

Proficiency Testing

Food Microbiology

January 2016

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Edition

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Proficiency Testing

Microbiology – Food

January 2016



1457
ISO/IEC 17043

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 Listeria Ottaviani & Agosti
APW 2%	Alcaline peptone water, 2 % NaCl
BriS	Brilliance Salmonella-agar
BPW	Buffered peptone water
CIN	Cefsulodin-irgasan-novobiocin-agar
CT-SMAC	Cefixime-tellurite-sorbitol-MacConkey-agar
LMBA	Listeria monocytogenes Blood-agar
MPCA	Milk Plate Count Agar
PSB	Phosphate-sorbitol-broth
PCA	Plate Count Agar
RVS	Rappaport-Vassiliadis-soya peptone-broth
SMAC	Sorbitol MacConkey agar
SPB	Salt-polymyxin-broth
TCBS	Thiosulfate citrate salt sucrose agar
XLD	Xylose lysine deoxycholate agar
VRBG	Violet Red Bile Glucose agar

Organisations

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 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 organised by the National Food Agency is accredited since early 2012, it is mandatory for the participating laboratories to give method information for all analyses for which they report results. Method information is sometimes difficult to interpret, e.g. several laboratories choose a medium that differs from that in the reported standard methods. Therefore, in the following section, results have been grouped according to the method or the medium used to perform the analysis.

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 participant's results.

Tables and figures legend

Tables

n	number of laboratory that performed the analysis
m	results mean value in \log_{10} cfu/ml (false results and outliers excluded)
s	results 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 all analytical results obtained for each mixture are presented. The mean value of the analysis 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 2016

General outcome

Samples were sent to 169 laboratories, 33 in Sweden, 116 in other European countries, and 20 outside Europe. Out of the 163 laboratories that reported results, 67 (41 %) provided at least one result that received an annotation. In the previous round (January 2015) with similar analyses, the proportion was 30 %.

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

Table 1 Microorganisms in each mixture and % of deviating results (F%: false positive or false negative, X%: outliers).

	Mixture A			Mixture B			Mixture C		
% participants with	1%	0%		2%	0%		2%	1%	
0 annotation	8%			10%			28%		
1 annotation									
2 annotations									
>2 annotations									
Organisms	<i>Micrococcus sp.</i> <i>Escherichia coli</i> <i>Salmonella Stockholm</i> <i>Yersinia enterocolitica</i>			<i>Staph. saprophyticus</i> <i>Vibrio cholera</i> <i>Hafnia alvei</i> <i>Listeria ivanovii</i> <i>Listeria seeligeri</i> <i>Salmonella Enteritidis</i>			<i>Aeromonas hydrophila</i> <i>Listeria monocytogenes</i> <i>Campylobacter coli</i> <i>Escherichia coli O157</i>		
Analysis	Target	F%	X%	Target	F%	X%	Target	F%	X%
Aerob. microorg, 30 °C	<i>Micrococcus</i> <i>E. coli</i>	0	1	<i>S. saprophyticus</i> <i>H. alvei</i>	0	1	<i>A. hydrophila</i>	1	4
Enterobacteriaceae	<i>E. coli</i>	0	2	<i>H. alvei</i>	2	2	(<i>A. hydrophila</i>) <i>E. coli O157</i>	28	0
Thermotol. campylo- bacter	(E. coli)	0	0	-	0	0	<i>C. coli</i>	8	0
Qual.		7	-		3	-		7	-
<i>L. mono- cytogenes</i>	-	0	0	<i>(L. ivanovii)</i> <i>(L. seeligeri)</i>	6	0	<i>L. monocytogenes</i>	3	9
Quant.		3	-		9	-		0	-
<i>Salmonella</i>	<i>S. Stockholm</i>	1	-	<i>S. Enteritidis</i>	2	-	-	2	-
<i>E. coli O157</i>	-	11	-	-	4	-	<i>E. coli O157</i>	7	-
Path. <i>Vibrio</i> spp.	-	0	-	<i>V. cholera</i>	5	-	-	4	-
<i>Y. enterocolitica</i>	<i>Y. enterocolitica</i>	19	-	-	0	-	-	6	-

- no target organism or no value; (*microorganism*)

() The organism is false positive on the primary growth medium

Aerobic microorganisms, 30 °C

Mixture A

Strains *Micrococcus sp.* and *Escherichia coli* occurred in the highest concentrations in the mixture and thus represented the majority of the colonies in the analysis.

Mixture B

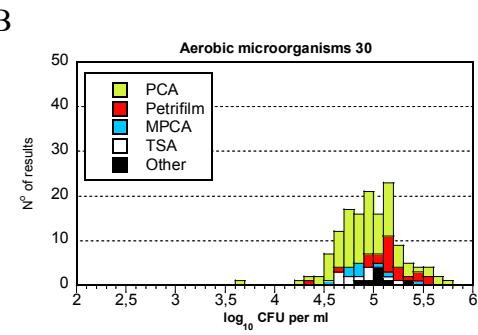
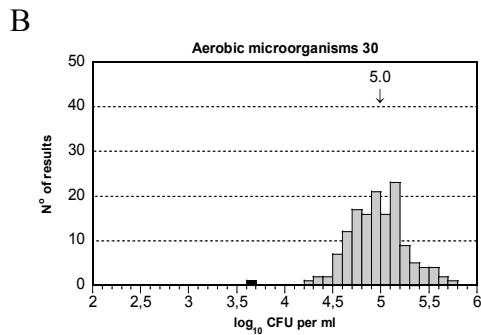
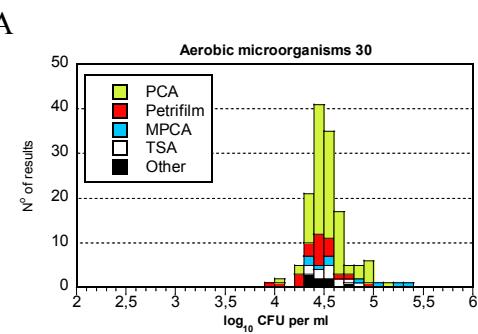
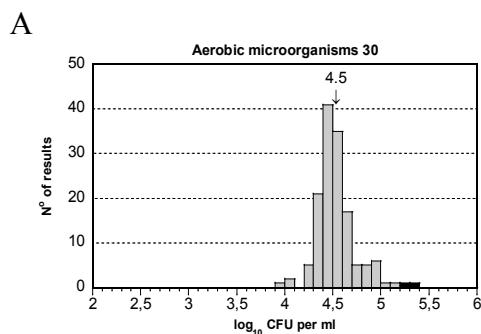
Most colonies in the analysis consist of *Staphylococcus saprophyticus* and *Hafnia alvei* strains.

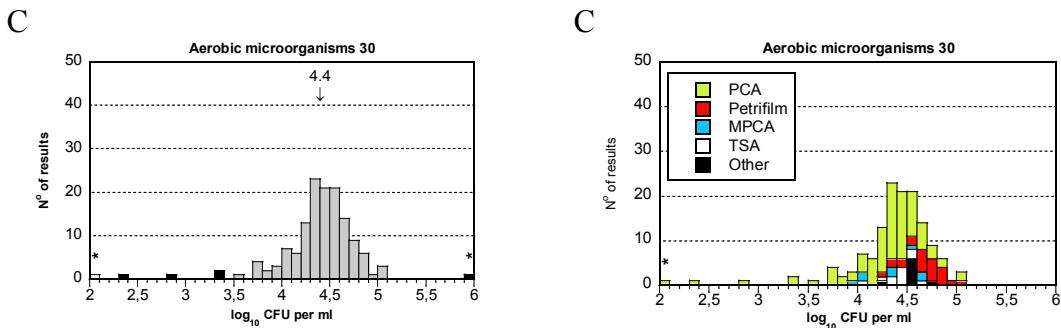
Mixture C

Aeromonas hydrophila occurred in the highest concentration and therefore was the most abundant among the colonies that grew.

Results of aerobic microorganisms analysis

Medium	A				B				C									
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	142	4.52	0.19	0	0	2	143	4.97	0.28	0	1	0	140	4.42	0.28	1	4	1
PCA	92	4.54	0.18	0	0	0	92	4.94	0.29	0	1	0	90	4.35	0.28	1	4	1
Petrifilm™	22	4.43	0.22	0	0	0	23	5.13	0.27	0	0	0	23	4.66	0.20	0	0	0
MPCA	9	4.59	0.26	0	0	2	9	4.91	0.27	0	0	0	8	4.33	0.28	0	0	0
TSA	11	4.55	0.15	0	0	0	11	4.86	0.21	0	0	0	11	4.40	0.18	0	0	0
Other	8	-	-	0	0	0	8	-	-	0	0	0	8	-	-	0	0	0





There is no difference in results due to choice of media. However, comparing Petrifilm™ and other media, the results are slightly lower than the mean value for mixture A and slightly higher than the mean value for mixture B and C on Petrifilm™. In case of mixture A, one possible explanation is that in some cases it is more difficult to distinguish between colonies when different colony types with different sizes exist. It means that smaller colonies with diffuse edges could be obscured by larger colonies and thus not be counted. The reason for the slightly higher values in B and C mixture is that the grids on Petrifilm™ can facilitate the counting of normal sized colonies, compared to other media.

The results were more scattered in the histogram – both with and without deviating results – for mixture B and C than for A. In mixture C where the *A. hydrophila* was most abundant, the distribution of results was more scattered on PCA media. The reason for that is unclear.

Enterobacteriaceae

Mixture A

Escherichia coli was the target organism for this analysis and showed no problem.

Mixture B

Hafnia alvei was the target organism for this analysis and no problem occurred.

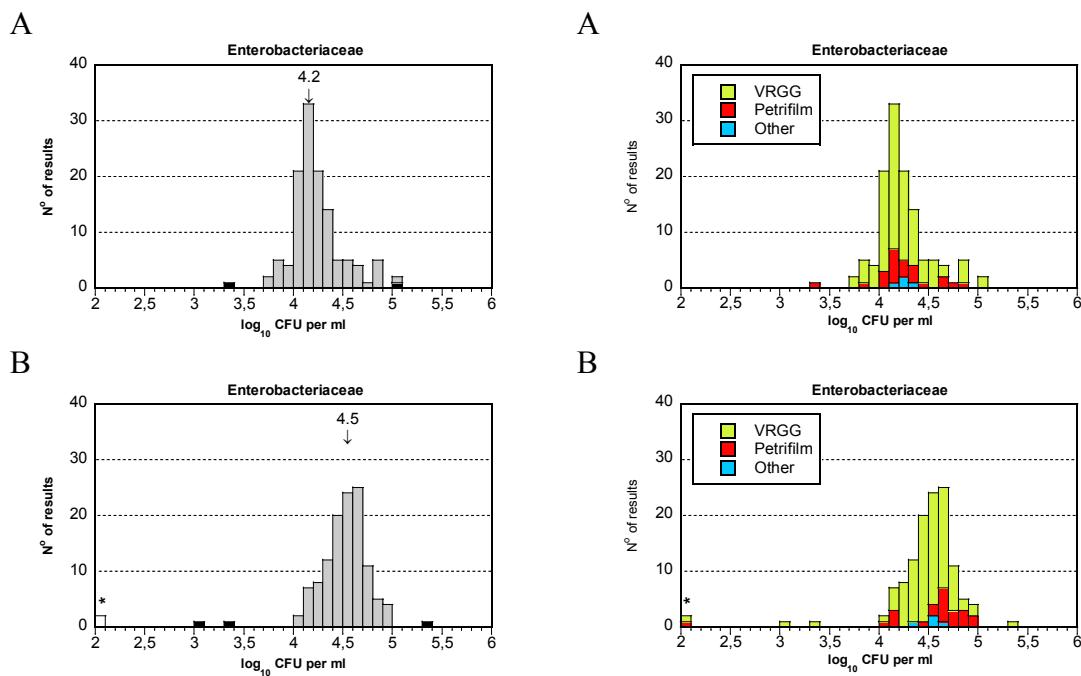
Mixture C

Approximately 28 % of the laboratories failed in the analysis of mixture C. The mixture contained $4.8 \log_{10}$ cfu/ml of an *Aeromonas hydrophila* strain and very low concentration, 10 cfu/ml, of a strain of *Escherichia coli* O157. When performing the analysis according the recommended dilution ranges only *A. hydrophila* grows on the plates with colonies which can be interpreted as Enterobacteriaceae. *A. hydrophila* is an oxidase positive strain which is used as a false-positive organism for the analysis. Such a false positive result indicates an erroneous or skipped confirmation. For the 34 false positive results, 8 laboratories reported the use of confirmation and 26 didn't perform any confirmation.

There were no uncertainties regarding the confirmation of *A. hydrophila* in National Food Agency. Analysis, following the recommended dilution ranges led to the growth of pure culture *A. hydrophila*, which was oxidase positive.

Results of Enterobacteriaceae analysis

Medium	A				B				C			
	n	m	s	F < >	n	m	s	F < >	n	m	s	F < >
Total	123	4.23	0.24	0 1 1	123	4.52	0.20	2 2 1	121	1.13	0.15	- 0 34
VRGG	97	4.22	0.25	0 0 1	97	4.47	0.51	1 2 1	95	1	-	- 0 21
Petrifilm™	22	4.24	0.32	0 1 0	22	4.40	1.04	1 0 0	22	1.20	0.14	- 0 12
Other	4	-	-	0 0 0	4	-	-	0 0 0	4	-	-	- - 1



Medium choice for the analysis of Enterobacteriaceae seemed to have insignificant impact for the mixture A and B. However, Petrifilm™ showed slightly higher average comparing to VRGG. It is possible that the indicator dye present in Petrifilm™ facilitated the reading of colonies.

In case of mixture C, which contained *E. coli* O157 and *A. hydrophila* as a false positive strain, 22 laboratories used Petrifilm™ and 14 of them reported positive results. In 2 cases out of those 14 the concentration of Enterobacteriaceae was found to be up to 1.5 \log_{10} cfu/ml. It suggests even lower dilutions than recommended used and thus counting *E. coli* O157 as Enterobacteriaceae, which is absolutely correct. The remaining 12 laboratories reported a concentration higher than 2.0 \log_{10} cfu/ml which suggests that they counted *A. hydrophila* as Enterobacteriaceae. These results are reckoned as high outliers but can also be considered as false positive ones within the recommended dilution ranges. Two laboratories out of those 12, which counted *A. hydrophila* as Enterobacteriaceae on Petrifilm™, stated they had confirmed their results.

For those who used VRGG, 21 out of 95 answers were high outliers/false positive results with the recommended dilutions. They indicated a content more than 2.0 \log_{10} cfu/ml, which suggests that they counted *A. hydrophila* as Enterobacteriaceae.

Only one laboratory among those who used VRGG stated the content of Enterobacteriaceae corresponding to that of *E. coli* O157. This implies the use of lower dilutions than those recommended which enabled them to identify *E. coli* O157 from *A. hydrophila*, which in this case is correct.

Six laboratories which reported high outliers stated they had confirmed the results, indicating an unsuccessful confirmation. The remaining 15 laboratories didn't perform any confirmation.

Thermotolerant campylobacter

Mixture A

The mixture didn't contain any thermotolerant campylobacter. One strain of *E. coli* was used as false positive. In both qualitative and quantitative analysis at NFA, the strain appeared with atypical white colonies. Furthermore, two false positive results occurred in the qualitative analysis. Confirmation was performed in these cases.

Mixture B

The mixture didn't contain any thermotolerant campylobacter or false positive strain. However one false positive result is reported for qualitative analysis.

Mixture C

The mixture contains *Campylobacter coli*. A few false positive results were reported.

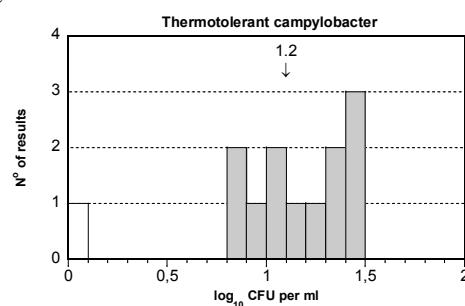
Quantitative results of thermotolerant campylobacter analysis

Method	A				B				C			
	n	m	s	F < >	n	m	s	F < >	n	m	s	F < >
Total	13	-	-	0 - -	13	-	-	0 - -	13	1.16	0.25	1 0 0
ISO	7	-	-	0 - -	7	-	-	0 - -	7	1.19	0.21	0 0 0
NMKL	6	-	-	0 - -	6	-	-	0 - -	6	1.12	0.31	1 0 0

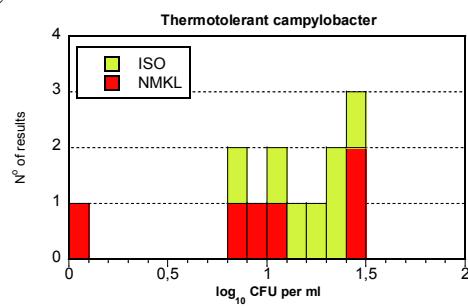
Qualitative results of thermotolerant campylobacter analysis

Method	A			B			C		
	n	+/-	F < >	n	+/-	F < >	n	+/-	F < >
Total	29	neg	2 - -	29	neg	1 - -	30	pos	2 - -
ISO	8	neg	0 - -	8	neg	0 - -	9	pos	0 - -
NMKL	15	neg	1 - -	15	neg	0 - -	15	pos	2 - -
Other	6	neg	1 - -	6	neg	1 - -	6	pos	0 - -

C



C



Very few laboratories performed the analysis; therefore it is not possible to draw any conclusion about the choices of method and media.

Listeria monocytogenes

Mixture A

There was no target organism or false positive organism in the mixture.

Mixture B

Although *Listeria monocytogenes* wasn't present in the mixture, 13 false positive results were reported.

Listeria seeligeri and *Listeria ivanovii* were present in the mixture. The colonies of *Listeria ivanovii* on ALOA media and also on some chromogenic media could be misinterpreted as colonies of *L. monocytogenes*. On the other hand, both *L. seeligeri* and *L. ivanovii* produce colonies resembling those of *L. monocytogenes* on blood-based media (LMBA) and media that demonstrate esculin hydrolysis (PALCAM and Oxford). However, confirmation can separate the above mentioned strains from strains of *L. monocytogenes*. Both *L. seeligeri* and *L. ivanovii* ferment xylose in contrary to *L. monocytogenes*.

Mixture C

Mixture C contained $2.8 \log_{10}$ cfu/ml *Listeria monocytogenes*. Some outliers were present and also two false positive results.

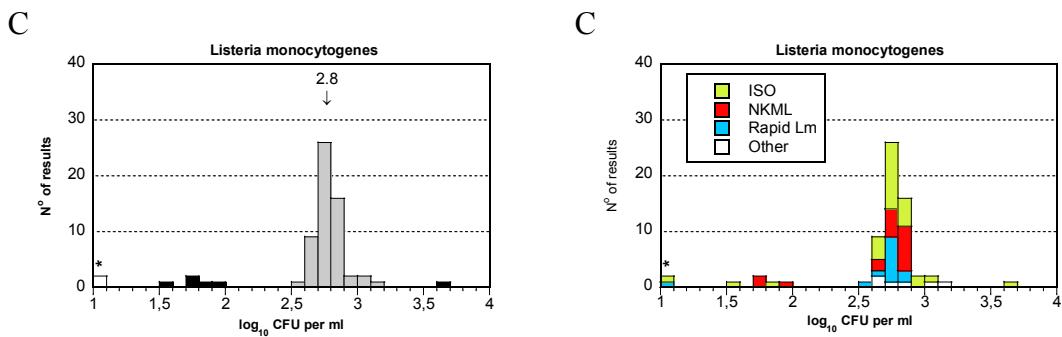
Quantitative results of L. monocytogenes analysis

Method	A				B				C			
	n	m	s	F < >	n	m	s	F < >	n	m	s	F < >
Total	63	-	-	0 - -	64	-	-	4 - -	65	2.78	0.10	2 5 1
ISO 11290-2	28	-	-	0 - -	28	-	-	1 - -	28	2.78	0.10	1 2 1
NMKL 136	18	-	-	0 - -	18	-	-	2 - -	18	2.77	0.08	0 3 0
Rapid L.m.	12	-	-	0 - -	12	-	-	0 - -	13	2.74	0.07	1 0 0
Other	5	-	-	0 - -	6	-	-	1 - -	6	-	-	0 0 0

Qualitative results of L. monocytogenes analysis

Method	A			B			C		
	n	+/-	F < >	n	+/-	F < >	n	+/-	F < >
Total	100	neg	3 - -	101	neg	9 - -	101	pos	0 - -
ISO 11290-2	28	neg	2 - -	28	neg	2 - -	28	pos	0 - -
NMKL 136	16	neg	0 - -	16	neg	2 - -	16	pos	0 - -
VIDAS	22	neg	0 - -	22	neg	1 - -	22	pos	0 - -
Rapid L.m.	14	neg	0 - -	14	neg	1 - -	14	pos	0 - -
PCR	9	neg	1 - -	9	neg	2 - -	9	pos	0 - -
Other	11	neg	0 - -	12	neg	1 - -	12	pos	0 - -

Most of the laboratories used chromogenic media for selection. The false results cannot be related to the method. When it comes to the methods, the low outliers are too few to indicate any differences. However, NFA has observed a difference between the ALOA and OCLA media. Development of colonies on OCLA, and even their zones, is slower than on ALOA.



Therefore, it is important to emphasize that it could be difficult to see the zone around the colonies on OCLA after 24 hours. Even after 48 hours a very careful examination of plates might be required.

In both the ISO 11290-2 and NMKL 136 methods and when it comes to the confirmation stage where haemolysis zone is studied on blood agar, it is important to complete the streak by stabbing the loop into the agar to get better haemolysis.

Salmonella

Mixture A

Mixture contained $0.8 \log_{10}$ cfu/ml of the strain *Salmonella* Stockholm. At NFA the strain appeared with typical colonies on both XLD and Brilliance Salmonella agar.

Mixture B

Mixture contained $1.3 \log_{10}$ cfu/ml of strain *Salmonella* Enteritidis. At NFA the strain appeared with typical colonies on both XLD and Brilliance Salmonella agar.

Mixture C

There was no target organism or false positive organism in the mixture.

Qualitative results of Salmonella analysis

Method	A			B			C		
	n	+/-	F < >	n	+/-	F < >	n	+/-	F < >
Total	127	pos	1 - -	127	pos	2 - -	126	neg	2 - -
ISO 6579	26	pos	0 - -	26	pos	0 - -	26	neg	0 - -
NMKL 71	40	pos	0 - -	40	pos	1 - -	39	neg	2 - -
NMKL 187	6	pos	0 - -	6	pos	0 - -	6	neg	0 - -
VIDAS	19	pos	1 - -	19	pos	0 - -	19	neg	0 - -
PCR	17	pos	0 - -	17	pos	0 - -	17	neg	0 - -
Other	19	pos	0 - -	19	pos	1 - -	19	neg	0 - -

It is not possible to explain the false results by comparing the methods. The majority used XLD along with another medium for isolation. There is no correlation between the method or media and the false results.

Escherichia coli O157

Mixture A

The mixture didn't contain any *E. coli* O157 but one strain of *E. coli* that unlike *E. coli* O157 can ferment sorbitol and form pink colonies on SMAC. At NFA, *E. coli* formed isolated pink colonies even on CT-SMAC. These colonies should be dropped after the confirmation by agglutination test.

Mixture B

There was no target organism in the mixture. There was one strain of *Hafnia alvei*, which similar to *E. coli* O157 cannot ferment sorbitol. It can form beige colonies on SMAC and CT-SMAC but it can be separated from *E. coli* O157 by the confirmation.

Mixture C

The mixture contained *E. coli* O157 in concentration of $1.0 \log_{10}$ cfu/ml.

Qualitative results of E. coli O157 analysis

Method	A			B			C		
	n	+/-	F < >	n	+/-	F < >	n	+/-	F < >
Total	28	neg	3 - -	27	neg	1 - -	29	pos	2 - -
ISO	9	neg	1 - -	8	neg	0 - -	10	pos	0 - -
NMKL	5	neg	0 - -	5	neg	0 - -	5	pos	0 - -
Other	14	neg	2 - -	14	neg	1 - -	14	pos	2 - -

The majority of laboratories which reported the method information used CT-SMAC for isolation, along with another media such as Chromagar. Half of all the laboratories reported that they used Modified Trypton Soya Broth (mTSB) in the enrichment step. There is no correlation between the method or medium and the false results. However, it is important to emphasize that the method for analysis of *E. coli* is not appropriate for analysis of *E. coli* O157.

Pathogenic Vibrio spp.

Mixture A

The mixture didn't contain any target organism. In NFA, yellow colonies were observed on TCBS after enrichment in APV 2 % and in SP broth. *Vibrio spp.* was not detected in the confirmation step, as anticipated. All the strains in the mixture were checked without enrichment regarding their growth on TCBS: *S. Stockholm* formed green colonies, *E. coli* formed yellow colonies in the primary streak only, whereas neither *Micrococcus* nor *Y. enterocolitica* formed any colonies.

Mixture B

The mixture contained *Vibrio cholera* in high concentration, $4.7 \log_{10}$ cfu/ml. When checked at NFA, a pure culture of characteristic yellow colonies, flat and about 2-3 mm in diameter appeared on TCBS from both APV 2 % or SP broth.

Mixture C

The mixture didn't contain any target organism. At NFA, no growth was observed on TCBS after enrichment in SP broth but tiny green colonies appeared on the primary

streak after enrichment in APV 2 %. *Vibrio spp.* was not detected in the confirmation, as anticipated.

E.coli O157 has been checked in a previous testing round for growth on TCBS without enrichment. Unlike ordinary *E. coli*, *E. coli O157* formed greenish colonies only in the primary streak. Most likely, it is an inhibited *E. coli O157* emerging in the analysis for *Vibrio spp.*

Qualitative results of pathogenic Vibrio spp. analysis

Method	Tot n	A			B			C		
		n	+/-	F < >	n	+/-	F < >	n	+/-	F < >
Total		23	neg	0 - -	22	pos	1 - -	23	neg	1 - -
ISO/TS 21872-1		9	neg	0 - -	8	pos	1 - -	9	neg	1 - -
NMKL 156		11	neg	0 - -	11	pos	0 - -	11	neg	0 - -
Other		3	neg	0 - -	3	pos	0 - -	3	neg	0 - -

All laboratories except three used APV 2 % for enrichment and all used TCBS agar for isolation.

Yersinia enterocolitica

Mixture A

Mixture contained $1.3 \log_{10}$ cfu/ml of a strain of *Y. enterocolitica*. Typical colonies grew on CIN-agar at NFA after 3 weeks enrichment in PSB at 4 °C from all the 15 tested vials.

Mixture B

There was no target organism or false positive organism in the mixture.

Mixture C

There was no target organism or false positive organism in the mixture.

Qualitative results of Y. enterocolitica analysis

Method	Tot n	A			B			C		
		n	+/-	F < >	n	+/-	F < >	n	+/-	F < >
Total		16	pos	3 - -	16	neg	0 - -	17	neg	1 - -
ISO 10273		8	pos	2 - -	8	neg	0 - -	9	neg	1 - -
NMKL 117		3	pos	1 - -	3	neg	0 - -	3	neg	1 - -
Other		5	pos	0 - -	5	neg	0 - -	5	neg	1 - -

The mixture A contained a low concentration of *Y. enterocolitica*. However, too few laboratories participated in the analysis for us to be able to evaluate the results regarding the methods. Particularly, with such a low start concentration the long cold incubation period of three weeks is probably required to detect *Y. enterocolitica*.

Outcome of the results of individual laboratory - assessment

In order to allow comparison of the results from different analyses and mixtures, all the results of the analyses were 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. For qualitative analyses, a z-score of zero is attributed for a correct answer. The z-scores obtained, which are listed in Annex 2, can be used as a tool by laboratories when following up on the results.

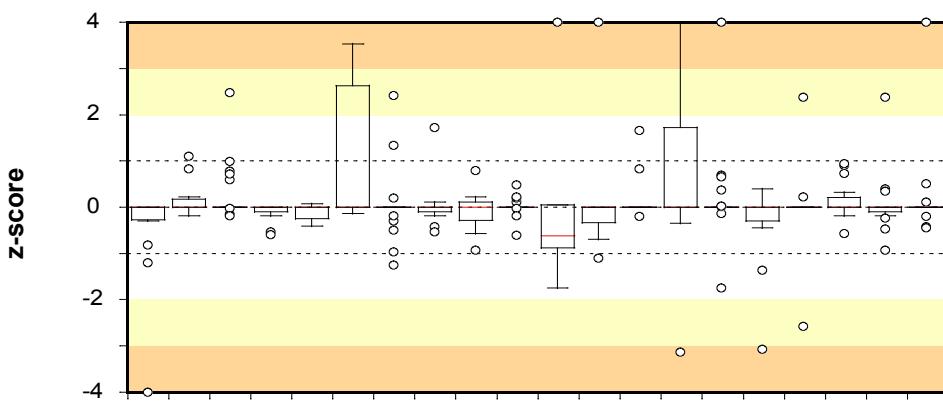
All the results from each laboratory – outliers included and false results excluded – were compiled into a box plot based on their z-scores. The smaller and more centred around zero the box of a laboratory is, the closer its results are to the general mean values calculated for all laboratory results.

The laboratories were not grouped or ranked based on their results. However, for each laboratory, the numbers of false results and outliers are presented below the box plots. These results are also highlighted in Annex 1, where all the reported results are listed, and the minimum and maximum accepted values for each analysis are stated.

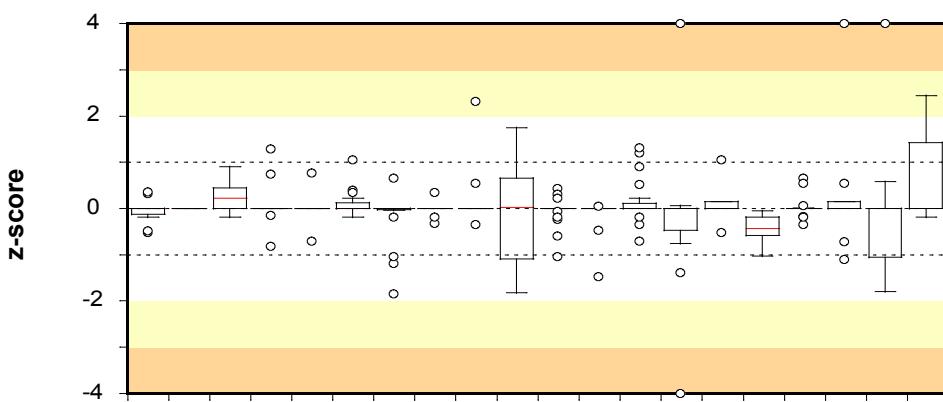
Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol (2). Samples for follow-up can be ordered, free of charge via our website: www.livsmedelsverket.se/en/PT-extra

Box plots and numbers of deviating results for each laboratory

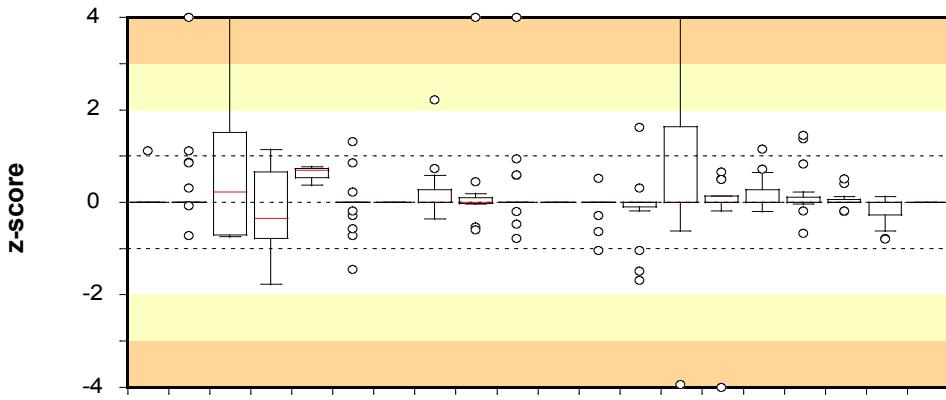
- *The plots are based on the laboratory results from all analyses transformed into z-scores calculated according to the formula: $z = (x-m)/s$, where x is the result of the individual laboratory, m is the mean and s is the standard deviation of the results of all participating laboratories.*
- *Correct results for quantitative analyses without target organism and for qualitative analyses generate a z-value of 0.*
- *The outliers are included in the plots after recalculation to standardised values with the same standard deviation (s) as for the rest of the results.*
- *False results do not generate z-scores and are not included in 'No. of results'.*
- *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.*
- *Very deviating results are represented by circles and are calculated as follow: the lowest result in the box – $1.5 \times (\text{the highest result in the box} - \text{the lowest result in the box})$ or the highest result in the box + $1.5 \times (\text{the highest result in the box} - \text{the lowest result in the box})$. z-scores higher than +4 and less than -4 are positioned at +4 and -4, respectively, in the plot.*
- *The background is divided by lines and shaded fields to indicate ranges in order to simplify location of laboratory results.*



Lab no	1254	1594	1970	2035	2050	2058	2072	2129	2151	2221	2324	2344	2386	2402	2458	2637	2670	2704	2745	2764
No. of results	15	12	21	12	9	6	24	15	8	18	5	24	9	9	27	15	9	15	14	
False positive	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	1	
False negative	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	
Low outliers	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
High outliers	-	-	-	-	-	-	-	-	-	-	1	1	-	1	1	-	-	-	1	

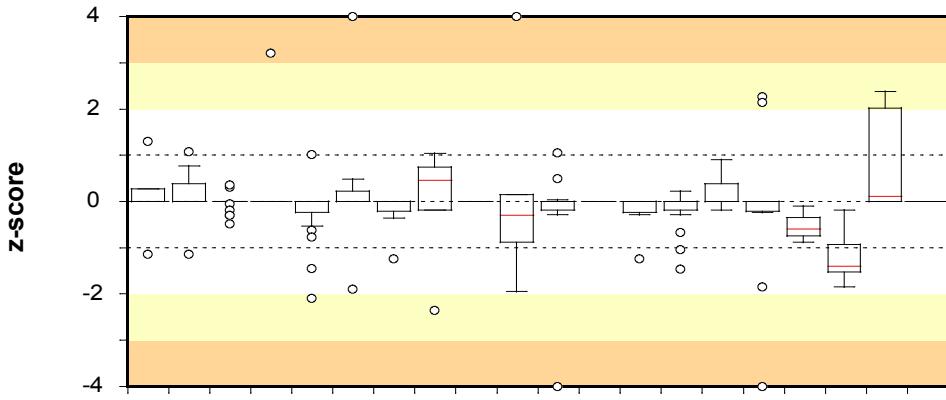


Lab no	2797	2842	2920	2944	3126	3159	3305	3457	3533	3587	3626	3825	3868	3923	3925	4064	4100	4171	4246	4288
No. of results	12	12	9	13	8	15	21	14	9	6	21	12	20	12	6	6	15	12	12	12
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
False negative	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	



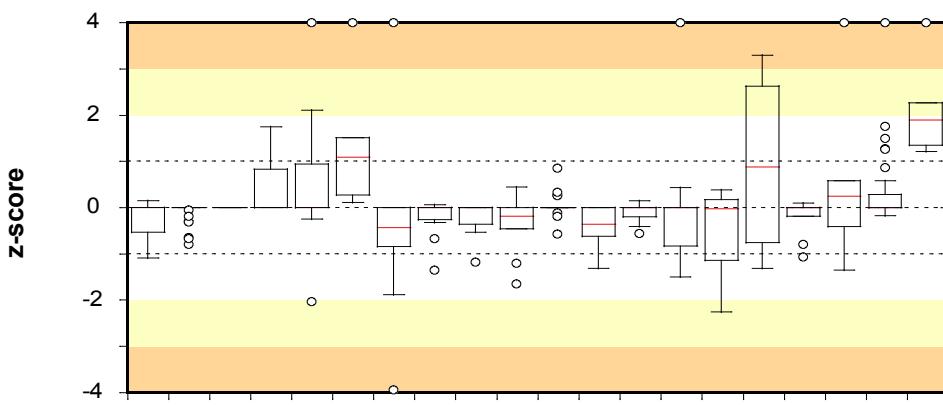
Lab no

4339	14
4352	22
4400	6
4449	6
4557	3
4562	30
4605	3
4633	15
4635	12
4664	18
4683	-
4817	24
4840	15
4879	12
4889	13
4944	15
4955	15
4980	19
5018	3
5028	-



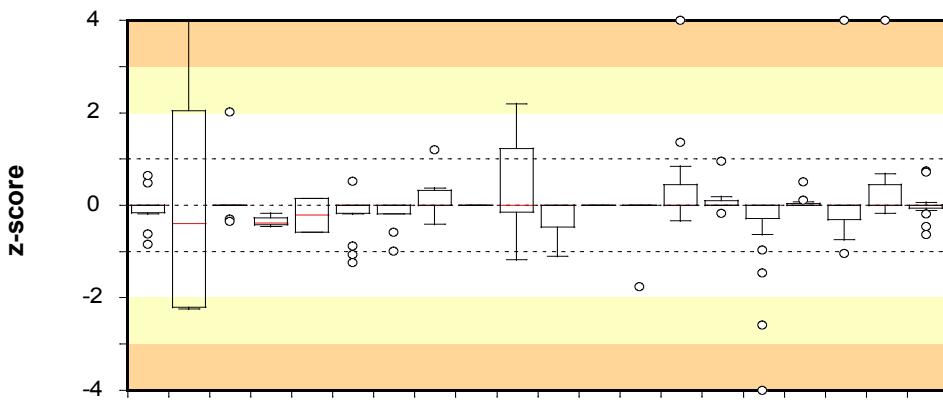
Lab no

5100	6
5128	8
5188	24
5200	9
5204	21
5220	9
5304	7
5329	6
5333	6
5342	6
5352	13
5447	3
5545	8
5553	21
5615	12
5632	12
5701	3
5801	6
5808	6
5856	-



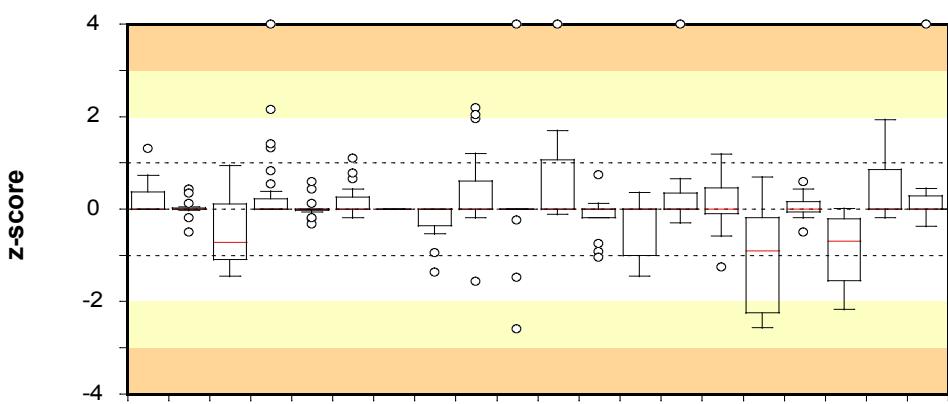
Lab no 5883 5950 5993 6109 6175 6224 6232 6253 6343 6352 6368 6443 6456 6594 6647 6658 6686 6762 6860 6971

No. of results	15	29	3	9	9	6	9	12	9	18	9	11	12	3	4	10	6	28	6
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2	-	-
False negative	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
Low outliers	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	1	1	1	-	-	-	-	-	1	-	-	-	1	1

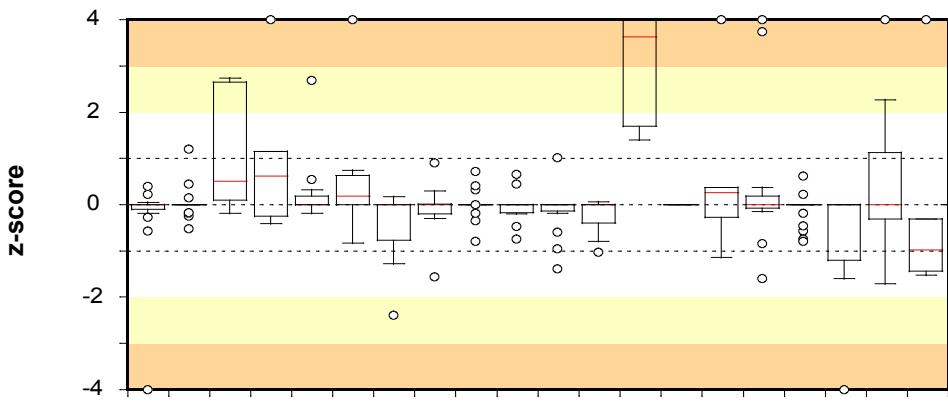


Lab no 7096 7182 7191 7232 7242 7248 7253 7282 7302 7330 7334 7543 7564 7596 7627 7688 7728 7750 7825 7876

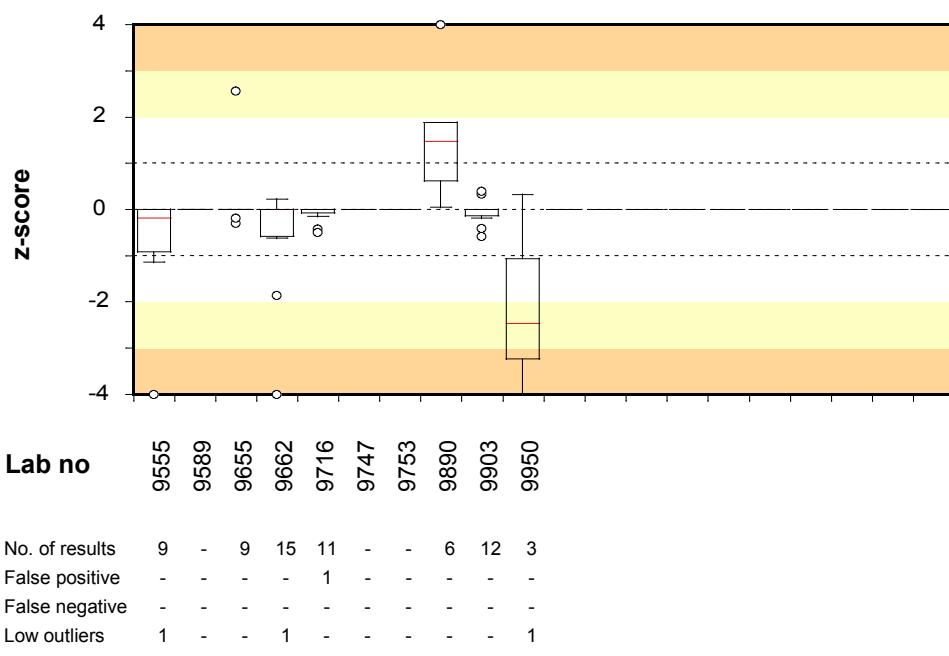
No. of results	12	6	9	3	2	18	12	12	9	9	7	-	6	18	7	24	12	11	15	15
False positive	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	1	-	-
False negative	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	1	-



Lab no	7882	7930	7940	7946	7962	7968	8042	8066	8068	8165	8252	8260	8313	8333	8397	8430	8435	8528	8529	8568
No. of results	8	15	3	27	15	18	3	12	15	18	12	13	12	12	12	6	12	4	15	12
False positive	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	1	-	-	-	-	-	1	1	-	-	1	-	-	-	-	-	1



Lab no	8626	8628	8657	8734	8742	8756	8766	8918	8955	9002	9034	9051	9078	9086	9217	9429	9436	9441	9453	9512
No. of results	15	18	6	5	12	8	17	12	27	15	15	12	5	-	6	12	24	15	12	6
False positive	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
High outliers	-	-	-	-	-	1	-	-	-	-	-	-	3	-	1	1	-	2	1	-



Test material and quality control

Test material

Each laboratory received three freeze-dried microbial mixtures designated A-C. The manufactured test material was freeze-dried in portions of 0.5 ml in vials, as described by Peterz and Steneryd (3). Before analysing the samples, the contents of each vial had to be dissolved in 254 ml of diluent. The organisms present in the mixtures are listed in Table 2.

Table 2. Microorganisms present in mixture A-C supplied to participants

Mixture ¹	Microorganism	Strain collection no.		Log cfu/ ml
		SLV (own)	CCUG ²	
A	<i>Micrococcus sp.</i>	SLV-055	CCUG 35073	4.6
	<i>Escherichia coli</i>	SLV-558	-	4.2
	<i>Salmonella</i> Stockholm	SLV-390	-	0.8
	<i>Yersinia enterocolitica</i>	SLV-408	CCUG 45643	1.3
B	<i>Staphylococcus saprophyticus</i>	SLV-013	CCUG 45100	5.0
	<i>Hafnia alvei</i>	SLV-015	CCUG 45642	4.7
	<i>Listeria seeligeri</i>	SLV-347	-	3.0 ³
	<i>Listeria ivanovii</i>	SLV-348	-	3.0 ³
	<i>Salmonella enteritidis</i>	SLV-436	-	1.3
	<i>Vibrio cholera</i>	SLV-530	CCUG 45388	4.7
C	<i>Aeromonas hydrophila</i>	SLV-454	CCUG 30208	4.8
	<i>Campylobacter coli</i>	SLV-271	CCUG 45147	2.0
	<i>Listeria monocytogenes</i>	SLV-444	CCUG 48089	2.8
	<i>Escherichia coli</i> O157	SLV-479	-	1.0

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

²Culture Collection University of Gothenburg, Sweden

³Content is only calculated together with another microorganism

Quality control of the mixtures

It is essential to have aliquots of homogeneous mixture and equal volume in all vials in order to allow comparison of all freeze-dried samples from one mixture. Quality control is performed on 10 randomly chosen vials in conjunction with manufacturing of the mixtures or on 5 vials if an “old” mixture was used and the last quality control was performed more than 6 months before the testing round. Homogeneity of a 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 exceed simultaneously 2.6 and 2.0, respectively.

Table 3. Concentration means (m), T and I_2 values from the quality control of the mixtures; m is expressed in \log_{10} cfu (colony forming units) per ml of sample.

Analysis and method	A			B			C		
	m	T	I_2	m	T	I_2	m	T	I_2
Aerobic microorganisms 30 °C NMKL-method no. 86	4.573	1.51	1.61	4.961	1.40	1.28	4.765	1.53	2.41
Enterobacteriaceae NMKL-method no. 144	4.183	1.24	0.86	4.692	2.20	9.20	-	-	-
Thermotolerant campylobacter, quant. NMKL method no. 119	-	-	-	-	-	-	1.950	2.12	1.13
Thermotolerant campylobacter, qual. NMKL method no. 119	neg	-	-	neg	-	-	pos	-	-
<i>Listeria monocytogenes</i> , quant. NMKL method no. 136	-	-	-	-	-	-	2.827	1.17	0.38
<i>Listeria monocytogenes</i> , qual. NMKL method no. 136	neg	-	-	neg	-	-	pos	-	-
<i>Salmonella</i> NMKL method no. 71	0.807*	1.12	0.26*	1.303	1.38	0.69	neg	-	-
<i>Escherichia coli</i> O157 NMKL method no. 164	neg	-	-	neg	-	-	0.969*	1.41	0.66
Pathogenic <i>Vibrio</i> spp. NMKL-method no. 156	neg	-	-	4.737	1.28	1.01	neg	-	-
<i>Yersinia enterocolitica</i> NMKL-method no. 117	1.310	1.28	0.78	neg	-	-	neg	-	-

- No target organism

* Internal values based on the analyses results of parallel mixtures

References

1. Kelly, K. 1990. Outlier detection in collaborative studies. *J. Assoc. Off. Anal. Chem.* 73:58-64.
2. Anonymous, 2015. Protocol. Microbiology. Drinking Water & Food. The National Food Agency.
3. Peterz. M. Steneryd. A.C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *J. Appl. Bacteriol.* 74:143-148.

Annex 1 Results from the participating laboratories - January 2016

All results are expressed in \log_{10} cfu per ml sample.

Results reported as "<value" were regarded as zero (negative).

Results reported as " > value" were excluded of the calculations.

A dash in the table indicates that the analysis was not performed.

Outliers and false results are highlighted and summarized for each analysis at the end of the table.

Lab no.	vial	Aerobic microorganisms 30 °C			Enterobacteriaceae			Thermotolerant campylobacter			Listeria monocytogenes			Thermotolerant campylobacter			Listeria monocytogenes			Salmonella			Escherichia coli O157 (VT-neg)			Pathogenic Vibrio spp			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							
4400	3 1 2	4.65	4.77	4.84	4.17	4.37	4.24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4400						
4449	1 2 3	4.42	5.15	4.2	3.81	4.75	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4449						
4557	1 2 3	4.65	5.18	4.52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4557						
4562	3 1 2	4.38	4.56	4.34	4.28	4.69	<1	<1	<1	1.48	<1	<1	2.72	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Neg	Pos	Neg	Pos	Neg	4562					
4605	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	-	-	-	-	-	4605							
4633	1 3 2	4.45	5.13	4.4	4.4	4.63	<2	-	-	-	<1	<1	3	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	4633				
4635	3 2 1	4.51	5.02	4.54	4.1	4.4	3.43	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	4635						
4664	1 3 2	4.37	5.13	4.68	4.18	4.64	4.08	-	-	-	<1	<1	2.73	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	Neg	Pos	Neg	-	-	-	-	4664				
4683	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4683						
4817	2 1 3	4.32	4.79	4.34	-	-	-	-	-	-	<1	<1	2.83	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Neg	Pos	Neg	Pos	Neg	4817					
4840	3 1 2	4.32	4.55	4.87	4.3	4.18	<2	-	-	-	<1	<1	<1	Pos	Pos	Pos	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	4840				
4879	1 2 3	4.4	5.4	4.9	3.3	4.9	3	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	4879				
4889	1 3 2	4.54	5.11	4.6	4.26	4.62	0	-	-	-	0	0	1.71	-	-	-	Neg	Neg	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	4889				
4944	2 3 1	4.64	4.94	4.74	4.18	4.63	<2	-	-	-	<1	<1	2.85	-	-	-	Neg	Neg	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	4944				
4955	2 3 1	4.51	5.2	4.8	4.28	4.81	<2	-	-	-	<0	<0	2.71	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	4955				
4980	1 3 2	4.53	5.08	4.56	4.18	4.53	<2	-	-	-	<1	<1	2.79	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	4980				
5018	3 2 1	4.37	4.87	4.31	4.08	4.36	<1	-	-	-	<1	<1	2.79	Pos	Neg	Neg	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	5018					
5028	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5028						
5100	3 1 2	4.57	5.33	4.1	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	Pos	Pos	Neg	-	-	-	-	-	-	-	-	5100				
5128	2 1 3	4.3	5.18	4.72	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	5128						
5188	1 3 2	4.46	4.95	4.51	4.11	4.59	<2	<1	<1	<1	<1	<1	2.75	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Pos	Neg	Pos	Neg	Neg	Pos	Neg	5188					
5200	1 2 3	-	-	-	-	-	-	-	-	-	<0	<0	3.1	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	5200				
5204	3 2 1	4.4	4.9	4.7	4.1	4.1	<2	<1	<1	0.8	<1	<1	2.7	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	5204				
5220	1 2 3	4.61	5.03	3.89	-	-	-	-	-	-	0	0	3.65	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	5220				
5304	3 2 1	4.45	4.62	4.4	-	-	-	-	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	5304						
5329	1 2 3	4.66	5.1	3.76	4.47	4.61	<2	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	5329			
5333	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	5333						
5342	2 1 3	4.46	4.72	4.46	4.16	4.13	2.3	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	5342			
5352	3 1 2	4.61	4.98	4.37	4.16	4.73	<2	-	-	-	<1	1.18	1.97	-	-	-	Neg	Pos	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	5352			
5447	1 2 3	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	-	-	-	Neg	Pos	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	5447			
5545	3 1 2	-	-	-	4.16	4.27	0	-	-	-	-	-	-	Neg	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	5545			
5553	2 1 3	4.56	4.78	4.35	4.16	4.31	<1	-	-	-	<1	<1	2.63	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	-	-	-	-	-	-	-	-	5553		
5615	3 1 2	4.58	5.11	4.67	4.28	4.61	<1	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	Pos	Pos	Neg	-	-	-	-	-	-	-	-	5615				
5632	2 1 3	4.95	4.9	3.3	4.73	4.15	<1	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	Pos	Pos	Neg	-	-	-	-	-	-	-	-	5632				
5701	2 3 1	4.35	4.8	4.39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5701					
5801	2 1 3	4.34	4.54	4	3.92	4.15	<2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5801				
5808	2 3 1	4.97	5.53	4.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	-	5808		
5856	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5856			
5883	1 3 2	4.31	4.85	4.24	4.06	4.55	<2	-	-	-	0	0	2.69	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	5883			
5950	2 1 3	4.46	4.88	<2	4.04	4.51	<2	<1	<1	1	<1	<1	2.71	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Neg	Pos	Neg	Pos	Neg	5950					
5993	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	-	-	-	5993
6109	1 2 3	4.85	5.26	4.65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	Neg	Neg	Pos	-	-	-	-	-	-	-	-	6109	
6175	2 3 1	4.92	5.23	3.85	4.17	4.6	3.2	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	-	6175		
6224	1 3 2	4.57	5	4.84	4.41	4.8	4.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6224			
6232	2 3 1	4.44	4.73	3.32	3.79	4.38	3.71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6232			
6253	1 2 3	4.53	4.78	4.04	4.15	4.49	<2	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	6253			
6343	3 2 1	4.45	4.82	4.09	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	6343			
6352	1 3 2	4.43	4.63	4.54	3.84	4.46	<2	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	6352			
6368	3 1 2	4.57	5.04	4.51	4.2	4.69	<2	-	-	-	<0	<0	2.72	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	Neg	Pos	Neg	-	-	-	-	-	6368		
6443	3 1 2	4.45	4.6	4.27	4.08	4.26	<2	-	-</																										

Lab no.	vial	Aerobic microorganisms 30 °C			Enterobacteriaceae			Thermotolerant campylobacter			Listeria monocytogenes			Thermotolerant campylobacter			Listeria monocytogenes			Salmonella			Escherichia coli O157 (VT-neg)			Pathogenic Vibrio spp			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			
6647	3 2 1	4.59	4.96	3.79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6647		
6658	1 3 2	4.89	-	4.05	5	-	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6658		
6686	2 3 1	-	-	-	4.04	4.54	<1	-	-	-	<1	2.73	2.67	-	-	-	Neg	Pos	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	6686	
6762	3 1 2	4.26	4.98	4.58	4.13	4.61	1.3	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	6762	
6860	2 3 1	4.76	5.21	4.77	4.64	4.82	4.46	<1	<1	1.3	<1	<1	2.76	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Neg	Neg	Neg	6860			
6971	1 3 2	4.75	5.56	5.05	4.62	4.79	4.58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6971			
7096	2 1 3	4.4	5.1	4.38	4.03	4.65	<2	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	7096	
7182	2 3 1	4.09	4.35	4.31	4.13	4.93	4.58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7182			
7191	3 1 2	4.46	5.53	4.32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	-	7191			
7232	2 3 1	4.43	4.92	4.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7232			
7242	1 3 2	-	4.81	-	-	4.55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7242			
7248	3 1 2	4.35	4.67	4.37	4.2	4.27	<1	-	-	-	<1	<1	2.83	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	7248		
7253	3 1 2	-	-	-	4.09	4.32	<1	-	-	-	<1	<1	2.76	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	7253	
7282	1 3 2	4.44	5.07	4.5	4.51	4.59	<1	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	7282	
7302	3 1 2	-	-	-	-	-	-	-	-	-	<0	<0	2.6	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	-	-	-	-	-	-	7302
7330	3 1 2	4.49	5.31	4.09	4.55	4.96	<1	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	Pos	Neg	-	-	-	7330			
7334	2 3 1	4.48	4.76	4.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	Pos	Neg	-	-	-	7334			
7543	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7543			
7564	1 2 3	-	-	-	-	-	-	-	-	-	<0	<0	2.6	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	7564	
7596	1 3 2	4.68	5.19	4.33	4.33	4.79	4.15	-	-	-	0	0	2.77	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	Neg	Pos	Neg	-	7596		
7627	3 1 2	4.7	4.92	4.47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	Neg	Pos	-	-	-	7627			
7688	2 1 3	4.24	4.79	4.31	4	4	<1	-	-	-	<1	<1	1.83	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	-	-	-	7688			
7728	1 3 2	4.54	4.99	4.56	-	-	-	-	-	-	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	7728				
7750	2 1 3	4.32	4.76	4.37	4.19	4.43	3.72	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	Pos	-	-	-	7750				
7825	2 3 1	4.55	5.11	4.52	4.19	4.66	3.06	-	-	-	<1	<1	2.84	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	7825	
7876	2 1 3	4.53	4.84	4.24	4.2	4.67	<1	-	-	-	<1	<1	2.85	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	7876	
7882	2 1 3	4.52	5.17	4.79	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Neg	-	-	-	-	-	-	-	7882		
7930	1 2 3	4.6	4.83	4.41	4.31	4.53	<2	-	-	-	<0	<1	2.79	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	7930	
7940	2 1 3	4.38	4.56	4.68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7940			
7946	3 1 2	4.77	5.2	5.02	4.24	4.63	3.37	<1	<1	1.25	<1	<1	2.92	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Neg	Neg	Pos	7946			
7962	1 3 2	4.6	4.95	4.36	4.15	4.64	0	-	-	-	<1	<1	2.79	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	7962	
7968	2 3 1	4.6	5.15	4.49	4.41	4.74	<1	-	-	-	<1	<1	2.79	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	7968		
8042	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	-	-	-	-	-	8042			
8066	1 3 2	-	-	-	4.1	4.33	0	-	-	-	0	0	2.64	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	8066	
8068	1 2 3	4.89	5.58	3.98	4.51	4.93	0	-	-	-	0	0	2.76	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	8068	
8165	2 3 1	-	-	-	3.88	4	2.96	<1	<1	1.1	-	-	-	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	-	-	-	8165			
8252	1 3 2	4.6	5.32	4.66	4.2	4.86	4.48	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	8252	
8260	1 3 2	4.66	4.71	4.21	4.24	4.31	<1	-	-	-	<1	2.72	2.79	-	-	-	Neg	Pos	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	8260	
8313	2 1 3	4.24	4.67	4.52	3.99	4.32	<2	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	8313	
8333	2 1 3	4.54	5.15	4.45	4.36	4.46	3.35	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	Neg	Pos	-	-	-	8333				
8397	1 2 3	4.28	5.15	4.75	4.09	4.6	<1	-	-	-	<1	<1	2.83	-	-	-	Neg	Neg	Pos	-	-	-	-	-	-	-	8397				
8430	2 1 3	4.03	4.71	4.61	3.7	4.34	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8430				
8435	2 3 1	4.6	5.06	4.28	4.2	4.64	<1	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	8435	
8528	3 1 2	4.34	4.36	-	4.23	4.43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8528				
8529	1 3 2	4.79	5.32	4.59	4.68	4.72	<2	-	-	-	<1	<1	2.85	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	8529	
8568	1 2 3	4.58	4.94	4.54	4.14	4.57	3.92	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	Neg	Pos	-	-	-	8568				
8626	2 3 1	4.41	4.89	4.48	4.32	4.53	0	-	-	-	0	0	1.73	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	8626	
8628	2 3 1	4.42	5.09	4.46	4.17	4.76	<2	-	-	-	<0	<0	2.76	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	Neg	Pos	Neg	-	8628		
8657	1 3 2	5.04	5.06	4.61	4.85	4.54	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8657				
8734	2 3 1	4.44	5.14	4.74	4.17	<1	1.1	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	8734	
8742	1 2 3	4.58	4.98	4.57	4.86	4.49	<1	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	8742	
8756	2 1 3	4.36	5.03	4.56	4.26	4.67	4.28	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	Pos	-	-	-	-	-	8756			
m																															

Lab no.	vial	Aerobic microorganisms 30 °C			Enterobacteriaceae			Thermotolerant campylobacter			Listeria monocytogenes			Thermotolerant campylobacter			Listeria monocytogenes			Salmonella			Escherichia coli O157 (VT-neg)			Pathogenic Vibrio spp			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			
8766	2 1 3	4.55	4.61	3.75	4	4.27	0	-	-	-	0	0	2.7	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	Neg	Neg	Neg	8766	
8918	3 1 2	4.46	4.91	4.67	4.23	4.58	<2	-	-	-	0	0	2.62	-	-	-	Neg	Neg	Pos	-	-	-	-	-	-	-	-	-	-	-	8918
8955	3 1 2	4.58	5.08	4.32	4.04	4.52	<2	-	-	-	<1	<1	2.85	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Neg	Pos	Neg	8955	
9002	2 3 1	4.49	4.76	4.6	4.18	4.61	<2	-	-	-	0	0	2.73	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	9002
9034	3 2 1	4.5	4.7	4.7	3.9	4.4	<2	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	Pos	Neg	Neg	9034	
9051	2 1 3	4.53	4.95	4.13	4.04	4.4	0	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	9051
9078	3 2 1	5.31	5.44	-	5.08	4.8	4.35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9078		
9086	1 2 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9086		
9217	1 2 3	4.3	4.89	4.52	4.3	4.56	3.92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9217		
9429	3 2 1	5.23	4.73	4.52	3.85	4.49	1	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	9429
9436	3 2 1	4.41	4.76	4.48	4.12	4.36	<1	-	-	-	<1	<1	2.84	Neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Neg	Pos	Neg	-	-	9436	
9441	2 3 1	4.34	4.63	2.3	3.85	4.28	<2	-	-	-	<1	<1	2.63	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	9441
9453	2 3 1	4.4	5.6	3.94	4.06	5.36	3.71	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	9453
9512	1 3 2	4.37	4.54	4.33	3.95	4.23	3.94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9512		
9555	2 1 3	4.47	4.65	4.16	4.19	3.35	<1	-	-	-	-	-	-	-	-	-	Pos	Pos	Neg	-	-	-	-	-	-	-	-	-	-	9555	
9589	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9589		
9655	1 3 2	-	-	-	4.83	4.46	<1	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	9655
9662	2 1 3	4.41	3.63	4.48	4.08	4.4	<2	-	-	-	<0	<0	2.59	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	9662
9716	3 1 2	4.49	4.85	4.28	-	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	9716
9747	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9747		
9753	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9753		
9890	2 3 1	4.83	5.14	4.43	4.67	4.78	3.78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9890		
9903	2 1 3	4.44	4.94	4.51	4.09	4.6	<2	-	-	-	-	-	-	-	-	-	Neg	Neg	Pos	Pos	Pos	Neg	-	-	-	-	-	-	-	-	9903
9950	3 2 1	4.58	4.28	2.85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9950		

n	142	143	140	123	123	121	13	13	13	63	64	65	29	29	30	100	101	101	127	127	126	28	27	29	23	22	23	16	16	17	n
Min	3.92	3.63	0.00	3.30	0.00	0.00	0	0	0.00	0	0	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Min		
Max	5.31	5.70	6.48	5.08	5.36	4.58	0	0	1.48	0	2.73	3.65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Max			
median	4.50	4.96	4.43	4.18	4.55	0.00	0	0	1.18	0	0	2.78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	median			
m	4.518	4.967	4.417	4.226	4.519	0.039	0	0	1.157	0	0	2.777	neg	neg	pos	neg	neg	pos	pos	pos	neg	neg	pos	neg	pos	neg	pos	m			
s	0.191	0.279	0.279	0.235	0.201	0.209	0	0	0.246	0	0	0.101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	s			
F+	0	0	0	0	0	0	0	0	0	0	4	0	2	1	0	3	9	0	0	0	2	3	1	0	0	0	1	F+			
F-	0	0	1	0	2	0	0	0	1	0	0	2	0	0	2	0	0	0	1	2	0	0	0	2	0	1	0	F-			
<	0	1	4	1	2	0	0	0	0	0	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<				
>	2	0	1	1	1	34	0	0	0	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>				
< OK	3.92	4.28	3.56	3.70	4.00	0.00	0	0	0.80	0	0	2.59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< OK				
> OK	5.19	5.70	5.08	5.00	4.96	1.50	0	0	1.48	0	0	3.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	> OK				

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

Annex 2. z-scores of all participants - January 2016

z-scores were calculated according the formula : $z = (x-m)/s$.

x: result of the individual laboratory, m: mean of the results of all participating laboratories , s: standard deviation of the results of all participating laboratories .

Correct negative results in quantitative analyses and correct results in qualitative analyses obtained a z-score of zero.

False results did not generate a z-score.

■ $2 < |z| \leq 3$, ■ $|z| > 3$

Lab no.	sample	Aerobic microorganisms 30 °C			Enterobacteriaceae			Thermotolerant campylobacter			Listeria monocytogenes			Thermotolerant campylobacter			Listeria monocytogenes			Salmonella			Escherichia coli O157 (VT-neg)			Pathogenic Vibrio spp			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		
1254	2 3 1	-0.249	-1.205	-0.813	-0.027	-0.295	-0.187				0	0	-4.000				0	0	0	0	0	0							1254	
1594	2 3 1	0.118	0.836	-0.061	0.228	1.100	-0.187				0	0	0.991	0	0	0.724	0	0	0	0	0	0							1594	
1970	3 2 1	-0.144	2.482	-0.025	0.781	0.602	-0.187				0	0		0	0	0.724	0	0	0	0	0	0							1970	
2035	2 3 1				-0.538	-0.594	-0.187				0	0		0	0	0	0	0	0	0	0	0							2035	
2050	3 1 2	-0.407	0.084	-0.240	-0.282	0.054	-0.187				0	0		0	0	2.415	0	0	0	0	0	0							2050	
2058	2 3 1	3.528	2.626	-0.133							0	0		0	0	2.415	0	0	0	0	0	0							2058	
2072	2 3 1	-0.302	1.337	-0.491	-0.963	0.203	-0.187				0	0	-1.249	0	0	2.415	0	0	0	0	0	0							2072	
2129	1 2 3	-0.092	-0.417	0.118	-0.538	-0.096	-0.187				0	0		0	0	1.719	0	0	0	0	0	0							2129	
2151	1 2 3	-0.932	0.800	-0.563												0.227	0			0	0	0							2151	
2221	1 2 3	0.118	0.478	-0.598	-0.027	0.203	-0.187				0	0	0.227	0	0		0	0	0	0	0	0						2221		
2324	2 3 1	-0.617	-1.742	0.047	-0.878		4.000				0	0		0	0		0	0	0	0	0	0						2324		
2344	2 1 3	-0.459	-1.097	-0.563	-0.197	-0.694	4.000			0	0	-0.638	0	0	-0.568	0	0	0	0	0	0							2344		
2386	3 1 2	-0.197	0.836	1.659							0	0		0	0		0	0	0	0	0	0						2386		
2402	2 1 3	-3.135	1.731	1.372	2.568	-0.345	4.000				0	0		0	0		0	0	0	0	0	0					2402			
2458	1 3 2	0.695	0.370	-0.133	0.015	-1.739	4.000			0	0	0.665	0	0	0.028	0	0	0	0	0	0							2458		
2637	1 3 2	-0.040	-0.417	-3.071	0.398	-0.444	-0.187				0	0	-1.364	0	0		0	0	0	0	0	0						2637		
2670	1 3 2	2.374	0.227	-2.569							0	0		0	0	0.327	0	0	0	0	0	0						2670		
2704	1 2 3	-0.564	0.728	0.907	0.100	0.950	-0.187				0	0		0	0	-0.469	0	0	0	0	0	0						2704		
2745	1 3 2	-0.932	-0.238	2.376	0.398	0.353	-0.187				0	0		0	0		0	0	0	0	0	0						2745		
2764	1 3 2	0.118	-0.417	0.512	-0.197	-0.444	4.000				0	0		0	0		0	0	0	0	0	0						2764		
2797	3 1 2	-0.066	0.324	-0.520	-0.478	0.358	-0.187				0	0		0	0		0	0	0	0	0	0						2797		
2842	2 3 1										0	0	0				0	0	0	0	0	0							2842	
2920	3 1 2	0.223	0.263	0.907	0.483	0.452	-0.187				0	0		0	0		0	0	0	0	0	0						2920		
2944	1 3 2	0.748	-0.811		1.292	-0.146											0	0	0	0	0	0							2944	
3126	3 2 1		0.764	-0.706													0	0	0	0	0	0								3126
3159	3 1 2	-0.144	0.227	1.050	0.398	0.353	-0.187				0	0	0.028	0	0		0	0	0	0	0	0						3159		
3305	3 2 1	-0.040	-1.849	-0.025	0.654	-1.192	-0.187				0	0	-1.044	0	0	0	0	0	0	0	0	0						3305		
3457	2 1 3				-0.325	0.353	-0.187				0	0		0	0	0	0	0	0	0	0	0						3457		
3533	2 1 3	2.322	0.549	-0.348							0	0		0	0		0	0	0	0	0	0						3533		
3587	2 3 1	1.744	0.657	-1.817	0.228	-1.092	-0.187				0	0		0	0		0	0	0	0	0	0						3587		
3626	2 1 3	0.433	-0.238	-0.061	0.313	-0.594	-0.187				0	0	-1.045	0	0	0.227	0	0	0	0	0	0						3626		
3825	3 1 2	-0.465	0.048	-1.466							0	0		0	0		0	0	0	0	0	0						3825		
3868	2 3 1	0.223	-0.704	0.907	1.207	-0.345	-0.187				0	0	1.316	0	0	0.526	0	0	0	0	0	0						3868		
3923	3 1 2	0.065	-1.384	4.000	-0.750	-4.000	-0.187				0	0		0	0		0	0	0	0	0	0						3923		
3925	2 1 3	-0.512	1.051	0.154							0	0		-0.580	-0.046	-0.187				0	0	0							3925	
4064	2 3 1	-0.407	-0.453	-1.028							0	0		0.654	0.552	-0.187				0	0	0							4064	
4100	2 1 3	0.065	-0.167	-0.348	0.143	0.552	4.000				0	0	0.010	0	0	0	0	0	0	0	0	0						4100		
4171	3 2 1	-0.722	-1.097	0.154							0	0		0	0		0	0	0	0	0	0						4171		
4246	1 3 2	-1.404	-1.384	0.584	-0.708	-1.789	4.000				0	0		0	0		0	0	0	0	0	0						4246		
4288	3 2 1	2.374	1.659	0.548	2.440	1.199	-0.187				0	0		0	0	1.122	0	0	0	0	0	0						4288		
4339	3 2 1										0	0		0	0		0	0	0	0	0	0						4339		
4352	2 3 1	-0.722	1.122	0.871	0.313	0.851	4.000				0	0	-0.071	0	0	0	0	0	0	0	0	0						4352		
4400	3 1 2	0.695	-0.704	1.516	-0.240	-0.743	4.000				0	0		0	0		0	0	0	0	0	0						4400		
4449	1 2 3	-0.512	0.657	-0.778	-1.771	1.149	-0.187				0	0		0	0		0	0	0	0	0	0						4449		
4557	1 2 3	0.695	0.764	0.369							0	0	1.316	0	0	-0.568	0	0	0	0	0	0						4557		
4562	3 1 2	-0.722	-1.455	-0.276	0.228	0.851	-0.187				0	0		0	0		0	0	0	0	0	0						4562		
4605	3 1 2										0	0		0	0	2.216	0	0	0	0	0	0						4605		
4633	1 3 2	-0.354	0.585	-0.061	0.739	0.552	-0.187				0	0		0	0		0	0	0	0	0	0						4633		
4635	2 3 1	-0.040	0.191	0.441	-0.538	-0.594	4.000				0	0		0	0		0	0	0	0	0	0						4635		

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: www2.slv.se/absint

The National Food Agency's reference material

As a complement to the proficiency testing, National Food Agency produces also reference material (RM) for internal quality control: a total of 8 RM for food and drinking water microbiological analyses, including pathogens, are available.

Information available on our website: www.livsmedelsverket.se/en/RM-micro