SADCMET Water PT 2017 Evaluation Workshop

Dr. K. Luden

ISO 13843:2017

Water quality - Requirements for establishing performance characteristics of quantitative microbiological methods

Contents

1. Scope

2. Normative References

3. Terms and Definitions

4. Basic Concepts

5. Specifications: **some guideline values**

6. Designs for determining performance characteristics of a method

7. Designs for single laboratory verification of a method

Annexes

A. Mathematical models of variation (*informative*)

B. Assessment of the lower limits (*normative*)

C. Assessment of the upper limits (*normative*)

D. Determination of the operational variability in repeatability and intralaboratory reproducibility conditions (*normative*)

E. uncertainty in counting (*normative*)

F. Determination of the operational variability (interlaboratory reproducibility) in a collaborative performance study (*normative*)

G. Glossary of principal symbols (*informative*)

Scope

This document deals with the **characterization of microbiological methods**.

In terms of this document, characterization means the study of parameters that can be measured to describe how the method is likely to perform in a given set of conditions, which can be described as performance characteristics.

The document describes procedures for the determination of performance characteristics which can be used for subsequent **validation** or **verification** of methods.

applies to selective quantitative methods (all types of water)

Definitions (41)

robustness insensitivity of an analytical method to small changes in the procedure

sensitivity fraction of total number of positive cultures or colonies correctly assigned in the presumptive inspection

specificity fraction of total number of negative cultures or colonies correctly assigned in the presumptive inspection

verification performance of a second characterization by a different laboratory to confirm the results of the original characterization

Performance characteristics

Parameter	Definition	required for performance characteristics	required for single laboratory verification
Sensitivity	fraction of the total positives correctly assigned in the presumptive count	X	X
Specificity	fraction of the total negatives correctly assigned in the presumptive count	X	X
False positive	fraction of positive results (e.g. typical colonies) that are subsequentliy shown to be due to non-	X	X
False negative	fraction of negative results (e.g. atypical colonies) shown to be target organisms	X	X
Selectivity	ratio of the number of target colonies to the total number of colonies in the sample volume	X	X
Efficiency	fraction of total colonies correctly assigned in the presumptive count	X	X

Performance characteristics

Parameter	Definition	required for performance characteristics	required for single laboratory verification
Upper limit	upper end of the working range for which the method is useful (i.e. the maximum countable colonies per plate or other detection system)	X	
Repeatability	precision under repeatability conditions (same operator, same…)	X	X
Reproducibility	precision under intralaboratory reproducibility conditions	X	
Robustness	measure of the capacity of a test to remain unaffected by small but deliberate variations in testing conditions (e.g. temperature)	X	
Relative recovery	efficiency with which a method recovers target organisms from a sample when compared with another procedure	X	
Uncertainty of counting	relative standard deviation of replicate counts of the target obtained by repeated counting (plate) under stipulated conditions	X	X

Validation?

Term is not mentioned because it is not clearly defined!!

Initial characterization can be carried out in a single laboratory that needs to have considerable experience in other microbiological methods.

Includes an unambiguous description of the target of interest (such as positive colony or tube)

Verification

- Implementation of a method developed elsewhere into one laboratory.

- Gathering evidence that the laboratory is able to generate performance data <u>similar</u> to those established in the primary characterization

- Natural samples are the optimal test materials and the work need only addess those aspects of the method performance that are of interest to the laboratory.

Specification guideline values

Parameter	Definition	guidance value
Sensitivity	fraction of the total positives correctly assigned in the presumptive count	> 90%
Specificity	fraction of the total negatives correctly assigned in the presumptive count	> 80 %
Selectivity	ratio of the number of target colonies to the total number of colonies in the sample volume	results usually not valid if <10 %

How to…

1. Samples need to contain 20-80 CFU (10-60 target organisms)

   - preferably naturally contaminated

2. all colonies (typical and atypical) are counted

3. all colonies are identified by indipendent method (biochemical tests, DNA sequencing…)

at least 20 Samples from different sources

for spiking samples with surface or wastewater at least 3 different sources

		presumptive count		
		+	**-**	
confirmed	**+**	a	b	a+b
count	**-**	c	d	c+d
		a+c	**b+d**	**n**

a true positives   (confirmed by secondary identification)

b false negatives

c false positives

d true negatives

		presumptive count		
		+	**-**	
confirmed	**+**	a	b	a+b
count	**-**	c	d	c+d
		a+c	**b+d**	**n**

sensitivity = a / (a+b)	fraction of the total positives correctly assigned in the presumptive count
Specificity = d / (c+d)	fraction of the total negatives correctly assigned in the presumptive count
false positive rate = c / (a+c)	fraction of positive results (e.g. typical colonies) that are subsequentliy shown to be due to non-target organisms
False negatie rate = b / (b+d)	fraction of negative results (e.g. atypical colonies) shown to be target organisms
Selectivity = a / n	ratio of the number of target colonies to the total number of colonies in the sample volume
Efficiency = (a+d) / n	fraction of total colonies correctly assigned in the presumptive count

Repeatability

- 10 replicates analysed under repeatability conditions (same operator, same equipment, same time…)

- minimum of 3 sets

- minimum of 3 sources of target organism

Sample	1	2	3	4	5	6	7	8	9	10
1										
2										
3										

Uncertainty in counting

- Read the same plates repeatedly under uniform conditions (short intervall of time = max 1 hour) (single person or intralaboratory/more than one)

- Randomly selected plates with more than 20 colonies (no unusual plates)

- count 30 plates

Plate	A1	A2	B1	B2	B3	m	s	$u_{rel,L}$	$u^2_{rel,L}$
1									
2									
3									

working groups

Implementation of CCA (ISO 9308-1:2014), TSC (ISO 14189)

- What sources can be used for the 20 samples required for presumptive/confirmed count

		presumptive count		
		+	-	
confirmed	+	a	b	a+b
count	-	c	d	c+d
		a+c	b+d	n

- What kind of secondary identification should be used

- what kind of samples would you count for uncertainty of counting

What sources can be used for the 20 samples required for presumptive/confirmed count?

Water types possible containing the target organisms E. coli or C. perfringens respectively)

- surface water (ponds, rivers, wells, lakes)

- borehole water

- waste/sewage water

- bottled water

- secondary quality control material (PT)

18

What kind of secondary identification can be used?

for E. coli

- biochemical tests (API)

- Oxidase test +vIndole+ Gram stain

- Colilert medium used as p/a test in smaller portions for identification of subcultures

- Vitec2

- BGBB

- MALDI-TOF

- target specific PCR

19

What kind of secondary identification can be used?

for C. perfringens:

- biochemical tests (API)

- Gram stain + sporestain

- Acid Phosphatase

- Ammonium-Hydroxide

- CAMP Test

- MALDI-TOF

- target specific PCR

20

What sources can be used for the 30 samples required for assessing uncertainty of counting?

Water types possible containing the target organisms E. coli or C. perfringens respectively)

-

- same as for question 1! No need for additional sources.