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Proficiency Testing

Microbiology – Food

January 2021

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
BA	Blood agar
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
HEA	Hektoen enteric agar
ITC	Irgasan ticarcillin potassium chlorate broth
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 EC	TEMPO® E. coli
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	Swedish Food Agency, Sweden

Contents

General information on results evaluation

Statistical evaluation of the results

For analyses, where more than 20 laboratories have reported results, outliers are identified with statistical methods. Values that after $\log_{10}$ transformation do not belong to a strictly normal distribution are for this purpose identified as outliers with Grubbs' test modified by Kelly (1). When fewer than 20 laboratories have reported results, as well as in some individual cases, subjective adjustments are instead made to set outlier limits based on knowledge of the samples contents.

Mean values and standard deviations are normally provided for the different analyses. For analyses with fewer than 20 reported results, the median is provided instead of the mean value. Normally, for method groups with fewer than 5 results, only the number of false results and outliers are provided. Outliers and false results are not included in the calculations of mean values and standard deviations. Results reported as "> value" are not evaluated. Results reported as "< value" are interpreted as zero (negative result).

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. This method information is sometimes difficult to interpret, for example when laboratories state a medium that that is not included in the standard method they refer to. In such cases, as a general rule, the medium stated by the laboratory is nevertheless used in method comparisons. Method data from laboratories that are in other ways contradictory or difficult to interpret are normally either excluded or added to the group "Other", together with results from methods and media that are only used by 1-2 laboratories.

Uncertainty of measurement for the assigned values

The measurement uncertainty 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 is the mean value of the participants' results with outliers and false results excluded.

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

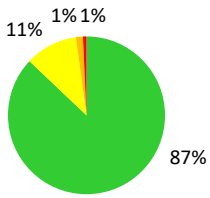
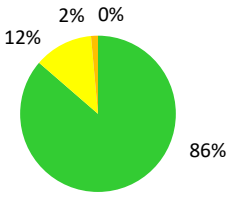
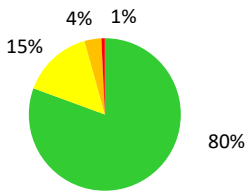
Results of the PT round January 2021

General outcome

Samples were sent to 144 laboratories, 30 in Sweden, 98 in other European countries, and 16 outside of Europe. Of the 139 laboratories that reported results, 44 (32 %) provided at least one result that received an annotation. In the previous round with similar analyses (January 2020) the proportion was 24 %.

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	*Campylobacter coli* *Citrobacter freundii* *E. coli* O157 *Listeria monocytogenes*	*Escherichia coli* *Salmonella* Stockholm *Staphylococcus aureus* *Vibrio cholerae* *Yersinia enterocolitica*	*Campylobacter jejuni* *Proteus mirabilis* *Salmonella* Enteritidis *Vibrio parahaemolyticus*

Analysis		**Target**	**N**	**F**	**X**	**Target**	**N**	**F**	**X**	**Target**	**N**	**F**	**X**
Aerobic micro-organisms 30 °C		***C. freundii***	114	0%	4%	***S. aureus*** ***E. coli***	112	0%	3%	***P. mirabilis***	114	0%	4%
Enterobacteriaceae		***C. freundii***	98	3%	3%	***E. coli***	97	0%	2%	***P. mirabilis***	97	4%	13%
Thermotol. *Campylo-bacter*	Quant.	***C. coli***	17	6%	0%	*(E. coli)*	16	6%	0%	***C. jejuni***	17	18%	0%
	Qual.		23	4%	-		23	0%	-		23	4%	-
L. mono-cytogenes	Quant.	***L. mono-cytogenes***	58	0%	5%	-	58	0%	0%	-	58	0%	0%
	Qual.		94	0%	-		94	1%	-		94	0%	-
Salmonella		*(C. freundii)*	104	0%	-	***S.* Stockholm**	104	4%	-	***S.* Enteritidis**	104	4%	-
E. coli O157		***E. coli* O157**	29	17%	-	*(E. coli)*	29	24%	-	-	29	10%	-
Pathogenic *Vibrio* spp.		*(E. coli* O157*)*	20	5%	-	***V. cholerae***	20	10%	-	***V. para-haemolyticus***	20	5%	-
Y. enterocolitica		*(C. freundii)*	12	0%	-	***Y. enterocolitica***	12	0%	-	-	13	8%	-

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

Aerobic microorganisms, 30 °C

Sample A

The strain of *C. freundii* was present in the highest concentration and was thus the main target organism.

The mean values for Petrifilm AC and TSA were somewhat higher compared to the mean values of other media. Somewhat higher results are relatively often seen for Petrifilm AC and can therefore be considered as normal. In contrast, the high mean value for TSA is due to one high result that causes an artificially high mean value.

Sample B

The strains of *S. aureus* and *E. coli were* present in the highest concentrations and were thus the main target organisms.

Sample C

The strain of *P. mirabilis* was present in the highest concentration and was thus the main target organism.

Swarming *P. mirabilis* sometimes cause problems for the participants, and low results are in these cases often reported. However in this PT round, only two low results were reported.

General remarks

As in previous proficiency testing rounds, the laboratories mainly followed NMKL 86 (different versions), ISO 4833 (different versions) or 3M Petrifilm. Both NMKL 86 and ISO 4833 are based on incubation on PCA or MCPA at 30 °C for 72 h. Users of Petrifilm AC can choose between times/temperatures, depending on which method validation that is followed. For example, AOAC ® 990.12 prescribes incubation at 35 °C for 48 h while AFNOR 3M 01/1-09/89 prescribes 30 °C for 72 h.

One of the laboratories that incubated on PCA stated that they followed the method for contaminating microorganisms in dairy products (ISO 13559 / IDF 153:2002). However this method does use the same incubation time and temperature as NMKL 86:2013 and ISO 4833-1:2013.

The majority of the laboratories incubated on either PCA or Petrifilm AC. Incubation on MPCA was mainly done by laboratories in 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	114	109	3.88	0.17	0	2	3	112	109	4.64	0.10	0	3	0	114	110	4.22	0.13	0	2	2
PCA	62	59	3.79	0.12	0	1	2	60	59	4.62	0.09	0	1	0	62	60	4.18	0.11	0	1	1
Petrifilm AC	23	22	4.06	0.11	0	1	0	23	22	4.65	0.10	0	1	0	23	22	4.32	0.12	0	1	0
TSA	9	8	4.06	0.18	0	0	1	9	8	4.72	0.05	0	1	0	9	9	4.25	0.07	0	0	0
MPCA	6	6	3.82	0.10	0	0	0	6	6	4.68	0.12	0	0	0	6	6	4.18	0.18	0	0	0
TEMPO AC	5	5	3.97	0.18	0	0	0	5	5	4.72	0.11	0	0	0	5	5	4.30	0.14	0	0	0
TGE	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0
Compact Dry TC	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	2	-	-	0	0	1
Other	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0

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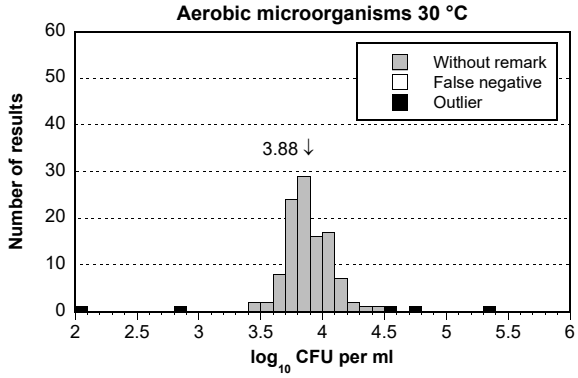

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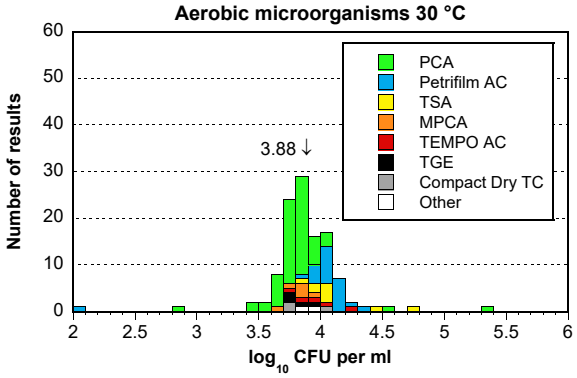

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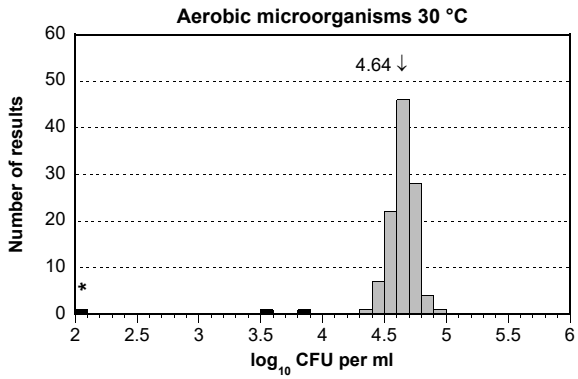

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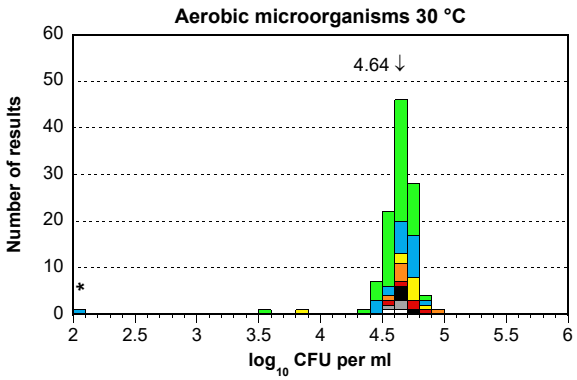

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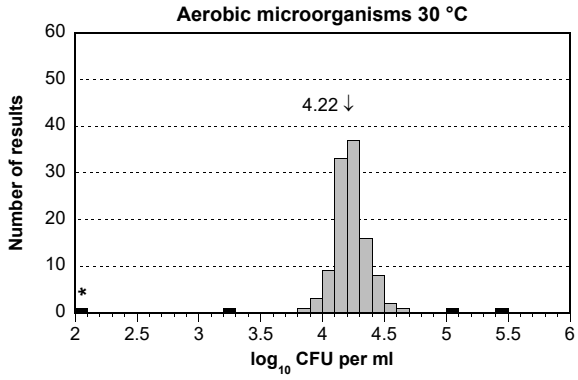

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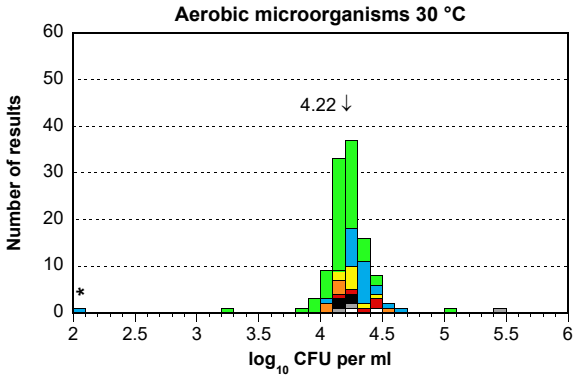

Enterobacteriaceae

Sample A

The strains of *C. freundii* and *E. coli* O157 belong to Enterobacteriaceae. The strain of *C. freundii* was however present in considerably higher concentration than *E. coli* O157 and was thus the main target organism. On VRBG, the strain of *C. freundii* forms typical red colonies surrounded by a bile salt precipitation zone. The strain is oxidase-negative.

Sample B

The strain of *E. coli* was target organism. In the Swedish Food Agency's quality control on VRBG, it formed typical colonies surrounded by a precipitation zone. The strain is oxidase-negative.

Sample C

The strains of *P. mirabilis* and *S.* Enteritidis belong to Enterobacteriaceae. The strain of *P. mirabilis* was however present in considerably higher concentration than *S.* Enteritidis and was thus the main target organism.

Twelve laboratories reported low outliers, likely due to swarming of *P. mirabilis*. The majority of the low outliers were reported by laboratories that incubated on VRBG.

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 (45 %) or a method with Petrifilm EB (23 %), while the ISO methods (different versions) were used by in total 17 %. ISO 21528-2:2017 is based on colony count, while ISO 21528-1:2017 is based on MPN. The latter method is recommended when the expected concentration of Enterobacteriaceae is lower than 100 cfu g^{-1}.

The number of users was higher for ISO 21528-2:2017 than for ISO 21528-2:2004 (9 % and 5 %, respectively). In comparison, three laboratories (3 %) stated following the older ISO 21528-1:2004.

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. The majority of the laboratories that performed a confirmation specified that this consisted of an oxidase test.

With exception for the low outliers in sample C, the results for the different methods and media were similar, and only a few outliers and false results were reported. Somewhat higher results for TEMPO EB have been reported in previous proficiency testing rounds. In the current proficiency testing round only four laboratories used TEMPO EB and the reported results were similar to those from other methods and media, with a possible exception with somewhat higher results for sample A.

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	98	92	3.61	0.24	3	2	1	97	95	4.03	0.22	0	2	0	97	80	4.08	0.15	4	12	1
VRBG	65	62	3.56	0.25	1	1	1	64	63	4.00	0.24	0	1	0	64	52	4.07	0.13	1	10	1
Petrifilm EB	22	19	3.74	0.10	2	1	0	22	21	4.13	0.11	0	1	0	22	18	4.06	0.18	2	2	0
TEMPO EB	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0
TSA/VRBG	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0
Compact Dry ETB	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
RAPID'Eb*	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	0	-	-	1	0	0

* RAPID'Enterobacteriaceae.

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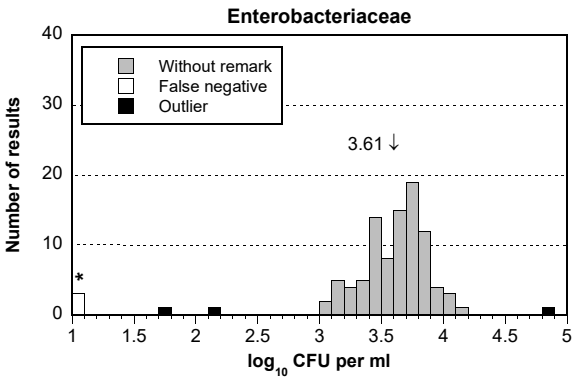

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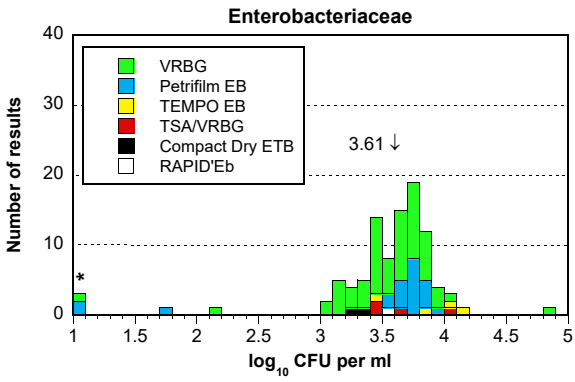
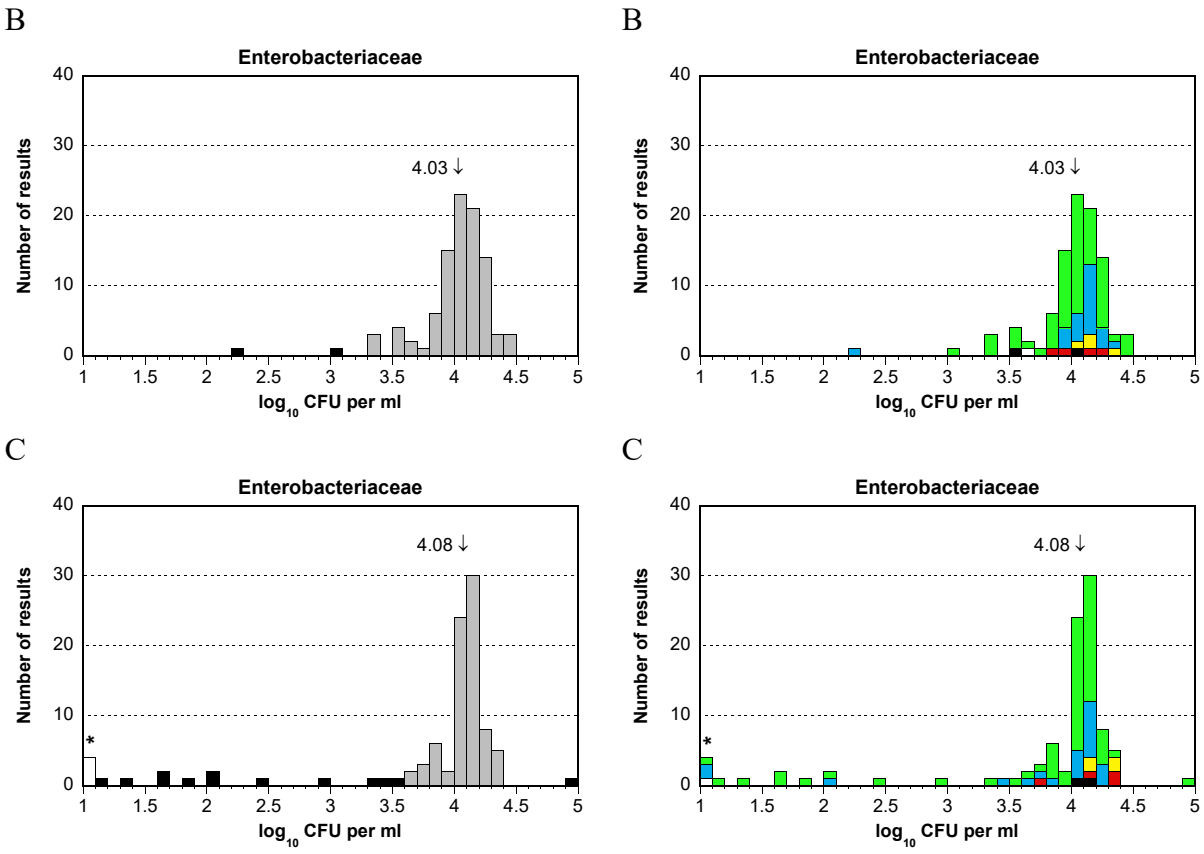




Thermotolerant *Campylobacter*

Sample A

The strain of *C. coli* was target organism. On mCCDA it may possibly form both smaller and larger colonies. The strain is oxidase-positive and catalase-positive. It is also positive for the hydrolysis of indoxyl acetate, negative for the hydrolysis of hippurate, and has a for *Campylobacter* typical appearance under a microscope.

The results in the quantitative analysis had a fairly wide distribution, which is not unusual for this parameter. Since only 17 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 23 laboratories. One of these was a false negative result.

Sample B

No target organism was present in the sample. It did however contain a strain of *E. coli*, which is false positive for the analysis. In the Swedish Food Agency's quality control, it formed grey colonies on mCCDA. During subsequent confirmation on BA it formed colonies surrounded by a distinct zone of haemolysis. The strain is oxidase-negative and catalase-positive. Under the microscope, it is easily distinguished from *Campylobacter*.

The only deviating result was a false positive result in the quantitative analysis.

Sample C

The strain of *C. jejuni* was target organism. It forms typical colonies on mCCDA. It is positive for hydrolysis of indoxyl acetate and hippurate, and has a for *Campylobacter* typical appearance under a microscope.

Three false negative results were reported in the quantitative analysis and one in the quantitative analysis.

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/white colour and a glossy surface. Confirmation is often 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 in both the quantitative and qualitative analysis by all except one laboratory. The most common types of confirmation were a motility test and/or an oxidase test.

NMKL 119:2007, ISO 10272-1:2017 (qualitative) and ISO 10272-2:2017 (quantitative) were the most common methods. In the qualitative analysis, one laboratory stated that they followed ISO 17995, which is a method for detection of *Campylobacter* in water samples.

In the qualitative analysis, the majority of the laboratories (78 %) used Bolton broth for the enrichment, but the use of Preston broth and CampyFood® was also reported. For the selective step, most laboratories (87 %) used mCCDA, but Brilliance™ CampyCount agar and Abeyta-Hunt Bark agar were also used with good results.

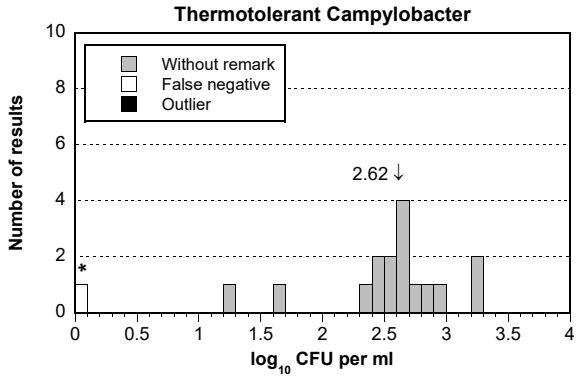
In the qualitative analysis, 13 of 17 laboratories incubated on mCCDA. Abeyta-Hunt Bark agar and RAPID'Campylobacter were used by one laboratory each, with correct results.

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	17	16	2.62	0.51	1	0	0	16	15	-	-	1	-	-	17	14	1.98	0.31	3	0	0
ISO 10272-2:2017	10	9	2.63	0.62	1	0	0	9	8	-	-	1	-	-	10	7	2.03	0.39	3	0	0
NMKL 119:2007	5	5	2.56	0.36	0	0	0	5	5	-	-	0	-	-	5	5	2.00	0.24	0	0	0
ISO 10272-2:2006	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0
Other	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0

* Med = median

A



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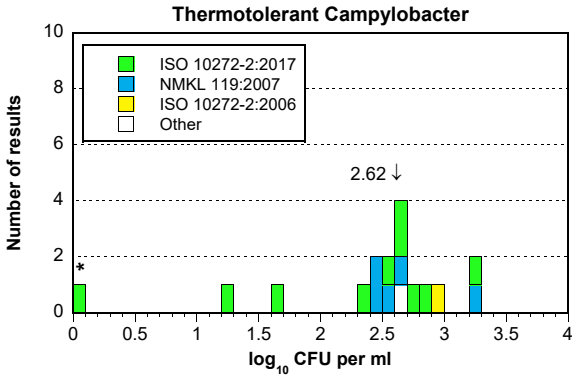

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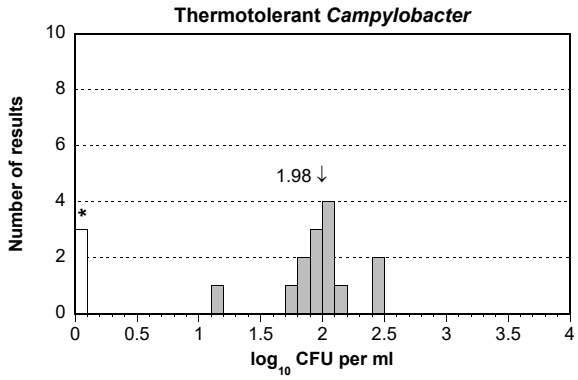

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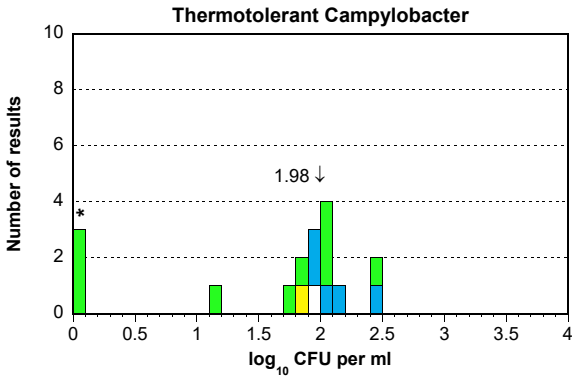


Results from qualitative analysis of thermotolerant Campylobacter

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
All results	23	22	Pos.	1	23	23	Neg.	0	23	22	Pos.	1
NMKL 119:2007	12	11	Pos.	1	12	12	Neg.	0	12	12	Pos.	0
ISO 10272-1:2017	7	7	Pos.	0	7	7	Neg.	0	7	6	Pos.	1
ISO 10272-1:2006	1	1	Pos.	0	1	1	Neg.	0	1	1	Pos.	0
Other*	3	3	Pos.	0	3	3	Neg.	0	3	3	Pos.	0

* Includes ISO 17995 (water method), VIDAS, and a PCR method.

Listeria monocytogenes

Sample A

The strain of *L. monocytogenes* was target organism. On ALOA it forms characteristic blue-green colonies, surrounded by a distinct opaque halo. The strain is catalase-positive, displays β-haemolysis on blood agar, and ferments rhamnose but not xylose.

Sample B

No target organism was present in the sample.

Only one deviating result was reported; a false positive result in the qualitative analysis.

Sample C

No target organism was present in the sample.

No false positive results were reported, neither in the quantitative nor in the qualitative analysis.

General remarks

ISO 11290 (different versions), NMKL 136:2010 and RAPID'L.mono were the main methods used in both the quantitative and in the qualitative analysis. In the qualitative analysis, VIDAS® and different PCR methods were also common.

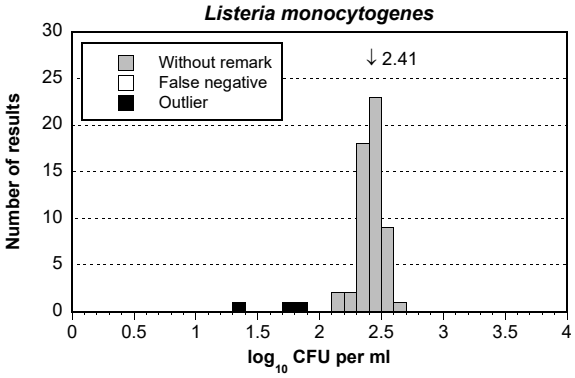
NMKL 136:2010 includes both detection and enumeration of *L. monocytogenes*. In comparison, ISO 11290-1 (qualitative) and ISO 11290-2 (quantitative) detect/enumerate both *Listeria* spp. and *L. monocytogenes*. All of the methods mainly use ALOA for the isolation, on which *L. monocytogenes* form blue-green colonies due to β-glucosidase activity. The colonies are also 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. RAPID'L.mono is based on a chromogenic medium that identifies the enzyme PI-PLC in *L. monocytogenes*. It identifies both *Listeria* spp. and *L. monocytogenes* based on their inability to metabolise xylose. Similarly, Listeria Precis™ is based on the chromogenic medium Brilliance™ Listeria, on which *Listeria* spp. and *L. monocytogenes* form blue colonies due to their β-glucosidase activity. SwabSURE ListeriaP is a test based on swab sampling, for detection of *L. monocytogenes* and *L. ivanovii* in surface samples. In comparison, VIDAS® is based on detection of specific *L. monocytogenes* antigen, in a method based on ELFA (*Enzyme Linked Fluorescent Assay*). The alternative methods are all validated by AFNOR and/or NordVal. In addition to the previously mentioned media, many laboratories used either of Oxoid Brilliance™ Listeria agar (previously OCLA), PALCAM, Oxford Listeria selective agar and/or LMBA.

L. monocytogenes is often 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). Confirmation was performed by 81 % 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	58	55	2.41	0.10	0	3	0	58	58	-	-	0	-	-	58	58	-	-	0	-	-
RAPID'L.mono	16	16	2.36	0.11	0	0	0	16	16	-	-	0	-	-	16	16	-	-	0	-	-
NMKL 136:2010	15	14	2.45	0.07	0	1	0	15	15	-	-	0	-	-	15	15	-	-	0	-	-
ISO 11290-2:2017	14	13	2.42	0.08	0	1	0	14	14	-	-	0	-	-	14	14	-	-	0	-	-
ISO 11290-2:1998/Amd 1:2004	7	6	2.44	0.09	0	1	0	7	7	-	-	0	-	-	7	7	-	-	0	-	-
ISO 11290-2:1998	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	-	-
Other	5	5	2.42	0.11	0	0	0	5	5	-	-	0	-	-	5	5	-	-	0	-	-

A



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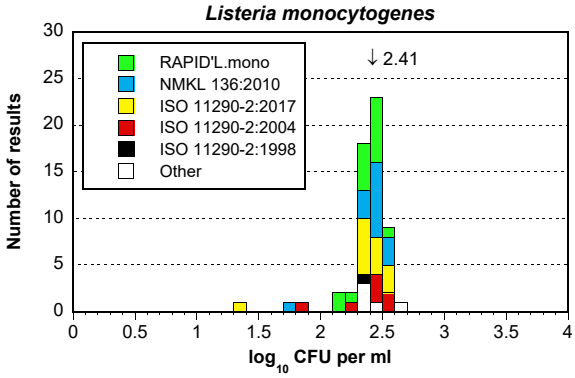


Results from qualitative analysis of Listeria monocytogenes

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
All results	94	94	Pos.	0	94	93	Neg.	1	94	94	Neg.	0
RAPID'L.mono	16	16	Pos.	0	16	16	Neg.	0	16	16	Neg.	0
PCR method	16	16	Pos.	0	16	16	Neg.	0	16	16	Neg.	0
VIDAS	14	14	Pos.	0	14	14	Neg.	0	14	14	Neg.	0
ISO 11290-1:2017	13	13	Pos.	0	13	13	Neg.	0	13	13	Neg.	0
NMKL 136:2010	11	11	Pos.	0	11	11	Neg.	0	11	11	Neg.	0
ISO 11290-1/Amd 1:2004	8	8	Pos.	0	8	8	Neg.	0	8	8	Neg.	0
Listeria Precis™	3	3	Pos.	0	3	3	Neg.	0	3	3	Neg.	0
SwabSURE™ ListeriaP	3	3	Pos.	0	3	3	Neg.	0	3	3	Neg.	0
Other	10	10	Pos.	0	10	9	Neg.	1	10	10	Neg.	0

Salmonella

Sample A

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

Sample B

The strain of *S.* Stockholm was target organism for the analysis. 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.

Four false negative results were reported.

Sample C

The strain of *S.* Enteritidis was target organism for the analysis. 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.

Four false negative results were reported.

General remarks

The two most common methods were NMKL 71:1999 (23 %) and ISO 6579-1:2017 (21 %), which are very similar. Both are based on pre-incubation in BPW, followed by selective enrichment in RVS. 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*. With both methods, incubation is mainly on XLD, and confirmation is by biochemical (e.g. mannitol and urea) and serological (e.g. *Salmonella* polyvalent O and H antisera) tests. Confirmation of some kind was performed by the majority (94 %) of the laboratories.

Relatively many laboratories followed either of the older ISO 6579:2002 or ISO 6579:2002/Amd 1:2007. The most important changes in the method from 2017 include that detection of β-galactosidase and indole are 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, Harlequin ® Salmonella ABC Medium and HEA.

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.

It should be mentioned that four of the five laboratories that used RAPID' Salmonella reported one false negative result each. The method is based on detection of the enzyme C8 esterase in *Salmonella*. Its activity releases a chromophore in RAPID' Salmonella, which causes *Salmonella* to form red/purple colonies on the plates. Other micro-organisms are inhibited, or form blue or transparent colonies. It is unclear why users of RAPID' Salmonella did not perform as well as other laboratories, but it does not appear to be due to any specific problem with the method. This is since two of the four laboratories reported a false negative result for sample B, but not for sample C, whereas the opposite was true for the two other laboratories. A possible explanation could be that the four laboratories that reported a false negative result incubated only on RAPID' Salmonella. The fifth laboratory incubated in parallel on XLD. Other laboratories that used methods based on the detection of *Salmonella* esterase activity (e.g. Brilliance™ Salmonella) also performed well. Similarly, these laboratories in general incubated on another medium in parallel, most commonly XLD.

Results from analysis of Salmonella

Method	Sample A				Sample B				Sample C			
	N	n	+/-	F	N	n	+/-	F	N	n	+/-	F
All results	104	104	Neg.	0	104	100	Pos.	4	104	100	Pos.	4
NMKL 71:1999	24	24	Neg.	0	24	23	Pos.	1	24	24	Pos.	0
ISO 6579-1:2017	22	22	Neg.	0	22	22	Pos.	0	22	22	Pos.	0
PCR method	20	20	Neg.	0	20	19	Pos.	1	20	20	Pos.	0
VIDAS*	13	13	Neg.	0	13	13	Pos.	0	13	13	Pos.	0
RAPID'Salmonella	5	5	Neg.	0	5	3	Pos.	2	5	3	Pos.	2
ISO 6579:2002	5	5	Neg.	0	5	5	Pos.	0	5	5	Pos.	0
ISO 6579:2002/Amd1:2007	4	4	Neg.	0	4	4	Pos.	0	4	4	Pos.	0
NMKL 187**	3	3	Neg.	0	3	3	Pos.	0	3	3	Pos.	0
Reveal 2.0 Salmonella	2	2	Neg.	0	2	2	Pos.	0	2	1	Pos.	1
Salmonella Precis™	1	1	Neg.	0	1	1	Pos.	0	1	1	Pos.	0
Other***	5	5	Neg.	0	5	5	Pos.	0	5	4	Pos.	1

* The group VIDAS includes two laboratories that used MINI VIDAS®.
** Includes both NMKL 187:2007 and NMKL 187:2016.
*** Includes ambiguous methods, as well as national and company-specific methods.

Escherichia coli O157

Sample A

The strain of *E. coli* O157 was target organism for the analysis. 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.

Five false-negative results were reported. Three of these can be attributed to the use of inappropriate methods.

Sample B

No target organism was present in the sample. The strain of *E. coli* was however false positive for the analysis. In the Swedish Food Agency's quality control it formed red colonies on SMAC. No colonies were observed on CT-SMAC.

Seven false-negative results were reported. Three of these can be attributed to the use of inappropriate methods, and one to a not clearly defined method.

Sample C

No target organism was present in the sample. In the Swedish Food Agency's quality control, atypical red colonies were observed on SMAC. No colonies were observed on CT-SMAC.

Three false positive results were reported.

General remarks

Only 29 laboratories performed the analysis. Statistical evaluation of the results is therefore difficult. However many of the false results appear to be due to the use of inappropriate methods.

In total, 38 % 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 version, 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).

As already mentioned, at least four of the participants used methods and or media that are 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*). These results are included among "Other" in the results summary. The parameters *E. coli* and coliform bacteria should however be analysed in the April and October proficiency testing rounds, respectively. The two laboratories that used TEMPO, as well as the laboratory that followed NMKL 44, reported two false results each.

As in previous proficiency testing rounds, the most frequently used media were CT-SMAC, SMAC and CHROMagar™ O157. CT-SMAC and SMAC distinguish between bacteria that ferment sorbitol (most non-pathogenic *E. coli*) are those that do not (most

E. coli O157). On these media, sorbitol-negative *E. coli* O157 form transparent colonies with a dark center, whereas sorbitol-positive *E. coli* instead form red colonies. One laboratory used Harlequin™ SMAC-BCIG. This is similar to SMAC, and contains the chromogenic substrate BGIC which causes sorbitol-negative and β-glucuronidase--positive *E. coli* to form blue/green colonies. In comparison, on CHROMagar™ *E. coli* O157 form mauve (purple) colonies that can be distinguished from coliform (blue) or other bacteria (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	29	24	Pos.	5	29	22	Neg.	7	29	26	Neg.	3
ISO 16654:2001*	8	8	Pos.	0	8	7	Neg.	1	8	7	Neg.	1
PCR method	6	5	Pos.	1	6	6	Neg.	0	6	5	Neg.	1
NMKL 164:2005	3	2	Pos.	1	3	1	Neg.	2	3	3	Neg.	0
VIDAS	2	2	Pos.	0	2	2	Neg.	0	2	2	Neg.	0
Other**	10	7	Pos.	3	10	6	Neg.	4	10	9	Neg.	1

* Includes laboratories that used ISO 16654:2001/Amd 1:2017.
** Includes four laboratories have used inappropriate or not clearly defined methods.

Pathogenic *Vibrio* spp.

Sample A

No target organism was present in the sample. The strain of *E. coli* O157 may possibly form colonies on TCBS.

Sample B

The strain of *V. cholerae* was target organism. On TCBS, it forms typical grey/yellow colonies. It is oxidase-positive and sensitive to vibriostatic agent O129. The strain of *E. coli* may form yellow and oxidase-negative colonies on TCBS. The strain of *S.* Stockholm may also form colonies on TCBS. All atypical colonies that were observed in the Swedish Food Agency's quality control on TCBS were oxidase-negative upon confirmation.

Sample C

The strain of *V. parahaemolyticus* was target organism. It forms typical blue/green colonies on TCBS. It is oxidase-positive and sensitive to vibriostatic agent O129.

In a first test of sample C, *P. mirabilis* formed atypical small light green colonies on TCBS. These colonies were however oxidase-negative, and could therefore be distinguished from *V. parahaemolyticus* after confirmation.

General remarks

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

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. In this proficiency testing round, more laboratories followed the new ISO 21872-1:2017 compared to the older ISO/TS 21872-1:2007.

ISO 21872-1:2017 contains several changes, including how to perform confirmation with biochemical and/or PCR methods. However, it mainly follows the same principle as the previous versions. Primary and secondary enrichment in APW 2% is followed by inoculation onto 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 TCBS, *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	19	Neg.	1	20	18	Pos.	2	20	19	Pos.	1
NMKL 156:1997	8	8	Neg.	0	8	8	Pos.	0	8	7	Pos.	1
ISO 21872-1:2017	7	6	Neg.	1	7	5	Pos.	2	7	7	Pos.	0
ISO/TS 21872-1:2007	4	4	Neg.	0	4	4	Pos.	0	4	4	Pos.	0
AOAC 988.20:1988*	1	1	Neg.	0	1	1	Pos.	0	1	1	Pos.	0

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

Yersinia enterocolitica

Sample A

No target organism was present in the sample. The strain of *C. freundii* was however false positive for the analysis. In the Swedish Food Agency's quality control, it formed atypical pink colonies on CIN and yellow colonies on BS. The strain of *C. freundii* is oxidase-negative, and does not display agglutination against O:3 and O:9 antisera.

Sample B

The strain of *Y. enterocolitica* was target organism for the analysis. 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 was present in the sample. In the Swedish Food Agency's quality control, no colonies were observed on CIN.

General remarks

Most laboratories followed ISO 10273, mainly ISO 10273:2017 but also the older 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.

NMKL 117 has also been thoroughly revised and will be published in a new version in 2021. The major changes to the method have made it more similar to ISO 10273, for example with parallel enrichment in PSB and ITC. Cold enrichment has also been made optional and the procedure for this has been revised.

On CIN, colonies of *Y. enterocolitica* have a typical appearance; a dark 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. With this, enrichment in semi-selective PSB or in non-selective TSBY 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. 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	13	12	Neg.	1
ISO 10273:2017	4	4	Neg.	0	4	4	Pos.	0	5	4	Neg.	1
ISO 10273:2003*	2	2	Neg.	0	2	2	Pos.	0	2	2	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
ISO 18867:2015**	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 used a modified version of ISO 10273:2003.

** The laboratory stated following ISO 18867:2015, with confirmation according to ISO 10273:2017.

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

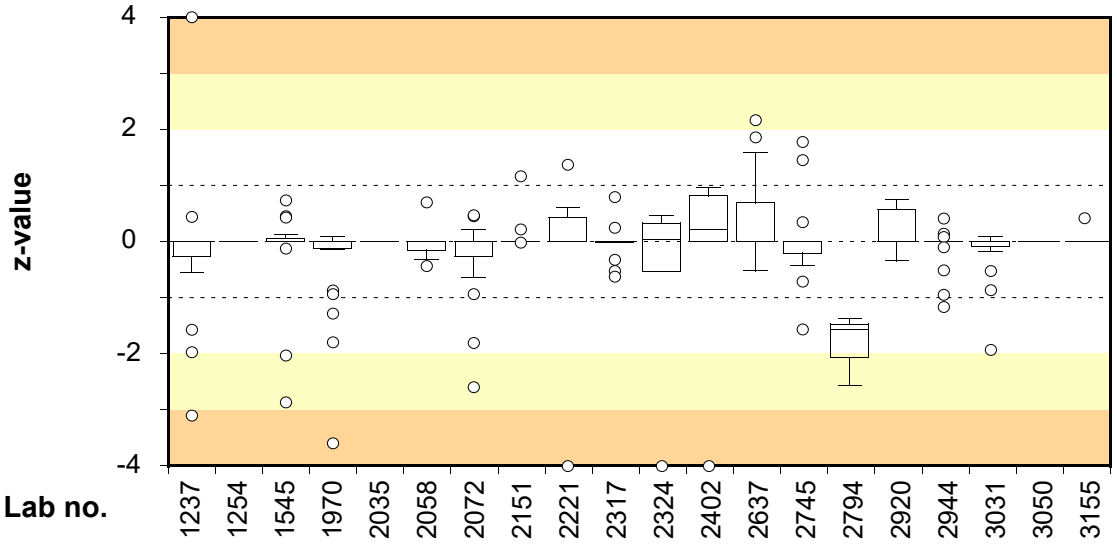
In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated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

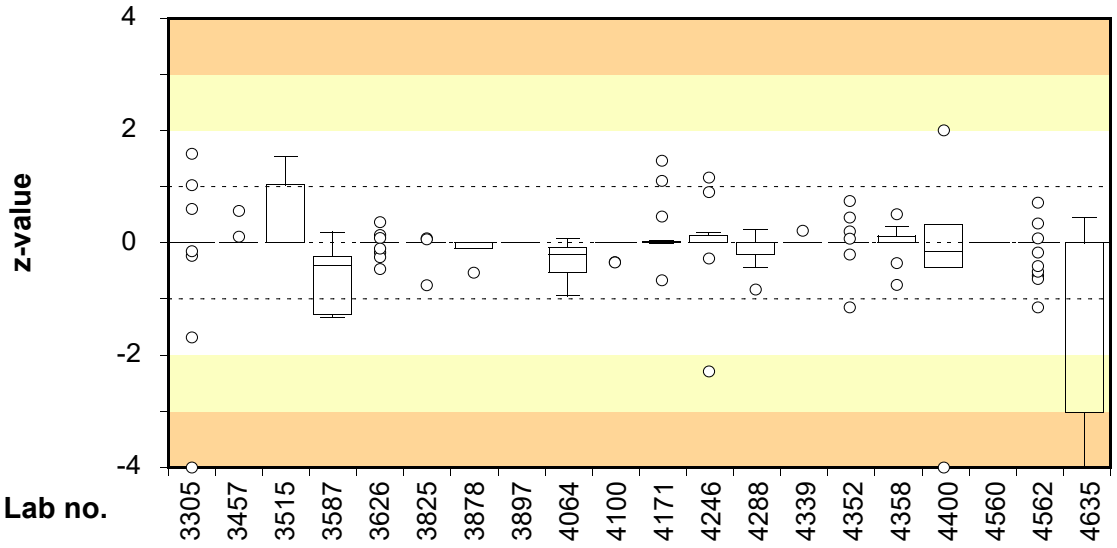
Box plots and numbers of deviating results for each laboratory

- *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.*

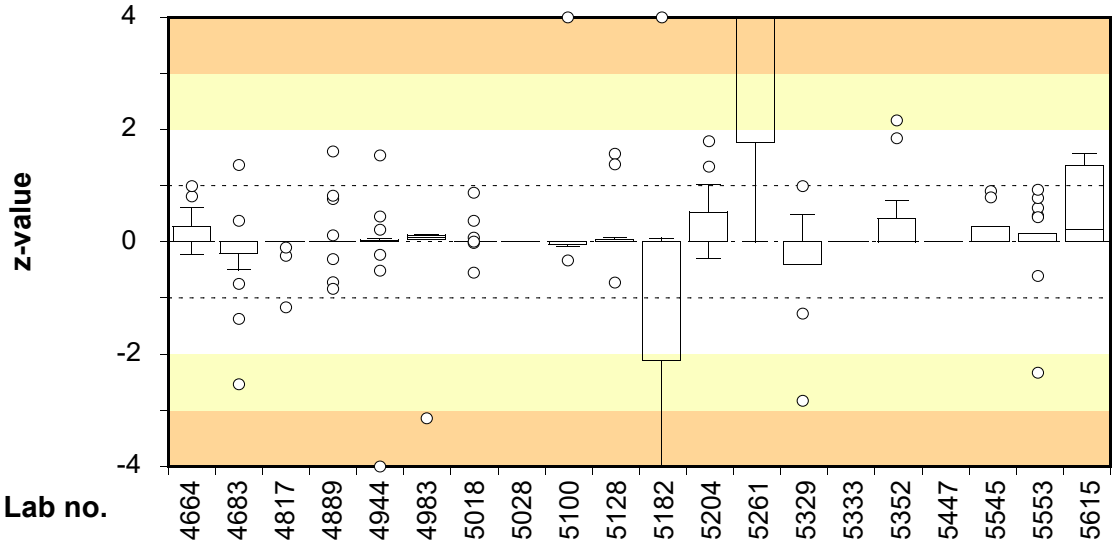
* *< [lowest value in the box −1,5 × (highest value in the box− lowest value in the box)]*
or
> [highest value in the box + 1,5 × (highest value in the box − lowest value in the box)].



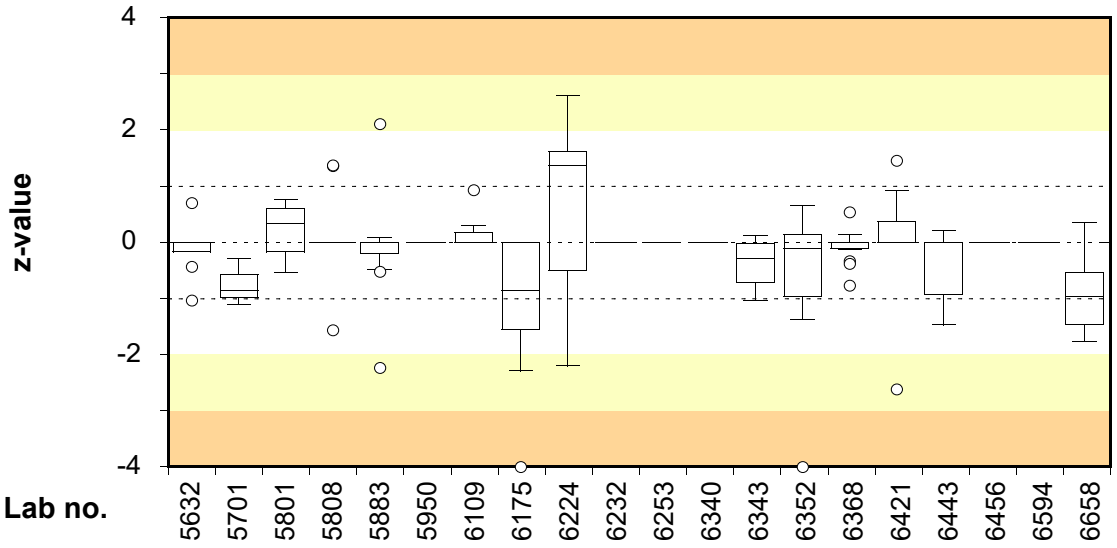
No. of results	19	6	15	24	-	12	24	12	15	14	6	9	15	15	3	9	18	15	9	9
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
False negative	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
Low outliers	-	-	-	1	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-	-
High outliers	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



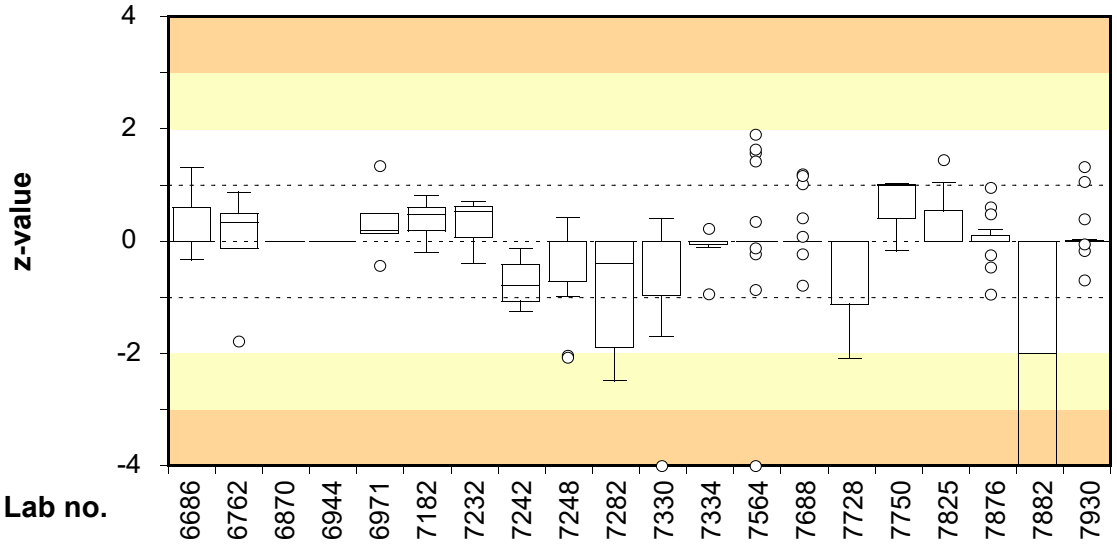
Lab no.	3305	3457	3515	3587	3626	3825	3878	3897	4064	4100	4171	4246	4288	4339	4352	4358	4400	4560	4562	4635
No. of results	21	12	13	6	19	12	5	-	6	12	15	12	12	15	24	9	6	6	30	12
False positive	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	3	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	3
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



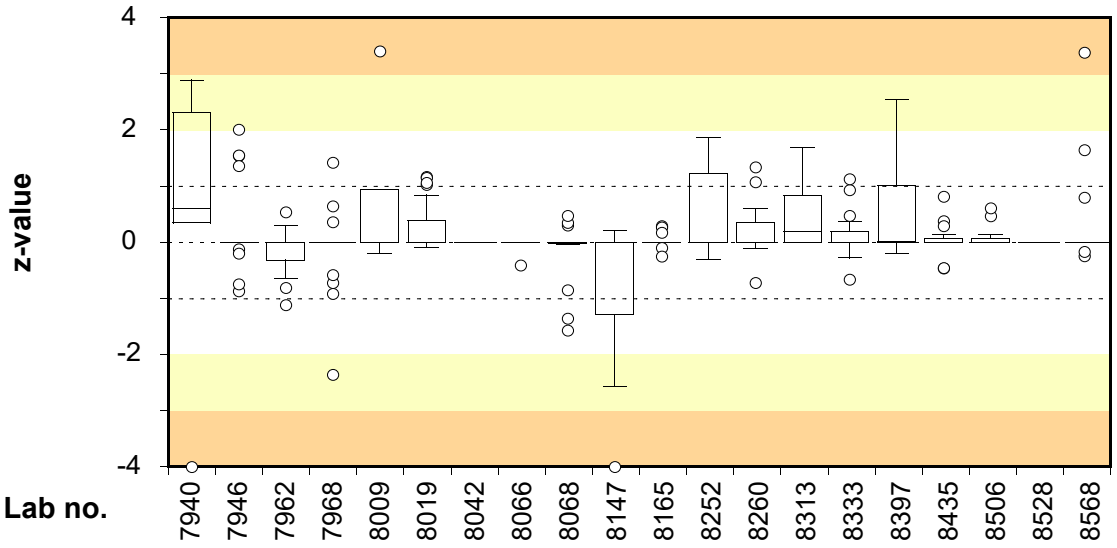
Lab no.	4664	4683	4817	4889	4944	4983	5018	5028	5100	5128	5182	5204	5261	5329	5333	5352	5447	5545	5553	5615
No. of results	18	23	21	18	15	6	23	3	7	12	9	21	7	9	5	15	3	9	21	12
False positive	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-
False negative	-	1	-	-	-	-	1	-	1	-	-	-	1	-	1	-	-	-	-	-
Low outliers	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	1	-	1	-	4	-	-	-	-	-	-	-



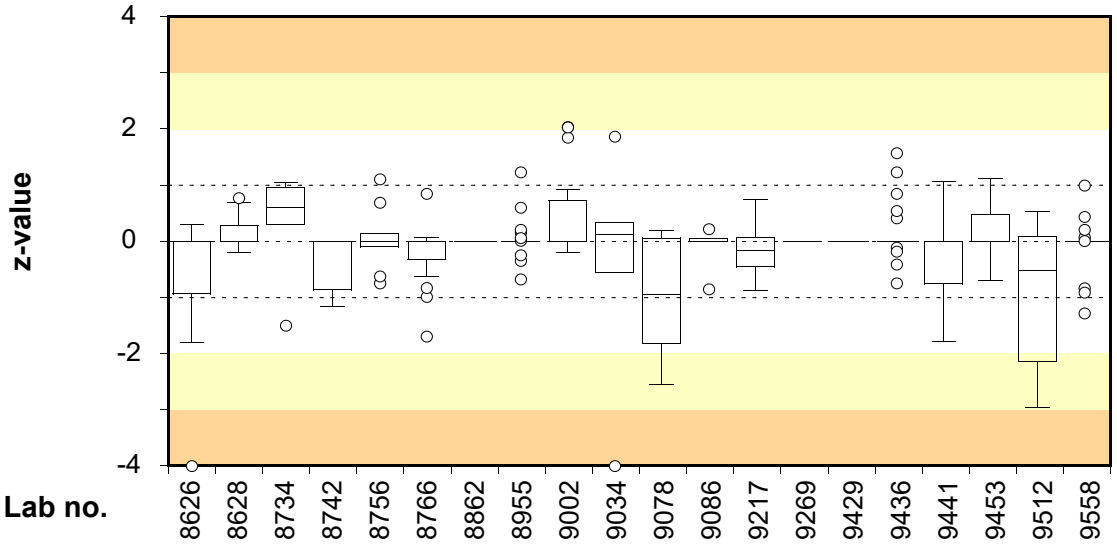
Lab no.	5632	5701	5801	5808	5883	5950	6109	6175	6224	6232	6253	6340	6343	6352	6368	6421	6443	6456	6594	6658
No. of results	10	3	6	9	15	-	8	11	6	-	6	6	6	8	18	18	6	6	-	6
False positive	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



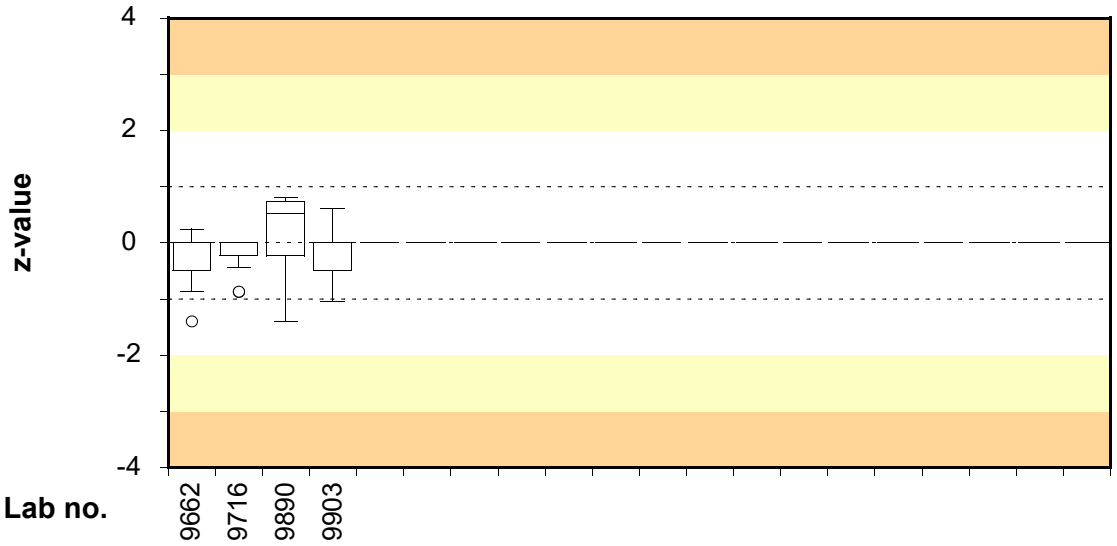
Lab no.	6686	6762	6870	6944	6971	7182	7232	7242	7248	7282	7330	7334	7564	7688	7728	7750	7825	7876	7882	7930
No. of results	13	6	3	3	6	6	3	4	18	9	15	8	21	23	15	3	9	15	12	15
False positive	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
False negative	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	6	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Lab no.	7940	7946	7962	7968	8009	8019	8042	8066	8068	8147	8165	8252	8260	8313	8333	8397	8435	8506	8528	8568
No. of results	6	25	15	20	6	24	3	9	15	15	20	14	15	12	15	12	15	12	2	14
False positive	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	2	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	1
Low outliers	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Lab no.	8626	8628	8734	8742	8756	8766	8862	8955	9002	9034	9078	9086	9217	9269	9429	9436	9441	9453	9512	9558
No. of results	15	18	6	9	10	15	3	28	16	6	6	6	7	3	6	24	15	12	6	28
False positive	-	-	-	-	1	-	-	1	1	-	-	-	2	-	-	-	-	-	-	-
False negative	-	-	-	-	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-	2
Low outliers	2	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



No. of results	15	12	5	12
False positive	-	-	-	-
False negative	-	-	1	-
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	SLV-no.[2]	Origin	Reference[3]
A	*Campylobacter coli*	SLV-271	faeces, hen	CCUG 45147
	Citrobacter freundii	SLV-091	-	CCUG 43597
	Escherichia coli O157	SLV-479	-	SMI 811 86
	Listeria monocytogenes	SLV-513	milk	CCUG 44510
B	*Escherichia coli*	SLV-558	-	-
	Salmonella Stockholm	SLV-390	chocolate powder	-
	Staphylococcus aureus	SLV-280	egg	-
	Vibrio cholerae	SLV-507	-	CCUG 34649
	Yersinia enterocolitica	SLV-408	dog food	CCUG 45643
C	*Campylobacter jejuni*	SLV-540	chicken	-
	Proteus mirabilis	SLV-374	-	CCUG 43605
	Salmonella Enteritidis	SLV-436	-	-
	Vibrio parahaemolyticus	SLV-529	-	CCUG 38981

[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] 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 ml^{-1}.*

Analysis and method	A[1]			B[2]			C[1]		
	m	**I_2**	**T**	**m**	**I_2**	**T**	**m**	**I_2**	**T**
Aerobic microorganisms 30 ˚C NMKL-method no. 86:2013	3,93	1,06	1,24	4,65	1,49	1,40	4,31	0,45	1,33
Enterobacteriaceae NMKL-method no. 144:2005	3,60	0,49	1,26	4,10	**6,75**	1,93	4,20	0,49	1,43
Thermotolerant *Campylobacter,* quant. NMKL-method no. 119:2007	3,16	**8,14**	1,93	-	-	-	2,61	**3,47**	1,72
Thermotolerant *Campylobacter,* qual. NMKL-method no. 119:2007	Pos.	-	-	Neg.	-	-	Pos.	-	-
Listeria monocytogenes, quant. NMKL-method no. 136:2010	2,48	1,41	1,54	-	-	-	-	-	-
Listeria monocytogenes, qual. NMKL-method no. 136:2010	Pos.	-	-	Neg.	-	-	Neg.	-	-
Salmonella NMKL-method no. 71:1999	Neg.	-	-	2,21*	**2,12***	1,59*	2,02*	0,16*	1,17*
Escherichia coli O157 NMKL-method no. 164:2019	1,60*	0,21*	1,14*	Neg.	-	-	Neg.	-	-
Pathogenic *Vibrio* spp. NMKL-method no. 156:1997	Neg.	-	-	2,16*	1,90*	1,58*	2,83*	1,55*	1,91*
Yersinia enterocolitica NMKL-method no. 117:1996	Neg.	-	-	2,59*	0,78*	1,30*	Neg.	-	-

– No target organism and therefore no value
[1] n = 5 vials analysed in duplicate
[2] n = 10 vials analysed in duplicate
* From analysis of a parallel sample mixture

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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