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Increasing Accessibility to Monitoring Ammonia Levels in the Body Through Development of a Novel Point-Of-Care Breath Ammonia Diagnostic Device

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INCREASING ACCESSIBILITY TO MONITORING AMMONIA LEVELS IN THE BODY THROUGH DEVELOPMENT OF A NOVEL POINT-OF-CARE BREATH AMMONIA DIAGNOSTIC DEVICE

By
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TW

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DEDICATION

This thesis is dedicated to our friends and family who have supported us throughout our four years at the University of Mississippi.

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We would like to thank everyone who has been with us throughout this project. We would also especially like to thank the family members who have inspired this project, provided us with insight into their lives, and answered any questions we have had along the way. We would also like to thank our other group member, Noah Jones, for his collaboration along the way. Additionally, we would like to thank our advisor, Troy Drewry, for allowing us to further develop this idea by providing us with effective tools to put the theory of designing a medical device into actual practice while guiding us along the way.

ABSTRACT

INCREASING ACCESSIBILITY TO MONITORING AMMONIA LEVELS IN THE BODY THROUGH DEVELOPMENT OF A NOVEL POINT-OF-CARE BREATH AMMONIA DIAGNOSTIC DEVICE
(Under the directions of Troy Drewry)

Hyperammonemia is a life-threatening metabolic condition that is characterized by elevated levels of ammonia concentrations in the blood. Resulting from serious conditions such as chronic kidney disease or liver failure, the limited functions of the liver and kidney lead to an increase in blood urea nitrogen (BUN) within the body. Currently, there are a wide variety of treatments for this condition, ranging from various medications to surgical procedures such as liver transplants. However, there is a lack of variety in diagnostic testing methods to determine a patient's ammonia levels. In most cases, patients will need to schedule a doctor's appointment to perform a blood test; blood samples are sent to a lab to then be analyzed for ammonia concentrations. Though this method is sufficient, it is often extremely complicated, costly, and time-consuming. Moreover, patients with hemophobia or sensitivity to being pricked often find this testing procedure extremely uncomfortable. Thus, our biomedical engineering senior design team sought to develop a non-invasive screening device that detects ammonia in real-time. There is a reasonable correlation between blood urea concentration and mouth-exhaled ammonia concentration. The device contains an ammonia sensor that allows for the detection and display of ammonia levels through mouth exhalation. This sensor, when in the presence of ammonia, rises in conductivity along with the rising gas concentration. With the device, a detailed instruction pamphlet will be included which will provide safe operating instructions for the user. The device is currently in

development as proof of concept. With sufficient funding, resources, and research the potential for more accurate detection sensors is viable.

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Introduction

Part of the degree requirements for Biomedical Engineering includes taking a two-semester sequence during senior year which consists of Senior Design I and Senior Design II. In Senior Design I, all students are tasked with finding 3 clinical needs and then pitching them to the class. Four of these clinical needs were chosen, and then the class split up into teams to start researching and brainstorming to solve these problems.

When trying to find unmet clinical needs, many students asked family and friends what bothered them or what would make their lives easier when receiving medical treatment. As it turns out, one of our family members, who is a four-year-old female, was in and out of the hospital for a couple of months before being diagnosed with a urea cycle disorder (UCD). This is a genetic disorder where the body can not convert ammonia into urea, thus creating a build-up of ammonia. While there are certain medications and diet plans she can follow to keep ammonia levels suppressed, any imbalance in diet, medication, or behavioral activity can trigger higher levels of ammonia and this can occur for the rest of her life. To ensure ammonia levels stay in a safe range, blood has to be drawn every two weeks or a month depending on where previous levels fell. Due to the blood having to be drawn and prepared in a specific way to ensure correct results for certain tests, this testing has to be done in a hospital setting where the test can be run quickly or properly separated and stored to be sent off to a lab where results normally come back in a week. This is very inconvenient not only because she doesn't like needles but also because by the time the test results come in, levels could have increased even

more. This current process does not provide a satisfactory way to measure ammonia results in real-time because there have been times in between bloodwork appointments when she has had to go to the emergency room to get treated for hyperammonemia which is where ammonia levels have exceeded the upper limit of the normal range. As a result, she has had to get IV infusions and in extreme cases hemodialysis to remove the excess ammonia from her system. Had the increase of levels been caught earlier while they were still low enough to decrease them by adjusting diet and medication from home, it probably would have prevented the hospital visits for hyperammonemia. Because of the difficulties facing the four-year-old female and her parents, the idea to create an at-home ammonia diagnostic device came about so that easily monitoring levels to provide adequate treatment when needed would be available.

Our device, Ammonia-lyzer, aims to increase accessibility to monitoring ammonia levels in the body through the development of a novel point-of-care breath ammonia diagnostic device. Throughout this two-course sequence, we have been developing this product through researching the disease state of hyperammonemia, current diagnostic methods and those in development, the problems with the current diagnostic options, and the market potential. Hyperammonemia, if not treated quickly, can cause irreversible brain damage and even death. If patients aren't getting accurate ammonia levels in real-time, levels can increase over time in the background and trigger one of these hyperammonemic episodes. In an effort to prevent hyperammonemic events from occurring, we developed Ammonia-lyzer: a breath ammonia diagnostic device that allows more convenient and frequent testing to be performed in the home while being less invasive than traditional blood ammonia testing performed in hospitals.

What is Hyperammonemia?

Ammonia is produced naturally as a result of the breakdown of amino acids in the colon, small intestines, and skeletal muscle. While it is naturally produced, in high concentrations, ammonia is extremely toxic. After it is produced, ammonia is then transferred to the liver where it gets converted into urea, which is a less toxic chemical, by way of the urea cycle. The kidneys are then able to easily excrete the urea in the urine due to it being water-soluble [1]. If any step in this process is interrupted or unable to be carried out, it can lead to abnormally high levels of ammonia that exceed the normal physiological range. As a result, detrimental and irreversible neurological effects and even death can occur. When plasma ammonium levels are elevated to levels outside of the normal physiological range, the resulting effect is called hyperammonemia [2].

What Levels are Considered Hyperammonemic?

When ammonia levels initially increase in the body, glutamine is produced in other organs to briefly protect the body from the toxic effects of ammonia. In addition, more ammonia is temporarily excreted in urine which allows for less absorption into the blood. Unfortunately, these protections do not last long as hyperammonemia becomes more severe [3].

Different age groups have varying ranges of standard blood ammonia levels. Infants from age 0 to 14 days have a range of levels of 56-107 μmol/L. From the age of 15 days to less than 2 years old, the standard range is 40-80 μmol/L. Children and adults

older than 2 years show between 11-35 μmol/L as being normal. The lab starts providing courtesy alerts if results go above 80 μmol/L [4].

Breath Ammonia levels in a normal individual have a range from 50 to 2,000 parts per billion (ppb) [5]. A scientific study that tested the correlation between ammonia levels and protein intake by using both breath and blood ammonia testing, discovered that mean breath ammonia ranged from 490 to 704 ppb at baseline and from 920 to 1642 at maximum. Meanwhile, mean blood ammonia ranged from 107 to 171 μg/dL at baseline and from 218 to 342 μg/dL at maximum levels [6]. By converting the breath ammonia reading from ppb to μg/dL the levels ranged from 49 to 70.4 μg/dL at baseline and from 92 to 164.2 μg/dL at maximum. The blood ammonia levels are around 2.25 times higher than the breath ammonia levels.

What Conditions Cause Hyperammonemia?

While hyperammonemia is a symptom of many conditions, such as genetic defects in the urea cycle, hepatic damage or disorders, and kidney failure, if left unrecognized and untreated immediately, significant damage can occur. While hyperammonemia occurs as a symptom of many disease states, genetic defects in the urea cycle disorder lead to the most severe cases of hyperammonemia [7].

At What Age Do Hyperammonemic Episodes First Occur?

In a study from 1983 to 2003, researchers followed 260 patients for 21 years and over that time, there were 975 instances of hospitalization where an injection of NaPA/NaBZ Injection 10%/10% as a treatment to control a hyperammonemia episode.

While this study was for the FDA regulatory process of the drug Ammonul®, which is included in many emergency treatments for hospitalizations now, it provides a good overview of the frequency and severity of hyperammonemic episodes in patients with UCDs. Of the 260 patients in this study, this is the breakdown of when the first hyperammonemia episode was experienced: 34% were 0-30 days old, 18% were 31 days to 2 years old, 28% were 2 to 12 years old, 10% were 12-16 years old, and 10% were 16+ years old. The age range was 1 day to 53 years and the median age was 2 years old [8]. Figure 1 shows the number of participants in this study characterized by UCDs diagnosis. The data is further broken down to include the age of their first hyperammonemia episode.

Why Do Hyperammonemic Episodes Reoccur After Diagnosis of Initial Disease?

Many children that have UCDs can have a hyperammonemia episode at any point in their life due to an imbalance in nitrogen. Increased intake of protein, prolonged fasting, a fever, or increased exercise can trigger a hyperammonemia episode [9]. Therefore many children with UCDs have a special diet plan and list of medications created by a dietician and doctor to try to keep ammonia levels at bay. An example of such a plan can be found in Appendix A. However if this plan isn't followed as prescribed, events can be triggered. 58% of all hyperammonemic episodes had a reported illness prior to the episode. Additionally, 15% were due to not following the prescribed diet which could entail receiving too much high protein or too few calories. 10% were due to non-compliance with medication which could entail missing a dose of medication or not taking it at the prescribed time. Finally, 10% of the hyperammonemic episodes

were due to major life events such as school stress, surgery, or accidents [8]. Because of the variety of factors that can trigger a hyperammonemia episode at any time, it is especially important to monitor ammonia levels in UCD patients to intervene with treatment before levels get too high that symptoms of a hyperammonemia episode occur.

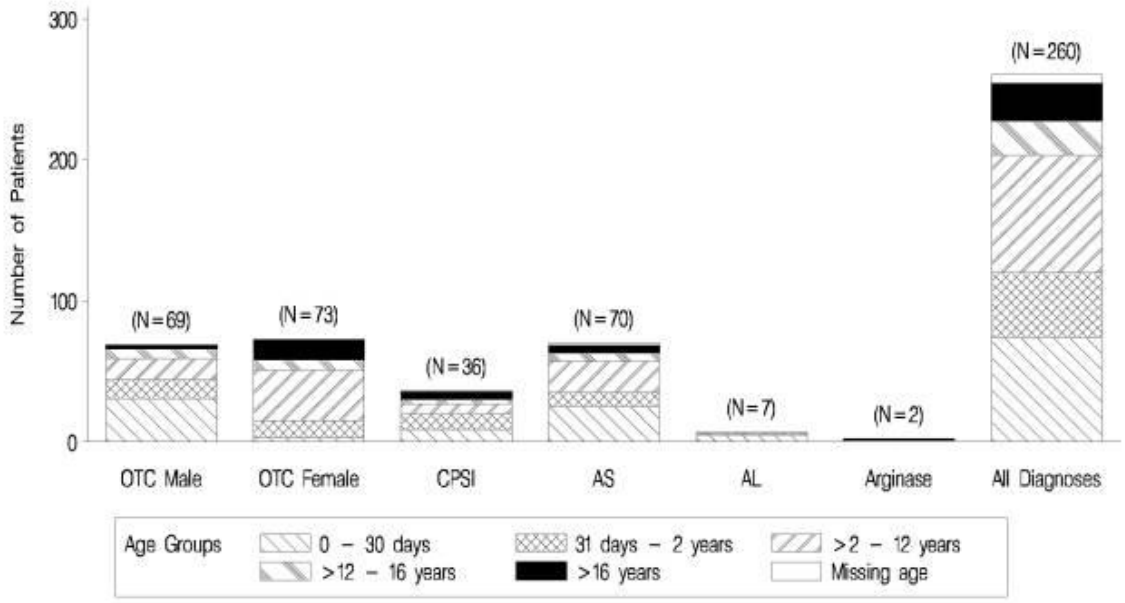


Figure 1: Number of patients by diagnosis and age at first episode [8]

Frequency of Hyperammonemic Episodes

While previously mentioned that hyperammonemic episodes hail from a multitude of disorders, it is hard to get an accurate picture of how many episodes actually occur each year. Around 1 in 35,000 people on average are born each year with a urea cycle disorder [10]. According to the Center for Disease Control, in 2020 the birthrate was around 3,613,000 in the United States (US) [11]. That comes out to around 103 people born with UCDs each year just in the US alone. In the 1982-2003 study, many patients experienced multiple episodes of hyperammonemia over a period of 21 years.

For patients that experienced 5 or more episodes throughout the 21 years of this study, the average number ranged from 2.2 to 2.8 depending on the specific UCD the patient had and the sex of the patient. The range of episodes per year varied from 0.6 to 8 episodes per year. For males with UCDs around 70% of hyperammonemia, episodes happened between 31 days and 12 years while in females 92% of episodes occurred between 2 and 12 years old [8].

Figure 2 shows the number of episodes documented in this study characterized by UCDs diagnosis. The data is further broken down to include the age of the patient at the time of the hyperammonemic episode.

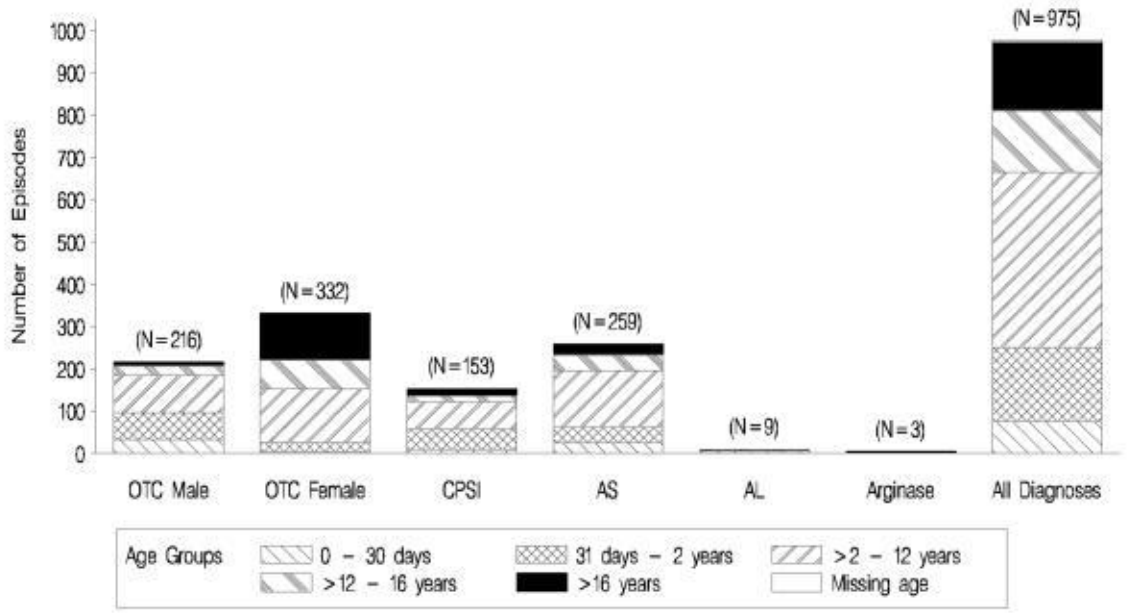


Figure 2: Number of patients by diagnosis and age at the time of episode [8]

Symptoms of Hyperammonemia

Many of the symptoms associated with hyperammonemia are neurological since ammonia is toxic to the brain but not to other tissues in the body [3]. 80% of episodes

documented a neurological symptom, and 33% of episodes documented gastrointestinal symptoms [8]. Some of the symptoms of hyperammonemia include vomiting and lethargy, but the most concerning effect if this is left untreated is brain damage or even death [12]. Many of these symptoms develop slowly over a few days going from vomiting and lethargy to disorientation and coma if not treated [3]. The symptoms of such a disease range with the age and level of ammonium. Infants normally show poor suck, hypotonia, vomiting, lethargy, grunting respirations, and seizures. Older children normally present a range of symptoms from behavioral changes to a frank coma. It is generally harder to diagnose because a lot of the symptoms are nondescript such as vomiting, headache, increased respiratory rate, and poor appetite are confused with other problems [12].

Children with Urea Cycle Disorders (UCD) who have survived several hyperammonemic episodes show focal cortical necrosis and ventriculomegaly and can even have deficient myelination. The severity of these effects corresponds with the length of the hyperammonemic events [2].

How Are Hyperammonemic Episodes Treated?

When treating hyperammonemia, the goal is to correct imbalanced biochemical levels and reinstate proper nutritional intake. Patients with UCDs normally have an emergency treatment plan, developed by their doctor, on the steps to take in the event of a hyperammonemia episode, which is when levels reach 3 times the reference range [13]. A standard treatment plan is listed in Appendix B. The first step of this treatment is to immediately stop protein intake and consult with the doctor and the dietician. Many times

if the episode is caught early, this step can reverse the ammonia build-up without hospital intervention. Step 2 is to provide intravenous fluids, D10W+NS to provide calories [14]. If hyperammonemia is confirmed by a blood ammonia test, the next step is to give Intralipid which provides calories and essential fatty acids for nutrition [15]. Next, ammonia scavenger medicines including sodium benzoate, arginine, phenylacetate, and phenylbutyrate are used to remove the nitrogen by transforming it into other products that can be excreted [1]. After the administering of the ammonia scavenger medications, the ammonium levels in the blood should be checked in addition to every 8 hours. If the levels do not decrease in 8 hours, hemodialysis should be started to rapidly dispose of ammonia and other nitrogenous wastes. Newborns with a standard of levels higher than 300 μmol/L should be immediately administered hemodialysis; however, in older patients, there is no ammonia level cutoff that has been established. Therefore, clinical judgment is to be used.

The current therapeutic options to treat hyperammonemia focus on targeting either the reduction of ammoniagenesis and its rate of absorption in the GI tract or by activating ammonia removal by upregulating ureagenesis through treatment with N-carbamylglutamate or supplementing with urea cycle intermediates and glutamine synthesis [16]. Front-line therapy options for patients are primarily nonabsorbable disaccharides. The most prevalent example is the complete metabolization of lactulose into lactic, formic, and acetic acid in the colon by B-galactosidase from colonic bacteria. This causes increased acidification in the colon and osmotic pressure, thus inhibiting the growth of urease-active bacteria. Rifaximin is currently the most effective antibiotic in treating hyperammonemia. A nonsystemic, GI site-specific antibiotic, Rifaximin results

from adding a nonabsorbable pyridoimidazole ring. However, it still retains the potential to cross the cell wall of Gram-negative bacteria and inhibits RNA synthesis by binding to the B subunit of the bacterial DNA-dependent RNA polymerase enzyme. Table 1 explores current various treatment options [16].

These various treatment options are the most commonly available to patients in need. However, these options also reveal a more concerning picture - the lack of affordable and readily available treatments. Most medicines do not cure hyperammonemia, they rather serve as long-term options that must be taken daily. For example, peritoneal dialysis requires patients to set aside three to five hours for treatment per week for approximately $53,000 [17]. Permanent treatments such as liver transplants are also incredibly expensive. The estimated mean cost of a U.S liver transplantation was approximately $163,438 [18].

Table 1: Current Treatments of Hyperammonemia [16]

Names of Medicines	**Pharmaceutical Name**	**Mechanism of Action**	**Drawbacks**
Lactulose	Enulose	Acidification of colonic contents, increase in osmotic pressure, cathartic effect	Abdominal cramping, bloating, flatulence, electrolyte imbalances
Rifaximin	Xifaxan	Inhibition of RNA synthesis in intestinal bacteria.	High cost, nausea, bloating, diarrhea
Sodium Benzoate	Ammonul	Decrease glycine degradation, increase glycine elimination	Headache, nausea, impaired mental status
Sodium phenylacetate/ phenylbutyrate	Bupenyl	Decrease glutamine degradation, increase glutamine elimination	Complication for patients with hypertension
L-arginine/L-citrulline	L-arginine/ L-citrulline	Activation of UC	Gastrointestinal distress, diarrhea
Carglumic Acid	Carglumic Acid	Activation of UC through N-acetylglutamate restorement	Chills, body aches, flu symptoms, sores in mouth and throat
Albumin-based dialysis	Prometheus, Hepa Wash, MARS	elimination of albumin-bound substances	mild thrombocytopenia
Peritoneal dialysis	Peritoneal dialysis	decrease of blood ammonia by transporting ammonia from vascular system to peritoneal cavity	mild to moderate nausea and vomiting
Neomycin	Neomycin	inhibition of protein synthesis in intestinal bacteria	oto-, neuro-, nephrotoxicity

Diagnostic Methods and Shortcomings

Traditional Diagnostic Methods and Problems

Plasma ammonia levels are needed to diagnose or confirm hyperammonemia, but getting an accurate reading is a challenge sometimes. The standard current practice is based on the method of the amino reduction of alpha-ketoglutarate. This method requires a reduced nicotinamide adenine dinucleotide phosphate (NADPH). While this method is an indirect method of measuring ammonia, the reduction of the a-ketoglutarate is proportionally correlated with the amount of ammonia in the blood. Spectrometry is used to measure the concentration of the alpha-ketoglutarate [19].

While the method of using amino reduction of α-ketoglutarate is the standard method used in laboratory analysis, it does have many limitations. One of which is the collection and handling of the sample. Because this blood is not tested immediately, it has to be venously collected [19]. For this method to yield accurate results, the blood samples must be taken in a 3 mL lavender-top (K2 EDTA) tube [4], which is shown in Figure 3.

Additionally, the sample must be taken without using a tourniquet or clenching the fist because muscle contractions normally elevate ammonia levels taken from a vein. If these collection procedures are not followed, the results will be invalid. However, the collection of the sample is not the only source of error. Because ammonia is stable for less than 15 minutes at 4 C°, samples must be immediately put on ice and the plasma must be separated from the cells within 15 minutes and then frozen immediately. The

specimen is not allowed to sit at room temperature or even be refrigerated. If the sample is not frozen or received somewhat partially thawed, the test will be rejected [20].

Figure 3: 3 mL lavender-top K2 EDTA tube

Smaller laboratories that might not have the correct equipment or methods to handle the sample, use a faster and cheaper method. This method involves putting whole blood on a dry chemistry strip that contains bromocresol green. The level of ammonia is measured by reacting bromocresol green and measuring the range of color. However, if levels are above 286 micromol/L the results are unreliable.

Due to the mishandling and delay of the samples before testing, many tests come back with increased false levels of ammonia. If mishandling is suspected, the test has to be run again.

Currently, testing the blood for ammonia typically involves drawing the blood from a vein, transporting the sample cold-stored to a lab, then waiting hours for the sample to be centrifuged and subjected to a biochemical assay. Furthermore, the testing process can often be inconclusive, resulting in more samples to be taken and analyzed.

As such, this invasive time-consuming procedure can be an unnerving experience for patients who are uncomfortable with drawing blood.

Diagnostic Methods in Development and Problems

As diagnostic methods are being investigated for more non-invasive procedures, testing the measurement of ammonia in exhalation is gaining traction. Helicobacter Pylori (HP) is a bacteria that lives in the stomach. HP will secrete the urease, which will split urea into carbon dioxide and ammonia [21]. Currently, the standard and most used breath test is the C13 urea test. C13 is a rare but natural isotope of carbon dioxide; there is no urease in uninfected humans, so detection of urease activity is an indication of an HP infection. When testing, a patient is given a small amount of urea by mouth, which is marked with C13. The carbon dioxide produced by urease will then also be marked by C13, thus indicating individuals with C13. This is a basis for modern ammonia breath testing, which instead focuses on detecting the ammonia rather than the carbon dioxide [22]. Thus, the availability of a completely noninvasive breath testing methodology provides a straightforward alternative for detecting ammonia levels.

Other diagnostic tests are being researched and improved to lessen the invasiveness. For example, researchers at Stanford are developing a glucometer-like device for measuring blood ammonia which can be seen in Figure 4. Reimagining the traditional ammonia gas sensor, it is paired with test strips that can isolate ammonia from the blood once dabbled with capillary blood. The device also measures and reports the levels in less than a minute while only requiring one drop of blood, which is almost less than 1 percent of the blood required for a standard lab test [23]. However, this device is

still in development and is not available to the market yet. In addition, while it is less invasive than drawing blood venously, it is still somewhat invasive in that a finger still has to be pricked.

Figure 4: Stanford startup blood ammonia reader

Current At-home Ammonia Diagnostic Devices "On the Market" and Problems

The primary issue underlying "at home" diagnostic options for ammonia levels is ultimately the lack of available, cost-effective devices. For example, PocketCHEM BA Blood Ammonia Analyser, developed by Woodley Laboratory Diagnostics [24], is a portable blood ammonia measurement device that enables immediate testing and delivers results in approximately 3 minutes and 30 seconds. An image of this device can be seen in Figure 5. However, it is not readily available for purchase for residential or private use, only being permitted in hospitals, pediatric units, healthcare settings, and laboratories. Additionally, it is only available in certain countries. With these devices only available commercially and limited, they are often far too expensive at a cost of $3,000 for an everyday user to obtain.

Figure 5: PocketCHEM BA [24]

Ultimately, in the medical industry, breath ammonia monitoring is extremely rare due to the high costs and complexity associated with the detection instruments. Currently, a device named AmBeR, which can be found in Figure 6, is being developed for use in both clinical and home settings. This device will consist of a new ammonia sensing material, polyaniline, a sensitive-to-ammonia conductor of electricity that can be deposited on the device using low-cost printing methods [25]. However, it is still in testing and development and the availability of AmBeR is still unseen in today's markets.

Figure 6: AmBeR by BreathDX

Design and Development Process for Ammonia-lyzer

Literature Review

After discovering this unmet clinical need for creating an at-home ammonia diagnostic device (Ammonia-lyzer) so people who were predisposed to having hyperammonemic episodes could routinely monitor their ammonia levels, the next step was to perform a literature review. The literature review that was performed can be found in Appendix C. This document focuses on five areas which include the disease state, the current diagnostic options, the user needs, market research, and the competitive landscape. This document provides much of the foundation for the rest of the project.

While the disease state and current diagnostic options have been explained in depth in previous sections of this document, we will now focus on the unmet user needs. Since ammonia levels build up over time and symptoms severity increases with levels, it is important to provide a point-of-care option to people that have a predisposition to hyperammonemic episodes, such as those with UCDs. Because a hyperammonemia episode can be triggered by subtle changes in diet, exercise, or even stresses in these patients, it is important to be able to monitor the levels of ammonia frequently to assess if changes to any of these areas increase levels so that an increase in levels can be caught and addressed before symptoms progress so where hospital intervention is needed or so that adjustments to lifestyle can also be made to prevent hyperammonemic episodes in the future. One of the unmet user needs is easier accessibility. A more convenient

option to get ammonia results is needed instead of having to travel to a hospital that has the ability to correctly take the sample and correct equipment to run a test. To get blood tests done, the four-year-old female family member and her mother have to go to downtown Houston which is an hour both ways from where they live. After a hyperammonemia episode or changing any part of her medication or diet, her levels have to be monitored every 2 weeks until another change in medication or diet or the labs look good. In addition, if all is well, testing transitions to monthly. From the period of December to mid-March they were getting blood tested every 2 weeks. Therefore, that is an average of about 15 hours not to mention the amount of time in the doctor's office every time her levels need to be monitored. While the female family member diagnosed with a UCD is only 4, in August she will be going to school, and it will be much harder for her to get those tests done to monitor levels. Additionally, traditional ammonia testing normally reports results about a week after the sample is sent to the lab. However, by then, levels could have drastically changed. Therefore faster testing coupled with convenient testing places are needed to get an accurate level count. Another user need is a less invasive option. Many UCD patients are children who are growing so their medication amounts and diet changes are changing frequently. This means that they are getting many blood ammonia tests taken. If the child does not like needles, which many of them do not, it can be a challenge to get a venous blood sample which is required for the traditional ammonia tests. To summarize, some of the preliminary user needs we found included better accessibility, faster response and reporting time, and a less invasive way to get the measurement. This device should be useful to parents who have children who are more susceptible to higher ammonia level diseases and disorders. An at-home

blood ammonia checker would allow caregivers and parents to be able to monitor their child's ammonia level without bringing them into a clinic, and this would help diagnose rare genetic disorders or other potential disorders due to a high ammonia concentration within the blood.

The next thing that was looked into was market research. Around 140 million people were born in 2020 [26]. By using the 1 in 35,000 UCD births per year, that is around 4,000 people born each year that will at some point in their lives need to monitor their ammonia levels. Over the past 20 years, that is around 60,000 people that need to monitor their levels worldwide. While we are designing this device with UCD patients in mind, the opportunity for this device to measure ammonia in other contexts is vast. While this device would primarily be used to prevent hyperammonemic events and provide data to the patient on how their changes in medication and diet as prescribed by a doctor are affecting their ammonia levels, there are many more implications. This device could in the future be used to monitor the effectiveness of hemodialysis, assess asthma, diagnose hepatic encephalopathy, detect Helicobacter pylori, and analyze halitosis [5]. There could also be sports training applications in exercise physiology and studies of drug metabolism [27]. All of these applications lead to an ever-growing market of at-home ammonia tests.

Blood ammonia tests are most prominent in the medical field and have been widely used in hospitals and doctor offices. Companies have started creating portable blood ammonia checkers that would allow patients to be able to check their ammonia levels. PocketChem can produce results in up to 3 minutes and 20 seconds. For blood ammonia-related purposes, PocketChem has been the top provider for patients and hospitals. But, its uses are typically for doctors' offices, health clinics, hospitals,

laboratories, military settings, etc. [24]. AmBeR is another device created by BreathDX that measures the ammonia levels of your breath [28]. A new prototype for a blood ammonia checker is being created by Dr. Chu, which guarantees results within a minute. It uses a chemical process to isolate ammonia from the blood. This new device needs a single drop of blood to detect and measure ammonia levels in the blood using an integrated sensor. However, the device is still in prototype form [23]. Ultimately, while there are options available, currently they are extremely expensive and still in prototyping phases. Nonetheless, a vacuum exists for a portable blood ammonia reader that can be affordably obtained and simplistically used in the comfort of a patient's home.

Prior Art Search

After completing the Literature Review, the next step in our process was to conduct a search on prior art. The Prior Art document can be found in Appendix D. This task consisted of going through and finding both US and international patents to see what devices were out there that had qualities that we wanted to in some way mimic in our design. All of the initial prior art searches were for blood ammonia testing. These searches yielded several patent applications for blood ammonia detection. Patents such as US10591495B2 [29] and US9625443B2 detailed methods for rapid detection of blood ammonia and/or hyperammonemia. Patent US9625443B2 is the patent from the Stanford research group that partnered with Aza Technology. Schematics for this device can be found in Figure 7 and Figure 8. Figure 7 represents the standard industrial ammonia sensor they purchased for around $200. Figure 8 shows the schematic of the blood

ammonia device with label 14 representing the sensor while label 12 represents where the blood sample area resides. [23]

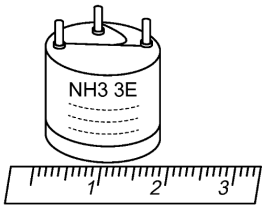


Figure 7: NH_3 sensor used in Stanford startup blood ammonia reader [23]

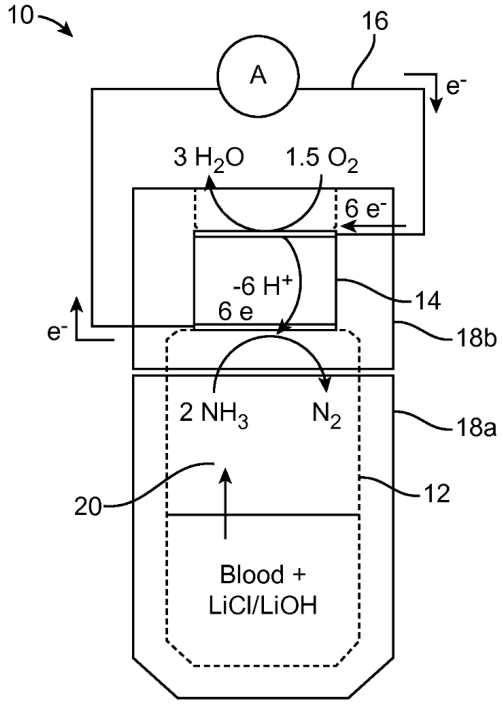


Figure 8: Internal schematic of Stanford startup blood ammonia reader [23]

These patents often used alkali buffer solutions and reagents that required blood or body fluid samples. Various other international patents often revealed a similar

methodology. While the device patents were impressive in their technology, we wanted our device to be completely non-invasive and found that the feasibility of developing a minimally invasive device with similar chemical technology was outside the scope of our budget and resources. Further research into potential non-invasive detection methods highlighted a relatively obscure diagnostic option which included detecting ammonia from human breath exhalation. Upon switching our main focus to breath ammonia, we found several patent applications for methods and apparatuses for detecting ammonia from exhaled breath. For example, US Patent 20050171449A1 [30] details an apparatus for detecting ammonia in exhaled breath through a breath capture device that contains a selected Lewis acid dye, which when deposited produces a detectable spectral or reflectance response in the presence of ammonia. As shown in Figure 9, this apparatus would be connected to a computer and receive the breath-component signals from the analyzer, and memory and data programs in the computer would store the signals and provide a response for the patient based on the correlating inputs.

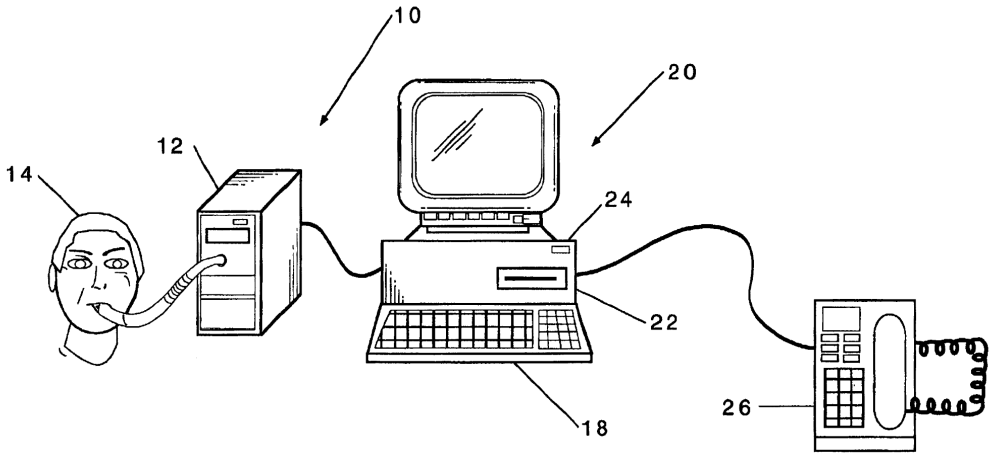


Figure 9: U.S Patent 20050171449A1 [30]

Design and Development Plan

After conducting the prior art search, the next step in the design process was to form the design and development plan which can be found in Appendix E. This document touches on the problem, a needs statement, literature review, prior art search, competition and differentiation, value proposition, the anticipated regulatory pathway, reimbursement strategy, estimated costs, potential market impact, indications for use, patient population, materials, features, and components. This document consisted of putting all of our previous work into one document as well as adding additional considerations.

While the problem, literature review, and prior art search have previously been covered in-depth, we will move on to the major competition and what we initially decided would differentiate our product. BreathDX would be considered our main competitor since they have developed a breath ammonia detection device called AmBeR, which can detect the content of ammonia in just eight breaths. The device uses a glucose-like test strip which is inserted into the device and comes into contact with polyaniline to measure the ammonia found. For our prototype, we plan to have an easy-to-use, portable, cheap blood ammonia test that differs from our competitors. Our prototype will include a tube, in which you exhale through your mouth. This will then lead into a small box that encompasses the gas sensor that will detect the concentration of ammonia found within the exhalation. In the presence of ammonia, the sensor's conductivity will increase with correlation to the increase in the gas concentration. The device will have a built-in circuit that will allow for the readings of the changes in

conductivity which will then display an approximate ammonia concentration reading on an LCD screen. The sensor reads in parts per million (ppb) but the output from the sensor can be converted through formulas in the code to the standard measurement of ammonia which is μmol/L. By using a common gas sensor instead of expensive equipment that is normally found in a lab, we are creating a device that people can afford to monitor their or their children's ammonia levels even if insurance does not decide to pay for the device.

When constructing the Design and Development Plan, we also looked at what value our device would have to consumers. An at-home breath ammonia reader such as Ammonia-lyzer will allow people to test ammonia levels in the comfort of their home. The idea of transitioning testing from hospital to home allows patients a point-of-care option to address treatment similar to the way a glucometer measures blood sugar. As a result of instant results, treatment action can be taken immediately if needed. Instead of waiting long times for lab samples, patients can accurately get their ammonia level in minutes. This would provide patients with a "video" of health of where ammonia levels are instead of giving them a "photo" of health once every two weeks. This would also provide peace of mind to consumers since ammonia buildup can cause serious side effects if not caught.

As with any medical device, eventually, the plan would be to apply for FDA approval. The class of this device is anticipated to be a Class II device, similar to the diagnostic device of a non-invasive glucometer. The regulatory pathway we plan to pursue is the 510k submission process. This will expedite the process in which the FDA reviews the application and shouldn't require a clinical study.

While it was mentioned that the goal is for this product to be affordable for patients even if insurance decided not to pay for it, we do plan to use the cost-based reimbursement strategy. This states that the payer will agree to reimburse the provider for the costs incurred in providing services to the population. After trying to find the cost of an ammonia test online we came across a test for $59 at both Quest and LabCorp [31]. If a patient were to take a test at least every month for a year and maybe 6 additional tests to monitor changing medications, the 18 tests per year would cost around $1,062 per year. This device would be a preventative diagnostic tool to help monitor levels and detect changes rapidly to try to prevent hyperammonemic episodes. After being hospitalized for a hyperammonemic episode last year for three days, the four-year-old female family member racked up a bill of $200,000 that luckily was billed to insurance. We plan for the insurance companies to see the value of this device as a preventative care option to reduce reactive care costs. In addition to being available for home and private use, we aim to bring our product to the medical world in the future in which medics, physicians, hospitals, and alike will utilize the product to provide more options in where patients can get tested.

Our device will be marketed towards everyday people who can use this device from the safety of their own homes to check their ammonia levels. Children and infants have higher abnormalities in their ammonia levels. Because of this, it would be useful to parents who have children who are more susceptible to hyperammonemic events. An at-home blood ammonia checker would allow caregivers and parents to be able to monitor their child’s ammonia level without bringing them into a clinic. This would help form treatment plans to adjust medication and diet while also alerting when levels rise to

a threshold that could trigger a hyperammonemic episode. In the future, it could be used to test for rare genetic disorders such as UCDs at birth since it is a non-invasive and fast option. It could also be used to measure the effectiveness of hemodialysis. Around 750,000 people live with kidney disease in the United States and while this accounts for 1% of Medicare's patients, the dialysis costs that these patients receive account for around 7% of the total Medicare budget. This contributes to around $28 billion annually [32]. While most of these markets are potential markets to tap into, we do plan to focus on patients with UCDs where frequent monitoring is significantly needed. At the moment, we will initially launch in the United States before reviewing plans for a global launch due to varying international medical restrictions.

Design Summary Matrix

After developing more of the design features and business considerations of our device and how those would set us apart from our competitors, we came up with and more formally defined the user needs in the Design Summary Matrix document, which can be found in Appendix F. We found seven primary user needs to keep in mind when designing our product. The list is as follows: portable, safe, accurate, affordable, easy to use, repeatable results, comfortable, and long-lasting.

With each of these needs, we went through and defined what the design input and output would be for each need. Testing methods were also considered to verify and validate the product. Verification activities would be performed to make sure the device measures the values we want it to, but validation activities would also need to be performed to ensure that the measured value is, in fact, the correct thing to measure.

When designing the Ammonia-lyzer to meet the first user's need for portability, we decided to make the device small enough to transport. We defined this as being no bigger than the size of a tablet so that it can be transported easily and can be taken on longer trips. Since creating the device and developing our prototype, the design has transitioned into the size of a 6”x 6” cube. All of the electrical components fit within this 6” x 6” profile of the box.

Safety is something else that was considered when designing the Ammonia-lyzer. Since electrical components will be utilized, there needs to be adequate grounding to ensure there is no electrical shock to the user if any part in the circuit shorts. To test the safety of the device, the plan is to put the device through rigorous testing after prototyping to ensure we find failures before releasing the product. To further evaluate the safety of our device and eliminate the failures through design efforts, we performed a Risk Analysis Plan and a Failure Mode Effects Analysis (FMEA) document both of which will be discussed later.

Since this device is a diagnostic monitoring device, accuracy was defined as the third user need. The main component that would test this is the actual reading of the gas ammonia sensor. Because hyperammonemic episodes are treated at three times the normal levels, we decided the device needs to be accurate to ± 10 μmol/L to accurately show the changes due to lifestyle changes and so that changes that could trigger a hyperammonemic episode could be monitored and recorded. To verify that the levels are accurate, future testing would include having varying known concentrations of ammonia in a gas form and flowing it into the device. This will show if the sensor reads what the actual concentration of ammonia is in the air. Validation on the other hand would require

clinically testing breath ammonia on the device and getting those levels while also getting blood tested the traditional way to ensure that the levels match. Of course, breath ammonia and blood ammonia calculations are different but the correlation calculations that we use in determining the blood ammonia from the breath can be checked to ensure the levels are equivalent.

The fourth user need was affordability. As previously mentioned, the device needs to be affordable to the average person. The cost of the device to the consumer after reimbursement by the insurance company is intended to be less than $500. Therefore, we intend to use standard common materials that are easily accessible and aren't expensive. As of right now, the ammonia gas sensor is the most expensive of all the parts we have ordered at $34.95. In total, we have spent around $120 for the prototype; however, this cost should be much less because we will not be using all of the parts that we initially ordered. There are problems with the sensor that will be discussed later, but for now, a new sensor with a smaller minimum detection limit is needed. Prices for the intended new sensor are much more expensive at $687. However, this sensor might not be needed if another design route with chemical test strips where a regular gas ammonia sensor could be used is considered. This would put us more in line with competitors as far as detection methods. By getting insurance to pay at least half of the cost and ensuring that the consumer pays no more than $500 with insurance, $1,000 per device could be charged. If the desired profit margin is 35%, $650 is available for the cost of the device. In the future, if this device was to be mass-produced, the costs of material would be cheaper due to economies of scale.

Because this device will be used in homes, it is imperative that the device be easy to use. The consumers that will be using this device do not have medical device training, and thus can not be expected to provide inputs other than their external breath into this device. This means that the device will need to prompt the user as much as possible to complete the next steps. To satisfy the ease of use, users need a training manual that can be read in less than five minutes. This would be provided when this product is released to the market. To verify this user need, we will provide labels on the device and create the training manual to allow the user to be aware of how to work the device. To validate this need, field studies with users unfamiliar with the device could be conducted. The measurement of this test would be to have them assess the ease with which they were using the device.

Additionally, because this device will be used in homes and with many young children it is extremely important for the device to be comfortable. We defined this as minimally invasive. To satisfy this requirement, the initial idea mimicked a glucometer using a drop of capillary blood to detect ammonia amounts. However, a less invasive technique was considered, and it was decided to test ammonia by exhalation of the breath. No child enjoys being stuck with a needle even if it's just a prick in the finger. Over time, if levels were being tested every day or two, it's much easier to breathe into the device than to have to prick a finger. Additionally, this option doesn't involve biological waste such as blood, which might ease user hesitations in handling this fluid.

The final user need that was determined was the ability for the device to be long-lasting which means being able to be reused. Customers don't want to shell out money for a new device every time they need a test. With this need in mind, a sensor with

a reliable life span of 200 tests, which would allow a test every other day for a little over a year, was chosen If tested less frequently, the sensor would last even longer. In addition, a rechargeable battery was used so regular batteries would not be an additional expense. The goal of these reusable design features is to ensure that parents are not spending unnecessary amounts of money. To verify this user need, bench testing would be performed to see how many tests the sensor and battery will actually perform and to validate the accuracy of these tests the data would be assessed to see how performance is affected over testing cycles.

Creating the Design/Brainstorming

During the first semester, the idea was to design a blood ammonia diagnostic device similar to a glucose meter, but over time this idea evolved into a device that would measure breath ammonia similar to a breathalyzer. Initially, the first design was a tube with a chemical in it that is known to react with ammonia. The tube would have valves similar to the tricuspid valve in the heart in the sense that it would only open one way to allow the breath to flow through, but for no reagent to come out. Nessler's reagent was originally chosen since it reacted with ammonia and changed fluorescence based on the concentration of ammonia. After use, the reagent would be contaminated, so a pod cartridge system would be utilized so that the main device could be reused while only the pods had to be replaced. The idea was to calibrate this device with known concentrations of ammonia and capture the intensity of the fluorescence with a good imaging system. With these known fluorescence intensities in the system, patients could blow into the tube, let it react and image the tube and let the software tell them the levels. However,

this whole process has been a learning experience, and we would be remiss if we didn't mention that we followed our adviser's advice and in fact "failed often and failed fast." The first problem that we ran into when trying to make this idea work was when we got the full information on Nessler's Reagent. As it turns out, Nessler's Reagent is extremely toxic. We moved on to other designs due to difficulties in handling the chemical and the possibility of inhalation of the chemical when breathing into the tube.

Trying to steer away from using chemicals due to problems in handling and disposal, we looked into actual blood alcohol concentration (BAC) readers also known as breathalyzers. After picking a breathalyzer apart, the components and circuitry were analyzed, helping us realize that we could revamp existing breathalyzer technology from detecting alcohol to ammonia. The current prototype consists of an Arduino board, an LCD screen, an MQ-137 Ammonia sensor, a 6" x 6" 3D printed box, and a rechargeable battery. The device is shown in Figure 10 and Figure 11.

Figure 10: Ammonia-lyzer Consumer View

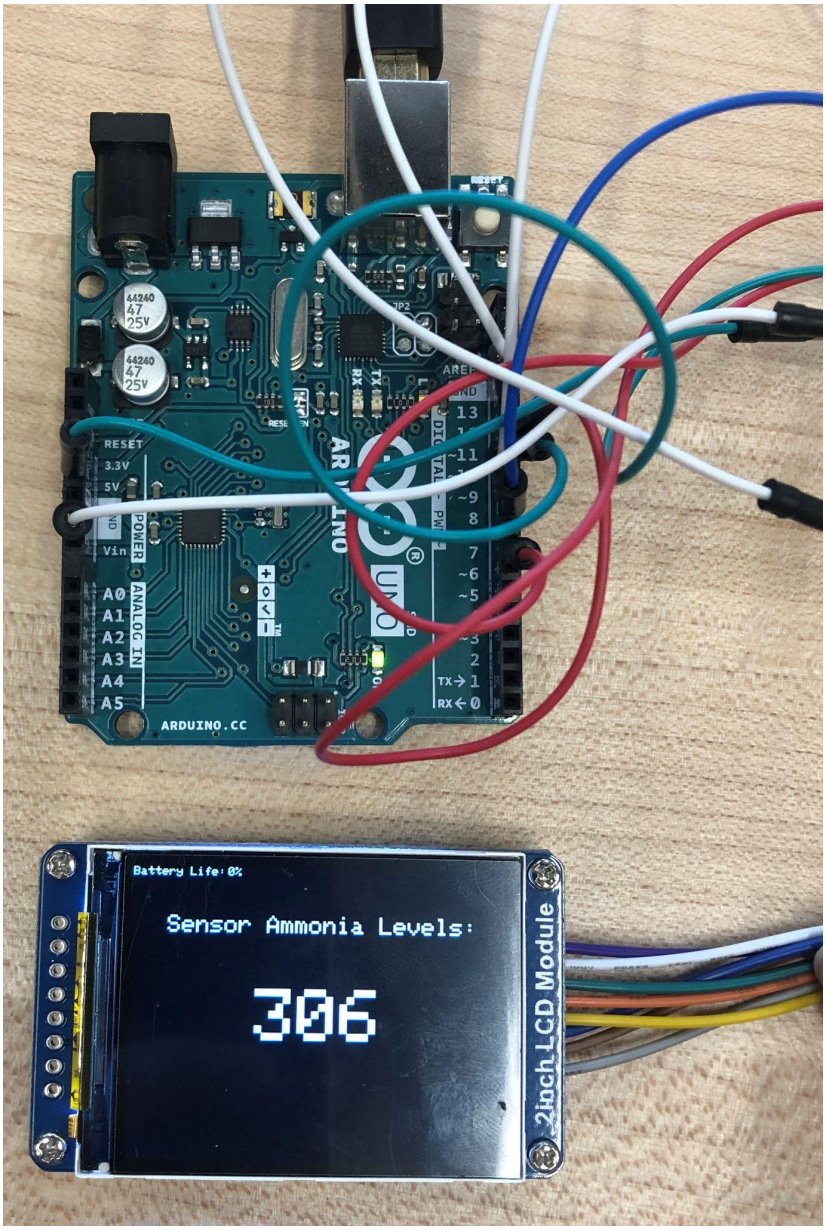


Figure 11: Ammonia-lyzer Circuit and LCD

At the core of a breathalyzer was essentially a basic circuit that was hooked up to an MQ alcohol sensor. This is a simple gas sensor that when in the presence of alcohol conductivity of the sensor rises along with the rising gas concentration. To our advantage, we found that MQ ammonia sensors also exist. This would allow the detection of

ammonia by measuring the changes in conductivity. Tutorials were discovered on how to create an alcohol breathalyzer using an alcohol gas sensor and an Arduino board for the circuitry. Because the schematics for a traditional 6 pin MQ sensor are the same between the alcohol and ammonia sensor, we were able to follow along with these tutorials. Figure 12 details the circuitry schematics.

The sensor requires two voltage inputs, a heater voltage, and a circuit voltage. The heater voltage is used to supply standard working temperatures to the sensor and can be adopted with DC or AC power, while VRL is the voltage of the load resistance, RL, which is in series with the sensor. The circuit voltage supplies the detection voltage to the load resistance and adopts the DC power. Figure 4 displays the resistivity of the sensor in measuring ammonia. In Figure 4, the Y-Axis is the resistance ratio of the sensor and the X-Axis is the varying concentration of gasses. [33]

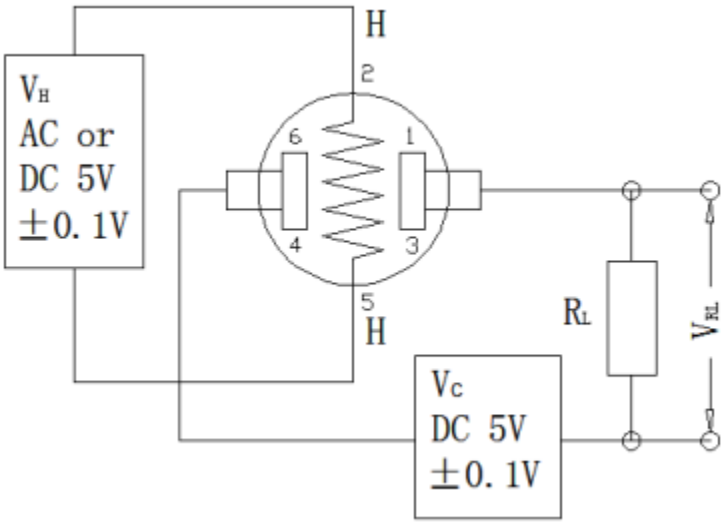


Figure 12: MQ-137 Test Circuit [33]

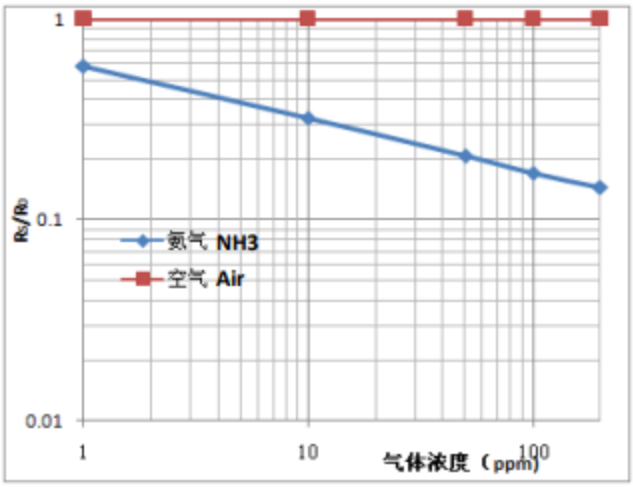


Figure 13: NH_3 Sensitivity Curve [33]

Risk Management Plan

As previously mentioned, one of the user needs was safety. For any medical device, a Risk Management Plan along with a Failure Modes And Effects Analysis (FMEA) should be completed to ensure that everything that can go wrong is accounted for and assessed. The product might get misused by the consumer, so it is vital to design out all of the possible ways the device could fail or be misused so that the patient won't end up getting harmed. While we were completing these documents before finishing the prototype, we tried to come up with all the ways we thought the device could fail. However, if testing were to be conducted on the prototype, many more failures would probably present themselves. The Risk Management Plan, which can be found in Appendix G, was very helpful in considering things that could potentially go wrong and as a result, hurt the consumer or give inaccurate information. The document asked prompting questions to allow us to analyze how the device could be misused or how it

could fail due to components, user error, design error, environmental factors, and manufacturing.

After developing the Risk Management Plan, the next task was to perform a Failure Modes and Effects Analysis. This FMEA document can be found in Appendix H. In this document, the potential mode of failure and possible causes are outlined along with controls in place to prevent the cause or failure mode. The effects of the failure are ranked by how severe the impact is to the product or consumer from one to five using Table 2. The occurrences are also quantified using Table 3 to determine how often this failure occurs. The severity and occurrence rankings are then multiplied to come up with a risk index as shown in Table 4 where the failures are then ranked. The higher risk indexes should be an initial point of concern and actions should be taken to reduce the risk indexes.

Table 2: Failure Mode Effect Analysis Severity Rankings

Severity (SEV)		
Ranking	Definition	Effect
5	Catastrophic	Device failure or defect may cause death or permanent injury with or without warning of failure
4	Severe	Device failure or defect will cause severe injury which would necessitate revision surgery
3	Moderate	Failure renders device useless or will result in a minor injury of a non-permanent nature
2	Minor	Failure will result in no loss of product performance but may create some annoyance to user
1	None	No effect

Table 3: Failure Mode Effect Analysis Occurrence Rankings

Occurrence (OCC)		
Ranking	Definition	Frequency
5	Extremely High	Failure almost inevitable
4	High	Repeated failure
3	Likely	Occasional failure
2	Rare	Failure unlikely
1	Remote	Remote chance of failure

Table 4: Failure Mode Effect Analysis Risk Index Rankings

Risk Index Table							
			Hazard Severity Level (S)				
			None	Minor	Moderate	Severe	Catastrophic
			1	2	3	4	5
Occurrence	Extremely High	5	5	10	15	20	25
	High	4	4	8	12	16	20
	Likely	3	3	6	9	12	15
	Rare	2	2	4	6	8	10
	Remote	1	1	2	3	4	5

While we have developed potential risks that may arise during the use of our product, Ammonia-lyzer, we are unable to comment on the FMEA occurrence and severity of these risks as we have not entered the testing phase of our device yet.

However, we believe that the risks we've identified carry little to no safety concern to the user. The primary risks identified pertain to the small lithium-ion battery, as with any case of lithium-ion batteries pose flammability issues. However, we have designed our product with this in mind and developed a special enclosure for the battery within the housing to address potential overheating.

Future developments for viability

One of the problems we ran into was after ordering the MQ-137 ammonia sensor. When trying to figure out how to write the program to convert the sensor's electrical output to a numerical ammonia concentration, the datasheet was consulted again. It was noticed that the sensor had a range of 5-500 parts per million (ppm). Ppm can be expressed as mg/L, so the range would be from 5-500 mg/L of ammonia. The molar mass of NH_3, which is 17.031 g/mol, was used along with standard g to mg and mol to μmol conversions making the conversion from ppm to μmol/L which are the standard units for measuring ammonia both in the breath and blood. The simplified conversion rate is 1 ppm equals 58.71 μmol/L. It was determined that the sensor measured 293.58 μmol/L at the lowest possible range which was originally 5 ppm. Seeing as how breath ammonia ranges start at 50 ppb, this sensor would not be able to measure the ammonia in the breath. This sensor might give a reading when the exhaled breath is inputted, but it will not be reliable at all.

After realizing this, ammonia sensors with a lower minimum range were considered. One was found that measures 0-500 PPM; however it was $637 [34] and would take a while to be shipped. Figuring out that the sensor had a similar pin configuration, the plan was revised. Due to limited funds and time constraints, the new strategy would be to go ahead and construct the device with the MQ-137 sensor with a range of 5-500 ppm. Then large amounts of ammonia that are within the 5 - 500 ppm range with known concentrations would be inputted into the sensor. If the test levels

show correct concentrations from the input, it is known that the rest of the components in the device can be assumed to be working properly together. If it worked with testing larger amounts within the range of the MQ-137 sensor, the next plan would be to order a new sensor with a lower minimum range, preferably one that reads ppb, and change it out with the one with the 5-500 ppm range. Additional testing would need to be completed to ensure that this change in the sensor didn't drastically affect the performance of the device.

Future Developments to Increase Scope

While the initial goal was to get a working prototype for a breath ammonia diagnostic device, there are ways the device could be improved for added benefit. The prototype that is currently in development has no place to internally store the results of each test after the test is completed, and the device is turned off. The ammonia reading would need to be written down by the consumer if they wanted to keep track of it. The future goal of this project would be to create an app that could be interfaced with the device. This would provide a convenient place for data to be collected and tracked over time. Additional features of this app could include diet, medication, and behavioral activity to be tracked as well to try to pinpoint a root cause if levels do start to rise. This information could also be automatically sent to doctors if levels get out of range ensuring that treatment could begin almost immediately instead of having to book an appointment and drive to the hospital to get blood drawn. It would help catch rises in ammonia before levels got to a point where hospital intervention had to take place to treat hyperammonemia. Instead, a patient and doctor could be notified when levels start to rise, ensuring that a sick day plan could begin immediately instead of waiting until the levels increase a couple of days later when the patient starts to present early symptoms to start treatment. It would help catch rises in ammonia before levels got to a point where hospital intervention had to take place to treat hyperammonemia.

If this app were developed and used alongside this breath ammonia testing device, it could provide valuable insight into how to prevent hyperammonemic episodes

altogether. A centralized database of medications, diets, behavioral activities, and how those activities correlate with the levels of ammonia could be used to better understand how all of these factors come together and integrate into causing or preventing hyperammonemic episodes.

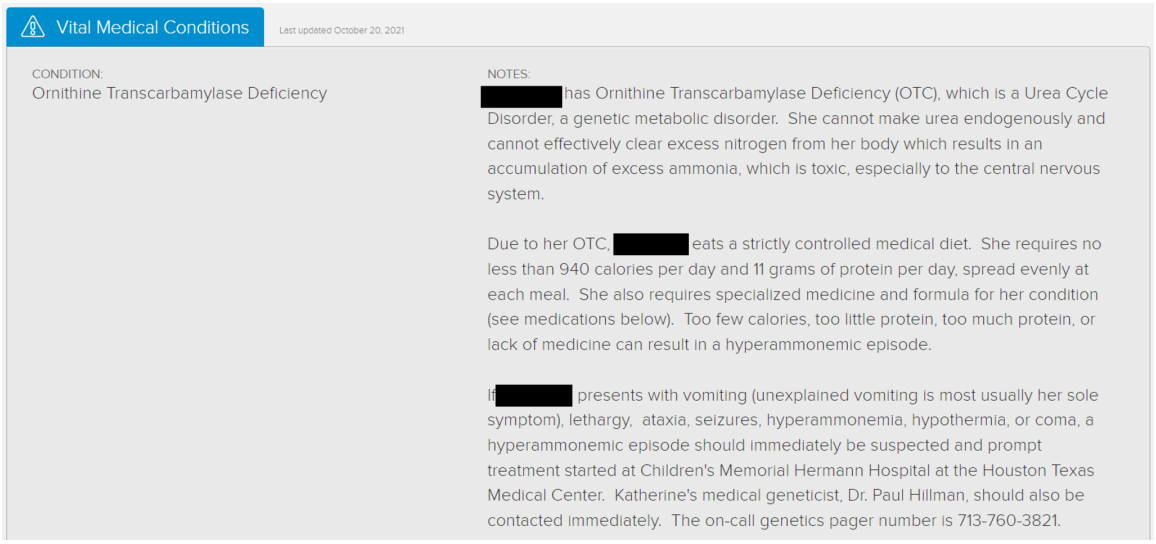
In today's age of technology, healthcare information can be easily shared by patients and doctors making treatment easier than ever; however, with that comes certain considerations that need to be taken into account. Because medical information is extremely sensitive, it needs to be handled with care. With increases in technology, hacking has also become prevalent. This app would need to be designed to prevent not only hacking into logged patient data but prevent them from hacking into the app and changing the test results.

Conclusion

Elevated ammonia levels pose a significant health risk and can be potentially fatal. From liver disease to various metabolic disorders, systemic ammonia issues must be continually and effectively monitored in real-time. Nonetheless, the lack of effective diagnostics outside of the hospital is especially alarming. In an era where blood pressure, glucose levels, heart rate, and VO2 max levels can be tested at home with minimal costs, ammonia level testing must rid itself of the complexity and cost from the bureaucracy of the American healthcare system. Existing blood ammonia testing diagnostics are complicated and not viable for point-of-care or high-volume testing. The human breath contains an abundance of analytes and data to measure health, and its non-invasive nature will allow for easier detection and faster ascertaining of clinical decisions. While current breath testing technology will require further research to perfect ammonia sensors, it is of utmost importance to bridge this gap in the medical device industry for the well-being of all.

Appendices

Appendix A



Vital Medical Conditions

Last updated October 20, 2021

CONDITION:
Ornithine Transcarbamylase Deficiency

NOTES:
[redacted] has Ornithine Transcarbamylase Deficiency (OTC), which is a Urea Cycle Disorder, a genetic metabolic disorder. She cannot make urea endogenously and cannot effectively clear excess nitrogen from her body which results in an accumulation of excess ammonia, which is toxic, especially to the central nervous system.

Due to her OTC, [redacted] eats a strictly controlled medical diet. She requires no less than 940 calories per day and 11 grams of protein per day, spread evenly at each meal. She also requires specialized medicine and formula for her condition (see medications below). Too few calories, too little protein, too much protein, or lack of medicine can result in a hyperammonemic episode.

If [redacted] presents with vomiting (unexplained vomiting is most usually her sole symptom), lethargy, ataxia, seizures, hyperammonemia, hypothermia, or coma, a hyperammonemic episode should immediately be suspected and prompt treatment started at Children's Memorial Hermann Hospital at the Houston Texas Medical Center. Katherine's medical geneticist, Dr. Paul Hillman, should also be contacted immediately. The on-call genetics pager number is 713-760-3821.

Medications

Last updated December 08, 2021

NAME:	DOSAGE:	FREQUENCY:
Ravicti Oral Liquid	2.7 ml	3 times daily

NOTES:
[redacted] requires 2.7ml of Ravicti 3 times daily, usually immediately following her meals.

NAME:	DOSAGE:	FREQUENCY:
UCD Trio	35 gr	1 time daily

NOTES:
UCD Trio (35.0g), ProPhree (25.0g), and Citrulline (2.4g) are mixed together with 1.5oz of rice milk (or other non-protein liquid). 1/2 of the mixture/shake (~40ml) is given immediately following breakfast (~9am) and the other half of the mixture shake is given immediately following dinner (~7pm).

NAME:	DOSAGE:	FREQUENCY:
ProPhree	25 gr	1 time daily

NOTES:
UCD Trio (35.0g), ProPhree (25.0g), and Citrulline (2.4g) are mixed together with 1.5oz of rice milk (or other non-protein liquid). 1/2 of the mixture/shake (~40ml) is given immediately following breakfast (~9am) and the other half of the mixture shake is given immediately following dinner (~7pm).

NAME:	DOSAGE:	FREQUENCY:
Citrulline	3.8 gr	1 time daily

NOTES:
Total Citrulline daily dose of 3.8g is split between morning/night formula (2.4g), lunch EAA formula (0.7g), and early afternoon EAA formula (0.7g).

Morning/Night formula: UCD Trio (35.0g), ProPhree (25.0g), and Citrulline (2.4g) are mixed together with 1.5oz of rice milk (or other non-protein liquid). 1/2 of the mixture/shake (~40ml) is given immediately following breakfast (~9am) and the other half of the mixture shake is given immediately following dinner (~7pm).

Lunch & Early Afternoon EAA formula: EAA packet (9g) and Citrulline (0.7g) are mixed with approximately 10ml of pineapple juice (or water in a pinch) and shaken well. The entire mixture (about 13ml) is given immediately after lunch (~1pm) and again in the early afternoon (~4pm).

NAME:	DOSAGE:	FREQUENCY:
EAA Supplement	18 gr	1 time daily

NOTES:
Lunch & Early Afternoon EAA formula: EAA packet (9g) and Citrulline (0.7g) are mixed with approximately 10ml of pineapple juice (or water in a pinch) and shaken well. The entire mixture (about 13ml) is given immediately after lunch (~1pm) and again in the early afternoon (~4pm).

Appendix B

UT★Physicians

Pediatrics Division of Medical Genetics

6410 Fannin Street, Suite 500
Houston,TX. 77030
(832) 325-6516
www.utphysicians.com

[redacted]

[redacted]

MRN: [redacted]
DOB: [redacted]
Home: [redacted]
Work:

Date of Service: 11/06/2019
Letter

Letter of Emergency Protocol

Patient: [redacted]
DOB: [redacted]
MRN: [redacted]
Address: [redacted]
City, State, Zip: [redacted]
Phone #: [redacted]

Medical Condition: Ornithine Transcarbamylase (OTC) Deficiency
Geneticist: [redacted]
On-Call Medical Genetics Pager: [redacted]

To Whom It May Concern;

My patient, [redacted] has been diagnosed with X-linked OTC deficiency, one of the urea cycle disorders. Specifically, OTC deficiency is caused by a defect in the mitochondrial enzyme ornithine transcarbamylase. Patients with these disorders cannot make urea endogenously and thus cannot effectively clear excess nitrogen from their bodies. This results in accumulation of **excess ammonia** which has toxic effects, most severe in the central nervous system causing **cerebral edema**.

If [redacted] presents with lethargy, vomiting, ataxia, seizures, hyperammonemia, hypothermia, or coma, a **hyperammonemic episode** should immediately be suspected and prompt treatment started. Please note that these episodes can be precipitated by

underlying infection. [redacted] medical geneticist should also be contacted immediately.

IMMEDIATE TREATMENT:

1. **Stop all protein intake**. Before re-introducing feedings, a medical geneticist and a metabolic dietitian should be consulted.
2. Provide **intravenous fluids**: D10W+NS or D10W+1/2 NS at 1.5 times maintenance rate.
3. If hyperammonemia confirmed, give **intralipid** at 2-3 g/kg in addition to IV fluids to prevent catabolism,
4. Provide **ammonia scavenger** medications as detailed below.
   a) IV Ammonul® (sodium benzoate and sodium phenylacetate):
      - Dose for patients <20kg: 2.5 mL/Kg prior to mixture with dextrose solution. This will provide 250 mg/kg of sodium phenylacetate and 250 mg/kg of sodium benzoate.
      - Nausea & Vomiting are the most common side effects: control with Zofran prior to or during infusion.
      - Special considerations: **sodium should not be provided in supplemental IV fluids** when IV Ammonul® is given since this solution contains sufficient amounts of sodium. Otherwise, hypernatremia may result.

   b) PO citrulline: Citrulline supplementation is the first line amino acid to supplement as it is the metabolite immediately downstream of OTC. The dosing is 170 mg/kg/day divided TID and can be given via NG tube. If citrulline not available or patient not able to take PO/enteral medications, then arginine is second-line therapy.
   c) IV Arginine: This is second-line to citrulline when PO not tolerated or citrulline not available. IV dosing is 600mg/kg/day divided TID to provide supplemental arginine which can stimulate the urea cycle. Since arginine comes as arginine hydrochloride, acidosis is a possible side effect.

5. Prepare for **possible hemodialysis**.
   Hemodialysis is the most effective way of rapidly disposing of excess ammonia and is far superior to other methods of dialysis. Moreover, it has the added benefit of removing amino acids such as glutamine and, in that way, disposing of additional

waste nitrogen from the body. **Administer IV Ammonul until hemodialysis is instituted**.
- ⇒ A newborn or infant with plasma ammonia >300 µmol/L should have hemodialysis ASAP. Clinical judgment should be used in older patients, as no ammonia level cut off has been established.

6. Treat the underlying precipitant.

INITIAL LABWORK SHOULD INCLUDE:

1. Ammonia: from peripheral or central line only, in a Na-Heparin tube and sent STAT on ice
   - ⇒ If blood ammonia is >100 µmol/L repeat the level.
   - ⇒ If confirmed, give **D10W+NS or higher glucose solution AND Intralipid at 2-3g/kg**.
   - ⇒ Trend ammonia q4h.
2. Blood glucose: high IV dextrose solutions should not be decreased or stopped in the face of hyperglycemia. The goal is to keep the level from rising above 150 mg/dl. If hyperglycemia occurs while IV dextrose is supplied for added calories, an IV insulin drip at 0.01 units/kg/hour should be started to maintain plasma glucose between 100 and 150 mg%. Insulin may be increased by 0.01-0.03 units/kg/hour until desired effect is obtained.
3. Electrolytes
4. VBG
5. LFTs
6. Plasma amino acids (choose option that says "Baylor" in parentheses)
7. Lactate level
8. Treat/test for any intercurrent illness per primary team. **Note: do not perform a lumbar puncture before evaluating for the presence of cerebral edema.**

MEDICATIONS TO AVOID:

- × Valproic Acid
- × Haloperidol
- × Systemic corticosteroids

UT★Physicians

Patient: [redacted]
MRN: [redacted]
DOB: [redacted]
Date of Service: [redacted]
Letter

Sincerely,

[redacted]
Assistant Professor
Division of Genetics
Department of Pediatrics
McGovern Medical School
The University of Texas Health Science Center at Houston

Electronically signed by: [redacted] Nov 15 2019 3:55PM CST Author

6410 Fannin Street, Suite 500
Houston, TX 77030
(832) 325-6516

Appendix C

Emily Wright, Noah Jones, Ashwin Sivalingam
Home Blood Ammonia Detector
BME 461 – Fall 2021
Literature Review

BACKGROUND

♦ **Summary:**

Hyperammonemia is an elevation in plasma ammonium concentration and is correlated with many conditions such as urea cycle disorders, liver disease, and Reye syndrome. At the normal pH in the body, 7.4, ammonia is 98% ammonium ion (NH4+) which doesn't easily cross barriers. But as the pH increases, ammonium is changed into ammonia (NH3) which crosses berries much easier. This increase in permeability is a concern when it comes to the blood brain barrier which makes ammonia toxic. Some of the symptoms include vomiting, lethargy, but the most concerning symptom if this is left untreated is brain damage or even death. [1] While there are treatments to manage and decrease ammonia levels in conditions such as urea cycle disorders, they must be maintained for the duration of a patient's lifetime. Therefore it is of vital importance to continue monitoring blood ammonia levels as the potential for spikes in ammonia if treatment isn't working is inevitable. Before 1960, blood samples were volitized after adding an alkaline solution to separate and release ammonia from the sample. This was met with skepticism as it was believed that excess ammonia had been released from labile amides in the blood while in the alkaline solution. Now, the most common method for measuring ammonia is to react with ammonia with glutamine dehydrogenase and some other compounds that form glutamic acid. This reaction has been widely used as the standard assay for measuring blood ammonia. To test ammonia levels there are generally two steps: capturing ammonium ions from the sample or releasing ammonia gas and quantifying the amount of freed gas or captured ions. [2] When drawing blood to determine ammonia levels, several steps must be taken to ensure the sample won't give false increased or decreased results. Ammonia concentration can be influenced by factors such as clenched fists, fasting, use of tourniquets during withdrawal, intensive exercise, medication, and failure to separate plasma and put on ice immediately. [3] Due to these factors, ammonia tests are generally done at hospitals to ensure standardization and more accurate reports of ammonia levels.

♦ **Search Terms:**

hyperammonemia, measuring ammonia in blood, blood ammonia collection, preanalytical increase ammonia

♦ **References:**

[1] M. Batshaw. "Hyperammonemia". Current Problems in Pediatrics, Volume 14, Issue 11, 1984, Pages 6-69, ISSN 0045-9380, https://www.sciencedirect.com/science/article/pii/0045938084900471 [Accessed: 18-Oct-2021]

[2] R. Barsotti, "Measurement of ammonia in blood". The Journal of Pediatrics, Volume 138, Issue 1, Supplement, 2001, Pages S11-S20, ISSN 0022-3476, https://www.sciencedirect.com/science/article/pii/S0022347601844188 [Accessed: 19-Oct-2021]

[3] N. Nikolac, J. Omazic, A. Simundic, "The evidence based practice for optimal sample quality for ammonia measurement". Clinical Biochemistry, Volume 47, Issue 12, 2014, Pages 991-995, ISSN 0009-9120, https://www.sciencedirect.com/science/article/pii/S0009912014003920 [Accessed: 18-Oct-2021]

CURRENT TREATMENT OPTIONS

♦ **Summary:**

The most common current detection methods for increased levels of blood ammonia cause delayed treatment due to the strain of specific preparation of the sample which is difficult to do without proper training and give inaccurate results if done incorrectly. In addition, lack of mass spectroscopy machines in smaller areas make it challenging to get accurate results in a timely manner. Before drawing samples, a patient must fast for 6 hours, avoid certain medications, avoid smoking, and avoid strenuous activity. When drawing the samples, a tourniquet can't be used and clenching of fists isn't allowed which can make it difficult to take the sample. [1] After drawing the blood, they must be separated into plasma and placed on ice immediately to avoid increased concentrations of ammonia which occur in whole standing blood. [2]

The current detection methods involve two steps: capturing ammonium ions from the sample or releasing ammonia gas and quantifying the amount of freed gas or captured ions. The first step of capturing ions or releasing gas is performed by one of the following methods: distillation, aeration/microdiffusion, ion-exchange chromatography, and deproteinization. Some pos include being faster than previous tests, reducing potential effects of other components interacting with the ammonia while cons of these methods include being quickly outdated, and liberating ammonia from other blood components. Once the separation is performed one of the quantification methods such as titration, colorimetric/fluorometric reactions, gas-sensing electrode, or an enzymatic method. Depending on the method some of the pros include low loss, speed, and simplicity. Some cons include longer wait times, insensitivity, and large blood samples. Most labs use the enzymatic method to quantify ammonia levels. [3]

♦ **Search Terms:**

current blood ammonia detection, measuring blood ammonia

♦ **References:**

[1] Ayyub, O. B., Behrens, A. M., Heligman, B. T., Natoli, M. E., Ayoub, J. J., et al. (2015). "Simple and inexpensive quantification of ammonia in whole blood. Molecular Genetics and Metabolism". 115(2-3), 95-100. https://www-ncbi-nlm-nih-gov.umiss.idm.oclc.org/pmc/articles/PMC4462127/?tool=pmcentrez&report=abstract [Accessed: 18-Oct-2021]

[2] N. Nikolac, J. Omazic, A. Simundic, "The evidence based practice for optimal sample quality for ammonia measurement". Clinical Biochemistry, Volume 47, Issue 12, 2014, Pages 991-995, ISSN 0009-9120, https://www.sciencedirect.com/science/article/pii/S0009912014003920 [Accessed: 18-Oct-2021]

[3] R. Barsotti, "Measurement of ammonia in blood". The Journal of Pediatrics, Volume 138, Issue 1, Supplement, 2001, Pages S11-S20, ISSN 0022-3476, https://www.sciencedirect.com/science/article/pii/S0022347601844188 [Accessed: 19-Oct-2021]

IDENTIFY USER NEEDS

- **Summary:**

An ammonia level test measures the level of ammonia in a user's blood. Ammonia is a byproduct waste made by the body during protein digestion. If a user's body cannot process or eliminate ammonia, accumulation occurs in the bloodstream. High ammonia levels can contribute to serious health problems, including brain damage, coma, and death. Typically, high ammonia levels in the blood are caused by liver disease, kidney failure, and genetic disorders. Thus, users need ammonia testing when experiencing signs and symptoms of elevated ammonia levels, such as mental changes, disorientation, changes in consciousness or comas. Moreover, newborns also require testing provided they are experiencing symptoms of seizures, vomiting, or lethargy within the first few days after birth.

Users will require an ammonia analyser that enables immediate testing and delivers results in minutes. Compared to traditional ammonia testing, it will eliminate the need for costly pre-processes such as centrifugal separation. There is a need for measurements that can replicate the accuracy of clinical settings while eliminating the high costs associated with it. Traditional ammonia testing requires lab analysis and results that can take weeks. Users will be able to frequently test ammonia levels with only a small sample volume while simultaneously providing easy maintenance and ease of use .

- **Search Terms:**

"home blood ammonia reader, blood ammonia importance, blood ammonia effects"

- **References:**

[1] "Ammonia levels: Medlineplus medical test," MedlinePlus, 09-Sep-2021. [Online]. Available: https://medlineplus.gov/lab-tests/ammonia-levels/. [Accessed: 19-Oct-2021].

[2] "Ammonia," Lab Tests Online, 25-Mar-2021. [Online]. Available: https://labtestsonline.org/tests/ammonia. [Accessed: 19-Oct-2021].

[[3] S. Felson, Ed., "Ammonia test: Purpose, procedure, preparation, & results," WebMD, 04-Feb-2021. [Online]. Available: https://www.webmd.com/a-to-z-guides/ammonia-test. [Accessed: 18-Oct-2021].

[4] "Ammonium Blood Test," ucsfhealth.org, 06-Oct-2020. [Online]. Available: https://www.ucsfhealth.org/medical-tests/ammonia-blood-test. [Accessed: 18-Oct-2021].

MARKET RESEARCH

- **Summary:**

There are currently little to no accessible ammonia tests that everyday people or even patients could use within their homes. Most of the tests that are available are at the hands of doctors and other healthcare professionals. There is a growing potential for blood ammonia checkers that are portable, accessible, and cheap. In addition, there is a need for urgent results, as the current state of testing requires days to weeks to receive lab results. People who are in need or want ammonia tests have to go to the hospital or doctor's office to receive the test. As such, few if any of the current options are suitable for point of care testing.

The rising rates of urinary tract infections, as well as, hyperammonemia, lead to an ever growing market of at-home blood ammonia tests. Children and infants especially have higher abnormalities in their ammonia levels. Because of this, it would be useful to parents who have children who are more susceptible to higher ammonia level diseases and disorders. An at-home blood ammonia checker would allow care-givers and parents to be able to monitor their child's ammonia level without bringing them into a clinic, and this would help diagnose rare genetic disorders, or other potential disorders due to a high ammonia concentration within the blood.

♦ **Search Terms:**

"blood ammonia market, market analysis for blood ammonia tests, blood ammonia testing market"

♦ **References:**

[1] R. Goggs, S. Serrano, B. Szladovits, I. Keir, R. Ong, and D. Hughes, "Clinical investigation of a point-of-care blood ammonia analyzer," Wiley Online Library, 18-Apr-2008. [Online]. Available: https://onlinelibrary.wiley.com/doi/10.1111/j.1939-165X.2008.00024.x. [Accessed: 21-Oct-2021].

[2] "Ammonia testing market," Future Market Insights (FMI) | Smart Market Intelligence for Smarter you. [Online]. Available: https://www.futuremarketinsights.com/reports/ammonia-testing-market. [Accessed: 21-Oct-2021].

[3] N. Brannelly, "The development of a point of care device for measuring blood ammonia," UWE Bristol Research Repository Home, 01-Jan-2017. [Online]. Available: https://uwe-repository.worktribe.com/output/900674/the-development-of-a-point-of-care-device-for-measuring-blood-ammonia. [Accessed: 20-Oct-2021].

COMPETITIVE LANDSCAPE

♦ **Summary:**

Blood ammonia tests are most prominent in the medical field and have been widely used in hospitals and doctor offices. Companies have started creating portable blood ammonia checkers that would allow patients to be able to check their ammonia levels. PocketChem BAC is the biggest provider for at-home ammonia testing. This device can produce results in up to 3 minutes and 20 seconds. AmBeR is another device created by BreathDX that measures the ammonia levels of your breath. For blood ammonia related purposes, PocketChem has been the top provider for patients and hospitals. But, its uses are typically for doctors offices, health clinics, hospitals, laboratories, military settings, and etc. A new prototype for a blood ammonia checker is being created by Dr. Chu, which guarantees results within a minute. It uses a chemical process to isolate the ammonia from the blood. This new device needs a single drop of blood to detect and measure ammonia levels in the blood using an integrated sensor. However, the device is still in the prototype form. Ultimately, while there are options available, currently they are extremely expensive and still in prototyping phases. Nonetheless, a vacuum exists for a portable blood ammonia reader that can be affordably obtained and simplistically used in the comfort of a patient's home.

♦ **Search Terms:**

"blood ammonia checker, blood ammonia companies, portable ammonia checker, PocketChem"

♦ **References:**

[1] T. R. Veltman, C. J. Tsai, N. Gomez-Ospina, M. W. Kanan, and G. Chu, "Point-of-care analysis of blood ammonia with a gas-phase sensor," ACS Publications. [Online]. Available: https://pubs.acs.org/doi/10.1021/acssensors.0c00480. [Accessed: 21-Oct-2021].

[2] A. T. Kubota, A. E. Digitale, and A. B. Goldman, "Device could help patients test blood ammonia levels at home," Scope, 24-Jul-2020. [Online]. Available: https://scopeblog.stanford.edu/2020/07/24/device-could-help-patients-test-blood-ammonia-levels-at-home/. [Accessed: 21-Oct-2021].

[3] R. Goggs, S. Serrano, B. Szladovits, I. Keir, R. Ong, and D. Hughes, "Clinical investigation of a point-of-care blood ammonia analyzer," Veterinary clinical pathology. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/18533920/. [Accessed: 21-Oct-2021].

Appendix D

BIOMEDICAL ENGINEERING
UNIVERSITY OF MISSISSIPPI

Blood & Boujee
Emily Wright, Noah Jones, Ashwin Sivalingam
BME 461 – Fall 2021
Prior Art Search

I would like a minimum of two (2) prior art references to be from international sources – i.e., outside the US Patent Office and from international journals or sources.

Prior Art Reference #1 (*CN1778946A Determination of blood ammonia content and blood ammonia diagnostic reagent kit*)

- **Search Terms:**
  "home blood ammonia reader"
- **Summary:**
  This patent includes a reagent kit that will determine the blood ammonia content using a chemical process to isolate the ammonia in the blood. The reagent box is consisted of buffer solution, 2-ketoglutarate, reduced coenzyme, phosphoenolpyruvate, glutamate dehydrogenase, glutamate decarboxylase, phosphoenolpyruvate carboxylase, malate dehydrogenase and stabilizer. To determine the blood ammonia content, a test strip with the patient's blood is inserted into the device. After the test strip is inserted, an enzyme-coupled reaction occurs. After the reaction, the final reactant is placed under a biochemical analyzer where the absorbance variational situation or speed of the dominant wavelength is measured. This is used to calculate the level of blood ammonia in the patient. This specific test is primarily used in hospitals and offices, and has high sensitivity and precision, and can produce results relatively quickly.
- **Source:**
  https://patents.google.com/patent/CN1778946A/en

Prior Art Reference #2 (*US10591495B2 Device and methods of using device for detection of hyperammonemia*)

- **Search Terms:**
  "blood ammonia reader"
- **Summary:**
  Dr. Omar Bilal Ayyub and his team of researchers sought out new ways to detect the hyperammonemia levels within bodily fluids. Current testing methods are often quite expensive and tedious and could take days to produce reliable results. Dr. Ayyub and his team developed a biosensor that would identify the levels of ammonia or ammonium ions in any sample. The biosensor is composed of vessels with a perfluorinated membrane across them. Each vessel within the device has a different purpose, varying from leading to an alkali buffer solution to receiving the blood or bodily fluid sample. A test strip, chip,or cartridge is used to collect the bodily fluid, mainly for whole blood. The test strip is then inserted into the device and the fluid comes into contact with hypophalite, the alkali buffer solution, a catalyst, and one indophenol reagent. The reaction results in a current across the first electrode, which in turn, corresponds to the concentration of ammonia within the sample. This specific biosensor was designed without relying on gas chromatography. The sensor has a response time of 30 minutes.
- **Source:**
  Device and methods of using device for detection of hyperammonemia - US10591495B2 | PatentGuru

Prior Art Reference #3 (*US9625443 Rapid small volume detection of blood ammonia*)

- **Search Terms:**

  "Blood ammonia detection"

- **Summary:**

  Dr. Chu and his team of researchers invented a new device that could easily and rapidly detect the blood ammonia content within the patient. Because hyperammonemia is a dangerous condition, Dr. Chu decided to create a device that could easily detect it. Hyperammonemia is characterized as an excess of ammonia in the blood. Many cases of hyperammonemia go undetected until it is too late for the patient. This device uses a test strip containing up to 20 microliters of blood. The test strip is brought on top of an ammonia gas sensor. The blood mixes with an alkaline substance which creates a current across the gas-ammonia sensor. This specific current is used to measure the concentration of ammonia in the blood. The alkaline substance is used to hasten the ammonia leaving the blood. This device is typically used at a patient's bedside in hospitals, nursing homes, or doctor's offices. Results are generated typically within a few minutes to a few seconds.

- **Source:**

  https://patents.google.com/patent/US9625443B2/en
  https://patents.justia.com/patent/9625443

Prior Art Reference #4 (*GB 1417538 A Process and Reagent for the Determination of Blood Ammonia*)

- **Search Terms:**

  "Blood ammonia patent"

- **Summary:**

  This invention utilizes glutamate dehydrogenase, alpha ketoglutarate, and NADPH to determine blood ammonia by utilizing non-deproteinized plasma which allows for high speed and low cost. This invention claims to be able to perform tests on a large amount of samples which makes it good for routine laboratory use. Compared to NADH which earlier methods use, NADPH allows for reactions to proceed more quickly and with few side reactions that don't have to be accounted for. The pH for this invention is deemed to range from 7.0 to 9.5. This invention claims to not need a pre-incubation period meaning that five minutes is all that is needed to get a stable read.

- **Source:**

  https://www.lens.org/lens/patent/169-519-601-113-394/frontpage

Prior Art Reference #5 (*EP 0 351 774 B1 Determination of blood ammonia levels*)

- **Search Terms:**
  "Blood ammonia international patent"

- **Summary:**
  This kit utilizes separately packaged reagents such as glutamine dehydrogenase, alpha-ketoglutarate, and reduced nicotinamide hypoxanthine dinucleotide phosphate (NHxDPH) along with a buffer that combine to determine the amount of ammonia in a sample. This invention claims that this system could be easily converted into a diagnostic system that could be automated which would allow accurate and precise results. The technology utilizes spectrophotometry to determine the absorbance change which can be used to find the amount of ammonia in the sample. It is claimed that this assay that contains NHxDPH, compared to previous assays that contain NADPH, allows the reaction to occur more efficiently and faster.

- **Source:**
  https://patentimages.storage.googleapis.com/cd/79/d5/cbe33c868fab6e/EP0351774B1.pdf

Prior Art Reference #6 (*CN 1,746,677 A Blood ammonia determination and kit thereof*)

- **Search Terms:**
  "Blood ammonia international patent"

- **Summary:**
  This invention contains reagents such as a buffer solution, 2-ketoglutarate, reduced coenzyme, glutamate dehydrogenase and stabilizer to determine the amount of ammonia. To measure the amount of ammonia, samples and assays must be mixed in a certain volume ratio to allow them to react to form an enzyme couple reaction. After the reaction is performed, it is placed under a biochemical analyzer which reads changes in absorbance which can be used to calculate how much ammonia is in the blood. It is claimed that the speed of this test is fast and accuracy is high.

- **Source:**
  https://patents.google.com/patent/CN1746677A/en

Appendix E

BIOMEDICAL ENGINEERING UNIVERSITY OF MISSISSIPPI Document Type: Form	QD006F01, Version B **Design and Development Plan**

Project Name: Blood and Boujee Noah Jones, Ashwin Sivalingam, Emily Wright	DHF #DDP2122	D&D Plan Revision:B

Description of the Product

Executive Summary	A vacuum exists for a portable blood ammonia reader that can be affordably obtained and simplistically used in the comfort of a patient's home. Current blood ammonia detectors are mainly used by healthcare professionals and can take an abnormally long time to receive the results, as well as an expensive cost associated with it. We plan to build and manufacture a portable breath ammonia detector that utilizes a MQ-137 Ammonia Gas Sensor. Our product will be cheaper, quicker, and be accessible to everyday people to be able to conduct at-home tests.
Description of the Problem to be Solved	There is currently no way to monitor blood ammonia levels real time and at home. Adults and children living with conditions where ammonia buildup is a real concern are forced to get levels tested at the hospital for constant monitoring. It can sometimes take up to a week to get the results back due to having been sent to a lab to perform the testing. At that point, the ammonia levels will have changed and that is not an accurate depiction of the current levels. If not, many symptoms show and the ammonia levels get too high, it can potentially lead to brain damage, comas, and even death.
Needs Statement	Blood ammonia tests are not readily available to everyday people and to be able to get an accurate test, one would have to go to the hospital or local clinic. Testing for ammonia levels in blood requires that blood be drawn, put on ice, and sent to a lab for analysis. The process is overly cumbersome and samples can be rejected if not done correctly, and life threatening as for example, in newborns, elevated blood ammonia levels can cause brain damage within hours. Essentially, it is a way to address the monitoring of ammonia levels in patients where high and dangerous levels are reoccurring that allows them to get more accurate and frequent updates on levels.
Literature Review	Blood ammonia detectors are vastly growing in popularity with the rising rates of hyperammonemia. There has been an increase in the need for easy to use and portable blood ammonia detectors. Unfortunately, most of the tests that are available are at the hands of doctors and other healthcare professionals. To get an accurate blood ammonia test, one must go to the local doctor's office or hospital to receive the test. There is a need for measurements that can replicate the accuracy of clinical settings while eliminating the high costs associated with it. Current blood ammonia detectors cause delayed treatment due to the strain of specific preparation of the blood sample which is difficult to do without proper training and give

Project Name: Blood and Boujee	DHF #DDP2122	D&D Plan Revision:B

	inaccurate results if done incorrectly. Portable ammonia readers are in need in order to monitor ammonia levels at home and possibly help diagnose rare genetic disorders, or other disorders associated with high ammonia concentration. BME 461 - Literature Review - FALL 2021 - Home Blood Ammonia Detector - Wright, Jones, Sivalingam.docx - Google Docs
Prior Art Search, Assessment, & Patentability	Blood ammonia detectors have been ever growing in the medical field and various prototypes have been created to counteract the high cost associated with blood ammonia tests within the healthcare field. Many of these researchers sought out new ways to detect hyperammonemia and through the use of chemical reactions, they have created a variety of devices that can be used to accurately detect the concentration of ammonia found within bodily fluids and gases. Biosensors have been widely used by these researchers to determine the blood ammonia content following the chemical reactions. BME 461 - Prior Art Search - FALL 2021.docx - Google Docs
Competition & Differentiation	BreathDX is our main competitor. They have created a breath ammonia detection device called AmBeR, which can detect the content of ammonia in just eight breaths. The device uses a glucose test strip which is inserted into the device and comes into contact with polyaniline to measure the ammonia found. For our prototype, we plan to have an easy to use, portable, cheap blood ammonia test that differs from our competitors. Our prototype will include a tube, in which you exhale into through your nostril, this will then lead into a small box that encompasses the gas sensor that will detect the concentration of ammonia found within the exhalation. In the presence of ammonia, the sensor's conductivity will increase with correlation to the increase in the gas concentration. The device will have a built-in circuit that will allow for the readings of the changes in conductivity which will then display an approximate ammonia concentration reading on an LCD screen.
Value Proposition & Differentiation	A Home Blood Ammonia Reader can allow people to test ammonia levels at home, similar to a glucometer for measuring blood sugar. Instead of waiting long times for lab samples, patients can accurately find their ammonia level in minutes. This would provide patients with a "video" of health of where ammonia levels are instead of giving them a "photo" of health once every two weeks. This would also provide a peace of mind to patients or parents of patients since ammonia buildup can cause serious side effects if not caught.
Anticipated Regulatory Pathway	The regulatory pathway we plan to pursue is the 510k submission process. This will expedite the process in which the FDA reviews the application and shouldn't require a clinical study. The class of this device is anticipated to be a Class II device, similar to non-invasive glucose monitors.
Reimbursement Strategy	We plan to use the cost-based reimbursement. This states that the payer will agree to reimburse the provider for the costs incurred in providing services to the population. In addition to being available for home and private use, we

	aim to bring our product to the medical world in which medics, physicians, hospitals, and alike will utilize the product.
Estimated Manufacturing Cost	Ammonia Gas Sensor: $34.95 Cast Aluminum Enclosure: $4.50 - $13.25 (depending on the size needed) Arduino Micro Pro, FTDI Cable: $20.70, $20.25 (depending on length) USB Lipo Charger: $2-$12.99 5V DC to DC step up (transformer): $4.95 -$8.99 3.6v, 1200 mAh Lipo battery: $10.49 128x64 Positive LCD with RGB backlight: $30.60 - 128x64 RGB Backlit Graphic LCD from Crystalfontz
Potential Market & Global Impact	Our device will market towards everyday people who can use this device from the safety of their own homes to check their ammonia levels. Children and infants especially have higher abnormalities in their ammonia levels. Because of this, it would be useful to parents who have children who are more susceptible to higher ammonia level diseases and disorders. An at-home blood ammonia checker would allow care-givers and parents to be able to monitor their child's ammonia level without bringing them into a clinic, and this would help diagnose rare genetic disorders, or other potential disorders due to a high ammonia concentration within the blood. At the moment, we will initially launch in the United States before reviewing plans for a global launch due to varying international medical restrictions.
Intended Use / Indications for Use	Our product will be used to detect the concentration of ammonia found in a breath.
Patient Population	Our device will be targeted to those who recently had children and have abnormal ammonia levels. Our device is suited to high-risk adult patients as well.
Materials	Ammonia Gas Sensor, Cast Aluminum Enclosure, Arduino Micro Pro, FTDI Cable, USB Lipo Charger, 5V DC to DC step up, 3.6v, 1200 mAh Lipo battery, 128x64 Positive LCD with RGB backlight
Features	Screen that indicates approximate ammonia levels
Components	Gas sensor, portable revamped breathalyzer used to detect ammonia concentration

Add Rows as needed

User Needs

Transfer User Need # and Design Input to QD0006F02, Design Summary Matrix.

If a user's need will not be fulfilled, provide a rationale for not fulfilling the need.

User Needs #	Description (User request)	Design Input or Rationale for Not Fulfilling Need
U1	Portable	Small, wireless device

U2	Safe	No adverse human reaction
U3	Accurate	Accurate to a whole number
U4	Affordable	Common materials
U5	Easy to Use	Can be learned in 20 minutes
U6	Repeatability	Minimally invasive
U7	Comfort	Minimally invasive
U8	Durability	Main device can be reused

Add Rows as needed

Part Number

Part Number	Description	UDI[1]

[1] Document UDI if UDI needs to be included on the CAD and/or etched on the physical part.

Add Rows as needed or attach excel list.

Timeline

Attach a project timeline that defines at a minimum the project tasks, the name of the responsible team member, milestones, and the start date, and the due dates. The project timeline should be updated throughout the project and a copy of the current timeline should be reviewed during design review meetings. It is acceptable to use Excel, Project, or other project management tools.

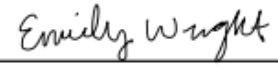
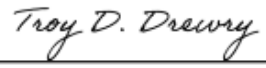
Project Team

Function Required	Name
Product Development	Noah Jones
Quality Assurance	Emily Wright
Regulatory Affairs	Ashwin Sivalingam
Independent Reviewer	Troy Drewry
Additional Functions As Needed	
Manufacturing	Ashwin Sivalingam
Sterilization	Emily Wright
Packaging	Noah Jones

Add Rows as needed

Approvals

Project Name: Blood and Boujee	DHF #DDP2122	D&D Plan Revision:B

Title	Name	Signature	Date
Product Development	Noah Jones		08 Feb 2022
Quality Assurance	Emily Wright		08 Feb 2022
Regulatory Affairs	Ashwin Sivalingam		08 Feb 2022
Independent Reviewer	Troy Drewry		08 Feb 2022

Description of Design and Development Plan revisions.

Revision	Effective Date	Author	Description of Change
A	30 Nov 2021	Team	Initial Draft
B	08 Feb 2022	Team	For Design Review 1

Revision History (Form)

Version	**CR number**	**Approval Date**
A	113021	30 Nov 2021
B	020822	08 Feb 2022

Appendix F

BIOMEDICAL ENGINEERING UNIVERSITY OF MISSISSIPPI
Document Type: Form

QD006F02, Version B

Design Summary Matrix

Project Name:Blood & Boujee	DHF #DSM2122	Matrix Revision:B

User Need #[1]	Design Input[2]	Design Output[3]	Essential Req[4] (Yes/No)	Verification Activity[5]	Validation Activity[6]
U1. Portable	Small, wireless device	Set Dimensions	Yes	Tolerance Stack-Up	Visual Inspection
U2.Safe	No adverse human reaction	Safe electrical conditions; grounded electrical components	Yes	Electrical Testing	Visual Inspection
U3.Accurate	Ammonia Gas Sensor	Accurate to a Whole Number	Yes	Electrical Testing; Chemical Testing	Clinical Studies
U4.Affordable	Common materials	less than $200 or is able to be reimbursed by insurance	No	Mechanical Testing; Packaging	Product Cost Documents and Desired Profit Margins
U5.Easy to use	Can be learned in 20 minutes	User training manual	Yes	Labeling Reviews, Packaging,	Visual Inspection
U6.Repeatability	Gas sensor is able to	Multiple readings close together	Yes	Chemical Testing; Electrical Testing	Clinical Studies

Project Name: Blood & Boujee	DHF #DSM2122	Matrix Revision:B

	recalibrate after each test				
U7.Comfort	Minimally invasive	Use of breath from mouth or nostrils	Yes	Mechanical Testing	Clinical Studies; Visual Inspection
U8.Long-lasting	Main device can be reused	ability to be used 200+ times before replacement	Yes	Mechanical Testing; Tolerance Stack-Up;	Clinical Studies; Visual Inspection

Add rows as needed.

[1]Need # from QD006F01, Design and Development Plan

[2]Design Inputs are to be reviewed by team to ensure they are complete, not ambiguous, and do not conflict.

[3]Design outputs should include catalog numbers, drawings/specifications, material specifications, sterilization, packaging, labeling, features/components of the device, etc.

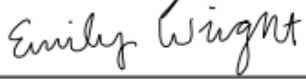
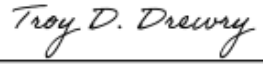
[4]Essential design requirements include those that if they are not met the product could cause harm to a patient or the device could malfunction. The essential design requirements are the features of the design that are deemed critical for function of the component. For these features, validation of the final parts should be performed or alternatively, 100% inspection of the essential design output requirement features may be performed.

[5]Verification activities could include mechanical testing, animal testing, review of drawings/specifications, tolerance stack-ups, labeling reviews, packaging, etc. List applicable document numbers and document names.

[6]Validation activities could include animal testing, clinical studies, saw bone labs, cadaver studies, visual inspection of product, etc. List applicable document numbers and document names.

Project Name: Blood & Boujee	DHF #DSM2122	Matrix Revision:B

Add Rows as needed

Approvals			
Title	Name	Signature	Date
Product Development	Noah Jones		08 Feb 2022
Quality Assurance	Emily Wright		08 Feb 2022
Regulatory Affairs	Ashwin Sivalingam		08 Feb 2022
Independent Reviewer	Troy Drewry		08 Feb 2022

Add Rows as needed

Description of matrix revisions.

Revision	Effective Date	Author	Description of Change
A	30 Nov 2021	Team	Initial Draft
B	08 Feb 2022	Team	For Design Review 1

Project Name: Blood & Boujee	DHF #DSM2122	Matrix Revision:B

Revision History (Form)

Version	CR number	Approval Date
A	113021	30 Nov 2021
B	020822	08 Feb 2022

Appendix G

BIOMEDICAL ENGINEERING UNIVERSITY OF MISSISSIPPI
Document Type Form

QD009F02, Version A

Risk Management Plan and Report

Project Name: Blood and Boujeee

1. Purpose of Revision

X Risk Management Plan (initial) ☐ Risk Management Report

☐ Modification to Risk Management Plan ☐ Modification to Risk Management Report

2. Plan and Report Approvals

Revision	Team Member Function	Team Member Name (printed)	Team Member Approval Signature	Date
A	Product Development	Noah Jones	Noah Jones	3/10/22
	Quality Assurance	Emily Wright	Emily Wright	3/10/22
	Regulatory Affairs	Ashwin Sivalingam	Ashwin Sivalingam	3/10/22
	Executive Management	Troy Drewry	Troy D. Drewry	3/10/22
	Other			

3. Risk Management Details

Risk Management Plan: This Risk Management Plan outlines Risk Management activities for the lifecycle of the products listed in Table 1-3 from the initial product development through post market surveillance. Post market surveillance will be performed as needed, but at a minimum an annual review is required for each product, as outlined in QD006, Design and Development.

Table 1: Part Number

These are the essential parts to the device.

Part Number	Description
Arduino	Motherboard/coding device
MQ-137 Ammonia Sensor	The sensor that detects ammonia readings
LCD Screen	Displays readings
Lithium Ion Battery	Provides power to the device

Add rows as needed or attach list.

Table 2: Indications for Use

Indications for Use	Measuring the ammonia captured in a breath
Foreseeable Misuse (In what way(s) might the medical device be deliberately misused?)	Because the product will bear the breathalyzer name, the device may be misused as an alcohol breathalyzer. There will be sufficient wording and/or graphics on the device to indicate its use for ammonia readings. Moreover, while the device could bear some modifiable design elements such as sharp edges, we believe it will pose no additional risk to the health, safety or well being of the user.

Table 3: Description of the Product

Risk Item	Description
Materials and / components	The lithium ion battery could combust. Electrical components could short and cause some type of electrical hazard such as electrocution. LCD screen could break causing results not to be read. The 3d printed casing around the device could crack or break causing sharp edges or corners. If the sensor stops working or isn't picking up all of the ammonia in the breath, the device will not work.
Energy delivered to and/or extracted	Energy supplied by lithium ion battery which has risk to catch on fire. The sensor reading is sent to the Arduino to calculate the amount of ammonia in the breath which is then sent to the LCD screen. If that connection is broken due to wiring or a faulty part, the device will not work.
Substances delivered to and / or extracted from the patient	Ammonia is extracted from the user's breath and delivered to the ammonia sensor in the device. We will need to ensure that breathing a larger volume into the device doesn't create a higher ammonia readout. If it does we will need to develop a way to get a standard and consistent amount of exhaled gas, to be read by the sensor.
Duration of Use	Ideally, the device will deliver readings in under 30 seconds for each use. If the device takes longer than 3 minutes to give a readout, consumers might get impatient and turn the device off improperly or start pushing buttons that freezes the software up. This could cause problems with the device.
What is the lifetime of the device?	While we haven't been able to do any testing on it yet, the average life expectancy of a breathalyzer whose results depend on accuracy is around 6 months or 200 tests before the sensor needs to be replaced. While the aim of a medical devices lifetime of the device is approximately 7 years this device might have to be replaced earlier, but cyclic testing will need to be completed before determining how many tests can be done before accuracy is affected.
Biological materials processed by the device for subsequent re-use	The ammonia sensor will be reused within the device to read the ammonia levels extracted. With proper calibration, it should pose no issue in terms of reusability.

Risk Item	Description
Supplied sterile or intended to be sterilized by users	As of right now, since it is an external diagnostic device with no testing strips there is no need for the device to be sterilized. If in the future, if we decided to have a testing strip for the ammonia in the breath to react with, sterile packaging would be needed.
Intended to be routinely cleaned and disinfected by the user	Yes. The user should routinely clean the device to ensure accurate readings. As they will be breathing on it, disinfecting is important to also protect safe hygiene. To ensure proper cleaning, we could provide a guide on how to properly disinfect the device to reduce variability of cleaning and improper cleaning. This will ensure that the user is not getting improper readings or even getting sick.
Intended to modify the patient environment?	No. The device will not hold any bearings on the patient's environment or health and is used simply to check their existing ammonia levels. However, results need to be accurate because depending on the reading of the levels, consumers either might not react if the tested levels are within a normal range when in reality, they are high or might overreact if the tested levels are high but in reality their actual levels are within a normal range. The readouts could affect the forward treatment actions of the patient, so it is important they are correct.
Measurements?	The measurements will consist of ammonia breath readings in PPM. 1 PPM = 1 mg/L. We just need to ensure amount of ammonia in the breath is within the detection range of the sensor specifications. If it is not, the results will adversely be affected. Depending on what the normal ammonia ranges are for each age group, and if they don't fall within the detection range of the sensor, we either need to find a new sensor with a new detection range or restrict the age groups that use the product.
Is the device interpretative?	Yes. The readings will be displayed in units consistent with the standard of measuring ammonia.
Intended for use in conjunction with medicines or other medical technologies?	No. The device is solely utilized independent of other medical technologies and medicine. However, should the user's ammonia levels be concerning, they can seek additional physician care and follow their guidance. In the future, we might think about using an online platform where their tests can be stored to keep a track of levels over time and possibly send to a doctor. This would require some sort of cybersecurity measure since sensitive medical information is being used.
Unwanted outputs of energy or substances?	Battery or internal components might get overheated.
Is the device susceptible to environmental factors?	It can pick up ammonia readings in the presence of external ammonia gas. As of right now, we don't think it is susceptible to other gases in the breath or even humidity; however, we will have a better idea after testing. Some studies have shown that depending on what you eat

Risk Item	Description
	such as red meat or even if you exercise right before this, ammonia levels might be affected. This is a concern for caution.
Essential consumables or accessories associated with the device?	No. The device is a standalone breathalyzer and will not require additional accessories. However, if we decide to create a detachable tube where you blow into the chamber so that it can be changed depending on the user, we would hae to examine the risks. Some risks might include it getting stuck or beaking off and being unable to be removed, a choking hazard for small children, or contamination of testing.
Routine maintenance and/or calibration?	The sensor should be recalibrated or replaced every 200 tests or every 6 months, whichever comes first. Calibration directions will be included in the provided instruction pamphlet.
Software?	The device will use arduino software to provide the ammonia readings. We will be writing the code to convert the PPM reading from the sensor to a standard ammonia reading in umol/L. If the software is written incorrectly or gets corrupted, the readout wil be incorrect.
Restricted "shelf life"?	The shelf life of the ammonia breathalyzer will be approximately 12 months. Unused sensors can be used for up to 12 months.
Is the device subject to mechanical forces?	There are no moving parts or pieces within the device nor will the device be dependent on external mechanical forces to function. However, if a large object is placed or hits the LCD screen, the results will be unable to be read.
Is the device intended for single use?	No. The device is intended to be used many times and for the sensor to be recalibrated or replaced as suggested.
Is safe disposal of the medical device necessary?	The device does not contain any chemicals or hazards that need to be disposed of. However since the device contains a lithium-ion battery, it should be disposed according to those procedures.
Is installation or special training required?	Advanced special training or installation will not be required to use the product since it should be fairly straightforward. However, to ensure consumers use it properly, we will provide a user manual and a short video on how to use the device to ensure they get accurate results.
How will information for safe use be provided?	Safe use directions will be provided in an instructions pamphlet.
Can the user interface design features contribute to user error?	No. The internal components will not be accessible to the consumers, which would lead to user error. The safety and guide manual will have instructions on how to use the device
Is the medical device used in an environment where distractions can cause user error?	No. The device can be used in a variety of environments and will not be affected by any distractions that may be brought forth. However, we recommend that the user use the device in a safe and steady environment to ensure that the readings are most accurate.

Risk Item	Description
Will new manufacturing processes be established or introduced?	No. The device only contains select parts from specific companies. There will be no new manufacturing processes that will be needed to assemble or use the device.
Is device critically dependent on human factors such as user interface?	No. The device's interface is not interactable and only depends on the readings gathered by the sensor from the breath of the user.
Does device have connecting parts or accessories?	It has a USB cable to re-charge the device. Internally, there are also several wiring components and soldered points that could break off causing the circuit connection to be lost and the device to stop working correctly.
Does device have control interface?	No. The display interface is not controllable and is only programmable through the arduino.
Does device display information?	The sensor collects readings in PPM but the LCD screen will display ammonia readings in the standard units of umol/L. We also plan for the LCD sceen to display battery levels so users will know when to charge it.
Is device controlled by menu?	No. The device will simply display ammonia readings when turned on.
Will the medical device be used by persons with special needs?	Yes, it will contain accessibility options.
Can the user interface be used to initiate user actions?	No. The interface is only there to display readings and is not interactable.
Does the medical device use an alarm system?	No. The device does not consist of an alarm system and also will not make additional noise.
Does the medical device hold data critical to patient care?	Yes. Ammonia readings can provide insight into whether the patient should seek greater medical care for underlying health issues related to high ammonia levels. It will not store the reading after the device is turned off, so it must be recorded by the patient somewhere else.
Is device intended to be mobile or portable?	Yes. The device will be small and compact enough to be carried around and will fit into a purse or handbag.
Does the user of the medical device depend on essential performance?	The device is intended to be used as an at-home, quick and reliable way of testing the ammonia found in your breath. The primary market focus is for every-day citizens and people who suffer from hyperammonemia. The readouts could affect the forward treatment actions of the patient, so it is important they are correct.

Add Rows as needed

3.1. For each risk area, mitigation activities actions are defined that are typically examined as part of risk management. For each action, the appropriate evidence consists of several different items. The evidence documents (physical copies or references) are placed in the Design History File and/or Risk Management File.

3.2. The following documents, at a minimum, should be included in the Risk Management File for each product:

3.2.1. Complaint Review

3.2.2. Clinical / Literature Review

3.2.3. Risk Analysis

3.2.4. Trending related to product complaints, CAPAs, Non-Conforming Reports (NCR)

4. Risk Management Report

4.1. At the completion of the project, this document becomes the cover sheet for the Risk Management Report. Documents are compiled and approved to verify that risk mitigation evidence is complete or a rationale has been written to justify why the activity was not necessary. Any key assumptions should be included in the objective evidence or rationale. Mark the items included in the report. For items not included a rationale to justify why the activity is not necessary must be attached.

☐ Complaint Review

☐ Clinical / Literature Review

X Risk Analysis

☐ Trending related to product specific complaints, CAPAs and/or NCRs

For items not included provide a rationale to justify why activity was not necessary:

Comments: This is our first draft. Until we are able to deliver the product to the market, we cannot use a complaint review.

5. Risk Acceptance Criteria

5.1. Risk acceptance is defined in QD006, Design and Development and QD009F01, FMEA and document in the risk analysis.

6. Risk / Benefit Summary

6.1. Document an assessment of overall residual risk, if applicable.

6.2. Address the following questions:

6.2.1. Is the risk level acceptable? ■ Yes ☐ No

6.2.2. Do the benefits outweigh the potential risk? ■ Yes ☐ No

If risk level is not acceptable, document how the benefits outweigh the potential risk.

Comments: We are waiting to build part of the prototype to be able to do some testing on it to see where it fails and how much.

7. Post Market Surveillance

7.1. Post market surveillance will consist of periodic review and update, as needed, of applicable risk management documents, but at a minimum an annual review is required for each product, as outlined in QD006, Design and Development.

7.2. Specific post market surveillance activities will typically include complaint and adverse event analyses and review/update of appropriate risk analysis documents (i.e., FMEA).

8. Dates

8.1. Anticipated Launch Date: _____TBD_____

8.2. Next Risk Management Review (Month/Year): _____07APR2022_____

Revision History (Form)

Version	CR number	Approval Date
A	031022	10MAR2022

Appendix H

Failure Modes and Effects Analysis (FMEA)

Process or Product Name: Blood and Boujee Team
Responsible:

Prepared by: Blood and Boujee Team
FMEA Date (Orig): 3/10/22 (Rev:) 1 CR 1

Risk #	Feature / Function	Potential Failure Mode	Effects of Failure	SEV	Potential Causes	OCC	Current Controls	Risk Index	Recommended Actions (if needed)	Responsible Person(s)	Actions Taken	SEV	OCC	Risk Index
#	What is the feature/function under investigation?	In what ways does the key input go wrong?	What is the impact on the key output variables (customer requirements) or internal requirements?	How severe is the effect to the customer	What causes the key feature/function to go wrong?	How often does cause or failure mode occur	What are the existing controls that prevent either the cause or the failure mode?	Severity x Occurrence	What are the actions for reducing the RPN. Should have actions only on high RPN's or easy	Who is responsible for the recommended action?	What actions have been taken and date completed?			SEV * OCC
1	Lithium-ion battery	Could cause the circuit and device to overheat, and potentially cause an unexpected fire	cause fire	TBD	Overcharging/ storing in extreme temperatures	TBD	Plan to have a safety manual and warning of using a lithium battery	TBD	Waiting on device to be assembled		Waiting on device to be assembled	TBD	TBD	TBD
2	LCD Screen	The LCD screen could crack or fracture, which would cause the display to become faulty	screen bleeds/ unable to see readings	TBD	Misuse by customer; placing large heavy objects on top of device	TBD	Will encase the LCD screen in a mesh, or soft material to dampen the affects of colllision	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
3	3D printed cage	The 3D printed cast that the device would be housed in could contain sharp edges or corners that could potentially harm the consumer	could cut consumer	TBD	Design; 3D printed material breaking off creating sharp edges/corners	TBD	The edges and corners will be sanded to remove the sharpness	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
4	Wiring	Improper wiring could cause the circuit to become unfunctional, and potentially harm the consumer due to the wires not being properly grounded	could cause macroshocks	TBD	Improper Wiring; students not knowing what they are doing...	TBD	Will wrap the wires in electrical tape and tie them together to prevent any fraying	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
5	Wiring	Excessive use, overheating, and improper wire management could cause the wires to fray. This could cause the circuit to become unresponsive and potentially lead to an unexpected electricty hazard.	could short out the circuit	TBD	wear of the wiring or constant twisting/turning, placement/correct length of the wires	TBD	Will wrap the wires in electrical tape and tie them together to prevent any fraying	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
6	Arduino	The arduino could malfunction resulting in the reading not being displayed	The display would be unresponsive	TBD	Improper code or wiring	TBD	Will have a reset option so the arduino can resart	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
7	Charging Ports	The open charging ports could get contaminated with dust, dirt, etc. particles	unable to recharge so becomes inoperable after battery drains	TBD	user misuse; dust collection;	TBD	Create a plastic or rubber piece that will prevent dust/dirt particles from getting into the charging port	TBD	Waiting on device to be assembled		Waiting on device to be assembled	TBD	TBD	TBD
8	Sensor	The sensor could malfunction, and the reading would not be accurate	unable to detect ammonia	TBD	Incorrect calibration or defect in sensor	TBD	Along with the arduino, the senor will have the same reset option to prevent any inaccurate readings	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
9	Whole device	The device is not intended to be waterproof, or fireproof	shorts circuit and could cause shock	TBD	Customer misuse	TBD	The safety manual will dicuss and warn the consumers that the device is flammable and not waterproof	TBD	Waiting on device to be assembled		Waiting on device to be assembled	TBD	TBD	TBD
10	Controls	Readout doesn't match known control value	provides inaccurate readings to consumer	TBD	Arduino code or sensor malfunction	TBD	The reset or on/off switch will reset the device	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
11	Correct Parts Inputted	The dimensions of the parts ordered from various companies could be changed or altered	Incompatibility issues with other parts	TBD	Incompatibility could cause device to not work or give faulty results	TBD	incoming inspections	TBD	Waiting on device to be assembled		Waiting on device to be assembled	TBD	TBD	TBD
12	Speed	Arduino takes a while to send information to LCD screen	Could cause impatience in users	TBD	Arduino code and the type of wires used	TBD	Low battery indicator and proper wire management	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
13	Reading hard to see	LCD screen too small, too pixelated or too dim	Could cause eyesight strain	TBD	Design/arduino code doesn't increaase font size	TBD	Will increase the font size if needed	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
14	Low battery indicator affecting performance	Battery not properly charged or working	Device could shut down frequently, and the speed at which you recieve the reading could be affected	TBD	Low battery could affect the performance and speed at which the display is updated	TBD	Have a battery life indicator on the screen	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
15	Device not working	Fails to turn on or show readings	N/A	TBD	Each component could faill or it could be a comprehensive failure	TBD	Have the reset switch	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
16	Giving inconsistent readings	Arduino code incorrect,not proper calibration, sensor not working properly	Could lead to misleading readings	TBD	Arduino code could be miswritten, calibration of sensor could have been done incorrectly, the sensor was defective from the start or damaged	TBD	Have the reset switch	TBD	Waiting on device to be assembled	Noah	Waiting on device to be assembled	TBD	TBD	TBD
17	Lifetime	Depending on the number of tests that have been done, the accuracy of the sensor or other components might decrease.	Precision and accuracy of the sensor would be affected	TBD	The overuse of the sensor could potentially diminsh the accuracy of the sensor leading to an inaccurate reading	TBD	Offer a warranty plan that will allow the consumer to have the sensor replaced	TBD	Waiting on device to be assembled		Waiting on device to be assembled	TBD	TBD	TBD
18	Cleaning	If the device is not cleanned properly or at all, it might affect results.	Dust/Dirt particles could affect the accuracy of the sensor and speed at which the reading is displayed	TBD	The migration of dust and dirt on the motherboard, or other internal components could affect the speed at which the arduino produces the reading and	TBD	Provide consumers with guide on how to properly clean the equipment	TBD	Waiting on device to be assembled		Waiting on device to be assembled		TBD	TBD

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Figure 3: *Specimen collection container*. [Online]. Available: https://www.geisingermedicallabs.com/catalog/container_view.cfm?cont_itn=79. [Accessed: 29-Mar-2022].

Figure 4: *Stanford researchers develop a portable blood ammonia detector*, 21-Jul-2020. [Online]. Available: https://news.stanford.edu/press/view/35314#:~:text=July%2021%2C%202020-,Stanford%20researchers%20develop%20a%20portable%20blood%20ammonia%20detector,certain%20diseases%20and%20genetic%20conditions. [Accessed: 29-Mar-2022].

Figure 5: "Amber® - BreathDX - Breath Analysis Solutions," *BreathDX*, 12-Nov-2017. [Online]. Available: https://www.breathdx.com/solutions/amber/. [Accessed: 01-Apr-2022].