# Breath Biomarker Analysis: The Future Point of Care Non-invasive Diagnostic Technology

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**Abstract:** Medicine is the science and art of healing. It requires objective analysis of disease conditions to reach a meaningful conclusion. Laboratory medicine is the integral part of today's healthcare. A classical clinical laboratory requires sample collection, sample transport, sample analysis and transfer of the report to the end user and so vital information of critical parameters are available for patient care after quite some time. Therefore, considerable current interest has been generated for non-invasive diagnostics that can be performed at the point of care. Analysis of breath biomarkers is currently understood as a topic that will be the major future point of care diagnostic methodology and this aspect is reviewed in the current manuscript to highlight the work so far done on the subject.

**Keywords:** Breath analysis, laboratory medicine, non invasive diagnostics, point of care technology.

## INTRODUCTION

Developing potentially sensitive and specific non-invasive point of care techniques (POCT) for prompt disease diagnosis is unquestionably one of the biggest needs in the field of health care. The choice of potential non-invasive human samples for diagnosis of several diseased and physiological conditions in a clinical set up comprise exhaled out breath air, sputum, saliva, sweat, urine etc. Amongst the above mentioned, breath air indeed can serve as an attractive patient friendly option as it is the easiest biological sample to be collected from patients as well as individuals undergoing diagnosis. Successful identification and analysis of different metabolic breath biomarkers in the exhaled breath air may open up an avenue towards the horizon of point of care research. In fact, it is a new hope for non-invasive point of care worldwide.

Human breath consists of hundreds of volatile and non-volatile chemical components, all of which are the result of uncountable numbers of metabolic reactions taking place each moment in the body. In a normal human individual they are indicative of normal physiology, but get altered during any physiological malfunction such as in the course of infection or metabolic disorders [1]. Alteration of the composition of the exhaled breath air is believed to be very sensitive according to the altered metabolism. Because of this fact, detecting and measuring the changing pattern of these chemical components can easily give an inside view of a diseased condition. So, these significant informer chemicals, though exhaled out in very less amount can be identified as sensitive breath biomarkers according to different altered physiological conditions and this makes the basis of "breathometry" or "breathography" in diagnosis of diseases. Breathometry can produce and provide disease specific chemical fingerprints or breathprints for disease diagnosis by analyzing the above mentioned breath biomarkers.

Breath biomarker analysis will be able to boost up the patient compliance with the course of diagnostic procedures and treatment of diseases. A few of these boosting features include the ease of sample collection, ease of sample repetition without patient discomfort, painlessness due to non-invasiveness etc. Despite all these promising patient friendly features, exhaled breath sample analysis is very difficult, probably the most difficult technique which itself affects its sensitivity and specificity to a great extent.

Understanding and realization of the impact of the contribution of the breath biomarker analysis in disease diagnosis will definitely tell us about the much needed scientific and technological innovation in this matter. Here we review the impact of breath biomarker analysis in different infectious and non-infectious disease diagnosis.

## ASTHMA

Asthma is itself as one of the most common chronic respiratory illness to over 300 million people throughout the world [2]. Although, the disease asthma rarely extends itself up to a fatal consequence, it is an unimaginable agony provider. Moreover, the

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management of asthma has always been thought to be a great economic burden [3]. Till date, the diagnosis of asthma is largely based on classical asthmatic symptoms often supported by clinical laboratory lung function test (LFT) to determine possible airway obstruction, measurement of the blood eosinophil count, serum IgE level, skin prick test (SPT) for allergen detection, etc. [4-11]. So, a simple and rapid non-invasive diagnostic test for asthma diagnosis with high sensitivity and specificity is very much needed.

Exhaled breath air contains a complex mixture of thousands of volatile organic compounds (VOCs) and also the non-volatile ones. Their levels get altered according to the body's changing physiology as in the variety of diseased conditions and evaluation of these may be useful in disease diagnosis [1]. In case of asthma, the serving breath biomarkers can be nitric oxide (NO), carbon monoxide (CO), pentane, ethane, hydrogen peroxide ($H_2O_2$), thiobarbituric acid reactive products (TBARs), exhaled 8-isoprostane etc. [12-31]. Levels of exhaled NO, CO are increased in course of stable asthma [17, 18]. Exhaled level of pentane shows marked increment during exacerbations of acute asthma, but gets reduced up to the normal level when the patient is recovering [21, 24]. Level of ethane is also reported to be higher in patients suffering from mild steroid-naive asthma while it is comparatively stable in a much lower level in normal individuals and steroid treated patients [22]. In patients with nocturnal asthma, exhaled levels of NO and pentane are increased along with a reduced level of nitrite/nitrate while in obstructive sleep apnea, exhaled NO level is increased, but the level of pentane gets reduced [23, 24]. Thus, a suggestive or diagnostic breathprint is possible to get from breath analysis of these biomarker compounds. So, the future promise of a rapid, sensitive and non-invasive method for distinctive asthma diagnosis is lying in the hope shown by breath biomarker analysis or breathometry.

## CARDIAC CARE

Breath biomarker analysis has been reported to be an efficient means to differentiate between the patients with high risk cardiac chest pain and low risk group with no pain. It has also been proposed to be potent markers for detecting chest pain due to ischemic heart diseases by analyzing the high breath content of pentane, ethane, isoprene etc. as there the oxidative stress is markedly high [32-36]. A sensitive hydrogen breath test is also reported for effective diagnosis of lipid peroxidation [34, 35]. High level of pentane is also seen in patients of both myocardial infarction and congestive heart failure [37, 38]. A few years back, a study had done a comparison of breath methylated alkane contour (BMAC) between cardiac patients with unstable angina pectoris and normal, healthy individuals and marked it as a marker for the detection of unstable angina pectoris [36, 39]. BMAC shows significant alterations according to increasing age and assesses the cardiac risks. It is also a potent marker in oxygen breathing and heart transplant rejection [40, 41].

## CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

The most promising and distinctive COPD breath biomarkers are NO, 8-isoprostane, $H_2O_2$, pentane, isoprene and eicosanoids like leukotrines, prostanoids etc. [42-47]. Exhaled NO level is lowered in stable COPD in either case of smoking and non-smoking asthmatics [48-52]. In smokers, this reduction is probably due to the down-regulation of eNOS by tobacco smoking which in turn increases the risk of pulmonary and cardiovascular diseases in smokers [48, 53]. Level of ethane gets elevated in patients with COPD who practice extensive smoking, but pentane and isoprene levels are usually high in normal smokers too [54-57]. A marked increment in the level of 8-isoprostane is noticeable in both breath and urine [45, 58]. Distinctive breathometry fingerprint by the altered levels of above mentioned biomarkers can possibly be proven useful in the diagnosis or predicting prognosis of COPD.

## CYSTIC FIBROSIS (CF)

It has been seen that in cystic fibrosis (CF), the exhaled and nasal levels of NO get a marked reduction, though the airway neutrophilic inflammation is quite intensive. This neutrophilic inflammation in turn leads to formation of nitrate from NO by releasing superoxide anions [59-61]. Thus, patients with CF exhibit increased nitrate and nitrite detectable in the exhaled breath air and condensate [61-63]. Though, the level of NO has not been shown to have a clearly established association with disease severity [64]. Exhaled CO and ethane levels are reportedly quite high in breaths of CF patients [65-67]. Increased levels of nitrite/nitrate and nitrotyrosine are fairly high in both in the stable CF and exacerbations [51, 61, 62]. Moreover, elevation in the levels of 8-isoprostane, nitrite and S-nitrothiols are also noted in exhaled breath condensate of CF patients [51, 68, 69]. Increased breath level of $H_2O_2$ is also observed in patients with

CF [70, 71]. Moreover, exhaled breath detection and quantification of methyl thiocyanate and hydrogen cyanide (HCN) are shown to be diagnostic of *Pseudomonas aeruginosa* associated cystic fibrosis (CF) and chronic suppurative lung disease respectively [72, 73].

## DIABETES

Diabetes mellitus is a metabolic malfunction with persistent hyperglycaemic state as a result of relative or absolute deficiency of insulin. In 2013, it is estimated that 382 million people in this world suffering from diabetes making it the most prevalent metabolic disorder. Moreover, 592 million people are expected to have diabetes by 2035 [74]. Diabetes also contributes as an important predisposing factor to many other infectious diseases. Diagnosis and regular monitoring are very important in the management of diabetes, which in turn demand rapid, sensitive and cost effective non-invasive methods. Detection of high level of acetone in exhaled out breath air serve as a potent breath biomarker in diagnosis of diabetes [75-78]. The exhaled level of CO is increased in diabetes [79]. Other than these, altered levels of isoprene, methyl nitrate are the awaiting breath biomarker candidates need clinical analytical validation for being used for making breathprints for diabetes diagnosis [80].

## GASTRO-ENTERIC DISEASES

$H_2$ is considered to be a good breath biomarker in case of digestion and absorption related gastro-enteric diseases [81-83]. Pentane is an alkane generated during the peroxidation process of cellular fatty acids and has been shown to be capable of being utilized as an important non-invasive breath biomarker for determining inflammatory bowel disease (IBD) [84, 85]. Application of urea breath test (UBT) is an established and long known process in diagnosis of peptic ulcers and *Helicobacter pylori* infections [86-96]. The breath pepsin level is thought to be a significant biomarker for detection of gastro-esophegal reflux Disease (GERD). Elevated levels of pepsin, oxidative stress markers, e.g., NO metabolites (NOX) and total sulphydrile (TSH), magnesium (Mg) and calcium (Ca) in breath form the fingerprint of gastro-esophegal reflux disease (GERD) detection [97-100].

## HALITOSIS

Hydrogen sulfide ($H_2S$) is proved to be an efficient breath biomarker for diagnosis of halitosis, the bad breath [78]. It is important as the concern about halitosis is that it is thought to be one of the major causes demand serious dental care [101].

## HEPATIC DISEASES

Alcoholic and non-alcoholic liver diseases also comprise a major area of serious concern. Several numbers of volatile organic compounds (VOCs) are detected with significant alterations in patients with liver cirrhosis. These are identified to be different ketones, terpenes, sulfur and nitrogen compounds and alcohols. A gross diagnostic breath fingerprint can be plotted by the altered levels of 2-butanone, 2- or 3- pentanone, C8-ketone, C9-ketone, monoterpene, heptadienol, methanol, 2,4-heptadienol etc. [102]. Moreover, a sensitive $^{13}$C-methacetin breath test (MBT) has been proposed to be a reliable diagnostic test to diagnose nonalcoholic fatty liver diseases and microsomal impairment. $^{13}CO_2$ is detected and measured through this promising test which is generated through $^{13}$C-methacetin metabolism in liver [103].

## LUNG CANCER

Lung cancer is a leading cause of death in population worldwide. Successful treatment and management of lung cancer demands rapid, sensitive and cost effective early diagnosis. Present procedures are not as blessed as they are not sufficiently sensitive and specific and moreover seek either time or money or both [104]. In this context, effective breath biomarker analysis may promise these expected parameters in lung cancer diagnosis. Altered levels of some volatile and non-volatile organic chemicals in the breath have been shown to be promising in this regard [104-107].

Breath analysis of lung cancer patients has shown increased levels of NO and nitrite while with a marked decrease in the levels of isoprene, acetone and methanol [104, 108-110]. Other potential biomarkers include different alcohols, aldehydes, ketones and hydrocarbons [104, 111]. Some of these candidates are styrene, decane, benzene, undecane, 1-hexane, hexanal, methyl cyclopentane etc. [112]. Standardized fingerprints of altered levels of these may be capable of suggesting possible detection of lung cancer.

## RENAL DISORDERS

Trimethylamine (TMA) serves as a major breath biomarker in chronic kidney disease (CKD) as it is significantly elevated and detected in the breath of patients with CKD [113]. Altered levels of acetone, isoprene, and pentane along with TMA are also helpful

in the formation of a sensitive breath fingerprint for diagnosing and managing CKD [113]. Moreover, breath analysis of the altered levels of isoprene, acetone, pentane, benzene, ethanol, DMS etc. as potential biomarkers can be utilized to recognize and prevent the well known and worst possible detrimental effects of haemodialysis in patients with end stage renal disease (ESRD) [114].

## TUBERCULOSIS (TB)

Tuberculosis (TB) caused by acid fast *Mycobacterium tuberculosis* is one of the most dreadful bacterial killers throughout the globe affecting one third of the world population [115, 116]. Recently the WHO has estimated that, this tuberculosis alone accounts for 1.3 million deaths per year [117]. Present TB diagnosis demands a lot of time through comparatively less efficient conventional methods while the advanced diagnostic methods are not cost effective. These facts along with a long term treatment strategy comprise the cause of lack of patient compliance and in turn give rise to deadlier drug resistant strains [118]. This alarming situation is desperately shouting for rapid, sensitive and cost effective means of TB diagnosis. Possibly, the breath biomarker analysis can be the most promising candidate in this context. During the course of the disease tuberculosis (TB), quite a large number of distinctive volatile organic compounds (VOCs) along with the non-volatile ones are generated as a result of host as well as mycobacterial metabolism and infection induced increase in oxidative stress [1, 119-123]. An elevated NO level in the breath air or condensate is very much indicative of cases of almost all inflammatory and infectious pulmonary diseases including tuberculosis (TB) [124]. Distinctively identical VOCs are identified from both the patient's breath and mycobacterial cultures. More than hundred of such VOCs with high distinctive diagnostic values are detected through gas chromatography-mass spectrometry (GC-MS) and other related techniques. Amongst these, the most abundant and diagnostic VOCs are found to be naphthalene, 3-heptanone, methylcyclododecane, 1-methyl-, heptane, benzene, 1-methyl-4-(1-methylethyl)-, 2,2,4,6,6-pentamethyl-, cyclohexane, and 1,4- dimethyl- etc. with almost 100% sensitivity and specificity even in culture positive smear negative patients [123, 125-126]. Moreover, as the tuberculosis causing mycobacteria (*Mycobacterium tuberculosis*, *Mycobacterium bovis* etc.) are potent producers of enzyme urease, an efficient urea breath test (UBT) has been developed to diagnose pulmonary TB [127, 128]. So, here it is quite understandable that, breath biomarker analysis has the capability of giving the much waited diagnostic dimension in the near future to boost up our battle against tuberculosis (TB).

## VARIOUS OTHER BREATH BIOMARKERS IN MISCELLANEOUS DISEASES:

A remarkably high level of NO is distinctive in rhinitis. This fact possibly contributes by higher production of NO inside paranasal sinuses [129, 130]. A similar increase in exhaled NO level is also observable in bronchiectasis while primary ciliary dyskinesia (PCD) exhibits a very low level of exhaled NO [51, 131-134]. In interstitial lung diseases (ILDs) like systemic sclerosis, fibrosing alveolitis, sarcoidosis, altered levels of NO are shown to have some diagnostic importance. In systemic sclerosis, breath NO content is lowered, but in fibrosing alveolitis and sarcoidosis elevation in breath NO level in observed [135-140]. Breath samples from patients with pulmonary hypertension show high levels of exhaled NO [51, 141]. In several occupational asthma-like breath diseases such as laboratory animal allergy (LAA) and asthma-like symptoms in aluminium pot room workers and swine confinement workers, exhaled NO level is thought to be a helpful breath biomarker [142-145]. A sharp increase in exhaled NO level is noticeable in a wide range of bacterial and viral diseases along with many distinct VOCs of pathogen origin [146-150].

Some important breath biomarkers and their diagnostic applications in corresponding diseases are summarized below in a tabular format (see Table **1**).

Apart from the above discussed possible potent breath biomarkers, some other parameters are also believed to act helpful to support the breath analysis for disease diagnosis e.g., exhaled breath temperature, humidity, pH, smell, etc. Exhaled breath temperature seems to be quite high in patients of asthma because of an increase in bronchial blood flow provoked due to an airway cooling process which lead to a rapid heat resupply in breath of asthma patients [155-156]. Increased exhaled breath temperature and humidity are the commonest seen features in a wide range of respiratory complications, including asthma, COPD, pneumonia, pneumoconiosis, rhinitis etc. [156-157]. Thus, these features can be utilized as simple, non-specific and inexpensive means for household monitoring and treatment assessment in the course of these diseases. pH of exhaled breath condensate has also been proposed by studies to be a non-invasive

**Table 1: Promising Diagnostic Breath Biomarkers in Certain Diseases**

| Disease category or possible application | Diseases (/Infections) | Promising breath biomarker (s) | Reference (s) |
|---|---|---|---|
| Nasal / Upper respiratory complications | Influenza | NO↑ | [51, 129, 130, 146-150] |
| | Rhinitis | NO↑ | |
| | Upper respiratory tract with *Staphylococcus aureus* in active Wegener's granulomatosis | NO↓ | |
| Lung / Lower respiratory diseases | Asthma | NO↑, CO, H2O2, isoprostanes, nitrite/nitrate | [12-31, 42-73, 105-112, 123-128, 151-153] |
| | Bronchitis (Chronic) | NO (Stable) | |
| | Chronic obstructive pulmonary disease (COPD) | NO, $H_2O_2$, Pentane, Isoprene Eicosanoids (leukotrienes, prostanoids, isoprostanes) | |
| | Chronic cough | NO↑ | |
| | Cystic fibrosis | NO ↓ , CO ↑ , H2O2, Mthyl thiocyanate, HCN, isoprostanes, nitrite/nitrate | |
| | Diffuse panbronchitis (DPB) | Low nasal NO | |
| | Influenza | NO | |
| | Lung cancer | NO, Isoprene ↓ , acetone ↓ , methanol ↓ , styrene, decane, benzene, undecane, 1-hexane, hexanal, propyl benzene, 1,2,4-trimethyl benzene, heptanol, methyl cyclopentane | |
| | Lung injury | NO | |
| | Lung transplant rejection (acute) | Exhaled carbonyl sulphide | |
| | Pulmonary allograft dysfunction | NO | |
| | Tuberculosis | NO, naphthalene, 1-methyl-, 3-heptanone, heptane, methylcyclododecane, 1-methyl-4-(1-methylethyl)-, benzene, and cyclohexane, 2,2,4,6,6-pentamethyl-, and 1,4-dimethyl-, and $^{13}CO_2$ (detectable through Urea breath test) | |
| Interstitial lung diseases (ILDs) | Systemic sclerosis | NO ↓ | [51, 135-140] |
| | Fibrosing alveolitis | NO↑ | |
| | Sarcoidosis | NO ↑ | |
| Pulmonary hipertension | | NO ↑ | [51, 141] |
| Occupational breath diseases | Laboratory animal allergy (LAA) | NO | [51, 142-150] |
| | Asthma-like symptoms in aluminium potroom workers | NO | |
| | Asthma-like symptoms in swine confinement workers | NO | |

**Table 1 Contineu …**

| Disease category or possible application | Diseases (/Infections) | Promising breath biomarker (s) | Reference (s) |
|---|---|---|---|
| Gastroenteric diseases | Peptic ulcer | $^{13}CO_2$, $^{14}CO_2$ (detectable through Urea breath test) | [84-100] |
| | Inflammatory bowel disease (IBD) | Pentane | |
| | Gastroesophegal reflux disease (GERD) | Pepsin, NO metabolites (NOX), Total sulphydrile (TSH), Mg, Ca | |
| Kidney/Renal diseases | Chronic kidney disease (CKD) | Trimethylamine (TMA), Acetone, Isoprene, Pentane | [113, 114] |
| Hepatic (Liver) diseases | Liver cirrhosis | 2-butanone, 2- or 3- pentanone, C8-ketone, C9-ketone, monoterpene, heptadienol, methanol, 2,4-heptadienol | [102, 103] |
| Oxidative stress | Asthma and Adult Respiratory Distress Syndrome (ARDS) | $H_2O_2$, Breath methylated alkane contour (BMAC) | [32-41, 51] |
| | Bronchiectasis, | NO, CO, $H_2O_2$, Breath methylated alkane contour (BMAC) | |
| | Chronic obstructive pulmonary disease (COPD), | $H_2O_2$, Breath methylated alkane contour (BMAC) | |
| | Lipid peroxidation | Pentane, ethane | |
| Metabolic disorders | Diabetes | Acetone, Isoprene, Methyl nitrate | [75-80] |
| Exposure to VOCs | | Vinyl chloride, chloroform, trichloroethene *cis*-1,2-dichloroethene, bromodichloromethane etc. | [154] |

disease biomarker in airway inflammations in asthma, COPD etc. [158-162]. Smell is also an important parameter for suggesting several diseased conditions since the ancient days of medicine [163, 164]. Rotten apple like sweet smell in the breath is believed to be suggestive of uncontrolled diabetes while fishy and urine like smells are suggestive of liver disease and kidney failure respectively [164-166].

## ADVANTAGES OF BREATH BIOMARKER ANALYSIS OR BREATHOMETRY

As the volatile and non-volatile components of breath are the results of numerous metabolic reactions in the body, altered levels of these give exact information about blood constituents and the body's metabolic state [1, 167]. Breath sampling is a simple and non-invasive process that permits the needful repeats of the process without causing patient discomfort even when the patient is in sleep and during surgery. In case of respiratory system malfunction, it reveals the most suggestive diagnostic information [164, 167-173].

## PRESENT DAY LIMITATIONS

Though the collection of a breath sample is simple and advantageous, till date this breath biomarker analysis is confined to the advanced research and technologically competent laboratories. Basically, gas chromatography (GC) based methods are in use with advanced and sensitive spectrometry support [51, 167, 173]. Technological competency is very much needed because breath concentrations of maximum of these volatile and non-volatile substances are very less, range from nmol/L to pmol/L [167, 173]. This fact, in turn, is a major barrier for it for being used in a massive manner. Moreover, no standard analytical methods and standardized interpretation guidelines have been recommended so far for breathometry, which can be accepted universally [167]. These facts are the major barrier to the massive application of breathometry.

To overcome all these barriers, the breath biomarker analysis demands extensive research attention. Here at this point it is quite understandable that still a very long way ahead to go to explore the very promising new horizon of point of care with this breath biomarker analysis, but then too, this promising feature of breath analysis has already attracted the scientific interest of researchers all over the world for last few decades. The baseline breath biomarkers mentioned here in this review work are the honest evidences of this fact. In fact, the recent most breakthrough as the development of the incredible

"electronic nose" sensor based technology for rapid breath analysis is a milestone in this journey of POCT which is now under trial and evaluation [174-178]. But this expedition is still under a serious need of a long list of distinctive and reliable breath biomarkers which will enrich the breathprint database for future electronic applications in disease diagnosis.

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