

Publishable Summary for 18HLT03 SEPTIMET

Metrology to enable rapid and accurate clinical measurements in acute management of sepsis

Overview

The overall aim of SEPTIMET was to employ measurement science to improve accuracy and reproducibility of rapid diagnostic tests for the identification and treatment of sepsis and similar acute conditions. Sepsis is a life-threatening condition where time to diagnosis is critical to patient outcome. The project developed reference methods for testing for identifying the pathogenic causes of sepsis as well as a reference measurement procedure for detection of procalcitonin, a biomarker of infection used for sepsis diagnosis. It is through the availability of these supporting reference methods that improvements can be made to address more rapid and accurate clinical measurements in sepsis management. The outcomes of the project will support IVD (In-Vitro Diagnostic) manufacturers in meeting developments in EU diagnostic regulation.

Need

Sepsis must be treated within hours to avoid potentially high mortality or morbidity. Yet today's diagnostic deficit in terms of accurate and reproducible tools to diagnose and guide treatment of sepsis has been a major contributor to its devastating impact, resulting in ~700,000 European deaths a year as of 2023. Metrological support is needed to improve the performance of existing methods and enable the efficient translation of new near patient solutions.

The main method for guiding treatment, microbiological culture, is too slow to realistically help sepsis patients. The current Surviving Sepsis Campaign international guidelines are based on clinical scores only. Biomarkers could improve quick diagnosis of sepsis, but uncertainty over the accuracy of those proposed has led to variable uptake. Reference measurement procedures to support the traceability of such biomarker tests, alone and in combination, did not exist (objective 1), and the possibility of applying machine learning algorithms to identify solutions for sepsis management was still in the research phase by the time the project was proposed. A metrological framework was required to support faster laboratory tests to guide treatment (objective 2). Reference measurement procedures have been developed and will support rapid near-patient test manufacturers meet the new IVD Regulation 2017/746 (objective 3). The accuracy and metrological requirements of new and innovative 'omics' methods that could deliver more sensitive and specific tests to aid sepsis patient survival have been assessed (objective 4).

SEPTIMET ensured that metrological principles will contribute to a solution to this global health issue that affects 30 million people a year and leads to 6 million deaths. The clinically focussed consortium has achieved this by developing the underpinning metrological concepts to facilitate the development and application of the rapid, accurate tests needed to improve sepsis survival.

Objectives

The overall objective of the project was to develop traceable and reproducible measurements to support more rapid diagnosis for the treatment and management of sepsis. Current practice splits patient management into a two-step process using tests to i) identify patients with sepsis and ii) determine cause and therefore guide antibiotic treatment. Current approaches used for patient identification are non-specific with unclear reproducibility, while current methods to identify microbial cause lack sensitivity and speed. Both situations

Report Status: PU Public

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EMPIR  
The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

Publishable Summary

Issued: May 2023

lead to delay in optimal treatment, and it is this delay that contributes to high morbidity and mortality associated with sepsis. SEPTIMET focused on addressing these problems by delivering the following scientific and technical objectives:

1. To improve the traceability and accuracy of measurements of established biomarkers (e.g., C-reactive protein and procalcitonin) used for sepsis diagnosis. This included the development of validated methods with target improvements to measurement uncertainties of <20 % and traceable materials for single and simultaneous, multiple sepsis biomarker measurements, as well as the definition of reference ranges of biomarkers in patients who were at risk of sepsis.
2. To develop a metrological and quality assurance framework for current methods used to confirm the microbiological aetiology of sepsis. This included an evaluation of the accuracy and reproducibility of current methods and the quantification of target levels of accuracy and reproducibility required for quality assurance.
3. To develop improved reference methods to reduce uncertainties to <30 % and enhance reproducibility for of rapid near patient (point of care) testing for sepsis (diagnosis and to guide treatment). Such methods were to be suitable for accreditation and meet the EU IVD Regulation (2017/746). In addition, to develop an associated proficiency scheme for the point of care testing platforms, specifically for non-specialist users (e.g., healthcare workers without laboratory training).
4. To develop and qualify a metrological framework underpinning new and innovative methods for early sepsis diagnosis (e.g., transcriptomics) and treatment guidance (e.g., metagenomics). This included an evaluation of their accuracy and reproducibility and the identification of target levels of both, for each method.
5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain (Clinical Laboratories, Hospitals), standards developing organisations (ISO/TC 212, CCQM, SoGAT), and end users (e.g., ESCMID, ESICM, IFCC).

Progress beyond the state of the art

Improving the traceability and accuracy of measurements of established biomarkers

Previously, identification of sepsis relied on well-established clinical scores, based e.g. on parameters like blood pressure, which had poor specificity. More than 200 biomarkers have been explored in clinical studies to improve specificity and negative predictive value. Among the top eight is procalcitonin (PCT) allowing to differentiate between infectious and non-infectious causes, but despite this hospital uptake is variable. A possible reason for this is poor calibration leading to reproducibility problems. A previous EMPIR project (15HLT07 AntiMicroResist) introduced the first initiative to construct an internationally agreed reference system for PCT. SEPTIMET developed a primary reference measurement procedure to implement SI traceable measurement for PCT. SEPTIMET also investigated the accuracy of panels of multiplexed biomarkers and the use of uncertainty in the wider application of data science to develop algorithms using diagnostic data to better manage sepsis. For example, the measurement uncertainty for the reference methods was established, in protein biomarkers and pathogenic agents, this will allow the incorporation of these uncertainties into better understanding sepsis.

Development of a metrological and quality assurance framework for current methods used to confirm the microbiological aetiology of sepsis

Microbiological culture, the gold standard for identifying the cause of most bacterial infections takes more than 24 hours, which is too slow to guide treatment for most sepsis patients. A number of automated solutions have been developed that have fast turnaround times and can confirm microbiological cause with culture or directly from patient samples. However, the support for the development and application of these approaches (such as quantitative reference materials) is in its infancy. SEPTIMET developed a metrological quality assurance framework to support the routine application of these methodologies.

Development of improved reference methods for rapid near patient (point of care) testing for sepsis

Near patient testing is used routinely in many clinical scenarios in hospitals and the community. However, poor reproducibility and sensitivity currently limits their use in sepsis. SEPTIMET developed reference methods to enhance the application of such approaches for sepsis testing. This will support confident application of established tests. SEPTIMET developed sensitive analytical methods to conduct high accuracy measurements in the patient, providing IVD manufacturers with the results needed to design new tests.

Development and qualification of a metrological framework underpinning new and innovative methods

SEPTIMET focused on developing the underpinning metrology in three areas of innovation for sepsis management:

1. Frequently sepsis involves bacterial endotoxins released into the blood stream. SEPTIMET developed direct measurements of endotoxin in patient blood using a novel biosensor platform based on GFET (graphene field effect transistor)
2. Cost for gene sequencing have fallen dramatically in the past ten years. Direct metagenomics analysis has the potential to provide detailed information on the causative pathogen and its resistance to antibiotics when applied for whole genome sequencing. However, standard approaches are too slow for sepsis. SEPTIMET evaluated the likely metrological support needed for rapid sequencing technologies (e.g., the Oxford Nanopore MinION) that potentially provide direct results in less than four hours.
3. Characteristic changes of cell concentrations (e.g., leukocytes, endothelial cells) and cellular response to systemic infection (e.g., changes in antigens, RNA or neutrophil extracellular traps induced by endotoxins) are also among the top eight potential sepsis biomarkers. SEPTIMET explored the possibility to measure these cells using advanced flow cytometry, microscopy and transcriptomic strategies. The results for which demonstrated reference methods suitable for measuring the cellular response in sepsis.

Results

Objective 1: To improve the traceability and accuracy of measurements of established biomarkers

A recombinant protein procalcitonin (PCT) was sourced and characterized to produce a candidate protein primary calibrator. The purity and SI traceable concentration of this calibrator was determined. Development and validation of a SI traceable method for PCT quantification by isotope dilution mass spectrometry (ID-MS) in serum was achieved and published in Analytical Chemistry. The submission of the ID-MS method for nomination to JCTLM as a reference measurement procedure was performed. In addition, human serum materials were produced, and value was assigned by this method. These materials constituted candidate materials needed by the IFCC working group on Standardization of Procalcitonin assays (WG-PCT) to assess the current level of harmonisation of PCT assays. A commutability study, including these materials, is planned to be organised by the WG-PCT in Autumn 2023.

Meanwhile, a mass spectrometry-based multiplex method for detection of five relevant protein biomarkers out of the nine chosen initially was optimised. The method involved both peptide- and protein-based calibration for the selected biomarkers. Peptides to be used as primary calibrators were quantified by amino acid analysis and assessed for purity. Steps in the analytical process, from the sample preparation, chromatographic conditions for peptide separation and MS ionisation and fragmentation stages of the method were optimised to achieve the required sensitivity and accuracy. The analytical performance of the method and associated measurement uncertainty was completed, and this final method was applied to the simultaneous detection of the five proteins in pools of serum.

The objective of improving the traceability and accuracy of measurements of established biomarkers has therefore been partially met. The reference measurement procedure developed achieved relative expanded uncertainties of < 23 % which is suitable for the requirements of the stakeholders. The project developed a multiplex method for the quantification of several protein biomarkers of sepsis by mass spectrometry which was applied to the simultaneous detection of the 5 proteins in pools of serum samples produced.

Objective 2: To develop a metrological and quality assurance framework for current methods used to confirm the microbiological aetiology of sepsis.

Model pathogens including *E. coli*, MRSA, *N. meningitis* and *A. baumannii* were selected and materials developed to different levels of complexity, from synthetic constructs to whole cell materials to appropriate models. The pre-analytical stage of the methods for confirming and quantifying the pathogenic cause of sepsis was evaluated and optimal methods were selected moving forward. Performance characteristics of these methods, e.g., singleplex vs multiplex assay formats, platform comparisons, were completed. The development of a reference measurement procedure using digital PCR (Polymerase Chain Reaction) as a potential primary reference measurement procedure (RMP) was completed. Preliminary interlaboratory studies showed that the prototype RMP was performing on some of the whole cell materials with uncertainties which require improvement. Of note the methods were performing with acceptable expanded uncertainties (<20 %) when using genomic DNA preparations. Improvements in the protocol were investigated and applied to further interlaboratory studies. The performance of the prototype RMP was satisfactory with expanded uncertainties of ~ 30 %. The SARS-CoV-2 model was added as a further pathogen to be investigated following the COVID-19 pandemic as it is a cause of viral sepsis. The objective of developing a metrological and quality assurance framework for current methods used to confirm the microbiological aetiology of sepsis has been achieved.

Objective 3: Development of improved reference methods for rapid near patient (point of care) testing for sepsis

Materials and methods to support the investigation of rapid near patient testing for sepsis were developed as part of objective 2. These were applied as part of an available EQA (External Quality Assurance) scheme during May 2022 and also in November 2022 which involved ten different commercially available rapid molecular tests, as well as in house developed tests performed by 271 different laboratories. A preliminary study in these near patient approaches for detection of SARS-CoV-2 was performed as part of an EQA scheme using well defined materials. A biosensor prototype capitalising on a graphene-based field-effect transistor (GFET) to support the measurement of endotoxins from suitable blood was evaluated at Great Ormond Street Hospital. For protein biomarkers, a candidate reference method with measurement uncertainties below 30% has been developed for procalcitonin, a well-established biomarker for bacterial sepsis. The objective of the development of improved reference methods for rapid near patient (point of care) testing for sepsis has been achieved. The reference methods for the bacterial model, methicillin resistant *Staphylococcus aureus* (MRSA) has been completed.

Objective 4: Development and qualification of a metrological framework underpinning new and innovative methods for early sepsis diagnosis (e.g., transcriptomics) and treatment guidance (e.g., metagenomics)

Newer methods to determine the causative agent of sepsis were investigated using rapid portable sequencing platforms. Materials were modelled *in silico* to simulate clinical samples and were processed through metagenomics bioinformatics pipelines. The technical performance of the methods was validated using mock samples to determine the indicative technology requirements for application to clinical samples in a sepsis patient. Further clinical samples were processed through the method and cell-based sepsis markers were also identified for investigation in the project that would provide an additional route to support patient stratification. The objective of the development and qualification of a metrological framework underpinning new and innovative methods for early sepsis diagnosis (e.g., transcriptomics) and treatment guidance (e.g., metagenomics) has been achieved. An GFET sensor for the detection of endotoxins has been realized in laboratory scale, sources of uncertainty have been established for the metagenomic approaches and the framework for measuring cell based sepsis markers has been developed.

Impact

The aims and objectives of this project were discussed at internationally leading scientific fora, including the 18th International Metrology Congress, the Joint Committee for Traceability in Laboratory Medicine (JCTLM) workshop and JCTLM Accurate Results for Patient Care Workshop, the ESCMID Conference on Coronavirus Disease (ECCVID) and ECCMID (The European Congress of Clinical Microbiology and Infectious Diseases).

There were eight papers accepted and published from the project in peer review journals and three more papers have been submitted (Clinical Chemistry, British Medical Journal, Journal of Clinical Virology Plus, Analytical Chemistry, and Current Opinion in Pulmonary Medicine, PLOS ONE and Biosensors). The route to impact via presentations, workshops and webinars was demonstrated through thirty presentations, three workshops and open access publications and guidance documents. In addition, 30 stakeholders joined our mailing list having expressed an interest in our results.

SEPTIMET inherently responded to a specific need of the healthcare sector, where impact was strongest; this coordinated metrological programme have major long-term contributions to guiding rapid diagnosis and treatment of sepsis and therefore improved survival of septic patients. It also produced a range of secondary benefits applicable to a broader set of beneficiaries through continued attraction and investment in the continued professional development of experts by formal training and informal national and international peer networking and collaboration.

Impact on industrial and other user communities

SEPTIMET developed new international biochemical, molecular and cellular/immunological measurement capabilities and reference systems that will directly assist IVD manufacturers and clinical end users.

The project had a stakeholder network of 30 organisations that included representatives from healthcare, industry, university and clinical settings. Through committee participation in, for example as chair in the Standardisation of Procalcitonin assays working group within the IFCC, SoGAT and ESGMD (ESCMID Study Group for Genomic and Molecular Diagnostics), the project was actively engaged with relevant scientific networks.

This project assisted IVD manufacturers to transfer technology to the clinic (including near patient tests) by providing them with routes to better characterise analytical performance. Industrial stakeholders in the IVD domain can benefit from the development of SI traceable reference measurement procedures for biomarkers to identify sepsis patients and guide their treatment. IVD manufacturers are able to use project findings to support demonstration of test development and routine performance and meet regulatory requirements. In addition, manufacturers and providers of external quality assurance / proficiency testing schemes are also able to leverage such new measurement frameworks.

Impact on the metrology and scientific communities

This project brought together European NMIs, each contributing to their own area of expertise to complement a critical body of clinical and technological inputs guaranteeing the delivery and further development of the objectives through an improved system of metrology. It developed reference methods specifically targeted at clinical samples. These higher order measurements will define the accuracy of a variety of analytes speculated to be used in sepsis management.

The route to impact via presentations, workshops and webinars were providing mechanisms that ensured the projects' findings were being directed at, and integrated with, the research priorities identified by stakeholders. Metrological findings were incorporated into medical microbiology Master-level teaching and research programmes, ensuring the next generation of scientists are prepared for future diagnostic challenges.

Impact on relevant standards

SEPTIMET explored routes to support use of appropriate regulations for IVD (such as the new IVD Regulation (2017/746) and in assisting the transition process to this new regulation from the IVD Directive (98/79/EC), currently under way and due to be in place by spring 2022). An example is the need for traceability of the values assigned to IVD calibrators to reference materials and/or reference methods of higher order, which are not currently available for many tests used in sepsis management.

Partners held committee membership and/or Working Group convenor status in a range of relevant international organisations that were active in the area of enhancing the comparability of laboratory medicine including IFCC and JCTLM as well as sitting on ISO TC212 (Clinical laboratory testing and IVD test systems), with WG2 on reference systems and WG4 on microbiology and molecular diagnostics, and ISO TC276 with WG3 on analytical methods. Project outcomes addressed standards for newer technologies such as next generation sequencing for which guidelines were currently being drafted as part in ISO TC276 including ISO/WD 20397-3. For example, outputs from SEPTIMET had been used to guide the drafting of the fast tracked

Joint ISO/TC 212 - ISO/TC 276 (JWG 6) ISO/TS 5798. Partners were also involved in driving the development of specific working groups to address issues associated with sepsis (e.g. IFCC WG-PCT and CM-MD).

Longer-term economic, social and environmental impacts

As of 2023, the current incidence of sepsis in Europe is 3.4 million people a year. Sepsis is usually treatable if accurate timely diagnosis is available. Yet incorrect treatment often administered in the absence of such tests reduces the patient's chances of survival while also increasing the risk of antimicrobial resistance. The potential economic impact of improving sepsis outcomes in healthcare terms is stark. Sepsis accounts for ~50 % of intensive care unit (ICU) bed days, which costs ~1700 € per day, each sepsis patient in the ICU costs almost 30,000 € to treat. Sepsis is estimated to cost Europe an estimated 18 billion € a year. More accurate application of existing diagnostic methods, as well as efficient development of innovative cutting-edge solutions, will identify sepsis patients earlier, reducing treatment costs and those associated with prolonged hospital stay.

In addition to the economic benefits from the improved healthcare of the population, commercial benefits linked to IVD companies wishing to develop newer fast diagnostic assays for sepsis (and other medical emergencies such as meningitis) will benefit from improved validation frameworks and RMs developed within the project. This will empower both existing European commercial providers of centralised laboratory tests but also the growing number of providers of near patient point of care methods. Given the growing market size in the overall Med Tech sector, the lack of metrological concepts to de-risk pre-clinical research, and support translation and application of rapid diagnostic tests represents a significant bottleneck. The outputs of this project will aid in reducing the associated risks by implementing the required reference measurement systems at sufficiently large-scale European effort to support major downstream economic impact.

As well as patients, long-term beneficiaries from this project include doctors, nurses and other healthcare professionals who will benefit from improved outcomes of their clinical decisions and better health service performance. Epidemiologists and public health laboratories will benefit from improved diagnostic accuracy of patient identification due to better accuracy and comparability of the surveillance data across Europe.

Better management of sepsis patients, enabled by high accuracy measurements and underpinned by the metrological concepts developed by SEPTIMET, will help reduce the devastating mortality and morbidity they currently face.

List of publications

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This list is also available here: <https://www.euramet.org/repository/research-publications-repository-link/>

Project start date and duration:		September 2019, 42 months
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Internal Funded Partners:	External Funded Partners:	Unfunded Partners:
1. LGC, United Kingdom	7. APHP, France	
2. LNE, France	8. BGU, Israel	
3. METAS, Switzerland	9. CEA, France	
4. NIB, Slovenia	10. GOSH, United Kingdom	
5. NPL, United Kingdom	11. MUW, Poland	
6. PTB, Germany	12. RSFT, United Kingdom	
RMGs: -		