

BREATH TESTING DEVICES AND ANALYTICS

CROSS REFERENCE TO RELATED APPLICATION

1. The present application claims the benefit of U.S. provisional application Ser. No. 63/559,446, filed Feb. 29, 2024, which is hereby incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

2. The present application relates generally to a breath testing device and to methods of testing for multiple diseases of the gastrointestinal tract, the liver, the lungs, and the kidneys, along with testing for cancer, infections, and metabolic diseases. The present application also relates to a breath analyzer and breath test methods for detecting multiple gases in a human breath sample to determine the presence of diseases in a subject's digestive tract. In some instances, the disease can include, but is not limited to, *H. pylori*, *Celiac Disease*, *Metabolic dysfunction-associated steatohepatitis (MAHD)*, *Inflammatory Bowel Disease (IBD)*.

BACKGROUND OF THE INVENTION

3. Exhaled breath contains many gases and thousands of Volatile Organic Compounds (VOCs). Gases and VOCs in breath can be detected with the use of technologies like gas chromatography-mass spectroscopy (GC-MS), selected ion flow tube mass spectrometry (SIFT-MS), FTIR spectroscopy, ion mobility mass spectrometry, field asymmetric ion mobility spectroscopy, semiconductor chips, carbon nanotubes, metal oxides, doped and non-doped polymers and other types of conductive materials sensitive to various gases. Types of conductive materials are chemical sensitive field effect transistors or floating gate field effect transistors or any other field effect transistors. Other types of gas sensitive sensors are optical, electrochemical, thermochemical and surface acoustic wave (SAW) thin films deposited on conducting material (e.g., gold, platinum, palladium or other metals).
4. Gases in exhaled breath are mainly, oxygen (O₂) 16%, carbon dioxide (CO₂) 4%, nitrogen (N₂) 75% and water vapor (5%-6%). In addition the exhaled breath contains small amounts of argon, hydrogen (H₂), ammonia (NH₃), acetone, methanol, ethanol and methane (CH₄) which are some of the most commonly encountered organic volatile compounds. Other VOCs in exhaled breath are acetaldehyde, 2-propanol, acetonitrile, acrylonitrile, benzene, isoprene, pentane, methylethane, ethane, hydrogen sulfide, triethyl amine, trimethylamine, carbon disulfide, dimethyl

sulfide, 1-Heptene, 1-Octene, 1-Nonene, 1-Decene, octane, nonene, dodecane, cyclohexane, 2-butane, indole, ester, carbon disulfide, pentane, nitric oxide (NO), ethane and propane. Breath VOCs are metabolic byproducts of physiologic or pathophysiologic processes taking place within the digestive tract, small and large intestine, in healthy or in diseased individuals.

5. Metabolomics is the field which deals with the byproducts or metabolites of either physiologic or pathophysiologic processes within the human body. Such byproducts are, among others, the volatile organic compounds (VOCs) derived from breakdown of ingested substances by bacteria of the colon in healthy individuals and in individuals with diseases of the gastrointestinal tract, of the kidneys, of the lung, of metabolism, of the liver, and in individuals with metabolic syndrome, obesity, cancer and infections. Analysis of VOCs and their patterns reflects the composition of microbiota and are considered to be markers of these diseases. Detection of VOCs is currently done using technologies such as gas chromatography-mass spectroscopy (GC-MS), selective ion flow tube mass spectrometry (SIFT-MS), field asymmetric ion mobility spectrometry (FAIMS) and electronic noses (e-Nose).

6. Technologies like GC-MS, SIFT-MS, and FAIMS are efficacious in detecting VOCs especially derived from colon microbiota. However, the instruments are expensive, large and mostly stationary. The e-Nose technology, a pattern recognition technology, uses various sensors which are less gas-selective and unstable. None of these technologies use individual sensors which detect each VOC in exhaled breath, and none of these instruments are small, hand-held, inexpensive and available for use over-the-counter (OTC) by exhaling in the instrument directly through an opening of the instrument or a through a mouthpiece. Metal oxide sensors either on their own or coupled with GC-MS are not optimal because the majority of them do not operate at room temperature and most of them need to be heated to about 450 degrees C prior to use.

7. Celiac disease is an autoimmune enteropathy precipitated in susceptible individuals by the ingestion of gluten, specifically its immunotoxic component gliadin. Celiac disease enteropathy resolves with complete and lifelong gluten-free diet. Non-adherence or poor adherence to gluten-free diet may lead to small intestinal lymphoma, adenocarcinoma or other immunological diseases, such as diabetes, thyroiditis, hepatitis, etc. Individuals susceptible to celiac disease express autoantibodies such as antiendomysial and anti-tissue transglutaminase. In

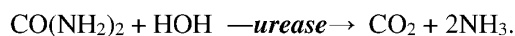
addition to those with confirmed Celiac disease, there are patients with subclinical or asymptomatic Celiac disease and family members of patients with Celiac Disease who have abnormal small intestinal histology without symptoms and who remain undiagnosed. The undiagnosed patients and those with delayed diagnosis remain on gluten-containing diet and risk serious damage to their intestinal mucosa and life-threatening consequences like cancer.

8. The prevalence of Celiac disease has been increasing in parts of the world, where consumption of gluten is common. Currently in the US, the prevalence of Celiac disease is 1%, much higher than its 0.02% prevalence in the early 1990s. There is also increasing prevalence of Non-Celiac Gluten Sensitivity (NCGS), estimated at 3-6% in the US and Europe. Patients with NCGS have no autoantibodies or intestinal mucosal changes of Celiac disease, but have similar symptoms with Celiac disease patients after ingestion of gluten, which improve on gluten-free diet.

9. The current gold standard in the diagnosis of Celiac disease is small bowel biopsies of the small intestinal mucosa which are obtained through upper endoscopy. Patients with Celiac disease, who must follow a strict, lifelong gluten-free diet, undergo follow-up endoscopies with biopsies to assess adherence to gluten-free diet and the health of the small bowel mucosa. Other diagnostic methods exist, including blood tests for antiendomysial and anti-transglutaminase antibodies. Currently there is no screening test for patients with symptomatic Celiac disease or with asymptomatic Celiac disease (subclinical Celiac disease and asymptomatic family members) who do not have the reason to undergo endoscopies. These asymptomatic patients however, are at high risk of developing co-morbidities consistent with those of symptomatic Celiac disease patients and potentially cancer. These asymptomatic patients who are mostly family members of patients with Celiac disease can benefit from certain embodiments of the present invention that offer a non-invasive breath test highly sensitive to the hydrogen in the breath after an eight to twelve (8-12) hour overnight fasting.

10. *Helicobacter Pylori* (*H. Pylori*), which affects two thirds of the world population, is a highly contagious, gram-negative bacterium which causes chronic gastritis, peptic ulcers and can cause gastric cancer and other malignancies like gastric lymphoma (MALToma). It is associated with extraintestinal diseases like anemia, liver disease, gallbladder disease and pulmonary disease like asthma. One of the properties of *H. Pylori* is its ability to hydrolyze urea into CO₂

and ammonia (NH₃) by using its abundant enzyme urease as in the following equation:



11. The gold standard in diagnosis of H. Pylori is a ¹³C urea breath test (UBT) which measures ¹³CO₂ in breath after ingestion of ¹³C labeled urea as in the following equation: ¹³CO(NH₂)₂ + HOH $\xrightarrow{\text{urease}}$ ¹³CO₂ + 2NH₃. The current ¹³C UBT requires the use of a relatively expensive kit and very expensive (several thousands of dollars) equipment which must be operated by professionals. There are other known methods to diagnose H. Pylori infection, including: 1) upper endoscopy with biopsies and culture of the tissue; 2) upper endoscopy with biopsies and rapid urease test (CLO test); and 3) serum antibodies to H. Pylori. Positive antibodies indicate infection with H. Pylori. However, the antibodies remain positive even after eradication of the bacterium. As a result, the serum antibodies cannot be used to confirm eradication as the ¹³C UBT (and the other methods) can. None of the existing diagnostic methods are inexpensive and hand-held, combining test and device in one.

12. Inflammatory bowel diseases are mainly Crohn's disease and Ulcerative colitis. There are differentiating features for each of these inflammatory bowel diseases as relating to 1) the part of the intestinal tract which is affected by inflammation in each of them (ulcerative colitis limited to the large intestine and Crohn's disease potentially affecting the entire digestive tract, but mainly the small intestine); 2) the histological changes in the intestinal mucosa; 3) the diagnosis; 4) the treatment; and 5) the prognosis.

13. The diagnosis of inflammatory bowel disease and the differentiation between Crohn's and ulcerative colitis is established with the use of endoscopies of the small and large intestine with biopsies and histological examination of the intestinal mucosa in addition to radiological studies (especially in Crohn's disease). After the diagnosis is established, appropriate treatment is instituted. Such treatment is lengthy and requires periodic follow-up with endoscopies and biopsies to assess efficacy of the treatment.

14. It has been observed that changes in the colon-derived metabolome in patients with inflammatory bowel disease (Crohn's and ulcerative colitis) contribute to the development of a pattern of Volatile Organic Compounds (VOCs) in the breath of these patients. These VOCs are acetaldehyde, 2-propanol, acetonitrile, acrylonitrile, benzene, isoprene, pentane, methylethane,

ethane, hydrogen sulfide, triethyl amine, trimethyl amine, carbon disulfide, dimethyl sulfide, 1-Heptene, 1-Octene, 1-Nonene and 1-Decene and others.

15. Small intestinal bacterial overgrowth (SIBO) occurs in predisposed individuals and presents with symptoms similar to inflammatory bowel diseases such as abdominal pain, diarrhea, anemia and weight loss. The small bowel normally contains a very small number of microbes as compared to the large bowel, which normally contains several trillion of microbes (estimated at 100 billion per milliliter). SIBO occurs when bacteria, which normally ferment carbohydrates in the large bowel, migrate to the small bowel. As a result, fermentation of carbohydrates takes place in the small bowel, which in turn creates the symptoms of abdominal pain, steatorrhea, and abnormally high production of hydrogen which is absorbed and released through the lungs in the breath.

16. Conditions which predispose to the development of SIBO are anatomic abnormalities, abnormal motility of the intestine as in pseudo-obstruction, absence of the migratory motor complexes, autonomic neuropathy as in diabetes, excessive bacterial load as in achlorhydria, fistula and loss of the ileocecal valve and immunological problems like immunodeficiency and malnutrition.

17. Diagnosis of SIBO is obtained with invasive or non-invasive tests. Invasive tests include small bowel endoscopy and aspiration of content for culture for aerobic and anaerobic bacteria and motility studies. Non-invasive tests are 72 hour stool collection for fecal fat, serum bile acids and breath tests. The most common breath test is the hydrogen breath test after consumption of lactulose. Lactulose, a non-absorbable sugar, provides the substrate for the colonic bacteria to produce hydrogen which results in increased hydrogen levels in breath. If the large bowel bacteria are present in the small bowel then the hydrogen production after ingestion of lactulose occurs much earlier than usual and an early and late peak of hydrogen production is observed during the breath test. The gold standard hydrogen breath test is performed with Quintron breath analyzer equipment. The individual undergoing breath testing for SIBO provides a baseline fasting breath sample and ingests a certain amount (about 7g/Kg) of lactulose. In cases of SIBO, post-lactulose excretion of hydrogen gives two peaks, one early because large bowel bacteria are in the small bowel, and another peak later when the lactulose reaches the large bowel.

18. Irritable bowel syndrome (IBS) is a diagnosis of exclusion and is given to individuals who present with symptoms of abdominal pain, diarrhea or constipation, bloating and flatulence. IBS is a functional disorder of the digestive tract which, despite symptoms similar to IBD, has no organic etiology and no histologic abnormality of the digestive tract has been associated with it. Intestinal motility abnormalities and food intolerance have been observed in certain individuals with IBS which can present with either diarrhea or constipation. Diagnosis of IBS is made after all organic causes for IBD, SIBO, infections and Celiac Disease have been ruled out. It is reported that analysis of fecal Volatile Organic Metabolites has a pattern consistent with IBS with diarrhea (IBS-D) which differentiates IBS-D from IBD and healthy individuals with a 94-96% sensitivity and 80-82% specificity. IBS-D fecal volatile organic compounds contain esters of short chain fatty acids such as cyclohexane carboxylic acids, butyrate, acetate and propionate. This observation can lead to developing the colon-derived breath metabolome as markers for IBS especially with diarrhea.

19. Lactose intolerance is intolerance to lactose, the carbohydrate contained in milk. Lactose intolerance presents with symptoms similar to Irritable Bowel Syndrome and other malabsorption problems. The symptoms are abdominal pain, diarrhea and flatulence and occur after ingestion of milk or milk-containing products. Lactose is a disaccharide which is broken down to the monosaccharides glucose and galactose with the use of the enzyme lactase which is an enzyme present at the intestinal brush border. Glucose and galactose are easily absorbed and usually cause no symptoms. In individuals in whom the enzyme lactase is absent or severely limited, as in genetic conditions or post infections involving the digestive tract like, viral enteritis or parasitic or microbial infections, lactose is not metabolized and as a result it causes symptoms similar to carbohydrate malabsorption, diarrhea, abdominal pain, bloating and flatulence. Fructose intolerance is the inability to tolerate intake of fructose, the carbohydrate found in fruits. As a result, individuals with such disabilities who ingest fruits experience similar symptoms as the individuals with lactose intolerance. It is estimated that the adult human intestine can tolerate up to 25g of fructose without symptoms. Ingesting more than 25 grams of fructose can cause diarrhea, flatulence and abdominal pain. Any amount of fructose can cause symptoms in individuals who have the genetic form of fructose intolerance due to genetic deficiency of the enzyme Aldolase B. Liver diseases are divided in two categories; genetic and

inherited liver diseases and acquired liver diseases. The genetic and inherited liver diseases include disorders of carbohydrate metabolism, disorders of amino acid metabolism, abnormalities in mitochondrial fatty acid β -oxidation, α 1-antitrypsin deficiency, disorders of the bile acid synthesis and metabolism, Wilson disease and others. The acquired liver diseases are mainly viral infections like hepatitis A, B, C and others which can lead to chronic hepatitis, cirrhosis and end-stage liver disease, Hepatocellular carcinoma (HCC) and other liver malignancies and diseases of the liver due to obesity, such as metabolic dysfunction associated steatotic liver disease (MASLD) aka NAFLD and metabolic dysfunction-associated steatohepatitis (MASH) aka NASH and metabolic abnormalities such as diabetes and others. Autoimmune hepatitis, which can lead to chronic hepatitis and cirrhosis, is an inflammatory hepatitis which is secondary to autoimmune diseases affecting more than one body organ. HCC can be the result of viral hepatitis and of steatohepatitis which is due to deposits of fat in its tissue. Steatohepatitis can be secondary to obesity, diabetes and alcoholic hepatitis. If untreated, steatohepatitis can lead to cirrhosis and end stage liver disease. Changes in metabolome have been observed in genetic liver diseases as well as in acquired liver disease and in end stage liver disease. Hyperammonemia induced encephalopathy is one of the symptoms of liver failure.

20. Renal diseases are diseases of the kidneys and are genetic-idiopathic and acquired. Genetic diseases of the kidneys include several types of glomerulonephritis such as IgA nephropathy, Alport syndrome, Good pasture disease, membranous glomerulopathy, lupus-induced glomerulonephritis and others and anatomic abnormalities such as polycystic disease of the kidneys and UPJ obstruction. Acquired kidney diseases are post-infection glomerulonephritis such as acute post-streptococcal glomerulonephritis, hemolytic uremic syndrome (HUS), drug-induced glomerulonephritis, post-traumatic renal disease and others. Symptoms of renal disease are hematuria, proteinuria, edema and poor growth in children. When renal disease becomes chronic and progresses to renal failure (kidney failure), hemodialysis and eventual kidney transplant are essential. Hyperammonemia is a key feature of end stage renal failure due to the inability of kidneys to excrete the accumulating ammonia.

21. More than 3,500 components (gases) make up the human breath and the majority are volatile organic compounds (VOCs) in very small amounts. In addition to nitric oxide (NO), which has become a biomarker for respiratory inflammation and asthma, a pattern of VOCs has emerged as prevalent within the population with allergic and inflammatory component asthma.

These compounds are nonane, 2,2,4,6,6-pentamethylheptane, decane, 3,6-dimethyldecane, dodecane, and tetradecane. The breath metabolome provides a characteristic of chronic obstructive pulmonary disease (COPD).

22. Metabolic diseases like diabetes and obesity affect the metabolome. Increased levels of acetone in breath of individuals with diabetes, is a finding consistent with undiagnosed diabetes or poorly managed diabetes in individuals who are already diagnosed. The concentration of breath acetone has been found to correlate with the β -hydroxybutyrate concentration of venous blood in fasting obese patients. Studies have identified eight specific metabolites such as isopropanol and 2,3,4-trimethylhexane, 2,6,8-trimethyldecane, tridecane and undecane as a more specific pattern for the presence of type 2 diabetes with a high sensitivity and specificity. Increase in VOCs has been described in patients with various types of cancer such as colon, lung, breast and others. Infections involving the gastrointestinal tract such as bacterial infections (salmonella, shigella) and parasitic infections alter the colonic bacteria, and as a result, alter the corresponding breath metabolome.

23. *Helicobacter pylori* (“*H. pylori*”) is a gram-negative bacterium found in the digestive tract that affects about two-thirds of the world’s population. Most people contract *H. pylori* infection during childhood and may never have any signs or symptoms of the infection. However, when signs or symptoms do occur with *H. pylori* infection, they may include a burning pain in the abdomen and chest, nausea, vomiting, frequent burping, bloating and weight loss. *H. pylori* causes gastritis and the majority of peptic ulcers, and is associated with other intestinal diseases like inflammatory bowel diseases, hepatobiliary and pancreatic diseases. *H. Pylori* is also associated with extraintestinal diseases, some of which are idiopathic thrombocytopenic purpura, iron deficiency anemia, renal diseases, and cardiovascular diseases like ischemic heart disease and atherosclerosis.

24. In addition, *H. Pylori* was declared a Class I carcinogen for humans in 1994 by the International Association for Research on Cancer (IARC) and has been strongly associated with the development of gastric cancer and with gastric MALToma, a type of lymphoma. *H. Pylori* has also been associated with other cancers like colon cancer and pancreatic cancer.

25. Current diagnostic methods for detecting *H. Pylori* infection have several drawbacks. Some existing diagnostic methods include endoscopy with biopsies and bacterial culture. Such

methods are expensive, uncomfortable, and carry risks for the subject to undergo. In addition, the results of such tests are not available until several days after the test has been performed. Furthermore, these invasive methods utilize large and expensive equipment operated by highly-trained personnel; require the use of either anesthesia, sedation, or both; and require the use of pathology and microbiology laboratories to render a diagnosis.

26. Currently available non-invasive methods for the detection of *H. Pylori* include the ^{13}C labeled urea breath test; detecting *H. pylori* antigens in stool; a stool test for *H. Pylori* DNA; and serum antibody testing. These known non-invasive methods are costly, not available for self-testing, and the testing results are unavailable until several hours or days after the test has been performed. Currently, these non-invasive methods utilize laboratory equipment for analysis of the breath sample for $^{13}\text{CO}_2$; to test blood for the *H. Pylori* antibody; and to examine stool for the *H. Pylori* antigen and/or for *H. Pylori* DNA. All of these methods require expensive equipment and qualified personnel to carry out the particular testing method.

27. ^{13}C -Urea breath tests are also known and have been used to detect *H. pylori* infection. In such known types of urea breath tests, the presence of *H. Pylori* is based on the measurement of the ratio of $^{13}\text{CO}_2$: $^{12}\text{CO}_2$ in the breath of a subject after ingestion of a urea substrate labeled with ^{13}C . The subject exhales into a bag, which is then attached to large equipment (e.g., a spectrometer) for analysis of the breath sample. If the $^{13}\text{CO}_2$: $^{12}\text{CO}_2$ ratio is above a certain value, the subject is considered positive for *H. pylori*. However, these known types of urea breath tests have several drawbacks. First, they use a urea substrate labeled with ^{13}C , which, though it is a naturally occurring isotope of carbon, is expensive in comparison to the also naturally occurring ^{12}C which is contained in the unlabeled urea. In addition, known ^{13}C -Urea breath tests require expensive instruments (e.g., infrared spectrometers) to analyze $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ levels.

28. As set forth in the present disclosure, it would be desirable to provide a breath test method that measures elevated levels of both ammonia and CO_2 (measured as $^{12}\text{CO}_2$ or $^{13}\text{CO}_2$; both $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$; or as a ratio of $^{13}\text{CO}_2$: $^{12}\text{CO}_2$) in the breath of a subject before and after the subject ingests a urea substrate. It would also be desirable to provide such a breath test that can utilize a urea substrate regardless of whether the urea is labeled or unlabeled. In addition, it would be desirable to provide a reliable point-of-care diagnostic test that can be self-administered using a

hand-held device to determine the presence of *H. pylori*. Still further, it would be desirable to provide an improved breath test that is less costly and more convenient than existing breath tests.

29. Breath testing for the detection and measurement of biomarkers for disease. The breath metabolome, which is a constellation of gases found in the breath, can be used as biomarkers for several diseases of the digestive tract, liver, pancreas, kidneys, as well as cancer and infections.

30. One breath biomarker is fasting breath hydrogen (FBH), a diagnostic biomarker for Celiac disease. Fasting breath hydrogen (FBH) is significantly higher in patients with Celiac disease than in patients with other malabsorption syndromes and remains elevated until the patient remains on strict gluten-free diet for at least 6 months. FBH can be used for monitoring of patients with Celiac disease on strict gluten-free diet because, it has been shown, that the level of FBH correlates with the histologic condition of the small bowel mucosa. The higher the FBH the more abnormal the histology of the small bowel, and the reverse, the lower the FBH the less abnormal the histology of the small bowel. When the FBH is within normal limits, the histology of the small bowel is normal.

31. Another breath biomarker is fasting breath ammonia (FBA). Metabolic dysfunction associated steatohepatitis (MASH) aka NASH is part of the spectrum of metabolic dysfunction associated steatotic liver disease (MASLD) aka NAFLD which has become the most common liver disease in the world due to worldwide increasing rates of obesity and metabolic syndrome⁶. MASH develops when lipotoxic lipids, accumulated due to fatty infiltration of the liver, cause significant hepatocellular injury.

lipotoxic lipids → *hepatocellular injury* → **MASH** → *fibrosis* → *cirrhosis* → *HCC*

Lipotoxic lipids (diacylglycerols, ceramides and others) mediate an array of intracellular processes (endoplasmic reticulum stress, mitochondrial dysfunction, inflammation and apoptosis) to produce the histologic phenotype of MASH. These intracellular processes are the stimuli for fibrogenesis and possibly hepatocellular carcinoma (HCC).

32. An estimated 2-5% of the US population or 20% of the population with obesity-induced MASLD will advance to MASH and cirrhosis without clearly established predictability criteria and MASH may soon become the leading indication for liver transplantation. The cumulative annual risk for developing HCC in patients with MASH-fibrosis, if untreated, ranges from 2.4% to 12.8% and the projected annual economic impact of MASLD-MASH is estimated at \$103

billion in the US. There is a dire need to identify and treat MASH before the development of cirrhosis.

33. Liver biopsy, an invasive procedure, is the gold standard in diagnosis and staging of NASH fibrosis along with elevated serum alanine transferase (ALT) ≥ 50 IU/L and imaging (ultrasonography-MRI) studies. Biomarkers are expected to improve the ability to stratify disease severity in MASLD and to identify additional pathways to target for treatment before it advances to MASH fibrosis and cirrhosis. Rigorous review of the literature revealed that several non-invasive biomarkers and panels of biomarkers with blood tests that reflect underlying disease pathways in MASH are being developed. Biomarkers such as Caspase-generated CK-18 fragment (CK-18), fibroblast growth factor 21(FGF21), insulin-like growth factor 2 (IGF-2) as well as epidermal growth factor receptor (EGFR) have moderate diagnostic and prognostic accuracy. Biomarker panels such as AST:ALT ratio, MAFLD fibrosis score, BARD score (BMI, AST:ALT ratio, Diabetes mellitus) are less accurate than specific fibrosis markers (FibroTest, FibroMeter). These specific fibrosis markers accurately predict the fibrosis stage, only, after fibrosis has long been developed. Hyaluronic acid (HA), a major component of extracellular matrix, is detected during advanced stages of fibrosis.

34. Imaging biomarkers such as FibroScan and point shear wave elastography (psWE) have moderate to high accuracy of diagnosing advanced fibrosis or cirrhosis but not early stages of fibrosis. Magnetic Resonance Elastography (MRE) has higher success rate and accuracy than ultrasound-based technologies but is limited by cost and availability. Genetic and genomic markers for assessment of disease susceptibility and disease severity are being developed but with limitations in accuracy and reproducibility with regards to prediction of MAFLD and MASH and its severity. Metabolomic studies are underway and studies of the microbiome profile produced an algorithm that could predict advanced fibrosis suggesting that a test based on it would be a useful marker. However, such marker would not predict early fibrogenesis. So far, despite promising clinical studies, an accurate, reproducible non-invasive method to predict early NASH has not been identified.

35. There is a need for accurate, non-invasive, reproducible, and specific biomarker with high predictive value of MASH fibrosis at the earliest possible stage of fibrogenesis.

36. Ammonia in MASH: None of the existing biomarkers is a breath test, the simplest method of testing for a potential biomarker. Recent, compelling evidence in animal studies and in humans shows that hepatocellular injury causes structural changes (gene expression, activity) in the urea cycle enzymes ornithine transcarbamylase (OTC) and carbamoyl phosphate synthetase (PS), thus impairing ammonia's conversion to urea resulting in hyperammonemia. Studies have shown a **direct link between increased ammonia concentration and the development of fibrosis**. Ammonia at concentrations of 50-300 $\mu\text{M/L}$ activates the usually quiescent hepatic stellate cells (HSC) which then become highly proliferative and synthesize a fibrotic matrix rich in type I collagen becoming key for the development of fibrosis, portal hypertension and HCC. **Hyperammonemia** due to hepatocellular injury is among the earliest stimuli for the development of fibrosis in MASH.

37. Breath ammonia as biomarker for MASH: The toxicity of hyperammonemia makes monitoring of ammonia a priority. However, measurement of blood ammonia is considered non-reliable, as per AASLD, due to inaccuracies associated with blood drawing methods (arterial blood is a preferable but painful procedure and requires professional training- Venous blood often gives inaccurate results) and errors with the transport of the specimen to the lab. Measurement of breath ammonia, a simpler and more accurate procedure than blood ammonia, can replace blood ammonia especially since ammonia is in equilibrium in the lungs and studies have shown that blood ammonia correlates with breath ammonia.

SUMMARY OF THE INVENTION

38. Certain embodiments of the present disclosure provide a universal, electrochemical multi-analyte breathalyzer device for the detection of gases in human breath of individuals with gastrointestinal, renal, respiratory, metabolic and inflammatory diseases, cancer, and infections. The universal electrochemical device can comprise a main body which houses multiple electrochemical sensors, the electronics to support the operation of the device, a power source, a USB port, and Bluetooth technology to transfer data wirelessly to another device or computer. The universal electrochemical device, which can have a display for input by the user and for output of the results, accepts the user's breath through either an opening of the body of the device (which acts as mouthpiece) or through a removable mouthpiece.

39. Certain other embodiments provide a breath analyzer that includes an input, a first sensor, a second sensor, a processor, and an electrical circuit. The input receives the breath sample. The first sensor contacts the breath sample and includes polyaniline and a conductive material. The polyaniline contacts the conductive material and is doped with a first dopant that increases pH sensitivity of the polyaniline. The polyaniline has a resistivity that increases in response to increased concentration of ammonia. The second sensor also contacts the breath sample. The second sensor includes polypyrrole and a conductive material. The polypyrrole contacts the conductive material and is doped with a second dopant that increases pH sensitivity of the polypyrrole. The polypyrrole has a resistivity that increases in response to increased concentration of carbon dioxide. The electrical circuit operably connects the first and second sensors to the processor. The processor detects resistivity in the electrical circuit and uses the resistivity to calculate a total concentration of ammonia and a total concentration of carbon dioxide in the breath sample.

40. Other embodiments provide a handheld, portable breath analyzer that includes a removable mouthpiece and a main body. The main body includes a first sensor, a second sensor, a processor, and an electrical circuit. The first sensor includes polyaniline, and the polyaniline is doped with a first dopant that increases pH sensitivity of the polyaniline. The polyaniline has a resistivity that increases in response to increased concentration of ammonia gas. The second sensor comprises polypyrrole, and the polypyrrole is doped with a second dopant that increases pH sensitivity of the polypyrrole. The polypyrrole has a resistivity that increases in response to increased concentration of carbon dioxide. The electrical circuit operably connects the first sensor and the second sensor to the processor. The processor detects resistivity of the first sensor and uses the resistivity to calculate a concentration of ammonia, and the processor detects resistivity of the second sensor and uses the resistivity to calculate a concentration of carbon dioxide.

41. Still other embodiments provide a breath test method. The breath test method includes the step of providing a portable breath analyzer that includes a removable mouthpiece and a main body. The main body includes a first sensor, a second sensor, a processor, and an electrical circuit. The first sensor includes ammonia selective material that has a resistivity that increases in response to increased concentration of ammonia gas. The second sensor comprises carbon dioxide selective material that has a resistivity that increases in response to increased concentration of carbon dioxide gas. The electrical circuit operably connects the first sensor and the second sensor to the

processor, and the processor measure resistivity of the first sensor and the second sensor. The method further includes prompting a subject to exhale a baseline breath sample into the removable mouthpiece, and allowing the processor to measure a resistivity of the first sensor that occurs when the baseline breath sample contacts the first sensor. The method also includes prompting a subject to exhale a post-urea breath sample into the removable mouthpiece, and allowing the processor to measure a resistivity of the first sensor that occurs when the post-urea breath sample contacts the first sensor. Additionally, the breath test method includes the step of comparing the measured resistivity of the baseline breath sample to the measured resistivity of the post-urea breath sample.

42. Certain other embodiments provide a method of detecting presence of *H. pylori* in a digestive tract of a subject. The method includes collecting a baseline breath sample from a subject and determining a total amount of ammonia and a total amount of carbon dioxide present in the baseline breath sample. The method further includes collecting a post-urea breath sample from the subject, and determining a total amount of ammonia and a total amount of carbon dioxide present in the post-urea breath sample. Additionally, the method includes the step of designating a presence of *H. pylori* in the digestive tract if the total amount of ammonia and the total amount of carbon dioxide present in the post-urea breath sample exceeds the total amount of ammonia and the total amount of carbon dioxide present in the baseline breath sample by a predetermined value.

43. Principal Component Analysis (PCA) is an analytical method, which can be used in medical testing to classify data from clinical studies. PCA can identify subjects with the same diagnosis and cluster them together on the basis of input of components included in the various studies.

44. Artificial intelligence and machine learning are being used in medicine to better interpret results of testing for diseases. Machine learning algorithms are being used to examine clustering of data and detect abnormalities with precision in way that was not previously accomplished with the standard statistical methods. K-means and DBSCAN algorithms are used to examine the data and derive conclusions about positive or negative disease outcome of a test.

45. In certain embodiments in step 6 of the device operational flow (Figs. 5 and 34), the recalled data are passed through a mathematical model code programmed into the microcontroller for calibration and (using this information) for calculation of the result. In step 7, after calibration and calculation of data, the result is displayed on the LED screen.

46. In step 6, the recalled data pass through a mathematical model which is coded in the microcontroller (fitting) and yield the multiple key parameters. The key parameters along with the user's input information (body weight, height, GI related survey, and etc) pass through a trained Machine Learning model or other classification model which was generated by KNN (20) patients' data and implanted in the microcontroller and return the classification result.

BRIEF DESCRIPTION OF THE DRAWINGS

47. Figure 1 shows one embodiment of the breathalyzer device. Depicted are the front of the device with the screen and the mouthpiece receptacle; the side of the device with the on/off switch; and the mouthpiece.

48. Figure 2 shows a schematic of the operation of the device when used by the user.

49. Figure 3 shows a schematic of the operation of the breathalyzer and the wireless capabilities

50. Figure 4 shows an embodiment of a 3-D printed body of breathalyzer device.

51. Figure 5 is a schematic showing the stages of breathalyzer device operation. Through steps 1-7 the breathalyzer performs the breath testing operation.

52. Figure 6 is a Schematic of a 3-D print out of two-channel breathalyzer device, bottom half. The figure shows the channels and this embodiment's specifications. Other embodiments have different specifications and measurements.

53. Figure 7 shows a schematic of the top half of the breathalyzer while the bottom is depicted in figure 6.

54. Figure 8 shows the sensor holder, which is located at the end of each channel depicted in figs. 6 and 7. In certain embodiments the sensor holder contains desiccant or filter; in other embodiments the sensor holder does not contain desiccant or filter.

55. Figure 9 shows a schematic of a single channel breathalyzer top half. Depicted is also the screen which is located on the top half of the breathalyzer device.

56. Figure 10 shows a schematic of the bottom half of the single channel breathalyzer device.

57. Figure 11 shows a schematic of the top and bottom halves of the breathalyzer device, assembled. In certain embodiments, 3-D printed breathalyzer device have two portions, one top and one bottom which complement each other and seal upon assembly. This figure also shows the cover of the battery located on the bottom half.
58. Figure 12 shows the chemical structure of polyaniline.
59. Figure 13 is an example of a sensor, in this embodiment PANI-CSA sensor. Depicted are the contacts pads which connect electrically to the circuit and the interdigitated fingers which are the electrodes which can be either gold or platinum.
60. Figure 14 shows the sensitivities of the PANI-CSA sensor to various gases. The graph demonstrates the high sensitivity of the sensor to ammonia.
61. Figure 15 shows the sensor mount and its specifications. The sensor of the embodiment of Fig. 14 is mounted with the interdigitated finger electrodes. In other embodiments the PANI-CSA can be placed on the mount without the electrodes.
62. Figure 16 shows the resistivity of PANI-CSA sensor to various concentrations of ammonia gas. As is shown, the resistivity changes even below 25ppb of ammonia.
63. Figure 17 shows the corresponding changes of current to the resistivity. The higher the resistivity, the lower the current.
64. Figure 18 shows the resistivity change of the PANI-CSA sensor in the presence of a sieve, which is a type of desiccant. The presence of sieve decreased the resistivity by 50% because the sieve absorbed ammonia. This graph makes the argument that the ammonia sensor, in this embodiment, has better sensitivity as measure of resistivity without the presence of sieve.
65. Figure 19 shows the signal derives from an ammonia sensor in relation to meals. This graph depicts the ammonia contained in the breath of a fasting (nothing to eat or drink except water for 8-12 hours) user, of a user in between meals and of a user 30 minutes after a meal. According to this graph and in this embodiment the fasting breath ammonia resistivity is the highest after 8-12 hours fasting.
66. Figure 20 shows calibration of the SPANI sensor. SPANI sensor is sensitive to CO₂ at concentrations 0.5% to 3%.

67. Figure 21 shows an embodiment of a multianalyte breathalyzer device with a mouthpiece already attached, but removable, a touchscreen display covering the entire front of the device and a power button.
68. Figure 22 shows an embodiment of an interior of a multi-analyte breathalyzer device having 21 sensors. The device can contain more sensors as needed. This version of the device has an opening which serves as mouthpiece and accepts the breath sample from the user who applies the mouth around the opening. The opening contains several desiccant crystals embedded in the wall at the opening but the mouth does not come in contact with the crystals. The interior of the device contains an electronic circuit and a microprocessor connected to each sensor, along with Bluetooth, a USB port, a battery and a small hole for release of breath as it exits the device.
69. Figure 23 shows the exterior of a multi-sensor, multi-analyte device
70. Figure 24 shows the interior of one version of the multi-analyte breathalyzer device which contains seven sensors. As an example, each sensor can detect each of the following gases: 2-propanol, acrylonitrile, carbon disulfide, dimethyl sulfide, ethanol, isoprene, trimethylamine or any combination of Volatile Organic Compounds (VOCs) which indicate the presence of irritable bowel disease (IBD)
71. Figure 25 shows a schematic of the interior of a two-sensor device.
72. Figure 26 shows a schematic of the interior of a seven-sensor device.
73. Figure 27 shows the screen of a multi-sensor, multi-analyte breathalyzer device with a drop-down menu and the various diseases the breathalyzer of this embodiment can test for.
74. Figure 28 shows the high sensitivity of PANI/DNNSA to two concentration of hydrogen gas, 20kppm and 6kppm
75. Figure 29 shows Principal Component Analysis (PCA) of data from breath testing for hydrogen in breath of individuals with suspected Celiac disease. The PCA method demonstrates clustering of the positive for Celiac disease breath tests.
76. Figure 30 shows the high sensitivity of SPANI to CO₂
77. Figure 31 shows capability of a breathalyzer device to analyze data from breath testing using artificial intelligence-machine learning. Machine learning algorithms filed within the

Arduino microprocessor of a breathalyzer device derive the results with better accuracy than PCA or standard statistical analysis.

78. Figure 32 shows capabilities of machine learning algorithms to better analyze the data obtained by using a breathalyzer device for breath testing.

79. Figure 33 shows 100% accuracy of identifying the correct diagnosis among users of breathalyzer device who are considered to have symptoms compatible with a certain disease.

80. Figure 34 shows the location for the intervention (steps 6 and 7) and insertion of learned file with algorithms in microprocessor to perform analysis of data from user of a breathalyzer device of this embodiment. In step 6, the recalled data pass through a mathematical model which is coded in the microcontroller (fitting) and yield the multiple key parameters. The key parameters along with the user's input information (body weight, height, GI related survey, and etc) pass through a trained Machine Learning model or other classification model which was generated by KNN (20) patients' data and implanted in the microcontroller and return the classification result.

81. Figure 35 shows a schematic of a circular tubular channel with multiple sensors on its periphery. The breath is inserted through a sealing opening of the channel where the mouthpiece is securely attached.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

82. The following detailed description is to be read with reference to the drawings, in which like elements in different drawings have like reference numerals. The drawings, which are not necessarily to scale, depict selected embodiments and are not intended to limit the scope of the invention. Skilled artisans will recognize that the examples provided herein have many useful alternatives that fall within the scope of the invention.

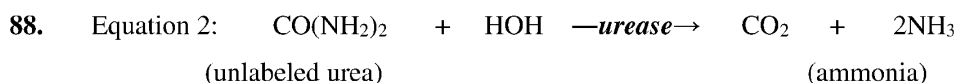
83. In certain embodiments, the present invention provides an improved breath analyzer and breath test method to determine the presence of *H. pylori* in a subject's digestive tract. The improved breath analyzer and breath test is less costly, more convenient, and more diagnostically accurate than existing methods and devices.

84. Exhaled human breath may contain 100 times more CO₂ than inhaled air. Exhaled CO₂ comes from various sources within the human body. One of these sources can be the presence of

H. Pylori in the gastrointestinal tract. Exhaled human breath contains about 3.8% CO₂ in healthy individuals who are not infected with *H. Pylori*. ¹³C is the naturally occurring isotope of elemental carbon. ¹²C is the more stable isotope of carbon-12 and is in CO₂. ¹²C exists in nature in abundance at 98.9% of the amount of element carbon. ¹³C, which is present in ¹³CO₂, is less abundant in nature and consists of only 1.1% of the natural element carbon.

85. *H. Pylori* is a genotypically diverse bacterium that has the capacity to change its genetic makeup and mutate *in vivo* during colonization within a human subject's digestive tract. When *H. Pylori* is positive for the cytotoxin-associated gene A (CagA), the risk for development of stomach cancer increases relative to when *H. Pylori* is negative for CagA. In the western world, *H. Pylori* seropositivity for CagA is approximately 60% as opposed to the Asian countries and most of Africa where the seropositivity for CagA approaches 100% within the *H. Pylori*-affected population. The highly immunogenic CagA protein encoded by the CagA gene elicits serum antibody responses which can be detected by enzyme-linked immunosorbent assay (ELISA).

86. Despite differences in genetic makeup, all types of *H pylori* are able to hydrolyze urea (either ¹³C labeled or unlabeled) using its abundant enzyme urease to produce CO₂ (or ¹³CO₂) and ammonia (NH₃) gas. Once produced, ammonia and carbon dioxide (CO₂ or ¹³CO₂) are diffused in the bloodstream through the gastrointestinal mucosa and exhaled from the lungs through exhaled breath. The hydrolysis of urea, both in labeled and unlabeled form, is shown by Equations 1 and 2 below:



89. Prior urea breath tests require labeled urea substrates to detect the amount of labeled CO₂ in the breath. In contrast, the present breath analyzer and breath test method uses one or more sensors to detect the total amount of both ammonia and carbon dioxide present in a breath sample. Advantageously, the present breath analyzer and urea breath test method do not require a labeled substrate.

90. Referring to the drawings, and in particular, FIGS. 1, 2, and 9, there are shown schematics of a breath analyzer of the present disclosure. In some embodiments, the breath analyzer includes some or all of the components depicted in these figures. In other embodiments, the breath analyzer includes additional components, other than those depicted in these figures.

91. Certain embodiments of the breath analyzer detect presence and concentration of both ammonia and carbon dioxide in a breath sample. As described in greater detail below, the breath analyzer includes an input, one or more gas sensors, an electrical circuit, and a processor. The input receives a breath sample. The at least one gas sensor contacts the breath sample. At least one of the one or more gas sensors includes ammonia selective material (e.g., doped polyaniline) and a conductive material. The electrical circuit operably connects the conductive material to the processor. The processor detects changes in resistivity in the electrical circuit and uses the changes in resistivity to calculate a concentration of ammonia in the breath sample. The same or a different gas sensor includes carbon dioxide selective material (e.g., doped polypyrrole) and a conductive material. The electrical circuit operably connects this conductive material to the processor. The processor detects changes in resistivity in the electrical circuit and uses the changes in resistivity to calculate a total concentration of carbon dioxide in the breath sample.

92. The breath analyzer includes a mouthpiece that has open ends to allow a breath sample to move therethrough. The mouthpiece can comprise any suitable type of material, including, but not limited to, plastic or metal. The mouthpiece includes a first portion and a second portion. The first portion is configured as an input that receives a breath sample. The first portion can be sized and shaped to receive a user's lips, so that a user can blow exhaled breath into the mouthpiece.

93. In some cases, the mouthpiece includes a one-way valve. In such cases, a user blows exhaled breath into the mouthpiece. The exhaled breath moves forward pass the one-way valve and becomes trapped. In other words, the exhaled breath cannot move backward past the one-way valve and toward the first portion.

94. The breath analyzer also includes a main body. The mouthpiece is attached to the main body. In particular, the second portion of the mouthpiece is sized and shaped to removably connect to the main body. For example, the second portion can be snapped onto or perhaps screwed onto the main body. The main body can comprise plastic, metal, or any other suitable

material. In some cases, the main body and the mouthpiece comprise the same material. In other cases, the main body and the mouthpiece comprise different materials.

95. In certain embodiments, the mouthpiece is a single-use mouthpiece. A single-use mouthpiece is desirable because it can be replaced for use with each new user. Also, in some cases, components of the mouthpiece and/or main body in contact with exhaled breath can be made of an inert or non-reactive material (e.g., polytetrafluoroethylene (PTFE)) that does not interfere with ammonia absorption.

96. The mouthpiece can be integral with the main body, or can be a separate structure that is connected to the main body. In instances where the mouthpiece is a separate structure connected to the main body, the mouthpiece can be placed inside the main body through a hole in the main body using, for example, a push-in, screw-in, or tack-in motion.

97. In one embodiment, the mouthpiece is permanently attached to the main body. In such instances, the mouthpiece can be securely mounted on the main body, extending straight out from the main body or at an angle from the main body. These alternate configurations allow the eyes of the subject taking the breath sample to either directly face the main body or to face away from main body while taking the breath sample. The mouthpiece can be permanently mounted to an opening in the main body using a receptacle made of plastic or metal or any other material. In other cases, the mouthpiece can be permanently attached to the main body without the use of a receptacle.

98. FIGS. 10 and 11 illustrate an exemplary embodiment of the breath analyzer. FIG. 10 shows the breath analyzer with the mouthpiece attached to the main body, whereas FIG. 11 shows the breath analyzer with the mouthpiece detached from the main body. The breath analyzer in these embodiments is provided as a self-contained, portable hand-held device. In some cases, the mouthpiece is a single use mouthpiece that is disposed of after use, and that can be replaced with a new mouthpiece for each new user.

99. The mouthpiece can be attached to an exterior of the main body or can extend into the main body of the breath analyzer. The mouthpiece can be attached anywhere on or within the breath analyzer, provided that a first end of the mouthpiece is accessible to lips of the subject undergoing the breath test. The mouthpiece can attach to the breath analyzer via any suitable type of connection, including a straight connection, push-in connection, or screw-in connection, or have

another type of connection within the main body of the breath analyzer. In some cases, the mouthpiece can be glued or can use any other type of adhesive to adhere the mouthpiece to the main body.

100. The mouthpiece can have any desired shape. For example, the mouthpiece can be oblong, cylindrical, cone-shaped, or straw-shaped. The shape of the mouthpiece should be such that the lips of the subject are able to wrap around the mouthpiece in a tight manner. As disclosed above, the mouthpiece can optionally include a self-sealing, one-way valve to seal the breath sample from the surrounding air once the breath sample exits the mouthpiece and enters the main body of the breath analyzer.

101. The mouthpiece can optionally include a lining material positioned inside of the mouthpiece. Where provided, the lining material covers some or all of an interior surface of the mouthpiece. In some cases, the lining material is a desiccant that can trap humidity. The desiccant can comprise (consist of, or consist essentially of) silica, activated charcoal, calcium sulfate, calcium chloride, or any other type of desiccant. The desiccant can also include a combination of any one or more of these or other desiccants. The desiccant can optionally have a color indicator to indicate the amount of humidity that the desiccant has trapped. In other instances, the mouthpiece is devoid of any type of desiccant or other lining material.

102. In another embodiment, the mouthpiece includes a lining material configured to trap and absorb some of the gases contained in a breath sample, while allowing other gases to go through. As an example, the lining material on the mouthpiece can block H₂O, nitric oxide (NO), methane gas (CH₄), nitrogen gas (N₂), and other volatile organic compounds contained in human breath, while allowing CO₂ (or ¹³CO₂) and NH₃ to pass through the lining material and through the mouthpiece.

103. The breath analyzer can optionally include one or more filters (or “traps”). The one or more filters perform a similar function as the lining material for the mouthpiece. That is, the one or more filters will allow certain gases to pass through, while trapping other gases and preventing them from passing through. The material for the one or more filters can comprise sodium hydroxide, silica, activated charcoal, calcium sulfate, calcium chloride, or any other type of desiccant. The filter can also include a humidity sensor, such as a hygrometer. However, in some cases, the breath analyzer does not include any filters.

104. In one embodiment, the filter is a single filter configured to block the passage of certain gases that are present in human breath and that are not intended to be measured by the breath analyzer. As an example, the filter can block humidity, nitric oxide, methane, oxygen gas, and/or other volatile organic compounds present in a breath sample. This single filter allows the passage of ammonia (NH_3) and CO_2 and $^{13}\text{CO}_2$ or only ammonia or only CO_2 or only $^{13}\text{CO}_2$.

105. In another embodiment, the filter comprises multiple filters. The filters can have any desired shape and can comprise various types of materials. The shape of the filters is not limiting, and can be round, oblong, square or a combination of different shapes. Each of these filters can trap one or more of the undesirable gases (i.e., those gases not intended to be measured), and allow CO_2 , $^{13}\text{CO}_2$ and ammonia to pass through, either separately or together.

106. The breath analyzer includes at least one gas sensor, a processor and a power source. The at least one sensor, processor and power source are electrically connected via an electrical circuit.

107. The processor can be any desired processor known in the art. In some cases, the processor is a microcontroller. In certain cases, the processor is an Arduino microcontroller.

108. The at least one sensor can have any of the embodiments already described. In some cases, the at least one sensor includes the sensor shown in the embodiment of FIG.13. The at least one gas sensor is electrically connected to the electrical circuit using any desired connection mechanism. In some cases, the at least one sensor connects to the electrical circuit via an optional sensor mount. In such cases, the at least one sensor can be mounted directly onto the sensor mount. The sensor mount serves as an interface between the sensor and the electrical circuit. Thus, the sensor mount can be any structure known in the art that connects the electrodes of the at least one sensor to the electrical circuit. In some embodiments, the sensor mount is a printed circuit board.

109. In other cases, the at least one sensor is directly connected to the electrical circuit 4. For example, in some embodiments, the electrical circuit includes two metal clips that can be clamped onto the contact pads to create an electrical connection. A user can also replace an old sensor with a new sensor by pulling the old sensor out of the metal clips and inserting a new sensor into the clips.

110. The main body also includes an on/off button and the power source. The power source can be a portable power source, such as a battery. When the on/off button is activated, the power

source turns on. As shown, for example in FIG., the power source supplies voltage to a voltage regulator. In certain cases, the power source supplies volts to the voltage regulator. The voltage regulator regulates the amount of voltage sent to the sensor. In some cases, the voltage regulator supplies a voltage to the at least one sensor in the amount of between 0 volts to 5 volts. In certain cases, the voltage regulator supplies a voltage to the at last one sensor in the amount of about 5 volts. In one embodiment, the voltage regulator is an IC1 7805 voltage regulator, a product manufactured by Fairchild Electronics.

111. A resistor is also electrically connected to the at least one sensor and provides resistance to the at least one sensor. In some case, the resistor is a 10 k Ω resistor. When the breath sample contacts the at least one sensor, a change in resistivity occurs in the sensor that correlates to an amount of ammonia and/or amount of carbon dioxide present in the sample. The at least one sensor outputs voltage (along with the changes in resistivity) to the processor. The processor detects changes in resistivity in the at least one sensor and uses the changes in resistivity to calculate a concentration of ammonia and carbon dioxide in the breath sample. The processor can also compare a concentration of ammonia between two different breath samples. Similarly, the processor can compare a concentration of carbon dioxide between two different breath samples.

112. The gas sensor can comprise a single gas sensor or more than one gas sensor. Each gas sensor is configured to detect and measure one or more of ammonia, CO₂, and ¹³CO₂ in a human breath sample. Thus, at least one gas sensor 50 is sensitive to ammonia (NH₃), at least one gas sensor is sensitive to CO₂, and at least one gas sensor is sensitive to ¹³CO₂. For example, one gas sensor can be sensitive to ammonia, a different gas sensor can be sensitive to CO₂, and yet a different gas sensor can be sensitive to ¹³CO₂. Alternatively, a single gas sensor can be sensitive to any combination of these gases. As an example, one gas sensor can be sensitive to ammonia and to CO₂ and to ¹³CO₂; another gas sensor can be sensitive to ammonia (and not to CO₂ or ¹³CO₂); and another gas sensor can be sensitive to CO₂ and to ¹³CO₂ (and not to ammonia). One or more of gas sensors can measure the humidity in breath to assess its effect on the sensitivity of the gas sensor to the particular gas under detection.

113. The breath analyzer can measure ammonia and CO₂ (including ¹²CO₂, ¹³CO₂, and/or both ¹²CO₂ and ¹³CO₂) either simultaneously or in succession. Where the breath analyzer 10 measures ammonia and CO₂ in succession, the measurements occur within a short period of time (e.g., from

about 1-10 seconds) of each other. This enables the breath analyzer to measure and process the effect of each gas sensor simultaneously or nearly (i.e., substantially) simultaneously.

114. Each gas sensor includes a substrate, an electrically-conductive material, and a gas-selective material. The electrically-conductive material is deposited onto the substrate. The electrically-conductive material can comprise (consist of, or consist essentially of) any desired electrically-conductive material. In some cases, the electrically-conductive material is platinum. In other cases, the electrically-conductive material is gold.

115. In certain embodiments, the electrically-conductive material is an electrode arrangement. The electrode arrangement can be a single electrode or a plurality of electrodes. The electrodes can be spaced apart in any desired arrangement. In some cases, the electrodes are spaced less than about 250 μm apart, perhaps less than about 150 μm apart, such as about 100 μm apart. In certain cases, the electrodes are spaced less than about 10 μm apart, such as 5 μm apart. In some embodiments, such as the gas sensor shown in FIG. 13, the electrodes include interdigitated finger electrodes. In one embodiment, the gas sensor comprises interdigitated platinum finger electrodes with a line spacing of 100 μm apart or less.

116. The one or more gas sensors can operate at room temperature (i.e., 68-77°F, or 20-25°C) or at a higher temperature. The one or more gas sensors can be fabricated by any suitable method. Such methods can include spin coating, drop-coating, sol-gel, or any other known fabrication method. The one or more gas sensors can be fabricated for either single use or for multiple, repeated uses. The one or more gas sensors can comprise polyaniline or polypyrrole doped with a protonic acid. As non-limiting examples, the one or more gas sensors can comprise PANI/CSA (polyaniline/camphorsulfonic acid); PANI/DNNSA (polyaniline/dinonylnaphthalenesulfonic acid); or PPY/DBSA (polypyrrole/dodecylbenzynesulfonic acid). Applicant has found that a PANI/CSA gas sensor can be sensitive to detecting ammonia, and further that PANI/DNNSA and PPY/DBSA can be particularly sensitive to detecting $^{12}\text{CO}_2$ and/or $^{13}\text{CO}_2$.

117. The one or more gas sensors can comprise thin-film or thick-film sensors capable of sensing ammonia gas (NH_3) and/or carbon dioxide gas (CO_2 and/or $^{13}\text{CO}_2$) simultaneously or in succession. Such gas sensor or sensors can comprise polymer-thin film or polymer-thick film or polymer composite film. The one or more gas sensors can be metal oxide sensors such as zinc oxide (ZnO) or other metal oxide sensors. The one or more gas sensors can be a blend of polymer

with metal oxide (such as polyaniline, polypyrrole, or another polymer, in combination with zinc oxide, or another metal oxide). In some cases, the one or more gas sensors comprise sulfonated polyaniline or polyethylenimine (PEI) blended with polyelectrolytes. In other cases, the one or more gas sensors can be emeraldine-base polyaniline (EB-PANI) blended with poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS).

118. In some cases, the ammonia selective material includes doped polyaniline. Polyaniline exhibits three different oxidation states: leucoemeraldine (LEB, fully reduced), emeraldine (EB, half-oxidized), and pernigraniline (PNB, fully oxidized). Also, when polyaniline is in the emeraldine state, it can be in either an emeraldine salt or emeraldine base form. When in the emeraldine salt form, the polyaniline is conducting. The emeraldine salt form is usually obtained by protonating the basic amine and imine sites with strong acids. This process is reversible in that the emeraldine base form is obtained by deprotonating the amine groups. Thus, the emeraldine state of polyaniline transitions between an acid form and base form.

119. The inventor has discovered that the acid-base transition of polyaniline renders it pH sensitive and this characteristic allows it to be effectively used in ammonia detection. When ammonia contacts an emeraldine salt form of polyaniline, the ammonia deprotonates the amine groups and converts it to an emeraldine base, which also causes an increase in resistivity and a corresponding decrease in conductivity.

120. In some case, the one or more gas sensors can include a dopant to increase sensitivity of the gas sensor 50. For example, where polyaniline is used for the at least one gas sensor 50, the polyaniline can be doped with a protonic acid to increase its pH sensitivity. Polyaniline with increased pH sensitivity is desirable for ammonia detection because when ammonia converts the polyaniline to an emeraldine base, it causes an even larger increase in resistivity and corresponding decrease in conductivity. Larger increases in resistivity (and decreases in conductivity) are desirable because they are easier to detect, and thus increase the sensitivity of the polyaniline to ammonia.

121. Polyaniline doped with a protonic acid has increased pH sensitivity compared to undoped polyaniline. In some cases, the polyaniline can be doped with a protonic acid including ions such Cl^- and SO_4^{2-} to obtain pH sensitivity of around 59 mV. Certain protonic acids cause an even larger

increase in pH sensitivity. For example, polyaniline doped with camphor sulfonic acid has been shown to have a pH sensitivity of around 70 mV.

122. In some cases, the polyaniline comprises at least one dopant that increases pH sensitivity of the polyaniline. In some cases, the dopant is a protonic acid. In some embodiments, the dopant is hydrochloric acid. In other embodiments, the dopant is camphor sulfonic acid. In yet other embodiments, the dopant is both hydrochloric acid and camphor sulfonic acid. Other possible dopants include, but are not limited to, sulfuric acid, salicylic acid, acetic acid, citric acid, tartaric acid, oxalic acid, malonic acid, succinic acid, glutamic acid, adipic acid and phthalic acid. Also, in some cases, the polyaniline has a dopant that provides the polyaniline with a pH sensitivity of more than 59 mV. In one embodiment, the polyaniline has a camphor sulfonic acid dopant, which provides the polyaniline with a pH sensitivity of about 70 mV, which is a higher sensitivity observed than when using other dopants.

123. Also, the gas-selective material of the gas sensor can be deposited directly onto the electrically-conductive material using any desired deposition process. For example, the gas-selective material can be deposited onto the electrically-conductive material using a spin coating method, a drop coating method, a chemical vapor deposition method, or a sputtering method. In certain cases, the electrically-conductive material is coated with spun cast gas-selective material. In certain embodiments, the gas-selective material is doped polyaniline deposited directly onto the electrically-conductive material using a spin coating method.

124. As discussed above, the one or more gas sensors can detect and measure one, two, or more than two gases. At least one of the one or more gas sensors detects ammonia gas (NH_3) at very low ppb levels. In some cases, the one or more gas sensors 50 detect ammonia concentration levels in a range from 1 ppb up to 1 ppm. In some cases, the one or more gas sensors 50 detect ammonia gas in a concentration range of 10ppb and higher (e.g., in a range of from about 10 ppb up to about 50 ppb), or in a concentration range of 500 ppb or higher. In still other cases, the one or more gas sensors 50 detect breath ammonia at levels lower than 50 ppb and as high as 500 ppm.

125. As noted above, at least one of the one or more gas sensors detects CO_2 . The CO_2 detected can be $^{12}\text{CO}_2$, $^{13}\text{CO}_2$, or the ratio of $^{13}\text{CO}_2$: $^{12}\text{CO}_2$. In some cases, at least one of the gas sensors 50 detects CO_2 (or $^{13}\text{CO}_2$) at concentrations in a range of 10ppm-1500ppm and higher (Figure 6). In some cases, the at least one gas sensor detects CO_2 at levels as low as 1mM/min or 50-100ppm. In

addition, in some cases, the at least one gas sensor detects $^{13}\text{CO}_2$ at levels as low as low as 1mM/min or 50-100ppm.

126. The one or more gas sensors can comprise thin film polyaniline (PANI) doped with hydrochloric acid (HCl). Such a sensor can be prepared by chemical oxidative polymerization of aniline in aqueous acidic medium (1M HCl) with ammonium persulfate (APS) as an oxidant. The PANI sensor can be doped with acid dopants other than HCl. In some cases, the one or more gas sensors can comprise thin film polyaniline doped with camphorsulfonic acid (PANI-CSA) and further doped with HCl or another acid dopant.

127. In some cases, the one or more gas sensors can comprise thick film polymer pyrrole. Such sensors can be polymerized in the presence of an oxidant such as FeCl_3 or any other acid. Polymerized pyrrole (polypyrrole) can detect and measure CO_2 and $^{13}\text{CO}_2$ at concentration levels of 10 ppm and above.

128. In certain instances, the one or more than one gas sensors can comprise a fiber optic sensor capable of sensing ammonia, CO_2 and/or $^{13}\text{CO}_2$. It is envisioned that other gas sensors 50, other than those specifically mentioned herein, can be used as the one or more gas sensors 50 to detect and measure ammonia, CO_2 , and $^{13}\text{CO}_2$.

129. One non-limiting method for fabricating a PANI-CSA thin-film sensor is the following: The films can be prepared by spin coating the PANI-CSA solution on interdigitated array (IDA) electrodes. Prior to spin coating, the electrodes can be cleaned by rinsing in methanol followed by rinsing with deionized water and drying in a stream of dry nitrogen. PANI films can then be spun cast onto the IDA electrodes by adding 100 μl solutions at 500 RPM. The electrode pads can be cleaned, e.g., with a Q-tip dipped in methanol, to facilitate direct electrical contact with the breath analyzer.

130. The breath analyzer further includes contact pads and an electronic circuit 60. The one or more gas sensors are connected to the electronic circuit via the contact pads. The electronic circuit is electrically connected to the one or more gas sensors. The electronic circuit can function as shown in Figure 5, or can function as shown in Figure 23. The electronic circuit can register multiple successive values for each measured gas detected by the one or more gas sensors. In some cases, the electronic circuit will register the highest detected value or the average value for each gas detected by the one or more gas sensors. Through the use of software, the electronic circuit

will convert the measured resistivity of the gases into concentrations (ppb for ammonia and ppm for $^{13}\text{CO}_2$ and CO_2).

131. In some embodiments, the breathalyzer includes a display. The display is electrically connected to the processor. The display is configured to visually display the amount of ammonia, CO_2 , and $^{13}\text{CO}_2$ gas detected by the one or more gas sensors. The display can be a window display provided on the main body of the breath analyzer. In an alternative embodiment, the results can be displayed, with the use of Bluetooth technology or any other wireless data transmitter, through a computer portal or other device that can be either stationary or portable.

132. The display can display a single result that represents the additive result of all three gases (ammonia, $^{13}\text{CO}_2$ and CO_2) or can display one result for each respective gas (i.e., one output for ammonia, one output for CO_2 , and one output for $^{13}\text{CO}_2$). In some embodiments, the results are displayed in numerical values (e.g., from 1-1000, where a value of 1 represents no *H. Pylori* infection, and a value of 1000 represents a subject infected with *H. Pylori*) and/or in actual units of measured gases (e.g., ammonia concentration in ppb; and CO_2 and/or $^{13}\text{CO}_2$ concentration in ppm). In other embodiments, the results can be displayed as a yes or no indication, and/or in color using a light source. For example, green light on the display 30 can represent a negative test result and red light on the display can represent a positive test result. Any one or more of these results (e.g., numerical values, actual units of measured gases, color, yes/no indication) can be displayed on the display.

133. In some embodiments, the main body is configured as including a first compartment and a second compartment. In some cases, the first compartment is a chamber that houses the at least one gas sensor and a sensor mount, and the second compartment is an electrical housing that houses various components. In certain embodiments (not shown), a separate chamber can be provided for each of the at least one gas sensor.

134. In some cases, the chamber includes a lid or door that opens and shuts. When the door is closed, the chamber provides a closed, sealed environment around the at least one sensor. When the door is open, the at least one gas sensor is accessible through the door opening. A user can open the chamber door to remove and replace the at least one sensor as needed. The chamber also includes an outlet. The outlet includes a cap that can be opened to release a breath sample from the chamber and closed to trap a breath sample within the chamber.

135. The mouthpiece is connected to the chamber such that exhaled breath passes from the mouthpiece directly into the chamber. As discussed above, the breath analyzer 10 can include a filter. In some cases, the filter is a desiccant assembly. The desiccant assembly helps to remove excess moisture from the exhaled breath. In some embodiments, the desiccant assembly is provided inside of the mouthpiece. In other embodiments, the desiccant assembly is provided inside of the chamber. Exhaled breath first moves through the desiccant assembly before coming into contact with the at least one gas sensor.

136. In some cases, the desiccant assembly is provided as a tube upon which exhaled air flows through. The tube can have an interior filled with a plurality of desiccant beads. The desiccant beads can also be arranged such that a plurality of channels are created for exhaled air to flow through. In this embodiment, the tube can have an interior filled with a plurality of desiccant beads 64 arranged such that a single channel is created.

137. As discussed above, the breath analyzer can include one or more optional gas filters 13. The gas filter(s) help to remove a selected gas (e.g., nitrogen and/or hydrogen) from the exhaled breath. In some embodiments, the gas filter is provided inside of the mouthpiece. In other embodiments, the gas filter is provided inside of the chamber. Exhaled breath first moves through the gas filter before coming into contact with the sensor.

138. The main body can also include a second compartment configured as an electrical housing that houses various components. In the illustrated embodiment, the electrical housing houses the processor, parts of the electrical circuit, a power source, a display, and a wireless connector.

139. During use, a user turns the breath analyzer on by activating the on/off switch. The on/off switch can be located anywhere about an exterior surface of the main body. This on/off switch 26 in turn prompts the power source to supply voltage to the voltage regulator. The voltage regulator regulates and supplies voltage to the at least one gas sensor. The resistor also supplies resistance to the at least one gas sensor.

140. A user then blows a breath sample into the first portion of the mouthpiece. The breath sample moves past the one-way valve and through the optional desiccant/gas filter. The breath sample then moves out of the optional desiccant/gas filter and into the chamber where it contacts the at least one gas sensor. The breath sample causes a change in resistivity to occur in the at least one gas sensor. This change in resistivity is outputted to the processor.

141. The present disclosure also provides a urea breath test (“UBT”) method that is an improvement of existing urea breath test methods. This novel UBT uses the breath analyzer described above, which is simple to operate, non-invasive, inexpensive and essentially risk-free. The breath analyzer and related breath test method are available at the point-of-care for self-testing, and the results of the test become available instantaneously.

142. To undergo this novel UBT, all subjects who wish to be tested for the presence of *H. Pylori* infection will need to abstain from taking antibiotics, bismuth, sucralfate, and proton pump inhibitors for two weeks prior to using the breath analyzer and breath test method.

143. On the day of the breath test method, the subject will follow the following protocol:

144. In one embodiment the subject will abstain from food and drink intake for one hour prior to breathing into the breath analyzer. At the end of the one hour abstinence, the subject will exhale into the mouthpiece for the first time by wrapping his or her lips around the mouthpiece and exhaling breath air into the breath analyzer through the mouthpiece with a prolonged exhalation of approximately 5 seconds of time or over a range of 3-8 seconds of time depending on the age, height and weight of the subject. This provides the baseline exhalation.

145. After the first exhalation, the subject will ingest urea in quantity calculated as about 5mg/kg of the subject’s body weight or in standard dose of 300mg or in another standard dose of 125mg or 250mg or in another single dose or multiple doses depending on age and weight and general clinical status of the subject. The urea to be ingested can be in two forms. It can be either labeled urea ($^{13}\text{CO}(\text{NH}_2)_2$) or unlabeled urea ($\text{CO}(\text{NH}_2)_2$). Any dose of urea can be taken in a form of a capsule, which can be swallowed with a small amount of water, or can be contained in a small meal, which is either solid (e.g. pudding) or liquid (e.g. citric juice) and that is ingested orally.

146. In a span of ten to thirty minutes after ingestion of the urea (capsule or urea-containing meal), the subject will exhale for a second time into the device through the mouthpiece. The post-urea exhalation through the mouthpiece can be anywhere in a range of from 10 minutes after ingestion up to 30 minutes after ingestion of the urea. For example, the post-urea exhalation can be at 10 minutes after ingestion, at 15 minutes after ingestion, at 20 minutes after ingestion, or at 30 minutes after ingestion of the urea (labeled or unlabeled). In this embodiment, the second exhalation cannot be earlier than 10 minutes after ingestion of the urea (whether in capsule form

or as a urea-containing meal), and cannot be later than 100 minutes after ingestion of the urea (whether in capsule form or as a urea-containing meal). Most commonly, the second exhalation will take place at 15-30 minutes after ingestion of the urea or urea containing meal.

147. In an alternative embodiment, after the baseline exhalation, the subject will ingest a high-protein meal (e.g. hamburger, or high protein bar or equivalent). In a span of 20 to 120 minutes after ingestion of the high-protein meal, the subject will exhale into the mouthpiece 12 for the second time. The second exhalation can be at 20 minutes after ingestion, 30 minutes after ingestion, 50 minutes after ingestion, 80 minutes after ingestion, and 120 minutes after ingestion of the high-protein meal. Most commonly, the second exhalation will take place at 60 minutes after ingestion of the high-protein meal.

148. The exhaled breaths (baseline and post-urea) enter the main body of the breath analyzer through filter (if present), or directly through the mouthpiece where there is no filter. Each breath exits filter (or mouthpiece) and enters the one or more gas sensors. As the breath samples (baseline exhalation and post-urea exhalation) enter the one or more gas sensors of the breath analyzer, the resistivity of the one or more gas sensors changes in relation to the amount of ammonia and/or CO₂ and/or ¹³CO₂ in the breath under examination.

149. Where the at least one gas sensor is a single gas sensor, the sensor can detect three gases; ammonia, CO₂, and ¹³CO₂. Where the at least one gas sensor comprises two gas sensors, one of the gas sensors can detect ammonia, and the other of the gas sensors can detect CO₂ and/or ¹³CO₂. Where the at least one gas sensor comprises three sensors, one gas sensor can detect ammonia, one gas sensor can detect CO₂, and another gas sensor can detect ¹³CO₂. Where the at least one gas sensor comprises four gas sensors, one gas sensor can detect ammonia, a different gas sensor can detect CO₂, and yet another gas sensor can detect ¹³CO₂. Where the at least one gas sensor comprises four gas sensors, one gas sensor can detect ammonia, another gas sensor can detect CO₂, yet another gas sensor can detect ¹³CO₂, and still yet another sensor can detect humidity.

150. The change in resistivity due to the presence of ammonia in the breath sample is converted to electrical current and then to parts per billion (ppb) of ammonia. Similarly, the change in resistivity due to the presence of CO₂ or ¹³CO₂ is converted to electrical current and then to parts per million (ppm). The conversion of delta (post-urea minus baseline) resistivity (ΔR) to ppb and

to ppm and any measurement of combinations of the gases will be done with the use of software developed exclusively for the function of the breath analyzer and the electronic circuit.

151. In one embodiment, display will display a numerical value of the concentration of ammonia in ppb and the numerical value of CO₂ (and/or ¹³CO₂ and/or ¹³CO₂:CO₂) in ppm. In this embodiment, these numeral values corresponding to the gas concentrations will display through a window on the main body of the breath analyzer.

152. In another embodiment, the numerical value on display 30 will be a single value derived from calculation pertaining to the amount of ammonia plus the amount of CO₂ or ¹³CO₂ or ¹³CO₂:CO₂ in the breath of the subject at baseline exhalation and at post-urea exhalation. This calculation can be based on equation for linear predictor of outcome. As an example, the equation can have the form:

153. $(\log_{10} \text{ammonia} \times \text{constant for ammonia}) + (\text{CO}_2 \times \text{constant for CO}_2)$ or

154. $(\log_{10} \text{ammonia} \times \text{constant for ammonia}) + (^{13}\text{CO}_2 \times \text{constant for } ^{13}\text{CO}_2)$ or

155. $(\log_{10} \text{ammonia} \times \text{constant for ammonia}) + (\text{CO}_2 \times \text{constant for CO}_2) + (^{13}\text{CO}_2 \times \text{constant for } ^{13}\text{CO}_2)$ or

156. $(\log_{10} \text{ammonia} \times \text{constant for ammonia}) + (^{13}\text{CO}_2:\text{CO}_2 \times \text{constant for } ^{13}\text{CO}_2:\text{CO}_2)$ or

157. another form of polynomial equation where ammonia is in ppb and ¹³CO₂ and CO₂ are in ppm and ¹³CO₂:CO₂ is in numerical value.

158. The constant is derived from analysis (e.g. regression) of multiple values of ammonia, CO₂, and ¹³CO₂ within the various populations at risk for infection with *H. Pylori* and populations in general and within the various dietary habits of such populations and populations in general. The equation will be incorporated into software.

159. The equation for linear predictor of outcome can have the basic form:

160. $f(i) = \beta_0 + \beta_{1x_{i1}} + \dots + \beta_{px_{ip}}$ for data point i.

161. Below a certain number derived from this equation the subject will be found to be negative for the presence of infection with *H. Pylori*. Above a certain number derived from this equation, the subject will be found to be positive for the presence of active infection with *H. Pylori*. A small

number of inconclusive results will be referred for a repeat of the test and if the results remain inconclusive, the subject will be referred for further evaluation through additional means.

162. As discussed above, CO₂ (or ¹³CO₂) and ammonia, which are produced through hydrolysis of urea (unlabeled or labeled) by the *H. Pylori*-produced enzyme urease are diffused in the bloodstream through the mucosa and exhaled from the lungs through the exhaled breath. Consumption of urea, either labeled or unlabeled, by an infected subject undergoing testing using this invention will produce elevated amounts of CO₂ (or ¹³CO₂) and ammonia in the subject's breath.

163. As also discussed above, the subject undergoing testing using this method will exhale into the breath analyzer twice. The first (baseline) exhalation being after one hour abstinence of food and water, and the second (post-urea) exhalation being after the consumption of urea (labeled or unlabeled) or a high-protein meal or high-protein bar. At all times during the method, the breath analyzer 10 will detect and measure the amount of ammonia, CO₂ and/or ¹³CO₂.

164. When ammonia, CO₂ and/or ¹³CO₂ levels at post-urea exhalation are higher than at baseline, at a level greater than 100% above baseline levels, the subject being tested can be diagnosed as being infected with *H. Pylori*. In addition, when the combined ammonia and CO₂ (and/or ¹³CO₂) levels at post-urea exhalation are at a level greater than 50% above the baseline levels, the subject can be diagnosed as being infected with *H. Pylori*.

165. In diagnosing infection with *H. Pylori*, the sensitivity and specificity of the presently disclosed method is higher than with prior methods. This is because the presently disclosed method detects the combined amount of ammonia and CO₂ (and/or ¹³CO₂), rather than detecting ammonia independently of CO₂ (and/or ¹³CO₂), or CO₂ (and/or ¹³CO₂) independently of ammonia. Because the present method measures all products of urea hydrolysis (including ammonia, ¹²CO₂, and ¹³CO₂), the method of the present disclosure provides the most accurate method for detecting and diagnosing *H. Pylori* infection. Simultaneous measurement of CO₂ (or ¹³CO₂) and ammonia in breath significantly minimizes statistical and other errors which can occur when measuring either CO₂ (or ¹³CO₂) without ammonia, or ammonia without CO₂ (or ¹³CO₂).

166. The combined increase of ammonia and CO₂ (and or ¹³CO₂) in subjects infected with *H. Pylori* subjects is multiplicative (or exponential), not additive. Thus, even small differences

between the baseline exhalation and the post-urea exhalation would be diagnostic of *H. Pylori* infection hence significantly decreasing false negative results.

167. The combined increase of ammonia and CO₂ (and or ¹³CO₂) in infected subjects will be more specific and sensitive for *H. Pylori* infection than existing breath test methods because the combination of ammonia and CO₂ (and or ¹³CO₂) is indicative of, and diagnostic, only for *H. Pylori* infection (and no other infection) in individuals without end stage liver disease, encephalopathy, end stage renal disease, and metabolic disease (e.g. Krebs's cycle defect, urea cycle defect).

168. The following non-limiting examples are intended to show how the breath test method and breath analyzer of the present disclosure can be used to detect *H. Pylori* infection in humans, adults and children.

169. EXAMPLE 1 - Simultaneous calculation of the rise of ammonia and of CO₂ in breath after ingestion of unlabeled urea (CO(NH₂)₂) as diagnostic of *H. Pylori* infection.

170. The subject abstains from antibiotics, bismuth, proton pump inhibitors, and sucralfate for two weeks. At the end of the two week period, the subject abstains from food and drink for one hour. At the end of the one hour fast, the subject exhales into the breath analyzer through the mouthpiece. The exhalation can last from about 2-10 seconds. In another embodiment, the exhalation continues until a characteristic sound or light coming from the main body indicates that a sufficient amount of exhaled breath has entered the main body. By sufficient, it is meant that there is enough of the breath sample to come into contact with the one or more gas sensors.

171. When the baseline exhalation enters the main body, the breath sample passes through filter (when it is in place), where gases like NO, CH₄, N₂, volatile organic compounds, O₂, and humidity are blocked from entering the at least one gas sensor. Ammonia and CO₂ are not blocked by the filter and are allowed to contact the one or more gas sensors.

172. When the breath sample passes to the one or more gas sensors, the resistivity of the one or more gas sensors changes according to the amount of ammonia and CO₂ present in the exhaled breath. The resistivity of the at least one gas sensor is converted to ppb of ammonia and ppm of CO₂ with the use of the electronic circuit and software. The numerical value of ammonia and CO₂ obtained during the first exhalation can be stored in memory.

173. At the end of the baseline exhalation, the subject ingests a known quantity (e.g., 125 mg, 250mg, or 300mg, depending on the weight and age of the subject) of unlabeled urea either in tablet or in a small meal or drink. In a span of 10 to 120 minutes post ingestion of unlabeled urea, the subject exhales into the mouthpiece for the second time for the post-urea exhalation, following the same procedure as with the first exhalation. The post-urea exhalation takes place most commonly at 20 minutes post ingestion of unlabeled urea. The post-urea exhalation will not take place earlier than 10 minutes post ingestion, or later than 120 minutes post-urea ingestion of unlabeled urea. The values of ammonia in ppb and of CO₂ in ppm of the second exhalation are also stored in memory of the breath analyzer device 10.

174. The breath analyzer will calculate the final values of ammonia and CO₂ as follows:

175. Final ammonia= post-urea ammonia minus baseline ammonia

176. Final CO₂= post-urea CO₂ minus baseline CO₂.

177. Software can be used to convert the resistivity values to final ammonia concentration in ppb and final CO₂ concentration in ppm. In subjects who test positive for *H. Pylori* infection, the range of values for baseline ammonia would be 20ppb-200ppb and the range for post-urea ammonia would be 80ppb to 600ppb. Because of the overlap of the baseline and post-urea ammonia values, the actual percent change between baseline and post-urea ammonia will be the marker for the infection with *H. Pylori*. When the percent change between baseline and post-urea ammonia is greater than 200%, the subject is considered positive for *H. Pylori* infection. When the percent change between baseline and post-urea ammonia is lower than 200%, the subject is negative for *H. Pylori* infection.

178. In subjects who are positive for *H. Pylori*, the baseline CO₂ concentration is typically below 200 ppm, and the post-urea CO₂ concentration is typically above 200ppm (e.g., ranging from 200-1000 ppm). When the calculated percent change between baseline and post-urea CO₂ is greater than 1000%, the subject is considered positive for *H. Pylori* infection.

179. In one embodiment, the positive test result will be the value of percent change of ammonia above 200%, and the value of the percent change of CO₂ above 1000%, in a numerical value, in ppb and in ppm respectively, as determined using device specific software.

180. In another embodiment, the final result, which the device's software will convert into values recognizable by computer program, will be calculated using the equation which takes into account the percent or actual rise of ammonia and the percent or actual rise of CO₂ between baseline and post-urea breath samples. The equation can be a logarithmic or statistical equation with the values of two variables (ammonia and CO₂) corresponding to the subject being tested, the two constants, and one or more coefficients. The constants and the coefficients are calculated on the basis of demographics and characteristics (e.g. age, gender, race, height and weight) of subjects infected with *H. Pylori*.

181. In another embodiment, the positive *H. Pylori* result will be a color (e.g. red), and the negative *H. Pylori* result will be a color (e.g. green). In another embodiment, the positive result for *H. Pylori* infection will be a plus sign (+) and the negative for *H. Pylori* will be a minus sign (-).

182. The final result can be displayed through a window of the main body of the breath analyzer. The final result (whether indicating positive or negative, yes/no, different colored lights, or numerical values of ammonia and of CO₂) will be displayed through the window for the main body. The breath analyzer will include instructions for the subject undergoing testing so as to allow the subject to interpret the displayed results and provide the subject with recommendations for potential further examination by physician or equivalent personnel and potential treatment options.

183. EXAMPLE 2 - Simultaneous Calculation of the rise of ammonia and of ¹³CO₂ in breath after ingestion of ¹³C labeled urea as markers for the presence of *H. Pylori* infection.

184. In this example, the subject abstains from antibiotics, bismuth, proton pump inhibitors, and sucralfate for two weeks. At the end of the two-week period, the subject abstains from food and drink for one hour. At the end of the one-hour abstinence, the subject exhales into the mouthpiece. The exhalation lasts 2-10 seconds or until a characteristic sound or light coming from the main body of the breath analyzer indicates that a sufficient amount of exhaled breath has entered the main body through the mouthpiece. By sufficient, it is meant that there is enough of the breath sample to come into contact with the one or more gas sensors.

185. At the end of exhalation and when the sufficient amount of exhaled breath enters from the mouthpiece into the main body, it passes first through filter (when present) where gases such as NO, CH₄, N₂, volatile organic compounds, O₂, and humidity are blocked from entering the one or

more gas sensors. The filter does not block ammonia or $^{13}\text{CO}_2$, which gases are allowed to pass through and contact the one or more gas sensors.

186. In another embodiment, the exhaled breath enters into the main body from the mouthpiece directly to the one or more gas sensors. The resistivity of the one or more gas sensors changes according to the amount of ammonia and $^{13}\text{CO}_2$ present in the exhaled breath that comes into contact with the one or more gas sensors. The change in resistivity of the one or more gas sensors is converted to ppb of ammonia and to ppm of $^{13}\text{CO}_2$ through the use of the electronic circuit and software. The numerical value of ammonia and $^{13}\text{CO}_2$ obtained during the first exhalation are stored in the memory of the electronic circuit.

187. At the end of the baseline exhalation, the subject ingests a known quantity (e.g., 125mg or 250mg or 300mg depending on the weight and the age of the subject) of ^{13}C labeled urea, either in tablet form or in the form of a small meal or drink. In a span of 10 to 120 minutes post-ingestion of urea, the subject exhales into the mouthpiece for the post-urea exhalation. following the same procedure as with the baseline exhalation. The post-urea exhalation takes place most commonly at 20 minutes post-ingestion of labeled urea. The post-urea exhalation will not take place earlier than 10 minutes post ingestion or later than 120 minutes post ingestion of labeled urea. The concentration values of ammonia in ppb and of $^{13}\text{CO}_2$ in ppm are stored in the memory of the device.

188. The device will calculate the final values of ammonia and $^{13}\text{CO}_2$ as follows:

189. Final ammonia= post-urea ammonia minus baseline ammonia

190. Final $^{13}\text{CO}_2$ = post-urea $^{13}\text{CO}_2$

191. The designed software will convert the values of final ammonia to ppb and final $^{13}\text{CO}_2$ to ppm. In subjects who test positive for *H. Pylori* infection, the range of values for baseline ammonia is 20ppb-200ppb, and the range for post-urea ammonia is 80ppb to 600ppb. Because of the overlap of the baseline and post-urea ammonia values, the percent change between pre-urea and post-urea ammonia will be the marker for the infection with *H. Pylori*. When the percent change between pre-urea and post-urea ammonia is greater than 200%, the subject is considered positive for *H. Pylori* infection. When the percent change between baseline and post-urea ammonia is lower than 200%, the subject is negative for *H. Pylori* infection.

192. In subjects who are positive for *H. Pylori* infection, the baseline $^{13}\text{CO}_2$ concentration is typically below 200 ppm, and the post-urea $^{13}\text{CO}_2$ concentration is typically above 200ppm (and can range, e.g., from 200 ppm up to about 1000 ppm).

193. In one embodiment, the positive test result will be the value of percent change of ammonia above 200% plus the value of the percent change of $^{13}\text{CO}_2$ above 1000% as a numerical value determined using device specific software.

194. In another embodiment, the final result, which the device's software will convert into values recognizable by computer program, will be calculated using an equation which takes into account the percent rise of ammonia and of $^{13}\text{CO}_2$ between baseline and post-urea breath samples. The equation can be a logarithmic or statistical equation with the values of two variables (ammonia and $^{13}\text{CO}_2$) corresponding to the subject being tested and two constants and one or more coefficients.

195. In another embodiment, the positive *H. Pylori* result will be a color (e.g. red), and the negative *H. Pylori* result will be a color (e.g. green). In yet another embodiment, a positive *H. Pylori* result will be the plus sign (+) and a negative result for *H. Pylori* will be a minus sign (-).

196. The final result can be displayed through a window on the main body of the device. The final result (e.g., indicating positive or negative, yes/no, different colored lights, or numerical values of ammonia and of $^{13}\text{CO}_2$) will be displayed through the window on the main body. The device will include instructions to allow the subject undergoing testing to interpret the displayed results and provide the subject with recommendations for potential further examination by physician or equivalent personnel and potential treatment options.

197. EXAMPLE 3 - Calculation of the rise of the ratio $^{13}\text{CO}_2/^{12}\text{CO}_2$, as delta over baseline (DOB), in human breath at baseline and post-urea (^{13}C labeled Urea) and calculation of host-dependent urea hydrolysis rate as markers for *H. Pylori* infection.

198. In this example, the subject abstains from antibiotics, bismuth, proton pump inhibitors, and sucralfate for two weeks. At the end of the two week period, the subject abstains from food and drink for one hour. At the end of the one hour abstinence, the subject exhales into the mouthpiece. The baseline exhalation takes place over 2-10 seconds (or longer), or until a characteristic sound or light coming from the main body of the device indicates that a sufficient

amount of exhaled breath has entered the main body through the mouthpiece. By sufficient, it is meant that there is enough of the breath sample to come into contact with the one or more gas sensors. In another embodiment, the subject exhales into the mouthpiece for a short period of time, which can be shorter than 5 seconds and it can be a short burst of exhaled air which enters the device through the mouthpiece. In this embodiment, the short burst of exhaled air would be a sufficient amount of breath to enter the device through the mouthpiece. There could also be multiple (e.g., more than one) short bursts of exhaled air that will enter the breath analyzer through the mouthpiece.

199. When the sufficient amount of exhaled breath enters the main body of the device, and passes through the filter, undesirable gases (e.g., NH_3 , NO , CH_4 , N_2 , volatile organic compounds, and/or O_2) and humidity are trapped and do not pass through the filter. The filter does not block CO_2 gas, which gas then comes into contact with the one or more gas sensors.

200. In another embodiment, the device does not contain filter, and the sufficient amount of exhaled breath over 2-10 seconds (or bursts of exhaled breath) enters the device 10 through the mouthpiece and reaches the one or more gas sensors directly (i.e., without passing through a filter). In such instances, none of the gases contained in the exhaled breath are blocked when passing from the mouthpiece to the one or more gas sensors 50.

201. The resistivity of the at least one sensor changes according to the amount of CO_2 present in the exhaled breath which comes into contact with the at least one sensor. The change in resistivity is converted to ppm of CO_2 with the use of the electronic circuit 60 and software. The numerical value in ppm of CO_2 obtained during the first exhalation is stored in the memory of the device with the use of software.

202. In another embodiment, the resistivity of the sensor is not converted to ppm. It remains as a measurement of current that is generated by change in resistivity and is stored in the memory of the device after each exhalation (baseline and post-urea).

203. At the end of the baseline exhalation, the subject ingests a known quantity (e.g., 125mg or 250mg or 300mg) of ^{13}C labeled urea either in capsule or tablet form, or in the form of a small meal or drink. In a span of 10 to 120 minutes post ingestion of ^{13}C labeled urea, the subject exhales into the mouthpiece for a second time, following the same procedure as with the baseline exhalation. The post-urea exhalation takes place most commonly at 20 minutes post ingestion of

^{13}C labeled urea. The post-urea exhalation will not take place earlier than 10 minutes post ingestion or later than 120 minutes post ingestion of ^{13}C labeled urea. After the post-urea exhalation, the values of CO_2 in ppm and of $^{13}\text{CO}_2$ in ppm are stored in the memory of the device.

204. The device will then calculate the final values of CO_2 and $^{13}\text{CO}_2$ as follows:

205. Final CO_2 = post-urea CO_2 minus baseline CO_2

206. Final $^{13}\text{CO}_2$ = post-urea $^{13}\text{CO}_2$ minus baseline $^{13}\text{CO}_2$.

207. In one embodiment the positive test result will be the value of percent change between $^{13}\text{CO}_2/\text{CO}_2$ of the post-urea exhaled breath over the $^{13}\text{CO}_2/\text{CO}_2$ of the baseline exhaled breath sample of greater than a certain number which will be called the cut-off point. Above the cut-off point, the result will be positive for the presence of *H. Pylori* infection. Below the cut-off point, the result will be negative for *H. Pylori* infection. The cut-off point will be calculated during the clinical examination of patients with *H. Pylori* infection undergoing the present breath test. The highest level of $^{13}\text{CO}_2/\text{CO}_2$ demonstrated by the subjects (in clinical trials) who test negative for *H. Pylori* infection will be the cut-off point. Levels above this cut-off point will indicate positive for *H. Pylori* infection.

208. In another embodiment, the final result will be calculated using an equation which takes into account the percent rise of CO_2 between baseline and post-urea breath samples. The equation can be a logarithmic or statistical equation with the values of one variable (CO_2) corresponding to the metabolic rate of the subject being tested, one or more constants, and one or more coefficients. This equation will be used to discern the range of values above which the results will be positive for *H. Pylori* infection and below which the results will be negative for *H. Pylori* infection.

209. In another embodiment, the positive *H. Pylori* result will be a color (e.g. red), and the negative *H. Pylori* result will be a color (e.g. green). In another embodiment, the positive *H. Pylori* result will be a plus sign (+) and a negative *H. Pylori* result will be the minus sign (-).

210. The final result will be displayed through a window on the main body 14 of the device 10. The final result (e.g., indicating positive or negative, yes/no, different colored lights, or numerical values of the DOB (Delta over Baseline) for $^{13}\text{CO}_2/\text{CO}_2$) will be displayed through the window on the main body of the device. The device will include instructions to allow the subject undergoing

testing to interpret the displayed results and provide the subject with recommendations for potential further examination by physician or equivalent personnel and potential treatment options.

211. Calculation of host-dependent urea hydrolysis rate (UHR):

212. $\text{UHR} = \text{CO}_2 \text{ produced} \times \text{delta over baseline} \times 0.3463$, where delta over baseline is defined as the difference between baseline CO_2 (or $^{13}\text{CO}_2$) and post-urea CO_2 (or $^{13}\text{CO}_2$).

213. In certain embodiments, the present disclosure provides a multi-sensor breath analyzer (breathalyzer) device which analyzes the breath of an individual for the presence of gases including, but not limited to, acetaldehyde, 2-propanol, acetonitrile, acrylonitrile, benzene, isoprene, pentane, methylexane, ethane, hydrogen sulfide, triethyl amine, trimethyl amine, carbon disulfide, dimethyl sulfide, 1-Heptene, 1-Octene, 1-Nonene, 1-Decene, methane, ethanol, ammonia (NH_3), nitric oxide (NO), nitrogen (N_2), hydrogen (H_2), Oxygen (O_2), carbon dioxide (CO_2 or $^{13}\text{CO}_2$), carbon monoxide (CO), 2,2,4,6,6-pentamethylheptane, 3,6-dimethyldecane, dodecane, 2,3,4-trimethylhexane, 2,6,8-trimethyldecane, tridecane, undecane, tetradecane, as well as other gases.

214. In one embodiment, as shown, for example, a breathalyzer device is provided that comprises a main body that can be made of a durable and lightweight material. The main body can include a display (e.g., a touch screen display). The display can extend over (and cover) some or all of one side of the body (e.g., with unbreakable glass). The display can allow for input by the user and for output by the device after the device has finished analyzing the breath of the user.

215. The main body of breathalyzer (figure 21) can contain multiple sensors each of which is connected independently to an electronic circuit and microprocessor. The electronic circuit which is connected to each sensor through mechanical connection converts the sensor's resistivity and current to voltage. The microprocessor, which can be Arduino or another type of microprocessor constructed for use by this breathalyzer, analyzes the signal from the sensor and converts it to voltage. Through software, the voltage is converted to units of measurement for each gas to which each sensor is sensitive.

216. In one embodiment, the sensors of breathalyzer are electrochemical sensors. Electrochemical sensors have a conductive portion and a substrate portion. The conductive portion can include, but is not limited to, gold, platinum, palladium or a mixture of gold and platinum. The

substrate portion can include, but is not limited to, doped polymers such as doped polyaniline, doped polypyrrole and others. Polyaniline, when doped with protonic acids such as dinonaphthalenesulfonic acid (DNNSA), Camphorsulfonic acid (CSA), hydrochloric acid (HCL), sulfosalicylic acid (SSA) and 4-dodecylbenzenesulfonic acid (DBSA), is rendered highly sensitive to gases such as ammonia, hydrogen, nitrogen, methane and hydrogen sulfate. Polypyrrole doped with FeCl_3 , or with Aminobenzenesulfonic acid (ABSA) or with salicylic acid or another protonic acid is rendered sensitive to CO_2 and to $^{13}\text{CO}_2$. Electrochemical sensors such as polymer-based sensors (e.g., doped polyaniline and polypyrrole) are more effective and desirable for the breathalyzer because they are stable and operate at room temperature (about 70 degrees Fahrenheit), the temperature at which the present breath analyzer would optimally operate.

217. In another embodiment the sensors of breathalyzer are metal oxide nanosensors, such as ZnO , PbO -doped SnO_2 , which are sensitive to hydrogen, methanol, propanol and acetone. Other metal oxides can be used as the sensor material, either independently or in combination with other conductive material placed on metal finger electrodes (e.g., platinum or gold or both) or on floating gate field effect transistors (FGFET) or on carbon nanotubes or on nanowires.

218. In another embodiment, the sensors of the breathalyzer are polymers (e.g., polyaniline and/or polypyrrole) on conductive materials which are chemical sensitive field effect transistors or floating gate field effect transistors (FGFET) or any other field effect transistors (FETs).

219. In one embodiment the breathalyzer comprises a removable mouthpiece or an opening on one side of the breathalyzer 100 which is constructed in such manner for the mouth of the user to be wrapped around it. In one embodiment in which the opening of the device serves as mouthpiece, desiccant can be embedded into the wall of the device itself. In another embodiment in which there is a removable mouthpiece, desiccant can be embedded in the wall of the mouthpiece. The desiccant absorbs humidity at the desired level for the operation of the sensors. In addition or alternatively, a filter can be placed in front of some (or all) sensors in order to block gas potentially interfering with a particular sensor which is tasked to detect another gas. Such filter can be in the form of crystal or like silica crystal.

220. The display of breathalyzer, which can be a touch screen display, and which can occupy the entire side of the body of the breathalyzer much like the display of a smart phone, can include a drop down menu which provides the user with several options for testing. Such options can

include, but are not limited to, the following medical diagnoses: Celiac Disease, NCGS, SIBO, IBS, diabetes, asthma, COPD, Hyperammonemia, kidney disease, liver disease, lung disease, lactose intolerance, fructose intolerance and others. The user touches one or more than one medical diagnoses and the breathalyzer, through its software, asks for input of demographics and characteristics or symptoms compatible with the disease desired to be investigated. After this process is completed to the satisfaction of the preprogrammed algorithm, the user is prompted to exhale into the breathalyzer. The breathalyzer performs the predetermined task of analyzing the breath sample and provides the result with general recommendations.

221. In one embodiment, the invention provides a breathalyzer device which contains and utilizes 21 or more sensors each of which is sensitive to one gas. These gases can include acetaldehyde, 2-propanol, acetonitrile, acrylonitrile, benzene, isoprene, pentane, methylexane, ethane, hydrogen sulfide, triethyl amine, trimethyl amine, carbon disulfide, dimethyl sulfide, 1-Heptene, 1-Octene, 1-Nonene, 1-Decene, methane, ethanol, ammonia (NH₃), nitric oxide (NO), nitrogen (N₂), hydrogen (H₂), Oxygen (O₂), carbon dioxide (CO₂ or ¹³CO₂), carbon monoxide (CO), 2,2,4,6,6-pentamethylheptane, 3,6-dimethyldecane, dodecane, 2,3,4-trimethylhexane, 2,6,8-trimethyldecane, tridecane, undecane, tetradecane and H₂O. Under this embodiment, the breathalyzer 100 can be used for screening and monitoring of celiac disease, non-celiac gluten sensitivity (NCGS), IBD (ulcerative colitis and Crohn's disease), IBS, SIBO, lactose intolerance, fructose intolerance, asthma, COPD, liver disease (steatohepatitis, end stage), H. Pylori infection, kidney failure and metabolic disease (diabetes, genetic).

222. In another embodiment, the invention provides a breathalyzer which contains 21 or more sensors and electronically utilizes, on command, all or fewer of the sensors each of which is sensitive to one gas. These gases include but are not limited to acetaldehyde, 2-propanol, acetonitrile, acrylonitrile, benzene, isoprene, pentane, methylexane, ethane, hydrogen sulfide, triethyl amine, trimethyl amine, carbon disulfide, dimethyl sulfide, 1-Heptene, 1-Octene, 1-Nonene, 1-Decene, methane, ethanol, ammonia (NH₃), nitric oxide (NO), nitrogen (N₂), hydrogen (H₂), Oxygen (O₂), carbon dioxide (CO₂ or ¹³CO₂), carbon monoxide (CO), 2,2,4,6,6-pentamethylheptane, 3,6-dimethyldecane, dodecane, 2,3,4-trimethylhexane, 2,6,8-trimethyldecane, tridecane, undecane, tetradecane.

223. In another embodiment, the invention provides a breathalyzer which contains 21 or more sensors and electronically utilizes, on command, 17 or fewer of the sensors which are sensitive to one gas each. These gases can include, but are not limited to, 2-propanol, acetonitrile, acrylonitrile, benzene, isoprene, pentane, methylethane, ethane, hydrogen sulfide, triethyl amine, trimethyl amine, carbon disulfide, dimethyl sulfide, 1-Heptene, 1-Octene, 1-Nonene, 1-Decene.

224. In another embodiment, the invention provides a breathalyzer which contains 21 or more sensors and electronically utilizes, on command, 7 or fewer of the sensors which detect one gas each. These gases can include 2-propanol, acrylonitrile, carbon disulfide, dimethylsulfide, ethanol, isoprene, trimethylamine.

225. In another embodiment, the invention provides a breathalyzer which contains 21 or more sensors and electronically utilizes, on command two or fewer sensors which detect one gas each. Under this embodiment one gas or two gases are detected. These gases include but are not limited to hydrogen (H₂), nitric oxide (NO), ammonia, acetone, CO₂ or ¹³CO₂. Under this embodiment, the diseases for which to screen and monitor include but are not limited to celiac disease, NCGS, IBS, diabetes, H. Pylori infection, asthma, and kidney failure.

226. In another embodiment the invention provides a breathalyzer device which contains one or two or more than two sensors each of which is sensitive to either one gas or more than one gas.

227. In yet another embodiment, the invention provides a breathalyzer device which contains seven or more than seven sensors each of which is sensitive to one gas or more than one gas.

228. In one embodiment the mouthpiece of the breathalyzer is an opening on one of the side of the breathalyzer device. In another embodiment, the mouthpiece is a separate cylindrical piece which can be removably attached to the device.

229. In one embodiment, the breathalyzer device contains desiccant (e.g., in the form of crystals) to remove a predetermined amount of humidity from the breath sample and/or a filter to block certain gas or gases from coming into contact with the operating sensors. The crystals are placed either in the interior of the device in the vicinity of the sensor(s) or are placed on or within the wall of the mouthpiece or under the mouthpiece or in the interior of the device.

230. The following non-limiting examples illustrate certain methods that can be used with breathalyzer.

231. EXAMPLE 4

232. The user wishes to examine whether he/she has celiac disease by using the present breathalyzer, and can do so using the following procedure:

233. 1. The user presses the power button on the front of the device and the device responds by giving a greeting on the screen and asks the user for information (e.g., name, height, weight, age, gender) and guides him/her to tap the drop-down menu.

234. 2. The user selects (e.g., taps) the drop down menu and finds the celiac/SIBO icon.

235. 3. The user selects (e.g., taps) the celiac/SIBO icon and then the celiac icon.

236. 4. The device responds with a process which the user follows in order to prepare for the test.

237. A) The preparation which the device gives out states:

238. B) Abstain from high amount of carbohydrate for 48 hrs. and take nothing by mouth for 8hrs., overnight, before exhaling once into the device while fasting; and brush your teeth but don't use mouthwash.

239. 5. When the user is ready, he/she turns the device on, selects (e.g., taps) the drop-down menu and selects (e.g., taps) celiac disease.

240. 6. The device asks if the user is ready.

241. 7. The user selects (e.g., taps) yes (or no if not ready).

242. 8. The device asks the user to exhale through the mouthpiece for about 5 seconds and wait.

243. 9. The user exhales for 5 seconds.

244. 10. The device which is programmed through software to test for celiac disease by utilizing the hydrogen sensor blocks all other sensors and operates as a single sensor device.

245. 11. The device returns a quantitative result to the user for the amount of hydrogen in the user's breath sample and offers an assessment of whether the value is within normal limits. The device prompts the user to discuss the finding with his/her healthcare provider.

246. EXAMPLE 5:

247. The user wishes to have a full profile of his/her metabolomics, and can do so by performing the following steps. In this case, the breathalyzer device utilizes all sensors simultaneously:

- 248.** 1. The user turns the device on by pressing on the power button.
- 249.** 2. The device gives the greeting and asks the user to select (e.g., tap) the drop-down menu.
- 250.** 3. From the menu, the user selects (e.g., taps) “total profile.”
- 251.** 4. The device responds with the following process for the user to follow:
 - 252.** A) Asks for information and whether the user has used antibiotics or other types of medications.
 - 253.** B) Depending on the responses, the device will either ask the user to wait for two weeks or would recommend overnight fasting (8hrs.) and return for the test in the morning.
- 254.** 5. When the user is ready in the morning, he/she has to brush teeth but not use mouthwash.
- 255.** 6. The user turns on the device.
- 256.** 7. The user selects (e.g., taps) the menu.
- 257.** 8. The user selects (e.g., taps) “total profile”
- 258.** 9. The device asks the user to exhale through the mouthpiece for 5 seconds and then wait.
- 259.** 10. The user exhales for 5 seconds.
- 260.** 11. The device utilizes all sensors simultaneously and responds with readout for all detected gases and offers an assessment of whether the values are within normal limits. The device prompts the user to discuss the findings with his/her healthcare provider.

261. EXAMPLE 6:

- 262.** The user wishes to examine whether he/she has symptoms compatible with inflammatory bowel disease (IBD), and can do so by performing the following steps:
- 263.** 1. The user turns the breathalyzer device on by pressing on the power button.
 - 264.** 2. The device gives a greeting and asks the user to select (e.g., tap) the drop-down menu.
 - 265.** 3. From the menu the user selects IBD.

- 266.** 4. The device responds with the following process:
- 267.** A) Asks for information and whether the user has used antibiotics or any other medications which treat IBD;
- 268.** B) Depending on the answer, the device responds with the process.
- 269.** 5. The user returns after overnight fast (8hrs.) in the morning after brushing teeth but not using mouthwash.
- 270.** 6. The user turns the device on.
- 271.** 7. The user selects (e.g., taps) the menu.
- 272.** 8. The user selects (e.g., taps) IBD.
- 273.** 9. The device asks if the user is ready.
- 274.** 10. The user responds yes.
- 275.** 11. The device asks the user to exhale for 5 seconds and wait.
- 276.** 12. The user exhales for 5 seconds.
- 277.** 13. The device blocks all sensors except those which are programmed by the software to operate under the IBD request.
- 278.** 14. The device responds with the measurement of each of these gases and an assessment of whether they are within normal limits. The device prompts the user to discuss the findings with his/her healthcare provider.
- 279.** EXAMPLE 7:
- 280.** The user suffers from asthma with an inflammatory component and is required to monitor the efficacy of the treatment and wishes to prevent flare-ups of the disease. In this case, the user seeks to measure nitric oxide in his/her breath, and can do so by performing the following steps:
- 281.** 1. The user turns on the breathalyzer device 100 by pressing on the power button.
- 282.** 2. The device provides a greeting and asks the user to select (e.g., tap) the drop-down menu icon.
- 283.** 3. On the menu, the user selects (e.g., taps) asthma/Nitric Oxide.

- 284.** 4. The device returns with instructions as to how to proceed with the breath test, in this case to breathe for about 5-10 seconds into the device through its mouth piece or opening and asks the user if he or she is ready.
- 285.** 5. The user selects (e.g., taps) yes.
- 286.** 6. The device asks for input of data such as name, age, gender, height and weight.
- 287.** 7. After the input, the device blocks all sensors except for the one which detects nitric oxide.
- 288.** 8. The device measures the nitric oxide in the user's breath sample.
- 289.** 9. The device stores the result and compares it to previous results from the same user.
- 290.** 10. The device outputs the result on the screen and gives the trendline from the previous results from tests of the past 1-365 days and provides an assessment indicating whether the results are within normal limits or not.
- 291.** 11. The device prompts the user to discuss the findings with his/her health care provider.
- 292.** While some preferred embodiments of the invention have been described, it should be understood that various changes, adaptations and modifications may be made therein without departing from the spirit of the invention and the scope of the appended claims.

CLAIMS

What is claimed:

1. An autonomous, standalone, hand-held and battery-operated breath analyzer device, which accepts exhaled breath through a replaceable mouth piece and comprises:
 - a. one or more than one sensor; wherein the sensor contacts the exhaled breath and wherein the sensor comprises a chemical material and a conductive material; wherein the chemical material is a polymer that has a resistivity that increases in response to the increase of gas in the exhaled breath sample; wherein the chemical material contacts the conductive material
 - b. a microprocessor; wherein recalled data pass through a mathematical model which is coded in the microcontroller (fitting) and yield multiple key parameters; wherein the key parameters along with the user's input information pass through a trained Machine Learning model or other classification model which is generated patients' data and implanted in the microcontroller and return the classification result;
 - c. an electrical circuit; wherein the electrical circuit operably connects the sensor to the microprocessor; wherein the microprocessor detects resistivity in the electrical circuit and uses the resistivity to calculate concentration of gas in the exhaled breath sample;
2. The breathalyzer of claim 1 wherein the sensor has a resistivity that increases with the increase of hydrogen in the breath sample;
3. The breathalyzer of claim 1 wherein the sensor has a resistivity that increases with the increase of ammonia in the breath sample;
4. The breathalyzer of claim 1 wherein the sensor has a resistivity that increases with the increase of CO₂ and ¹³CO₂ in the breath sample;
5. The breathalyzer of claim 1 wherein the sensor has a resistivity that increases with the increase of methane in the breath sample;
6. The breathalyzer of claim 1 wherein the sensor has a resistivity that increases with the increase of ethanol in the breath sample;
7. The breathalyzer of claim 1 wherein the sensor has a resistivity that increases with the increase of acetone in the breath sample;
8. The breathalyzer of claim 1 wherein the replaceable mouthpiece is securely attached to the body of the breathalyzer;

9. The breathalyzer of claim 1 wherein the sensors have a resistivity that increases with the increase of acetaldehyde, 2-propanol, acetonitrile, acrylonitrile, benzene, isoprene, pentane, methylethane, ethane, hydrogen sulfide, triethyl amine, trimethylamine, carbon disulfide, dimethyl sulfide, 1-Heptene, 1-Octene, 1-Nonene, 1-Decene, octane, nonene, dodecane, cyclohexane, 2-butane, indole, ester, carbon disulfide, pentane, nitric oxide (NO), ethane and propane in the breath sample
10. The breathalyzer of claim 1 wherein the polymer is doped polyaniline;
11. The breathalyzer of claim 1 wherein the polymer is doped polypyrrole;
12. The breathalyzer of claim 1 wherein the polymer is sulfonated polyaniline;
13. The breathalyzer of claim 1 wherein the conductive material comprises a plurality of electrodes;
14. The breathalyzer of claim 13 wherein the plurality of electrodes is finger electrodes separated by distance between them
15. The breathalyzer of claim 1, wherein the trained file catalogs the exhaled breath that contacts the resistivity of the sensor that contacts the exhaled breath;
16. The breathalyzer of claim 15, wherein the microprocessor and the integrated circuit predict the presence of disease in the person whose exhaled breath contacts the sensor.