FINAL PUBLISHABLE REPORT

Grant Agreement number	18HLT03
Project short name	SEPTIMET
Project full title	Metrology to enable rapid and accurate clinical measurements in acute management of sepsis

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

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1 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.

2 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.

3 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 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).

4 Results

Objective 1: To improve the traceability and accuracy of measurements of established biomarkers (e.g. C-reactive protein and procalcitonin) used for sepsis diagnosis. This will include 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 are at risk of sepsis.

Work to achieve this objective was performed by LNE, CEA, RSCH, GOSH, MUW, APHP and LGC.

Protein biomarkers are valuable tools to help clinicians make clinical decisions for treating diseases. For sepsis diagnosis, two biomarkers are currently used by clinicians in addition to other clinical diagnoses, the C-reactive protein (CRP) and the procalcitonin (PCT). However, using these biomarkers as a single diagnosis tool for sepsis is not an appropriate solution as they are non-specific for sepsis or may lack result comparability and thus varies between hospitals or even physicians (e.g. PCT for which there is no higher order method or materials). Therefore, improving diagnosis accuracy and specificity will support clinicians and improve patient recovery. Thus, the SEPTIMET project developed candidate reference method and materials for the quantification of PCT on the one hand and developed a method for the accurate quantification of several sepsis protein biomarkers on the other hand.

PCT is a recognised sepsis biomarker specific of bacterial infection allowing patient stratification and antibiotic therapy management. Different clinical decision cut-offs were established (e.g., 0.5 µg/L for sepsis diagnosis and 0.25 µg/L for antibiotic initiation or discontinuation for a patient with moderate or mild illness outside ICU). PCT measurement has been integrated into clinical guidelines and antimicrobial stewardship programs. Thus, reliable and accurate measurements of this biomarker are critical for sepsis diagnosis, guide treatment decisions, patient monitoring. A proposed route to improve results comparability and accuracy is developing reference calibration materials, which have been value-assigned with a higher-order reference measurement procedure (RMP). Such materials and method were lacking before the SEPTIMET project. To meet this need, LNE, with the support of CEA, developed and validated a candidate reference measurement procedure (RMP) for PCT quantification in human serum by Liquid Chromatography/Isotope Dilution-Mass Spectrometry1,2 (Figure 1). A bottom-up strategy performs the determination of the mass concentration of Procalcitonin through the quantification of the SALESSPADPATLSEDEAR and FHTFPQTAIGVGAPGK tryptic peptides.

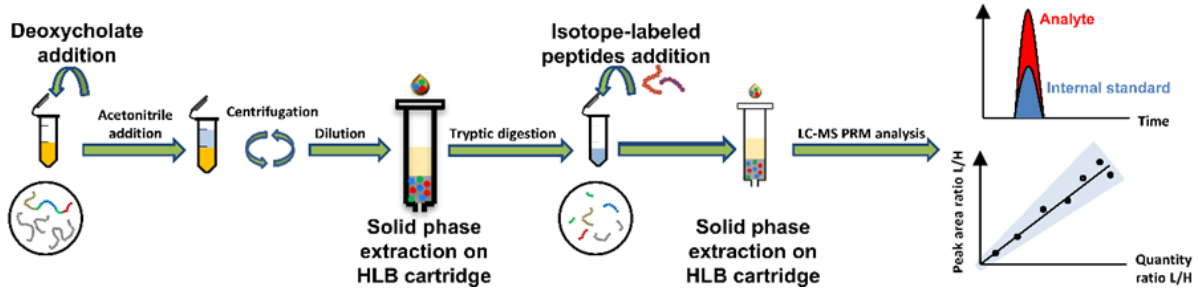


Figure 1: *Analytical workflow of the candidate reference measurement procedure for the quantification of PCT in human serum.*

For the SALESSPADPATLSEDEAR peptide, the developed method allows the quantification of PCT within the concentration range 0.25 – 132 µg/L with relative expanded uncertainties (k=2) ≤11 % at concentration ≥0.5 µg/L and 17 % at a concentration of 0.25 µg/L. For the FHTFPQTAIGVGAPGK peptide, the developed method allows the quantification of PCT within the concentration range 0.5 – 132 µg/L with relative expanded uncertainties (k=2) are ≤16 % at concentration ≥1 µg/L, and 30 % at a concentration of 0.5 µg/L. The quantification of samples with a PCT concentration between 0.25 µg/L and 0.5 µg/L relies on the quantification of the SALESSPADPATLSEDEAR peptide only. The quantification of samples with a PCT concentration between 0.5 µg/L and 132 µg/L relies on the quantification of both SALESSPADPATLSEDEAR and FHTFPQTAIGVGAPGK peptides (mean concentration). Considering an uncertainty component between peptides, the relative expanded uncertainties (k=2) are ≤13 % at a concentration ≥1 µg/L and 23 % at a concentration of 0.5 µg/L. To measure samples with a concentration between 13.74 and 132 µg/L, a dilution of the samples (up to 20-fold) with blank serum was performed.

For this candidate reference method, the quantification of PCT relies on a protein-based calibration strategy with a recombinant PCT protein as the primary calibrator and the corresponding isotope-labelled recombinant protein as an internal standard. LNE purchased the recombinant protein to be used as the primary calibrator and performed its characterisation by Peptide Impurity Corrected Amino Acid Analysis (Figure 2), with the support of LGC. This characterised primary calibrator ensures S.I. traceability of the measurement results of the candidate reference measurement procedure developed.

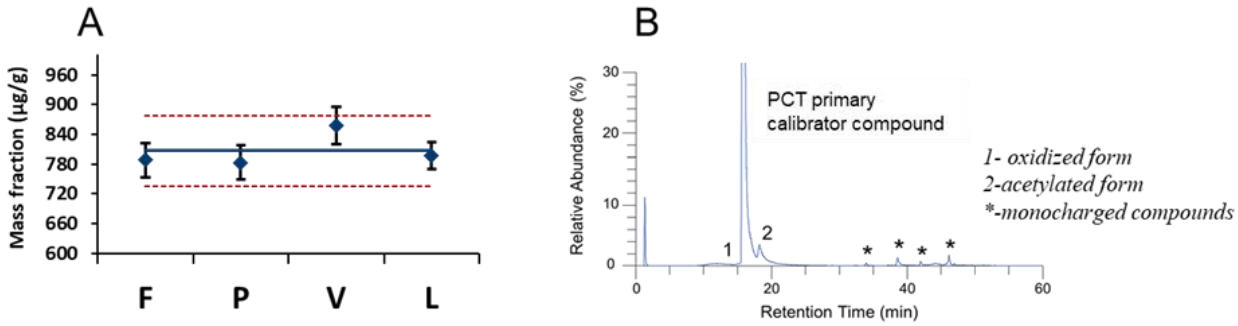


Figure 2: Characterisation of the PCT primary calibrator by amino acid analysis (A) and LC-HRMS analysis (B). (A) Mass fraction of the primary calibrator by quantification of the phenylalanine (F), Proline (P), Valine (V) and Leucine (L). (B) Extracted ion chromatogram obtained by injecting 5 µg of the protein.

In addition, LNE prepared seven matrix-matched materials covering the PCT concentration range of 0.25 – 20 ng/mL by pooling some of the clinical samples recruited within the project by APHP, RSCH and MUW. The close collaboration between these three partners of the project was crucial for producing materials (pools) with the intended concentration in a sufficient amount. To this aim, RSCH implemented PCT measurements in their facilities to provide single donations with a known initial PCT concentration. This allows LNE to pool the most suitable samples together to reach the target concentrations and volumes of the matrix-matched materials. For their part, MUW implemented a protocol designed by LNE to produce the pooled materials directly in their facilities at the time of sample collection. These secondary materials were value-assigned by the candidate RMP and constitute candidate materials needed by the IFCC working group on standardisation of PCT assays (WG-PCT) to assess the current level of harmonisation of PCT assays. First these materials will be included in a commutability study organised by the WG-PCT. Then, commutable materials will be used by the WG-PCT as external quality assessment (EQA) material to assess comparability of commercially available PCT assays and thus evaluate the current harmonisation status of PCT measurements.

The samples collected by APHP (single donation of large amount, ~4.5 mL), also constitute panels of real patient samples needed for the necessary for the commutability to be organized by the WG-PCT.

To meet the needs of a specific diagnosis of sepsis, the development of a multiple sepsis biomarker measurement by mass spectrometry was conducted by CEA with the support of LNE. A literature review was conducted beforehand by LNE and CEA. This review has resulted in a list of 56 diagnostic biomarkers for sepsis. This list was proposed to the clinical partners of the SEPTIMET project (APHP, RSCH, MUW) and the in-vitro diagnostic manufacturers, members of the WG-PCT. This consultation allowed the identification of biomarkers of current and future interest for diagnosing sepsis. The evaluation of the metrological needs and the analytical feasibility, in terms of the concentration level, the molecular mass of the proteins and the laboratory's prior knowledge, allowed for establishing a list of nine proteins.

Following the selection of protein biomarkers, a method for their multiplex quantification by liquid chromatography-mass spectrometry was developed. This method is also based on a bottom-up strategy. Unlike the PCT method, the calibration strategy uses synthetic peptides as primary calibrators and their isotope-labelled analogues as internal standards, except for PCT and CRP. For these proteins, primary calibrators were available. The peptides of interest for quantifying each protein were identified and selected. The corresponding peptides were purchased, quantified by amino acid analysis and characterised by LC-HRMS to ensure the traceability of measurement results to the SI.

Optimisation of each step of the protocol, from sample preparation to mass spectrometry quantification, was performed. Compromises had to be made because the physicochemical properties of the proteins were very different. Nevertheless, the best conditions allowed the quantification of five biomarkers in human serum out of the nine initially selected. The method's performance was evaluated regarding the limit of quantification, linearity and measurement uncertainty under intermediate fidelity conditions. This method was used to simultaneously detect all five proteins of interest in the seven materials produced by LNE.

With a view to the application of the method in clinics, a feasibility study has been performed to shorten the global method runtime by testing different conditions for faster digestion and faster chromatographic separation. However, these conditions deteriorated the method's performance in terms of the limit of quantification and constrained the implementation of the method in routine for sepsis diagnosis.

Towards achievement of objective 1, the work performed within SEPTIMET allowed to:

- produce the first primary reference measurement procedure allowing S.I. traceable PCT measurements,
- produce the first candidate reference materials (primary calibrator and matrix-matched materials) for PCT,
- demonstrate the feasibility of the detection of five proteins biomarkers in pools of serum samples from sepsis patients

For PCT, the outputs of the project will allow to improve the reliability of measurement performed in routine in clinics. Indeed, major lacks of the traceability chain has been addressed. Most importantly, the matrix-matched materials produced will be used by the IFCC working group on standardisation of PCT assays (WG-PCT) that constitute a direct uptake of SEPTIMET outputs.

For the quantification of multiple sepsis biomarkers the work performed within SEPTIMET constitute the first attempt to develop a multiplex accurate quantification by LC-MS/MS, with the use of SI-traceable primary calibrators. There is still development and optimisation to undertake toward an effective implementation in clinics as the sample preparation is still time-consuming and does not allow reaching a rapid diagnosis of sepsis compared to the current immunoassays.

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 This will include an evaluation of the accuracy and reproducibility of current methods and the quantification of target levels of accuracy and reproducibility required for quality assurance.

Work to achieve this objective was performed by LGC, PTB, NIB, METAS, RSCH, GOSH, MUW, BGU, LNE, NPL, APHP and CEA.

The microbiological gold standard for confirmation of aetiology (cause of infection) for sepsis is culture. However, this takes >24 hours which is simply not fast enough for most sepsis patients. Methods such a MALDI-TOF are used to speed up culture, but the time reduction offered is marginal as also they require ~6 hours. Consequently they are useful in many infections, but not sufficiently enough to impact a sepsis patient. The last 5-10 years has seen the development of a number of automated microbial molecular diagnostic solutions that have fast turnaround times; typically taking around one hour. These approaches require minimal hands on time or technical expertise, are currently used for sepsis in combination with culture reducing the turnaround time to ~10 hours. Commercially available PCR based multiplex sepsis diagnostic methods also exist that do not require culture with in similar turnaround times of 8-10 hours.

While quality assurance programmes exist to support the use of molecular platforms they do not typically represent the complexity for the intended use; often molecular EQAs are performed with existing formats for culture without confirmation if they are fit for use. Furthermore, unlike culture, molecular quantification is typically capable of uncertainties of <20 %, yet as this is typically performed over six orders of magnitude. As reference materials with defined uncertainty do not exist, test accuracy and reproducibility is highly problematic.

SEPTIMET developed a metrological quality assurance framework to support the routine application of these rapid methodologies. This framework comprised of the development of SI traceable reference measurement procedures and prototype reference materials with wide dissemination of EMPIR the protocols to develop them beyond the scope of the project. Such a resource can enable assessment of end user laboratory performance to support accreditation. The knowledge generated by meeting this objective will also support development and use of rapid near patient tests for bacterial cause. The models chosen following stakeholder consultation were: *Staphylococcus aurerus, Neisseria meningitidis, Escherchia coli, Streptococcus pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* SARS-CoV-2 was added as an additional model due to the circumstance of the COVID-19 pandemic.

Digital PCR (dPCR) assay suitable for the detection of *N. meningitidis*. Eight TaqMan PCR assays specific for *N. meningitidis* were evaluated regarding assay specificity and efficiency. These assays included four assays *rplF*, *zupT*, *tolC*, and *nhhA* designed by METAS, two assays *ctrA* and *porA* designed by LGC and previously published assays by Diene *et al.* 2016 (DOI: 10.1016/j.cmi.2016.03.022): *tauE* and *metA*. Quantitative PCR (qPCR) assay optimization experiments showed promising results for the *porA, metA,* and *rplF* assays, which were selected for further validation on the Naica® System 3-color digital PCR (Stilla).

For this, a double-stranded DNA fragment of 910 base pairs (bp) length was developed as positive control, using a puc57 vector (Figure 3). The fragment contains concatenated DNA sequences of the eight above mentioned loci, back-to-back, in the following order: *ctrA*, *porA*, *tau*, *metA*, *rplF*, *zupT*, *tolC*, and *nhhA*. To ensure confirmatory Sanger sequencing of the DNA can produce a complete DNA sequence of the template, flanking sequences of 20 bp were added to each end of the DNA templates (5'-GCACGTGGTACGGTTTCTGT, 3'-CTAGCAAAGGTTACATTTGT).

Figure 3: Positive control plasmid containing concatenated synthetic Neisseria loci. Total length: 910 bp, including eight loci of 870 bp and two flanking regions of 20 bp each.

A 10-step dilution series of the *Neisseria* template synthetic DNA fragment was used for the dPCR optimisation and quantification including measurement uncertainty estimation for precision and accuracy. For each assay (*porA, metA* and *rplF*), the estimated concentration of each dilution step was determined using Naica dPCR. To calculate precision and accuracy, three replicates per run and three runs per dilution were measured. A concentration of 3 copies/µL was used as positive control. Naica results were visualised using 2D-plots as

indicated, data shown for *porA* in Figure 4. Here, METAS benefited greatly from capacity building support of NIB Slovenia in carrying out these experiments and analysis.

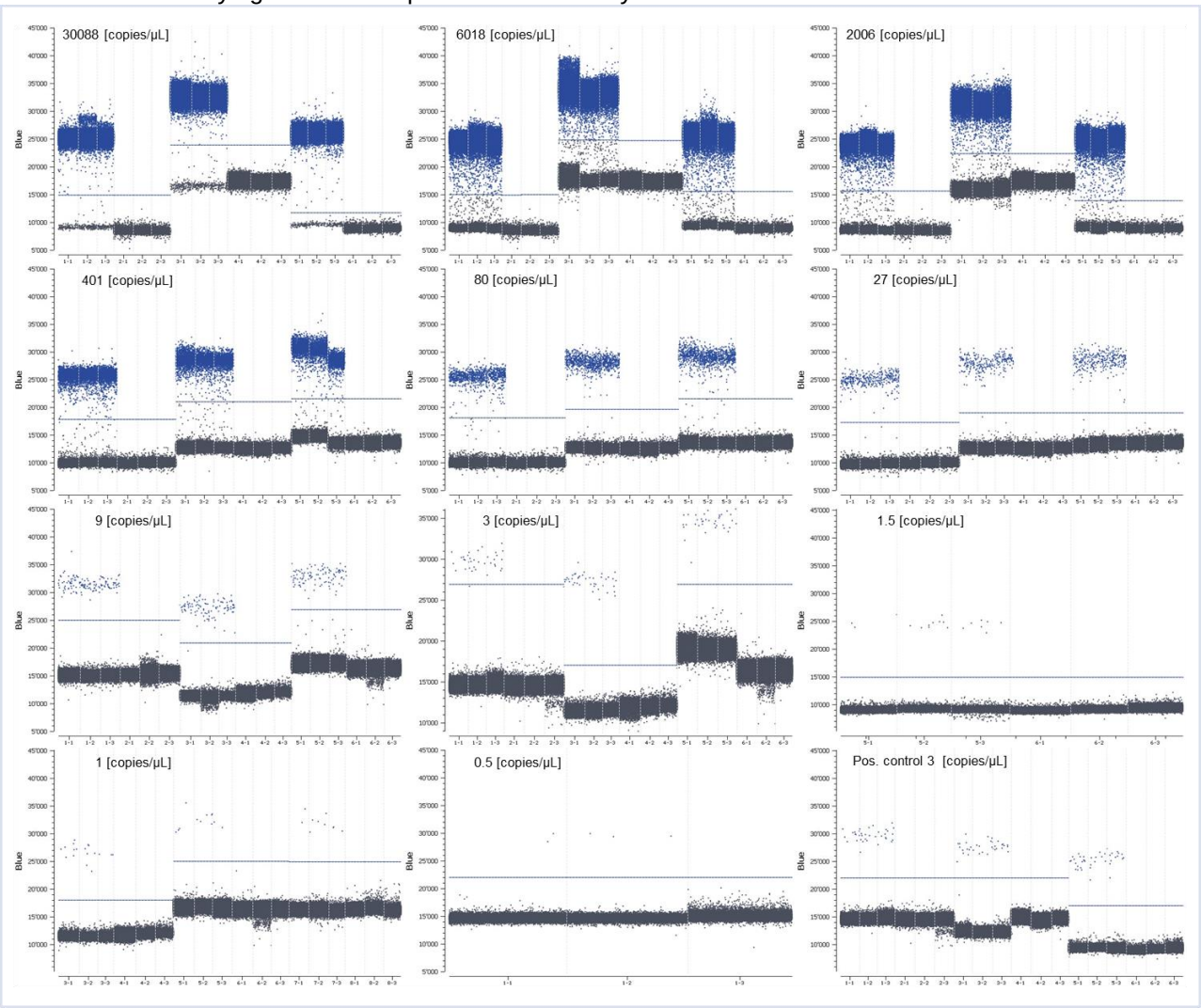


Figure 4 2D-plots of dPCR showing fluorescence intensity of both negative and positive droplets from the porA assay at each dilution step. Target concentrations indicated for each dilution step.

Table 1 reports determined DNA concentrations and the associated expanded measurement uncertainties (MU). The three assays are comparable in their respective limits of detection (LOD) and quantification (LOQ): with 13 copies per µL for the *metA* and *rplF* assay and 14 copies per µL for the *porA* assay, LOQ are almost identical. The LOD values were 5 copies per µL for the *porA and metA* assay, and 4 copies per µL for the *rplF assay.*

	porA (LGC)		***metA*** (Diene *et al.*, 2019)		***rplF*** (METAS)	
Estimated DNA concentration [copies/µL]	**Measured DNA concentration** [copies/µL]	**Expanded MU** [%]	**Measured DNA concentration** [copies/µL]	**Expanded MU** [%]	**Measured DNA concentration** [copies/µL]	**Expanded MU** [%]
30088	44262	6.4	45101	3.5	43616	6.4
6018	9507	2.3	9235	5.1	8894	4.9
2006	3156	10.5	2896	9.3	2910	8.9
401	599	5.5	586	4.4	583	5.4
80	133	8.4	124	6.7	115	11.1

	27	41	16.1	41	11.3	40	11.0
LOQ	9	14	10.4	13	21.8	13	24.9
LOD	3	5	29.5	5	48.8	4	41.7
	1.5	3	*n.a.*	2	*n.a.*	4	*n.a.*
	1	2	*n.a.*	2	*n.a.*	2	*n.a.*
	0.5	1	*n.a.*	0	*n.a.*	2	*n.a.*

Table 1: Measured DNA concentrations and expanded measurement uncertainties for the *N. meningitidis* assays *porA, metA* and *rplF*.

Estimated and observed DNA concentrations are significantly correlated in all assays with R^2 values above 0.99 for each assay, as presented in Figure 3.

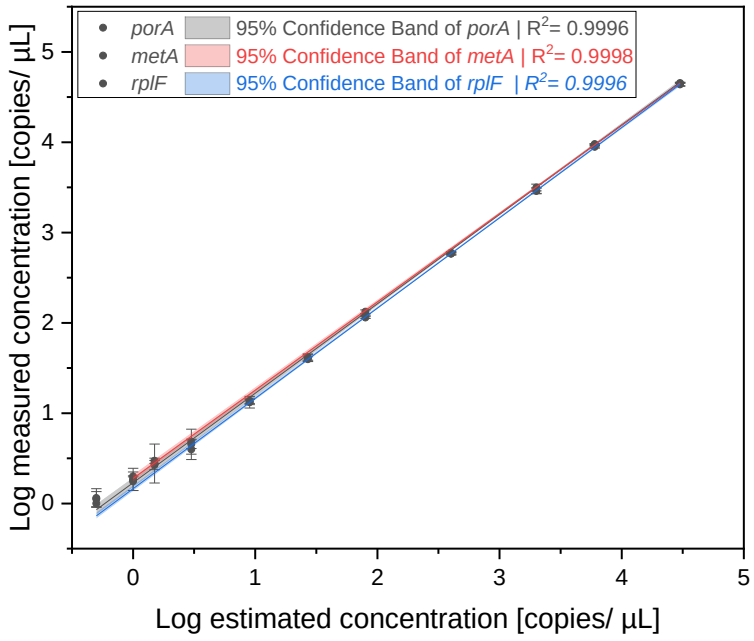


Figure 3: Correlation between the observed and expected concentrations of N. meningitis assays porA, metA and rplF.

Hospital acquired respiratory tract infections are a major cause of acquired sepsis. Three Gram-negative bacterial species that cause the majority of such infections are *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These bacterial species were chosen as some strains within these species have been shown to harbour multidrug and even pandrug resistance, making their accurate identification even more significant.
A pipeline that included a sequence analysis tool RUCS (doi:10.1093/bioinformatics/btx526) was developed based on Alič et al (doi:10.5958/2249-4677.2022.00023.8), enabling the development of highly specific dPCR assays for each of the chosen bacteria species. Two assays targeting *A. baumannii*, and 3 targeting *K. pneumoniae* and *P. aeruginosa* were developed. Additionally, a literature search was performed which gained eight, six and eighteen assays for *A. baumannii*, *K. pneumoniae* and *P. aeruginosa*, respectively. After an in depth in-silico specificity analysis only five were selected for further analysis.

Organism[1]	Assay Name	Gene	Amplicon length	Oligonucleotide sequence (F/R/P)[2]	Final concentration [nM]
A. baumannii	**AB_1**	***adeK***	**68**	**5'-GCTCGTGATTCGACTCAAATCA-3'** **5'-GCAAACGAATAATTTAACCATGCTT-3'** **5'-FAM-CTGATTAGCCAAGTTGC-MGBNFQ-3'**	**900** **900** **300**
	AB_2	*hemA*	66	5'-CTGCATTAAAAAAACGCCGTTA-3' 5'-CAATATCACGCGGTACAGCTAAA-3' 5'-FAM -AGCAAATGTTAATGGTCG- MGBNFQ-3'	900 900 300
	AB_3	*bap*	97	5'-CGCTGCAGCATCAAATCATG-3' 5'-TGGGTCAACCGAGAAAGTTACG-3' 5'-FAM-AGCACCTGCTGACACCACTCCACCA- BHQ-3'	900 900 300
K. pneumoniae	**KP_1**	***rssB***	**62**	**5'-CCCGCTGTGGTAATACCCTACT-3'** **5'-GCTGCGTCAGGCAAATCTTC-3'** **5'-FAM -CAGAGGGAACTGGC- MGBNFQ-3'**	**900** **900** **300**
	KP_2	*bioD*	65	5'-GGAACAGCTGCCGGTCATT-3' 5'-AAGCATTGCGTGGTTGATACAG-3' 5'-FAM -TGGTGGTGGGCGTTAA- MGBNFQ-3'	900 900 300
	KP_3	*bioD*	65	5'-ACAGCTGCCGGTCATTCTG-3' 5'-GGTAAGCATTGCGTGGTTGA-3' 5'-FAM -TGGTGGGCGTTAAGCTCGGCTGT-BHQ-3'	900 900 300
P. aeruginosa	**PA_1**	***dnaG***	**64**	**5'-TCTGCCGCGGGTTCTTC-3'** **5'-AGGATACCTACGCCCAGTTGCT-3'** **5'-FAM -CTTCCAGCAGGGACAC- MGBNFQ-3'**	**900** **900** **300**
	PA_2	*dnaG*	65	5'-TTCTTCTGCAGGGCTTCCA-3' 5'-TCGCCAGGGAGCAGGATAC-3' 5'-FAM -CAGGGACACCAGCAACTGGGCG- BHQ-3'	900 900 300
	PA_3	*rpoS*	57	5'-AGCTGCGTTGCGTCCAA-3' 5'-TCTTCCAAACAACACAAGCACAT-3' 5'-FAM -CGCGCGTGTAGTC- MGBNFQ-3'	900 900 300
	PA_4	*regA*	65	5'-TGCTGGTGGCACAGGACAT-3' 5'-TTGTTGGTGCAGTTCCTCATTG-3' 5'-FAM-CAGATGCTTTGCCTCAA- BHQ-3'	900 900 300
	PA_5	*ecfX*	64	5'-CGCATGCCTATCAGGCGTT-3' 5'-GAACTGCCCAGGTGCTTGC-3' 5'-FAM-ATGGCGAGTTGCTGCGCTTCCT- BHQ-3'	900 900 300
	PA_6	*gyrB*	125	5'-CCACAACAAGGTCTGGGAAC-3' 5'-CCAGGATGTCCCAACTGAAG-3' 5'-FAM-GGAGACCTTCAGCAACATCC- MGBNFQ-3'	900 900 300
	PA_7	*ecfX*	125	5'-TTCCATGGCGAGTTGCT-3' 5'-CGGGCGATCTGGAAAAGAA-3' 5'-FAM-GCTGAAATGGCCGGGCC- BHQ-3'	900 900 300

Table 2: List of assays, which passed in-silico evaluation.

To assess the best fit assay high-throughput qPCR (Fluidigm Biomark) was used. All assays were tested using three different mastermixes and genomic DNA extracted from type strains from the German Collection of Microorganisms and Cell Cultures (DSMZ) (*A. baumannii* – DSM No. 30007, *K. pneumoniae* - DSM No. 30104 and *P. aeruginosa* - DSM No. 50071). In this step in vitro specificity was also assessed. One assay was chosen for each bacterial species (marked in bold in table 2).

Assays were characterised using QX200 platform (BioRad), here repeatability, inter-mediate precision, selectivity, trueness, linearity and LOD and LOQ, were assessed. Where applicable measurement uncertainty was calculated. Characterisation was performed using synthetic gene fragments. All three assays showed wide linearity, with LODs ~ 10 cp/rnx and LOQ below 50 cp/rnx. MU was below 25% through the entire dynamic range. Additionally, analytical sensitivity was assessed on DNA extracted from sputum samples with spiked known amount of bacteria. Three such samples were prepared and in each all three bacteria were present in different concentrations (Table 3). Trueness was assessed on DNA extracted from 20 mock and clinical sputum samples provided by University Clinic Golnik. These samples were assessed with the golden standard method MALDI-TOF. The developed assays showed comparable results.

Organism	Measurement	Sample A	Sample B	Sample C
A. baumannii	McFarland cp/mL	5.5×10^{4}	5.5×10^{5}	5.5×10^{3}
	dPCR cp/mL	1.7×10^{5}	1.7×10^{6}	1.7×10^{4}
	MU %	12.77	11.89	19.30
K. pneumoniae	McFarland cp/mL	5.5×10^{5}	5.5×10^{3}	5.5×10^{4}
	dPCR cp/mL	8.5×10^{5}	8.7×10^{3}	8.7×10^{4}
	MU %	13.02	21.04	16.58
P. aeruginosa	McFarland cp/mL	5.5×10^{3}	5.5×10^{4}	5.5×10^{5}
	dPCR cp/mL	2.3×10^{4}	1.9×10^{5}	1.9×10^{6}
	MU %	25.03	13.30	14.52

Table 3: Analytical sensitivity as assessed by turbidity (McFarland) and dPCR complete with relative expanded measurement uncertainty (MU %).

Sputum samples spiked with each of the bacterial species were also used to assess the extraction methods (https://doi.org/10.3390/bios13040463). Five extraction methods were used: two automated methods using GXT NA extraction kit on an Arrow system and QuickPick genomic (g)DNA extraction kit on a KingFisher Duo system, two manual kit-based methods using QIAamp DNA mini kit and DNeasy UltraClean microbial kit, and an 'open' CTAB method. Extractions were evaluated based on yield and repeatability (figure 6).

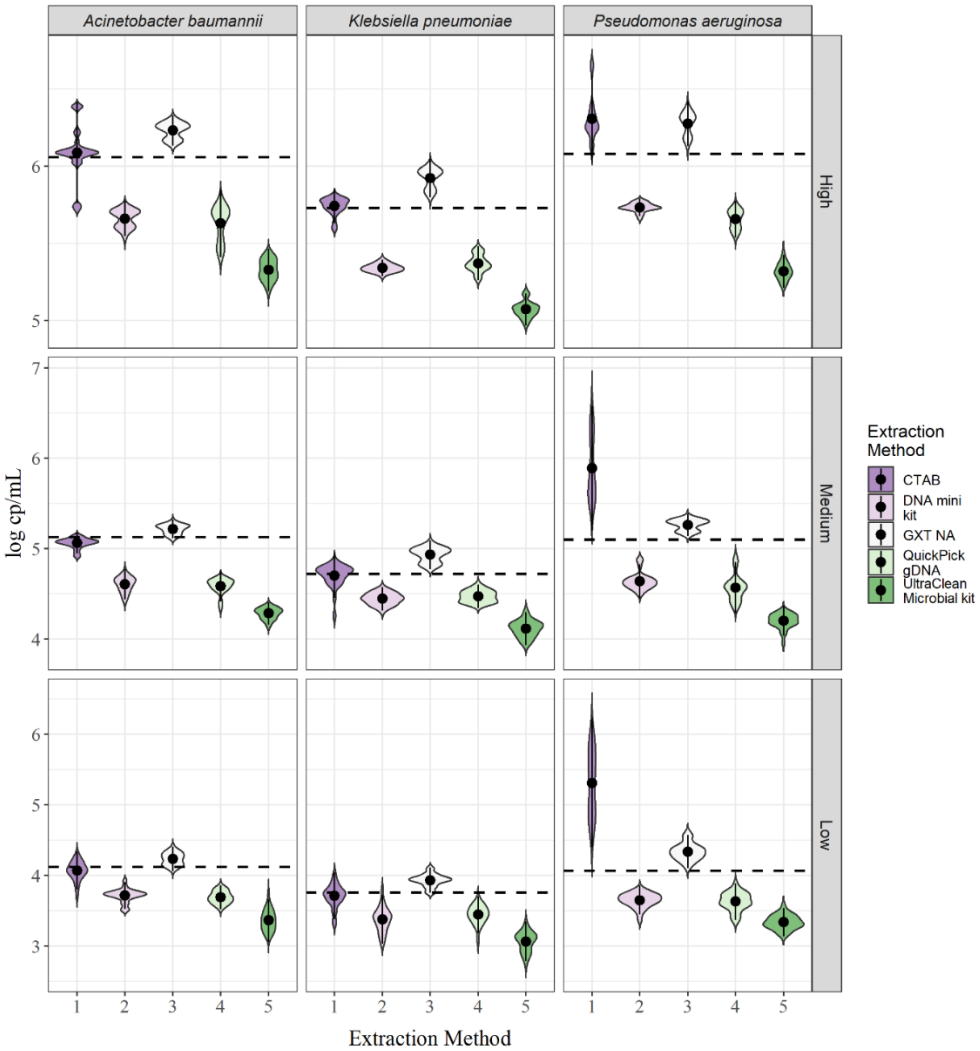


Figure 6. Yields for each of the target bacteria and DNA extraction methods. The dotted line represents the theoretical 100% yield.

Measurement uncertainties of results varied and were depending on the concentration, target bacteria and method (Figure 7). However even including extraction from a very complex sample, measurement uncertainty was below 30 % for 2-4 extraction method for each bacterial species (and each assay) for all three concentrations chosen.

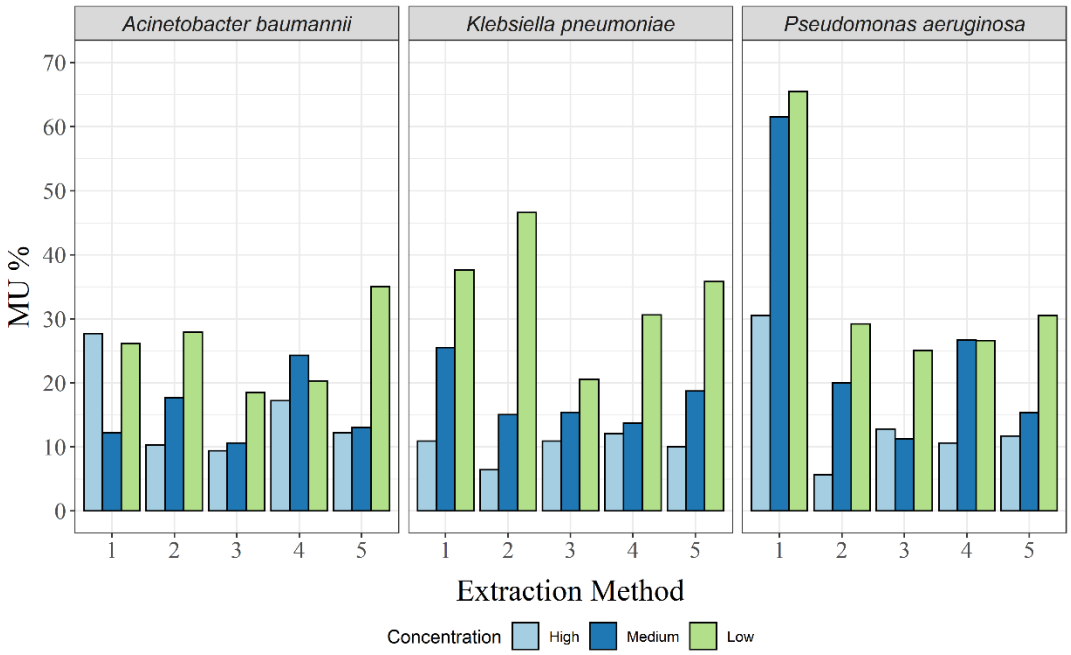


Figure 7. Relative expanded (k=2) measurement uncertainty for each of the three bacterial species and for all extraction methods: 1, CTAB; 2, QIAamp DNA mini kit; 3, GXT NA extraction kit; 4, QuickPick genomic DNA extraction kit; 5, DNeasy UltraClean microbial kit.

Additionally, within the consortium, reference methods were developed for detection of SARS-CoV-2 RNA. A fast-track pilot CCQM NAWG study was performed by the NMI's, co-led by the LGC, to demonstrate capability for reference measurement of SARS-CoV-2 RNA copy number quantification. The study was successful in demonstrating quantification by most laboratories of the materials within ± 40 % of the mean (https://www.bipm.org/en//-/2020-nmi-covid, accessed 29.03.2022). The report of this study is prepared and should be available shortly.

The work undertaken in SEPTIMET developed a metrological and quality assurance framework for current methods for detecting the microbiological cause of sepsis. These methods have been developed for detection of the following pathogenic organisms: *K. pneumoniae, E. coli, S. aureus, A. baumannii, P. aeruginosa*, N. *meningitidis* and SARS-CoV-2. Many of the approaches developed include the extraction of nucleic acid stages as well as the molecular detection of the targets. Sources of uncertainty such as extraction were investigated as well as cross platform comparisons performed. 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. 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 must 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).

Work to achieve this objective was performed by LGC, PTB, NIB, METAS, RSCH, GOSH, MUW, BGU, LNE, APHP, CEA and RSCH.

S. aureus was chosen as the bacterial model for one of the sepsis causing pathogens. The *S. aureus* model was selected based for its relevance to stakeholders, accessibility of standard materials and for clinical needs. A potential reference measurement procedure (RMP) was developed for accurate and fast detection of *S. aureus* as well as identification of *methicillin resistant S. aureus* (MRSA), which would support routine molecular testing in clinical practice.

The selection of *S. aureus* nucleic acid extraction method was investigated in the previous AntiMicroResist project. Re-evaluation of the previously selected extraction method for *S. aureus* was required since a

component of the lysis buffer, Triton X100, was banned in EU to protect the environment. Triton X100 is ecotoxic and its substitution for environmentally friendly alternatives is required by the European Chemicals Agency.

In this work, two different alternative detergents were identified and investigated, and the performance of *S. aureus* extraction using the Qiagen DNeasy® Blood & Tissue Mini Kit was evaluated. *S. aureus* material was obtained from a variety of sources. This included DSMZ whole bacterial *S. aureus*, clinical swabs and lyophilised whole MRSA from external quality assessment (EQA) schemes. Genomic DNA yield was determined by analysing the extracts using *S. aureus* specific assays consisting of Sa442 and coA on a dPCR platform. (Figure 8). A QX200 instrument (BIO-RAD) was used for these measurements.

Triton X100 reduced was found to give the highest yield compared to the NP40 using the Qiagen DNeasy® Blood & Tissue Mini Kit and was selected for the *S. aureus* extraction.

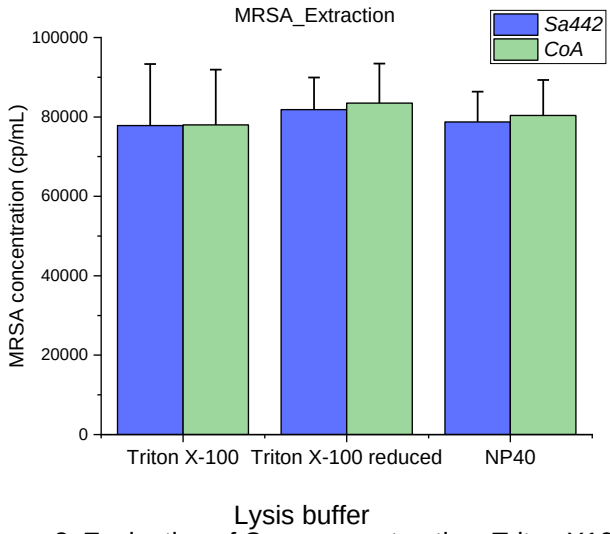
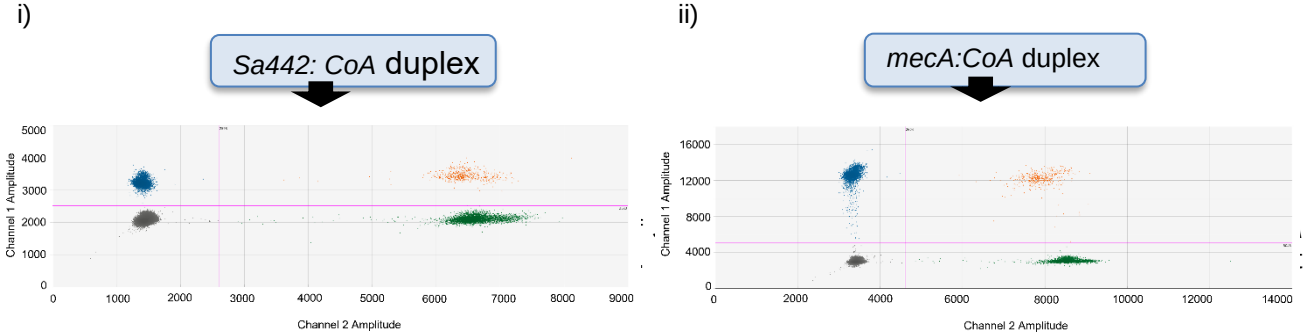


Figure 8: Evaluation of *S. aureus* extraction. Triton X100 reduced and NP40 as alternative detergents to Triton X 100 (previous standard detergent). DNA yields expressed as Sa442 and coA copy number per mililiter (cp/mL) were determined using dPCR. Results are presented as mean values based on triplicate technical replicates for each detergent analysed. Extraction was performed on three different days. Error bars represent standard deviation.

After re-evaluating the extraction method, multiple MRSA targets were chosen from three different gene/loci of the MRSA genome. The published assays from the literature were previously selected in the AMR project and consisted of *coagulase A* (coA) and *Staphylococcus aureus 442bp segment* (Sa442) for the identification of *S. aureus* and *methicillin resistance* (mecA) assay for the detection of methicillin resistance. All three assays were evaluated for technical parameters such as annealing temperature, positive and negative droplet separation and primer and probe concentration, as well as the analytical performance including dynamic range, linearity, repeatability, intermediate precision and LOD. Furthermore, two sets of duplex assays consisting of (coA:Sa442) and (coA:mecA) were evaluated. Performance characteristics such as appropriate annealing temperature, assay competition for reagents, rainy droplets were examined. An example of the amplification plot of the two duplex assays is presented in the Figure 9.



(Sa442 and *mecA*), green dots represent HEX-labelled targets (*coA*), orange dots represent both targets detected in a droplet, grey dots represent empty droplets without detected target gene sequence.

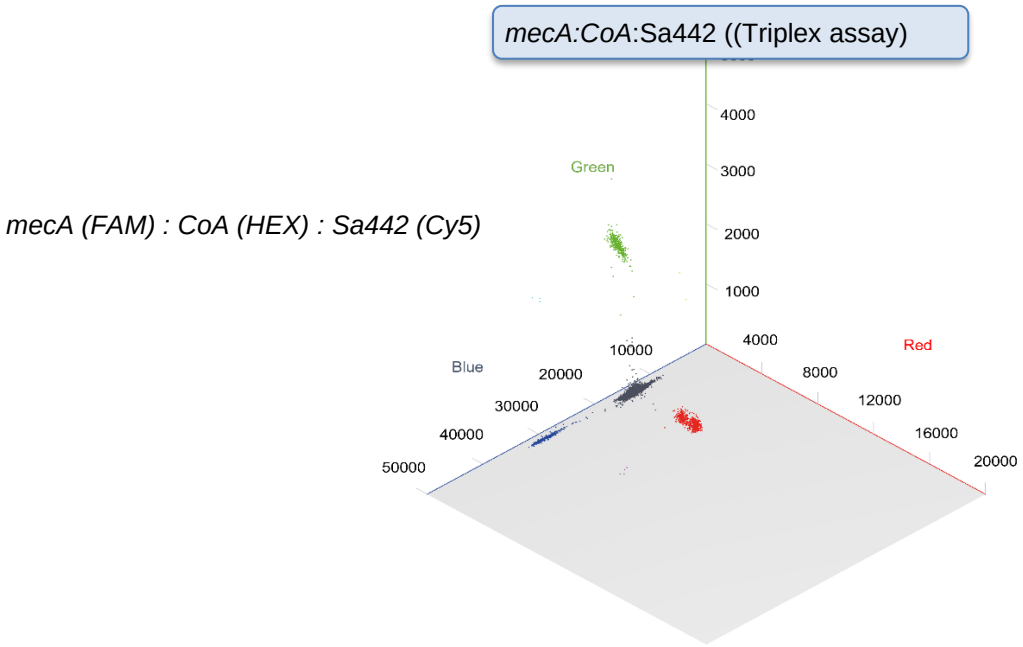


Figure 9: Naica digital PCR three dimensional plots showing detection of three targets: Sa442, *coA* and *mecA*. *S. aureus* genomic DNA from DSM 10227. Blue dots represent presence of FAM-labelled target *mecA*, green dots represent HEX-labelled targets coA, red dots represent Cy5-labelled *Sa442* and grey dots represent empty droplets without detected target gene sequence.

Both duplex assays were then validated and transferred to another dPCR platform, naica dPCR (Stilla), which allows the simultaneous detection of three targets. The two duplex assays were further characterised in a triplex assay and quantified on the naica dPCR platform as shown in Figure 9.

The developed RMP method was applied in an EQA program to determine if the method is fit for purpose. The inter-laboratory scheme organised by the German EQA provider INSTAND e.V. for MRSA/cMRSA genome detection (539). Four NMIs (PTB, NML/LGC, NIB and METAS) have participated in the two EQA programs (INSTAND code 539) from 2021 to 2022. The results of the inter-laboratory studies showed that the developed method performed well and showed good agreement between the NMI's for both quantitative and qualitative results. Despite the use of two different dPCR platforms (QX200 & naica dPCR), no significant differences or biases were found. The results of the interlaboratory studies and measurement uncertainty calculated using the data from three laboratories are shown in Table 4.

Table 4: Copies per mL of primary sample with standard (u_r) and expanded uncertainty (U_r) expressed as a %, with two degrees of freedom (k=4.3 95% confidence interval).:

Assay	Sample	Estimate (copies/mL)	u_r(%)	U_r(%)
coA	2215391	1.32E+05	8.19	35.2
mecA	2215391	1.48E+05	7.29	31.35
Sa442	2215391	1.56E+05	6.95	29.88
coA	2215392	2.67E+05	8.96	38.51
Sa442	2215392	2.86E+05	8.37	35.97
mecA	2215393	4.31E+05	4.28	18.41
coA	2215394	2.79E+06	4.64	19.95

mecA	2215394	2.24E+06	5.85	25.16
Sa442	2215394	3.04E+06	4.32	18.56
coA	2225391	2.99E+05	5.88	25.3
mecA	2225391	4.40E+05	4.03	17.34
Sa442	2225391	3.19E+05	5.57	23.94
coA	2225393	3.02E+04	7.34	31.57
mecA	2225393	5.27E+04	4.27	18.35
Sa442	2225393	5.42E+04	4.16	17.87
coA	2225394	1.54E+05	13.61	58.53
mecA	2225394	1.71E+05	12.3	52.9
Sa442	2225394	1.82E+05	11.57	49.77

The performance of the prototype RMP for detection of MRSA was satisfactory with expanded uncertainties of ~ 30 %.

The reference methods developed within the project can be applied to reduce the uncertainties of the current rapid near patient tests for 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. 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 should include an evaluation of their accuracy and reproducibility and the identification of target levels of both, for each method.

Work to achieve this objective was performed by PTB, NPL, MUW, RSCH, GOSH, LGC, BGU, LNE, METAS and APHP.

The aim of this part of the project was to identify emerging laboratory diagnostic approaches that are considered for early sepsis diagnosis and to guide treatment. The analytical methods used in this context will benefit from a metrological framework to characterize the analytical performance and harmonization of results obtained in routine labs by making available traceable measurements.

<u>Biosensors based on Surface Plasmon Resonance (SPR) or Graphene Field Effect Transistor (GFET)</u>
Inflammation induced by endotoxins can contribute significantly in sepsis caused by Gram-negative bacteria. The membrane of those bacteria contains a toxic protein than can be released for example by bacterial cell lysis by immune cells. Low limit of detection for endotoxins is routinely achieved using reagents (LAL) derived from the blood of horseshoe craps, which use them as part of their own immune system. This assay is, however, not suited for continuous monitoring. Optical biosensors that monitor an antibody-antigen reaction at the detector-sample interface can also be used to detect bacterial endotoxins.

A biosensor based on surface plasmon resonance (SPR) was successfully developed. This resonance reduces the light reflection in certain directions. Antibodies are mounted on the sensitive surface of the SPR sensor. If the toxic protein bounds to the antibodies this changes the optical properties at the sensor surface. This results in a shift of the plasmon resonance and changes the reflection pattern of the sensor. This change can be detected, e.g. using a camera, and can be applied to detect and quantify the amount of endotoxin in the sample.

The binding of target proteins to the antibodies not only changes the optical properties locally. At the interface the dielectric properties also change. This can be exploited to use electrical circuits to detect the presence of antigen and to quantify their concentration. Field-effect transistors are among the most sensitive electronic detectors for this purpose. A specific advantage of using electronic chips is the simplicity for integrating multiple electronic detectors that can be used to detect different targets.

Figure 10: 12 channel GFET sensor with fluidic interface.

The Covid-19 pandemic unfolded at the beginning of the project. Therefore, a detection zone was added that was coated with modified antibody able to detect the proteins of SARS-CoV-2 viruses. The respective antibodies are mounted on graphene platelets on top of the sensitive zone of the field effect transistor (GFET, Figure 10). A fluidic interface can be used to simplify sampling and washing steps. In principle microfluidic structures could be devised to specifically address all 12 integrated GFET sensors separately. The sensing device was successfully tested on SARS-CoV-2 positive samples in Great Ormond Street Hospital (GOSH, London). Based on this work NPL proposed a VAMAS project in TWA 40 (synthetic biomaterials) for "Validating biosensor kinetics for microorganism antigens" that is in progress since May 2022.

<u>Evaluation of (metagenomic) sequencing as innovative diagnostic tool for sepsis</u>

Early antibiotic treatment is important for treatment of sepsis. The initial selection of antibiotics is based on experience from previous cases. To improve selection of antibiotics the causative agent of the suspected infection has to be identified. Common approaches include the use of a broad range of PCR tests, and particularly using cell cultures derived from patient blood. These approaches can cover only a limited range of pathogens. An alternative is application of (metagenomic) sequencing. The aim was to develop and access metagenomic sequencing methods for application to patient samples as innovative diagnostic tool for detection of sepsis-causing microbes.

A number of enrichment and depletion methods were accessed which were intended to increase the fraction of genetic material from microbes relative to the human background. Those studies were carried out with contrived materials with known initial concentration of bacterial contaminants. Two commercially available method for host depletion were investigated; A and B. It was observed that the B kit achieved the greatest depletion of human DNA, as demonstrated by a ~3 log reduction in copies of PDH compared to no depletion, when analysing all the blood samples (Figure 11). The A method also achieved depletion of human DNA but to a lesser degree (~2 log compared to no depletion). The depletion methods also showed reductions in the amount of copies of *E. coli* extracted, ~1 and ~2 log for A and B respectively (Figure 4). This results in increases to the relative copies of E. coli/Human DNA present within the samples that had been depleted (7% for no depletion vs 49% and 61% for the A and B respectively).

The sequencing data shows a similar trend where the relative amounts of *E. coli*/Human reads increased for both the depleted samples, however the A shows a greater increase than the B, from 0.02% in the no depletion samples to 0.21% and 0.10% for the A and B respectively.

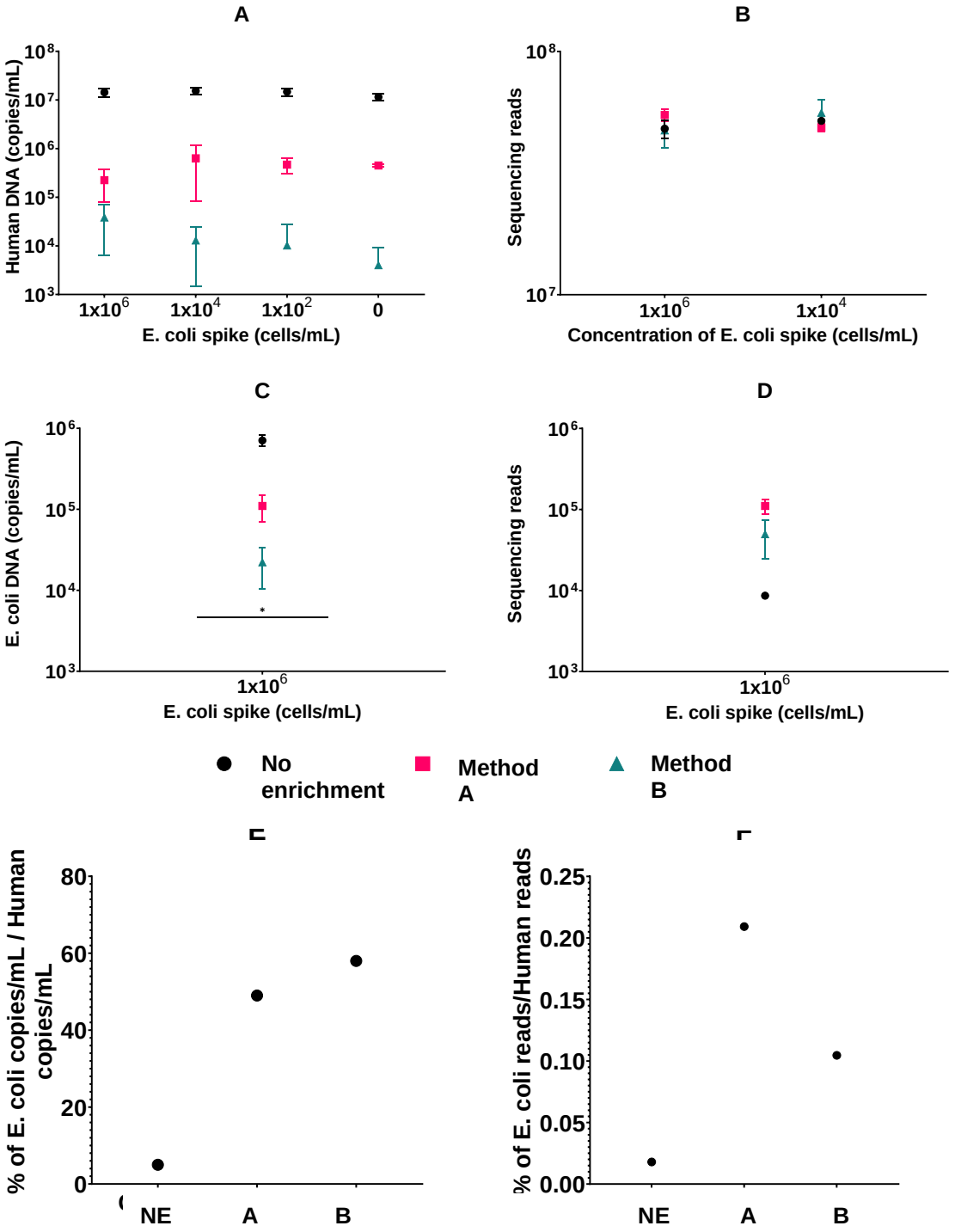


Figure 11: A – Depletion of human DNA from *E. coli* ST131 spiked blood samples extracted using a total DNA extraction (Qiagen QIAamp) and two host depletion methods (kit A and kit B). B – Sequencing reads of human DNA from *E. coli* ST131 spiked blood samples extracted via methods in A. C – The dPCR quantifications of *E. coli* ST131 spiked in whole blood and extracted by same methods in A, converted into copies/mL of the

initial samples. Horizontal line indicates the amplification that was present in whole blood extracted by these methods that contained no E. coli spike. D – Sequencing reads of *E. coli* DNA from *E. coli* ST131 spiked blood samples extracted via methods in A. E – Copy numbers of *E. coli* DNA relative to human DNA after performing the extraction methods. F – Sequencing reads of *E. coli* DNA relative to human DNA after performing the extraction methods. Graphs A-D – N1 = 3, error bars are standard error.

The bioinformatic pipeline was accessed with mock sample data based on numerical simulation of instrument reads. A key finding was that the mock data should include a sufficient amount of human DNA background reads, as such contributions are expected in the bioinformatic pipeline adjusted to analyse patient data.

Sufficient amount of sequencing library is required to complete sequencing. Unspecific fluorescence-based techniques or qPCR are commonly used for quantification of nucleic acid material. Work to objective 3 is based on application of digital PCR for quantification of DNA, as this method offers more reliable quantification than qPCR. Therefore, using digital PCR for library quantification was included in two currently developed CEN standards (CEN-TC140-WG3-WI00140151, In vitro diagnostic Next Generation Sequencing (NGS) workflows — Part 1: Human DNA examination; CEN-TC140-WG3-WI00140153, In vitro diagnostic Next Generation Sequencing (NGS) workflows — Part 2: Human RNA examination).

Metrology framework for cell-based sepsis markers

Traceability of measurement is of high importance for in-vitro diagnostic measurements as it allows to provide reliable and comparable results independent of time and, ideally, without the need to use calibration material that may degrade with time or get exhausted. Expression of CD64 and CD11b on neutrophils (and monocytes) were demonstrated to be potential markers for sepsis diagnosis and to guide treatment. The measurand applied for medical decision is the expression of these antigens on the cell surface.

Flow cytometric analysis carried out in the frame of the project included additional markers to properly identify the target cell population as well to explore potential additional markers (CD45, CD14, CD16, CD62, etc.). Based on approved ethics protocol measurements on three groups of human subjects were performed by MUW in Warsaw: patients with interstitial nephritis (inflammation without sepsis), patients diagnosed with urosepsis, and control patients (healthy volunteers). The key challenge for the metrological framework for cell-based sepsis markers is to develop a strategy that allows to establish traceability for quantification of marker expression. Measurement is based on applying fluorescently labelled antibodies. Therefore, two steps should be considered: conversion of the number of antigens on the cell surface to the number of fluorophores, and traceable quantification of the number fluorophores on the cell surface.

fluorophores on the cell surface.

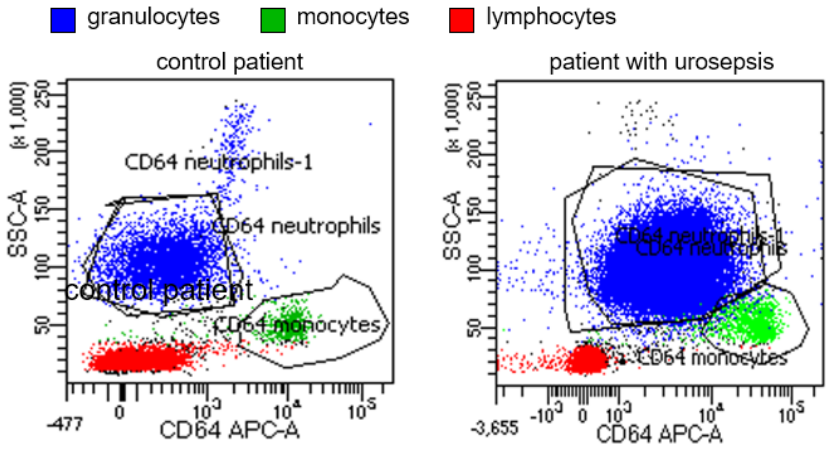


Figure 12: Scatter diagrams obtained by measuring CD64 expression

Figure 12 shows raw data obtained by MUW in a flow cytometric measurement of blood collected from a control patient and a patient with urosepsis. A significantly increased CD64 expression is evident for the patient with urosepsis, which is in line with the expectation of CD64 expression on neutrophils being increased in septic

patients. However, quantitative results cannot be derived directly. This requires calibration of the APC detection channel, which includes proper experimental design and the use of suitable calibration material.

Different methods have been developed to quantify the number of fluorophores on an antibody including mass spectroscopy and single molecule detection in microscopy. A simpler solution is to use fluorescent proteins as fluorescent labels, as those markers have only one dye molecule per antibody. In the project APC and PE were, therefore, selected as fluorescent labels for CD64 and CD11b. Molecular conjugates of those fluorescent protein were also used for other markers by MUW.

Suspensions of fluorescent beads are routinely used for instrument qualification in daily operation. Such beads can also be applied to calibrate the fluorescence detection channels. Traceability is obtained comparing the fluorescence of beads to the fluorescence of a known dye suspension in a fluorometer. For this purpose, the bead concentration is measured. Such bead material is commercially available including beads that can be used for several fluorescence channels (rainbow beads).

Using such fluorescence beads for calibration of the flow cytometer allows perform quantitative measurement of the fluorescence of the cell. A technical challenge can result from differences in the size of the beads and the cells. Typically, the bead size is in the range of 3 µm to 4 µm which is in the middle of size range of cells commonly measured using flow cytometry. Human monocytes and neutrophils are on the larger end of the cell size range. Therefore, the field of view available to register the fluorescence signal from the cell should be considered. An example comparing measurements with two flow cytometers operated at PTB is shown in Figure 13. The measurements were performed with the identical fluorescently labelled blood sample and identical spectral excitation and detection settings. Histograms show results gate on the monocyte cell population. The results from the microchip device were obtained with the detection geometry of an epifluorescence microscope. This system provides a large field of view, which mimics the situation during bead calibration.

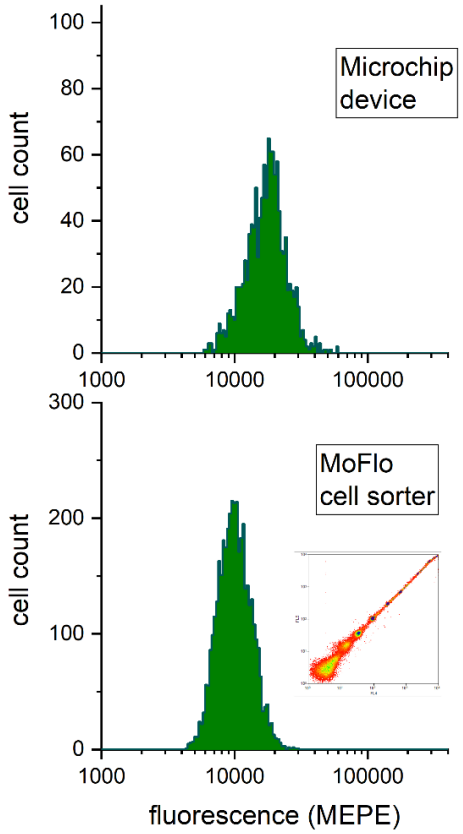


Figure 13: Apparent CD14 expression of monocytes measured labelling the cell surface with anti-CD14-PE. The cell sorter gives lower reading as result of detection geometry but distributions overlap. Insert shows scatterplot of calibration beads.

On the other hand, a large field of view is detrimental for high-speed cell counting as is required for cell sorters. The difference in laser excitation and fluorescence detection geometry in the MoFlo cell sorter is the reason for the apparently lower CD14 expression measured with this device (Figure 13). The average fluorescence signal from the monocytes is slightly less than two-times lower. The relative changes of the expression of the cell-based inflammation markers are expected to be higher in the development of sepsis so that the difference in detection efficiency will not result in fundamentally flawed results. However, the measurement bias introduced by the detection geometry of the flow cytometer should be considered for defining thresholds for medical decisions.

The overall key output achieved by objective 4 aimed at development of a metrological framework for three innovative approaches for early sepsis diagnosis. This includes an approach for chip-based detection of protein markers using plasmon resonance and field effect transistors, a quantitative measurement of inflammation markers of immune cells, and the exploration of metagenomic sequencing aimed at identification of the causative pathogens for sepsis. The first two examples use classical measurands (protein concentration, antibody binding capacity) that allow to establish SI traceable measurements. This was achieved in the setups developed in the frame of the project, and application to clinical samples was also demonstrated. However,

future efforts will be needed to complete the metrological framework by allowing harmonized measurements in routine clinical laboratories. A remaining challenge for future research is development of widely commutable reference materials to perform interlaboratory comparisons. For instance, the expression of cell-based antigen markers relevant for monitoring inflammation is known to change rapidly in life cells, making development of control materials difficult. The challenge for the metagenomic sequencing is even higher, as the primary result is obtained by nominal property evaluation rather than a measurement. The metrological terminology and the related uncertainty evaluation of nominal property evaluations is currently under development. Here, the key output provided by objective 4 was to evaluate aspects of metagenomic sequencing that are amenable to quantitation such as concentration ratios of detected sequences. Future efforts will be directed to uncertainty aspects of nominal property evaluation to establish a comparability of examinations similar to SI traceability for classical measurands. Therefore, 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.

5 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 (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.

6 List of publications

1. Huggett JF, Benes V, Bustin SA, Garson JA, Harris K, Kammel M, Kubista M, McHugh TD, Moran-Gilad J, Nolan T, Pfaffl MW, Salit M, Shipley G, Vallone PM, Vandesompele J, Wittwer C, Zeichhardt H., "Cautionary note on contamination of reagents used for molecular detection of SARS-CoV-2", Clin Chem., 2020 Sep, https://doi.org/10.1093/clinchem/hvaa214

2. Huggett J, Harris K, Moran-Gilad J, Zumla A., “Diagnostic tests for covid-19—improving accuracy and global harmonisation”, BMJ. 2020; May 6., https://blogs.bmj.com/bmj/2020/05/06/diagnostic-tests-for-covid-19-improving-accuracy-and-global-harmonisation/

3. Nathaniel Storey, Julianne R Brown, Rui P A Pereira, Denise M O'Sullivan, Jim F Huggett, Rachel Williams, Judith Breuer, Kathryn A Harris, “Single base mutations in the nucleocapsid gene of SARS-CoV-2 affects amplification efficiency of sequence variants and may lead to assay failure”, Journal of Clinical Virology Plus, Volume 1, Issue 3, 2021, https://doi.org/10.1016/j.jcvp.2021.100037

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