with a steady and consistent increase in liver regeneration. The liver regeneration remained steady throughout 11 days of observation. A social worker talked to the patient and she was then transferred to psychiatric care. When the woman’s condition improved, liver necrosis decreased, and liver function was stabilized, she was then discharged (8).

Figure 3.1 Chromatogram of 1.48 mg/dL standard showing ethanol and chloroform peaks. (Reprinted with permission from Journal of Analytical Toxicology.)

Figure 3.2 Serum biomarkers for liver necrosis and regeneration in chloroform poisoning. (Reprinted with permission from Journal of Analytical Toxicology.)
3.3 Acute Ethanol Intoxication in a 7-Month-Old Infant

A 7-month-old infant who accidentally ingested alcohol provided a unique opportunity to make observations on the elimination of ethanol in infants (9). There were no such reports in the scientific literature.

A female baby weighing 10.9 kg was brought to the emergency room by her grandmother, who accidentally mixed baby formula with unknown amount of vodka. After feeding the baby with this formula, the infant became hyperactive and behaved strangely. The baby vomited two times and the grandmother smelled alcohol in the emesis. The baby was brought to the emergency room, and the infant was noted to be active and alert. The baby was not in respiratory distress and was not irritable. The infant’s vital signs were normal. A stat serum ethanol was ordered. Infusion of normal saline was started. The baby was transferred to the nursing unit.

The serum ethanol on admission was 183 mg/dL. Subsequent ethanol levels in serum at 1 hour 45 minutes and at 8 hours 5 minutes were 96 mg/dL and no detectable amount, respectively. Vodka has 40% alcohol. Considering the baby’s weight and the volume of distribution for ethanol, it is calculated that the infant ingested 47.45 ml of vodka.

Alcohol toxicity in adults is well studied. At 50 to 100 mg/dL in blood, it causes central nervous system (CNS) depression, loss of coordination, loss of judgment, and loss of visual acuity. According to the published literature, death occurs at and above 400 mg/dL of ethanol in blood. In most U.S. states, the legal limit for blood alcohol is set at 80 mg/dL. Blood alcohol level above this is considered driving under the influence (DUI). In a normal healthy individual, the elimination of ethanol follows zero order kinetics. Approximately 20 mg/dL of ethanol is dissipated from an adult in 1 hour (10). Ethanol by itself causes insignificant toxicity when compared with its metabolites acetaldehyde and acetic acid. This metabolic conversion takes place due to the enzyme alcohol dehydrogenase (ADH) present in the cytosol, cytochrome P450 (CYP2E1) in microsomes, and catalase in peroxisomes. In turn, acetaldehyde is converted to acetic acid by aldehyde dehydrogenase in mitochondria (11). Since the infant did not exhibit any toxic effects due to the metabolites of alcohol conversion, it can be presumed that metabolic conversion of ethanol did not take place. In fact, CYP2E1 is only activated after birth and reaches 30 to 40% of the adult levels by one year (12). ADH follows similar developmental expression, with the infants aged 9 days to 2 months expressing 80% less ADH activity than adults. Adult activities are found after 5 years of age (13). These factors suggest that ethanol is rapidly cleared in infants (14,15).
3.4 Carry-Over Cocaine

The case is about Mrs. Roberta Chinchilla. She has history of asthma, which sometimes flares up severely. Her husband brought her to the emergency room as she developed breathing difficulties. In the emergency room, they immediately put her on oxygen and tried to stabilize her. Despite their best efforts, she developed cardiac arrest and died. The nursing notes indicated that the nurses were told that there was a party in her house in which she drank only Coca-Cola. The nursing notes instead abbreviated Coca-Cola and wrote that she had Coke at the party. Roberta was a non-smoker, but a few people were smoking at the party. After a couple of hours, Roberta developed respiratory distress, 911 was called, and the ambulance brought her to the emergency room. Stat serum alcohol and urine drug screens were requested. Serum was negative for ethanol. The urine was extracted and processed for drug analysis by GC-MS and an aliquot of the extract was drawn into a syringe and injected into the GC-MS instrument. Indeed, there were significant peaks, which were identified as that of cocaine and its metabolite. The technologist called the emergency room and talked to the nurses, and reported the presence of cocaine and its metabolite in the patient’s urine. Since the nursing notes indicated that the patient had Coke, both the nurse and the technologist were not surprised to see the presence of cocaine and its metabolites in the patient’s urine sample. Soon, the police got involved, visited the patient’s house, and questioned the husband as well as other relatives.

The husband hired an attorney, and strongly objected to this insinuation. He contended that his late wife was a God-fearing, church-going family woman with children and grandchildren. His wife never smoked or drank alcohol. She never abused drugs. He requested the laboratory to check whether there could be a mistake in the analysis. The analysis was repeated by GC-MS after re-extracting the urine sample. No cocaine or metabolites were found. The technologist noticed that the previous sample in the run was from a patient who was a confirmed chronic drug abuser with a history of jail time. His urine was analyzed before Mrs. Chinchilla’s urine sample. The urine extract of the previous sample from the drug addict had massive peaks of cocaine and its metabolite. Apparently, the syringe that was not washed enough and the same syringe was used to inject Mrs. Chinchilla’s extract. This contaminated the GC-MS spectra of the patient sample. This gave a false positive for cocaine and its metabolite in the GC-MS. Since then, the laboratory introduced two syringes and made sure that each syringe was washed before and after use with a solvent.
3.5 Nonalcoholic Beer and Blood Alcohol Levels

This case illustrates that metabolism of alcohol by the liver is slowed down considerably in cases of end-stage liver disease. Several patients with liver failure request liver transplants. However, in chronic alcoholics with substantial liver pathology or deficiency of ADH, the metabolism of ethanol is substantially reduced resulting in the elevation of blood alcohol levels. With the availability of nonalcoholic beer, it became evident that some patients with alcoholic liver disease were substituting nonalcoholic beer as a way of coping with their previous habit of alcohol consumption. Nonalcoholic beer contains 0.05% of alcohol. Even this small amount of alcohol is not metabolized by patients with end-stage liver disease. This case illustrates how a patient achieved a blood alcohol level of 57 mg/dL after consuming nonalcoholic beer.

A 33-year-old Caucasian male was diagnosed with liver failure, and referred to the transplant unit as a possible candidate for liver transplant. He reported a 14-year history of alcohol abuse, drinking six 12-ounce beers every day. An abdominal CT scan showed a small cirrhotic liver. Liver volume was not calculated. On examination at the clinic, he was noted to have alcoholic breath. Although he was asked not to drink alcohol, his primary care physician allowed him to drink nonalcoholic beer. He drank six 12-ounce cans of nonalcoholic beer the night before his appointment. He also drank three more cans of nonalcoholic beer the morning before he came to see the doctor at the transplant unit. His blood alcohol that morning was 57 mg/dL. Because of the absence of ADH in the liver, the oxidation of ethanol is taken over by catalase (14). Nonalcoholic beer contains a small amount of alcohol and even this small amount accumulated in the blood in the absence of ADH in the liver (16).

3.6 Phenylbutazone Poisoning

This case illustrates the role of the laboratory in the management of phenylbutazone poisoning with a successful outcome for the patient. This drug is discontinued in human medicine because of its toxicity and suppression of bone marrow. However, this drug is still used as a painkiller in veterinary medicine. Upon oral administration, the drug is rapidly absorbed and is protein bound. Peak drug levels in serum occur in 2 to 8 hours. It is eliminated mostly through urine. This drug is hepatotoxic as well as nephrotoxic (17).

This investigation employed a strategy for the identification of the drug and used a unique approach to estimate the amount of drug in serum and its elimination over a period of time during successful detoxification of the patient. Biomarkers for liver necrosis and liver regeneration were determined.
in sequential serum samples to guide the therapeutic approach in the management of a patient who tried to commit suicide (7). Brief details are given in the following case.

A 15-year-old female patient was brought to the emergency room of a community hospital. She was later transferred to a children’s hospital. According to her family, the patient was found unresponsive near her bed. A few minutes later, the patient had a seizure that lasted approximately one minute. It was reported that the patient had bluish discoloration of her lips. There were no empty bottles or pills in the room or near her bed. A sample of her urine was sent to the laboratory for drug screens, as well as for comprehensive drug screen determination.

The urine was negative for drugs of abuse. The comprehensive drug screen analysis by GC-MS showed the presence of several peaks and a peak identified as phenylbutazone. The physician taking care of the patient presented these findings to the mother. The mother told the doctor that the patient was involved in a fight with her classmates the previous day and was very upset. The patient helps in the family farm and takes care of horses. They give phenylbutazone to the horses to alleviate pain. The mother could not tell whether any tablets were missing. Clinical management of the patient included treatment for respiratory and cardiac depression. In addition, the clinicians focused on detoxifying the patient. Plasmapheresis was started on Day 3 of her admission. The patient regained consciousness and her condition improved considerably. The patient fully recovered by Day 7 and was discharged to psychiatric care.

The patient had acute renal failure and there was biochemical evidence for liver necrosis. Enzyme-multiplied immunoassay technique (EMIT) was used for drug screens in urine. On the day of admission, urine was found to be negative for normally abused drugs. Serum comprehensive drug screen by GC-MS showed several major and minor peaks. One major peak was identified as phenylbutazone by spectral library. The peak areas of internal standard, barbital, and that of phenylbutazone were measured and calculated ratios of phenylbutazone to barbital showed a decline from Day 3 to Day 7 post-admission, suggesting that plasmapheresis was effective in detoxifying the patient. These results are shown in the following table.

<table>
<thead>
<tr>
<th>Day (Post-Admission)</th>
<th>Peak Area of Control (A)</th>
<th>Peak Area of Drug (B)</th>
<th>Ratio (A/B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>179439009</td>
<td>42960031</td>
<td>4.2</td>
</tr>
<tr>
<td>5</td>
<td>49439734</td>
<td>22199712</td>
<td>2.2</td>
</tr>
<tr>
<td>6</td>
<td>11447700</td>
<td>22274784</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>17874160</td>
<td>24704758</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Figure 3.3 Serum biomarkers during phenylbutazone intoxication. [Reprinted with permission from *Journal of Toxicology Clinical Toxicology*.]

Serum biomarkers were measured in sequential samples every day, starting on Day 3 as shown earlier in the chloroform poisoning case. ALT and AST were selected for liver necrosis, and des γ-carboxy prothrombin (DCP), α-fetoprotein (AFP), and gamma glutamyl transpeptidase (GGT) were selected for liver regeneration. The values were normalized and markers for necrosis were grouped together. Similarly, the values for markers of liver regeneration (Figure 3.3) were grouped together and plotted against the day of admission (7). From the graph, it is apparent that by Day 4, liver necrosis declined and regeneration of the liver increased steeply. These findings show that it is possible to predict the outcome of a patient from acute liver toxicity by measuring the biomarkers in the sequential serum samples (18).

### 3.7 Unusual Requests

A toxicology laboratory in a modern medical center deals with unusual requests. Here is an example.

Two young women work in a human resources department. Sandy lives in Elizabeth Township and drives to work. She is paranoid about pollution and the presence of contaminants in the city water supply. She brings her own water from a well in her backyard. She keeps this water in a tightly closed jug and keeps it under her desk. The other young woman is Barbara who lives in the city with her parents. She comes to the office by local bus, and eats her lunch in the cafeteria. Sandy and Barbara argue with each other all the time. Not only are they not friends, they actually hate each other. Sandy went to
their supervisor and complained that the water she brings from her home
does not smell or taste good. In fact, Sandy suspects that Barbara might be
urinating in her jug of water when Sandy is away from her desk. The section
supervisor called the toxicology laboratory and asked them to determine
whether the water in Sandy’s jug was contaminated with urine. The absence
of creatinine in the drinking water in the jug ruled out urine contamination.

Sometimes the laboratory may be asked to determine whether IV bags
are tampered with or whether there is any suspicious dilution of narcotic
pain medications. Suspicious syringes found in patients’ rooms or doctors’
offices are brought in to have the contents identified.

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