Edition
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Proficiency Testing

Microbiology – Food

January 2020

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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Proficiency testing
ISO/IEC 17043

Abbreviations

Media

ALOA	Agar for *Listeria* according to Ottaviani & Agosti
APW 2%	Alcaline peptone water, 2 % NaCl
BGA	Brilliant green agar
BPW	Buffered peptone water
BS	Bromthymol blue saccharose agar
CIN	Cefsulodin irgasan novobiocin agar
Compact Dry ETB	Compact Dry™ Enterobacteriaceae
Compact Dry TC	Compact Dry™ Total Count
CT-SMAC	Cefixime tellurite sorbitol MacConkey agar
ITC	Irgasan ticarcillin potassium chlorate broth
Listeria Precis	Listeria Precis™
LMBA	*Listeria monocytogenes* blood agar
mCCDA	Modified charcoal cephoperazone deoxycholate agar
MKTTn	Muller-Kauffmann tetrathionate/novobiocin broth
MPCA	Milk plate count agar
MRB	Modified Rappaport broth
MSRV	Modified semi-solid Rappaport-Vassiliadis enrichment media
mTSB	Modified tryptone soya broth
OCLA	Oxoid Brilliance™ Listeria agar
PALCAM	Polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol agar
Petrifilm AC	3M™ Petrifilm™ aerobic count
Petrifilm EB	3M™ Petrifilm™ Enterobacteriaceae
PSB	Peptone sorbitol bile salts broth
PCA	Plate count agar
RVS	Rappaport-Vassiliadis Soy peptone broth
SMAC	Sorbitol MacConkey agar
SP	Salt Polymyxin broth
SSDC	*Salmonella/Shigella* sodium deoxycholate calcium chloride agar
TCBS	Thiosulphate citrate bile salts sucrose agar
TEMPO AC	TEMPO® Aerobic Count
TEMPO EB	TEMPO® Enterobacteriaceae
TGE	Tryptone glucose extract agar
TSA	Tryptic soya agar
TSBY	Tryptone soya broth with yeast extract
XLD	Xylose lysine deoxycholate agar
VRBG	Violet red bile glucose agar

Organisations

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

Contents

General information on results evaluation

Statistical evaluation of the results

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

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

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

Uncertainty of measurement for the assigned values

The uncertainty of measurement for an assigned value is calculated as the standard deviation divided by the square root of the number of correct results ("standard error"). The assigned value of evaluated parameters is the mean value of the participants results.

Table and figure legends

Tables

N	number of laboratories that performed the analysis
n	number of laboratories with satisfactory result
m	mean value in log_{10} cfu ml^{-1} (false results and outliers excluded)
s	standard deviation (false results and outliers excluded)
F	number of false positive or false negative results
<	number of low outliers
>	number of high outliers
	global results for the analysis
	values discussed in the text

Figures

Histograms of the analytical results for each mixture and parameter 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

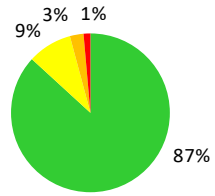
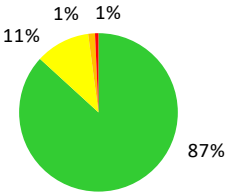
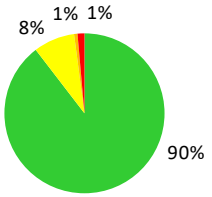
Results of the PT round January 2020

General outcome

Samples were sent to 147 laboratories, 30 in Sweden, 101 in other European countries, and 16 outside of Europe. Of the 144 laboratories that reported results, 34 (24 %) provided at least one result that received an annotation. In the previous round with similar analyses (January 2019) the proportion was 29 %.

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

***Table 1**. Composition of the test material and proportion of deviating results (N: number of reported results, F: false positive or false negative, X: outliers)*

	Sample A				Sample B				Sample C			
% participants with 0 annotations 1 annotation 2 annotations >2 annotations												
Microorganisms	*E. coli* O157 *Hafnia alvei* *Listeria monocytogenes* *Staphylococcus saprophyticus* *Vibrio cholerae* *Yersinia intermedia*				*Bacillus cereus* *Kocuria rhizophila* *Salmonella* Enteritidis *Vibrio cholerae* *Yersinia enterocolitica*				*Campylobacter coli* *Citrobacter freundii* *E. coli* O157 *Listeria monocytogenes*			
Analysis	**Target organism**	**N**	**F**	**X**	**Target organism**	**N**	**F**	**X**	**Target organism**	**N**	**F**	**X**
Aerobic micro-organisms 30 °C	***S. saprophyticus*** ***H. alvei***	123	0%	3%	***K. rhizophila***	123	1%	11%	***C. freundii*** ***E. coli* O157**	122	0%	2%
Enterobacteriaceae	***H. alvei***	109	1%	6%	***Y. enterocolitica*** ***S.* Enteritidis**	108	3%	3%	***C. freundii*** ***E. coli* O157**	109	1%	5%
Thermotol. *Campylo-bacter* Quant.	-	18	0%	0%	-	18	0%	0%	***C. coli***	18	6%	0%
Thermotol. *Campylo-bacter* Qual.		25	0%	-		25	0%	-		25	8%	-
L. mono-cytogenes Quant.	***L. mono-cytogenes***	67	3%	6%	-	67	0%	0%	***L. mono-cytogenes***	67	0%	6%
L. mono-cytogenes Qual.		98	2%	-		98	1%	-		98	1%	-
Salmonella	-	111	4%	-	***S.* Enteritidis**	112	0%	-	*(C. freundii)*	111	0%	-
E. coli O157	***E. coli O157***	28	18%	-	-	28	0%	-	***E. coli* O157**	28	11%	-
Pathogenic *Vibrio* spp.	***V. cholerae***	20	10%	-	***V. cholerae***	20	10%	-	*(E. coli* O157*)*	20	5%	-
Y. enterocolitica	*(Y. intermedia)*	12	0%	-	***Y. enterocolitica***	12	0%	-	*(C. freundii)*	12	0%	-

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

Aerobic microorganisms 30 °C

Sample A

S. saprophyticus and *H. alvei* were present in the highest concentrations (approximately $\log_{10}$ 4.7 and 4.4 cfu ml^{-1}, respectively) and were thus the main target organisms.

Sample B

The strain of *K. rhizophila* was present in the highest concentration (approximately $\log_{10}$ 4.5 cfu ml^{-1}) and was thus the main target organism.

Eleven low outliers were reported, which is more than usual for this analysis. Many low outliers were also reported in a previous proficiency testing round when the same sample mixture was used (January 2017).

Only *K. rhizophila* is present in a concentration higher than $\log_{10}$ 3.0 cfu ml^{-1} in the sample. It is therefore possible that low outliers are caused by not detecting this strain. Relatively many of the low outliers are reported by laboratories that used Petrifilm AC. At least two laboratories received low results with Petrifilm AC, but not with parallel incubation on PCA[1]. At least one of these laboratories only incubated for 48 h with Petrifilm AC, which is recommended for Petrifilm AC in some method validations. In subsequent tests at the Swedish Food Agency, *K. rhizophila* was observed to form very small colonies on Petrifilm AC, which were difficult to detect after 48 h. In these tests, the results for Petrifilm AC were approximately $\log_{10}$ 3.1 cfu ml^{-1} after 48 h at 30 °C and $\log_{10}$ 4.6 cfu ml^{-1} after 72 h vid 30 °C. ***Low results with Petrifilm AC after incubation for 48 h are therefore considered as accepted.***

[1] Information by personal communication.

Sample C

The strain of *C. freundii* was present in the highest concentration (approximately $\log_{10}$ 4.0 cfu ml^{-1}) and was thus the main target organism.

The mean values for Petrifilm AC and TEMPO AC were somewhat higher compared to the mean value for all results. Such somewhat higher results are relatively often seen for Petrifilm AC and TEMPO AC, and should therefore be considered as normal.

General remarks

The laboratories mainly followed NMKL 86:2013 (40 %), 3M Petrifilm (17 %) and ISO 4833-1:2013 (15 %). The older NMKL 86:2006 and ISO 4833:2003 were still used by 8 % and 3 % of the laboratories, respectively. The different methods are however similar, and are all based on incubation on PCA or MCPA at 30 °C for 72 h. Users of Petrifilm AC can however use a different time/temperature, depending on the method validation. For example, AOAC® 990.12 prescribes incubation at 35 °C for 48 h while AFNOR 3M 01/1-09/89 prescribes 30 °C for 72 h.

The majority of the laboratories incubated on either PCA or Petrifilm AC. Incubation on MPCA was mainly done by laboratories within the dairy industry. Incubation on TSA was mainly done by users of a company-specific method. A smaller number of laboratories used TEMPO AC, which is based on MPN (Most Probable Number). With this method, the sample is incubated in a card that contains different-sized wells. A substrate in the medium emits fluorescence with hydrolysed by the microorganisms. The number of microorganisms is determined by the number and size of the fluorescing wells.

Results from analysis of aerobic microorganisms, 30 °C

Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	123	119	4.81	0.15	0	4	0	123	109	4.67	0.12	1	11	2	122	120	3.87	0.21	0	2	0
PCA	73	72	4.77	0.14	0	1	0	73	69	4.69	0.10	1	3	0	72	72	3.79	0.16	0	0	0
Petrifilm AC	22	21	4.86	0.14	0	1	0	22	18	4.63	0.14	0	4	0	22	21	4.13	0.11	0	1	0
TSA	8	8	4.93	0.20	0	0	0	8	6	4.65	0.17	0	0	2	8	8	3.94	0.29	0	0	0
MPCA	6	6	4.84	0.21	0	0	0	6	6	4.61	0.08	0	0	0	6	6	3.80	0.07	0	0	0
TEMPO AC	5	5	4.86	0.11	0	0	0	5	5	4.65	0.18	0	0	0	5	5	4.16	0.12	0	0	0
TGE	4	4	-	-	0	0	0	4	3	-	-	0	1	0	4	4	-	-	0	0	0
Compact Dry TC	2	1	-	-	0	1	0	2	0	-	-	0	2	0	2	1	-	-	0	1	0
Other	3	2	-	-	0	1	0	3	2	-	-	0	1	0	3	3	-	-	0	0	0

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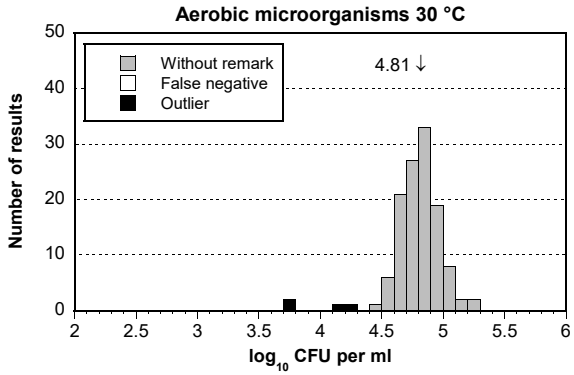

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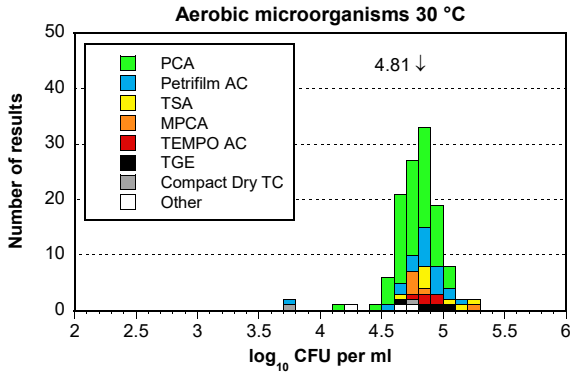

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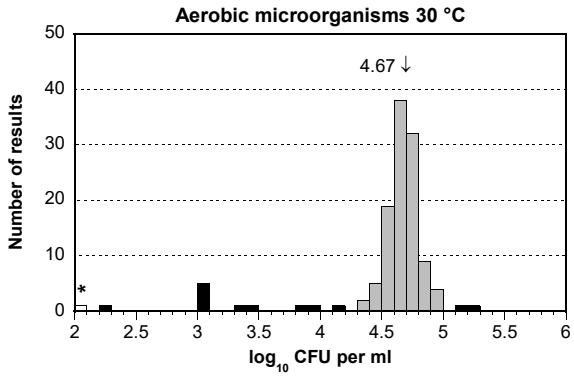

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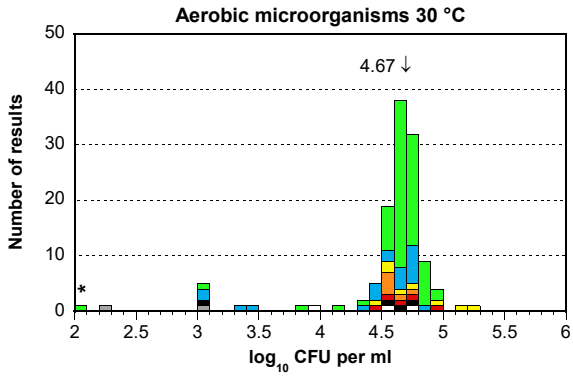

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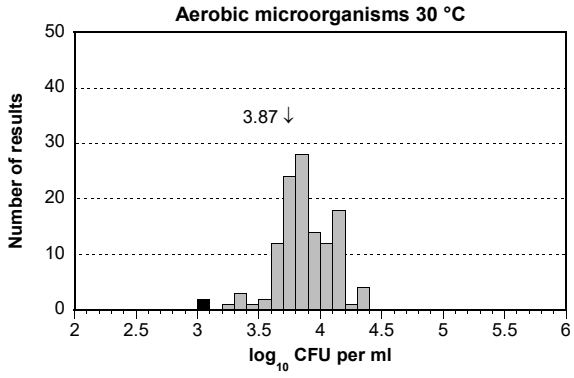

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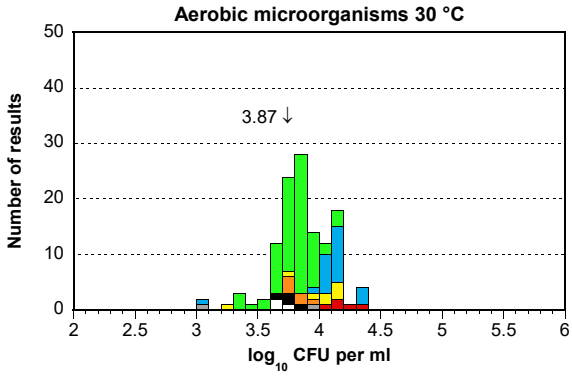

Enterobacteriaceae

Sample A

The strains of *H. alvei*, *E. coli* O157 and *Y. intermedia* belong to Enterobacteriaceae. The strain of *H. alvei* was however present in considerably higher concentration (approximately $\log_{10}$ 4.4 cfu ml^{-1}) than the other two strains (both below $\log_{10}$ 1.0 cfu ml^{-1}) and was thus the main target organism.

Sample B

The strains of *Y. enterocolitica* and *S.* Enteritidis were target organisms and were present in similar concentrations in the sample (approximately $\log_{10}$ 2.4 and $\log_{10}$ 2.0 cfu ml^{-1}, respectively).

On VRBG, both strains form typical red, oxidase-negative colonies, which were surrounded by a precipitation zone. The colonies of *Y. enterocolitica* are usually smaller than those of *S.* Enteritidis, and they may also have a less prominent precipitation zone.

Sample C

The strains of *C. freundii* and *E. coli* O157 were target organisms. The strain of *C. freundii* was present in considerably higher concentration than *E. coli* O157 (approximately $\log_{10}$ 4.0 and $\log_{10}$ 1.7 cfu ml^{-1}, respectively) and was thus the main target organism. At the Swedish Food Agency, *C. freundii* formed typical red, oxidase-negative colonies on VRBG, which were surrounded by a precipitation zone. The size of the precipitation zone varied somewhat between the colonies.

General remarks

Enterobacteriaceae are Gram-negative and oxidase-negative bacteria that ferment glucose with the formation of acid by-products. On VRBG, they therefore form pink/red colonies, with or without a bile salt precipitation zone. Enterobacteriaceae have a similar appearance on Petrifilm EB, which also contains a colour indicator that facilitates detection of acid by-products, and a plastic film for detection of gas production.

As in previous proficiency testing rounds, most laboratories followed either NMKL 144:2005 (46 %) or a method with Petrifilm EB (17 %), while the ISO methods (different versions) were used by in total 22 %. The number of users of the new ISO 21528-2:2017 was higher than ISO 21528-2:2004 (13 % and 5 %, respectively). Five

laboratories (5 %) reported using the older ISO 21528-1:2004. Another five laboratories used method based on detection of fluorescence (TEMPO EB).

ISO 21528-2:2017 is based on colony-count, while ISO 21528-1:2017 is based on MPN (Most Probable Number). The latter method is recommended when the expected concentration of Enterobacteriaceae is lower than 100 cfu g^{-1}.

With NMKL 144:2005, presumptive colonies on VRBG are confirmed with an oxidase test. With ISO 21528-2:2017, presumptive colonies are confirmed with both an oxidase test and with a test for glucose fermentation. In total, 74 % of the laboratories stated that they performed some kind of confirmation test; the majority of these specified that this consisted of an oxidase test.

No major differences could be seen between the different methods and media that were used. For sample C, there was however a tendency for higher results for TEMPO EB, compared to other media. Such somewhat higher results are relatively often seen for TEMPO EB, and should therefore be considered as normal.

Results from analysis of Enterobacteriaceae

Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	109	102	4.30	0.21	1	4	2	108	102	2.02	0.22	3	1	2	109	103	3.60	0.31	1	4	1
VRBG	75	72	4.28	0.22	1	1	1	75	70	2.02	0.21	2	1	2	75	71	3.58	0.30	1	3	0
Petrifilm EB	21	19	4.36	0.24	0	1	1	21	21	2.08	0.24	0	0	0	21	20	3.71	0.27	0	0	1
TEMPO EB	5	5	4.24	0.15	0	0	0	5	5	1.94	0.12	0	0	0	5	5	3.87	0.22	0	0	0
TSA/VRBG	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0
Compact Dry ETB	2	1	-	-	0	1	0	2	1	-	-	1	0	0	2	2	-	-	0	0	0
Other	2	1	-	-	0	1	0	1	1	-	-	0	0	0	2	1	-	-	0	1	0

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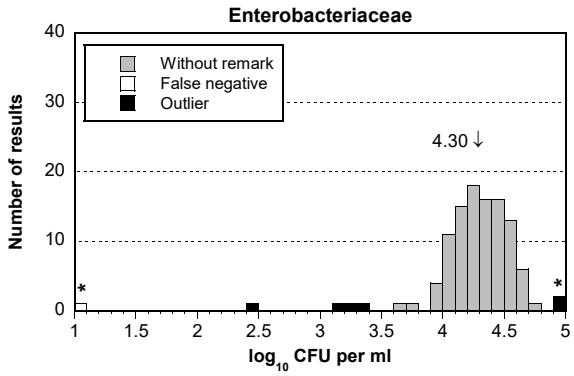

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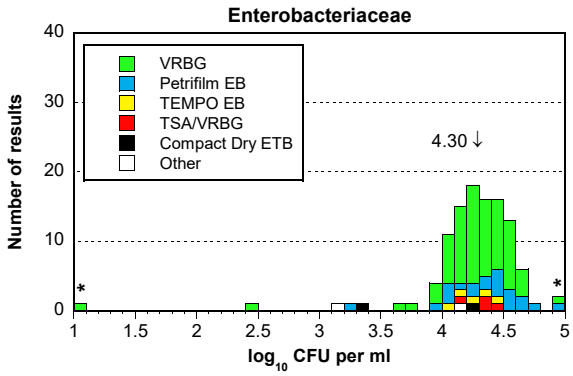
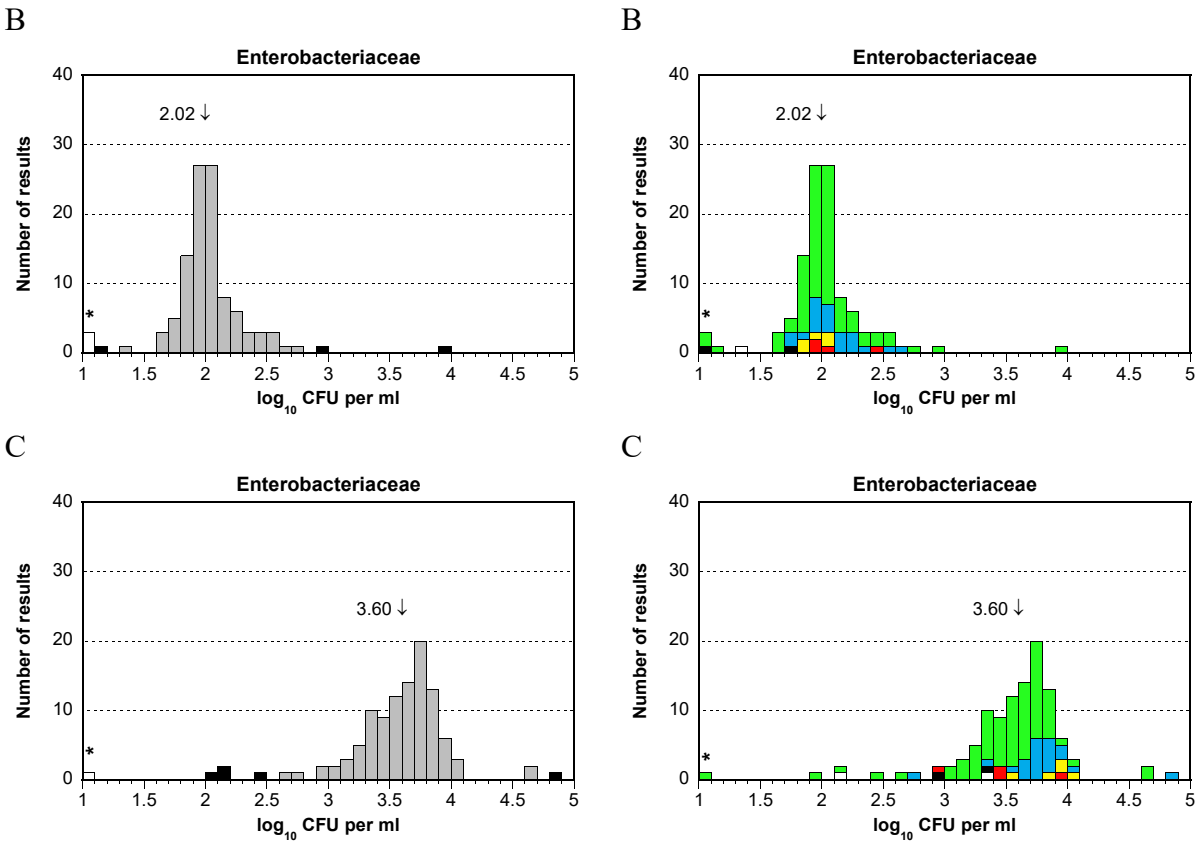




Thermotolerant *Campylobacter*

Sample A

No target organism was present in the sample. All results were correct negative, both in the quantitative and in the qualitative analysis.

Sample B

No target organism was present in the sample. All results were correct negative, both in the quantitative and in the qualitative analysis.

Sample C

The strain of *C. coli* was target organism and was present in approximately $\log_{10}$ 3.2 cfu ml^{-1} in the sample. In the Swedish Food Agency's quality control it formed smaller as well as larger colonies on mCCDA. Both colony types were however positive in subsequent oxidase, catalase and motility tests. Both were also positive for the hydrolysis of indoxyl acetate, and negative for the hydrolysis of hippurate.

The results in the quantitative analysis had a fairly wide distribution, which is not unusual for this parameter. Since only 18 laboratories performed the quantitative analysis, none of the results were considered as outliers. One false negative result was however reported.

In the qualitative analysis results were reported by 25 laboratories. Two of these were false negative results.

General remarks

Campylobacter spp. are gram-negative, oxidase-positive and catalase-positive bacteria. On mCCDA they normally form flat or convex colonies, with a grey or white colour and a glossy surface. Confirmation is usually done with an oxidase test or a catalase test, or phenotypically by microscopy. The bacteria normally have a spiral morphology, and display characteristic darting or corcscrew-like movements. In addition, *C. jejuni*, *C. coli* and *C. lari* can be separated by differences in their hydrolysis of hippurate and indoxyl acetate, and their sensitivity/resistance to nalidixic acid and cephalothin. Confirmation of some kind was performed by all laboratories in the quantitative analysis and by all except one laboratory in the qualitative analysis. The laboratory that did not perform a confirmation used a PCR-based method for detection of *Campylobacter*. In both analyses, the most common types of confirmation were a motility test and/or an oxidase test.

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

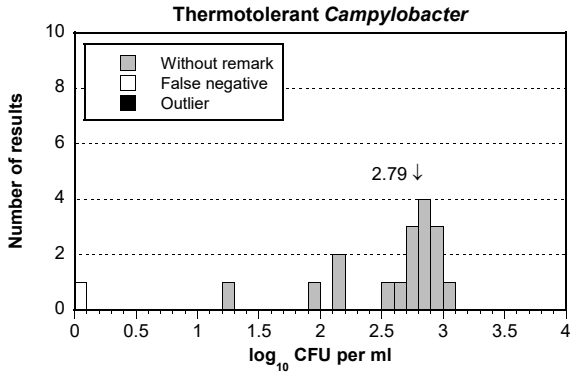
The majority of the laboratories (83 %) in the qualitative analysis used Bolton broth for the enrichment step, but the use of Preston broth was also reported. For the selective step, most laboratories (88 %) used mCCDA. In the quantitative analysis, 16 of 18 laboratories incubated on mCCDA. One laboratory incubated on Preston agar. This laboratory reported a false negative result for sample C.

Results from quantitative analysis of thermotolerant Campylobacter

Method	Sample A							Sample B							Sample C						
	N	n	Med*	s	F	<	>	N	n	Med*	s	F	<	>	N	n	Med*	s	F	<	>
All results	18	18	-	-	0	-	-	18	18	-	-	0	-	-	18	17	2.79	0.48	1	0	0
ISO 10272-2:2017	11	11	-	-	0	-	-	11	11	-	-	0	-	-	11	10	2.61	0.55	1	0	0
NMKL 119:2007	6	6	-	-	0	-	-	6	6	-	-	0	-	-	6	6	2.88	0.14	0	0	0
ISO 10272-2:2006	1	1	-	-	0	-	-	1	1	-	-	0	-	-	1	1	-	-	0	0	0

* Med = median

C



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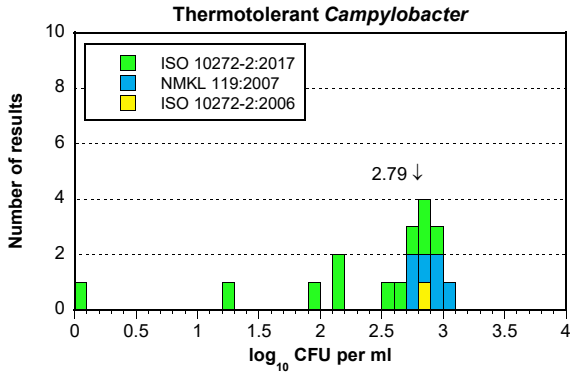


Results from qualitative analysis of thermotolerant Campylobacter

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
All results	25	25	Neg	0	25	25	Neg	0	25	23	Pos	2
NMKL 119:2007	13	13	Neg	0	13	13	Neg	0	13	13	Pos	0
ISO 10272-1:2017	7	7	Neg	0	7	7	Neg	0	7	5	Pos	2
ISO 10272-1:2006	2	2	Neg	0	2	2	Neg	0	2	2	Pos	0
Other*	3	3	Neg	0	3	3	Neg	0	3	3	Pos	0

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

Listeria monocytogenes

Sample A

The strain of *L. monocytogenes* was target organism and was present in approximately $\log_{10}$ 2.5 cfu ml^{-1} in the sample. In the Swedish Food Agency's quality control, it formed characteristic blue-green colonies on ALOA, which were surrounded by a distinct opaque halo. On the same plates, atypical, small, white/blue colonies could be seen. On PALCAM, the strain formed typical grey/green colonies, surrounded by a black/brown zone. The strain is catalase-positive, displays β-haemolysis on blood agar, and ferments rhamnose but not xylose.

Sample B

No target organism for the analysis was present in the sample.

Sample C

The strain of *L. monocytogenes* (not identical to the one in sample A) was target organism and was present in approximately $\log_{10}$ 2.5 cfu ml^{-1} in the sample. In the Swedish Food Agency's quality control, it formed characteristic green colonies on ALOA, which were surrounded by a distinct opaque halo. The strain is catalase-positive, displays β-haemolysis on blood agar, and ferments rhamnose but not xylose.

General remarks

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

Both ISO 11290-1 (qualitative) and ISO 11290-2 (quantitative) distinguish between detection of *Listeria* spp. and *L. monocytogenes*. The qualitative method ISO 11290-1:2017 is based on primary enrichment in half-Fraser broth, followed by secondary enrichment in Fraser broth. Aliquots from both enrichments are plated onto ALOA and onto another selective medium chosen by the laboratory.

In the quantitative method ISO 11290-2:2017 the sample is first suspended in BPW or in half-Fraser broth and material is then transferred from these to ALOA. The quantitative and qualitative methods used in NMKL 136:2010 are similar to the ones in the ISO methods.

On ALOA, *L. monocytogenes* form blue-green colonies due to β-glucosidase activity. The colonies are surrounded by an opaque halo due to hydrolysis of inositol in the medium. The halo is sometimes weak, or may not be present at all. *L. monocytogenes* can be confirmed by microscopy, catalase test, and by tests of β-haemolysis and

carbohydrate utilisation (fermentation of rhamnose and xylose). *L. monocytogenes* is catalase-positive, displays β-haemolysis on blood agar, and ferments rhamnose but not xylose. Confirmation can also be done by the increased and decreased β-haemolysis displayed by *L. monocytogenes* in the presence of *Staphylococcus aureus* and *Rhodococcus equi*, respectively (CAMP test).

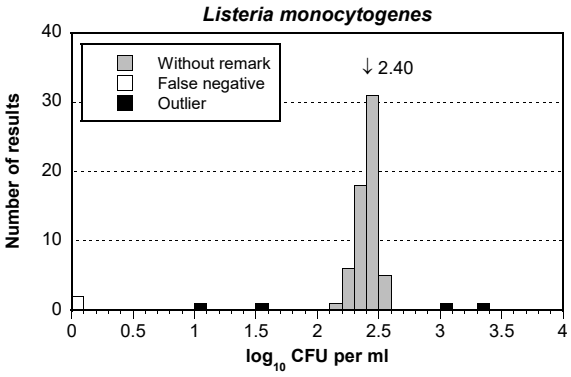
Different versions of ISO 11290-2 and ISO 11290-1 were the most common methods in both the quantitative and the qualitative analysis (39 % and 22 % of the laboratories, respectively). In addition to these, the use of NMKL 136:2010 and RAPID'L.mono was common, as well as VIDAS® and different PCR methods in the qualitative analysis. RAPID'L.mono uses a chromogenic medium that identifies the enzyme phosphatidyl-inositol phospholipase C (PI-PLC) in *L. monocytogenes*. The medium also identifies both *Listeria* spp. and *L. monocytogenes* by the fact that they do not metabolize xylose. The method in Listeria Precis™ in a similar way uses a chromogenic medium that detects *Listeria* spp. and *L. monocytogenes* β-glucosidase cleavage of X-glucoside in the medium Brilliance™ Listeria. In comparison, VIDAS® is based on detection of specific *L. monocytogenes* antigen, by a method based on ELFA (Enzyme Linked Fluorescent Assay). The alternative methods are all validated by AFNOR and/or NordVal.

ALOA, RAPID'L.mono and Oxoid Brilliance™ Listeria-agar (tidigare OCLA) were the most frequently used media, but the use of PALCAM, LMBA, Oxford Listeria selective agar, SwabSure™ ListeriaP and other types of chromogenic media was also reported. Confirmation of some kind was performed by 82 % of the laboratories in the quantitative analysis and by 86 % in the qualitative analysis.

Results from quantitative analysis of Listeria monocytogenes

Method	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	67	61	2.40	0.09	2	2	2	67	67	-	-	0	-	-	67	63	2.42	0.09	0	3	1
RAPID' L.mono	19	18	2.39	0.08	1	0	0	19	19	-	-	0	-	-	19	18	2.42	0.09	0	1	0
NMKL 136:2010	16	14	2.43	0.10	0	1	1	16	16	-	-	0	-	-	16	14	2.43	0.08	0	1	1
ISO 11290-2:2017	14	11	2.37	0.11	1	1	1	14	14	-	-	0	-	-	14	13	2.41	0.13	0	1	0
ISO 11290-2:1998 /Amd 1:2004	10	10	2.42	0.05	0	0	0	10	10	-	-	0	-	-	10	10	2.42	0.05	0	0	0
Listeria Precis	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
ISO 11290-2:1998	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	2	-	-	0	0	0
Other	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0

A



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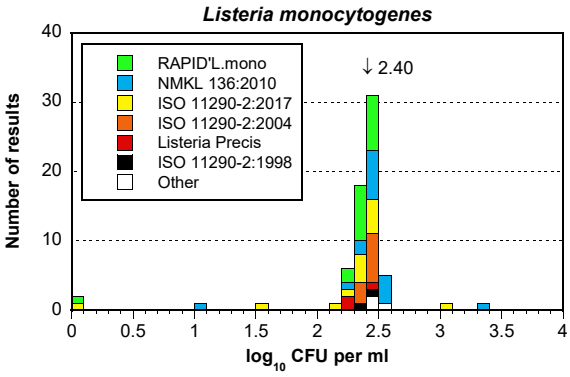

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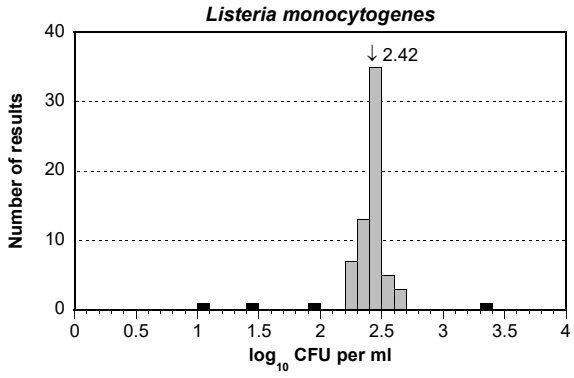
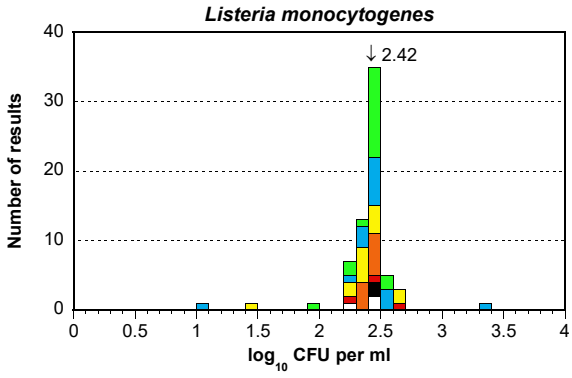


C



Results from qualitative analysis of Listeria monocytogenes

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
All results	98	96	Pos	2	98	97	Neg	1	98	97	Pos	1
RAPID' L.mono	18	17	Pos	1	18	17	Neg	1	18	18	Pos	0
VIDAS	16	16	Pos	0	16	16	Neg	0	16	16	Pos	0
PCR method	13	13	Pos	0	13	13	Neg	0	13	13	Pos	0
NMKL 136:2010	12	12	Pos	0	12	12	Neg	0	12	12	Pos	0
ISO 11290-1:2017	12	11	Pos	1	12	12	Neg	0	12	12	Pos	0
ISO 11290-1:1996 /Amd 1:2004	10	10	Pos	0	10	10	Neg	0	10	10	Pos	0
Listeria Precis	4	4	Pos	0	4	4	Neg	0	4	4	Pos	0
SwaBSure ListeriaP	3	3	Pos	0	3	3	Neg	0	3	2	Pos	1
Other	10	10	Pos	0	10	10	Neg	0	10	10	Pos	0

Salmonella

Sample A

No target organism for the analysis was present in the sample. Despite this, four false positive results were reported.

Sample B

The strain of *S.* Enteritidis was target organism and was present in approximately $\log_{10}$ 2.0 cfu ml^{-1} in the sample. On XLD, it forms typical red colonies with a black center. On Brilliance™ Salmonella, it forms typical purple colonies. The strain is positive for agglutination against both O and H antigen.

Sample C

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

General remarks

Most laboratories followed either NMKL 71:1999 (26 %) or ISO 6579-1:2017 (17 %), which are very similar methods. Both are based on pre-enrichment in BPW, followed by selective enrichment in RVS. Subsequent inoculation is on XLD and a second selective medium chosen by the laboratory. In contrast to NMKL 71:1999, ISO 6579-1:2017 also includes selective enrichment in MKTTn. With the ISO method, RVS can also be substituted with semi-solid MSRV for the analysis of motile *Salmonella*. Confirmation is by biochemical (e.g. mannitol and urea) and serological (e.g. *Salmonella* polyvalent O and H antisera) tests.

The majority of the 30 laboratories that analysed according to ISO 6579, stated that they followed the new ISO 6579-1:2017, but some laboratories still followed the older ISO 6579:2002 or ISO 6579:2002/Amd 1:2007. The most important changes in the 2017 version are that detection of β-galactosidase and indole is optional in the confirmation, and that positive results for agglutination against both O and H antigen is required for a strain to be considered as *Salmonella*.

Users of NMKL methods can in addition to NMKL 71:1999 also choose to follow NMKL 187:2016. The latter method is intended for detection of motile *Salmonella* and, similarly to ISO 6579-1:2017, uses MSRV instead of RVS during the selective enrichment step. Two of the three laboratories that followed NMKL 187 stated that they followed the older version NMKL 187:2006. The version from 2016 contains clarifications regarding the choice of the selective agar medium complementary to XLD, and the concentration of Novobiocin in MSRV. It also contains new paragraphs regarding pre-enrichment of samples from primary animal production, faecal samples and swab samples.

On XLD, which was used by the majority of the laboratories, typical *Salmonella* form transparent red colonies with a black center. As a complementary medium to XLD, the laboratories mainly used chromogenic media such as Brilliance™ Salmonella, BGA, Rambach™ agar and Harlequin® Salmonella ABC Medium. As in previous proficiency testing rounds, several laboratories chose to analyse with alternative methods like RAPID'Salmonella or VIDAS®, which are validated by AFNOR and/or NordVal against ISO 6579-1:2017. PCR-based methods were also frequently used. No obvious differences in the results could be seen between the different methods that were used.

Confirmation of some kind was performed by the majority (95 %) of the laboratories. Of the four false positive results for sample A, only one was from a laboratory that did not perform a confirmation.

Results from analysis of Salmonella

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
All results	111	107	Neg	4	112	112	Pos	0	111	111	Neg	0
NMKL 71:1999	29	29	Neg	0	29	29	Pos	0	29	29	Neg	0
ISO 6579-1:2017	19	18	Neg	1	19	19	Pos	0	19	19	Neg	0
PCR method	18	18	Neg	0	18	18	Pos	0	18	18	Neg	0
VIDAS*	16	15	Neg	1	16	16	Pos	0	16	16	Neg	0
RAPID' Salmonella	7	6	Neg	1	7	7	Pos	0	7	7	Neg	0
ISO 6579:2002	6	5	Neg	1	6	6	Pos	0	6	6	Neg	0
ISO 6579:2002 /Amd 1:2007	5	5	Neg	0	5	5	Pos	0	5	5	Neg	0
NMKL 187**	3	3	Neg	0	3	3	Pos	0	3	3	Neg	0
Other***	8	8	Neg	0	9	9	Pos	0	8	8	Neg	0

* The group VIDAS includes two laboratories that analysed with MINI VIDAS®.
** Includes both NMKL 187:2007 and NMKL 187:2016
*** The group Other includes Oxoid Salmonella Precis™, the water method ISO 19250, as well as national and/or company-specific methods.

Escherichia coli O157

Sample A

The strain of *E. coli* O157 was target organism and was present in approximately $\log_{10}$ 0.8 cfu ml^{-1} in the sample. On CT-SMAC, it forms typical sorbitol-negative transparent colonies with a dark center. The strain is positive for production of indole and for agglutination with *E. coli* O157 antiserum. It contains the gene *eae*, but no *stx* genes.

Sample B

No target organism for the analysis was present in the sample. All results were correct negative.

Sample C

The strain of *E. coli* O157 (not identical to the one in sample A) was target organism and was present in approximately $\log_{10}$ 1.7 cfu ml^{-1} in the sample. On CT-SMAC, it forms typical sorbitol-negative transparent colonies with a dark center. The strain is positive for production of indole and for agglutination with *E. coli* O157 antiserum. It contains the gene *eae*, but no *stx* genes.

General remarks

Only 28 laboratories performed the analysis. Statistical evaluation of the results is therefore difficult. The false results that were reported could not be attributed to the use of a specific method or medium.

In total, 43 % of the laboratories followed either NMKL 164:2005 or ISO 16654:2001, which are similar methods. Enrichment is done in mTSB with novobiocin, and is followed by immunomagnetic separation and isolation on CT-SMAC and another medium selected by the laboratory. Confirmation is by a test for indole production as well as agglutination with *E. coli* O157 antiserum. ISO 16654:2001 was last reviewed by ISO in 2018, and remains current. The NMKL method is present in a new edition,

NMKL 164:2019. The major change from the previous edition is that presumptive *E. coli* O157 shall be sent to a reference/expert laboratory for determination of the virulence profile (*eae* and *stx* genes).

At least three participants used methods and/or media not primarily designed for detection of *E. coli* O157. These include NMKL 44 (coliform bacteria), TEMPO EC (*E. coli*) and Compact Dry EC (coliform bacteria and *E. coli*). All of these laboratories reported at least one false result. These results have nevertheless, as an exception, been included in the results summary. The parameters *E. coli* and coliform bacteria should however be analysed in the April and October proficiency testing rounds, respectively.

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

Results from analysis of Escherichia coli *O157*

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
All results	28	23	Pos	5	28	28	Neg	0	28	25	Pos	3
ISO 16654:2001	8	7	Pos	1	8	8	Neg	0	8	7	Pos	1
PCR method	6	5	Pos	1	6	6	Neg	0	6	5	Pos	1
NMKL 164:2005	4	4	Pos	0	4	4	Neg	0	4	4	Pos	0
VIDAS	2	2	Pos	0	2	2	Neg	0	2	2	Pos	0
Other*	8	5	Pos	3	8	8	Neg	0	8	7	Pos	1

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

Pathogenic *Vibrio* spp.

Sample A

The strain of *V. cholerae* was target organism and was present in approximately $\log_{10}$ 3.4 cfu ml^{-1} in the sample. In the Swedish Food Agency's quality control, it formed typical yellow colonies on TCBS. The strain is oxidase-positive and sensitive to vibriostatic agent O129.

Sample B

The strain of *V. cholerae* (not identical to the one in sample A) was target organism and was present in approximately $\log_{10}$ 2.9 cfu ml^{-1} in the sample. The strain forms typical yellow colonies on TCBS. Upon confirmation, it is oxidase-positive and sensitive to vibriostatic agent O129.

The strain of *S.* Enteritidis in the sample may also have formed colonies on TCBS.

Sample C

No target organism for the analysis was present in the sample. The strain of *E. coli* O157 that is present in the sample may have formed colonies on TCBS.

General remarks

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

As in previous proficiency testing rounds, the majority of the laboratories followed either NMKL 156:1997 or a version of ISO 21872. The latest of these, ISO 21872-1:2017, replaces both ISO/TS 21872-1:2007 and ISO/TS 21872-2:2007. However, most of the participants in this proficiency testing round still followed either of the older versions.

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

All laboratories stated that colonies were isolated on TCBS. One laboratory reported parallel isolation on CHROMagar™ Vibrio. Bile salts in TCBS inhibit the growth of Gram-positive microorganisms, whereas a high pH promotes the growth of *V. cholerae*. On this medium, *Vibrio* spp. form either green of yellow colonies, depending on if they ferment sucrose or not. *V. parahaemolyticus* and *V. vulnificus* (sucrose-negative) normally form blue-green colonies, whereas *V. cholerae* (sucrose-positive) normally form yellow colonies.

Results from analysis of pathogenic Vibrio *spp.*

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
All results	20	18	Pos	2	20	18	Pos	2	20	19	Neg	1
NMKL 156:1997	8	7	Pos	1	8	8	Pos	0	8	8	Neg	0
ISO/TS 21872-1:2007	5	5	Pos	0	5	5	Pos	0	5	5	Neg	0
ISO 21872-1:2017	4	4	Pos	0	4	3	Pos	1	4	3	Neg	1
ISO/TS 21872-1:2007 /Cor 1:2008	1	1	Pos	0	1	1	Pos	0	1	1	Neg	0
AOAC 988.20:1988*	1	1	Pos	0	1	1	Pos	0	1	1	Neg	0
Other	1	0	Pos	1	1	0	Pos	1	1	1	Neg	0

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

Yersinia enterocolitica

Sample A

No target organism for the analysis was present in the sample. The sample did however contain a very low amount (approximately 1-2 cfu ml^{-1}) of *Y. intermedia*, which is false positive for the analysis. On BS, it forms typical yellow colonies. The strain is oxidase-negative, but does not display agglutination against neither O:3 nor O:9 antisera. It does not contain the gene *ail*, which is used for PCR-detection of *Y. enterocolitica*. The strain may be difficult to identify as *Y. intermedia* with API 20 E.

Since all laboratories appear to have used methods that include some form of enrichment step, *Y. intermedia* should have been possible to detect, despite the low concentration. It can however not be ruled out that some laboratories achieved a negative result simply due to the low concentration. During the quality control at the Swedish Food Agency, *Y. intermedia* was detected after 3 weeks cold incubation in PSB, followed by surface spreading on CIN. Such cold incubation is mandatory with NMKL 117:1996, but optional with ISO 10273:2017.

Sample B

The strain of *Y. enterocolitica* was target organism and was present in approximately log_{10} 2.4 cfu ml^{-1} in the sample. On CIN, it forms typical colonies with a dark red center, and an outer transparent zone. On BS, it forms typical yellow colonies. The strain is oxidase-negative, and displays agglutination against O:3 antiserum, but not against O:9 antiserum. The strain contains the gene *ail*.

Sample C

No target organism for the analysis was present in the sample. However in the Swedish Food Agency's quality control, *C. freundii* formed atypical pink colonies on CIN, and yellow colonies on BS.

General remarks

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

The majority of the laboratories followed ISO 10273, distributed almost evenly between ISO 10273:2017 and ISO 10273:2003. The new ISO 10273:2017 contains several important changes compared to the previous version. These include that characteristic *Y. enterocolitica* can be confirmed either by the traditional biochemical methods or by detection of the chromosomal virulence-associated gene *ail* by real-time PCR. Further, NMKL 117:1996 is currently being revised, and the new version will likely be very similar to ISO 10273:2017. There is however no estimated date for when the new NMKL version will be published.

The method in ISO 10273:2017 is based on parallel enrichment in PSB and ITC. Aliquots are subsequently inoculated onto CIN as well as (optionally) on a second chromogenic medium selected by the laboratory. Characteristic colonies are confirmed by biochemical methods or by real-time PCR. Cold enrichment can also be performed, but is not mandatory. The method in NMKL 117:1996 is instead based on pre- and cold enrichment in PSB, as well as selective enrichment in MRB. After the enrichment steps,

samples are inoculated onto CIN, but SSDC can also be used. Presumptive colonies are subcultured on BS and sucrose-positive colonies (yellow) are selected for confirmation.

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

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

Results from analysis of Yersinia enterocolitica

Method	Sample A				Sample B				Sample C			
	N	**n**	**+/-**	**F**	**N**	**n**	**+/-**	**F**	**N**	**n**	**+/-**	**F**
All results	12	12	Neg	0	12	12	Pos	0	12	12	Neg	0
ISO 10273:2017	4	4	Neg	0	4	4	Pos	0	4	4	Neg	0
ISO 10273:2003*	3	3	Neg	0	3	3	Pos	0	3	3	Neg	0
PCR method	2	2	Neg	0	2	2	Pos	0	2	2	Neg	0
NMKL 117:1996	1	1	Neg	0	1	1	Pos	0	1	1	Neg	0
Other	2	2	Neg	0	2	2	Pos	0	2	2	Neg	0

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

Outcome of the results of individual laboratory - assessment

Reporting and evaluation of results

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

It is the responsibility of the participating laboratories to correctly report results according to the instructions. When laboratories incorrectly report their results, for example by stating “pos” or “neg” for quantitative analyses, the results cannot be correctly processed. Such incorrectly reported results are normally excluded. Inclusion and further processing of such results may still be done, after manual assessment in each individual case.

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

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

Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol (4). Samples for follow-up can be ordered, free of charge via our website: www.livsmedelsverket.se/en/PT-extra

Z-scores, box plots and deviating results

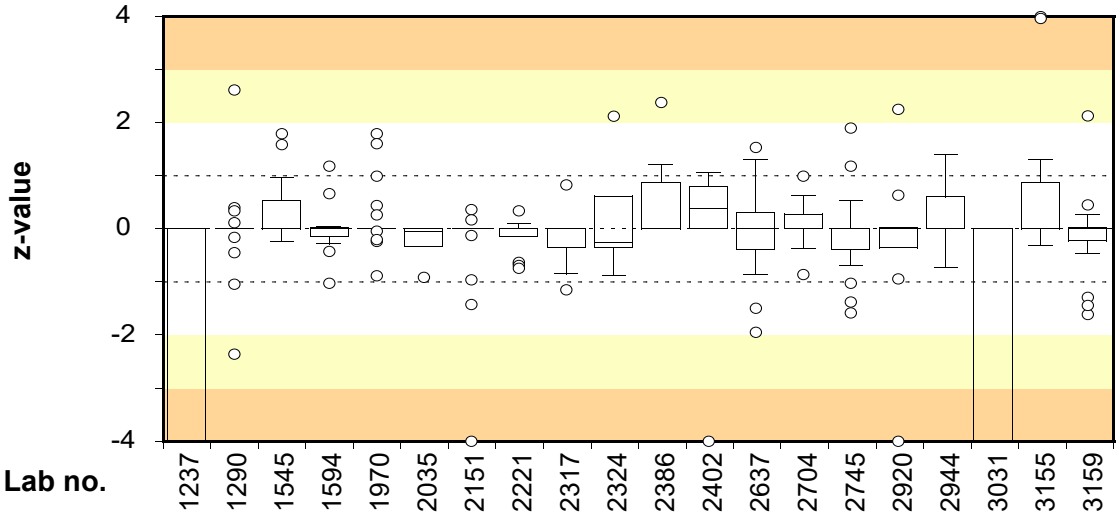
In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated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

The box plots are based on the z-scores listed in Annex 2, and give a comprehensive view of the achievement of each laboratory. A small box, centred around zero, indicates that the results of the individual laboratory, with false results excluded, are close to the general mean values calculated for all laboratory results. The range of z-scores is indicated by the size of the box and, for most laboratories, by lines and/or circles above and beneath the box. For each laboratory, the number of false results and outliers are also listed in the tables below the box plots.

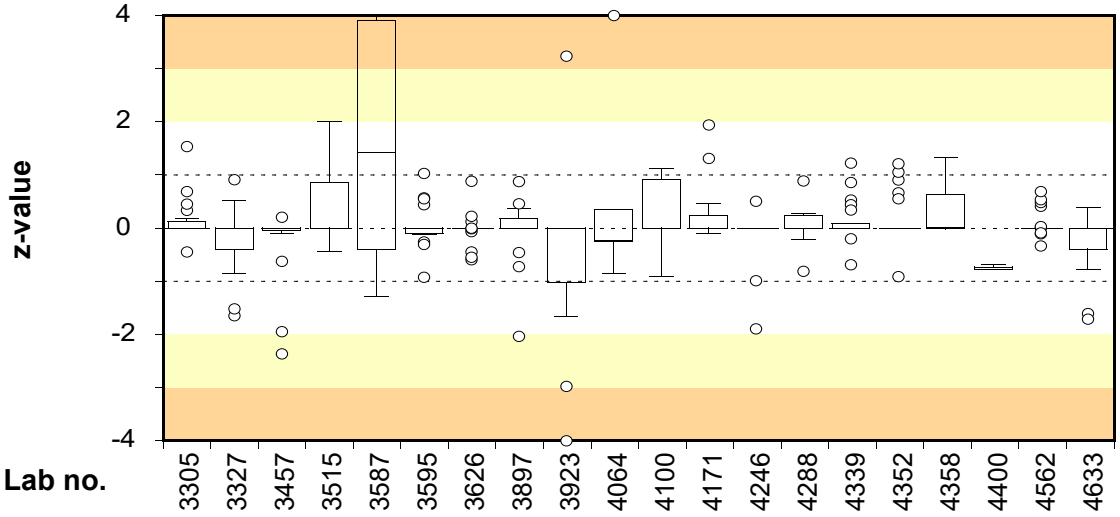
Box plots and numbers of deviating results for each laboratory

- *Z-scores are calculated according to the formula: z = (x-m)/s, where x is the result of the individual laboratory, m is the mean of the results of all participating laboratories, and s is the standard deviation of the participating laboratories, after removing outliers and false results.*
- *Outliers are included in the figures after being calculated to z-scores in the same way as for other results.*
- *False results do not generate any z-scores, and are not included in "No. of results".*
- *Correct results for qualitative analyses and correct negative results for quantitative analyses without target organism generate a z-score of 0.*
- *The laboratory median value is illustrated by a horizontal line in the box.*
- *The box includes 50 % of a laboratory's results (25 % of the results above the median and 25 % of the results below the median). The remaining 50 % are illustrated by lines and circles outside the box.*
- *A circle is for technical reasons shown in the plot when a value deviates to certain degree* from the other values. This does not by itself indicate that the value is an outlier.*
- *z-scores >+4 and <−4 are positioned at +4 and −4, respectively, in the plot.*
- *The background is divided by lines and shaded fields to simplify identifying the range in which the results are located.*

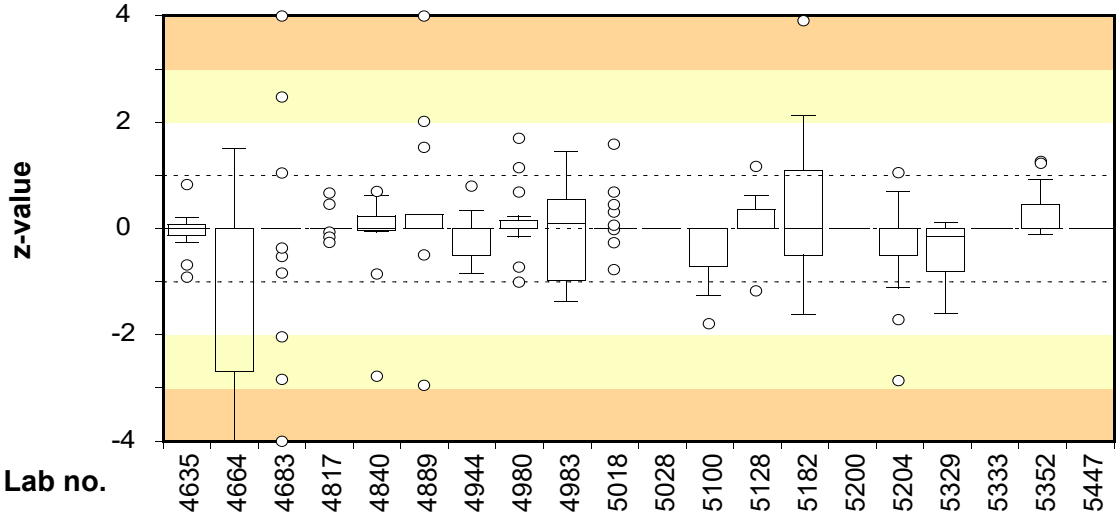
* *< [lowest value in the box −1,5 × (highest value in the box− lowest value in the box)]*
or
> [highest value in the box + 1,5 × (highest value in the box − lowest value in the box)].



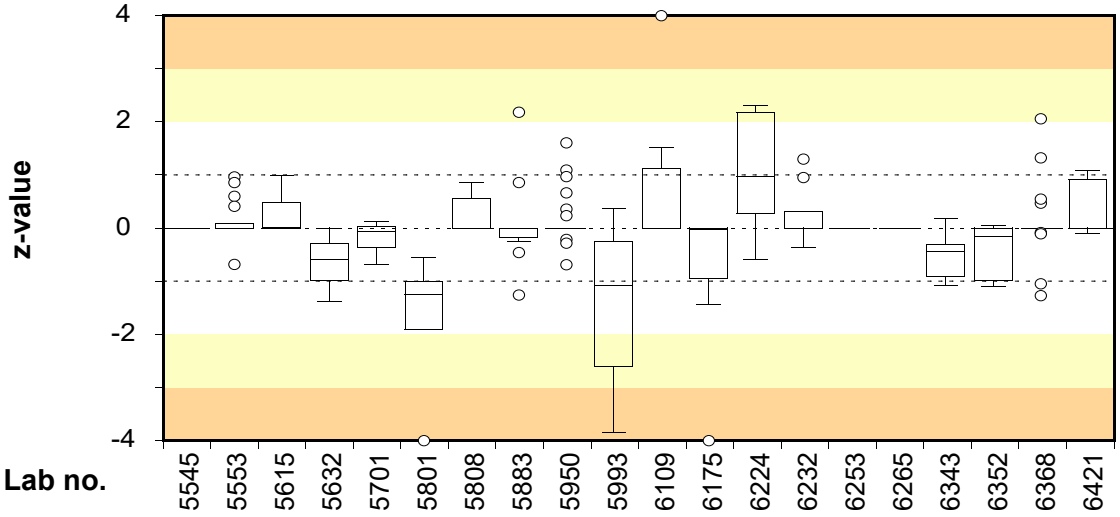
Lab no.	1237	1290	1545	1594	1970	2035	2151	2221	2317	2324	2386	2402	2637	2704	2745	2920	2944	3031	3155	3159
No. of results	13	21	15	12	24	6	18	18	15	5	9	9	15	15	15	9	18	15	15	15
False positive	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	6	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Low outliers	4	-	-	-	-	-	1	-	-	-	-	1	-	-	-	1	-	5	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-



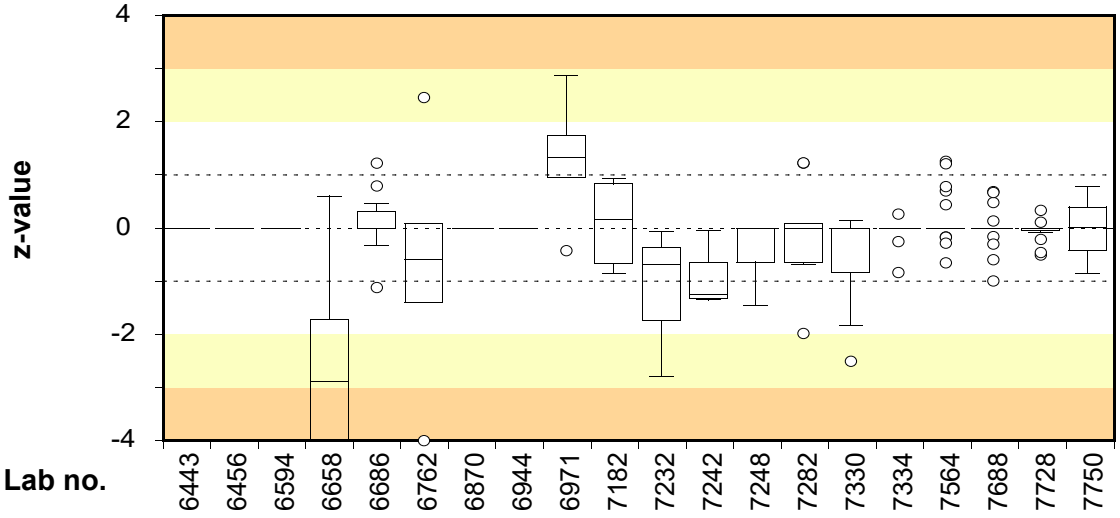
Lab no.	3305	3327	3457	3515	3587	3595	3626	3897	3923	4064	4100	4171	4246	4288	4339	4352	4358	4400	4562	4633
No. of results	21	21	15	12	6	18	21	15	15	6	15	12	9	12	21	24	9	5	30	12
False positive	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	-	3	2	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	2	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-



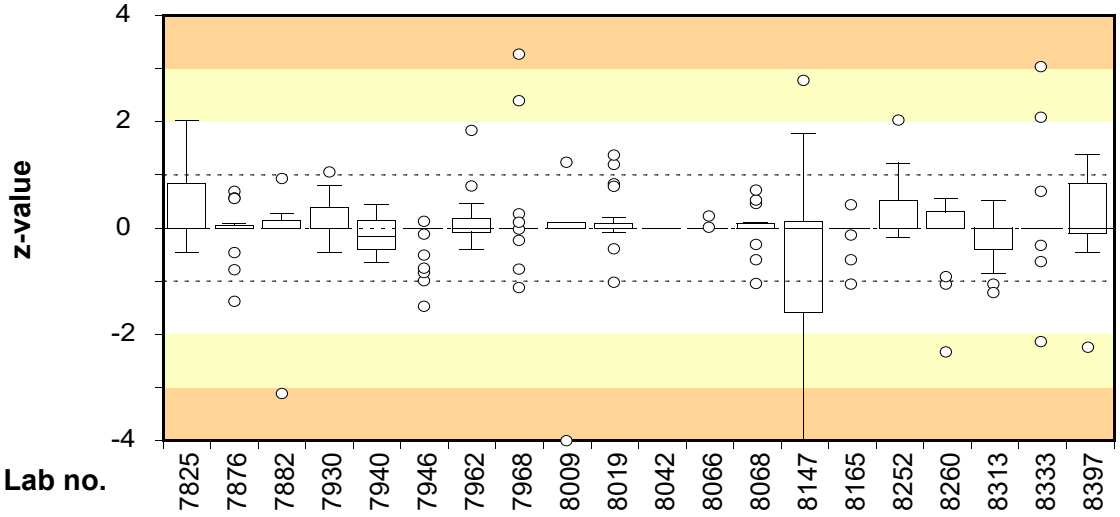
Lab no.	4635	4664	4683	4817	4840	4889	4944	4980	4983	5018	5028	5100	5128	5182	5200	5204	5329	5333	5352	5447
No. of results	12	18	25	21	12	18	15	15	6	24	3	8	12	9	6	21	8	6	15	3
False positive	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-
Low outliers	-	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	1	-	-	2	-	-	-	-	-	-	-	1	-	-	-	-	-	-



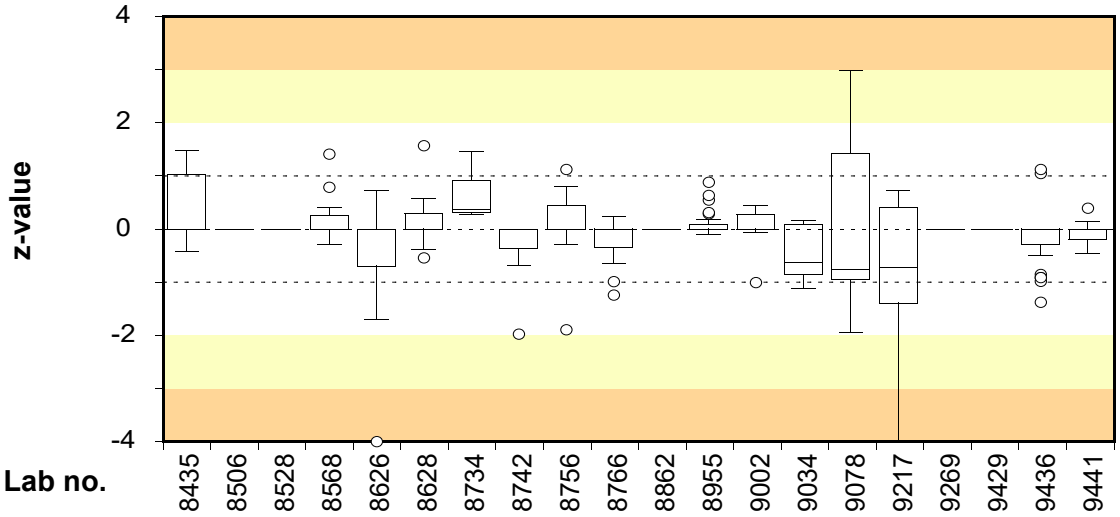
Lab no.	5545	5553	5615	5632	5701	5801	5808	5883	5950	5993	6109	6175	6224	6232	6253	6265	6343	6352	6368	6421
No. of results	6	21	12	3	3	6	8	15	30	6	9	12	6	9	6	-	6	9	18	18
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	1	-	-	-	1	-	1	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-



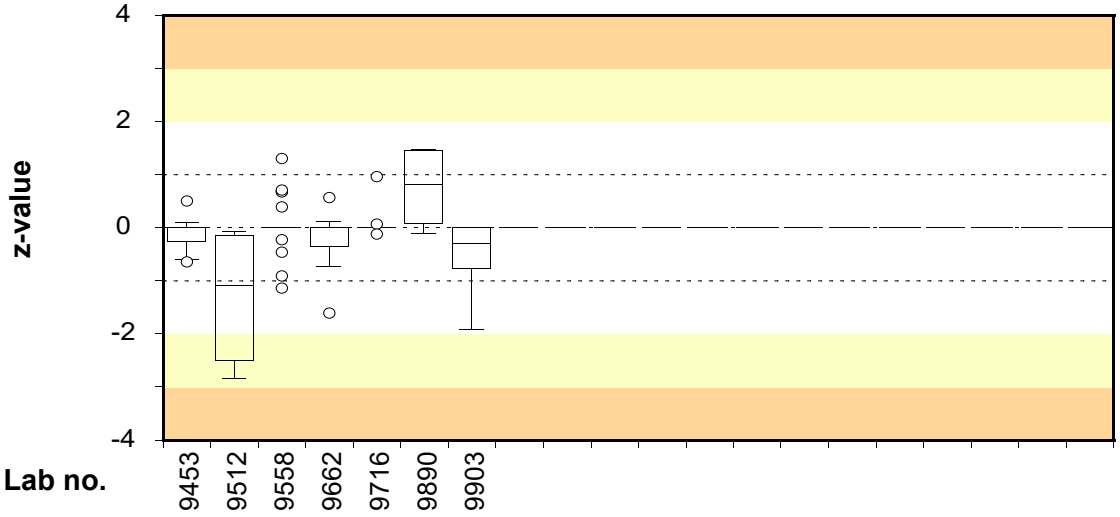
Lab no.	6443	6456	6594	6658	6686	6762	6870	6944	6971	7182	7232	7242	7248	7282	7330	7334	7564	7688	7728	7750
No. of results	3	6	-	6	15	6	3	3	6	6	3	4	18	9	15	9	21	24	15	3
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
Low outliers	-	-	-	3	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Lab no.	7825	7876	7882	7930	7940	7946	7962	7968	8009	8019	8042	8066	8068	8147	8165	8252	8260	8313	8333	8397
No. of results	15	15	8	15	3	26	15	18	6	20	3	9	15	14	21	15	15	15	15	12
False positive	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
False negative	-	-	-	-	-	4	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	1	-	-	-	-	2	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Lab no.	8435	8506	8528	8568	8626	8628	8734	8742	8756	8766	8862	8955	9002	9034	9078	9217	9269	9429	9436	9441
No. of results	12	-	3	15	15	17	3	9	10	15	3	30	18	6	6	6	3	6	24	15
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	1	-	-	2	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



No. of results	12	6	30	15	12	6	12
False positive	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-

Test material and quality control

Test material

Each laboratory received three sample mixtures with freeze-dried microorganisms, designated A-C. The test material was freeze-dried in portions of 0.5 ml in vials, as described by Peterz and Steneryd (5). Before analysing the samples, the contents of each vial should be dissolved in 254 ml of sterile diluent. The organisms present in the mixtures are listed in Table 2.

Table 2. *Microorganisms in the samples*

Sample[1]	Microorganism	Strain	
		SLV no.[2]	Reference[3]
A	*Escherichia coli* O157	SLV-528	CCUG 47557
	Hafnia alvei	SLV-015	CCUG 45642
	Listeria monocytogenes	SLV-361	smoked salmon
	Staphylococcus saprophyticus	SLV-013	CCUG 45100
	Vibrio cholerae	SLV-507	CCUG 34649
	Yersinia intermedia	SLV-472	ATCC 29909
B	*Bacillus cereus*	SLV-516	CCUG 44740
	Kocuria rhizophila	SLV-055	CCUG 35073
	Salmonella Enteritidis	SLV-436	-
	Vibrio cholerae	SLV-530	CCUG 45388
	Yersinia enterocolitica	SLV-408	CCUG 45643
C	*Campylobacter coli*	SLV-271	CCUG 45147
	Citrobacter freundii	SLV-091	CCUG 43597
	Escherichia coli O157	SLV-479	SMI 811 86
	Listeria monocytogenes	SLV-513	CCUG 44510

[1] The links between the mixtures and the randomised sample numbers are shown in Annex 1.
[2] Internal strain identification no. at the Swedish Food Agency
[3] Origin or culture collection (ATCC: American Type Culture Collection, CCUG: Culture Collection University of Gothenburg, Sweden, SMI: Public Health Agency of Sweden)

Quality control of the samples mixtures

In order to allow comparison of all freeze-dried samples, it is essential to have aliquots of homogeneous sample mixtures and equal volume in all vials. Quality control is performed on 10 randomly chosen vials in conjunction with manufacturing of the samples or on 5 vials if an "old" sample mixture was used and the last quality control was performed more than 6 months ago. Homogeneity of a sample mixture is approved if, for each analysis, the values obtained for the test of reproducibility (T) and the test "Index of dispersion" between vials (I_2) do not simultaneously exceed 2.6 and 2.0, respectively. (For definitions of T and I_2, see references 6 and 7 respectively.)

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

Analysis and method	A[1]			B[1]			C[2]		
	m	I_2	T	m	I_2	T	m	I_2	T
Aerobic microorganisms 30 °C NMKL method no. 86:2013	4.82	**2.65**	1.50	4.61	1.83	1.53	3.98	0.87	1.21
Enterobacteriaceae NMKL method no. 144:2005	4.37	**2.76**	1.94	2.09	**2.06**	2.05	3.59	**2.44**	1.64
Thermotolerant *Campylobacter*, quant. NMKL method no. 119:2007	Neg	-	-	Neg	-	-	3.18	**5.12**	1.44
Thermotolerant *Campylobacter*, qual. NMKL method no. 119:2007	Neg	-	-	Neg	-	-	Pos	-	-
Listeria monocytogenes, quant. NMKL method no. 136:2010	2.46	0.89	1.43	Neg	-	-	2.47	0.82	1.38
Listeria monocytogenes, qual. NMKL method no. 136:2010	Pos	-	-	Neg	-	-	Pos	-	-
Salmonella NMKL method no. 71:1999	Neg	-	-	1.96[3]	0.50[3]	1.37[3]	Neg	-	-
Escherichia coli O157 NMKL method no. 164:2005	0.81[3]	1.93[3]	1.34[3]	Neg	-	-	1.67[3]	1.13[3]	1.32[3]
Pathogenic *Vibrio* spp. NMKL method no. 156:1997	3.36[3]	**5.57**[3]	1.91[3]	2.89[3]	0.49[3]	1.36[3]	Neg	-	-
Yersinia enterocolitica NMKL method no. 117:1996	0.18[3,4]	**4.20**[3,4]	1.99[3,4]	2.37[3]	1.95[3]	1.44[3]	Neg	-	-

– No target organism and therefore no value
[1] n = 5 vials analysed in duplicate
[2] n = 10 vials analysed in duplicate
[3] From analysis of a parallel sample mixture
[4] Not a target organism for the analysis

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1. Kelly, K. 1990. Outlier detection in collaborative studies. J. Assoc. Off. Anal. Chem. 73:58–64.
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5. Peterz, M., Steneryd. A.C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *Journal of Applied Bacteriology*. 74:143–148.
6. Mooijman, K.M., During, M. & Nagelkerke, N.J.D. 2003. MICROCRM: Preparation and control of batches of microbiological materials consisting of capsules. RIVM report 250935001/2003. RIVM, Bilthoven, Holland.
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Internal and external control for microbiological analyses of food and drinking water

All analytical activities require work of a high standard that is accurately documented. For this purpose, most laboratories carry out some form of internal quality assurance, but their analytical work also has to be evaluated by an independent party. Such external quality control of laboratory competence is commonly required by accreditation bodies and can be done by taking part in proficiency testing (PT).

In a proficiency test, identical test material is analysed by a number of laboratories using their routine methods. The organiser evaluates the results and compiles them in a report.

The Swedish Food Agency's PT program offers

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

For more information, visit our website: https://www2.slv.se/absint

The Swedish Food Agency's reference material

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

For more information, visit our website: www.livsmedelsverket.se/en/RM-micro