

# Proficiency testing Food Microbiology

January 2025

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# Abbreviations

## Media

ALOA	Agar for <i>Listeria</i> according to Ottaviani & Agosti
APW 2%	Alcaline peptone water, 2 % NaCl
BA	Blood agar
BcsA	<i>Bacillus cereus</i> selective agar
BEA	Bile esculin agar
BGA	Brilliant green agar
BGLB	Brilliant green lactose bile broth
BP	Baird-Parker agar
BPW	Buffered peptone water
BS	Bromthymol blue saccharose agar
CBC	Oxoid Brilliance™ <i>Bacillus cereus</i> agar
CIN	Cefsulodin irgasan novobiocin agar
Compact Dry EC	Compact Dry™ <i>E. coli</i> and coliforms
Compact Dry ETB	Compact Dry™ Enterobacteriaceae
Compact Dry ETC	Compact Dry™ Enterococcus
Compact Dry TC	Compact Dry™ Total Count
COMPASS	COMPASS® Enterococcus agar
CT-SMAC	Cefixime tellurite sorbitol MacConkey agar
DG18	Dikloran glycerol agar
DRBC	Dikloran Rose-Bengal chloramphenicol agar
EC	<i>E. coli</i> broth
EMB	Eosin Methylene Blue agar
ENT	Slanetz & Bartley <i>Enterococcus</i> agar
HEA	Hektoen enteric agar
IA	Iron agar
ISA	Iron sulphite agar
ITC	Irgasan ticarcillin potassium chlorate broth
KEAA	Kanamycin esculin azide agar
LMBA	<i>Listeria monocytogenes</i> blood agar
LSB	Lauryl sulphate broth
LTLSB	Lactose tryptone lauryl sulphate broth
mCCDA	Modified charcoal cephaloperazone deoxycholate agar
mCP	Membrane <i>Clostridium perfringens</i> agar
MKTTn	Muller-Kauffmann tetrathionate/novobiocin broth
MLCB	Manitol Lysine Crystal violet Brilliant green agar
MPCA	Milk plate count agar
MRB	Modified Rappaport broth
MRS	de Man, Rogosa and Sharpe agar
MRS-aB	de Man, Rogosa and Sharpe agar with amphotericin
MRS-S	de Man, Rogosa and Sharpe agar with sorbic acid

MSRV	Modified semi-solid Rappaport-Vassiliadis enrichment media
mTSB	Modified tryptone soya broth
MYP	Mannitol egg yolk polymyxin agar
NAP	Nitrite actidione Polymyxin agar
OCLA	Oxoid Brilliance™ Listeria agar
OGYE	Oxytetracyclin glucose yeast extract agar
OPSP	Oleandomycin, Polymixin, Sulphadiazine, Perfringens agar
PAB	Perfringens agar base
PDA	Potato dextrose agar
PALCAM	Polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol agar
Petrifilm AC	3M™ Petrifilm™ Aerobic Count
Petrifilm CC	3M™ Petrifilm™ Coliform count
Petrifilm Disk	3M™ Petrifilm™ Staph Express Disk
Petrifilm EB	3M™ Petrifilm™ Enterobacteriaceae
Petrifilm EC/CC	3M™ Petrifilm™ <i>E. coli</i> /Coliform count
Petrifilm EL	3M™ Petrifilm™ Environmental Listeria
Petrifilm LAB	3M™ Petrifilm™ Lactic acid bacteria
Petrifilm RAC	3M™ Petrifilm™ Rapid Aerobic Count
Petrifilm REC	3M™ Petrifilm™ Rapid <i>E. coli</i> /Coliform count
Petrifilm SEC	3M™ Petrifilm™ Select <i>E. coli</i>
Petrifilm Staph	3M™ Petrifilm™ Staph Express
PEMBA	Polymyxin pyruvate egg yolk mannitol bromothymol blue agar
PSB	Peptone sorbitol bile salts broth
PCA	Plate count agar
RPFA	Baird-Parker agar with rabbit plasma fibrinogen
SFA	Sugar-free agar
RVS	Rappaport-Vassiliadis Soy peptone broth
Saubouraud	Saubouraud chloramphenicol agar
SC	Sulphite cycloserine agar
SCD	Soyabean Casein Digest agar
SFP	Shahidi-Ferguson Perfringens agar
SMAC	Sorbitol MacConkey agar
SP	Salt Polymyxin broth
SSDC	Salmonella/Shigella sodium deoxycholate calcium chloride agar
TBX	Tryptone bile X-glucuronide agar
TCBS	Thiosulphate citrate bile salts sucrose agar
TGE	Tryptone glucose extract agar
TEMPO AC	TEMPO® Aerobic count
TEMPO BC	TEMPO® <i>Bacillus cereus</i>
TEMPO CAM	TEMPO® Campylobacter
TEMPO CC	TEMPO® Coliform count
TEMPO EB	TEMPO® Enterobacteriaceae
TEMPO EC	TEMPO® <i>E. coli</i>
TEMPO RYM	TEMPO® Rapid Yeast/Mould
TEMPO STA	TEMPO® Coagulase-positive staphylococci

TEMPO YM	TEMPO® Yeast/Mould
TGE	Tryptone glucose extract agar
TS	Tryptose sulphite agar
TSA	Tryptic soya agar
TSC	Tryptose sulphite cycloserine agar
TSBY	Tryptone soya broth with yeast extract
XLD	Xylose lysine deoxycholate agar
VIDAS CAM	VIDAS® Campylobacter
VIDAS ECPT	VIDAS® UP E. coli O157 (including H7)
VIDAS LMX	VIDAS® Listeria monocytogens Xpress
VRB	Violet red bile agar
VRBG	Violet red bile glucose agar
YGC	Yeast extract glucose chloramphenicol agar

## Organisations

AFNOR	French National Standardization Association
AOAC	AOAC INTERNATIONAL
ATCC	American Type Culture Collection
CBS	Centraalbureau voor Schimmelcultures (Westerdijk Institute)
CCUG	Culture Collection University of Gothenburg
IDF	International Dairy Foundation
ISO	International Organization for Standardization
NMKL	Nordic-Baltic Committee on Food Analyses
NordVal	NordVal International - NMKL
SLV	Livsmedelsverket/Swedish Food Agency, Sweden
Fohm	Public Health Agency of Sweden

# Analyses in this PT round

## Quantitative analyses

Aerobic microorganisms, 30 °C

Enterobacteriaceae

Thermotolerant *Campylobacter*

*Listeria monocytogenes*

## Qualitative analyses

Thermotolerant *Campylobacter*

*Listeria monocytogenes*

*Salmonella*

*Escherichia coli* O157

Pathogenic *Vibrio* spp.

*Yersinia enterocolitica*

# Method

## Reporting of results and method information

It is the responsibility of the individual participants to correctly report results according to the instructions. Incorrectly reported results, for example results reported for the wrong sample, cannot be correctly processed. Incorrectly reported results are as a general rule excluded but may – after manual assessment by the Swedish Food Agency in each individual case – still be included and processed.

It is also mandatory for the participants to report method information for all analyses. This method information is sometimes contradictory or difficult to interpret. For example when participants state a medium that is not included in the standard method they refer to, or when manual comments by the participant contradict the reported method information. In such cases, the reported method information provided by the participants is generally used in method comparisons “as it is”. Alternatively, method data that are difficult to interpret may be excluded or added to the group “Other”, together with results from methods and media that are only used by 1–2 participants.

## Standard deviation and assigned value

Evaluation of the participants’ results and statistical calculations are carried out on the  $\log_{10}$  transformed results. Results reported by participants as “> value” are not evaluated. Results reported as “< value” are excluded from the evaluation, or occasionally treated as zero (negative result).

A robust statistical approach is used to determine the mean value and standard deviation. Algorithm A with iterated scale as described in ISO 13528:2022 [1] is used to determine the robust mean ( $m_{PT}$ ) and robust standard deviation ( $s_{PT}$ ) of the participants’ results. Results that are obviously erroneous are excluded prior to determining  $m_{PT}$  and  $s_{PT}$  (blunder removal). For evaluated parameters, the assigned value consists of  $m_{PT}$ . It is regarded as the true, normative value.

For small datasets, there is an increased uncertainty associated with determining the robust mean ( $m_{PT}$ ) and robust standard deviation ( $s_{PT}$ ) of the participants’ results. Therefore, when fewer than 12 participants have reported evaluated results, the statistical measures for performance evaluation will be provided *only as an information* to the participants.

## Outliers

Outliers are results that deviate from the other results in a way that cannot be explained by normal variation. Results within  $m_{PT} \pm 3s_{PT}$  are considered acceptable, whereas results outside this interval are considered as outliers. When fewer than 12 participants have reported results, as well as in some individual cases, subjective adjustments are made to set acceptance limits based on prior knowledge of the samples contents.



## Results from different methods

*Non-robust* median values (*Med*) and standard deviations (*s*) are calculated to assist in the evaluation of the results from different methods. These are shown in tables in the report, in connection with the respective analyses. In these instances, *Med* and *s* are calculated from the respective method groups' results, with outliers and false results excluded. For method groups with fewer than 5 results, only the number of false results and outliers are provided.

## Measurement uncertainty for the assigned values

The standard uncertainty ( $u_{PT}$ ) of the assigned value ( $m_{PT}$ ) is estimated from the standard deviation ( $s_{PT}$ ) and the number of evaluated results ( $n$ ):

$$u_{PT} = 1.25 \times \frac{s_{PT}}{\sqrt{n}}$$

The measurement uncertainty is considered negligible compared to the standard deviation (which is used for evaluating the participants' results) when:

$$u_{PT} < 0.3s_{PT}$$

## Z-scores

To allow comparison of the results from different analyses and samples, results are transformed into standard values (z-scores). Z-scores are calculated as:

$$z = \frac{x_{lab} - m_{PT}}{s_{PT}}$$

where  $x_{lab}$  is the result of the individual participant.

Z-scores for individual analyses are shown in Appendix 2 and can be used as a tool by participants when following up on the results. For quantitative analyses, a z-score is either positive or negative, depending on whether the participants result is higher or lower than  $m_{PT}$ .

In evaluations of the analytical results, the following guidelines can be used:

- $|z| \leq 2$  indicates that the result is acceptable
- $2 < |z| < 3$  indicates a warning that the result may be deviating, and might motivate an action in the follow-up process
- $|z| \geq 3$  indicates that the result is regarded as deviating and should lead to an action in the follow-up process

## Table legends

- $N$  number of participants that reported results for the analysis
- $n$  number of participants with satisfactory result (false results and outliers excluded)
- $m_{PT}$  assigned value, robust mean value in  $\log_{10}$  cfu ml<sup>-1</sup>
- $s_{PT}$  robust standard deviation
- $u_{PT}$  standard uncertainty of the assigned value

- $F$  number of false positive or false negative results
- $<$  number of low outliers
- $>$  number of high outliers
- results deviating more than  $1 s_{PT}$  from  $m$ , or unusually many deviating results.

## Figure legends

- results within the interval of acceptance
- outlier
- false negative result
- \* value outside the x-axis scale

# Results

## General outcome

Samples were sent to 116 participants: 21 in Sweden, 83 in Europe, and 12 outside of Europe. Individual results are listed in Appendix 1. Z-scores for individual results are listed in Appendix 2.

**Table 1.** Composition of the test material and proportion of deviating results (*N*: number of reported results, *F*: false positive or false negative, *X*: outliers)

	Sample A				Sample B				Sample C			
<b>% participants with</b>												
<b>Microorganisms</b>	<i>Campylobacter coli</i> <i>Citrobacter freundii</i> <i>Listeria monocytogenes</i> <i>Vibrio parahaemolyticus</i> <i>Yersinia enterocolitica</i>				<i>Campylobacter coli</i> <i>Citrobacter freundii</i> <i>Listeria monocytogenes</i> <i>Vibrio parahaemolyticus</i> <i>Yersinia enterocolitica</i>				<i>Campylobacter jejuni</i> <i>Escherichia coli</i> <i>Escherichia coli O157</i> <i>Listeria monocytogenes</i> <i>Salmonella Stockholm</i>			
<b>Analysis</b>	<b>Target organism</b>	<b>N</b>	<b>F</b>	<b>X</b>	<b>Target organism</b>	<b>N</b>	<b>F</b>	<b>X</b>	<b>Target organism</b>	<b>N</b>	<b>F</b>	<b>X</b>
Aerobic microorganisms 30 °C	<i>C. freundii</i>	110	0	5	<i>C. freundii</i>	109	0	7	<i>E. coli</i>	109	0	5
Enterobacteriaceae	<i>C. freundii</i>	94	0	3	<i>C. freundii</i>	94	0	5	<i>E. coli</i>	93	0	4
Thermotol. campylobacter - Quantitative	<i>C. coli</i>	16	1	0	<i>C. coli</i>	16	0	0	<i>C. coli</i>	16	1	1
<i>Listeria monocytogenes</i> - Quantitative	<i>L. monocyt.</i>	56	0	5	<i>L. monocyt.</i>	56	0	8	<i>L. monocyt.</i>	56	0	4
Thermotol. campylobacter - Qualitative	<i>C. coli</i>	23	0	0	<i>C. coli</i>	23	0	0	<i>C. coli</i>	23	2	0
<i>Listeria monocytogenes</i> - Qualitative	<i>L. monocyt.</i>	99	0	0	<i>L. monocyt.</i>	96	2	0	<i>L. monocyt.</i>	100	3	0
<i>Salmonella</i>	-	127	2	0	-	125	1	0	<i>S. Stockholm</i>	125	1	0
<i>Escherichia coli</i> O157	-	22	0	0	-	22	0	0	<i>E. coli O157</i>	22	0	0
Pathogenic <i>Vibrio</i> spp.	<i>V. parahaemolyticus</i>	16	0	0	<i>V. parahaemolyticus</i>	16	0	0	-	17	0	0
<i>Yersinia enterocolitica</i>	<i>Y. enterocolitica</i>	10	1	0	<i>Y. enterocolitica</i>	10	1	0	-	10	1	0

- no target organism or no value; **microorganism** = main target organism; (*microorganism*) = false positive before confirmation

☐ The results are not evaluated.

# Aerobic microorganisms, 30 °C

## Sample A/B

*C. freundii* was present in the highest concentration and was thus the main target organism.

### Sample A

In total, 110 results were evaluated. Four low and one high outlier were identified.

### Sample B

In total, 109 results were evaluated. Six low and one high outlier were identified.

## Sample C

*E. coli* was present in the highest concentration and was thus the main target organism.

In total, 109 results were evaluated. Three low and two high outliers were identified.

## General remarks

Most participants followed either NMKL 86:2013, ISO 4833-1:2013 or used 3M Petrifilm AC. ISO 4833-1:2013 was last reviewed by ISO in 2019 and remains current. An amendment with a clarification on the scope of the method is available (ISO 4833-1:2013/Amd 1:2022). NMKL 86:2013 was last reviewed by NMKL in 2022 and remains current.

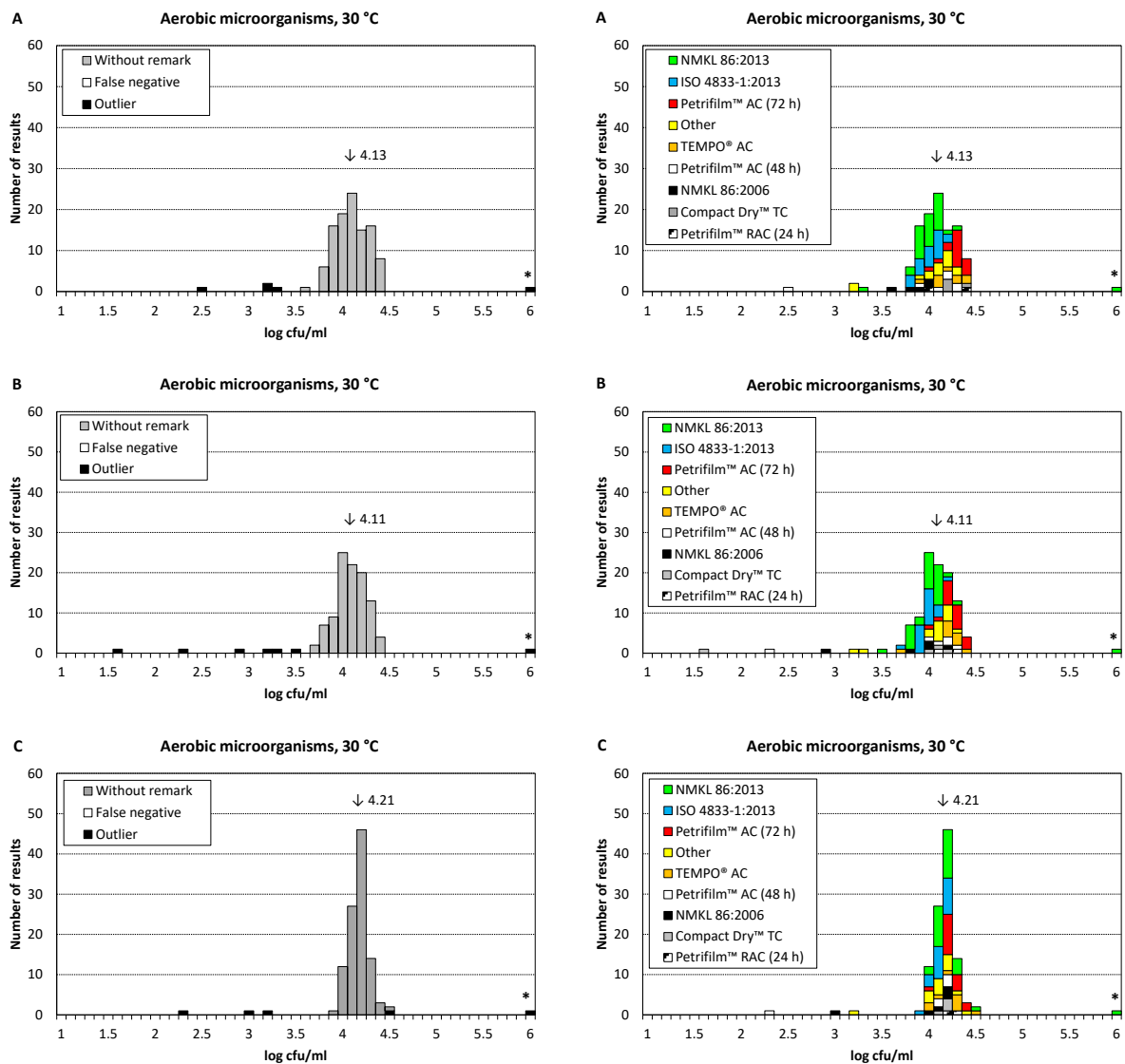
Both NMKL 86:2013 and ISO 4833-1:2013 are based on incubation on PCA or MPCA at 30 °C for 72 h. Users of Petrifilm™ AC can use different incubation times/temperatures, depending on the method validation. For example, AOAC® prescribes incubation at 35 °C for 48 h while AFNOR prescribes 30 °C for either 48 h or 72 h, depending on which product that is analysed.

For samples A and B, the results for Petrifilm™ AC and TEMPO® AC were somewhat higher compared to results from other methods. Differences of this magnitude are not uncommon for Petrifilm™ AC and TEMPO® AC and can be considered normal.

**Table 2.** Results from analysis of aerobic microorganisms, 30 °C.

Method	Sample A							Sample B							Sample C						
	N	n	$m_{PT}$	$s_{PT}$	F	<	>	N	n	$m_{PT}$	$s_{PT}$	F	<	>	N	n	$m_{PT}$	$s_{PT}$	F	<	>
<b>All results</b>	<b>110</b>	<b>105</b>	<b>4.13</b>	<b>0.18</b>	<b>0</b>	<b>4</b>	<b>1</b>	<b>109</b>	<b>102</b>	<b>4.11</b>	<b>0.18</b>	<b>0</b>	<b>6</b>	<b>1</b>	<b>109</b>	<b>104</b>	<b>4.21</b>	<b>0.11</b>	<b>0</b>	<b>3</b>	<b>2</b>
NMKL 86:2013	31	29	4.08	0.12	0	1	1	31	29	4.08	0.13	0	1	1	30	28	4.21	0.08	0	0	2
ISO 4833-1:2013	21	21	4.06	0.12	0	0	0	21	21	4.04	0.10	0	0	0	21	21	4.18	0.10	0	0	0
Petrifilm™ AC (72 h)	17	17	4.32	0.09	0	0	0	17	17	4.30	0.10	0	0	0	17	17	4.28	0.08	0	0	0
Other	14	12	4.19	0.10	0	2	0	14	12	4.14	0.09	0	2	0	14	13	4.18	0.10	0	1	0
TEMPO® AC	9	9	4.25	0.17	0	0	0	9	9	4.28	0.20	0	0	0	9	9	4.32	0.17	0	0	0
Petrifilm™ AC (48 h)	7	6	4.26	0.13	0	1	0	6	5	4.20	0.09	0	1	0	6	5	4.20	0.07	0	1	0
NMKL 86:2006	5	5	3.99	0.20	0	0	0	5	4	-	-	0	1	0	6	5	4.22	0.10	0	1	0
Compact Dry™ TC	4	4	-	-	0	0	0	4	3	-	-	0	1	0	4	4	-	-	0	0	0
Petrifilm™ RAC (24 h)	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0

For individual methods:  $m_{PT}$  = median value and  $s_{PT}$  = standard deviation for the particular method (outliers and false results excluded).



**Figure 1.** Results from analysis of aerobic microorganisms, 30 °C.

# Enterobacteriaceae

## Sample A/B

The strain of *C. freundii* was target organism. On VRBG, it forms typical red/purple colonies surrounded by a bile salt precipitation zone. The strain is oxidase-negative.

### Sample A

In total, 94 results were evaluated. Two low and one high outlier were identified.

### Sample B

In total, 94 results were evaluated. Four low and one high outlier were identified.

## Sample C

The strain of *E. coli* was target organism. On VRBG, it forms typical red/purple colonies surrounded by a bile salt precipitation zone. The strain is oxidase-negative.

In total, 93 results were evaluated. Three low and one high outlier were identified.

## General remarks

Enterobacteriaceae are Gram-negative and oxidase-negative bacteria that ferment glucose with the production of acid by-products. On VRBG they therefore form pink/red colonies, with or without a bile salt precipitation zone. The appearance is similar on Petrifilm EB, which also includes a colour indicator for acid by-products and a plastic film for detection of gas production.

The most common methods were NMKL 144:2005, a method with Petrifilm EB and ISO 21528-2:2017. ISO 21528-2:2017 was last reviewed by ISO in 2022 and remains current.

Most methods used by the participants stipulate a 37 °C incubation temperature. With Petrifilm EB, both 30 °C and 37 °C is possible to use. Here, two participants used the lower incubation temperature. One of these reported a low outlier for sample A, but it is difficult to determine if this was due to the incubation temperature or some other factor.

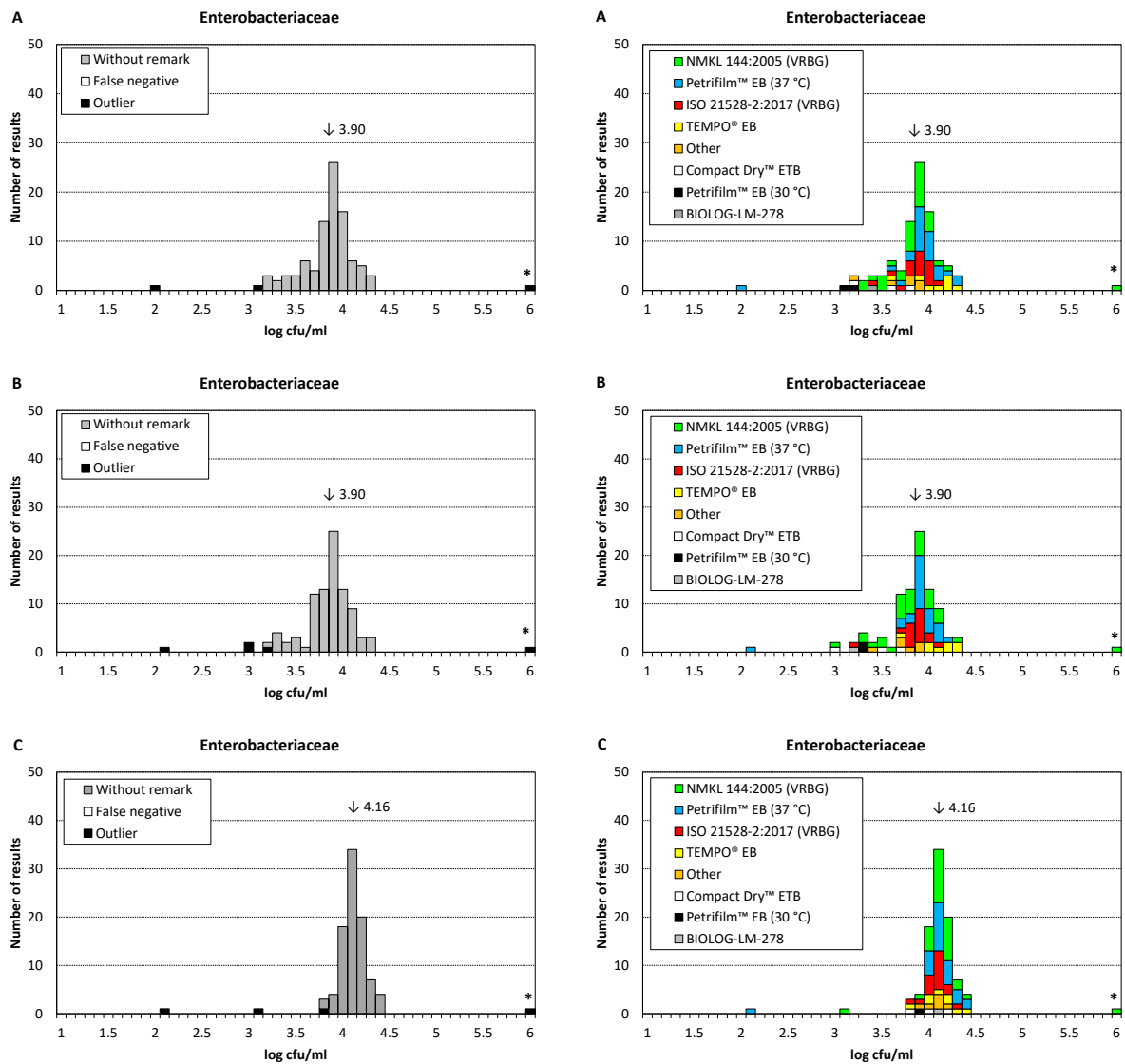
For samples A and B, the results for TEMPO® EB were somewhat higher compared to results from other methods. Differences of this magnitude are not uncommon for TEMPO® EB and can be considered normal.

**Table 3.** Results from analysis of Enterobacteriaceae.

Method	Sample A							Sample B							Sample C						
	N	n	<i>m</i> <sub>PT</sub>	<i>s</i> <sub>PT</sub>	F	<	>	N	n	<i>m</i> <sub>PT</sub>	<i>s</i> <sub>PT</sub>	F	<	>	N	n	<i>m</i> <sub>PT</sub>	<i>s</i> <sub>PT</sub>	F	<	>
<b>All results</b>	<b>94</b>	<b>91</b>	<b>3.90</b>	<b>0.23</b>	<b>0</b>	<b>2</b>	<b>1</b>	<b>94</b>	<b>89</b>	<b>3.90</b>	<b>0.22</b>	<b>0</b>	<b>4</b>	<b>1</b>	<b>93</b>	<b>89</b>	<b>4.16</b>	<b>0.11</b>	<b>0</b>	<b>3</b>	<b>1</b>
NMKL 144:2005 (VRBG)	31	30	3.89	0.22	0	0	1	31	29	3.87	0.24	0	1	1	31	29	4.18	0.11	0	1	1
Petrifilm™ EB (37 °C)*	26	25	3.99	0.15	0	1	0	26	25	3.98	0.12	0	1	0	26	25	4.18	0.10	0	1	0
ISO 21528-2:2017 (VRBG)	17	17	3.93	0.18	0	0	0	17	17	3.91	0.19	0	0	0	17	16	4.16	0.10	0	1	0
TEMPO® EB	8	8	4.20	0.22	0	0	0	8	8	4.19	0.20	0	0	0	8	8	4.21	0.17	0	0	0
Other	6	6	3.81	0.24	0	0	0	6	6	3.76	0.20	0	0	0	6	6	4.13	0.08	0	0	0
Compact Dry™ ETB	3	3	-	-	0	0	0	3	2	-	-	0	1	0	3	3	-	-	0	0	0
Petrifilm™ EB (30 °C)	2	1	-	-	0	1	0	2	2	-	-	0	0	0	1	1	-	-	0	0	0
BIOLOG-LM-278	1	1	-	-	0	0	0	1	0	-	-	0	1	0	1	1	-	-	0	0	0

For individual methods: *m*<sub>PT</sub> = median value and *s*<sub>PT</sub> = standard deviation for the particular method (outliers and false results excluded).

\* For Petrifilm™ EB (37 °C) one of the participants incubated at 35 °C.



**Figure 2.** Results from analysis of Enterobacteriaceae.

# Thermotolerant *Campylobacter*

## Sample A/B

The strain of *C. coli* was target organism. On mCCDA it forms typical grey colonies. The strain is oxidase-positive and catalase-positive. It is also positive for the hydrolysis of indoxyl acetate, negative for the hydrolysis of hippurate, and has a for *Campylobacter* typical appearance under a microscope.

### Sample A

In the quantitative analysis, 16 participants reported results. No statistically significant  $m_{PT}$  and  $s_{PT}$  could be identified. All positive results are therefore considered acceptable. One false negative result was reported.

**Note:** the  $m_{PT}$  and  $s_{PT}$  in Appendix 1 refer to the non-robust median and standard deviation of the 15 positive results, respectively. The uncertainty of the assigned value ( $u_{PT}$ ) in Appendix 1 and the z-scores in Appendix 2 should be treated only as informational.

In the qualitative analysis, 23 participants reported results. All results were correct positive.

### Sample B

In the quantitative analysis, 16 participants reported results. No outliers were identified, and no false results were reported.

**Note:** The uncertainty of the assigned value ( $u_{PT}$ ) is not negligible. The evaluation of the results could therefore be affected, and Z-scores in Appendix 2 should be interpreted with caution. The lower and upper acceptance limits were manually adjusted (see below) which resulted in the inclusion of one high result, initially considered as an outlier.

In the qualitative analysis, 23 participants reported results. All results were correct positive.

## Sample C

The strain of *C. jejuni* was target organism. On mCCDA, it forms typical grey-white colonies. The strain is oxidase-positive and catalase-positive. It is also positive for the hydrolysis of indoxyl acetate and hippurate and has a for *Campylobacter* typical appearance under a microscope.

In the quantitative analysis, 16 participants reported results. One low outlier was reported, as well as one false negative result.

**Note:** The uncertainty of the assigned value ( $u_{PT}$ ) is not negligible. The evaluation of the results could therefore be affected, and Z-scores in Appendix 2 should be interpreted with caution.

In the qualitative analysis, 23 participants reported results. Two false negative results were reported.



## General remarks

*Campylobacter* spp. are gram-negative, oxidase-positive and catalase-positive bacteria. On mCCDA they normally form flat or convex colonies, with a grey/white colour and a glossy surface. Confirmation is often done with an oxidase test or a catalase test, or phenotypically by microscopy. The bacteria normally have a spiral morphology and display characteristic darting or corkscrew-like movements. In addition, *C. jejuni*, *C. coli* and *C. lari* can be separated by differences in their hydrolysis of hippurate and indoxyl acetate, and their sensitivity/resistance to nalidixic acid and cephalothin. Confirmation often also consists of a motility test and/or an oxidase test.

NMKL 119:2007 (qualitative/quantitative), ISO 10272-1:2017 (qualitative) and ISO 10272-2:2017 (quantitative) were the most common methods. Amendments are available for the ISO methods (ISO 10272-1:2017/Amd 1:2023 and ISO 10272-2:2017/Amd 1:2023). They contain alternative confirmation of thermotolerant *Campylobacter* spp. with PCR and changes in the performance testing of the culture media.

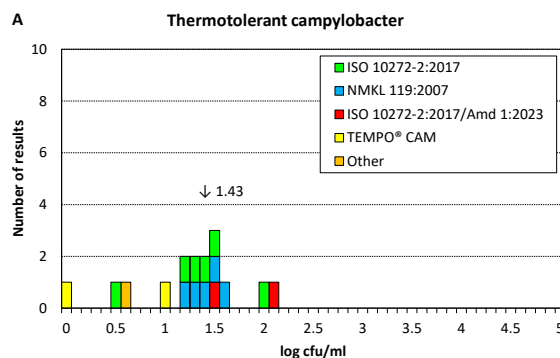
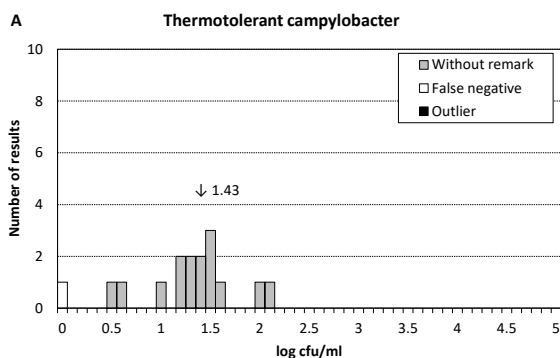
In the qualitative analysis, one participant followed ISO 17995:2019, which is a method for detection of *Campylobacter* in water samples.

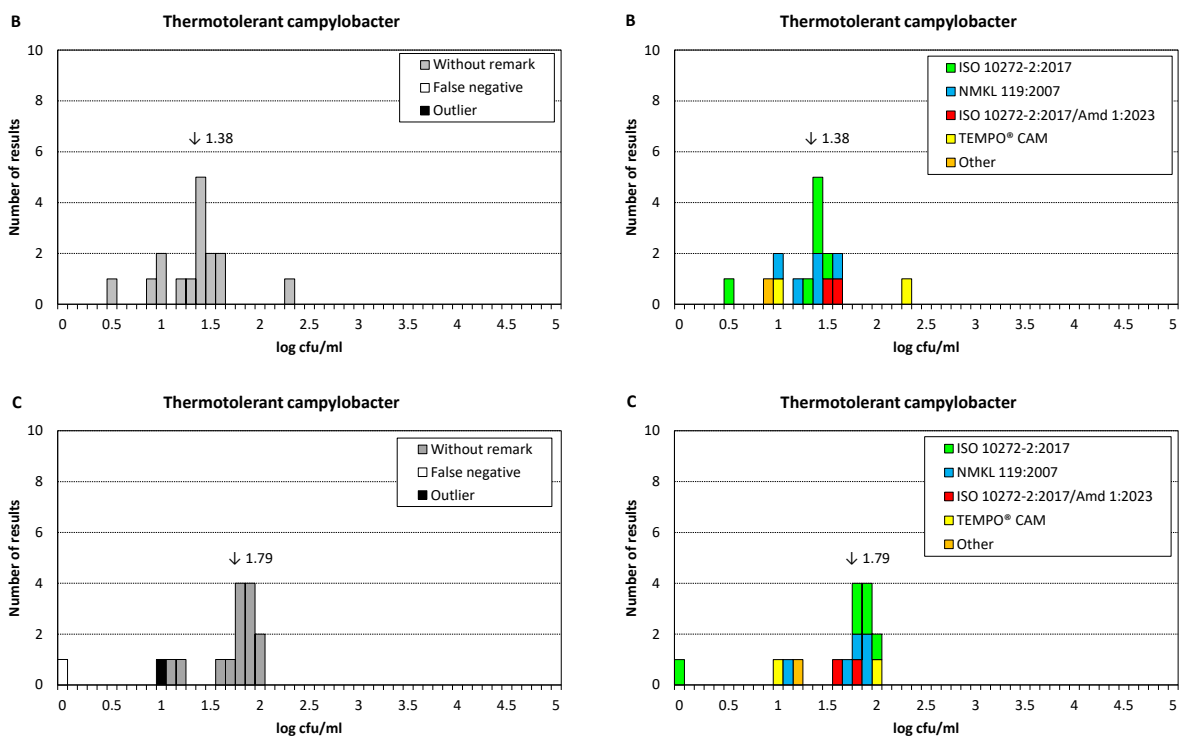
**Note:** The statistical analysis could not identify robust  $m_{PT}$  and  $s_{PT}$  for sample A. The measurement uncertainty ( $u_{PT}$ ) of the assigned value was also large, for both samples A and B. Since the samples were identical, the results were therefore combined into a single dataset and evaluated this way as well. This approach yielded  $m_{PT} = 1.37$  and  $s_{PT} = 0.35$  for the combined dataset, with a negligible uncertainty ( $u_{PT} = 0.08$ ). Based on this, the lower and upper acceptance values were adjusted to 0.31 and 2.42  $\log_{10}$  cfu  $ml^{-1}$ , respectively, for samples A and B.

**Table 4.** Results from quantitative analysis of thermotolerant *Campylobacter*.

Method	Sample A							Sample B							Sample C						
	N	n	$m_{PT}$	$s_{PT}$	F	<	>	N	n	$m_{PT}$	$s_{PT}$	F	<	>	N	n	$m_{PT}$	$s_{PT}$	F	<	>
<b>All results</b>	<b>16</b>	<b>15</b>	<b>1.43</b>	<b>0.41</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>16</b>	<b>16</b>	<b>1.38</b>	<b>0.31</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>16</b>	<b>14</b>	<b>1.79</b>	<b>0.24</b>	<b>1</b>	<b>1</b>	<b>0</b>
ISO 10272-2:2017	6	6	1.41	0.46	0	0	0	6	6	1.49	0.39	0	0	0	6	5	1.92	0.08	1	0	0
NMKL 119:2007	5	5	1.46	0.16	0	0	0	5	5	1.43	0.21	0	0	0	5	5	1.83	0.33	0	0	0
ISO 10272-2:2017/ Amd 1:2023	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
TEMPO® CAM	2	1	-	-	1	0	0	2	2	-	-	0	0	0	2	1	-	-	0	1	0
Other	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0

For individual methods:  $m_{PT}$  = median value and  $s_{PT}$  = standard deviation for the particular method (outliers and false results excluded).





**Figure 3.** Results from quantitative analysis of thermotolerant *Campylobacter*.

**Table 5.** Results from qualitative analysis of thermotolerant *Campylobacter*.

Method	Sample A			Sample B			Sample C		
	<i>N</i>	<i>n</i>	<i>F</i>	<i>N</i>	<i>n</i>	<i>F</i>	<i>N</i>	<i>n</i>	<i>F</i>
<b>All results</b>	<b>23</b>	<b>23</b>	<b>0</b>	<b>23</b>	<b>23</b>	<b>0</b>	<b>23</b>	<b>21</b>	<b>2</b>
NMKL 119:2007	10	10	0	10	10	0	10	8	2
ISO 10272-1:2017/Amd 1:2023	4	4	0	5	5	0	4	4	0
ISO 10272-1:2017	3	3	0	3	3	0	3	3	0
VIDAS® CAM	3	3	0	2	2	0	3	3	0
Other	2	2	0	2	2	0	2	2	0
ISO 17995:2019	1	1	0	1	1	0	1	1	0

# *Listeria monocytogenes*

## Sample A/B

The strain of *L. monocytogenes* was target organism. On ALOA it forms characteristic blue-green colonies, surrounded by a distinct opaque halo. The strain is catalase-positive, displays  $\beta$ -haemolysis on blood agar, and ferments rhamnose but not xylose.

### Sample A

In the quantitative analysis, 56 participants reported results. Three low and two high outliers were identified.

In the qualitative analysis, 99 participants reported results. All results were correct positive.

### Sample B

In the quantitative analysis, 56 participants reported results. Six low and two high outliers were identified.

In the qualitative analysis, 96 participants reported results. Two false negative results were reported.

## Sample C

The strain of *L. monocytogenes* (not identical to the one in samples A/B) was target organism. On ALOA it forms characteristic blue-green colonies, surrounded by a distinct opaque halo. The strain is catalase-positive, displays  $\beta$ -haemolysis on blood agar, and ferments rhamnose but not xylose.

In the quantitative analysis, 56 participants reported results. Three low and one high outlier were reported.

In the qualitative analysis, 100 participants reported results. Three false negative results were reported.

## General remarks

ISO 11290-1:2017 (qual.), ISO 11290-2:2017 (quant.), NMKL 136:2010 and RAPID'L.mono were the most common methods used by the participants. Both ISO methods were last reviewed by ISO in 2022 and remain current.

NMKL 136:2010 describes both detection and enumeration of *L. monocytogenes*. In comparison, ISO 11290-1:2017 and ISO 11290-2:2017 detect/enumerate both *Listeria* spp. and *L. monocytogenes*. All of these methods mainly use ALOA for the isolation, on which *L. monocytogenes* form blue-green colonies due to  $\beta$ -glucosidase activity. The colonies are also surrounded by an opaque halo due to hydrolysis of inositol in the medium. The halo is sometimes weak or may not be present at all.

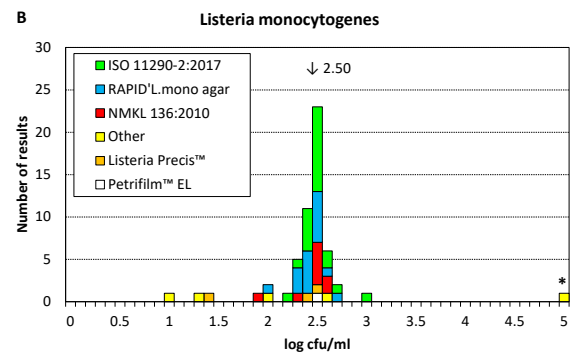
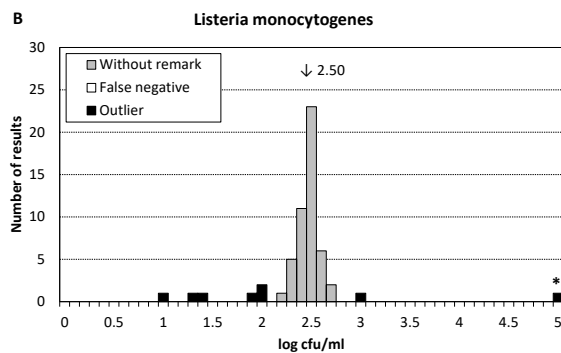
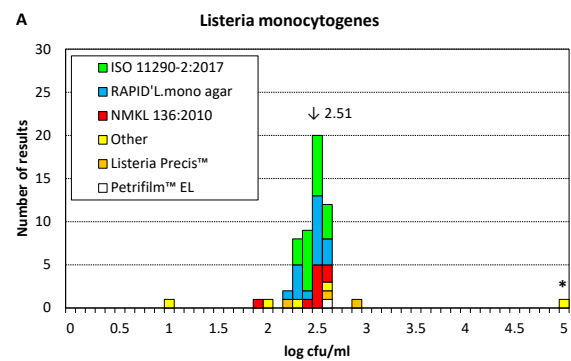
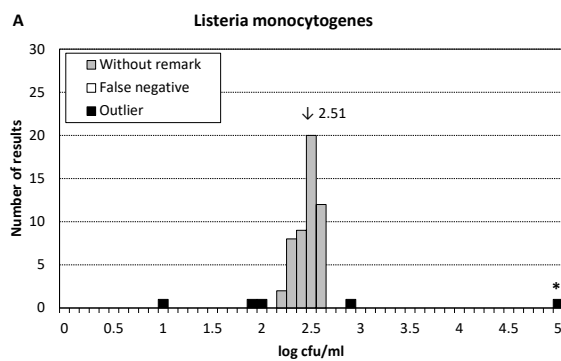
RAPID'L.mono is based on a chromogenic medium that identifies the enzyme PI-PLC in *L. monocytogenes*. It identifies both *Listeria* spp. and *L. monocytogenes* based on their inability to metabolise xylose. Similarly, Listeria Preci<sup>TM</sup> is based on the chromogenic medium. Brilliance<sup>TM</sup> Listeria, on which *Listeria* spp. and *L. monocytogenes* form blue colonies due to their  $\beta$ -glucosidase

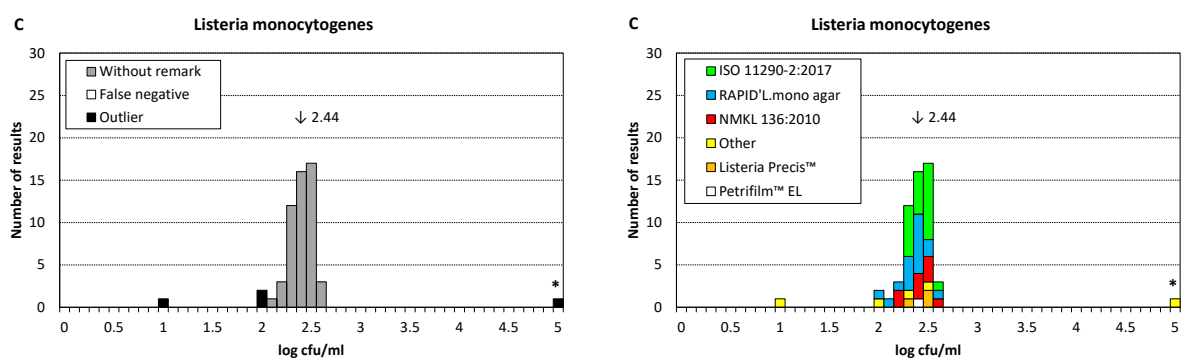
activity. SwabSURE ListeriaP is a test based on swab sampling, for detection of *L. monocytogenes* and *L. ivanovii* in surface samples. In comparison, VIDAS® is based on detection of specific *L. monocytogenes* antigen, in a method based on ELFA (Enzyme Linked Fluorescent Assay). Different variants of the VIDAS® method exist for *Listeria* spp. and/or *L. monocytogenes*. The alternative methods are all validated by AFNOR and/or NordVal.

**Table 6.** Results from quantitative analysis of *Listeria monocytogenes*.

Method	Sample A							Sample B							Sample C						
	N	n	m <sub>PT</sub>	s <sub>PT</sub>	F	<	>	N	n	m <sub>PT</sub>	s <sub>PT</sub>	F	<	>	N	n	m <sub>PT</sub>	s <sub>PT</sub>	F	<	>
<b>All results</b>	<b>56</b>	<b>51</b>	<b>2.51</b>	<b>0.13</b>	<b>0</b>	<b>3</b>	<b>2</b>	<b>56</b>	<b>48</b>	<b>2.50</b>	<b>0.13</b>	<b>0</b>	<b>6</b>	<b>2</b>	<b>56</b>	<b>52</b>	<b>2.44</b>	<b>0.13</b>	<b>0</b>	<b>3</b>	<b>1</b>
ISO 11290-2:2017	21	21	2.51	0.10	0	0	0	21	20	2.54	0.10	0	0	1	21	21	2.48	0.10	0	0	0
RAPID'L.mono agar	17	17	2.56	0.12	0	0	0	17	16	2.50	0.10	0	1	0	17	16	2.46	0.13	0	1	0
NMKL 136:2010	9	8	2.53	0.07	0	1	0	9	8	2.56	0.08	0	1	0	9	9	2.49	0.13	0	0	0
Other	5	2	-	-	0	2	1	5	1	-	-	0	3	1	5	2	-	-	0	2	1
Listeria Precis™	3	2	-	-	0	0	1	3	2	-	-	0	1	0	3	3	-	-	0	0	0
Petrifilm™ EL	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0

For individual methods: m<sub>PT</sub> = median value and s<sub>PT</sub> = standard deviation for the particular method (outliers and false results excluded).





**Figure 4.** Results from quantitative analysis of *Listeria monocytogenes*.

**Table 7.** Results from qualitative analysis of *Listeria monocytogenes*.

Method	Sample A			Sample B			Sample C		
	<i>N</i>	<i>n</i>	<i>F</i>	<i>N</i>	<i>n</i>	<i>F</i>	<i>N</i>	<i>n</i>	<i>F</i>
<b>All results</b>	<b>99</b>	<b>99</b>	<b>0</b>	<b>96</b>	<b>94</b>	<b>2</b>	<b>100</b>	<b>97</b>	<b>3</b>
ISO 11290-1:2017	21	21	0	22	22	0	21	21	0
Other	19	19	0	18	17	1	20	18	2
RAPID'L.mono agar	18	18	0	18	17	1	18	17	1
VIDAS® LMX	13	13	0	12	12	0	13	13	0
NMKL 136:2010	5	5	0	5	5	0	5	5	0
VIDAS® LMO	4	4	0	4	4	0	4	4	0
Listeria Precis™	4	4	0	4	4	0	4	4	0
iQ-Check L. monocytogenes II (PCR)	4	4	0	4	4	0	4	4	0
VIDAS® LIS	4	4	0	3	3	0	4	4	0
SwabSURE ListeriaP	3	3	0	3	3	0	3	3	0
BAX® Q7 (PCR)	2	2	0	2	2	0	2	2	0
VIDAS® LDUO	1	1	0	1	1	0	1	1	0
SureTect™ L. monocytogenes	1	1	0	0	0	0	1	1	0

# Salmonella

## Sample A/B

No target organism was present in the sample. *C. freundii* is capable of forming atypical white colonies on XLD and Brilliance™ Salmonella.

### Sample A

In total, 127 participants reported results. Two false positive results were reported.

### Sample B

In total, 125 participants reported results. One false positive result was reported.

## Sample C

The strain of *Salmonella* Stockholm was target organism. On XLD, it forms typical red colonies with a black centre. On Brilliance™ Salmonella, it forms typical purple colonies. The strain is positive for agglutination against both O and H antigen.

In total, 125 participants reported results. One false negative result was reported.

## General remarks

Most notably, the most commonly reported method was “Other”, which was often not specified further by the participants. Disregarding this, the most common methods were NMKL 71:1999, ISO 6579-1:2017 and VIDAS® UP Salmonella (SPT).

NMKL 71:1999 and ISO 6579-1:2017 are very similar. Both are based on pre-incubation in BPW, followed by selective enrichment in RVS. ISO 6579-1:2017 also includes selective enrichment in MKTTn. With the ISO method, RVS can also be substituted with semi-solid MSRV for the analysis of motile *Salmonella*. With both methods, incubation is mainly on XLD, and confirmation is by biochemical (e.g. mannitol and urea) and serological (e.g. *Salmonella* polyvalent O and H antisera) tests. ISO 6579-1:2017 was last reviewed by ISO in 2022 and remains current. The amendment ISO 6579-1:2017/Amd 1:2020 includes important changes, including wider intervals for incubation temperatures and corrections to the composition of some of the media.

VIDAS® UP Salmonella (SPT) is validated by AFNOR and AOAC against the reference ISO 6579-1:2017/Amd 1:2020. It uses an enzyme-linked fluorescent assay (ELFA) for the initial detection of presumptive *Salmonella*. Confirmation is done by isolation on ChromID™ Salmonella, followed by API® 20 E, ID 32 E or VITEK 2 GN, and biochemical and serological confirmation.

Users of NMKL methods can in addition to NMKL 71:1999 also choose to follow NMKL 187:2016. The latter method is intended for detection of motile *Salmonella* and, similarly to ISO 6579-1:2017, uses MSRV instead of RVS during the selective enrichment step.

**Table 8.** Results from analysis of Salmonella.

Method	Sample A			Sample B			Sample C		
	<i>N</i>	<i>n</i>	<i>F</i>	<i>N</i>	<i>n</i>	<i>F</i>	<i>N</i>	<i>n</i>	<i>F</i>
<b>All results</b>	<b>127</b>	<b>125</b>	<b>2</b>	<b>125</b>	<b>124</b>	<b>1</b>	<b>125</b>	<b>124</b>	<b>1</b>
Other	30	30	0	28	28	0	27	27	0
NMKL 71:1999	22	22	0	22	22	0	23	23	0
ISO 6579-1:2017/Amd 1:2020	21	21	0	21	20	1	22	22	0
VIDAS® UP Salmonella (SPT)	18	18	0	18	18	0	18	18	0
ISO 6579-1:2017	9	8	1	9	9	0	8	7	1
iQ-Check Salmonella II (PCR)	7	7	0	7	7	0	7	7	0
RAPID'Salmonella	7	6	1	7	7	0	7	7	0
NMKL 187:2016 (MSRV)	3	3	0	3	3	0	3	3	0
BAX® Q7 (PCR)	3	3	0	3	3	0	3	3	0
MTP-06	2	2	0	2	2	0	2	2	0
SureTect™ Salmonella	2	2	0	2	2	0	2	2	0
ISO/TR 6579-3:2014	1	1	0	1	1	0	1	1	0
Salmonella Velox (qPCR)	1	1	0	1	1	0	1	1	0
BIOLOG-LM-469	1	1	0	1	1	0	1	1	0

# *Escherichia coli* O157

## Sample A/B

No target organism was present in the sample.

### **Sample A**

In total, 22 participants reported results. All results were correct negative.

### **Sample B**

In total, 22 participants reported results. All results were correct negative.

## Sample C

The strain of *E. coli* O157 was target organism. On CT-SMAC, it forms typical sorbitol-negative transparent colonies with a dark centre. The strain is positive for production of indole and for agglutination with *E. coli* O157 antiserum. It contains the gene *eae*, but no *stx* genes.

In total, 22 participants reported results. All results were correct positive.

**Note:** For one participant it was ambiguous if they analysed only for *E. coli* or specifically for *E. coli* O157. Since this is of no practical consequence (only *E. coli* O157 is present in the sample) this result was still included in the evaluation.

## General remarks

Like the analysis of *Salmonella*, most of the reported methods fall into the group “Other”. Still, all reported results were assessed as correct.

The methods that were specified were NMKL 164:2019, VIDAS® UP *E. coli* O157 (ECPT) and ISO 16654:2001/Amd 1:2017.

NMKL 164:2019 and ISO 16654:2001/Amd 1:2017 are similar methods; enrichment is done in mTSB with novobiocin and is followed by immunomagnetic separation and isolation on CT-SMAC and another medium selected by the laboratory. Confirmation is by a test for indole production as well as agglutination with *E. coli* O157 antiserum. With NMKL 164:2019, the virulence profile of presumptive *E. coli* O157 (*eae* and *stx* genes) is also determined. ISO 16654:2001 was last reviewed by ISO in 2024 and remains current. It has two published amendments; Amd 1:2017 (results of interlaboratory studies) and Amd 2:2023 (performance testing of culture media and reagents).

VIDAS® ECPT is based on detection of *E. coli* O157-specific receptors using an enzyme-linked fluorescent assay (ELFA). Confirmation is done by immunoconcentration followed by isolation on ChromID™ O157:H7 agar or CT-SMAC. It is validated against ISO 16654:2001/Amd 1:2017 by AFNOR and AOAC.



**Table 9.** Results from analysis of *Escherichia coli* O157.

Method	Sample A			Sample B			Sample C		
	<i>N</i>	<i>n</i>	<i>F</i>	<i>N</i>	<i>n</i>	<i>F</i>	<i>N</i>	<i>n</i>	<i>F</i>
<b>All results</b>	<b>22</b>	<b>22</b>	<b>0</b>	<b>22</b>	<b>22</b>	<b>0</b>	<b>22</b>	<b>22</b>	<b>0</b>
Other	15	15	0	16	16	0	16	16	0
VIDAS® UP E. coli O157 (ECPT)	3	3	0	3	3	0	3	3	0
NMKL 164:2005	3	3	0	2	2	0	2	2	0
ISO 16654:2001/Amd 1:2017	1	1	0	1	1	0	1	1	0

# Pathogenic *Vibrio* spp.

## Sample A/B

The strain of *V. parahaemolyticus* was target organism. It is oxidase-positive and forms yellow/green colonies on TCBS. At the Swedish Food Agency, colonies were more frequent on TCBS after enrichment in alkaline peptone water (APW 2 %) compared to salt polymyxin broth (SP). The strain is sensitive to vibriostatic agent O129, though at the Swedish Food Agency the results have sometimes also been negative.

### Sample A

In total, 16 participants reported results. Fifteen results were correct positive. One negative result was reported but was not evaluated due to the occasional negative results with vibriostatic agent O129 at the Swedish Food Agency.

### Sample B

In total, 16 participants reported results. Fifteen results were correct positive. One negative result was reported but was not evaluated due to the occasional negative results with vibriostatic agent O129 at the Swedish Food Agency.

## Sample C

No target organism was present in the sample, but *E. coli* may form small green colonies on TCBS.

In total, 17 participants reported results. All results were correct negative.

## General remarks

Most participants followed either ISO 21872-1:2017 or NMKL 156:1997. ISO 21872-1:2017 was last reviewed by ISO in 2023 and remains current. Though it follows the same main principle as the 2007 version, it contains several changes, including how to perform confirmation with biochemical and/or PCR methods. Primary and secondary enrichment in APW 2 % is followed by inoculation onto TCBS. The procedure in NMKL 156:1997 is similar to ISO 21872-1:2017, but also includes enrichment in SP. In addition, the NMKL method only utilizes biochemical confirmation tests.

Bile salts in TCBS inhibit the growth of Gram-positive microorganisms, whereas a high pH promotes the growth of *V. cholerae*. On TCBS, *Vibrio* spp. form either green or yellow colonies, depending on if they ferment sucrose or not. *V. parahaemolyticus* and *V. vulnificus* (sucrose-negative) normally form blue-green colonies, whereas *V. cholerae* (sucrose-positive) normally form yellow colonies.

The two negative results for sample A/B were reported by two separate participants, using two different methods; NMKL 156:1997 for sample A and ISO 21872-1:2017 for sample B. In effect, these two participants thus reported both positive and negative results for the identical samples. The affected participants are encouraged to evaluate this discrepancy; i.e. if the negative results are a consequence of

the properties of the specific strain in sample A/B, the general performance of the analysis as the laboratory, or simple typing errors during the reporting.

**Table 10.** Results from analysis of pathogenic *Vibrio* spp.

Method	Sample A			Sample B			Sample C		
	<i>N</i>	<i>n</i>	<i>F</i>	<i>N</i>	<i>n</i>	<i>F</i>	<i>N</i>	<i>n</i>	<i>F</i>
<b>All results</b>	<b>16</b>	<b>16</b>	<b>0</b>	<b>16</b>	<b>16</b>	<b>0</b>	<b>17</b>	<b>17</b>	<b>0</b>
ISO 21872-1:2017	8	8	0	7	7	0	8	8	0
NMKL 156:1997	6	6	0	7	7	0	7	7	0
Other	2	2	0	2	2	0	2	2	0

# *Yersinia enterocolitica*

## Sample A/B

The strain of *Y. enterocolitica* was target organism. On CIN, it forms typical colonies with a red/pink centre, and an outer transparent zone. On BS, it forms typical yellow colonies. The strain is oxidase-negative, and displays agglutination against O:3 antiserum, but not against O:9 antiserum. The strain contains the gene *ail*.

### Sample A

In total, 10 participants reported results. One false negative result was reported.

### Sample B

In total, 10 participants reported results. One false negative result was reported.

## Sample C

No target organism was present in the sample.

In total, 10 participants reported results. One false positive result was reported.

## General remarks

The participants followed either ISO 10273:2017, NMKL 117:1996 or ISO/TS 18867:2015.

ISO 10273:2017 is based on direct incubation on CIN, as well as parallel incubation in PSB and ITC, followed by isolation on CIN. Suspected colonies are further analysed by traditional biochemical methods or by detection of the chromosomal virulence-associated gene *ail* by real-time PCR. The method was last reviewed by ISO in 2022 and remains current.

The three participants that followed NMKL 117:1996 should be aware that a revised version of this was published in 2022; NMKL 117:2022. The new method contains many changes compared to the previous version and is aimed specifically at the detection of pathogenic bioserotypes of *Y. enterocolitica*. Overall, the method follows a similar principle as ISO 10273:2017.

ISO/TS 18867:2015 is a PCR-based method for the detection of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis*. It was last reviewed by ISO in 2022 and remains current.

**Table 11.** Results from analysis of *Yersinia enterocolitica*.

Method	Sample A			Sample B			Sample C		
	N	n	F	N	n	F	N	n	F
<b>All results</b>	<b>10</b>	<b>9</b>	<b>1</b>	<b>10</b>	<b>9</b>	<b>1</b>	<b>10</b>	<b>9</b>	<b>1</b>
ISO 10273:2017	3	2	1	3	2	1	3	3	0
NMKL 117:1996	3	3	0	3	3	0	3	3	0
ISO/TS 18867:2015	2	2	0	2	2	0	2	1	1
Other	2	2	0	2	2	0	2	2	0

# Outcome of the results of individual participants - assessment

## Reporting and evaluation of results

The results of all participants are listed in Appendix 1, together with the minimum and maximum accepted values for each analytical parameter. Outliers and false results are highlighted in yellow and red, respectively, with bold font.

Participants are not grouped or ranked based on their results. The performance of an individual participant can be broadly assessed by the numbers of outliers and false results, and by the  $z$ -scores.

Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol [2].

Samples for follow-up analyses can be ordered at: <https://laboratory.livsmedelsverket.se>

## Box plots and numbers of deviating results for each participant

Box plots are based on the  $z$ -scores listed in Appendix 2 and give a comprehensive view of the performance of each participant. The range of  $z$ -scores is indicated by the size of the box and, for most participants, by lines and/or circles above and beneath the box. A small range of values, centred around zero, indicates that the results of the individual participant are in general close to  $m_{PT}$  for the different analyses. For each participant, the number of false results and outliers are also listed in the tables below the box plots.

The different parts of a box plot are shown in figure 5.

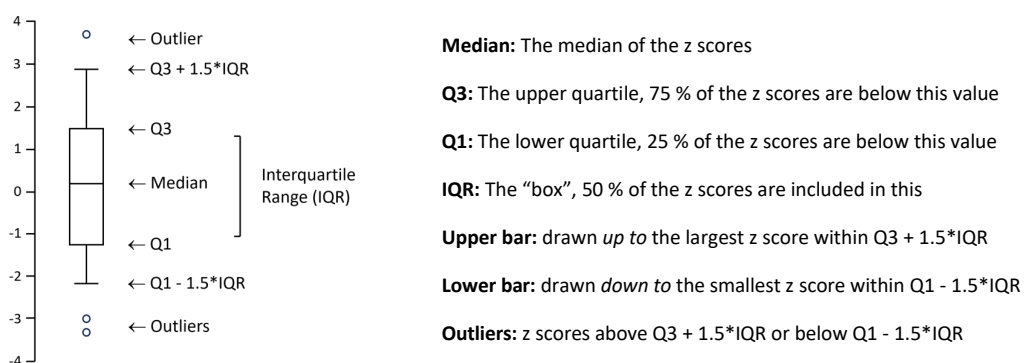
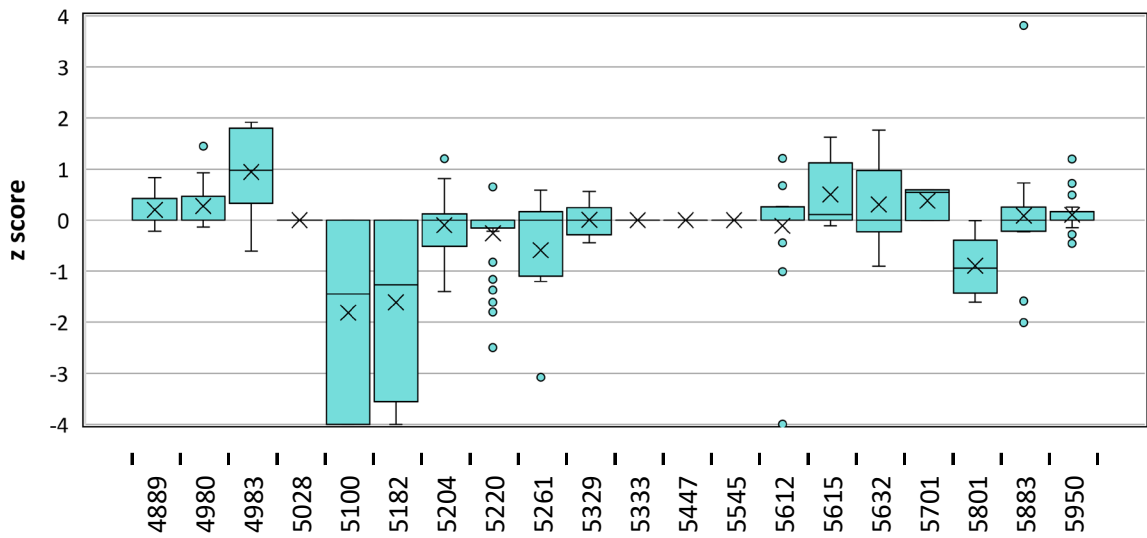
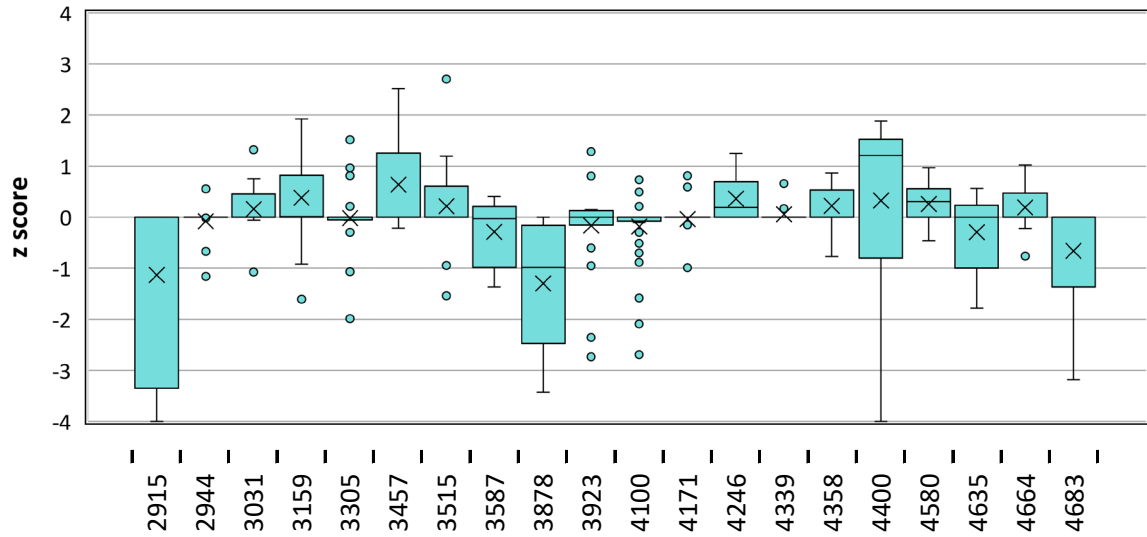
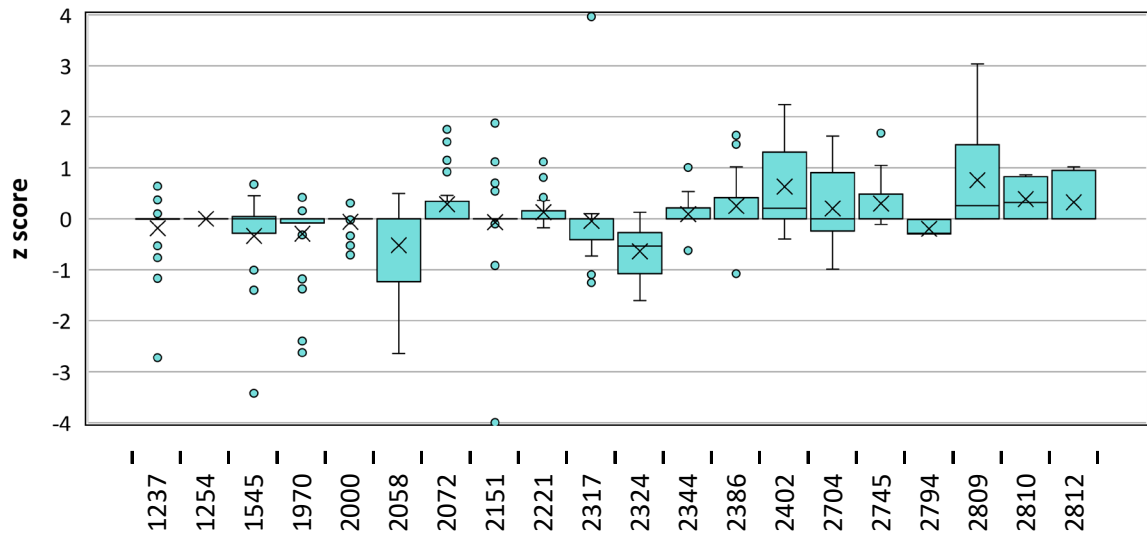
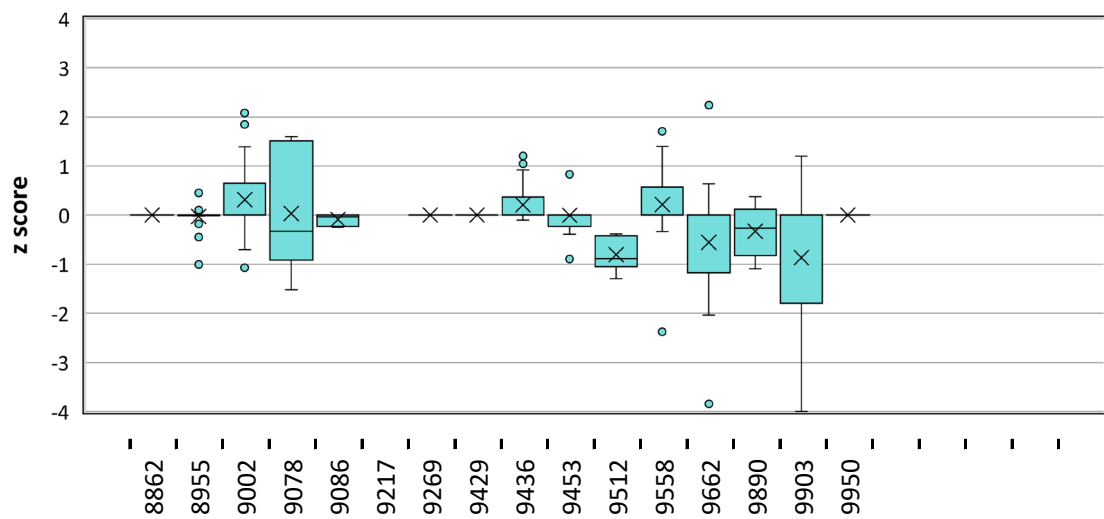
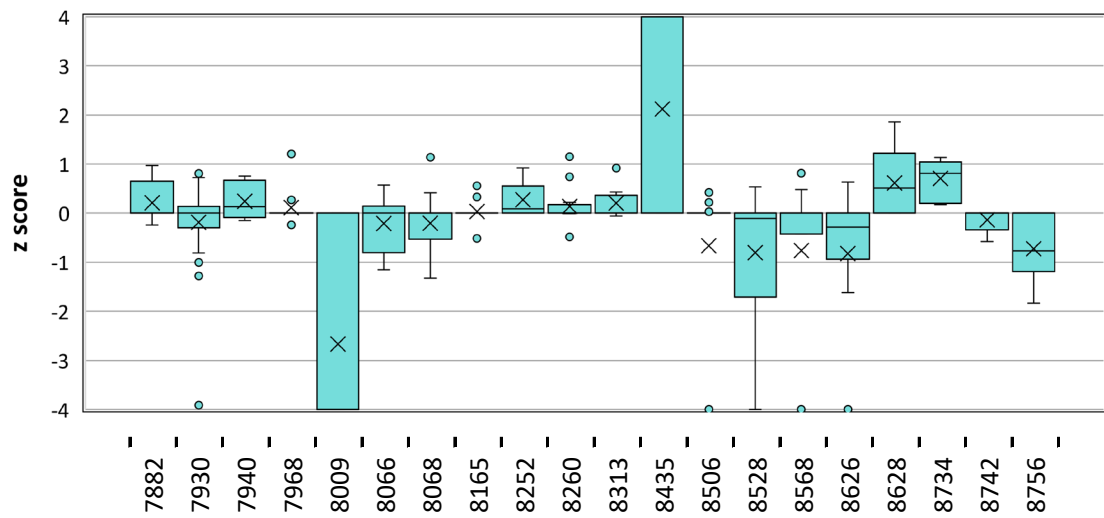
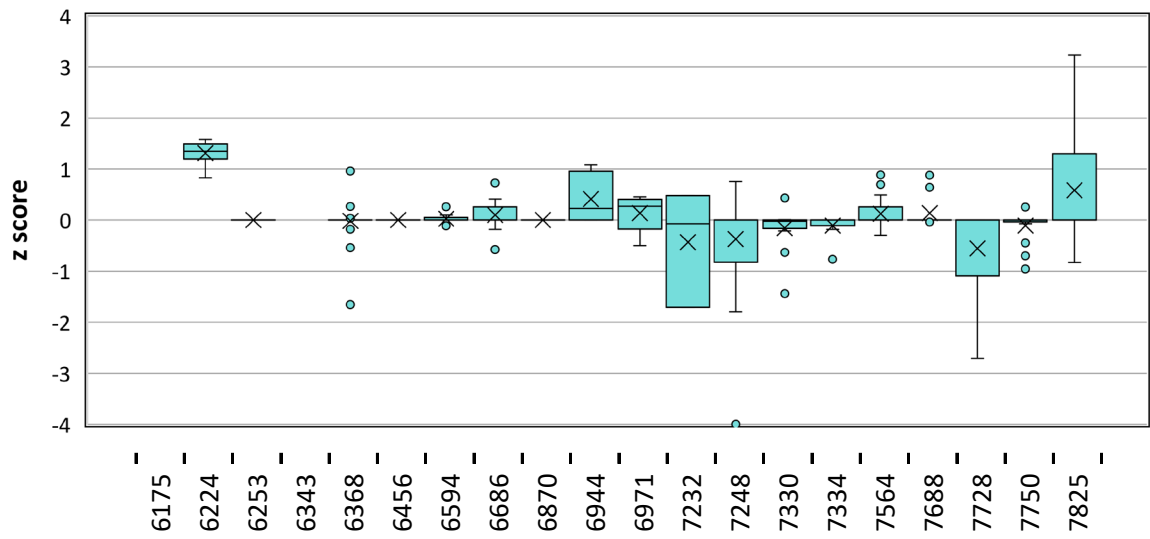


Figure 5. Schematic explanation of a box plot.





# Test material and quality control

## Test material

Each participant received three samples with freeze-dried microorganisms, designated A–C. The test material was freeze-dried in 0.5 ml portions in glass vials, as described by Peterz and Steneryd [3]. Before analysing the samples, the contents of each vial should be reconstituted in 254 ml of sterile diluent. The microorganism content of the samples and the concentrations determined at the Swedish Food Agency are listed in table 12.

**Table 12.** Microorganisms and approximate concentrations in the samples.

Sample	Microorganism	Strain			
		SLV no. <sup>1</sup>	Isolated from	Reference <sup>2</sup>	log <sub>10</sub> cfu ml <sup>-1</sup>
A/B	<i>Campylobacter coli</i>	SLV-271	Hen, faeces	CCUG 45147	1.9
	<i>Citrobacter freundii</i>	SLV-091	-	CCUG 43597	4.0
	<i>Listeria monocytogenes</i>	SLV-513	Milk	CCUG 44510	2.6
	<i>Vibrio parahaemolyticus</i>	SLV-125	-	-	2.5
	<i>Yersinia enterocolitica</i>	SLV-408	Frozen dog food	CCUG 45643	3.2
C	<i>Campylobacter jejuni</i>	SLV-540	Chicken	-	2.2
	<i>Escherichia coli</i>	SLV-477	Cheese	CCUG 43601	4.2
	<i>Escherichia coli</i> O157	SLV-479	Ear infection	-	1.6
	<i>Listeria monocytogenes</i>	SLV-361	Smoked salmon	-	2.5
	<i>Salmonella</i> Stockholm	SLV-390	Chocolate powder	-	1.8

<sup>1</sup> Internal strain identification no. at the Swedish Food Agency.

<sup>2</sup> Culture collection. ATCC: American Type Culture Collection, CBS: Centraalbureau voor Schimmelcultures (Westerdijk Institute), CCUG: Culture Collection University of Gothenburg, Sweden; SMI: Public Health Agency of Sweden.



## Quality control of the samples

Quality control and evaluation of sample homogeneity is performed on 10 randomly chosen vials in conjunction with manufacture, or on 5 vials if an “old” batch of samples is used. Homogeneity of a test material is approved if, for each analysis, the  $p$  value of a one-way analysis of variance (ANOVA) fulfils the criterion  $p \geq 0.05$ . If the ANOVA yields  $p < 0.05$ , the PT test item batch is still considered homogenous, if  $s_{bb} < s_R/3$ , where:

$s_{bb}$ : the between-vial standard deviation from the ANOVA

$s_R$ : the expected laboratory variation, generally assumed to be 0.25 for the Food scheme.

See the Scheme protocol [2] for more information regarding the evaluation of homogeneity.

**Table 13.** Concentration mean ( $m$ ), between-vial variation ( $s_{bb}$ ) and  $p$  values from the quality control of the samples;  $m$  is expressed in  $\log_{10}$  cfu (colony forming units) per ml of sample.

Analysis and method	A <sup>1</sup>			B <sup>1</sup>			C <sup>2</sup>		
	$m$	$s_{bb}$	$p$	$m$	$s_{bb}$	$p$	$m$	$s_{bb}$	$p$
Aerobic microorganisms 30 °C NMKL method no. 86:2013	4.17	0.01	0.48	4.17	0.01	0.48	4.26	0.06	<b>0.02</b>
Enterobacteriaceae NMKL method no. 144:2005	3.70	0.03	0.17	3.70	0.03	0.17	4.20	0.00	0.77
Thermotolerant campylobacter NMKL method no. 119:2007	1.85	0.04	0.17	1.85	0.04	0.17	2.20	0.02	0.47
<i>Listeria monocytogenes</i> NMKL method no. 136:2010	2.60	0.03	0.30	2.60	0.03	0.30	2.49	0.00	0.89
<i>Salmonella</i> NMKL method no. 71:1999	-	-	-	-	-	-	1.83	0.00	0.86
<i>Escherichia coli</i> O157 NMKL method no. 164:2019	-	-	-	-	-	-	1.56	0.00	0.91
Pathogenic <i>Vibrio</i> spp. NMKL method no. 156:1997	2.48	0.03	0.06	2.48	0.03	0.06	-	-	-
<i>Yersinia enterocolitica</i> NMKL method no. 117:1996	3.25	0.00	0.94	3.25	0.00	0.94	-	-	-

– No target organism or no value

<sup>1</sup>  $n = 10$  vials analysed in duplicate

<sup>2</sup>  $n = 5$  vials analysed in duplicate

# References

1. ISO 13528:2022 Statistical methods for use in proficiency testing by interlaboratory comparison.
2. Ilbäck J and Blom L. 2024. Protocol – Microbiological Proficiency Testing, Swedish Food Agency.
3. Peterz M and Steneryd AC. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *Journal of Applied Bacteriology*. 74:143–148.















## Appendix 1. Results of the participating laboratories

Lab no.	Aerobic microorganisms, 30 °C			Enterobacteriaceae			Thermotolerant campylobacter			Listeria monocytogenes			Thermotolerant campylobacter			Listeria monocytogenes			Salmonella			Escherichia coli O157			Pathogenic Vibrio spp.			Yersinia enterocolitica		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
<b>N</b>	110	109	109	94	94	93	16	16	16	56	56	56	23	23	23	99	96	100	127	125	125	22	22	22	16	16	17	10	10	10
<b>n</b>	105	102	104	91	89	89	15	16	14	51	48	52	23	23	21	99	94	97	125	124	124	22	22	22	16	16	17	9	9	9
<b>Min</b>	2.55	1.64	2.34	2.07	2.12	2.13	0	0.54	0	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<b>Max</b>	4.48	4.49	4.56	4.34	4.32	4.43	2.11	2.32	2.04	2.99	3.00	2.63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<b>Med</b>	4.14	4.11	4.22	3.93	3.95	4.18	1.43	1.47	1.87	2.53	2.53	2.46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<b>m<sub>PT</sub></b>	<b>4.13</b>	<b>4.11</b>	<b>4.21</b>	<b>3.90</b>	<b>3.90</b>	<b>4.16</b>	<b>1.43</b>	<b>1.38</b>	<b>1.79</b>	<b>2.51</b>	<b>2.50</b>	<b>2.44</b>	<b>Pos</b>	<b>Pos</b>	<b>Pos</b>	<b>Pos</b>	<b>Pos</b>	<b>Pos</b>	<b>Neg</b>	<b>Neg</b>	<b>Pos</b>	<b>Neg</b>	<b>Neg</b>	<b>Pos</b>	<b>Pos</b>	<b>Pos</b>	<b>Neg</b>	<b>Pos</b>	<b>Pos</b>	<b>Neg</b>
<b>s<sub>PT</sub></b>	<b>0.18</b>	<b>0.18</b>	<b>0.11</b>	<b>0.23</b>	<b>0.22</b>	<b>0.11</b>	<b>0.41</b>	<b>0.31</b>	<b>0.24</b>	<b>0.13</b>	<b>0.13</b>	<b>0.13</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<b>u<sub>PT</sub></b>	0.022	0.022	0.013	0.029	0.028	0.014	0.133	0.097	0.076	0.021	0.021	0.022	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>F+</b>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	1
<b>F-</b>	0	0	0	0	0	0	1	0	1	0	0	0	0	0	2	0	2	3	0	0	1	0	0	0	0	0	0	1	1	0
<b>&lt;</b>	4	6	3	2	4	3	0	0	1	3	6	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>&gt;</b>	1	1	2	1	1	1	0	0	0	2	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Lower</b>	3.58	3.57	3.89	3.23	3.24	3.83	0.31	0.31	1.08	2.13	2.12	2.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Upper</b>	4.68	4.66	4.53	4.58	4.56	4.48	2.42	2.42	2.50	2.89	2.88	2.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

N = number of reported results  
n = results without annotation

Min = lowest reported result  
Max = highest reported result

Med = median value  
m<sub>PT</sub> = assigned value

s<sub>PT</sub> = standard deviation  
u<sub>PT</sub> = measurement uncertainty

F+ = false positive  
F- = false negative

< = low outlier  
> = high outlier

Lower = lowest accepted value  
Upper = highest accepted value

- False positive or false negative
- Outside the acceptance limits
- Results "larger than" are not evaluated
- The parameter is not evaluated
- The result is not evaluated
- u<sub>PT</sub> > 0,3 s<sub>PT</sub> and/or > 20% outliers and/or fewer than 12 evaluated results













## Appendix 2. Z-scores of all participants

Lab no.	Aerobic microorganisms, 30 °C			Enterobacteriaceae			Thermotolerant campylobacter			Listeria monocytogenes			Thermotolerant campylobacter			Listeria monocytogenes			Salmonella			Escherichia coli O157			Pathogenic Vibrio spp.			Yersinia enterocolitica		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C			

- $|z| \geq 3,0$  ("Unacceptable" or "Action")
- $2,0 < |z| < 3,0$  ("Warning")
- The parameter is not evaluated
- The result is not evaluated





## Internal and external control for microbiological analyses of food and drinking water

All analytical activities require work of a high standard that is accurately documented. For this purpose, most participants carry out some form of internal quality assurance, but the analytical work also needs to be evaluated by an independent party. Such external quality control of laboratory competence is commonly required by accreditation bodies and can be done by taking part in proficiency testing (PT).

In a PT, identical test material is analysed by a number of participants. After reporting of results by the participants, the organiser evaluates the results and compiles them in a report.

### The Swedish Food Agency's PT program offers

- External and independent evaluation of participants' analytical competence.
- Improved knowledge of analytical methods with respect to various types of organisms.
- Expert support.
- Tool for inspections regarding accreditation.

For more information, visit our website: [www.livsmedelsverket.se/en/PT-micro](http://www.livsmedelsverket.se/en/PT-micro)

### The Swedish Food Agency's reference material

As a complement to the proficiency testing, but without specific accreditation, the Swedish Food Agency also manufactures a number of reference materials (RM) for internal quality control of food and drinking water microbiological analyses, including pathogens.

For more information, visit our website: [www.livsmedelsverket.se/en/RM-micro](http://www.livsmedelsverket.se/en/RM-micro)