# Publishable Summary for 22HLT06 GenomeMET
# Metrology for genomic profiling to support early cancer detection and precision medicine

## Overview

Cancer is a major burden on European society. Advances in genomics, driven by technologies such as Next Generation Sequencing (NGS) are transforming cancer care, enabling earlier and more accurate diagnosis, guiding therapy selection and driving development of targeted therapies (precision medicine), which improves patient outcomes and health system effectiveness. However, the quality and comparability of genomic profiling currently varies significantly and development of standards and metrological means to support the field are in their infancy. This project aims to address these needs by applying metrological principles to develop reference measurement systems (RMS) to support cancer genomic diagnostics in compliance with the In-vitro Diagnostic Device Regulation (IVDR EU 2017/746).

## Need

Cancer is one of the most significant challenges for European societies and healthcare systems, being the second largest cause of death with more than 1.9 million deaths per year. Horizon Europe's Mission on Cancer has identified earlier diagnosis and implementation of precision medicine as key priorities for reducing deaths, improving health and the cost-effectiveness of health systems.

Precision medicine relies on molecular characterisation of a patient's disease, with genomic profiling central to new treatment models, enabling earlier and more accurate diagnosis/stratification and guiding targeted therapies. The EU Beating Cancer plan recommends genomic profiling for all cancer patients, with the "Cancer Diagnostic and Treatment for All" initiative improving access to new genomic diagnostics.

High quality genomic testing using technologies such as NGS and liquid biopsies is vital for successful implementation of precision medicine. However, NGS relies on complex multi-step workflows to simultaneously analyse large numbers of genomic variants. These are susceptible to major and poorly understood sources of uncertainty, resulting in significant variability and a current lack of comparability thereby impacting patient care and hindering wider implementation.

The standards and RMS to support assay validation and Quality Assurance (QA), including reference measurement procedures (RMP) and higher order methods, i.e., high-accuracy methods with low uncertainty that can be used as reference methods or for value assignment of reference materials, SI-traceable reference materials (RM) and measurement uncertainty (MU) guidance have yet to be established and are urgently needed to support new test development and approval under IVDR EU 2017/746 and implementation by clinical laboratories accredited to quality standards such as ISO 15189 or ISO 17025.

Developing and establishing novel metrological concepts, capabilities and RMS for genomic profiling will require a large-scale, multi-disciplinary and coordinated approach in collaboration with key end-user stakeholders to achieve the collective goals.

## Objectives

The overall objective of this project is to develop metrological capability and to establish metrology frameworks to improve quality and reproducibility of critical processes within genomic profiling workflows as well as RMS for high accuracy SI-traceable cancer gene measurement to improve comparability and support assay validation as required by the IVDR (EU) 2017/746.

The specific objectives are:

**Report Status:**
**PU –** Public, fully open

**Publishable Summary**

Issued: April 2025

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or EURAMET. Neither the European Union nor the granting authority can be held responsible for them.

European Partnership Co-funded by the European Union

The project has received funding from the European Partnership on Metrology, co-financed from the European Union's Horizon Europe Research and Innovation Programme and by the Participating States.

METROLOGY PARTNERSHIP EURAMET

1. To demonstrate the application of Reference Measurement Systems (RMS) to support development, validation, and quality assurance (QA) and external quality assessment (EQA) of genomic IVDs in accordance with EU IVDR 2017/746, including i) the establishment of an initial baseline framework (using outcomes from Objectives 2, 3 and 4), and ii) demonstration of proof of concept using key cancer genomic profiling models (NGS).
2. To establish Reference Measurement Procedures (RMPs) for high accuracy (VCs < 20 %) SI-traceable (to N=1) measurement of key cancer biomarkers and higher order methods to measure critical Quality Control (QC) parameters within genomic profiling workflows to support genomic RMS development.
3. To develop and characterise Reference Materials (RM) and external quality assessment (EQA) materials for genomic profiling in line with ISO 15194, ISO 15711 and JCTLM, with SI-traceable reference values and sequencing datasets, and to use these to establish a framework for SI traceable value assignment and commutability assessment of reference and EQA materials to support genomic IVDs.
4. To develop a framework for determining the measurement uncertainty (MU) in quantitative genomic data and nominal output data in multiparametric genomic profiles.
5. To facilitate take up of the measurement infrastructure, methods and materials developed in the project by the measurement supply chain (via EMN TLM), standards developing organisations (e.g., CEN TC 140 and ISO TC 212), and end users (e.g., healthcare, and medical laboratories, IVD developers, genomics/cancer/pathology institutes, EQA providers, RM producers, instrument/reagent developers, regulators).

**Progress beyond the state of the art and results**

GenomeMET will progress the state of the art by initiating development of novel metrological concepts, RMS and standards needed to support analytical validation and QA of genomic profiling IVDs for cancer patients. This will help enable implementation of accurate, comparable, and traceable genomic profiling for improved diagnosis, targeted treatment, and management of cancer. Some progress has been made since the beginning of the project. This includes the following advances described in the “Progress beyond the state of the art” sections:

Objective 1: To develop and demonstrate the application of Reference Measurement Systems (RMS) to support development, validation, and quality assurance of genomic IVD.

*Current state of the art*

Global and European efforts are underway to develop guidelines and standards to support the validation and QA/EQA of genomic profiling. However, current guidance for genomic test validation and QA lacks routes for independent comparability and assessment of analytical performance criteria.

*Progress beyond the state of the art*

This project aims to develop traceable methods for assessing critical quality attributes across key steps of the NGS-based genomic profiling workflow. These include nucleic acid (NA) isolation (yield and quality) from clinical samples—such as tissue and liquid biopsies from cancer patients—and NGS library preparation (yield and coverage uniformity), feeding into frameworks that support assay quality assurance.

To date, the Consortium has prioritised clinically relevant applications, including molecular stratification (disease sub-typing), minimal residual disease (MRD) testing, and advanced cancer treatment selection. Lung and colorectal cancers have been selected as the primary cancer types of focus.
Critical Quality Metrics (CQMs) have been defined and prioritised to support laboratory workflow QA/QC, assay validation, and associated examinands and measurands, covering both the pre-analytical and analytical phases. These metrics include fragment size distribution, sample purity, instrument performance, procedural consistency, and adherence to standardised protocols.
An extensive review has been carried out, covering genomic methodologies, sample requirements, sequencing parameters, and data analysis workflows for both tissue and liquid biopsies. This review also considered recent ISO standards, key scientific publications, and initiatives in early cancer detection.
Genomic test methods—both commercial kits and laboratory-developed assays—have been selected, with platform specifications, performance characteristics, and associated chemical and procedural risks assessed

and documented. A clinical sample planning document has been finalised to guide the collection, sharing, and secure storage of samples within the project.
A study protocol and patient information/informed consent form (ICF) for prospective sample collection have been prepared and approved by the local Ethics Committee. The availability of tissue, xenograft-derived, and liquid biopsy samples for genomic workflows has been reviewed, and Material Transfer Agreements (MTAs) have been signed.
Plans for NSCLC tissue sample collection and liquid biopsy sampling for gastrointestinal cancers are ongoing. Genomic workflows for both tissue and liquid biopsy testing have been implemented, and reference materials (RMs) and quality control materials (QCMs) for performance evaluation have been selected. Bioinformatic pipelines for data analysis have been chosen, and the experimental design for validation activities is currently under discussion.

Objective 2: To establish reference Measurement Procedures (RMP) for cancer biomarkers, and higher order methods.

*Current state of the art*

To date, there is only one primary RMP for quantification of a single cancer genetic variant in JCTLM DB, which is limited in scope to synthetic DNA controls.

*Progress beyond the state of the art*

The project will develop Reference Measurement Procedures (RMPs) for high-accuracy, SI-traceable quantification of key cancer biomarkers. It will assess the performance of these RMPs using contrived reference materials (RMs) and demonstrate their applicability in validating genomic profiling workflows. In parallel, novel sequencing strategies (NGS and Sanger) will be established for the orthogonal validation of genomic variant calls, as well as for identity and purity certification of genomic RMs and external quality assessment (EQA) materials.

To date, the project has identified key cancer biomarkers—including *KRAS*, *BRAF*, and *PIK3CA*—through an extensive literature review. Relevant information on their role in early cancer detection and their quantification using nucleic acid amplification-based methods, including digital PCR (dPCR), has been compiled into newly created reference tables. Based on this review, digital PCR has been selected as the most suitable methodology for developing RMPs for both cancer gene variant quantification and total DNA amount/fragment size determination.
Human-specific reference genes for total DNA quantification have been identified through literature surveys and stakeholder consultations. Sequencing approaches for the development of RMPs for nominal properties and RM characterisation have also been reviewed. High-accuracy NGS methods have been selected as the preferred sequencing platform to explore their potential as RMPs. Both new and existing RMPs are being optimised or verified, and candidate methods are undergoing evaluation using complex DNA templates.
The project has also defined the pre-analytical steps in nucleic acid profiling technologies, including the selection of amplification-based methods tailored to biomarker quantification. Candidate methods for assessing nucleic acid quality, integrity, and fragment size have been comprehensively evaluated based on published data and internal validation studies. The highest-performing methods have been selected for further characterisation to ensure analytical robustness, forming a strong foundation for the standardisation of laboratory workflows and assay validation.
A new CCQM KC study "Measurement of Single Nucleotide Variation (SNV) and Small Deletion in Cancer Biomarker of PIK3CA and EGFR" (K189) has been performed to support reference measurement services for cancer variant measurements by NMIs. Preliminary results have been discussed during the CCQM NAWG meeting 2025 in Paris.

Objective 3: To establish SI-traceable frameworks for the development and characterisation of Reference Materials (RMs) and EQA materials for genomic profiling.

*Current state of the art*

WHO International Standards and commercial contrived RM/QCMs are only available for selected individual cancer biomarkers. Higher order RMs currently only exist for germline materials and only sequence identity is

certified. In addition, these materials lack traceability to higher order standards and may not be commutable because frameworks for assessing the commutability of genomic RMs have not yet been established.

*Progress beyond the state of the art*

This project supports the development and implementation of improved and new external quality assessment (EQA) schemes for cancer genomic profiling, by providing reference values to ensure traceability and comparability across schemes. It aims to establish methodologies for assessing the commutability of complex, multi-analyte genomic reference materials (RMs), taking into account both nominal (variant identity) and quantitative properties, such as variant allele frequency (vAF). The development of novel cell- and tissue-based RM formats is also a key focus.
To date, the Consortium has conducted a comprehensive review of the availability of test, control, and reference materials relevant to selected cancer models, applications, and biomarkers. This effort has resulted in the compilation of several informative reference tables. Materials suitable for the development and evaluation of RMPs for both specific biomarkers/variant types and total nucleic acid (NA) quantity and quality have been selected.
Relevant EQA schemes associated with the chosen cancer models, biomarkers, and/or technologies have been identified. In addition, areas where new EQA schemes are needed to support cancer genomic testing have been highlighted and reported. A targeted survey on standardisation needs in cancer genomic profiling was distributed to relevant stakeholders, primarily EQA scheme participants, across multiple countries (such as, but not only, France, Ireland, Portugal, Poland, Italy, Spain, Greece and Germany in Europe, and Brazil, Colombia, Australia and Hong Kong, outside Europe). Feedback from 95 laboratories has provided the GenomeMET Consortium with valuable insights into current priorities for standardisation across genomic workflows, including reference genes, guidelines, and QA practices.
The Consortium has initiated the development of new RMs and quality control materials (QCMs) for cancer models and applications where such materials are currently lacking. A potential novel cell-based RM, designed for enrichment and genomic analysis of circulating tumour cells (CTCs) in liquid biopsy samples, has been developed and is undergoing validation. In parallel, cell culture systems are being optimised to support the production of further cell-based RMs.
Work has also begun on designing a new EQA scheme to accommodate emerging testing modalities such as liquid biopsies. This includes the development and production of EQA materials and prototype samples. A pre-commutability study is currently underway to provide proof of principle, prior to launching a more comprehensive commutability assessment. Profiling platforms for evaluating RM and EQA sample commutability have been selected, and sequencing data will be generated accordingly. Prototype samples and materials for the new EQA schemes are already in development.

Objective 4: To develop a framework for determining measurement uncertainty (MU) of genomic profiling

*Current state of the art*

Traditional MU approaches for clinical chemistry and genetic testing focuses on single analytes which are incongruent with multiparametric genomic testing.

*Progress beyond the state of the art*

This project is establishing a robust framework based on statistical approaches to assess measurement uncertainty (MU) in multi-parametric genomic profiling assays, incorporating both quantitative (e.g. read count, variant allele frequency [vAF]) and qualitative (e.g. sequence or variant identity) parameters.

To date, the Consortium has made significant progress by reviewing statistical methodologies for evaluating MU and identifying sources of variation in multi-parametric genomic datasets within cancer genomic profiling workflows. Fishbone diagrams have been developed to visualise potential sources of uncertainty. A number of suitable datasets have been identified, curated, and uploaded to the project database, accompanied by detailed metadata describing variable factors, output data, and associated variant information.
In addition, the Consortium is currently investigating variability in both qualitative and quantitative data outputs arising from library preparation processes. Statistical approaches for expressing confidence in nominal data (e.g. variant identity) have been assessed. Two genomic reference materials (RMs) have been selected for sequencing using multiple technologies, and a consensus truth set is being compiled, with a comprehensive summary of sequence characterisation currently under development.

A review of the literature and software repositories has been conducted to identify relevant bioinformatics pipelines and best practices applicable to cancer genomic profiling in the GenomeMET context. A systematic assessment of available raw sequencing data and matched test/reference datasets has been initiated, with the aim of evaluating the qualitative accuracy of different bioinformatics workflows. Software tools capable of assessing the qualitative accuracy of genomic datasets are also under evaluation.
Uncertainty associated with specific modules of bioinformatics workflows—such as sequence alignment and variant calling—is being analysed, along with the impact of different pipeline configurations on data accuracy. In parallel, performance parameters related to specific cancer variant types and their relevant measurands have been reviewed to ensure a comprehensive evaluation of sequencing platforms and bioinformatics pipelines for cancer genomic profiling.

**Outcomes and impact**

The results developed by the project have been effectively disseminated through a robust stakeholder network established with contributions from the entire consortium. We proactively engage with individuals who have joined the network to share outputs and understand their needs. Communication with the wider stakeholder community has been facilitated via mailing lists, social media, meetings, and a dedicated Stakeholder area on the GenomeMET website. Key findings have been shared through technical reports made publicly available online, including a survey on EQA schemes and genomic testing practices, literature reviews, and new databases on biomarkers, reference materials, methodologies, statistical tools, and quality metrics. The project has also contributed discussions on the need for new nucleic acid amplification-based RMPs and the first commutability study in genomic testing.

Project participants participated in over 45 standardisation and regulatory activities at international, European, and national levels, including involvement in 9 technical committees and several working groups within ISO, CEN, ASI, BSI, and IFCC. Contributions focused on *in vitro* diagnostics, molecular methods (qPCR, dPCR, NGS), AI in medical labs, and quality management. Highlights include the revision of ISO 20395, proposals for NGS oncology workflow standards, and standards on pre-analytics and liquid biopsy diagnostics.

The project was showcased at 2 national and 9 international events through 8 posters and 3 oral presentations, including the “Human and Medical Genetics Conference 2024”, “EMBL Cancer Genomics”, and the ISLB24 annual conference. Thirteen training activities were delivered, such as a course on statistical analysis, an interactive webinar on digital PCR in cancer detection, and an invited seminar at ELITech Group. To further increase visibility, the project website and LinkedIn page are regularly updated. Dissemination has also included participation in discussions, newsletters, social media outreach, press releases, and media articles.

Outcomes for industrial and other user communities

This project’s outcomes support the implementation of precision medicine for cancer patients. It is envisaged that these will have impacts across multiple key stakeholder communities including, but not limited to:

- IVD developers - RMS will support the generation of enhanced performance validation data incorporating MU and metrological traceability, enabling genomic IVD developers to better demonstrate performance in line with the IVDR. This will lead to improved quality and comparability of IVDs and faster translation to market through more streamlined and consistent regulatory submissions.

- Clinical laboratories – Higher order methods and QC materials for monitoring key workflow quality metrics and performance will enable clinical laboratories to establish improved and standardised QA frameworks, resulting in better quality and more comparable genomic profiling across laboratories and supporting accreditation (ISO 15189 or 17025).

- Healthcare providers – Frameworks for assessing analytical performance will enable healthcare providers to undertake improved Health Technology Assessments (HTA) of novel genomic IVDs, incorporating more robust data with defined uncertainties to support future test performance specifications and uptake of genomic profiling into health practice.

- RM producers – Frameworks for improved characterisation and SI-traceable value assignment of genomic RMs will enable RM producers to demonstrate metrological traceability and commutability in line with the IVDR and ISO 17511, leading to more streamlined RM development and a wider range of high quality RMs. A collaboration agreement with a major RM producer who is now actively working with the project and is involved in the Consortium meetings.

- EQA providers – Provision of SI-traceable reference values will enable EQA providers to demonstrate long term comparability and traceability of EQA materials and schemes, reducing reliance on arbitrary consensus values. This will improve robustness and quality of genomic EQAs, and support development of new schemes and harmonisation of EQAs in molecular pathology. In the first nine months of the project, the Consortium worked hard to plan two new EQA schemes which will be performed in the coming months.

- Drug developers will be able to undertake more streamlined development of targeted therapies through improved quality of genomic data from clinical trials, enabling more accurate selection of responders/non responders, leading to reduced development times, fewer failures, lower costs, and more effective cancer therapies.

- Clinical researchers will be able to generate more robust, reliable and reproducible genomic datasets, helping to address the current reproducibility crisis in clinical translational research, supporting faster translation of novel biomarkers to the clinic.

- Regulators – RMS and guidance for assay validation, incorporating metrological traceability, will inform IVD competent authorities /regulators / reference laboratories on performance metrics for genomic profiling assays, enabling more streamlined assessment of new IVDs and development of recommendations for implementation of genomic approaches in clinical practice.

Outcomes for the metrology and scientific communities

This project will provide a vehicle for joint activity, inter-laboratory comparisons, and knowledge sharing to support development of novel metrological concepts and capability for clinical genomics. Outcomes will support improved EU metrology infrastructure enabling provision of new RMS and measurement/calibration services allowing NMI/DIs to provide more reliable SI-traceable reference values and improving agreement between different laboratories worldwide. Outcomes include:

- Improved NMI/DI capabilities for quantification of cancer genomic biomarkers, quantification of total Nucleic acids and detection of panels of genomic variants, demonstrated through inter-laboratory comparisons. GenomeMET participants participated in a new CCQM study: K189 “Measurement of Single Nucleotide Variation (SNV) and Small Deletion in Cancer Biomarker of PIK3CA and EGFR.” This will demonstrate cancer gene variants quantification capability, under development in GenomeMET, through recognised CCQM studies, leading to added value for the metrology and wider communities.

- Dissemination of case studies to advance development of metrological frameworks for multi-analyte clinical genomic profiling.

- Submission of new RMP for quantification of cancer genomic biomarkers and SI-traceable RMs to JCTLM database.

The metrological capabilities developed in this project will also support the wider clinical genomics sector (e.g., rare diseases and non-invasive prenatal testing (NIPT)) where Next Generation Sequencing (NGS) profiling is being applied and complement metrology development for other ‘omics sectors where multi parametric testing is needed, e.g., transcriptomics, proteomics, and metabolomics.

Outcomes for relevant standards

The RMS to support assay validation will enable IVD developers, clinical laboratories, and other end-users, e.g., EQA providers and RMs producers, to better comply with regulations and standards in the IVD field e.g., IVDR and ISO 15189, ISO 17025 and ISO 17511 through generation of more robust and comparable datasets incorporating metrological traceability and MU.

Higher order methods, i.e., high-accuracy methods with low uncertainty that can be used as reference methods or for value assignment of reference materials, e.g., dPCR and materials (RMs/EQA materials) will support stakeholder-driven standardisation initiatives, linked to GenomeMET, e.g., INSTAND-NGS4P project by providing the underpinning methods/materials required to assess performance.

Outputs from this project will be incorporated into relevant CEN TC 140 and ISO TC 212 standards in development for NGS and liquid biopsies through participant representation on drafting committees, and into periodic revisions of standards such as ISO 15193, ISO 15194 and ISO 20914.

Finally, proposals for new standards under CEN TC 140 or ISO TC 212 are expected during the lifetime of the project in order to support improved validation and QA of genomic profiling and accurate quantification of cancer gene biomarkers. To date, a new standard has been proposed under ISO TC 212 WG4 to support "NGS-based oncology applications" with GenomeMET participants as part of the Expert Drafting Team. Particularly, the RMS being developed for actionable cancer biomarkers for lung and colorectal cancer in GenomeMET were presented by LGC to the ISO TC 212 WG4 committee to demonstrate how they can support standardisation and quality assurance of genomic profiling and genomic IVD assays.

Longer-term economic, social and environmental impacts

Outputs from the project will support earlier cancer detection and implementation of precision medicine, through confident and valid uptake of genomic profiling. Cancer is the second largest cause of death in Europe, with more than 3.7 million new cases and 1.9 million deaths each year and carries an economic burden of €141.8 billion/pa (1.07 % of GDP). Earlier detection and genomics-guided targeted therapies with greater efficacy and less toxicity compared to traditional systemic therapies will significantly reduce healthcare costs and improve patient outcomes. High quality genomic testing will result in fewer diagnostic errors e.g., missed/incorrect diagnosis and support provision of the “right drug to the right patient at the right time” reducing the economic burden of cancer and allowing citizens to live longer and healthier lives.

The project’s outputs will also support growth of the European IVD and oncology therapeutics markets, valued at 33 billion Euros/pa and 75 billion Euros/pa respectively, through more streamlined routes for approval of new companion and precision genomic diagnostics, and improved genomic data from clinical trials resulting in more accurate selection of responders and more streamlined development of novel targeted therapies.

Environmental impacts include a reduction in the use of medical tools/devices and diagnostic kits/ components through more accurate “right first time” testing. These components are often single-use plastic products, the disposal of which presents an environmental risk.

### List of publications

Bronkhorst, A.J. and Holdenrieder, S., (2023) 'The changing face of circulating tumor DNA (ctDNA) profiling: Factors that shape the landscape of methodologies, technologies, and commercialization', Medizinische Genetik,35(4) p.201-235. Available at https://doi.org/10.1515/medgen-2023-2065.

| | | |
|---|---|---|
| Project start date and duration: | 36 months | |
| Coordinator: Carla Divieto, INRIM Tel: +390113919971 E-mail: c.divieto@inrim.it<br>Project website address: www.genomemet.org | | |
| Internal Beneficiaries:<br>1. INRIM, Italy<br>2. LNE, France<br>3. NIB, Slovenia<br>4. PTB, Germany<br>5. TUBITAK, Türkiye | External Beneficiaries:<br>6. CEA, France<br>7. FPO, Italy<br>8. INSTAND, Germany<br>9. MUG, Austria<br>10. UNITO, Italy | |
| Associated Partners: 11. BIO-RAD, United States, 12. GenQA, United Kingdom, 13. LGC, United Kingdom, 14. METAS, Switzerland, 15. MHRA, United Kingdom, 16. ULE, United Kingdom | | |