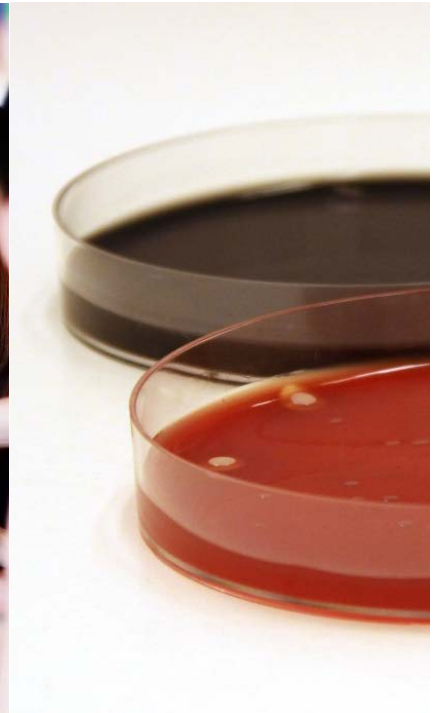


## Food Microbiology

January 2014

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*Proficiency Testing*  
**Microbiology – Food**  
January 2014



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*

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## Abbreviations

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### Media

ALOA	Agar Listeria Ottaviani & Agosti
APW 2%	Alcaline Peptone Water, 2 % NaCl
CIN	Cefsulodin-Irgasan-Novobiocin-agar
CT-SMAC	Cefixime-Tellurite-Sorbitol-MacConkey-agar
mCCDA	Charcoal Cefoperazone Deoxycholate modified Agar base
MPCA	Milk Plate Count Agar
MRB	Modified Rappaport Broth
PSB	Phosphate-Sorbitol-Broth
PCA	Plate Count Agar
SMAC	Sorbitol MacConkey agar
TSA	Tryptone Soya Agar
TCBS	Thiosulfate citrate Bile salts 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. some 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 participants results.




## Tables and figures legend

### Tables

n	number of laboratory that performed the analysis
m	results mean value in log <sub>10</sub> 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

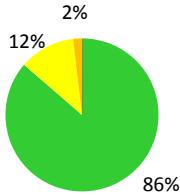
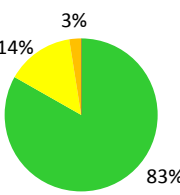
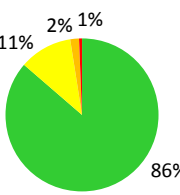
## Results of the PT round January 2014

### General outcome

Samples were sent to 166 laboratories, 34 in Sweden, 116 in other European countries, and 16 outside Europe. 161 laboratories reported results, 52 (32 %) provided at least one result with annotation. In the previous round with similar analyses (January 2013), the proportion was 65 %. (56% of the laboratories had reported a result with annotation for the enterobacteriaceae analysis where *Y. enterocolitica* was target organism).

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](http://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										
<b>Organisms</b>		<i>Micrococcus sp</i> <i>Escherichia coli</i> <i>Salmonella</i> Stockholm <i>Yersinia enterocolitica</i>			<i>Klebisella peumoniae</i> <i>Campylobacter jejuni</i> <i>Listeria monocytogenes</i> <i>Listeria innocua</i> <i>Salmonella bovismorbificans</i> <i>Escherichia coli</i> O157			<i>Citrobacter freundii</i> <i>Listeria monocytogenes</i> <i>Vibrio parahaemolyticus</i> <i>Vibrio cholera</i>		
<b>Analysis</b>		<b>Target</b>	<b>F%</b>	<b>Out</b>	<b>Target</b>	<b>F%</b>	<b>Out</b>	<b>Target</b>	<b>F%</b>	<b>Out</b>
Aerob. microorg, 30 °C		<i>Micrococcus</i> <i>E. coli</i>	0	4	<i>K. peumoniae</i>	0	3	<i>C. freundii</i>	1	3
Enterobacteriaceae		<i>E. coli</i>	0	3	<i>K. peumoniae</i>	4	1	<i>C. freundii</i>	4	2
Thermo. camp.	Quant.	<i>(E. coli)</i>	18	-	<i>C. jejuni</i>	0	0	-	0	-
	Qual.		11	-		4	-		0	-
<i>L. mono-</i> <i>cytogenes</i>	Quant.	-	0	-	<i>L. mono-</i> <i>cytogenes</i>	2	3	<i>L. mono-</i> <i>cytogenes</i>	6	3
	Qual.		1	-		7	-		4	-
<i>Salmonella</i>		<i>S. Stockholm</i>	5	-	<i>S. bovis-</i> <i>morbificans</i>	5	-	<i>(C. freundii)</i>	4	-
<i>E. coli</i> O157		-	7	-	<i>E. coli</i> O157	11	-	-	0	-
Path. <i>Vibrio</i> spp.		<i>(S. Stockholm)</i>	6	-	-	0	-	<i>V. para-</i> <i>haemolyticus</i> <i>V. cholera</i>	0	-
<i>Y. enterocolitica</i>		<i>Y. enterocolitica</i>	0	-	-	0	-	<i>(C. freundii)</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 *Micrococcus sp* and *Escherichia coli* present at the highest concentration in mixture A.

### Mixture B

The colonies counted for this analysis were mainly from the strain of *Klebsiella pneumoniae* present at the highest concentration in mixture B.

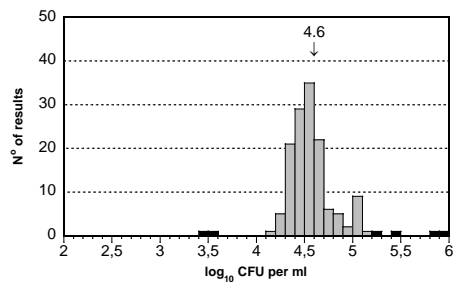
### Mixture C

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

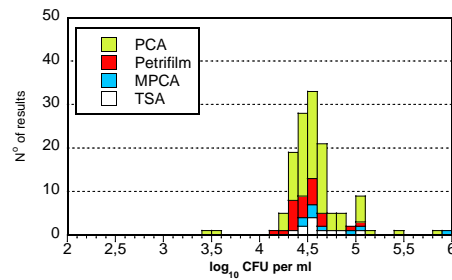
### Results of aerobic microorganisms analysis

Medium	Mixture A					Mixture B					Mixture C				
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
Total	142	4.56	0.20	0	2 4	143	4.63	0.25	0	3 2	142	3.77	0.19	1	3 1
PCA	89	4.57	0.20	0	2 2	90	4.59	0.25	0	1 2	89	3.72	0.18	1	2 0
Petrifilm™	25	4.49	0.19	0	0 0	25	4.73	0.21	0	1 0	25	3.93	0.12	0	1 1
TSA	11	4.60	0.21	0	0 0	11	4.54	0.35	0	1 0	11	3.78	0.21	0	0 0
MPCA	9	4.64	0.22	0	0 1	9	4.77	0.20	0	0 0	9	3.77	0.14	0	0 0

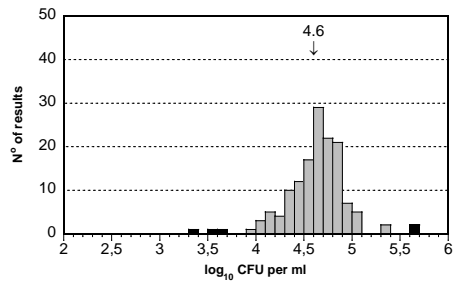
A



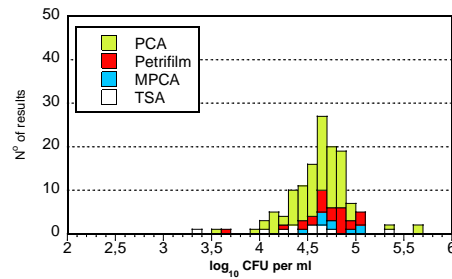
A



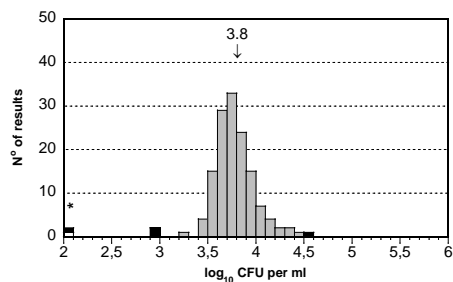
B



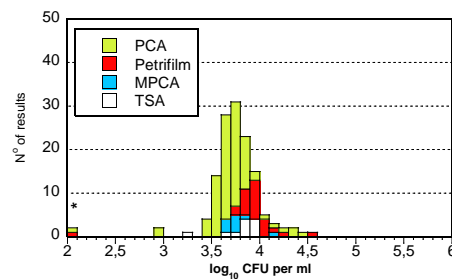
B



C



C





Results from mixture A are spread with a tail of higher value, but it cannot be linked to any method or medium used to perform the analysis.

Results from mixtures B and C are also quite spread with a tail of lower and higher values, respectively, linked mainly to the use of PCA. For these mixtures, results obtained with the use of Petrifilm™ tend to be higher than the general results average. This suggests that in these cases, the indicator dye present in Petrifilm™ could facilitate the enumeration of colonies and therefore lead to higher counts.

## Enterobacteriaceae

### Mixture A

*Escherichia coli* was target organism for this analysis.

### Mixture B

*Klebsiella pneumoniae* was target organism for this analysis. As for the analysis of aerobic microorganisms, the results are spread with a tail of lower values.

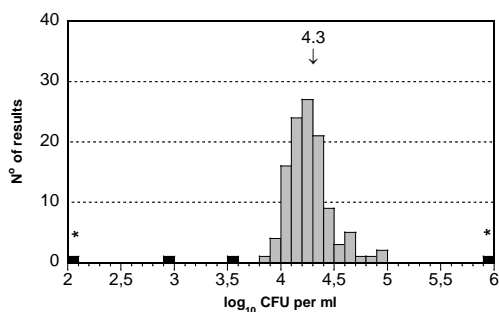
### Mixture C

*Citrobacter freundii* was target organism for this analysis.

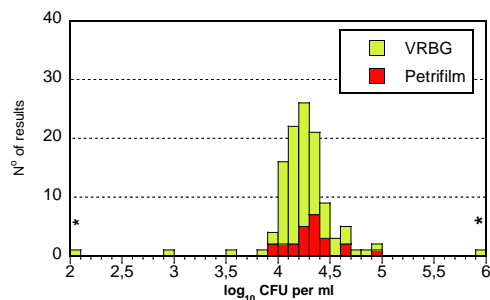
#### Results of enterobacteriaceae analysis

Medium	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	118	4.26	0.20	0	3	1	121	4.57	0.24	5	1	0	120	3.45	0.25	5	1	1
VRBG	91	4.25	0.19	0	3	1	94	4.57	0.22	5	1	0	93	3.41	0.26	5	1	1
Petrifilm™	24	4.31	0.22	0	0	0	24	4.58	0.29	0	0	0	24	3.62	0.14	0	0	0

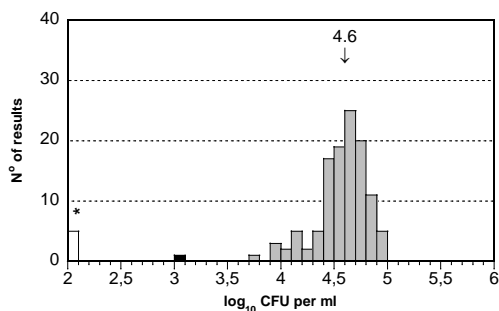
A



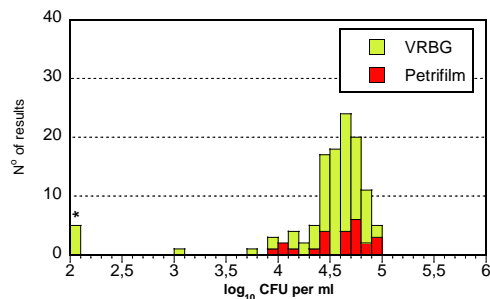
A



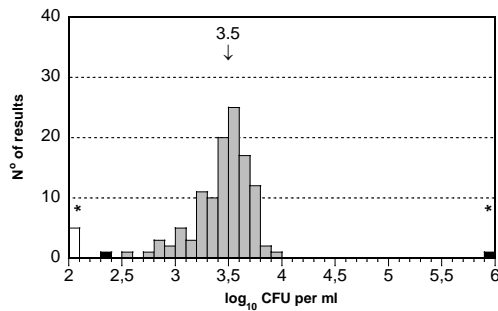
B



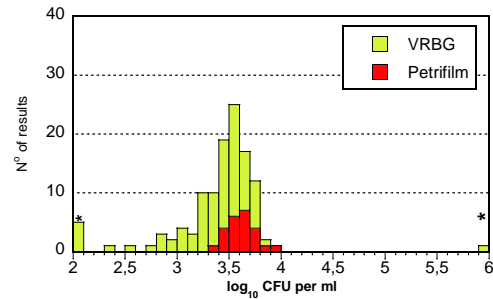
B



C



C



No significant differences are seen depending on the method, mainly ISO 21528-2 and NMKL 144, or the medium used. For mixture C, laboratories using Petrifilm™ reported values slightly higher and less spread than those using VRBG. As for the analysis of aerobic microorganisms, it is possible that the indicator dye present in Petrifilm™ facilitated the reading of *C. freundii* colonies and therefore led to a higher and more reproducible count for mixture C.

## Thermotolerant campylobacter

### Mixture A

Mixture A did not contain any strain of thermotolerant campylobacter but a strain of *E. coli* which can form colonies on mCCDA medium after incubation at 41.5°C in microaerobic condition. At NFA, this strain formed atypical white colonies on mCCDA both for the quantitative and qualitative analysis of thermotolerant campylobacter.

### Mixture B

A strain of *Campylobacter jejuni* was present in mixture B. The analysis did not cause difficulties but the results distribution is big.

### Mixture C

Mixture C did not contain any strain of thermotolerant campylobacter.

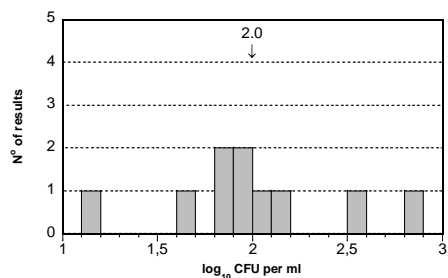
#### Results of thermotolerant campylobacter quantitative analysis

Method	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	11	-	-	0	-	-	10	1.99	0.46	0	0	0	11	-	-	0	-	-
ISO10272-2:2006	6	-	-	1	-	-	5	2.17	0.49	0	0	0	6	-	-	0	-	-
NMKL 119:2007	5	-	-	1	-	-	5	1.80	0.39	0	0	0	5	-	-	0	-	-

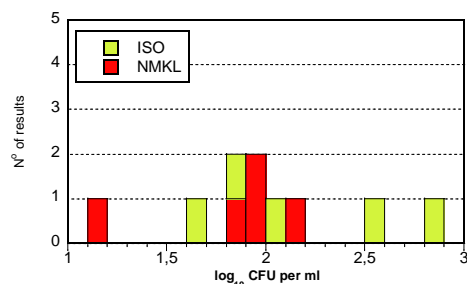
#### Results of thermotolerant campylobacter qualitative analysis

Method	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	28	-	-	3	-	-	28	-	-	1	-	-	28	-	-	0	-	-
ISO10272-1:2006	8	-	-	1	-	-	7	-	-	0	-	-	8	-	-	0	-	-
NMKL 119:2007	15	-	-	2	-	-	16	-	-	0	-	-	15	-	-	0	-	-

B



B



Few laboratories participate in the quantitative analysis of thermotolerant campylobacter; it is therefore quite difficult to draw any conclusion regarding the use of different methods. It seems however, that results obtained by following the method ISO 10272-2 tend to be higher than those obtained with the method NMKL 119. This could be linked to the fact that the ISO method prescribes the analysis of 0.1ml and 1ml of the sample while the NMKL method prescribes only the analysis of 0.1ml.

## *Listeria monocytogenes*

### Mixture A

There was no target organism for this analysis in mixture A.

### Mixture B

The mixture contained a strain of *Listeria monocytogenes* and a strain of *Listeria innocua*. AT NFA, mixed cultures were obtained on ALOA for the quantitative and qualitative analysis of *L. monocytogenes*. Colonies of *L. innocua* were atypical without precipitation zone and therefore could easily be differentiated from colonies of *L. monocytogenes*.

### Mixture C

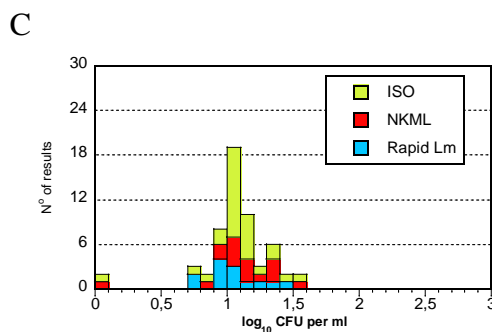
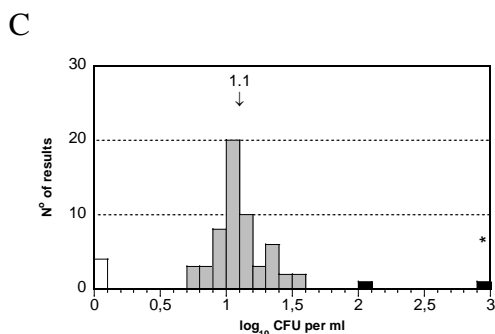
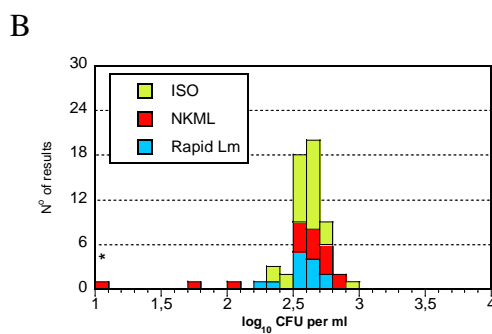
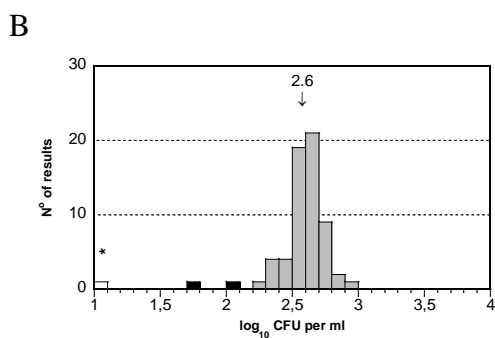
The strain of *L. monocytogenes* in mixture B was also target organism for the analysis in mixture C. However, here the strain was present at a lower concentration which could be the explanation for the report of few false negative results.

#### *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	62	-	-	0	- -	64	2.60	0.12	1	2	0	63	1.08	0.19	4	0	2
ISO 11290-2	28	-	-	0	- -	29	2.61	0.12	0	0	0	28	1.10	0.18	1	0	0
NMKL 136:2010	16	-	-	0	- -	16	2.67	0.09	1	2	0	16	1.12	0.19	1	0	0
Rapid L.m	13	-	-	0	- -	13	2.58	0.13	0	0	0	13	1.02	0.22	0	0	0

#### *Results of L. monocytogenes qualitative analysis*

Method	Mixture A					Mixture B					Mixture C				
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
Total	101	-	-	1	- -	102	-	-	7	- -	101	-	-	4	- -
ISO ISO 11290-1	28	-	-	0	- -	28	-	-	3	- -	28	-	-	0	- -
NMKL 136:2010	13	-	-	0	- -	13	-	-	2	- -	13	-	-	1	- -
Rapid L.m	18	-	-	0	- -	18	-	-	0	- -	18	-	-	0	- -
VIDAS method	19	-	-	1	- -	20	-	-	1	- -	19	-	-	1	- -
PCR method	9	-	-	0	- -	9	-	-	0	- -	9	-	-	0	- -



Most of the laboratories used a medium detecting the biochemical characteristics of *L. monocytogenes*. No correlation between method used and results of the quantitative analysis can be concluded.

In mixture B, *L. innocua* was present at a lower concentration than *L. monocytogenes* but has a faster growth rate and could outnumber *L. monocytogenes* in the enrichment steps of the qualitative analysis. On medium detecting esculin hydrolysis (PALCAM and Oxford) *L. innocua* show a positive reaction similar to *L. monocytogenes*. This could explain the false negative results reported, if no further confirmation was performed or only colonies of *L. innocua* were confirmed. Unlike *L. monocytogenes*, *L. innocua* does not show zone of hemolysis on blood-based medium.

## *Salmonella*

### Mixture A

Mixture A contained a strain of *Salmonella* Stockholm at a concentration of  $0.8 \log_{10} \text{cfu ml}^{-1}$ . At NFA, the strain formed typical colonies on XLD and Brilliance Salmonella agar. Six false negative results were reported.

### Mixture B

Mixture B contained a strain of *Salmonella* bovisorbificans at a concentration of  $1.0 \log_{10} \text{cfu ml}^{-1}$ . *K. pneumoniae* present in the mixture formed colonies on both XLD and Brilliance Salmonella agar after the enrichment steps. However, these colonies were atypical and easily to differentiate from the colonies of *S. bovisorbificans*. Six false negative results were reported.

## Mixture C

Eventhough mixture C did not contain any salmonella strain, some false positive results were reported. *Citrobacter freundii* present in mixture C form atypical yellow colonies on XLD and brownish colonies on Brilliance Salmonella agar; that differentiate from black and violet colonies of salmonella on the same media.

### Results of Salmonella qualitative analysis

Method	Mixture A					Mixture B					Mixture C				
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
Total	126	-	-	6	- -	127	-	-	6	- -	125	-	-	5	- -
ISO 6579:2002	26	-	-	1	- -	26	-	-	0	- -	26	-	-	1	- -
NMKL 71:1999	37	-	-	0	- -	37	-	-	1	- -	37	-	-	1	- -
NMKL 187:2007	7	-	-	1	- -	7	-	-	0	- -	7	-	-	0	- -
VIDAS method	19	-	-	3	- -	20	-	-	3	- -	18	-	-	0	- -
PCR method	17	-	-	1	- -	17	-	-	0	- -	17	-	-	1	- -

Most of the laboratories (84%) used XLD-agar together with another medium for the isolation step of the analysis. For mixture A and B, 3 of the 6 laboratories that reported a false negative result used a VIDAS method. For mixture C, 4 of the 5 laboratories that reported a false positive result did not perform any confirmation.

## Escherichia coli O157

### Mixture A

Mixture A did not contain any *E. coli* O157 strain but a strain of *E. coli* which, unlike *E. coli* O157, ferment sorbitol and form atypical pink colonies on SMAC.

### Mixture B

The mixture contains a strain of *E. coli* O157 at a concentration of  $1.5 \log_{10} \text{cfu ml}^{-1}$ . At NFA, the strain formed typical colonies both on SMAC and CT-SMAC after enrichment and immuno-separation steps.

### Mixture C

Mixture C did not contain any *E. coli* O157 strain.

### Results of E. coli O157 qualitative analysis

Method	Mixture A					Mixture B					Mixture C				
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
Total	28	-	-	2	- -	28	-	-	3	- -	28	-	-	0	- -
ISO 16654:2001	10	-	-	1	- -	10	-	-	0	- -	10	-	-	0	- -
NMKL 164:2005	7	-	-	1	- -	7	-	-	0	- -	7	-	-	0	- -
PCR method	6	-	-	0	- -	6	-	-	0	- -	6	-	-	0	- -

Almost all laboratories (75%) used CT-SMAC together with another medium for the isolation step of the analysis. No link between method/medium used and false results can be concluded. As a general comment it is important to point that analysis methods for the detection of *E. coli* are not applicable for the detection and identification of *E. coli* O157.

## ***Pathogenic Vibrio spp.***

---

### **Mixture A**

Mixture A did not contain any target organism for this analysis. At NFA, we observed yellow colonies on TCBS after enrichment in APW 2% and, as expected, the confirmation step did not identify *Vibrio spp.* Strains included in mixture A were tested directly for growth on TCBS (without enrichment step): *Micrococcus* and *Y. enterocolitica* did not grow; *S. Stockholm* formed green colonies, while *E. coli* grew yellow colonies in the primary streak.

### **Mixture B**

There was no target organism for this analysis in mixture B.

### **Mixture C**

Mixture C contained a strain of *Vibrio cholera* ( $2.8 \log_{10}$  cfu ml<sup>-1</sup>) and a strain of *Vibrio parahaemolyticus* ( $2.9 \log_{10}$  cfu ml<sup>-1</sup>) which are both target organism for this analysis.

#### *Results of pathogenic Vibrio spp. qualitative analysis*

Method	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	16	-	-	1	-	-	15	-	-	0	-	-	15	-	-	0	-	-
ISO/TS 21872-1:2007	6	-	-	1	-	-	5	-	-	0	-	-	5	-	-	0	-	-
NMKL 156:1997	8	-	-	0	-	-	8	-	-	0	-	-	8	-	-	0	-	-

All laboratories used APW for enrichment and TCBS agar for isolation.

## ***Yersinia enterocolitica***

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### **Mixture A**

A strain of *Yersinia enterocolitica* was included in mixture A at a concentration of  $1.4 \log_{10}$  cfu ml<sup>-1</sup>. At NFA, during the quality control of the mixture, typical colonies grew on CIN plates (i) after 3 hours in PSB at room temperature for 10% of the tested vials, (ii) after 8 days in PSB at 4°C and enrichment in MRB for 80% of the tested vials, and (iii) after 3 weeks in PSB at 4°C for 100% of the tested vials.

### **Mixture B**

There was no target organism for this analysis in mixture B.

### **Mixture C**

There was no target organism for this analysis in mixture C. At NFA, *C. freundii*, present in the mixture, formed pink colonies on CIN after incubation in PSB, 3 hours at room temperature and 3 weeks at 4°C. The strain was easily differentiated from *Y. enterocolitica* after confirmation.

#### *Results of Y. enterocolitica qualitative analysis*

Method	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	11	-	-	0	-	-	11	-	-	0	-	-	12	-	-	0	-	-
ISO 10273:2003	5	-	-	0	-	-	5	-	-	0	-	-	6	-	-	0	-	-
NMKL 117:1996	2	-	-	0	-	-	2	-	-	0	-	-	2	-	-	0	-	-
PCR method	3	-	-	0	-	-	3	-	-	0	-	-	3	-	-	0	-	-

Most laboratories use 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.

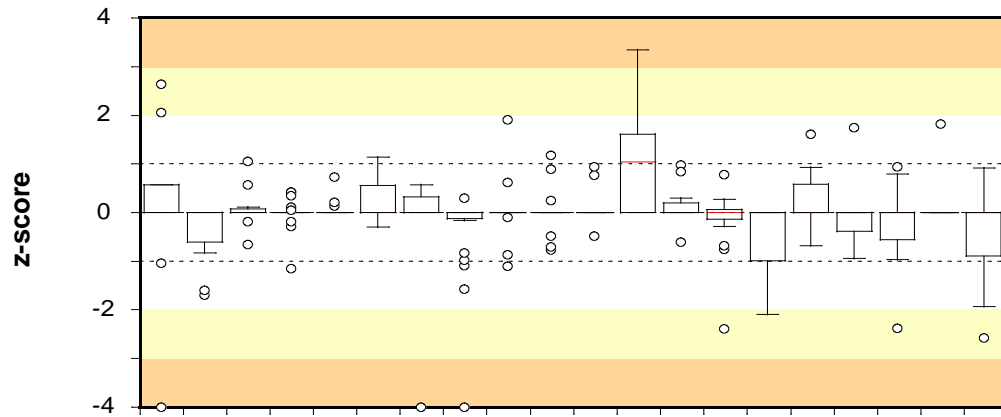
All the results from each laboratory – outliers included and false results excluded – were compiled into a box plot based on their z-scores. The smaller and more centred round zero the box of a laboratory is, the closer its results are to the general mean values calculated for all laboratory results.

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.

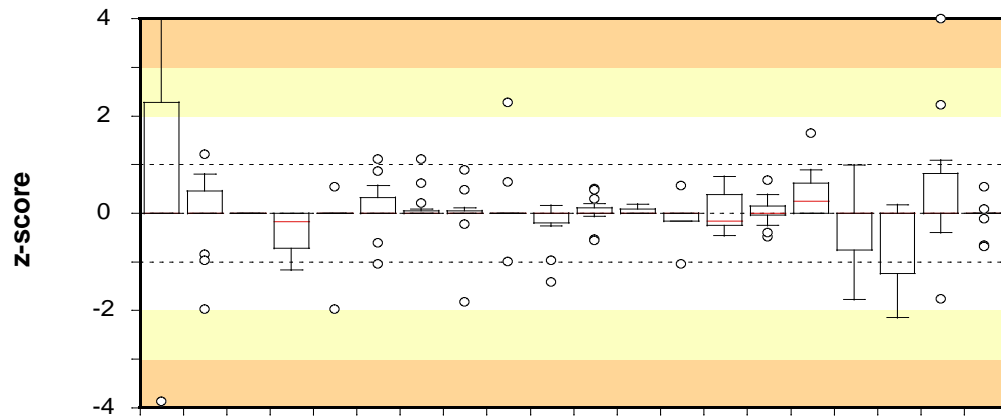
Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol (2). Samples for follow-up can be ordered, free of charge via our website: [www.slv.se/pt\\_extra](http://www.slv.se/pt_extra)

### **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.*

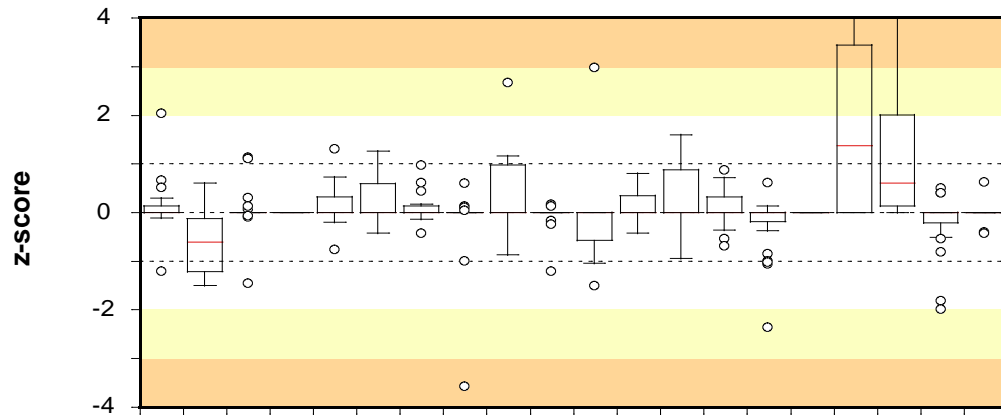


Lab no	1081	1254	1594	1970	2035	2050	2058	2072	2151	2324	2386	2402	2553	2637	2670	2704	2745	2764	2842	2920	
No. of results	9	18	12	23	15	9	6	23	14	14	9	9	14	15	5	15	15	14	5	9	
False positive	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
False negative	-	-	-	2	-	-	-	-	-	1	-	-	-	-	1	-	-	-	1	-	-
Low outliers	1	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

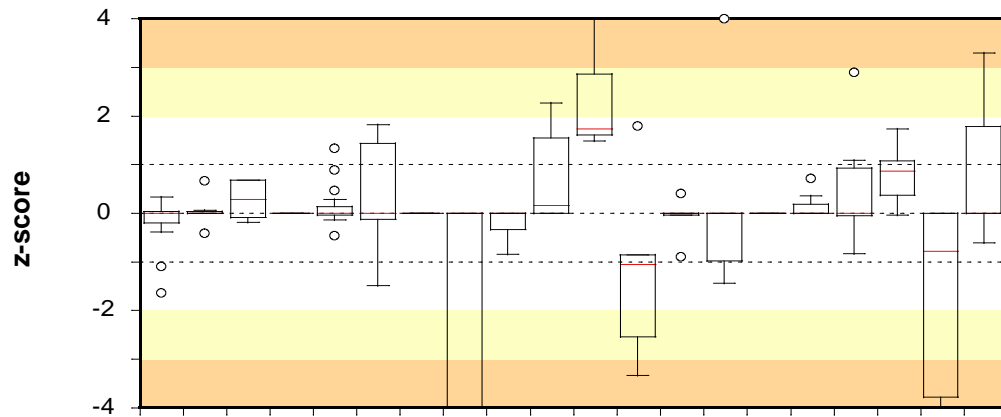


Lab no	3126	3159	3225	3305	3327	3346	3457	3511	3533	3588	3626	3829	3925	4064	4100	4153	4171	4246	4288	4339
No. of results	6	15	-	6	9	17	15	12	9	15	21	3	6	6	15	14	9	12	11	18
False positive	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
False negative	2	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	-	-	1	-
Low outliers	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-

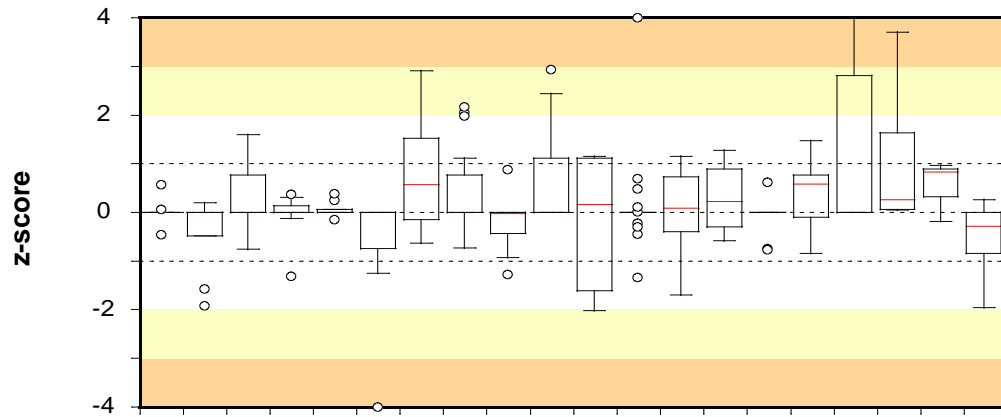




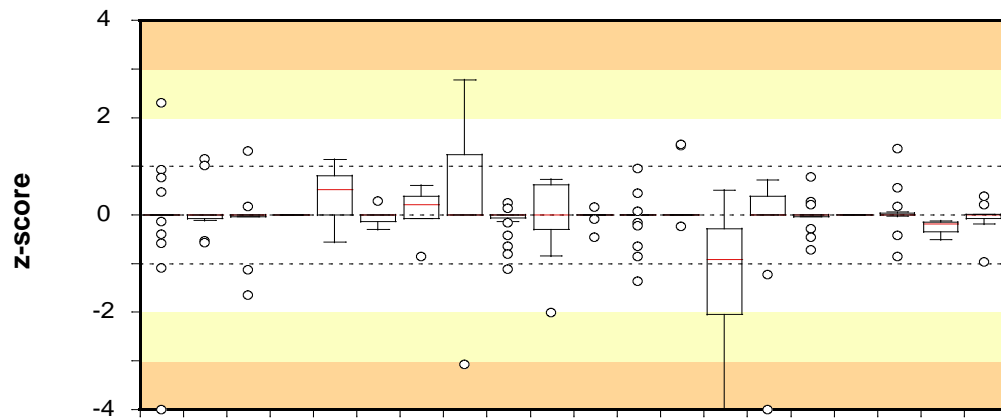
Lab no	4352	4400	4562	4605	4633	4635	4664	4683	4689	4817	4840	4889	4955	4980	5018	5028	5100	5197	5204	5220	
No. of results	23	6	29	3	14	12	18	21	7	24	17	15	15	15	24	3	5	7	24	9	
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	1	-	1	-	1	-	-	-	1	-	1	-	-	-	-	-	1	2	-	-	
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	



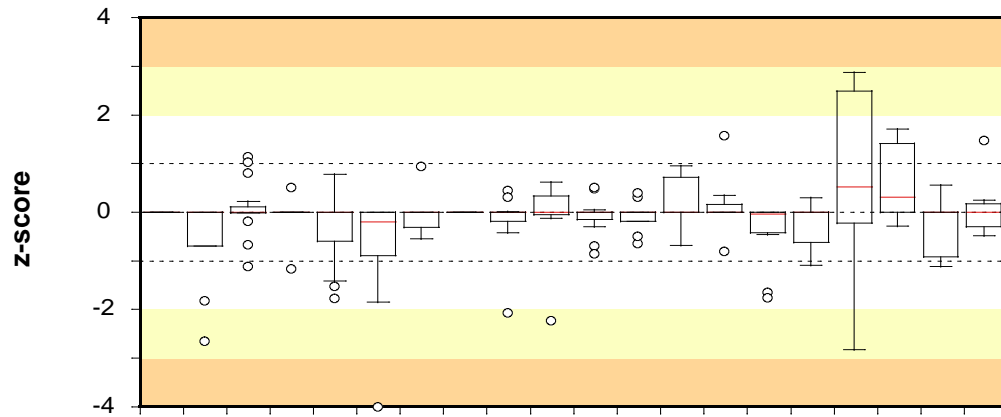
Lab no	5221	5304	5329	5333	5352	5380	5447	5545	5553	5615	5701	5801	5808	5883	5993	6109	6175	6224	6232	6253
No. of results	12	8	6	6	15	10	3	8	19	12	3	6	6	15	-	9	7	6	9	10
False positive	-	1	-	-	-	1	-	1	-	-	-	-	-	-	-	-	1	-	-	1
False negative	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Low outliers	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	3	-
High outliers	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-



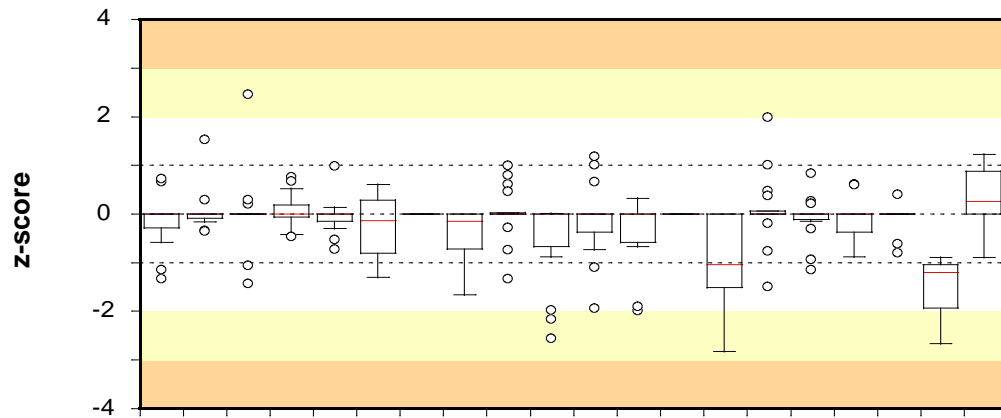
Lab no	6343	6352	6368	6443	6456	6594	6658	6707	6720	6751	6762	6860	6971	7024	7096	7182	7191	7207	7232	7242	
No. of results	9	9	18	9	12	9	5	15	14	18	6	27	6	6	9	6	9	6	3	8	
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	3	1	-	-	1	-	-	-	-	-	-	-	-	-	-	1
Low outliers	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	2	1	-	-	-



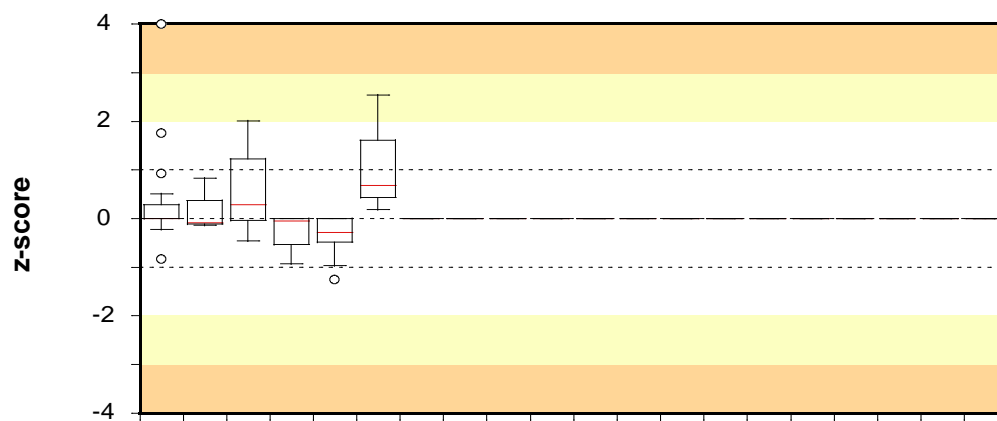
Lab no	7248	7253	7282	7302	7330	7334	7449	7543	7564	7596	7627	7688	7728	7750	7825	7876	7882	7930	7940	7962	
No. of results	21	17	9	9	9	6	6	8	27	12	9	24	11	6	15	14	4	15	3	9	
False positive	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	1	2	-	-	-	-
Low outliers	1	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Lab no	8042	8066	8068	8255	8260	8313	8333	8352	8380	8397	8428	8435	8529	8568	8626	8628	8657	8734	8742	8756
No. of results	3	10	15	9	15	12	12	-	17	12	15	12	15	10	11	15	6	9	11	9
False positive	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
False negative	-	2	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	1	-
Low outliers	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Lab no	8766	8918	8955	9002	9034	9217	9245	9429	9436	9441	9451	9453	9512	9555	9569	9589	9662	9716	9747	9753
No. of results	18	12	22	15	15	6	-	15	18	15	15	12	-	8	17	17	12	11	3	9
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Lab no	9763	9783	9890	9903	9923	9950
No. of results	18	3	6	12	9	3
False positive	1	-	-	-	-	-
False negative	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-
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 (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 <sup>1</sup>	Microorganism	Strain no.
A	<i>Micrococcus sp.</i>	SLV-055
	<i>Escherichia coli</i>	SLV-558
	<i>Salmonella</i> Stockholm	SLV-390
	<i>Yersinia enterocolitica</i>	SLV-408
B	<i>Klebsiella pneumoniae</i>	SLV-537
	<i>Campylobacter jejuni</i>	SLV-540
	<i>Listeria monocytogenes</i>	SLV-444
	<i>Listeria innocua</i>	SLV-312
	<i>Salmonella</i> bovismorbificans	SLV-443
	<i>Escherichia coli</i> O157	SLV-515
C	<i>Citrobacter freundii</i>	SLV-091
	<i>Listeria monocytogenes</i>	SLV-444
	<i>Vibrio parahaemolyticus</i>	SLV-529
	<i>Vibrio cholera</i>	SLV-530

<sup>1</sup>The 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 (2). 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 log<sub>10</sub> units and 0.5 log<sub>10</sub> units, respectively.

**Table 3.** Concentration mean (*m*) and standard deviation (*s*) from analyses of 10 randomly selected vials per mixture, expressed in log<sub>10</sub> 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	4.57	0.06	4.38	0.08	3.83	0.06
Enterobacteriaceae NMKL-method no. 144	4.22	0.05	4.46	0.09	3.69	0.06
Thermotolerant campylobacter, quant. NMKL method no. 119	-	-	2.84	0.14	-	-
Thermotolerant campylobacter, qual. NMKL method no. 119	-	-	pos	-	neg	-
<i>Listeria monocytogenes</i> , quant. NMKL method no. 136	-	-	2.68	0.04	1.13	0.06
<i>Listeria monocytogenes</i> , qual. NMKL method no. 136	neg	-	pos	-	pos	-
<i>Salmonella</i> NMKL method no. 71	0.83*	0.04*	1.00*	0.04*	neg	-
<i>Escherichia coli</i> O157 NMKL method no. 164	-	-	1.50*	0.03*	neg	-
<i>Yersinia enterocolitica</i> NMKL-method no. 117	1.37*	0.05*	neg	-	neg	-
Patogena <i>Vibrio</i> spp. <i>V. parahaemolyticus</i> NMKL-metod nr. 156 <i>V. cholera</i>	neg	-	neg	-	2.95*	0.07*
					2.84*	0.06*

- 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, 2012. Protocol. Microbiology. Drinking Water & Food. The National Food Agency. [www.slv.se/absint](http://www.slv.se/absint)
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.











Lab nr.	Provrnr.	Aeroba microorganisms 30 °C			Enterobacteriaceae			Thermotolerant campylobacter			Listeria monocytogenes			Thermotolerant campylobacter			Listeria monocytogenes			Salmonella			Escherichia coli O157 (VT-neg)			Pathogenic Vibrio spp			Yersinia enterocolitica			Lab nr.
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C				
n		142	143	142	118	121	120	11	10	11	62	64	63	28	28	28	101	102	101	126	127	125	28	28	28	16	15	15	11	11	12	n
Min		3.48	3.39	0	1	0	0	0	1.15	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Min	
Max		5.9	5.68	4.57	6.9	4.97	6.62	5	2.83	0	0	2.97	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Max	
median		4.52	4.65	3.75	4.24	4.6	3.5	0	1.935	0	0	2.62	1.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	median	
m		4.557	4.630	3.774	4.258	4.569	3.447	0	1.985	0	0	2.603	1.082	neg	pos	neg	neg	pos	pos	pos	pos	pos	neg	neg	pos	neg	neg	neg	pos	neg	neg	m
s		0.198	0.248	0.187	0.195	0.237	0.254	0	0.463	0	0	0.123	0.194	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	s	
F+		0	0	0	0	0	0	2	0	0	0	0	0	3	0	0	1	0	0	0	0	5	2	0	0	1	0	0	0	0	0	F+
F-		0	0	1	0	5	5	0	0	0	0	1	4	0	1	0	0	7	4	6	6	0	0	3	0	0	0	0	0	0	0	F-
<		2	3	3	3	1	1	0	0	0	0	2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<	
>		4	2	1	1	0	1	0	0	0	0	0	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>	
< OK		4.19	3.97	3.2	3.86	3.78	2.54	0	1.15	0	0	2.29	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	< OK	
> OK		5.13	5.36	4.42	4.91	4.97	3.95	0	2.83	0	0	2.97	1.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	> 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













## **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: [www.slv.se/absint](http://www.slv.se/absint)



1457  
ISO/IEC 17043

### **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.slv.se/RM-micro](http://www.slv.se/RM-micro)

