Rapid point-of-care breath test for tuberculosis

Michael Phillips MD, FACP, FRCP
Volatile biomarkers of pulmonary tuberculosis in the breath

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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 (52)
- Healthy controls (59)
  - Collect breath VOCs

**Analyze VOCs with gas chromatography/mass spectrometry (GC/MS)**
The BCA 5.0
- All-electronic controls
- Digital display guides the user through every step
<table>
<thead>
<tr>
<th>RT</th>
<th>VOC</th>
<th>Area</th>
<th>Quality</th>
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<tbody>
<tr>
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<td>Culture (in vitro)</td>
<td>Breath (fuzzy logic)</td>
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<td>Naphthalene, 1-methyl-</td>
<td>Cyclohexane, 1,3-dimethyl-, trans-</td>
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<td>3-Heptanone</td>
<td>Benzene, 1,4-dichloro-</td>
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<td>Methylcyclooctadecane</td>
<td>Cyclohexane, 1,4-dimethyl-</td>
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<tr>
<td>Heptane, 2,2,4,6,6-pentamethyl-</td>
<td>1-Octanol, 2-butyl-</td>
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<td>Benzene, 1-methyl-4-(1-methylethyl)-</td>
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<td>Naphthalene, 1-methyl-</td>
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<tr>
<td>3,5-dimethylamphetamine</td>
<td>Camphene</td>
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<td>Butanal, 3-methyl-</td>
<td>Decane, 4-methyl-</td>
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<td>Bicyclo_3_1_1_hept-2-ene, 3,6,6-trimethyl-</td>
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<td>l-<em>beta</em>-Pinene</td>
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## Identical VOCs

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<tr>
<th>Culture (in vitro)</th>
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<tr>
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<td>Camphene</td>
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<td>2-Hexene</td>
<td>Decane, 4-methyl-</td>
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<td>Trans-anti-1-methyl-decahydronaphthalene</td>
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<td>Octane, 2,6-dimethyl-</td>
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<td>Benzene, 1,2,3,4-tetramethyl-</td>
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<td></td>
<td>Bicyclo_3_1_1_hept-2-ene, 3,6,6-trimethyl-</td>
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<tr>
<td></td>
<td>Cyclohexane, 1-ethyl-4-methyl-, trans-</td>
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<td>l__beta__Pinene</td>
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</table>
### Identical and Similar VOCs

**Culture (in vitro)**
- Naphthalene, 1-methyl-
- 3-Heptanone
- Methylcyclohexadecane
- 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

**VOC manufactured by Mycobacteria**

**Excreted in breath: unchanged or as a metabolite**
### 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
- 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

**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

226 high-risk patients
All symptomatic
e.g. cough, fever, night sweats, weight loss

INVESTIGATIONS
Sputum culture  *(Bactec 460, Becton Dickinson)*
Sputum microscopy  *(AFB staining)*
Chest X-ray

BREATH TEST
Breath VOCs:

Correlation with different diagnostic criteria
Positive for sputum smear and sputum culture and chest x-ray

AUC = 0.85

sensitivity = 84.0%
specificity = 64.7%
2010: Lab-based breath test for TB

Advantages:
- ID biomarkers with GC/MS
- ID disease - 85% accuracy

Disadvantages:
- Inconvenient – ship samples to lab
- Expensive – GC/MS ~$200K + highly trained staff
- Slow turnaround time
Point-of-care breath test for active pulmonary TB
The evolution of a successful diagnostic test

**Pregnancy tests**

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

- **1960** Immunological tests
  - Faster
  - Cost $$

- **1976** First home test kit
  - Fastest
  - Cost ~$10
**Lab Test**

- Basic R&D (GC/MS) → identify biomarkers

**Point-of-care test**

- Migrate biomarkers to POC instrument → cheaper, quicker

**Personal test**

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

- Collects breath volatile organic compounds (VOCs)
- Concentrates VOCs
- Separates VOCs with GC
- Detects VOCs with SAW
- Interpretation in <10 minutes
**BreathLink™**

- Breath analysis anywhere in the world
  - Upload data over internet
    - JONES, J
- Menssana Research, Inc. analysis of breath data
  - Final report
    - Sent over internet
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
Study: Active pulmonary TB

Results available in USA within 10 minutes of breath sample collection anywhere in the world.
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
Criteria for active pulmonary TB

Positive sputum culture
and/or
positive sputum smear microscopy
and/or
chest x-ray consistent with active pulmonary TB.
## Control group

<table>
<thead>
<tr>
<th>Site</th>
<th>No. recruited</th>
<th>Unsatisfactory</th>
<th>Satisfactory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cavite</td>
<td>47</td>
<td>5</td>
<td>42</td>
</tr>
<tr>
<td>London</td>
<td>17</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Manila</td>
<td>52</td>
<td>6</td>
<td>46</td>
</tr>
<tr>
<td>Mumbai</td>
<td>22</td>
<td>2</td>
<td>20</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>138</strong></td>
<td><strong>17</strong></td>
<td><strong>121</strong></td>
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## Disease group

<table>
<thead>
<tr>
<th>Site</th>
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<th>Technical quality of breath test</th>
<th>Smear positive</th>
<th>Culture positive</th>
<th>Chest x-ray positive</th>
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<td></td>
<td>Not OK</td>
<td>OK</td>
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<td>4</td>
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<td>7</td>
<td>41</td>
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<tr>
<td>Total</td>
<td>141</td>
<td>11</td>
<td>130</td>
<td>126</td>
<td>96</td>
</tr>
</tbody>
</table>
Analysis of data

Segment the chromatograms into series of 100 candidate biomarkers by retention times compared to alkane standards

Kovats Index (KI) windows
Is it a new biomarker?  
Or is it just a face in the clouds?

- Costs of assays falling
- BUT...costs of human and animal studies rising
- > 1,000 candidate biomarkers, <100 subjects
  - high risk of over-fitting data in statistical models
  - false-positive IDs of biomarkers
    - seeing “faces in the clouds”
A rigorous method for biomarker identification

1. What was the experimental accuracy of a candidate VOC?
   Area under curve (AUC) of receiver operating characteristic (ROC) curve
   Range: 0.5 (coin-flip) to 1.0 (perfect accuracy)

2. What was its accuracy by chance alone?
   Multiple Monte Carlo simulations
   Assign random diagnosis (disease or no disease)
   → determine AUC of the ROC curve
   → repeat 40 times
   → mean chance accuracy

3. Compare the outcomes
No. candidate biomarkers

1.0

Chance accuracy with random diagnosis

True accuracy with correct diagnosis

Biomarkers with accuracy better than chance

AUC of biomarker ROC curve
Disease group
Technical quality of breath test
Smear positive
Culture positive
Chest x-ray positive
Total positive**

<table>
<thead>
<tr>
<th>Site</th>
<th>No. recruited</th>
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<tr>
<td>Cavite</td>
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</tr>
<tr>
<td>London</td>
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<td>Manila</td>
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<td>Total</td>
<td>141</td>
<td>11</td>
<td>130</td>
</tr>
</tbody>
</table>

Mean of multiple Monte Carlo simulations

No. KI windows vs. C-statistic cutoff value

Correct
Random
Random + 1 SD
<table>
<thead>
<tr>
<th>Biomarker number</th>
<th>KI window</th>
<th>Breath biomarker VOCs</th>
<th>M. tuberculosis in vitro VOCs</th>
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<tbody>
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<td>1 &amp; 2</td>
<td>965-1030</td>
<td>camphene; l-beta-pinene; benzene, 1,3,5-trimethyl-</td>
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<td>3 &amp; 4</td>
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<td>naphthalene, 1-methyl-; tridecane; 1-octanol, 2-butyl; dodecane, 4-methyl</td>
<td>naphthalene, 1-methyl-</td>
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</tbody>
</table>
Accuracy = 84% in age-matched subgroups

Sensitivity = 84.7%
Specificity = 66.7%

Is that good enough for clinical use?
Clinical role of BreathLink

“Rule out” test for primary screening

NOT a “rule in” test for final diagnosis
Predicted outcome of using BreathLink

Screen 10,000 people
Assume prevalence of active TB = 5%
Screen 10,000 people
Assume prevalence of active TB = 5%

Breath test

- Disease: 500
  - True positives 424
  - False negatives 76

- No disease: 9,500
  - True negatives 6,337
  - False positives 3164

Predictive values

Positive PV = 424/3588 = 11.8%

Negative PV = 6337/6413 = 98.8%
Staged case finding

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

**Step 2**
- If negative: reassure – no further testing
- If positive: sputum testing
Staged diagnosis with two sequential tests

Test 1
Test 2
Disease

Screened population
Number of sputum tests

With BreathLink: 3,588
Without BreathLink: 10,000

Reduction: 64%
Breath tests and sputum tests are complementary, NOT competitive.
Comparison: BreathLink breath test and Xpert sputum test

<table>
<thead>
<tr>
<th></th>
<th>BreathLink</th>
<th>Xpert</th>
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<tbody>
<tr>
<td>Sensitivity</td>
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<td>86.1</td>
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<tr>
<td>Specificity</td>
<td>66.7</td>
<td>95.0</td>
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<tr>
<td>Speed</td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td>Cost</td>
<td>Low</td>
<td>High</td>
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</tbody>
</table>

Current practice:  
Sputum testing in everyone

A safe alternative strategy:  
BreathLink breath test in everyone

• If negative:  
  reassure  →  retest in future

• If positive:  
  →  sputum testing
Outcome

Case discovery rate: No change

Cost of case finding: Reduced +++
Other potential applications of BreathLink

Prospective studies
  – response to treatment?

MDR strains
  – ID infections?
Does the breath VOC signal change with treatment?

**Rationale:** VOC products of MTB in breath

**Proposal:** Perform prospective study of BreathLink in treated patients
Is the breath VOC signal changed in MDR infection?

Proposal: Planned in-vitro study of MDR strains
Two-dimensional gas chromatography and time-of-flight mass spectrometry
BCG and M. gordonii

- Comparing Media & MG, BCG – Response in MG & BCG has higher level than Media
Thanks for listening!