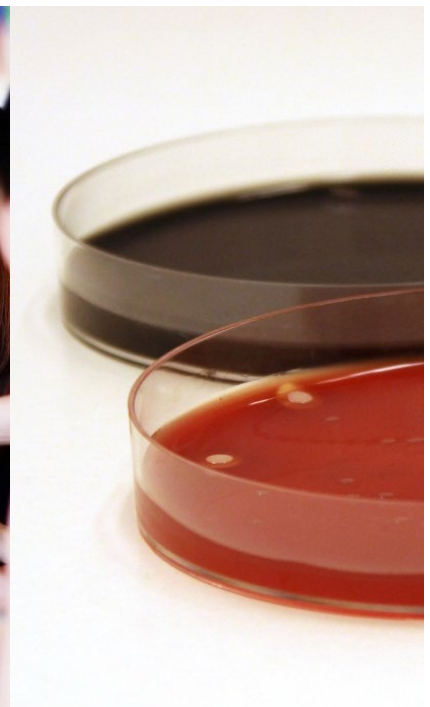


Food Microbiology

October 2020

Jonas Ilbäck



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Proficiency Testing
Microbiology – Food
October 2020

Quantitative analyses

- Aerobic microorganisms, 30 °C
- Aerobic microorganisms, 20 °C
- Contaminating microorganisms in dairy products
- Enterobacteriaceae
- Coliform bacteria, 30 °C
- Coliform bacteria, 37 °C
- Thermotolerant coliform bacteria
- *Escherichia coli*
- Presumptive *Bacillus cereus*
- Coagulase-positive staphylococci
- Enterococci

Qualitative analyses

- Gram-negative bacteria in pasteurised milk and cream

Abbreviations

Media

BA	Blood agar
BEA	Bile esculin agar
BcsA	<i>Bacillus cereus</i> selective agar
BGLB	Brilliant green lactose bile broth
BHI	Brain heart infusion broth
BP	Baird-Parker agar
CBC	Oxoid Brilliance™ <i>Bacillus cereus</i> agar
Compact Dry ETC	Compact Dry™ <i>Enterococcus</i>
COMPASS	COMPASS® <i>Enterococcus</i> agar
EC	<i>E. coli</i> broth
ENT	Slanetz & Bartley <i>Enterococcus</i> agar
IA	Iron agar
KEAA	Kanamycin esculin azide agar
LSB	Lauryl sulphate broth
LTLNB	Lactose tryptone lauryl sulphate broth
MPCA	Milk plate count agar
MYP	Mannitol egg yolk polymyxin agar
PCA	Plate count agar
PEMBA	Polymyxin pyruvate egg yolk mannitol bromothymol blue agar
Petrifilm AC	3M™ Petrifilm™ Aerobic Count
Petrifilm EB	3M™ Petrifilm™ Enterobacteriaceae
Petrifilm EC/CC	3M™ Petrifilm™ <i>E. coli</i> /Coliform Count
Petrifilm RCC	3M™ Petrifilm™ Rapid Coliform Count
Petrifilm REC	3M™ Petrifilm™ Rapid <i>E. coli</i> /Coliform Count
Petrifilm SEC	3M™ Petrifilm™ Select <i>E. coli</i>
Petrifilm Staph	3M™ Petrifilm™ Staph Express
Petrifilm Disk	3M™ Petrifilm™ Staph Express Disk
RPFA	Rabbit plasma fibrinogen agar
SFA	Sugar-free agar
TBX	Tryptone bile X-glucuronide agar
TEMPO AC	TEMPO® Aerobic Count
TEMPO BC	TEMPO® <i>Bacillus cereus</i>
TEMPO CC	TEMPO® Coliforms Count
TEMPO EB	TEMPO® Enterobacteriaceae
TEMPO EC	TEMPO® <i>E. coli</i>
TEMPO STA	TEMPO® Coagulase-positive staphylococci
TGE	Tryptone glucose extract agar
TSA	Tryptone soya agar
VRB	Violet red bile 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	Livsmedelsverket/Swedish Food Agency, Sweden

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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 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

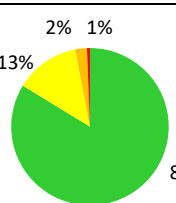
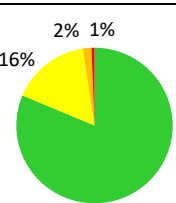
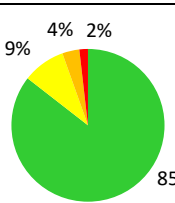
Results of the PT round October 2020

General outcome

Samples were sent to 174 laboratories, 45 in Sweden, 114 in other European countries, and 15 outside of Europe. Of the 166 laboratories that reported results, 60 (34 %) provided at least one result that received an annotation. In the previous round with similar analyses (October 2019) the proportion was 48 %.

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												
Microorganisms	<i>Enterococcus hirae</i> <i>Klebsiella pneumoniae</i> <i>Kocuria rhizophila</i>				<i>Bacillus cereus</i> <i>Enterococcus faecium</i> <i>Staphylococcus xylosum</i>				<i>Escherichia coli</i> <i>Serratia marcescens</i> <i>Staphylococcus aureus</i>			
Analysis	Target	N	F	X	Target	N	F	X	Target	N	F	X
Aerobic microorganisms, 30 °C	All	152	0%	4%	All	152	0%	4%	All	152	0%	5%
Aerobic microorganisms, 20 °C	All	32	0%	6%	All	32	0%	0%	All	33	0%	3%
Contaminating microorganisms	All	13	0%	0%	All	13	0%	0%	All	13	0%	0%
Enterobacteriaceae	<i>K. pneumoniae</i>	142	1%	2%	-	141	3%	0%	<i>E. coli</i> <i>S.marcescens</i>	141	1%	4%
Coliform bacteria, 30 °C	<i>K. pneumoniae</i>	46	0%	2%	-	46	0%	0%	<i>E. coli</i> (<i>S. marcescens</i>)	45	0%	2%
Coliform bacteria, 37 °C	<i>K. pneumoniae</i>	87	0%	3%	-	86	1%	0%	<i>E. coli</i> (<i>S. marcescens</i>)	86	1%	2%
Thermotolerant coliform bacteria	<i>K. pneumoniae</i>	43	2%	2%	-	45	0%	0%	<i>E. coli</i>	45	0%	2%
<i>Escherichia coli</i>	(<i>K. pneumoniae</i>)	112	6%	0%	-	112	0%	0%	<i>E. coli</i>	107	0%	2%
Presumptive <i>B. cereus</i>	-	106	1%	0%	<i>B. cereus</i> (<i>S. xylosum</i>)	105	1%	2%	(<i>S. marcescens</i>) (<i>S. aureus</i>)	106	2%	0%
Coagulase-positive staphylococci	-	95	1%	0%	(<i>S. xylosum</i>)	94	15%	0%	<i>S. aureus</i>	95	1%	6%
Enterococci	<i>E. hirae</i>	62	2%	6%	<i>E. faecium</i>	63	0%	11%	-	63	0%	0%
Gram-negative bacteria in milk products	<i>K. pneumoniae</i>	11	-	-	-	10	-	-	<i>E. coli</i> <i>S.marcescens</i>	11	-	-

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

Aerobic microorganisms, 30 °C and 20 °C

Sample A

All strains in the sample were target organisms. The strain of *K. rhizophila* was present in somewhat higher concentration than *K. pneumoniae* and *E. hirae*.

Sample B

All strains in the sample were target organisms. The strain of *S. xylosus* was present in higher concentration than *B. cereus* and *E. faecium*.

Sample C

All strains in the sample were target organisms. The strain of *S. marcescens* was present in somewhat lower concentration than *S. aureus* and *E. coli*.

General remarks

As in previous proficiency testing rounds most laboratories used NMKL 86 (different versions), ISO 4833 (different versions) or 3M Petrifilm. Both NMKL 86 and ISO 4833 are based on incubation on PCA or MPCA at 30 °C for 72 h. Users of Petrifilm AC can 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.

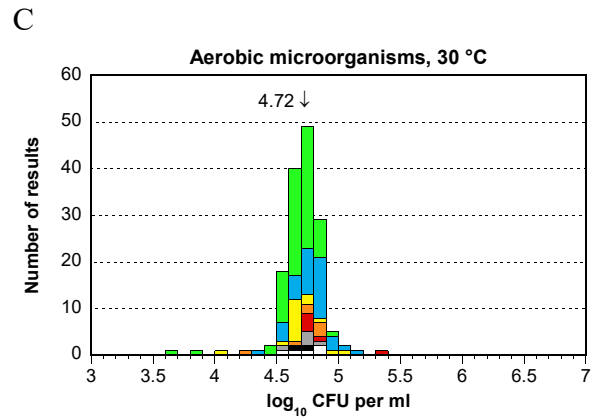
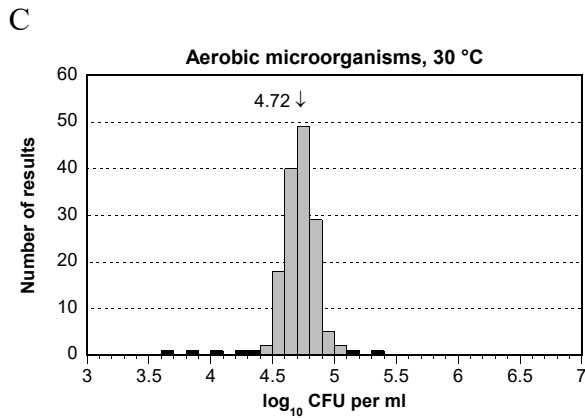
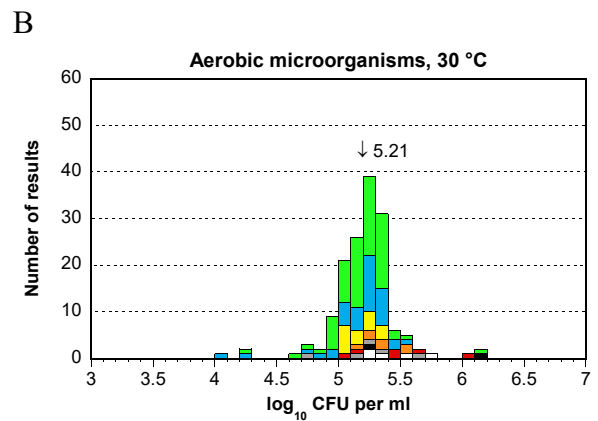
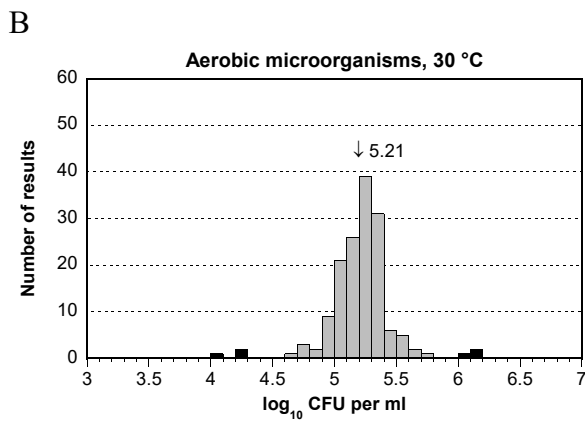
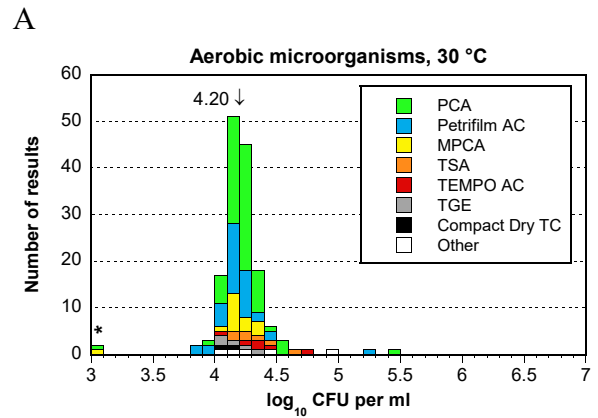
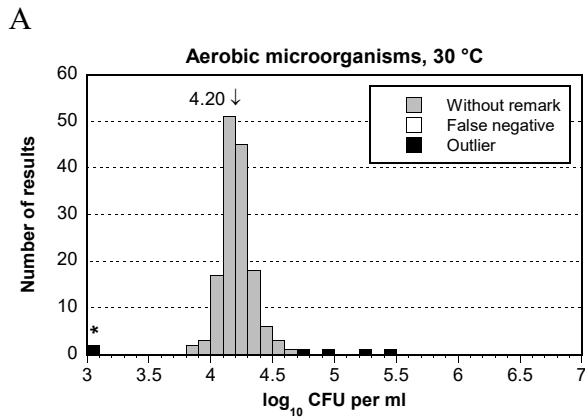
The majority of the laboratories incubated on PCA or Petrifilm AC. Incubation on MPCA was mainly done by laboratories within the dairy industry. Incubation on TSA was mainly done by users of a company-specific method. At 20 °C, incubation on IA was done by laboratories that followed NMKL 184. This method is adapted for aerobic microorganisms and specific spoilage microorganisms in fish and fish products.

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.

Comment: For the analysis at 30 °C one laboratory stated following ISO 4832:2006 (coliform bacteria), and another stated ISO 13559/IDF 153 (contaminating microorganisms). However both of these laboratories used media suited for the analysis of aerobic microorganisms.

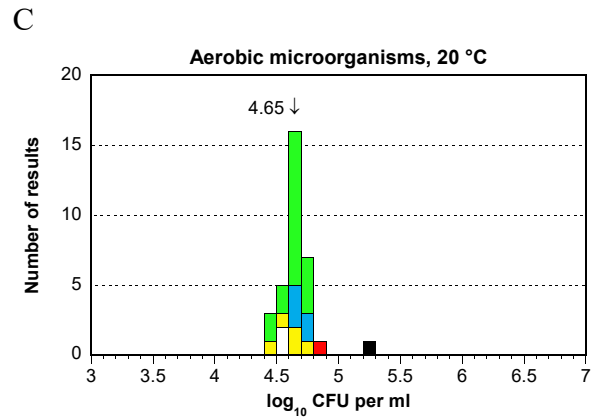
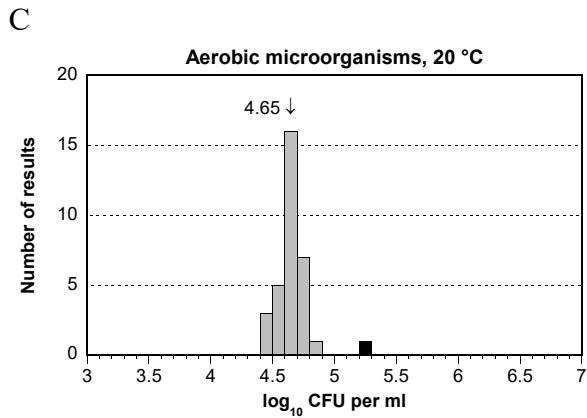
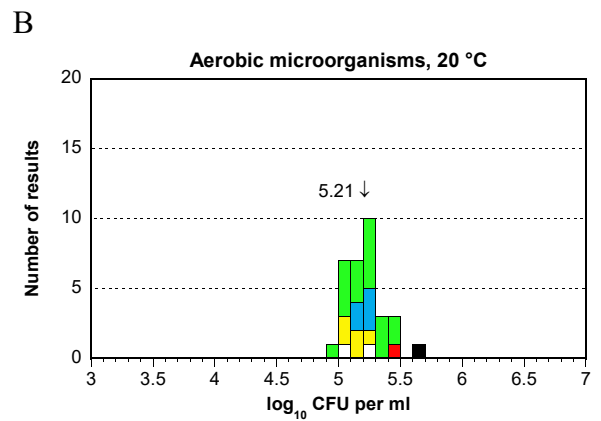
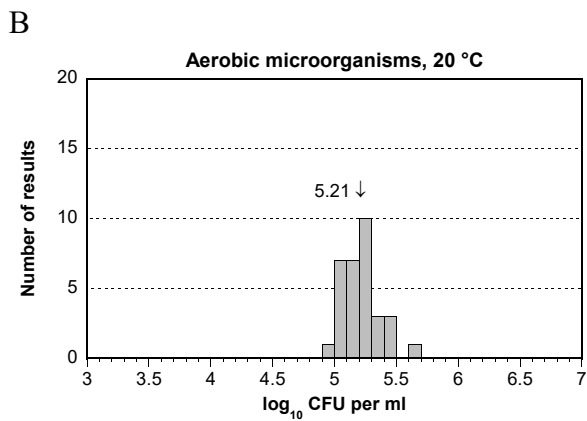
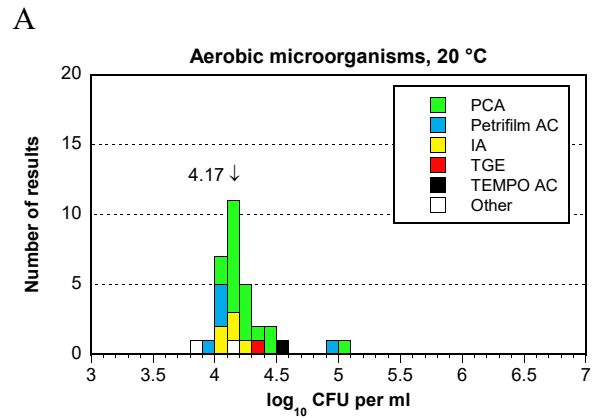
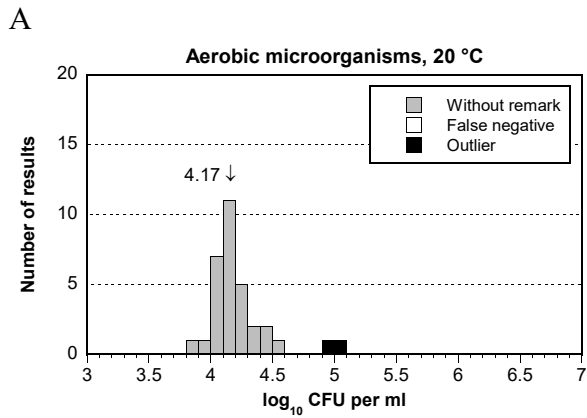
Results from analysis of aerobic microorganisms, 30 °C

Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	152	146	4.20	0.13	0	2	4	152	146	5.21	0.18	0	3	3	152	145	4.72	0.11	0	5	2
PCA	72	70	4.21	0.11	0	1	1	72	70	5.18	0.17	0	1	1	73	71	4.69	0.09	0	2	0
Petrifilm AC	39	38	4.15	0.14	0	0	1	39	37	5.20	0.17	0	2	0	38	36	4.77	0.12	0	1	1
MPCA	16	15	4.19	0.08	0	1	0	16	16	5.17	0.12	0	0	0	16	15	4.73	0.14	0	1	0
TSA	7	7	4.30	0.17	0	0	0	7	7	5.35	0.16	0	0	0	7	6	4.77	0.09	0	1	0
TEMPO AC	7	6	4.32	0.14	0	0	1	7	6	5.40	0.24	0	0	1	7	6	4.78	0.07	0	0	1
TGE	5	5	4.17	0.10	0	0	0	5	5	5.25	0.33	0	0	0	5	5	4.71	0.12	0	0	0
Compact Dry TC	2	2	-	-	0	0	0	2	1	-	-	0	0	1	2	2	-	-	0	0	0
Other	4	3	-	-	0	0	1	4	4	-	-	0	0	0	4	4	-	-	0	0	0



Results from analysis of aerobic microorganisms, 20 °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	32	30	4.17	0.16	0	0 2	32	32	5.21	0.15	0	0 0	33	32	4.65	0.09	0	0 1
PCA	18	17	4.21	0.13	0	0 1	18	18	5.20	0.14	0	0 0	19	19	4.65	0.08	0	0 0
Petrifilm AC	5	4	4.03	0.06	0	0 1	5	5	5.22	0.05	0	0 0	5	5	4.68	0.07	0	0 0
IA	5	5	4.12	0.08	0	0 0	5	5	5.13	0.10	0	0 0	5	5	4.63	0.11	0	0 0
TGE	1	1	-	-	0	0 0	1	1	-	-	0	0 0	1	1	-	-	0	0 0
TEMPO AC	1	1	-	-	0	0 0	1	1	-	-	0	0 0	1	0	-	-	0	0 1
Other	2	2	-	-	0	0 0	2	2	-	-	0	0 0	2	2	-	-	0	0 0



Contaminating microorganisms in dairy products

Sample A

All strains in the sample can form colonies on SFA. The strain of *K. rhizophila* was present in somewhat higher concentration than *K. pneumoniae* and *E. hirae*. *E. hirae* is catalase-negative and may therefore have been excluded after confirmation.

Sample B

In the quality control at the Swedish Food Agency, all strains in the sample formed colonies on SFA. The strain of *S. xylosum* was present in a higher concentration than *B. cereus* and *E. faecium*. *E. faecium* is catalase-negative and may therefore have been excluded after confirmation.

Sample C

In the quality control at the Swedish Food Agency, all strains in the sample formed colonies on SFA. The strain of *S. marcescens* was present in somewhat lower concentration than *S. aureus* and *E. coli*.

General remarks

Only 13 laboratories performed the analysis and the results were therefore difficult to evaluate statistically. Outliers have therefore been determined manually. When determining outliers, consideration was taken to the species and concentration of target organisms (Table 3), the mean value of all laboratories, and the distribution of results that is normally seen in this analysis. Based on this, no results were considered as outliers.

Eight of the 13 laboratories followed ISO 13559:2002 / IDF 153:2002. This was last reviewed by ISO in 2019 and remains current. One laboratory followed a modified version of the older IDF 153:1999. Other laboratories either followed internal methods, or did not specify further which method they used. All laboratories except one used the medium SFA.

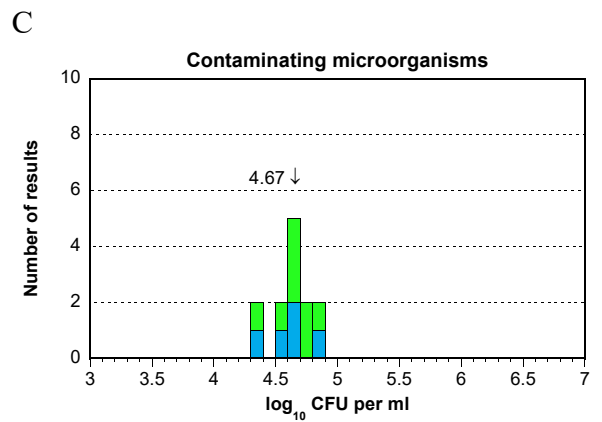
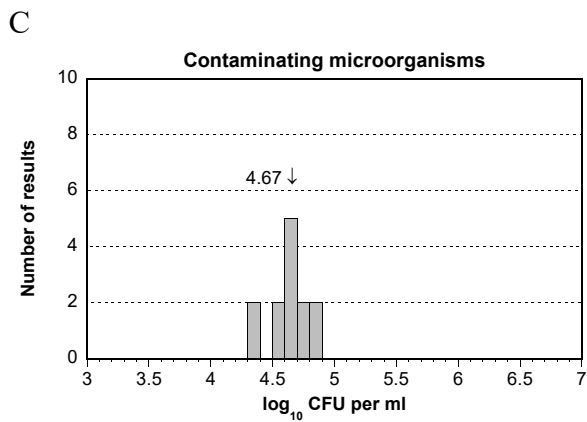
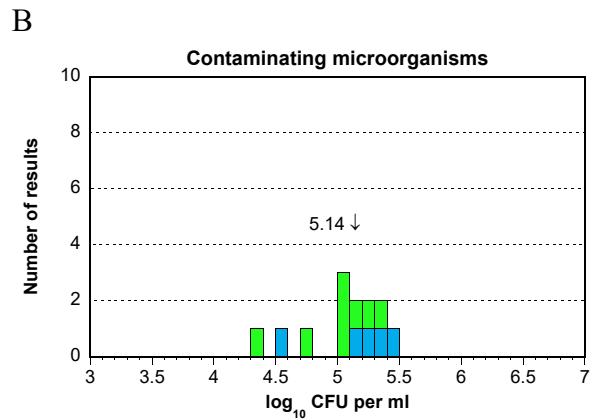
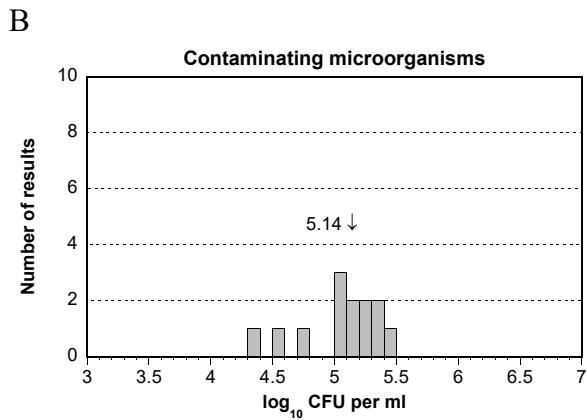
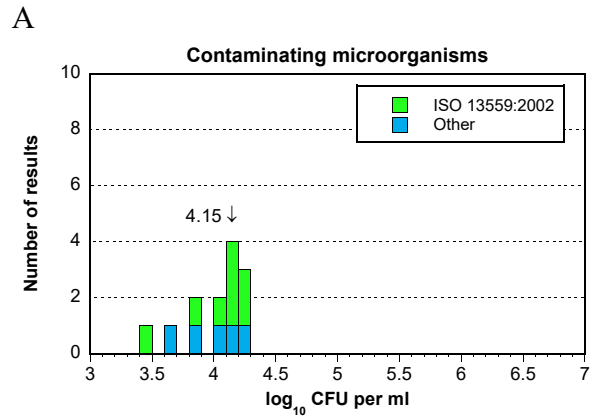
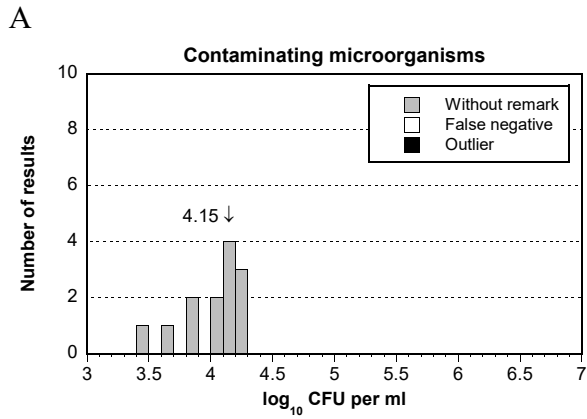
The goal of the analysis is to identify potential contaminating microorganisms in dairy products. For these products, lactic acid bacteria are generally not considered as contaminating microorganisms. Lactic acid bacteria are catalase-negative and some laboratories therefore use confirmation with a catalase test. Such a test is however not necessary with ISO 13559:2002 / IDF 153:2002, and the method only specifies the enumeration of "characteristic contaminating microorganisms". In total, five of the 13 laboratories stated performing a confirmation with a catalase test.

Results from analysis of contaminating microorganisms

Method	Sample A						Sample B						Sample C					
	N	n	Med*	s	F	< >	N	n	Med*	s	F	< >	N	n	Med*	s	F	< >
All results	13	13	4.15	0.26	0	0 0	13	13	5.14	0.33	0	0 0	13	13	4.67	0.16	0	0 0
ISO 13559:2002 / IDF 153:2002**	8	8	4.16	0.29	0	0 0	8	8	5.07	0.33	0	0 0	8	8	4.68	0.15	0	0 0
Other	5	5	4.08	0.25	0	0 0	5	5	5.21	0.35	0	0 0	5	5	4.65	0.19	0	0 0

* Med = median

** One of these laboratories stated "IDF 153:1991, modified"



Enterobacteriaceae

Sample A

The strain of *K. pneumoniae* was the target organism. It is oxidase-negative upon confirmation.

All of the deviating results were reported by laboratories that incubated on VRBG.

Sample B

No target organism was present in the sample.

Three of the four false positive results were reported by laboratories that used Petrifilm EB.

Sample C

The strains of *E. coli* and *S. marcescens* were the target organisms. In the Swedish Food Agency's quality control they both formed colonies on VRBG. The bile salt precipitation zone was less prominent for the colonies of *S. marcescens* compared to the colonies of *E. coli*. Both strains were oxidase-negative upon confirmation.

The majority of the deviating results were reported by laboratories that incubated on VRBG.

General remarks

Enterobacteriaceae are Gram-negative and oxidase-negative bacteria that ferment glucose with the production of acid by-products. On VRBG they therefore form pink/red colonies, with or without a bile salt precipitation zone. The appearance is similar on Petrifilm EB, which also includes a colour indicator for 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 (43 %) or a method with Petrifilm EB (28 %), while the ISO methods (various versions) were used by 20 %. 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 level of Enterobacteriaceae is lower than 100 cfu g⁻¹.

The number of users of ISO 21528-2:2017 was somewhat higher compared to ISO 21528-2:2004 (9 % and 6 %, respectively). In comparison, four laboratories (3 %) stated the older ISO 21528-1:2004, while three (2 %) stated the new ISO 21528-1:2017.

NMKL 144:2005 stipulates confirmation of presumptive colonies with an oxidase test. ISO 21528-2:2017 stipulates confirmation of presumptive colonies with both an oxidase test and with a test for glucose fermentation. The majority of the laboratories that stated they performed a confirmation test specified that this consisted of an oxidase test.

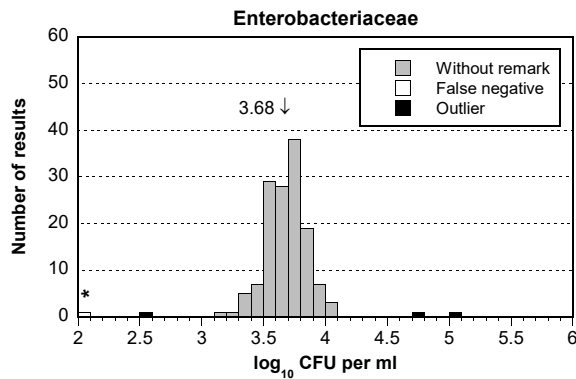
With the exception of what is mentioned above, the results for the different methods and media that were used were similar. In total, only a small number of outliers and false results were reported. Even though the majority of the deviating results could be attributed to VRBG and Petrifilm EB, these were at the same time the most used media by the participants. Somewhat higher results have often been noted for TEMPO EB in previous proficiency testing rounds, but such a trend was not apparent this time.

Results from analysis of Enterobacteriaceae

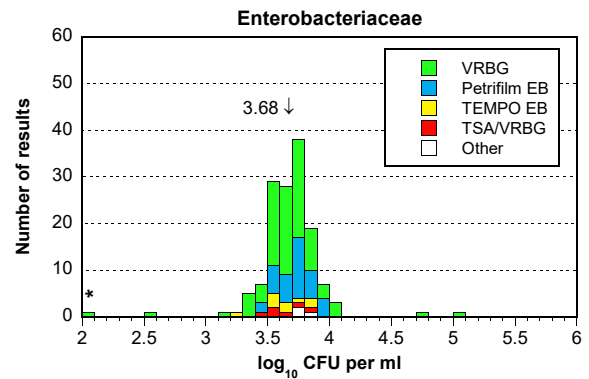
Medium	Sample A					Sample B					Sample C				
	N	n	m	s	F < >	N	n	m	s	F < >	N	n	m	s	F < >
All results	142	138	3.68	0.15	1 1 2	141	137	-	-	4 - -	141	134	4.17	0.15	1 4 2
VRBG	87	83	3.66	0.16	1 1 2	87	86	-	-	1 - -	86	81	4.16	0.16	0 4 1
Petrifilm EB	37	37	3.71	0.13	0 0 0	36	33	-	-	3 - -	37	36	4.17	0.14	0 0 1
TEMPO EB	9	9	3.63	0.17	0 0 0	9	9	-	-	0 - -	9	9	4.28	0.20	0 0 0
TSA/VRBG	6	6	3.63	0.17	0 0 0	6	6	-	-	0 - -	6	5	4.13	0.12	1 0 0
Other*	3	3	-	-	0 0 0	3	3	-	-	0 - -	3	3	-	-	0 0 0

* Includes Compact Dry ETB.

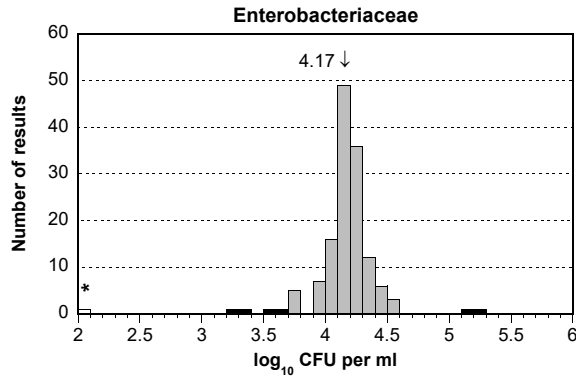
A



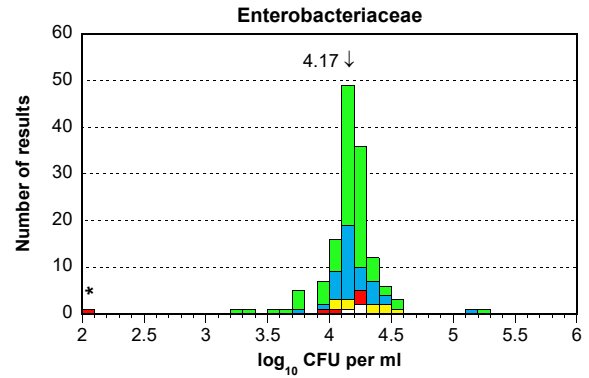
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C



C



Coliform bacteria, 30 °C and 37 °C

Sample A

The strain of *K. pneumoniae* was the target organism at both temperatures. On VRB, it forms typical red colonies with a bile salt precipitation zone.

Sample B

No target organism was present in the sample.

Sample C

The strain of *E. coli* was the target organism. On VRB, it forms typical red colonies with a bile salt precipitation zone. In the Swedish Food Agency's quality control the strain of *S. marcescens* also formed colonies on VRB. Both strains are oxidase-negative, but *S. marcescens* can be excluded after confirmation since in contrast to *E. coli*, it does not produce gas in BGLB.

General remarks

Coliform bacteria are Gram-negative rods that ferment lactose with the production of gas and acid by-products. On VRB they form characteristic red colonies due to uptake of crystal violet and neutral red from the medium. The colonies are normally surrounded by a red/pink precipitation zone, which is formed due to the precipitation of bile salts when the pH decreases. Petrifilm CC and Petrifilm EC/CC are based on VRB, but also have a plastic film that facilitates detection of gas production.

The most common methods were NMKL 44:2004 and ISO 4832:2006. At 37 °C, 3M™ Petrifilm™ was also used by many laboratories. Both NMKL 44:2004 and ISO 4832:2006 prescribe incubation on VRB, but the confirmation steps differ somewhat. With NMKL 44:2004 all presumptive colonies are confirmed with BGLB. In contrast, with ISO 4832:2006 only atypical colonies require further confirmation. A few laboratories also performed a pre-incubation on TSA, which is recommended by some methods if the sample is suspected to contain stressed coliform bacteria.

LSB in combination with BGLB was used by laboratories that followed the MPN-based methods ISO 4831 and NMKL 96 (various editions). ISO 4831:2006 is adapted for use when the expected concentration of coliform bacteria is lower than or equal to 100 cfu g⁻¹. NMKL 96 is adapted for the analysis of coliform bacteria in fish and seafood. It is recommended when the expected concentration of microorganisms is lower than or equal to 300 cfu g⁻¹. In some previous proficiency testing rounds, users of these methods have reported somewhat deviating results, likely since they are not adapted for the concentrations in the PT samples. For the analysis at 37 °C in this proficiency testing round, slightly higher results were reported for these methods for sample C.

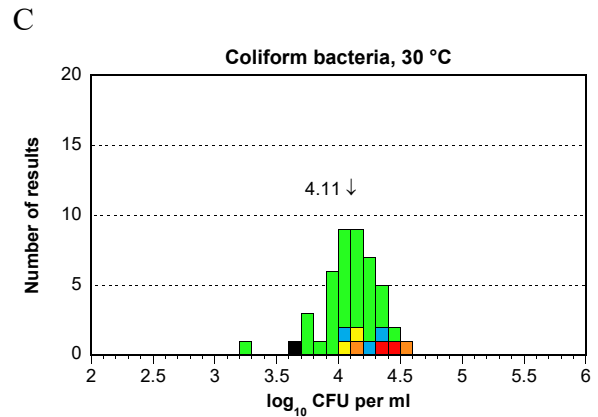
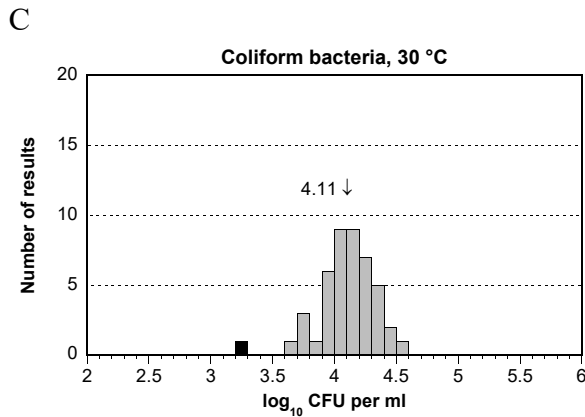
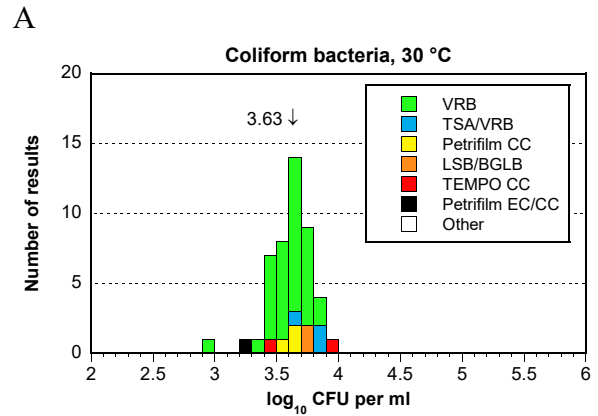
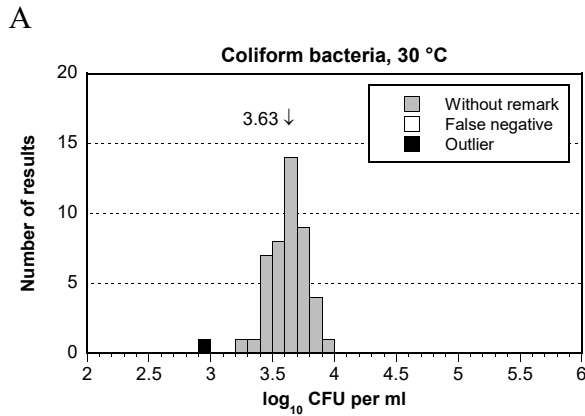
A wider range of media were used at 37 °C, compared to at 30 °C. At 37 °C, three laboratories used RAPID'E. coli 2 agar, which is a chromogenic medium that detects β-galactosidase and β-glucuronidase activity. On this medium, coliform bacteria (Gal+/Gluc-) form blue/green colonies, while *E. coli* (Gal+/Gluc+) form pink/purple colonies. Two laboratories used Compact Dry EC, on which coliform bacteria form red or red/violet colonies, while *E. coli* forms blue colonies.

At 37 °C, confirmation of some kind was performed by 78 % of the laboratories that followed an NMKL or ISO method. At 30 °C, the corresponding number was 84 %.

Confirmation was less often reported by laboratories that used Petrifilm CC and Petrifilm EC/CC, which is reasonable since confirmation is not required with those methods.

Results from analysis of coliform bacteria, 30 °C

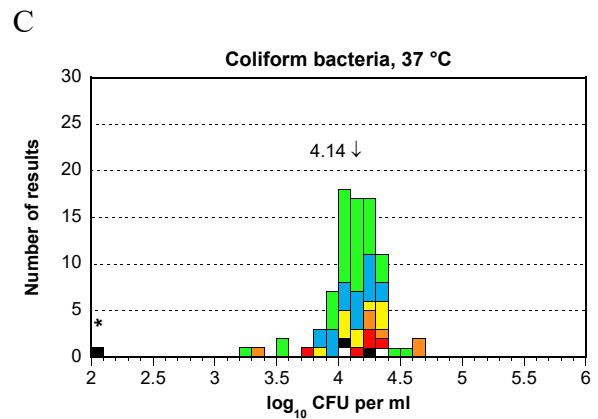
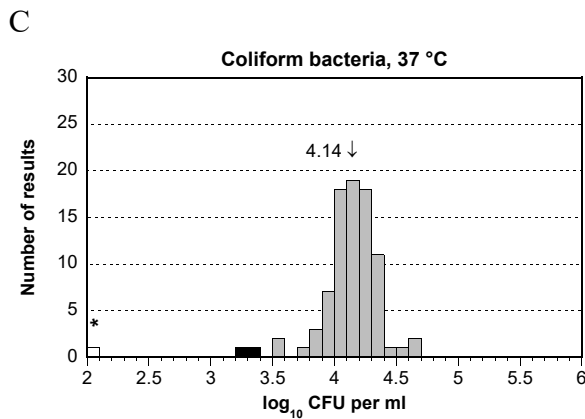
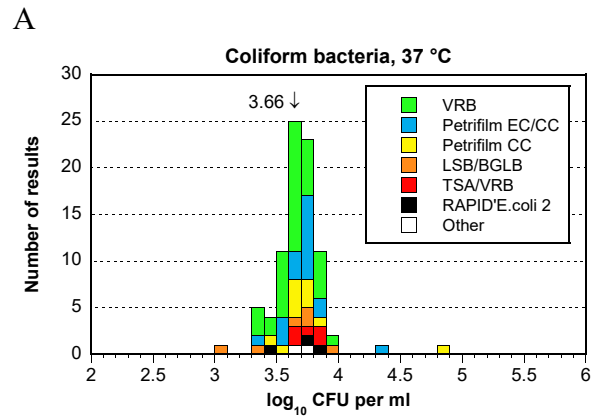
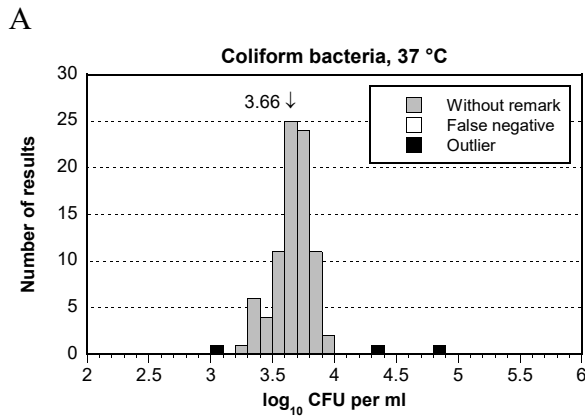
Medium	Sample A						Sample B						Sample C					
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >
All results	46	45	3.63	0.15	0	1 0	46	46	-	-	0	- -	45	44	4.11	0.19	0	1 0
VRB	35	34	3.61	0.13	0	1 0	35	35	-	-	0	- -	35	34	4.08	0.17	0	1 0
TSA/VRB	3	3	-	-	0	0 0	3	3	-	-	0	- -	3	3	-	-	0	0 0
Petrifilm CC	3	3	-	-	0	0 0	3	3	-	-	0	- -	2	2	-	-	0	0 0
LSB/BGLB	2	2	-	-	0	0 0	2	2	-	-	0	- -	2	2	-	-	0	0 0
TEMPO CC	2	2	-	-	0	0 0	2	2	-	-	0	- -	2	2	-	-	0	0 0
Petrifilm EC/CC	1	1	-	-	0	0 0	1	1	-	-	0	- -	1	1	-	-	0	0 0
Other	0	0	-	-	0	0 0	0	0	-	-	0	- -	0	0	-	-	0	0 0



Results from analysis of coliform bacteria, 37 °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	87	84	3.66	0.15	0	1	2	86	85	-	-	1	-	-	86	83	4.14	0.18	1	2	0
VRB	38	38	3.64	0.14	0	0	0	38	38	-	-	0	-	-	38	37	4.11	0.19	0	1	0
Petrifilm EC/CC	19	18	3.68	0.12	0	0	1	19	18	-	-	1	-	-	19	19	4.11	0.14	0	0	0
Petrifilm CC	11	10	3.66	0.11	0	0	1	10	10	-	-	0	-	-	10	10	4.14	0.16	0	0	0
LSB/BGLB	6	5	3.70	0.21	0	1	0	6	6	-	-	0	-	-	6	5	4.43	0.22	0	1	0
TSA/VRB	5	5	3.78	0.11	0	0	0	5	5	-	-	0	-	-	5	5	4.14	0.22	0	0	0
RAPID' E.coli2	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	2	-	-	1	0	0
Compact Dry EC	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	2	-	-	0	0	0
TEMPO CC	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0
Other*	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	2	-	-	0	0	0

* Includes one laboratory that incubated on ECC ChromoSelect Selective Agar. Also includes one laboratory that incubated on Petrifilm EB, followed by confirmation with an oxidase test.



Thermotolerant coliform bacteria and *Escherichia coli*

Sample A

The strain of *K. pneumoniae* was the target organism for the analysis of thermotolerant coliform bacteria, but not for *E. coli*. It produces gas but not indole in LTLSB, and can therefore be excluded in the analysis of *E. coli*.

In the analysis of *E. coli* seven false positive results were reported. They correspond to the concentration of *K. pneumoniae* in the sample (approximately \log_{10} 3.6 cfu ml⁻¹). Five of the seven false positive results were reported by laboratories that followed NMKL 125:2005. This however appears to be a coincidence; in previous proficiency testing rounds when the same sample has been used, three and four false positive results were reported, respectively, and mainly from laboratories using other methods than NMKL 125:2005.

Sample B

No target organism was present in the sample. In the Swedish Food Agency's quality control, no colonies were detected on VRB.

Sample C

The strain of *E. coli* was the target organism for both analyses. On VRB, it forms typical red colonies with a bile salt precipitation zone. In the Swedish Food Agency's quality control, no other colonies were detected on VRB. Upon confirmation, the strain produces both gas and indole in LTLSB. The strain is oxidase-negative, and positive for β -glucuronidase.

General remarks

At the Swedish Food Agency, thermotolerant coliform bacteria are analysed by pre-incubation on TSA, followed by an overlay with VRB and incubation at 44 °C. On VRB, typical thermotolerant coliform bacteria form dark red colonies, which are surrounded by a red zone of precipitation. During subsequent inoculation in either EC or LTLSB they produce gas as a consequence of lactose fermentation. *E. coli* can be distinguished from other thermotolerant coliform bacteria, since they also produce indole in either LTLSB or in tryptone broth.

NMKL 125:2005 was the most commonly used method for the analysis of thermotolerant coliform bacteria (60 % of the laboratories). It is based on TSA/VRB and describes the analysis of both thermotolerant coliform bacteria and of *E. coli*. For the analysis of *E. coli*, most laboratories used methods based on 3M™ Petrifilm™ (either Petrifilm EC/CC or Petrifilm SEC), followed by NMKL 125:2005 and ISO 16649-2:2001. Both Petrifilm EC/CC and Petrifilm SEC include substrates that facilitate detection of β -glucuronidase, and thus *E. coli* form blue-green colonies on these media. The plastic film in Petrifilm EC/CC and Petrifilm SEC also facilitates detection of gas production due to lactose fermentation. ISO 16649-2:2001 is also based on detection of β -glucuronidase activity. The method uses TBX, on which *E. coli* form typical blue colonies. Further confirmation of β -glucuronidase positive colonies is not required according to ISO 16649-2:2001.

It can here be mentioned that NMKL 125 is being revised, and the new version will likely be more similar to ISO 16649-2. ISO 16649-2:2001 was last reviewed by ISO in 2019 and remains current.

Among the less frequently used methods were ISO 7251:2005 and NMKL 96:2009. ISO 7251 is an MPN-based method for the detection of *E. coli*. NMKL 96 is also based on MPN, and is adapted for the analysis of coliform bacteria, thermotolerant coliform bacteria and *E. coli* in fish and seafood. Laboratories that followed these methods normally incubated in LSB/EC.

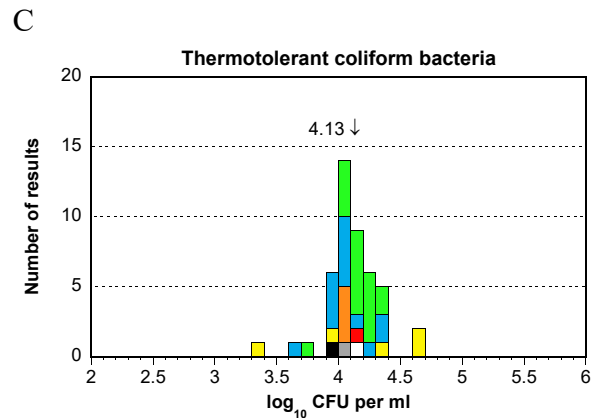
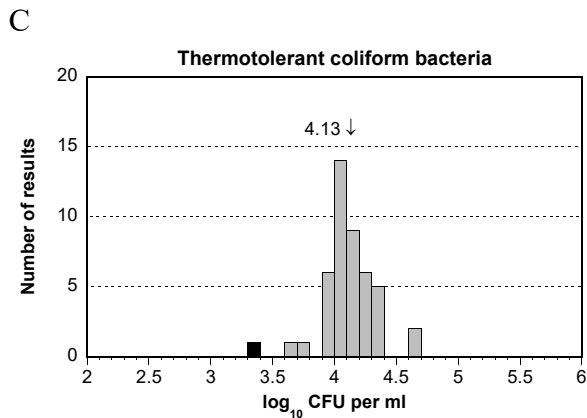
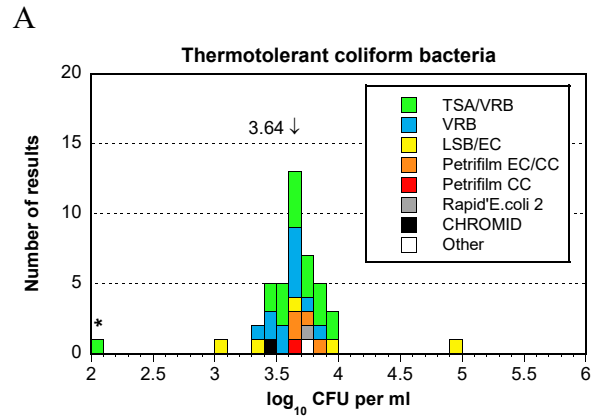
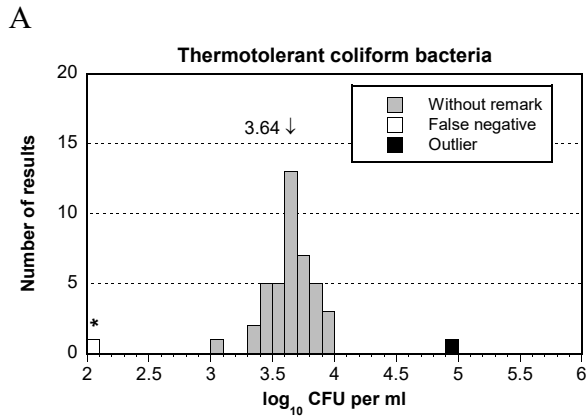
In the analysis of *E. coli*, a few laboratories used TEMPO EC. This was associated with somewhat higher results, compared to other methods/media. This was seen also in PT October 2019, and to a lesser extent in PT April 2020. Somewhat higher results for TEMPO EC can therefore be considered normal. In previous proficiency testing rounds, the results for *E. coli* have occasionally been somewhat lower for TBX, and somewhat higher for TSA/VRB, compared to other media. At those times, the differences have been assumed to be due to performing, or not performing, a pre-incubation at a lower temperature. Here, the mean values for TSA/VRB and TBX did not deviate significantly from other media, and the results were within one standard deviation from the mean value of all results.

In the analysis of *E. coli* incubation was normally done either at 42-44 °C or at 35-37 °C, depending on which method that was used. The mean values of the two temperature groups did however not differ, and the number of outliers and false results were also relatively evenly distributed between the two groups.

In general, confirmation appears to have been performed by the laboratories when required by the method. For example, 84 % of the laboratories that followed NMKL 125:2005 for the analysis of *E. coli* performed a confirmation test, usually a test for the production of gas or indole. Confirmation was less often carried out by laboratories that used Petrifilm or that followed ISO 16649-2:2001, which is reasonable, since these methods do not require a confirmation.

Results from analysis of thermotolerant coliform bacteria

Medium	Sample A								Sample B								Sample C							
	N	n	m	s	F	<	>		N	n	m	s	F	<	>		N	n	m	s	F	<	>	
All results	43	41	3.64	0.18	1	0	1		45	45	-	-	0	-	-		45	44	4.13	0.19	0	1	0	
TSA/VRB	18	17	3.69	0.16	1	0	0		18	18	-	-	0	-	-		18	18	4.14	0.13	0	0	0	
VRB	12	12	3.60	0.12	0	0	0		13	13	-	-	0	-	-		14	14	4.07	0.19	0	0	0	
LSB/EC	5	4	3.51	0.40	0	0	1		6	6	-	-	0	-	-		5	4	4.40	0.35	0	1	0	
Petrifilm EC/CC	4	4	-	-	0	0	0		4	4	-	-	0	-	-		4	4	-	-	0	0	0	
Petrifilm CC	1	1	-	-	0	0	0		1	1	-	-	0	-	-		1	1	-	-	0	0	0	
RAPID'E. coli	1	1	-	-	0	0	0		1	1	-	-	0	-	-		1	1	-	-	0	0	0	
CHROMID®	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	

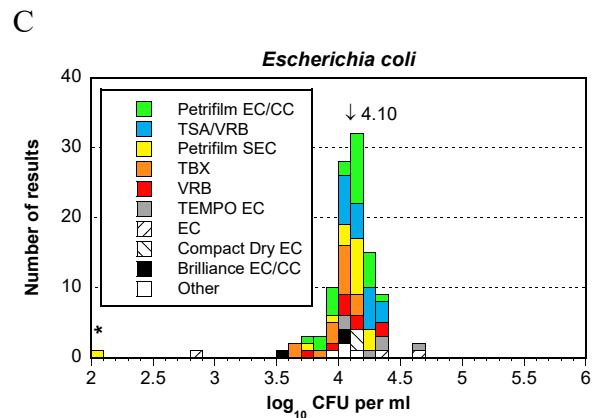
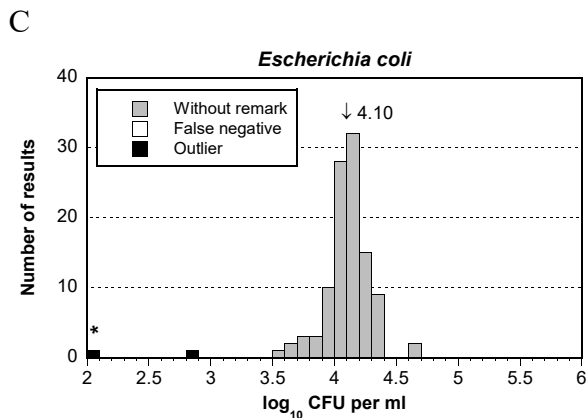


Results from analysis of *Escherichia coli*

Medium	Sample A						Sample B						Sample C						
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >	
All results	112	105	-	-	7	- -	112	112	-	-	0	- -	107	105	4.10	0.17	0	2	0
Petrifilm EC/CC	25	24	-	-	1	- -	25	25	-	-	0	- -	25	25	4.09	0.14	0	0	0
TSA/VRB*	21	18	-	-	3	- -	21	21	-	-	0	- -	21	21	4.16	0.11	0	0	0
Petrifilm SEC	18	18	-	-	0	- -	18	18	-	-	0	- -	17	16	4.09	0.12	0	1	0
TBX	18	17	-	-	1	- -	18	18	-	-	0	- -	16	16	4.00	0.16	0	0	0
VRB	9	7	-	-	2	- -	9	9	-	-	0	- -	9	9	4.11	0.18	0	0	0
TEMPO EC	6	6	-	-	0	- -	6	6	-	-	0	- -	6	6	4.28	0.21	0	0	0
EC	3	3	-	-	0	- -	3	3	-	-	0	- -	3	2	-	-	0	1	0
Compact Dry EC	3	3	-	-	0	- -	3	3	-	-	0	- -	3	3	-	-	0	0	0
Brilliance EC/CC	3	3	-	-	0	- -	3	3	-	-	0	- -	3	3	-	-	0	0	0
Other**	6	6	-	-	0	- -	6	6	-	-	0	- -	4	4	-	-	0	0	0

* Includes three laboratories that used TSA/VRBQ.

** Includes e.g. Rapid'E.coli 2, Petrifilm RCC, Petrifilm REC and Rebecca agar.



Presumptive *Bacillus cereus*

Sample A

No target organism was present in the sample.

Sample B

The strain of *B. cereus* was the target organism. In the Swedish Food Agency's quality control on BA, it formed typical grey colonies with a zone of haemolysis. On BcsA, it formed typical blue colonies surrounded by a blue zone of precipitation. The strain of *S. xylosus* in the sample can also form colonies on BA. It can however be excluded after confirmation on BcsA.

Sample C

No target organism was present in the sample. The strains of *S. marcescens* and *S. aureus* can however form colonies on BA. In the Swedish Food Agency's quality control, only atypical colonies were detected on BA. They could also easily be excluded after confirmation on BcsA.

General remarks

Most laboratories followed either NMKL 67:2010 (56 %) or ISO 7932:2004 (24 %), which differ somewhat. NMKL 67:2010 is based on primary incubation on BA, and colonies are confirmed either on BcsA or on Cereus-Ident agar. In comparison, ISO 7932:2004 prescribes incubation on MYP, which is followed by confirmation of haemolysis on BA. One laboratory stated following the MPN-method ISO 21871:2006, which is adapted for analysis of low number of *B. cereus*.

An amendment was recently published for ISO 7932:2004 (Amd 1:2020). It contains optional tests, including for PCR detection of *cytK* genes. It can also be mentioned that NMKL 67 is currently under revision.

On BA, *B. cereus* forms large, irregular grey colonies, surrounded by a distinct zone of haemolysis. On BcsA, presumptive *B. cereus* form bluish colonies that are surrounded by a blue zone of precipitation, due to lecithinase activity on egg yolk present in the medium. On Cereus-Ident agar, presumptive *B. cereus* are blue/turquoise and possibly surrounded by a blue ring. The colour is a result of *B. cereus* phosphatidylinositol

phospholipase C (PI-PLC) cleavage of the chromogenic substrate X-myoinositol-1-phosphate. On MYP, presumptive *B. cereus* form large pink colonies that are normally surrounded by a zone of precipitation, again as a consequence of lecithinase activity.

In addition to BA, BcsA and MYP, the chromogenic medium CBC was used by five laboratories. Cleavage of the substrate X-Gluc present in CBC by *B. cereus* β -glucuronidase results in white colonies with a blue/green centre. Other media that were used to a lesser extent were Compact Dry X-BC, TEMPO BC, COMPASS® *Bacillus cereus* agar and BACARA™.

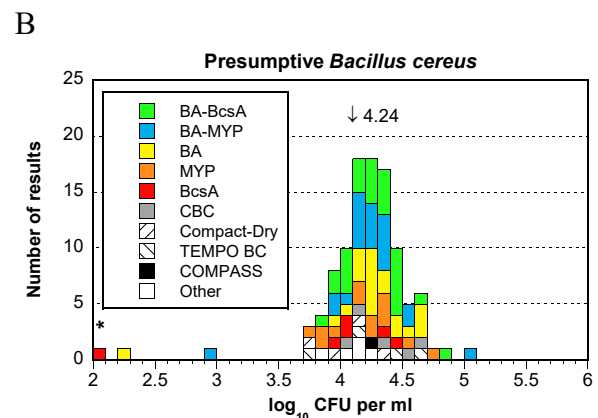
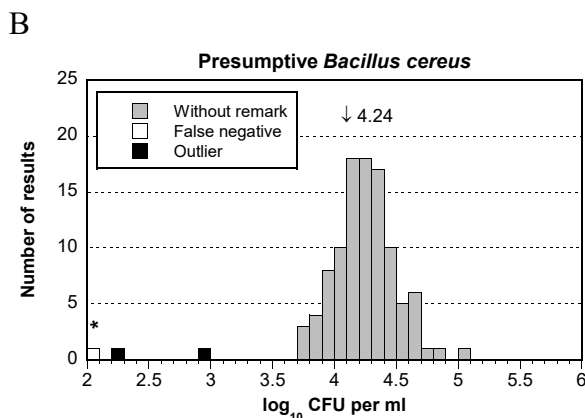
As in previous proficiency testing rounds the reporting of method data for *B. cereus* was in several cases ambiguous, or difficult to interpret. For example, several laboratories reported combinations of method and media that are incompatible. Despite these uncertainties, the results and mean values for the different methods and media were very similar.

Results from analysis of presumptive Bacillus cereus

Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	106	105	-	-	1	-	-	105	102	4.24	0.24	1	2	0	106	104	-	-	2	-	-
BA-BcsA	26	26	-	-	0	-	-	26	26	4.27	0.22	0	0	0	26	26	-	-	0	-	-
BA-MYP	21	21	-	-	0	-	-	21	20	4.27	0.25	0	1	0	21	20	-	-	1	-	-
BA	20	20	-	-	0	-	-	20	19	4.30	0.21	0	1	0	20	19	-	-	1	-	-
MYP	14	14	-	-	0	-	-	13	13	4.18	0.29	0	0	0	14	14	-	-	0	-	-
BcsA*	6	5	-	-	1	-	-	6	5	4.15	0.19	1	0	0	6	6	-	-	0	-	-
CBC	5	5	-	-	0	-	-	5	5	4.34	0.24	0	0	0	5	5	-	-	0	-	-
Compact Dry X-BC	4	4	-	-	0	-	-	4	4	-	-	0	0	0	4	4	-	-	0	-	-
TEMPO BC	4	4	-	-	0	-	-	4	4	-	-	0	0	0	4	4	-	-	0	-	-
COMPASS <i>B. cereus</i>	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	1	-	-	0	-	-
Other**	5	5	-	-	0	-	-	5	5	4.08	0.16	0	0	0	5	5	-	-	0	-	-

* Includes laboratories that stated PEMBA.

** Includes e.g. TSA/BA and BACARA™.



Coagulase-positive staphylococci

Sample A

No target organism was present in the sample.

Sample B

No target organism was present in the sample. The coagulase-negative strain of *S. xylosus* was however false-positive for the analysis. In the Swedish Food Agency's quality control on RPFA, it formed atypical blue-grey colonies without a coagulase zone. The strain may also form grey colonies on BP.

In total, 14 false positive results were reported. Users of Petrifilm Staph appeared to be somewhat overrepresented among the false positive results, compared to laboratories that incubated on RPFA or BP. In previous proficiency testing rounds when the same strain has been included, false positive results have mainly been reported by users of BP and Petrifilm Staph. All except one of the laboratories that reported a false positive result had performed some kind of confirmation, which is more frequent compared to all laboratories (93 % and 70 %, respectively).

Sample C

The strain of *S. aureus* was the target organism. In the Swedish Food Agency's quality control, it formed typical colonies on RPFA. The surrounding coagulase zone was less prominent after 24 hours incubation, compared to after 48 hours incubation.

General remarks

Most laboratories (42 %) followed NMKL 66:2009. Other major methods were 3M™ Petrifilm™ (19 %), ISO 6888-1:1999 (14 %) and ISO 6888-2:1999 (12 %). Both ISO 6888-1:1999 (based on BP) and ISO 6888-2:1999 (based on RPFA) were last reviewed by ISO in 2015 and remain current. An alternative confirmation by stab-culture in RPFA was added in 2018 for ISO 6888-1 (ISO 6888-1:1999/Amd 2:2018). Two laboratories followed the MPN-based ISO 6888-3:2003, which is adapted for use when low numbers of stressed coagulase-positive staphylococci are expected.

With NMKL 66:2009 incubation is done on BP and/or RPFA. With BP, colonies are confirmed by a positive result in a coagulase test. When using RPFA, the coagulase activity is instead tested directly in the medium. In comparison, ISO 6888-1:1999 stipulates surface spreading on BP followed by confirmation with a coagulase test, whereas 6888-2:1999 stipulates the use of RPFA. On BP, *S. aureus* forms characteristic convex, shiny colonies that have a grey/black colour due to reduction of tellurite in the medium. The colonies are usually surrounded by a clear zone, due to proteolysis of egg yolk in the medium (lecithinase activity). An opaque halo may also form near the colony, due to precipitation caused by lipase activity. Petrifilm Staph is based on a modified Baird-Parker agar. It also contains a chromogenic indicator that causes *S. aureus* to form red/purple colonies.

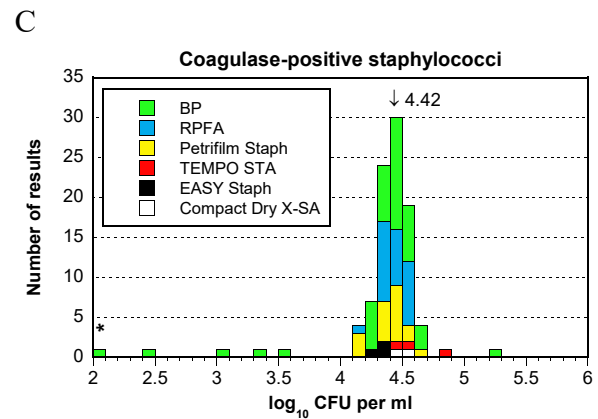
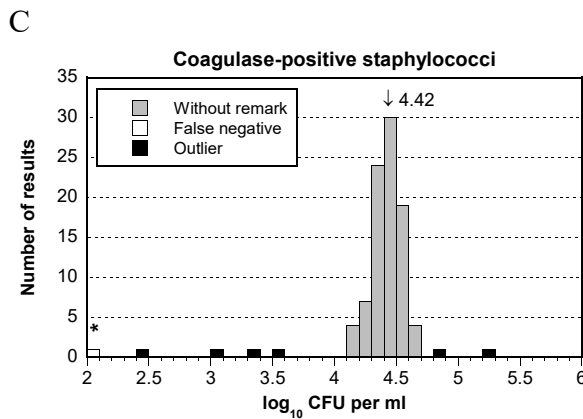
Taken together, the results were very similar for the most common media BP, RPFA and Petrifilm Staph, in all three samples. The only exception to this was the false positive results in sample A, which were reported to a somewhat higher degree by users of Petrifilm Staph. Somewhat lower mean values have in previous proficiency testing rounds sometimes been seen for Petrifilm Staph, but this was not evident this time. The

media EASY Staph®, TEMPO STA and Compact Dry™ X-SA were only used by a small number of laboratories, which makes them difficult to evaluate.

In total, 70 % of the laboratories stated that they performed some kind of confirmation. When using BP, this usually consisted of a tube coagulase test, while users of Petrifilm Staph mainly used Petrifilm Disk for confirmation. Traditionally, confirmation of coagulase-positive staphylococci is by detection of extracellular or bound coagulase (tube coagulase test and slide coagulase test respectively). Another common confirmation is a latex agglutination test. This is based on latex particles coated either with fibrinogen or with IgG that binds to protein A on the bacterial cell surface. Antibodies targeted against polysaccharides on the bacterial cell surface are also used in variations of this test. Confirmation with Petrifilm Disk is based on detection of extracellular DNase, which is produced by the majority of coagulase-positive *S. aureus*, but also by the coagulase-positive staphylococci *S. intermedius* and *S. hyicus*. Toluidin blue O in the disks visualises DNase activity as a pink zone around the colonies.

Results from analysis of coagulase-positive staphylococci

Medium	Sample A						Sample B						Sample C						
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >	
All results	95	94	-	-	1	- -	94	80	-	-	14	- -	95	88	4.42	0.11	1	4	2
BP	43	43	-	-	0	- -	42	36	-	-	6	- -	43	37	4.43	0.11	1	4	1
RPFA	25	25	-	-	0	- -	25	22	-	-	3	- -	26	26	4.43	0.11	0	0	0
Petrifilm Staph	19	18	-	-	1	- -	19	14	-	-	5	- -	18	18	4.38	0.12	0	0	0
TEMPO STA	3	3	-	-	0	- -	3	3	-	-	0	- -	3	2	-	-	0	0	1
EASY Staph	3	3	-	-	0	- -	3	3	-	-	0	- -	3	3	-	-	0	0	0
Compact Dry X-SA	2	2	-	-	0	- -	2	2	-	-	0	- -	2	2	-	-	0	0	0



Enterococci

Sample A

The strain of *E. hirae* was the target organism. In the Swedish Food Agency's quality control on ENT, it formed typical small, somewhat raised, dark red colonies. Upon confirmation on BEA, the strain usually causes a distinct black colour in the medium after 2 hours.

Sample B

The strain of *E. faecium* was the target organism. In the Swedish Food Agency's quality control on ENT, it formed typical small, somewhat raised, dark red colonies. Upon confirmation on BEA, the strain caused a faint black colour in the medium after 2 hours, and a distinct black colour after 24 hours. The strain is catalase-negative.

Seven low outliers were reported. Six of these were from laboratories that incubated at 37 °C. In total, 74 % of the laboratories incubated at 44 °C, while only 26 % incubated at 37 °C. Therefore, it cannot be excluded that the strain was difficult to identify at 37 °C. However at the same time, the strain was identified in correct concentration by the majority of the 17 laboratories that incubated at this temperature. Further, it cannot be ruled out that a short incubation time on BEA/KEAA may have contributed to low results.

Comment: According to communication by one of the participants, one of the reported low outliers for sample B is incorrect. According to this laboratory, the correct result is within the acceptance limits.

Sample C

No target organism was present in the sample. In the Swedish Food Agency's quality control, no colonies were detected on ENT.

General remarks

A clear majority of the laboratories (65 %) followed NMKL 68:2011. Among the less frequently used methods were the drinking water method ISO 7899-2:2000 (9 %), IDF 149A:1997 (6 %) and the older NMKL 68:2004 (3 %). Most of the remaining laboratories used company-specific methods, or methods that were not further specified. ISO 7899-2:2000 was last reviewed by ISO in 2016 and remains current. IDF 149A:1997 has been replaced by ISO 27205:2010/IDF 149:2010. This was last reviewed by ISO in 2020 and remains current.

With NMKL 68:2011, incubation is on ENT at 44 °C, possibly after a pre-incubation on TSA. Confirmation of atypical colonies is on BEA. The drinking water method ISO 7899-2:2000 is based on membrane filtration and incubation is on ENT at 37 °C. Confirmation is done similarly to the NMKL method, but by transfer of the entire membrane filter from ENT to BEA (possibly with the addition of azide). With the older NMKL 68:2004 (which was used by two laboratories) confirmation is not with BEA, but with a catalase test, and with tests for growth in BHI with 6.5 % salt and growth in BHI with pH 9.6.

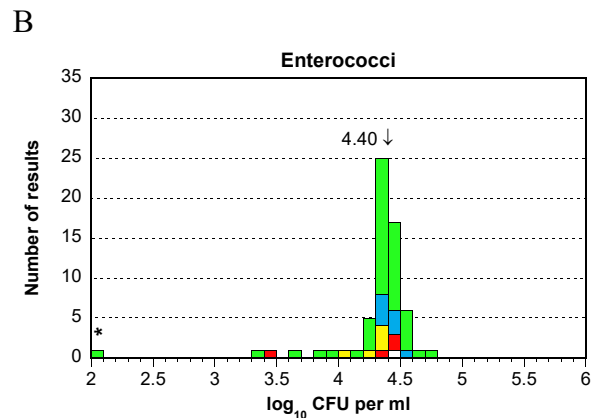
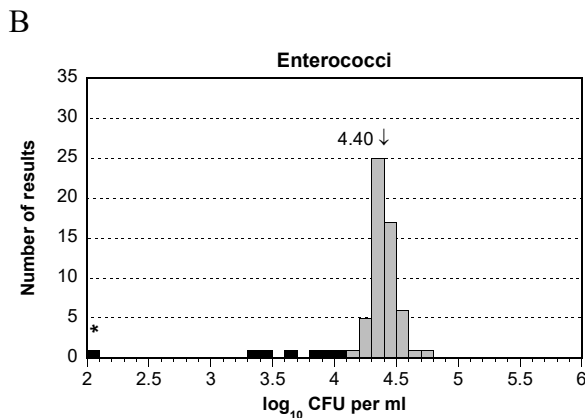
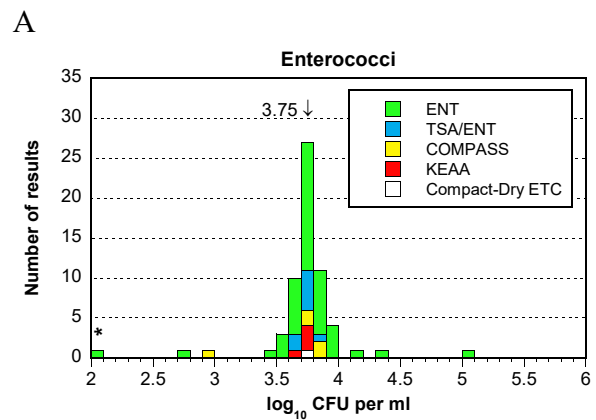
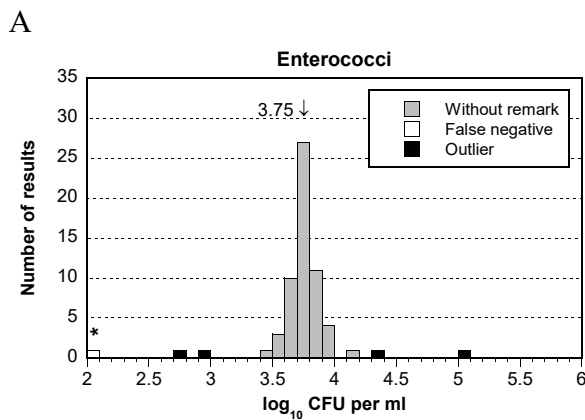
Enterococci are normally defined as Gram-positive, catalase-negative and oval cocci that hydrolyse esculin at 44 °C. On ENT they reduce the colourless substrate 2,3,5-trifenylnitroimidazole chloride to red formazan and form slightly raised colonies with a pink/red/maroon colour. They can sometimes also have a colourless edge. Upon confirmation on BEA, enterococci cause a tan/black colour in the medium after 2-24

hours. The colour comes from β -glucosidase hydrolysis of esculin in BEA. This produces esculetin and glucose, which together with iron ions in the medium form a black precipitate.

In total, 83 % of the laboratories incubated either on ENT or on TSA/ENT. A smaller number of laboratories used KEAA, COMPASS[®] Enterococcus agar or Compact Dry ETC. KEAA was used by laboratories that followed IDF 149A:1997. With KEAA, hydrolysis of esculin is detected directly in this medium. Similar to BEA, COMPASS also detects β -glucosidase activity, but is instead based on the substrate X-Gluc. On this medium, enterococci therefore form blue colonies. The majority of the laboratories that incubated on COMPASS stated that they also performed a confirmation on BEA. In total, 78 % of the laboratories stated that they performed some kind of confirmation.

Results from analysis of enterococci

Medium	Sample A						Sample B						Sample C						
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >	
All results	62	57	3.75	0.11	1	2 2	63	56	4.40	0.10	0	7 0	63	63	-	-	0	-	-
ENT	44	40	3.75	0.13	1	1 2	45	40	4.40	0.11	0	5 0	45	45	-	-	0	-	-
TSA/ENT	8	8	3.75	0.07	0	0 0	8	8	4.42	0.07	0	0 0	8	8	-	-	0	-	-
COMPASS	5	4	-	-	0	1 0	5	4	-	-	0	1 0	5	5	-	-	0	-	-
KEAA	4	4	-	-	0	0 0	4	3	-	-	0	1 0	4	4	-	-	0	-	-
Compact Dry ETC	1	1	-	-	0	0 0	1	1	-	-	0	0 0	1	1	-	-	0	-	-



Gram-negative bacteria in pasteurised milk and cream

Sample A

The strain of *K. pneumoniae* is Gram-negative.

Sample B

No target organism was present in the sample.

Sample C

The strains of *E. coli* and *S. marcescens* are Gram-negative.

General remarks

All reported results were correct. Eight laboratories followed NMKL 192:2011. One laboratory followed the ISO method for Enterobacteriaceae, ISO 21528-2:2017. The remaining two laboratories followed a company-specific method. Ten of the eleven laboratories incubated on VRBG, while one used MacConkey agar.

NMKL 192:2011 is a qualitative method for detecting recontamination by Gram-negative bacteria in pasteurised milk and cream. These bacteria do not survive high temperature/short time pasteurisation (HTST), where the temperature is raised to 72 °C for at least 15 seconds. Presence of Gram-negative bacteria therefore indicates recontamination, something that may limit the shelf-life of the product. With the method the unopened product is pre-incubated at 25 °C, which is followed by plating on VRBG. Presence of five or more colonies on VRBG is considered a positive result. When needed, confirmation is done with potassium hydroxide (KOH).

Results from analysis of Gram-negative bacteria

Method	Sample A			Sample B			Sample C		
	N	n	F	N	n	F	N	n	F
All results	11	11	0	10	10	0	11	11	0
NMKL 192:2011	8	8	0	8	8	0	8	8	0
ISO 21528-2:2017	1	1	0	0	0	0	1	1	0
Other	2	2	0	2	2	0	2	2	0

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 (2). 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

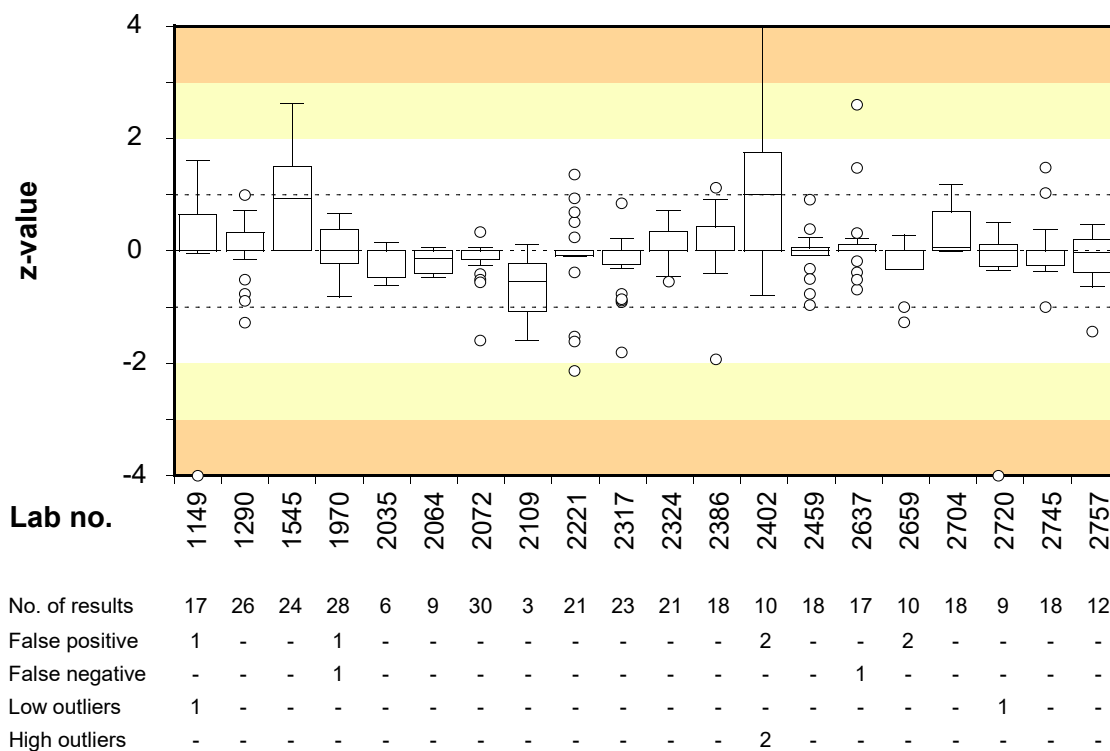
In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated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

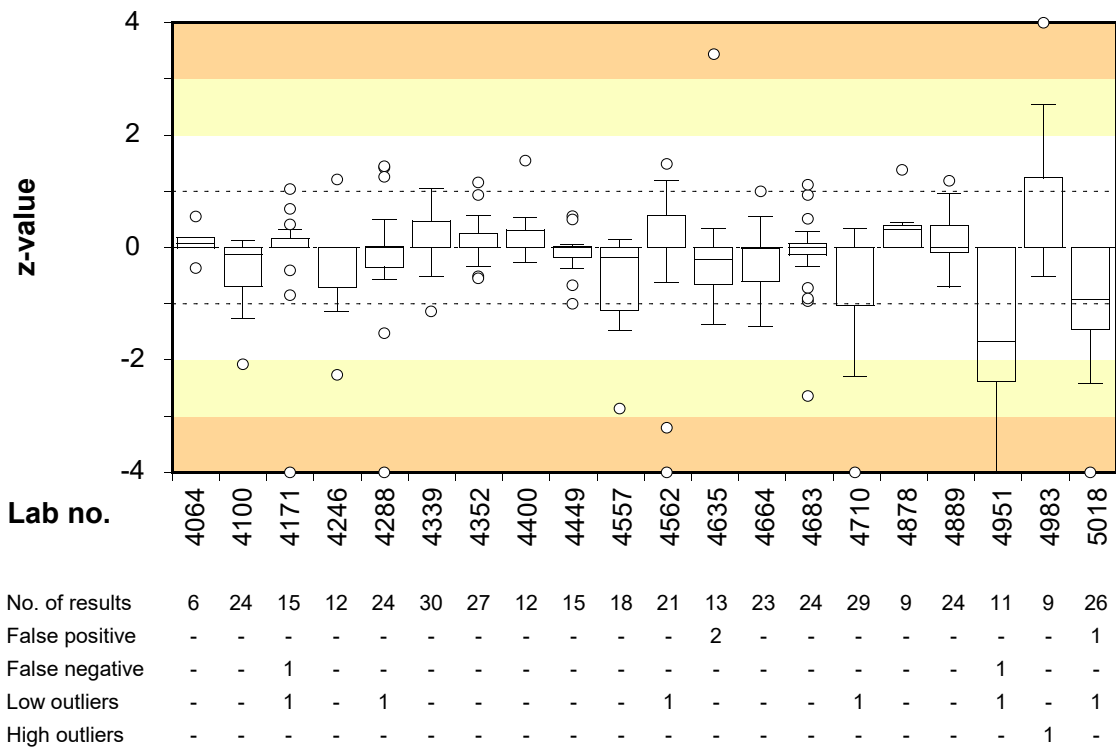
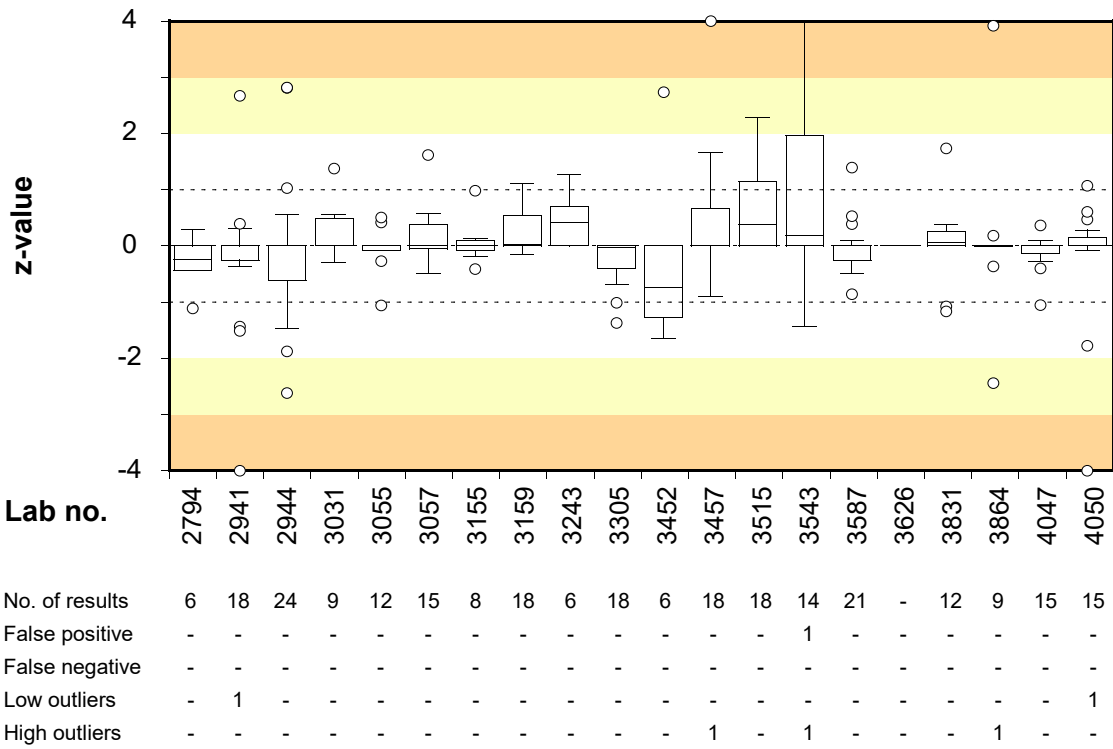
The box plots are based on the z-scores listed in Annex 2, and give a comprehensive view of the achievement of each laboratory. A small box, centred around zero, indicates that the results of the individual laboratory, with false results excluded, are close to the general mean values calculated for all 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.

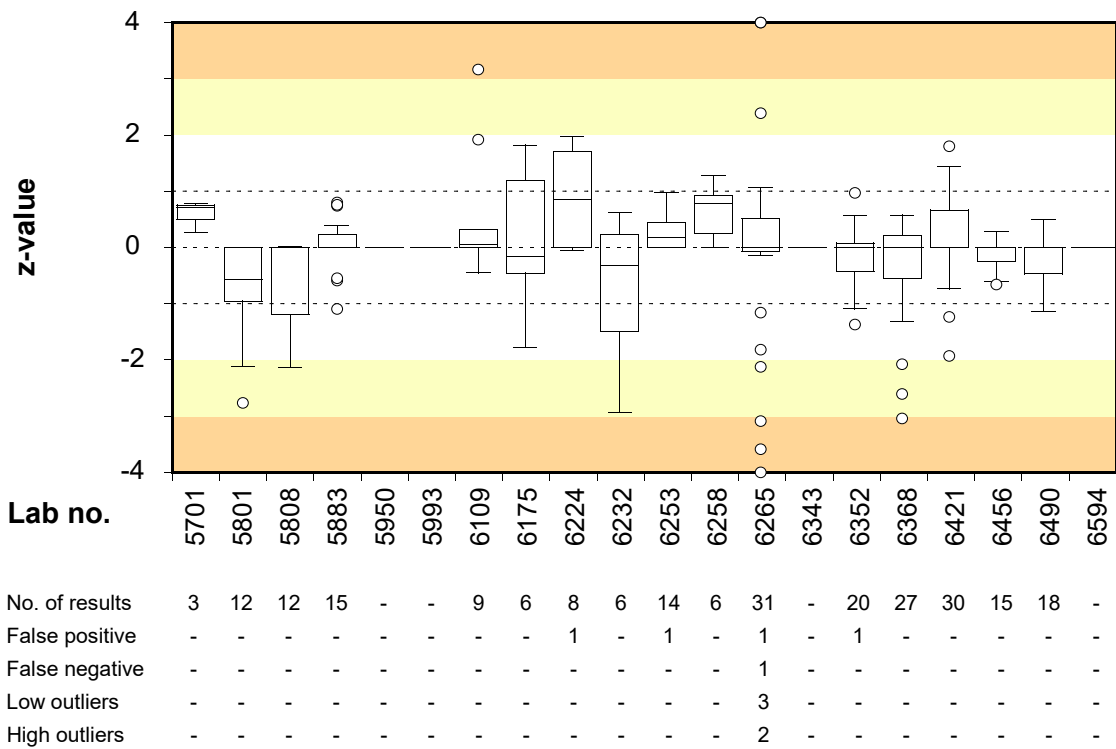
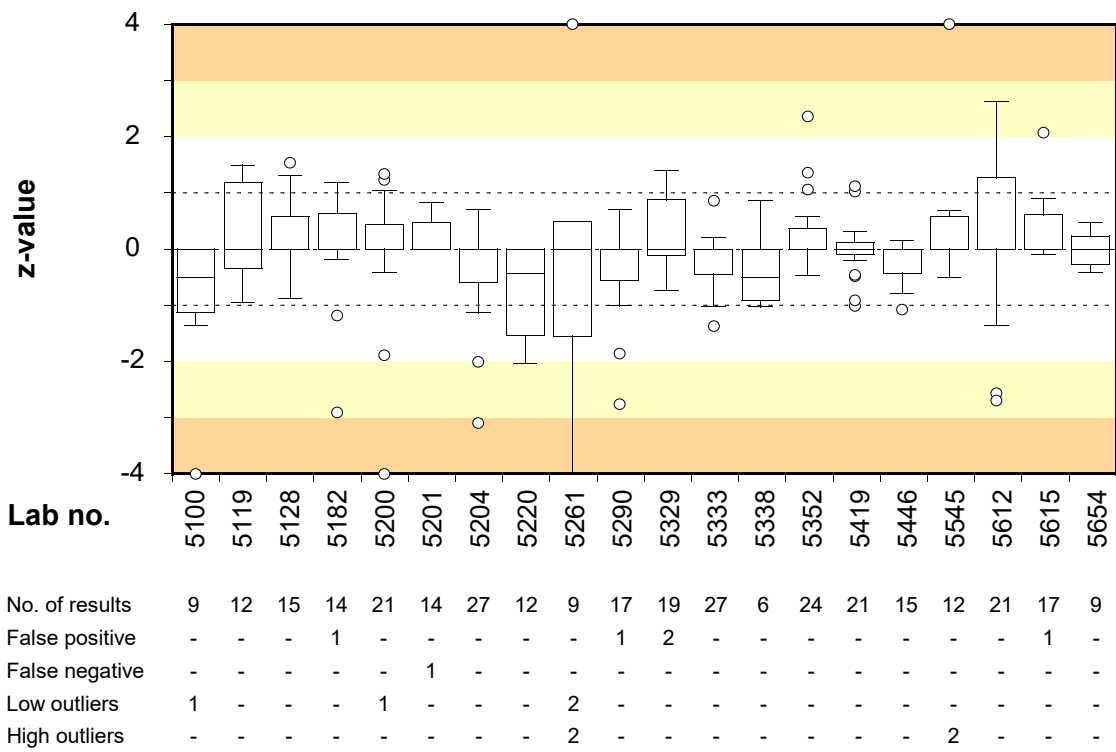
Box plots and numbers of deviating results for each laboratory

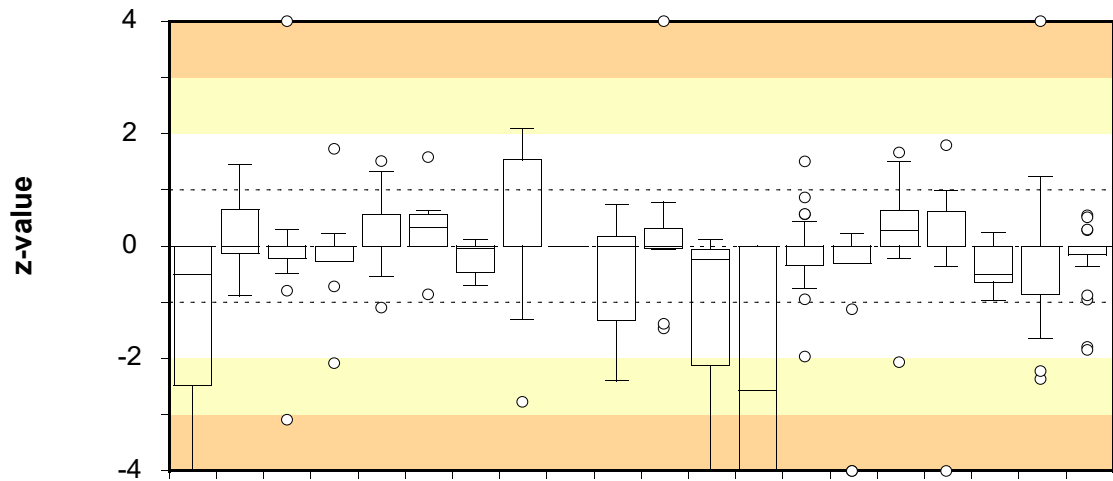
- Z-scores are calculated according to the formula: $z = (x-m)/s$, where x is the result of the individual laboratory, m is the mean of the results of all participating laboratories, and s is the standard deviation of the participating laboratories, after removing outliers and false results.
- Outliers 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.

* $< [\text{lowest value in the box} - 1,5 \times (\text{highest value in the box} - \text{lowest value in the box})]$
or
 $> [\text{highest value in the box} + 1,5 \times (\text{highest value in the box} - \text{lowest value in the box})]$.

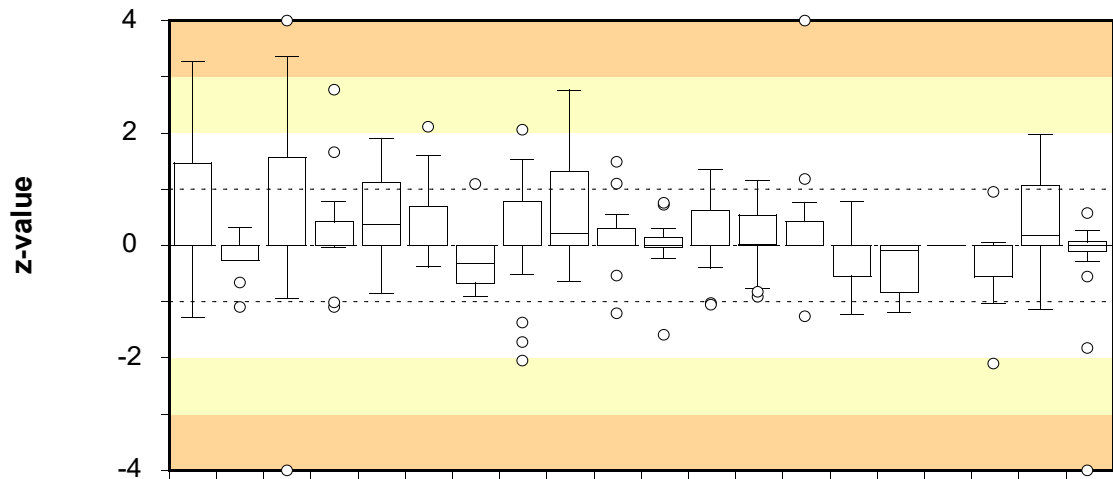




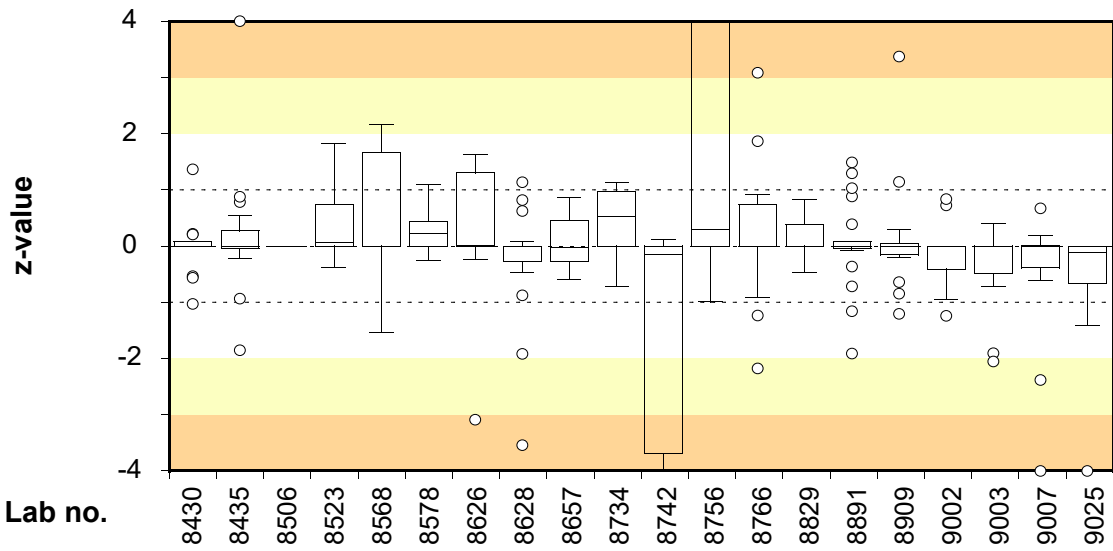




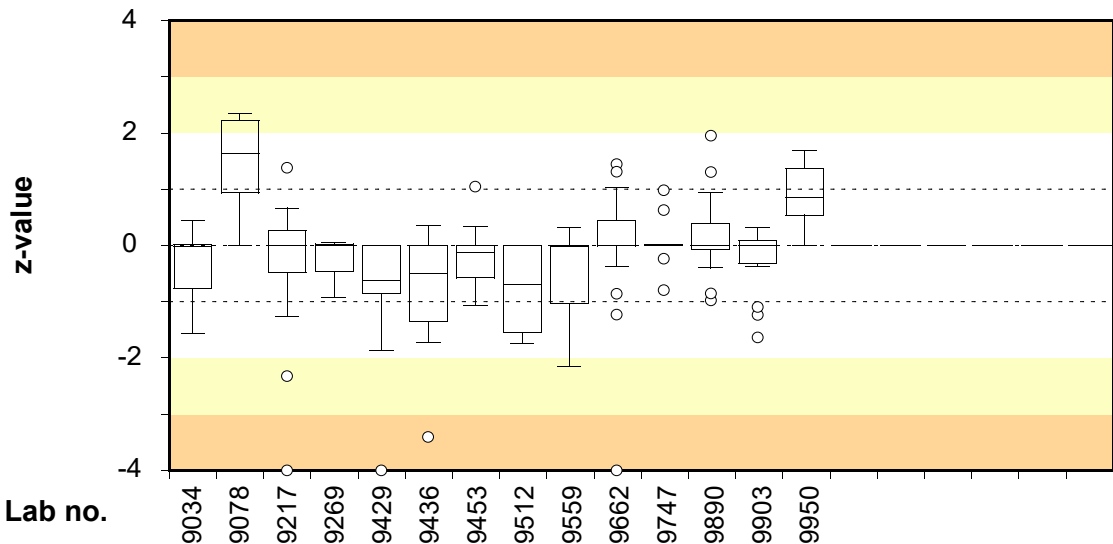
Lab no.	6658	6686	6728	6762	6885	6944	6958	6971	6992	7182	7207	7232	7242	7248	7334	7564	7617	7631	7640	7688	
No. of results	12	20	14	9	21	9	9	9	-	21	12	3	6	33	14	18	15	9	33	27	
False positive	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	2	-	-	-	-	-	-	-	-	-	-	1	3	-	2	-	1	-	-	-	-
High outliers	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-



Lab no.	7728	7750	7825	7876	7882	7930	7940	7962	7968	7984	8009	8019	8068	8105	8147	8213	8260	8313	8333	8397	
No. of results	24	9	14	17	14	27	6	27	30	12	12	27	30	15	9	12	-	21	15	17	
False positive	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
False negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
High outliers	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-



No. of results	14	29	-	12	15	9	13	30	6	6	15	18	17	15	21	18	15	15	12	12
False positive	1	1	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	1	-	-	4	-	-	-	-	-	-	-	1	1
High outliers	-	1	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-



No. of results	12	6	15	3	18	27	18	9	24	26	9	20	18	6
False positive	-	-	-	-	-	-	-	-	-	1	-	1	-	-
False negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	1	-	1	-	-	-	-	1	-	-	-	-
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 (3). 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	<i>Enterococcus hirae</i>	SLV-536	water	CCUG 46536
	<i>Kocuria rhizophila</i>	SLV-055	-	CCUG 35073
	<i>Klebsiella pneumoniae</i>	SLV-186	vegetarian kebab	CCUG 45102
B	<i>Bacillus cereus</i>	SLV-518	couscous	CCUG 44741
	<i>Enterococcus faecium</i>	SLV-459	-	CCUG 35172
	<i>Staphylococcus xylosum</i>	SLV-283	cheese	-
C	<i>Escherichia coli</i>	SLV-477	cheese	CCUG 43601
	<i>Serratia marcescens</i>	SLV-040	pond water	ATCC 13880
	<i>Staphylococcus aureus</i>	SLV-280	egg	-

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)

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 4 and 5 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 ²		
	M	I_2	T	m	I_2	T	m	I_2	T
Aerobic microorganisms, 30 °C NMKL method no. 86:2013	4,22	2,79	1,41	5,29	3,94	1,54	4,74	3,68	1,46
Aerobic microorganisms, 20 °C NMKL method no. 86:2013	4,21	0,98	1,25	5,25	1,39	1,28	4,72	0,90	1,30
Contaminating microorganisms ISO method no. 13559:2002 IDF method no. 153:2002	4,20	1,28	1,82	5,15	6,19	1,88	4,79	0,66	1,23
Enterobacteriaceae NMKL method no. 144:2005	3,70	3,01	1,72	-	-	-	4,29	0,63	1,17
Coliform bacteria, 30 °C NMKL method no. 44:2004	3,69	1,74	1,43	-	-	-	4,21	2,71	1,43
Coliform bacteria, 37 °C NMKL method no. 44:2004	3,67	3,20	1,67	-	-	-	4,19	1,95	1,37
Thermotolerant coliform bacteria NMKL method no. 125:2005	3,77	0,39	1,18	-	-	-	4,23	1,40	1,28
<i>Escherichia coli</i> NMKL method no. 125:2005	-	-	-	-	-	-	4,23	1,40	1,28
Presumptive <i>Bacillus cereus</i> NMKL method no. 67:2010	-	-	-	4,33	3,54	2,04	4,75 ³	0,31 ³	1,15 ³
Coagulase-positive staphylococci NMKL method no. 66:2009	-	-	-	5,18 ³	0,54 ³	1,18 ³	4,53	0,85	1,37
Enterococci NMKL method no. 68:2011	3,77	1,08	1,29	4,48	0,95	1,42	-	-	-
Gram-negative bacteria in pasteurised milk and cream. Detection of recontamination. NMKL method no. 192:2011	Pos	-	-	Neg	-	-	Pos	-	-

- No target organism and therefore no value

¹ n = 5 vials analysed in duplicate

² n = 10 vials analysed in duplicate

³ False positive for the analysis

References

1. Kelly, K. 1990. Outlier detection in collaborative studies. *J. Assoc. Off. Anal. Chem.* 73:58–64.
2. Anonymous, 2018. Protocol. Microbiology. Drinking water & Food, Swedish Food Agency.
3. 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.
4. 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.
5. Heisterkamp, S.H., Hoekstra, J.A., van Strijp-Lockfeer, N.G.W.M., Havelaar, A.H., Mooijman, K.A., in't Veld, P.H., Notermans, S.H.W., Maier, E.A. ; Griepink, B. 1993. Statistical analysis of certification trials for microbiological reference materials. Luxembourg: Commission of the European Communities, Report EUR 15008 EN.

Annex 1 Results of the participating laboratories - October 2020

All results are in log₁₀ cfu per ml sample. Results reported as "< value" have been regarded as zero. Results reported as "> value" are excluded from the calculations. A dash indicates the analysis was not performed. Outliers and false results are highlighted and summarized for each analysis at the end of the table.

Lab no.	Vial	Aerobic microorg. 30 °C			Aerobic microorg. 20 °C			Contaminating microorganisms			Enterobacteriaceae			Coliform bacteria 30 °C			Coliform bacteria 37 °C			Thermotolerant coliform bacteria			Escherichia coli			Presumptive Bacillus cereus			Coagulase-positive Staphylococci			Enterococci			Gram-neg bacteria in dairy prod.			Lab no.			
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C				
1149	2 1 3	4.28	5.34	4.73	-	-	-	-	-	-	3.79	0	4.2	-	-	-	3.65	0	4.26	-	-	-	0	0	4.26	0	4.63	0	0	5.28	3	-	-	-	-	-	-	1149			
1290	3 1 2	4.29	5.39	4.58	4.26	5.23	4.68	-	-	-	3.71	<1	4.28	3.72	<1	4.23	3.67	<1	4	-	<1	3.96	<1	<1	4.08	<1	4.12	<1	<1	<1	4.43	-	-	-	-	-	-	1290			
1545	2 1 3	4.32	5.69	4.8	4.32	5.49	4.81	-	-	-	3.78	<0	4.56	-	-	-	-	-	-	3.84	<0	4.37	<0	<0	4.37	<1	4.59	<1	<1	<1	4.6	3.91	4.53	<1	-	-	-	-	1545		
1970	3 1 2	4.16	5.3	4.63	4.06	5.3	4.65	-	-	-	3.58	<1	4.08	3.61	<1	4.2	3.68	<1	4.23	3.54	<1	4.21	3.54	<1	4.21	3.54	<1	4.32	<1	<1	<1	4.49	<1	4.32	<1	-	-	-	-	1970	
2035	1 2 3	-	-	-	-	-	-	-	-	-	3.7	<1	4.1	-	-	-	-	-	-	-	-	-	<1	<1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	2035		
2064	1 3 2	4.15	5.22	4.67	-	-	-	-	-	-	3.64	0	4.15	-	-	-	-	-	-	-	-	-	-	-	-	0	4.13	0	-	-	-	-	-	-	-	-	-	-	-	2064	
2072	1 3 2	4.2	5.2	4.71	4.11	5.18	4.68	-	-	-	3.68	<1	4.18	3.63	<1	4.08	3.63	<1	4.15	3.63	<1	4.08	<1	<1	4.08	<1	3.86	<1	<1	<1	4.36	3.76	4.34	<1	-	-	-	-	2072		
2109	2 3 1	4	5.23	4.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2109		
2221	3 1 2	4.2	5.21	4.71	-	-	-	3.62	4.52	4.3	3.66	<1	4.25	-	-	-	-	-	-	-	-	-	<1	<1	4.04	<1	4.3	<1	<1	<1	4.52	3.83	4.53	<1	-	-	-	-	2221		
2317	3 2 1	4.18	5.22	4.62	-	-	-	-	-	-	3.54	<1	4.15	3.66	<1	4.1	3.64	<1	4	-	-	-	<1	<1	4.05	<1	-	<1	<1	<1	4.51	3.55	4.31	<1	-	-	-	-	2317		
2324	2 1 3	4.15	5.3	4.66	-	-	-	4.19	5.29	4.68	3.73	<1	4.17	-	-	-	-	-	-	-	-	-	<1	<1	4.17	<1	4.29	<1	<1	<1	4.47	3.79	4.35	<1	-	-	-	-	2324		
2386	2 1 3	4.25	5.26	4.8	-	-	-	-	-	-	3.85	<1	4.11	-	-	-	3.73	<1	4.31	3.72	<1	4.09	-	-	-	<1	3.78	<1	<1	<1	4.44	-	-	-	-	-	-	-	2386		
2402	1 3 2	4.1	5.53	4.74	-	-	-	-	-	-	3.88	3.58	5.19	-	-	-	4.36	0	4.3	-	-	-	4.36	0	4.3	-	-	-	-	-	-	-	-	-	-	-	-	-	2402		
2459	2 1 3	4.19	5.28	4.82	-	-	-	-	-	-	3.71	0	4.18	-	-	-	3.61	0	4	-	-	-	0	0	4.02	0	4.3	0	0	0	4.31	-	-	-	-	-	-	-	-	2459	
2637	3 1 2	4.23	5.48	4.7	-	-	-	-	-	-	3.57	<1	4.57	-	-	-	-	-	-	<1	<1	4.15	<1	<1	4.04	<1	4.32	<1	<1	<1	4.36	-	-	-	-	-	-	-	2637		
2659	2 3 1	4.04	5.26	-	-	-	-	-	-	-	3.66	0	-	3.58	0	-	3.51	0	-	-	-	-	0	0	-	-	-	-	2.04	5	-	-	-	-	-	-	-	-	2659		
2704	1 2 3	4.31	5.3	4.83	-	-	-	-	-	-	3.86	<1	4.17	-	-	-	3.8	<1	4.16	-	-	-	<1	<1	4.16	<1	4.41	<1	<1	<1	4.46	-	-	-	-	-	-	-	2704		
2720	1 3 2	2.94	5.23	4.69	-	-	-	-	-	-	3.71	<1	4.25	-	-	-	-	-	-	-	-	-	-	-	-	<1	4.16	<1	-	-	-	-	-	-	-	-	-	-	-	2720	
2745	2 1 3	4.17	5.14	4.61	-	-	-	-	-	-	3.64	<0	4.23	-	-	-	-	-	-	3.59	<0	4.06	<0	<0	4.06	<1	4.49	<1	<1	<1	4.58	-	-	-	-	-	-	-	2745		
2757	3 1 2	4.26	5.09	4.77	3.95	5.15	4.64	-	-	-	3.74	0	4.17	3.62	0	4.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2757		
2794	3 1 2	4.06	5.17	4.69	-	-	-	-	-	-	-	-	-	-	-	-	3.7	0	4.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2794	
2941	1 2 3	4.25	5.14	4.09	-	-	-	-	-	-	4.09	0	4.22	3.42	0	4.06	-	-	-	-	-	-	0	0	4.12	0	4.22	0	0	0	4.25	-	-	-	-	-	-	-	-	2941	
2944	2 3 1	4.17	5.31	4.59	-	-	-	-	-	-	3.52	<1	4.06	-	-	-	3.38	<1	4.66	3.38	<1	4.66	<1	<1	3.66	<1	4.49	<1	<1	<1	4.36	3.74	4.41	<1	-	-	-	-	2944		
3031	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.86	0	4.23	3.72	0	4.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3031	
3055	1 2 3	4.25	5.19	4.6	-	-	-	-	-	-	3.76	0	4.16	-	-	-	-	-	-	-	-	-	-	-	0	4.18	0	-	-	-	-	-	-	-	-	-	-	-	-	Pos Neg Pos	3055
3057	3 2 1	-	-	4.78	-	-	4.7	-	-	-	3.66	0	4.18	-	-	4.15	-	-	4.05	-	-	4.08	0	0	4.38	0	4.38	0	-	-	4.38	-	-	-	-	-	-	-	-	3057	
3155	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.67	-	4.32	3.61	<1	4.05	<1	<1	4.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3155	
3159	3 2 1	4.18	5.25	4.79	-	-	-	-	-	-	3.76	<1	4.2	-	-	-	3.82	<1	4.15	3.78	<1	4.11	<1	<1	4.18	-	-	-	<2	<2	4.49	-	-	-	-	-	-	-	-	3159	
3243	1 3 2	4.26	5.21	4.86	-	-	-	-	-	-	3.73	0	4.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3243	
3305	2 1 3	4.2	5.2	4.57	-	-	-	-	-	-	3.57	<1	4.11	-	-	-	-	-	-	3.63	<1	4.04	<1	<1	4.04	<1	4	<1	<1	<1	4.4	-	-	-	-	-	-	-	-	3305	
3452	1 3 2	4.04	5.71	4.54	-	-	-	-	-	-	-	-	-	3.55	0	3.93	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3452	
3457	2 1 3	-	-	-	4.94	5.26	4.71	-	-	-	3.8	0	4.27	-	-	-	3.74	0	3.99	-	-	-	<1	<1	3.95	-	-	-	<1	<1	4.6	3.69	4.47	<1	-	-	-	-	-	3457	
3515	3 1 2	4.34	5.42	4.72	-	-	-	-	-	-	3.82	0	4.36	3.96	0	4.41	-	-	-	-	-	-	0	0	4.29	0	4.43	0	0	0	4.56	-	-	-	-	-	-	-	-	3515	
3543	1 2 3	4.48	5.57	4.83	-	-	-	-	-	-	3.59	0	4.34	-	-	-	-	-	-	-	-	-	-	-	0	3.9	0	0	5.04	4.46	5.02	4.71	0	-	-	-	-	-	-	3543	
3587	3 2 1	4.19	5.28	4.68	-	-	-	-	-	-	3.69	<1	4.13	-	-	-	3.53	<1	4.05	-	-	-	<1	<1	4.06	<1	4.37	<1	<1	<1	4.57	3.72	4.37	<1	-	-	-	-	-	3587	
3626	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3626	
3831	1 3 2	4.42	5.01	4.72	4.21	5.04	4.66	-	-	-	-	-	-	-	-	-	3.7	0	4.21	-	-	-	0	0	4.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3831	
3864	1 3 2	4.2	4.76	5.15	-	-	-	-	-	-	3.62	0	4.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos Neg Pos	3864	
4047	3 1 2	4.15	5.16	4.76	-	-	-	-	-	-	3.68	<1	4.13	-	-	-	-	-	-	-	-	-	<1	<1	4.12	<1	3.99	<1	<1	<1	4.42	-	-	-	-	-	-	-	-	4047	
m		4.200	5.208	4.720	4.174	5.209	4.650	4.021	5.051	4.642	3.676	0	4.172	3.628	0	4.109	3.657	0	4.140	3.643	0	4.128	0	0	4.104	0	4.243	0	0	0	4.416	3.753	4.395	0	pos	neg	pos	m			
s		0.126	0.184	0.110	0.156	0.146	0.088	0.263	0.329	0.160	0.155	0	0.153	0.145	0	0.193	0.147	0	0.185	0.178	0	0.189	0	0	0.170	0	0.240	0	0	0	0.110	0.112	0.099	0	-	-	-	s			

Lab no.	Vial			Aerobic microorg. 30 °C			Aerobic microorg. 20 °C			Contaminating microorganisms			Enterobacteriaceae			Coliform bacteria 30 °C			Coliform bacteria 37 °C			Thermotolerant coliform bacteria			Escherichia coli			Presumptive Bacillus cereus			Coagulase-positive Staphylococci			Enterococci			Gram-neg bacteria in dairy prod.			Lab no.
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
5808	1	2	3	4.12	5.09	4.52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5808	
5883	2	3	1	4.21	5.1	4.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5883	
5950	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5950		
5993	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5993		
6109	1	3	2	4.6	5.56	4.74	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6109		
6175	1	3	2	4.16	4.88	4.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6175		
6224	3	1	2	4.45	5.34	4.83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6224		
6232	3	1	2	4.23	5.32	4.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6232		
6253	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6253		
6258	2	3	1	4.29	5.36	4.86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6258		
6265	3	1	2	4.32	6.19	4.78	4.34	4.9	4.49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos Neg Pos	6265		
6343	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6343		
6352	3	1	2	4.22	5.16	4.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6352		
6368	3	1	2	4.22	5.26	4.75	4.06	5.27	4.62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6368		
6421	3	1	2	4.3	5.33	4.75	4.06	5.28	4.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6421		
6456	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6456		
6490	1	3	2	4.12	5.07	4.69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6490		
6594	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6594		
6658	2	1	3	3.8	4.88	4.34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos Neg Pos	6658		
6686	3	1	2	4.3	5.15	4.79	4.28	5.08	4.68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6686		
6728	3	2	1	4.1	6.1	4.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6728		
6762	2	1	3	4.11	5.2	4.91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6762		
6885	3	2	1	4.21	5.11	4.83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6885		
6944	1	2	3	-	-	-	4.04	5.26	4.79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos Neg Pos	6944	
6958	1	2	3	4.14	5.08	4.65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6958		
6971	2	3	1	3.85	5.49	4.95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6971		
6992	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6992		
7182	3	1	2	4.25	5.24	4.74	4.04	5.18	4.62	4.21	5.14	4.76	3.44	<1	4.07	3.28	<1	3.65	3.38	<1	3.88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7182		
7207	3	1	2	4.92	5.29	4.74	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7207		
7232	1	3	2	4.17	5.23	4.22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7232		
7242	3	1	2	3.08	5	3.64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7242		
7248	2	3	1	4.12	5.07	4.68	4.13	5.17	4.66	4.17	5.04	4.64	3.53	<1	4.2	3.56	<1	4.4	3.6	<1	4.3	3.58	<1	4.15	<1	<1	4.18	<1	4.38	<1	<1	<1	4.2	3.74	4.32	<1	-	7248		
7334	3	2	1	4.16	4.2	4.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7334		
7564	2	1	3	-	-	-	4.14	5.28	4.76	4.19	5.21	4.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7564		
7617	1	3	2	4.33	5.3	4.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7617		
7631	3	1	2	4.13	5.03	4.65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7631		
7640	2	1	3	4.2	4.98	4.79	5.04	5	4.76	3.4	4.32	4.38	3.52	<1	4.36	3.64	<1	4.04	3.65	<1	3.99	3.49	<1	3.91	<1	<1	4	<1	4.54	<1	<1	<1	4.3	3.72	4.32	<1	-	7640		
7688	1	2	3	4.08	5.18	4.78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7688		
7728	3	1	2	4.18	5.45	4.58	4.26	5.3	4.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7728		
7750	1	2	3	4.2	5.16	4.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7750		
7825	3	1	2	4.52	5.5	4.78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7825		
7876	1	2	3	4.3	5.3	4.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7876		
7882	1	2	3	4.44	5.05	4.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7882		
m				4.200	5.208	4.720	4.174	5.209	4.650	4.021	5.051	4.642	3.676	0	4.172	3.628	0	4.109	3.657	0	4.140	3.643	0	4.128	0	0	4.104	0	4.243	0	0	0	4.416	3.753	4.395	0	pos neg pos	m		
s				0.126	0.184	0.110	0.156	0.146	0.088	0.263	0.329	0.160	0.155	0	0.153	0.145	0	0.193	0.147	0	0.185	0.178	0	0.189	0	0	0.170	0	0.240	0	0	0	0.110	0.112	0.099	0	-	s		

Lab no.	Vial			Aerobic microorg. 30 °C			Aerobic microorg. 20 °C			Contaminating microorganisms			Enterobacteriaceae			Coliform bacteria 30 °C			Coliform bacteria 37 °C			Thermotolerant coliform bacteria			Escherichia coli			Presumptive Bacillus cereus			Coagulase-positive Staphylococci			Enterococci			Gram-neg bacteria in dairy prod.			Lab no.
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
9559	2	3	1	4.02	5.05	4.6	4.13	5.04	4.51	-	-	-	3.53	0	3.92	-	-	-	3.65	0	3.99	-	-	-	0	0	3.95	0	4.32	0	0	0	4.18	-	-	-	Pos	Neg	Pos	9559
9662	2	3	1	4.2	5.23	4.81	4.13	5.03	4.69	-	-	-	3.9	0	4.26	2.96	0	4.11	3.85	0	4.26	-	-	-	0	0	4.04	0	4.28	0	0	4.38	4.53	3.72	4.31	0	-	-	-	9662
9747	1	2	3	4.28	5.21	4.72	-	-	-	-	-	-	3.64	0	4.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9747
9890	1	3	2	4.15	5.26	4.76	4.48	5.4	4.62	-	-	-	3.74	4.28	4.15	-	-	-	3.76	0	3.96	-	-	-	0	0	3.96	0	4.26	0	0	0	4.52	-	-	-	-	-	-	9890
9903	1	2	3	4.16	5.14	4.73	-	-	-	-	-	-	3.69	<0	4.22	-	-	-	-	-	-	-	-	-	<0	<0	4.16	<1	3.85	<1	<1	<1	4.28	3.63	4.41	<1	-	-	-	9903
9950	2	1	3	4.29	5.39	4.87	-	-	-	-	-	-	3.76	0	4.43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9950	

N	152	152	152	32	32	33	13	13	13	142	141	141	46	46	45	87	86	86	43	45	45	112	112	107	106	105	106	95	94	95	62	63	63	11	10	11	N
Min	2.94	4.00	3.64	3.85	4.90	4.48	3.40	4.32	4.30	0	0	0	2.96	0	3.26	3.04	0	0	0	0	3.30	0	0	0.30	0	0	0	0	0	0	1.54	0	-	-	-	Min	
Max	5.47	6.19	5.36	5.04	5.60	5.25	4.29	5.40	4.85	5.10	4.28	5.24	3.96	0	4.57	4.81	3.63	4.66	4.94	0	4.66	4.36	0	4.66	3.63	5.07	4.62	2.04	5.28	5.23	5.02	4.71	0	-	-	-	Max
Med	4.20	5.22	4.72	4.14	5.21	4.66	4.15	5.14	4.67	3.69	0	4.18	3.63	0	4.10	3.67	0	4.15	3.63	0	4.10	0	0	4.10	0	4.23	0	0	0	4.43	3.75	4.39	0	-	-	-	Med
m	4.200	5.208	4.720	4.174	5.209	4.650	4.021	5.051	4.642	3.676	0	4.172	3.628	0	4.109	3.657	0	4.140	3.643	0	4.128	0	0	4.104	0	4.243	0	0	0	4.416	3.753	4.395	0	pos	neg	pos	m
s	0.126	0.184	0.110	0.156	0.146	0.088	0.263	0.329	0.160	0.155	0	0.153	0.145	0	0.193	0.147	0	0.185	0.178	0	0.189	0	0	0.170	0	0.240	0	0	0	0.110	0.112	0.099	0	-	-	-	s
u_(lg)	0.010	0.015	0.009	0.028	0.026	0.016	0.073	0.091	0.044	0.013	0	0.013	0.022	0	0.029	0.016	0	0.020	0.028	0	0.028	0	0	0.017	0	0.024	0	0	0	0.012	0.015	0.013	0	-	-	-	u_(lg)
F+	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	1	0	0	0	0	7	0	0	1	0	2	1	14	0	0	0	0	0	0	0	F+
F-	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	F-
<	2	3	5	0	0	0	0	0	0	1	0	4	1	0	1	1	0	2	0	0	1	0	0	2	0	2	0	0	4	2	7	0	-	-	-	<	
>	4	3	2	2	0	1	0	0	0	2	0	2	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	2	2	0	0	-	-	-	>	
< OK	3.80	4.62	4.49	3.85	4.62	4.48	3.40	4.32	4.30	3.15	0	3.70	3.28	0	3.65	3.26	0	3.57	3.04	0	3.63	0	0	3.54	0	3.72	0	0	0	4.10	3.46	4.19	0	-	-	-	< OK
> OK	4.60	5.71	5.09	4.57	5.60	4.81	4.29	5.40	5.29	4.09	0	4.57	3.96	0	4.57	3.97	0	4.66	3.97	0	4.66	0	0	4.66	0	5.07	0	0	0	4.65	4.13	4.71	0	-	-	-	> OK

N = number of analyses performed Max = highest reported result m = mean value F+ = false positive < = low outlier < OK = lowest accepted value u_(lg) = measurement uncertainty for assigned value (m)
Min = lowest reported result Med = median value s = standard deviation F- = false negative > = high outlier > OK = highest accepted value

	The results are not evaluated
	Outlier, false positive or false negative
	Results "larger than" are not evaluated

Lab no.	Vial			Aerobic microorganisms 30 °C			Aerobic microorganisms 20 °C			Contaminating microorganisms			Enterobacteriaceae			Coliform bacteria 30 °C			Coliform bacteria 37 °C			Thermotolerant coliform bacteria			Escherichia coli			Presumptive Bacillus cereus			Coagulase-positive Staphylococci			Enterococci			Gram-neg bacteria in dairy prod.			Lab no.
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
4557	1	3	2	-0.319	-0.042	-1.184	-0.539	0.142	-0.004				-0.751	0	-1.121	-0.792	0	-1.466				0	0	-1.377				0	0	-2.867							4557			
4562	3	1	2	0.948	-3.200	0.092						-0.622	0	-0.141			0.566	0	1.190				0	0	0.270	0	0.697	0	0	0	0.939	1.492	-4.000	0			4562			
4635	2	3	1	-0.952	-1.077	-1.366						-0.557	0	-0.206																							4635			
4664	2	1	3	0.077	0.557	1.002	-0.923	-0.544	-0.910			-0.751	0	-0.206	-1.403	0	-0.761	-0.129	0	-0.412	0	0				0	0	-0.420	0.334	-0.658	0						4664			
4683	2	1	3	-0.715	0.938	0.001						0.154	0	0.513	-0.953	0	0.214	0.294	0	-0.328	-0.072	0	-2.638	0	0	-0.906	0	-0.178	0	0	0	1.120					4683			
4710	1	2	3	-0.161	-0.587	-1.366	-0.027	0.005	0.335			-1.269	0	-0.468	-0.953	0		-2.285	0		-1.475	0	-0.836	0	0	-1.024	0	-2.176	0	0	0	-1.055		-4.000	0	0	0	4710		
4878	2	3	1	0.394	0.339	0.456						1.382	0	0.252													0	0.322	0								4878			
4889	2	1	3	0.235	-0.173	-0.701						0.561	0	0.703			0.824	0	-0.539	-0.488	0	-0.460	0	0	-0.371	0	0.784	0	0	0	0.132	1.189	0.968	0			4889			
4951	1	2	3	-0.873	-2.329	-1.912						-2.432	0	-4.000			-1.674	0					0	0	-3.318												4951			
4983	3	2	1	4.000	-0.511	2.551						-0.215	0	1.245													0	0.243	0								4983			
5018	3	1	2	-0.398	-1.567	-1.457						-2.303	0	-1.383	-1.848	0	-1.859	-2.421	0	-1.303	-1.082	0	-0.942	0	0	-0.906	0	-0.552	0	0	-1.055	-1.270	-4.000	0			5018			
5100	3	2	1	-0.081	-1.349	-0.910											-1.131	0	-4.000																			5100		
5119	1	3	2	1.422	-0.914	0.547						-0.687	0	1.494	-0.953	0	1.094																					5119		
5128	2	1	3	-0.715	1.536	0.365						-0.127	0	1.302													0	0	0.800	0	1.030	0	0	0	-0.873			5128		
5182	3	2	1	0.948	0.992	0.638						0.477	0	0.317			-0.181	0	1.190							0	-2.906	0	-1.177	0							5182			
5200	1	2	3	1.232	1.036	0.037	-0.417	1.336	-1.884			0.438	0	0.010	0.713	0	0.400	0.511	0	0.388																		5200		
5201	2	3	1	-0.002	0.829	0.274						0.477	0													0	0.682	0	0.322	0	0	0	0.576					5201		
5204	2	1	3	-0.398	-0.532	-2.003						-0.040	0	-1.056	0.700	0	-1.133	-0.656	0	-3.092	0.433	0	-0.147	0	0	-0.024	0	0.322	0	0	0	-0.330	-0.824	-0.759	0			5204		
5220	1	3	2	-1.419	-1.659	-2.031						-1.715	0	-1.141												0	0	-0.636										5220		
5261	1	3	2	4.000	0.493	-4.000						4.000	0	-4.000																								5261		
5290	2	1	3	0.710	0.121	-0.546						-1.010	0	-2.756	-0.746	0	-1.859									0	-0.553	0	0	-0.420							5290			
5329	1	3	2	0.299	-0.319	-0.697						-0.479	0	1.117													0	1.402	0	-0.742	0	0	0.925	1.092	0.828	0			5329	
5333	2	1	3	-0.240	-1.022	-0.819	-0.497	-0.883	0.860			0.089	0	-0.402	-0.058	0	-0.045	-0.181	0	-0.490						0	0.212	0	-0.677	0	0	0	0.123	-0.023	-1.365	0			5333	
5338	1	3	2	-1.031	-0.914	-0.546						0.865	0	-0.468																									5338	
5352	2	3	1	0.077	0.284	0.365						1.059	0	-0.337			0.091	0	-0.165	-0.185	0	0.383	0	0	0.564	0	2.362	0	0	0	0.576	-0.468	1.362	0			5352			
5419	1	2	3	0.077	-0.478	-0.091	1.024	0.119	0.235			0.219	0	0.317												0	0	-0.200	0	-1.010	0	0	1.120	-0.914	-0.456	0			5419	
5446	3	2	1	-0.556	-1.077	-0.091						0.154	0	-0.533			-0.792	0	-0.328							0	0	-0.024										5446		
5545	1	2	3									4.000	0	4.000																									5545	
5612	3	2	1	1.581	2.625	1.549						-2.562	0	2.017			-2.692	0	0.215	-1.363	0	-0.094	0	0	1.270	0	1.654	0	0	0.576	0.690	-0.355	0				5612			
5615	1	3	2	-0.002	0.611	-0.091						0.607	0	0.709			0.905	0	0.052						0	0.270	0	2.071	0	0	0.305							5615		
5654	2	1	3	0.235	-0.424	-0.273						0.477	0	-0.337													0	0.239	0									5654		
5701	3	1	2	0.789	0.720	0.274																																	5701	
5801	1	2	3	-0.715	-2.765	-1.093						-0.557	0	-0.598	-0.815	0	-2.118																						5801	
5808	1	2	3	-0.604	-0.625	-1.830											-1.756	0	0.014							0	0	0.000	0	-2.126	0								5808	
5883	2	3	1	0.077	-0.587	-0.546						0.800	0	0.382												0	0	0.741	0	-1.094	0	0	0.758						5883	
5950	2	3	1																																				5950	
5993	2	3	1																																					5993
6109	1	3	2	3.164	1.918	0.183																																	6109	
6175	1	3	2	-0.319	-1.785	-0.455						1.188	0	1.820																									6175	
6224	3	1	2	1.976	0.720	1.002						1.447	0	1.951																									6224	
6232	3	1	2	0.235	0.622	-1.493						-0.629	0	-2.926																									6232	
6253	1	2	3									0.865	0	0.382	0.975	0	0.214																						6253	
6258	2	3	1	0.719	0.838	1.278											0.257	0	0.929																				6258	
6265	3	1	2	0.948	4.000	0.547	1.061	-2.121	-1.816			0.089	0	-3.082	0.906	0	2.389	0.701	0	0.485	4.000	0	-1.154	0	0	-0.142	0	0	0	-4.000	-4.000	-3.588	0	0	0	0	0	6265		
6343	1	3	2																																					6343
6352	3	1	2	0.156	-0.260	0.274						0.542	0	-0.860			0.973	0	-0.599							0	-1.094	0	0	0.576	-1.003	-1.365	0					6352		
6368	3	1	2	0.156	0.284	0.274	-0.731	0.417	-0.344			-0.881	0	-1.317			-0.996	0	-3.037	-0.353	0	0.383	0	0	0.564	0	0.405	0	0	0.576	-2.606	-2.072	0				6368			
6421	3	1	2	0.789	0.665	0.274	-0.731	0.485	-1.929			1.253	0	-0.010	1.801	0	1.094	0.837	0	0.865	1.443	0	0.330	0	0	0.506	0	0.572	0	0	-1.236	-0.112	-0.052	0			6421			
6456	2	1	3									-0.363	0	0.186	0.286	0	-0.460	-0.113	0	-0.599					0	0	-0.142											6456		
6490	1	3	2	-0.635	-0.750	-0.273						0.348	0	-0.141			0.498	0	-0.544																					

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