

## Falsely Elevated Ethanol Results Using Hospital Enzymatic Assay Blood Testing

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BRAKES, SQUEAL, SMOKE, SCREAM, CRASH!! Whether it is an ambulance with emergency medical services (EMS) or a Life Flight team, saving the client's life is all that matters after an accident. However, after recovery, many clients face serious criminal charges which rely on forensically unacceptable evidence. This article aims to expose a forensically unacceptable method of testing blood for alcohol performed by hospitals through enzymatic assay testing.

Before examining the problems with hospital enzymatic assay blood testing, the science and the process involved must be understood. Whole blood is drawn from the arm via (1) a syringe and then injected into a test tube; or (2) a Vacutainer which allows the needle to draw directly into the test tube. Whole blood is great for gas chromatography, which is usually the preferred testing method in forensic laboratories.<sup>1</sup> However, if a hospital tests your blood for alcohol, it is normally not for prosecution, but rather to determine any reactions with necessary medicine or procedures. The hospital only provides the District Attorney's office with the blood alcohol results pursuant to a subpoena.

In hospital enzymatic assay blood testing, the test tube does not contain sodium fluoride (preservative) or potassium oxalate (anti-coagulant) required in the grey topped test tubes used in forensic samples.<sup>2</sup> The test tube only contains your client's whole blood. Hospitals do not test whole blood for alcohol using enzymatic assay testing.<sup>3</sup> Only plasma or serum is used.<sup>4</sup> In order to obtain plasma or serum, the test tube is centrifuged to separate the plasma or serum from the cellular material.<sup>5</sup> A centrifuge spins a test tube at an angle at a high rate of speed so

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<sup>1</sup> See Nine, Jeffrey S., *Serum-Ethanol Determination: Comparison of Lactate and Lactate Dehydrogenase Interference in Three Enzymatic Assays*, 19 J. Analytical Toxicology 192, 192 (1995).

<sup>2</sup> Hospitals may sometimes use these grey topped tubes or may use red topped tubes with clot activators or nothing. Always request and examine which type of tube was used and whether anything was in it. Special thanks to Justin McShane for his edits. See also Citron, Joseph, *Hospital Laboratory Testing Lacks Forensic Reliability*, 20 J. of Legal Nurse Consulting 1, 3-4 (2009)

<sup>3</sup> See Garriott, James C., *Medicolegal Aspects of Alcohol* Fifth Edition, p. 258 (2008). See also Citron at 4; Courtney, Max, *Utilizing Results of Various Blood Alcohol Determination Methodologies in Predicting Intoxication*, Forensic Consultant Services, p. 2.

<sup>4</sup> *Id.*

<sup>5</sup> *Id.* See also Courtney at p. 2.

that all the cellular material collects at the bottom of the tube and the plasma is left at the top.<sup>6</sup> The plasma appears as a viscous, yellowish liquid at the top of the tube.<sup>7</sup> Serum is the whole blood without the cellular material or the clotting element.<sup>8</sup> Serum appears as a clearer, yellow liquid that surfaces above the clotted, red, cellular material.<sup>9</sup> Serum is rarely used because the whole blood must be allowed to stand and clot before being centrifuged.<sup>10</sup> Time being a luxury when the client has serious injuries, the hospital usually prefers plasma over serum to avoid waiting for the blood to clot.<sup>11</sup>

In more detail, a deproteinizing agent, Trichloroacetic Acid (TCA), which strips the protein from the whole blood, is added to the whole blood and then the tube is centrifuged.<sup>12</sup> That leaves you with: (1) supernate – top stuff (*plasma*) and alcohol, and (2) precipitate – red corpuscles, white corpuscles, platelets, TCA protein pellet and red blood cells.<sup>13</sup> The supernate is then poured or pipetted off, which is also called aspirating, and the precipitate is thrown out.<sup>14</sup> Now, the hospital is ready to test for alcohol using the plasma/supernate.

There are only two ways to measure amounts: (1) the direct way – for example stand on a scale and measure your weight; or (2) the indirect way – jump on someone's back, then weigh both of you and subtract the other person's weight.<sup>15</sup> Hospital enzymatic assay blood testing measures the alcohol in the blood through an indirect method of seeing how much of a substance is produced as a reaction with alcohol.<sup>16</sup> Then this substance is measured using a color chart.<sup>17</sup>

More specifically, spectrometry is a colorimetric response used to analyze light going in versus light coming out, also known as Beer-Lambert Law.<sup>18</sup> This is also the same law used in the Intoxilyzer 5000 breath testing machine. A spectrophotometer is a device that measures the

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<sup>6</sup> <http://www.differencebetween.net/science/health/difference-between-plasma-and-serum/>;  
<http://www.buzzle.com/articles/serum-vs-plasma.html>

<sup>7</sup> Thank you to Justin McShane for his help editing this work;  
<http://www.differencebetween.net/science/health/difference-between-plasma-and-serum/>;  
<http://www.buzzle.com/articles/serum-vs-plasma.html>

<sup>8</sup> See Citron at 4. See also <http://www.differencebetween.net/science/health/difference-between-plasma-and-serum/>;  
<http://www.buzzle.com/articles/serum-vs-plasma.html>

<sup>9</sup> <http://www.differencebetween.net/science/health/difference-between-plasma-and-serum/>;  
<http://www.buzzle.com/articles/serum-vs-plasma.html>

<sup>10</sup> See Courtney, Max, *Utilizing Results of Various Blood Alcohol Determination Methodologies in Predicting Intoxication*, Forensic Consultant Services, p. 1-2.

<sup>11</sup> *Id.*

<sup>12</sup> See McShane, Justin, *Hospital Blood Testing in Enzymatic Assay in a Nutshell*, page 6.

<sup>13</sup> *Id.*

<sup>14</sup> *Id.*

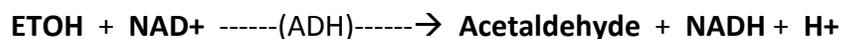
<sup>15</sup> *Id.* See also Citron at 4.

<sup>16</sup> See Citron at 4.

<sup>17</sup> See Courtney at p. 1-2.

<sup>18</sup> See The Beer-Lambert Law and <http://www.rrcap.unep.org/male/manual/national/11Chapter11.pdf>, page 67.

light intensity (photometer) as a function of a color or a wavelength of light.<sup>19</sup> So, the analyst will place a sample of the plasma on a slide and into the analytical device or autoanalyzer machine.<sup>20</sup> However, in order to get ethanol (ETOH) to react and produce a measurable response, a known quantity of an enzyme, Nicotinamide Adenine Dinucleotide (NAD<sup>+</sup>), and Alcohol Dehydrogenase (ADH) is added to the plasma on the slide, which catalyzes the metabolism of alcohol to acetaldehyde.<sup>21</sup> ADH oxidizes ETOH to Acetaldehyde using the coenzyme NAD, which is concurrently reduced to form NADH (2,3).<sup>22</sup>



Depending on what substance is being measured, it is essential to know the spectral bandwidth and linear range of absorption measurement of the spectrophotometer. A light source shines light/energy into the monochromator, which determines the particular wavelength and that wavelength is beamed at the sample.<sup>23</sup> When testing for levels of NADH, the specific wavelength is 340 nanometers.<sup>24</sup> The sample absorbs the energy and the photodetector on the other end measures how much energy actually made it through.<sup>25</sup> If the analyte, NADH, is present then it interferes with the energy emitted and the photodetector detects less energy.<sup>26</sup> Then a comparison is made between what was expected and measured and a colorimetric response is produced, which is lighter or darker based upon the concentrate of the NADH.<sup>27</sup>

Enzymatic assay testing does not actually test the ethanol in the blood, like GC does.<sup>28</sup> Instead, the machine measures the amount of NADH produced, which should be directly proportionate to the amount of ethanol present. However, NADH is not specific for ethanol to the exclusion of others.<sup>29</sup> Remember the client was rushed to the hospital with traumatic injuries. In the course of making his condition stable or saving his life, EMS or hospital staff will administer whatever is necessary and the body will produce natural compounds in an effort to preserve and save the organs.

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<sup>19</sup> See <http://en.wikipedia.org/wiki/Spectrophotometry>

<sup>20</sup> See Courtney at p. 2.

<sup>21</sup> See Courtney at p. 2. See also Rose, Stefan M.D., *How False Positive Serum Ethanol Results Occur Using Enzyme-Assay Hospital Tests*, p.1; University Medical and Forensic Consultants, Inc. 10130 Northlake Boulevard, Suite 214-300, West Palm Beach, Florida 33412.

<sup>22</sup> *Id.*

<sup>23</sup> See <http://en.wikipedia.org/wiki/Monochromator>

<sup>24</sup> See Nine at p. 192.

<sup>25</sup> See [http://chemistrybyscott.org/Worksheets%20&%20Handouts/AP%20Chem/Notes/Spectrophotometer-What\\_Goes\\_On\\_Inside.pdf](http://chemistrybyscott.org/Worksheets%20&%20Handouts/AP%20Chem/Notes/Spectrophotometer-What_Goes_On_Inside.pdf)

<sup>26</sup> See Garriott's at 256-58.

<sup>27</sup> See Citron at 4.

<sup>28</sup> *Id.*

<sup>29</sup> *Id.*

In cases with trauma, Lactated Ringers Solution is a common substance administered intravenously to combat acidosis, which is a chemical imbalance as a result of acute fluid loss or renal failure.<sup>30</sup> Additionally, lactate is a compound formed by the body as a result of trauma and hypoxia where the tissue is deprived of oxygen.<sup>31</sup> Furthermore, Lactate Dehydrogenase (LDH) is naturally in the muscle cells to breakdown lactate formed after anaerobic exercise, but is also released into the blood stream after trauma or a car crash.<sup>32</sup>

Problems arise after a car crash or a traumatic injury when the hospital tests the blood for ethanol using enzymatic assay testing. LDH oxidizes Lactate, whether it is produced naturally or introduced through a solution, to Pyruvate using the coenzyme NAD, which is concurrently reduced to form NADH (2,3).<sup>33</sup>

**ETOH + NAD<sup>+</sup> + Ringer, Lactate, LDH ----(ADH/LDH)----> Acetaldehyde + NADH (but way more) + H<sup>+</sup>**

A higher NADH concentration will result in a higher ethanol result.<sup>34</sup> Why is the client's alcohol result so high? Simple, look at how much NADH is now produced. Similar to ethanol oxidizing to acetaldehyde and producing NADH, lactate oxidizes to pyruvate also producing NADH.<sup>35</sup> The photodetector is simply measuring the amount of energy that makes it through and is not absorbed by NADH. However, it cannot differentiate between the energy absorbed by NADH from the oxidation of ethanol or lactate.<sup>36</sup> Just like when you stand on a scale and the scale doesn't know if you are naked or wearing shorts with gold bars in your pockets.<sup>37</sup> The machine may be "accurate" in the measurement, but the measurement is always relative to the individual, the environment, and any unique circumstances.

Since NADH is not specific to ethanol, there is no way to determine what level of NADH is a result of ethanol and what level is due to Lactate Ringer's Solution, Lactate, or LDH.<sup>38</sup> Ethanol combined with any of these additions will produce a falsely elevated ethanol result with undeterminable error.<sup>39</sup> Additionally, there is no way to convert this method of testing to a whole blood measurement, which is required under the definition of intoxication. TEX. PENAL

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<sup>30</sup> See Rose at p.1.

<sup>31</sup> *Id.* See also Powers, Robert H., *Evaluation of Potential Lactate/Lactate Dehydrogenase Interference with an Enzymatic Alcohol Analysis*, 33 J. of Analytical Toxicology 561, 561 (2009).

<sup>32</sup> *Id.*

<sup>33</sup> *Id.*

<sup>34</sup> See Nine at 193-194.

<sup>35</sup> See Rose at p.1.

<sup>36</sup> *Id.*

<sup>37</sup> Thanks to Justin McShane for his analogy.

<sup>38</sup> See Rose p. 1.

<sup>39</sup> See Nine at 196; Rose at p. 1.

CODE § 49.01(1)(b).<sup>40</sup> In the end, hospital enzymatic assay testing is not specific for ethanol, not forensically acceptable under the *Kelly* test, and ultimately does not belong in a courtroom.

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<sup>40</sup> See Citron at p. 5.