

# Proficiency testing Food Microbiology

January 2024

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*Edition*

Final report, Version 2 (2024-03-20). This version replaces version 1 (2024-03-18) with the following changes:

- In Table 3, “TEMPO® EC” has been corrected to “TEMPO® EB”.

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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
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
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*

*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 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 142 participants: 29 in Sweden, 96 in Europe, and 17 outside of Europe. In total, 134 participants (94 %) reported results, of which 51 (38 %) provided at least one result that received a remark.

Individual results are listed in Appendix 1 and on the website: <https://www2.slv.se/absint>. 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>Escherichia coli</i> O157 <i>Listeria monocytogenes</i>				<i>Escherichia coli</i> <i>Salmonella</i> Stockholm <i>Staphylococcus aureus</i> <i>Vibrio cholerae</i> <i>Yersinia enterocolitica</i>				<i>Campylobacter jejuni</i> <i>Escherichia coli</i> <i>Escherichia coli</i> O157 <i>Listeria monocytogenes</i> <i>Salmonella</i> Stockholm			
<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> <i>L. monocyt.</i>	114	0	8	<i>S. aureus</i> <i>E. coli</i>	114	0	9	<i>E. coli</i>	114	0	11
Enterobacteriaceae	<i>C. freundii</i> <i>E. coli</i> O157	98	3	5	<i>E. coli</i>	98	0	7	<i>E. coli</i>	99	0	8
Thermotol. <i>Campylobacter</i> - Quantitative	<i>C. coli</i>	19	1	0	-	18	0	0	<i>C. jejuni</i>	19	0	0
<i>Listeria monocytogenes</i> - Quantitative	<i>L. monocyt.</i>	61	0	8	-	60	1	0	<i>L. monocyt.</i>	61	0	5
Thermotol. <i>Campylobacter</i> - Qualitative	<i>C. coli</i>	25	2	0	-	24	0	0	<i>C. jejuni</i>	24	3	0
<i>Listeria monocytogenes</i> - Qualitative	<i>L. monocyt.</i>	93	2	0	-	93	1	0	<i>L. monocyt.</i>	93	0	0
<i>Salmonella</i>	-	104	2	0	<i>S. Stockholm</i>	105	0	0	<i>S. Stockholm</i>	105	1	0
<i>Escherichia coli</i> O157	<i>E. coli</i> O157	22	2	0	( <i>E. coli</i> )	22	4	0	<i>E. coli</i> O157	21	4	0
Pathogenic <i>Vibrio</i> spp.	-	21	0	0	<i>V. cholerae</i>	21	1	0	-	21	1	0
<i>Yersinia enterocolitica</i>	-	12	1	0	<i>Y. entero-colitica</i>	13	2	0	-	12	2	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

*C. freundii* was present in a much higher concentration than the other microorganisms, and was thus the main target organism.

In total 114 participants reported results. Three low and five high outliers were reported.

## Sample B

*E. coli* and *S. aureus* were present in considerably higher concentrations than the other strains, and were thus the main target organisms.

In total 114 participants reported results. Five low and four high outliers were reported.

## Sample C

*E. coli* was present in considerably higher concentrations than the other strains, and was thus the main target organism.

In total 114 participants reported results. Six low and five high outliers were reported.

## General remarks

Most participants followed either NMKL 86:2013, ISO 4833-1:2013 or used 3M Petrifilm AC. The withdrawn NMKL 86:2006 and ISO 4833:2003 were still used by six and four participants, respectively.

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<sup>®</sup> 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. 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.

The majority of the participants incubated on PCA, but Petrifilm AC was also common. Incubation on MPCA was mainly done by laboratories within the dairy industry. Incubation on TSA was mainly done by users of a company-specific method.

For sample A, the results for Petrifilm AC were somewhat higher compared to other media. This has been seen previously for Petrifilm AC, and can be considered normal.

A few participants used TEMPO AC, which is based on MPN (Most Probable Number). With this method, the sample is incubated in a card that contains different-sized wells. A substrate in the medium emits fluorescence when hydrolysed by the microorganisms. The number of microorganisms is determined statistically by the number and size of the fluorescing wells. For sample A, the mean value

from participants that used TEMPO AC was somewhat higher compared to participants that used other methods. Differences of this magnitude are not uncommon for TEMPO methods, and can be considered normal.

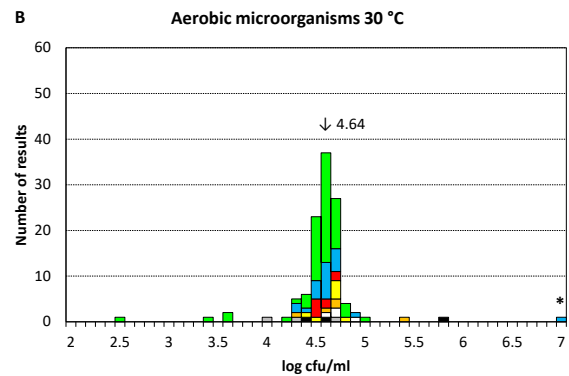
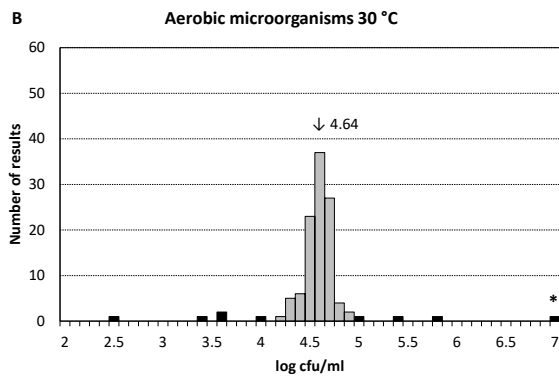
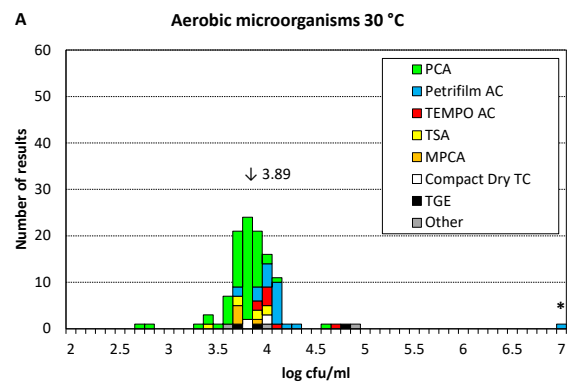
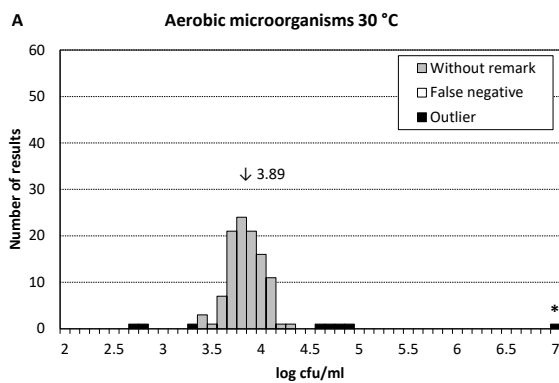
One participant followed ISO 13559/IDF 153, which is adapted for the enumeration of contaminating microorganisms in butter, fermented milks and fresh cheese. Since the participant incubated on PCA, the results have still been included in the evaluation.

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

Medium	Sample A							Sample B							Sample C						
	<i>N</i>	<i>n</i>	<i>m</i> <sub>PT</sub>	<i>s</i> <sub>PT</sub>	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> <sub>PT</sub>	<i>s</i> <sub>PT</sub>	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> <sub>PT</sub>	<i>s</i> <sub>PT</sub>	<i>F</i>	<	>
<b>All results</b>	<b>114</b>	<b>106</b>	<b>3.89</b>	<b>0.18</b>	<b>0</b>	<b>3</b>	<b>5</b>	<b>114</b>	<b>105</b>	<b>4.64</b>	<b>0.12</b>	<b>0</b>	<b>5</b>	<b>4</b>	<b>114</b>	<b>103</b>	<b>4.24</b>	<b>0.10</b>	<b>0</b>	<b>6</b>	<b>5</b>
PCA	62	58	3.83	0.13	0	3	1	62	57	4.64	0.11	0	4	1	62	56	4.23	0.08	0	5	1
Petrifilm AC <sup>1</sup>	22	21	4.10	0.13	0	0	1	22	21	4.63	0.13	0	0	1	22	21	4.26	0.10	0	0	1
TEMPO AC	8	7	4.08	0.10	0	0	1	8	8	4.60	0.09	0	0	0	8	7	4.18	0.11	0	0	1
TSA	7	7	3.91	0.21	0	0	0	7	7	4.72	0.14	0	0	0	7	7	4.27	0.08	0	0	0
MPCA	5	5	3.79	0.06	0	0	0	5	4	-	-	0	0	1	5	5	4.21	0.11	0	0	0
Compact Dry TC	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	3	-	-	0	0	1
TGE	3	2	-	-	0	0	1	3	2	-	-	0	0	1	3	2	-	-	0	0	1
Other	3	2	-	-	0	0	1	3	2	-	-	0	1	0	3	2	-	-	0	1	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).

<sup>1</sup> "Petrifilm AC" includes two participants that incubated on Petrifilm RAC.



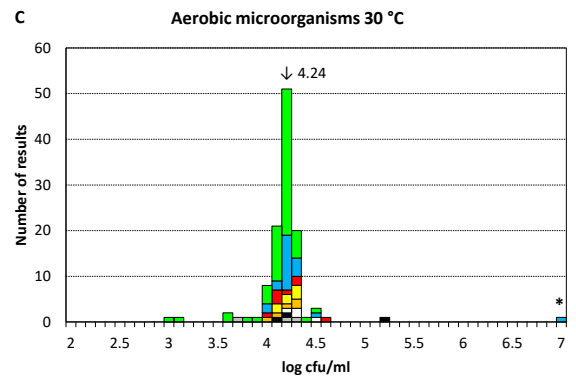
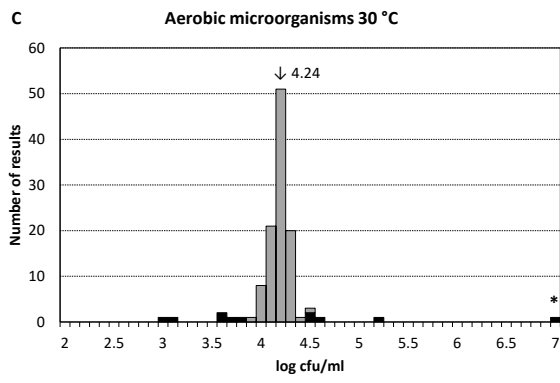


Figure 1. Results from analysis of aerobic microorganisms, 30 °C.

# Enterobacteriaceae

## Sample A

*C. freundii* and *E. coli* O157 both belong to Enterobacteriaceae. The strain of *C. freundii* was however present in considerably higher concentration than *E. coli* O157 and was thus the main target organism. On VRBG, the strain of *C. freundii* forms typical red colonies surrounded by a bile salt precipitation zone. The strain is oxidase-negative.

In total 98 participants reported results. Three low and two high outliers were reported, as well as three false negative results.

## Sample B

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

In total 98 participants reported results. Four low and three high outliers were reported.

## Sample C

The strain of *E. coli* (not identical to that in sample B) was target organism. On VRBG, it forms typical red/purple colonies surrounded by a precipitation zone. The strain is oxidase-negative.

In total 99 participants reported results. Three low and five high outliers were reported.

## 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.

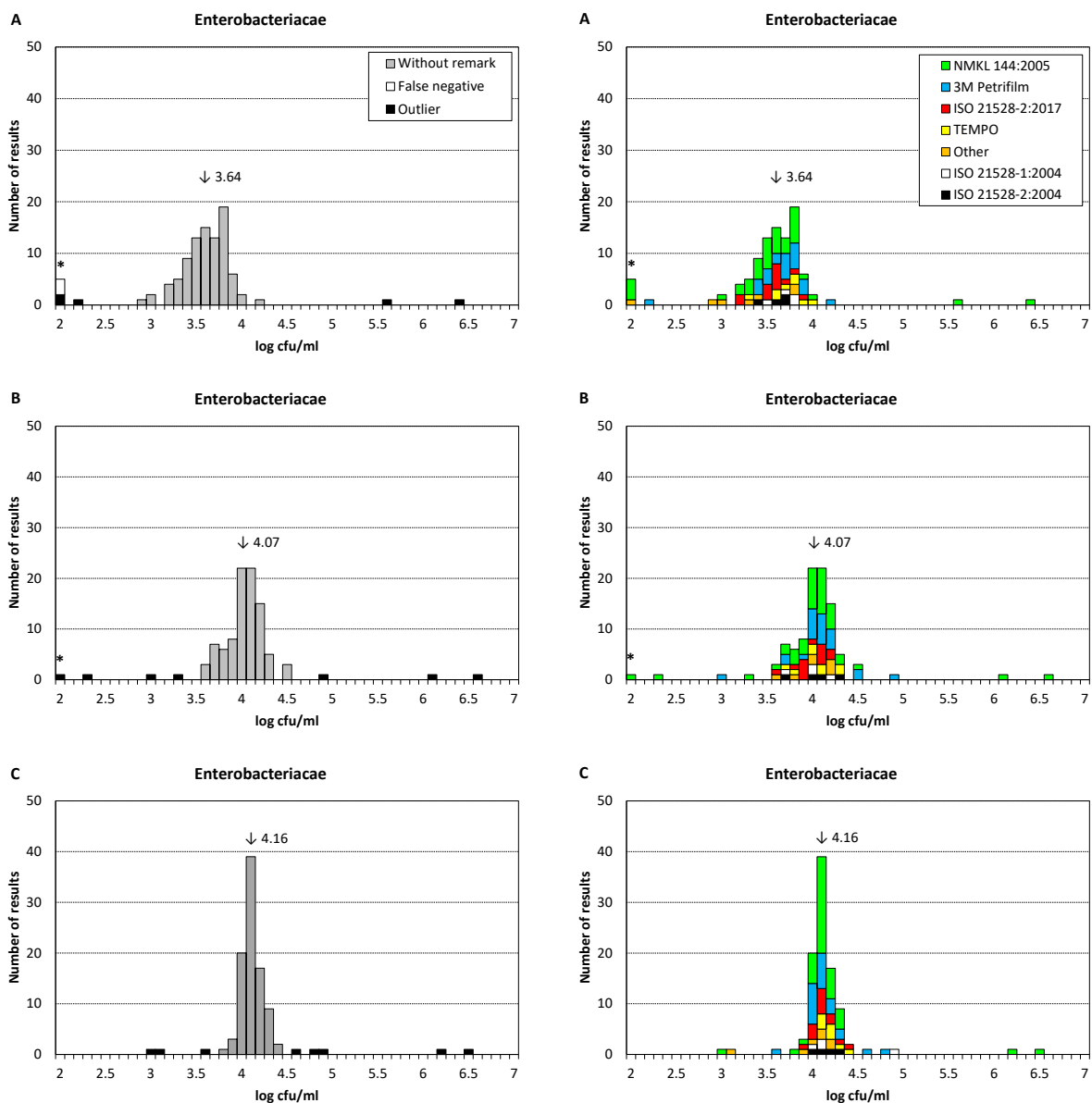
Most participants followed either NMKL 144:2005, a method with Petrifilm EB, or an ISO method. Among the latter, the majority followed ISO 21528-2:2017, which is based on colony-count. In comparison, ISO 21528-1:2017 is based on MPN. The latter method is recommended when the expected level of Enterobacteriaceae is lower than 100 cfu g<sup>-1</sup>. Both ISO standards were last reviewed by ISO in 2022 and remain current. Eight participants still followed either of the previous – and now withdrawn – ISO 21528-2:2004 and ISO 21528-1:2004.

Confirmation was performed by 60 % of the participants, and most often consisted of an oxidase test.

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

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	<	>
All results	98	90	3.64	0.24	3	3	2	98	91	4.07	0.20	0	4	3	99	91	4.16	0.12	0	3	5
NMKL 144:2005	39	33	3.62	0.23	3	1	2	39	34	4.09	0.18	0	3	2	40	37	4.15	0.11	0	1	2
3M Petrifilm	23	22	3.76	0.20	0	1	0	23	21	4.13	0.20	0	1	1	23	20	4.12	0.09	0	1	2
ISO 21528-2:2017	13	13	3.60	0.19	0	0	0	13	13	4.00	0.18	0	0	0	13	13	4.11	0.12	0	0	0
TEMPO® EB	8	8	3.78	0.23	0	0	0	8	8	4.13	0.20	0	0	0	8	8	4.23	0.08	0	0	0
Other	7	6	3.36	0.37	0	1	0	7	7	4.06	0.22	0	0	0	7	6	4.15	0.12	0	1	0
ISO 21528-1:2004	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	3	-	-	0	0	1
ISO 21528-2:2004	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	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 2.** Results from analysis of Enterobacteriaceae



# Thermotolerant *Campylobacter*

## Sample A

The strain of *C. coli* was target organism. On mCCDA it may form both smaller and larger 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.

In the quantitative analysis, 19 participants reported results. One false negative result was reported.

In the qualitative analysis, 25 participants reported results. Two false negative results were reported.

## Sample B

No target organism was present in the sample. *E. coli* is however false positive for the analysis. It may form atypical white/grey colonies on mCCDA. The strain is oxidase-negative and catalase-positive. It is easily distinguished from *Campylobacter* under a microscope.

In total, 18 and 24 participants reported results in the quantitative and qualitative analysis, respectively. No false positive results were reported.

## 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, 19 participants reported results. No outliers were reported. Due to the low mean value, and the high standard deviation, one zero result is included among the accepted results.

In the qualitative analysis, 24 participants reported results. Three 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 by the participants often consist of a motility test and/or an oxidase test.

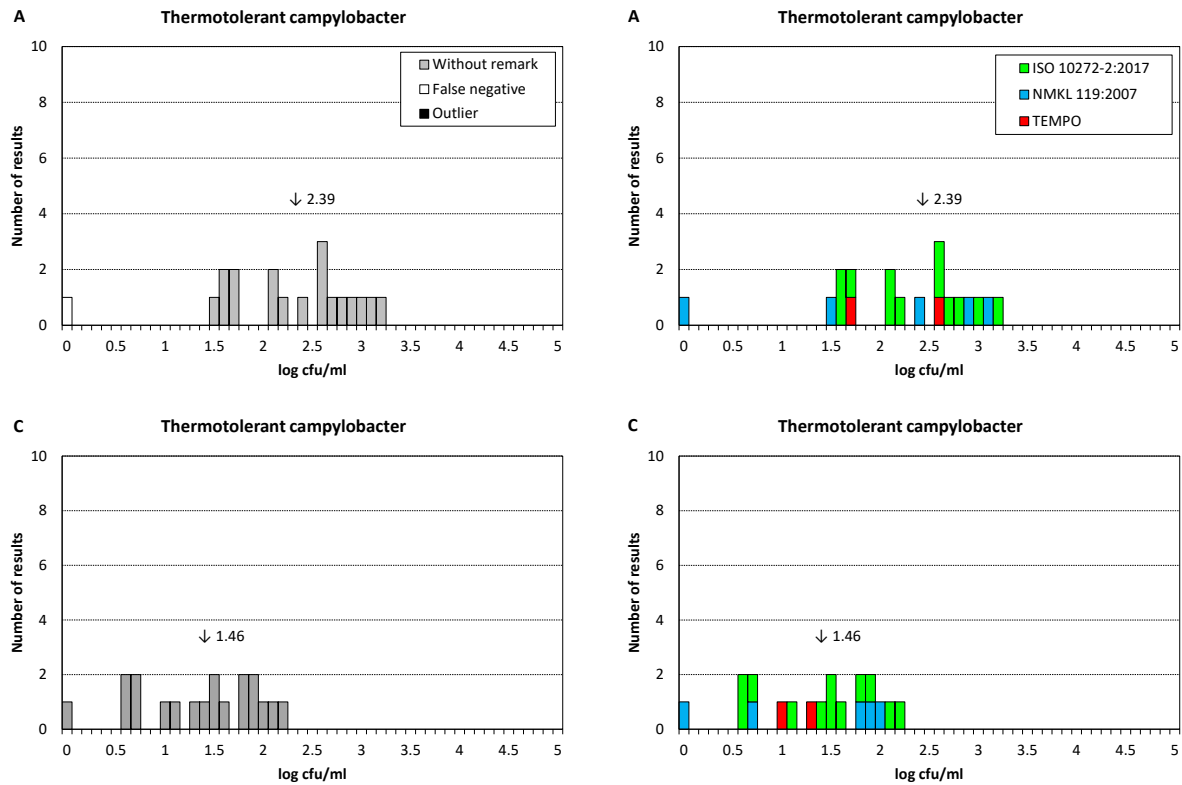
NMKL 119:2007 (qualitative/quantitative), ISO 10272-1:2017 (qualitative) and ISO 10272-2:2017 (quantitative) were the most common methods. In the qualitative analysis, one participant followed the withdrawn ISO 10272-1:2006. Also in the qualitative analysis, one participant followed ISO 17995:2019, which is a method for detection of *Campylobacter* in water samples.

In the qualitative analysis, the majority of the participants (80 %) used Bolton broth for the enrichment, but the use of Preston broth and CampyFood® was also reported. For the selective step, most participants (84 %) used mCCDA, but CampyFood®, Brilliance™ CampyCount agar and Abeyta-Hunt Bark agar were also used. Similarly, in the quantitative analysis, 79 % of the participants incubated on mCCDA, but TEMPO®CAM and Abeyta-Hunt Bark agar were reportedly also used.

**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	<	>
All results	19	18	2.39	0.63	1	0	0	18	18	-	-	0	-	-	19	19	1.46	0.60	0	0	0
ISO 10272-2:2017	12	12	2.45	0.55	0	0	0	11	11	-	-	0	-	-	12	12	1.52	0.55	0	0	0
NMKL 119:2007	5	4	-	-	1	0	0	5	5	-	-	0	-	-	5	5	1.80	0.89	0	0	0
TEMPO	2	2	-	-	0	0	0	2	2	-	-	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 3.** Results from quantitative analysis of thermotolerant *Campylobacter*.

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

Medium	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>25</b>	<b>23</b>	<b>2</b>	<b>24</b>	<b>24</b>	<b>0</b>	<b>24</b>	<b>21</b>	<b>3</b>
ISO 10272-1:2017	10	8	2	10	10	0	10	9	1
NMKL 119:2007	10	10	0	10	10	0	10	9	1
CampyFood®	1	1	0	1	1	0	1	1	0
ISO 17995:2019	1	1	0	1	1	0	1	1	0
ISO 10272-1:2006	1	1	0	1	1	0	1	0	1
PCR method	1	1	0	1	1	0	1	1	0
Other	1	1	0	0	0	0	0	0	0

# *Listeria monocytogenes*

## Sample A

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.

In the quantitative analysis, 61 participants reported results. Four low and four high outliers were reported.

In the qualitative analysis, 93 participants reported results. Two false negative results were reported.

## Sample B

No target organism was present in the sample.

In the quantitative analysis, 60 participants reported results. One false positive result was reported.

In the qualitative analysis, 93 participants reported results. One false positive result was reported.

## Sample C

The strain of *L. monocytogenes* (not identical to that in sample A) 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, 61 participants reported results. Three low and two high outliers were reported.

In the qualitative analysis, 93 participants reported results. All results were correct positive.

## General remarks

ISO 11290-2:2017 and RAPID'L.mono were the main methods used in the quantitative analysis. In contrast, in the qualitative analysis, VIDAS<sup>®</sup> and RAPID'L.mono were the most common methods. ISO 11290-1:2017 (qualitative) and ISO 11290-2:2017 (quantitative) were last reviewed by ISO in 2022, and remain current.

NMKL 136:2010 was also used by many participants. It 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* Precis<sup>™</sup> is based on the chromogenic medium

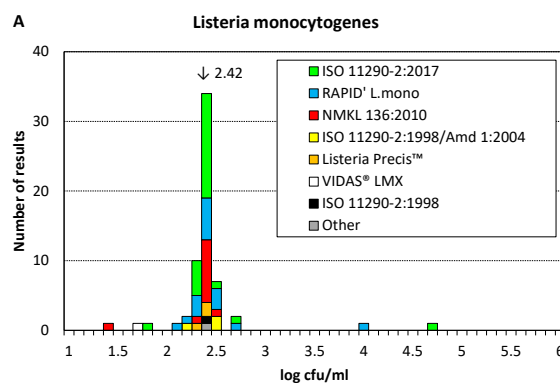
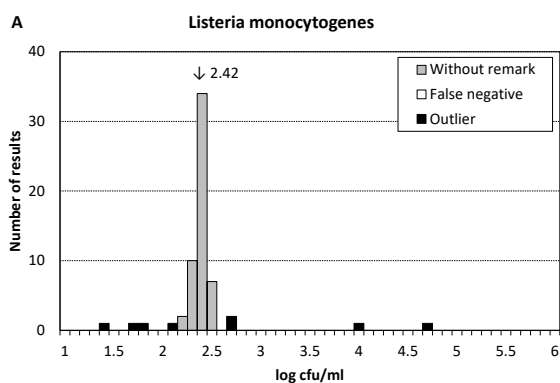
Brilliance™ Listeria, on which *Listeria* spp. and *L. monocytogenes* form blue colonies due to their  $\beta$ -glucosidase activity. SwabSURE ListeriaP is a test based on swab sampling, for detection of *L. monocytogenes* and *L. ivanovii* in surface samples. In comparison, VIDAS® is based on detection 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.

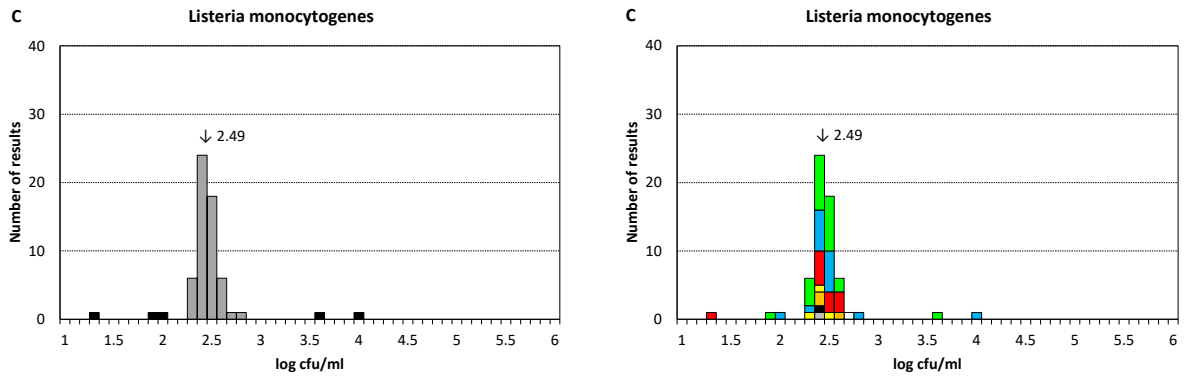
*L. monocytogenes* is often confirmed by microscopy, catalase test, and by tests of  $\beta$ -haemolysis and carbohydrate utilisation (fermentation of rhamnose and xylose). *L. monocytogenes* is catalase-positive, displays  $\beta$ -haemolysis on blood agar, and ferments rhamnose but not xylose. Confirmation can also be done by the increased and decreased  $\beta$ -haemolysis displayed by *L. monocytogenes* in the presence of *Staphylococcus aureus* and *Rhodococcus equi*, respectively (CAMP test).

**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>61</b>	<b>53</b>	<b>2.42</b>	<b>0.08</b>	<b>0</b>	<b>4</b>	<b>4</b>	<b>60</b>	<b>59</b>	-	-	<b>1</b>	-	-	<b>61</b>	<b>56</b>	<b>2.49</b>	<b>0.11</b>	<b>0</b>	<b>3</b>	<b>2</b>
ISO 11290-2:2017	24	21	2.41	0.06	0	1	2	23	22	-	-	1	-	-	24	22	2.47	0.10	0	1	1
RAPID' L.mono	16	13	2.41	0.08	0	1	2	16	16	-	-	0	-	-	16	14	2.52	0.11	0	1	1
NMKL 136:2010	12	11	2.44	0.04	0	1	0	12	12	-	-	0	-	-	12	11	2.51	0.07	0	1	0
ISO 11290-2:1998/Amd 1:2004	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
Listeria Precis™	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
VIDAS® LMX	1	0	-	-	0	1	0	2	2	-	-	0	-	-	1	1	-	-	0	0	0
ISO 11290-2:1998	1	1	-	-	0	0	0	0	0	-	-	0	-	-	1	1	-	-	0	0	0
Other	1	1	-	-	0	0	0	1	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).





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

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

Medium	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>93</b>	<b>91</b>	<b>2</b>	<b>93</b>	<b>92</b>	<b>1</b>	<b>93</b>	<b>93</b>	<b>0</b>
VIDAS®	19	19	0	19	19	0	19	19	0
RAPID' L.mono	17	17	0	17	17	0	17	17	0
ISO 11290-1:2017	14	13	1	14	13	1	14	14	0
PCR method	14	14	0	14	14	0	14	14	0
Other	12	11	1	12	12	0	12	12	0
NMKL 136:2010	7	7	0	7	7	0	7	7	0
ISO 11290-1:1996/Amd 1:2004	5	5	0	5	5	0	5	5	0
SwabSURE ListeriaP	2	2	0	2	2	0	2	2	0
Listeria Precis™	2	2	0	2	2	0	2	2	0
ALOA® One Day	1	1	0	1	1	0	1	1	0

# Salmonella

## Sample A

No target organism was present in the sample. *C. freundii* was however false positive for the analysis. In the Swedish Food Agency's initial quality control, it formed atypical white colonies on XLD and Brilliance™ Salmonella.

In total 104 participants reported results. Two false positive results were reported.

## Sample B

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 105 participants reported results. All results were correct positive.

## Sample C

The strain of *Salmonella* Stockholm (identical to that in sample B) 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 105 participants reported results. One false negative result was reported.

## General remarks

The two most common methods were NMKL 71:1999 and ISO 6579-1:2017, which 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 MSR/V 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.

Other common methods used by the participants were VIDAS® SPT and RAPID'Salmonella. Both are validated by AFNOR and AOAC against ISO 6579-1:2017. PCR-based methods were also frequently used. The withdrawn methods ISO 6579:2002/Amd 1:2007 and ISO 6579:2002 were followed by three and two participants, respectively. 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 MSR/V instead of RVS during the selective enrichment step.

On XLD, which was used by the majority of the participants, typical *Salmonella* form transparent red colonies with a black centre. As a complementary medium to XLD, the participants mainly used chromogenic media such as Brilliance™ Salmonella or BGA.

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

Medium	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>104</b>	<b>102</b>	<b>2</b>	<b>105</b>	<b>105</b>	<b>0</b>	<b>105</b>	<b>104</b>	<b>1</b>
NMKL 71:1999	22	22	0	23	23	0	23	23	0
ISO 6579-1:2017	22	21	1	22	22	0	22	22	0
VIDAS® SPT <sup>1</sup>	18	17	1	18	18	0	18	18	0
PCR method	17	17	0	17	17	0	17	16	1
Other	9	9	0	9	9	0	9	9	0
RAPID'Salmonella	7	7	0	7	7	0	7	7	0
ISO 6579:2002/Amd 1:2007	3	3	0	3	3	0	3	3	0
NMKL 187:2016 <sup>2</sup>	3	3	0	3	3	0	3	3	0
ISO 6579:2002	2	2	0	2	2	0	2	2	0
Neogen Reveal 2.0 Salmonella	1	1	0	1	1	0	1	1	0

<sup>1</sup> The group VIDAS includes two participants that used MINI VIDAS®.

<sup>2</sup> Includes both NMKL 187:2007 and NMKL 187:2016.



# *Escherichia coli* O157

## Sample A

The strain of *E. coli* O157 was target organism for the analysis. 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. Two false negative results were reported.

## Sample B

No target organism was present in the sample. In the Swedish Food Agency's quality control, the false-positive strain of *E. coli* formed red colonies on SMAC. No colonies were observed on CT-SMAC.

In total 22 participants reported results. Four false positive results were reported.

Note: One participant stated that they only analysed *E. coli* to the species level, but not the strain level. This is likely why this participant reported a false positive result for sample B.

## Sample C

The strain of *E. coli* O157 (identical to that in sample A) was target organism for the analysis. 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 21 participants reported results. Four false negative results were reported.

## General remarks

Only 22 participants reported evaluated results, and many of the reported methods fall into the group "Other" and "PCR method", which are in general not specified in detail. An assessment of the general performance is therefore challenging. Also, the false positive and false negative results are not connected to the use of a specific method, but appear to be randomly distributed evenly among the different method groups.

Among the specified methods are NMKL 164:2019 (though most participants specified the withdrawn NMKL 164:2005) and ISO 16654:2001. These 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 and Amd 2:2023.

VIDAS® ECPT was used by four participants. It is a phage ligand assay which detects *E. coli* O157-specific receptors using ELFA (Enzyme Linked Fluorescent Assay). The method is validated against ISO 16654:2001 by AFNOR and AOAC.

The most frequently specified media for isolation were CT-SMAC, SMAC and CHROMagar™ O157. CT-SMAC and SMAC distinguish between bacteria that ferment sorbitol (most non-pathogenic *E. coli*) are those that do not (most *E. coli* O157). On these media, sorbitol-negative *E. coli* O157 form transparent colonies with a dark centre, whereas sorbitol-positive *E. coli* instead form red colonies. Harlequin™ SMAC-BCIG is another medium that is sometimes used by participants. It is similar to SMAC, and contains the chromogenic substrate BGIC that causes sorbitol-negative and βglucuronidase-positive *E. coli* to form blue/green colonies. In comparison, on CHROMagar™ *E. coli* O157 form mauve (purple) colonies that can be distinguished from coliform (blue) or other bacteria (colourless) that may grow on this medium.

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

Medium	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>20</b>	<b>2</b>	<b>22</b>	<b>18</b>	<b>4</b>	<b>21</b>	<b>17</b>	<b>4</b>
ISO 16654:2001	6	5	1	6	5	1	6	5	1
PCR method	4	4	0	4	4	0	4	3	1
VIDAS® ECPT	4	4	0	4	4	0	3	2	1
Other	4	4	0	4	3	1	4	3	1
NMKL 164:2005	3	2	1	3	2	1	3	3	0
NMKL 164:2019	1	1	0	1	0	1	1	1	0

# Pathogenic *Vibrio* spp.

## Sample A

No target organism was present in the sample. The strain of *E. coli* O157 may possibly form colonies on TCBS.

In total 21 participants reported results. All results were correct negative.

## Sample B

The strain of *V. cholerae* was target organism. On TCBS, it forms typical green/yellow colonies. It is oxidase-positive and sensitive to vibriostatic agent O129. *E. coli* and *S. Stockholm* may also form colonies on TCBS. All atypical colonies in the Swedish Food Agency's initial quality control on TCBS were oxidase-negative upon confirmation.

In total 21 participants reported results. One false negative result was reported.

## Sample C

No target organism was present in the sample. In the Swedish Food Agency's quality control, *E. coli* formed small green colonies on TCBS. They were easily distinguished from *Vibrio* spp. upon confirmation.

In total 21 participants reported results. One false positive result was reported.

## General remarks

The majority of the 21 participants reported correct results. The two false results were reported by participants that used NMKL 156:1997. Since this was one of the most used methods, it is difficult to draw any conclusions from this observation.

Most participants followed either NMKL 156:1997 or ISO 21872-1:2017. Four participants followed the withdrawn ISO/TS 21872-1:2007. ISO 21872-1:2017 was last reviewed by ISO in 2023 and remains current. It contains several changes, including how to perform confirmation with biochemical and/or PCR methods, though it mainly follows the same principle as the previous version. 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.

All except one participant isolated colonies on TCBS. 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. One participant incubated on HardyCHROM™ *Vibrio* agar,

which is a chromogenic medium that can differentiate between *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*.

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

Medium	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>21</b>	<b>21</b>	<b>0</b>	<b>21</b>	<b>20</b>	<b>1</b>	<b>21</b>	<b>20</b>	<b>1</b>
ISO 21872-1:2017	8	8	0	8	8	0	8	8	0
NMKL 156:1997	8	8	0	8	7	1	8	7	1
ISO/TS 21872-1:2007	4	4	0	4	4	0	4	4	0
Other	1	1	0	1	1	0	1	1	0

# *Yersinia enterocolitica*

## Sample A

No target organism was present in the sample. *C. freundii* was however false positive for the analysis. In the Swedish Food Agency's quality control, it formed atypical pink colonies on CIN and yellow colonies on BS. The strain of *C. freundii* is oxidase-negative, and does not display agglutination against O:3 and O:9 antisera.

In total 12 participants reported results. One false positive result was reported.

## Sample B

The strain of *Y. enterocolitica* was target organism. On CIN, it forms typical colonies with a dark red 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*.

In total 13 participants reported results. Two false negative results were reported.

## Sample C

No target organism was present in the sample.

In total 12 participants reported results. Two false positive results were reported.

## General remarks

Most participants followed ISO 10273:2017. One participant followed the withdrawn 10273:2003. ISO 10273:2017 contains several important changes compared to the previous version. These include that characteristic *Y. enterocolitica* can be confirmed either by the traditional biochemical methods or by detection of the chromosomal virulence-associated gene *ail* by real-time PCR. It was last reviewed by ISO in 2022 and remains current.

One participant followed NMKL 117:1996. 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*.

All except one participant isolated colonies on CIN, in some cases in combination with another medium. On CIN, colonies of *Y. enterocolitica* have a typical appearance; a dark red "bull's eye" centre and an outer transparent zone.

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

Medium	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>12</b>	<b>11</b>	<b>1</b>	<b>13</b>	<b>11</b>	<b>2</b>	<b>12</b>	<b>10</b>	<b>2</b>
ISO 10273:2017	7	7	0	8	6	2	7	6	1
PCR method	2	2	0	2	2	0	2	2	0
NMKL 117:1996	1	1	0	1	1	0	1	0	1
ISO 10273:2003	1	0	1	1	1	0	1	1	0
Other	1	1	0	1	1	0	1	1	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: [www.livsmedelsverket.se/en/PT-extra](http://www.livsmedelsverket.se/en/PT-extra)

## 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.

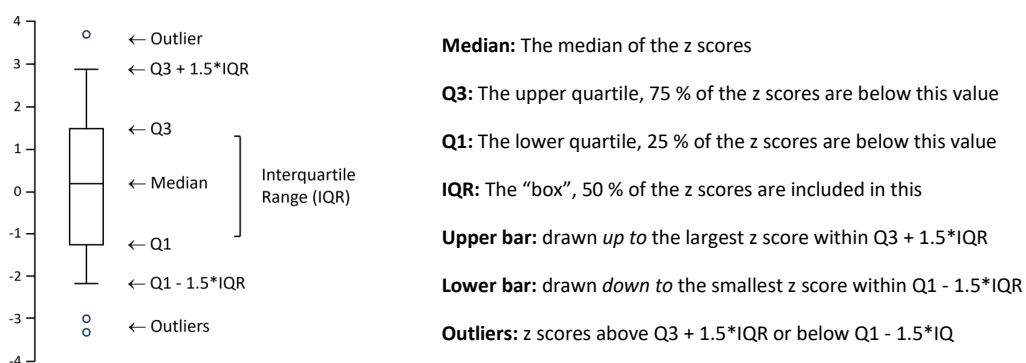
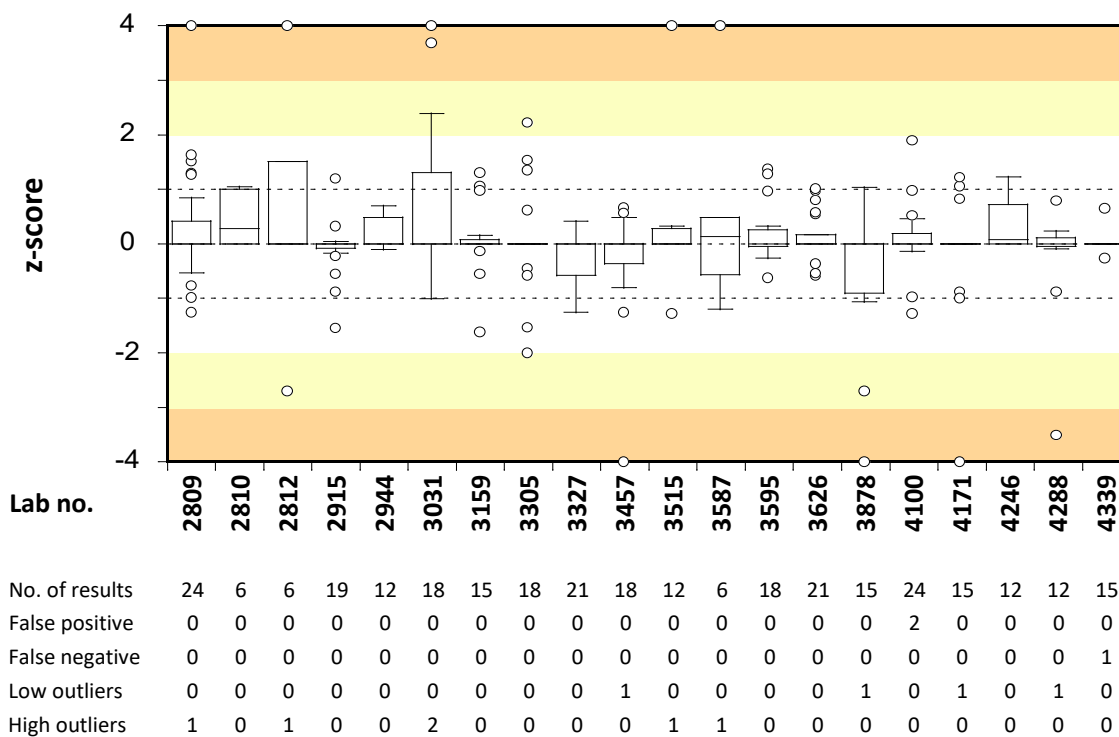
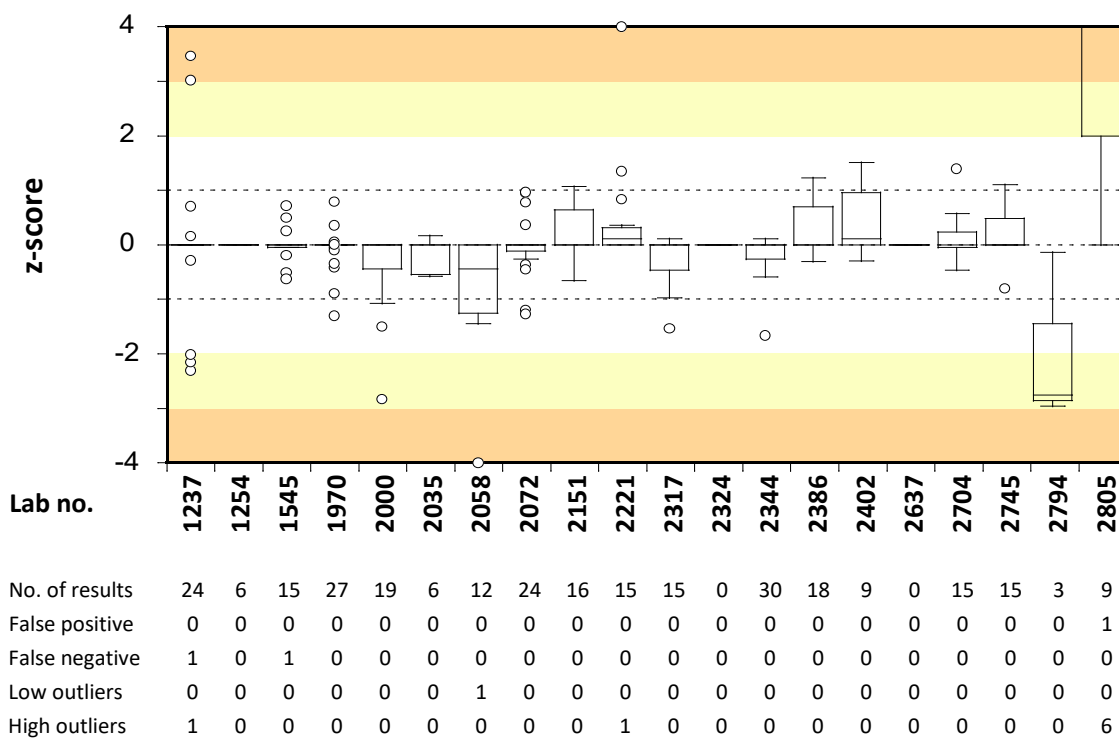
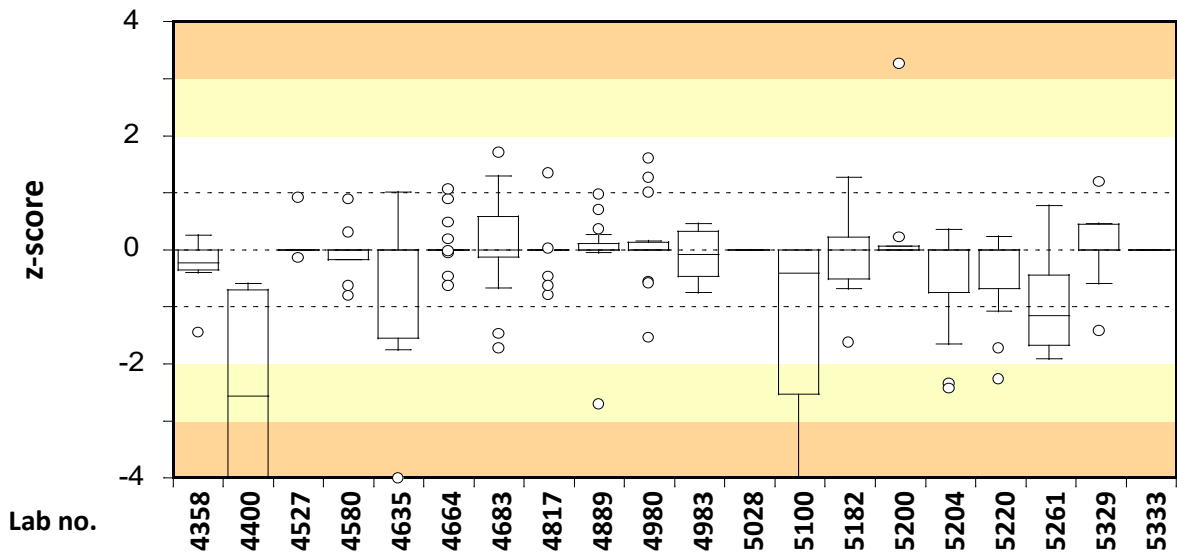


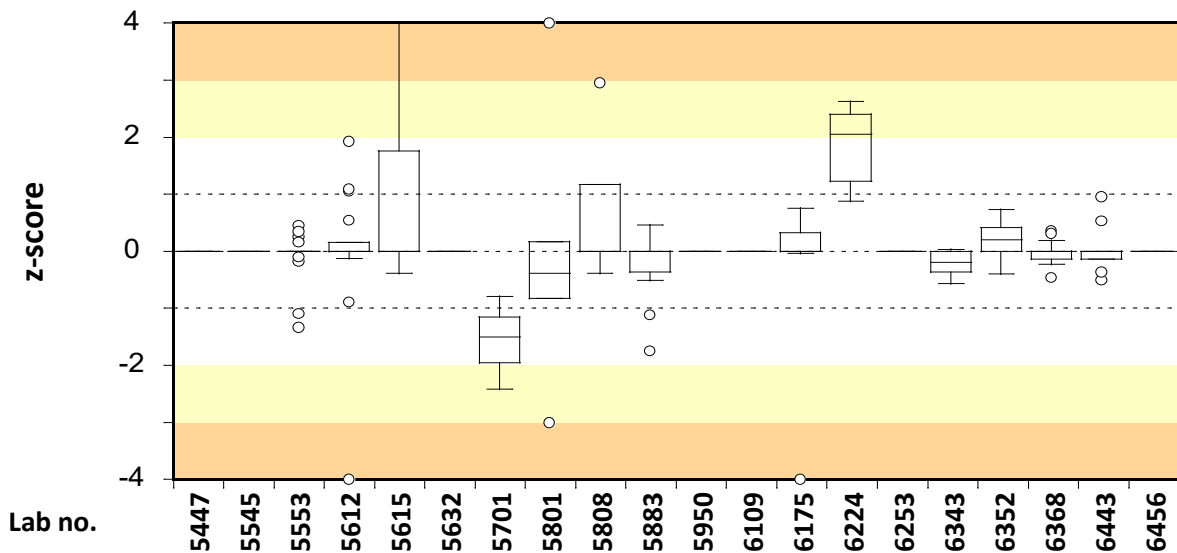
Figure 5. Schematic explanation of a box plot.



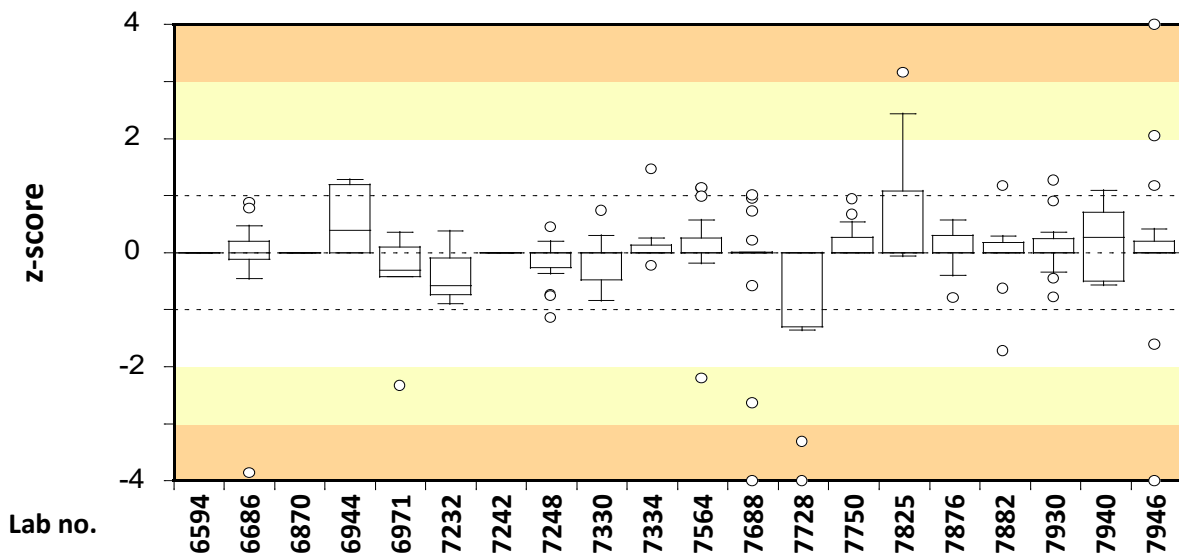




Lab no.	4358	4400	4527	4580	4635	4664	4683	4817	4889	4980	4983	5028	5100	5182	5200	5204	5220	5261	5329	5333
No. of results	8	6	9	9	12	18	15	21	18	15	6	1	6	9	9	21	19	9	9	6
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
False negative	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0
Low outliers	0	3	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
High outliers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

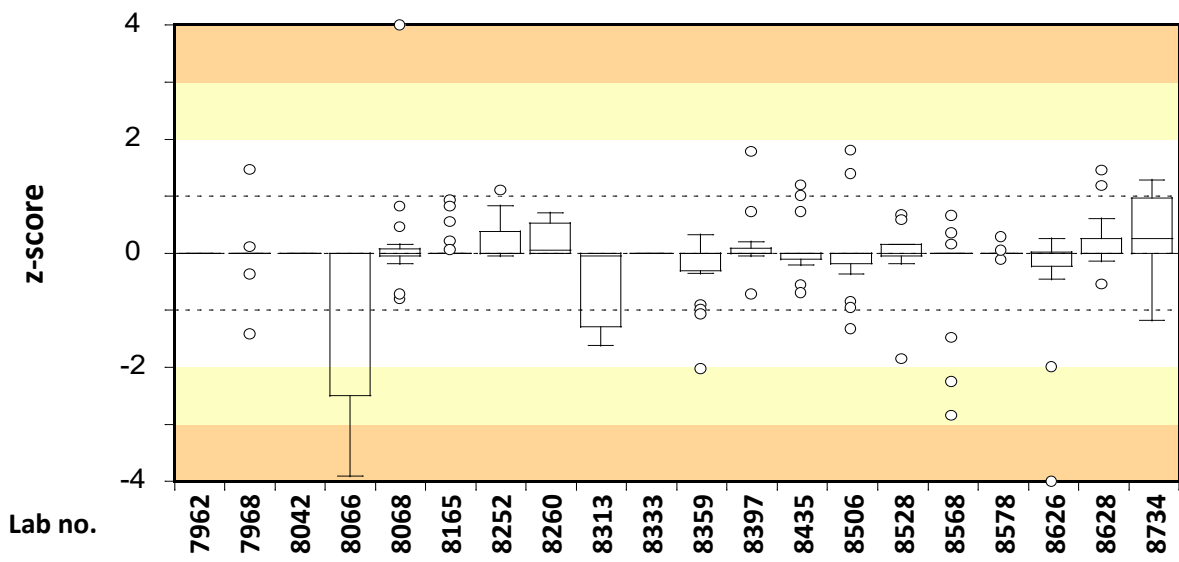


Lab no.	5447	5545	5553	5612	5615	5632	5701	5801	5808	5883	5950	6109	6175	6224	6253	6343	6352	6368	6443	6456
No. of results	3	6	21	18	12	3	3	6	6	15	0	0	9	6	6	9	9	18	9	6
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False negative	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Low outliers	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
High outliers	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0



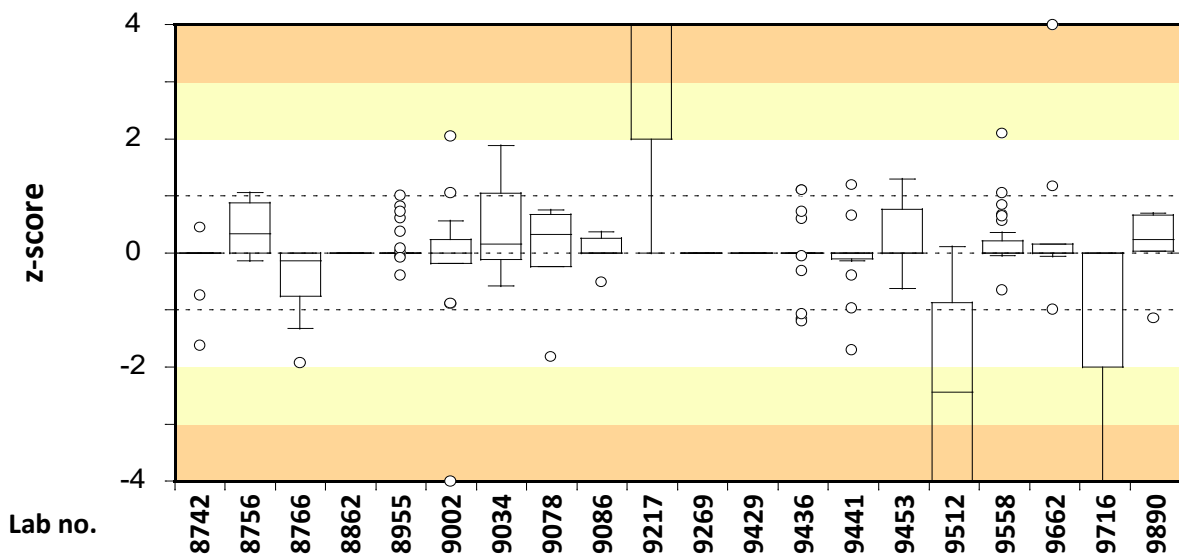
Lab no.

No. of results	0	15	3	6	6	3	0	18	15	9	21	21	18	12	15	15	12	15	6	27
False positive	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	4
False negative	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	4
Low outliers	0	1	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	2
High outliers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2

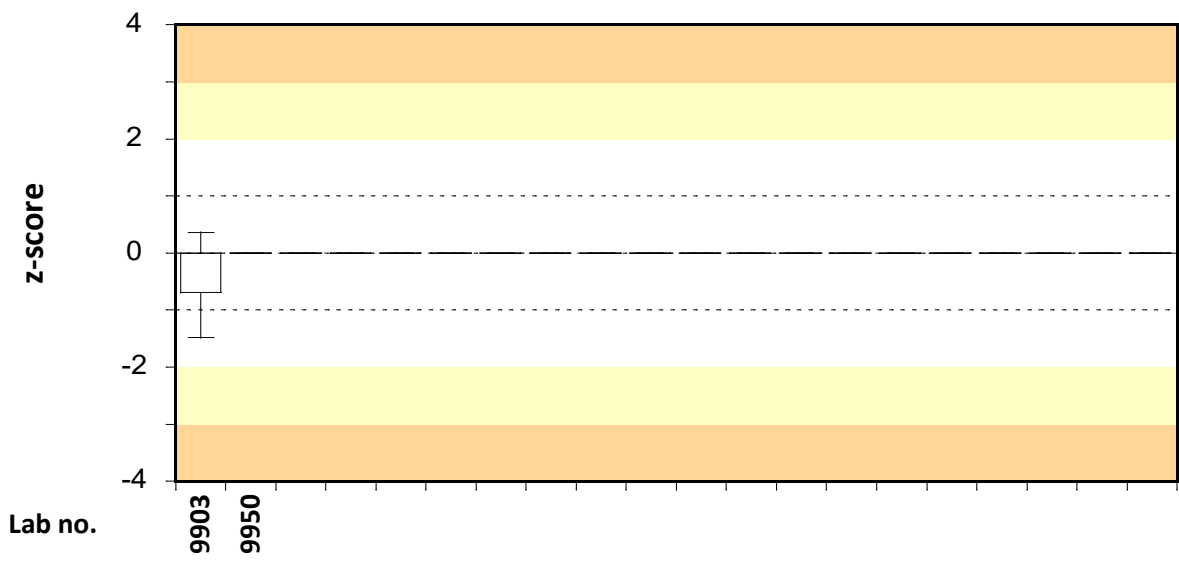


Lab no.

No. of results	0	13	3	6	15	21	12	12	12	0	21	15	15	15	9	15	12	15	18	9
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False negative	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0
Low outliers	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
High outliers	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



Lab no.	8742	8756	8766	8862	8955	9002	9034	9078	9086	9217	9269	9429	9436	9441	9453	9512	9558	9662	9716	9890
No. of results	9	9	15	3	30	18	6	6	6	9	3	6	21	15	12	6	30	15	12	6
False positive	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
False negative	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Low outliers	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	3	0	0	3	0
High outliers	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	2	0	0



Lab no.	9903	9950
No. of results	15	3
False positive	0	0
False negative	0	0
Low outliers	0	0
High outliers	0	0

# 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 the table below.

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

Sample	Microorganism	Strain			
		SLV no. <sup>1</sup>	Origin	Reference <sup>2</sup>	log <sub>10</sub> cfu ml <sup>-1</sup>
A	<i>Campylobacter coli</i>	SLV-271	Hen, faeces	CCUG 45147	2.7
	<i>Citrobacter freundii</i>	SLV-091	-	CCUG 43597	3.0
	<i>Escherichia coli</i> O157	SLV-479	Ear infection	SMI 811 86	1.5
	<i>Listeria monocytogenes</i>	SLV-513	Milk	CCUG 44510	2.5
B	<i>Escherichia coli</i>	SLV-558	-	-	4.1
	<i>Salmonella</i> Stockholm	SLV-390	Chocolate	-	2.2
	<i>Staphylococcus aureus</i>	SLV-280	Egg	-	4.4
	<i>Vibrio cholerae</i>	SLV-507	Ear infection	CCUG 34649	2.1
	<i>Yersinia enterocolitica</i>	SLV-408	Dog food	CCUG 45643	2.6
C	<i>Campylobacter jejuni</i>	SLV-540	Chicken	-	2.3
	<i>Escherichia coli</i>	SLV-477	Cheese	CCUG 43601	4.2
	<i>Escherichia coli</i> O157	SLV-479	Ear infection	SMI 811 86	1.5
	<i>Listeria monocytogenes</i>	SLV-361	Salmon	-	2.5
	<i>Salmonella</i> Stockholm	SLV-390	Chocolate	-	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

In order to allow comparison of the freeze-dried samples, it is essential to have aliquots of homogeneous test material and equal volume in all vials. Quality control is performed on 10 randomly chosen vials in conjunction with manufacturing of the samples or on 5 vials if an “old” sample mixture was used and the last quality control was performed more than 6 months ago. Homogeneity of a test material is approved if, for each analysis, the values obtained for the test for “Index of dispersion” between vials ( $I_2$ ) and the test for reproducibility ( $T$ ) do not simultaneously exceed 2.0 and 2.6, respectively. (For definitions of  $I_2$ , and  $T$ , see references [4] and [5] respectively.)

**Table 13.** Concentration mean ( $m$ ),  $I_2$  and  $T$  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>		
	$m$	$I_2$	$T$	$m$	$I_2$	$T$	$m$	$I_2$	$T$
Aerobic microorganisms 30 °C NMKL method no. 86:2013	3.94	0.58	1.17	4.65	1.35	1.42	4.26	0.76	1.19
Enterobacteriaceae NMKL method no. 144:2005	2.96	0.12	1.24	4.12	<b>2.82</b>	1.51	4.13	1.97	1.41
Thermotolerant <i>Campylobacter</i> NMKL method no. 119:2007	2.73	0.72	1.25	-	-	-	2.25	<b>2.21</b>	1.57
<i>Listeria monocytogenes</i> NMKL method no. 136:2010	2.48	0.42	1.27	-	-	-	2.50	0.80	1.39
<i>Salmonella</i> NMKL method no. 71:1999	-	-	-	2.22	0.82	1.32	1.78	0.71	1.21
<i>Escherichia coli</i> O157 NMKL method no. 164:2019	1.57	0.29	1.17	-	-	-	1.53	0.70	1.29
Pathogenic <i>Vibrio</i> spp. NMKL method no. 156:1997	-	-	-	2.57	0.43	1.21	-	-	-
<i>Yersinia enterocolitica</i> NMKL method no. 117:1996	-	-	-	2.06	0.62	1.34	-	-	-

– No target organism or no value

<sup>1</sup> n = 5 vials analysed in duplicate

<sup>2</sup> n = 10 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. 2023. 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.
4. Heisterkamp SH, Hoekstra JA, van Strijp-Lockefeer NGWM, Havelaar AH, Mooijman KA, in't Veld PH, Notermans SHW, Maier EA and Griepink B. 1993. Statistical analysis of certification trials for microbiological reference materials. Luxembourg: Commission of the European Communities, Report EUR 15008 EN.
5. Mooijman KM, During M and Nagelkerke NJD 2003. MICROCRM: Preparation and control of batches of microbiological materials consisting of capsules. RIVM report 250935001/2003. RIVM, Bilthoven, Holland.

## 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: <https://www2.slv.se/absint>

### 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)