Introduction

• Exhaled breath condensate (EBC) carries abundant information on airway health and disease, as well as systemic disease.  
• Current EBC samplers focus on volatile compounds and protein biomarkers.  
• This approach does not allow for analysis of biomolecules (e.g. DNA, proteins, metabolites).  
• We are developing an optimised EBC biomolecule sampler and analyser using EBC microbiomics (infectious disease) as a validation approach.

EBC bioanalytics: requirements

• This requires large EBC volume collection.  
• Cannot currently discriminate upper from lower airway components (Fig. 1).  
• Must eliminate environmental contamination.  
• Must prevent sample loss.

Figure 1: Origin of biomarkers from the human airways. Although the airways are typically separated into upper and lower section, diseases affect specific sites across the respiratory tract. E.g. Tuberculosis affects immune cells of the alveolar sacs, asthma affects the bronchi and bronchioles and viral infections can affect various parts of the airways. Current EBC devices collect all exhaled air and are thus unable to select biomarker signals from specific airway regions. As a result, the disease-relevant signal might be too diluted in a volume of irrelevant data arising from respiration.

Materials and Methods

• Total EBC was collected using a First Generation device. Samples were then removed from cartridge and returned to room temperature.  
• EBC volume was measured by pipetting (Fig. 2) using a healthy adult 35 yo white male sampled across 5 consecutive days.  
• DNA extraction was performed using a liquid-based bacteriolytic protocol and purification was carried out using the Life Technologies Purelink genomic DNA extraction kit.  
• PCR amplification was carried out using the KAPA Biosystems KAPA HiFi Polymerase and primers for the V3 region of the 16S rRNA gene.  
• Sequencing template was prepared using the Ion Torrent One Touch and sequencing was carried out using the Ion Torrent Personal Genome Machine and 318 chip according to the manufacturer’s instructions. Data was analysed by QIIME according to RDP and plotted using MEGANS.

PulmoScreen Design, Volumetric & Microbiome Data:

A research-grade airway biomarker collection device for laboratory-based testing:  
• Simple components for in-lab assembly or kit commercialization (Fig. 3).  
• Compact design for community / field use.  
• Compatible with all analytic technologies for volatile and biological compounds.  
• Leverages >20 years of pharmaceutical technology development to provide airway compartmentalization solutions (2nd generation).  
• Consistent sample collection performance (Fig. 2).  
• Efficient detection of airway microbiome in EBC using standardised phylogenetic methods (Fig. 4).

Figure 2: Sample collection performance. Single operator me (A, D) and EBC volume collection (B, C) metrics across a range of sample collection temperatures. Reproducibility across 3-5 breaths is shown, with performance at optimal temperature range depicted in (C) and (D). Data relevant to single airway compartment only, obtained across 5 consecutive days in a controlled environment using a single subject.

Figure 3: PulmoScreen design. The first generation device (A) is a total EBC collection system engineered to isolate EBC from environmental contamination and prevent salivary contamination through inertial impaction (red surface). A retractable drawer arrangement (B) facilitates sample isolation preventing both sample loss and environmental contamination. The second generation device (B) utilises the same sample isolation principle in cascade impactor array format to enable sample separation by particle size. A single impaction stage drawer assembly is shown, with the lid retracted. The lid is equipped in a separated housing to ensure EBC collection stage isolation after sampling. This opens up use for segmented airway bioanalysis as well as non-invasive inhaled pharmaceutical deposition metrics.

Figure 4: Detection of airway microbiome by 16S next generation sequencing using the first generation instrument. PCR amplification of the V3 variable region of the microbial ribosomal RNA gene increases according to EBC sample size (total no. of breaths analysed). A 16S V3 amplicon denotes detection of bacteria. No amplicon was detected in either PCR reaction control or filtered-air condensates, indicating signal arises from the airway microbiome. Phylogenetic analysis of airway microbiome population resembles observations with invasive techniques suggesting a dominance of airway microbiome signal. Sequencing was carried out using a 318 chip on the Ion Torrent Personal Genome Machine; chip loading efficiency (C), read quality (D) and read length (E) metrics are shown. Comparison of four independent EBC microbiome signatures (EBC A-D) to paired salivary microbiome samples (SAL A-D) demonstrates distinct microbial populations in the two loci. Rarefaction analysis confirms adequate microbial population sampling (D).

Progress to commercialisation:

• Rapid prototype device successfully produced.  
• Prototype tested to demonstrate sample sealing and isolation from environmental contamination (Fig. 5).  
• Patent awarded and patent pending in preparation for commercial production.  
• 3rd generation device development under way.

Figure 5: Liquid immersion tests demonstrate sampler isolation from environmental contamination. Ingress of liquids (A) causes bubble formation, indicating sample contamination risk. Appropriate modification of components (B and C) ensure complete isolation between the collector and lid (A), mouthpiece assembly (B) and system housing (C).

Conclusions

• PulmoScreen is an efficient system for EBC sample collection and laboratory based sample analysis.  
• Our technology enables EBC segregation across airway compartments.  
• Analytical technique high sensitivity permits microbiomic analyses in as little as a single breath.  
• General improvements have projected the next design phase that will include several modular, non-invasive modifications such as:

3rd Generation

✓ Integrated T control and sensor circuitry.  
✓ On-the-fly sample extraction technology.

References

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4. Craig Clements, Amie Wilkinson, Kavit Shah and Sterghios A. Moschos