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Foreword

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This document was prepared by Technical Committee ISO/TC 201, *Surface chemical analysis*.

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Introduction

Biofilms represent a predominant form of microbial life on our planet. These are aggregates of microorganisms, which are embedded in a self-produced matrix formed by extracellular polymeric substances (EPS). Biofilms are capable of forming on virtually every surface in aqueous, humid and non-sterile environments. They can adapt to the most extreme environments from hot springs to frozen glaciers, from very acidic to very alkaline environments. Biofilms can colonise nearly all interfaces and affect many fields of life. Therefore, detailed knowledge of microorganisms enclosed in biofilms as well as the chemical composition, structure, and functions of the complex biofilm matrix and their changes at different stages of the biofilm formation and under various physical and chemical conditions is necessary. Globally, a significant drive for research in microbial studies comes from the healthcare and biomedical sectors, including diagnostics and pharmaceutical industries. Biofilm characterization is also relevant to the needs of the medical devices industry by providing understanding of the barriers that need to be overcome. Biofilm formation creates a problem in water and oil pipelines as well as microbial-induced corrosion in marine environment. Biofilm characterization is the most important and fundamental activity both for understanding the risk associated with the accumulation of reservoirs of antimicrobial resistant pathogen build-up within biofilms that can affect healthcare (e.g. on indwelling devices such as catheters), domestic (e.g. washing machines) and commercial (e.g. in food industry pipelines) environments. Consultations have been carried with experts from major European initiatives, specifically New Drugs for Bad Bugs (ND4BB), a large consortium funded by the Innovative Medicine Initiative (IMI), as well as the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR). From these discussions, the following key needs for advancing measurement capability and metrology have been identified:

— well-controlled model systems to allow cross-platform measurement of bacterial components and bacterial processes in single cells, suspended cellular aggregates and in biofilm communities;

— metrology for 3D chemical imaging of microbial samples allowing measurements with high sensitivity, with high-spatial resolution at a single cell level and allowing qualitative measurements and sample components detection and identification;

— reliable, reproducible, and traceable quantitative measurements of the vertical concentration profiles of antibacterial agents in bacteria and biofilms;

— measurements to be performed in liquid and near ambient pressure necessitating innovation in instrumentation;

— methodology, based on cryogenic preparation methods, to enable analysis of hydrated samples in the vacuum of high-performance metrology instruments without ultrastructural reorganisation and translocation of exogenous and endogenous molecules;

— advancements in measurement capabilities and metrology to image surface macromolecules, such as porins or metal-transport proteins, to study the efflux mechanisms in bacteria and to give real-time quantitative measurements of drug-uptake in bacteria and biofilms;

— signal enhancement strategies, such as surface nanofabrication, to aid the applicability of existing analytical methods to the analysis of microbial samples;

— numerical modelling and algorithms to support measurement in complex biological environments.

This document is based on work of the 15HLT01 MetVBadBugs project funded by the European Metrology Programme for Innovation and Research (EMPIR) under Horizon 2020. The MetVBadBugs project was formulated in response to global challenge of antimicrobial resistance and the objectives of the project were to advance the measurement capability by providing urgently needed essential metrology to quantitatively measure and image the localisation of antibiotics and to understand the antibiotic penetration and efflux processes in bacteria and biofilms. The project tested, advanced and developed a range of physical methods and techniques with a focus on spectroscopical methods. This report gives an overview of these methods and summarises their applicability to measurement of microbial samples.