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Proficiency Testing
Microbiology – Food
January 2019

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*

Abbreviations

Media

ALOA	Agar for <i>Listeria</i> according to Ottaviani & Agosti
APW 2%	Alcaline peptone water, 2 % NaCl
BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
BS	Bromthymol blue Saccharose agar
CIN	Cefsulodin Irgasan Novobiocin agar
CT-SMAC	Cefixime Tellurite Sorbitol MacConkey agar
ITC	Irgasan Ticarcillin potassium Chlorate broth
LMBA	<i>Listeria monocytogenes</i> Blood Agar
mCCDA	Modified Charcoal Cephoperazone Deoxycholate Agar
MKTTn	Muller-Kauffmann Tetrathionate/novobiocin broth
MPCA	Milk Plate Count Agar
MRB	Modified Rappaport Broth
MSRV	Modified Semi-solid Rappaport-Vassiliadis enrichment media
mTSB	Modified Tryptone Soya Broth
OCLA	Oxoid Brilliance™ <i>Listeria</i> agar
PSB	Peptone Sorbitol Bile salts broth
PCA	Plate Count Agar
RVS	Rappaport-Vassiliadis Soy peptone broth
SMAC	Sorbitol MacConkey agar
SP	Salt Polymyxin broth
SSDC	<i>Salmonella/Shigella</i> Sodium Deoxycholate Calcium chloride agar
TCBS	Thiosulphate Citrate Bile salts Sucrose agar
TGE	Tryptone Glucose Extract agar
TSA	Tryptic Soya Agar
TSBY	Tryptone Soya Broth with Yeast extract
XLD	Xylose Lysine Deoxycholate agar
VRBG	Violet Red Bile Glucose agar

Organisations

AOAC	AOAC International
AFNOR	French National Standardisation Association
ISO	International Organization for Standardization
NMKL	Nordic Committee for Food Analyses
SLV/NFA	Livsmedelsverket/National Food Agency, Sweden

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General information on results evaluation

Statistical evaluation of the results

Highly deviating values that did not belong to a strictly normal distribution after \log_{10} transformation were identified as statistical outliers (Grubbs' test modified by Kelly (1)). In some cases, subjective adjustments were made to set limits based on knowledge of the mixture's contents. Outliers and false results were not included in the calculations of means and standard deviations. Results reported as "> value" were excluded from the evaluation. Results reported as "< value" were interpreted as being zero (negative result). All reported results are presented in Annex 1.

According to EN ISO/IEC 17043, for which the proficiency testing programme is accredited, it is mandatory for the participating laboratories to report method information for all their analyses. Method information is sometimes difficult to interpret, since many laboratories report a medium that is not included in the standard method they refer to. Results from laboratories that report contradictory data on methods/media have either been excluded from the method analysis, or been added to the group of "Others", together with results from methods and media that are only used by 1-2 laboratories.



Mean values and standard deviations are normally provided for the different analyses. When the total number of reported results for an analysis is fewer than 20, the median is provided instead of the mean value. For method groups with fewer than 5 results, only the number of false results and outliers are provided.

Uncertainty of measurement for the assigned values

The uncertainty of measurement 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 of evaluated parameters is the mean value of the participants' results.




Table and figure legends

Tables

N	number of laboratories that performed the analysis
n	number of laboratories with satisfactory result
m	mean value in \log_{10} cfu ml ⁻¹ (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
	global results for the analysis
	values discussed in the text

Figures

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

	values within the interval of acceptance (Annex 1)
	outliers
	false negative results
*	values outside of the x-axis scale

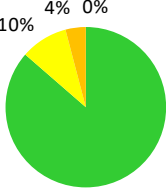
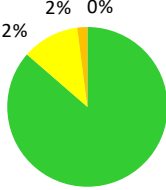
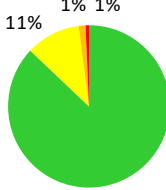
Results of the PT round January 2019

General outcome

Samples were sent to 151 laboratories, 31 in Sweden, 99 in other European countries, and 21 outside Europe. Of the 148 laboratories that reported results, 42 (29 %) provided at least one result that received an annotation. In the previous round with similar analyses (January 2018), the proportion was 31 %.

Individual results for each analysis of 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 Microorganisms in each mixture and % 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>Campylobacter jejuni</i> <i>Proteus mirabilis</i> <i>Salmonella</i> Enteritidis <i>Vibrio parahaemolyticus</i>				<i>Campylobacter jejuni</i> <i>Escherichia coli</i> O157 <i>Kocuria rhizophila</i> <i>Salmonella</i> Dublin <i>Yersinia enterocolitica</i>				<i>Escherichia coli</i> O157 <i>Hafnia alvei</i> <i>Listeria monocytogenes</i> <i>Staphylococcus saprophyticus</i> <i>Vibrio cholerae</i> <i>Yersinia intermedia</i>			
Analysis		Target organism	N	F%	X%	Target organism	N	F%	X%	Target organism	N	F%	X%
Aerobic micro-organisms 30 °C		All	119	0	3	All	121	1	8	All	121	0	6
Enterobacteriaceae		<i>P. mirabilis</i> <i>S. Enteritidis</i>	102	3	10	<i>Y. enterocolitica</i> <i>S. Dublin</i> <i>E. coli</i> O157	102	23*	0*	<i>H. alvei</i> <i>E. coli</i> O157 <i>Y. intermedia</i>	102	1	0
Thermotol <i>Campylobacter</i>	Quant.	<i>C. jejuni</i>	16	0	0	<i>C. jejuni</i>	16	31*	0	-	16	0	-
	Qual.		23	4	-		23	0	-		23	0	-
<i>L. monocytogenes</i>	Quant.	-	59	0	-	-	59	0	-	<i>L. monocytogenes</i>	59	2	8
	Qual.		97	0	-		97	1	-		97	3	-
<i>Salmonella</i>		<i>S. Enteritidis</i>	114	4	-	<i>S. Dublin</i>	114	6	-	-	114	1	-
<i>E. coli</i> O157		-	28	0	-	<i>E. coli</i> O157	28	11	-	<i>E. coli</i> O157	28	11	-
Pathogen. <i>Vibrio</i> spp.		<i>V. parahaemolyticus</i>	20	10	-	-	20	5	-	<i>V. cholerae</i>	20	10	-
<i>Y. enterocolitica</i>		-	12	0	-	<i>Y. enterocolitica</i>	12	0	-	(<i>Y. intermedia</i>)	12	0	-

- no target organism or no value
(*microorganism*) = false positive before confirmation
Microorganism = main target organism
* the results are not evaluated

Aerobic microorganisms 30 °C

Sample A

The strain of *P. mirabilis* was present in the highest concentration and was thus the main target organism. Two laboratories reported problems with swarming colonies on the agar plates; however, this does not appear to have had an impact on the results for the majority of the laboratories. Two low and two high outliers were reported.

Sample B

The strains of *K. rhizophila* and *Y. enterocolitica* were present in the highest concentrations and were thus the main target organisms. Five low and five high outliers were reported, as well as one false negative result.

Sample C

The strains of *H. alvei* and *S. saprophyticus* were present in the highest concentrations and were thus the main target organisms. Four low and three high outliers were reported.

General remarks

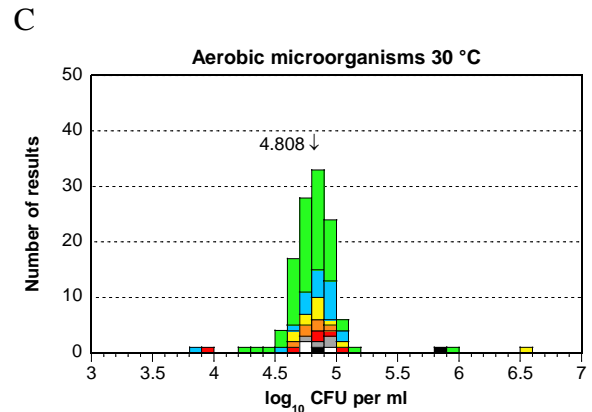
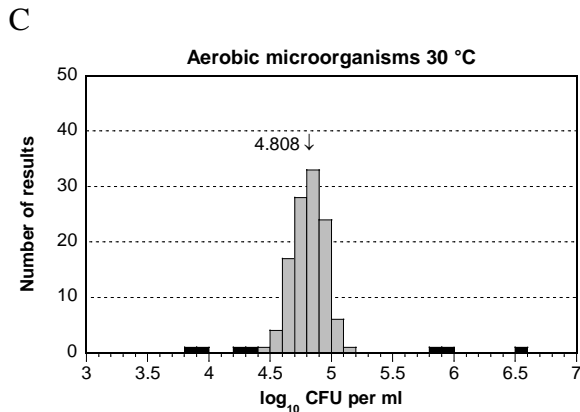
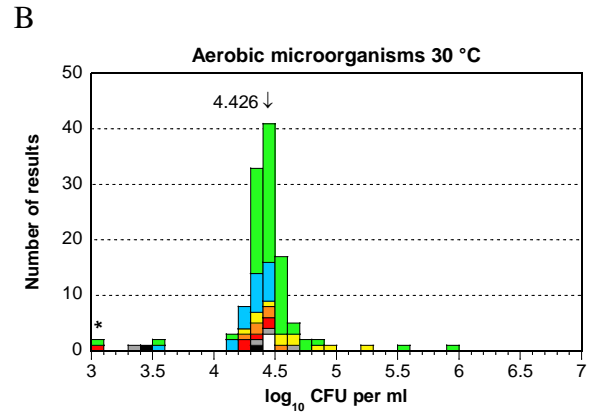
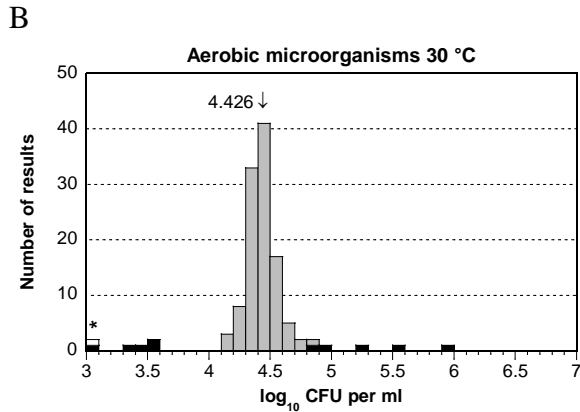
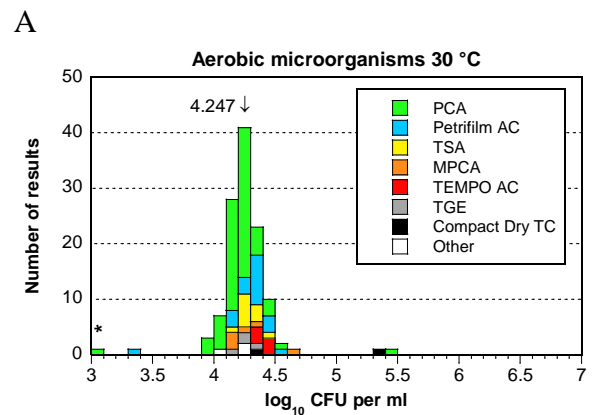
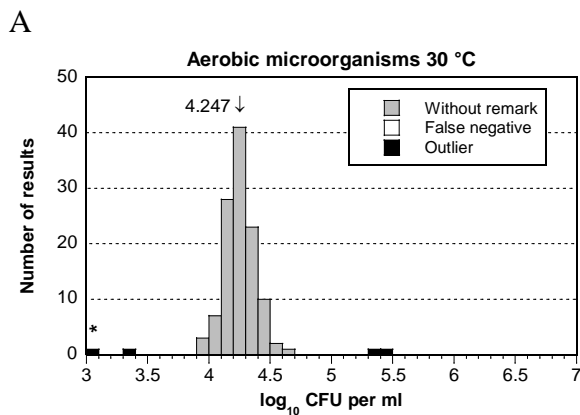
As a whole, the analyses were without problem. The mean values for the different methods and media were also very similar. The small number of outliers that were reported could not be attributed to the use of a specific method or medium.

The majority of the laboratories followed either NMKL 86:2013 (33 %), ISO 4833-1:2013 (14 %) or used 3M™ Petrifilm™ Aerobic Count (17 %). A smaller number of laboratories stated that they followed the older versions NMKL 86:2006 or ISO 4833:2003. The use of ISO 4833-2:2013, which is based on surface-spreading, was also reported. Six laboratories used TEMPO® AC (bioMérieux® SA, Marcy l'Étoile, France), which is based on MPN (Most Probable Number). With this method, the sample is incubated in a card that contains wells with different volumes. A substrate in the wells emits fluorescence when hydrolysed by the microorganisms. The concentration is determined by the number and size of the fluorescent wells.

Both NMKL 86:2013 and ISO 4833-1:2013 are based on a pour-plate method with plate count agar (PCA), and incubation at 30 °C for 72 h. Laboratories that use Petrifilm AC may however use different times/temperatures, depending on which method is followed. For example, AOAC® 990.12 prescribes incubation at 35 °C for 48 h while AFNOR 3M 01/1-09/89 prescribes 30 °C for 72 h. In addition to PCA and Petrifilm AC, the use of tryptone soya agar (TSA), milk plate count agar (MPCA), tryptone glucose extract agar (TGE), and Compact Dry™ TC was reported.

Results from analysis of aerobic microorganisms, 30 °C

Medium	N	Sample A					Sample B					Sample C				
		n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
All results	119	115	4.247	0.126	0	2 2	110	4.426	0.122	1	5 5	114	4.808	0.127	0	4 3
PCA	67	65	4.203	0.113	0	1 1	63	4.447	0.105	1	1 3	65	4.786	0.132	0	2 1
Petrifilm AC	20	19	4.324	0.109	0	1 0	20	4.330	0.108	0	1 0	20	4.851	0.121	0	1 0
TSA	11	11	4.287	0.069	0	0 0	9	4.530	0.174	0	0 2	10	4.807	0.135	0	0 1
MPCA	6	6	4.283	0.202	0	0 0	6	4.407	0.076	0	0 0	6	4.815	0.098	0	0 0
TEMPO AC	6	6	4.409	0.056	0	0 0	5	4.360	0.111	0	1 0	5	4.856	0.133	0	1 0
TGE	4	4	-	-	0	0 0	3	-	-	0	1 0	4	-	-	0	0 0
Compact Dry TC	2	1	-	-	0	0 1	1	-	-	0	1 0	1	-	-	0	0 1
Other	3	3	-	-	0	0 0	3	-	-	0	0 0	3	-	-	0	0 0



Enterobacteriaceae

Sample A

The strains of *P. mirabilis* and *S. Enteritidis* are members of Enterobacteriaceae. The strain of *P. mirabilis* was however present in a much higher concentration than *S. Enteritidis*, and was thus the main target organism. Eight low and two high outliers were reported, as well as three false negative results.

Sample B

The strains of *Y. enterocolitica*, *S. Dublin* and *E. coli* O157 are members of Enterobacteriaceae. The strain of *Y. enterocolitica* was however present in a much higher concentration (estimated \log_{10} 3.5 cfu ml⁻¹) than the other two strains (both approximately \log_{10} 1.2 cfu ml⁻¹) and was therefore the main target organism. At the National Food Agency, the strain of *Y. enterocolitica* formed very small colonies on violet red bile glucose agar (VRBG), and a magnifying lens was required when counting the colonies. At the same time, the low concentrations of *S. Dublin* and *E. coli* O157 were likely difficult to detect when analysing the recommended dilutions. (Based on the concentration of *Y. enterocolitica*, analysis of the dilutions 10⁻¹ – 10⁻⁴ was recommended.)

The results had a very wide distribution. In total, 102 laboratories reported results. Of these, 23 reported negative results and 29 reported results lower than \log_{10} 2.0 cfu ml⁻¹. Due to the above circumstances, the results for sample B are not evaluated.

Comment: The results for sample B are not statistically evaluated. Therefore, no z-scores have been calculated for these results, and they are also not included in the tables located below the box plots.

Sample C

The strains of *H. alvei*, *E. coli* O157 and *Y. intermedia* are members of Enterobacteriaceae. The strain of *H. alvei* was however present in a much higher concentration (approximately \log_{10} 4.5 cfu ml⁻¹) than the other two strains (both below \log_{10} 1.0 cfu ml⁻¹) and was therefore the main target organism. No outliers were reported, but one false negative result.

General remarks

As in previous proficiency testing rounds, most laboratories followed NMKL 144:2005 (51 %) or used 3M™ Petrifilm™ Enterobacteriaceae (20 %). ISO 21528-2 was used by 13 % of the laboratories, divided almost equally between the new ISO 21528-2:2017 and the older ISO 21528-2:2004 (7 % and 6 % respectively). An additional four laboratories followed the older ISO 21528-1:2004, which is based on MPN (Most Probable Number). The new version of the MPN method, ISO 21528-1:2017, is recommended when the expected level of Enterobacteriaceae is lower than 100 cfu g⁻¹. As in the analysis of aerobic microorganisms, a few laboratories used methods based on detection of fluorescence (TEMPO® Enterobacteriaceae). One laboratory used RAPID'Enterobacteriaceae (RAPID' EB), which is validated against ISO 21528-2:2017 (AFNOR BRD: 07/24 - 11/13).

Enterobacteriaceae are gram-negative and oxidase-negative bacteria that ferment glucose with the production of acid by-products. Both NMKL 144 and ISO 21528-2 are based on a pour-plate method with VRBG. With this medium, Enterobacteriaceae form pink/red colonies, with or without a bile 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.

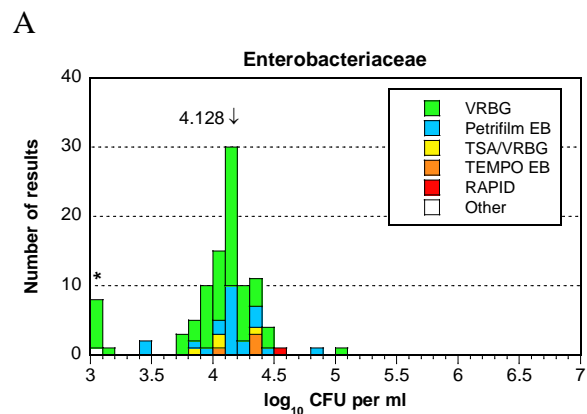
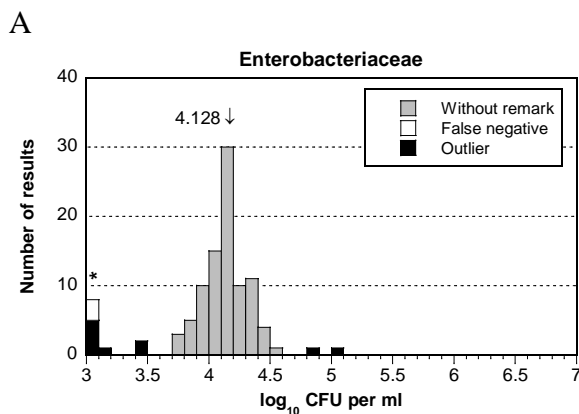
With NMKL 144:2005, presumptive colonies isolated on VRBG are confirmed with an oxidase test. With ISO 21528-2:2017, colonies are instead confirmed both with an oxidase test and with a test for glucose fermentation. Oxidase-negative colonies that also ferment glucose in glucose oxidation/fermentation (OF) medium are confirmed as Enterobacteriaceae. In total, 74 % of the laboratories stated that they performed some kind of confirmation test. The vast majority of these specified that this consisted of an oxidase test. A test for fermentation of glucose was mainly performed by laboratories that followed ISO 21528-2:2017.

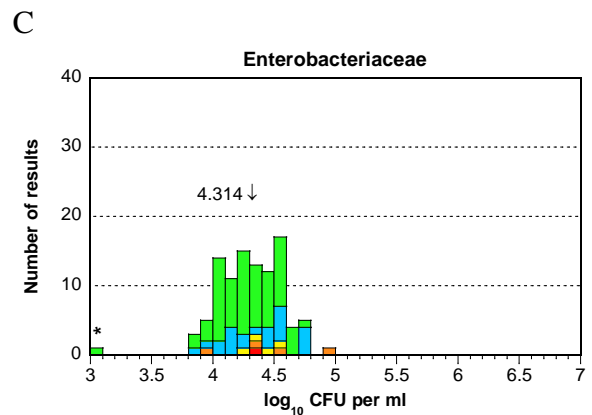
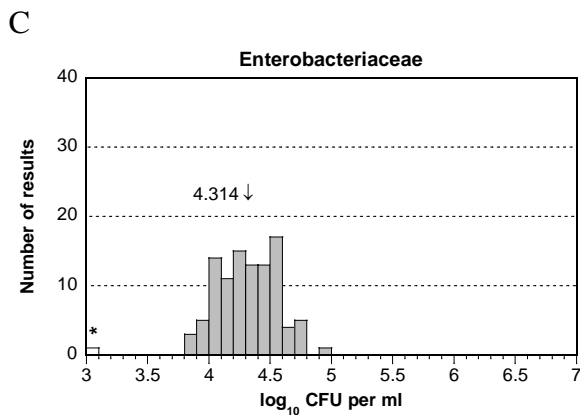
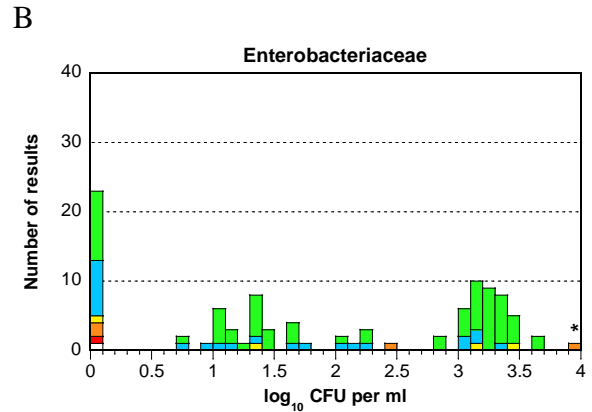
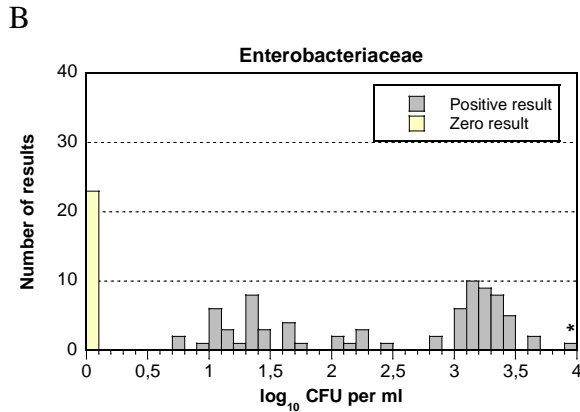
The results for the different methods and media were similar for samples A and C. For sample B however, users of VRBG as a whole appear to have succeeded better than users of Petrifilm EB. For this sample, users of VRBG reported both relatively fewer false negative results and more results near the expected concentration of \log_{10} 3.5 cfu ml⁻¹. It is therefore possible that the strain of *Y. enterocolitica* was more easily identified with VRBG than with Petrifilm EB. The remaining media were used by too few laboratories to be evaluated. It can also be mentioned that none of the laboratories that used the MPN method ISO 21528-1:2004 reported false-negative results for sample B. As mentioned previously, the method is adapted for detection of low levels of Enterobacteriaceae.

Results from analysis of Enterobacteriaceae

Medium	N	Sample A						Sample B*						Sample C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	102	89	4.128	0.169	3	8	2	102	1.882	1.339	23	0	0	101	4.314	0.235	1	0	0
VRBG	69	60	4.103	0.162	2	6	1	70	2.121	1.254	10	0	0	68	4.285	0.213	1	0	0
Petrifilm EB	23	20	4.165	0.153	0	2	1	23	1.331	1.240	8	0	0	23	4.355	0.272	0	0	0
TSA/VRBG	4	4	-	-	0	0	0	4	-	-	1	0	0	4	-	-	0	0	0
TEMPO EB	4	4	-	-	0	0	0	4	-	-	2	0	0	4	-	-	0	0	0
RAPID' EB	1	1	-	-	0	0	0	1	-	-	1	0	0	1	-	-	0	0	0
Other	1	0	-	-	1	0	0	1	-	-	1	0	0	1	-	-	0	0	0

* The results for sample B are not evaluated.





Thermotolerant *Campylobacter*

Sample A

The strain of *C. jejuni* was target organism and was present at approximately log₁₀ 2.3 cfu ml⁻¹ in the sample. No false negative results were reported in the quantitative analysis. One false negative result was reported in the qualitative analysis.

Sample B

The same strain of *C. jejuni* as in sample A was target organism, but was here instead present at approximately log₁₀ 1.3 cfu ml⁻¹ in the sample (approximately 22 cfu ml⁻¹).

Five false negative results were reported in the quantitative analysis. Depending on the choice of method and the volume that was inoculated on the plates, the low concentration of *C. jejuni* may have been difficult to detect. The amount of *Campylobacter* spp. in a freeze-dried sample may also decrease somewhat during prolonged transport at room temperature, as indicated by tests at the National Food Agency. The quantitative analysis is therefore not evaluated.

It should also be mentioned that *Campylobacter* spp. are sensitive to mechanical stress and to dehydration. Another explanation for low (or false-negative) results can therefore be a too harsh surface spreading. At the National Food Agency, *Campylobacter* spp. are carefully spread onto pre-warmed plates and the final drying of the bacterial suspension is done by leaving the lids of the plates slightly open (maximum five minutes).

A total of 23 laboratories reported results in the qualitative analysis. In contrast to the quantitative analysis, no false results were reported. This is likely since the qualitative methods normally contain an enrichment step.

Comment: The results from the quantitative analysis of sample B are not statistically evaluated. Therefore, no z-scores have been calculated for these results, and they are also not included in the tables located below the box plots.

Sample C

No target organism was present in the sample. No false positive results were reported, neither in the quantitative nor in the qualitative analysis.

General remarks

Only 16 and 23 laboratories performed the quantitative and qualitative analysis, respectively. Most of them used similar methods and media. The results are therefore difficult to evaluate statistically.

Campylobacter spp. are gram-negative, oxidase-positive and catalase-positive bacteria. On modified charcoal cephaloperazone deoxycholate agar (mCCDA) they normally form flat or convex colonies, with a grey or white colour and a glossy surface. Confirmation is usually 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. The vast majority of laboratories in both the quantitative and the qualitative analysis reported performing some kind of confirmation, most commonly a motility test and/or an oxidase test.

NMKL 119 and ISO 10272 (various versions) were the most commonly used methods in both the quantitative and the qualitative analysis. Most laboratories were now using the new ISO 10272-2:2017 and 10272-1:2017 instead of the older ISO 10272-2:2006 and 10272-1:2006. In the qualitative analysis, one laboratory reported following ISO 17995, which is a method for detection of *Campylobacter* in water samples. It can also be mentioned that NMKL 119:2007 is being revised, and that the new version will likely be more similar to ISO 10272-2:2017 and 10272-1:2017.

The majority of the laboratories (83 %) in the qualitative analysis used Bolton broth for the enrichment step, but the use of Preston broth was also reported. For the selective step, most laboratories (87 %) used mCCDA (87 %). In the quantitative analysis, all laboratories except one reported using mCCDA.

Results from quantitative analysis of thermotolerant *Campylobacter*

Method	N	Sample A					Sample B**					Sample C								
		n	Med*	s	F	<	>	n	Med*	s	F	<	>	n	Med*	s	F	<	>	
All results	16	16	2.11	0.33	0	0	0	16	0.60	0.49	5	0	0	0	16	-	-	0	-	-
ISO 10272-2:2017	10	10	2.07	0.31	0	0	0	10	0.40	0.52	4	0	0	0	10	-	-	0	-	-
NMKL 119:2007	5	5	2.10	0.37	0	0	0	5	0.70	0.36	1	0	0	0	5	-	-	0	-	-
ISO 10272-2:2006	1	1	-	-	0	0	0	1	-	-	0	0	0	0	1	-	-	0	-	-

* Med = median

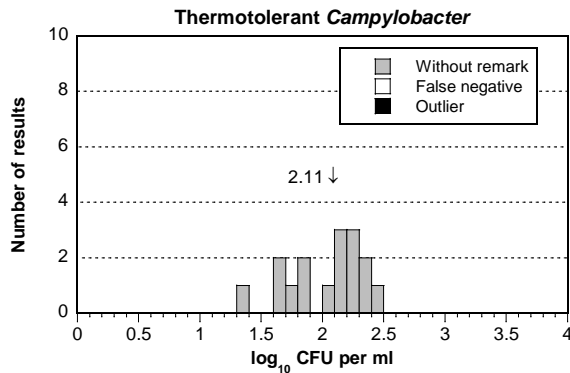
** The results for sample B are not evaluated.

Results from qualitative analysis of thermotolerant Campylobacter

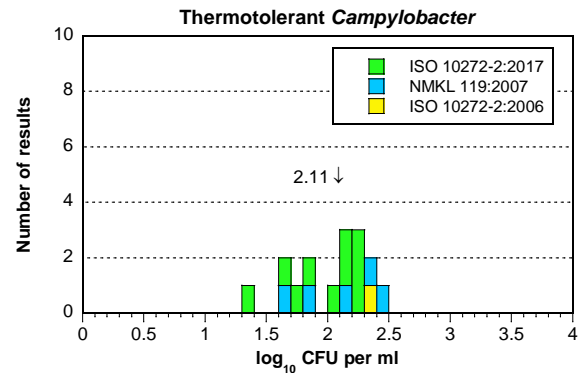
Method	N	Sample A			Sample B			Sample C		
		n	+/-	F	n	+/-	F	n	+/-	F
All results	23	22	Pos	1	23	Pos	0	23	Neg	0
NMKL 119:2007	11	11	Pos	0	11	Pos	0	11	Neg	0
ISO 10272-1:2017	6	5	Pos	1	6	Pos	0	6	Neg	0
ISO 10272-1:2006	3	3	Pos	0	3	Pos	0	3	Neg	0
Other*	3	3	Pos	0	3	Pos	0	3	Neg	0

* The group Other includes ISO 17995 (water method), VIDAS, and a PCR method.

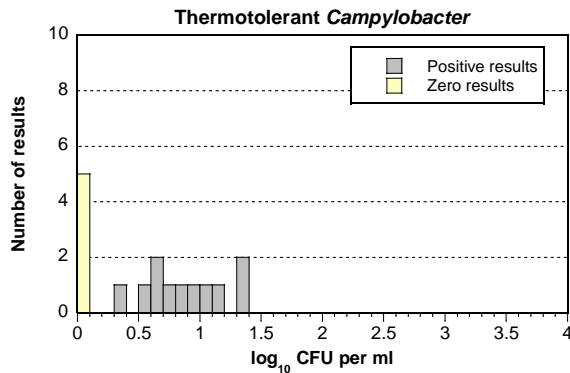
A



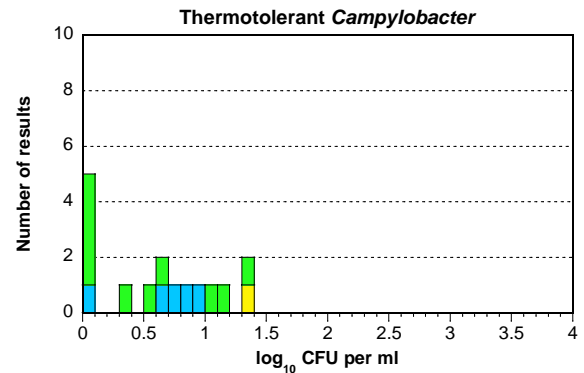
A



B



B



Listeria monocytogenes

Sample A

No target organism was present in the sample. No false negative results were reported, neither in the quantitative nor in the qualitative analysis.

Sample B

No target organism was present in the sample. One false positive result was reported in the qualitative analysis.

Sample C

The strain of *L. monocytogenes* was target organism and was present at approximately $\log_{10} 2.5 \text{ cfu ml}^{-1}$ in the sample. Five low outliers and one false negative result were

reported in the quantitative analysis. Three false negative results were reported in the qualitative analysis.

General remarks

As a whole, the analyses were without problem for the laboratories. Outliers and false results could not be attributed to the use of a specific method or medium.

New versions of ISO 11290-1 (qualitative) and ISO 11290-2 (quantitative) were published in 2017. The revised methods distinguish between detection/enumeration of *Listeria* spp. and *Listeria monocytogenes*. Changes have also been made in which confirmation tests that should be performed. The qualitative method ISO 11290-1:2017 is based on primary enrichment in half-Fraser broth, followed by secondary enrichment in Fraser broth. Aliquots from both enrichments are plated onto selective agar for *Listeria* according to Ottaviani & Agosti (ALOA) and onto another selective medium chosen by the laboratory. In the quantitative method ISO 11290-2:2017 the sample is first suspended in buffered peptone water (BPW) or in half-Fraser broth and material is then transferred from these to ALOA. The quantitative and qualitative methods used in NMKL 136:2010 are similar to the ones in the ISO methods.

On ALOA, *L. monocytogenes* form blue-green colonies due to β -glucosidase activity. The colonies are 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. *L. monocytogenes* can be confirmed by microscopy, catalase test, and by tests of β -haemolysis and carbohydrate utilisation (fermentation of rhamnose and xylose). *L. monocytogenes* is catalase-positive, displays β -haemolysis on blood agar, and ferments rhamnose but not xylose. Confirmation can also be done by the increased and decreased β -haemolysis displayed by *L. monocytogenes* in the presence of *Staphylococcus aureus* and *Rhodococcus equi* respectively (CAMP test).

Taken together, different versions of ISO 11290-2 and ISO 11290-1 were the most used methods in both the quantitative and the qualitative analysis (42 % and 26 % of the laboratories respectively). The two versions were used by similar numbers of laboratories. In addition to ISO 11290, the use of NMKL 136:2010, RAPID'L.mono and VIDAS[®] was common. The use of *Listeria* Precis[™] was also reported. RAPID'L.mono uses a chromogenic medium that identifies the enzyme phosphatidylinositol phospholipase C (PI-PLC) in *L. monocytogenes*. The medium also identifies both *Listeria* spp. and *L. monocytogenes* by the fact that they do not metabolize xylose. The method in *Listeria* Precis[™] in a similar way uses a chromogenic medium that detects *Listeria* spp. and *L. monocytogenes* β -glucosidase cleavage of X-glucoside in the medium Brilliance[™] *Listeria*. In comparison, VIDAS[®] is based on detection of specific *L. monocytogenes* antigen, by a method based on ELFA (Enzyme Linked Fluorescent Assay). The alternative methods are all validated by AFNOR and/or NordVal.

ALOA and Oxoid Brilliance[™] *Listeria*-agar (previously OCLA) were the most commonly used media, but PALCAM, *Listeria monocytogenes* blood agar (LMBA), Oxford *Listeria* selective agar, and other types of chromogenic media were also used. Confirmation was carried out by most laboratories. In total, confirmation (in various forms) was carried out by 86 % and 85 % of the laboratories in the quantitative and qualitative analyses respectively.

Results from quantitative analysis of Listeria monocytogenes

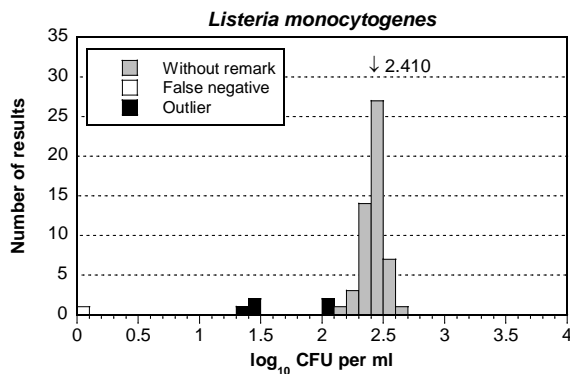
Method	N	Sample A					Sample B					Sample C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	59	59	-	-	0	-	-	59	-	-	0	-	-	53	2.410	0.086	1	5	0
NMKL 136:2010	15	15	-	-	0	-	-	15	-	-	0	-	-	14	2.401	0.094	0	1	0
RAPID' L.mono	14	14	-	-	0	-	-	14	-	-	0	-	-	12	2.432	0.087	0	2	0
ISO 11290-2:1998 /Amd 1:2004	13	13	-	-	0	-	-	13	-	-	0	-	-	12	2.413	0.074	0	1	0
ISO 11290-2:2017	10	10	-	-	0	-	-	10	-	-	0	-	-	10	2.380	0.095	0	0	0
Listeria Precis™	3	3	-	-	0	-	-	3	-	-	0	-	-	1	-	-	1	1	0
ISO 11290-2:1998	2	2	-	-	0	-	-	2	-	-	0	-	-	2	-	-	0	0	0
Other	2	2	-	-	0	-	-	2	-	-	0	-	-	2	-	-	0	0	0

Results from qualitative analysis of Listeria monocytogenes

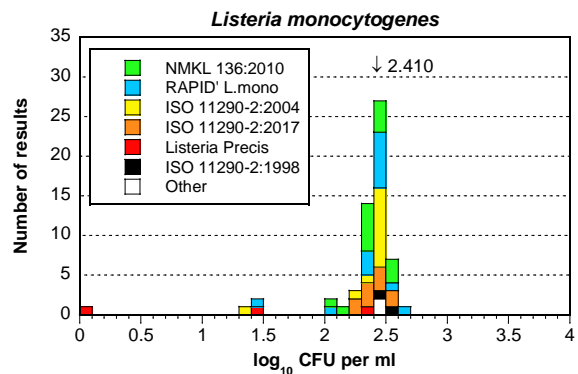
Method	N	Sample A			Sample B			Sample C		
		n	+/-	F	n	+/-	F	n	+/-	F
All results	97	97	Neg	0	96	Neg	1	94	Pos	3
RAPID' L.mono	17	17	Neg	0	17	Neg	0	17	Pos	0
VIDAS	17	17	Neg	0	17	Neg	0	16	Pos	1
NMKL 136:2010	12	12	Neg	0	11	Neg	1	11	Pos	1
PCR method	11	11	Neg	0	11	Neg	0	11	Pos	0
ISO 11290-1:2017	10	10	Neg	0	10	Neg	0	10	Pos	0
ISO 11290-1:1996 /Amd 1:2004	10	10	Neg	0	10	Neg	0	10	Pos	0
Listeria Precis	5	5	Neg	0	5	Neg	0	4	Pos	1
ISO 11290-1:1996	5	5	Neg	0	5	Neg	0	5	Pos	0
Other	10	10	Neg	0	10	Neg	0	10	Pos	0

* The group Other includes SwabSure Listeria P, IDF Standard 143A:1995, as well as national and/or company-specific methods.

C



C



Salmonella

Sample A

The strain of *S. Enteritidis* was target organism and was present at approximately \log_{10} 1.9 cfu ml⁻¹ in the sample. Four false negative results were reported.

Sample B

The strain of *S. Dublin* was target organism and was present at approximately \log_{10} 1.2 cfu ml⁻¹ in the sample. At the National Food Agency, on Brilliance™ *Salmonella* agar it has been observed to form atypical flat colonies, which have a white or slightly pink colour. On xylose lysine deoxycholate agar (XLD) it however forms typical transparent red colonies with a black center. Seven false negative results were reported.

Sample C

No target organisms was present in the sample. One false negative result was reported.

General remarks

Most laboratories followed either ISO 6579-1:2017 or NMKL 71:1999, which are very similar. Both are based on pre-enrichment in buffered peptone water (BPW), followed by selective enrichment in Rappaport-Vassiliadis soy peptone broth (RVS). Subsequent inoculation is on XLD and a second selective medium chosen by the laboratory. In contrast to NMKL 71:1999, ISO 6579-1:2017 also includes selective enrichment in Muller-Kauffmann tetrathionate/novobiocin broth (MKTn). With the ISO method, RVS can also be replaced by modified semi-solid Rappaport-Vassiliadis enrichment media (MSRV) for the analysis of motile *Salmonella*. Confirmation is by biochemical (e.g. mannitol and urea) and serological (e.g. *Salmonella* polyvalent O and H antisera) tests. Confirmation (in various forms) was in this proficiency testing carried out by the majority (94 %) of the laboratories.

The new ISO 6579-1:2017 was published in the beginning of 2017 and replaced previous ISO methods. The majority of the 36 laboratories that analysed according to ISO however still followed the older ISO 6579:2002 or ISO 6579:2002/Amd 1:2007. Among the changes in the new version from 2017 are that detection of β -galactosidase and indole is optional in the confirmation, whereas 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 MSRVS instead of RVS during the selective enrichment step. Notably, both of the two laboratories that followed NMKL 187 stated that they followed the older version NMKL 187:2006. The new version from 2016 contains clarifications regarding the choice of the selective agar medium complementary to XLD, and the concentration of Novobiocin in MSRVS. 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 center. As a complementary medium to XLD, the laboratories mainly used chromogenic media such as Brilliance™ *Salmonella*, Rambach™ agar and brilliant green agar (BGA). As in previous proficiency testing rounds, several laboratories chose to analyse with alternative methods like RAPID[®] *Salmonella* or VIDAS[®], which are validated by AFNOR and/or NordVal

against ISO 6579 1:2017. PCR-based methods were also common. No obvious differences in the results could be seen between the different methods that were used. There were also no false results reported by the few laboratories that did not perform a confirmation.

Results from analysis of Salmonella

Method	N	Sample A			Sample B			Sample C		
		n	+/-	F	n	+/-	F	n	+/-	F
All results	114	110	Pos	4	107	Pos	7	113	Neg	1
NMKL 71:1999	30	29	Pos	1	28	Pos	2	29	Neg	1
ISO 6579-1:2017	17	17	Pos	0	17	Pos	0	17	Neg	0
PCR	16	16	Pos	0	16	Pos	0	16	Neg	0
VIDAS*	15	14	Pos	1	14	Pos	1	15	Neg	0
ISO 6579:2002	10	9	Pos	1	10	Pos	0	10	Neg	0
ISO 6579:2002/Amd 1:2007	9	9	Pos	0	8	Pos	1	9	Neg	0
RAPID [®] Salmonella	7	6	Pos	1	5	Pos	2	7	Neg	0
NMKL 187:2007	2	2	Pos	0	2	Pos	0	2	Neg	0
Other**	8	8	Pos	0	7	Pos	1	8	Neg	0

* The group VIDAS includes three laboratories that analysed with MINI VIDAS[®].

** The group Other include Neogen[®] Reveal[®] 2.0 Salmonella, Oxoid Salmonella PreciS[™] as well as national and/or company-specific methods.

***Escherichia coli* O157**

Sample A

No target organism was present in the sample. No false positive results were reported.

Sample B

The strain of *E. coli* O157 was target organism and was present at approximately log₁₀ 1.2 cfu ml⁻¹ in the sample. Three false negative results were reported.

Sample C

A strain of *E. coli* O157 (not identical to the one in sample B) was target organism and was present at approximately log₁₀ 0.8 cfu ml⁻¹ in the sample. Three false negative results were reported.

General remarks

Only 28 laboratories performed the analysis. Statistical evaluation of the results is therefore difficult, in particular since very few false results were reported. Differences between the various methods and media that were used are also difficult to discern. Confirmation (in various forms) was performed by 22 laboratories (79 %), which is similar compared to the January PT round 2018.

In total, 39 % of the laboratories followed either of the traditional methods NMKL 164:2005 or ISO 16654:2001, which are similar. Enrichment is done in modified tryptone soya broth (mTSB), and is followed by immunomagnetic separation and isolation on cefixime tellurite sorbitol MacConkey agar (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. NMKL 164:2005 is undergoing revision – there is however no estimated date for when a new version will be available. In addition to the NMKL and ISO methods, the use of PCR-based methods and VIDAS® was common.

Three of the participating laboratories have stated that they have followed methods that are not primarily designed for detection of *E. coli* O157. As an example, NMKL 44 is adapted for analysis of coliform bacteria. One of the laboratories stated that they are aware that they do not detect *E. coli* O157, but for the other two laboratories, it is unclear if additional tests for the specific detection of *E. coli* O157 have been performed. These results have nevertheless, as an exception, been included in the results summary. The parameters *E. coli* and coliform bacteria should however be analysed in the April and October proficiency testing rounds, respectively.

As in previous proficiency testing rounds, the most commonly used media were CT-SMAC, sorbitol MacConkey agar (SMAC) and CHROMagar™ O157. On CT-SMAC and SMAC, bacteria that ferment sorbitol (most non-pathogenic *E. coli*) are distinguished from those that do not (most *E. coli* O157). The inclusion of cefimixin and tellurite in CT-SMAC makes it more selective compared to SMAC, and it inhibits growth of many *Proteus* spp. and *Aeromonas* spp., which often are sorbitol-negative. On CT-SMAC and SMAC, sorbitol-negative *E. coli* O157 form transparent colonies, approximately 1-2 mm in diameter and with a dark center. Sorbitol-positive *E. coli* instead form red colonies on these media. In comparison, on CHROMagar™ *E. coli* O157 form mauve (purple) colonies that can be distinguished from other colonies (blue or colourless) that may grow on this medium.

Results from analysis of *Escherichia coli* O157

Method	N	Sample A			Sample B			Sample C		
		n	+/-	F	n	+/-	F	n	+/-	F
All results	28	28	Neg	0	25	Pos	3	25	Pos	3
ISO 16654:2001	7	7	Neg	0	6	Pos	1	5	Pos	2
PCR method	6	6	Neg	0	6	Pos	0	6	Pos	0
NMKL 164:2005	4	4	Neg	0	4	Pos	0	4	Pos	0
VIDAS	3	3	Neg	0	3	Pos	0	3	Pos	0
Other*	8	8	Neg	0	6	Pos	2	7	Pos	1

* The group Other includes national and/or company-specific methods, as well as laboratories for which it is unclear if they have used methods specific for *E. coli* O157.

Pathogenic *Vibrio* sp.

Sample A

The strain of *V. parahaemolyticus* was target organism and was present at approximately \log_{10} 2.8 cfu ml⁻¹ in the sample. The strain forms typical blue-green colonies on thiosulphate citrate bile salts sucrose agar (TCBS). Two false negative results were reported.

In an initial test of sample A, *P. mirabilis* formed small, atypical and light-green colonies on TCBS. These colonies were however oxidase negative, and could therefore be distinguished from *V. parahaemolyticus* in the confirmation.

Sample B

No target organism was present in the sample. One false positive result was reported.

During the initial quality control of the sample mixture at the National Food Agency, after enrichment in alkaline peptone water with 2 % NaCl (APW 2 %), atypical small light-green and oxidase negative colonies were observed on thiosulphate citrate bile salts sucrose agar (TCBS). Such colonies were however not observed on TCBS when the enrichment was done in salt polymyxin broth (SP).

Sample C

The strain of *V. cholerae* was target organism and was present at approximately \log_{10} 3.2 cfu ml⁻¹ in the sample. Two false positive results were reported.

During the quality control at the National Food Agency, large as well as small colonies formed on TCBS. All of these had a yellow colour typical for *V. cholerae*. Subsequent confirmation with API 20 NE also showed that all tested colonies were *V. cholerae*. A similar observation was made in a previous proficiency testing round where the same sample mixture was used (January 2018). At that time, all tested colonies were confirmed as *V. cholerae* with API 20 NE, and the tested colonies were also oxidase positive and sensitive to vibriostaticum O129.

General remarks

Only 20 laboratories performed the analysis and most used similar methods and media. The majority also reported correct results. The results are therefore difficult to evaluate statistically. All laboratories except one (95 %) stated that they performed some kind of confirmation.

As in previous proficiency testing rounds, the majority of the laboratories followed either NMKL 156:1997 or ISO/TS 21872-1:2007. A new version of the ISO method, ISO 21872-1:2017, is however available. It replaces both ISO/TS 21872-1:2007 and ISO/TS 21872-2:2007. Compared to the proficiency testing round January 2018, more laboratories had now started using the new ISO 21872-1:2017.

ISO 21872-1:2017 contains several changes, including the performance of confirmation with biochemical and/or PCR methods. However it mainly follows the same principle as the previous versions. Primary and secondary enrichment is in APW 2 % and is followed by inoculation onto TCBS. Another medium, chosen by the laboratory, is inoculated in parallel to TCBS. Subcultured colonies are subsequently confirmed by biochemical tests, PCR and/or real-time PCR. The procedure in NMKL 156:1997 is similar to ISO 21872-1:2017, but also includes enrichment in SP. The NMKL method also only includes biochemical confirmation tests.

All laboratories stated that colonies were isolated on TCBS. One laboratory reported parallel isolation on CHROMagar™ Vibrio. Bile salts in TCBS inhibit the growth of Gram-positive microorganisms, whereas a high pH promotes the growth of *V. cholerae*. On this medium, *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, 2-3 mm in diameter, whereas *V. cholerae* (sucrose-positive) normally form yellow colonies, 1-2 mm in diameter.

Results from analysis of pathogenic Vibrio sp.

Method	N	Sample A			Sample B			Sample C		
		n	+/-	F	n	+/-	F	n	+/-	F
All results	20	18	Pos	2	19	Neg	1	18	Pos	2
ISO/TS 21872-1:2007	7	7	Pos	0	7	Neg	0	6	Pos	1
NMKL 156:1997	6	5	Pos	1	6	Neg	0	6	Pos	0
ISO 21872-1:2017	4	4	Pos	0	4	Neg	0	3	Pos	1
ISO/TS 21872-1:2007/Cor 1:2008	2	1	Pos	1	1	Neg	1	2	Pos	0
AOAC 988.20:1988*	1	1	Pos	0	1	Neg	0	1	Pos	0

* The laboratory has stated they used a modified version of AOAC 988.20:1988.

Yersinia enterocolitica

Sample A

No target organism was present in the sample. All laboratories reported a correct negative result.

Sample B

The strain of *Y. enterocolitica* was target organism. On cefsulodin irgasan novobiocin agar (CIN) the strain forms typical colonies with a dark red center (“bull’s eye”), surrounded by an outer transparent zone. All laboratories reported a correct positive result.

Sample C

No target organism was present in the sample. The sample however contained a strain of *Y. intermedia* – which is false positive for the analysis – at a concentration of approximately 3 cfu ml⁻¹. Since all laboratories appear to have used methods that include some form of enrichment step, *Y. intermedia* should have been possible to detect, despite its low concentration. It can however not be ruled out that some laboratories achieved a negative result simply due to the low concentration of *Y. intermedia*. During the quality control at the National Food Agency, *Y. intermedia* was detected after 3 weeks cold incubation in peptone sorbitol bile salts broth (PSB) that was followed by surface spreading on CIN. Such cold incubation is mandatory with NMKL 117:1996, but optional with ISO 10273:2017. In a previous PT round at the National Food Agency (January 2018), the strain was characterised as oxidase negative and it did not display agglutination against neither O:3 nor O:9 antisera. The strain may be difficult to identify as *Y. intermedia* with API 20 E. All laboratories reported a correct negative result.

General remarks

Only 12 laboratories performed the analysis and all reported correct results. The results can therefore not be evaluated statistically. All laboratories except one stated that they performed some kind of confirmation.

The majority of the laboratories followed ISO 10273, distributed evenly between ISO 10273:2017 and ISO 10273:2003. The new 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. Here, it can be mentioned that NMKL 117:1996 is currently being revised, and the new version will likely be very similar to ISO 10273:2017. There is however no estimated date for when the new NMKL version will be published.

The method in ISO 10273:2017 is based on parallel enrichment in PSB and irgasan ticarcillin potassium chlorate broth (ITC). Aliquots are subsequently inoculated onto CIN as well as (optionally) on a second chromogenic medium selected by the laboratory. Characteristic colonies are confirmed by biochemical methods or by real-time PCR. Cold enrichment can also be performed, but is not mandatory. The method in NMKL 117:1996 differs somewhat and is instead based on pre- and cold enrichment in PSB, as well as selective enrichment in modified Rappaport broth (MRB). After the enrichment steps, samples are inoculated onto CIN, but *Salmonella/Shigella* sodium deoxycholate calcium chloride agar (SSDC) can also be used. Presumptive colonies are subcultured on bromthymol blue saccharose agar (BS) and sucrose-positive colonies (yellow) are selected for confirmation.

On CIN, colonies of *Y. enterocolitica* have a typical appearance; a red “bull’s eye” center and an outer transparent zone. All laboratories in this proficiency testing reported incubating 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. The sample is here enriched in semi-selective PSB or in non-selective tryptone soya broth with yeast extract (TSBY). The enrichment step 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. Inoculation from the enrichment broth onto CIN is optional. 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 low suspected levels of *Y. enterocolitica*.

Results from analysis of *Yersinia enterocolitica*

Method	N	Sample A			Sample B			Sample C		
		n	+/-	F	n	+/-	F	n	+/-	F
All results	12	12	Neg	0	12	Pos	0	12	Neg	0
ISO 10273:2017	4	4	Neg	0	4	Pos	0	4	Neg	0
ISO 10273:2003*	4	4	Neg	0	4	Pos	0	4	Neg	0
PCR method	2	2	Neg	0	2	Pos	0	2	Neg	0
NMKL 117:1996	1	1	Neg	0	1	Pos	0	1	Neg	0
Other	1	1	Neg	0	1	Pos	0	1	Neg	0

* One of the laboratories stated that they used a modified version of ISO 10273:2003.

Outcome of the results of individual laboratory - assessment

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 stating “pos” or “neg” for quantitative analyses, the results cannot be correctly processed. Such incorrectly reported results are normally excluded. Inclusion and further processing of such results may still be done, after manual assessment in each individual case.

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 only 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 can be ordered, free of charge via our website: www.livsmedelsverket.se/en/PT-extra

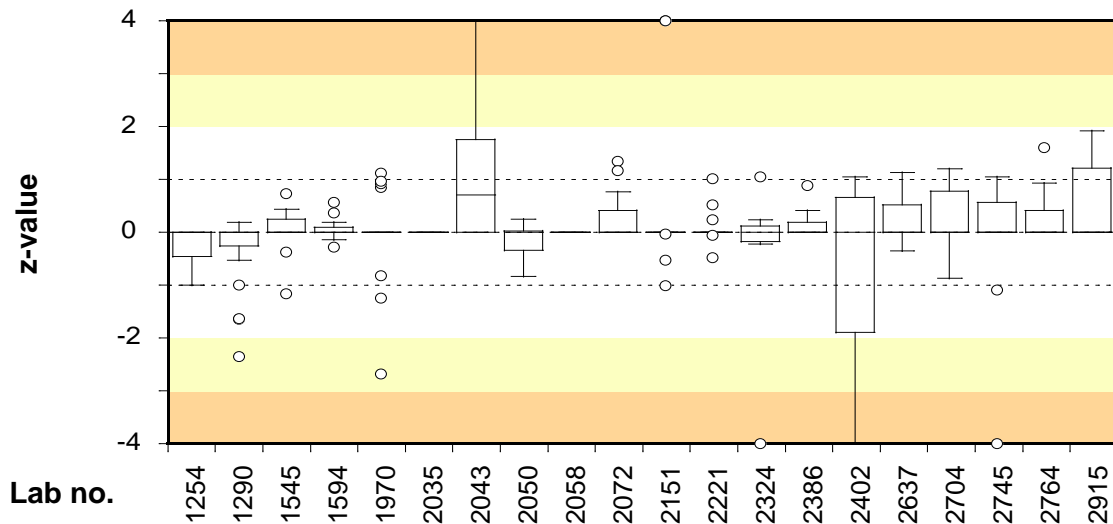
In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated 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 achievement of each laboratory. A small box, 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. 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. For each laboratory, the number of false results and outliers are also listed in the tables below the box plots.

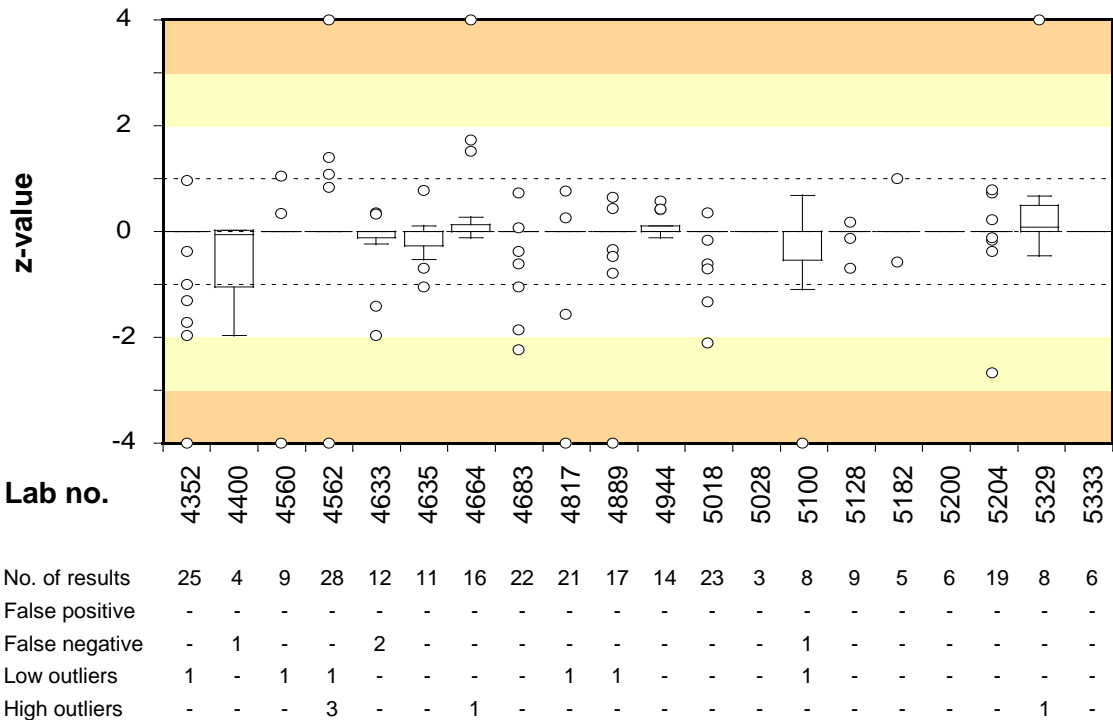
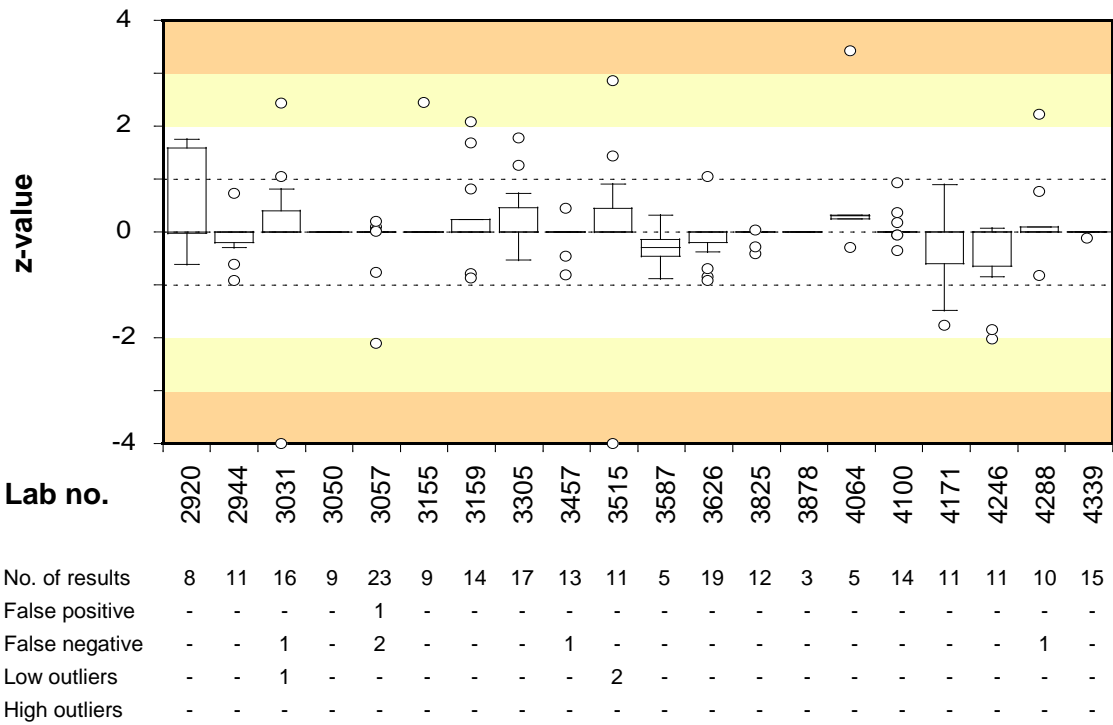
Box plots and numbers of deviating results for each laboratory

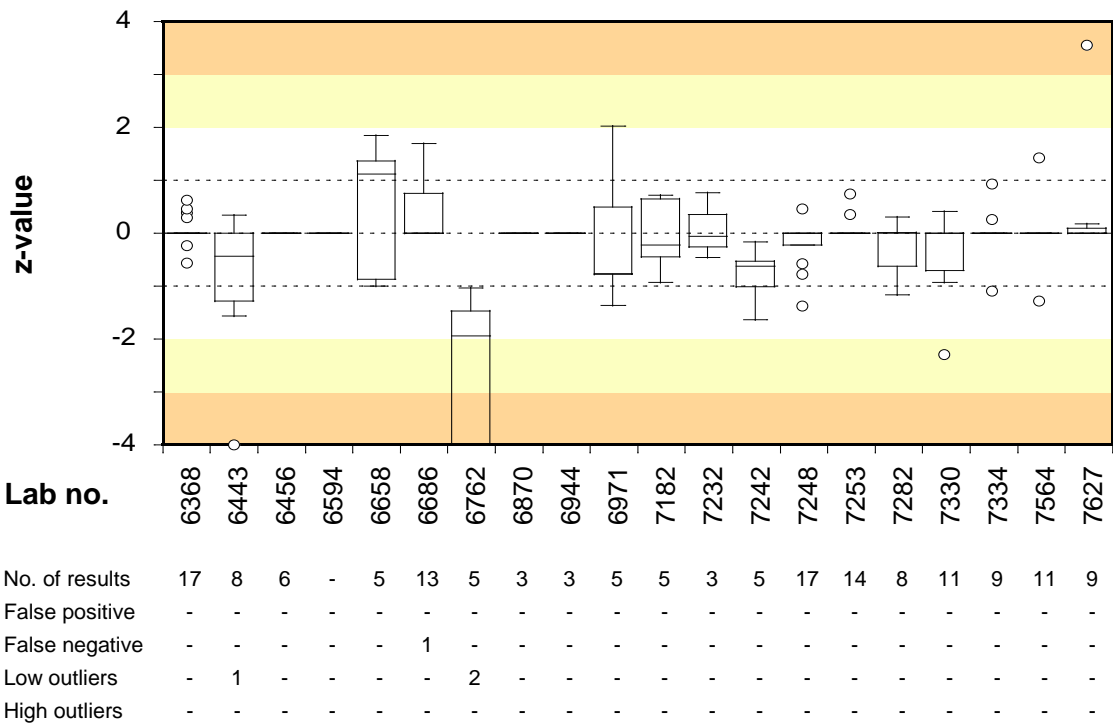
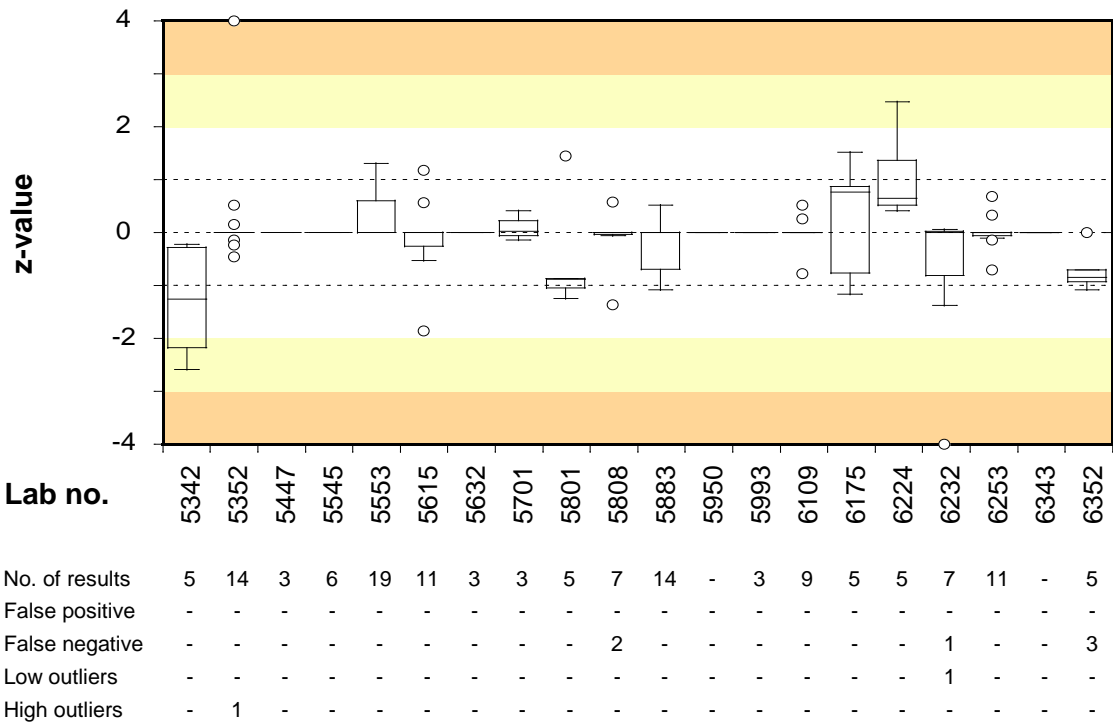
- Z-scores are calculated according to the formula: $z = (x-m)/s$, where x is the result of the individual laboratory, m is the mean of the results of all participating laboratories, and s is the standard deviation of the participating laboratories, after removing outliers and false results.
- Outliers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

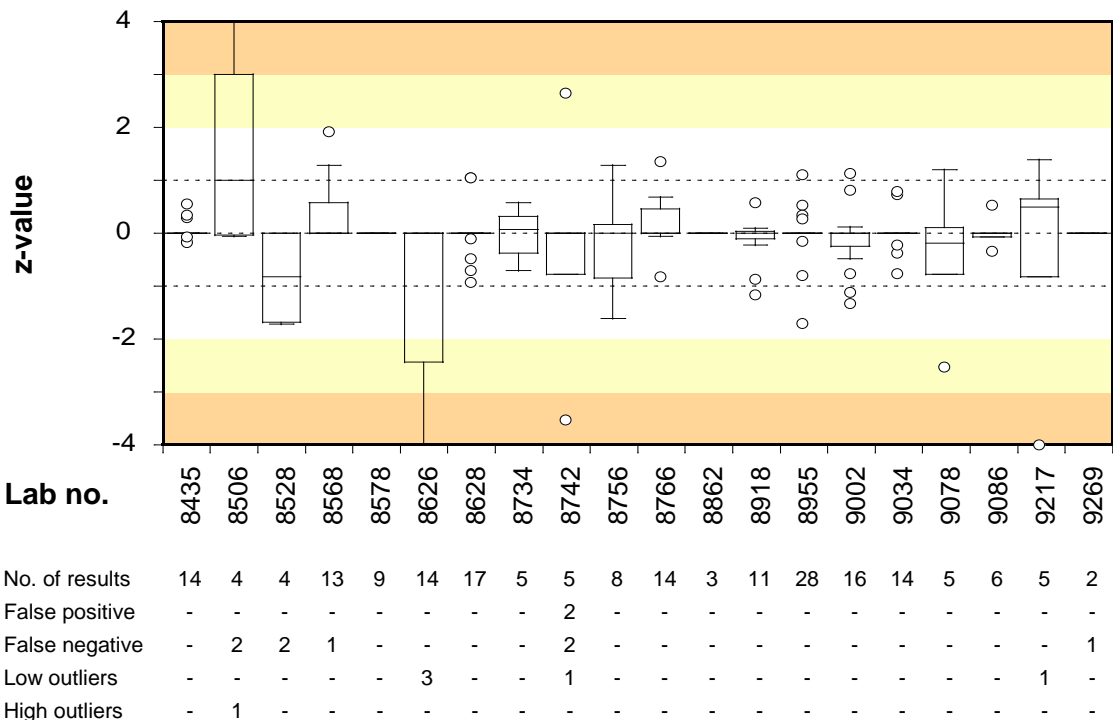
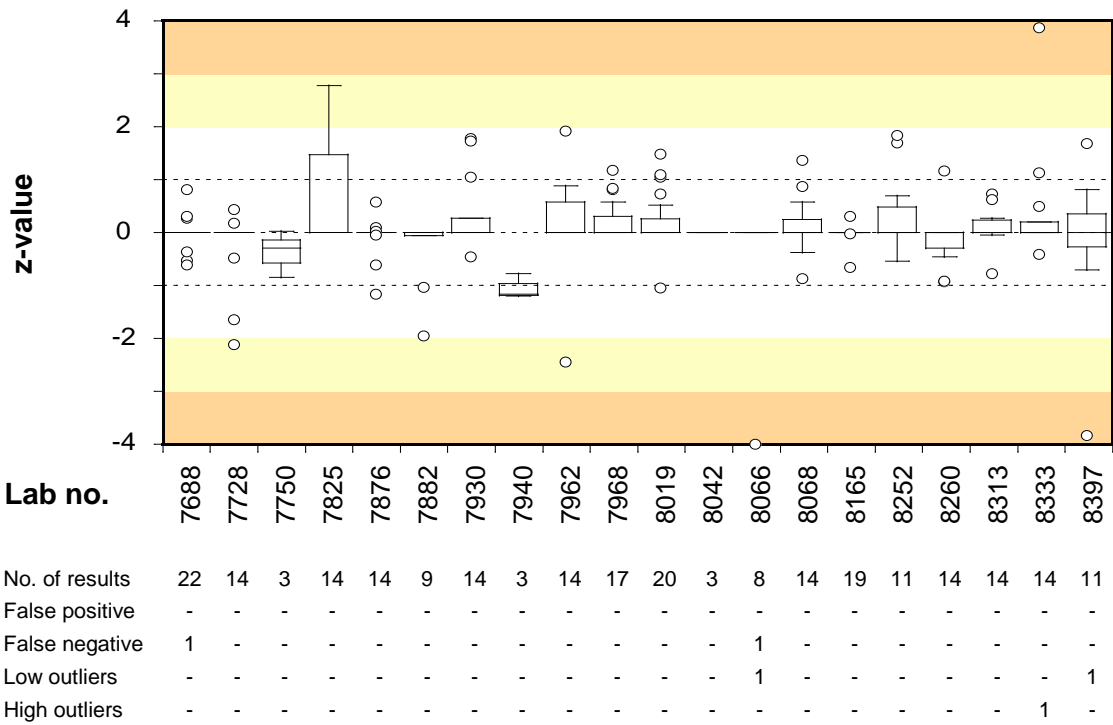
* $< [lowest\ value\ in\ the\ box - 1,5 \times (highest\ value\ in\ the\ box - lowest\ value\ in\ the\ box)]$ or
 $> [highest\ value\ in\ the\ box + 1,5 \times (highest\ value\ in\ the\ box - lowest\ value\ in\ the\ box)]$.

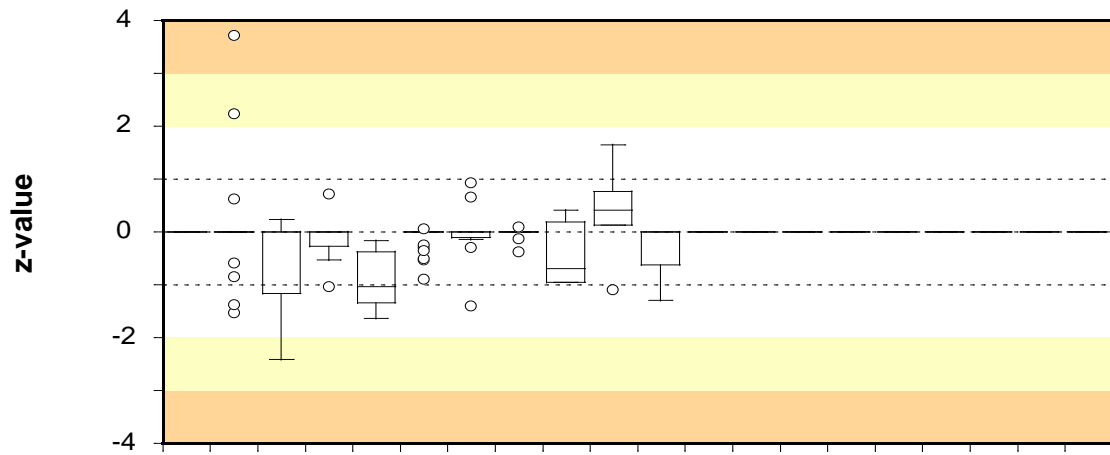


Lab no.	1254	1290	1545	1594	1970	2035	2043	2050	2058	2072	2151	2221	2324	2386	2402	2637	2704	2745	2764	2915
No. of results	14	20	14	11	25	3	6	8	-	22	17	16	8	9	8	14	14	14	14	11
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2	-	-	1	-	-
High outliers	-	-	-	-	-	-	1	-	-	-	2	-	-	-	-	-	-	-	-	-









Lab no.	9429	9436	9441	9453	9512	9558	9662	9716	9747	9890	9903
No. of results	6	22	14	11	5	27	13	12	5	5	11
False positive	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	1	1	-	-	-	-
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 had to be dissolved in 254 ml of sterile diluent. The organisms present in the mixtures are listed in Table 2.

Table 2. *Microorganisms present in sample mixtures A-C.*

Sample ¹	Microorganism	Strain	
		SLV ²	Reference ³
A	<i>Campylobacter jejuni</i>	SLV-540	Chicken, 2003
	<i>Proteus mirabilis</i>	SLV-374	CCUG 43605
	<i>Salmonella</i> Enteritidis	SLV-436	-
	<i>Vibrio parahaemolyticus</i>	SLV-529	CCUG 38981
B	<i>Campylobacter jejuni</i>	SLV-540	Chicken, 2003
	<i>Escherichia coli</i> O157	SLV-479	SMI 81186
	<i>Kocuria rhizophila</i>	SLV-055	ATCC 9341
	<i>Salmonella</i> Dublin	SLV-242	CCUG 35631
	<i>Yersinia enterocolitica</i>	SLV-408	CCUG 45643
C	<i>Escherichia coli</i> O157	SLV-528	CCUG 47557
	<i>Hafnia alvei</i>	SLV-015	CCUG 45642
	<i>Listeria monocytogenes</i>	SLV-361	Smoked salmon
	<i>Staphylococcus saprophyticus</i>	SLV-013	CCUG 45100
	<i>Vibrio cholerae</i>	SLV-507	CCUG 34649
	<i>Yersinia intermedia</i>	SLV-472	CCUG 39927

¹ The links between the mixtures and the randomised sample numbers are shown in Annex 1.

² Internal strain identification no. at the National Food Agency.

³ Origin or culture collection reference (CCUG: Culture Collection University of Gothenburg, Sweden; ATCC: American Type Culture Collection; SMI: Public Health Agency of Sweden)

Quality control of the samples

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 of reproducibility (T) and the test “Index of dispersion” between vials (I_2) do not simultaneously exceed 2.6 and 2.0, respectively. (For definitions of T and I_2 , see references 6 and 7 respectively.)

Table 3. Concentration mean (*m*), *T* and I_2 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 ¹			B ¹			C ¹		
	m	T	I_2	m	T	I_2	m	T	I_2
Aerobic microorganisms 30 °C NMKL method no. 86:2013	4.37	1.45	0.82	4.49	1.36	0.78	4.88	1.22	0.75
Enterobacteriaceae NMKL method no. 144:2005	4.26	1.33	0.38	3.46	1.42	1.01	4.46	1.91	2.95
Thermotolerant <i>Campylobacter</i> , quant. NMKL method no. 119:2007	2.33	2.47	4.87	1.34	1.67	1.75	-	-	-
Thermotolerant <i>Campylobacter</i> , qual. NMKL method no. 119:2007	Pos	-	-	Pos	-	-	Neg	-	-
<i>Listeria monocytogenes</i> , quant. NMKL method no. 136:2010	-	-	-	-	-	-	2.50	1.86	2.97
<i>Listeria monocytogenes</i> , qual. NMKL method no. 136:2010	Neg	-	-	Neg	-	-	Pos	-	-
<i>Salmonella</i> NMKL method no. 71:1999	1.92	1.34	0.41	1.16	1.15	0.46	Neg	-	-
<i>Escherichia coli</i> O157 NMKL method no. 164:2005	Neg	-	-	1.19	1.24	0.48	0.84	1.35	2.01
Pathogenic <i>Vibrio</i> spp. NMKL method no. 156:1997	2.82	1.44	0.55	Neg	-	-	3.25	1.35	1.01
<i>Yersinia enterocolitica</i> NMKL method no. 117:1996	Neg	-	-	2.27	2.11	3.30	(0.39)	(1.31)	(1.18)

– No target organism and therefore no value

¹ n = 5 vials analysed in duplicate

(result) = false positive for the analysis

References

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7. Heisterkamp, S.H., Hoekstra, J.A., van Strijp-Lockefeer, N.G.W.M., Havelaar, A.H., Mooijman, K.A., in't Veld, P.H., Notermans, S.H.W., Maier, E.A., Griepink, B. 1993. Statistical analysis of certification trials for microbiological reference materials. Luxembourg: Commission of the European Communities, Report EUR 15008 EN.


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	
8252	3 2 1	4.46	4.36	5.04	4.18	2.23	4.48	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	-	8252		
8260	1 2 3	4.24	4.39	4.69	4.05	3.28	4.1	-	-	-	<1	<1	2.51	-	-	-	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	8260			
8313	2 1 3	4.15	4.46	4.9	4.12	3.21	4.46	-	-	-	<1	<1	2.43	-	-	-	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	8313			
8333	3 1 2	4.39	4.9	4.87	4.06	3.13	4.36	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Pos	-	-	-	-	-	-	8333			
8397	1 2 3	4.46	4.36	4.91	3.48	<1	4.15	-	-	-	<1	<1	2.47	-	-	-	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	8397			
8435	1 2 3	4.23	4.41	4.85	4.19	<1	4.3	-	-	-	0	0	2.46	-	-	-	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	8435			
8506	3 2 1	4.24	5.2	5.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Neg	Neg	-	-	-	-	-	-	8506			
8528	2 1 3	4.04	<1	4.59	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Neg	-	-	-	-	-	-	-	-	-	8528			
8568	2 1 3	4.32	4.66	4.97	<0	3.4	4.53	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Pos	-	-	-	8568			
8578	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Neg	Pos	-	-	8578			
8626	3 2 1	3.94	4.36	4.26	2.6	0	3.83	-	-	-	0	0	2	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	8626			
8628	1 3 2	4.38	4.34	4.69	4.11	1.6	4.2	-	-	-	0	0	2.5	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	8628			
8734	2 1 3	4.32	4.34	4.76	4.14	0	4.39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8734			
8742	1 3 2	4.15	4.75	4.36	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	Pos	Neg	Pos	-	-	-	-	-	-	8742			
8756	1 2 3	4.41	4.23	4.85	4.09	2.48	3.97	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	8756			
8766	1 2 3	4.24	4.51	4.98	3.99	1	4.45	-	-	-	0	0	2.45	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	8766			
8862	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	-	-	-	-	-	-	-	8862		
8918	1 3 2	4.32	4.4	4.82	4.14	1.3	4.11	-	-	-	<0	<0	2.31	-	-	-	Neg	Neg	Pos	-	-	-	-	-	-	-	-	-	8918			
8955	1 3 2	4.15	4.49	4.85	3.84	1.3	4.28	2.11	<1	<1	<1	<1	2.51	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Pos	-	-	-	8955			
9002	2 1 3	4.35	4.29	4.64	4.32	<1	4.2	1.78	<1	<1	<1	<1	2.42	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	9002			
9034	2 1 3	4.2	4.4	4.9	4	3.4	4.5	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	9034			
9078	1 2 3	4.15	4.44	4.96	3.7	0	4.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9078			
9086	2 1 3	4.2	4.49	4.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	-	-	-	-	-	-	-	9086		
9217	3 2 1	4.31	3.33	4.89	3.99	3.34	4.64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9217			
9269	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9269		
9429	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9429		
9436	3 1 2	4.53	4.88	4.7	3.87	<1	3.99	2.23	0.3	<1	<1	<1	2.36	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Pos	-	-	-	9436			
9441	2 1 3	3.97	4.34	4.66	3.72	0.7	4.04	-	-	-	0	0	2.43	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	9441			
9453	1 3 2	4.24	4.3	4.74	4.25	2.08	4.2	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	9453			
9512	1 2 3	4.2	4.3	4.6	4.1	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9512			
9558	1 2 3	4.26	4.36	4.77	3.98	3.4	4.26	1.86	<1	<1	<1	<1	2.38	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Neg	Neg	Pos	Pos	-	-	-	-	9558			
9662	2 3 1	4.07	4.39	4.79	4.11	3.04	4.47	-	-	-	0	0	2.49	-	-	-	Neg	Neg	Pos	Pos	Neg	Neg	-	-	-	-	-	-	9662			
9716	1 2 3	4.2	4.41	4.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9716		
9747	2 3 1	4.3	4.31	4.72	4.16	3.24	4.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9747		
9890	2 3 1	4.11	4.52	4.86	4.15	0	4.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9890		
9903	3 1 2	4.11	4.37	4.71	3.91	<1	4.22	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	9903		

N	119	121	121	102	102	102	16	16	16	59	59	59	23	23	23	97	97	97	114	114	114	28	28	28	20	20	20	12	12	12	N
Min	2.03	0	3.81	0	0	0	1.30	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Min	
Max	5.40	5.91	6.55	5.03	4.53	4.99	2.49	1.34	0	0	0	2.62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Max	
Med	4.24	4.43	4.82	4.12	1.65	4.34	2.11	0.60	0	0	0	2.43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Med	
m	4.247	4.426	4.808	4.128	1.882	4.314	2.027	0.576	0	0	0	2.410	pos	pos	neg	neg	neg	pos	pos	pos	neg	neg	pos	pos	pos	neg	neg	pos	neg	m	
s	0.126	0.122	0.127	0.169	1.339	0.235	0.326	0.485	0	0	0	0.086	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	s	
F+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	F+
F-	0	1	0	3	0	1	0	0	0	0	0	0	1	1	0	0	0	3	4	7	0	0	3	3	2	0	2	0	0	0	F-
<	2	5	4	8	0	0	0	0	0	0	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<	
>	2	5	3	2	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>	
< OK	3.94	4.10	4.48	3.70	0	3.82	1.30	0	0	0	0	2.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< OK	
> OK	4.68	4.86	5.11	4.54	4.54	4.99	2.49	1.34	0	0	0	2.62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	> OK	

N = number of analyses performed Max = highest reported result m = mean value F+ = false positive < = low outlier < OK = lowest accepted value
 Min = lowest reported result Median = median value s = standard deviation F- = false negative > = high outlier > OK = highest accepted value

- The analysis is not evaluated
- Outlier, false positive or false negative
- Results "larger than" are not evaluated

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	A	B	C		
7688	1	3	2	-0.533	0.275	-0.613	0.306			-0.359			0.000	0.000	0.817	0	0	0	0	0	0	0	0	0	0	0	0				0	0	0	7688	
7728	1	3	2	-2.117	0.439	0.176	-1.645			-0.486						0	0	0	0	0	0	0	0	0	0	0							7728		
7750	1	3	2	0.022	-0.298	-0.850																												7750	
7825	2	1	3	1.677	2.781	2.339	1.092			1.052			0.000	0.000	1.470				0	0	0	0	0	0									7825		
7876	3	2	1	0.101	0.030	-0.613	-0.049			-1.168			0.000	0.000	0.584				0	0	0	0	0	0									7876		
7882	1	3	2	-0.058	-1.035	-1.954													0	0	0	0	0	0									7882		
7930	1	2	3	-0.454	0.275	1.044	1.783			1.730			0.000	0.000	0.001				0	0	0	0	0	0									7930		
7940	3	2	1	-0.771	-1.199	-1.165																												7940	
7962	2	1	3	0.180	1.913	0.887	0.779			0.579			0.000	0.000	-2.448				0	0	0	0	0	0									7962		
7968	2	3	1	0.101	1.176	0.808	0.306			0.835			0.000	0.000	0.584	0	0	0	0	0	0	0	0										7968		
8019	2	3	1	0.734	0.521	1.044	1.488			1.090			0.000	0.000	-1.049	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8019		
8042	1	3	2																															8042	
8066	3	2	1										0.000	0.000	-4.000				0	0	0	0	0	0									8066		
8068	3	2	1	-0.375	-0.871	0.255	1.370			0.877			0.000	0.000	0.584				0	0	0	0	0	0									8068		
8165	3	2	1				0.306			-0.657	-0.020	0.000				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8165		
8252	3	2	1	1.701	-0.535	1.841	0.282			0.694									0	0	0	0	0	0									8252		
8260	1	2	3	-0.058	-0.298	-0.929	-0.463			-0.913			0.000	0.000	1.167				0	0	0	0	0	0									8260		
8313	2	1	3	-0.771	0.275	0.729	-0.049			0.622			0.000	0.000	0.234				0	0	0	0	0	0									8313		
8333	3	1	2	1.130	3.878	0.492	-0.404			0.196			0.000	0.000	0.700				0	0	0	0	0	0	0	0	0						8333		
8397	1	2	3	1.685	-0.544	0.808	-3.832			-0.700			0.000	0.000	0.549				0	0	0	0	0	0									8397		
8435	1	2	3	-0.161	-0.175	0.295	0.347			-0.069			0.000	0.000	0.549				0	0	0	0	0	0									8435		
8506	3	2	1	-0.058	4.000	1.991																												8506	
8528	2	1	3	-1.642		-1.718													0		0													8528	
8568	2	1	3	0.576	1.913	1.281			0.920										0	0	0	0	0	0	0	0	0	0						8568	
8578	2	1	3																															8578	
8626	3	2	1	-2.434	-0.544	-4.000	-4.000			-2.063			0.000	0.000	-4.000				0	0	0	0	0	0									8626		
8628	1	3	2	1.051	-0.707	-0.929	-0.108			-0.486			0.000	0.000	1.050				0	0	0	0	0	0									8628		
8734	2	1	3	0.576	-0.707	-0.376	0.069			0.323																								8734	
8742	1	3	2	-0.771	2.650	-3.533													0															8742	
8756	1	2	3	1.289	-1.608	0.334	-0.226			-1.467									0	0	0	0	0	0									8756		
8766	1	2	3	-0.058	0.685	1.360	-0.817			0.579			0.000	0.000	0.467				0	0	0	0	0	0									8766		
8862	1	2	3																															8862	
8918	1	3	2	0.576	-0.216	0.097	0.069			-0.870			0.000	0.000	-1.165				0	0	0	0	0	0									8918		
8955	1	3	2	-0.802	0.529	0.342	-1.710			-0.150	0.268	0.000	0.000	0.000	1.109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8955		
9002	2	1	3	0.814	-1.117	-1.323	1.133			-0.486	-0.757	0.000	0.000	0.000	0.117				0	0	0	0	0	0									9002		
9034	2	1	3	-0.375	-0.216	0.729	-0.758			0.792									0	0	0	0	0	0									9034		
9078	1	2	3	-0.771	0.112	1.202	-2.531			-0.188																								9078	
9086	2	1	3	-0.343	0.529	-0.068													0	0	0	0	0	0										9086	
9217	3	2	1	0.497	-4.000	0.650	-0.817			1.389																								9217	
9269	1	2	3																																9269
9429	1	3	2																																9429
9436	3	1	2	2.239	3.714	-0.850	-1.527			-1.381	0.624	0.000	0.000	0.000	-0.582	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9436		
9441	2	1	3	-2.196	-0.707	-1.165	-2.413			-1.168			0.000	0.000	0.234				0	0	0	0	0	0									9441		
9453	1	3	2	-0.058	-1.035	-0.534	0.719			-0.486									0	0	0	0	0	0										9453	
9512	1	2	3	-0.375	-1.035	-1.639	-0.167			-1.339																									9512
9558	1	2	3	0.063	-0.530	-0.291	-0.890			-0.251	-0.501	0.000	0.000	0.000	-0.347	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9558		
9662	2	3	1	-1.404	-0.298	-0.139	-0.108			0.664			0.000	0.000	0.934				0	0	0	0	0	0									9662		
9716	1	2	3	-0.375	-0.134	0.097																													9716
9747	2	3	1	0.418	-0.953	-0.692	0.188			-0.955																									9747
9890	2	3	1	-1.087	0.767	0.413	0.128			1.644																									9890
9903	3	1	2	-1.087	-0.462	-0.771	-1.290			-0.401									0	0	0	0	0	0										9903	

 The analysis is not evaluated

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

All analytical activities require work of a high standard that is accurately documented. For this purpose, most 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 National 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 National Food Agency's reference material

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

More information is available on our website: www.livsmedelsverket.se/en/RM-micro