Volatile organic compound signature from co-culture of lung epithelial cell line with *Pseudomonas aeruginosa*

Oluwasola Lawal\(^1\)\(^5\), Hugo Knobel\(^2\), Hans Weda\(^3\), Lieuwe D. Bos\(^4\), Tamara M.E. Nijsen\(^3\), Royston Goodacre\(^5\), Stephen J. Fowler\(^1\)\(^6\) on behalf of the BreathDx consortium

\(^1\)Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom

\(^2\)Philips Innovation Labs, Philips Lighting, Eindhoven, The Netherlands

\(^3\)Philips Research, Royal Philips B.V., Eindhoven, The Netherlands

\(^4\)Department of Respiratory Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

\(^5\)School of Chemistry, Manchester Institute of Biotechnology, University of Manchester, United Kingdom

\(^6\)Manchester Academic Health Science Centre, The University of Manchester and Manchester University NHS Foundation Trust, Manchester, United Kingdom

SUPPLEMENTARY MATERIAL
A549-bacterial optimisation

A multiplicity of infection (MOI) of 50 and 100 were tested on A549 cells seeded in T25 flasks. After cell count by Trypan blue dye exclusion, both MOIs yielded a comparable number of viable cells (Fig S1). A MOI of 100 was subsequently used for the infection experiment.

**Fig S1.** Viable cell count by Trypan blue exclusion after infection of A549 cells in T25 flasks. The averages of three replicates are shown for each condition and the error bars represent standard errors (SE).
Treatment of A549 cells with H$_2$O$_2$

A549 cells were treated with varying concentrations of H$_2$O$_2$ and cell viability was determined using the alamarBlue™ assay. High fluorescence intensity indicates a large proportion of viable cells. Decreasing fluorescence intensity indicating cell death was observed from 10 mM dose (Fig S2).

**Fig S2.** Cell viability of A549 epithelial cell determined by alamarBlue assay after treatment with varying concentrations of H$_2$O$_2$. The error bars represent SE of eight repeats.

**Viable cell count after hydrogen peroxide treatment**

A representative viable cell count is shown following 100 mM H$_2$O$_2$ treatment of A549 epithelial cells in glass bottles and counted after headspace collection (Fig S3).
**Fig S3.** Viable cell count by Trypan blue exclusion after treatment of A549 cell in glass bottles with 100 mM H$_2$O$_2$. The averages of three replicates are shown for each condition and the error bars represent SE.

**PC-DFA loadings plot**

The loadings plots from PC-DFA analysis are shown in Fig S4. The variables at the extreme ends of the loading plots were investigated to determine those features that contributed most to the observed separation in the scores plot (Fig 6). Fragments 1038 and 506 belongs to 3-methyl-1-butanol and ethylidenecyclopropane respectively (Fig S4A) and fragment 94 to methyl tert butyl ether (Fig S4B). Fragments 16, 730 and 478 represent tert-butyl ethyl ether. The identity of the other fragments is still unknown.
**Fig S4.** PC-DFA loadings plot for (A) DF1, (B) DF2.