

DIN 38411-25:2025-11 (E)

German standard methods for the examination of water, waste water and sludge -
Microbiological methods (group K) - Part 25: Protocol for the validation of alternative
confirmation procedures and for verification of validated alternative confirmation
procedures (K 25)

Contents	Page
Foreword	5
Introduction	7
1 Scope	9
2 Normative references	9
3 Terms and definitions	10
4 Designation	13
5 General principles for the validation of confirmation procedures	13
6 Strains	14
7 Method comparison study	14
7.1 General	14
7.2 Selection of test organisms	15
7.3 Inclusivity study	15
7.3.1 Testing of target strains	15
7.3.2 Family level	15
7.3.3 Genus level	16
7.3.4 Species level	16
7.3.5 Sub-type level	16
7.4 Exclusivity study	16
7.4.1 Testing of non-target strains	16
7.4.2 Family level	16
7.4.3 Genus level	16
7.4.4 Species level	17
7.4.5 Sub-type level	17
7.5 Expression and interpretation of results	17
7.5.1 Expression and interpretation of results for inclusivity	17
7.5.2 Expression and interpretation of results for exclusivity	19
7.6 Overall evaluation	20
7.7 Requirements for the validation report	21
8 Interlaboratory comparison study	22
8.1 General	22
8.2 Required data sets	22
8.3 Protocol	23
8.4 Expression of results	23
8.5 Interpretation and evaluation	24
9 Validated alternative confirmation and typing procedures -- Technical protocol for verification	25
9.1 General	25
9.2 Experimental design	26

9.2.1	General	26
9.2.2	Strain selection	26
9.3	Evaluation of the results	26
9.4	Acceptance limit	27
9.5	Root cause analysis	27
Annex A (normative) Points to be considered when selecting strains for testing inclusivity and exclusivity		28
A.1	General	28
A.2	Target group categories	28
A.3	Target group selection in inclusivity study	28
A.4	Non-target group selection in exclusivity study	28
Annex B (normative) Overview of the minimum number of strains to be tested as well as the acceptability limits		30
Annex C (informative) Validation of the identification of microorganisms with a spectrometric or spectroscopic method in a single laboratory or a laboratory network		32
C.1	Validation of the identification of microorganisms with a spectrometric or spectroscopic method in a single laboratory or a laboratory network	32
C.2	Example of the recommended minimum sample size of the target parameter and non-target parameter for determination of the performance indicators to be reached for spectroscopic or spectrometric applications	33
C.3	Data from validation of a single laboratory using the example of <i>Clostridium perfringens</i>	34
Annex D (informative) Validation of Legionellaceae confirmation with qPCR		37
D.1	General	37
D.2	Method comparison study	37
D.2.1	Purpose of validation	37
D.2.2	Protocol for validation	37
D.2.3	Strain selection	37
D.2.4	Results	38
D.2.5	Root cause analysis	38
D.3	Interlaboratory comparison study	38
D.3.1	Organizing laboratory	38
D.3.2	Participating laboratories	38
D.3.3	Required data sets	38
D.3.4	Material	39
D.3.5	Presentation and evaluation of the results	39
D.3.6	Interpretation of the results	40
D.4	Final evaluation and approval of the method	40
Annex E (informative) Validation of the confirmation of coliform bacteria by means of MALDI- TOF- MS as an example of single laboratory validation on family level		41
E.1	General	41
E.2	Purpose of the validation study	42
E.3	Protocol for validation	42
E.4	Strain selection	42
E.5	Results	42
E.6	Root cause analysis	43
E.7	Final evaluation	48
E.8	Approval of the procedure	49
E.9	Exclusion	49
Annex F (informative) Example of verification of an alternative confirmation procedure		50
F.1	General	50
F.2	Experimental design	50

F.3	Evaluation of the results	50
F.4	Final evaluation of the verification	52
Annex G (informative) Renewal of the validation with a changed database		53
G.1	General	53
G.2	Database update in combination with commercially available validations	53
G.3	Database update with individual laboratory validations	53
G.4	Expansion of the database due to user entries	54
Bibliography		55
Tables Table 1 -- Example of the presentation of the results for inclusivity		18
Table 2 -- Interpretation of the results between the reference and alternative procedures for the inclusivity study		18
Table 3 -- Example of the presentation of the results for exclusivity		19
Table 4 -- Interpretation of the results between the reference and alternative procedures for the exclusivity study		20
Table 5 -- Example of a summary of the results in the method comparison study		20
Table 6 -- Example of the evaluation of the results in the method comparison study		21
Table 7 -- Presentation of the results for the interlaboratory study		24
Table 8 -- Summary of the results in the interlaboratory study		24
Table 9 -- Evaluation of the results in the interlaboratory study		25
Table 10 -- Number of strains for verification of validated alternative confirmation or typing procedures		26
Table 11 -- Overview of verification results for a validated alternative confirmation or typing procedure		27
Table B.1 -- Minimum number of strains to be tested and the acceptability limits for the method comparison study		30
Table B.2 -- Number of required results as well as the acceptability limits for the interlaboratory comparison study		31
Table C.1 -- Performance parameters and sample sizes		34
Table C.2 -- Example of a method description in a tabular validation report for identification of <i>Clostridium perfringens</i>		35
Table C.3 -- Example of the interpretation in a tabular validation report for identification of <i>Clostridium perfringens</i>		36
Table D.1 -- Summary of the results from the method comparison study to a family level		38
Table D.2 -- Summary of the results from the interlaboratory comparison study to the family level ..		39
Table D.3 -- Evaluation of the results on the family level		40
Table E.1 -- Summary of the inclusivity and exclusivity tests from the 1st data set		43
Table E.2 -- Summary of the inclusivity and exclusivity tests from the 2nd data set		43

Table E.3 -- Root cause analysis inclusivity deviation 1st data set -- Colonies tested directly from the CCA plate	45
Table E.4 -- Root cause analysis exclusivity deviation 1st data set -- Colonies tested directly from the CCA plate	46
Table E.5 -- Root cause analysis inclusivity deviation 2nd data set -- Colonies of subculture tested on TSA	47
Table E.6 -- Summary of the results 1st data set	48
Table E.7 -- Evaluation of results 1st data set	48
Table E.8 -- Summary of the results 2nd data set	49
Table E.9 -- Evaluation of results 2nd data set	49
Table F.1 -- Evaluation of the results of the verification of an alternative confirmation procedure	50