



Technical Report

ISO/TR 4550

Surface chemical analysis — Surface chemical analysis of bacteria and biofilms

*Analyse chimique des surfaces — Analyse chimique des surfaces
des bactéries et des biofilms*

**First edition
2026-01**

Document Preview
iTech Standards
(<https://standards.iteh.ai>)

ISO/TR 4550:2026

<https://standards.iteh.ai/catalog/standards/iso/0be637f7-4cfa-44ab-bf97-9d556629004c/iso-tr-4550-2026>

iTeh Standards
(<https://standards.itih.ai>)
Document Preview

ISO/TR 4550:2026

<https://standards.itih.ai/catalog/standards/iso/0be637f7-4cfa-44ab-bf97-9d556629004c/iso-tr-4550-2026>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2026

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Symbols and abbreviated terms	1
5 X-ray photoelectron spectroscopy	2
5.1 General	2
5.2 XPS on freeze-dried bacteria	2
5.3 Bacterial surface characterization by Cryo-XPS	4
5.4 Bacterial and biofilm surface characterisation by near-ambient pressure XPS	4
6 Fourier-transform infrared spectroscopy	5
6.1 General	5
6.2 State of the art	6
6.3 Beyond state of the art	6
6.4 Quantitative analysis of triclosan uptake in <i>E. coli</i> biofilms by FTIR	6
7 X-ray fluorescence spectroscopy and related X-ray spectroscopy	8
7.1 General	8
7.2 Calibration of quantitative XRF measurements	9
7.3 XRF analysis of bacteria and biofilms	9
8 Secondary ion mass spectrometry	11
8.1 General	11
8.2 SIMS imaging of dehydrated biofilms	11
8.3 SIMS imaging of frozen-hydrated bio-samples	12
9 Raman-spectroscopy	13
9.1 General	13
9.2 Dielectrophoresis-Raman analysis of bacteria in a liquid matrix	14
9.3 Raman biofilm analysis	15
9.4 Quantitative Raman spectroscopy on freeze-dried bacteria	16
10 Super-resolution microscopy	17
10.1 General	17
10.2 Single-molecule tracking techniques	17
10.3 Single-molecule localization microscopy techniques	18
10.4 Highly inclined and laminated optical sheet microscopy	20
11 Concluding remarks	21
Bibliography	25

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

ISO/TR 4550:2026

<https://standards.itech.ai/catalog/standards/iso/0be637f7-4cfa-44ab-bf97-9d556629004c/iso-tr-4550-2026>

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.