

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 <i>Escherichia coli</i>	51
Annex B	(informative) Example of an interpretation of quantitative PCR results for <i>Legionella pneumophila</i> 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