Technical Specification

ISO/TS 16099

First edition
2025-07

Water quality — Polymerase chain reaction (PCR) for the detection and quantification of microorganisms and viruses — General requirements, quality assurance and validation

Qualité de l'eau — Réaction de polymérisation en chaîne (PCR) pour la détection et la quantification des microorganismes et des virus — Exigences générales, assurance de la qualité et validation

iTeh Standards
(https://standards.iteh.ai)
Document Preview
ISO/TS 16099:2025
https://standards.iteh.ai/catalog/standards/iso/59eb4323-dc51-4e9e-8ca9-aef093868f00/iso-ts-16099-2025

Reference number
ISO/TS 16099:2025(en)

© ISO 2025

iTeh Standards
(https://standards.iteh.ai)
Document Preview

ISO/TS 16099:2025
https://standards.iteh.ai/catalog/standards/iso/59eb4323-dc51-4e9e-8ca9-aef093868f00/iso-ts-16099-2025

COPYRIGHT PROTECTED DOCUMENT

© ISO 2025

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 ..... **vi**
Introduction ..... **vii**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **1**
4 Principle **11**
4.1 General 11
4.2 Test material 11
4.3 Sampling, transport and storage 12
4.4 Preparation of the sample 12
4.4.1 General 12
4.4.2 Preparation and concentration of samples 12
4.4.3 Treatment of samples containing heavy metal particles 13
5 Nucleic acid extraction **14**
5.1 General 14
5.2 Nucleic acid removal or inactivation from damaged microorganism(s) 14
5.3 Nucleic acid quantity and quality 15
5.4 Stability of nucleic acid extracts 15
6 PCR-based methods **16**
6.1 General 16
6.2 Qualitative PCR-based methods 17
6.3 Quantitative PCR-based methods 17
6.4 Digital PCR 17
7 Laboratory setup **18**
7.1 General 18
7.2 Layout of laboratory areas and workflow 18
7.3 Working environment 19
7.3.1 General 19
7.3.2 Reagents preparation area 20
7.3.3 Sample preparation area 20
7.3.4 PCR area 21
7.4 Cleaning of laboratory 21
7.5 Environmental monitoring for PCR 21
8 Equipment **21**
8.1 General 21
8.2 Biological safety cabinet 22
8.3 Centrifuge 22
8.4 Digital PCR system 22
8.5 Filtration setup 23
8.6 Freezer and ultra-low temperature freezer 23
8.7 Heating block module 23
8.8 PCR workstation 23
8.9 Pipettes 24
8.10 Pipetting robots (optional) 24
8.11 Refrigerators 24
8.12 Thermal cycler 24
8.13 Spectrophotometry or fluorometry instrument 25
8.14 On-site PCR systems 25
9 Reagents and consumables **25**
9.1 General 25
9.2 Primers and probes 26

iTeh Standards
(https://standards.iteh.ai)
Document Preview
ISO/TS 16099:2025
https://standards.iteh.ai/catalog/standards/sist/eb4323-dc51-4e9e-8ca9-aef093868f00/iso-ts-16099-2025

© ISO 2025 – All rights reserved

9.2.1 General ... 26
9.2.2 Quality control ... 26
9.2.3 Storage ... 26
9.3 Lyophilized PCR reagents ... 26
9.4 Ammonium oxalate solution ... 27
9.5 Pipetting tips ... 27
9.6 Membrane filters ... 27
9.7 PCR plates and tubes ... 27
9.8 Calibration standard ... 27
9.9 Master mix ... 28
9.9.1 General ... 28
9.9.2 Commercially available master mixes ... 28
9.9.3 Master mix prepared by user ... 28
9.10 Chemicals and consumables for nucleic acid extraction kits ... 29
9.10.1 General ... 29
9.10.2 Commercially available extraction kits ... 29
9.10.3 Nucleic acid extraction chemicals prepared by user ... 29
9.11 On-site PCR ... 29

10 Procedure ... 30
10.1 Controls ... 30
10.1.1 General ... 30
10.1.2 Negative process control ... 30
10.1.3 Positive process control ... 31
10.1.4 Internal process control ... 31
10.1.5 Amplification control ... 31
10.1.6 Positive PCR control ... 32
10.1.7 Negative PCR control ... 32
10.1.8 Required controls for dPCR ... 32
10.2 Data analysis of results ... 33
10.2.1 Data analysis for real-time PCR ... 33
10.2.2 Data analysis for dPCR ... 33
10.3 Evaluation of results ... 34
10.3.1 General ... 34
10.3.2 Evaluation of positive controls using control charts ... 35
10.3.3 Standard curve evaluation ... 35
10.3.4 Absolute quantification (real-time PCR and dPCR) ... 36
10.3.5 Relative quantification ... 36
10.4 Test report ... 37

11 Validation and verification of PCR-based methods ... 37
11.1 General ... 37
11.2 Pre-validation ... 38
11.3 Validation ... 39
11.3.1 General ... 39
11.3.2 Method comparison studies ... 39
11.3.3 Validation without method comparison ... 40
11.4 Sample preparation ... 40
11.5 Water matrices ... 41
11.6 Performance characteristics for validation ... 41
11.7 Validation of the PCR step ... 43
11.7.1 General ... 43
11.7.2 Multiplex PCR-related methods ... 43
11.7.3 Calibration of standard curve ... 43
11.7.4 Measurement range ... 44
11.7.5 Inclusivity and exclusivity ... 44
11.8 Validation of qualitative PCR-based methods ... 45
11.8.1 General ... 45
11.8.2 Sensitivity ... 45
11.8.3 (Relative) trueness ... 45

iTeh Standards
(https://standards.iteh.ai)
Document Preview
ISO/TS 16099:2025
https://standards.iteh.ai/catalog/standards/sist/eb4323-dc51-4e9e-8ca9-aef093868f00/iso-ts-16099-2025

© ISO 2025 – All rights reserved

11.8.4 (Relative) limit of detection 46
11.9 Validation of quantitative PCR-based methods 46
11.9.1 General 46
11.9.2 (Relative) trueness 46
11.9.3 (Relative) limit of quantification 47
11.9.4 (Relative) limit of detection 47
11.9.5 Linearity 47
11.9.6 Specificity and sensitivity 48
11.9.7 Precision 48
11.9.8 Robustness 48
11.10 Controls and validation 49
11.11 Interlaboratory study 49
11.12 Verification of PCR-based methods 49

Annex A (informative) **Example of an interpretation of qualitative PCR results for *Escherichia coli*** **51**

Annex B (informative) **Example of an interpretation of quantitative PCR results for *Legionella pneumophila* with an internal control** **53**

Annex C (informative) **Example of an interpretation of dPCR results for SARS-CoV-2** **57**

Annex D (informative) **Verification of the calibration function of the quantitative PCR phase** **60**

Bibliography **68**

iTeh Standards
(https://standards.iteh.ai)
Document Preview

ISO/TS 16099:2025
https://standards.iteh.ai/catalog/standards/iso/59eb4323-dc51-4e9e-8ca9-aef093868f00/iso-ts-16099-2025

© ISO 2025 – All rights reserved

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 147, *Water quality*, Subcommittee SC 4, *Microbiological methods.*

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.

iTeh Standards
(https://standards.iteh.ai)
Document Preview
ISO/TS 16099:2025
https://standards.iteh.ai/catalog/standards/iso/59eb4323-dc51-4e9e-8ca9-aef093868f00/iso-ts-16099-2025

© ISO 2025 – All rights reserved

Introduction

PCR-based methods are developed for the detection and/or quantification of, for example, pathogenic bacteria, for rapid and reliable outcomes as an alternative to culture-based methods. For example, for the screening on the presence of *Legionella* or faecal-related microorganisms in water, see References [26], [29], [43], [49] and [56] for further information.

Performing nucleic acid quantification assays to a high standard of analytical quality can be challenging. For example, it is well known that impure or degraded nucleic acid extracts can affect the accuracy of quantification. Similarly, a poorly designed quantitative polymerase chain reaction (qPCR) assay with poor amplification efficiency and poor primer specificity will impact the quantification accuracy of nucleic acid targets.

In addition, aspects such as the water matrix and standard curves can have a significant influence on the accuracy of quantitative measurements of nucleic acid targets. Therefore, it is important to improve the reliability of data by setting general requirements for PCR-based methods.

iTeh Standards
(https://standards.iteh.ai)
Document Preview

ISO/TS 16099:2025
https://standards.iteh.ai/catalog/standards/iso/59eb4323-dc51-4e9e-8ca9-aef093868f00/iso-ts-16099-2025

© ISO 2025 – All rights reserved