Publishable Summary for 15HLT01 MetVBadBugs
Quantitative measurement and imaging of drug-uptake by bacteria with antimicrobial resistance

Overview

The innate resistance of Gram-negative bacteria to antibiotics is a consequence of the combinatorial effects of two permeability barriers: the outer and inner bacterial cell membranes, their ability to efflux antibiotics out of the cell and their capacity to form antibiotic tolerant biofilms that are up to 100 times more resistant than planktonic bacterial cells. The objectives of this project are to advance the measurement capability by providing the urgently needed essential metrology to quantitatively measure and image the localisation of antibiotics and to understand the penetration and efflux processes in bacteria and biofilms.

Need

It is universally acknowledged that the threat of antimicrobial resistance (AMR) to the health and prosperity of Europe and the world is real. The European Union has a major initiative to fight AMR. The Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) is developing a strategic research agenda and is co-ordinating European research in Horizon 2020, in the Innovative Medicines Initiative (IMI) and in the EC's ERA-NET funding scheme. For example, the New Drugs for Bad Bugs (ND4BB) programme of the IMI involves nine large pharmaceutical companies in seven ND4BB projects, with a total committed budget of more than €600 million. They have identified a metrology gap that EMPIR is uniquely placed to fill. The ND4BB project TRANSLOCATE has stated that *"At present, there are no reliable and general methods for measuring these [drug] penetration processes in Gram-negative bacteria and this bottleneck substantially hinders the ability of scientists to optimise antimicrobial activity in intact bacterial cells"*. Furthermore, they identify the key need to quantify and image the penetration of drugs into bacteria and to measure the efflux processes. MetVBadBugs is directly focused at these metrology challenges. There is clearly no single technique that can deliver all the measurement answers needed by scientists studying AMR and developing new antibiotics. A robust metrology framework is needed, which is built on fundamental studies of the techniques as well as being combined with cross measurement platform validation including pre-normative studies. The objectives of this project are clearly aimed at addressing these needs.

Objectives

This project addresses the following scientific and technical objectives:

1. To develop urgently needed new metrological capabilities for:
    - the label-free 3D imaging of antibacterial agents in bacteria. This requires a new 3D chemical imaging instrument with 100 times better sensitivity and a high-spatial resolution (100 nm). The instrument will have a mass resolution of >100 000 and the ability to sample from sub-micron areas, simultaneously.
    - the traceable quantification of the vertical concentration profile of antibacterial agents in bacteria and biofilms. Measurements will be performed in liquid and at near ambient pressure.
    - imaging surface macromolecules, such as porins or metal-transport proteins, to study the efflux mechanisms in Gram-negative bacteria and to give real-time quantitative measurements of drug-uptake in bacteria and biofilms. Numerical modelling and algorithms will be developed to support measurements in complex biological environments.
2. To develop well-controlled model systems to allow cross-platform measurement of penetration, accumulation and efflux of antibacterial agents in single cells, in suspended cellular aggregates, as well as in biofilm communities including binding to biofilm matrix components. The efficacy of novel antibacterial agents and efflux pump inhibitors will be investigated.
3. To develop signal enhancement strategies and advanced sample preparation methods for studying antibacterial agents in bacteria and biofilms including:

- advanced cryo preparation methods to enable 'liquid' (vitrified) water to be present in the vacuum of high-performance metrology instruments without ultrastructural reorganisation and translocation of exo/endo -genous molecules.
- novel methods to nano-sculpt bacteria for chemical imaging at 50 nm resolution.
- nano structured substrates for enhanced sensitivity.

4. To facilitate the take up of the technology and measurement infrastructure developed by the project by healthcare professionals (hospitals and health centres) and industry (pharmaceutical companies), in order to fight the threat from antimicrobial resistance to the health and prosperity of Europe.

Progress beyond the state of the art

To address this project's needs, metrological innovation is necessary. This project will develop the technology and methods needed to enable an unprecedented 3D imaging of biocides in bacteria and biofilms. This will be undertaken using a unique 3D nanoSIMS instrument with a mass resolution > 240,000, a mass accuracy < 1 ppm, and an image resolution for organics (< 1 micron for the argon cluster source, < 100 nm Bi nanoprobe). The high performance MS/MS capability of this instrument will aid molecular identification in complex biological matrices. Novel laser post-ionisation will be applied allowing neutral molecules to be ionised in order to enhance the method's sensitivity and to reduce or eliminate matrix effects in complex biological samples.

Advanced sample cryo-preparation and cryo-handling capabilities will be developed, such as the high pressure freezing of the biofilm for analysis in high-vacuum methods. This will enable the high resolution chemical imaging of frozen-hydrated bacteria and biofilms without ultrastructural reorganisation within the studied samples.

An innovative concept involving the fusion of s-SNOM with SIMS for 3D nano-FTIR will be investigated to study antibiotic drug localisation in bacteria by providing chemical and topographical information simultaneously.

Development of STORM/PALM with inclined illumination and beam shaping will push the spatial resolution of the techniques below 20 nm in biofilms. These optical advancements will allow the accurate imaging of membrane proteins on single bacteria as well as the dynamic tracking of antibiotics / biocides within the biofilm and at the surface of bacteria.

Signal enhancement strategies and advanced sample preparation methods will be developed; specially engineered and optimised tips for biological samples and corresponding signal enhancement will be made for TERS experiments. Novel nanoengineered SERS substrates will be employed to increase the sensitivity for antibiotic / biocide susceptibility measurements.

X-ray based techniques will be made applicable to bacterial samples in their natural environment. The use of an innovative NAP-XPS and synchrotron NAP-HAXPES instrument will allow the extended in-depth analysis of irregularly-surfaced samples such as biofilms. Also, a novel liquid cell will be used to enable quantitative chemical in-depth sample analysis (in their wet state). Quantitative NAP-XPS will be made traceable to the SI by calibration using reference-free synchrotron (GI)-XRF.

The progress beyond-state-of-the-art of this project will be demonstrated by four biologically led case studies, where new capabilities will be used to answer fundamental questions in health-care and the pharmaceutical and medical device industries.

Results

1. *To develop urgently needed new metrological capabilities for: the label-free 3D imaging of antibacterial agents in bacteria. This requires a new 3D chemical imaging instrument with 100 times better sensitivity and a high-spatial resolution (100 nm). The instrument will have a mass resolution of >100 000 and the ability to sample from sub-micron areas, simultaneously. The traceable quantification of the vertical concentration profile of antibacterial agents in bacteria and biofilms. Measurements will be performed in liquid and at near ambient pressure. Imaging surface macromolecules, such as porins or metal-transport proteins, to study the efflux mechanisms in Gram-negative bacteria and to give real-time quantitative measurements of drug-uptake in bacteria and biofilms. Numerical modelling and algorithms will be developed to support measurements in complex biological environments.*

Significant progress has been made since the last report. After much method development we have demonstrated the first high-resolution 3D SIMS imaging of a hydrated and *Pseudomonas aeruginosa* biofilm where the structure is preserved using high-pressure freezing. The Orbitrap mass spectrum (MS) data have a mass resolution of > 200,000 which significantly exceeded our original target with a spatial resolution of < 2 µm. In the time-of-flight (ToF) MS images, individual bacteria can be resolved. This exciting capability will now be used to study the localisation of quorum sensing molecules registered with bacteria locations in 3D. NPL has installed and commissioned a CAMECA NanoSIMS 50L, which enables SIMS imaging of elements with a resolution of < 50 nm and ppb sensitivity. This is the only NanoSIMS instrument in a National Metrology Institute, worldwide. We have demonstrated the ability to image individual *Mycobacterium bovis* bacteria and the uptake of an antibiotic.

PTB have advanced the XRF metrology for iodine measurement. The energy dependent absorption of iodine L-lines in the Celgard calibration sample has been measured. An absorption difference of the iodine L-lines is clearly visible, which potentially allows the penetration depth of iodine in a bacterial film of 10 µm thickness to be determined if small exiting angles can be realised. PTB has optimised the synchrotron radiation source resulting in signal enhancement of about 200 %. Also, the conditioning of the optical output, using a spectral and polarisation filter, has led to further enhancement of up to 2000 %. BAM and SPECS have tested NAP-XPS measurement capabilities on both artificial and biological samples, including a biofilm of E. coli. Alginate (exopolysaccharide) was characterised and quantified based on measurements performed under near ambient pressure and ultra-high vacuum. Progress in sample preparation optimised for NAP-XPS has been made. Planktonic bacteria and early stage biofilms on glass and silicon wafers have been prepared and characterised with SEM, and successfully measured with NAP-XPS. At LENS, super-resolution imaging of the distribution of a fluorescent derivative of penicillin (Bocillin FL) on the surface of planktonic bacteria has been performed successfully. Progress in sample preparation optimised for NAP-XPS has also been made: planktonic bacteria and early stage biofilms on glass and silicon wafers have been prepared and characterised with SEM and successfully measured with NAP-XPS.

2. *To develop well-controlled model systems to allow cross-platform measurement of penetration, accumulation and efflux of antibacterial agents in single cells, in suspended cellular aggregates, as well as in biofilm communities including binding to biofilm matrix components. The efficacy of novel antibacterial agents and efflux pump inhibitors will be investigated.*

Iodine and triclosan are effective biocides and two calibration samples have been successfully prepared. Firstly, a polymer membrane material (Celgard-separator 2325®) with added iodo-benzoic-acid, PVP-iodine, and Triclosan with quantification by reference-free X-ray fluorescence measurements. Secondly, highly oriented pyrolithic graphite (HOPG) with an implanted dose of iodine. The implanted HOPG sample has been successfully quantified for a 7 keV excitation energy and quantification of the Celgard separator samples and an ionic liquid sample is in progress and will be finished in October 2018.

Optimisation of artificial biofilms is ongoing. Partners have assessed a set of different antimicrobial agents with different characteristics to select the best ones for their respective platform. A range of approaches are also being investigated by UNOTT, FCI, GSK, SNM, DoH and NPL to create reproducible biofilm systems that are appropriate for more than two platforms, including functionalising surfaces for attachment, minimising observation on platforms and utilising polysaccharides purified directly from bacterial species. It has been possible to measure the same antimicrobial by microscopy and IR spectroscopy and also by microscopy and mass spectroscopy.

3. *To develop signal enhancement strategies and advanced sample preparation methods for studying antibacterial agents in bacteria and biofilms including: advanced cryo preparation methods to enable 'liquid' (vitrified) water to be present in the vacuum of high-performance metrology instruments without ultrastructural reorganisation and translocation of exo/endo -genous molecules. Novel methods to nano-sculpt bacteria for chemical imaging at 50 nm resolution. Nano structured substrates for enhanced sensitivity.*

DoH have developed protocols for the freezing of planktonic bacteria and biofilms by high pressure freezing using the ammonium formate cryoprotectant and assessed quality of the vitreous samples by cryo-TEM and SEM. Procedures for the sectioning of frozen biofilms into vitreous sections for analysis have been developed and samples sent for analysis. Samples frozen with ammonium formate cryoprotectant have been found to be suitable for SIMS instruments and analysis. The 3D OrbiSIMS instrument with a cryo-stage and cryo transfer system has been further improved. Sample holders have been optimised so that they fit onto the cryogenic stage to accommodate sapphire disks and high-pressure freezing planchettes in two different sizes (3 mm and

6 mm diameter). The cryo-sample transfer procedure was successful at preventing ice formation at the surface and samples could be maintained in a frozen-hydrated state during the analysis. A draft standard operating procedure (SOP) has been developed.

To increase SIMS sensitivity, we have developed a method for in situ matrix deposition using the argon gas cluster ion beam (GCIB) sputtering of a reservoir of DHB matrix molecules between SIMS imaging and sputtering cycles. The method has been automated to improve repeatability, which has been tested on a tissue homogenate sample dosed with the drug amiodarone. For 5 repeat measurements an average amiodarone [M+H]+ enhancement was a factor of 2.89 with a relative standard deviation of 3 %. Optimisation of the matrix could lead to higher enhancements.

With the aim of performing nano-FTIR spectroscopy with enhanced sensitivity at the surface of bacterial membranes and cross-sections, INRIM nano-fabricated two different shaped gold tips, with support from CMI and LENS.

4. To facilitate the take up of the technology and measurement infrastructure developed by the project by healthcare professionals (hospitals and health centres) and industry (pharmaceutical companies), in order to fight the threat from antimicrobial resistance to the health and prosperity of Europe.

To ensure uptake of the results of this project and to promote the topic within the scientific community, NPL and NIBSC have submitted a proposal for an IUVSTA workshop “Biological and soft matter sample preparation for high-resolution imaging by high-vacuum techniques”. The proposal was successful and this 89th IUVSTA workshop will be held in Poland in May 2019. This is a perfect timing with the end of this project.

With advice from industry stakeholders, three industry relevant case studies have been selected to be taken further (i) determine the effect of using antagonists/agonists that target bacterial cell-cell communication on a biofilm, (ii) track the penetration of the antimicrobial iodine in a biofilm and bacteria and (iii) investigate how triclosan affects resistance to other antimicrobials.

Impact

To promote the uptake of the results generated in MetVBadBugs, results were shared with a variety of different stakeholders in conferences, workshops and training sessions. Since the M18 report, we have organised a successful focus meeting ‘Cool tools for microbial imaging’ at the Microbiology Society Annual Meeting 2018. The meeting highlighted that in recent years there have been extraordinary advances in the technologies available to study microbial biology. The development of new techniques to probe individual cells and molecules is a major driver of scientific advance. The focus meeting covered and showcased a number of new advanced techniques that have been successfully applied to microbiology, including mass spectrometry imaging, light and electron microscopy and Raman spectroscopy. These have diverse applications which attracted a broad audience across all the Microbiology Society divisions.

Sample preparation is one of the most critical steps for reproducible high-resolution imaging. To ensure uptake of the results of this project and to promote the topic within the scientific community, NPL and NIBSC have submitted a proposal for an IUVSTA workshop “Biological and soft matter sample preparation for high-resolution imaging by high-vacuum techniques”. The proposal was successful and this 89th IUVSTA workshop will be held in Poland in May 2019. This is a perfect timing with the end of this project.

To date, the partners have successfully met the peer reviewed publication target of at least 15 papers and have given 29 presentations at conferences (many of which are invited). In addition, we have given 41 other dissemination activities including an interview on BBC World service ‘The Inquiry’ about antimicrobial resistance, seminars, communication with the public through “Massacre the microbes activity” at the University of Nottingham and a Schools study Day at Nottingham Castle.

Impact on relevant standards

The project’s progress and engagement will be reported at all ISO TC 201, VAMAS and CCQM-SAWG and CCQM-CAWG meetings. The project will enable the partners to provide input to the development of the existing and new standards. These will include: Contribution to a draft standard for the measurement of sputtering yield for organic materials; Participation in a CCQM pilot study on ‘the amount of substances at a buried interface’; Participation in a VAMAS interlaboratory study on measurements of ‘the lateral resolution in mass spectrometry imaging’; Project proposal for a VAMAS interlaboratory study for ‘chemical depth profiling

of hydrated films'; An exploitation plan has been made and it is regularly updated. The adaptation of the liquid cell for a potential application to analyse biofilms using quantitative reference-free XRF was given as an outlook in the talk "Characterisation of bio-molecular nano-layers by means of reference-free X-ray Spectrometry".

SPECS held a presentation at the annual workshop on AP-XPS in December 2017 about the development of instrumentation for measuring biofilms with NAP-XPS, and recently published two application notes related to the subject. Partners are regularly involved in networking activities as user meetings and participation in standard- and technical committees: ISO TC 201, NA 062-08-16 AA and BIPM/CCQM SAWG. BAM have led the development of NWIP for a Technical Report on surface chemical analysis of biofilms. The report includes contributions from partners on the following techniques: XRF (PTB), FTIR/SEIRA (PTB), 3D OrbiSIMS (NPL), NAP-XPS (SPECS, BAM), Raman (INRIM). The technical report is a practical guide that summarises the current state of the art for the analysis of biofilms. This will be presented at the 27th Plenary Meeting of ISO/TC 201, Mexico, September 2018.

Impact on industrial and other user communities

This project will advance the measurement capability and will provide the essential metrology needed for measuring drugs in bacteria and biofilms. It will have an impact across healthcare and industry sectors, including large pharma, clinicians, wound and infection centres and instrument vendors. This research is all pre-competitive and will support the ethos of knowledge-sharing. The metrology outputs will be, in the first instance, targeted at the above listed industries, addressing the main issues in the field of antimicrobial resistance and the study of bacteria.

The implementation of the 3D OrbiSIMS instrument will have an impact on research in all areas of drug discovery. It will enable researchers to perform high-resolution and high-sensitivity label-free 3D imaging of antibacterial agents in bacteria at the sub-cellular scale. This unprecedented measurement capability could be further translated to studies of animal and human samples and have impact on other life science sectors.

NPL has started offering the 3D OrbiSIMS instrument as a measurement service. For example, a large pharmaceutical company has been using the methodology developed at NPL and expertise to study biofilms.

The adaptation of the liquid cell will allow traceable quantification of the vertical concentration profiles of antibiotics in bacteria and biofilms. This will have an impact on the wide range of biological and biomedical research as it will enable studies of biological samples by XRF methods, which has not been possible thus far due to the high-vacuum requirements of these techniques.

The well-controlled bacterial model systems not only will give insights into the mechanisms of antimicrobial resistance but will also permit the same processes to be studied using different techniques, thus providing complementary information. In addition, analysts will benefit from the availability of artificial biofilm model samples as a quality assurance tool for improved reliability and credibility. Likewise, analysis instrument vendors will benefit from the availability of quantifiable reference samples for the benchmarking of methods and, in turn, advancing measurement capability.

Also, the sensitivity of Raman techniques for complex biological samples will be vastly enhanced through the nanofabrication of nano-structured substrates (for SERS) and specialised tips (for TERS). Novel methods to nano-sculpt bacteria will aid chemical imaging at 50 nm resolution using 3DNanoSIMS and SNOM. These methods will be designed for studying bacterial samples but end users of the techniques are also expected to benefit as the developments could be translated and tailored to study other complex biological samples.

Impact on the metrology and scientific communities

The development of novel, beyond the state-of-the-art capabilities: 3D OrbiSIMS, super-resolution 3D imagingquantitative XRF using a wet cell and metrology for ambient-pressure and SI-traceable NAP-XPS will have an important impact on the scientific communities. The 3D OrbiSIMS is revolutionary and is at least two leaps ahead of current technology. Successful outcomes have strong potential for publications in high-profile peer-reviewed and trade journals. Good practice guides for well-controlled bacterial model systems and for cryogenic sample preparation will be produced, which will be of significant impact to the wider community. To maximise the dissemination of the outputs of the project, the consortium intends to organise workshops, conferences and symposia as well as special sessions at high-profile EU conferences. The project will have a strong role in training, including e-Learning and webinars and teaching. The CCQM-SAWG will conduct a pilot

study in 2017 to measure the amount of substance in a buried layer. The research in this project featured in a lecture at the International School of Physics (Varenna, Italy) in association with BIPM.

Longer-term economic, social and environmental impacts

This project is providing better, faster and more reliable tools for the scientific and industrial communities in sectors such as medical, pharmaceutical, medical devices and biomedical discovery:

Economic benefits:

The O'Neill review on antimicrobial resistance (https://amr-review.org/) commissioned two independent studies by RAND Europe and KPMG, who modelled two scenarios for the situation in 2050. Using the available data, which under-reports AMR effects, they conservatively estimate that "300 million people are expected to die prematurely because of drug resistance over the next 35 years". The drop in the world's GDP will be between 2 % to 3.5 % with a corresponding loss of economic output between 60 and 100 trillion USD.

As an example, the Adult Intensive Care Unit (ICU) at the Queen's Medical Centre cares for >3500 critically ill patients every year. A single patient day in an intensive care unit costs the NHS more than £2 k, and mortality rates average approximately 20 %. The annual budget for Critical Care within just one hospital is more than £15 M. The potential benefit of interventions that reduce the length of stay and or clinical risk within this environment is huge.

The antibiotic resistance of bacteria, in the biofilm mode of growth, contribute to the chronicity of infections such as those associated with medical devices. Biofilms have become a common cause of medical, difficult-to-treat infections. From EMPIR perspective on the impact that this project could have can be seen from its potential benefit in treating chronic infections caused by biofilms. Chronic infections such as wounds cost the healthcare industry billions of dollars each year.

This project is also expected to aid the pharmaceutical industry overall. With its annual output of € 220 B, its approximately 800,000 employees and as the world's major trader in medicinal products, the EU pharmaceutical industry is of strategic importance to the European economy. It is a major asset with regard to its contributions to economic growth, the labour market and the European science and technology base (estimated € 30,000 million in R&D in Europe in 2012). The world market for medical products is expected to reach nearly $1.17 trillion by 2017. It is therefore essential for the EU to maintain its competitive edge.

Social benefits**:**

The O'Neill review states that "Antimicrobial-resistant infections currently claim at least 50,000 lives each year across Europe and the US alone"

As a result of AMR, modern health systems and treatments that rely heavily on antibiotics could be severely undermined". Surgery would become far more dangerous and too risky to undertake. Modern chemotherapy drugs give increasingly successful patient outcomes but suppress the patient's immune system, making them susceptible to infections. In the last century, the world has witnessed a 50-fold decrease in maternal deaths and it is, more-or-less, taken for granted that childbirth is safe. Without action on AMR this modern-day concept will change.

Bacterial infection and biofilms are a major source of complications and even death upon severe surgical treatment and chemotherapy. Especially biofilms of implants are difficult to detect and treatment often fails due to the complex structure of the biofilm. This often requires the costly exchange of the implant and an impairment of life quality for years.

Environmental benefits:

The effects of antibiotics on the environment are still not entirely understood. However, one major concern is the rise of antibiotic-resistant strains of bacteria due to increased antibiotic use in humans and animals. This can critically disturb natural bacterial ecosystems and lead to a serious threat to human health. Biocidal Product Regulation (EU 528/2012) now requires evidence that a biocidal product will not give rise to microbial resistance. Increased research into new antibiotics and antibiotic alternatives is urgently needed to prevent the resistance from forming.

The developed technologies and methodologies will be transferable to other biological systems or soft matter samples such as cells, tissues or food, and this will support uptake of the developed metrology by other academic and industry sectors, e.g. veterinary sciences, food production, marine biology, etc.

List of publications

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2. Hermann, P., Kästner, B., Hoehl, A., Kashcheyevs, V., Patoka, P., Ulrich, G., Feikes, J., Ries, M., Tydecks, T., Beckhoff, B., Rühl, E., and Ulm, G. (2017) Enhancing the sensitivity of nano-FTIR spectroscopy. *Opt. Express 25*, 16574–16588.
3. T. Vignolini, L. Gardini, V. Curcio, M. Capitanio and F. S. Pavone OPTIMIZATION OF HIGHLY INCLINED OPTICAL SHEET ILLUMINATION FOR SUPER-RESOLUTION MICROSCOPY Biophysical Journal
4. P Sanjuan-Alberte, MR Alexander, RJM Hague, FJ Rawson Electrochemically stimulating developments in bioelectronic medicine Bioelectronic Medicine
5. SA Khaled, MR Alexander, DJ Irvine, RD Wildman, MJ Wallace, S Sharpe Extrusion 3D printing of unique geometries of paracetamol single formulation with tunable release profiles AAPS PharmSciTech
6. ID Styliari, C Conte, AK Pearce, A Hüsler, RJ Cavanagh, MJ Limo High-Throughput Miniaturized Screening of Nanoparticle Formation via Inkjet Printing Macromolecular Materials and Engineering, 1800146
7. J Meurs, MR Alexander, PA Levkin, S Widmaier, J Bunch, DA Barrett Improved extraction repeatability and spectral reproducibility for liquid extraction surface analysis–mass spectrometry using superhydrophobic–superhydrophilic patt... Analytical Chemistry 90 (10), 6001-6005
8. A Hüsler, S Haas, L Parry, M Romero, T Nisisako, P Williams Effect of surfactant on Pseudomonas aeruginosa colonization of polymer microparticles and flat films RSC Advances 8 (28), 15352-15357
9. SA Khaled, MR Alexander, RD Wildman, MJ Wallace, S Sharpe, J Yoo, 3D extrusion printing of high drug loading immediate release paracetamol tablets International journal of pharmaceutics 538 (1-2), 223-230
10. I Louzao, B Koch, V Taresco, L Ruiz-Cantu, DJ Irvine, CJ Roberts, C Tuck Identification of Novel “Inks” for 3D Printing Using High-Throughput Screening: Bioresorbable Photocurable Polymers for Controlled Drug Delivery ACS applied materials & interfaces 10 (8), 6841-6848
11. M. Kjærvik, K. Schwibbert, P. Dietrich, A. Thissen, W.E.S. Unger Surface characterisation of Escherichia coli under various conditions by near-ambient pressure XPS Surface and Interface Analysis
12. Eleonora Cara, Luisa Mandrile, Federico Ferrarese Lupi, Andrea Mario Giovannozzi, Masoud Dialameh, Chiara Portesi, Katia Sparnacci, Natascia De Leo, Andrea Mario Rossi & Luca Boarino Influence of the long-range ordering of gold-coated Si nanowires on SERS Scientific Report
13. Alessio Sacco, Dario Imbraguglio, Andrea M. Giovannozzi, Chiara Portesi and Andrea M. Rossia Development of a candidate reference sample for the characterization of tip-enhanced Raman spectroscopy spatial resolution RSC Advances

Project start date and duration:	01 May 2016, 36 months	
Coordinator: Paulina D Rakowska PhD, NPL Tel: +44 208 943 7111 E-mail: paulina.rakowska@npl.co.uk Project website address: http://empir.npl.co.uk/metvbadbugs/		
Internal Funded Partners: 1 NPL, United Kingdom 2 BAM, Germany 3 CMI, Czech Republic 4 DoH, United Kingdom 5 INRIM, Italy 6 PTB, Germany	External Funded Partners: 7 LENS, Italy 8 UNOTT, United Kingdom	Unfunded Partners: 9 FCI, United Kingdom 10 GSK, United Kingdom 11 ION-TOF, Germany 12 SNM, United Kingdom 13 SPECS, Germany