

# Proficiency testing Food Microbiology

January 2022

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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
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
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 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
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
IDF	International Dairy Foundation
ISO	International Organization for Standardization
NMKL	Nordic Committee for Food Analyses
NordVal	NordVal International - NMKL
SLV	Livsmedelsverket/Swedish Food Agency, 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

## Statistical evaluation of the results

For analyses where more than 20 participants have reported results, outliers are identified with statistical methods. Values that after  $\log_{10}$  transformation do not belong to a strictly normal distribution are for this purpose identified as outliers with Grubbs' test modified by Kelly [1]. When fewer than 20 participants have reported results, as well as in some individual cases, subjective adjustments are made to set outlier limits based on knowledge of the samples contents.


Mean values and standard deviations are normally provided for the different analyses. For analyses with fewer than 20 reported results, the median is provided instead of the mean value. Normally, for method groups with fewer than 5 results, only the number of false results and outliers are provided. Outliers and false results are not included in the calculations of mean values and standard deviations. Results reported as "> value" are not evaluated. Results reported as "< value" are interpreted as zero (negative result).

According to EN ISO/IEC 17043, for which the proficiency testing (PT) programme is accredited, it is mandatory for the participants to report method information for all their 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 nevertheless 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.

## Uncertainty of measurement for the assigned values

The measurement uncertainty for an assigned value is calculated as the standard deviation divided by the square root of the number of correct results ("standard error"). The assigned value is the mean value of the participants' results with outliers and false results excluded.

## Table legends

N	number of laboratories that performed the analysis
n	number of laboratories with satisfactory result
m	mean value in $\log_{10}$ cfu ml <sup>-1</sup> (false results and outliers excluded)
s	standard deviation (false results and outliers excluded)
F	number of false positive or false negative results
<	number of low outliers
>	number of high outliers
	results deviating more than 1 s from m, or unusually many deviating results.



## Figure legends

Histograms of the analytical results for each mixture and parameter are presented. The mean value of the analysis results is indicated in each histogram.

- values within the interval of acceptance
- outliers
- false negative results
- \* values outside of the x-axis scale

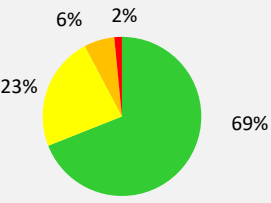
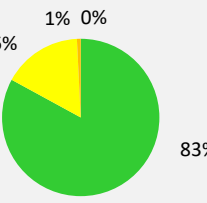
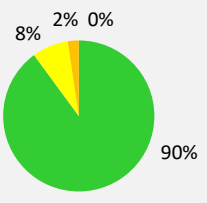
# Results

## General outcome

Samples were sent to 144 laboratories; 29 in Sweden, 98 in other European countries, and 17 outside of Europe. Of the 129 laboratories that reported results, 53 (41 %) provided at least one result that received an annotation. In the previous PT round with similar analyses (January 2021) the proportion was 32 %.

Individual results for each analysis in the PT round are listed in Annex 1 and are also available on the website after logging in: <https://www2.slv.se/absint>.

**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			
% participants with													
Microorganisms		<i>Aeromonas hydrophila</i> <i>Campylobacter coli</i> <i>Escherichia coli</i> O157 <i>Listeria monocytogenes</i>				<i>Bacillus cereus</i> <i>Kocuria rhizophila</i> <i>Salmonella</i> Enteritidis <i>Vibrio cholerae</i> <i>Yersinia enterocolitica</i>				<i>Campylobacter coli</i> <i>Citrobacter freundii</i> <i>Escherichia coli</i> O157 <i>Listeria monocytogenes</i>			
Analysis		Target organism	N	F	X	Target organism	N	F	X	Target organism	N	F	X
Aerobic micro-organisms 30 °C		<i>A. hydrophila</i>	107	0%	2%	<i>K. rhizophila</i>	107	0%	14%	<i>C. freundii</i> <i>E. coli</i> O157	106	0%	2%
Enterobacteriaceae		<i>E. coli</i> O157 ( <i>A. hydrophila</i> )	93	0%	31% <sup>1</sup>	<i>Y. enterocolitica</i> <i>S. Enteritidis</i>	94	1%	4%	<i>C. freundii</i> <i>E. coli</i> O157	93	0%	2%
Thermotol. <i>Campylobacter</i>	Quant.	<i>C. coli</i>	17	29%	12%	-	17	0%	0%	<i>C. coli</i>	16	6%	0%
	Qual.		20	5%	-		21	5%	-		19	5%	-
<i>L. monocytogenes</i>	Quant.	<i>L. monocytogenes</i>	55	2%	4%	-	56	0%	0%	<i>L. monocytogenes</i>	54	0%	4%
	Qual.		86	2%	-		86	0%	-		85	1%	-
<i>Salmonella</i>		-	99	3%	-	<i>S. Enteritidis</i>	99	0%	-	( <i>C. freundii</i> )	98	1%	-
<i>E. coli</i> O157		<i>E. coli</i> O157	22	14%	-	-	22	5%	-	<i>E. coli</i> O157	22	9%	-
Pathogenic <i>Vibrio</i> spp.		-	19	11%	-	<i>V. cholerae</i>	19	5%	-	( <i>E. coli</i> O157)	19	5%	-
<i>Y. enterocolitica</i>		-	11	0%	-	<i>Y. enterocolitica</i>	11	0%	-	( <i>C. freundii</i> )	11	18%	-

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

<sup>1</sup> High outliers should be regarded as false positive results

# Aerobic microorganisms 30 °C

## Sample A

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

## Sample B

*K. rhizophila* was present in a much higher concentration than the other microorganisms, and was thus the main target organism. Low outliers are therefore also likely caused by not detecting this strain.

Relatively many outliers were reported by laboratories that used Petrifilm AC, and by all laboratories that used the similar medium Compact Dry TC. In previous tests at the Swedish Food Agency, *K. rhizophila* formed very small colonies on Petrifilm AC that were difficult to detect after 48 h. In these tests, the results for Petrifilm AC were approximately 3.1 and 4.6 log<sub>10</sub> cfu ml<sup>-1</sup> after incubation at 30 °C for 48 h and 72 h, respectively. **Low results with Petrifilm AC and Compact Dry TC after incubation for 48 h are therefore considered as accepted.**

Somewhat high results were reported by laboratories that incubated on TSA. The only deviating result by users of this medium was a high outlier.

## Sample C

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

The mean value for Petrifilm AC was slightly higher compared to the mean values of other media. This is relatively often seen for Petrifilm AC and can be considered as normal.

## General remarks

Most participants followed either NMKL 86:2013, ISO 4833-1:2013 or used 3M Petrifilm AC. 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 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.

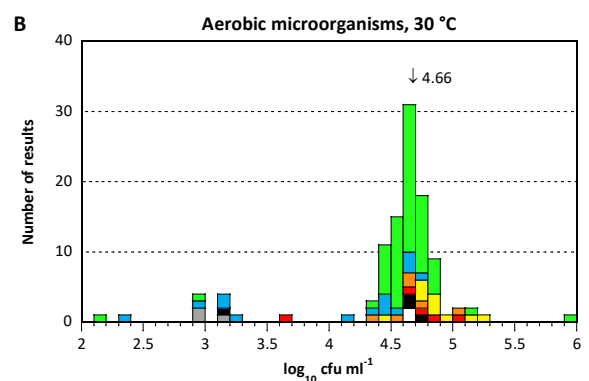
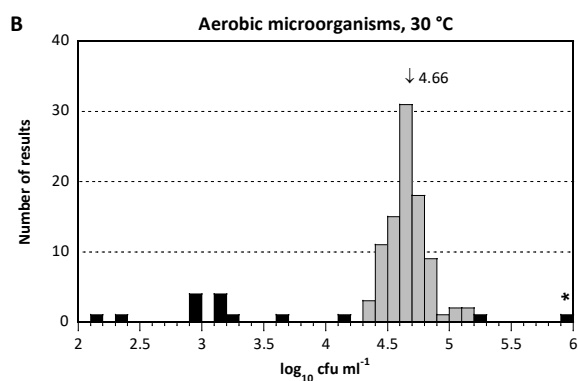
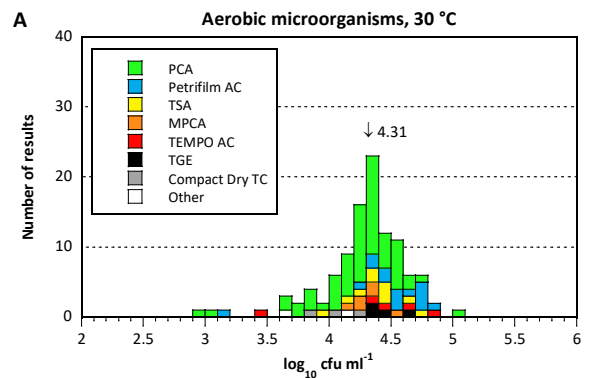
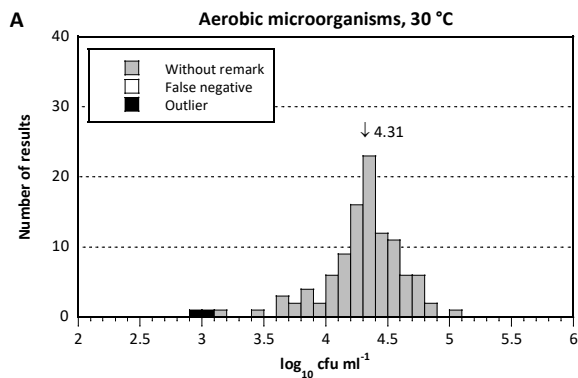
The majority of the participants incubated on PCA, but Petrifilm AC was also fairly common. Incubation on MPCA was mainly done by laboratories within the dairy industry. Incubation on TSA was mainly done by users of a company-specific method. A few laboratories used TEMPO AC, which is based on MPN (Most 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.

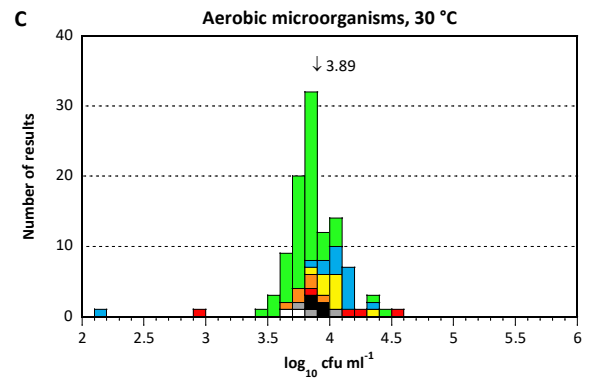
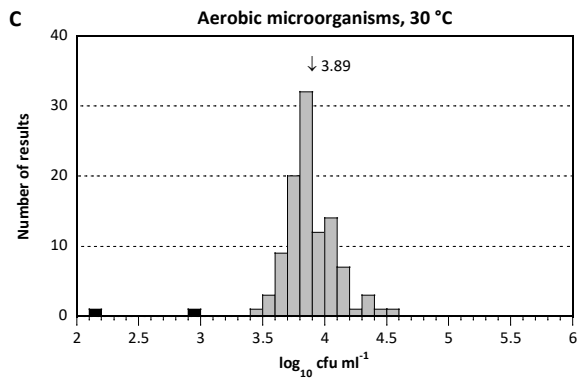
**Comment:** One laboratory followed ISO 13559/IDF 153 (contaminating microorganisms). Since the laboratory incubated on PCA, the results were still included in the evaluation.

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

Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	107	105	4.31	0.30	0	2	0	107	92	4.66	0.15	0	13	2	106	104	3.89	0.19	0	2	0
PCA	62	60	4.27	0.27	0	2	0	62	59	4.64	0.13	0	2	1	61	61	3.81	0.16	0	0	0
Petrifilm AC <sup>1</sup>	15	15	4.46	0.40	0	0	0	15	9	4.55	0.13	0	6	0	15	14	4.08	0.13	0	1	0
TSA	10	10	4.37	0.23	0	0	0	10	9	4.81	0.18	0	0	1	10	10	4.03	0.14	0	0	0
MPCA	6	6	4.32	0.12	0	0	0	6	6	4.65	0.23	0	0	0	6	6	3.82	0.10	0	0	0
TEMPO AC	5	5	4.37	0.53	0	0	0	5	4	-	-	0	1	0	5	4	-	-	0	1	0
TGE	4	4	-	-	0	0	0	4	3	-	-	0	1	0	4	4	-	-	0	0	0
Compact Dry TC	3	3	-	-	0	0	0	3	0	-	-	0	3	0	3	3	-	-	0	0	0
Other	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0

<sup>1</sup> Includes one laboratory that used Petrifilm RAC.





# Enterobacteriaceae

## Sample A

*E. coli* O157 belongs to Enterobacteriaceae. It was present in a very low concentration; approximately 8 cells ml<sup>-1</sup> in the undiluted sample. On VRBG, it forms typical red/purple colonies surrounded by a precipitation zone.

In total 93 laboratories reported results. Only five laboratories reported concentrations corresponding to *E. coli* O157 (median = 0.78 log<sub>10</sub> cfu ml<sup>-1</sup>). Of the remaining laboratories, 29 reported high outliers, and 59 reported negative (zero) results.

*A. hydrophila* was present at approximately 4.5 log<sub>10</sub> cfu ml<sup>-1</sup>. It can form colonies on VRBG that can be interpreted as belonging to Enterobacteriaceae. *A. hydrophila* should be distinguished from Enterobacteriaceae in subsequent confirmation, since it is oxidase-positive. Identification of *A. hydrophila* as Enterobacteriaceae may be a consequence of not confirming or of having problems with the confirmation.

**Due to the low concentration of *E. coli* O157 in the sample, zero results are considered as correct.**

**High outliers should be regarded as false positive results.**

## Sample B

*Y. enterocolitica* and *S. Enteritidis* were target organisms and were present in similar concentrations in the sample (approximately 2.3 and 2.0 log<sub>10</sub> cfu ml<sup>-1</sup>, respectively). On VRBG, both strains form typical red, oxidase-negative colonies, surrounded by a precipitation zone. The colonies of *Y. enterocolitica* are usually smaller than those of *S. Enteritidis*, and they may also have a less prominent precipitation zone.

## Sample C

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

## 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 laboratories followed either NMKL 144:2005 (45 %) or a method with Petrifilm EB (20 %), while the ISO methods (various versions) were used by 22 %. ISO 21528-2:2017 is based on colony-count, while 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>.

The number of users of ISO 21528-2:2017 was higher compared to the withdrawn ISO 21528-2:2004 (14 and 4 laboratories, respectively). In contrast, three laboratories followed the withdrawn ISO 21528-1:2004, while none had adopted the new ISO 21528-1:2017. The reported results from the different ISO methods were similar.

NMKL 144:2005 stipulates confirmation of presumptive colonies with an oxidase test. ISO 21528-2:2017 stipulates confirmation of presumptive colonies with both an oxidase test and with a test for glucose fermentation. Here, the majority of the laboratories that performed a confirmation test specified that it consisted of an oxidase test. Of the 29 laboratories that reported high outliers for sample A, only 31 % performed a confirmation, compared to 71 % of the participants overall.

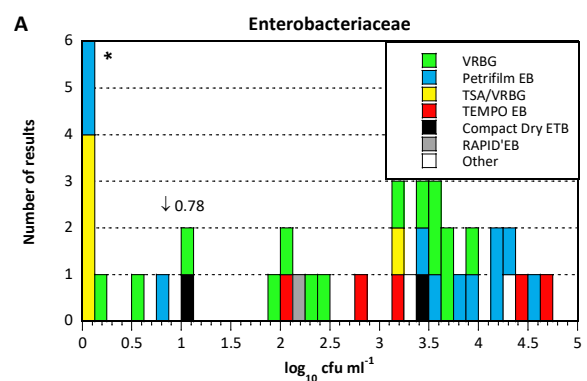
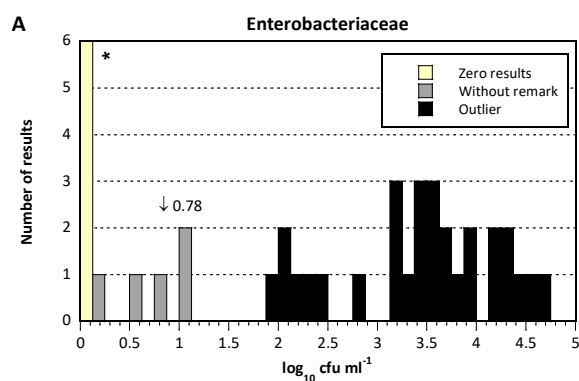
Overall, no large differences could be seen between the various methods, media and types of confirmation that were used by the laboratories. The only exception would be that all five participants that used TEMPO EB reported high outliers in sample A.

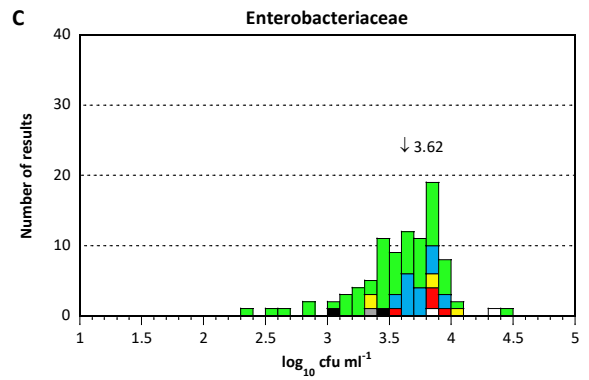
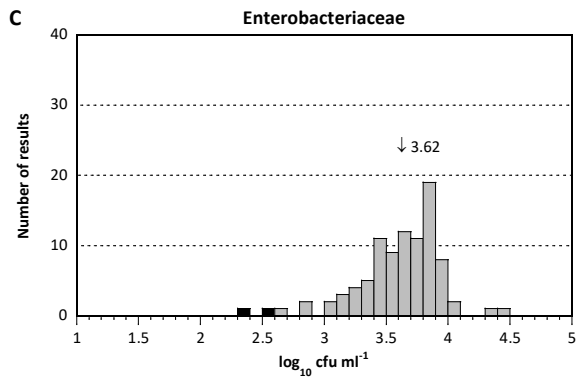
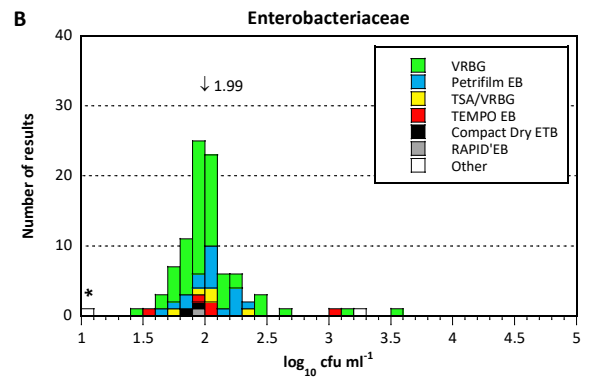
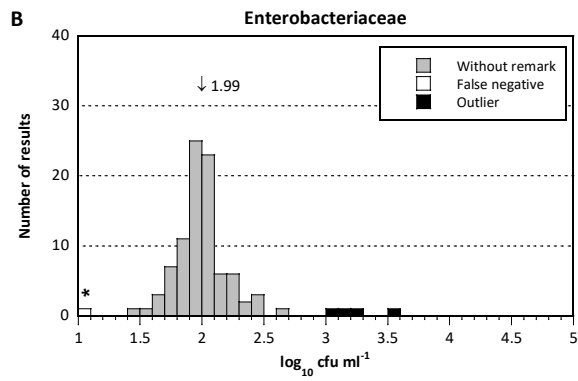
**Table 3.** Results from analysis of Enterobacteriaceae.

Medium	Sample A							Sample B							Sample C						
	N	n	Med <sup>1</sup>	s <sup>1</sup>	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	93	64	0.78	0.36	0	0	29 <sup>2</sup>	94	89	1.99	0.20	1	0	4	93	91	3.62	0.31	0	2	0
VRBG	61	50	-	-	0	0	11	61	59	1.98	0.20	0	0	2	60	58	3.56	0.33	0	2	0
Petrifilm EB	17	9	-	-	0	0	8	18	18	2.05	0.21	0	0	0	18	18	3.73	0.13	0	0	0
TSA/VRBG	5	4	-	-	0	0	1	5	5	2.00	0.20	0	0	0	5	5	3.67	0.32	0	0	0
TEMPO EB	5	0	-	-	0	0	5	5	4	-	-	0	0	1	5	5	-	-	0	0	0
Compact Dry ETB	2	1	-	-	0	0	1	2	2	-	-	0	0	0	2	2	-	-	0	0	0
RAPID'EB	1	0	-	-	0	0	1	1	1	-	-	0	0	0	1	1	-	-	0	0	0
Other	2	2	-	-	0	0	2	2	0	-	-	1	0	1	2	2	-	-	0	0	0

<sup>1</sup> Med = median. Median and s are based only on the 5 positive results.

<sup>2</sup> High outliers should be regarded as false positive results







# Thermotolerant *Campylobacter*

## Sample A

*C. coli* was target organism. On mCCDA it may possibly 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.

## Sample B

No target organism was present in the sample.

## Sample C

*C. coli* (identical strain to that in sample A) was target organism. On mCCDA it may possibly 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.

## 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 of some kind was performed in both the quantitative and qualitative analysis by all except one laboratory. The most common types of confirmation were a motility test and/or an oxidase test, but a catalase test was also fairly common.

NMKL 119:2007, ISO 10272-1:2017 (qualitative) and ISO 10272-2:2017 (quantitative) were the most common methods. In the qualitative analysis, one participant stated that they followed ISO 17995, which is a method for detection of *Campylobacter* in water samples. The retracted methods ISO 10272-1:2006 and ISO 10272-2:2006 were used by two and one laboratories, respectively.

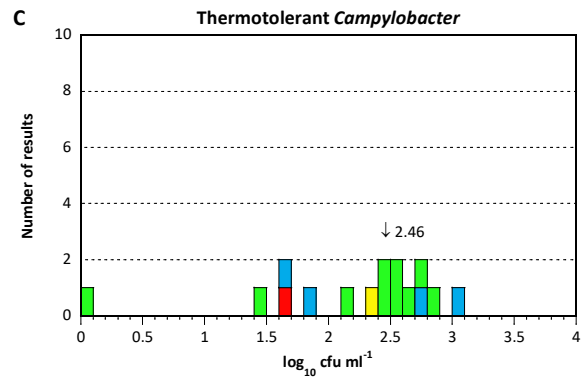
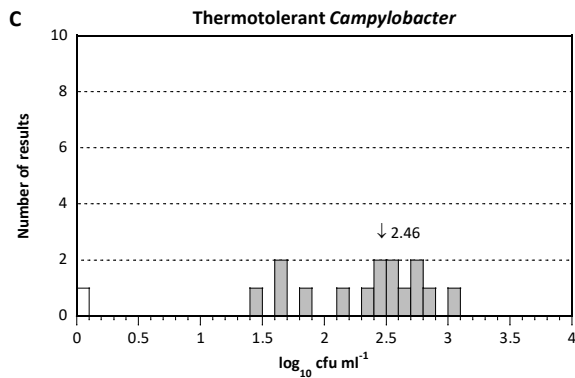
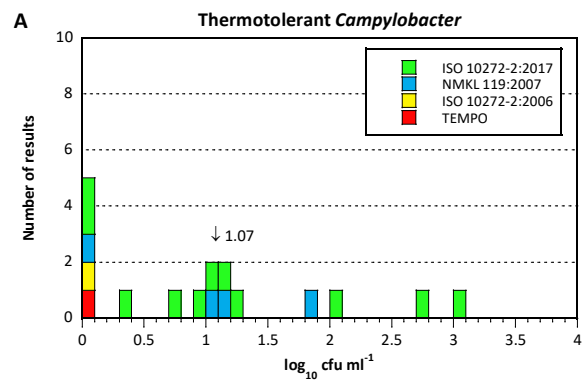
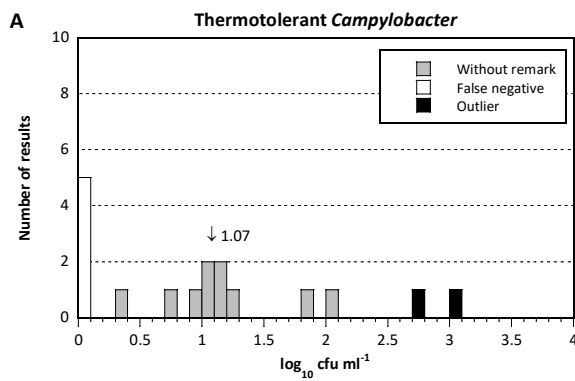
In the qualitative analysis, the majority of the participants (71 %) used Bolton broth for the enrichment, but the use of Preston broth and CampyFood® was also reported. For the selective step, most participants (87 %) used mCCDA, but CampyFood® and Abeyta-Hunt Bark agar were also used.

Similarly, in the quantitative analysis, 76 % of the participants incubated on mCCDA. Preston agar, Abeyta-Hunt Bark agar, RAPID'Campylobacter and TEMPO®CAM were used by one laboratory each.

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

Method	Sample A						Sample B						Sample C					
	N	n	Med <sup>1</sup>	s	F	< >	N	n	Med <sup>1</sup>	s	F	< >	N	n	Med <sup>1</sup>	s	F	< >
<b>All results</b>	<b>17</b>	<b>10</b>	<b>1.07</b>	<b>0.49</b>	<b>5</b>	<b>0 2</b>	<b>17</b>	<b>17</b>	-	-	<b>0</b>	- -	<b>16</b>	<b>15</b>	<b>2.46</b>	<b>0.48</b>	<b>1</b>	<b>0 0</b>
ISO 10272-2:2017	11	7	1.04	0.52	2	0 2	11	11	-	-	0	- -	10	9	2.56	0.40	1	0 0
NMKL 119:2007	4	3	-	-	1	0 0	4	4	-	-	0	- -	4	4	-	-	0	0 0
ISO 10272-2:2006	1	0	-	-	1	0 0	1	1	-	-	0	- -	1	1	-	-	0	0 0
TEMPO	1	0	-	-	1	0 0	1	1	-	-	0	- -	1	1	-	-	0	0 0

<sup>1</sup> Med = median.



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

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
<b>All results</b>	<b>20</b>	<b>19</b>	<b>Pos.</b>	<b>1</b>	<b>21</b>	<b>20</b>	<b>Neg.</b>	<b>1</b>	<b>19</b>	<b>18</b>	<b>Pos.</b>	<b>1</b>
NMKL 119:2007	9	9	Pos.	0	9	9	Neg.	0	8	8	Pos.	0
ISO 10272-1:2017	5	4	Pos.	1	6	6	Neg.	0	5	4	Pos.	1
ISO 10272-1:2006	2	2	Pos.	0	2	2	Neg.	0	2	2	Pos.	0
VIDAS	2	2	Pos.	0	2	1	Neg.	1	2	2	Pos.	0
ISO 17995	1	1	Pos.	0	1	1	Neg.	0	1	1	Pos.	0
PCR method	1	1	Pos.	0	1	1	Neg.	0	1	1	Pos.	0

# *Listeria monocytogenes*

## Sample A

*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 B

No target organism was present in the sample.

## Sample C

*L. monocytogenes* (strain 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.

## General remarks

ISO 11290 (different versions), NMKL 136:2010 and RAPID'L.mono were the main methods used in both the quantitative and in the qualitative analysis. In the qualitative analysis, VIDAS® and different PCR-based methods were also common.

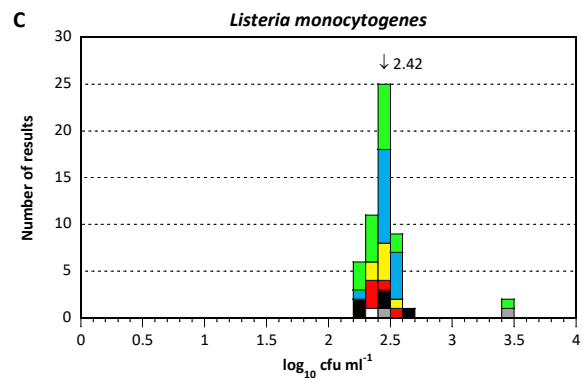
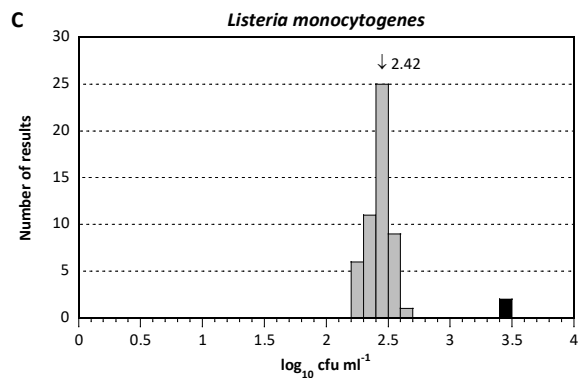
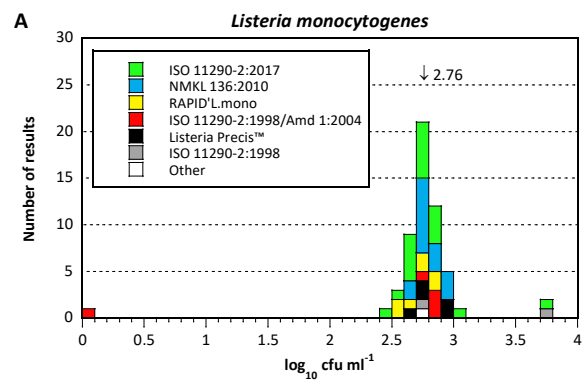
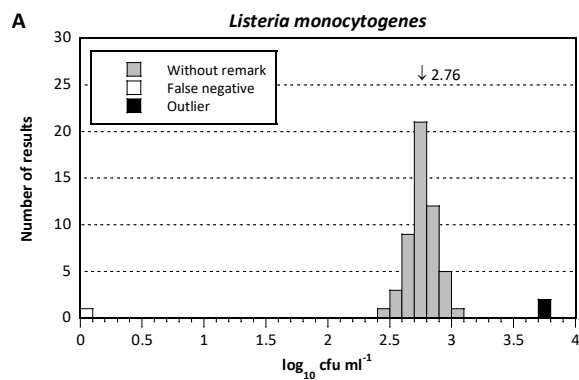
NMKL 136:2010 describes both detection and enumeration of *L. monocytogenes*. In comparison, ISO 11290-1:2017 (qualitative) and ISO 11290-2:2017 (quantitative) detect/enumerate both *Listeria* spp. and *L. monocytogenes*. All of these methods mainly use ALOA for the isolation, on which *L. monocytogenes* 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™ 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*). The alternative methods are all validated by AFNOR and/or NordVal. In addition to the previously mentioned media, many laboratories used either of Oxoid Brilliance™ Listeria agar (previously OCLA), PALCAM, Oxford Listeria selective agar and/or LMBA.

*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). Confirmation was performed by 91 % of the laboratories in the quantitative analysis and by 90 % in the qualitative analysis.

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

Method	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
<b>All results</b>	<b>55</b>	<b>52</b>	<b>2.76</b>	<b>0.11</b>	<b>1</b>	<b>0</b>	<b>2</b>	<b>56</b>	<b>56</b>	<b>-</b>	<b>-</b>	<b>0</b>	<b>-</b>	<b>-</b>	<b>54</b>	<b>52</b>	<b>2.42</b>	<b>0.09</b>	<b>0</b>	<b>0</b>	<b>2</b>
ISO 11290-2:2017	19	18	2.74	0.13	0	0	1	19	19	-	-	0	-	-	18	17	2.40	0.11	0	0	1
NMKL 136:2010	16	16	2.78	0.09	0	0	0	16	16	-	-	0	-	-	16	16	2.46	0.06	0	0	0
RAPID' L.mono	7	7	2.70	0.12	0	0	0	7	7	-	-	0	-	-	7	7	2.44	0.06	0	0	0
ISO 11290-2:1998/Amd 1:2004	5	4	2.80	0.02	1	0	0	5	5	-	-	0	-	-	5	5	2.42	0.06	0	0	0
Listeria Precis™	5	5	2.80	0.14	0	0	0	5	5	-	-	0	-	-	5	5	2.39	0.15	0	0	0
ISO 11290-2:1998	2	1	-	-	0	0	1	2	2	-	-	0	-	-	2	1	-	-	0	0	1
Other	1	1	-	-	0	0	0	2	2	-	-	0	-	-	1	1	-	-	0	0	0



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

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
<b>All results</b>	<b>86</b>	<b>86</b>	<b>Pos.</b>	<b>2</b>	<b>86</b>	<b>86</b>	<b>Neg.</b>	<b>0</b>	<b>85</b>	<b>85</b>	<b>Pos.</b>	<b>1</b>
ISO 11290-1:2017	14	14	Pos.	0	14	14	Neg.	0	13	13	Pos.	0
VIDAS	13	13	Pos.	0	13	13	Neg.	0	13	13	Pos.	0
RAPID <sup>®</sup> L.mono	13	13	Pos.	0	13	13	Neg.	0	13	13	Pos.	0
PCR method	12	11	Pos.	1	12	12	Neg.	0	12	12	Pos.	0
NMKL 136:2010	11	11	Pos.	0	11	11	Neg.	0	11	11	Pos.	0
ISO 11290-1/Amd 1:2004	5	5	Pos.	0	5	5	Neg.	0	5	5	Pos.	0
Listeria Precis <sup>™</sup>	4	4	Pos.	0	4	4	Neg.	0	4	4	Pos.	0
SwabSURE <sup>™</sup> ListeriaP	3	2	Pos.	1	3	3	Neg.	0	3	2	Pos.	1
Other	11	11	Pos.	0	11	11	Neg.	0	11	11	Pos.	0

# Salmonella

## Sample A

No target organism was present in the sample.

## Sample B

*S. Enteritidis* was target organism and was present in approximately  $2.0 \log_{10}$  cfu ml<sup>-1</sup> in the sample. 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.

## Sample C

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

## General remarks

The two most common methods were NMKL 71:1999 (23 %) and ISO 6579-1:2017 (21 %), 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.

Notably, the withdrawn methods ISO 6579:2002/Amd 1:2007 and ISO 6579:2002 were followed by three and four participants, respectively. The new ISO 6579-1:2017 includes important changes, including that detection of  $\beta$ -galactosidase and indole are optional in the confirmation, and that positive results for agglutination against both O and H antigen is required for a strain to be considered as *Salmonella*.

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. Two of the three laboratories that followed NMKL 187 stated that they followed the withdrawn NMKL 187:2007. The new NMKL 187:2016 contains clarifications regarding the choice of the selective agar medium complementary to XLD, and the concentration of Novobiocin in MSR/V. It also contains new paragraphs regarding pre-enrichment of samples from primary animal production, faecal samples and swab samples.

On XLD, which was used by the majority of the laboratories, 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, BGA, Rambach™ agar, RAPID'Salmonella and Harlequin® Salmonella ABC Medium.

Several laboratories chose to analyse with alternative methods like VIDAS® or RAPID'Salmonella, which are validated by AFNOR and/or NordVal against ISO 6579-1:2017. PCR-based methods were also frequently used.

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

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
<b>All results</b>	<b>99</b>	<b>96</b>	<b>Neg.</b>	<b>3</b>	<b>99</b>	<b>99</b>	<b>Pos.</b>	<b>0</b>	<b>98</b>	<b>97</b>	<b>Neg.</b>	<b>1</b>
NMKL 71:1999	23	23	Neg.	0	23	23	Pos.	0	23	23	Neg.	0
ISO 6579-1:2017	21	20	Neg.	1	21	21	Pos.	0	20	19	Neg.	1
VIDAS <sup>1</sup>	15	15	Neg.	0	15	15	Pos.	0	15	15	Neg.	0
PCR method	14	14	Neg.	0	14	14	Pos.	0	14	14	Neg.	0
RAPID'Salmonella	6	4	Neg.	2	6	6	Pos.	0	6	6	Neg.	0
ISO 6579:2002	4	4	Neg.	0	4	4	Pos.	0	4	4	Neg.	0
ISO 6579:2002/Amd 1:2007	3	3	Neg.	0	3	3	Pos.	0	3	3	Neg.	0
NMKL 187 <sup>2</sup>	3	3	Neg.	0	3	3	Pos.	0	3	3	Neg.	0
Other	10	10	Neg.	0	10	10	Pos.	0	10	10	Neg.	0

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

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

# *Escherichia coli* O157

## Sample A

*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.

## Sample B

No target organism was present in the sample.

## Sample C

*E. coli* O157 (strain 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.

## General remarks

Only 22 laboratories performed the analysis. Statistical evaluation of the results is therefore difficult. Several of the false results appear to be due to the use of inappropriate methods.

In total, 36 % of the laboratories followed either NMKL 164:2005 or ISO 16654:2001, which 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. ISO 16654:2001 was last reviewed by ISO in 2018 and remains current. The NMKL method is present in a new version, NMKL 164:2019. The major change from the previous edition is that presumptive *E. coli* O157 shall be sent to a reference/expert laboratory for determination of the virulence profile (*eae* and *stx* genes).

At least two of the participants used methods and/or media that are not primarily designed for detection of *E. coli* O157. These included TEMPO EC and RAPID'E.coli 2 agar, and are included among "Other" in the results summary. The two laboratories that used TEMPO EC and RAPID'E.coli 2 reported negative results for all three samples. A RAPID medium specifically aimed for detection of O157 is available; RAPID'E.coli O157:H7.

The most frequently used media 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. This is similar to SMAC, and contains the chromogenic substrate BGIC that causes sorbitol-negative and β-glucuronidase-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.

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
<b>All results</b>	<b>22</b>	<b>19</b>	<b>Pos.</b>	<b>3</b>	<b>22</b>	<b>21</b>	<b>Neg.</b>	<b>1</b>	<b>22</b>	<b>20</b>	<b>Pos.</b>	<b>2</b>
ISO 16654:2001 <sup>a</sup>	5	5	Pos.	0	5	4	Neg.	1	5	5	Pos.	0
PCR method	4	4	Pos.	0	4	4	Neg.	0	4	4	Pos.	0
VIDAS	3	3	Pos.	0	3	3	Neg.	0	3	3	Pos.	0
NMKL 164:2005	3	2	Pos.	1	3	3	Neg.	0	3	3	Pos.	0
Other	7	5	Pos.	2	7	7	Neg.	0	7	5	Pos.	2

<sup>a</sup> Includes laboratories that stated ISO 16654:2001/Amd 1:2017.

# Pathogenic *Vibrio* spp.

## Sample A

No target organism was present in the sample.

## Sample B

*V. cholerae* was target organism and was present in approximately 2.8 log<sub>10</sub> cfu ml<sup>-1</sup> in the sample. The strain forms typical yellow colonies on TCBS. Upon confirmation, it is oxidase-positive and sensitive to vibriostatic agent O129.

## Sample C

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

## General remarks

Only 19 laboratories performed the analysis, and most used similar methods and media. The majority of the laboratories reported correct results. All laboratories except two (89 %) also stated that they performed some kind of confirmation. The results are therefore difficult to evaluate statistically.

The majority of the laboratories followed either NMKL 156:1997 or ISO 21872-1:2017. Four laboratories followed the retracted ISO/TS 21872-1:2007. ISO 21872-1:2017 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 laboratories stated that colonies were isolated 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.

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

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
<b>All results</b>	<b>19</b>	<b>17</b>	<b>Neg.</b>	<b>2</b>	<b>19</b>	<b>18</b>	<b>Pos.</b>	<b>1</b>	<b>19</b>	<b>18</b>	<b>Neg.</b>	<b>1</b>
NMKL 156:1997	8	8	Neg.	0	8	8	Pos.	0	8	8	Neg.	0
ISO 21872-1:2017	6	5	Neg.	1	6	5	Pos.	1	6	5	Neg.	1
ISO/TS 21872-1:2007	4	3	Neg.	1	4	4	Pos.	0	4	4	Neg.	0
AOAC 988.20:1988 <sup>a</sup>	1	1	Neg.	0	1	1	Pos.	0	1	1	Neg.	0

<sup>a</sup> The laboratory used a modified version of AOAC 988.20:1988.

# *Yersinia enterocolitica*

## Sample A

No target organism was present in the sample.

## Sample B

*Y. enterocolitica* was target organism and was present in approximately  $2.3 \log_{10}$  cfu ml<sup>-1</sup> in the sample. 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*.

## Sample C

No target organism was present in the sample. *C. freundii* was 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.

## General remarks

Most of the participants followed ISO 10273:2017. Two participants followed the retracted 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.

One laboratory followed NMKL 117:1996. A revised version of this was scheduled for publication in 2021, but this appears to have been delayed. The new method will likely be more similar to ISO 10273, for example with parallel enrichment in PSB and ITC. Cold enrichment will also likely be optional and the procedure for this revised.

On CIN, colonies of *Y. enterocolitica* have a typical appearance; a dark red "bull's eye" centre and an outer transparent zone. All participating laboratories except one isolated colonies on CIN, in some cases in combination with another medium. Chromogenic media that can be used in parallel with CIN are for example YECA [2], YeCM [3] and CHROMagar™ *Y. enterocolitica*.

Laboratories that use NMKL methods can also choose a method based on real-time PCR, NMKL 163:2013. With this, enrichment in semi-selective PSB or in non-selective TSBY is followed by DNA extraction and real-time PCR aimed at the *ail* gene in *Y. enterocolitica*, in a similar way as in ISO 10273:2017. NMKL 163:2013 is suitable when high contamination levels are suspected, and the use of NMKL 117:1996 or the ISO method is recommended for samples with suspected low levels of *Y. enterocolitica*.

**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>11</b>	<b>11</b>	<b>Neg.</b>	<b>0</b>	<b>11</b>	<b>11</b>	<b>Pos.</b>	<b>0</b>	<b>11</b>	<b>9</b>	<b>Neg.</b>	<b>2</b>
ISO 10273:2017	5	5	Neg.	0	5	5	Pos.	0	5	5	Neg.	0
ISO 10273:2003	2	2	Neg.	0	2	2	Pos.	0	2	1	Neg.	1
NMKL 117:1996	1	1	Neg.	0	1	1	Pos.	0	1	0	Neg.	1
PCR method	1	1	Neg.	0	1	1	Pos.	0	1	1	Neg.	0
Other	2	2	Neg.	0	2	2	Pos.	0	2	2	Neg.	0

<sup>a</sup> One of the laboratories used a modified version of ISO 10273:2003.

# Outcome of the results of individual laboratory - assessment

## Reporting and evaluation of results

The reported results of all participating laboratories are listed in Annex 1, together with the minimum and maximum accepted values for each analysis. Results that received a remark (false results and outliers) are highlighted in yellow, with bold font.

It is the responsibility of the participating laboratories to correctly report results according to the instructions. When laboratories incorrectly report their results, for example by reporting results for the wrong sample, the results 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.

Z-scores (see below) for individual analyses are shown in Annex 2 and can be used as a tool by laboratories when following up on the results.

The laboratories are not grouped or ranked based on their results. The performance of a laboratory as a whole can be evaluated from the number of false results and outliers that are listed in Annex 1 and below the box plots.

Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol [4]. Samples for follow-up analyses can be ordered at: [www.livsmedelsverket.se/en/PT-extra](http://www.livsmedelsverket.se/en/PT-extra)

## Z-scores, box plots and deviating results

In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated from all laboratory results for each analysis.

The box plots are based on the z-scores listed in Annex 2, and give a comprehensive view of the performance of each laboratory. The range of z-scores is indicated by the size of the box and, for most laboratories, 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 laboratory, with false results excluded, are close to the general mean values calculated for all laboratory results. For each laboratory, the number of false results and outliers are also listed in the tables below the box plots.

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

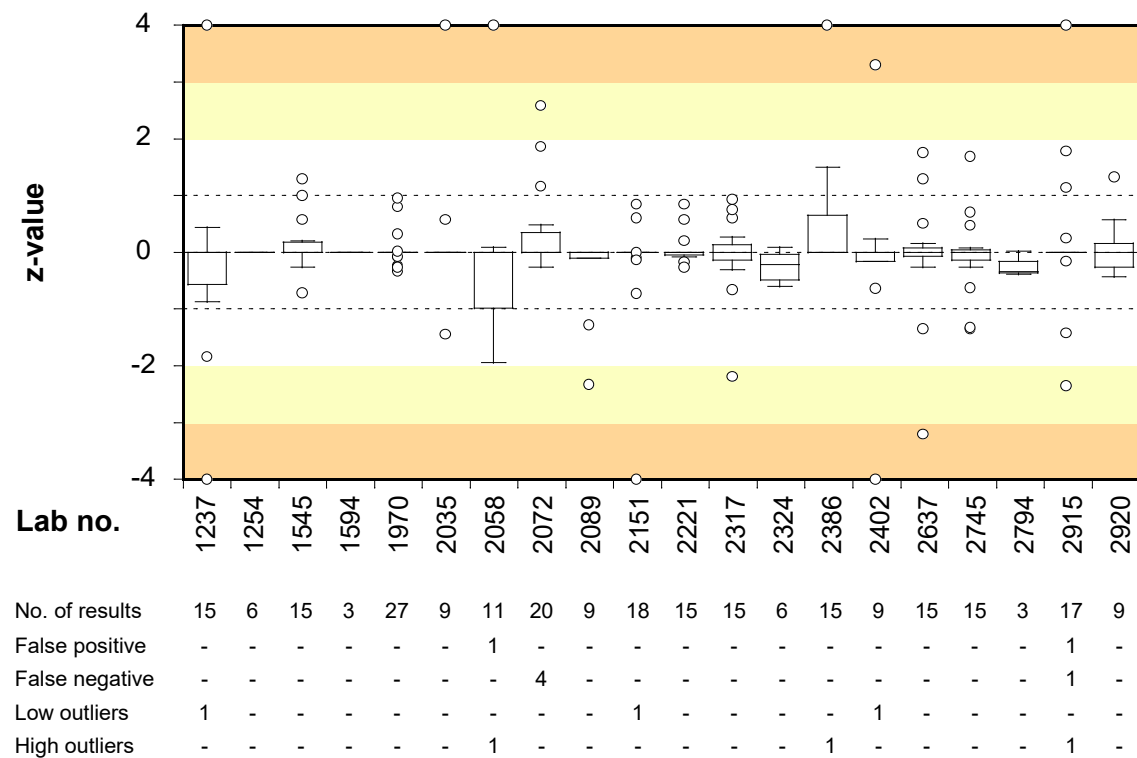
Z-scores are calculated according to the formula:  $z = (x-m)/s$ , where x is the result of the individual laboratory, m is the mean of the results of all participating laboratories, and s is the standard deviation of the participating laboratories, after removing outliers and false results.

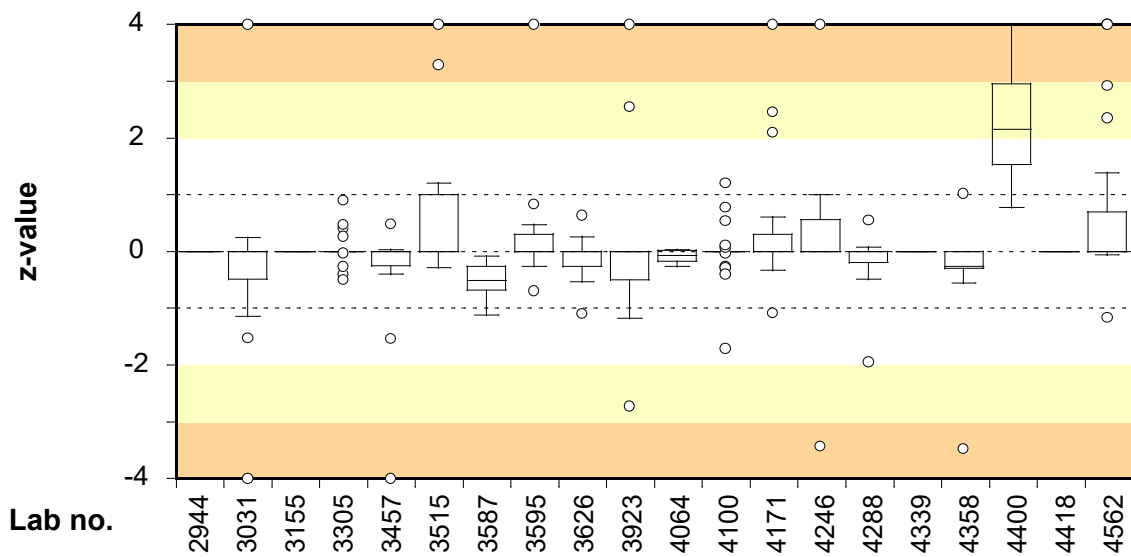
Outliers are included in the figures after being calculated to z-scores in the same way as for other results. False results do not generate any z-scores, and are not included in “No. of results”. Correct results for qualitative analyses and correct negative results for quantitative analyses without target organism generate a z-score of 0.

The laboratory median value is illustrated by a horizontal line in the box. The box includes 50 % of a laboratory’s results (25 % of the results above the median and 25 % of the results below the median). The remaining 50 % are illustrated by lines and circles outside the box. A circle is for technical reasons shown in the plot when a value deviates to certain degree\* from the other values. This does not by itself indicate that the value is an outlier.

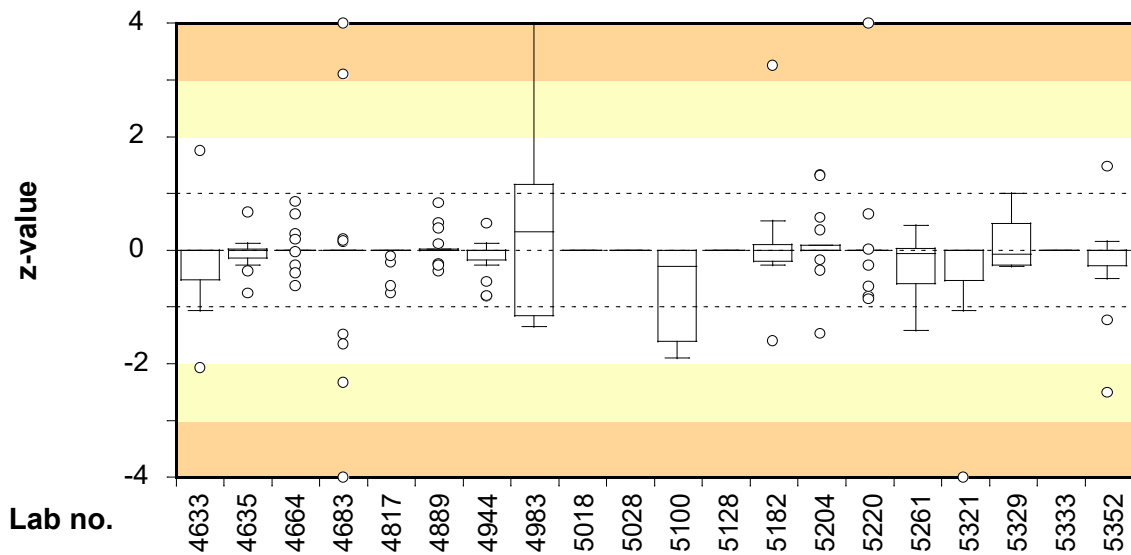
z-scores  $>+4$  and  $<-4$  are positioned at +4 and  $-4$ , respectively, in the plot. The background is divided by lines and shaded fields to simplify identifying the range in which the results are located.

\*  $< [\text{lowest value in the box} - 1,5 \times (\text{highest value in the box} - \text{lowest value in the box})]$   
or  
 $> [\text{highest value in the box} + 1,5 \times (\text{highest value in the box} - \text{lowest value in the box})]$ .

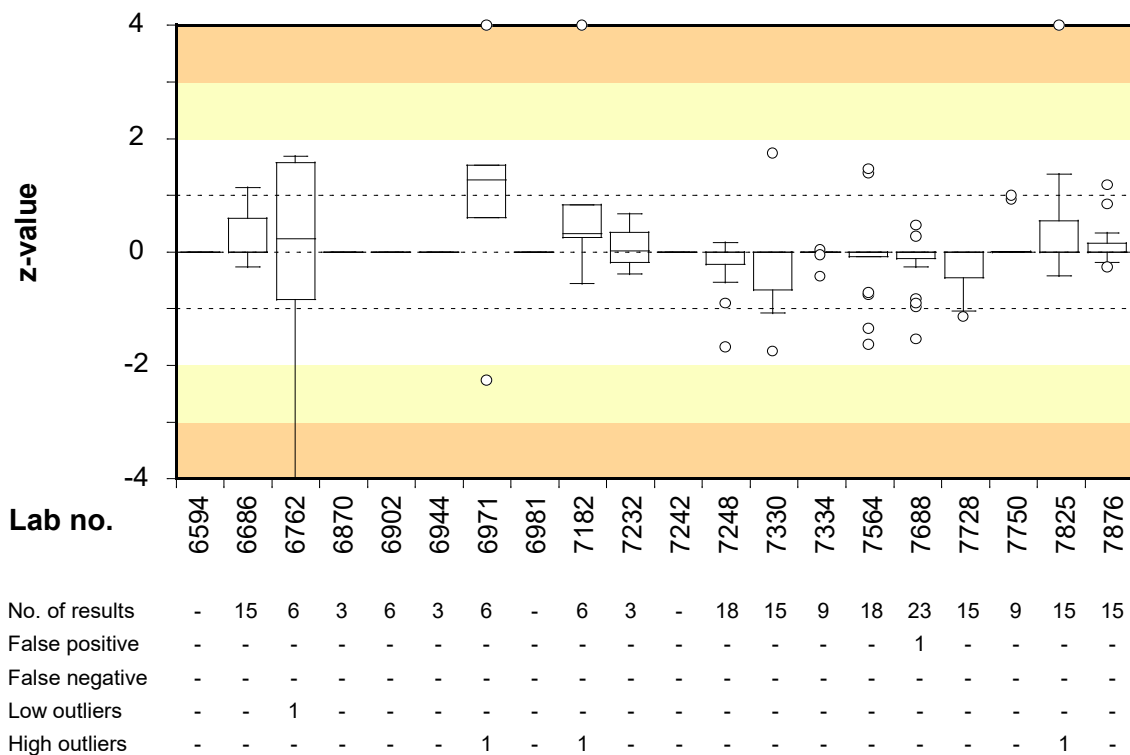
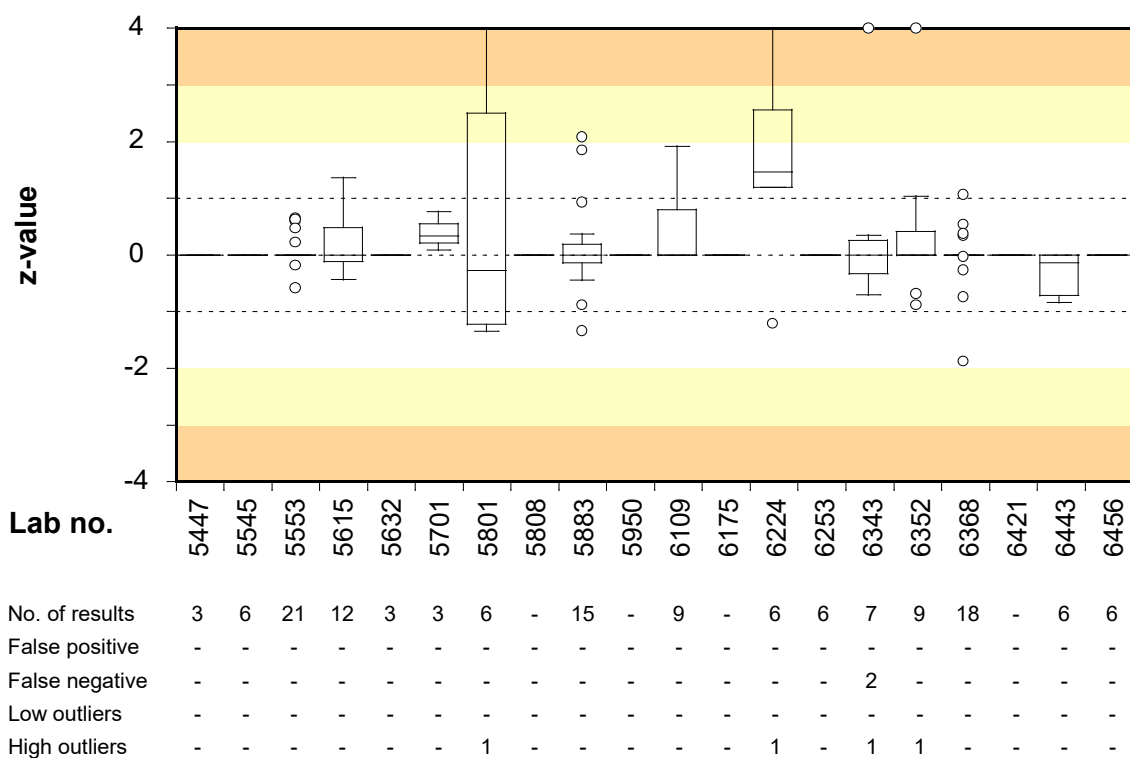




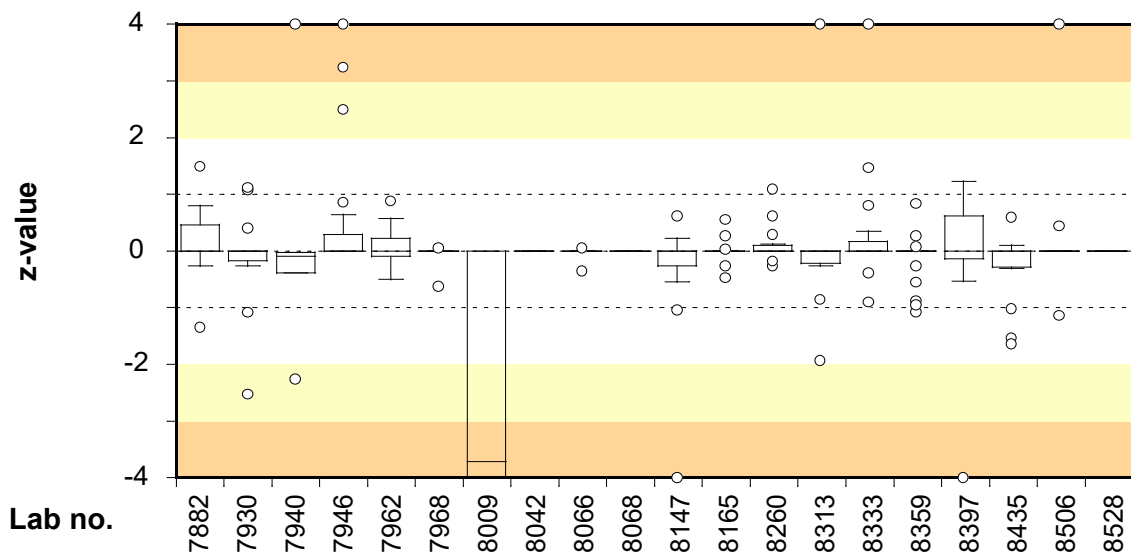
No. of results	-	17	-	18	18	12	6	12	21	14	6	23	15	12	12	-	9	5	-	27
False positive	-	1	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	2
False negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Low outliers	-	1	-	-	1	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-
High outliers	-	1	-	-	-	1	-	1	-	1	-	-	1	1	-	-	-	1	-	4



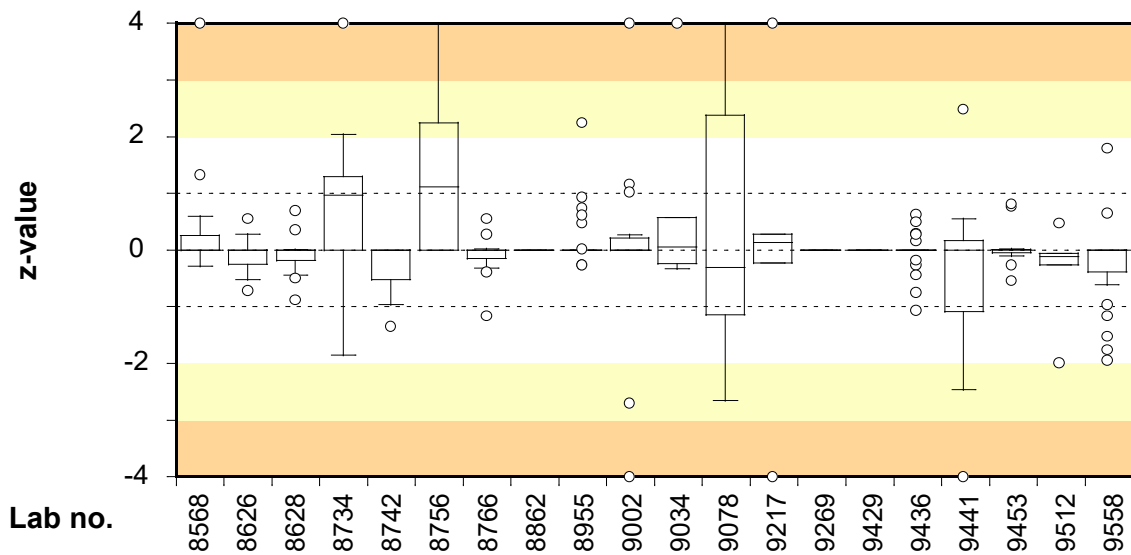
No. of results	15	12	18	21	19	18	15	6	-	3	6	-	9	18	18	8	12	8	6	15
False positive	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	2	2	-	-	-	-	-	-	-	-	1	-	1	-	1	-	-
Low outliers	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
High outliers	-	-	-	1	-	-	-	1	-	-	-	-	-	-	2	-	-	-	-	-



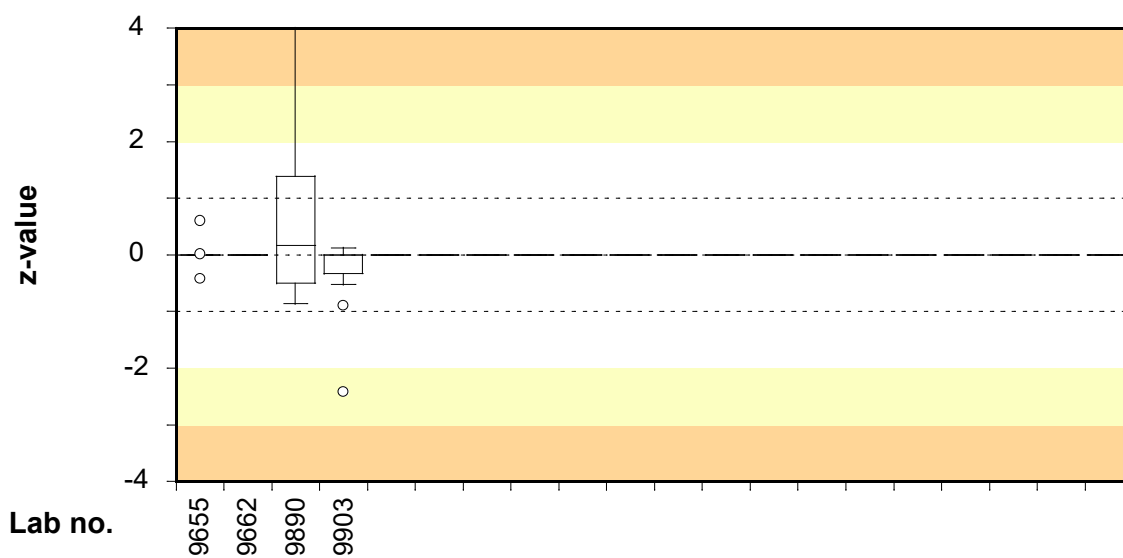




No. of results	12	15	6	29	15	12	5	3	9	-	15	21	15	12	15	21	12	15	12	3	
False positive	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	2	-	-	-	1	-	-	-	-	-	1	-	-	-	
High outliers	-	-	1	3	-	-	-	-	-	-	-	-	1	1	-	-	-	-	1	-	



No. of results	15	15	18	9	9	10	15	3	27	18	6	6	6	3	6	24	15	12	6	27	
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
False negative	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	2	-	-	-	1	-	-	-	1	-	-	-	-
High outliers	1	-	-	1	-	2	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-



Lab no.	9655	9662	9890	9903
No. of results	9	-	6	15
False positive	-	-	-	-
False negative	-	-	-	-
Low outliers	-	-	-	-
High outliers	-	-	1	-

# Test material and quality control

## Test material

Each laboratory received three sample mixtures with freeze-dried microorganisms, designated A-C. The test material was freeze-dried in portions of 0.5 ml in vials, as described by Peterz and Steneryd [5]. Before analysing the samples, the contents of each vial should be dissolved in 254 ml of sterile diluent. The organisms present in the mixtures are listed in the table below.

**Table 12.** Microorganisms in the samples

Sample <sup>1</sup>	Microorganism	Strain		
		SLV no. <sup>2</sup>	Origin	Reference <sup>3</sup>
A	<i>Aeromonas hydrophila</i>	SLV-454	-	CCUG 30208
	<i>Campylobacter coli</i>	SLV-271	faeces, hen	CCUG 45147
	<i>Escherichia coli</i> O157	SLV-479	-	SMI 81186
	<i>Listeria monocytogenes</i>	SLV-444	hamburger meat	CCUG 69007
B	<i>Bacillus cereus</i>	SLV-516	caramel pudding	CCUG 44740
	<i>Kocuria rhizophila</i>	SLV-055	-	CCUG 35073
	<i>Salmonella</i> Enteritidis	SLV-436	-	-
	<i>Vibrio cholerae</i>	SLV-530	-	CCUG 45388
	<i>Yersinia enterocolitica</i>	SLV-408	dog food	CCUG 45643
C	<i>Campylobacter coli</i>	SLV-271	faeces, hen	CCUG 45147
	<i>Citrobacter freundii</i>	SLV-091	-	CCUG 43597
	<i>Escherichia coli</i> O157	SLV-479	-	SMI 811 86
	<i>Listeria monocytogenes</i>	SLV-513	milk	CCUG 44510

<sup>1</sup> The links between the mixtures and the randomised sample numbers are shown in Annex 1.

<sup>2</sup> Internal strain identification no. at the Swedish Food Agency

<sup>3</sup> Culture collection (ATCC: American Type Culture Collection, CCUG: Culture Collection University of Gothenburg, Sweden; SMI: Public Health Agency of Sweden)

## Quality control of the samples mixtures

In order to allow comparison of all freeze-dried samples, it is essential to have aliquots of homogeneous sample mixtures 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 sample mixture 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 [6] and [7] respectively.)

**Table 13.** Concentration mean (m),  $I_2$  and T values from the quality control of the sample mixtures; 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>1</sup>		
	m	$I_2$	T	m	$I_2$	T	m	$I_2$	T
Aerobic microorganisms 30 °C NMKL method no. 86:2013	4.64	0.94	1.20	4.74	<b>3.46</b>	1.64	3.92	<b>3.98</b>	1.54
Enterobacteriaceae NMKL method no. 144:2005	0.90	1.48	1.71	2.03	1.45	2.04	3.43	1.83	1.68
Thermotolerant <i>Campylobacter</i> , quant. NMKL method no. 119:2007	1.80	<b>9.59</b>	1.61	-	-	-	3.07	<b>2.33</b>	1.50
Thermotolerant <i>Campylobacter</i> , qual. NMKL method no. 119:2007	Pos.	-	-	Neg.	-	-	Pos.	-	-
<i>Listeria monocytogenes</i> , quant. NMKL method no. 136:2010	2.81	0.79	1.25	-	-	-	2.44	0.50	1.31
<i>Listeria monocytogenes</i> , qual. NMKL method no. 136:2010	Pos.	-	-	Neg.	-	-	Pos.	-	-
<i>Salmonella</i> NMKL method no. 71:1999	Neg.	-	-	Pos.	-	-	Neg.	-	-
<i>Escherichia coli</i> O157 NMKL method no. 164:2019	Pos.	-	-	Neg.	-	-	Pos.	-	-
Pathogenic <i>Vibrio</i> spp. NMKL method no. 156:1997	Neg.	-	-	Pos.	-	-	Neg.	-	-
<i>Yersinia enterocolitica</i> NMKL method no. 117:1996	Neg.	-	-	Pos.	-	-	Neg.	-	-

– No target organism and therefore no value

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

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Lab no.	Vial	Aerobic micro-organisms 30 °C			Enterobacteriaceae			Thermotolerant Campylobacter			Listeria monocytogenes			Thermotolerant Campylobacter			Listeria monocytogenes			Salmonella			Escherichia coli O157 (VT-neg)			Pathogenic Vibrio spp.			Yersinia enterocolitica			Lab no.				
		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					
5028	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	5028	
5100	3 1 2	3.82	4.57	3.52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	5100		
5128	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5128		
5182	3 1 2	4.461	5.155	3.904	<1	1.947	3.13	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	5182		
5204	3 2 1	4.71	4.67	4.14	-	2.1	3.73	<1	<1	1.62	-	-	-	Pos	Neg	-	-	Pos	Neg	Pos	Neg	Neg	Pos	Neg	-	-	-	-	-	-	-	-	5204			
5220	2 3 1	4.06	4.66	3.72	0	1.86	3.82	-	-	-	2.72	<1	2.41	Pos	Neg	Pos	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	-	-	5220		
5261	3 1 2	4.03	4.64	3.61	0	2	3.76	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	-	-	-	-	-	-	-	5261		
5321	2 1 3	3.06	2.17	3.68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	-	-	-	-	-	-	5321		
5329	1 3 2	4.23	4.8	3.83	<1	1.96	3.93	-	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	-	-	-	-	-	-	-	-	-	-	-	-	-	5329		
5333	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	5333	
5352	3 1 2	4.27	4.68	3.79	0	1.49	3.24	-	-	-	2.92	0	2.4	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	5352		
5447	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5447	
5545	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	5545	
5553	2 1 3	4.13	4.75	3.85	0.2	2.11	3.69	1.13	<1	2.56	-	-	-	Pos	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Neg	Pos	Neg	Pos	-	-	-	-	-	-	-	5553		
5615	1 2 3	4.6	4.62	4.15	<1	1.9	3.97	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	5615	
5632	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5632	
5701	2 1 3	4.54	4.67	3.95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5701	
5801	1 2 3	4.36	4.45	3.65	1.92	2.48	3.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5801	
5808	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5808
5883	2 1 3	4.42	4.8	3.8	0	1.81	3.21	-	-	-	2.96	0	2.61	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	5883	
5950	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5950	
6109	3 2 1	4.6	4.95	4.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6109	
6175	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6175
6224	3 1 2	4.76	4.47	4.38	4.24	2.27	3.99	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6224	
6253	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	6253
6343	3 2 1	4.36	4.71	3.75	3.45	1.97	3.44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Neg	Neg	-	-	-	-	-	-	-	-	-	6343	
6352	2 1 3	4.62	4.52	3.94	3.47	1.85	3.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6352	
6368	3 2 1	4.47	4.71	3.88	<1	1.84	3.04	-	-	-	2.8	<1	2.52	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	Neg	Pos	Neg	-	-	-	-	-	-	6368	
6421	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6421
6443	1 2 3	-	-	-	0	1.82	3.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6443
6456	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	6456
6594	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6594
6686	2 3 1	4.56	4.83	4.04	<1	2.04	3.88	-	-	-	2.79	<1	2.46	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	-	6686
6762	1 2 3	4.82	3.26	4.19	<1	1.82	3.85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6762
6870	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6870
6902	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	-	6902
6944	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6944
6971	3 1 2	4.77	4.31	4.11	3.92	2.26	3.81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6971
6981	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6981
7182	1 2 3	4.56	4.57	3.95	4.16	2.05	3.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7182
7232	3 2 1	4.19	4.76	3.89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7232
7242	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7242
7248	3 2 1	4.24	4.4	3.86	<1	1.88	3.34	-	-	-	2.77	<1	2.44	Pos	Neg	Pos	Pos	Neg	Pos	Neg	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	7248
7330	1 3 2	4.11	4.57	3.7	<1	1.64	3.41	-	-	-	2.64	<1	2.58	-	-	-	Pos	Neg	Pos	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	7330
7334	2 1 3	4.32	4.59	3.88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7334
7564	3 1 2	4.28	4.45	3.74	-	-	-	1.81	<1	3.04	2.68	<1	2.28	Pos	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	7564
7688	2 1 3	3.84	4.53	3.7	<1	1.94	3.34	-	-	-	2.81	<1	2.45	Pos	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Neg	Pos	Neg	7688
7728	3 2 1	4.18	4.54	3.79	0	1.76	3.3	-	-	-	-	-	-	Pos	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	7728
7750	3 2 1	4.31	4.8	4.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7750
7825	1 2 3	4.38	4.59	3.87	2.12	2.11	4.01	-	-	-	2.91	<1	2.47	-	-	-	Pos	Neg	Pos	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	7825
7876	1 3 2	4.29	4.68	3.95	0	1.95	3.67	-	-	-	2.85	0	2.53	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	-	7876
7882	2 1 3	4.43	4.45	4.04	0	2.28	3.78	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	-	7882
7930	3 1 2	4.43	4.49	3.87	<1	2.2	3.54	-	-	-	2.88	<1	2.2	-	-	-	Pos	Neg	Pos	Pos	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	7930
7940	3 2 1	4.3	4.31	3.87	3.6	1.91	3.59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7940
m	s	4.306	4.656	3.885	0.058	1.985	3.621	1.129	0																											

Lab no.	Vial	Aerobic micro-organisms 30 °C			Enterobacteriaceae			Thermotolerant Campylobacter			Listeria monocytogenes			Thermotolerant Campylobacter			Listeria monocytogenes			Salmonella			Escherichia coli O157 (VT-neg)			Pathogenic Vibrio spp.			Yersinia enterocolitica			Lab no.																		
		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																			
7946	3 1 2	4.5	4.7	4.05	4.29	3.21	4.39	2.7	0	2.46	2.79	0	2.47	Pos	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	7946
7962	1 3 2	4.48	4.79	3.84	0	1.95	3.76	-	-	-	2.81	0	2.38	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	7962
7968	1 2 3	-	-	-	-	-	-	-	-	-	2.69	0	2.43	Pos	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	7968	
8009	1 3 2	3.18	2.38	2.11	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8009			
8042	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8042			
8066	1 3 2	-	-	-	-	-	-	-	-	-	2.72	0	2.43	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8066		
8068	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8068			
8147	2 1 3	4.25	4.69	3.78	<1	1.78	2.34	-	-	-	2.73	<1	2.48	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8147		
8165	3 2 1	-	-	-	<1	2.04	3.63	0.9	<1	2.6	-	-	-	Pos	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8165			
8260	1 3 2	4.33	4.7	3.91	0	1.95	3.96	-	-	-	2.76	0	2.48	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8260				
8313	2 3 1	3.72	4.63	3.72	0	3.18	3.59	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8313				
8333	3 1 2	4.41	4.88	4.04	3.2	1.91	3.34	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8333				
8359	1 3 2	4.04	4.57	3.9	<1	2.04	3.88	-	-	-	2.64	<1	2.34	Pos	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8359					
8397	1 3 2	4.5	3.1	4	0	2	4	-	-	-	2.7	0	2.5	-	-	-	Pos	Neg	Pos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8397				
8435	3 1 2	4.3	4.67	4	0	1.79	3.15	-	-	-	2.72	<1	2.28	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8435				
8506	2 1 3	3.96	5.28	3.97	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8506				
8528	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8528			
8568	3 2 1	4.22	4.86	4	3.74	2.03	3.71	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8568				
8626	1 3 2	4.36	4.74	3.83	0	1.94	3.46	-	-	-	2.68	0	2.45	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8626				
8628	1 3 2	4.31	4.52	4.02	0	1.95	3.73	-	-	-	2.71	0	2.38	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8628				
8734	3 1 2	4.7	4.66	4.11	4.56	2.39	3.92	-	-	-	2.57	0	2.26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8734			
8742	3 2 1	4.15	4.45	3.7	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8742				
8756	2 1 3	4.87	5	4.16	4.4	3.07	3.87	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	8756				
8766	2 3 1	4.21	4.74	3.88	0	1.91	3.26	-	-	-	2.76	0	2.45	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8766				
8862	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8862			
8955	3 2 1	4.59	4.77	3.89	<1	2.43	3.54	-	-	-	2.81	<1	2.48	Pos	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	8955			
9002	1 2 3	3.49	3.61	2.96	3.18	2.04	3.98	1.23	0	2.83	2.76	0	2.43	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	9002				
9034	3 2 1	4.35	4.65	3.84	3.69	1.92	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9034			
9078	2 3 1	4.27	5.02	3.79	2.34	1.76	2.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9078			
9217	3 1 2	4.38	3.19	3.94	2.49	1.99	3.55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9217			
9269	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9269		
9429	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	9429				
9436	1 2 3	4.08	4.7	3.68	<1	2.11	3.67	1.04	<1	2.57	2.71	<1	2.45	Pos	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	9436					
9441	3 1 2	2.99	4.74	3.45	0.6	2.05	2.86	-	-	-	2.52	0	2.46	-	-	-	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	9441				
9453	2 1 3	4.54	4.64	3.78	<1	1.99	3.87	-	-	-</																																								



Lab no.	Vial	Aerobic micro-organisms 30 °C			Enterobacteriaceae			Thermotolerant <i>Campylobacter</i>			<i>Listeria monocytogenes</i>			Thermotolerant <i>Campylobacter</i>			<i>Listeria monocytogenes</i>			<i>Salmonella</i>			<i>Escherichia coli</i> O157 (VT-neg)			Pathogenic <i>Vibrio</i> spp.			<i>Yersinia enterocolitica</i>			Lab no.
		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	

N = number of analyses performed  
Min = lowest reported result

Max = highest reported result  
Median = median value

m = mean value  
s = standard deviation

F+ = false positive  
F- = false negative

< = low outlier  
> = high outlier

< OK = lowest accepted value  
> OK = highest accepted value

- High outliers should be regarded as false positive results
- Outlier, false positive or false negative
- Results "larger than" are not evaluated

**Annex 2 Z-scores of all participants - January 2022**

Z-scores are calculated according to the formula:  $z = (x-m)/s$ , where  $x$  = result of the individual laboratory,  $m$  = mean of the results of all participating laboratories,  $s$  = standard deviation of the results from all participating laboratories. Correct negative results in quantitative analyses and correct results in qualitative analyses have obtained a z-score of zero. False results did not generate a z-score. Z-scores from outliers are not real z-scores, but are a practical means to express the results from the outliers. Very low and high z-scores are here limited to -4 and +4 respectively.

2 < |z| ≤ 3, |z| > 3

Lab no.	Vial			Aerobic micro-organisms 30 °C			Enterobacteriaceae			Thermotolerant <i>Campylobacter</i>			<i>Listeria monocytogenes</i>			Thermotolerant <i>Campylobacter</i>			<i>Listeria monocytogenes</i>			<i>Salmonella</i>			<i>Escherichia coli</i> O157 (VT-neg)			Pathogenic <i>Vibrio</i> spp.			<i>Yersinia enterocolitica</i>			Lab no.
	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	A	B	C	
1237	1	3	2	-0.749	-4.000	-0.375	4.000	-0.872	-1.836				0.441	0	0.074				0	0	0	0	0	0							1237			
1254	3	1	2																0	0	0	0	0	0							1254			
1545	2	1	3	1.003	0.157	0.128	-0.263	0.579	-0.714				0.202	0	1.295				0	0	0	0	0	0							1545			
1594	3	2	1																0	0	0	0	0	0							1594			
1970	3	2	1	-0.086	-0.234	-0.080	-0.263	-0.331	0.322	-0.265	0	0.805	0.019	0	0.958	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1970		
2035	2	1	3				4.000	-1.442	0.581																0	0	0		0	0	0	2035		
2058	3	1	2	-1.604	0.092	-1.945	4.000	-1.948	-0.358										0	0	0	0	0	0							2058			
2072	1	2	3	-0.020	0.222	0.490	-0.263	0.478	1.163	0			2.578	0	1.857	0			0	0	0	0	0	0		0	0	0			2072			
2089	3	1	2	-2.330	-0.104	-1.271													0	0	0	0	0	0							2089			
2151	2	3	1	-0.103	-4.000	0.604				-0.723	0	0.850	0.001	0	-0.132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2151			
2221	3	1	2	-0.053	-0.169	-0.028	-0.263	-0.078	0.581				0.202	0	0.846				0	0	0	0	0	0							2221			
2317	3	1	2	0.937	-0.300	-0.650	-0.263	0.275	0.613				0.750	0	-2.189				0	0	0	0	0	0							2317			
2324	2	3	1	-0.482	0.092	-0.598	-0.263	-0.179	-0.034																							2324		
2386	3	2	1	1.498	0.418	1.008	4.000	0.478	0.839										0	0	0	0	0	0		0	0	0			2386			
2402	2	3	1	-0.020	-4.000	0.231	3.307	-0.634	-0.164																0	0	0					2402		
2637	2	3	1	0.508	0.157	-0.131	-0.263	-1.341	-3.206				1.756	0	1.295				0	0	0	0	0	0							2637			
2745	3	2	1	0.079	-1.343	-1.323	-0.263	1.690	0.710				0.476	0	-0.615				0	0	0	0	0	0							2745			
2794	1	2	3	-0.383	0.027	-0.339																										2794		
2915	3	1	2	0.244	1.135	1.786	4.000	-2.352	-0.164	0	-1.423	0				0	0	0	0	0	0	0	0								2915			
2920	3	2	1	0.574	1.331	-0.390	-0.263	-0.432	0.160																0	0	0					2920		
2944	2	1	3																													2944		
3031	2	1	3	-1.521	-4.000	-0.484	4.000	-0.159	-0.837				-1.142	0	0.250				0	0	0	0	0	0							3031			
3155	2	1	3																													3155		
3305	3	1	2	-0.020	0.418	-0.390	-0.263	0.275	0.904				0.476	0	-0.503	0	0	0	0	0	0	0	0								3305			
3457	1	2	3	-0.251	-4.000	-0.080	-0.263	0.478	0.031				-1.535	0	-0.390				0	0	0	0	0	0		0	0	0			3457			
3515	3	1	2	1.201	0.483	3.288	4.000	-0.280	0.807										0	0	0	0	0	0							3515			
3587	1	3	2	-0.449	-0.560	-1.116	-0.263	-0.078	-0.681																							3587		
3595	1	2	3	0.838	-0.691		-0.263	0.124		4.000	0		0.476	0					0	0		0	0								3595			
3626	3	2	1	0.640	-0.365	-0.442	-0.263	0.073	0.257	-0.059	0	-1.093	-0.530	0	-0.278	0	0	0	0	0	0	0	0								3626			
3923	2	1	3	-1.175	-0.300	-0.857	-0.263	4.000	2.555				-2.723	0	-0.503																	3923		
4064	2	1	3	-0.053	-0.169	0.024	-0.263	-0.078	0.031																							4064		
4100	1	2	3	0.541	1.200	-0.028	-0.263	0.073	0.775	-1.707	0	-0.289	0.110	0	-0.390				0	0	0	0	0	0		0	0	0			4100			
4171	2	1	3	0.607	-1.082	2.459	4.000	2.094	-0.326										0	0	0	0	0	0	0	0	0	0			4171			
4246	2	1	3	0.541	-3.430	1.008	4.000	0.528	0.581										0	0	0	0	0	0							4246			
4288	2	3	1	0.079	0.548	-0.131	-0.263	-1.948	-0.487										0	0	0	0	0	0							4288			
4339	3	2	1																													4339		
4358	2	1	3	1.029	-0.293	-0.287	-0.263	-0.558	-3.481																0	0	0					4358		
4400	3	2	1	1.531	2.961	2.148	4.000		0.775															0	0	0						4400		
4418	1	2	3																													4418		
4562	1	2	3	2.356	4.000	2.925	4.000	1.387	-1.167	0	-0.062	4.000	0	4.000	0	0	0	0	0	0	0	0	0	0	0	0	0	0			4562			
4633	3	2	1	-2.066	-0.821	-1.064	-0.263	-0.533	-0.520				1.756	0	-0.166				0	0	0	0	0	0	0	0	0	0			4633			
4635	2	1	3	0.046	-0.365	-0.753	-0.263	0.124	0.678										0	0	0	0	0	0							4635			
4664	1	2	3	0.640	0.287	0.853	-0.263	-0.028	0.192				-0.621	0	-0.390				0	0	0	0	0	0		0	0	0			4664			

Lab no.	Vial			Aerobic micro-organisms 30 °C			Enterobacteriaceae			Thermotolerant Campylobacter			Listeria monocytogenes			Thermotolerant Campylobacter			Listeria monocytogenes			Salmonella			Escherichia coli O157 (VT-neg)			Pathogenic Vibrio spp.			Yersinia enterocolitica			Lab no.
	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				
4683	2	3	1	-2.330	-4.000	-1.478	4.000	3.105	-1.652				0.202	0	0.172				0	0	0	0	0	0				0	0	0	4683			
4817	1	2	3	-0.218	-0.104	-0.753													0	0	0	0	0	0	0	0	0	0	0	0	4817			
4889	2	1	3	0.112	-0.365	-0.235	-0.263	0.478	0.839				0.019	0	0.396				0	0	0	0	0	0				0	0	0	4889			
4944	1	2	3	-0.812	-0.560	-0.805	-0.263	0.124	-0.067				0.476	0	-0.053				0	0	0	0	0	0							4944			
4983	2	1	3	0.112	-1.147	1.164	4.000	-1.341	0.548																						4983			
5018	3	1	2																												5018			
5028	3	1	2																												5028			
5100	3	1	2	-1.604	-0.560	-1.893																0	0	0							5100			
5128	3	1	2																												5128			
5182	3	1	2	0.511	3.255	0.097	-0.263	-0.195	-1.588													0	0	0							5182			
5204	3	2	1	1.333	0.092	1.319				0	-1.465	-0.347	0	-0.166	0	0					0	0	0	0	0	0				5204				
5220	2	3	1	-0.812	0.027	-0.857	-0.263	-0.634	0.645			4.000	0	4.000								0	0	0							5220			
5261	3	1	2	-0.927	-0.114	-1.412	-0.263	0.073	0.438																0	0	0				5261			
5321	2	1	3	-4.000	-4.000	-1.064																0	0	0							5321			
5329	1	3	2	-0.251	0.940	-0.287	-0.263	-0.129	1.001													0	0	0							5329			
5333	1	2	3																			0	0	0							5333			
5352	3	1	2	-0.119	0.157	-0.494	-0.263	-2.503	-1.232			1.482	0	-0.278								0	0	0							5352			
5447	3	1	2												0	0	0														5447			
5545	2	1	3																		0	0	0	0	0	0					5545			
5553	2	1	3		0.614	-0.183	0.652	0.629	0.225	0.003	0	0.475									0	0	0	0	0	0	0	0	0		5553			
5615	1	2	3	0.970	-0.234	1.371	-0.263	-0.432	1.131													0	0	0							5615			
5632	1	3	2																			0	0	0							5632			
5701	2	1	3	0.772	0.092	0.335																									5701			
5801	1	2	3	0.178	-1.343	-1.219	4.000	2.498	-0.714																						5801			
5808	2	1	3																												5808			
5883	2	1	3	0.376	0.940	-0.442	-0.263	-0.887	-1.329			1.847	0	2.082								0	0	0							5883			
5950	3	1	2																												5950			
6109	3	2	1	0.970	1.918	0.801																0	0	0							6109			
6175	1	3	2																												6175			
6224	3	1	2	1.498	-1.213	2.563	4.000	1.437	1.195																						6224			
6253	2	1	3																			0	0	0							6253			
6343	3	2	1	0.178	0.353	-0.701	4.000	-0.078	-0.584																						6343			
6352	2	1	3	1.036	-0.887	0.283	4.000	-0.685	0.419													0	0	0							6352			
6368	3	2	1	0.541	0.353	-0.028	-0.263	-0.735	-1.879			0.385	0	1.071								0	0	0							6368			
6421	3	2	1																												6421			
6443	1	2	3				-0.263	-0.836	-0.714													0	0	0							6443			
6456	2	1	3																			0	0	0							6456			
6594	3	1	2																												6594			
6686	2	3	1	0.838	1.135	0.801	-0.263	0.275	0.839			0.293	0	0.396								0	0	0							6686			
6762	1	2	3	1.696	-4.000	1.578	-0.263	-0.836	0.742																						6762			
6870	1	3	2																			0	0	0							6870			
6902	1	2	3																			0	0	0							6902			
6944	2	1	3																			0	0	0							6944			
6971	3	1	2	1.531	-2.256	1.164	4.000	1.387	0.613																						6971			
6981	1	3	2																												6981			
7182	1	2	3	0.838	-0.560	0.335	4.000	0.326	0.257																						7182			
7232	3	2	1	-0.383	0.679	0.024																									7232			
7242	1	3	2																												7242			
7248	3	2	1	-0.218	-1.669	-0.131	-0.263	-0.533	-0.908			0.110	0	0.172	0	0	0					0	0	0						7248				
7330	1	3	2	-0.647	-0.560	-0.960	-0.263	-1.746	-0.681			-1.078	0	1.745								0	0	0							7330			
7334	2	1	3	0.046	-0.437	-0.054																			0	0	0				7334			





## 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 laboratories carry out some form of internal quality assurance, but their analytical work also has 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 proficiency test, identical test material is analysed by a number of laboratories using their routine methods. The organiser evaluates the results and compiles them in a report.

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

- External and independent evaluation of laboratories analytical competence.
- Improved knowledge of analytical methods with respect to various types of organisms.
- Expert support.
- Tool for inspections regarding accreditation.
- Free extra material for follow-up analyses.

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)