

Volatilomics for Medical Microbiology Diagnostics using Standard Bacterial Headspace Sampling

Mehmet Berk Bilgin¹, Norma Toccacino¹, Emma Slack^{2,3,4}, Adrian Egli⁵, Andreas Güntner¹

¹ Human-centered Sensing Laboratory, D-MAVT, ETH Zürich, Zürich, Switzerland

² Laboratory for Mucosal Immunology, Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland

³ Botnar Institute of Immune Engineering, Basel, Switzerland

⁴ Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

⁵ Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland
andreas.guentner@hsl.ethz.ch

Summary:

This presentation advocates for the use of volatilomics as an alternative rapid microbiological testing method. A critical bottleneck is the reliable and controlled sampling of volatile biomarkers in the headspace of bacterial species. We will show exemplarily volatilome measurement with an online headspace sampling setup to track metabolic activity via headspace microbial volatile compound (mVOC) concentrations by mass spectrometry. We identify key translational gaps impeding clinical adoption.

Keywords: Volatilomics, Headspace sampling, Antibiotic susceptibility testing, Microbiological sensing, Novel diagnostics.

Background, Motivation and Objective

Detection and identification of bacteria based on their mVOC emissions is a promising approach for rapid diagnostics [1,2]. Although some bacterial odors have been utilized in infection diagnostics since early modern times[3], the advent of high-resolution analytical techniques, like mass spectrometry, has enabled accurate mVOC quantification, paving the way for targeted biomarker analysis to reduce the time-to-result for AST from 48 hours to just a few hours (Figure 1) using compact, selective, and sensitive sensors [4–6]. Rapid point-of-care testing can drastically improve the disease prognosis and significantly contribute to the antibiotic stewardship efforts. Such clinical devices for fast pathogen discrimination can only be realizable with representative and reproducible sampling of the headspace to extract the volatilome at different timepoints. However, the critical role of sampling has been overlooked, and the variability in sampling methodologies has hindered direct comparability across studies. To date, no approach has established a standardized, robust method for longitudinal volatilome monitoring. This inconsistency undermines reproducibility and has left the relationship between volatilome composition and microbial metabolism insufficiently understood. The presented system aims to close the critical gap between mVOCs and microbial functions by proposing a reliable way of controlled volatilome extraction for both mass spectrometric and

chemoresistive sensor measurements. The presentation starts with the outline of the envisioned workflow for an alternative microbiological diagnostic test. Individual steps for a successful test are discussed with an emphasis on the sampling approach. The recent advancements in the field are discussed with a future outlook.

Description of the Approach

We start with the proposed rapid volatilomics pathway as a diagnostic alternative. Sampling is the first step of the analysis: the extracted headspace volume must be a representative part of the bacterial volatilome. To ensure accuracy, we present the online headspace sampling setup for reliable and reproducible controlled volume extraction. As a crucial part of the system, parametric sweep of the dilution ratio was performed to meet the dynamic range limits of the analytical tool. The sampling accuracy is validated with mass spectrometry and analyte gas standards. Headspace mVOC concentrations are obtained from preliminary bacteria measurements for biomarker identification, which are particularly important for onsite testing, as target biomarker information must be available for selective detection of volatiles using compact sensors. The resulting multidimensional sensor data can be processed with machine learning algorithms to create a diagnostic report.

Results

The proposed setup is validated with acetone, ethanol, benzene, and acetaldehyde gas standards for reliable volume extraction down to ppb-level concentrations. *Escherichia coli* metabolic changes across different stages of incubation, and these changes are reflected in the preliminary headspace concentrations (Figure 2). Although the acetaldehyde concentrations serve as the main indicator of the growth phase, the results show that tracking similar volatiles with mass spectrometry and sensors can yield bacterial metabolic information for pathogen characterization. Finally, we emphasize the role of machine learning for the interpretation of such complex data for diagnostics.

Conclusion:

Volatilomics holds exceptional promise for rapid microbiological diagnostics, particularly in rapid antibiotic susceptibility testing. By establishing a standardized, reproducible headspace sampling platform compatible with both analytics and point-of-care sensors, our work bridges a critical gap between volatilome dynamics and microbial physiology. Combining the presented system with compact, sensitive, and selective sensor to unlock complex volatile data using machine learning algorithms, we envision volatilomics as an enabler for same-hour clinical diagnostics, antimicrobial stewardship enhancement, and a significant improvement of clinical infection management.

Figures

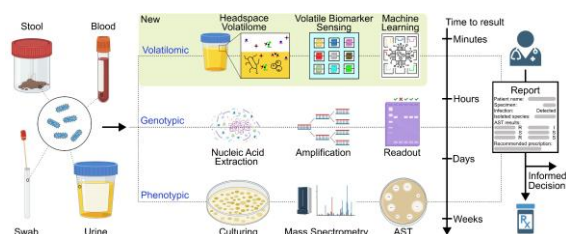


Fig. 1. Envisioned volatilomics and current workflows in medical microbiological pathogen and AST diagnostics [6]

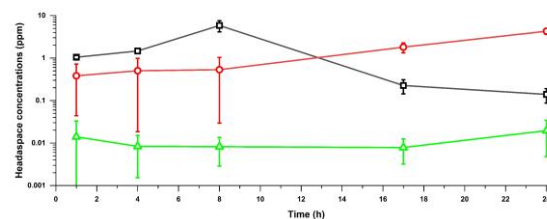


Fig. 2. Temporal profiles of the headspace concentrations of selected molecules from *Escherichia coli* culture

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