



## **NordVal**

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# **Protocol for the validation of alternative microbiological methods**

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## Scope

This NordVal protocol describes the technical procedures for validation of alternative methods for microbiological analyses of food, water, animal faeces, feed, samples from equipment and processing areas and environmental samples.

## Definitions

### General terms:

#### Alternative method

An alternative method is a method of analysis that demonstrates or estimates, for a given category of products, the same analyte as is measured using the corresponding reference method.

#### Analyte

The analyte is the component demonstrated or measured by the method of analysis. It may be the microorganism, its components or products.

#### Collaborative study

Study of the performance of the alternative method using common samples in several laboratories and controlled by the expert laboratory.

#### Expert laboratory

Laboratory having qualified staff and skills to perform the method comparison study and organise the collaborative study. The availability of an experienced statistician is essential for the analysis of the results.

#### Method comparison study

Study performed by the expert laboratory of the alternative method against the reference method.

#### Negative deviation

The alternative method presents a negative deviation if it gives a negative result when the reference method gives a positive result.

#### Positive deviation

The alternative method presents a positive deviation if it gives a positive result when the reference method gives a negative result.

#### Qualitative method

A qualitative method is a method of analysis whose response is either the presence or absence of the analyte in a certain amount of sample.

#### Quantitative method

A quantitative method is a method of analysis whose response is the amount of the analyte measured either directly or indirectly in a certain amount of sample.

#### Relative accuracy

The relative accuracy is the degree of correspondence between the response obtained by the alternative method and the reference method on artificially inoculated samples.

#### Relative detection level

Documentation must be provided that the detection level of the alternative method is equal to that of the reference method.

### Selectivity

The selectivity is a measure of a) the inclusivity: detection of the target microorganism from a wide range of strains, and b) the exclusivity: the lack of interference from a relevant range of non-target microorganisms.

### Relative sensitivity

The relative sensitivity is the ability of the alternative method to detect the analyte compared to the reference method.

### Relative specificity

The relative specificity is the ability of the alternative method not to detect the target microorganism when it is not detected by the reference method.

### Reference method

A reference method is a method which is internationally recognised and accepted (e.g. NMKL, ISO, CEN and AOAC International methods, methods given in EU/national legislations and certain national standards of equivalent standing).

### Validation of an alternative method

The validation of an alternative method is the procedure to demonstrate if the alternative method provides equivalent or better results compared to the reference methods.

## **Statistical terms:**

### Repeatability

The repeatability is the closeness of agreement between successive and independent results obtained by the same method on identical test material under the same conditions (apparatus, operator, laboratory and short intervals of time).

### Repeatability limit (r)

The repeatability limit is the value less than or equal to which the absolute difference between two tests results obtained under repeatability conditions is expected to be with a probability of 95%.

Note: If the difference between two results exceeds  $r$ , the results should be considered as suspect.

### Reproducibility

The reproducibility is the closeness of agreement between single test results on identical test material using the same method and obtained by operators in different laboratories using different equipment.

### Reproducibility limit (R)

The reproducibility limit is the value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions is expected to be with a probability of 95%.

Note: If the difference between two results from different laboratories exceeds  $R$ , the results must be considered suspect.

## Phases of validation

The validation of qualitative and quantitative methods comprises two phases:

- A: A method comparison study of the alternative method against a reference method (performed by an expert laboratory).
- B: A collaborative study of the alternative method (organised by an expert laboratory).

## Test characteristics

The test characteristics for alternative methods are shown in the following table:

Qualitative methods	Quantitative methods
<ul style="list-style-type: none"><li>1. Selectivity (inclusivity/ exclusivity)</li><li>2. Relative accuracy</li><li>3. Detection level</li><li>4. Relative sensitivity</li><li>5. Relative specificity</li><li>6. The agreement between the methods, <math>\kappa</math></li></ul>	<ul style="list-style-type: none"><li>1. Selectivity (inclusivity/ exclusivity)</li><li>2. Lowest validated level with satisfactory precision</li><li>3. Repeatability</li><li>4. Reproducibility</li><li>5. Uncertainty of the method</li></ul>

## Part 1: Qualitative methods

### A. Method comparison study

#### 1) Selectivity

The relative selectivity is a measure of a) the inclusivity: detection of the target microorganism from a wide range of strains, and b) the exclusivity: the lack of interference from a relevant range of non-target microorganisms.

##### a) inclusivity

Select 50 pure cultures of target microorganisms relevant to the alternative method and the food matrices.

From each test strain a growth medium is inoculated with a level of 10 to 100 times greater than the detection level. The analyses are carried out according to the respective method descriptions.

##### Results

Microorganism	Alternative method +/-	Reference method +/-
Strain 1		
Strain 2		
Strain 3		
Etc.		
TOTAL		

##### b) exclusivity

Select 30 pure cultures of non-target microorganisms chosen from both the strains known to cause interference with the target microorganism and from strains naturally present in the food matrices.

From each test strain a growth medium is inoculated with a level similar to the greatest level of contamination expected to occur in the food matrices.

##### Results

Microorganism	Alternative method	Reference method
Strain 1		
Strain 2		
Strain 3		
Etc.		
TOTAL		

Note: Criteria for the selection of test strains for measurement of selectivity see Annex G in ISO 16140 : 2003 - Protocol for the validation of alternative methods.

## 2) Relative accuracy

The relative accuracy is the degree of correspondence between the response obtained by the alternative method and the reference method on identical samples.

As far as possible naturally contaminated samples should be used. However, in the Nordic countries such samples are seldom available in sufficient numbers for extensive validations.

Therefore, artificial contamination of samples is permitted. As a general rule three inoculation levels are used: 0= negative control, 1-10 cells per 25 g sample and 10-100 cells per 25 g sample usually 2 strains relevant for the matrix are selected.

In consideration of the physical status of the matrix presented for validation the use of strains stressed by either heating, freezing or chilling at selected time/temperature combinations might be required.

If validation is requested for all food matrices, at least 5 relevant food matrices are selected from the NordVal matrix-lists. Samples from a food matrix should be representative by taking samples from several of the sub-matrices indicated in NV-DOC: F. Matrices for feed of other than plant origin, should include meat- and bonemeal and fishmeal. As regards Salmonella 2 relevant strains for these matrices should be used. Matrices for faeces should include faeces from poultry, swine and cattle unless a specific matrix is chosen. For other categories of matrices a relevant number of matrices are selected.

For each matrix at least 60 samples are analysed to give approximately 30 positive and 30 negative results by the reference method. Each sample is analysed once by the reference method and once by the alternative method.

Identical samples should be used as far as possible. However, when different pre-enrichments are used, duplication of samples is necessary.

*Note:* See guidance in Annex D in EN ISO 16140:2003 - Protocol for the validation of alternative methods.

Tabulate the data from the accuracy measurement after screening.

<b>Responses</b>	<b>Reference method positive (+/ )</b>	<b>Reference method negative (-/ )</b>
<b>Alternative method positive ( /+)</b>	+/+ positive agreement (PA)	-/+ positive deviation (PD)
<b>Alternative method negative ( /-)</b>	+/- negative deviation (ND)	-/- negative agreement (NA)

$$\text{Relative accuracy, \%} = \frac{(\text{PA} + \text{NA}) \cdot 100}{\text{PA} + \text{NA} + \text{PD} + \text{ND}}$$

Tabulate the data from the accuracy measurement after confirmation.

<b>Responses</b>	<b>Reference method positive (+/ )</b>	<b>Reference method negative (-/ )</b>
<b>Alternative method positive ( /+)</b>	+/+ positive agreement (PA)	-/+ true positives (TP)
<b>Alternative method negative ( /-)</b>	+/- false negatives (FN)	-/- negative agreement (NA) + false positives (FP)

$$\text{Relative accuracy, \%} = \frac{(\text{PA} + \text{NA} + \text{FP}) \cdot 100}{\text{PA} + \text{NA} + \text{TP} + \text{FN} + \text{FP}}$$

#### Positive deviation (PD)

The alternative method presents a positive deviation if it gives a positive result when the reference method gives a negative result.

A positive deviation must be confirmed. A positive deviation (PD) becomes a false positive (FP) result when the true result can be proven as being negative.

A positive deviation is considered as a true positive (TP) result when the true result can be proven as being positive.

#### Negative deviation (ND)

The alternative method presents a negative deviation if it gives a negative result when the reference method gives a positive result.

A negative deviation becomes as a false negative (FN) when the true result can be proven as being positive.

It is generally assumed that the reference method gives the true result. However, experience has shown that poor accuracy can be due to poor performance of either the alternative method or the reference method.

### **3) Detection level**

Documentation must be provided that the detection level of the alternative method can detect levels as low as the reference method.

For the determination of limit of detection, it is recommended to use LOD<sub>50</sub>, the level at which 50% of the replicates are positive or negative, as an independent performance parameter for qualitative methods. Calculation of LOD<sub>50</sub> is based on the Spearman-Kärber method.

$$\text{LOD}_{50} = e^m$$

$$\text{where } m = \sum_{i=1}^{k-1} (p(i) + (p(i+1) - p(i)) \frac{(x(i) + x(i+1))}{2})$$

k = number of levels

p(i) = the proportion of positives for the level i (i = 1, 2, 3) – the sensitivity for each level

x(i) = the log concentration i

Use the results of the comparison study to calculate the LOD.

#### 4) Relative sensitivity and specificity

How to calculate the relative sensitivity and specificity after a screening and a confirmation respectively are given in the tables below.

After screening:

Matrices	PA	NA	ND	PD	Sum	Relative Accuracy AC (%)	Relative sensitivity SE (%)	Relative specificity SP (%)
					N	$\frac{(PA+NA) \times 100}{N}$	$\frac{PA \times 100}{PA+ND}$	$\frac{NA \times 100}{PD+NA}$
Food cat. 1								
Food cat. 2								
Food cat. 3								
Food cat. 4								
Food cat. 5								
TOTAL								

$$N = PA + NA + PD + ND$$

PA, positive agreement; ND, negative deviation; PD, positive deviation; NA, negative agreement

After confirmation:

Matrices	PA	NA	FN	TP	FP	Sum	Relative Accuracy AC (%)	Relative sensitivity SE (%)	Relative specificity SP (%)
						N	$\frac{(PA+NA+FP) \times 100}{N}$	$\frac{(PA+TP) \times 100}{PA+FN}$	$\frac{NA \times 100}{NA+FP}$
Food cat. 1									
Food cat. 2									
Food cat. 3									
Food cat. 4									
Food cat. 5									
TOTAL									

$$N = PA + NA + FN + TP + FP$$

PA, positive agreement; ND, negative deviation; PD, positive deviation; NA, negative agreement; FP, false positive; FN, false negative.

Interpretation: Generally, values above 95% are considered acceptable.

## B. Collaborative study

The aim of the collaborative study is to determine the variability of the results obtained by the alternative method in different laboratories using identical samples.

The expert laboratory shall include the alternative method and the reference method in its study.

Results from at least 8 laboratories with valid results must be available for the calculations. (Therefore, it is advisable to select 10-12 labs). In case of very expensive instruments results from 5 laboratories are considered acceptable.

One relevant food matrix is selected from the NordVal matrix list to prepare the test samples. Artificial inoculation is used at 3 levels (O = negative control, 1-10 cells per 25 g and 10-100 cells per 25 g). Duplicates are prepared for each laboratory to be analysed by the alternative method.

If the alternative method comprises more than one method procedure a relevant food matrix for each procedure should be selected for the study.

The expert laboratory shall determine which data from the participating laboratories are suitable for the calculation of the precision data.

Note: Guidelines for the expert laboratory conducting the collaborative study see: Annex H and L in EN ISO 16140:2003 – Protocol for the validation of alternative methods.

### Calculation.

Tabulate the data obtained with each method as follows:

#### Positive results by the expert laboratory

Laboratories	Contamination level		
	L <sub>0</sub>	L <sub>1</sub>	L <sub>2</sub>
Reference method	/2	/2	/2
Alternative method	/2	/2	/2

L<sub>0</sub> = negative control  
L<sub>1</sub> = 1-10 cells per 25 g  
L<sub>2</sub> = 10-100 cells per 25 g

#### Positive results by the alternative method from the collaborative study

Laboratories	Contamination level		
	L <sub>0</sub>	L <sub>1</sub>	L <sub>2</sub>
Laboratory 1	/2	/2	/2
Laboratory 2	/2	/2	/2
Laboratory 3	/2	/2	/2
Etc. etc	/2	/2	/2
Total	FP <sup>a</sup>	TP <sup>b</sup>	TP <sup>c</sup>

a False positive by the alternative method  
b True positive at level 1 by alternative method  
c True positive at level 2 by the alternative method

For level L<sub>0</sub> and each method, calculate the percentage specificity SP

$$(1) \text{ SP} = \left( 1 - \left( \frac{\text{FP}}{\text{N}^-} \right) \right) \cdot 100\%$$

Where:

N - is the total number of all L<sub>0</sub> tests;

FP is the number of false positive.

For each positive contamination level L<sub>1</sub> + L<sub>2</sub> and for each method, calculate the percentage of sensitivity SE

$$(2) \text{ SE} = \frac{\text{TP}}{\text{N}^+} \cdot 100\%$$

Where:

N+ is the total number of all L<sub>1</sub> or L<sub>2</sub> tests respectively;

TP is the number of true positive.

For each level of contamination and the totality of the results, compare the alternative method and the reference method in order to calculate the relative accuracy in the study carried out by the expert laboratory. Each pair of results from a sample measured by the alternative and the reference method shall be reported in a cross table as follows:

Alternative method	Reference method		Total
	+	-	
+	PA	TP	n+
-	FN	NA+ FP	n-
Total	N+	N-	N

Calculate the relative accuracy AC expressed in percentage

$$\text{AC} = \frac{(\text{PA} + \text{NA} + \text{FP})}{\text{N}} \cdot 100\%$$

Where:

N is the number of tested samples (for the level L<sub>1</sub> or all levels);

PA is the number of positive agreement;

NA + FP is the number of negative agreement.

### **Interpretation.**

Compare AC, SE and SP with their relative counterparts obtained within the comparative study.

### Checking the degree of agreement:

The degree of agreement might be quantified by kappa,  $\kappa$ . If all the observed values lie on the diagonal of the cross table, there is perfect agreement between the two methods. On the other hand, more values placed outside the table diagonal, indicate less agreement between the methods. To get an estimate of the agreement between the methods, Cohen's kappa may be calculated as follows:

The observed proportion of agreement, the accuracy, AC:

$$p_o = AC = \frac{PA + NA + FP}{N}$$

The expected frequency of agreement, the expected accuracy, or repeatability by chance is:

$$p_e = \frac{(N+ \cdot N-) + (n+ \cdot n-)}{N^2}$$

To measure the agreement between the methods, Cohen's  $\kappa$  (kappa) can be applied:

$$\kappa = \frac{p_o - p_e}{1 - p_e}$$

In general, the following  $\kappa$  values are used in the interpretation of kappa:

- $\kappa \leq 0.20$  → Poor agreement
- $\kappa \in \{0.21 - 0.40\}$  → Fair agreement
- $\kappa \in \{0.41 - 0.60\}$  → Moderate agreement
- $\kappa \in \{0.61 - 0.80\}$  → Good agreement
- $\kappa > 0.80$  → Very good agreement

For method validation, considering the overall agreement (number of test results are relatively high), "very good agreement" is often required, i.e.  $\kappa > 0.80$ .

## Part 2: Quantitative methods

### A. Method comparison study

#### 1) Selectivity

The selectivity is a measure of a) the inclusivity: detection of the target microorganism from a wide range of strains, and b) the exclusivity: the lack of interference from a relevant range of non-target microorganisms.

##### a) inclusivity

Select at least 30 pure cultures of target microorganisms relevant to the alternative method and the food matrices. These strains shall be representative of the most relevant strains for the matrix of interest.

From each test strain an appropriate growth medium is cultured overnight before the alternative medium and the reference medium are inoculated. For both the alternative medium and the reference medium the complete protocol is used

##### Results

Microorganism	Alternative method +/-	Reference method +/-
Strain 1		
Strain 2		
Strain 3		
etc.		
TOTAL		

##### b) exclusivity

Select at least 20 pure cultures of non-target microorganisms chosen from both the strains known to cause interference with the target microorganism and from strains naturally present in the food matrices. These strains shall be representative of the most common strains in the Nordic countries.

From each test strain an appropriate growth medium is cultured overnight before the alternative medium and the reference medium are inoculated. For both the alternative methods and the reference method the complete protocol is used.

##### Results

Microorganism	Alternative method	Reference method
Strain 1		
Strain 2		
Strain 3		
etc.		
TOTAL		

Note: Criteria for the selection of test strains for measurement of selectivity see Annex G in EN ISO 16140 : 2003 - Protocol for the validation of alternative methods.

## 2) Reliability of the method

For examining the reliability of the method, a minimum of five different levels of analyte in each food type is required. For horizontal methods, five food types are required. The levels should cover the range of interest (low, intermediate and maximum levels). The number of analysis per level for the alternative and the reference method, respectively, are five.

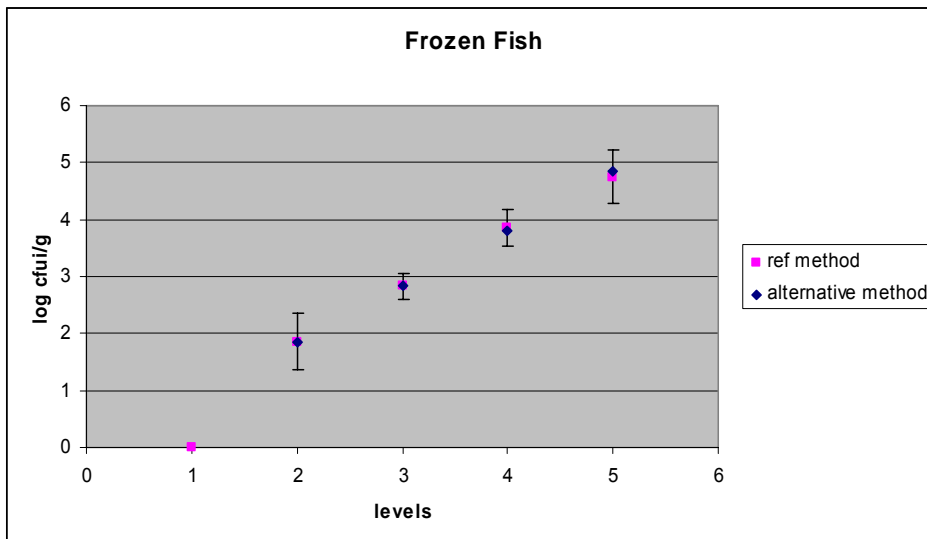
For each level, food type and method calculate the mean (in log cfu/g) and the standard deviation (in log cfu/g) as illustrated in the table below.

Food type	I				
Level	A	B	C	D	E
Reference method					
Results	X <sub>A1</sub> X <sub>A2</sub> X <sub>A3</sub> X <sub>A4</sub> X <sub>A5</sub>	X <sub>B1</sub> X <sub>B2</sub> X <sub>B3</sub> X <sub>B4</sub> X <sub>B5</sub>	X <sub>C1</sub> X <sub>C2</sub> X <sub>C3</sub> X <sub>C4</sub> X <sub>C5</sub>	X <sub>D1</sub> X <sub>D2</sub> X <sub>D3</sub> X <sub>D4</sub> X <sub>D5</sub>	X <sub>E1</sub> X <sub>E2</sub> X <sub>E3</sub> X <sub>E4</sub> X <sub>E5</sub>
Mean	$\bar{X}_{Ref} = \frac{X_{A1} + X_{A2} + X_{A3} + X_{A4} + X_{A5}}{5}$				
Standard deviation, s	$S_{Ref} = \sqrt{\frac{\sum_{i=1}^5 (X_i - \bar{X})^2}{4}}$				
Confidence level: ± 2s	2 · S <sub>Ref</sub>				
Alternative method					
Results	X <sub>A1</sub> X <sub>A2</sub> X <sub>A3</sub> X <sub>A4</sub> X <sub>A5</sub>	X <sub>B1</sub> X <sub>B2</sub> X <sub>B3</sub> X <sub>B4</sub> X <sub>B5</sub>	X <sub>C1</sub> X <sub>C2</sub> X <sub>C3</sub> X <sub>C4</sub> X <sub>C5</sub>	X <sub>D1</sub> X <sub>D2</sub> X <sub>D3</sub> X <sub>D4</sub> X <sub>D5</sub>	X <sub>E1</sub> X <sub>E2</sub> X <sub>E3</sub> X <sub>E4</sub> X <sub>E5</sub>
Mean	$\bar{X}_{Alt} = \frac{X_{A1} + X_{A2} + X_{A3} + X_{A4} + X_{A5}}{5}$				
Standard deviation, s	$S_{Alt} = \sqrt{\frac{\sum_{i=1}^5 (X_i - \bar{X})^2}{4}}$				

Calculate the mean and the standard deviation for each level, matrix and method.

Draw a diagram for each matrix. Use Excel spread sheet. Plot the results obtained by the reference and the alternative method; the levels makes the x-axis and the means are illustrated on the y-axis. Include the confidence interval (±2s) of the reference method for each level in the graph. If the mean results obtained by the alternative method fall within the relevant confidence intervals, there are no significant difference between the methods. That is, if the standard deviation of the reference method and the alternative method are satisfactory. As a rule of thumb, a satisfactory standard deviation (according to NMKL Procedure No 8) is 0.4 log cfu/g or lower. The lowest validated level with satisfactory precision should also be stated in the study report.

An example of such a graphical presentation is given below.



## B. Collaborative study

A collaborative study establishes the following values:

1. Standard deviation of repeatability (sr)
2. Standard deviation of reproducibility (SR)
3. The uncertainty of the methods

The aim of the collaborative study is to determine the variability of the results obtained by the alternative method in different laboratories using identical samples by calculating the repeatability and the reproducibility.

One relevant food matrix is selected to prepare the test samples. Artificial inoculation is used at 4 levels (from 0 to  $10^4$  per 1 g). For each laboratory duplicate samples are prepared and analysed by the alternative method and by the reference method.

Results from at least 8 laboratories with valid results must be available for the calculations. (Therefore, it is advisable to select 10-12 labs). In case of very expensive instruments results from 5 laboratories are considered acceptable.

If the alternative method comprises more than one protocol a relevant food matrix for each protocol should be selected for the study.

Note: Guidelines for the expert laboratory conducting the collaborative study see: Annex H in EN ISO 16140:2003 - Protocol for the validation of alternative methods.

### Calculation

Tabulate the data obtained with each method for statistical analyses for the calculation of standard-deviation of repeatability (sr), repeatability limit (r), standard-deviation of reproducibility (SR) and reproducibility limit (R).

**Standard deviation of repeatability,  $s_r$ :** is carried out as follows, for each food matrix (i) and contamination level (j),

Food matrix (i) Contamination level (j)	Duplicates (log <sub>10</sub> )		$M_k$	$SD_k$
	1	2		
Laboratory (k) : 1	$y_{k1}$	$y_{k2}$	$\frac{(y_{k1} + y_{k2})}{2}$	$\frac{ y_{k1} - y_{k2} }{\sqrt{2}}$
2	...	...	...	...
...	...	...	...	...
K	...	...	...	...
MEDIAN :			MED $\{M_k\}$	MED $\{SD_k\}$

$y_{k1}$  (or  $y_{k2}$ ) is the result transformed in log, obtained by the laboratory k for the duplicate 1 (or 2) of the combination (food matrix i, contamination level j).

$$s_r = k_2 \text{MED} \{SD_k\}$$

Where :

- $k_2$  is a constant,  $k_2 = 1.4836$
- $\text{MED} \{SD_k\}$  is the median of the standard-deviations of duplicates for the K laboratories.

**The repeatability limit,  $r = 2\sqrt{2} s_r \approx 2.8 s_r$**

**Standard deviation of reproducibility,  $s_R$ :**

Calculate the recursive median  $s_d = 1.1926 \times S_n$

where  $S_n = \text{MED}_k \{ \text{MED}_j |M_k - M_j| \}$

For calculation of  $S_n$ , see Annex I.

Standard deviation of reproducibility is  $s_R = \sqrt{s_d^2 + \frac{s_r^2}{2}}$

**The reproducibility limit,  $R = 2\sqrt{2} s_R \approx 2,8 s_R$**

Check if the obtained results of the alternative method fall within the confidence interval ( $\pm 2s$ ) of the reference method. If so there is no significant deviation between the methods. Calculate the combined uncertainty for the alternative and the reference method respectively,  $u$ ,

$$u = \sqrt{\frac{\sum (n_i - 1) s_{R_i}^2}{\sum (n_i - 1)}}$$

A combined uncertainty of less than 0.4 log cfu/g illustrates satisfactory precision of the studies.

NordVal will evaluate information provided by the manufacturer based on results for the above mentioned characteristics obtained by using international recognised protocols.

**References:**

AFNOR – 2002: Requirements relating to preliminary and collaborative studies conducted by an expert laboratory.

AOAC – 1999: Methods Committee Guidelines for Validation of Qualitative and Quantitative Microbiological Methods.

ISO N 543 – 2003: Statistics of Analytical Data – Protocol for the Establishment of Precision Characteristics of Microbiological Quantitative methods by Interlaboratory Studies.

ISO 16140 – 2003: Protocol for the Validation of Alternative Microbiological Methods.

NMKL Procedure No 23, 2008: Guide on quality assurance for microbiological laboratories.

NMKL Procedure No 8, Version 4, 2008: Measurement of uncertainty in quantitative microbiological examination of foods.

NMKL Procedure No 20, 2007: Evaluation of results from qualitative methods.

## Annex 1 – Robust estimator of dispersion based on the recursive median Sn from Rousseeuw

from Microbiology of food and animal feeding stuffs –Protocol for the establishment of precision characteristics of quantitative methods by inter-laboratory studies, elaborated by the leadership of Bertrand Lombard.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	
1																					
2																					
3				A	b	e	f	g	h	I	j	k	l	m	n	o	p	q	r		
4			<b>Transpose</b>																		
5		<b>DATA</b>	<b>→</b>	5,24	5,80	5,15	5,73	6,66	4,00	3,30	6,08	3,78	5,81	5,35	7,92	5,93	5,05	4,87	4,03		
6		<b>↓</b>		=D\$5-\$B7	=E\$5-\$B7	=E\$5-\$B7	etc.														
7	a	5,24	D\$5-\$B7 =		0,56	0,09	0,49	1,42	1,24	1,94	0,84	1,46	0,57	0,11	2,68	0,69	0,20	0,38	1,21		
8	b	5,80	D\$5-\$B8 =	0,56		0,65	0,07	0,86	1,80	2,50	0,28	2,02	0,00	0,45	2,12	0,13	0,76	0,94	1,77		
9	e	5,15	D\$5-\$B9 =	0,09	0,65		0,58	1,51	1,15	1,85	0,93	1,37	0,65	0,20	2,77	0,78	0,11	0,29	1,12		
10	f	5,73	etc.	0,49	0,07	0,58		0,93	1,73	2,43	0,35	1,95	0,07	0,38	2,19	0,20	0,69	0,87	1,70		
11	g	6,66		1,42	0,86	1,51	0,93		2,66	3,36	0,58	2,88	0,85	1,31	1,27	0,73	1,61	1,79	2,63		
12	h	4,00		1,24	1,80	1,15	1,73	2,66		0,70	2,08	0,22	1,81	1,35	3,92	1,93	1,05	0,87	0,03		
13	i	3,30		1,94	2,50	1,85	2,43	3,36	0,70		2,78	0,48	2,51	2,05	4,62	2,63	1,75	1,57	0,73		
14	j	6,08		0,84	0,28	0,93	0,35	0,58	2,08	2,78		2,30	0,27	0,73	1,85	0,15	1,03	1,21	2,05		
15	k	3,78		1,46	2,02	1,37	1,95	2,88	0,22	0,48	2,30		2,03	1,57	4,14	2,15	1,27	1,09	0,25		
16	l	5,81		0,57	0,00	0,65	0,07	0,85	1,81	2,51	0,27	2,03		0,46	2,12	0,13	0,76	0,94	1,78		
17	m	5,35		0,11	0,45	0,20	0,38	1,31	1,35	2,05	0,73	1,57	0,46		2,57	0,58	0,31	0,48	1,32		
18	n	7,92		2,68	2,12	2,77	2,19	1,27	3,92	4,62	1,85	4,14	2,12	2,57		1,99	2,88	3,06	3,89		
19	o	5,93		0,69	0,13	0,78	0,20	0,73	1,93	2,63	0,15	2,15	0,13	0,58	1,99		0,89	1,07	1,90		
20	p	5,05		0,20	0,76	0,11	0,69	1,61	1,05	1,75	1,03	1,27	0,76	0,31	2,88	0,89		0,18	1,02		
21	q	4,87		0,38	0,94	0,29	0,87	1,79	0,87	1,57	1,21	1,09	0,94	0,48	3,06	1,07	0,18		0,84		
22	r	4,03		1,21	1,77	1,12	1,70	2,63	0,03	0,73	2,05	0,25	1,78	1,32	3,89	1,90	1,02	0,84			
23	n	=	MED =	0,69	0,76	0,78	0,69	1,42	1,35	2,05	0,93	1,57	0,76	0,58	2,68	0,78	0,89	0,94	1,32		
24			<b>↓</b>		= MEDIAN (D7 : D22)				...	... =MEDIAN (K7 : K22) etc.				etc.							
25			MED =	0,91	= MEDIAN (D23 : S23) = Sn																
26			SD(Sn) =	1,08	= 1,1926*D25																
27																					
28			<b>Operations (Excel 7.0) :</b>																		
29			1) Tranpose the original data column B7:B22 in D5:55																		
30			2) In the central square table, write the formula for the absolute deviation of the 1 <sup>st</sup> cell : =ABS(D\$5-\$B7)																		
31			3) Copy it in all the table																		
32			4) Delete the main diagonal																		
33			5) Under the 1 <sup>st</sup> column, write the median formula : =MEDIAN (D7 : D22), and copy it under all columns																		
34			6) Write the final recursive median Sn : =MEDIAN (D23 : S23), and its corresponding SD in multiplyingt Sn by 1,1926																		