ISO/TS 16099:2025-07 (E)

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

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
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

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**