

Proficiency testing

Food Microbiology

– January 2013

by Laurence Nachin, Christina Normark and Irina Boriak



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 used by laboratories 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: www.slv.se/absint/index.aspx

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 7 RM for food and drinking water microbiological analyses, including pathogens, are available.

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

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- 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*

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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. For this PT round, between 52 and 98 % of the participants reported which method and/or medium they used for the different analyses. Method information is sometimes difficult to interpret, e.g. many 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.




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
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

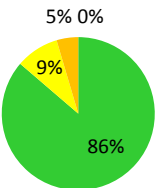
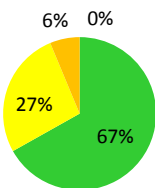
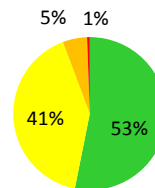
Results of the PT round January 2013

General outcome

Samples were sent to 180 laboratories, 34 in Sweden, 126 in other European countries, and 20 outside Europe. 175 laboratories reported results, 114 (65 %) provided at least one result that received an annotation. In the previous round (January 2011) with similar analyses, the proportion was 38 %.

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

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

		Mixture A			Mixture B			Mixture C		
% participants with										
Organisms		<i>Staphylococcus saprophyticus</i> <i>Hafnia alvei</i> <i>Listeria seeligeri</i> <i>Listeria ivanovii</i> <i>Salmonella</i> Enteritidis <i>Vibrio cholera</i>			<i>Micrococcus</i> sp. <i>Aeromonas caviae</i> <i>Campylobacter lari</i> <i>Listeria monocytogenes</i> <i>Vibrio parahaemolyticus</i>			<i>Micrococcus</i> sp. <i>Yersinia enterocolitica</i> <i>Campylobacter jejuni</i> <i>Salmonella</i> Dublin <i>Escherichia coli</i> O157		
Analysis		Target	F%	Out	Target	F%	Out	Target	F%	Out
Aerob. microorg, 30 °C		<i>S. saprophyticus</i> <i>H. alvei</i>	0	4	<i>Micrococcus</i> <i>A. caviae</i>	0	3	<i>Micrococcus</i>	1	7
Enterobacteriaceae		<i>H. alvei</i>	1	3	(<i>A. caviae</i>)	28	-	<i>Y. enterocolitica</i>	40	16
Thermo. camp.	Quant.	-	1	-	<i>C. lari</i>	45	0	<i>C. jejuni</i>	10	0
	Qual.	-	2	-		17	-		7	-
<i>L. mono-</i> <i>cytogenes</i>	Quant.	(<i>L. ivanovii</i>) (<i>L. seeligeri</i>)	6	-	<i>L. monocytogenes</i>	0	5	-	0	-
	Qual.	(<i>L. ivanovii</i>) (<i>L. seeligeri</i>)	11	-		1	-		3	-
<i>Salmonella</i>		<i>S. Enteritidis</i>	0	-	-	2	-	<i>S. Dublin</i>	5	-
<i>E. coli</i> O157		-	3	-	-	3	-	<i>E. coli</i> O157	3	-
Path. <i>Vibrio</i> spp.		<i>V. cholera</i>	11	-	<i>V. parahaemolyticus</i>	33	-	-	6	-
<i>Y. enterocolitica</i>		-	0	-	-	0	-	<i>Y. enterocolitica</i>	0	-

- : no target organism or no value; (*microorganism*): false positive

Aerobic microorganisms, 30 °C

Mixture A

The colonies counted for this analysis were mainly from the strains of *Staphylococcus saprophyticus* and *Hafnia alvei* present at the highest concentration in mixture A.

Mixture B

The colonies counted for this analysis were mainly from the strains of *Micrococcus sp.* and *Aeromonas caviae* present at the highest concentration in mixture B.

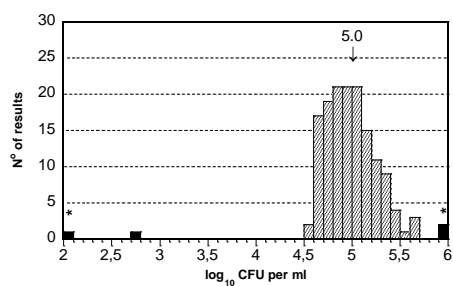
Mixture C

The colonies counted for this analysis were mainly from the strain of *Micrococcus sp.* present at the highest concentration in mixture C.

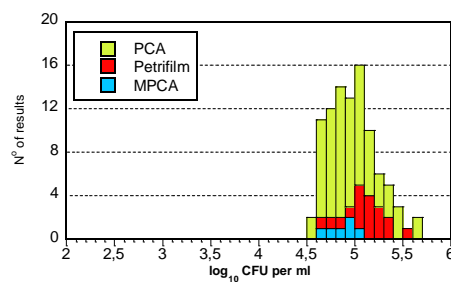
Results of aerobic microorganisms analysis

Medium	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	150	4.98	0.25	0	2	4	151	4.67	0.27	0	2	3	151	4.46	0.14	1	4	6
PCA	68	4.95	0.26	0	1	2	68	4.61	0.24	0	1	2	68	4.46	0.12	1	2	2
Petrifilm™	19	5.10	0.21	0	0	1	19	4.77	0.33	0	0	0	19	4.39	0.16	0	1	1
MPCA	6	4.85	0.12	0	0	0	6	4.63	0.19	0	0	0	6	4.46	0.10	0	0	0

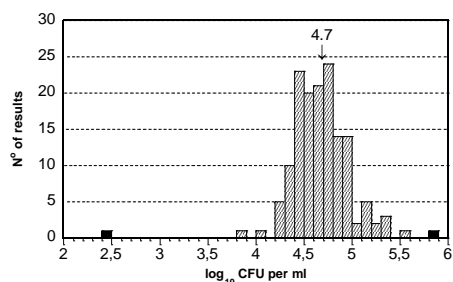
A



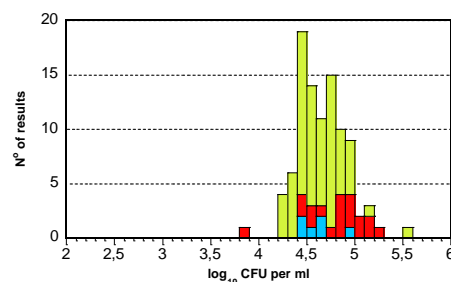
A



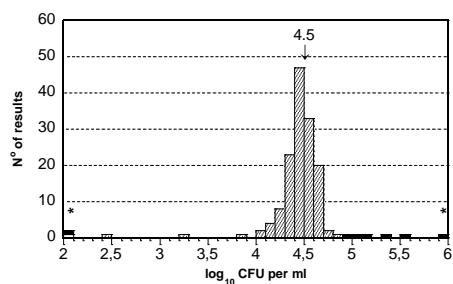
B



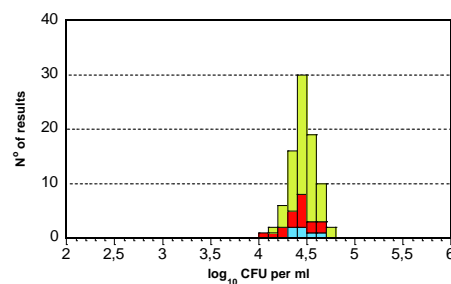
B



C



C



No obvious differences in the results can be seen depending on the medium used for this analysis. However, the results are noticeably more spread for the mixture A and B than for mixture C. In the two first mixtures, counted colonies are from two different microorganisms while only from one in mixture C. It is not obvious that this is the reason for the differences in results but it can be speculated that a bigger variability in colonies appearance can lead to a higher variation in colonies enumeration.

Enterobacteriaceae

Mixture A

Hafnia avei was the target organism for this analysis which did not reveal special difficulty.

Mixture B

Mixture B contained a strain of *Aeromonas caviae* which forms small red colonies on VRBG but is oxidase positive and therefore differentiates from enterobacteriaceae. The 34 laboratories that reported a false positive result have failed or not performed the confirmation test.

Mixture C

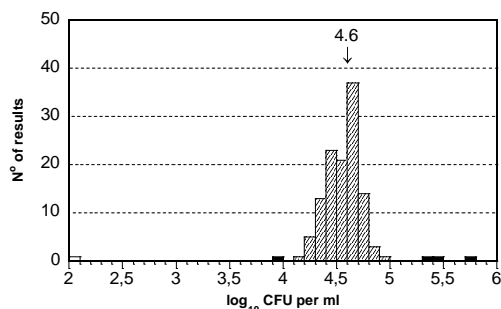
Yersinia enterocolitica was the main target organism for the analysis but single colonies of *Salmonella* Dublin could appear as well if non- or low-diluted sample was analysed. Even though the concentration of *Y. enterocolitica* in mixture C was 3.5 log₁₀ cfu/ml, 40% of the laboratories reported a false negative result. Moreover, many laboratories reported results considered as low outliers, which probably accounted for the enumeration of *S. Dublin* colonies only.

At NFA, *Y. enterocolitica* formed typical but small colonies on VRBG and their enumeration did not cause difficulty after 24±2 hours incubation at 37 °C. However the small size of the colonies could explain the very high dispersion of the results as well as the high amount of false results if plates were incubated for a shorter period.

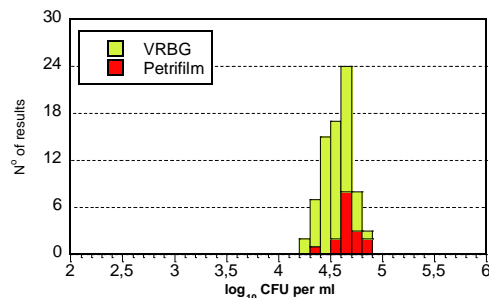
Results of enterobacteriaceae analysis

Medium	Mixture A					Mixture B					Mixture C				
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
Total	123	4.55	0.15	1	1 3	121	-	-	34	- -	121	3.26	0.14	48	19 1
VRBG	62	4.53	0.13	0	1 1	60	-	-	11	- -	60	3.25	0.16	22	7 1
Petrifilm™	17	4.65	0.12	0	0 1	17	-	-	7	- -	16	3.14	0.12	7	6 0
Other	6	-	-	0	0 0	6	-	-	1	- -	6	-	-	4	0 0

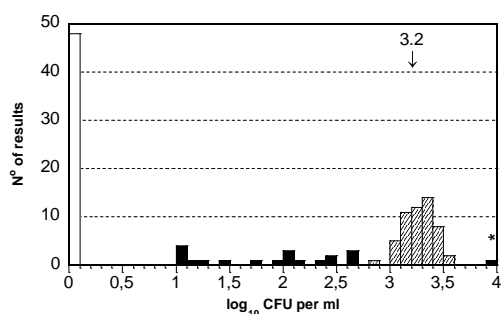
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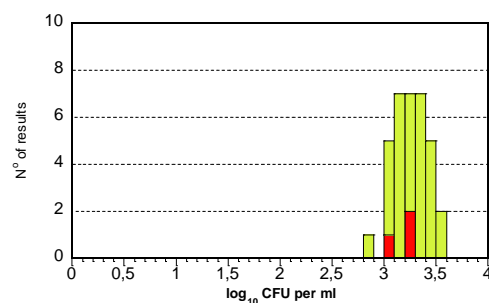
A



C



C



Most of the laboratories used VRBG plate or Petrifilm™ for the analysis of enterobacteriaceae which did not lead to significant results differences when analysing mixture A. On the other hand, the use of Petrifilm™ is almost exclusively linked to false negative results or low outliers for the analysis of mixture C. The reason for this correlation is difficult to assess, but it might be possible that the strain of *Y. enterocolitica* present in mixture C grew slower or formed colonies difficult to see on Petrifilm™.

Thermotolerant campylobacter

Mixture A

Mixture A did not contain any strain of thermotolerant campylobacter

Mixture B

A strain of *Campylobacter lari* was target organism for this analysis but was present at low concentration in mixture B ($1.4 \log_{10}$ cfu/ml). It was the first time this strain was used in the PT program. At NFA, we noticed that it grew slower than other *Campylobacter* strains. This, together with the low concentration, can explain the big dispersion of the results and the false negative results obtained for both the quantitative and qualitative analysis.

Mixture C

Mixture C contained a strain of *Campylobacter jejuni* at a concentration of $1.5 \log_{10}$ cfu/ml. The analysis did not cause special difficulties but the results distribution is big.

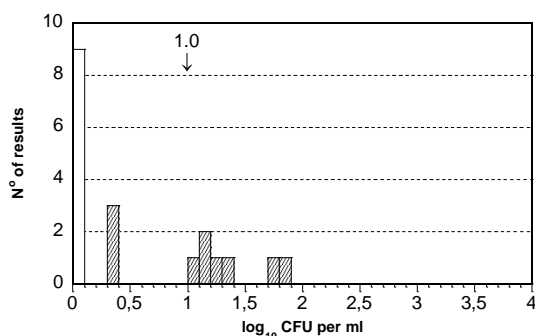
Results of thermotolerant campylobacter quantitative analysis

Quantitative	Mixture A					Mixture B					Mixture C				
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
Total	19	-	-	1	- -	19	1.03	0.57	9	0 0	19	0.92	0.36	1	0 0
ISO	5	-	-	0	- -	5	0.90	0.53	2	0 0	5	0.98	0.15	0	0 0
NMKL	5	-	-	1	- -	5	0.85	0.49	2	0 0	5	0.75	0.53	0	0 0

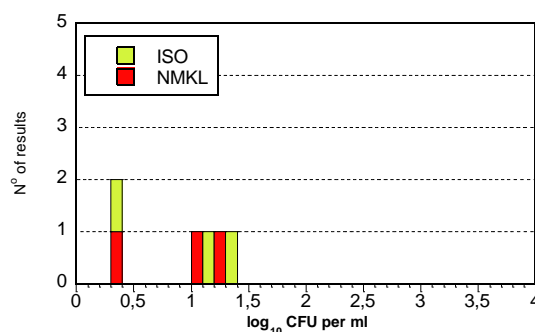
Results of thermotolerant campylobacter qualitative analysis

Qualitative	n	F	n	F	n	F
Total	39	1	39	7	39	2
ISO	3	0	3	0	3	1
NMKL	9	0	9	1	9	0

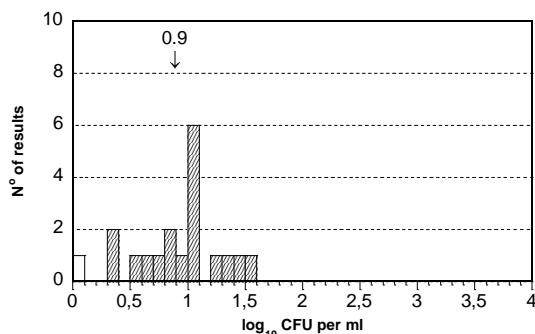
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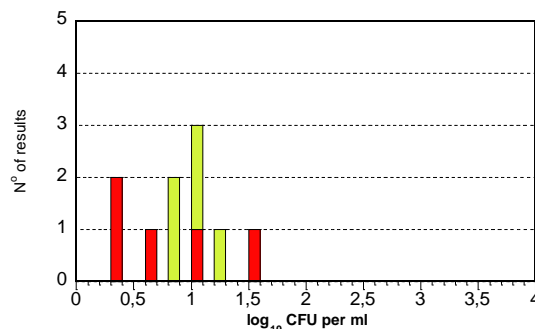
B



C



C



Few laboratories participate in the analysis of thermotolerant campylobacter, it is therefore quite difficult to draw any conclusion regarding the use of different methods. As a general comment, for both mixture B and C, quantitative results obtained by NFA are higher than the average results of the participants: 1.36 versus 1.03 and 1.52 versus 0.92, respectively.

The moisture of the medium can have an influence on the result: campylobacter cells are sensitive to dry plates; therefore, it is preferable to use moist plates and let the sample dry on the plate before incubation. However, if the plates are too moist, colonies tend to flow together which makes the reading more difficult. Moreover, the surface spreading on plates should be done carefully. Studies at NFA have shown that strong surface spreading gives fewer colonies on the plates than careful spreading. At NFA, a spiral spreader is used, which eliminates the variation caused by manual spreading, and can explain the higher results obtained.

Listeria monocytogenes

Mixture A

A strain of *Listeria seeligeri* and of *Listeria ivanovii* were included in mixture A. On ALOA medium and other chromogenic medium, colonies of *L. ivanovii* can be misjudged as *L. monocytogenes*. On blood-based medium (LMBA), and medium revealing esculine hydrolysis (PALCAM and Oxford) both *L. seeligeri* and *L. ivanovii* form colonies similar to *L. monocytogenes*.

However, upon confirmation, these strains can be differentiated: *L. seeligeri* and *L. ivanovii* ferment xylose while *L. monocytogenes* does not.

Mixture B

A strain of *L. monocytogenes* was present in mixture B.

Mixture C

No target organism was present in mixture C for this analysis.

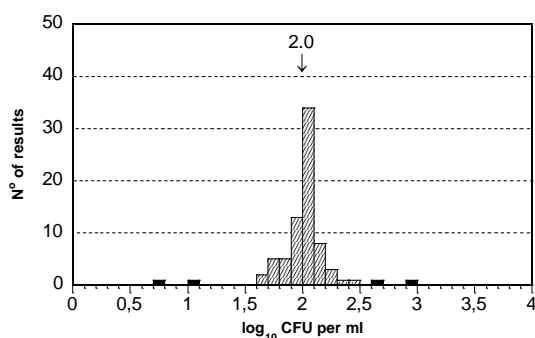
Results of *L. monocytogenes* quantitative analysis

Method	Mixture A					Mixture B					Mixture C				
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
Total	73	-	-	4	- -	76	2.00	0.14	0	2 2	74	-	-	0	- -
ISO	17			1		18	2.03	0.11	0	1 0	17			0	
NMKL	12			2		13	1.98	0.12	0	1 1	12			0	
Rapid L.m	16			2		15	2.00	0.12	0	0 1	15			0	

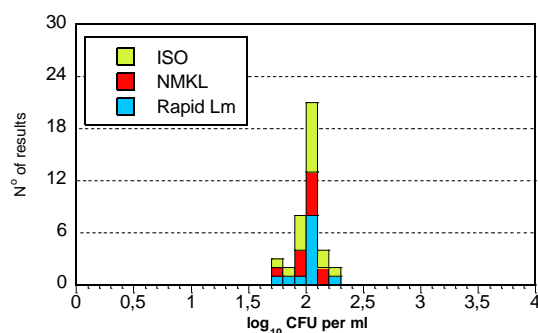
Results of *L. monocytogenes* qualitative analysis

Method	n	F	n	F	n	F
Total	108	11	109	0	109	2
ISO	19	2	19	0	19	0
NMKL	12	2	12	0	12	0
Rapid L.m	14	2	14	0	14	0
PCR	7	0	7	0	7	0

B



B



Most of the laboratories used a chromogenic medium for isolation. No correlation between method used and false results or outliers can be concluded.

Salmonella

Mixture A

Mixture A contained a strain of *Salmonella* Enteritidis.

Mixture B

Mixture B did not contain any *Salmonella* strain. Few atypical colonies could appear on XLD medium.

Mixture C

A strain of *Salmonella* Dublin, at a concentration of 13 cfu/ml, was target organism for this analysis. At NFA, this strain formed typical colonies on XLD medium but atypical white colonies on BriS chromogenic medium, after enrichment in BPW and RVS. Moreover, this strain is sensitive to temperature above 42 °C and to high concentration of MgCl₂ in RVS medium (2). According to NMKL method this concentration should not be higher than 29 g/l. These characteristics might explain the report of 7 false negative results.

Results of Salmonella qualitative analysis

Method	Mixture A		Mixture B		Mixture C	
	n	F	n	F	n	F
Total	141	0	142	3	141	7
ISO	26	0	25	0	25	0
NMKL	32	0	32	0	32	3
VIDAS	16	0	16	0	16	1
PCR	11	0	11	0	11	0

Most of the laboratories used XLD agar together with another medium for the isolation step. No correlation between the method used and false negative result can be concluded.

Escherichia coli O157

Mixture A

Mixture A did not contain any *E. coli* O157 strain but a strain of *Hafnia alvei* which, as *E. coli* O157, does not ferment sorbitol and can form beige colonies on SMAC or CT-SMAC. However, *H. alvei* differentiates from *E. coli* O157 upon confirmation.

Mixture B

Mixture B did not contain any *E. coli* O157 strain but a strain of *Aeromonas caviae* which, as *E. coli* O157, does not ferment sorbitol. Although it can form beige colonies on SMAC or CT-SMAC upon direct isolation after enrichment, *A. caviae* differentiates from *E. coli* O157 in the confirmation steps of the analysis. However, at NFA, no such colonies were observed if an immuno-separation step was performed between enrichment and isolation. Only atypical pink colonies grew on SMAC plates.

Mixture C

Mixture C contained an *E. coli* O157 strain at a concentration of 16 cfu/ml.

Results of E. coli O157 qualitative analysis

Method	Mixture A		Mixture B		Mixture C	
	n	F	n	F	n	F
Total	33	1	33	1	33	1
ISO	5	0	5	0	5	0
NMKL	5	0	5	1	5	0

Almost all laboratories that reported method information for this analysis used CT-SMAC together with another medium for the isolation step. No link between method/medium used and false results can be concluded.

Pathogenic *Vibrio* spp.

Mixture A

A strain of *Vibrio cholera* was target organism for this analysis and was present at a concentration of 5.0 log₁₀ cfu/ml in mixture A. At NFA, the strain formed typical yellow colonies on TCBS plate after enrichment in APW 2 % or SPB.

Mixture B

A strain of *Vibrio parahaemolyticus* was target organism for this analysis. Although the strain was present at a concentration of 4.3 log₁₀ cfu/ml in mixture B, one third of the laboratories that performed the analysis reported a false negative result. Upon the quality control performed at NFA, the strain formed typical blue-green colonies on TCBS plate after enrichment in APW 2 % or SPB.

Mixture C

No target organism was present in mixture C for this analysis.

Results of pathogenic Vibrio spp. qualitative analysis

Method	Mixture A		Mixture B		Mixture C	
	n	F	n	F	n	F
Total	18	2	18	6	18	1
ISO	6	1	6	1	6	0
NMKL	6	1	6	2	6	0

Almost all laboratories that performed the analysis used APW 2% for enrichment and TCBS agar for isolation. No correlation between method used and false results can be concluded.

Yersinia enterocolitica

Mixture A

No target organism was present in mixture A.

Mixture B

No target organism was present in mixture B.

Mixture C

Mixture C contained a *Yersinia enterocolitica* strain at a concentration of 3.5 log₁₀ cfu/ml, which was also target organism for the analysis of enterobacteriaceae.

Results of Y. enterocolitica qualitative analysis

Method	Mixture A		Mixture B		Mixture C	
	n	F	n	F	n	F
Total	15	0	15	0	15	0
ISO	5	0	5	0	5	0
NMKL	4	0	4	0	4	0

Most laboratories used PSB for enrichment and CIN as isolation medium.

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.

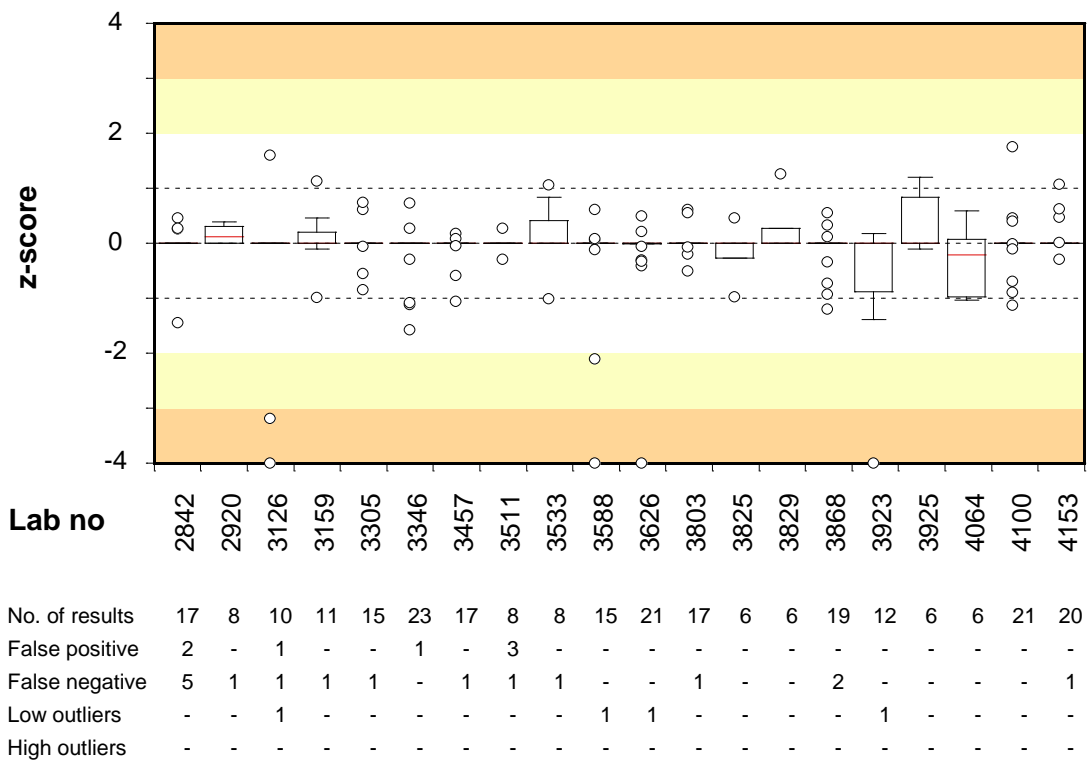
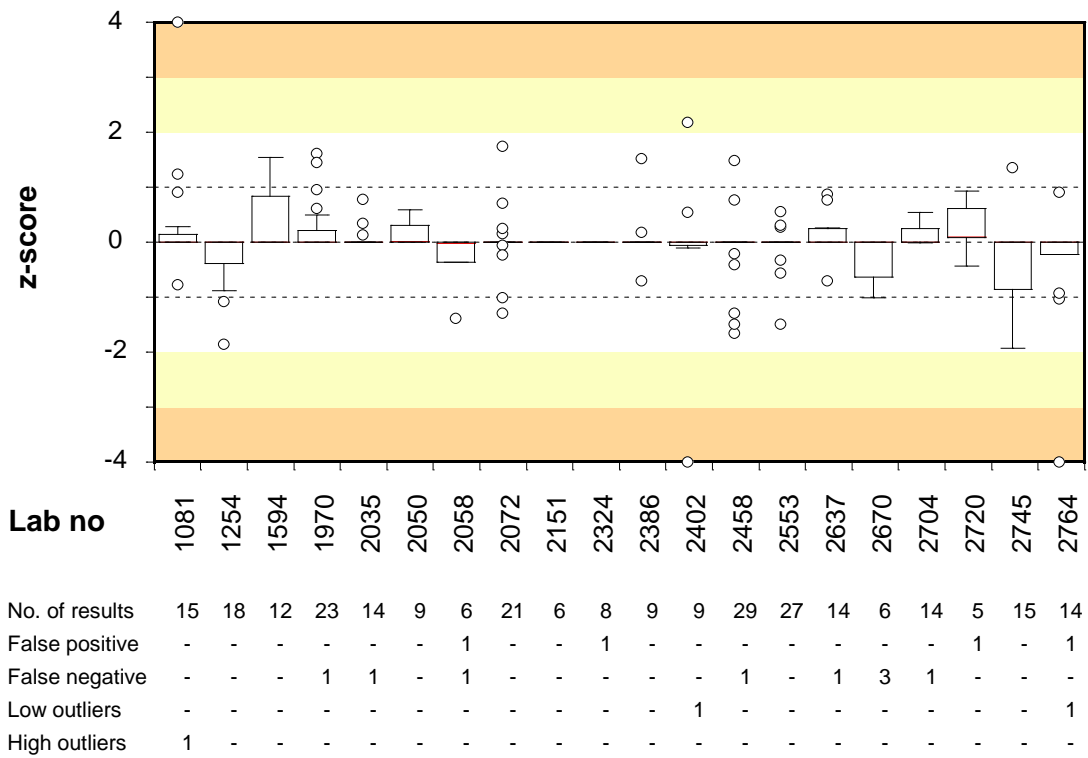
All the results from each laboratory – outliers included and false results excluded – were compiled into a box plot (Figure 1) based on their z-scores. The smaller and more centred round zero the box of a laboratory is, the closer its results are to the general mean values calculated for all laboratory results.

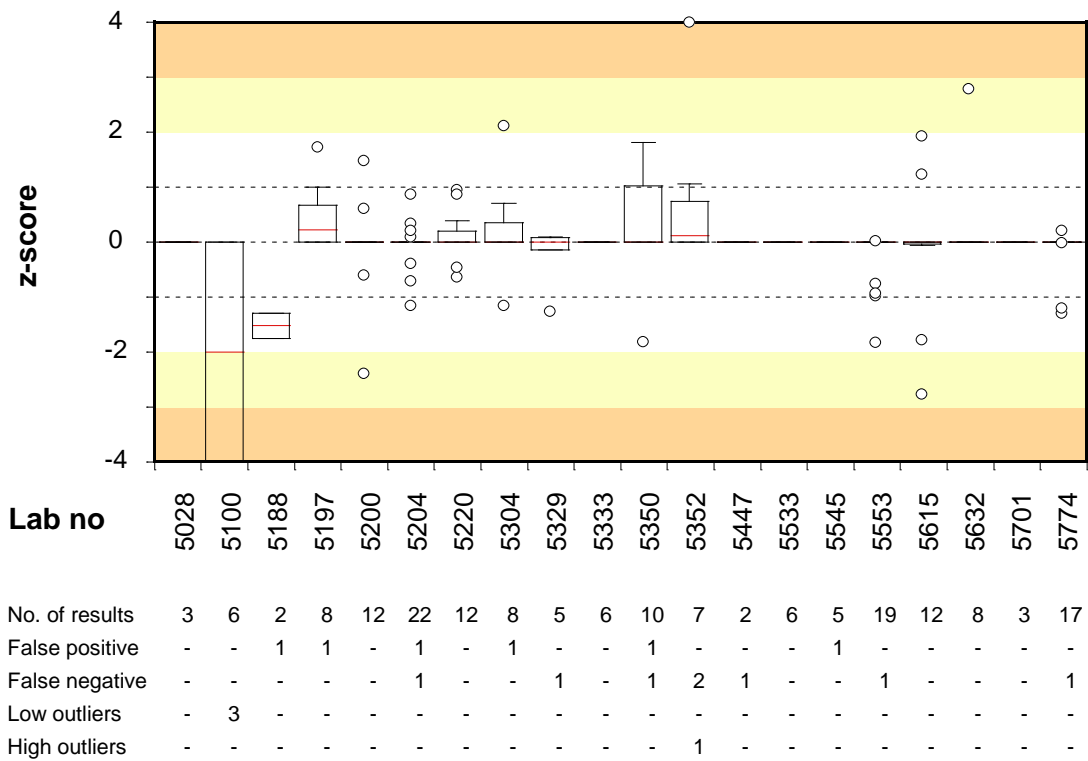
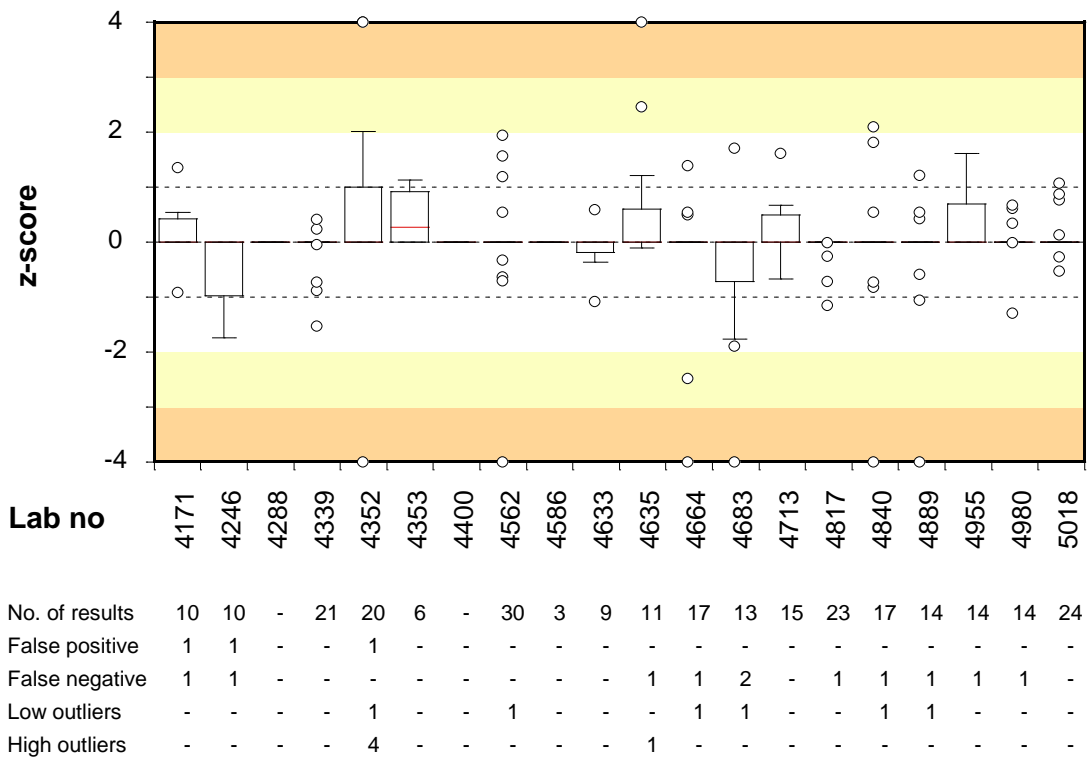
The laboratories were not grouped or ranked based on their results. However, for each laboratory, the numbers of false results and outliers are presented below the box plots. These results are also highlighted in Annex 1, where all the reported results are listed, and the minimum and maximum accepted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

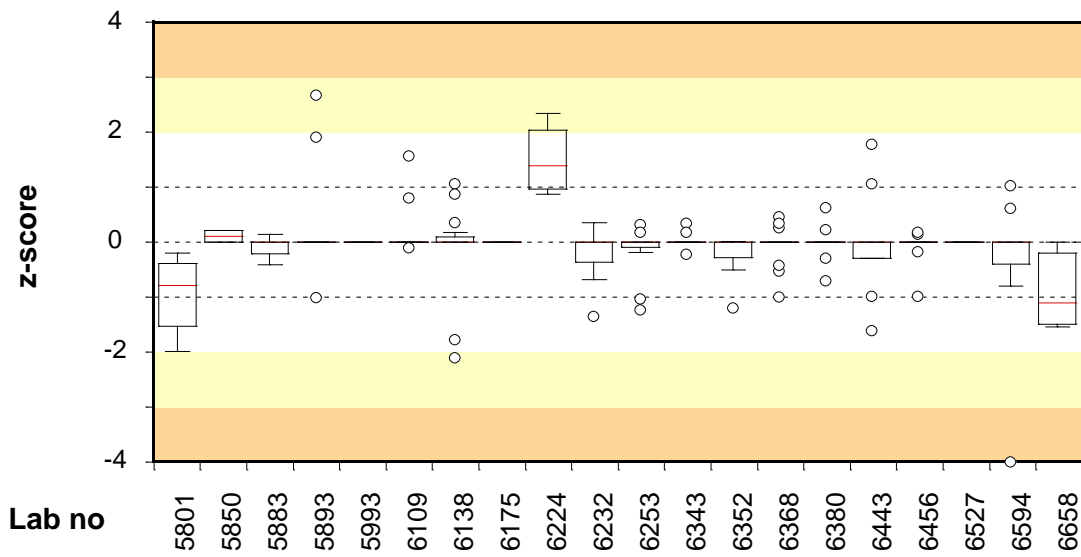
Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol (3). Samples for follow-up can be ordered, free of charge via our website: www.slv.se/pt_extra

Box plots and numbers of deviating results for each laboratory

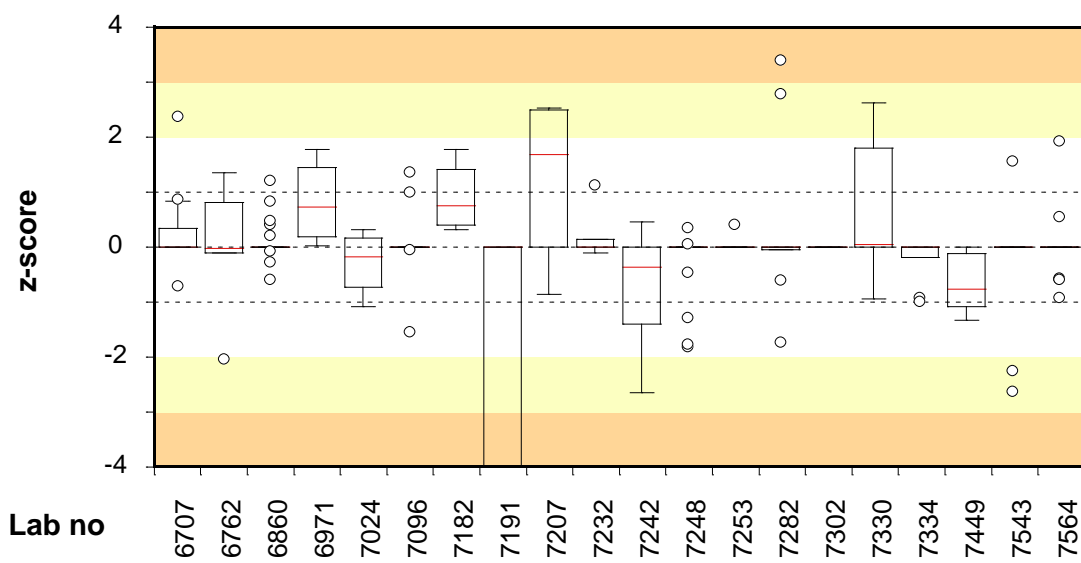
- *The plots are based on the laboratory results from all analyses transformed into z-scores calculated according to the formula: $z = (x-m)/s$, where x is the result of the individual laboratory, m is the mean of the results of all participating laboratories, and s is the standard deviation.*
- *Correct results for quantitative analyses without target organism and for qualitative analyses generate a z-value 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.*
- *Very deviating results are represented by circles and are calculated as follow: the lowest result in the box $- 1.5 \times$ (the highest result in the box $-$ the lowest result in the box) or the highest result in the box $+ 1.5 \times$ (the highest result in the box $-$ 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.*



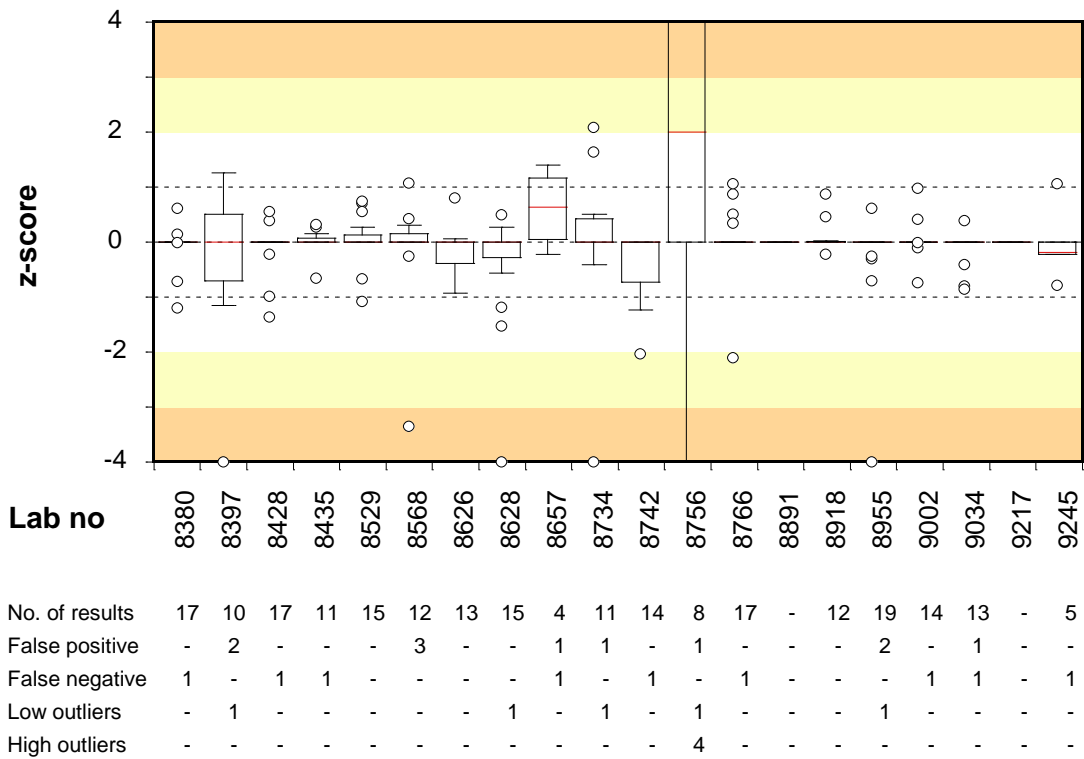
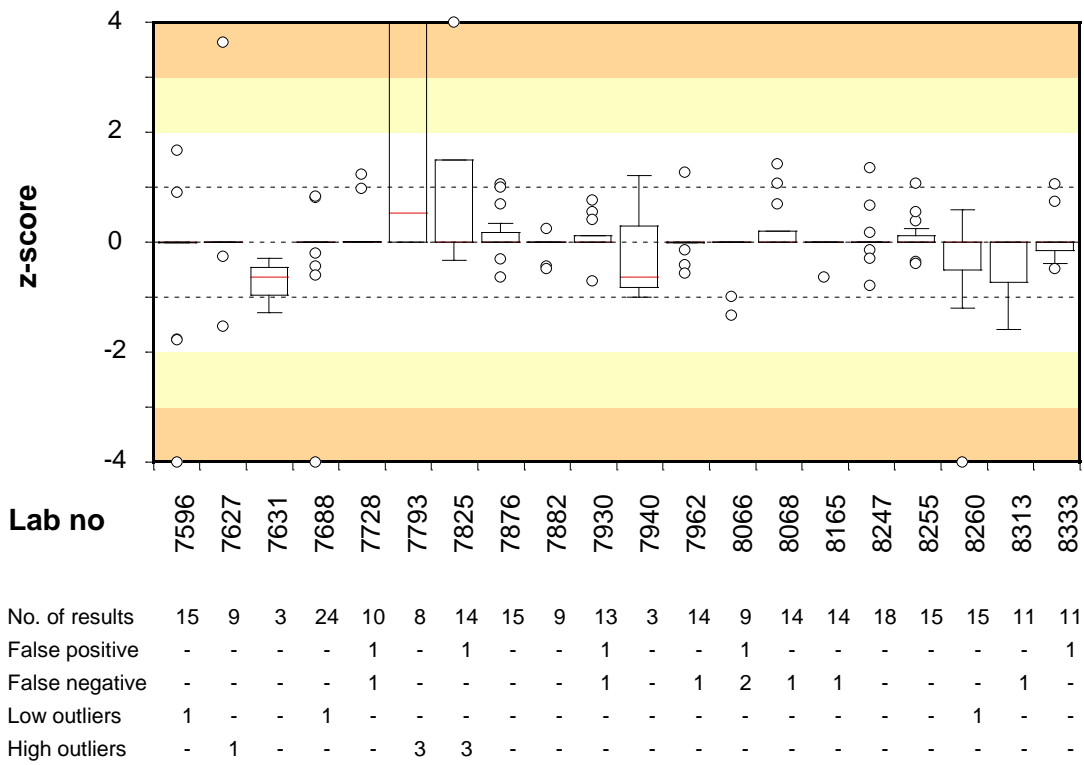


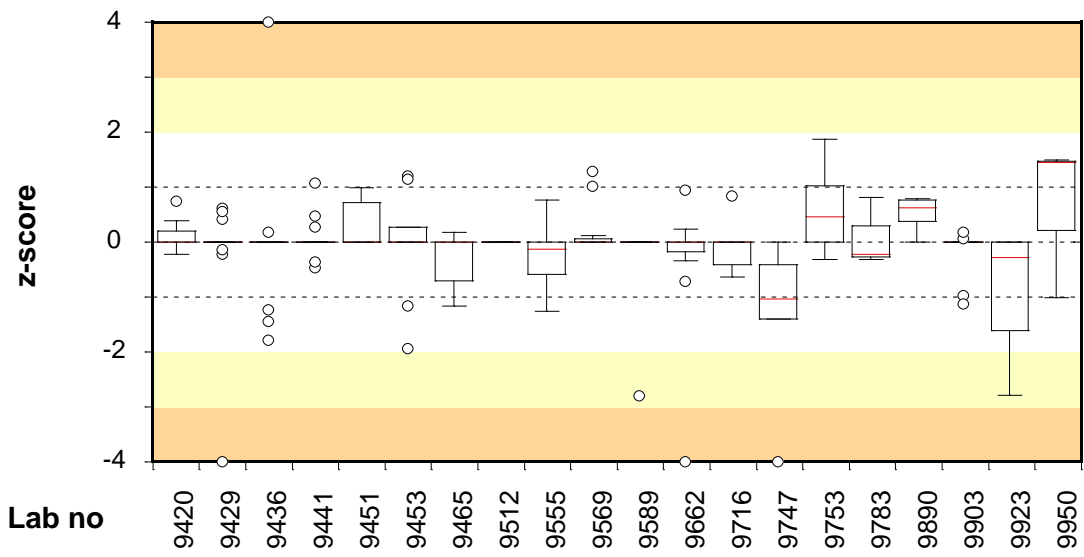


Lab no	5801	5850	5883	5893	5993	6109	6138	6175	6224	6232	6253	6343	6352	6368	6380	6443	6456	6527	6594	6658
No. of results	5	2	15	10	3	9	15	3	4	7	12	9	7	18	10	9	10	6	11	6
False positive	1	-	-	1	-	-	-	-	1	1	-	-	1	-	2	-	1	-	1	-
False negative	-	1	-	1	-	-	-	-	1	1	-	-	1	-	-	-	1	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Lab no	6707	6762	6860	6971	7024	7096	7182	7191	7207	7232	7242	7248	7253	7282	7302	7330	7334	7449	7543	7564
No. of results	14	5	29	4	4	11	4	7	5	6	8	18	12	10	6	8	9	6	12	26
False positive	-	1	1	1	1	1	1	-	-	-	1	-	-	1	-	1	-	-	-	2
False negative	-	-	-	1	1	-	1	2	1	-	-	3	-	-	-	-	-	-	-	2
Low outliers	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-





Lab no	9420	9429	9436	9441	9451	9453	9465	9512	9555	9569	9589	9662	9716	9747	9753	9783	9890	9903	9923	9950	
No. of results	8	15	17	14	15	10	9	-	8	17	12	12	6	6	8	3	6	11	8	3	
False positive	-	-	-	-	-	1	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-
False negative	1	-	1	1	-	1	-	-	-	1	-	-	-	-	-	-	-	1	1	-	
Low outliers	-	1	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	
High outliers	-	-	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 (4). 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 no.
A	<i>Staphylococcus saprophyticus</i>	SLV-013
	<i>Hafnia alvei</i>	SLV-015
	<i>Listeria seeligeri</i>	SLV-347
	<i>Listeria ivanovii</i>	SLV-348
	<i>Salmonella</i> Enteritidis	SLV-436
	<i>Vibrio cholera</i>	SLV-530
B	<i>Micrococcus</i> sp.	SLV-055
	<i>Aeromonas caviae</i>	SLV-206
	<i>Campylobacter lari</i>	SLV-559
	<i>Listeria monocytogenes</i>	SLV-361
	<i>Vibrio parahaemolyticus</i>	SLV-529
C	<i>Micrococcus</i> sp.	SLV-055
	<i>Yersinia enterocolitica</i>	SLV-408
	<i>Campylobacter jejuni</i>	SLV-540
	<i>Salmonella</i> Dublin	SLV-242
	<i>Escherichia coli</i> O157	SLV-479

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

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 was performed in conjunction with manufacturing of the mixtures according to Scheme Protocol (3). The results are presented in Table 3. Homogeneity requires that the standard deviation and the difference between the highest and lowest value of results from 10 samples analysed do not exceed 0.15 log₁₀ units and 0.5 log₁₀ units, respectively.

Table 3. Concentration mean (*m*) and standard deviation (*s*) from analyses of 10 randomly selected vials per mixture, expressed in log₁₀ cfu (colony forming units) per ml of sample.

Analysis and method	A		B		C	
	m	s	m	s	m	s
Aerobic microorganisms 30 °C NMKL-method no. 86	5.17	0.04	4.89	0.10	4.48	0.05
Enterobacteriaceae NMKL-method no. 144	4.36	0.05	–	–	3.50	0.06
Thermotolerant campylobacter, quant. NMKL method no. 119	–	–	1.36	0.14	1.52	0.15
Thermotolerant campylobacter, qual. NMKL method no. 119	–	–	pos	–	pos	–
<i>Listeria monocytogenes</i> , quant. NMKL method no. 136	–	–	2.02	0.12	–	–
<i>Listeria monocytogenes</i> , qual. NMKL method no. 136	–	–	pos	–	–	–
<i>Salmonella</i> NMKL method no. 71	1.22*	0.15*	–	–	1.12*	0.04*
<i>Escherichia coli</i> O157 NMKL method no. 164	–	–	–	–	1.22**	0.03**
Pathogenic <i>Vibrio</i> spp. NMKL-method no. 156	5.01*	0.08*	4.27*	0.19*	–	–
<i>Yersinia enterocolitica</i> NMKL-method no. 117	–	–	–	–	3.50	0.06

– No target organism

* Internal values based on the analyses results of parallel mixtures

** Values based on the analyses results of thermotolerant coliform bacteria and *E. coli* (NMKL method no 125)

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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			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	
9420	1 2 3	4.92	4.77	4.46	4.66	<2	<2	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	-	-	-	-	-	-	-	-	-	9420		
9429	1 2 3	4.92	4.63	4.54	4.61	<1	1	-	-	-	<1	2.08	<1	-	-	-	Neg	Pos	Neg	Pos	Neg	Pos	-	-	-	-	-	-	-	9429		
9436	1 2 3	4.62	4.34	4.48	4.28	<1	<1	-	-	-	<1	2.61	<1	-	-	-	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Neg	Pos	-	-	-	-	9436		
9441	2 1 3	4.86	4.57	4.52	4.71	<2	<2	-	-	-	<1	2.04	<1	-	-	-	Neg	Pos	Neg	Pos	Neg	Pos	-	-	-	-	-	-	-	9441		
9451	2 3 1	5.15	4.93	4.52	4.69	<1	3.36	-	-	-	<1	2.11	<1	-	-	-	Neg	Pos	Neg	Pos	Neg	Pos	-	-	-	-	-	-	-	9451		
9453	3 2 1	4.5	4.36	4.62	4.59	3.01	3.42	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	Pos	Neg	Neg	-	-	-	-	-	-	-	9453		
9465	3 2 1	4.76	4.36	4.36	4.48	<1	3.28	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	-	-	-	-	-	-	-	-	9465		
9512	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9512	
9555	2 1 3	4.85	4.87	4.42	4.36	2.74	3.16	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	-	-	-	-	-	-	-	-	-	9555	
9569	3 1 2	5	4.7	4.63	4.7	<1	<1	-	-	-	<1	2.01	<1	Neg	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Neg	Pos	-	-	-	-	-	-	-	9569	
9589	3 2 1	-	-	-	-	-	-	-	-	-	<1	1.6	<1	Neg	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Neg	Pos	-	-	-	-	-	-	-	9589	
9662	2 3 1	4.8	4.73	4.41	4.69	<1	1.04	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	Pos	Neg	Pos	-	-	-	-	-	-	-	-	9662	
9716	1 3 2	4.82	4.56	4.57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	-	-	-	-	-	-	-	-	-	9716	
9747	1 3 2	4.63	4.49	4.4	4.34	<1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9747	
9753	2 3 1	5.2	4.93	4.6	4.83	4.28	3.21	-	-	-	-	-	-	-	-	-	-	-	Pos	Neg	Pos	-	-	-	-	-	-	-	-	-	9753	
9783	3 1 2	4.92	4.88	4.41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9783	
9890	2 3 1	5.17	4.82	4.56	4.65	0	3.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9890	
9903	1 3 2	4.99	4.41	4.48	4.38	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9903	
9923	2 3 1	4.65	4.52	4.2	4.13	<2	<2	-	-	-	-	-	-	-	-	-	-	-	Neg	Pos	Neg	Pos	Neg	Pos	-	-	-	-	-	-	9923	
9950	2 3 1	5.33	4.4	4.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9950	
n		150	151	151	123	121	121	19	19	19	73	76	74	39	39	39	108	109	109	141	142	141	33	33	33	18	18	18	15	15	15	n
Min		1.99	1.98	0	0	0	0	0	0	0	0	0.70	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Min	
Max		6.08	6.60	6.26	5.73	4.58	4.00	0.60	1.85	1.54	3.03	2.94	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Max	
median		4.95	4.65	4.47	4.59	0	3.28	0	1.14	1.00	0	2.01	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	median	
m		4.98	4.67	4.46	4.55	0	3.26	0	1.03	0.92	0	2.00	0	neg	pos	pos	neg	pos	neg	pos	neg	pos	neg	neg	pos	pos	pos	neg	neg	pos	m	
s		0.25	0.27	0.14	0.15	0	0.14	0	0.57	0.36	0	0.14	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	s	
F+		0	0	0	0	34	0	1	0	0	4	0	0	1	0	0	11	0	2	0	3	0	1	1	0	0	0	1	0	0	0	F+
F-		0	0	1	1	0	48	0	9	1	0	0	0	0	7	2	0	0	0	0	0	7	0	0	1	2	6	0	0	0	0	F-
<		2	2	4	1	0	19	0	0	0	0	2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<	
>		4	3	6	3	0	1	0	0	0	0	2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>	
< OK		4.50	3.82	4.00	4.13	0	2.88	0	0.30	0.30	0	1.60	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< OK	
> OK		5.66	5.57	4.80	4.90	0	3.51	0	1.85	1.55	0	2.40	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	> OK	

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

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