**European Partnership on Metrology**
**Call 2025 – Health, Integrated European Metrology, Regulation and Research Potential**

Selected Research Topic number: **SRT-h08**
Version: 1.0

# Title: Metrology for breath gas analysis


## Abstract

The analysis of exhaled human breath (breath gas analysis) offers a non-invasive method for detecting biomarkers linked to various health conditions, with the potential to revolutionise clinical diagnostics, disease monitoring, personalised medicine, and therapeutic drug monitoring. To ensure reliable adoption in clinical and non-clinical applications advancements in metrology are essential. Proposals addressing this topic should focus on key objectives - accuracy, traceability, reproducibility, calibration, standardised protocols, and uncertainty evaluation - to improve the reliability and comparability of breath gas analysis. These advancements will enable broader clinical use and support the development of consumer health technologies, making breath analysis a robust tool for both medical and everyday applications.



## Keywords

Breath Gas Analysis, Metrology, Breath Sampling, volatile organic compounds (VOCs), proton transfer reaction mass spectrometry (PTR-MS), Spectroscopy, Disease Diagnostics, Disease Monitoring, Personalised Medicine


## Background to the Metrological Challenges

Analysis of exhaled human breath, or breath gas analysis (BGA), holds significant promise as a transformative tool in clinical diagnostics, therapeutic drug monitoring, and broader applications such as fitness tracking or the measurement of oxidative or mental stress. BGA offers a non-invasive, rapid, and potentially cost-effective alternative to conventional diagnostic methods by detecting biomarkers in exhaled breath. BGA must overcome substantial challenges related to accuracy, traceability, and comparability of results to fulfil its potential in clinical diagnostics.

The variability in existing measurement technologies (e.g., mass spectrometry (MS), gas chromatography (GC), or photonics) and their outputs creates uncertainty in detecting, identifying and quantifying biomarkers, especially at the low concentration (amount-of-substance fraction) levels required for early disease detection, and limits a consensus agreement within the community. Biomarkers such as volatile organic compounds (VOCs), ammonia ($NH_3$), and fraction of exhaled nitric oxide ($F_ENO$) are often present at trace concentrations, requiring precise and reproducible detection methods. In this context, traceability - ideally, SI traceability - is urgently needed. The iMERA-Plus project T2.J02 - Breath analysis as a diagnostic tool for early disease detection - focused on absorption spectroscopy in combination with techniques like cavity ringdown spectroscopy (CRDS) and demonstrated detection limits at the ppq-level (part per quadrillion or $10^{-15}$). SI-traceability can be realised directly, and uncertainties can be calculated. Other methods mainly used in BGA rely on calibrations (ideally before and after each measurement) and absolute uncertainties are rarely addressed with appropriate metrological care (within the framework of the Guides to the expression of uncertainty in measurement (GUM), JCGM 100:2008 and related documents). Target uncertainties depend on marker molecules, corresponding concentrations (amount fraction), analytical technologies, gas compositions, and potential applications, but it is considered that should remain below 5% ($k$=1).

Some progress has been made regarding reference materials through "*breath in a bottle*" by NIST in the USA and a breath surrogate delivery system to create matrix-matched materials that mimic the relevant characteristics of breath. Many relevant biomarkers in exhaled breath are volatile organic compounds (VOCs). The main issue of these species is their high reactivity causing every contact with possible surfaces to be a potential loss of substance making sampling, storage and analysis of breath even more complex. The previous


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EMRP projects ENV56 KEY-VOCs and ENV52 HIGHGAS, the EMPIR project 19ENV06 MetClimVOC and the Metrology Partnership project 21GRD10 quantiAGREMI have set a firm foundation for static and dynamic production of reference gas mixtures for a selected group of VOCs and ammonia.

A primary concern, aside from technological strides like the lack of SI-traceability, is the lack of standardisation in breath sampling, storage, and transportation protocols. Variations in these procedures are leading to inconsistent results, making it difficult to compare findings across different studies and laboratories. These issues have been highlighted in recent publications by The European Metrology Network (EMN) for Traceability in Laboratory Medicine. Another factor affecting new BGA technology is the corresponding large amount of data that is generated with new digital technologies making it difficult to analyse and compared among types of outputs. Nevertheless, large amounts of data are important for machine learning and the possibility of measuring the same breath samples with several redundant technologies and different sampling protocols offers the key to making the AI-supported measurement results potentially explainable in such a way that they ideally also provide valid uncertainties.

Despite many promising developments, the current state-of-the-art in BGA is not yet fully equipped to meet the rigorous demands of clinical diagnostics and other applications. Without standardisation and traceability based on universally accepted reference materials the variability in results undermines confidence among medical practitioners, researchers, and patients. Addressing these challenges is essential to fully realise the potential of BGA in improving health outcomes and advancing personalised medicine.

## Objectives

Proposers should address the objectives stated below, which are based on the PRT submissions. Proposers may identify amendments to the objectives or choose to address a subset of them in order to maximise the overall impact, or address budgetary or scientific / technical constraints, but the reasons for this should be clearly stated in the protocol.

The proposal shall focus on the traceable measurement and characterisation of breath gas analysis.

The specific objectives are

1. To establish traceability to SI units for measurement technologies on mass spectrometry (MS) (e.g. proton transfer reaction (PTR), selected ion flow tube (SIFT), secondary electrospray ionisation (SESI), gas chromatography (GC), ion mobility spectrometry (IMS), laser absorption spectroscopy (LAS)) used to detect potential biomarkers, such as volatile organic compounds (VOCs), labelled carbon dioxide ($^{13}CO_2$), ammonia ($NH_3$), fraction of exhaled nitric oxide ($F_ENO$) at trace concentrations, down to the nmol mol$^{-1}$ range. This should include a target uncertainty below 5 % ($k$=1).
2. To develop a metrological calibration infrastructure for measurement technologies addressed in Objective 1. This should include developing static and dynamic reference gas materials in breath relevant matrices to ensure accurate quantification of markers that are tied to universally recognised reference standards.
3. To evaluate existing and novel breath sampling, storage, and transportation protocols while minimising variability and bias, ensuring comprehensive documentation of relevant metadata. This evaluation should include environmental factors (e.g., temperature, humidity, and environmental contaminations), medications, physiological influences (e.g., breath pattern, posture, and time since last eating or drinking), and sample conditioning processes (e.g., pre-concentration or water vapour removal).
4. To verify and validate measurement accuracies, repeatability and reproducibility within and among laboratories, and to quantify uncertainties to enable comparison across methods and technologies, achieving consistent results by different methods (GC-MS, PTR-MS, SIFT-MS, SESI-MS, IMS, LAS, and other sensors). This should include data modelling and analysis as well as testing the feasibility of improvements through AI-based support.
5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain, standards developing organisations, other stakeholders (such as medical non-profit organisations (International Association of Breath Research (IABR), the European Respiratory Society (ERS)) and end users (e.g. clinical stakeholders, manufacturers of medical and healthcare products).

These objectives will require large-scale approaches that are beyond the capabilities of single National Metrology Institutes and Designated Institutes, and it is expected that multidisciplinary teams will be required. To enhance the impact of the research, the involvement of the appropriate user community such as medical

practitioners, medical (academic) hospitals and industry is strongly recommended, both prior to and during methodology development. Where relevant, proposals are encouraged to build on, or seek collaboration with, existing projects and develop synergies with other relevant European, national or regional initiatives and funding programmes. In particular, links are encouraged with (i) the projects funded under earlier relevant topics of the Horizon Europe programme; or (ii) other relevant European Partnerships.

Proposers should establish the current state of the art and explain how their proposed project goes beyond this. In particular, proposers should outline the achievements of the iMERA-Plus project T2.J02, the EMRP projects ENV56 KEY-VOCs and ENV52 HIGHGAS, the EMPIR project 19ENV06 MetClimVOC and the Metrology Partnership project 21GRD10 quantiAGREMI and how the proposal will build on those.

Proposers should note that the programme funds the activity of researchers to develop the capability, not the required infrastructure and capital equipment, which must be provided from other sources.

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 2.1 M€ and has defined an upper limit of 2.6 M€ for this proposal.

EURAMET also expects the EU Contribution to the external funded beneficiaries to not exceed 35 % of the total EU Contribution across all selected projects in this TP.

Any industrial beneficiaries that will receive significant benefit from the results of the proposed project are expected to be beneficiaries without receiving funding or associated partners.

## Potential Impact

Proposals must demonstrate adequate and appropriate participation/links to the 'end user' community, describing how the project partners will engage with relevant communities during the project to facilitate knowledge transfer and accelerate the uptake of project outputs. Evidence of support from the "end user" community (e.g. letters of support) is also encouraged.

You should detail how your proposal's results are going to:

- Address the SRT objectives and deliver solutions to the documented needs,
- Feed into the development of urgent documentary standards through appropriate standards bodies,
- Facilitate improved industrial capability, or improved quality of life for European citizens in terms of personal health, protection of the environment and the climate, or energy security,
- Transfer knowledge to the healthcare, diagnostics, and drug monitoring sectors.

You should detail other impacts of your proposed JRP as specified in the document "Guide 4: Writing Joint Research Projects (JRPs)"

You should also detail how your approach to realising the objectives will further the aim of the Metrology Partnership to develop a coherent approach at the European level in the field of metrology and include the best available contributions from across the metrology community. Specifically, the opportunities for:

- improvement of the efficiency of use of available resources to better meet metrological needs and to assure the traceability of national standards
- the metrology capacity of EURAMET Member States whose metrology programmes are at an early stage of development to be increased
- organisations other than NMIs and DIs to be involved in the work.

## Timescale

The project should be of up to 3 years duration.

## Additional information

The links provided in this section are only correct at the time of publication up until the end of the Call year.

These references have been provided by EURAMET.

[1]. EMN Traceability in Laboratory Medicine (TraceLabMed) Orientation paper 2025 Health https://metpart.eu/component/edocman/call-2025-orientation-emn-tlm-health/download.html?Itemid=0

[2]. EMN Mathmet Orientation paper 2025 Health https://metpart.eu/component/edocman/call-2025-orientation-emn-mathmet-all/download.html?Itemid=0

[3]. EMN Mathmet Strategic Research Agenda
https://www.euramet.org/research-innovation/metrology-partnership/strategic-research-and-innovation-agendas

[4]. EMN for Advanced Manufacturing Orientation paper 2025 Health
https://metpart.eu/component/edocman/call-2025-orientation-emn-advanced-manu-health/download.html?Itemid=0