

TITLE

A Photonic Method and Apparatus for Detecting Compounds and Pathogens in a Respiratory Sample

CROSS-REFERENCE TO RELATED APPLICATIONS

U.S. Provisional Patent Application No. 63021054 filed May 6, 2020

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Non-Applicable

SPECIFICATION

Field of the Invention

5 The present invention relates generally to a means of detecting a target molecule, compound or substance through target-specific attraction with a dual-labeled probe whereby said probe expresses a high affinity for a distinct target and is an “excitable”, fluorophore capable of luminescence in a determinable light wave range. Specifically, the oligonucleotide sequence defining the target-specific binding site, takes the form of a dual-labeled probe forming a
10 quenched, stem-loop structure in its native state which fluoresces upon hybridization to a target molecule nucleotide sequence. Said hybridization allows for optical detection of localized probe-conjugate combinations through a directed, filtered and focused light source. In the presence of a target molecule, nano-molecular probe-target conjugates illuminate and are made both identifiable (qualifiable) and quantifiable via luminescence (i.e., light wave excitation) and subsequent photo
15 detection. The presence and quantity of the target molecule may thereby be both detectable and quantifiable through improved optics utilizing ellipsoid reflection enhancing both sensitivity and specificity.

Background

20 The ability to detect and localize (i.e., qualify), monitor and quantify the expression of specific viral gene expressions in real-time offers unparalleled opportunities for advancements in molecular biology, disease pathophysiology, disease detection and medical diagnostics. Nonetheless, methods currently employed (e.g., selective amplification through multistep

Polymerase Chain Reaction sample amplification and “saturation binding” in the case of a microassay such as ELISA) achieve variable rates of sensitivity and specificity with defined shortcomings in their applicability and practicality.

Specifically, in the case of PCR a particular sample must undergo a denaturation (i.e., separation) and synthesis process, with required sequential variations in temperature, for successful completion of each respective step which ultimately results in millions to billions of replicated copies. This process may be repeated on the order of 20 to 40 times (cycles) through the beginning stages of sample procurement, into exponential amplification and sequential thermal cycling resulting in sample isolation and magnification. And while this process is undeniably advantageous in terms of its sensitivity and ability to quantify the amount or number of a specific antigen molecules, the process is also limiting in that (1) the PCR method is multi-step and time-dependent (i.e., non-rapid) thus requiring highly trained personnel and sophisticated and dedicated equipment for sample processing where (2) the target sequence must be first determined and (3) mutations and contaminations are an underlying and ongoing concern.

Currently, in the case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the identity of the etiological causative agent of coronavirus disease 2019 (COVID-19) is accomplished through a variation of PCR, real-time reverse transcription–polymerase chain reaction (RT–PCR). Through the testing of nasopharyngeal and oropharyngeal fluids, virus shedding is found to be in largest concentrations 5-7 days after symptom onset and continuing in peak concentrations for several weeks after onset. Functionally, swab-derived viral RNA is isolated and converted to complementary DNA (cDNA) which is then amplified (through cycling as above) using *Taq* DNA polymerase and a series of specific heating and cooling (thermo cycling) events. Manifestly, RT–PCR remains the definitive means of detection, owing to evidenced

proven sensitivity, precision and specificity for viral detection over and above available serological tests. But this specificity comes at a cost of (a) time, wherein turnaround time can take from hours to days, and (b) immobility, test being commonly performed in a laboratory setting. Other limitations in RT-PCT include sample storage requirements, sample contamination and, ultimately, cost.

Serological microassays (e.g., ELISA, EIA (enzyme immunoassay) and RT-LAMP (loop-mediated isothermal amplification) in saliva) are a commonly utilized analytical assay for qualitatively and quantitatively measuring and assessing the presence of a target entity within a sample by implementing qualitative detection of IgG or IgM antibodies. Such tests may verify or accompany RT-PCR results, determine a previous immune response against the viral spike (S) protein, include a quantitative aspect and prove helpful to assess acquired immunity against subsequent viral exposure, a basis for assessing reinfection and/or for contact tracing purposes. And while these assays have proven indispensable in modern medical technology, the detection of target molecules is in no way simple. The process entails a multi-step process consisting of pre- and post-analytic steps exclusive of the actual analytic steps of the assay – steps which are numerous and exacting – which rely on a fixed or mobile antigens, enzyme-linked antibodies and enzymatic substrates to produce a subsequent reaction that is in some way detectable or observable. This typically involves several applications with reagents, detergents and a final washing step to remove all non-specific or unbound antibodies which is finally followed by an enzyme facilitated chemical conversion (with a substrate) which results in an absorbance or fluorescence in a specific range or spectrum identifying the adherence of a selective, tagged-antibody to a target molecule or antigen. Though exhibiting a well-documented and lengthy track record, assays are however in need of a laboratory, dedicated equipment and trained personnel, as seen above in PCR testing,

but also necessitate binding and washing steps which require exacting, multi-step procedures in order to determine (1) presence or absence of desired target and/or (2) the overall quantify (e.g., concentrations) of said target. This lends more to a retrospective analysis in which antibodies are utilized to better detect the later stages of infection or past infection.

5 And while antibodies are a logically and naturally derived means of antigen (target) detection, responsible for identification and “tagging” of these antigens typically for a directed, immunological destruction of these targets through a series of actions including neutralization, agglutination, precipitation and complement activation, the antibody detection is only part of the process. To be observable, these antibodies must also be linked to an enzyme and exposed to a
10 substrate specific to the enzyme wherein the resultant reaction (between enzyme and substrate) produces a detectable signal (e.g., a chromogenic change). As is the case with OTC pregnancy tests and rapid Strep tests, this method is particularly helpful to determine the presence or absence of a target but lacks sensitivity when a small number of targets are present. Equally, this method relies upon an appreciable color change for determinations which may indicate the existence of
15 the target without informing the actual quantity of target molecule. What’s more, relatively large amounts of substrate must be hydrolyzed by the bound enzyme before any appreciable detection can be achieved.

To overcome this infirmity, enzyme-linked fluorescence assays (ELFA) was introduced to improve sensitivity in samples exhibiting greatly lowered concentrations as compared to those
20 substrates yielding strictly colored products (as above). Whereas ELISA is chromogenic, the substrate used in ELFA is fluorogenic and results in sensitivity rates which are on the order of 100 times more sensitive than ELISA. And, while greater sensitivity rates are achievable, the immunochromatographic process continues to rely on multi-step procedures (including the steps

of procuring samples, fixing antibodies, antigen binding, and washing) and therefore requires many steps – both in preparation, actual analysis and reading of results.

And although a multi-step preparation and analysis is untenable in a rapid detection format, the use of fluorescence and chemiluminescence is of particular appeal and importance to inventors. Specifically, although these assay processes are highly labor and equipment intensive and require samples to be collected via blood collection and transported to laboratories in order to determine results, luminescence as a marker or indicator has seen use in the serological detection of disease for a number of years and bears a long track record in this regard. In fact, chemiluminescence or decay from an excited electronic state to a lower energy level resulting in an emitted light, has seen applications in gas analysis for poisons or other impurities, analysis of organic metabolisms, combustion analysis and biomolecule detection. This is especially interesting in the detection of biological markers (e.g., in forensic science) wherein the heme in blood may be utilized to detect even trace amounts of blood. Moreover, fluorescence has the unique ability to both qualify and quantify the amount of a target molecule which is over and above the capability of simple ELISA.

And while serological testing complements “rapid” viral detection, as an indication of current or past exposure, the development of pivotal ‘first-line’ methods of detection, especially in respiratory samples (seeing the highest concentrations during initial stages and peaking in the second week), that are truly “*rapid*” is an absolute imperative.

It is therefore chiefly critical to rapid detection that inventors have determined a means to provide improved detection methods, evidencing enhanced sensitivity and specificity, through a combination of (1) highly sensitive biological receptivity to a specific target molecule (biomarkers, compounds, pathogens (organic and inorganic), drug substances and poisons alike), whereby target binding produces near-instantaneous duplex formation and subsequent conformational changes

resulting in a observable, single-step bio-illumination, in opposite of micro assays, that (2) can be optically detected *without* sequential (and multi-step) amplification, as in PCR, all via a non-invasive procedure. Moreover, it is equally advantageous for such a (1) biological binding and (2) optical detection unit to require little to no skilled technical intervention or reliance upon a laboratory (other than confirmatory results) which is completely contained within one device that is mechanically simple, largely reusable and capable of being reduced to a hand-held apparatus informed exclusively by utilizing exhaled breath.

DESCRIPTION OF THE RELATED ART

While immunology (more singularly serology) and fluorescence may find their roots as far back as the 19th century, the combination of the two is only in its nascent stages of development when used for detecting and analyzing respiratory samples for content of specific target substances, molecules and compounds. In fact, breath gas analysis, or “breathprints”, have only seen development starting as late as 1971 when Linus Pauling first demonstrated that human respiration contains a series of gases and compounds that may be utilized to *indirectly* analyze, monitor and diagnosis patients for any number of biomarkers correlated with diabetes, lung cancer, head and neck cancer, asthma, COPD and cirrhosis. What is more, breath analysis itself has only recently become recognized institutionally as evidenced by the International Association of Breath Research (IABR) only first established in 2005 and having its first breath analysis summit in 2007 (First Breath Analysis Summit/3rd annual meeting of IABR in 2007).

Manifestly, while there have been attempts to detect compounds that are a byproduct or secondary indicator of disease, these endeavors have failed to move beyond secondary analysis and on to a solid correlation to directly observe and analyze causative agents non-invasively and in real time.

Thus, there is a significant, well recognized, and yet unmet need in the art for a means to detect a target molecule, compound or substance through nanomaterial-based technology wherein, for example, a target-specific, oligonucleotide hybridization probe (molecular beacon: MB) acts as an antibody alternative (i.e., an antibody mimetic/probe) which expresses a high affinity for a distinct target molecule, in the present case a viral infective agent. And although the present invention is a viral pathogen, molecular sequestration and visualization can easily be utilized for a compound, biomarker, bacterial pathogen, poison, chemo or biohazard or any other genetic sequence capable of target-specific binding and resultant molecular conformational change. Said MB is particularly useful in the detection and quantification of target molecules where said probe is capable of luminescence after binding, in a specific light wave range, which may be observed and analyzed optically via the methods disclosed herein.

It is the introduction of nucleic acid probes in the late 1990s, by the likes of Tyagi and Kramer, which provides just the alternative to antibody detection, and antibodies themselves, via an oligonucleotide hybridization probe, consisting of short DNA or RNA oligomer sequences, capable of effectively binding to a target molecule and providing a duplex formation with discrete conformational changes. The molecular beacon (MB) itself is a hairpin-shaped structure which is typically 20 to 40 nucleotides in length evidencing a “loop”, “stems” and fluorophore and quencher “end cap” portions. The “loop” structure is roughly 18–30 base pairs which complements the target molecule. Each “stem” portion attaches to the termini of the “loop” structure and are made of short (5 to 7 nucleotides) and reciprocal base pairs ensuring an essentially “closed” conformation. At the 5′ end of the molecular beacon strand is covalently bound a fluorescent dye (i.e., fluorophore or fluorochrome) and at the 3′ end exists a covalently bound quencher dye, which does not fluoresce. In this “closed” conformation, the fluorescence of the sensor is *quenched* due to the

close proximity of the quencher to the fluorophore, ideally completely with very little residual fluorescence, through fluorescence resonance energy transfer (FRET) in the stem-closed formation wherein FRET is used to describe the transfer of energy between the fluorophore and the quencher in a non-radiative process, which is distance dependent. Concisely, the protein-
5 induced conformational change in the molecular beacon resulted in a change of proximity between the fluorescence probes attached to the ends of the probe generating a fluorescence signal change from the “de-quenched” fluorescent dye.

In operation, once the complementary target nucleic acid sequence is “recognized” by the loop structure, the affinity of the loop nucleotides for the reciprocal target nucleotides supersedes
10 the affinity of the base pairs in the stem, due in no small part to the larger ratio of loop structure nucleotides to stem nucleotides, thus forming a duplex with the target molecule. The molecular probe then transitions from an unstable “closed” configuration to the more stable “open” conformation wherein the fluorescent dye (fluorophore) and quencher dye are separated. A process called hybridization.

15 Therefore, in the absence of a target molecule, the higher stability of bonds between corresponding nucleotides in the stem greatly reduces the chances of a non-specific opening of the MB. Yet, with the introduction of a target molecule, and its recognizable nucleotide sequence, subsequent MB -target binding and hybridization occurs, the combined MB and target form a duplex, said duplex undergoes a conformational change thereby causing the fluorophore to
20 separate from the quencher. The fluorescence signature thereby increases significantly due to diminished energy capture by the quencher caused by increased distance between fluorophore and quencher in the “stem-open” arrangement thus producing observable illumination in the presence of a specific target molecule.

SUMMARY OF THE INVENTION

The present invention seeks to address, mitigate and/or alleviate the above referenced infirmities and disadvantages of the current state in the art of target molecule detection and to allow for (1) direct observation of a target molecule, (2) through the binding of an illuminable molecular beacon and target compound, that (3) fluoresces, post binding, and is excitable to a specific light range (4) detectable via a specifically emitted wavelength, (5) observable via directed, filtered beams in an optical scanner - all in a (6) reusable, handheld device that gives (7) near-instantaneous results.

In the present invention's broadest terms, the disclosed device allows the rapid detection and measurement of target molecules in an exhaled breath condensate through target-specific "tagging" by a selective probe or beacon, subsequent collection of breath condensate containing said combined duplex of target and "tag" (e.g., probe) and detection and observation of the presence (and quantity) or absence of "tagged" molecules in the collected sample through enhanced light projection to a receiving detector. Succinctly, the present invention is dependent upon both target detection and a conformation-induced fluorescence which is selectively excitable to allow for target-specific recognition (qualification) and quantification through improved optical observation techniques. Such observation utilizes a non-energy dependent reflective, ellipsoid surface to "amplify", direct and focus fluorescent light toward a sensor/detector for observation, data collection, analysis and conveyance of said data for medical diagnostics.

As well, inventors contemplate similar sensor beacons (e.g., quantum dot (QD) semiconductor particles) to be of particular interest whereby QD photoluminescence has greatly enhanced brightness, in the order of 20 to 100 times the brightness of a fluorescent dye, and high extension coefficient as a potential substitute for conventional donor fluorophores. Further QD

size may have implications on coloration and the potential duration of fluorescence which may afford a greater variability between and among QDs as opposed to fluorescent dyes. Yet, although potentially promising, this technology bears some concerns as to safety and toxicity that may potentially adversely affect the test administrator thereby decreasing their utility.

5 Taking the foregoing into consideration, the term probe (i.e., molecular beacons, quantum dots, antibodies and antibody-like substances including mimetics or other organic or inorganic molecules capable of fluorescence or phosphorescing), in most cases, may be used interchangeably as performing the same basic function of detection and signaling, but as can be seen, each does so in very discrete and disparate ways. It is these differences which informs the present invention in
10 terms of both form and function (i.e., unique operability).

 The biological functions of target-probe conjugation and subsequent fluorescence, while integral to the functioning of the present invention provides, though, affords an incomplete analysis of the unique character of the present invention. In addition to the novel use of molecular beacons in non-invasive identification of a target molecule, in this case a specific virus, is further
15 complemented with a new and novel means of biochemical detection. In opposite of a conventional spectrophotometer, where light (illumination) is provided to a sample and the sample emits light in an omnidirectionally and indiscriminately to a detector, the present means of detection directs, focuses and “pinpoints” light toward the detector (via an ellipsoid “concaving” mirror) which efficiently concentrates the detectable light which allows for conservative uses and the ability to
20 detect even very small concentrations of fluorescence.

 The present invention itself is thus comprised of (1) a biological component, (2) a collection reservoir and (3) an optical detection device (the later reservoir and device serving a

dual purpose). And while each may be viewed independently, it is their relationship to one another, taken as a whole, that provides the combined novelty and utility to the present invention.

- 5 A. Biological Components: a target-specific molecular probe (molecular beacon - MB) and target molecule, described herein as a duplex, consist of (a) molecular beacon, (b) a target molecule and (c) a resultant detectable MB-target complex which becomes detectable after undergoing binding, hybridization and a conformational change as to provide sufficient distance between a fluorophore and quencher to allow for luminescence. Further, the amount of target substance may be, in addition to qualification, may be quantified wherein normal ranges are calculated and determined
- 10 (from absence to presence) and test samples may be extrapolated to evidence a percent of detected substance that is measurable and equated to total body titers (i.e., a control).
- 15 B. Collection Reservoir: a hand-held or stationary receiving device (in the form of a receptacle or chamber) consisting of a mouthpiece, a tapered condensate receiving chamber, an exhaust portal and a collection reservoir, capable of both capturing and channeling, with the assistance of gravity, exhaled breath condensate (EBC) into a receiving collection receptacle. The producer of the EBC, the patient, exhales a sufficient quantity of exhalant to coat and saturate an interior chamber of the collection reservoir and cause condensate to exceed the adhesive forces of the receiving chamber and to be collected in an attached reservoir. The collection of condensate may be
- 20 facilitated through several mechanisms including (1) an induced vortex flow within said chamber better distributing condensate about the interior of the chamber, (2) a hydrophobic surface on the interior of the chamber repelling adhesion and inducing condensate to precipitate down the chamber wall and move in the direction of

gravitational flow to the collection receptacle and (3) exogenous application of a refrigerant, either externally applied or internally supplied, before or during exhalant capture.

- 5 C. Optical Detection Device (ODD): an instrument (the sensor), which is made to detect a target-specific beacon (once activated through binding) within a collection receptacle of a collected, patient derived respiratory sample wherein the patient's forced breath, exhaled, either contains or does not contain the desired target. Introduced breath, delivered through the present device, thereby allows the target to bind to molecular beacons which may reside on the interior of the chamber of within the collection
- 10 reservoir. In the case of a MB-target complex, conformational changes induced by MB and target coupling produces bio-illumination which is then emitted directionally (focused), thorough the use of an integrated ellipsoid mirror, to deliver a concentrated luminescence to a receiving detection sensor. In the alternative enzymatically-linked fluorescence induced monoclonal antibody-target duplex, the sensor is capable of
- 15 detecting an enzyme-antibody-target complex which is illuminable upon excitation with an exogenous light source. This excitation may take the form of an electromagnetic radiation or an absorbed light source dispersing an excitatory wavelength causing said antibody-enzyme-target to emit photons confirming detection. In either case, fluorescence, via fluorescent dye or enzyme linked antibody, can be
- 20 designed in order to emit specific frequencies of light depending on the designated target, sensor and/or detector requirements, based on a frequency, wherein distinct frequencies or wavelengths may be designated for identifying a particular target within a single sample or several targets within a mixed sample.

And while each one of these above advancements alone constitutes new and novel improvements individually, it is the combination of each of the biological, physical and optical processes and methods together which delivers a truly innovative means of rapid, repeatable and non-invasive target detection.

5 In a particular preferred embodiments, fluorescence or phosphorescence, can be induced through enzyme-linked bioassay, via conformational changes prompted by target-MB binding and resultant luminescence and or MB with the addition of quantum dots replacing the functional organic fluorophore. It is also within the contemplation of inventors that illumination, fluorescence or phosphorescence may be achieved through one of several means: chemiluminescence, a
10 chemical reaction (the addition of the enzymes to a substrate containing reagent), electrochemiluminescence, radioluminescence (power derived a radiation source), electroluminescence, mechanoluminescence: a mechanical triggering event (ex. as a result of the physical binding of target to probe), piezoluminescence (ex. changing pressures), sonoluminescence (sonic pressure), thermoluminescence, singly, or by a combination thereof.

15 In addition to the MB-target, fluorescence and sensor device, the present invention apparatus, method of use and system employs one or more ellipsoidal mirrors in the focused collection of detection data. Ellipsoidal mirrors are currently used in various applications like focusing light into optical fiber, focusing lasers, reflective microscopy, spectroscopy, and focusing x-rays and are employed here to exponentially increase detection passively, through reflected light,
20 resulting in an energy independent amplification thereby increasing target sensitivity requiring no additional resources for labeling, illuminating or identifying target compounds. Expressly, this application and use of ellipsoidal reflective properties is utilized to focus and concentrate small amounts of light emitted from a sample, to amplify and increase detection, qualify and quantify

the emitted light and thereby allow the present system to identify and measure even minute amounts of target complexes directly. Advantages again exist here with MB-target duplexes over enzyme-linked immunoassay wherein fluorescence is autonomous bioluminescence resulting from conformational changes requiring no exogenous light source for excitation.

5 Too, the patient interface would consist of a disposable tube containing a sensor, into which the patient would expel breath at a certain rate and volume. Inventors contemplate a means to measure exhalation rates which may be consist of a manual peak flow meter, an auditory indicator (e.g., a whistle) or any automated device, manual or electrical, which determines force and/or length of exhalation.

10 In operation, if the patient's breath contains the desired target, said target would react with the sensor (i.e., the affinity of the MB, antibody or other antibody mimetic to a target would allow for target binding and hybridization). The tagged-target complex would undergo an illumination event (as described above). The amount of tagged-target present in the volume of breath can be directly "observed", collected and recorded as related to the amount of light detected. This measure
15 of complex illumination (i.e., measurable light units) may then be used to determine the quantity of the target substance whereby the amount may signify a 'level' that allows the observer to determine the presence of target substance, the amount of target substance, or both, in order to determine the effects of the presence and amount of said target substance.

20 The sensor device may consist of a PCR collection tube or tubes, or similar device(s), or and of a plurality of probes which may be coated with the specific MB or probe designed to bind with the target sample. It is in the further contemplation of inventors to have the interior surface of the collection chamber coated with target-specific probes wherein a specific target, if present, would bind to a probe, and the resulting complex may precipitate into a collection reservoir for

detection and analysis. Ideally, the sensor is integrated into a hand-held device, and when incorporated into or inserted into the device, or the device is placed into a collection receptacle having same, the collection is suspended at one focal point of an ellipsoidal mirror.

The patient interface is disposable and may be designed specifically for a single substance
5 or a plurality of substances, sequentially or contemporaneously. The optical detection device can be reused, sensors interchangeable and each can be designed for detecting a variety of substance molecules, light frequencies and wavelengths within the same device. The device may be designed to identify a specific patient interface and calibrate its ability to analyze a patient's breath accordingly (factoring variables, including but not limited to, sensor particle size, target size,
10 complex weight, breath volume and the like).

BRIEF DESCRIPTION OF THE DRAWINGS

While the novel features and method of use of the application are set forth above, the application itself, as well as a preferred method of use, and advantages thereof, will best be understood by referencing the following detailed description when read in conjunction with the
15 accompanying drawings (in view of the appended claims), wherein:

- Fig. 1** depicts molecular beacon (MB), MB-target bound and MB target unbound, in both "closed", unbound, conformation and "open", MB-target bound, conformation.
- 20 **Fig. 2** illustrates the operational key components and functionality of the present device.

- 5
- Fig. 3** depicts an expanded view of the TE (thermo electric) Cooler.
- Fig. 4** shows a block diagram of one embodiment with 2 components (1) a disposable tube and sensor and (2) reusable test instrument.
- Fig. 5** is a representational block diagram of another embodiment of a disposable unit and detection device.
- Fig. 6a** depicts a flow diagram of EBC flow and detection process.
- Fig. 6b** represents a EBC flow, integral system components and computer analysis.
- Fig. 7** evidences a flow diagram of typical target detection and procedures.
- 10 **Fig. 8** represents a sample and directed fluorescent light via reflective ellipsoid mirror and detection in the present device.
- Fig. 9** is an ellipsoidal mirror and the functional components of sample analysis and photon detection.
- Fig. 10** depicts a detailed representation of a collection device and detection device utilizing an ellipsoidal mirror.

15

And while the present invention, integrated system and method of use are amendable to modifications and alternative configurations, embodiments thereof have been shown, by way of example only, in the drawings and are described herein in adequate detail to teach those having skill in the art how to make and practice the same. It should, however, be understood that the above

20 description and preferred embodiments disclosed, are not intended to limit the invention to the particular embodiment disclosed, but on the contrary, the invention disclosure is intended to cover

all modifications, alternatives and equivalents falling within the spirit and scope of the invention as defined within the claim's broadest reasonable interpretation that is consistent with the specification.

DETAILED DESCRIPTION

5 The following description and accompanying drawings are illustrative and are not to be interpreted as limiting. Various key features are described to provide those having skill in the art the requisite description to make and use the same. However, it is to be understood, that certain features that are routine in the art are not described in detail that would prove pedantic to the skilled artisan. It bears including that the present invention may be practiced, in various cases, in one,
10 some or all embodiments, preferred or simply described, with or without some of all details or features without departing from the spirit and scope of the invention as described.

 References to "an embodiment", "preferred embodiment", "one embodiment", "another embodiment" and "yet another embodiment" means that said "embodiments" are inclusive of all other "embodiments" in that a key feature, structure or iteration may be borne across all
15 embodiments where their inclusion is not obviated by physical or structural inabilities to incorporate or combine said features, structures, or iterations or where their incorporation would not render the present invention inoperable for its intended use. To the contrary, "embodiments" as they are described herein are inclusive of all embodiments and not mutually exclusive of other embodiments. It is also in the understanding of inventors that not all features may be included in
20 all embodiments while several features may be included in one embodiment or across embodiments.

Terms used within the present application are to be read as having their ordinary meanings in the art, as defined by the disclosure, and within the context of the surrounding defining language. Those terms, used by inventors, may as well be defined using specific terminology and descriptions are provided in the disclosure enumerating such terms and may have those meanings
5 ascribed to each term as defined by those terms' accompanying language.

Section headings, titles and subtitles may be used strictly for organizational purposes and should not be interpreted as limiting the disclosed subject matter in any way. And while certain descriptions or embodiments may appear under a certain heading, title or subtitle, these descriptions and embodiments pertain to the invention and disclosure on the whole and should be
10 read to apply to the entire description.

The present invention relates to the detection (qualification), analysis and quantification of a specific target molecule within an exhaled volume of breath, through and probe-reporter-detector model and process whereby occurs: (1) a high affinity attachment of said target to a fluorescent-tagged beacon (e.g., probe) to form a beacon-target complex, more technically a duplex, (2)
15 conformational-induced, or enzyme-substrate facilitated fluorescence of said beacon or tag (making said duplex identifiable), (3) collection and/or concentration of said into a collection receptacle, (4) focusing endogenously or exogenously produced fluorescent light toward a detector which senses either the absence or presence of fluorescence and, if present, may use the overall measurable target-probe (via number or light intensity generated to quantify duplex
20 populations through correlations with overall in vivo target concentrations.

As depicted in **Fig. 1**, a molecular beacon (MB) **100**, commonly described as an oligonucleotide hybridization probe, consists of short chain nucleotides (i.e., oligomeric sequence), having a high binding affinity to a target molecule **150**, providing a duplex formation

180 exhibiting resultant conformational changes causing the combination to fluoresce. The molecular beacon **100**, as depicted, is a hairpin-shaped structure which is typically 20 to 40 nucleotides in length consisting of a loop **120** and attached stems **130** to which are attached a donator fluorophore (F) **122** and acceptor quencher (Q) **125** existing at the terminus of each stem

5 **130**. The loop **120** is approximately 18–30 base pairs which complements (binds to) the target molecule **150**. Each stem **130** attaches to the termini of said loop **120** and each are made of short (5 to 7 nucleotides) consisting of reciprocal base pairs (ex. A-T or C-G) ensuring an essentially “closed” conformation **110** wherein, for example, TTTTGGGG may form stem **132** and CCCCAAAA may form stem **134**, or vice versa. At the 5′ end **140** of the molecular beacon **100** is

10 covalently bound an organic fluorescent dye (F) **122** (i.e., fluorophore or fluorochrome) and at the 3′ end **145** exists a covalently bound quencher dye (Q) **125**, which does not fluoresce (although the existence of two organic fluorescent dyes have also been used historically). In this “closed” conformation **110**, the fluorescence of the sensor is “*quenched*” due to the close proximity of the quencher **125** to the fluorophore **122**, ideally completely with very little residual fluorescence,

15 through fluorescence resonance energy transfer (FRET) in the stem-closed **110** formation wherein FRET is used to describe the transfer of energy between the fluorophore **122** and the quencher **125** in a non-radiative process, which is ultimately distance dependent. Concisely, the protein-induced conformational change in the molecular beacon **100** results in a change of proximity (increased distance) between the fluorescence dye **122** attached to the end of the molecular beacon **100** probe

20 stem **132** generating a fluorescence signal change (illustrated in duplex formation **180**) to a “de-quenched” fluorescent dye **122** effectively converting target **150** (depicted as a viral target) recognition into a fluorescence signal **170**.

In operation, once the complementary target **150** nucleic acid sequence is “recognized” by the loop **120** , the affinity of the loop **120** nucleotides for the reciprocal target **150** nucleotides supersedes the affinity of the base pairs (A-T and C-G) in the stem **130**, due to the number of loop **120** nucleotides in relation to stem **130** nucleotides, thus forming a duplex **180** with the target **150** molecule wherein the molecular beacon (MB) **100** is hybridized to the target **150**. The MB-target duplex **180** then transitions from an unstable “closed” configuration **110** to the more stable “open” conformation **160** wherein the fluorescent dye (fluorophore) **122** and quencher **125** are separated due to hybridization.

Therefore, in the absence of a target molecule **150**, the higher stability of nucleotide bonds between corresponding A-T and C-G nucleotides in the stem **130** greatly reduces the chances of a non-specific opening of the MB **100**. Yet, with the introduction of a target molecule **150**, and its recognizable nucleotide sequence, subsequent MB-target binding, duplex **180** formation and hybridization occurs, the combined MB **100** and target molecule **150** form as duplex **180**, said duplex **180** undergoes a conformational change thereby causing the fluorophore dye **122** and quencher **125** to separate. The fluorescence signature (fluorescence signal **170**) thereby increases significantly due to diminished energy capture by quencher **125** with co-temporal increased distance between fluorophore dye **122** and quencher **125** in the “stem-open” arrangement **160** thus producing observable illumination (via fluorescence signal **170**) exclusively in the presence of a specific target **150** molecule.

In the present application, the target (MB **100**), here a viral pathogen, is recognized by the probe (MB **100**) wherein the target **150** (virus) and the probe **100** bind to form duplex **180**. This binding is target-specific and only occurs in the presence of said virus target **150**. In the absence of target **150**, corresponding A-T and C-G nucleotides in the stem **130** would provide the most

stable energy state and would remain in the “closed” conformation **110**, providing no probe fluorescence (via fluorescence signal **170**).

As provided in **Fig. 2**, duplex **180** formation, as in **Fig. 1**, may occur either in a collection chamber **200** or in collection reservoir **220**. As depicted in **Fig. 1**, molecular beacon probe **100** would reside in collection reservoir **220**, as shown in **Fig. 2**, whereby condensate **210**, provided for by exhaled breath condensate (EBC), enters device **200** through breath tube **225** whereby exhalent enters the device chamber **208** and circles the internal perimeter **230** of device chamber **208** through cyclonic (vortex) action **240** within said chamber **208**. It is to be noted that breath tube **225** may be fitted with a one-way valve (not shown) providing a means to supply ECB into the unit without potential reflux. Structurally, internal surface **250** of the device chamber **208** is rounded and tapers as to provide the greatest surface area for EBC (i.e., condensate **210**) adhesion. Ideally, internal surface **250** is hydrophobic, repelling condensate **210**, and causing condensate **210** to avoid adherence to the internal surface **250**. Increased amounts of condensate **210** accumulate on this interior surface **250** and conglomerate (adhere to one another) due to both hydrophobic-induced negative interior surface **250** adhesion and natural cohesion between water molecules (the primary vehicle for EBC). Understanding that the natural form of a water droplet occurs during the “lowest energy state”, the state where the atoms in the molecule are using the least amount of energy, preferable to water, is when a water molecule is surrounded on all sides by other water molecules, creating a “cohesive” sphere or ball. Adding in gravitational pull **260** forces, the individual molecules in the droplet will bind to one another until the droplet (here condensate **210**) itself becomes so large wherein the bonds between water molecules (and those bonds to the inner chamber surface **250**) is less than natural gravitational pull **260**. The droplet then follows gravitational pull **260** to an awaiting collection receptacle **220**. And, just as the device

breath tube **225**, chamber **208** and collection reservoir **220** forms a closed system allowing only a discrete volume of EBC, exhaust valve **270** is incorporated at the superior portion of device **200** to allow for exhalant to escape chamber **275**, followed by replacement exhalant **280** supplied so long as the patient continues to exhale EBC. The exhaust filter **270**, given the potentially antigenic
5 properties of the exhalant, would exhibit a filter **290**, at a point most superior to the present device **200**, designated area **A**, with a sufficient micron pore size as to create a containment exhaust posing no risk to the operating technician or other medical personnel in the immediate area.

The collection reservoir **220**, which may take the form of a “PCR tube” or similarly translucent collection reservoir **220**, is positioned at the most inferior portion, designated area **B**,
10 or “trough” area of the present device **200**, and opposite from area **A** and filter **290**. It is contemplated that MB **100**, or similar probes, may reside on the interior surface of chamber **250**, within collection reservoir **220** or a combination thereof.

Completing the present device is the externally applied cooling system of **Fig. 3**. As may be readily understood, externally supplied “cooling” temperature (i.e., those temperatures below
15 ambient air) may hasten the condensation process. In conjunction with a hydrophobic inner chamber surface **250**, exogenous cooling facilitates an accelerated condensation and thus a more “rapid” detection device. The exogenously supplied coolant can be supplied through (1) pre-freezing of collection chamber **208**, (2) cold application to the external surface of collection chamber **208**, (3) or via a dual luminal collection chamber **208** (not shown) whereby a cooling
20 agent may be supplied between an internal lumen surrounded by an exterior capsule (allowing for cooling of the space between the internal lumen and outer capsule. Coolant may supply through pre-refrigeration /pre-freezing, ice, thermoelectric cooling, vapor compression cooling, refrigerant gels, cooling liquid, “cold pack” cooling (i.e., those coolants using water and ammonium nitrate,

calcium ammonium nitrate or urea causing an endothermic reaction) and/or phase change gels, singly or a in combination. As depicted, the cooling apparatus **300** of **Fig. 2**, is not to scale and is merely representational of a cooling apparatus as illuminated further in **Fig. 3**.

Fig. 3 depicts a thermoelectric external TE cooling apparatus **300** whereby Radiator **305**,
5 dissipates heat removed from cyclonic section **330** (corresponding to collection chamber **208** of **Fig. 2**) wherein said external Thermal Electric cooler **300** is attached to said cyclonic section **330** of breath tube **225** wherein vortex condensation unit **320** is facilitated in its action by heat collection, heat conduction **350** and/or cooling beyond ambient air **360**, conduction and cyclonic section **330** which moves heat from cyclonic section **300** (e.g. collection chamber **208**) and into
10 TE Cooler **310**.

Fig. 4 depicts a block diagram of the present invention, testing system and method of use. The system can be divided into two main components shown by **400** (containing photo tag **402** and disposable tube and sensor **403**) and **410**, which contain the testing device instrumentation, respectively. The disposable tube system has an additional data tag **102**. The data tag **402** is an
15 electronic integrated circuit, which may be integrated or insertable, that contains identifying information about the target-specific test, manufacturer, lot number, expiration and other defining data as may be desirable to include (and may be a QR code). Also, within the disposable breath tube **403** (i.e., disposable tube and sensor **403**) for the actual detection and collection of target substances or compounds. The testing device/instrument **410** contains several subsystems,
20 including photo collection **415**, photo detection and application **420**, signal processing **425**, display **430**, and communication **440** to an exterior computer for receipt of collected data, monitoring, analysis, presentation and storage of tests results.

One preferred embodiment, as shown in **Fig. 5**, is of the disposable breath tube **225** represented as disposable breath tube system **500** of present device **200** containing a breath tube

unit **510**, a sensor unit **520**, a spirometer unit **525**, a target (virus) filter unit **530** and a data tag **540** (which may be either a barcode or QR code). The disposable breath tube system **500** and test system **505** are diagrammatically adjoined and may be reversibly connectable as to allow for disposal of subunit **500** and retention of subunit **505**. Subunit **500** is defined by its components, indicated by disposable breath tube system **500** of present device **200** containing a breath tube unit **510**, a sensor unit **520**, a spirometer unit **525**, a target (virus) filter unit **530** and a data tag **540**, wherein sensor **520** and data tag **540** may be integrated, replaceable or removable. Moreover, spirometer **525** and virus filter **530** may be integrated or replaceable depending on variable perimeters (e.g., anticipated expiration rate due to health and age, virus particle size, and the like).

The test system and present invention **505** and its individual components are defined and indicated by photon collection device **545**, photodetector **550**, amplifier **560**, analog to digital converter **570**, and microprocessor **580**. While inventors have depicted two subunits, **500** and **505**, designed for reversible attachment and detachment, it is within contemplation of the inventors that the two subunits, **500** and **505**, may exist as a single unit.

In operation, the device user places a rubber tube, mouthpiece, cover or like apparatus (not shown) at the opening of the **225** or like breath collection device, in patient's mouth and exhales, via a self-actuated air movement either normally or forcibly. The user's exhalation (ECB) collects on the collection chamber interior **250**, accumulates, aggregates (i.e., precipitates) and "pools" into the collection reservoir **220** – serving as a target sensor **520**. The patient's exhalant continues in a vortex/cyclonic collector (see **Fig. 2** and **Fig. 3**), creating condensate **210** ultimately collecting in the collection reservoir **220** (serving as sensor **520**). ECB, unencumbered by the weight of condensate **210**, thus follows the path of least resistance up through chamber **208**, through exhaust valve **270**, followed by replacement exhalant **280**, traversing upper filter **290** and finally existing device **200** through escape chamber **275** wherein patient's expelled air is ultimately exhausted from the disposable tube system **200**.

In one preferred method, the sensor **520** contain MBs **100** that are specific for a particular target **150** (e.g., bacteria, virus, biomarker or inorganic marker). If the invention's user's breath (EBC) contains the desired target (i.e., pathogens or other sought particles or compounds) specific to the molecular beacon **100**, the target **150** will bind to the molecular beacon **100** and form the duplex described above. The photon emission (as seen in fluorescence signal **170** of **Fig. 1**) may be the result of a chemical reaction, electrical energy (i.e., via light wave introduction), conformational changes or the like. Light, either endogenously produced or exogenously excited then collected by a photon collector **545**, in subunit **505** of **Fig. 3**. The photon collector **545** focuses photon light emitted from the sensor **520** onto a photodetector **550**, where the detected photons may be converted to an electrical current. An electronic amplifier **560** amplifies the signal (electronic current) where the signal is then converted to a digital signal resulting in a digital indication of test results by an analog-to-digital converter **570**. The converted digital signal and resultant number is then transmitted to and sampled by a microprocessor **580** for data analysis (see specifically **Fig. 6b**).

Fig. 6a illustrates the path of the ECB. As shown breath tube **225** is fitted with mouthpiece **600** wherein said mouthpiece **600** is sterilized prior to shipment and is made of a hypoallergenic material. Further, depending on application, breath tube **225** may as well be sterilized prior to use. It is also acknowledged by inventors that a one-way valve (allowing breath into device **200** but not retrograde out of device **200**) which may be placed within mouthpiece **600**, breath tube **225**, or at a defined by the junction between said mouthpiece **600** and said breath tube **225**. The ECB (exhaled breath condensate) then exits breath tube **225** and enters collection chamber **208** wherein the ECB allows for circulation of air via a vortex or cyclone-type action **240**. Condensate **210** then accumulates via 'vortex condensation' within collection chamber **208**. Exhaled air is then allowed to escape through exhaust filter **290** wherein said exhaust filter **290** filters exiting air or expended exhaled breath condensate (eEBC) from viral, bacterial and other small particles) but does not significantly inhibit air flow. Exhaust filter **290** may be any micron size sufficient to ensure the

capture of harmful exhalant. Exhalant then exits the through escape chamber **275**. Condensate **210** then accumulates in collection reservoir **220** whereby said collection reservoir **220** serves as sensor **520** for EBC condensate **210** analysis via the Multi-pixel Photon Counter (MPPC/SiPM 6 mm X 6 mm) Sub Assembly **610**.

Fig. 6b provides a block diagram of the present device **200** and system architecture. As can be seen in the descending left column: Box **A** is the device's collection chamber (**208**) and primary enclosure, Box **B** signifies the thermoelectric (TE) Cooler Sub Assembly (illustrated further in **Fig. 3**) for accelerating condensation of condensate **210** (which could also be pre-refrigeration /pre-freezing, ice, thermoelectric (TE) cooling, vapor compression cooling, refrigerant gels, cooling liquid, "cold pack" cooling (i.e., those coolants using water and ammonium nitrate, calcium ammonium nitrate or urea causing an endothermic reaction) and/or phase change gels), Box **C** the mounting structure into what the device is placed for analysis, Box **D** is the Ellipsoid Mirror (see also **Figs. 8 -10** and ellipsoid mirror **801**), Box **E** is the Multi-pixel Photon Counter (MPPC/SiPM 6 mm X 6 mm) Sub Assembly **610** used as the photosensitive mechanism in the present device **200** (currently manufactured by Hamamatsu) and Box **F** represents the devices Firmware. As is evident in the descending right column: Box **G** is signifies the Sample Tube Volume (ex. 0.15ml PCR tube BrandTech® wherein 0.33% is filled plus or minus 1mm), also signified as a collection tube **220**, which may be included with the device **200** as sterilized breath tubes or supplied separately (and handled by laboratory technicians trained in handling biological substances), Box **H** is the operator controlled or operator informing a Display or displays for providing a graphical representation indicating test readiness, test progress and test completion, Box **I** is the Micro Controller Board for acquiring sensor information and guiding and informing the test operator and then uploading exam results to an externally residing computer (Box **M**) via WiFi® or Bluetooth® **650** wherein said computer (Box **M**) may have programmed into its software an application specific for the present devices' operation (see Box **N**), Box **J** is the Sensor Board, Box **K** is the High-Definition Camera for scanning a 5 mm QR Code (or bar code) at 8 inches,

Box **L** is the operable Battery and Charger controlling operable equipment, ideally for 8 hours, in Boxes **D**, **E**, **F**, **H**, **I**, **J** and **K** wherein said Battery or Batteries may be chemical, lithium expendable or rechargeable and, where rechargeable, said Charger supports domestic and international power.

5 As illustrated in **Fig. 7** a flow chart is provided for conducting a typical target detection. By way of example, from Start, a QR code is scanned from the breath tube label (step **701**) to verify that the breath tube is compatible with the device and is coded for the patient, a new breath tube is inserted into the present device (invention) (step **703**), the device then indicates when calibration is completed (step **705**). The breath tube is either placed in patient's mouth or the
10 patient is guided to the breath tube (**707**) wherein, once the patient is fixed in an upright position (step **709**) wherein the device remains $\pm 20^\circ$ from the vertical position and patient is coached to remain in an upright position. The patient is then encouraged to form a tight seal with patient's mouth around mouthpiece and to exhale, either normally or forcefully, dependent upon the parameters of the test, and the test is initiated or "triggered" (step **711**). If the technician fails to
15 "trigger" the device, operations are returned to the previous step (step **709**). If the "trigger" (step **711**) is successful, the process moves to the next step (step **713**) whereby the technician either places the complete collection device into a detector or sample is simply removed from the inferior portion of the device and placed into a detector and technician then monitors sample volume, patient status, initiates adsorption LEDs and samples MPPC output where appropriate. Once
20 sample volume is met (step **715**), the process moves to (B). If the sample volume is not met or the test times out (step **717**) the test may continue until step **715** (sample volume met) is successful. Yet, if the sample has timed out (due to a patient's inability to produce adequate sample or to complete the test within a set time) the test itself has failed (step **719**) and must be repeated (R) which returns to Start. Step **715** is also the determinative step (as depicted in **FIG. 1**) wherein those
25 probes specific to a single antigen (or other molecular substance desired) would undergo bonding and hybridization in the presence of said antigen. Alternatively, if the sample volume is met (step

715) the collection of sample is complete (step 721) and time of incubation, if any, begins (step 723). This step 723 may be either the time period in which the process takes to complete or the time period for confirmatory results. Once the incubation period times ends (step 725), the sample is tested via MPPC (step 727) and the determination is made as to whether a target is absent or target is present (wherein a pre-set quantification (e.g., determination of a viral load) may be conducted) (step 729). If the target (e.g., virus) is present, but quantification has not occurred (step 729), then incubation time may be extended returning to step 725. If the incubation time in step 725 yields a negative result, those results are analyzed and recorded where data is transferred and uploaded into a computer (upload MPPC output, upload breath test state, upload QR label data in step 731) or if step 725 continues to determine presence of a target (i.e., virus) in steps 727 and 729, those data are then transferred and uploaded to a computer the process is complete (END).

As depicted in **Fig. 8** sample Y tube is positioned within the ellipsoid mirror 801 with a focus point 805 (focus point 1) is imaged to the MPPC 815 located to the right of the ellipsoid mirror focus (focus point 2) 810 wherein mirror 801 is utilized to direct the fluorescent light through aperture 820, through condenser lens 830 to optical bandpass filter 840 to MPPC 815 wherein photons are counted up to and including single photon detection with high photon detection efficiency rates that expresses high resistance to excess light and limited to no distortion due to magnetic fields, allowing for not only the detection of the presence of fluorescent light but also each occurrence.

Equally in **Fig. 9**, the optical representation of the focus 900 of sample Y is fixed at focus point 810 wherein ellipsoid mirror 801 is used to direct the fluorescent light of sample Y through aperture 820, through condenser lens 830 to optical bandpass filter 840 and to Multi-Pixel Photon Counters (i.e., photomultiplier) 815 wherein the fluorescent light is conserved and concentrated for transmittance and detection by the MPPC.

Fig. 10 shows in greater detail the optical bandpass filter **840** of **Figs. 8** and **9** whereby light focused at point **810** is transmitted through aperture **820**, through collimation lens **902** and onto photo detector array **901**. Light occurring at the biofilter **906** resides at the bottom of PCR tube **907** for exhaled breath condensate, represented above as the collection reservoir **220** of **Fig. 2**, which is then reflected across the surface of ellipsoid mirror **801** to focus point **810** supplying non-energy consuming, reflective amplification.

From the above disclosure, many alterations and modifications, including preferred embodiments, of the present invention to a person having requisite skill in the art. Therefore, descriptions should be interpreted as merely illustrative and exemplary as providing the best mode or modes contemplated by inventors instructing the skilled artisan to make and use same which is susceptible to broad utility and application. Expressly features, structures, sizes, variations and configurations may be amended, restructured and reconfigured without departing from the scope and spirit of the present application.

The foregoing disclosure is not to be viewed in a limiting sense or to otherwise exclude any embodiments, adaptations or equivalent arrangements naturally following from disclosure of the present application. In opposite, the claims of present invention should be read giving the broadest interpretation to the inventive aspects of the invention described herein and only limited by the claims appended hereto including equivalents thereof.