



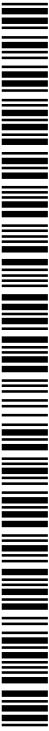
- (51) **International Patent Classification:**  
G01N 33/497 (2006.01) G01N 21/39 (2006.01)
- (21) **International Application Number:**  
PCT/US2013/074629
- (22) **International Filing Date:**  
12 December 2013 (12.12.2013)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**  
61/736,239 12 December 2012 (12.12.2012) US
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- (81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

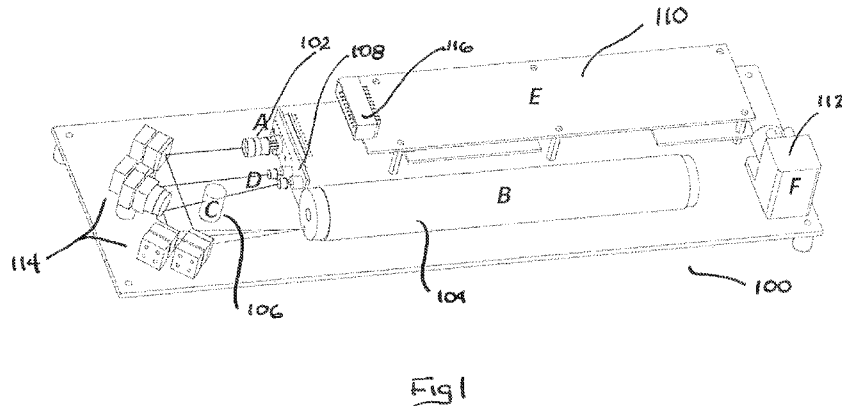
**Published:**

— with international search report (Art. 21(3))



WO 2014/093604 A1

(54) **Title:** DETERMINATION OF LOCATION OF BACTERIAL LOAD IN THE LUNGS



(57) **Abstract:** The present invention is direct to methods of determining the location of a bacterial load in the lungs of a subject.

DETERMINATION OF LOCATION OF BACTERIAL LOAD IN THE LUNGS

CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of U.S. Provisional Application No. 61/736,239, filed December 12, 2012, the entirety of which is incorporated by reference herein.

TECHNICAL FIELD

**[0002]** The invention is directed to methods of detecting the presence and location of bacterial load in the lungs of a subject.

BACKGROUND

**[0003]** Bacteria are naturally present in the lungs and if the bacterial load remains low, these bacteria will not adversely affect normal respiratory function. The presence of bacteria is called colonization, rather than infection. When the bacterial load increases in the upper airway, it may still be colonization and is generally not life threatening, but increased colonization may precede infection so measures may be started to decrease colonization before severe infection occurs. Increased colonization can be treated using non-aggressive methods, for example, by increasing airway clearance or by administering oral or inhaled broad-spectrum antibiotics.

**[0004]** Deeper into the lower airways of lung, bacteria are less common. An increase in bacterial load in the lower airways is often associated with infection - with the bacteria being more invasive. Increased bacterial load in the lower airways ("lower respiratory tract") can be life-threatening, resulting in infections such as pneumonia. Increased bacterial load in the lower airways requires more aggressive treatment, for example, broad spectrum intravenous antibiotics.

**[0005]** A rapid test that can determine whether an increased bacterial load is in the upper or lower respiratory tract would be helpful in determining an appropriate treatment.

SUMMARY

**[0006]** The present invention is directed to methods for determining the presence or absence and location of a bacterial load in the respiratory system of a subject comprising: administering to the subject, an effective amount of a <sup>13</sup>C-isotopically-labeled compound that produces <sup>13</sup>CO<sub>2</sub> upon bacterial metabolism; collecting a plurality of samples of exhaled breath from the subject; at least one of said samples comprising breath from the upper respiratory tract of the subject; and at least one of said samples comprising breath from the lower respiratory tract of the subject; conducting at least some of the samples to a sample chamber of a detection

apparatus; evaluating the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  present in each of the at least some samples; and relating the isotopic ratios thus ascertained to the location in the respiratory system from which said samples conducted to the sample chamber were collected.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0007]** Figure 1 is a plan view of an exemplary laser absorbance device for use in accordance with some embodiments of this invention.

**[0008]** Figure 2 illustrates a preferred jump scanning regime.

#### DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

**[0009]** Methods of determining whether a subject has a bacterial lung infection have been previously described. These methods include, for example, administering to the subject a  $^{13}\text{C}$ -isotopically-labeled compound that produces  $^{13}\text{CO}_2$  upon bacterial metabolism. Samples of exhaled breath are then collected and analyzed to determine the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  present in the samples. An increase in the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in the exhaled breath samples, as compared to a control sample, is indicative of a bacterial lung infection. *See, e.g.*, U.S. Provisional Application No. 61/715,992 and U.S. 7,771,857.

**[0010]** Those prior methods, however, did not provide any information about the location of the infection in the lung. Those methods also could not differentiate between colonization and infection. The present invention is directed to methods for determining the location of increased bacterial load in the lung. Any bacteria that can convert the  $^{13}\text{C}$ -isotopically-labeled compounds of the invention into  $^{13}\text{CO}_2$  can be detected using the methods of the invention. Examples of such bacteria include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Acenitobacter baumannii*, *Klebsiella pneumonia*, *Francisella tularensis*, *Proteus mirabilis*, and *Aspergillus species*.

**[0011]** An entire exhaled breath sample from a subject will include air from both the upper and lower airways. Air from the lower airways has a higher concentration of carbon dioxide than air from the upper airways. It is generally understood that air from the lower airways of a healthy adult has a pressure of  $\text{CO}_2$  of about 40 mm Hg. John F. Murray, *The Normal Lung*, 2d ed. W.B. Saunders Company, Philadelphia 1986, page 184. Air from the upper airways generally has a  $\text{CO}_2$  pressure of less than 1 mm Hg. *Id.* As such, one skilled in the art can determine whether an exhaled breath sample came from the upper or lower airways by determining the  $\text{CO}_2$  concentration. Samples having a higher  $\text{CO}_2$  concentration come from lower airways, whereas samples having lower  $\text{CO}_2$  concentration come from upper airways.

[0012] “Capnography” is known in the art as the monitoring of the concentration or partial pressure of carbon dioxide in respiratory gases. Apparatuses and methods for performing that monitoring are known by those of skill in the art. *See, e.g.*, U.S. 3,830,630; U.S. 7,122,154; and Schubert J.K., et al. CO<sub>2</sub>-controlled sampling of alveolar gas in mechanically ventilated patients. *J. Appl. Physiol.* (1985). 2001 Feb;90(2):486-92.

[0013] Active pressure sensing can also be used to determine from where in the lung an exhaled breath originated. *See, e.g.*, WO 2008/060165; U.S. 7,547,285. Alternatively, passive pressure sensing can be used to channel and isolate samples from the upper and lower respiratory tract. *See, e.g.*, Bio-VOC™ Breath Sampler (Markes International Limited, United Kingdom); U.S. 3,734,692; WO 1994/018885; WO 2003/049595; and WO 2004/032727.

[0014] Determination of the origin of an exhaled breath sample can also be achieved by measuring the temperature of the breath sample. *See, e.g.*, U.S. 4,248,245. Alternatively, exhaled breath is monitored using transthoracic impedance methods, which are known in the art.

[0015] It is also understood in the art that in an entire exhaled breath, samples exhaled first will be from the upper airways, while samples exhaled later in time will be from the lower airways. As a result, the skilled person can correlate the location of the exhaled breath sample to the point in time during the entire exhalation at which the sample was collected.

[0016] The methods of the invention include administering to the subject, an effective amount of a <sup>13</sup>C-isotopically-labeled compound that produces <sup>13</sup>CO<sub>2</sub> upon bacterial metabolism. Exemplary examples of such compounds include isotopically labeled urea, isotopically labeled glycine, isotopically labeled citrulline, or a mixture thereof. Administration of the <sup>13</sup>C-isotopically-labeled compound can be achieved by any known means. Preferred methods of administration include inhalation and ingestion. Administration *via* injection, *i.e.*, intramuscular, subcutaneous, peritoneal, and intradermal injection, is also within the scope of the invention.

[0017] In some embodiments of the invention, the <sup>13</sup>C-isotopically-labeled compound is administered to a specific area of the respiratory tract. For example, in certain embodiments, the <sup>13</sup>C-isotopically-labeled compound is delivered to the lower regions of the lungs, *i.e.*, alveolar regions. In some embodiments, the <sup>13</sup>C-isotopically-labeled compound is delivered to the upper regions of the lungs. In other embodiments, the <sup>13</sup>C-isotopically-labeled compound is delivered to the bronchial areas of the lungs. In yet other embodiments, the <sup>13</sup>C-isotopically-labeled compound is delivered to peripheral areas of the lungs. Methods and devices for targeting delivery of compounds to specific areas of the respiratory tract are known in the art. *See, e.g.*, U.S. 8,534,277.

[0018] Within the scope of the invention, one or more exhaled breath samples from the subject can be collected before administration of the  $^{13}\text{C}$ -isotopically-labeled compound. Such samples can be used as control samples in the methods of the invention. Alternatively, the control samples can include the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  present in exhaled breath of a population that has not been administered the  $^{13}\text{C}$ -isotopically-labeled compound.

[0019] Following a suitable time period after administration of the  $^{13}\text{C}$ -isotopically-labeled compound, a plurality of samples of exhaled breath are collected from the subject. A "suitable time period" refers to the length of time required for the compound to be converted to carbon dioxide by a bacteria. Preferably, the samples are collected after no more than 40-70 minutes following administration.

[0020] In some embodiments, the time required by the subject to complete a substantially complete exhalation can be evaluated. The subject's breathing patterns can also be evaluated. Those evaluations can be used to, for example, determine preselected time periods during exhalation for sampling.

[0021] Samples can be collected in any vessel suitable for containing samples of exhaled breath, for example, a bag or vial. Samples may also be directly exhaled into the device by using a suitable mouthpiece. Samples can also be directed exhaled into the sample chamber of a detection apparatus device by being collected using a nasal cannula from a suitable port on other respiratory equipment, for example, a ventilator.

[0022] At least one of the exhaled breath samples will be from the upper respiratory tract and at least one of the exhaled breath samples will be from the lower respiratory tract of the subject. The skilled person can identify the origin of the exhaled breath sample by determining the relative carbon dioxide concentration of the sample. A higher carbon dioxide concentration is indicative of the sample originating from the lower airways. A lower carbon dioxide concentration is indicative of the sample originating from the upper airways.

[0023] Alternatively, the skilled person can correlate the origin of the exhaled breath sample to the point in time of sample collection. A sample collected at or near the beginning of the entire exhalation will have originated from the upper airways. A sample collected at or near the end of the entire exhalation will have originated from the lower airways.

[0024] The origin of the exhaled breath can also be determined using any of the methods known in the art, such as, for example, capnography, active pressure sensing, passive pressure sensing, temperature sensing, and transthoracic impedance.

[0025] The samples are analyzed to determine the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in the samples. Preferably, at least a majority of the exhaled breaths, and most preferably every

exhaled breath, is sampled for a given time period or until the determination of the level of activity has reached a preset accuracy. By correlating the isotopic ratio of the sample to the sample origin, the skilled person can determine whether there is an increase in bacterial load and whether that increase is in the upper or lower airways.

**[0026]** The sample is conducted to a sample chamber of a detection apparatus. A laser light source of the detection apparatus is actuated to emit one or more of the wavelength pairs 2054.37 and 2052.42; 2054.96 and 2051.67; or 2760.53 and 2760.08 nanometers. The laser light thus actuated is directed through the sample in the sample chamber to impinge upon a detector for such wavelengths. The isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  present in the sample can then be ascertained.

**[0027]** A graph or curve may be generated showing the ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in the breath of the tested subject as a function of time. A curve showing an increase in the ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  over time is evidence of the existence of a bacterial infection.

**[0028]** The concentrations or amounts (ratio) of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  is compared to a standard concentration (ratio) of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in a healthy subject and a curve is conveniently generated. From the curve, the presence or absence of increased bacterial load may be determined or diagnosed directly. Other methods for comparing the output ratio to ratios expected from healthy subjects may also be employed.

**[0029]** In exemplary embodiments, a curve may be fitted to these measured concentrations and is then analyzed, preferably by determining the rate of rise of the curve. Such an analysis (rising rate) indicates the level of activity of bacterial load in the subject, which can be used to diagnose the presence and extent of bacterial load in the subject. This same approach may be used, with modification, to determine the effectiveness of therapy and the prognosis for inhibition and/or a cure of infection or colonization.

**[0030]** Within the scope of the invention are methods of detecting the presence or absence of a bacterial load in a subject by comparing the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in the exhaled breath samples obtained after administration of the  $^{13}\text{C}$ -isotopically labeled compound to the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in an exhaled breath sample obtained from the subject prior to the administration of the  $^{13}\text{C}$ -isotopically labeled compound.

**[0031]** Within the scope of the invention, an increase in the ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in the exhaled breath samples obtained after inhalation of the  $^{13}\text{C}$ -isotopically labeled compound to the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in the exhaled breath sample obtained from the subject prior to the inhalation of the  $^{13}\text{C}$ -isotopically labeled compound indicates the presence a bacterial load. If that sample originated from the upper airways, colonization is likely present in the upper

airways. If that sample originated from the lower airways, infection is likely present in the lower airways.

**[0032]** Once it is determined whether the increased bacterial load is in the upper or lower airways, appropriate therapies can be initiated. For example, if the increased bacterial load is in the upper airways, increased airway clearance in the respiratory tract of the subject can be initiated. Oral or inhaled antibiotics, or other ther suitable therapeutic agents , can also be administered.

**[0033]** If the increased bacterial load is in the lower airways, more aggressive treatment can be considered. Such treatments may include, for example, administering therapeutic agents. Such agents include, for example, antibiotics such as broad spectrum, intravenous antibiotics.

**[0034]** Detection apparatuses useful in the present invention will include a sample chamber, into which breath samples can be conducted. These devices will also include a laser light source actuated to emit one or more of the wavelength pairs 2054.37 and 2052.42; 2054.96 and 2051.67; or 2760.53 and 2760.08 nanometers. These devices will also include a detector for detection of one or more of the wavelength pairs.

**[0035]** The detection apparatuses useful in the present invention can include small, extremely low power, near infrared diode lasers to attain field portable, battery operated  $\delta^{13}\text{CO}_2$  measurement instruments with high degrees of accuracy and sensitivity. These devices and the methodologies which employ them may be used to determine  $\delta^{13}\text{CO}_2$  in exhaled breath samples of subjects having, or suspected of having, a bacterial colonization or infection.

**[0036]** Preferred detection apparatuses will analyze carbon isotope ratios in exhaled carbon dioxide samples without being adversely affected by temperature changes. The accuracy and precision of measuring carbon dioxide isotope ratios can be affected by changes in the ground state population of carbon dioxide. The origins of the isotopic differences in samples may be diverse and are not the subject of the present invention. Rather, it is recognized that ascertaining the value of the isotopic ratio is inherently important and commercially useful.

**[0037]** Optical absorption spectroscopy is based on the well-known Beer-Lambert Law. Gas concentrations are determined by measuring the change in the laser beam intensity,  $I_0$ , due to optical absorption of the beam by a sample of the gas. If a sample cell is used for the analysis, such that the path length of the beam and inherent characteristics of the measuring device are constant, absorbance measurements allow calculation of the gas number density,  $n$ , or gas concentration.

**[0038]** Gas phase diode laser absorption measurements interrogate individual absorption lines of gas molecules. These absorption lines correspond to the transition of the gas

molecule, e.g. carbon dioxide, from a ground energy state to a higher excited energy state by absorption of a photon of light. The lines are typically quite narrow at reduced sample gas pressure thereby permitting selective detection of a gas in the presence of other background gases such as water vapor. The isotopes of CO<sub>2</sub> have distinct absorption lines that occur at shifted wavelengths with respect to each other due to the mass difference between <sup>12</sup>C and <sup>13</sup>C.

**[0039]** Absorbance measurements are affected by the gas temperature and the magnitude of this temperature sensitivity varies depending on absorption line selection and the total ground state energy of the optical transition. A collection of molecules at room temperature is distributed over many discrete molecular energy states that vary in total energy according to how fast the molecules rotate and vibrate. That is, the ground state molecular population is distributed about discrete rotational and vibrational energy states according to a Boltzmann distribution.

**[0040]** A temperature dependence of  $\Delta\delta^{13}\text{CO}_2$  can affect the long term stability and sensitivity of diode laser based isotopic measurements of carbon dioxide. [references 2 - 6 ] <sup>13</sup>CO<sub>2</sub> and <sup>12</sup>CO<sub>2</sub> absorption lines with near equal ground state energies can be useful in attaining relative temperature insensitivity for isotopic ratio measurements.

**[0041]** Vertical cavity surface emitting lasers (VCSELs) have been shown to attain scan ranges of 10 to 15 cm<sup>-1</sup>. These have been used to give rise to rugged, high precision field instruments as exemplified by a laser hygrometer manufactured by Southwest Sciences, Inc and a handheld methane leak detector manufactured by the Southern Cross Company. Accordingly, for certain apparatuses for use in the invention, VCSELs can be used that may be scanned over the desired spectral wavelengths, at a useful scan rate in the context of an overall testing apparatus as to give rise to some or all of the desired benefits of the present invention. In some embodiments, the VCSEL devices are caused to scan in the kilohertz scan rate or greater over approximately 10 cm<sup>-1</sup> ranges.

**[0042]** Suitable laser sources may also be formed from a plurality, usually a pair of laser emitters. Such emitters may be fabricated to emit at one of the preferred wavelengths of a wavelength pair. VCSEL devices useful in the invention may be ordered from Vertilas GmbH of Germany and can also be made by other sources of laser emitters.

**[0043]** Pairs of <sup>13</sup>CO<sub>2</sub> and <sup>12</sup>CO<sub>2</sub> spectral lines have been identified, each pair of which has near zero ground state energy difference, a line separation less than 12 cm<sup>-1</sup>, and is substantially free of water interference. It is now been discovered that these pairs of lines are highly useful in the ascertainment of <sup>13</sup>CO<sub>2</sub> / <sup>12</sup>CO<sub>2</sub> isotopic ratios in gas samples. The temperature dependence of measurement using these pairs is desirably low.

**[0044]** The spectral line pairs as follows are highly useful in making carbon dioxide isotopic absorption measurements using VCSELs in gas cells in analyzing exhaled breath samples:

<sup>12</sup> CO <sub>2</sub> wavelength ( nm )	<sup>13</sup> CO <sub>2</sub> wavelength ( nm )
2054.37	2052.42
2054.96	2051.67
2760.53	2760.08

**[0045]** It will be appreciated that the wavelengths identified in the foregoing line pairs are nominal and that some variation from the listed values may be useful. In this regard, it will be understood that useful wavelengths will be those which are sufficiently close to the recited values as to provide one or more of the benefits of the present invention. Thus, such wavelengths will confer either improved accuracy, improved temperature stability or another of the desirable properties set forth herein to the measurement of CO<sub>2</sub> isotopic ratios. In general, preferred wavelengths will be within 0.5 of a nanometer of the recited values.

**[0046]** In addition to the laser light source operating at the desired wavelengths, the apparatuses useful with the present invention include a sample container for holding the gas sample, which container is configured to provide a relatively long light path through the sample by way of mirrors. One or more signal detectors are included as is control circuitry for controlling the laser and for collecting and manipulating the output signal from the detector or detectors. Other equipment to facilitate sample collection, sample preparation, data interpretation and display and other things may also be included in systems and kits provided by this invention. All such components are preferably sufficiently rugged as to permit the deployment of the devices outside of a laboratory and even in a hand held context.

**[0047]** The present apparatuses are also useful in a system or kit. Components of the system or kit may include sample collection containers, such as gas tight bags, preferably ones featuring injection ports, syringes, and other items which facilitate sample collection and transfer to the sample chamber of the apparatus. Such sample collection elements may assume different configurations depending upon the source of the gas to be sampled. Thus, the same may, for example, be useful for collecting breath of a subject, such as when sampling headspace gases from the stomach of a subject.

[0048] Portable devices and systems are known having a general arrangement of elements suitable for use in some of the embodiments of the present invention. For example, the '96 Hawk hand-held methane leak detector system sold by Southern Cross Corp. provides sample container, mirror assemblies, power supply, sample handling and other components which may be adapted for use in the invention. Such systems, however, are not otherwise amenable for such use. Thus, the provision of diode laser sources which are capable of scanning the requisite spectral line pairs with effective frequency, stability and accuracy must be accomplished. Likewise, detectors for sensing optical absorption in the selected line pairs with needed accuracy as well as data collection, storage, manipulation and display or reporting devices and/or software is needed.

[0049] Figure 1 depicts certain aspects of one device that can be used with the presenting invention. A CO<sub>2</sub> optical absorption measurement device is depicted 100, which comprises a diode laser source, mirrors 114, and gas sample chamber 104. Taken together, these form an optical path in conjunction with preferred reflective surfaces inside the sample chamber, not shown. The optical path, which is effectively many times longer than the physical length of the chamber, permits the enhanced absorption of laser light by gas samples in the chamber. One or more gas pumps, 112 are conveniently included to transport gas sample into and out of the sample chamber which may, likewise, be provided with one or more pressure gauges. Preferably, a reference gas chamber, 106 is also employed together with mirrors, 114 for directing laser light through the reference gas chamber 106. The light paths through the sample and reference chambers are directed to one or more detectors, 108 for assessing the intensity of laser light. Processor or processors in control module, 110 determine the amount of absorption of incident laser light by the sample in the sample chamber, by reference to the reference sample in the reference chamber. This determination may be performed by routine software of firmware, either on board the device or external to it. Preferably, electrical connections, 116 are provided enabling either signals or processed data from the device to be ported to external display or data collection and manipulation devices. In accordance with certain preferred embodiments, some or all of the elements making up apparatuses and systems of the invention and the functions they perform are operated under the control of a controller. Such controller, which may be on board the instrument or external to it, may be a general purpose digital computational device or a special purpose digital or digital – analog device or devices. Control by the controller may be of, for example, power supplies for the laser, detector, gas sample pump, processors and other components.

**[0050]** In operation, a gas sample suspected of containing carbon dioxide is placed into the sample chamber of the devices of the invention. The laser light source or sources is then caused to transit the sample chamber, preferably via a recurring pathway so as to increase the overall path length and improve the measurement sensitivity. The light source is then directed to one or more sensors and the sensor readings interpreted to give rise to a value for wavelength absorption by the sample. The methodologies for making this determination are well known in the art, and include, for example, direct absorption spectroscopy, wavelength modulation spectroscopy, cavity ringdown spectroscopy, and other alternatives. By comparing the absorption of light having each of the chosen pair of wavelengths, values for the carbon 12 and carbon 13 isotopes in the carbon dioxide sample become known. Perforce, their ratio may be calculated. For some of the preferred embodiments of the invention, a reference gas sample is provided and the same irradiated, detected and the signal interpreted. The data thus obtained is used to standardize the data arising from irradiation of the sample chamber.

**[0051]** The mechanics of the apparatus including the supply of power to the laser light source or sources, to the detectors and to any data storage, presentation and manipulation elements is preferably under the control of a controller, whether digital or analog. A digital computer may also or in addition be used. Such computer may be on board or connected via a control interface.

**[0052]** It is preferred that determination of light absorption in accordance with the present invention be accomplished by wavelength modulation spectroscopy (WMS). While WMS has been used previously for  $\delta^{13}\text{CO}_2$  measurements [17], it has never been performed for the line pairs that have now been determined to be used for isotopic ratios determinations in carbon dioxide.

**[0053]** WMS is preferred to direct absorption spectroscopy for use in the present invention, although direct measurement may be used if desired. For direct absorbance measurements the laser current is ramped so that the wavelength output is repeatedly scanned across a gas absorption line and the spectra generated are co-averaged. Analysis of direct absorption spectra involves detecting small changes on a large detector signal. For very low concentration changes this is problematic. To perform WMS, a small high-frequency sinusoidal modulation is superimposed on the diode laser current ramp. This current modulation produces a modulation of the laser wavelength at the same high frequency. Absorption by the target gas converts the wavelength modulation to an amplitude modulation of the laser intensity incident on the detector, adding AC components to the detector photocurrent. The detector photocurrent is demodulated at twice the modulation frequency,  $2f$  detection. This selectively amplifies only the

AC components (a zero background measurement) and shifts the measurement from near DC to higher frequencies where laser noise is reduced. Spectral noise is greatly reduced by performing signal detection at frequencies ( $>10$  kHz) high enough to avoid fluctuations in the laser output power, laser excess ( $1/f$ ) noise. In carefully optimized laboratory setups, WMS has measured absorbances as low as  $1 \times 10^{-7}$ , which is near the detector noise limit. However, in compact field instrumentation, background artifacts typically limit the minimum detectable absorbance  $\alpha_{\min}$  to  $1 \times 10^{-5} \text{ s}^{-1/2}$ . The value for  $\alpha_{\min}$  can be improved by longer time averaging of the  $2f$  signal with the improvement scaling as  $t^{1/2}$  for periods of 100 to 300 seconds.

**[0054]** The  $^{13}\text{CO}_2$  and  $^{12}\text{CO}_2$  absorption line pairs described herein give rise to relatively temperature insensitive  $\delta^{13}\text{CO}_2$  isotopic ratio determinations in gas samples are separated by several absorption lines that do not need to be measured. Instead of continuously scanning the laser wavelength between the two peaks of interest in each pair, the electronics is caused to operate the laser in a jump scan fashion. This is illustrated in Figure 2. The laser current scan is programmed to have a discontinuity that will rapidly change the wavelength. The first few data points after the jump are preferably not used, as the laser wavelength may not be stable immediately after the current jump. VCSELs used in the present invention may be operated in this way even with four current jumps in order to measure five different absorption lines simultaneously with no undue reduction in sensitivity.

**[0055]** Compositions for oral administration or inhalation, i.e., pulmonary, administration are as otherwise described herein. Oral compositions include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners, diluents, flavorings, dispersing aids, emulsifiers or binders may be desirable. Compositions for pulmonary administration include a pharmaceutically acceptable carrier, additive or excipient, as well as a propellant and optionally, a solvent and/or a dispersant to facilitate pulmonary delivery to the subject.

**[0056]** Sterile compositions for injection can be prepared according to methods known in the art.

**[0057]** While the present invention has been set forth with reference to numerous embodiments and alternatives, the present specification is not to be taken to be limiting. The invention is solely measured by its claims.

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  9. U.S. Patent 5,929,442
- All references cited herein are incorporated by reference in their entireties.

What is Claimed:

1. A method for determining the presence or absence and location of a bacterial load in the respiratory system of a subject comprising:
  - a. administering to the subject, an effective amount of a  $^{13}\text{C}$ -isotopically-labeled compound that produces  $^{13}\text{CO}_2$  upon bacterial metabolism;
  - b. collecting a plurality of samples of exhaled breath from the subject;
    - i. at least one of said samples comprising breath from the upper respiratory tract of the subject; and
    - ii. at least one of said samples comprising breath from the lower respiratory tract of the subject;
  - c. conducting at least some of the samples to a sample chamber of a detection apparatus;
  - d. evaluating the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  present in each of the at least some samples; and
  - e. relating the isotopic ratios thus ascertained to the location in the respiratory system from which said samples conducted to the sample chamber were collected.
2. The method of claim 1 wherein the isotopic ratios of at least some of the samples conducted to the sample chamber are determinative of the presence or absence of the bacterial load at the locations in the respiratory system from which the respective samples were collected.
3. The method of claim 1 further comprising
  - a. actuating a laser light source of the detection apparatus to emit one or more of the wavelength pairs 2054.37 and 2052.42; 2054.96 and 2051.67; or 2760.53 and 2760.08 nanometers; and
  - b. directing the laser light thus actuated through the sample in the sample chamber to impinge upon a detector for such wavelengths.
4. The method of claim 1 further comprising comparing the isotopic ratio of at least one sample conducted to the sample chamber with the isotopic ratio of a control sample to effect said determination.
5. The method of claim 4, wherein the control sample comprises at least one sample of exhaled breath from the subject prior to administration of the  $^{13}\text{C}$ -isotopically-labeled compound.

6. The method of claim 4, wherein the control sample includes the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  present in exhaled breath of a population that has not been administered the  $^{13}\text{C}$ -isotopically-labeled compound.
7. The method of claim 1 wherein the location of said samples is determined by collecting each of said samples during a preselected time period during exhalation by the subject.
8. The method of claim 7 wherein the time period for collection of the plurality of samples is determined following an evaluation of the breathing pattern of the subject.
9. The method of claim 8 wherein said evaluation comprises measurement of the time required by the subject to complete a substantially complete exhalation.
10. The method of claim 1 wherein the location in the respiratory system of at least some of said samples conducted to the sample chamber is determined by ascertaining the total carbon dioxide level in the breath of the subject in the samples.
11. The method of claim 1, wherein the bacterial load is in the lung.
12. The method of claim 1, wherein the  $^{13}\text{C}$ -isotopically-labeled compound is administered by inhalation.
13. The method of claim 1, wherein the  $^{13}\text{C}$ -isotopically-labeled compound is administered by ingestion.
14. The method of claim 1, wherein the  $^{13}\text{C}$ -isotopically-labeled compound is administered by injection.
15. The method of claim 1, the determination being of the presence of a bacterial load of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Acenitobacter baumannii*, *Klebsiella pneumonia*, *Francisella tularensis*, *Proteus mirabilis*, or *Aspergillus species*.
16. The method of claim 3, wherein the apparatus further comprises a processor for interpreting or presenting the signals received by the detector.
17. The method of claim 3, wherein the apparatus further comprises one or more of power supply, gas pump, pressure gauge, signal processor, and reference gas chamber.

18. The method of claim 3, wherein the laser light source of the apparatus scans the pair of wavelengths using wavelength modulation spectroscopy.
19. The method of claim 3, wherein the wavelength pair is 2054.37 and 2052.42 nanometers.
20. The method of claim 3, wherein the wavelength pair is 2051.67 and 2054.96 nanometers.
21. The method of claim 3, wherein the wavelength pair is 2760.53 and 2760.08 nanometers.
22. The method of claim 3, wherein the laser light source of the apparatus comprises a pair of laser emitters.
23. The method of claim 3, wherein the laser light source of the apparatus is a vertical cavity surface emitting laser.
24. The method of claim 1, wherein the  $^{13}\text{C}$ -isotopically-labeled compound is isotopically labeled urea, isotopically labeled glycine, isotopically labeled citrulline, or mixture thereof.
25. The method of claim 1 wherein the isotopically labeled compound is  $^{13}\text{C}$ -labeled urea.
26. The method of claim 1 wherein the isotopically labeled compound is a mixture of  $^{13}\text{C}$ -labeled urea and  $^{13}\text{C}$ -labeled glycine.
27. The method of claim 1 further comprising comparing the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in the evaluated exhaled breath samples obtained after administration of the  $^{13}\text{C}$ -isotopically labeled compound to the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in at least one exhaled breath sample obtained from the subject prior to the administration of the  $^{13}\text{C}$ -isotopically labeled compound.
28. The method of claim 1, wherein an increase in the ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in at least some of the samples conducted to the sample chamber before inhalation of the  $^{13}\text{C}$ -isotopically labeled compound to the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in the at least one exhaled breath sample obtained from the subject prior to the inhalation of the  $^{13}\text{C}$ -isotopically labeled compound indicates the presence of a bacterial load in a lung of the subject.
29. The method of claim 1, wherein an increase in the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  before inhalation of the  $^{13}\text{C}$ -isotopically labeled compound to the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in the at least one exhaled breath sample from the upper respiratory tract obtained from the subject indicates the presence of bacterial colonization in the upper respiratory tract of the subject.

30. The method of claim 1, wherein an increase in the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  before inhalation of the  $^{13}\text{C}$ -isotopically labeled compound to the isotopic ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in the at least one exhaled breath sample from the lower respiratory tract obtained from the subject indicates the presence of a bacterial infection in the lower respiratory tract of the subject.
31. The method of claim 29, further comprising the step of increasing airway clearance in the respiratory tract of the subject.
32. The method of claim 29 or 30, further comprising the step of administering to the subject a therapeutic agent for reducing said colonization or infection.

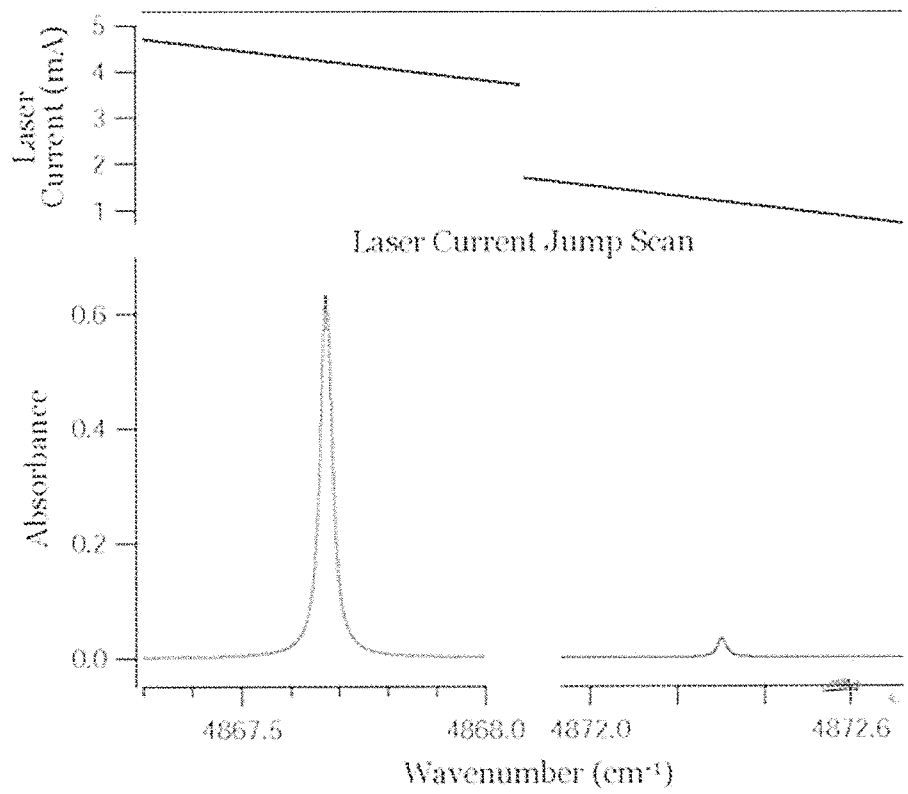
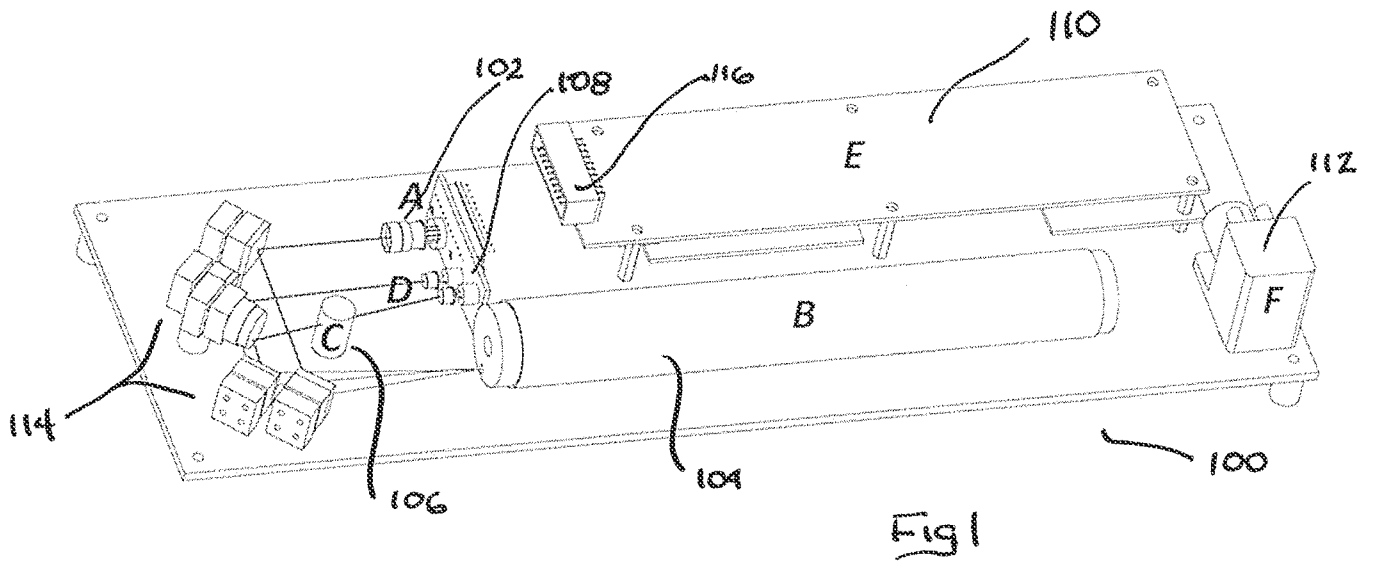


Fig 2

**INTERNATIONAL SEARCH REPORT**

International application No  
PCT/US2013/074629

**A. CLASSIFICATION OF SUBJECT MATTER**  
 INV. G01N33/497 G01N21/39  
 ADD.  
 According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**  
 Minimum documentation searched (classification system followed by classification symbols)  
 G01N  
 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 EPO-Internal, BIOSIS, WPI Data, INSPEC, COMPENDEX

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MAIGA MAMOUDOU ET AL: "Current tuberculosis diagnostic tools & role of urease breath test", INDIAN JOURNAL OF MEDICAL RESEARCH, vol. 135, no. 5, 1 May 2012 (2012-05-01), pages 731-736, XP002721175, ISSN: 0971-5916 the whole document ----- -/--	1,2, 7-17,24, 25,28-32

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search <b>6 March 2014</b>	Date of mailing of the international search report <b>01/04/2014</b>
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer <b>Joyce, David</b>

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2013/074629

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Frederick J. Miller, Julia S. Kimbell: "Chapter Ten:Regional Dosimetry of Inhaled Reactive Gases" In: Roger O. Mc Clellan, Rogene F. Henderson: "Concepts in INHALATION TOXICOLOGY Second Edition", 25 September 1995 (1995-09-25), Taylor & Francis, XP002721176, ISBN: 1-56032-368-X pages 257-258, page 258, paragraph 2nd -----	1,2, 7-17,24, 25,28-32
A	WO 2012/162695 A2 (SOUTHWEST SCIENCES INC [US]; MASSICK STEVEN MICHAEL [US]; PETERSON KRI) 29 November 2012 (2012-11-29) sentence 10, paragraph 0006 - sentence 13 page 3, line 1, paragraph 0011 - page 4, line 6; figure 1 -----	3,18-23
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