Rapid point-of-care breath tests for biomarkers of disease

Michael Phillips MD, FACP, FRCP
Hippocrates ~400 BC: Smell your patient’s breath!

Diabetes
Kidney failure
Liver failure
Lung abscess
Nutrients

rotten apples
urine-like
fetor hepaticus
sewer-like
garlic, alcohol
France, late 1700s
Lavoisier - the “father of chemistry”
Discovered carbon dioxide in breath
1971: USA
Linus Pauling: Two Nobel Prizes AND first microanalysis of breath

Concentrated breath in a cold trap
Analyzed with GC

>100 volatile organic compounds (VOCs)

An amazing discovery!
Alveolar membrane: ~120 M²

Tennis court: 195 M²
What’s going on here?

Lavoisier – proof of principle 1780’s
Alcohol breath tests – 1950’s
Linus Pauling – VOC microanalysis 1971

It’s 2014!
Why isn’t everyone using breath tests?
Collect breath VOCs

Preconcentrate breath VOCs

Analyze breath VOCs

Interpret the data

\textbf{Breath VOC analysis is technically difficult!}
Collect breath VOCs

Preconcentrate breath VOCs

Analyze breath VOCs

Interpret the data

Contamination challenge:
Compensate for room air VOCs

Detection challenge:
VOCs in parts per trillion

Technology challenge:
GC? Mass spectrometry?

Statistical challenge:
Find the signal in the noise
Breath Tests in Medicine

Since antiquity, it has been known that the breath contains clues to many diseases. Researchers are developing a new generation of breath tests as an alternative to invasive diagnostic procedures.

by Michael Phillips

Summarized the state of the art...
The state of the art in 1992
Breath collection apparatus

Simple to use

Digital display guides the user through every step
Library ID:
Undecane

- Robust method
- Reproducible results
- User-friendly
- Allowed the first large multicenter studies of breath testing
<table>
<thead>
<tr>
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<th>RT</th>
<th>VOC</th>
<th>Area</th>
<th>Quality</th>
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Breath tests using 1D GC MS

Demonstrated proof of principle in:
- Lung cancer
- Breast cancer
- Heart transplant rejection
- Pulmonary tuberculosis
- Environmental toxicology
- Influenza
...and around 2010 → 2D GC TOF MS
Breath tests can now detect ~2,000 different VOCs in a sample of breath.
The evolution of a successful diagnostic test

**Pregnancy tests**

- **Lab Test**
  - 1927 Ascheim Zondek test
    - Slow
    - Cost $$$

- **Point-of-care test**
  - 1960 Immunological tests
    - Faster
    - Cost $$

- **Personal test**
  - 1976 First home test kit
    - Fastest
    - Cost~$10
Basic R&D (GC/MS) → identify biomarkers

Migrate biomarkers to POC instrument → cheaper, quicker

Shrink the instrument → cell phone app?
BreathLink system
Point-of-care breath testing

Collect alveolar breath VOCs
→ concentrate on trap
→ analyze with GC SAW
Test complete in 6 minutes
BreathLink™

breath analysis anywhere in the world

upload data over internet

final report

sent over internet

Mensanna Research, Inc. analysis of breath data
BreathLink™

• Rapid, safe, and painless
• Patient-friendly and user-friendly
• Works anywhere in world - internet or phone
• Near real-time monitoring of patient and instrument
Point-of-care breath test for active pulmonary TB
Volatile biomarkers of pulmonary tuberculosis in the breath

Michael Phillips\textsuperscript{a,b,*}, Renee N. Cataneo\textsuperscript{a}, Rany Condos\textsuperscript{c}, Gerry Ring Erickson\textsuperscript{d}, Joel Greenberg\textsuperscript{a,*}, Vincent La Bombardi\textsuperscript{e}, Muhammad I. Munawar\textsuperscript{a}, Olaf Tietje\textsuperscript{f}

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\textsuperscript{d}Inforometrix, Inc, Woodinville, WA, USA
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**SBIR Phase I pilot study – NIH/NIAID**

**New York University, New York Medical College**

**IN VITRO**
- Culture reference strains of *M. tuberculosis*
- Collect VOCs from head-space air above culture

**HUMAN**
- High-risk hospitalized patients → sputum cultures for MTB
- Healthy controls
- Collect breath VOCs

**Analyze VOCs with gas chromatography/mass spectrometry (GC/MS)**
<table>
<thead>
<tr>
<th><strong>Culture (in vitro)</strong></th>
<th><strong>HUMAN</strong></th>
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</thead>
<tbody>
<tr>
<td>Naphthalene, 1-methyl-3-Heptanone</td>
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<tr>
<td>Methylcyclooctadecane</td>
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<td>Heptane, 2,2,4,6,6-pentamethyl-</td>
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<td>Benzene, 1-methyl-4-(1-methylethyl)-</td>
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<tr>
<td>Cyclohexane, 1,4-dimethyl-3,5-dimethylamphetamine</td>
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<tr>
<td>Butanal, 3-methyl-2-Hexene</td>
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<tr>
<td>Trans-anti-1-methyl-decahydronaphthalene</td>
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<tr>
<td><strong>Breath (fuzzy logic)</strong></td>
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<tr>
<td>Cyclohexane, 1,3-dimethyl-, trans-</td>
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<td>Benzene, 1,4-dichloro-</td>
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<td>Cyclohexane, 1,4-dimethyl-</td>
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<td>2-Butanone</td>
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<td>Naphthalene, 1-methyl-</td>
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<td>Camphene</td>
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<td>Decane, 4-methyl-</td>
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<tr>
<td>l-<em>beta</em>-Pinene</td>
<td></td>
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</table>
### Identical VOCs

**Culture (in vitro)**
- Naphthalene, 1-methyl-
- 3-Heptanone
- Methylcyclododecane
- Heptane, 2,2,4,6,6-pentamethyl-
- Benzene, 1-methyl-4-(1-methylethyl)-
- **Cyclohexane, 1,4-dimethyl-**
  - 3,5-dimethylamphetamine
  - Butanal, 3-methyl-
  - 2-Hexene
  - Trans-anti-1-methyl-decahydronaphthalene

**Breath (fuzzy logic)**
- Cyclohexane, 1,3-dimethyl-, trans-
- Benzene, 1,4-dichloro-
- **Cyclohexane, 1,4-dimethyl-**
  - 1-Octanol, 2-butyl-
  - 2-Butanone
- Naphthalene, 1-methyl-
- Camphene
- Decane, 4-methyl-
- Heptane, 3-ethyl-2-methyl-
- Octane, 2,6-dimethyl-
- Benzene, 1,2,3,4-tetramethyl-
- Bicyclo_3_1_1_hept-2-ene, 3,6,6-trimethyl-
- Cyclohexane, 1-ethyl-4-methyl-, trans-
- l_-beta_-Pinene
Identical and Similar VOCs

**Culture (in vitro)**
- Naphthalene, 1-methyl-
- 3-Heptanone
- Methylcycloododecane
- Heptane, 2,2,4,6,6-pentamethyl-
- Benzene, 1-methyl-4-(1-methylethyl)-
- Cyclohexane, 1,4-dimethyl-
- 3,5-dimethylamphetamine
- Butanal, 3-methyl-
- 2-Hexene
- Trans-anti-1-methyl-decahydronaphtha

**Breath (fuzzy logic)**
- Cyclohexane, 1,3-dimethyl-, trans-
- Benzene, 1,4-dichloro-
- Cyclohexane, 1,4-dimethyl-
- 1-Octanol, 2-butyl-
- 2-Butanone
- Naphthalene, 1-methyl-
- Camphene
- Decane, 4-methyl-
- Heptane, 3-ethyl-2-methyl-
- Octane, 2,6-dimethyl-
- Benzene, 1,2,3,4-tetramethyl-
- Bicyclo_3_1_1_hept-2-ene, 3,6,6-trimethyl-
- Cyclohexane, 1-ethyl-4-methyl-, trans-
- _l_-beta_-Pinene

VOC manufactured by Mycobacteria

Excreted in breath: unchanged or as a metabolite
### Oxidative stress products in breath

**Culture (in vitro)**
- Naphthalene, 1-methyl-
- 3-Heptanone
- Methylcyclooctadecane
- Heptane, 2,2,4,6,6-pentamethyl-
- Benzene, 1-methyl-4-(1-methylethyl)-
- Cyclohexane, 1,4-dimethyl-
- 3,5-dimethylnaphthalene
- Butanol, 3-methyl-
- 2-Hexene
- Trans-anti-1-methyl-decahydronaphthalene

**Breath (fuzzy logic)**
- Cyclohexane, 1,3-dimethyl-, trans-
- Benzene, 1,4-dichloro-
- Cyclohexane, 1,4-dimethyl-
- 1-Octanol, 2-butyl-
- 2-Butanone
- Naphthalene, 1-methyl-
- Camphene
- Decane, 4-methyl-
- Heptane, 3-ethyl-2-methyl-
- Octane, 2,6-dimethyl-
- Benzene, 1,2,3,4-tetramethyl-
- Bicyclo_3_1_1_hept-2-ene, 3,6,6-trimethyl-
- Cyclohexane, 1-ethyl-4-methyl-, trans-
- l_-beta_-Pinene

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**Breath signal derived from two sources:**

**Bacterial metabolites AND host response**
SBIR Phase II study – NIH/NIAID

Collaboration:

• University of California San Diego

International multicenter study:

• USA – San Diego
• UK - London
• Philippines – Manila and Cavite

Receiver operating characteristic (ROC) curve

- % true positives
- % false positives
Biomarker accuracy

true positive rate (sensitivity)

false positive rate (1-specificity)

Area under curve
0.5

Worthless test
- no better than flipping a coin
true positive rate

Fair test
alse positive rate

Area under curve
0.6
Area under curve
0.7

true positive rate

false positive rate

Good test
true positive rate

false positive rate

Area under curve
0.8

Very good
true positive rate

false positive rate

Area under curve
0.9

Excellent test
True positive rate = 100%
False positive rate = zero

true positive rate

false positive rate

Area under curve
1.0

Perfect test
Positive for sputum smear and sputum culture and chest x-ray

AUC = 0.85

sensitivity = 84.0%
specificity = 64.7%
Point-of-care breath test
Overview

• Recruit controls and disease group
  → 279 patients

• Breath test in ~6 min
  → upload data to US lab via internet

• Monte Carlo analysis of data
  → biomarker identification

• Combine biomarkers
  → multivariate algorithm
Accuracy = 84% in age-matched subgroups

How useful for finding new cases of TB?
Recruit 1,000 people in a high-burden country
Assume prevalence of TB = 5% → 50 with disease
Perform a screening breath test

Positive (358)

Negative (642)
People with TB

Positive (358)

Negative (642)
True positives

False negatives

Positive (358)

Negative (642)
Positive predictive value (PPV) = true positives/all positives

Negative predictive value (NPV) = true negatives/all negatives
PPV = probability that a person with a positive breath test has TB

NPV = probability that a person with a negative breath test does NOT have TB
Positive breath test
→ High risk group
Risk is 2.4 X higher than pre-test probability of TB

Negative breath test
→ Low risk group
Very low probability of disease

PPV = 11.8%

NPV = 98.8%
A new paradigm for TB case finding?

**Step 1**
Screen population with BreathLink breath test

**Step 2**
- If negative: reassure – no further testing needed
- If positive: sputum testing
Potential benefits

- Reduce sputum testing by ~64%
- Reduce discomfort of sputum induction
- Reduce costs of case finding
  → $ benefit to developing countries
BreathLink breath test for breast cancer

Previous studies in different labs ➔ proof of principle

Breath assays with:
  GC MS
  nanoparticle arrays
  sniffing dogs
HIGH-RISK GENOTYPE
Cytochrome p450 polymorphs
e.g. CYP19, CYP1A1, CYP1B1, and CYP3A4

HIGH-RISK PHENOTYPE
Induced activity: aromatase
other P450 enzymes

C19 ANDROGENS

C18 ESTROGENS

ENZYME INDUCTION

BREAST CANCER

C19 ANDROGENS

C18 ESTROGENS

Detectable changes in breath VOCs

Normal metabolism

HYPOTHESIS
HIGH- RISK GENOTYPE
Cytochrome p450 polymorphs
e.g. CYP19, CYP1A1, CYP1B1, and CYP3A4

HIGH- RISK PHENOTYPE
Induced activity: aromatase other P450 enzymes

C19 ANDROGENS

C18 ESTROGENS

ENZYME INDUCTION

HYPOTHESIS

NORMAL METABOLISM

VOC PRODUCTS

Detectable changes in breath VOCs

BREAST CANCER
The prevalence of breast cancer is low: ~ 4 in 1,000

Screening mammography has a low yield

0.4% → Positive for breast cancer: 0.4 %

Reassurance comes with costs

• Radiation
• Discomfort
• Anxiety
• $$

Rapid Point-Of-Care Breath Test for Biomarkers of Breast Cancer and Abnormal Mammograms

Michael Phillips\textsuperscript{1,2,\*}, J. David Beatty\textsuperscript{3}, Renee N. Cataneo\textsuperscript{1}, Jan Huston\textsuperscript{4}, Peter D. Kaplan\textsuperscript{1}, Roy I. Lalisan\textsuperscript{5}, Philippe Lambin\textsuperscript{6}, Marc B. I. Lobbes\textsuperscript{7}, Mayur Mundada\textsuperscript{1}, Nadine Pappas\textsuperscript{8}, Urvish Patel\textsuperscript{1}

NIH Phase 1 SBIR award
Swedish Cancer Center, Seattle, WA
St. Michael’s Med Center, Newark, NJ
Maastricht University, Netherlands
Methods:

• 244 women:
  
  Screening mammogram or a breast biopsy
  ➔ breath tests with BreathLink system

• Chromatograms segmented into a time series
  ➔ ID significant biomarkers
  ➔ multivariate predictive algorithms
Screening mammography group (n=130)
- Normal (BIRADS 1-2) 93
- Abnormal (BIRADS 3-6) 37

Breast biopsy group (n=114)
- Cancer negative 79
- Cancer positive 35
  - Invasive ductal carcinoma 20
  - Ductal carcinoma in situ 8
  - Invasive lobular carcinoma 5
  - Sarcomatoid carcinoma 1
  - Atypical papillary lesion 1
Normal screening mammogram vs breast cancer on biopsy

sensitivity = 81.8%
specificity = 70.0%
AUC = 0.79

cross-validation with LOO
AUC = 0.73
How good is that?

**AUC of ROC curve**

- Breath test 0.79
- **film mammography** 0.74
- **digital mammography** 0.78

Normal vs abnormal screening mammogram

sensitivity = 86.5%
specificity = 66.7%
AUC = 0.83
LOO AUC = 0.62
Cancer vs no cancer on breast biopsy

sensitivity = 75.8 %
specificity = 74.0 %
AUC = 0.78
LOO AUC = 0.67
Putting it all together:

- Breast cancer $\rightarrow$ VOC biomarkers in breath
- Proof of principle in previous studies
  other researchers/other methods
- Rapid POC breath test identified:
  breast cancer vs no cancer
  abnormal vs normal mammogram
Screen 1 million women for breast cancer with BreathLink test

Assume prevalence = 3.95 cancers per 1000

\[
PPV = 1.07\%
\]

High risk

2.7 X pre-test

\[
NPV = 99.9\%
\]

Low probability of disease
A new paradigm for breast cancer screening?

Step 1
Screen population with BreathLink breath test

Step 2
If negative: reassure – no further testing
If positive: → mammogram
Potential benefits

- Reduce screening mammograms by ~70%
- Reduce radiation exposure
- Reduce discomfort
- Reduce costs
Why can’t I get a screening breath test today?

- **Validation studies** – breast cancer, TB
- **More statistical power in blinded studies**
  - convince the world
- **Regulatory approval and insurance payment**
  - FDA, rest of world
Breathtaking technology