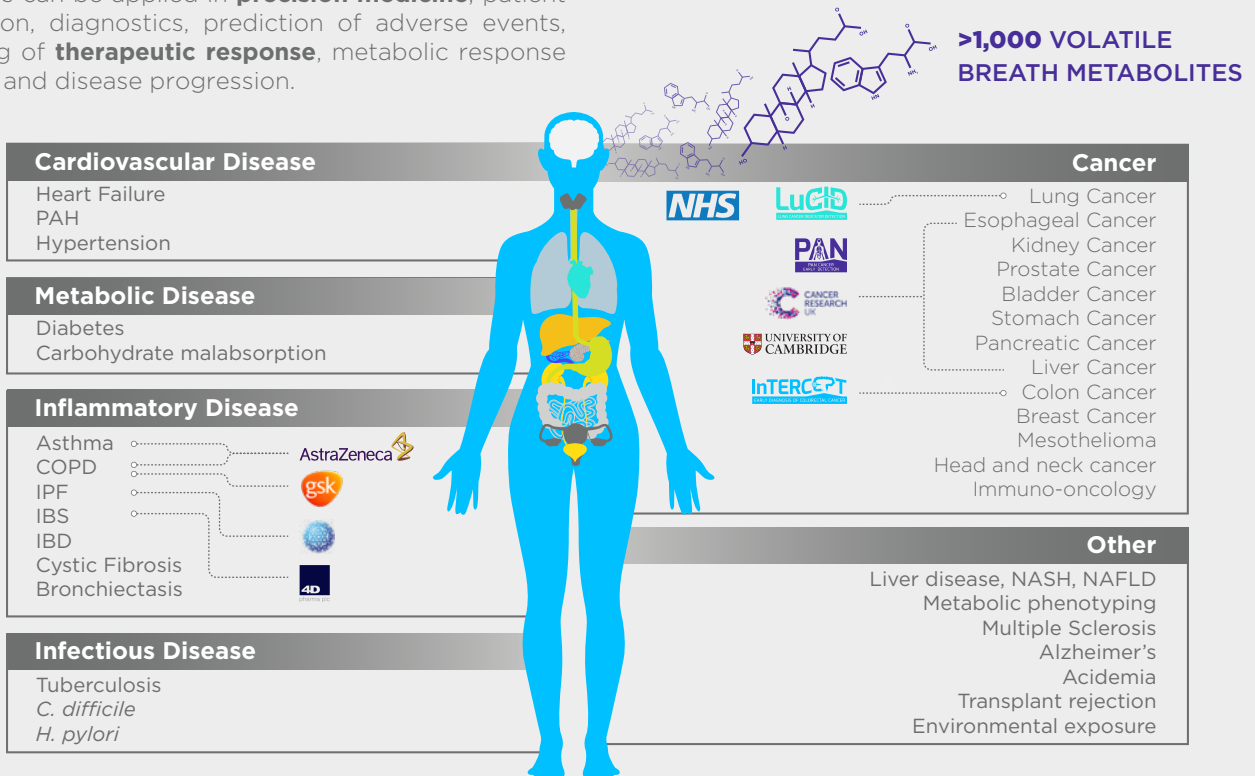


## Breath Biopsy Clinical Trials - A Wide Range of Application Areas

Breath VOCs have been reported for a wide range of applications. The diagram below shows the Breath Biopsy clinical trials completed or in progress, and additional areas where Breath Biopsy is applicable. Breath biomarkers can be applied in **precision medicine**, patient stratification, diagnostics, prediction of adverse events, monitoring of **therapeutic response**, metabolic response to stimuli, and disease progression.

## Integrate Breath Biopsy Into Your Clinical Trial

Our Breath Biopsy Services integrate seamlessly into clinical trials, enabling you to discover breath-based biomarkers for early disease detection and precision medicine applications.



### Volatile Chemicals in Breath

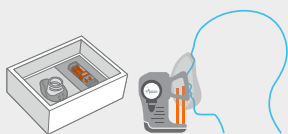
- Endogenous metabolites, some of which originate from the airways, but most of which are derived from the blood.
- Exogenous compounds related to drug metabolites, diet, air pollutants and metabolites from the microbiome.

### Why Breath?

- A real-time dynamic source of valuable information about the health of an individual, as well as lifestyle and environmental factors.
- Completely non-invasive sample collection; samples shipped under ambient conditions.

### Our Breath Biopsy Services combine breath collection with analysis in our established Breath Biopsy Laboratory

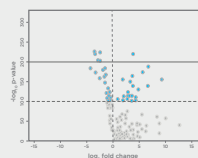
The Breath Biopsy Laboratory analyzes breath samples shipped from clinical sites around the world, including our own clinical trials of up to 4,000 patients.



Install **Breath Biopsy Collection Station** with **ReCIVA Breath Sampler** at clinical trial sites; **Breath Biopsy Kits** provided to sites for sample collection



Send Breath Biopsy samples to Owlstone Medical for **comprehensive VOC analysis**



**Owlstone Medical** performs statistical analysis such as **machine learning** and delivers a report including **biological interpretation**

Learn more at:

[owlstonemedical.com/services](http://owlstonemedical.com/services)

# Breath Biopsy Services: Three Steps to Robust Breath Biomarker Discovery

## Breath Collection

We install the Breath Biopsy Collection Station including ReCIVA Breath Sampler at clinical sites and provide everything required for reliable, reproducible breath collection in clinic, including training and support of trial staff, and scheduled maintenance throughout the study lifetime.

- Pre-concentrates VOCs onto a Breath Biopsy Cartridge for high sensitivity.
- High patient safety and comfort.
- CE Marked.
- In use in the world's largest breath-based clinical trials, at over 100 clinical sites around the world.
- CASPER Portable Air Supply provided to minimize contamination of breath samples by external VOCs.
- We provide site initiation (installation and training) and site support.



## Sample Analysis

We provide a regular supply of **2.0 Breath Biopsy Discovery VOC Kits**, manufactured and quality checked to the exacting standards required for the analysis of VOC biomarkers in breath. After sampling, the Breath Biopsy Cartridges are returned to Owlstone Medical for analysis.

Each Breath Biopsy Discovery VOC Kit includes:

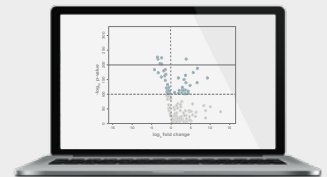
- One conditioned Breath Biopsy Cartridge.
- One disposable Breath Biopsy Mask.
- Comprehensive VOC analysis performed in Owlstone Medical's Breath Biopsy Laboratory using our thermal desorption gas chromatography mass spectrometry (TD-GC-MS) Breath Biopsy platform.



## Data Analysis

Our data science team uses statistical analysis including **machine learning algorithms** to analyze the VOC profile, combined with subject medical history and clinical labels, in order to distinguish between patient populations. Choose from the following three types of analysis:

- **Cross sectional** (identification of differences between Group A vs. Group B).
- **Longitudinal** analysis of changes in the VOC profile of a subject over time (studies including more than 2 samples per subject).
- **Pre-/Post-** (analysis of differences between samples collected from the same subject e.g. pre- vs. post-intervention).



We provide a comprehensive report detailing the results of our analysis including **biological interpretation** of the results. Our data analysis includes the following:

- Univariate feature exploration including volcano plots and box plots.
- Machine Learning such as Random Forest and Partial Least Squares Discriminant Analysis.
- Consideration of potential covariates including clinical and sampling variables.

Download the Example Report:

[owlstonemedical.com/discovery-report](https://owlstonemedical.com/discovery-report)

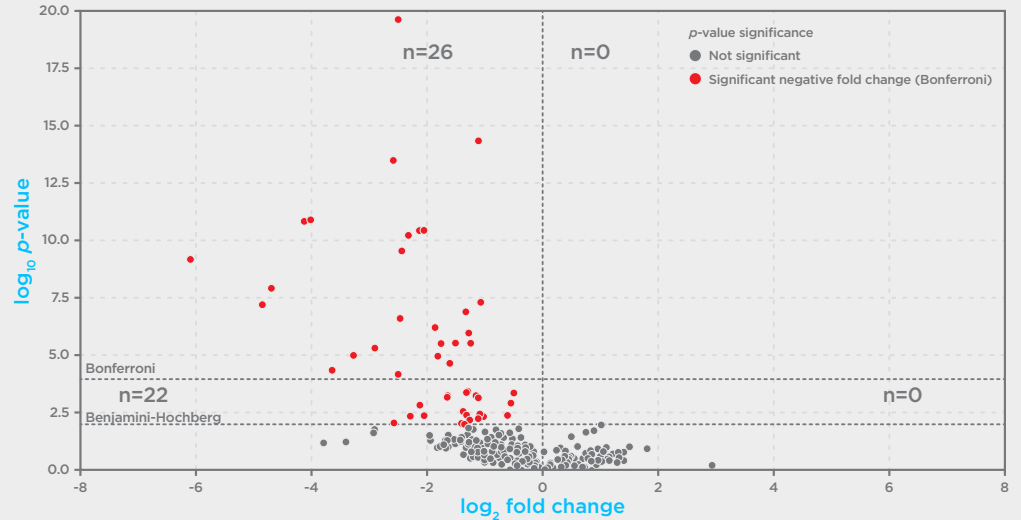


# Case Study: Breath Biomarkers Discriminate Non-Smokers vs. Smokers

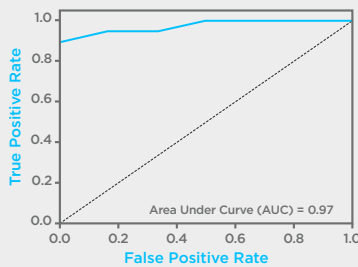
We analyzed 475 Molecular Features (MFs) extracted from the exhaled breath samples of 73 subjects (56 Non-smokers and 17 Smokers). We found that 26 MFs were significantly different between the groups. Combinations of MFs, obtained by different machine learning algorithms (Random Forest and Partial Least Squares Discriminant Analysis), discriminated between the groups with an accuracy of 97% and 96%, respectively, and were validated by a 10-fold cross-validation method.

<b>1. SUMMARY</b>
<b>2. UNIVARIATE FEATURES EXPLORATION</b>
a. Features Table
b. Volcano plot and box plot
<b>3. MACHINE LEARNING</b>
a. Random Forest
• ROC Curve k fold Validation
• Model Evaluation
• Contributing Features
• Shuffled Labels
• Confusion Matrix Details
b. Partial Least Squares Discriminant Analysis
• ROC Curve k fold Validation
• Model Evaluation
• Shuffled labels
• Confusion Matrix Details
c. Dummy Classifiers
<b>4. COVARIATES ANALYSIS</b>
a. Principal Component Analysis

A Volcano plot enables visual identification of those features that display statistically significant, large magnitude fold changes between classes.



A ROC Curve is used to assess the performance of the classifier after multiple rounds of cross-validation.



Box plots visualize the distribution of the peak area for individual features. A tentative ID is assigned based on comparison against the NIST unit mass spectral library.



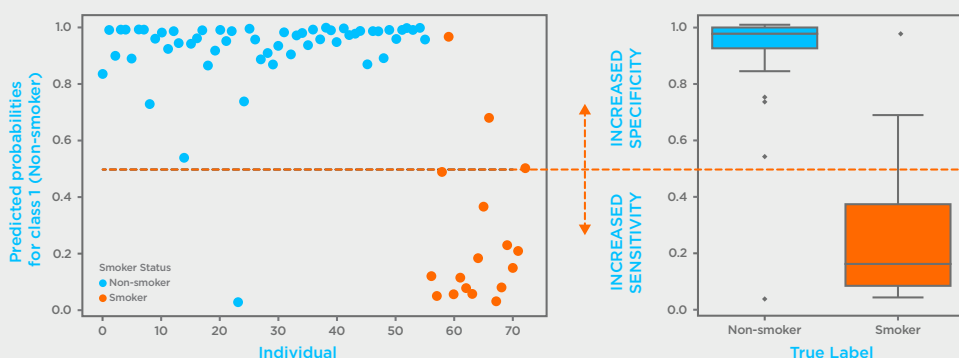
The tables on the right show  $p$ -value and log<sub>2</sub> fold change between classes (non-smokers vs. smokers) for specific MFs. Statistically significant  $p$ -values below the Benjamini-Hochberg cut-off are shown in yellow.

## Subject classification at Non-smoker threshold 0.5

		Breath Test (Predicted Label)		
		Smoker	Non-smoker	Total
True label	Smoker	15 True Negatives	2 False Positives	17
	Non-smoker	1 False Negatives	55 True Positives	56
Total		16	57	73

## Performance of the Classifier

Sensitivity (55/56) ..... 98%  
 Specificity (15/17) ..... 88%  
 Positive Predictive Value (55/57) ..... 96%  
 Negative Predictive Value (15/16) ..... 94%



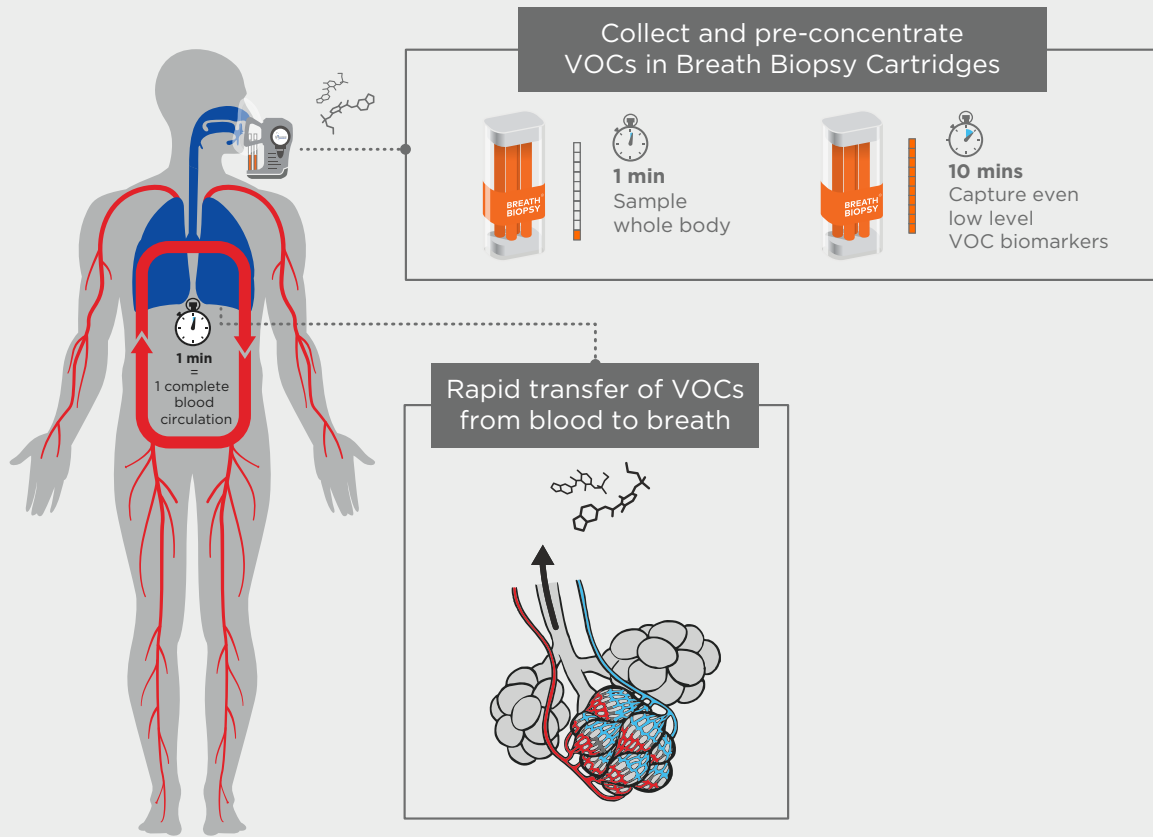
Confusion matrix describing the performance of the Random Forest Classification Model to classify the samples (top). True labels are based on self-reported smoking status. The figures describe the prediction probabilities of individual samples (bottom left), and box plots of each class (bottom right). Dashed line represents the Non-smoker threshold (0.5 was selected as optimal).

# Analyze Systemic VOC Biomarkers with Breath Biopsy

VOCs are produced throughout the body and circulate via the bloodstream. The lungs efficiently exchange volatile metabolites from blood to breath.

The Breath Biopsy Platform therefore enables the collection and analysis of VOCs originating from throughout the body, as well as VOCs originating from local airways tissue.

- Systemic and local VOC biomarkers can be analyzed.
- High sensitivity for the detection of breath biomarkers due to pre-concentration of VOCs in the Breath Biopsy Cartridge.



## Breath Biopsy Services at a Glance

- Seamless integration into your clinical trials
- Standardized breath collection parameters across entire study
- Dedicated project manager and consultation with our experts throughout the study
- Full support, training and maintenance of Breath Biopsy Collection Stations in the clinic
- World's first and only Breath Biopsy Laboratory with state-of-the-art workflows optimized for breath analysis
- Rigorous QC processes ensure high quality data
- Data science team provides statistical analysis including machine learning
- Comprehensive report including biological interpretation

**BREATH BIOPSY**

[owlstonemedical.com/services](https://owlstonemedical.com/services)

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